Assessment of pollen viability and germinability in some European chestnut genotypes (*Castanea sativa* L.)

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ABSTRACT: Pollen viability and germinability in some European chestnut genotypes was assessed in this study. In 10 chestnut genotypes, percentages of pollen viability were generally high and often around or over 80%. The pollen germination percentages of the genotypes were significantly affected by media sucrose concentrations. At optimum sucrose concentrations pollen germination percentages varied between 21.97 and 43.68% in 2004, 3.95 and 31.97% in 2005 and 6.79 and 31.03% in 2006, across all genotypes. The highest pollen germination percentage was obtained from 10% sucrose concentration in all years. Although, in 2006, a highly marked positive correlation ($r = 0.80$) was determined for the viability and germination percentages, no significant relation between the viability and germination percentages ($r = -0.54$ and $r = -0.05$, respectively) was found in 2004 and 2005. In 2005 and 2006, germination percentages declined compared to 2004.

Keywords: *Castanea sativa* L.; pollen viability; pollen germination

Chestnuts are monoecious and the flowers are borne on the current year's growth. Two types of inflorescence are found, the unisexual staminate catkins, which are on the lower parts of the shoot, and the bisexual catkins towards the terminal end of the shoots. Flowering occurs after the first leaves have fully expanded and varies somewhat according to species, clone, and season. Chestnut trees flower in very late spring and early summer (Jaynes 1975; Rutter et al. 1990).

Most chestnut varieties are self-incompatible. Before planting, one must ascertain that the varieties to be planted are genetically compatible and that they blossom at the same time. Varieties with long stamens produce abundant quantities of pollen, while varieties like Marrone have completely sterile male flowers. To ensure good cross pollination, it is important to plant more than two inter-compatible varieties. Generally the chestnut is wind pollinated although insects, especially bees, play a role in pollination (Bounous, Beccaro 2002).

In chestnut, as in most flowering plants, fertilization is required to form fruit and seed in the absence of parthenocarpy. A successful fertilization greatly depends on the quality of pollen, viability and germination capacity (Beyhan 1993; Beyhan, Odabas 1995). Therefore, pollen quality assessment is a necessary procedure to determine the suitability of cultivar combinations and pollinizer cultivars in commercial plantings. For artificial pollination, breeding and incompatibility experiments, it is also important to understand of sterility problems in hybridization programs, and evolutionary ecology (Rodriguez-Riano, Dafni 2000).

Various methods can be used for estimation of pollen viability and germinability in horticultural crops. Viability is defined as "having the capacity to live, grow, germinate or develop", but viable pollen grains may not actually germinate (*in vitro* or *in vivo*) if the conditions are not suitable. Many pollen grains can germinate in water or aqueous solutions of sucrose with no additives. However, the pollen of some species (such as trinucleated pollen grains) needs special substrates for germination. Germination of pollen grains in a sucrose solution series (usually between 0% and 50%) may serve to evaluate the osmotic relations of the pollen grains and to disclose the optimal concentration for germinability tests for each species (Dafni et al. 2005). The composition of the germination medium can dramatically affect pollen metabolism (Taylor, Hepler 1997).

Nuclear and vital dyes (Alexander's procedure, acetocarmine, aniline blue in lactophenol, FDA, TTC, MTT, X-Gal and FCR), which indicate the presence

of cytoplasm or enzymes, respectively, were used to determine the pollen viability (Rodriguez-Riano, Dafni 2000; Nepi et al. 2005). The pollen germination results can also be used as an indicator of pollen viability.

There have been several flower morphology and fertilization biology studies in chestnut (Soylu 1981, 1992; Valdiviesso et al. 1993; Bounous, Torello Marinoni 2005; Mert, Soylu 2007). However, to date there have been fewer studies as compared to some of the other temperate zone fruit species. In addition, pollen germination potential and quality may differ from genotype to genotype. Therefore, the objective of this study was to investigate the viability and germination percentages of pollen collected from different genotypes.

