Original article

In vitro pollen germination and tube growth of some European chestnut genotypes (*Castanea sativa* Mill.)

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In vitro pollen germination and tube growth of some European chestnut genotypes (*Castanea sativa* Mill.).

Abstract — Introduction. Investigation of pollen performance is essential in breeding experiments or artificial pollination procedures, especially in a crop such as chestnut, some genotypes of which are characterized by male sterility. Our study was carried out to determine the pollen germination rate and the pollen tube growth of some European chestnut genotypes. **Materials and methods**. Seven Turkish advanced chestnut selections (SE3-12, SE18-2, SE21-2, SE21-9, 552-8, 552-10 and 554-14), and a cultivar (Sariaslama), growing in two different locations, were studied in the central Black Sea region of Turkey in 2006. For this purpose, *in vitro* pollen germination tests were performed on media with four different concentrations of sucrose [(0, 5, 10 and 15)%]. **Results and discussion**. The pollen germination rates of the eight genotypes studied were significantly different due to the sucrose concentrations. The best pollen germination rate was found in the 554-14 chestnut genotype (35.80%). The best sucrose concentration for a high germination rate and pollen tube growth was 10%. For the same genotype, the results varied due to the two different study locations (Terme and Fatsa). A linear relationship between the sucrose concentrations and pollen tube length was found to be significant in most genotypes. The longest pollen tube length was found in the 554-14 genotype (74 µm).

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Germination du pollen et croissance du tube pollinique *in vitro* pour quelques génotypes de châtaigniers européens (*Castanea sativa* Mill.).

Résumé — Introduction. Des recherches sur les performances du pollen sont importantes pour la sélection des plantes ou pour la réalisation de pollinisations artificielles, et cela particulièrement pour une production comme le châtaignier dont certains génotypes sont mâles stériles. Notre étude a été effectuée pour déterminer le taux de germination du pollen et la croissance des tubes polliniques de quelques génotypes de châtaigniers. **Matériel et méthodes**. Sept sélections turques, confirmées, de châtaignier (SE3-12, SE18-2, SE21-2, SE21-9, 552-8, 552-10 et 554-14) et un cultivar (Sariaslama), localisés dans deux emplacements différents ont été étudiés dans la région centrale de la Mer Noire en Turquie, en 2006. À cette fin, des essais de germination de pollen *in vitro* ont été réalisés sur des milieux présentant quatre concentrations différentes de saccharose [(0, 5, 10 et 15) %]. **Résultats et discussion**. Les taux de germination du pollen des huit génotypes étudiés ont différé de façon significative en fonction des concentrations en saccharose du milieu. Le meilleur taux de germination du pollen a été trouvé dans le génotype 554-14 (35.80 %). La meilleure concentration en saccharose apte à induire un taux élevé de germination du pollen et de croissance du tube pollinique a été de 10%. Pour un même génotype, les résultats ont varié en raison de la localisation différente des zones d'étude (Terme et Fatsa). Pour la plupart des génotypes, le rapport entre les concentrations en saccharose et la longueur de tube pollinique s'est avéré significativement linéaire. Le tube pollinique le plus long a été trouvé pour le génotype 554-14 (74 µm).

Turquie / *Castanea sativa* / germination du pollen / tube du pollen / croissance

1. Introduction

Turkey is one of the countries where the European chestnut originated and has a wide range of variability. Since 1975, cultivar selection studies have been conducted on native chestnut populations in various parts of Turkey. Some promising genotypes have been identified in the central Black Sea region. It is important to know the flower morphology and fertilization biology of these new genotypes.

In nut crops such as the chestnut, fertilization is an essential process for the fruit set and seed development [1, 2]. Because of flowers maturing at different times, widely existing self-incompatibility and male-sterile cultivar pollens often being aborted or sterile are the major yield-limiting factors in the monoecious species chestnut; cross-pollination is essential. At least two different pollinizer cultivars together with one main cultivar in one orchard are recommended to ensure a high level of cross-pollination [3–5]. Therefore, the pollen quality of the pollinizer cultivar is the most important factor for adequate fruit set and seed development. Understanding the pollen quality of the cultivars is not only important for the selection of a suitable pollinizer while the orchard is being established, but also to increase the efficiency of the breeding program.

