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# Pollen Performance of 'Red Lake' and 'Rosenthal' Currant (*Ribes rubrum*) Cultivars

## Red Lake' ve 'Rosenthal' Frenk Üzüm Çeşitlerinin Polen Performansları

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## ABSTRACT

**Objective:** The aim of this study is to test the performance of the pollen grains of 'Red Lake' and 'Rosenthal' blackcurrant varieties. Pollen morphological homogeneity ratio, pollen viability ratio and germination rate at different temperatures were investigated.

**Material and Methods:** In this study, pollen germination, viability and quality of pollens belongs to 'Red Lake' and 'Rosenthal' currant cultivars were studied. For the fertilization biology studies, phenologic observations were first noted. In order to determine the pollen performance, morphological homogeneity, pollen viability (TTC staining test) and germination rates (agar in plate) were determined. In addition, the effect of different temperature conditions (15 °C, 20 $^{0}$C, 25 $^{0C,\ 30\ 0}$C) on pollen germination was noted and the most suitable germination temperature was determined for each cultivar. The effect of different incubation times (6h, 12h, 24h, 48h) on germination capacity was also determined.

**Results:** Morphological homogeneity value of the pollen of the two cultivars was quite high and higher pollen viability should be obtained from morphologically homogenous pollens. Effect of different temperatures on pollen germination percentage was found to be statistically different ($p<0.05$) for both cultivars.

**Conclusions**: 15$^{0}$C was the optimum temperature to obtain the highest pollen germination for 'Red Lake' while 20$^{0}$C was the optimum for 'Rosenthal' pollen grains. Increasing incubation period for both cultivars increased overall pollen germination rate.

**Keywords:**

Blackcurrant, pollen germination, morphological homogeneity, TTC

## ÖZ

**Amaç :** Bu çalışmanın amacı 'Red Lake' ve 'Rosenthal' frenk üzümü çeşitlerinin polen performanslarının belirlenmesidir. Bu amaçla iki çeşide ait çiçek tozlarında çiçek tozu morfolojik homojenlik oranı, çiçek tozu canlılık oranı ve farklı sıcaklıklarda çiçek tozu çimlenme oranı araştırılmıştır.

**Materyal ve Metod:** Çalışmanın bitkisel materyalini 'Red Lake' ve 'Rosenthal' frenk üzümüne ait çiçek tozları oluşturmaktadır. Döllenme biyolojileri çalışmaları için ilk once fenolojik kayıtlar tutulmuştur. Polen performansını belirlemek amacıyla, morfolojik homojenlik, polen canlılığı (TTC boyama testi) ve çimlenme oranları (petride agar) belirlenmiştir. Ayrıca, farklı sıcaklık koşullarının (15C$^{0}$, 20C$^{0}$, 25C$^{0}$, 30C$^{0}$) polen çimlenmesi üzerine etkileri yapılmış ve çeşitler için en uygun çimlenme sıcaklığı belirlenmiştir. Farklı inkübasyon sürelerinin (6 saat, 12 saat, 24 saat, 48 saat) çimlenme kapasitesi üzerindeki etkisi de belirlenmiştir.

**Bulgular :** Çeşitlerin morfolojik homojenlik değerleri oldukça yüksekti ve morfolojik olarak homojen polenlerden daha yüksek polen canlılığı elde edildi. Her iki çeşidin çimlenme yüzdesine göre farklı sıcaklıkların etkisi istatistiksel olarak farklı bulundu ($p <0.05$).

**Sonuçlar:** 15C$^{0}$, 'Red Lake' çeşidi için en uygun çiçlenme sıcaklığı olurken, 20 C$^{0}$, 'Rosenthal' çeşidi için optimum çimlenme sıcaklığı olarak bulunmuştur. İnkubasyon süresinin artmasıyla her iki çeşit içinde çiçek tozu çimlenme oranları artmıştır.

**Anahtar Sözcükler:**

Frenk üzümü, çiçek tozu çimlenmesi, morfolojik homojenlik, TTC

## INTRODUCTION

The first condition of formation of seed and fruit is developing healthy male and female organs of the flower and cells, except for an apparent partenocarpy of some cultivars. Pollen performance includes pollen produced in a flower, pollen morphological homogeneity,pollen germination, pollen tube growth and pollen competition; it is an important component of fertilization success in fruit trees (Thompson 2004; Hedly et al., 2004; Khan and Anjum, 2014; Sulusoglu and Cavusoglu, 2014).