MATERIALS AND METHODS

This study was carried out in Fatsa district of Samsun, Turkey in 2004-2006. Selected chestnut genotypes from The Black Sea Region namely SE3-12, SE18-2, SE21-2, SE21-9, 552-8, 552-10, 554-14, 556-8 and SA5-1 (Serdar 1999; Serdar, Soylu 1999), Sarıaslama cv. (Ayfer, Soylu 1995) were used in the study.

Mature catkins were randomly collected from three trees of each cultivar at full bloom stage in June. Samples were collected in the morning between 8 and 9 a.m. In this experiment, only male catkins were used as bisexual catkins often produce non-functional pollen. The catkins were laid on clean black paper in the laboratory.

The viability of freshly collected pollen grains was determined using a 1% TTC (2,3,5-triphenyl tetrazolium chloride) solution (Stanley, Linskens 1985). One or two drops of TTC solution was put on a clean microslide and pollen grains were sprinkled on these drops with a brush. Then, the drop was carefully covered by a cover glass without trapping air and kept for 2 h at ambient conditions. Then it was analyzed with a light microscope at a magnification of 400×. In the viability tests, 16 counts were made on four different fields in each of 4 microslides of all genotypes. At least 100–150 pollens were counted per observation field. A total of 2,000 pollen were counted per chestnut genotypes in this study of pollen viability. The pollen grain was considered viable if it turned red in colour.

Pollen germination was measured using methods described by Beyhan and Odabas (1995). Sucrose solutions of different concentration were added to basic agar of 1%. Four different sucrose concentrations, 5%, 10%, 15% and 20%, were tested in 2004. As the results obtained from the sucrose concentration of 20% were very low, this concentration was excluded from the study in the following years. However, the concentration of 5% gave high results, so the 0% concentration was also studied in 2005 and 2006. Approximately 10 ml of the medium was dispensed into glass Petri dishes, 8 cm in diameter. After it cooled and became semi-solid, the pollen grains were sieved through a screen with a mesh size of 0.149 mm onto the solidified medium to allow a uniform distribution of grains on the surface of the medium. The plates were then covered and incubated at 30°C for 24 h. After germination, pollens in the Petri dishes were refrigerated until counted. Two Petri dishes were used per sucrose concentration and eight observation areas were chosen randomly for each Petri dish. At least 100–150 pollens per observation field were counted in the germination tests. A total of 8,000 pollen grains were counted to estimate the germination percentage for each genotype. Pollen was considered as germinated if the pollen tube was at least as long as the length of the pollen grain.

Data were analyzed using the General Linear Models (GLM) procedure of SPSS (10.0). The experimental design is a factorial experiment with two factors (Sources; A, B and A × B) and 4 replicates. Means were separated by using Duncan's multiple comparison. Data of pollen viability and germination percentages were transformed using the arcsin transformation before statistical analysis.

RESULTS AND DISCUSSION

Pollen viability of the genotypes tested with TTC is presented in Table 1. Within each year, significant differences were found among the pollen viability rates of the chestnut genotypes. Viability percentages were generally high, not less than 80%. In general, in some previous studies, similar results for pollen viability were reported in Rubus species (12–59%) by Nybom (1985), in chestnut (67%) by Seiidov (1988), in hazelnut (49–97%) by Beyhan and Odabas (1995), in apricot (76–86%), sweet cherry (67–81%), sour cherry (71%) by Bolat and Pirlak (1999), in walnut (81–94%) by Sutyemez (2007).

There were also differences in pollen viability depending on experimental years (Table 1). For instance, whereas the genotype 552-8 had the highest pollen viability rate in 2005, it had the lowest pollen viability rates in 2004 and 2006. On the other hand, the genotype SE18-2 had the lowest pollen viability rate in 2004 while it had the highest pollen viability rate both in 2005 and 2006. The genotypes 554-14

Table 1. Percentage of pollen viability of chestnut genotypes between the years of 2004–2006 (%)