The pollen quality of the genotypes has been an important aspect for the self- and cross-pollination processes in breeding programs. In chestnut, chestnut canker disease [*Cryphonectria parasitica* (Murr.) Barr] is a very serious problem for all the world's chestnut forests and plantations. The Asian species *C. mollissima, C. crenata* and *C. henryi* have good resistance to this fungus. Therefore, in recent years, research in chestnut breeding has mainly been focusing on resistant Asian trees which are crossed with susceptible European and American varieties [6].

There have been several studies on flower morphology and fertilization biology for the chestnut [5, 7–10]; however, there are fewer than for some other temperate zone fruit species. In addition, the pollen germination potential and quality may differ from genotype to genotype. The aim of our study was to evaluate, for some European chestnut genotypes, the effects of various sucrose levels on the pollen germination and pollen tube growth in pollen samples taken from two different locations.

2. Materials and methods

Our study was carried out in the Terme district of Samsun and Fatsa district of Ordu (Turkey) in 2006. The seven chestnut genotypes SE3-12, SE18-2, SE21-2, SE21-9, 552-8, 552-10 and 554-14 were selected from the Black Sea coast [11, 12] and the Saralama cv. [13] was also studied in our experiment.

Mature catkins were randomly collected from three trees of each individual genotype at the full blooming stage around June. Only male (unisexual) catkins were used because bisexual catkins often produce non-functional pollen grains. The catkins collected were brought to the lab in the afternoon and laid on a clean black piece of paper.

In the pollen germination tests, the Petri dish agar method was used [2]. Sucrose solutions with four different concentrations (0%, 5%, 10% and 15%) were added to basic 1% agar medium. Approximately 10 mL of the medium was dispensed into 8-cm glass Petri dishes. When the medium became cold and semi-solid, the pollen grains were sieved, through a screen with a 0.149-mm mesh size, onto the solidified medium to allow a uniform distribution of grains on its surface. The plates were then covered and incubated at 30 °C for 24 h. After 24 h of germination, pollens in the Petri dishes were counted. Pollen was considered germinated if the pollen tube was at least as long as the length of the pollen grain. Each genotype was tested on four different sucrose-concentration media. Since two Petri dishes were used for each sucrose concentration tested, eight Petri dishes per genotype studied were used in total. In each Petri dish, eight fields were delimited. Therefore, each genotype was evaluated from the observation of 64 fields. In each field, 100–150 pollen grains and, therefore, for each genotype, from 6400 (64 × 100) pollen grains to 9600 (64 × 150) pollen grains were counted. Each Petri dish

Table I.
Effect of different sucrose concentrations on the germination rate (%) of chestnut pollen in the Terme zone, district of Samsun (Turkey).

Chestnut genotype		Sucrose concentrations (%)			
		0	5	10	15
Advanced selections	SE3-12	7.63 jkl	15.57 de	15.35 de	13.93 efg
	SE18-2	15.68 de	20.54 c	21.23 c	14.91 def
	SE21-2	7.12 jkl	8.00 jkl	14.82 def	12.49 efg
	SE21-9	18.47 cd	20.27 c	19.66 c	9.06 hij
	552-8	10.83 ghi	6.70 jkl	12.32 efg	6.14 kl
	552-10	5.96 l	7.82 jkl	11.60 fgh	14.06 ef
	554-14	34.92 a	27.32 b	35.80 a	21.10 c
Cultivar	Sariaslama	6.17 kl	6.27 kl	8.70 ijk	17.95 cd

Means followed by the same letter in the columns are not significantly different ($P < 0.01$).

surface was divided into two fields, and each field was considered one replication. Each replication field consisted of an average of four different observed data of half a Petri dish.

