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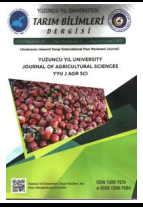

Araştırma Makalesi

Crossing of Alphonse Lavallee and Regent Grape Cultivars for Downy Mildew Resistant Genotypes. 1. Seed Germination and Seedling Growth

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Abstract: Crossing for disease resistance of grape cultivars is one of the main interests of grape breeding. Downy mildew is one of the destructive diseases of grapevines. *V. vinifera* has superior characteristics for wine or fresh consumption but mildew sensitive. *Vinifera* grapevines are cross with American wild grapes to obtain resistant cultivars. But some hybrid grapes have low seed germination. The main goal of this study was to develop hybrid table grape F1 offsprings with resistance to downy mildew disease and to test seed characteristics of them. Alphonse Lavalle (susceptible) and Regent (resistant) grape cultivars were crossed to obtain resistant F1 progenies. Hybrid seeds were soaked in gibberellic acid (GA_3, 1 000 ppm), Benzylaminopurine (BAP, 1 000 ppm) and hydrogen peroxide (H_2O_2, 1 M) solutions and water (control) for 24 hours after stratification (4 months at 5°C) and then sown in Perlite : Peat moss (1:1) potting soil. Germination was carried out in the plastic boxes with constant temperature (27°C) and relative humidity (99%). Total germination ranged from 60.39% to 78.32% in the control and GA3 treated seeds respectively. Also, GA3 significantly increased total germination when compared to the other treatments. Optimization of growth chamber, greenhouse and open field conditions for seed germination and seedling growth accelerated shoot growth up to 2 m in offsprings at the first year.

Mildiyö Hastalığına Dayanıklı genotipler oluşturmak için Alphonse Lavallee ve Regent Üzüm Çeşitlerinin Melezlenmesi.1.Tohum Çimlendirme ve Fide Gelişimi

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Anahtar kelimeler
Islah,
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V.vinifera

Öz: Üzüm ıslahının ilgilendiği ana konulardan biri üzüm çeşitlerinin hastalığa dayanıklılık amacıyla melezlenmesidir. Mildiyö asmaların yıkıcı hastalıklarından biridir. *V. vinifera* şaraplık ve sofralık özellikler açısından üstün olmasına karşın hastalıklara dayanıksızdır. *Vinifera* türüne ait asmalar dayanıklı çeşitler elde etmek amacıyla Amerikan yabani asmaları ile melezlenir. Ancak bazı melez üzümler düşük tohum çimlenmesine sahiptir. Bu çalışmanın ana amacı mildiyö hastalığına dayanıklı F1melez sofralık üzüm dölleri geliştirmek ve bunların tohum özelliklerini incelemektir. Alphonse Lavalle (hassas) ve Regent (dayanıklı) F_1 dölleri elde etmek amacıyla melezlenmiştir. Melez tohumlar katlamayı takiben (5°C da 4 ay) 24 saat süreyle gibberellik asit (GA_3,1 000ppm), benzilaminopurin (BAP,1 000 ppm) hidrojen peroksit (H_2O_2, 1M), ve su (kontrol)'ya batırıldı ve sonra perlit:torf (1:1) harcı içeren saksılara ekildi.

Çimlenme sabit sıcaklık (27ºC) ve nem (%99) içeren plastik kutularda gerçekleştirildi. Toplam çimlenme kontrol ve GA_3 uygulanan tohumlarda sırasıyla %60.39 ve %78.32 olarak değişti. GA_3 toplam çimlenmeyi diğer uygulamalarla karşılaştırıldığında önemli ölçüde arttırdı. Tohum çimlenmesi ve fide gelişimi için gelişme odası, sera ve tarla koşullarının optimizasyonu, melez döllerde ilk yıl sürgün gelişimini 2m'ye kadar çıkarmıştır.

1. Introduction

Turkey is one of the world's largest producers of grape, fifth in the ranking of planted surface area (480.000 ha) and sixth in production of grape (4 million tonnes) in 2016 (Anonym, 2017a). Turkey exports roughly 250 000 ton each of fresh and raisin grapes annually valued totally US$ 600 million (Anonymous, 2017b; Anonymous, 2018).