Recently, the crops within the "berries" including *Fragaria, Rubus, Ribes, Morus, Vaccinium* etc. have been the subject of increased interest by both industry and individuals worldwide, mainly due to the perceived health benefits of consumption of anthocyanins, phenolics, vitamins, minerals, sugars, organic acids etc. associated with these fruits (Henriques et al., 2004). *From among these berries, Ribes* is usually regarded as a member of the Saxifragaceae, and as such it has few related crop genera. However, a more recent study by Sinnott (1985) places the genus in the Grossulariaceae because of its floral morphology. All of the species in the family are diploids, and considerable debate has been generated over the past century as to whether the currants and gooseberries form a single genus, *Ribes*, or two, *Ribes* and *Grossularia*. A single genus is now the most common consensus, based on morphology, cross compatibility and molecular grounds (Senters and Soltis, 2003). The genus *Ribes* is native to the high latitudes of the northern hemisphere. Europe, Asia, and North America all have native species. Most commercial production is concentrated in Europe and the Russia Federation, and most cultivars have been derived from species native to these areas. Currant cultivation has been practiced at least since the 1500's in Europe, and the late 1700's in N. America when the first colonists arrived (Brennan, 2008).

Production of blackcurrant in Europe is on large plantations with a fully mechanized harvest, and much of the fruit is now used for juice production and other processing applications. However, there is a small but expanding fresh market sector, including some protected cropping in some countries of Europe such as Belgium. The cultivars used for processing are generally different from fresh market types, with widely divergent characteristics and requirements. Poland is the largest world producer of blackcurrant in recent years, followed by Russia, the United Kingdom and Scandinavia, with some production also taking place in New Zealand (Brennan, 2008).

By definition, pollen performance includes pollen produced in a flower, pollen morphological homogeneity, pollen germination, pollen tube growth and pollen viability and it is an important component for fertilization success, cross breeding and fruit cultivation in fruit trees (Engin and Hepaksoy, 2003;Thompson, 2004; Hedly et al., 2004, Tosun and Koyuncu, 2007; Güçlü et al., 2015). Pollen-pistil interactions and environmental factors also affect pollen performance (Dafni and Firmage, 2000). Temperature is one of the most important environmental factors for pollen germination, fruit set and seed set. Temperature has been shown to affect the chemical composition of pollen, pollen viability, and pollen tube growth (Johanson and Stephanson, 1998). The quality and quantity of *Ribes* bush yield depends on both agronomic factors and pollination processes. Pollination and fertilization are quite complex in blackcurrant. The degree of flower pollination crucially depends on the kind of pollen vector, while fertilization also depends on the number of pollen grains reaching the stigma (Denisow, 2002).

To determine pollen performance of many crops, plant growers test *in vitro* pollen viability, pollen germination and pollen tube growth because these tests are fast, inexpensive and the results are reliable and easy to understand. It is critical to these growing programs to identify favorable cultivars and genotypes that will be used as pollinizers in establishing orchards and in achieving breeding objectives (Sharafi et al. 2010; Gadze et al., 2011).

The aim of this work was to determine the pollination requirements of two commonly cultivated crops, blackcurrant 'Rosenthal' and redcurrant 'Red Lake', in Turkey. In this context we also tried to determine the pollen performance in two currant cultivars.

## MATERIAL and METHOD

**Plant material**: In 2013, plants of *Ribes nigrum* L. 'Rosenthal' and *Ribes rubrum* L. 'Red Lake' growing at the experimental field of the Sivasli district of Usak province were used for the experiment.

**Phenological observations**: First leaf date, full leaf date, first blooming date, the end of blooming date, fruit set ratio, verasion, and harvest date are determined.

**Pollen performances**: In this study, morphological homogeneity of pollen grains, pollen viability tests and germination tests were carried out. In addition, pollen germination rate at different temperatures was determined for pollen performance. For this purpose, pollens were obtained from flowers of the above-mentioned cultivars at the beginning of the flowering period. The flowers were harvested in the field and transferred to the laboratory immediately. Anthers were removed and placed into a dark-colored bottle to promote dehiscence at room temperature. Morphological homogeneity rates were determined first. Imperfectly shaped pollen grains were considered as aborted pollen (Tosun & Koyuncu, 2007). The final percentage of morphological homogeneity (MH) was defined as:

$$MH = \frac{\text{(number of normal shaped pollen)} - \text{(number of aborted pollen) per area}}{\text{Total number of pollen area}} \times 100$$