Genotypes	2004		2005		2006		Means
	mean	SD	mean	SD	mean	SD	
SE3-12	85.52 abcde	± 3.93	93.30 ab	± 2.01	81.35 de	± 7.85	86.72 A
SE18-2	61.90 g	± 11.55	91.08 abc	± 6.06	86.65 abcde	± 3.38	79.88 BC
SE21-2	88.88 abcd	± 4.00	88.89 abcd	± 2.79	78.71 ef	± 4.65	85.49 AB
SE21-9	82.28 cde	± 10.79	90.66 abc	± 6.50	85.36 bcde	± 3.31	86.10 AB
552-8	63.65 g	± 10.95	92.78 ab	± 2.23	69.12 fg	± 7.20	75.18 C
552-10	86.88 abcd	± 11.43	93.79 a	± 3.03	62.70 g	± 6.79	81.12 AB
554-14	88.06 abcde	± 2.14	68.27 fg	± 4.94	91.14 abc	± 3.36	82.49 AB
556-8	86.84 abcde	± 3.41	93.57 ab	± 1.57	79.80 de	± 7.03	86.74 A
SA5-1	85.38 bcde	± 3.14	80.65 de	± 4.30	87.26 abcde	± 3.69	84.43 AB
Sarıaslama	87.80 abcde	± 5.64	68.50 fg	± 4.93	87.27 abcde	± 5.13	81.19 ABC
Means	81.72 B		86.15 A		80.94 B		

Means followed by the same letter are not significantly different ($P = 0.01$), SD – standard deviation

and Sarıaslama had higher pollen viability rates in 2004 and 2006 compared to 2005. The differences between data of the three years (2004, 2005 and 2006) can be explained by changing environmental conditions. Genotypic differences must also be taken into account in determining pollen quality traits (Dafni et al. 2005).

The pollen germination percentages of the genotypes varied significantly trough the range of sucrose concentrations (Tables 2, 3, 4). In 2004, the highest pollen germinations were from SE18-2 genotype (43.68 and 42.53%) with 5 and 10% sucrose concentration, respectively, and SE21-9 (38.19%) with 10% (Table 2). In 2005, SE18-2 with 0% sucrose concentration had the highest pollen germination (31.97%) (Table 3). In both 2004 and 2005, SE18-2 had the highest pollen germination, although in 2006 it was significantly lower than some other genotypes. In 2006, 554-14 with 5 and 10% sucrose concentration had the highest pollen germination (31.03 and 29.05%) (Table 4). On the other hand, in 2004, 554-14 had the lowest germination. 556-8 had the lowest germination in 2005 and 2006, having an average rate in 2004. In the first two years, Sarıaslama had one of the lowest pollen germination rates but this genotype showed one of the highest germination rates in 2006.

In the present study, in 2005 and 2006, pollen germination declined compared to 2004. Pollen quality in the spring period of 2005 and 2006 was more adversely affected by climatic conditions than in 2004. Therefore, this study indicates that the pollen germination performance of the examined chestnut genotypes can vary with years.

Pollen germination at the optimal sucrose concentrations varied between 21.97 and 43.68% in 2004, 3.95 and 31.97% in 2005, 6.79 and 31.03% in 2006. Maximum pollen germination did not exceed 44%. Therefore, with the fact of having over 20% germination at least in two years, SE18-2, 554-14, SE3-12, SE21-9, Sarıaslama and SA5-1 can be recommended as promising male parents and pollinizers. However, Jaynes (1975) indicated that it was very difficult to reach over 60% in chestnut pollen germination. The highest pollen germination percentages in chestnut were reported to be 38.3% (Valdiviesso et al. 1993), 47.11% (Zhifang et al. 2005) and 33% (Fernando et al. 2006). However, Soylu (1981) and Mert and Soylu (2007) reported that the highest pollen germination percentages were 80.8 and 78%, respectively. These differences may be due to genotypic variations and different environmental conditions during growth.

The results obtained from the present experiment for pollen germination were generally lower than those found in the previous studies with other temperate fruit species (Eti 1991; Beyhan, Odabas 1995; Bolat, Pirlak 1999; Sutyemez 2007). It was also reported that germination success may depend on the humidity to which the pollen grains were exposed prior to the germination test and on the pollen age (Dafni et al. 2005). Moreover, it was noted that the rate of pollen germination *in vitro* depends largely on the experimenter's success in finding the optimal medium. In addition, weather conditions, position of the flowers on the canopy, genetic variations between individuals and timing at the season were referred to be the reasons of intraspecific variations.

