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Full Length Article

Influences of Self- and Cross-pollinations on Berry Set, Seed Characteristics and Germination Progress of Grape (*Vitis vinifera* cv. Italia)

ALİ SABIR[1]
Selcuk University Agriculture Faculty Horticulture Department, Konya, Turkey
[1]Corresponding author's e-mail: asabir@selcuk.edu.tr

ABSTRACT

The effects of self- and cross-pollinations of Italia grape cultivar (*Vitis vinifera* L.) on berry set, seed number per berry, viable seed rate, seed sizes, germination rate and seed germination period were investigated. For cross-pollination, Rupestris du Lot (*V. rupestris*), 1103 P and 140 Ru rootstocks (*V. berlandieri* × *V. rupestris*) were used as pollen sources. Variation in berry setting and seed number per berry were non-significant, while significant differences were found in other seed characteristics. The highest viable seed rate was obtained from the clusters pollinated with 1103 P (91.7%). One hundred seed weight was between 3.62 (self-pollination) and 5.76 (Italia × 140 Ru). 140 Ru and 1103 P pollens induced to produce significantly heavier seeds than other pollen sources. The germination rates of seeds were significantly higher when 1103 P and Rupestris du Lot rootstocks were used as pollinator (60.6 & 56.7%, respectively). The pollen sources had also significant effects on seed width, height and thickness of Italia. The highest seed width and thickness values were measured in crosses of Italia × 140 Ru (4.95 & 4.12 mm, respectively). Seed germination commenced at 12nd day after the seeds were put to germinate. The first germination appeared in the seeds of the crosses of Italia with rootstocks. Italia x Italia seeds began to germinate 14 days after putting into the dishes. In conclusion, pollen sources had significant effects on quantitative and qualitative characteristics of Italia seeds. © 2011 Friends Science Publishers

Key Word: Berry set; Italia cultivar; Pollination; Seed germination

INTRODUCTION

The genus *Vitis* consists of two subgenera, *Euvitis* Planch. (bunch grapes) and *Muscadinia* Planch. (muscadine grapes) (Galet, 1998). *Vitis vinifera* is predominant grape species grown worldwide for fresh or processed fruits. The most valuable characteristics of *V. vinifera* species relevant to the breeding of new genotypes are its great productivity, high fruit quality, ease of propagation from cuttings, large clusters and berry size, and some seedlessness (Alleweldt & Possingham, 1988). However, the vine, when cultivated in new habitats, has been very susceptible to fungus diseases, noxious pests, phylloxera and nematodes (Herbert *et al*., 2010). Certain American species have great potential of using the gene resources as a donor to introduce resistance or immunity to a wide range of pests that cause extensive loss in yield and quality of *V. vinifera* fruit (Mahanil *et al*., 2007). They also possess the most remarkable combination of desirable characters to counterbalance the deficiencies of *V. vinifera* vines (Sabir *et al*., 2010). A number of breeding programs aimed at developing new varieties resistant to diseases using complex hybrids of European and American species of *Vitis.* Attempts to produce interspecific hybrids that combine good fruit quality and disease resistance have been made by grape breeders for many years (Dalbó *et al*., 2000; Doucleff *et al*., 2004). Limited success was only reported when the *V. vinifera* was used as seed parents. Lu *et al*. (2000) crossed the grapes in more than 50 cross combinations between *V. vinifera* and *V. rotundifolia*. Limited number of seedlings was achieved in some crosses and only two hybrids were produced from the crosses.

Modern biotechnology methods may aid to plan new strategies of breeding procedures. However, literature investigations indicate that conventional hybridization methods will still be employed by grape breeders in the future studies (Bouquet *et al*., 2000; Notsuka *et al*., 2001). Therefore efforts should be made to obtain a thorough knowledge issues dealing with hybridization studies. Factually, breeders should consider the principles of crossing steps such as flower types, pollen fertilization capacity, and embryo growth and their dependence on environmental conditions.

Over the previous decades, detailed investigations on floral development in grape have contributed valuable knowledge to botanical sciences (Agaoglu, 1971; Cresti &

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Ciampolini, 1999; Marasali & Baydar, 2001; Kelen & Demirtas, 2003). These efforts also contributed clearer approaches of the facts of inflorescence and flower ontogeny as well as pollen morphology and pollination physiology of grapevines. However, there are still a number of questions, which have not been adequately addressed. Although the developmental morphology of the inflorescence is better understood, little information is available about the relationships within the varieties with respect to crossing compatibility and potential.

