Selcuk J Agr Food Sci, (2017) 31 (2), 92-97
ISSN: 2458-8377 DOI: 10.15316/SJAFS.2017.25

Selcuk Journal of Agriculture and Food Sciences

Fertilization Biology of Ancient Grapevine Variety 'Ekşi Kara' (*Vitis Vinifera* L.)

Zeki KARA[1], Ali SABIR[1], Kevser YAZAR[1], Osman DOĞAN[1], Ahmed Jalal Khaleel KHALEEL[1]

[1]Selcuk University Agriculture Faculty Horticulture Department, Konya, Turkey

ARTICLE INFO

Article history:
Received date: 19.06.2017
Accepted date: 19.07.2017

Keywords:
Grapevine
Vitis vinifera L.
Functional female flowers
Self-pollination
Pollen viability
Honey bee

ABSRACT

In some institutions in Turkey, grape breeding programs such as hybridization breeding between superior genotypes of *Vitis vinifera* L. varieties or clonal selection of local genotypes are carried out. Antique and autochthone grape varieties such as 'Ekşi Kara' (*Vitis vinifera* L.) are grown since ancient times in Konya-Karaman provinces and middle Taurus Mountains. Its economic value of this variety is high in the production area and is well adapted in the region. It is more indispensable than many other varieties in the region. It is also promising for similar ecologies. The development requirement of this very productive variety has been identified as a need. The flower type of the variety is functional female. A pollinator is a required for a good fruit set. Another ancient and autochthone grape variety, 'Gök Üzüm', is used as a pollen source in the region. Clusters and berry sizes the 'Ekşi Kara' variety in the producer vineyards are closely related to hens and chicken berry development percentage, berry growth, weather conditions (precipitation) during flowering and / or pollination period due to the honey bee activity.

This research was carried out under the producer vineyards conditions in which selected in the clone candidates (CC) were identified by the 'Ekşi Kara' grape variety clone selection project of the Selçuk University Faculty of Agriculture, Department of Horticulture and the laboratory conditions. Fertilization biology of 220 selected head CC were examined. The purpose of the study was to search the existence of self-fertile clones among the head CC. For this purpose, the head CC were self-pollinated in covered clusters, freely pollinated with 'Gök Üzüm' grape variety and pollens were tested for viability, pollen germination and tube growth of the germinated pollens.

All of the head CC did not have any seeded fruit set in the covered flower clusters. Pollen grains were alive in different percentage but in laboratory conditions in all of the tested pollens germination was practically less than 3%. A self-fertile clone candidate has not been identified. Honey bees and some other pollinator insects were the main vectors for pollen transport from 'Gök Üzüm' to 'Ekşi Kara'. The development of ampelographic and genetic databases will greatly contribute to genetic stocks that have not yet been evaluated in culturally diverse cultures or collections throughout the country, so that the correct identification of genotypes in general will be increased.

This manuscript has been produced from the master thesis project of Ahmed Jalal Khaleel KHALEEL is titled 'Fertilization Biology of Ancient Grape 'Vitis Vinifera L.' supported by Selcuk University Scientific Research Projects Board Project number is 17201043.

1. Introduction

Many grape breeding programs have been conducted in certain institutions as cross breeding between superior genotypes or clonal selection of local accessions of *Vitis vinifera* L. varieties in Turkey. 'Ekşi Kara' (*Vitis vinifera* L.) is an ancient and autochthone grapevine cultivar intensively grown in Konya due to its well-adaptation to the ecology. Thus, it has been promising with its unique characteristics peculiar to similar ecologies. This cultivar is robust and very fruitful in comparison with many other *V. vinifera* varieties in the region. The sex of the flowers is functionally female, and need a pollinator, for a good fruit set. 'Gök

* Corresponding author *zkara@selcuk.edu.tr*

Üzüm' (*Vitis vinifera* L.), another ancient and autochthonous grape variety is being suggested as a primary pollinizer with hermaphroditic flowers. Open pollination effected fruit set, and berry shape, hens and chicken rates. A clonal selection study has been continuing on the variety at the Selcuk University Faculty of Agriculture Department of Horticulture since 2010. 230 head CC are selected in 15 producer vineyards in different elevation, cultural practices, training systems in Konya and Karaman provinces in middle Taurus Mountains. The aim of the study is to search the self-fertile clone(s), fruit set without cross pollination among the 220 selected CC of 'Ekşi Kara' (*Vitis vinifera* L.).

