INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 8, p. 108-114, 2014

RESEARCH PAPER OPEN ACCESS

In vitro pollen viability and pollen germination of service tree (*Sorbus domestica* L.)

Melekber Sulusoglu[1,2*], Aysun Cavusoglu[1,2]

[1]*Kocaeli University, Arslanbey Agricultural Vocational School, TR-41285, Kocaeli/Turkey*

[2]*Kocaeli University, Graduate School of Natural and Applied Sciences, Department of Horticulture, TR-41380, Kocaeli/Turkey*

Key words: *Sorbus domestica* L., service tree, pollen viability, pollen germination, boric acid.

http://dx.doi.org/10.12692/ijb/5.8.108-114 Article published on October 23, 2014

Abstract

We investigated the pollen viability and germination of naturally grown service tree (Sorbus *domestica* L.). This is the first study on the pollen germination of service tree. Two staining tests, TTC (2,3,5-triphenyl tetrazolium chloride) and IKI (Iodine potassium iodide) were used. There were clear differences among the staining tests and IKI gave the highest viability percentages. In the initial studies, different sucrose concentrations (0, 5, 10, 15 or 20%) were added into the media and 15% sucrose gave the highest pollen germination. In the next study, 0, 25, 50, 75 or 100 mg/l boric acid added into media together with 1% agar and 15% sucrose to determine the effects of boric acid concentrations. The highest pollen germination has occurred in boric acid-free media. Pollen germination was inhibited in the higher boric acid concentrations and pollen tube development has continued until 75 mg/l concentration.

* **Corresponding Author:** Melekber Sulusoglu ✉ meleksl@kocaeli.edu.tr

Introduction

The service tree (2n=34) belongs to the genus *Sorbus* and the only one species of its subgenus *Cormus* (Miko and Gazo 2004; Brus *et al.*, 2011). Service tree (*Sorbus domestica* L.) is one of less known economically valuable forest fruit-bearing species. Nature growing populations of service tree are spread on large territory from Black Sea, Caucasus region to Western, Central and Southern Europe, Northwest Africa and Southwest Asia (Miko and Gazo 2004, Paganova 2008, Brindza *et al.,* 2009, Brus *et al.* 2011). In most European countries, it is considered as endangered species and listed one of the most endangered plants in Switzerland's Red Book (Brindza *et al.* 2009). It grows well in semi-shade and direct sun, and is tolerant to higher temperatures and low humidity (Rotach 2003, Pagonova 2008). The trees could be classed as long-living fruit tree (Pagan and Paganova 2000), grows slow and reach reproductive maturity only at ten to fifteen years old. Fruits are suitable for fresh consumption when overripe and processing as canned fruit or jam (Kacaniova and Fikselova, 2007, Miletic and Paunovic 2012). Previous studies have already proved the potent antioxidant capacity of fruits against to diabetic diseases (Termentzi *et al.* 2006). High-value timber of service tree is used for furniture, instrument and tool construction too (Miletic and Paunovic 2012). Service tree is a deciduous tree with white, rarely pink flowers in late spring. The flowers are cymose corymbose inflorescence and looks best in spring time (Hampton and Kay 1995, Öz-Atasever and Gerçekçioğlu 2013).

Concerning the possible changes of global climate, attention is given to rare fruitful plants which were not commercially important for horticulture in the past.

Investigation of geographical distribution area of service tree (Paganova 2008) and selecting good quality types was undergoing in the recent years (Nikolic *et al.* 1996, Gerçekçioğlu *et al.* 1997, Balaninin *et al.* 2006, Öz-Atasever and Gerçekçioğlu 2013). Research is undertaken on the breeding and conservation of service tree and improvement of methods for vegetative mass propagation including cutting propagation (Miko and Gazo 2004) and *in vitro* methods (Tsvetkov *et al.* 2007, Piagnani *et al.* 2012). Service trees grow very slow and produce non-uniform yields (Miletic and Paunovic 2012); so needs to selection and breeding for commercially production. In this perspective, we could not reach any information on pollen biology of service trees in the literature review. The quality of pollen is assessed on the basis of viability and vigor of the pollen grain and pollen viability is critical for the crop improvement and breeding programmes (Heslop-Harrison *et al.*, 1984, Shivanna and Rangaswamy 1992). Regarding future larger utilization of service tree, fertilization biology of this species was investigated in this study.

Materials and methods

Collection of pollen

The study was conducted in the years 2013 and 2014 and included one nature grown service tree pollen to evaluate the pollen quality. Unopened flowers were collected at white balloon stage in April from all sides of the tree. Petals and sepals were separated and anthers were isolated from flower buds and placed on a black paper under an incandescent lamp on a table overnight. In the next day, the pollen grains were collected in a small glass vial.