The length of the pollen tube was measured using a micrometer eyepiece (calibrated using a stage micrometer). A total of 100 pollen tubes was measured for each genotype. Linear regression analysis was used for evaluating the relationship between pollen tube length and sucrose concentration.

Data were analyzed using the General Linear models (GLM) procedure of SPSS (10.0). The experimental design for the germination test was a factorial experiment with two factors (genotype and dose) and four replicates. Means were separated by using Duncan multiple comparisons. Data of the pollen germination percentages were transformed using the arcsine transformation before statistical analysis.

3. Results and discussion

The pollen germination rates of the genotypes were generally low and varied significantly due to sucrose concentrations (*table I, II*).

For the genotypes from the Terme location, the highest pollen germination was obtained with the 554-14 genotype [(34.92 and 35.80)% of germination] with 0% and 10% sucrose concentrations, respectively (*table I*). This was followed by SE18-2 [(20.54% and 21.23)%] with 5% and 10% sucrose concentrations, then SE21-9 [(18.47, 20.27 and 19.66)%] with 0%, 5% and 10% sucrose concentrations, respectively. Germination rates for the other genotypes were lower than 20%.

For the genotypes from the Fatsa location, the highest pollen germination rate was with the 554-14 genotype [(29.05 and 31.03)% of germination] with 5% and 10% sucrose concentrations, respectively (*table II*). This was followed by Sariaslama (23.91% and 26.50%) with 10% and 15% sucrose concentrations. The 552-8 and 552-10 genotypes showed the lowest rates of germination.

In both ecologies, the 552-8 genotype had the lowest results and the 554-14 genotype had the highest results. Sariaslama had the lowest germination rate in the Terme location, although it had the highest one in the Fatsa location. Different germination rates were obtained for the same genotype according to whether it was from the Fatsa or from the Terme location. This may be due to different developmental circumstances of catkins and pollens because of variable climatic and soil conditions in the two locations.

Table II.
Effect of different sucrose concentrations on the germination rate (%) of chestnut pollen in the Fatsa zone, district of Ordu (Turkey).

Chestnut genotype		Sucrose concentrations (%)			
		0	5	10	15
Advanced selections	SE3-12	10.45 jk	20.44 def	15.52 hi	19.80 def
	SE18-2	21.92 de	14.79 hi	15.62 hi	12.37 ij
	SE21-2	7.04 lm	8.14 kl	6.90 lm	6.74 lm
	SE21-9	19.66 defg	17.86 efgh	17.69 efgh	6.82 lm
	552-8	5.89 lm	5.15 m	6.89 lm	5.25 m
	552-10	7.01 lm	5.06 m	6.19 lm	7.07 lm
	554-14	17.91 efgh	29.05 ab	31.03 a	18.80 efgh
Cultivar	Sariaslama	14.98 hi	16.75 fgh	23.91 cd	26.50 bc

Means followed by the same letter in the columns are not significantly different ($P < 0.01$).

According to Soylu, the pollen germination rates of chestnut varied between 36% and 80.8% [7]; for Valdiviesso *et al.*, it was between 0% and 38.3% [9]; for Fernando *et al.*, between 33% and 48% (American chestnuts) [14]; and for Mert and Soylu, between 11% and 78% [10]. The results for different genotypes and ecologies vary widely.

Soylu determined a maximum pollen germination rate for the Sariaslama cultivar of 37% in 1977 and 37.8% in 1978 in the Marmara region [7]. Mert and Soylu determined an 11% pollen germination rate for this same Sariaslama cultivar [10]. In our study, the maximum pollen germination rate obtained from the Sariaslama cultivar was 26.50%. According to these data, it appears that the results can vary for the same genotype due to different ecological conditions.