V. vinifera L., species cultivars set parthenocarpic or stenospermocarpic fruits genetically or occasionally based on specific physiological-environmental conditions (Kelen and Demirtas 2003; Stosser, et al. 1996). Pollination and fertilization are the basic factors affecting fruit setting volume and the most important goal of fruit growers is obtaining high quantity and quality yield in horticultural industry which depend on sufficient fruit setting. A linear relation between pollen viability and germination capability in many fruit species have been reported [(Wang, et al. 1993); (Chkhartishvili, et al. 2015) (Perveen and Ali 2010; Vouillamoz, et al. 2006) (Sharafi and Bahmani 2010) (Sharafi, et al. 2010; Sharafi and Bahmani 2011)]. Therefore, knowledge about fertilisation biology traits of the species and cultivars is one of the main issues for growers and breeders (Hancock, et al. 2003; Szabó 2003). For successful pollination, the high quantities and qualities of pollen must be transferred to the stigma when it is receptive (Sharafi 2011; Taylor and Hepler 1997; Wang, et al. 1993).

Pollen viability levels, germination capability of pollen is related to cultivars, nutrition conditions, and environmental factors (Bolat and Pirlak 1999; Dafni and Firmage 2000; Dantas, et al. 2005; Kelen and Demirtas 2003). There is a big variation in optimum germination conditions of pollen among plant species and cultivars (Kelen and Demirtas 2003). Many researchers have been performed to determine quantitatively and qualitatively the components necessary for the best composition of culture medium in pollen grain germination for different species pollens (Abreu, et al. 2006; Dane, et al. 2004; Eti 1991; Eti, et al. 1998; Kelen and Demirtas 2003; Liu and Zhu 1985; Sharafi 2011).

While aceto-carmine is useful to indicate the proportion of aborted grains, it does not appear to indicate reliably the viability of the pollen. Germination of pollen in *vitro* is said to be a reliable method of predicting the performance of the pollen in setting fruit (Nagarajan, et al. 1965). The usual germination method is to add grape pollen to a hanging drop culture of water with 20%' sucrose at 25-30 °C or at unspecified temperatures (Olmo 1942; Weaver and McCune 1960; Winkler 1926). Boric acid (5-20 ppm) has been added to increase germination (Bamzai and Randhawa 1967). Incubation times are given as six to 12 hours (Bamzai and Randhawa 1967; Olmo 1942). Agar (0.5-2.0%) with 5-10% sucrose and incubation for two to 24 hours at 26 °C have been used (Gollmick 1942; Mayer 1964; Nebel and Ruttle 1936). Some of these investigators stain pollen tubes with chrome-cresol-green or lacmoid; other stains can be used.

2. Material and Method

2.1. Plant material

In this study fertilisation biology of 230 selected CC of 'Ekşi Kara' (Vitis vinifera L.) were searched by covered flower clusters and self-pollination to obtain the fruit setting capacity. Stamens of functionally female flowers of the variety were differed between erect and turned-down. Matured clusters were different range of both seeded and parthenocarpic berry depend on the pollination. 'Gök Üzüm' (Vitis vinifera L.) has hermaphroditic or perfect flowers the stamens were erect with the anthers producing functional pollen and the pistils were functional, used for open pollination for CC.

2.2. Self-fertility tests

First inflorescences of all selected CC of 5 shoots were covered by bags 7-10 days before blooming, vibrated during the active pollination times about ten o'clock. Covering bags were opened two weeks after fruit set, parthenocarpic and seeded berries were counted the harvest time.

2.3. Pollen Tests

In May 2016, pollen was collected from well-developed newly opened flowers from each CC, paper bags were used for transport to the laboratory following the literature to determine pollen viability, pollen germination and pollen tube growth rates. (Winkler 1926) allowed anthers to dehisce in vials. Barrett and (Barrett and Arisumi 1952) stripped flowers from the cluster, dried them on a glass plate, then sifted out the pollen. Olmo (1942) harvested clusters on which half of the flowers had opened and tapped the clusters against a clean glass plate. The dry pollen was scraped up with a razor blade and put into small bottles. The equipment was cleaned with alcohol to kill unwanted pollen. Harvested pollens were maintained in desiccator's in refrigerator 4°C during the test preparation.

