Developing of Seedless Grape Varieties

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Abstract. In this study, it was planned to identify the seedless genotypes by screening with molecular markers for early selection among the F1 genotypes obtained in the hybridization study. In addition, it was aimed to determine the appropriate sampling time by applying the embryo rescue technique for F1 genotypes obtained from these combinations in which seedless varieties were used as the female parent. When the conversion rates into plants were examined, 50% success was achieved in the Yalova Seedless X Glenora combination, while this rate was 62.5% in the Yalova Seedless X Philipp combination. In the study, F1 genotypes obtained from these were screened with the *VvAGL11* marker. When the appropriate sampling times were examined, while the 8th week was prominent in the Yalova Seedless X Philipp combination, the best results were obtained from the samples taken at the 9th week in the Yalova Seedless X Glenora combination.

1 Introduction

Grape cultivation is one of the most widespread and lucrative agricultural practices worldwide. It occurs across several continents, from the temperate regions of Europe and the Americas to the landscapes of the Middle East and Australia. In essence, grape production is an integral part of the global agricultural fabric. Grapes are cultivated for diverse uses, including fresh consumption (table grapes), dried fruit (raisins), juice production, and wine production. The global grape production reached an astonishing 73 million tons. Asia, Europe, and the Americas are the principal grape-growing regions, with China, Italy, Spain, the United States, France and Türkiye leading the charge. When the grape area in the world are examined, Spain ranks first with approximately 930k hectares. Turkey, on the other hand, ranks 5th after France, Italy and China with an area of 390 hectares. Likewise, when analyzed in terms of production amount, China ranks first with 11.2 million tons of production. Türkiye ranks 6th after France with 3.6 million tons of production [1]. Grapes are a popular fruit consumed worldwide for their sweet taste, variability, and nutritional value. Seedless has been one of the most valuable quality attributes in grapevines for direct consumption as fresh fruit or raisins. Seedless grapes have become increasingly popular over the years [2]. In recent years, it is aimed to obtain large and high quality seedless varieties in breeding programs. In this context, studies are carried out for early selection of seedless grape varieties with mapping studies based on molecular marker systems instead of classical breeding methods that take a long time [3]. In this study, it was planned to identify the seedless genotypes by screening with molecular markers for early selection among the F1 genotypes obtained in the hybridization study. In addition, it was aimed to determine the appropriate sampling time by applying the embryo rescue technique for F1 genotypes obtained from the combinations of seedless varieties used as female parent.

2 Material

2.1 Plant materials

The *V. vinifera* L. “Yalova Seedless” grape ripens during mid-season; has the berry is oval, white, large (4-5 gr.) and thin-shelled. The clusters of this grape, which is seedless (stenospermocarpic), are medium-large (250-300 gr), conical and medium-firm [4]. Glenora has is blue-black skin. The flesh is melting and the flavor delicate, sweet, refreshing, and not noticeably labrusca in character. Glenora fruits respond very favorably to gibberellin treatment [5]. Philipp’s berries are relatively firmly attached to the structure. The dark blue berries color very well. The resistance properties against powdery mildew diseases are good, the variety shows a strong growth pattern. If the berries are overripe, however, they can easily burst, the berry flesh becomes tender and quickly loses the fruit acid, so timely harvesting is necessary [6].

2.2 Molecular Marker

Meija et al. stated that the *VvAGL11* gene may be the gene responsible for seedlessness in table grape varieties. Accordingly this, researchers stated that the *VvAGL11*

(F:5'CTCCCTTTCCCTCTCCCTCT-3; R:5'AAACGCG TATCCCAATGAAG-3') marker can be used to determine seedlessness [7].

2.3 E20A Media for Embryo Rescue

E20A medium was realized as stated in the study of Tangolar et al. in 1998 [8].

3 Method

3.1 Hybridization

Emasculation was performed 2-3 days before female flowers became receptive. After that, they were placed in breathable bags to prevent unwanted pollination. The previously collected and prepared pollen was pollinated 3 times every two days after the flowers became receptive.

3.2 Embryo rescue

Samples were taken for embryo rescue studies from the hybridized clusters 6th, 7th, 8th, 9th and 10th weeks after pollination. Embryo rescue studies were carried out as planned in the hybridizations. After sterilization, the ovules have been isolated and planted in E20A nutrient medium. Embryos were carried out in a climate room controlled at 25±1°C, in a photoperiod of 8 hours dark and 16 hours light, under lamps giving 4000 lux luminous intensity.