A pollen grain on the stigma surface hydrates and germinates to produce a pollen tube which enters the style. In *in vitro* germination tests, the semi-solid germination media was prepared by adding to agar an osmoticant and a carbon source, such as sucrose, and some mineral elements (Ca, Mg, K and B) [15, 16]. Stanley and Linskens mentioned the capacity for carbohydrate absorption by pollen and such advantages as having the aerobic conditions at agar level, controlled optimum temperature and humidity when using agar + sucrose mixtures in *in vitro* pollen germination tests [17]. However, Eti and Preuss *et al.* stated that the different tests for pollen viability and germination may not be successful at the same level for every fruit species and cultivar [18, 19]. Taylor and Hepler expressed the impossibility of the dynamic interaction between the pollen and pistil in artificial germination media; *in vitro* germination does not completely mimic *in vivo* growth [16]. According to Johnson and Preuss, pollen tubes grow faster, longer and more homogenously *in vivo* than *in vitro* [20]. Read *et al.* reported that, even with highly optimized germination media, *in vitro* tubes reach only 30–40% of *in vivo* lengths [21]. Stosser *et al.* pointed out that the relation between fruit set and viability tests is not clear and that the most effective method is fruit production by hand-pollination [22].

The pollen germination rates of the examined chestnuts were affected by the sucrose concentrations at different levels. According to Rutter *et al.*, a 1% sucrose concentration was sufficient for pollen germination in chestnuts [23]. Soylu used 0%, 1%, 5%, 10% and 15% concentrations [7]. Valdiviesso *et al.* [9], Fernando *et al.* [14], and Mert and Soylu [10] preferred to use only one concentration in their studies; 15%, 5% and 10%, respectively. In our study, the best results were generally obtained by using a 10% sucrose concentration.