Table 2. Effect of different sucrose concentrations on the germination rate (%) of chestnut pollen in 2004

Genotypes	Sucrose concentrations (%)								Means for genotypes
	5		10		15		20		
	mean	SD	mean	SD	mean	SD	mean	SD	
SE3-12	16.48 klmno	± 2.59	30.72 cde	± 4.87	17.36 jklmn	± 4.03	7.56 q	± 1.39	18.03 C
SE18-2	42.53 a	± 3.30	43.68 a	± 6.15	21.38 ghijk	± 2.14	17.62 jklmn	± 5.36	31.30 A
SE21-2	22.92 fghij	± 3.28	24.92 efgh	± 1.62	13.67 mno	± 0.97	2.17 r	± 0.89	15.92 D
SE21-9	35.42 bc	± 4.02	38.19 ab	± 5.78	28.93 def	± 5.82	12.91 no	± 2.29	28.86 A
552-8	21.21 ghijk	± 3.11	26.29 defg	± 5.81	13.61 no	± 3.54	2.21 r	± 2.38	15.83 D
552-10	14.46 lmno	± 1.68	32.49 bcd	± 8.31	28.45 def	± 6.12	8.83 pq	± 2.57	21.06 B
554-14	18.81 ijklm	± 1.72	21.97 ghijk	± 6.99	6.28 q	± 2.73	1.21 r	± 0.46	12.07 E
556-8	19.72 hijkl	± 4.05	27.02 defg	± 4.73	19.49 hijkl	± 4.15	8.34 pq	± 1.24	18.64 BC
SA5-1	24.94 efgh	± 4.11	23.91 fghi	± 3.66	13.31 no	± 2.63	6.81 q	± 0.74	17.24 CD
Sarıaslama	8.72 pq	± 1.94	16.44 klmno	± 1.64	22.68 fghij	± 7.67	12.01 op	± 1.82	14.96 D
Means for sucrose	22.52 B		28.56 A		18.52 C		7.97 D		

Means followed by the same letter are not significantly different ($P = 0.01$), SD – standard deviation

Table 3. Effect of different sucrose concentrations on the germination rate (%) of chestnut pollen in 2005

Genotypes	Sucrose concentrations (%)								Means
	0		5		10		15		
	mean	SD	mean	SD	mean	SD	mean	SD	
SE3-12	6.36 lm	± 2.17	4.40 no	± 1.06	14.12 de	± 4.82	17.34 c	± 5.42	10.55 D
SE18-2	31.97 a	± 11.36	23.85 b	± 3.07	12.20 defg	± 1.46	9.65 ghij	± 3.72	19.42 A
SE21-2	7.49 jkl	± 2.09	3.51 nop	± 0.80	5.29 mn	± 0.76	3.15 op	± 1.44	4.86 F
SE21-9	9.08 hijk	± 2.06	14.86 cd	± 3.94	12.74 def	± 2.35	6.54 lm	± 1.27	10.81 CD
552-8	9.66 ghij	± 2.98	8.28 ijkl	± 2.39	9.53 ghij	± 2.39	3.88 nop	± 0.92	7.84 E
552-10	11.28 efgh	± 2.75	8.68 hijkl	± 1.86	12.04 defg	± 3.66	14.15 de	± 2.26	11.54 BC
554-14	10.95 fgh	± 2.11	14.52 cd	± 2.07	17.35 c	± 2.16	7.84 ijkl	± 1.17	12.67 B
556-8	3.95 nop	± 3.33	2.71 p	± 1.12	3.18 op	± 1.20	3.88 nop	± 0.67	3.43 G
SA5-1	22.22 b	± 5.06	10.10 fghi	± 1.98	10.06 fghij	± 1.45	6.72 klm	± 1.01	12.28 BC
Sarıaslama	3.05 op	± 0.91	3.55 nop	± 0.52	6.89 klm	± 2.60	9.95 fghij	± 1.68	5.86 F
Means	11.60 A		9.45 B		10.34 A		8.31 C		

