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The success of *in vitro* embryo rescue technique in hybridization of seedless grape varieties

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Abstract. In this study six seedless grape cultivars (Crimson Seedless, Sultan 7, Black Kishmish, Early Superior, White Kishmish and Sultana) were hybridized to evaluate the effects of genotype and sampling time on embryo survival. After 7, 8, 9 and 10 weeks after hybridization ovules were excised and cultured on E20A solid medium with PVP. Embryos were rescued 12 weeks after ovule inoculation. The percentage of embryos and transplantable plants were recorded. The highest percentage of embryo (64 %) was obtained from Crimson Seedless × Sultan 7 combination, while Sultan 7 × Black Kishmish combination gave the lowest (1%). The ovule excision time and genotype were important for the success of embryo viability. 243 plantlets were obtained from a total of 845 cultured ovules, from which 98 plantlets were transferred to acclimatization environment.

1. Introduction

Viticulture has been doing for almost 3500 years and there are a huge number of varieties of *Vitis vinifera* in Anatolia [1]. Within this diversity there are many individuals which featured with their special characters like seedlessness, stress resistance, earliness, different quality parameters and so on. These characters could be used for breeding purposes to develop new varieties. Seedlessness is one of the desired features and could be obtained by classical breeding methods or selection. On the other hand it is possible to achieve this goal by hybridizing two seedless cultivars. Most of the seedless grape varieties are stenospermocarpic and seed abortion occurs after fertilization primarily because the endosperm fails to develop properly [2]. This is a handicap for developing new seedless varieties. Embryo rescue technique besides to its wide range of applications [3] is used to overcome this problem. By using this technique, the percentage of seedless individuals in F1 population has raised up to 44–94% [4–6]. Embryo development depends on many factors like plant genotype, ovule size, stage of embryo, sampling time, medium composition, growth conditions [3].

In this study it was aimed to evaluate the success of embryo rescue technique for the varieties used. Ovule culture, embryo rescue and plantlets production steps were observed and each successive production was recorded.

2. Material and method

This study has been conducted in Manisa Viticulture Research Station vineyards. Six seedless grape varieties of *V. vinifera* have been used for hybridization. These were Sultana, Sultan 7 (newly registered variety obtained by selection from Sultana), Crimson Seedless, Black Kishmish, Early Superior and White Kishmish.

Hybridization procedures took place in May 18–25, 2015. The clusters were collected for ovule excision 7, 8, 9 and 10 weeks after pollination. Surface sterilization of the berries was done as 20 min washing under tap water, 1 min in 70% ethanol and 10 min in 20% sodium hypochlorite with a few drops of Tween-20. After 3 times rinse with sterile water, berries were cut longitudinally, ovules were excised and cultured on E20A solid medium with 0.01 mg l^{-1} IAA, 2% (*w/v*) sucrose, 8% (*w/v*) agar [7] and 10 mg l^{-1} PVP. pH was adjusted to 5.9 before autoclaving. Cultures were transferred in an incubation room at 25°C and 16 h photoperiod for twelve weeks. Embryos were excised from the ovules under stereomicroscope and cultured on E20A solid medium. Embryo germination occurred within

Table1. Grape cultivars (all *Vitis vinifera* L.).

Cultivar	Characteristics*
Sultana	Small, green-yellow, ellipsoidal berries, thin skin, large and compact clusters, ripening at midseason.
Sultan 7	Small, green-yellow, ellipsoidal berries, thin skin, large and compact clusters, ripening at midseason, higher yield than Sultana.
Crimson Seedless	Red, medium sized, elongated berries, large, compact clusters, ripening at late season.
Early Superior	Medium, green-yellow, ellipsoidal berries, ripening at early season.
White Kishmish	Medium, green-yellow, ellipsoidal berries, medium and conical clusters, ripening at midseason.
Black Kishmish	Small, black berries, medium and conical clusters, ripening at midseason.

*Refer to [9].

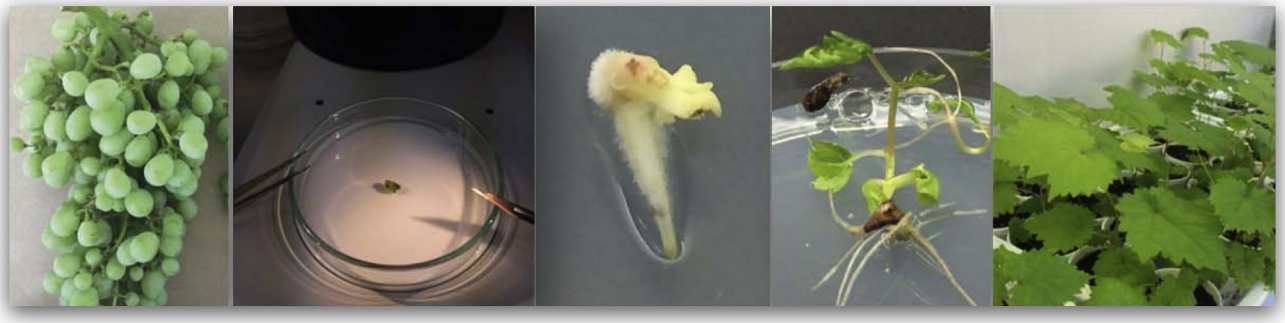

Figure 1. Embryo rescue procedure of seedless grape hybridization.