This paper reports the effects of different male rootstocks as pollen source on berry set, seed number per berry, viable seed rate, seed sizes, germination rate and germination period of Italia seeds. The results of self-and cross-pollination were considered from viticultural and cultivar improvement viewpoints.

MATERIALS AND METHODS

Nine healthy vines of Italia (*Vitis vinifera* L.) cultivar and three vines of each rootstocks Rupestris du Lot (*V. rupestris*), 1103 P and 140 Ru (*V. berlandieri* × *V. rupestris*) were selected for the study. A total of 36 Italia clusters, borne on 9 vines, were selected on the basis of unity and their positions on the plant. The experiment conducted on three replicates consisted of 3 clusters each. The clusters consisting of about 250-300 flower buds were emasculated leaving 100 stigmas for each cluster. Emasculations were performed 5-6 d before bloom (Chkhartishvili *et al*., 2006). The anthers were removed together with calyptra carefully, using forceps with fine tips. The pistils were left on clusters at similar intervals.

Clusters were covered cheesecloth bags to prevent fertilization by external pollens. After emasculation, the clusters were left and checked for the appearance of a bead of moisture on the stigma, for 2-3 days so that the female parts mature further and attain peak receptivity. About one week before flowering, hermaphroditic F_1 hybrids were covered with cheesecloth bags. Flowers were allowed to bloom in the bags and self pollinate. Hand pollination was carried out for each cross-combination using a hairbrush with the designated male pollen, which had been collected and stored earlier. On the other hand, rootstock clusters as pollen source were also covered with bags to prevent the contamination of external pollens. Pollens were collected by shaking the clusters on petridishes when most of their flowers were at full bloom. As soon as emasculated cluster reached to receptivity, crossing was implemented by brushing the pollen onto the stigmas. After pollination, the stigmata were immediately covered with cheesecloth bags to avoid cross pollination. This was carried out two times (morning & afternoon in the same day). Brushes of each cross combinations and hands were sterilized with 70% ethanol between pollinations. The clusters were tagged at pollination and were harvested individually as they ripened. One week after berry set, the clusters were exposed to full sun throughout the fruit development and maturation. Two emasculated clusters were not pollinated to ensure that the emasculation was done at correct time and also to check the possible incidence of *claistogamie* (self polination of any flower without calyptra fall).

The number of fruit per cluster was recorded at maturity. Fruits were harvested when TSS contents were between 16 and 17 ºBrix. The seeds were extracted from mature fruit and washed to remove pulp. Seeds were dropped into water to separate unviable seeds from viable. The seed tended to float were skimmed off and discarded. The remainder were cleaned of the berry pulp and dried. For investigations, 60 seeds per combination were randomly taken. In order to ensure optimum and uniform germination, the seeds were stratified in polyethylene bags in the refrigerator for three months to meet their chilling requirements (Eris, 1976). After three month storage at 3±1ºC, the beak parts of the seeds were wounded to accelerate the water penetration into tissues. The seeds were put on a moist blotter in petridishes at constant temperature of 25±2 with around daily 12 h light and dark periods. The number of germinated seeds (about 2 mm radicyl elongation) was recorded each day up to the end of germination.

Data were subjected to one way analysis of variance (ANOVA). Statistical differences with p values under 0.05 were considered significant and means were compared by Tukey’s MSD (Minimum Significant Differences) test at 5% level, using SPSS program version 13.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

In this study, berry set occurred in 100% of the investigated clusters. Self-pollinated Italia clusters gave the least values in all investigations. Berry settings varied depending on the pollinators, although the differences were statistically non-significant (Fig. 1). The highest rate of berry set was obtained from Italia x 1103 P combination (44.1%). Italia x 140 Ru followed by a close rate of 43.2%, while the lowest percentage was noted in selfed plants (36.4%). The test carried out to check *cleistogamie* showed that fruit set did not occur on the clusters, which were only emasculated and not pollinated.

The pollen sources had significant effects on seed width, height and thickness of Italia (Table 1). The highest seed width and thickness values were measured in Italia x 140 Ru (4.95 & 4.12 mm, respectively). Indeed, seed sizes were almost similar when 140 Ru or 1103 P were used as pollination sources. But self-or cross-pollination with Rupestris du Lot resulted in the production of smaller seeds. All values of such seeds were always lower than the means. Seed size is an important aspect of seed quality as seeds of many species are commercially sorted by their sizes (Rivera *et al*., 2007). Seed size variation can have a genetic basis since each embryo will normally be genetically distinct

Table I: Influence of pollen sources on seed width, height and thickness of Italia seeds. Means followed by a different letter differ significantly at $p < 0.05$ by Tukey