Means followed by the same letter are not significantly different ($P = 0.01$), SD – standard deviation

Table 4. Effect of different sucrose concentrations on the germination rate (%) of chestnut pollen in 2006

Genotypes	Sucrose concentrations (%)								Means
	0		5		10		15		
	mean	SD	mean	SD	mean	SD	mean	SD	
SE3-12	10.45 jk	± 1.90	20.44 def	± 4.20	15.52 ghi	± 2.83	19.80 ef	± 4.33	16.55 C
SE18-2	21.92 de	± 4.40	14.79 hi	± 2.29	15.62 ghi	± 2.49	12.37 ij	± 2.48	16.18 C
SE21-2	7.04 lm	± 1.94	8.14 kl	± 1.95	6.90 lm	± 1.26	6.74 lm	± 2.14	7.21 E
SE21-9	19.66 ef	± 4.04	17.86 fgh	± 3.43	17.69 fgh	± 2.65	6.82 lm	± 1.39	15.51 C
552-8	5.89 lmno	± 1.69	5.15 mno	± 1.56	6.89 lm	± 2.45	5.25 mno	± 1.95	5.80 FG
552-10	7.01 lm	± 2.01	5.06 mno	± 1.30	6.19 lmn	± 1.66	7.07 lm	± 2.14	6.33 EF
554-14	17.91 fgh	± 5.32	29.05 ab	± 6.37	31.03 a	± 7.27	18.80 efg	± 3.78	24.20 A
556-8	4.21 no	± 0.71	3.73 o	± 1.78	6.79 lm	± 1.12	5.17 mno	± 1.44	4.89 G
SA5-1	5.55 mno	± 2.45	12.49 ij	± 2.17	16.61 fgh	± 3.46	8.23 kl	± 1.60	10.72 D
Sarıaslama	14.98 ghi	± 1.86	16.75 fgh	± 1.63	23.91 cd	± 2.03	26.50 bc	± 2.97	20.54 B
Means	11.46 C		13.35 B		14.72 A		11.68 C		

Means followed by the same letter are not significantly different ($P = 0.01$), SD – standard deviation

Soylu (1981) determined the maximum pollen germination percentage for Sarıaslama cultivar in Marmara Region as 37% in 1977 and 37.8% in 1978. Mert and Soylu (2007) determined 11% pollen germination for Sarıaslama. In our study, the maximum pollen germination obtained from Sarıaslama was estimated at 26.50%. Thus it appears that the results can vary for the same genotype due to different ecologies.

The results were found difficult to evaluate when the data of viability and germination ratios were analyzed together. SE18-2 had the highest germination and the lowest viability in 2004 (Tables 1 and 2). 554-14 had the highest pollen germination and the highest viability in 2006. As seen in Fig. 1, there was no significant relation between the viability and germination ratios in 2004 and 2005 ($r = -0.54$ and $r = -0.05$, respectively). However, in 2006, a marked positive correlation ($r = 0.80$) was obtained between the viability and germination ratios. The TTC staining test did not give a reliable estimation for pollen germinability of chestnut genotypes.

In general, a linear relationship is expected between viability and germination ability. In viability test of TTC, tetrazolium salt is converted to reddish formazan by dehydrogenase respiratory enzymes. For many species, tetrazolium tests are reliable indicators of pollen viability. However, for other species, they may yield false positive scores for viability when compared with results from germination tests (Stone et al. 1995; Dafni et al. 2005). For example, Nybom (1985) reported that the germination test was not significantly correlated with TTC staining test in some blackberry species. Similar results were reported by Parfitt and Ganeshan (1989) in pollens of some *Prunus* species. Furthermore, the dead pollens could also be dyed together with fresh pollens in the most common staining procedures (Parfitt, Ganeshan 1989; Rodriguez-Riano, Dafni 2000). Parfitt and Ganeshan (1989) found that staining percentage of the killed *Prunus* pollens in the TTC staining test was only 1%.