Mildew diseases, downy and powdery, are the main fungal diseases of grapevines. Breeding of grapevine resistance to downy mildew was initiated in early 19th century. Selected resistant offsprings of *Vitis labrusca* such as Catawba, Concord or Isabella are the pioneer of resistant cultivars. Lately, interspecific hybrid cultivar Regent is getting more popular as wine grape because of broad resistance to downy and powdery mildew diseases. It is among the most important fungal-resistant quality wine grape varieties in the world (Gessler et al., 2011).

Seeds of *Vitis riparia* and *Vitis rupestris* have higher germination percentage than that of *Vitis labrusca* and V*itis vinifera* (Rambough, 2002a). In addition, seeds of early ripening grape cultivars have poor germination rates (Ramming et al., 1990). The higher germination percentage of hybrid grapes decreases the breeding costs while contributing to the overall breeding success. Grape seeds are stratified for 3 months at 5ºC for breaking embryo dormancy. Stratification period can be increased up to 1 year without any deterioration in seeds. Ungerminated seeds can be stratified again (Rombough, 2002a; 2002b).

Growth regulators affect seed germination positively but overdoses have detrimental effects on seeds. Application of growth regulators to the seeds is recommended before or after stratification (Pal et al., 1976; Ergenoğlu et al., 1997; Conner, 2008; Rusdy, 2012; Akkurt et al., 2013;). But a double application of GA3 at the time of before and after stratification have also positive effects on seed germination (Selim et al., 1981). Ellis et al. (1983) found that H2O2 and GA3 have positive effects on seed germination of grapes and 2000 ppm GA3 was the most effective dose for breaking dormancy but caused death of some seeds. Conners (2008), indicated that higher doses than 1 000 ppm GA3 has adverse effects on germination. Grape seeds treated with 1 000 ppm GA3 or combination of H202 (0.5M) +GA3 (1 000ppm) germinated 58% and 63%, respectively. Pall et al. (1976) found that different forms of GA3 had contrastive effects on seed germination and treatment with 1 000 ppm GA4+7 inhibited shoot growth of seedlings. Akkurt et al. (2013) shown that BAP and GA3 applications to Kalecik Karası seeds increased germination up to 67%. Yalvaç (2006) treated grape seeds with 500, 1 000 and 1 500 ppm GA3 but the germination rate never exceeded 10%.

The main goal of this study was to investigate the effects of plant growth regulators on seed germination in plastic boxes of hybrid grapes which is obtained by crossing of Alphonse Lavalle and Regent and followed by seedling growth in the greenhouse and open field.

2. Materials and Methods

In the experiment, hybrid grape seeds obtained from the crossing of Alphonse Lavalle x Regent grape varieties were used. The Alphonse Lavalle variety has seeded, black and large berries. It is a well known table grape cultivar but sensitive to the powdery and downy mildew diseases. The Regent variety is a wine grape cultivar with black, small berries that are resistant to mildew diseases both. The necessary cultivation techniques were carried out to crossed grapevines during the summer months following the crossing in early May, and the grapes were harvested on 08.09.2016 and 20.09.2016, with the grapes being held on the vine until the stage of extreme maturity. In the laboratory, the seeds separated from the fruit flesh were thoroughly washed with the help of a strainer under the running tap water, and the fruit flesh remaining on the seeds was completely cleaned. Seeds

were laid on paper in the laboratory and dried for 1 day at room temperature. The next day, the water floating method was used to separate the seeds with germination capacity. The sinking seeds in the water were considered to be germinating and live embryos. Floaters were thrown away. Live seeds were sprayed with fungicide (50% Captan, dose: 3 g / l). Then, the seeds were put into the refrigerator (5oC, 4 months) by putting the ziplock plastic bags containing perlite moistened with the same fungicide. Seeds were removed from the refrigerator in January and the perlite was cleared away from the seeds. The floating method was used again to determine the seeds that could lose germination properties during folding. Sinking seeds were used in the experiment. Seeds were soaked into different plant growth regulators (PGRs) as GA_3 (1 000 ppm), BAP (1 000 ppm), H_2O_2 (1M), BAP+GA_3 (1 000ppm+1 000ppm), water (control) for 24 hours before sowing in the pots.