2.3.1. Pollen viability

The pollens taken from CC viability status were immediately tested by 2.3.5 triphenyl tetrazolium (1%) (Kelen and Demirtas 2003; Korkutal, et al. 2004) in the flowering period. Results was figured at the Fig. 1.

2.3.2. Pollen germination

Pollen germination were tested in 5 different media that were 1. 20% sucrose, 1% agar, 2. 20% sucrose, 1% agar, 10 ppm boric acid, 3. 20% sucrose, 1% agar, 50 ppm boric acid, 4. 20% sucrose, 1% agar, 10 ppm indole 3 butyric acid 5. 20% sucrose, 1% agar, 10 ppm indole 3 butyric acid, 5 ppm GA_3, and 12 hours incubation time was used.

2.3.3. Pollen tube growth

The pollen tube length measurements of germinated pollen were directly recorded in relation to the eye micrometer scale (µm) attached to the eye lens in the microscope in order to evaluate pollen germination and tube growth in germinated pollen.

Statistical analysis

A complete randomized block design with three replicates (consisted of four grafted vines) was established. Data were separately evaluated for each rootstock by analysis of variance (ANOVA) and treatment means were separated by Least Significant Differences (LSD) test at $P < 0.05$. Analysis was performed with SPSS program version 13.0 (SPSS Inc., Chicago, IL).

sation conditions. In the area the bunch size, density and length, and all berry characters highly depend on the pollination. There were well pollinated clusters have average cluster weights in a drip irrigated vineyard approximately 1 kg, on the other hand poor pollination in the non-irrigated vineyard cluster weights were up to ten-fold less. Self and open pollinated clusters in non-irrigated vineyards were smaller than irrigated vineyards.

Covered inflorescences were not seeded fruit set. Some seedless, parthenocarpic berry can develop in all covered clusters of all CC. There was only 3 seeded berry has 4 seed among tested CC were obtained.

Previous study indicated that the anthesis occurs most frequently between 6 and 9 a.m. with a rising air temperature, and may also occur from 2 to 4 p.m. (Pratt 1971). In this study observed that the honey bee was the main pollen carrier from ‘Gök Üzüm’ to ‘Ekşi Kara’ and pollination time was mainly belonged to honey bee activity during the blooming period.

3.2. Pollen tests

3.2.1. Alive pollen rates

Alive pollen rates (%) test indicated that all pollens of CC were alive, the range was between 38.5% (117 clone candidate number) and 96.83 (117 clone candidate number), that was relatively due to vigorous of the candidate clone, nutrition statues, irrigation and the other cultural practices (see Fig. 1).

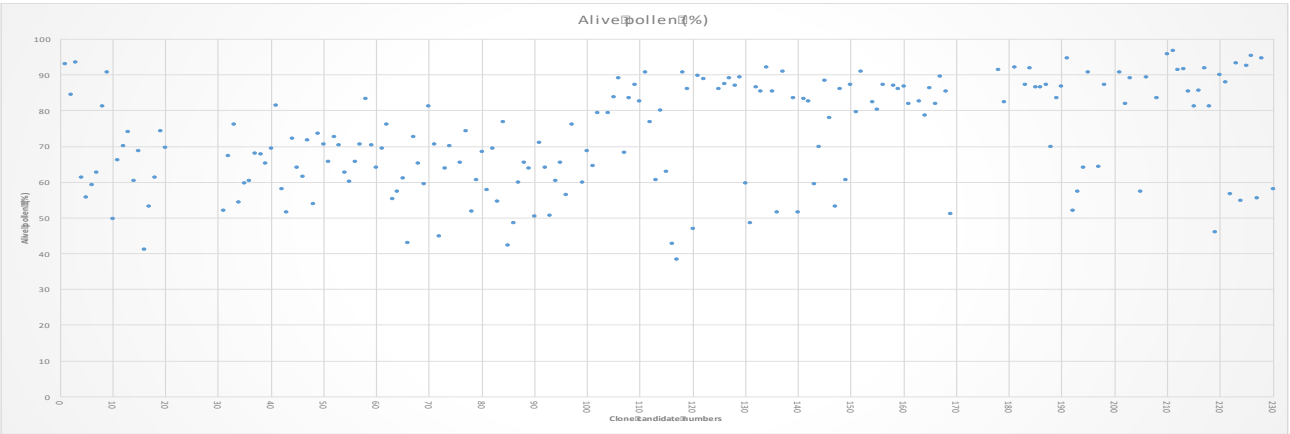

Fig. 1. Alive pollen rates (%)