	Width	Height	Thickness
Italia	4.13±0.02 b	6.54±0.17 b	3.19±0.02 c
Lot	4.34±0.14 b	6.56±0.09 b	3.73±0.17 b
140 Ru	4.95±0.08 a	7.88±0.16 a	4.12±0.12 a
1103 P	4.94±0.11 a	7.91±0.08 a	4.07±0.04 a
Mean	4.59	7.23	3.78
Tukey%5	0.31	0.34	0.29

Fig. 1: Influence of pollen sources on berry setting of Italia

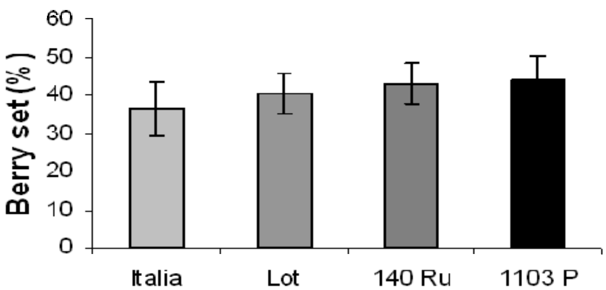


Fig. 2: Influence of pollen sources on seed number of Italia

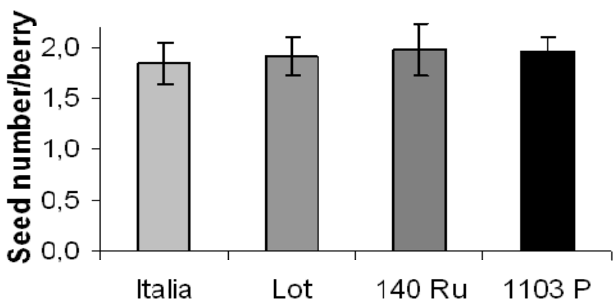
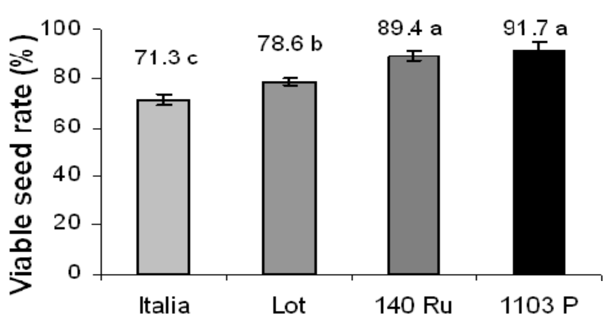


Fig. 3: Influence of pollen sources on viable seed rate of Italia. Means followed by a different letter differ significantly at $p < 0.05$ by Tukey



(Ristic & Iland, 2005). Therefore, seeds from un-favored donors may have more homozygous recessive lethal or deleterious alleles, resulting in more embryo abortion.

The pollinators had similar effects on seed numbers

Fig. 4: Influence of pollen sources on 100 seed weight of Italia. Means followed by a different letter differ significantly at $p < 0.05$ by Tukey

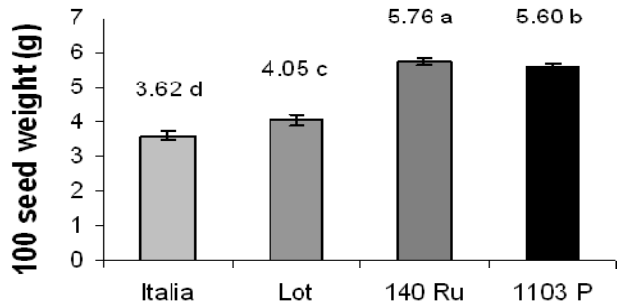


Fig. 5: Influence of pollen sources on seed germination rate of Italia seeds. Means followed by a different letter differ significantly at $p < 0.05$ by Tukey

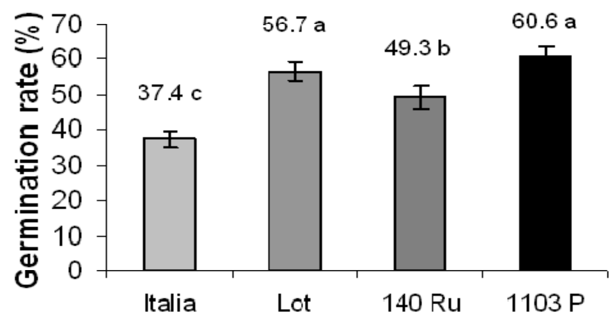
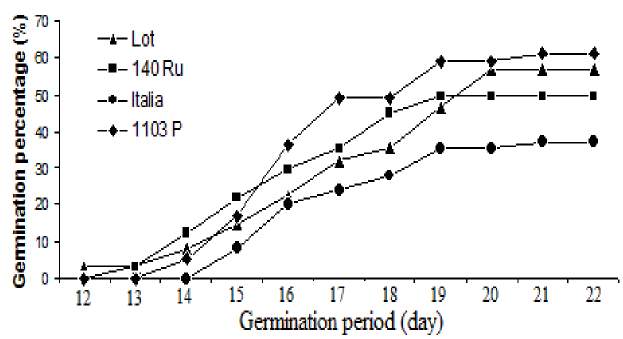