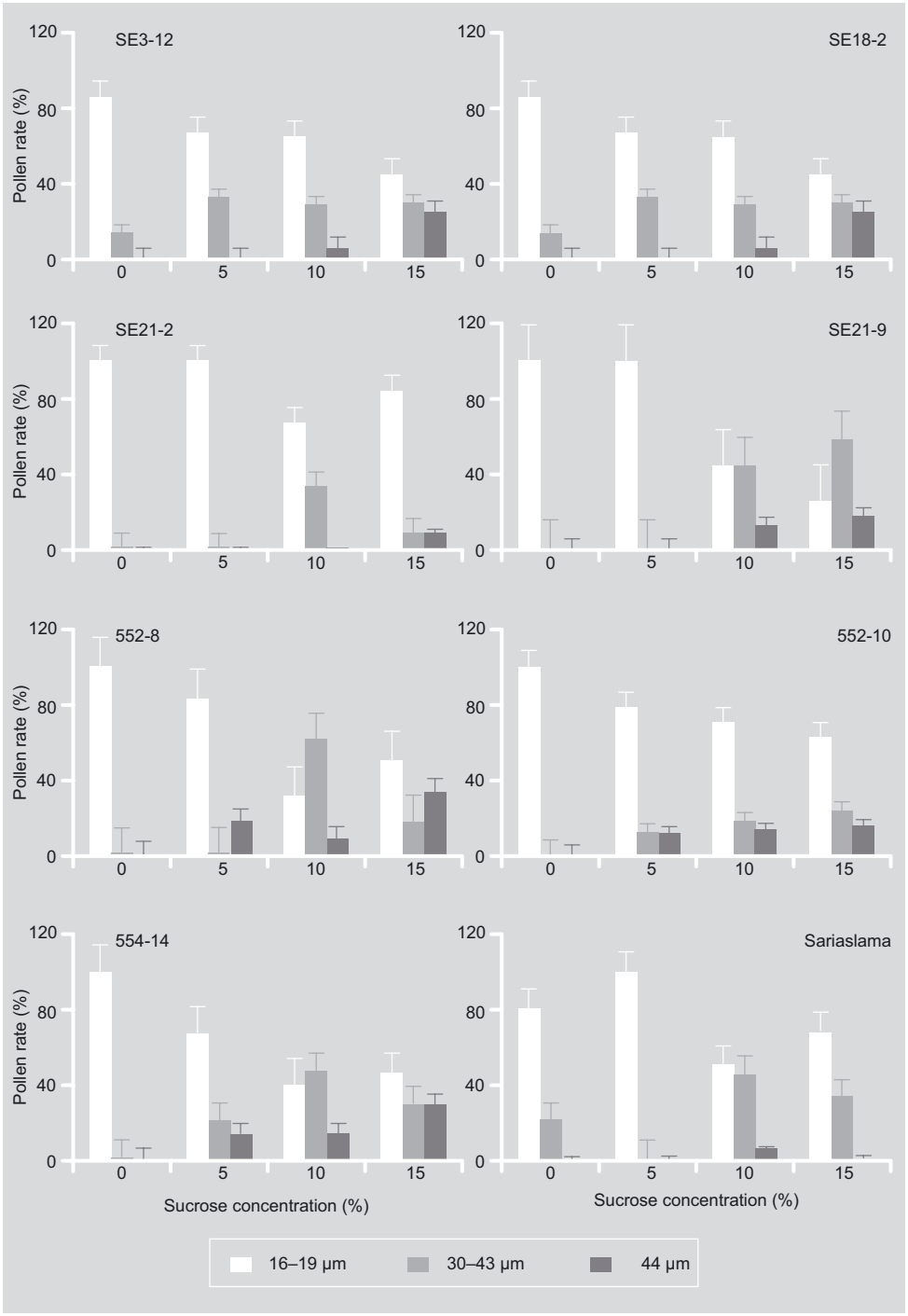

Figure 1.
The rate of chestnut germinated pollen distribution in three tube length categories for different sucrose concentrations in the Terme zone, district of Samsun (Turkey).

We also measured the pollen tube lengths, according to which the tubes were classified into three groups. For the first group, 69–100% of the pollen tubes were obtained with 0% sucrose concentration and 39–100% of the pollen tubes with 5% sucrose; their lengths ranged between (16 and 19) µm (*figures 1, 2*). With a 10% sucrose concentration, the percentage of pollen in this first group was 31–83%. In the