2.1. Germination

The seeds were sown in a compacted peat pot (top diameter 6 cm, bottom diameter 3.5 cm, depth 6.5 cm). The pots were filled with sterile peat: perlite mixture in a ratio of 1: 1 (v:v). Seeds are planted to a depth of about 1 cm. Following the planting, the pots were watered with a can and the excess water was drained completely by itself. The pots were then placed in plastic containers (45x65x34 cm). There were 50 pots in each container. The plastic containers were closed and placed in germination chamber with a temperature of 27 °C and a relative humidity of 40–50%. No additional lighting was made in the chamber but heated by electric heaters. Since the relative humidity in the room is very variable during the heating of the chamber, the relative humidity of the pot environment has been tried to be kept constant and high by placing the pots in the plastic containers. Thus, the pot environment was indirectly heated rather than directly heating. The relative humidity in the plastic containers placed in the germination chamber was fixed at 99%, and the chamber temperature and hence the box temperature was fixed at 27 °C. Since the containers and the peat were previously disinfected by the company, no disease was observed in the containers. The seeds were counted every day and germination status was recorded daily. Seeds appearing on the surface of the hypocotyl fold were considered germinated.

2.2. Plant development

The peat pots with germinated seeds in the plastic container were taken to the same size plastic container in the growth chamber. The chamber was illuminated and heated. However, the temperature of this chamber was set at 25-27 ^{o}C and the relative humidity level varied between 40-50%. The lids were kept slightly spaced so that the relative humidity in the containers was always above 90%. Thus, the amount of oxygen needed for plant development was provided. In addition, the plant growth chamber was ventilated 30 minutes a day to provide fresh air intake. LED light bulbs were used to illuminate the chamber (9W Spot LED, 24 pcs / m2). With this lighting system, 317µmol m-2s-1 photosynthetic active radiation (FAR) value was obtained at 10 cm distance from the bulbs. The plants were lighted for 16 hours of light + 8 hours of darkness. The plants were left in the containers until 2-3 true leaves emerged and then transferred to small plastic nursery bags (10x21cm) containing peat: perlite (1: 1; v: v) on 15 February 2017. The plants were kept in these bags and in the growth chamber for 2 weeks, followed by 1 week in a bright adaptation room. The plants were transferred to the greenhouse on March 7, 2017. The greenhouse was heated so that the temperature did not fall below 15°C. on May 9, 2017, the plants were transferred to larger plastic nursery bags (12x32 cm) which were filled in equal volume of media; peat: perlite:soil:manure (goat). They placed to a shaded area at outside of the greenhouse. These bags Since the plants were transferred into small bags, drip irrigation fertilizer [16 + 8 + 24 + 2MgO + TE (N + P + K + Mg + trace elements)] with a dose of 300 ppm (50 ml for each pot) was applied monthly. All bags were watered twice a week. Plants were not sprayed with any fungicides.

2.3. Germination parameters

Percentage of germination (%): Number of germinated seeds / number of seeds multiplied by x100 (Rusdy, 2012).

Germination period (days): Time between first germination and last germination (Rusdy, 2012).

2.4. Statistical analysis

Data were analyzed as a completely randomized plot design with three replications containing 50 seeds each. Percentage data was arcsine-square root transformed for statistical analysis, but raw data is reported. Statistical analysis was performed by the MINITAB software program. The significance of differences between the variants was evaluated by Tukey test.

3. Results

3.1. Properties of hybrid seeds

Hybrid grapes were harvested at two different times (8 and 20 September 2017) which are later than at normal maturity. The dates represent a period about 30-40 days later than the normal harvest date of the Alphonse Lavallee grape variety. The reason for this was to allow the endosperm and the embryo in the seeds to develop better. However, the vast majority of grapes (80.2%) were harvested on September 8, 2017. A total of 1 777 seeds were removed from 901 grape berries. In this case, about 2 seeds per berry were obtained. While 20% of the seeds floated on water, most of them (80%) sank in water (Table 1).

Table 1. Characteristics of seeds obtained by crossing.