6–8 weeks and the young plantlets were transferred to MS [8] hormone free medium. After 6 weeks plantlets were transplanted to small pots full with a synthetic soil matrix (perlite/turf 1:2) and transferred to acclimation room with 25°C, 16 h photoperiod and 80% humidity (Fig. 1).

3. Results and discussion

In this study total 845 ovules were excised and 98 hybrid plants were obtained (Table 2). Five combinations were evaluated for their sampling time and embryo formation efficiency. It is important to determine the sampling time for each cultivar as it has a significant effect on embryo formation. Embryos are aborted if sampling time is too late and it is hard to rescue if sampling time is too early [10]. The highest percentage of embryo formation (64%) was obtained in Crimson Seedless × Sultan 7 combination. The embryo formation percentage of Crimson Seedless × Black Kishmish combination is 39%, White Kishmish × Sultana is 53%, Early Superior × Sultana is

Table 2. Embryo rescues success for combinations and sampling times.

Combination	Week	Number of Excised Ovule	Number of Excised Embryo	Number of Germinated Embryo		Number of Plantlets	Number of Acclimated Plants	Embryo Percentage from Ovules	Plant Percentage from Ovules	Percentage of Acclimated Plants
				Pre	After					
Crimson Seedless × Sultan 7	7	24	3	1	1	7	0	17	29	0
	8	35	15	2	7	5	4	49	14	11
	9	89	48	3	12	26	17	57	29	19
	10	98	76	10	40	25	20	88	26	20
	Total	**246**	**142**	**16**	**60**	**63**	**41**	**64**	**26**	**17**
Crimson Seedless × Black Kishmish	7	37	4	0	0	0	0	11	0	0
	8	34	12	0	2	11	2	35	32	6
	9	44	16	0	4	9	2	36	20	5
	10	28	20	4	5	12	5	86	43	18
	Total	**143**	**52**	**4**	**11**	**32**	**9**	**39**	**22**	**6**
Sultan 7 × Black Kishmish	7	31	0	0	0	0	0	0	0	0
	9	28	1	0	1	1	1	4	4	4
	10	30	0	0	0	0	0	0	0	0
	Total	**89**	**1**	**0**	**1**	**1**	**1**	**1**	**1**	**1**
Early Superior × Sultana	7	20	2	5	0	3	1	10	15	5
	8	106	11	1	0	11	7	10	10	7
	9	26	4	0	0	3	2	16	12	8
	Total	**152**	**17**	**6**	**0**	**17**	**10**	**15**	**11**	**7**
White Kishmish × Sultana	7	66	33	5	8	37	12	58	66	18
	8	104	33	23	12	63	17	54	61	16
	9	45	21	0	11	30	8	46	67	28
	Total	**215**	**87**	**28**	**31**	**130**	**37**	**53**	**60**	**17**

11% and Sultan 7 × Black Kishmish is 1% average of all sampling times. The number of germinating ovules before embryo rescue is also included in these percentages. Some of the embryos germinated but became abnormal and did not develop properly (data not shown). Gray et al. [11] and Baharathy et al. [12] also reported the same result but on the medium containing BA (1 μM). Here we used low concentration of plant growth regulator to ensure the genetic diversity as suggested by Burger and Goussard [13]. Among the combinations where Crimson Seedless was female parent, embryo formation rate was 17% and 11% at 7th week as similar to Li et al. [14]. 10th week gave the highest result in respect of embryo yield (88% Crimson Seedless/Sultan 7 and 86% Crimson Seedless × Black Kishmish) and also acclimated plant number. On the other hand, both of Crimson Seedless combinations gave the higher embryo germination rate compared to Valdez [15]. This may be attributed to male parent genotype [16]. In Sultan 7 × Black Kishmish combination the embryo formation was very low still there were some in 9th week. Similar results were obtained from different researchers [17–19]. The 8th week samples of this combination were infected so the data is not shown here. Early Superior x Sultana combination gave the best embryo formation yield (16%) in 9th week. In White Kishmish × Sultana combination 7th week gave the highest embryo formation rate (58%).

On the other hand, plant percentage was highest at 9th week. This may be due to the germinating embryos did not develop properly to give plantlets at 7th week. In combinations where male genotype is the same (Sultana), White Kishmish × Sultana was better in respect to embryo formation and plant percentage compare to Early Superior × Sultana.

4. Conclusion

Cultivars are greatly influenced the embryo formation rate. In combinations where Crimson Seedless is used as female parent gave the best embryo formation rate. This may be the suitability of this cultivar for embryo rescue. Best sampling time for Crimson Seedless combinations is found 10th week after pollination. Sultan 7 × Black Kishmish combination gave the lowest embryo rate may be due to the sampling time is too late. The combinations where Sultana was male genotype also gave the sufficient result in respect to embryo formation rates. The best sampling time for these combinations was earlier than Crimson Seedless combinations.

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