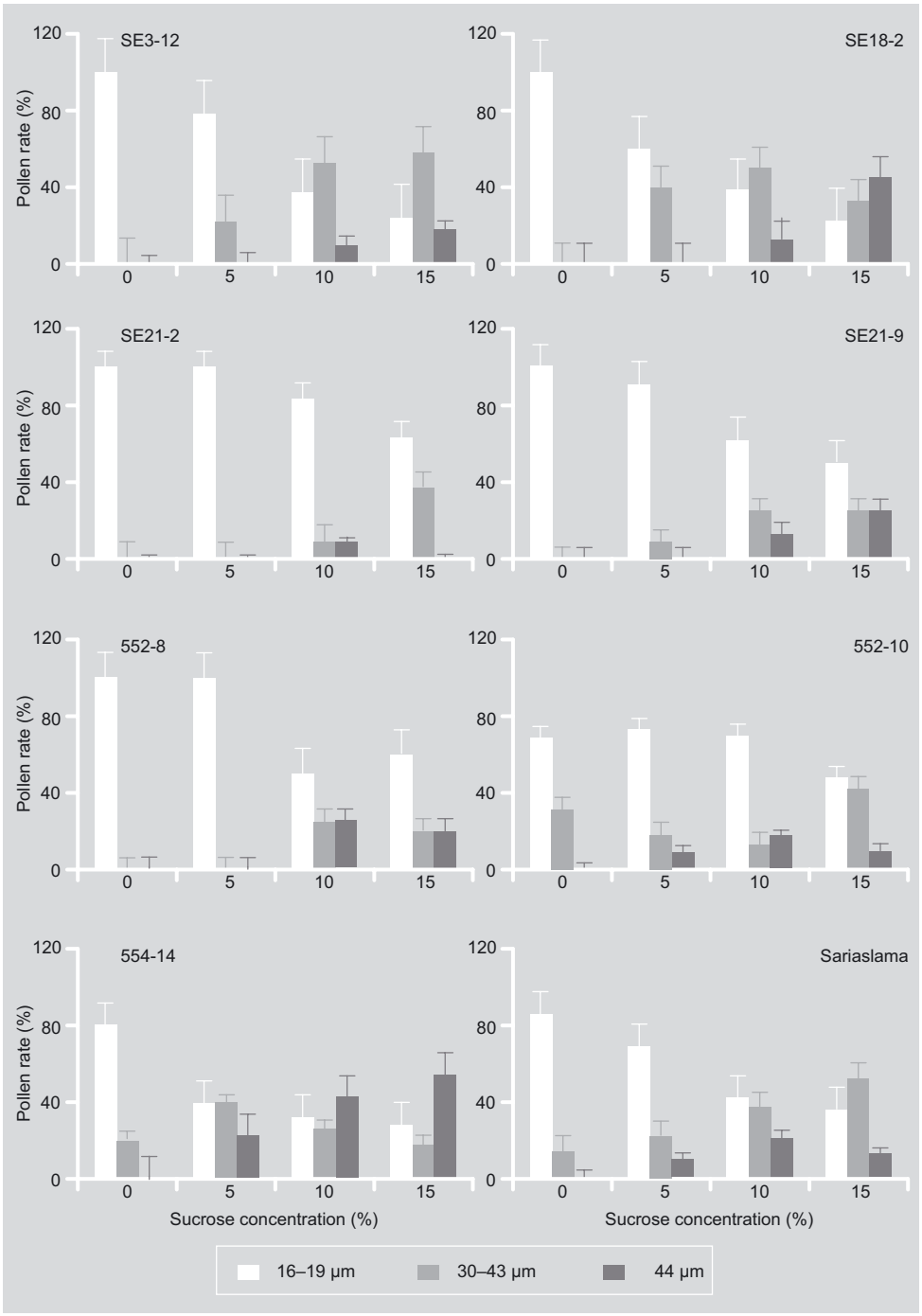

Figure 2.
The rate of chestnut germinated pollen distribution in three tube length categories for different sucrose concentrations in the Fatsa zone, district of Ordu (Turkey).

second group, the pollen percentage was 9–61% at the same concentration and tube lengths ranged between (30 and 43) µm. However, for this concentration, the number of pollen tubes from the third group was not more than 42%. Pollen tubes were longest for the 15% sucrose treatment. With 15% sucrose, 0–54% of pollen tubes were measured as 44 µm or longer. In our study, the longest pollen tube length was measured as

Table III.
Regression analysis between the sucrose concentrations of pollen germination media (%) and pollen tube length (µm) of the chestnut genotypes in Terme and Fatsa [regression model: $y = a + bx$; y = pollen tube length, x = sucrose (%)].

Chestnut genotype		Terme			Fatsa		
		a	b	r^2	a	b	r^2
Advanced selections	SE3-12	20.257 (0.549)**	0.936 (0.059)**	0.992	19.062 (1.670)**	1.167 (0.179)*	0.955
	SE18-2	18.627 (0.403)**	0.865 (0.043)**	0.995	20.714 (1.862)**	1.167 (0.199)*	0.945
	SE21-2	16.211 (0.410)**	0.625 (0.044)**	0.990	17.493 (1.491)**	0.572 (0.159)	0.865
	SE21-9	16.342 (0.748)**	1.256 (0.080)**	0.992	17.894 (0.256)**	0.805 (0.027)**	0.998
	552-8	21.653 (1.178)**	0.809 (0.126)*	0.954	16.738 (1.358)**	1.152 (0.145)*	0.969
	552-10	19.309 (1.343)**	0.792 (0.144)*	0.938	23.231 (0.164)**	0.549 (0.018)**	0.998
	554-14	22.446 (1.867)**	0.895 (0.200)*	0.909	26.214 (2.311)**	1.148 (0.247)*	0.915
Cultivar	Sariaslama	21.677 (2.613)*	0.498 (0.279)	0.614	24.781 (2.035)**	0.667 (0.218)	0.824

* Significant at 0.05% level, ** significant at 0.01% level.

