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Effects of Some Treatments Prior to Stratification on Germination in Kalecik Karası *(Vitis vinifera L.)* Seeds

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ABSTRACT: Kalecik Karası cv. (*Vitis vinifera* L.) is one of the main grapevine cultivars in Turkey that is commonly used in breeding programs. However, low germination of seeds is crucial problem for breeding programs. In order to increase the germination ratio of Kalecik Karası cv. seeds, they were stratified at +4°C for 60 and 90 days following some pre-treatments with growth regulators such as benzylaminopurine (BAP), gibberellic acid (GA_3), BAP + GA_3, and hydrogen peroxide (H_2O_2). Maximum germination ratio (66.67%) for 60-day stratification was recorded after pre-treatment with 1 g/l BAP + 3 g/l GA_3. In addition, for 90-day stratification, maximum germination ratio was also observed 64% after pre-treatment with 0.5 g/l BAP + 2 g/l GA_3. According to the results, the highest germination ratio in Kalecik Karası cv. seeds was realized in 0.5-1 g/L BAP + 2-3 g/L GA_3 treatments.

Keywords: Benzylaminopurine, gibberellic acid, hydrogen peroxide, stratification, germination

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Kalecik Karası *(Vitis vinifera L.)* Tohumlarında Katlama Öncesi Bazı Uygulamaların Çimlenme Üzerine Etkisi

ÖZET: Kalecik Karası, (*Vitis Vinifera* L.) Türkiye'de ıslah programlarında yaygın olarak kullanılan üzüm çeşitlerinden birisidir. Ancak, tohumlardaki düşük çimlenme oranı, ıslah çalışmalarında önemli bir sorundur. Kalecik Karası tohumlarında çimlenme oranını artırmak için tohumlar; benzilaminopürin (BAP), Gibberellik asit (GA_3), BAP+GA_3 ve Hidrojen peroksit (H_2O_2) gibi büyümeyi düzenleyici maddeler ile ön muamele yapıldıktan sonra +4°C de 60 ve 90 gün süreyle katlamaya tabi tutulmuştur. En yüksek çimlenme oranı (%66.67) 1 g/l BAP + 3 g/l GA_3 uygulaması ve 60 gün katlama süresinde elde edilmiştir. Bunun yanı sıra, 90 gün katlama süresinde ise en yüksek çimlenme oranı %64 ile 0.5 g/l BAP+2 g/l GA_3 uygulamasında elde edilmiştir. Bu sonuçlara göre, Kalecik Karası tohumlarında en yüksek çimlenme oranı 0.5-1 g/l BAP + 2-3 g/l GA_3 uygulamalarında gerçekleşmiştir.

Anahtar Kelimeler: Benzilaminopürin, giberalik asit, hidrojen peroksit, katlama, çimlenme

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INTRODUCTION

Grape (*Vitis* spp) is one of the most important fruits grown economically in the world because of its use in several ways such as wine, table and *raisin*. However, an increase in production requires for breeding practices to meet the recent world demand on grape. Therefore, improvement of new and more superior grape cultivars resistant to biotic and abiotic stress factors has become quite popular. Thus, most of researchers have aimed to improve resistant grape cultivar since the second half of 19th century. However, breeding efforts to generate new cultivars require *expensive* practices and intensive labor for many years.

Grapevine is mostly vegetative propagated *because* of segregation in generative propagation. However, generative reproduction is one of the indispensable methods in viticulture with regard to breeding studies. Grapevine seeds germinate with some difficulties. These difficulties are occurred especially in breeding studies. Therefore, determination of the practices that induce the germination ratio is quite important. In addition, germination capacities of hybrids (F_1) seeds also play an important role in the achievement of the cross-breeding studies.

Seed dormancy can be simply defined as a block to the completion of germination of seeds under favorable conditions. For germination of seeds in grape breeding programs, it is required to break seed dormancy. Main factors causing dormancy in seeds can be summarized as physical (mechanic) factors in the seed structure, internal (biochemical) factors within the seeds and external (environmental) factors outside the seeds. In general, folding, wetting, using of growth regulating substances, washing, drying, temperature, light, fight against oxidants, mechanical and acidic abrasion and combination of one or more of them are utilized to break dormancy (Yalvaç, 2006). This present study aims to determine effects of 16 pre-treatments and two (60 and 90 day) stratification periods to increase germination capability in 'Kalecik Karası' cv. seeds.