Estimation of pollen viability

Pollen viability was estimated using two staining techniques, TTC (2,3,5-triphenyl tetrazolium chloride) and IKI (Iodine potassium iodide) (Oberle and Watson 1953, Norton 1966, Baker and Baker 1979). In the first staining method, pollen viability counts were made after two hours then pollen were placed on 1% TTC solution (0.2 g. TTC and 12 g sucrose was dissolved in 20 ml distilled water). Pollen grains stained orange or bright red color were counted as alive. In the second staining method, 1 g potassium iodide and 0.5 g iodine were dissolved in 100 ml distilled water for the IKI solution. Pollen viability counts were made five minutes after pollen was placed on an IKI solution. Pollen grains stained

dark (dark red or brown color) were counted as alive.

Pollen germination tests

Pollen germination tests take much more time than pollen viability tests but, in some cases germination tests are necessary to observe the actual viability of pollen. We used to agarose-sucrose medium method for the pollen germination studies. In the first step of pollen germination treatments, different sucrose concentrations (0, 5, 10, 15 and 20%) were added into media together with 1% agar to determine the effects of sucrose concentrations. In the next study, different boric acid (H_3BO_3) concentrations (25, 50, 75 or 100 mg/l) were added together with 1% agar and 15 g/l sucrose. Petri dishes were placed in an oven, in dark condition and pollen germination was observed at the end of 24 hours incubation period. In both germination treatments, the pollen grains were considered germinated when the pollen tube length reached pollen diameter (Brown 1954).

Experimental design and statistical analysis

The experiments were designed as completely randomized blocks with three replications. Randomly selected visual areas, including about 100 pollen grains, were counted in each replicate. All observations of viability and germination were made at X100 magnification using a light microscope. Length of fifty pollen tubes was measured to calculate mean pollen tube length for each replicate. In both years (2013 and 2014), pollen viability and germination rates were very close to each other. All the data collected were combined and the means were used in the statistical analysis. Statistical analysis was performed in the Minitab 17 Statistical program. One-way ANOVA was used for the analysis of viability and germination rates and different means were compared with Duncan's Multiple Range Test at $P<0.05$. The values for viability and pollen germination were transformed to arcsin square root values before statistical analysis.

Results and discussion

Pollen viability rates

The results of TTC and IKI staining tests are shown in Table 1. There were clear differences in the pollen viability percentages in both staining tests and the differences were statistically significant. IKI gave the highest pollen viability rates. In 1% TTC test, semi-viable and dead pollen percentages were more than 50% of total pollen (Figure 1). This was previously reported in cornelian cherry and medlar where IKI test gave the higher viability rates and pollen stained better with IKI (Pırlak and Güleryüz 2005, Cavusoglu and Sulusoglu 2013).

Table 1. Pollen viability of service tree determined by TTC and IKI tests.

Staining test	Pollen viability (%)		
	Viable	Semi viable	Dead
TTC	37.71 b	39.45	22.84 a
IKI	72.06 a	26.61	1.33 b
Average	54.89	33.03	12.09
	P<0.05	ns.	P<0.05

Table 2. Effect of sucrose concentrations on agarose-sucrose media on pollen germination and pollen tube development of service tree.

Sucrose (%)	Pollen germination (%)	Pollen tube length (µm)
0	7.78 c	18.47 b
5	24.73 b	28.19 b
10	52.25 a	59.05 a
15	53.75 a	47.00 a
20	51.35 a	58.78 a
Average	37.97	42.30
	P<0.05	P<0.05

Effect of sucrose on pollen germination

Depending on the sucrose level, service tree pollen exhibited different germination rates and differences among sucrose concentrations was found statistically significant at $p<0.5$ level. Pollen germination rates increased up to 15% sucrose concentrations and decreased at 20% sucrose concentration. The differences between 10%, 15% and 20% sucrose concentrations were not statistically significant (Table 2). The same sucrose concentrations were increased the pollen tube length too (Figure 2). The lowest germination percentages (7.78%) were observed in sucrose free media and pollen grains developed only small pollen tube (18.47 µm) (Figure 2). Previous studies have been shown that the most suitable concentration of sucrose for fruit species pollen germination under *in vitro* condition was 15% sucrose and pollen germination percentages was decreased in sucrose free media (Pırlak and Güleryüz 2005, Bayazıt *et al.* 2012).

Tablo 3. Effect of boric acid concentrations on pollen germination and pollen tube development of service tree.