3. Results and Discussion

3.1. Self-fertility results

Open pollinated clusters were developed hens and chicken berry that was also belonging to pollination that was affected by weather (rainfall during the bloom), and cultural practices such as irrigation, fertili-

3.2.2. Pollen germination rates

Pollen germination were tested in 5 different media but the 20% sucrose, %1 agar gave the best results in

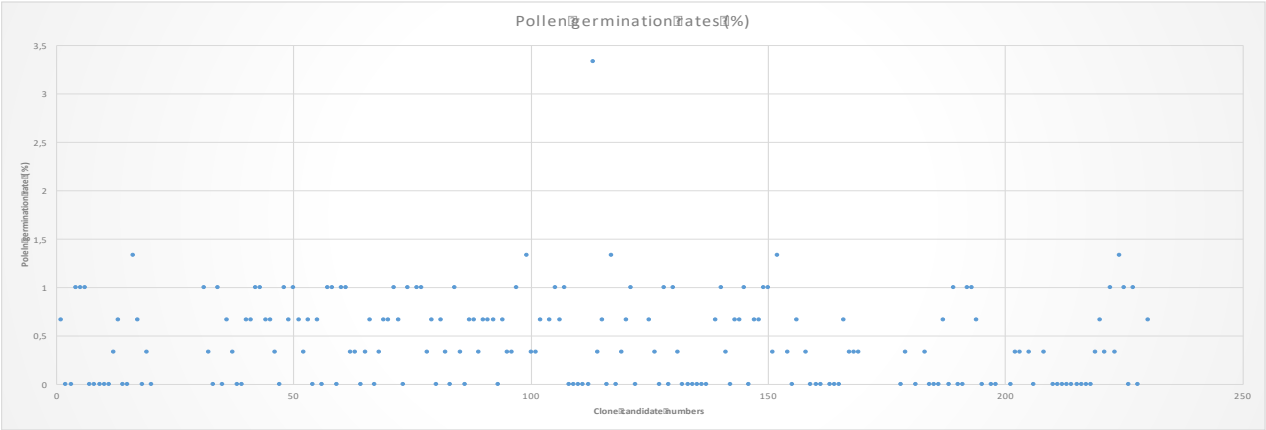

Fig. 2. Pollen germination rates (%)

all treatment, for this reason the only best results were figured at Fig. 2. Adding boric acid, Indole 3 butyric acid, and GA_3 were not promoted the pollen germination in 'Ekşi Kara' grape CC. Pollen germination rates (%) results were exactly different pollen alive results it was ranged between 0% (many of the CC) to 3.33% (at the 117-clone candidate number). Environment and cultural practices influence flowering, either directly or indirectly via their impact on photosynthesis and nutrient availability. Cultural practices encouraging light penetration into the canopy favour flower initiation, while practices resulting in shading have a detrimental impact (Díaz-Riquelme et al. 2009) but in this study these factors were not affected on 'Ekşi Kara' functional female flowers pollen germination.

(Mayer 1964), indicated that the method of pollination has been debated, but probably most hermaphroditic flowers are self-pollinated. The result of the self-pollination in cover clusters showed that flower sex of 'Eksi Kara' flowers is functionally female, and there was no significantly difference ($p < 0.05$) in CC was obtained. Pollens are alive between 38.5% (117 CC) and 96.83 (117 CC). Covered clusters can only parthenocarpic berry sets.

3.2.3. Pollen tube growth

Pollen tube growth were in all germinated pollens (see Fig. 3) these were recorded between 0% (many of the CC) to 123.33% (at the 33 CC number).