MATERIAL AND METHOD

Plant material and experimental design: In this research, 'Kalecik Karası' cv. seeds were used for all treatments. The seeds were obtained from harvested grapes, then cleared of fruit flesh, and later washed and float checked. The seeds were dried in the shade after treatment against to fungal infections. Dried seeds were stored in plastic bags at room temperature until pre-treatment. Then seeds were exposed to the following pre-treatments before stratification:

1) 24-h soak in 1 M H_2O_2

2) 24-h soak in 0.5, 1.0, and 2.0 g/l of BAP

3) 24-h soak in 1, 2, and 3 g/l of GA_3;

4) 24-h soak different doses BAP in combination with GA_3 (0.5 g/l BAP + 1.0 g/l GA_3, 1 g/l BAP + 1 g/l GA_3, 2 g/l BAP + 1 g/l GA_3, 0.5 g/l BAP + 2.0 g/l GA_3, 1 g/l BAP + 2 g/l GA_3, 2 g/l BAP + 2 g/l GA_3, 0.5 g/l BAP + 3 g/l GA_3, 1 g/l BAP + 3 g/l GA_3, 2 g/l BAP + 3 g/l GA_3).

Each treatment was performed 3 times using 50 seeds in each replication. Seeds were subjected to 200 ml of corresponding pre-treatment solution. Thereafter the seeds were rinsed three times with sterilized bidistilled water, and stratified in humid and sterile sand in an incubator at +4°C for 60 and 90-day periods.

After stratification, the seeds were washed and planted in 48×72×5 cm boxes containing germination medium of turf, vermiculite and perlite (1:1:1). Germination was realized under growth chamber conditions (28 ± 2°C temperature and 65% humidity). After 6-9 weeks, numbers of germinated seeds for each treatment were recorded. The indication of germinated seed was determined as the cotyledons emerging through the medium.

***Statistical analysis*:** Descriptive statistics were presented as mean and standard error of mean. After arcsin transformation for providing normal distribution, Two-way Factorial ANOVA was performed to determine differences among means of treatments and stratified periods, After Factorial ANOVA, Tukey multiple comparison test was carried out to determine different treatments. Statistical significant level was considered as 5% and SPSS (ver: 13) was used for all statistical computations.

RESULTS AND DISCUSSION

Dormancy breaking applications are very important to overcome the dormancy of grape seeds. In the literature, there are several reports that mention use of pre-chilling under cool conditions, and various chemicals for breaking the grape seed dormancy (Yeh et al., 1990; Ergenoğlu et al., 1997; Chuanli and Jing, 1999).

The data represent the pre-treatment effects of benzylaminopurine (BAP), gibberellic acid (GA_3), the combination of both at different doses and hydrogen peroxide (H_2O_2) applications to increase the germination ratio of 'Kalecik Karası' cv seeds (Table 1). According to results of ANOVA, statistically significant differences were found among some pre-treatments for 60 and 90 day stratified seeds ($p<0.05$).

Table 1. Descriptive statistics and comparison results for pre-treatments in 'Kalecik Karası' cv. (*Vitis vinifera* L.) seeds stratified for 60-90 day periods

Pre-treatments	Germination rate (%)	
	60 day	90 day
	Mean ± SEM	Mean ± SEM
Pure water (control)	12.00 ± 2.31 c #	37.33 ± 2.67 abcd
0.5 g/l BAP	54.67 ± 4.81 a	53.30 ± 11.90 ab
1 g/l BAP	52.00 ± 6.93 ab	54.67 ± 7.06 ab
2 g/l BAP	45.33 ± 4.81 abc	52.00 ± 4.00 abc
1 g/l GA_3	48.00 ± 1.01 ab #	18.67 ± 1.33 cd
2 g/l GA_3	41.33 ± 4.81 abc	24.00 ± 4.00 bcd
3 g/l GA_3	52.00 ± 6.11 ab #	21.98 ± 2.67 cd
0.5 g/l BAP + 1 g/ l GA_3	45.33 ± 1.33 abc #	29.33 ± 1.33 bcd
1 g/l BAP + 1 g/l GA_3	40.00 ± 8.33 abc	45.33 ± 3.53 abc
2 g/l BAP + 1 g/l GA_3	21.33 ± 1.33 bc	36.00 ± 6.93 abcd
0.5 g/l BAP + 2 g/l GA_3	49.33 ± 7.42 ab	64.00 ± 6.11 a
1 g/l BAP + 2 g/l GA_3	42.70 ± 9.10 abc	36.00 ± 0.00 abcd
2 g/l BAP + 2 g/l GA_3	36.00 ± 4.62 abc	34.67 ± 7.42 abcd
0.5 g/l BAP + 3 g/l GA_3	62.67 ± 3.53 a #	33.33 ± 7.06 abcd
1 g/l BAP + 3 g/l GA_3	66.67 ± 5.81 a	46.67 ± 5.81 abc
2 g/l BAP + 3 g/l GA_3	61.33 ± 3.53 a #	28.00 ± 8.11 bcd
1 M H_2O_2	41.33 ± 3.53 abc	48.00 ± 9.24 abc

Different lower cases in each column represent different pre-treatments ($p<0.05$)
#: Significant difference from 90 day