Boric acid (mg/l)	Pollen germination (%)	Pollen tube length (µm)
0	54.19 a	277.63 a
25	50.79 ab	277.92 a
50	48.76 ab	159.47 ab
75	32.98 b	203.84 a
100	31.75 b	57.20 b
Average	41.07	33.58
	P<0.05	P<0.05

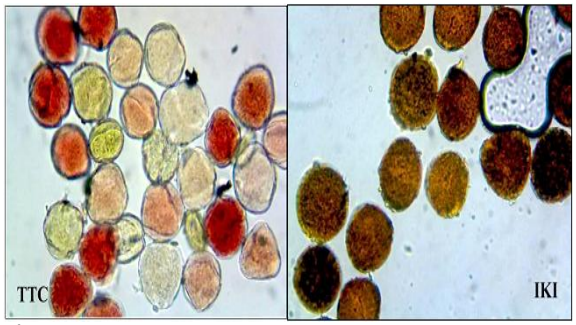

Fig. 1. TTC and IKI painted pollen of service tree (X400).

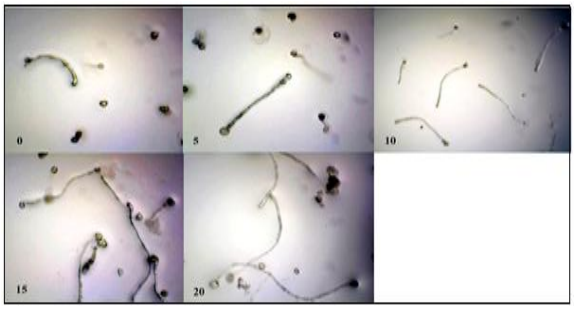

Fig. 2. Pollen germination in media containing different sucrose concentrations (0, 5, 10, 15 and 20%) after 24 hours germination (X100).

Effect of boric acid on pollen germination

Boric acid is important for *in vitro* pollen germination and generally used in the pollen germination studies. In the second step of the studies, boric acid effects were investigated. The highest germination percentages (54.19%) were observed in boric acid free media but this result was not statistically significant from germination values of 25 or 50 mg/l boric acid including media. 25 mg/l boric acid concentration gave the highest germination percentage among the boric acid concentrations and increasing concentrations of boric acid affected negatively the germination rate of pollen grains. The lowest germination percentage (31.75%) was observed in 100 mg/l boric acid including media (Table 3). Boric acid inhibitory effect is seen very often in many species (Pırlak and Güleryüz, 2005, Bolat and Pırlak 1999, Liu *et al.*, 2013) and its effect varies according to cultivars (Dalkiliç and Mestav 2011).

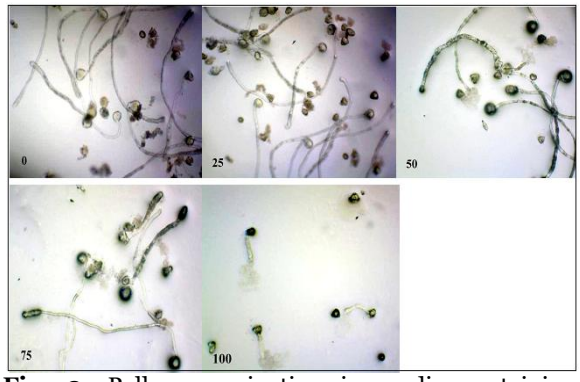

Fig. 3. Pollen germination in media containing different boric acid concentrations (0, 25, 50, 75 and 100 mg/l) after 24 hours germination (X100).

Differences among pollen tube lengths in boric acid concentrations was statistically significant at $p<0.05$ and pollen tube lengths dramatically decreased with the increasing concentrations of the boric acid (Figure 3). Previous studies have shown that the most suitable concentration of boric acid could change according to plant species. Liu *et al.*, (2013) observed that Areca need more boric acid in the pollen germination media and germination percentage was increased until 0.8 g/l boric acid concentration in the media while pollen tube length development was inhibited after 0.4 g/l concentration. Imani *et al.*, (2011) reported maximum germination for apple pollen in the media including 100 mg/l boric acid while this concentration was inhibited pollen germination and pollen tube development in our study.

Conclusion

In this study results showed that TTC stained pollen gave more close results to the pollen germination rates. Pollen germination of service tree was low. Sucrose has significant effects on service tree pollen germination and 15% sucrose was the best sucrose concentration for germination. We used higher concentrations of boric acid in here. It is known that boric acid can promote the germination and growth of pollen but need to try lower concentrations in the next studies to prevent the toxic effects. The results presented here are the first observation on the service tree pollen viability and germination rate that will help in artificial pollination studies.

Acknowledgements

The study was presented in the Balkan Agriculture Congress in 8-10 September 2014 as oral presentation.

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