In addition to the above mentioned statements, there were cases of pollen grains that stained many different colour tonalities, ranging from pale to deep red, and this made it difficult to distinguish viable pollens from nonviable ones (Parfitt, Ganeshan 1989; Stone et al. 1995; Kelen, Demirtas 2003). As mentioned previously, TTC staining test gave reliable estimations of pollen viability in many fruit species. As well as using TTC viability test, Kelen and Demirtas (2003) and Sutyemez (2007) also used FDA viability test for some grape and walnut

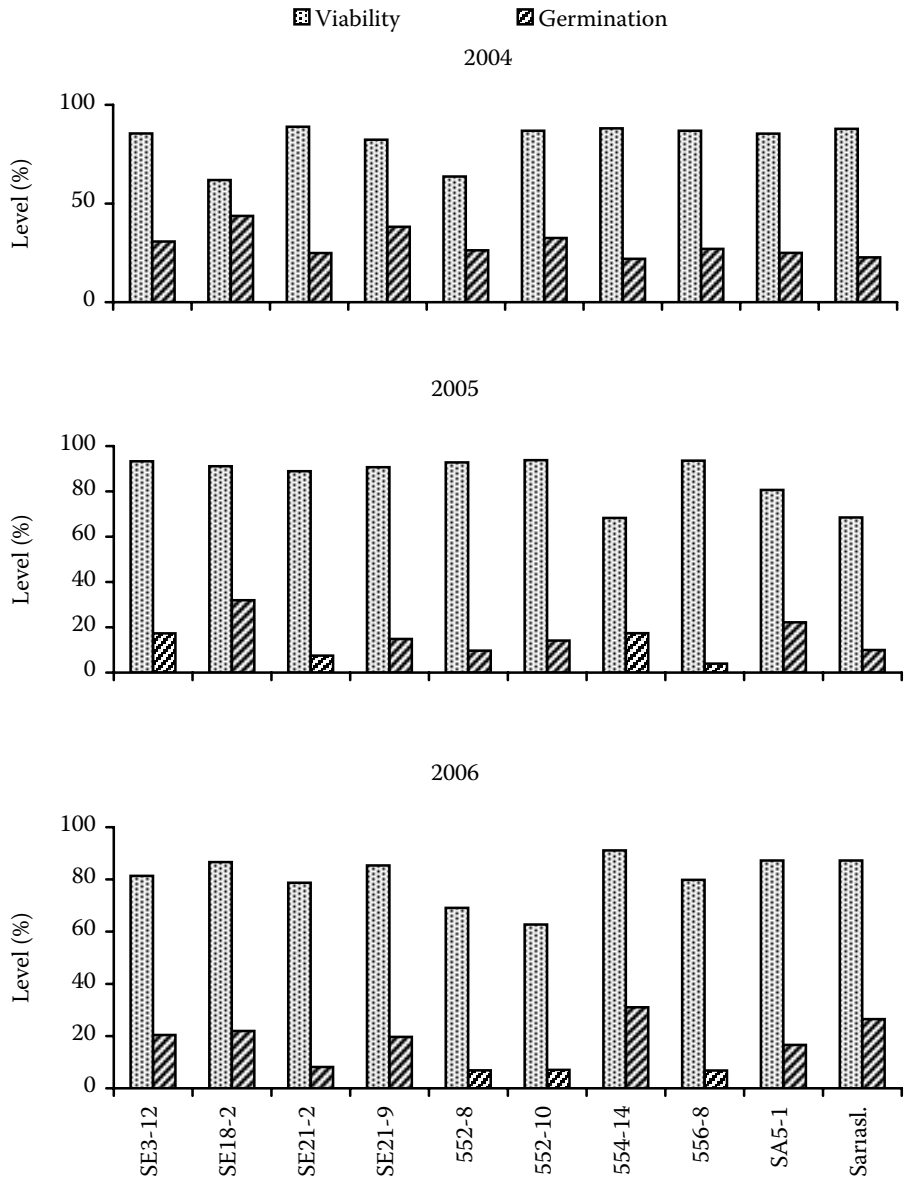

Fig. 1.Relation between the viability rates and germination rates in 2004 (r = –0.54), 2005 (r = –0.05) and 2006 (r = 0.80)**

cultivars, respectively. Moreover it was reported that both TTC and FDA viability tests gave similar results for pollen viability. It can be deduced from this result that TTC is reliable for determining pollen viability.