In the pollen viability test (stain tests), pollen viability was estimated by using TTC (2, 3, 5-triphenyl tetrazolium chloride) stains (Tosun & Koyuncu, 2007). Pollen grains were scattered onto TTC and solution and stained pollen grains were counted after 2 hours. To determine the pollen viability, pollen grains of each cultivar (of four different areas) were observed under a light microscope (100x magnification). The stained pollen was considered as viable in the TTC test. The 'agar in plate' method was used to evaluate pollen germination. According to preliminary studies (Tosun and Koyuncu, 2007; Koyuncu and Güçlü, 2009; Güçlü et al., 2018) for the *in vitro* test, 1%agar + 10% sucrose + 5 ppm $H_3BO_3$ (boric acid) medium was determined to be the best germination medium. Petri dishes were incubated at 15 $^0$C, 20 $^0$C, 25 $^0$C and 30 $^0$C to determine effects of different temperature on pollen germination. Statistical analyses were performed with the General Linear Model using SPSS (V.10; Statistical software, SPSS. Inc., USA). The differences were analyzed using the Duncan's multiple range test at $p< 0.05$ significance.

## RESULTS and DISCUSSION

**Phenological observation**: First leaf date, full leaf date, first blooming date, the end of blooming date, fruit set ratio, verasion and harvest date are shown in Table 1. As indicated in Table 1, first leaf date, full leaf date, first blooming date, the end of blooming date, fruit set ratio, verasion, and harvest date of 'Red Lake' and 'Rosenthal' were 7 March-20 February; 7 April-25 April; 16 April-13 April; 1 May-25 April; 25 April-2 May; 3 June-8 May and 25 June-14 July, respectively. The results revealed considerable variation in these phenological observations for both cultivars. These phenological stages observed for various fruit species are influenced by a combination of climate factors, including light, temperature, rainfall, and humidity and also by specific cultivar (Ercisli, 2007). Because of their close connection with climate, the timing of phenological stages can be accurate indicators of climate change as well. The first leaf date in these fruits relates to the timing of "early spring".

**Pollen performance:** Morphological homogeneity and pollen viability rate of pollens were quite high for both cultivars and there were no statistical differences between the cultivars in terms of pollen viability and morphological homogeneity ($p< 0.05$, Table 2). Morphological homogeneity level was 96% for 'Red Lake' while it was 98% for 'Rosenthal'. The pollen viability test of TTC (2, 3, 5-triphenyl tetrazoliumchloride ) showed that pollen viability of 'Red Lake' was 94%, and in 'Rosenthal' it was 97% (Table 1). The aim of the staining techniques was to determine the pollen enzyme activity, membrane integrity and stainability of the nucleus (Vizintin and Bohonec, 2004). Stain tests have advantages as indicators of pollen viability because they are faster and easier to perform than pollen germination. But, in some cases, differing results may be obtained in stain tests for some fruit species or cultivars. Therefore, to determine the actual amount of viable pollen, germination tests are necessary. Koyuncu (2006) studied strawberry pollen grains using TTC viability test and reported that pollen viability ratios reached from 82% for 'Allstar' and 'Elvira' to 86.5% for 'Chandler'. The viability and morphological homogeneity related to pollen quality. These two properties are among the most important properties for fruit trees and they provide useful information for plant breeders, geneticists and growers. However, an easy method for determining pollen viability is required not only to increase the efficiency of breeding programs but also in the selection of suitable pollinizer varieties when the orchard is being established.

The effect of different temperatures on pollen germination percentage is shown in Table 3. Effect of different temperatures was found to be statistically different ($p<0.05$) for both cultivars based on germination percentage (Table 3). As can be seen from Table 3, 'Red Lake' has reached its highest pollen germination at 15 $^0$C (88%). This was followed by, respectively, 20 and 25 $^0$C temperature regimes (75% and 70%, respectively). There was a dramatic decline in pollen germination at 30 °C with a decrease to 35% (Table 3). Optimum pollen germination temperature was found 20 $^0$C for 'Rosenthal' (83%). At 15 $^0$C, the germination percentage of pollen was 76% in this cultivar. With increasing temperature, pollen germination rate decreased and at 30 $^0$C it was found to be reduced to 41% for 'Rosenthal'. Our results supports the claim that global warming is indirectly a risk factor for fruit growing (reference). Optimum pollen germination temperature can vary between different fruit species and different cultivars. For example, in sweet cherry, 20 $^0$C and 25 $^0$C were found as optimum pollen germination temperatures (Tosun and Koyuncu, 2007); their research confirmed our conclusion that higher temperatures may reduce pollen germination for various fruit crops.. In another study that was conducted with strawberry, 25 $^0$C and 30 $^0$C were found to be optımum temperature for pollen germination (Koyuncu, 2006). Pham *et al.* (2015) conducted a study on Longan (*Dimocarpus longan*) pollen grains both in *in vivo* and *in vitro* and they found that pollen grains showed best germination performance at 23/24$^0$C *in vivo* conditions, but at 30 $^0$C in *in vitro* conditions.