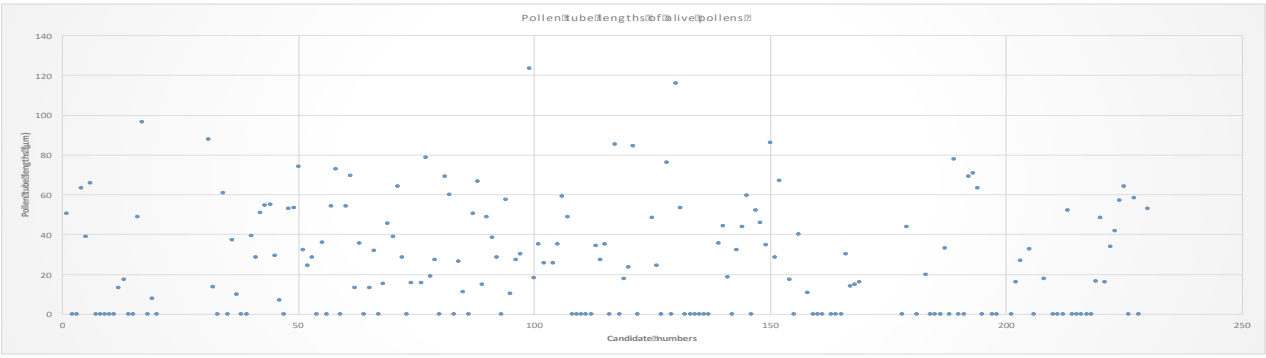

Fig. 3. Pollen tube lengths (µ)

4. Conclusion

CC of 'Ekşi Kara' pollens practically were not germinated, that means need to pollinator for vineyard establishments. 'Gök Üzüm' was a good pollen source to 'Ekşi Kara' CC for seeded, and high-quality fruit set. Honeybees are the main pollinators for 'Ekşi Kara' that should be kept during the blooming period in or around the vineyard plantations.

Further development of ampelographic and genetic data-bases will greatly contribute to the ancient cultivars and accessions nationwide, under cultivation or in

collections, thus increasing overall the accurate identification of varieties.

Acknowledgement

We would like to thank to University of Selcuk Scientific Research Board to supported this study by a grant from (SU BAP 17201043). We would like to thank to 'Ekşi Kara' clonal selection project researchers from Selcuk University Faculty of Agriculture Department of Horticulture, Bahri Dağdaş International Agricultural Research Institute, and Konya Soil, Water and Deserting Control Research Institute.

5. References

Abreu I, Costa I, Oliveira M, Cunha M, de Castro R. (2006). Ultrastructure and germination of Vitis vinifera cv. Loureiro pollen. *Protoplasma 228*(1): 131-135.

Bamzai RD, Randhawa GS. (1967). Effects of certain growth substances and boric acid on germination, tube growth and storage of grape pollen. *Vitis* 269-277.

Barrett HC, Arisumi T. (1952). Methods of pollen collection, emasculation, and pollination in fruit breeding. Proceedings of the American Society for Horticultural Science.

Bolat İ, Pirlak L. (1999). An investigation on pollen viability, germination and tube growth in some stone fruits. *Turkish Journal of Agriculture and Forestry* 23(4):383-388.

Díaz-Riquelme J, Lijavetzky D, Martínez-Zapater JM, Carmona MJ. (2009). Genome-wide analysis of MIKCC-type MADS box genes in grapevine. *Plant Physiology*, *149*(1), 354-369.

Chkhartishvili N, Vashakidze L, Gurasashvili V, Maghradze D. (2006). Type of pollination and indices of fruit set of some Georgian grapevine varieties. *Vitis-Geilweilerhof-*, *45*(4), 153.

Dafni A, Firmage D. (2000). Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Systematics and Evolution 222(1):113-132.

Dane F, Olgun G, Dalgiç Ö. (2004). In vitro pollen germination of some plant species in basic culture medium. *Journal of Cell & Molecular Biology 3*(2).

Dantas ACDM, Peixoto ML, Nodari RO, Guerra MP. (2005). Germination of pollen and the development of pollen tubes in apple (*Malus* spp.). *Revista Brasileira de Fruticultura 27*(3): 356-359.

Eti S. (1991). Determination of pollen viability and germination capability of some fruit species and cultivars by different in vitro tests. *J Agric Fac Cukurova Univ* 6:69-80.