Characteristics	First harvest (08 September 2016)	Second harvest (20 September 2016)	Total	Average
No. of hybrid berries	723	178	901	-
No. of hybrid seeds	1404	373	1777	-
No. of seed per berry	1.95	2.10	-	2.03
No. of sunk seeds	1106	302	1408	-
No. of floated seeds	298	71	369	-
Sinking seeds rate (%)	78.8	80.97	-	79.89
Floating seed rate (%)	21.2	19.03	-	20.11

3.2. Germination characteristics

During cold stratification, only a small proportion of the seeds germinated (2%) or cracked the testa (2,3%). However, the vast majority (95,7%) remain ungerminated. All of the ungerminated seeds sank in water (Table 2).

The seeds in the germination chamber began to germinate 11 days after sowing. The highest percentage of germination among the seeds treated with different PGRs was obtained from seeds applied GA3 (78.32%). The GA3 application significantly increased germination rate compared to other PGRs and control. No statistically significant difference was found between the other PGRs applications and the control application. The germination rate, which was 60.39% in control, increased to 64.35% in BAP application. There was no difference between applications in terms of germination period. The germination period varied between 11–18 days (Table 3).

Table 2. Characteristics of seeds obtained by crossing.

Characteristics	Amount (No.)	Rate (%)
No. of germinated seeds	28	2
No. of ungerminated but cracked seeds	32	2.3
No. of ungerninated seeds	1360	95.7
No. of sinked seeds	1360	100
No. of floated seeds	0	0

Table 3. Effects of PGRs on germination characteristics of hybrid seeds.

Treatments	Germination rate (%)	Germination period (day)
GA_3	78.32±7.52 a*	11.50±1.50
BAP	64.35±3.20 b	15.32±2.89
H_2O_2	62.00±0.80 b	12.67±3.51
BAP+GA_3	60.67 ±3.91 b	11.00±5.20
Water (Control)	60.39±3.18 b	18.00±1.73

*Means in the same column followed by the same letters were not significantly different at 0.05 level using Tukey test.

3.3. Shoot length

About 4 months after the transplanting of the plants, 41.64% of the plants reached shoot length up to 52 cm. Others had longer shoots. However, as the shoot length increased, the number of plants entering each class gradually decreased. It was found that some seedlings formed shoots more than 2 m but in small amounts (Table 4). In PGR applications, no abnormalities such as poor shoot growth or stopping of shoot growth have been observed in large part in terms of shoot development.

Table 4. Shoot lengths of hybrid plants (15.07.2017).

Class	Shoot lengt (cm)	Amount of plant (No.)	Rate (%)
1	3–52	296	41.64
2	53–102	258	35.61
3	103–152	108	15.20
4	153–202	44	6.17
5	202<	4	1.38

4. Discussions and conclusions

It is stated by Rombough (2002b) that the ratio of floating and sinking seeds in water differs according to species and varieties. The percentage of germination obtained with GA3 application in hybrid seeds can be considered quite high when compared to previous studies. In this study. the percentage of high germination obtained by GA3 application is significantly higher than that of germination rates of up to 66% in previous studies (Ergenoğlu et al.. 1997; Yalvaç. 2006; Conner. 2008; Akkurt et al.. 2013). The high germination percentage of seeds is thought to originate from the high (99%) and constant humidity provided in plastic containers during germination. In addition. the indirect heating process inside the chamber provided that the temperature and the humidity around the seeds were more stable. which was considered to have a positive effect on germination. However. since this environment is also ideal for the development of microorganisms. the materials and containers must be previously disinfected. The shoot lengths obtained are quite longer than that of Pal et al. (1976). as compared to the shoot length of 4.5 cm. Increasing the germination percentage of hybrid grape seeds is an important factor affecting the success and economics of breeding programs. In this respect. a simple. practical and effective seedling growth method has been developed. Hybrid grape seeds were soaked in GA3 solution at 1 000 ppm for 24 hours after stratification for 4 months at 5 °C in moist perlite. The seeds were then seeded in peat pots and germinated at 27 °C in plastic containers in the germination chamber. A very high germination percentage of up to 78% was detected with the GA3 application. Germinated plants were put in similar plastic containers and developed under light in growth chamber. The relative humidity in both plastic containers was 99%. Later. the seedlings were taken to the greenhouse and following to the shade place in the open field. They had long shoots with about 2 meters in the middle of the summer of the same year. We conclude that a constant combination of air temperature of 25–27 °C and high air humidity resulted in a positive impact on germination. Furthermore. 1 000 ppm GA3 application to the seeds after stratification significantly increased germination.

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