SEM: Standard Error of Mean

The seed coat is likely to have an effect on preventing germination in *Vitis* seeds. In order to overcome this, in our research, 1 M hydrogen peroxide (H_2O_2) was used as a pre-treatment before stratification as well. Our results showed that germination ratio increased from 12% (control) to 41.33% (1 M H_2O_2 pre-treatment) after 60 day stratification and from 37.33 % (control) to 48% (1 M H_2O_2 pre-treatment) after 90 day stratification. Moreover, for 60-day stratification, the highest germination rate was recorded as 66.67% in the pre-treated with "1 g/l BAP + 3 g/l GA_3" followed by "0.5 g/l BAP + 3 g/l GA_3" (62.67%) and "2 g/l BAP + 3 g/l GA_3" (61.33%). Similarly, at 90-day stratification, the highest germination rate was observed as 64.00% in "0.5 g/l BAP + 2 g/l GA_3" pre-treatment and followed by "1 g/l BAP" with 54.67%. After 60-day stratification, germination rate was increased from 12% (control) to 66.67% using 1 g/l BAP + 3 g/l GA_3 pre-treatment. Although these increases are not statistically significant, it can be state that pre-treatments cause tendency to increase of germination ratio.

Altuntoprak (1999) noted that germination rate of 'Kalecik Karası' cv. seeds were found 43.1% after 90 day stratification at 5°C. Likewise, Conner (2008) reported that maximum germination rate was obtained from the seeds that after 90-day stratification at 4°C in Muscadine (*Vitis rotundifolia*) grapes. Similarly, Forlani and Coppola (1977) noted that in Cabernet Franc with cold stratification, germination rate in-

creased from 29.4% to 42.4%. Furthermore, Pommer *et al*., (1988) studied the combination of 4, 13, 32, and 60 stratification periods with different GA_3 and emphasized that these treatments caused maximum germination rate in some treatments 32-day and over stratification periods. In the same way, ChiaWei and ShyiKuan (2003) noted that maximum germination rate was observed after 16-week stratification at 5°C in Kyoho grapes.

Several studies (Burrows, 1994; Ergenoğlu et al., 1997; ChiaWie and ShyiKuan, 2003; XueJun et al., 2010) about effect of gibberellic acid on germination rate have been mentioned positive effects of gibberellic acid. However, we are unable to find any research that aimed to determine increasing effects of treated by BAP (synthetic cytokinin) or combination with GA_3 (synthetic gibberellin) on germination rate in *Vitis* seeds. In our research, as compared with control, BAP + GA_3 combination provided higher germination rate in *Vitis* seeds after both 60 and 90 day stratification period. Similarly, Terzi and Kocaçalışkan (2010) reported that the most effective treatment in tomato and wheat was GA_3 + KIN combination.

Gibberellins regulate growth and influence various developmental processes such as elongation, germination, dormancy and flowering. Cytokinins are also promote cell division, stimulate shoot proliferation, activate gene expression and metabolic activity. Gan et al (2007) noted that gibberellins and cytokinins act antagonistically in leaf formation and meristem maintenance.

Similarly, David and Ori (2007) mentioned that GA and ABA play antagonistic roles in the regulation of numerous developmental processes. Whereas, GA is associated with the promotion of germination, growth, and flowering, ABA inhibits these processes. Furthermore, Razem et al. (2006) emphasized that the antagonistic relationship and the ratio between these two hormones regulate the transition from embryogenesis to seed germination. However, it is not clear whether there is antagonistic effect for the germination of grape seeds.

Effects of some treatments such as soaking in still water, soak in running water, peeling, sulphuric acid treatment were investigated by various researchers (Ellis et al., 1983; ChiaWei and ShyiKuan, 2003; Conner, 2008) and the reported results are in agreement with our study in that 1 M H_2O_2 treatment had an additive effect on germination rate after 60 day and 90 day stratification period. Similarly Ellis et al. (1983) specified that 0.5 M H_2O_2 as an additional pre-treatment increased the effect of GA_3. Furthermore, Conner (2008) indicated that H_2O_2 encourages the germination rate in *Vitis* seeds. This phenomenon is likely to result from the thinning effect of the seed coat (Chien and Lin, 1994; Keeley and Fotteringham, 1998) or the oxidant effect on germination inhibitors (Ogawa and Iwabuchi 2001). Recent researches have revealed the effect of H_2O_2 on plant cells more clearly. Liu et al., (2010) suggested that H_2O_2 is a signaling molecule in plant cells effective on dormancy and germination by providing a regulatory effect on abscisic acid (ABA) and GA_3 metabolism during water intake in Arabidopsis seeds. In addition, they reported that ABA and GA_3 concentrations were negatively correlated with the germination and dormancy period. Barba-Espin et al., (2010) noted that while there is a strong correlation between H_2O_2 and plant growth in pea seeds, H_2O_2 leads to decrease in ABA and Zeatin Ribozit (ZR) concentration of the cell.

CONCLUSION

As a result, it was found that a GA_3 and BAP combination (0.5-1 g/l BAP + 2-3 g/l GA_3) has a significant effect for increasing of germination rate in 'Kalecik Karası' cv. This significant effect can be valuable for plant breeders. In addition, these results can be transferred to other *Vitis vinifera* cultivars and considered to future studies.

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