Fig. 6: Influence of pollen sources on germination period of Italia seeds



per berry, ranging from 1.84 (selfing) to 1.98 (140 Ru) (Fig. 2). Pollination intensity was usually positively correlated with the number of seeds per fruits (Filler *et al*., 1994). Furthermore, viable seed rate is an important physiological factor affecting the quality and quantity of hybrid seedlessness. Therefore, the source of pollen often determines whether or not the berries will produce viable seeds. In the present study, viability of seed significantly varied among clusters pollinated with different pollen sources (Fig. 3). The highest viable seed rate was obtained from the clusters pollinated with 1103 P (91.7%). Besides, Italia pollinated with 140 Ru had also significantly higher viable seed rate (89.4%), almost equal in magnitude to those of 1103 P. On the other hand, the least rate was determined in Italia x Italia seeds. Such finding might indicate the existence of seed abortion at early stage of berry development. Golodriga (1953) stated that different cultivars of grapes (*V. vinifera*) reacted differently to pollinating agents. The author also stressed the importance of selecting the proper pollinators for grape cultivars.

One hundred seed weight ranged were between 3.62 g in self-pollinated to 5.76 g in 140 Ru (Fig. 4). Seed weight data indicated that 140 Ru and 1103 P pollens yielded significantly heavier seeds than other pollen sources. There was a trend for small seed with the self-pollination of Italia. Fotiric *et al*. (2003) also found significant differences in dependence of pollination combination regarding seed weight of Bagrina (*V. vinifera*) grape cultivar.

The germination rates of seeds were significantly higher when 1103 P and Rupestris du Lot rootstocks were used as pollinators (60.6 & 56.7%, respectively) (Fig. 5). However, seed germination rate of self-pollinated cluster was very low (37.4%). Also, there was a trend for lower seed germination rate (49.3%) when 140 Ru was used as pollen source. Sufficient germination rates indicate successful fertilization. Karatas and Agaoglu (2007) obtained 17.0 and 43.3% germination percentages, while investigating the effects of the pollinator grape cultivars Kalecik Karasi on the progeny fertility of grape cultivars.

Previous reports indicated that poor germination is common for grape seeds (Celik, 2001). Especially, the germination of grape seeds obtained from self pollinations are usually lower than those from open or cross pollinations (Doijode, 2001). If this was the case in this study, it was expected that the cross-pollinated seeds might have a significantly greater germination. Therefore, such failure in germination of the seeds from self-pollination may be due to prezygotic incompatibility mechanism.

As seen in Fig. 6, seed germination commenced at 12nd day after the seeds were placed in the petridishes. The first germination appeared among the seeds of the crosses of Italia with rootstocks. Italia × Italia seeds began to germinate 14 days after putting into the dishes. In seeds of Italia × 1103 P, the germination rate exhibited a rapid increase at 4 days later. Similar conditions were seen in seeds of interspecific hybridizations. However, the germination progressed quite slowly in Italia x Italia seeds and reached its maximum level 9 days after the first germination appeared.

Overall results indicate that general seed characteristics of Italia grape cultivar were solely affected by the pollen sources. Fruit set was slightly higher in cross-pollinated clusters than those of self-pollinated. Furthermore, cross-pollinations enhanced the general values relevant to seed observations. These findings imply that cross-pollination dominates in fertilization biology of Italia. The findings are likely to have practical usage for grape breeders in designing strategies for hybridization studies.