**Table 1.** Phenological observations in currant cultivars.
***Çizelge 1.*** *Frenk* üzümü çeşitlerinin *fenolojik kayıtları*.

| Cultivars | First leaf date | Full foliation date | First bloom | Blooming end | Fruit set | Verasion | Harvest date |
|---|---|---|---|---|---|---|---|
| 'Red Lake' | 07.03 | 07.04 | 16.04 | 01.05 | 25.04 | 03.06 | 25.06 |
| 'Rosenthal' | 20.02 | 25.04 | 13.04 | 25.04 | 02.05 | 08.05 | 14.07 |

**Table 2.** Morphological homogeneity and viability rate of pollen grains from 'Red Lake' and 'Rosenthal' currants.
***Çizelge 2.*** *Red Lake ve Rosenthall frenk* üzümü çeşitlerinin çiçek *tozlarına ait morfolojik homujenlik ve canlılık oranları.*

| | 'Red Lake' | 'Rosenthal' |
|---|---|---|
| Morphological homogeneity ratio | 96%$^{NS}$ | 98% |
| Viability ratio (TTC) | 94%$^{NS}$ | 97% |

NS: Non significant

**Table 3.** Pollen germination rate at different temperature regimes (%) of currant cultivars.
***Çizelge* 3.** Farklı sıcaklık değerleri için frenk üzüm çeşitlerine ait polenlerin çimlenme oranı.

| Cultivars | Temperature Regimes | | | |
|---|---|---|---|---|
| | 15$^0$C | 20$^0$C | 25$^0$C | 30$^0$C |
| 'Red Lake' | 88 a* | 75b | 70b | 35c |
| 'Rosenthal' | 67c | 83a | 76b | 42d |

*Values within a raw followed by different letters are significantly different ($P < 0.05$).

The *in vitro* germination of both 'Red Lake' and 'Rosenthal' pollen grains over different incubation periods (2, 12, 24 and 48 hours) at 15 $^0$C and 25 $^0$C are shown at Table 4. At 20 $^0$C, there was no pollen germination after 6 hours incubation for 'Red Lake' while 'Rosenthal' had 4% pollen germination. Pollen germination percentage increased with increasing incubation periods for both cultivars. Germination of both cultivar's pollen grains reached own maximum percentage in 48 hours. Our results closely paralleled the findings of Koyuncu and Güçlü (2009), who reported that germination percentage of eight sweet cherry cultivar pollen grains reached maximum germination after 48 hours incubation.

Pollen germination and pollen tube growth are important characteristics for morphological, physiological, biotechnological, ecological, evolutional, biochemical, systematic and molecular studies (Dane et al., 2004). Additionally, pollen performance could be a helpful criterion for evaluating seedling fruit progeny for breeding purposes, and especially for selecting which cultivars should be used by growers to insure good pollination and fruit set in the field. There are limited studies about fertilization biology of currant. Our results, discovered in this work, have determined that pollen performance can be a useful tool for studying fertilization biology of currant.

**Table 4.** *In vitro* pollen germination percentages of 'Red Lake' and 'Rosenthal' cultivars at 6, 12, 24 and 48-hour incubation periods.
***Çizelge 4.*** *'Red Lake' ve 'Rosenthal' frenk* üzümü çeşitlerinin *6,12,24 ve 48 saat sonar in vitro* çimlenme *oranları.*

| | Incubation period/pollen germination rate (%) | | | |
|---|---|---|---|---|
| | 6h | 12h | 24h | 48h |
| 15 $^0$C 'Red Lake' | 0.1c* | 19b | 88a | 91a |
| 20 $^0$C 'Rosenthal' | 4c | 27b | 83a | 89a |

*Values within a raw followed by different letters are significantly different ($P < 0.05$).

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