Eti S, Tangolar S, Gok S, Ergenoglu F. (1998). Investigations on pollen viability, germination capability and production and fruit set and quality of some grape varieties (in Turkish), National IV. In *Viticulture Symposium, Yalova* (pp. 349-353).

Gollmick F. (1942). The longevity of vine pollen. *Angew. Bot* 24:221-32.

Kelen M, Demirtas İ. (2003). Pollen viability, germination capability and pollen production level of some grape varieties (Vitis vinifera L.). Acta physiologiae plantarum 25(3):229-233.

Korkutal İ., Bahar, E., Demir, K. Ö. K., Çelik, S., & Selen, U. R. U. Ç. (2004). Bazı üzüm çeşitlerinde (*Vitis vinifera* L.) in vitro testler yardımıyla polen canlılığı ve çimlenme yeteneklerinin incelenmesi. *Trakya Univ J Sci 5*(2): 117-126.

Hancock JF. Floral biology, pollination and fertilisation in temperate zone fruit species and grape. Kozma P, Nyéki J, Soltész M, Szabó Z. (2003). Budapest: Akadémiai Kiadò. $98 (hardback). 621 pp. (2004): 763-763.

Liu CE, Zhu L. (1985). Studies on the pollen morphology and pollen germination morphology in grape cultivars. Acta Horticulturae Sinica (China).

Mayer G. (1964). Investigations into the causes of different germinabilities of various Vitis species. Mitteilungen (Klosterneuberg, Austria) Ser. A 14:118-132.

Nagarajan CR, Krishnamurthi S, Madhava Rao VN. (1965). Storage studies with grape pollen. *South Indian Hort* 13(1-2):1-14.

Nebel BR, Ruttle ML. (1936). Storage experiments with pollen of cultivated trees. *Jour. Pom. Hort. Sci* 15:347-359.

Olmo HP. (1942). Choice of parent as influencing seed germination in fruits. *Proc. Amer. Soc. Hort. Sci*, 41:171-175.

Perveen A, Shaukat A. (2010). Maintenance of pollen germination capacity of *Vitis vinifera* L. (*Vitaceae*). *Pakistan Journal of Botany* 42(5):3001-3004.

Pratt C. (1971). Reproductive anatomy in cultivated grapes-a review. *American Journal of Enology and Viticulture* 22(2):92-109.

Sharafi Y, Bahmani A. (2010). Study of pollen germination and tube growth in some Iranian Loquat cultivars and genotypes. *3th International Symposium on Loquat*, 2010, pp. 22-25.

Sharafi Y, Karimi M, Ghorbanifar M. (2010). Study of pollen tube growth, cross-compatibility and fruit set in some almond genotypes. *African Journal of Plant Science* 4(5):164-166.

Sharafi Y. (2011). An investigation on the pollen germination and tube growth in some Prunus persica genotypes and cultivars. *African Journal of Microbiology Research* 5(14):2003-2007.

Sharafi Y, Bahmani A. (2011). In vitro study of pollen traits after short storage in some almond, apricot and sweet cherry favorable genotypes. *Journal of Medicinal Plants Research* 5(2):266-269.

Stosser R, Hartman W, Anvari SF. (1996). General aspects of pollination and fertilization of pome and stone fruits. *Acta Hort* 423:15-21.

Szabó Z. (2003). Floral biology, pollination and fertilisation in temperate zone fruit species and grape: Akadémiai Kiadó.

Taylor LP, Hepler PK. (1997). Pollen germination and tube growth. *Annual review of plant biology 48*(1): 461-491.

Vouillamoz JF, McGovern PE, Ergul A, Söylemezoğlu G, Tevzadze G, Meredith CP, Grando MS. (2006). Genetic characterization and relationships of traditional grape cultivars from Transcaucasia and Anatolia. *Plant Genetic Resources 4*(2): 144-158.

Wang J, Horiuchi S, Matsui H. (1993). A histological study of seedlessness in seedless grapes. *Journal of the Japanese Society for Horticultural Science 62*(1): 1-7.

Weaver RJ, McCune SB. (1960). Further studies with gibberellin on Vitis vinifera grapes. *Botanical Gazette, 121*(3): 155-162.

Winkler A. (1926). The influence of pruning on the germinability of pollen and the set of berries in *Vitis vinifera*. *California Agriculture* 2(5):107-124.