REFERENCES

Agaoglu, Y.S., 1971. A study of the differentiation and the development of floral parts in grapes (*V. vinifera* L.). *Vitis,* 10: 20–26

Alleweldt, G. and J.V. Possingham, 1988. Progress in grapevine breeding. *Theor. Appl. Genet.,* 75: 669–673

Bouquet, A., Y. Danglot, M. Bongiovanni, P. Castagnone-Sereno, D. Esmenjaud, A. Dalmasso and L. Torregrosa, 2000. Breeding rootstocks resistant to grape fanleaf virus spread, using *Vitis x muscadinia* hybridization. *Acta Hortic*., 528: 523–532

Celik, H., 2001. Effect of bottom heating, germination medium and gibberellic acid treatments on germination of Isabella (*Vitis labrusca* L.) grape seed. *Pakistan J. Biol. Sci.,* 4: 953–957

Chkhartishvili, N., L. Vashakidze, V. Gurasashvili and D. Maghradze, 2006. Type of pollination and indices of fruit set of some Georgian grapevine varieties. *Vitis,* 45: 153–156

Cresti, M. and F. Ciampolini, 1999. Ultrastructural characteristic of pollen development in *Vitis vinifera* L. (cv. Sangiovese). *Vitis,* 38: 141–144

Dalbó, M.A., N.F. Weeden and B.I. Reisch, 2000. QTL analysis of disease resistance in interspecific hybrid grapes. *Acta Hortic.,* 528: 217–222

Doucleff, Y.J., F. Gao, S. Riaz, A.F. Krivanek and A. Walker, 2004. A genetic linkage map of grape, utilizing *Vitis rupestris* and *Vitis arizonica*. *Theor. Appl. Genet*., 109: 1178–1187

Doijode, S.D., 2001. *Seed Storage of Horticultural Crops*, 2nd edition, p: 339. Published by Haworth Press

Eris, A., 1976. On the influence of growth regulators and stratification on germination of seeds of the vine cv. Muscat Hamburg. *Mitteil. Rebe Wein Obstbau Fruchte*, 26: 85–90

Filler, D.M., J.J. Luby and P.D. Ascher, 1994. Incongruity in the interspecific crosses of *Vitis* reproductive expression in the F_1 grogeny. *Euphytica,* 78: 155–164

Galet, P., 1998. *Cépages et Vignobles de France, Tome 1. Les Vignes Américaines*, 2nd ed. Imprimerie Charles Déhan, Montpellier, France

Golodriga, P.I., 1953. On the selectıon of pollinizer varieties of grape. *Agrobiologiya,* 5: 105–110

Fotiric, M., M. Mulitinoviç, D. Nikoliç and V. Rakonjac, 2003. Pollenizer influence on berry and seed properties in grapevine cultivar 'Bagrina' (*Vitis vinifera* L.). *Acta Hortic*., 603: 775–777

Herbert, K.S., P.A. Umina, P.J. Mitrovski, K. Viduka and A.A. Hoffmann 2010. Clone lineages of grape phylloxera differ in their performance on *Vitis vinifera*. *Bull. Entomol. Res.,* 100: 671–678

Karatas, H. and Y.S. Agaoglu, 2007. The effects of the polinator grape cultivar Kalecik Karası on the progeny fertility of some grape cultivars. *Tarim Bilimleri Dergisi,* 13: 261–264

Kelen, M. and I. Demirtas, 2003. Fertilization biology of some grape varieties (*V. vinifera* L.). *Pakistan J. Biol. Sci.,* 6: 766–769

Lu, J., L. Schell and D.W. Ramming, 2000. Interspecific hybridization between *Vitis rotundifolia* and *Vitis vinifera* and evaluation of the hybrids. *Acta Hortic*., 528: 481–486

Mahanil, S., B.I. Reisch, C.L. Owens, P. Thipyapong and P. Laosuwan, 2007. Resistance gene analogs from *Vitis cinerea*, *Vitis rupestris* and *Vitis* hyrid Horizon. *American J. Enol. Vitic.,* 58: 484–493

Marasali, B. and N.G. Baydar, 2001. Studies on flower types and pollination properties of some Vitis species and cultivars used as rootstocks. *Turkish J. Agric. For.*, 25: 393–399

Notsuka, K., T. Tsuru, M. Shiraishi, 2001. Seedless-seedless hybridization via in-ovule embryo culture. *J. Japanese Soc. Hort. Sci*. 70: 7–15

Ristic, R. and P.G. Iland, 2005. Relationships between seed and berry development of *Vitis vinifera* L. cv Shiraz: Developmental changes in seed morphology and phenolic composition. *Australian J. Grape Wine Res.,* 11: 43–58

Rivera, D., B. Miralles, C. Obón, E. Carreńo and J.A. Palazón, 2007. Multivariate analysis of *Vitis* subgenus *Vitis* seed morphology. *Vitis,* 46: 158–167

Sabir, A., Y. Dogan, S. Tangolar and S. Kafkas, 2010. Analysis of genetic relatedness among grapevine rootstocks by AFLP (Amplified Fragment Length Polymorphism) markers. *J. Food Agric. Environ*., 8: 210–213

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