3.3 DNA Isolation and PCR

After the developing plants have formed 4-5 true leaves, DNA samples were taken from the fresh leaves on the shoot tip. DNA isolation was done by the method specified in QIAGEN Plant DNeasy kit. The quality and densities of the obtained DNAs were measured by nanodrop spectrophotometer (IMPLEN GMBH, NanoPhotometer N60 Touch). Then, PCR (BIO-RAD, T100 Thermal Cycler) was performed with the protocol specified for the *VvAGL11* marker. After the band images were obtained in gel electrophoresis, it was stated that the genotypes giving the same band as the reference cultivar could be seedless. The cycling profile was an initial heat activation step at 94°C for 3 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s, and a final extension at 72°C for 4 min [9]. The obtained PCR products were visualized with an imaging device (Vilber Loumart, 24 rue de LAMIRAULT).

4 Results and Discussion

As a result of the studies, 16 seeds at the 6th week, 31 seeds at the 7th week, 124 seeds at the 8th week, 60 seeds at the 9th week and 37 seeds at the 10th week were obtained from the Yalova Seedless X Glenora crossbreeding. While there were no embryos that turned into plants at 6th and 7th weeks, 1 plant was obtained at 8th week, 4 plants at 9th week, and 1 plant at 10th week.

In the Yalova Seedless X Philipp combination, 12 seeds were obtained in the 6th week, 110 seeds in the 7th week, 87 seeds in the 8th week, 52 seeds in the 9th week and 24 seeds in the 10th week. While there were no embryos that turned into plants at 6th, 9th and 10th weeks in this combination 1 plant was obtained at 7th week, 7 plants in 8th week.

In 2003, Liu et al., determined the best transformation from ovule to embryo occurred in the samples taken on the 60th and 70th days [10]. In the same way, study conducted by Li et al. in 2020, they hybridized the Ruby seedless grape variety with both itself (55 DAF) and the flame seedless (60 DAF) variety and tried to determine the most appropriate sampling time [11]. In this study, the highest rates were reached in the samples taken between 56 and 64 days. The data show parallelism in this context.

The genotypes directly affect the plant conversion rates. While Ji et al. 2013 used the same genotype as female parent, they used two different varieties as pollinators. (DA7 X Flame seedless = No plant formation DA7 X Jingyou (seeded)= % 15.7 Plant Growth) [12]. In this study, when the conversion rates to the plant were examined, the number of embryos that turned into plants was higher in the combination pollinated with the seeded variety Philipp (2.7%). On the other hand, this rate was 2.2% in the Yalova Seedless X Glenora combination.

Meija et al. [7] used the *VvAGL11* marker for the determination of seedlessness as in this study.

In the study conducted by Ulaş et al. in 2015, samples were taken 7th, 8th, 9th and 10th weeks after crossbreeding and placed in E20A nutrient medium. Sultan 7 X Black Kishmis =1% [13]. In this study, the performance of the E20A media was in parallel with the other study (Yalova Seedless X Glenora = 2.2% Yalova Seedless X Philipp = 2.8%).

The highest rate for the Yalova Seedless X Philipp combination is obtained from the samples taken at the 8th week, while the samples taken at the 9th week for the Yalova Seedless X Glenora combination stand out. And 4 genotype in Yalova Seedless X Philipp combination which have may be seedless after the electrophoresis image have been examined.

On the other hand, in the Yalova Seedless Glenora combination, it was observed that 2 out of 4 plants from the samples taken 9th weeks after pollination might be seedless.

5 Conclusion

The rate of conversion to plant in E20A medium was quite low. In some studies, this rate was increased with different media used. In the future, the exact efficiency of Genotypes can be determined by more extensive studies by conducting media trials. While these studies are carried out in regions where the severity of the disease is intense, it will be important to determine alternative control methods and to make a more effective disease

control, in terms of minimizing contamination in the laboratory environment. The development of marker systems may allow for more effective future breeding studies. The demand for new seedless grape varieties consumed as table in our country and in the world is increasing day by day. This demand has led to an increase in the number of breeding studies for the development of new seedless table grape varieties. Selection studies with classical breeding methods are less effective in terms of time and labor, and it can take 4-5 years for a hybrid genotype to be examined morphologically. Stenospermocarpic cultivars are effective to obtain seedlessness, it is possible to use them effectively in breeding studies thanks to embrio rescue technique. The long and costly nature of the classical breeding methods generally used in breeding studies has led the breeders to different methods where they can get better results in a shorter time. The most important of these is selection based on markers. By using different methods with previously determined markers, it can be determined whether genotypes carry those genes at the molecular level. With the marker-assisted selections researchers can evaluate a large number of hybrid genotypes in a short time. They can be successful in a shorter time in the studies of obtaining seedless varieties.

As a result of the studies carried out, it has been determined that it has been revealed that classical breeding studies should be supported by biotechnological methods such as molecular markers.

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