The pollen germination of the examined chestnuts was affected by differing sucrose concentrations. Besides, this interaction varied as affected by years. The highest pollen germination rates were obtained from 10% sucrose concentration for all years. 1% of sucrose concentration was reported to be enough for pollen germination in chestnuts (Rutter et al. 1990). However, Soylu (1981) used 0%, 1%, 5%, 10% and 15% concentrations for *in vitro* pollen germination. Valdiviesso et al. (1993), Fernando et al. (2006) and Mert and Soylu (2007) each preferred to use only one concentration in their studies as 15%, 5% and 10%, respectively.

Stanley and Linskens (1985) mentioned the capability for the absorption of carbohydrate by the pollen and the advantages such as having the aerobic conditions at agar level, controlled optimum temperature and humidity when using agar + sucrose mixtures in pollen germination test. However, Eti (1991) stated that the different tests for pollen viability and germination may not be successful at the same level for every fruit species and cultivar. Expressing the impossibility of the dynamic interaction between pollen and pistil in artificial germination mediums, Taylor and Hepler (1997) stated that *in vitro* pollen germination tests were not good in some species. Stösser et al. (1996) pointed out that

the relation between fruit set and viability tests was not clear and that the most effective method was the fruit production by hand pollination.

CONCLUSIONS

The highest pollen germination rates were obtained at 10% sucrose for all years. In connection with this, SE18-2, 554-14, SE3-12, SE21-9, Sarıaslama and SA5-1 can be recommended as promising male parents and pollinizers. The pollen germination capability of the examined chestnut genotypes varied between years. The nutritional physiology and the negatives effects of the rainfall during the blooming period might have been the reasons for this result. In all genotypes, pollen viability was found to be higher than the germination percentage. For data over three years, there was no significant relationship between the viability and germinability in the first two years (2004 and 2005). To estimate the pollen viability of the chestnut, an adequate method should be found as an alternative to TCC and therefore more viability tests must be conducted in further investigations. Further examinations should be done extensively on the reproduction biology of chestnut since it has some floral features to limit hybridization and fruit set such as dichogamy and sexual incompatibility. Barriers to interspecific hybridization in chestnut breeding may be overcome by further improved efforts.

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Hodnocení životnosti a klíčivosti pylů u vybraných evropských genotypů kaštanu (*Castanea sativa* L.)

ABSTRAKT: Studie hodnotila životnost a klíčivost pylů u vybraných evropských genotypů kaštanu. U deseti genotypů kaštanu bylo procento životnosti pylu obecně vysoké, často dosahovalo 80 % i více. Procenta klíčivosti pylu u zkoumaných genotypů byla významně ovlivněna koncentrací sukrózy v médiu. Při optimálních koncentracích se klíčivost pylu u všech genotypů pohybovala mezi 21,97 a 43,68 % v roce 2004, 3,95 a 31,97 % v roce 2005 a 6,79 a 31,03 % v roce 2006. Nejvyšší klíčivosti pylu (v procentech) bylo dosaženo na médiu s 10 % sukrózy ve všech letech. V roce 2006 byla pozorována výrazná pozitivní korelace (r = 0,80) mezi hodnotami životnosti a klíčivosti pylu na rozdíl od roku 2004 (r = –0,54) a 2005 (r = –0,05), kdy mezi těmito parametry žádná významná souvislost zjištěna nebyla. Při srovnání s rokem 2004 procento klíčivosti v letech 2005 a 2006 klesalo.

Klíčová slova: *Castanea sativa* L.; životnost pylu; klíčivost pylu

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