74 µm. Generally, an increasing sucrose concentration in the germination media increased the length of the pollen tubes.

The results obtained from the study of the regression analysis between the sucrose concentrations of the pollen germination media (%) and the pollen tube length (µm) of the chestnut genotypes show that the relationship between these two parameters is linear and significant for all genotypes, except for Sariaslama in both ecologies and SE21-2 in Fatsa (*table III*). For most of the genotypes, the tube length of the germinated pollen increased with the increase in the sucrose concentrations of germination media. Finally, the results of this study can only be binding for sucrose concentrations not exceeding 15%. Loguercio *et al.* reported that tobacco pollen germination was completely blocked in sucrose concentrations above 40% and they found maximal tube length when pollen was cultured with a sucrose concentration between 7.5% and 20% at room temperature over 16 h [24]. Similar results were also reported by Bolat and Pirlak for the sweet cherry [25]. They obtained the longest tube length with a 15% sucrose concentration and reported that sucrose concentrations of 20–25% had an inhibitory effect on pollen germination in hanging drop tests.

In our experiments regarding the chestnut pollen grain sizes, the average pollen length and width were (20.22 and 12.76) µm, respectively. Seiidov investigated *Castanea sativa* pollen morphology and reported that some pollens were large (13.8–15.4) µm in diameter and that some were small (11.9–13.0) µm [26]. Bounous *et al.* reported that pollen length varied from (14 to 18) µm, and width from (10 to 14) µm in some *Castanea* species [27]. According to Mert and Soylu, pollen length and width ranged from (13.33 to 21.30) µm to (8.72 to 11.78) µm, respectively [10]. Our results were similar to the previous reports.

Chestnut pollen sizes and tube lengths are smaller than for other fruit species. Pirlak stated that the pollen tube length was

between 230 µm and 423 µm in the sweet cherry, and between 218 µm and 299 µm in the apricot, in a medium of 15% sucrose and 1.5% agar [28]. Yasin and Gozlekci investigated the sizes of pollen in the sweet cherry, loquat, carob and indian fig pollen [29]; in their study, the carob was found to have the smallest pollen (16.58–23.70) µm and the Indian fig was found to have the largest pollen (52.10–68.10) µm.

In conclusion, pollen germination and tube growth varied according to the genotypes, sucrose concentrations of the germination medium and the influence of environmental factors upon growing conditions. In our study, the 554-14 chestnut genotype was found to have the best pollen germination rate and the relationship between the sucrose concentrations and pollen tube length was linearly significant in most genotypes. The best sucrose concentration for the germination rate and pollen tube growth is 10%.

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Germinación del polen y crecimiento del tubo de polen *in vitro* para ciertos genotipos de castaños europeos (*Castanea sativa* Mill.).

Resumen — Introducción. Resultan importantes las investigaciones sobre los rendimientos del polen para la selección de las plantas o para la realización de polinizaciones artificiales, y esto particularmente para una reproducción como el castaño, del cual, ciertos genotipos son machos estériles. Nuestro estudio se efectuó para determinar el índice de germinación del polen así como el crecimiento de los tubos de polen de ciertos genotipos de castaños. **Material y métodos**. Se estudiaron en 2006 siete selecciones turcas confirmadas de castaño (SE3-12, SE18-2, SE21-2, SE21-9, 552-8, 552-10 y 554-14), así como un cultivar (Sariaslama), localizados en dos lugares diferentes en la región central del Mar Negro en Turquía. Se realizaron con este fin unos experimentos de germinación de polen *in vitro* en medios que presentaban cuatro concentraciones diferentes de sacarosa [(0, 5, 10 y 15) %]. **Resultados y discusión**. Los índices de germinación del polen de los ocho genotipos estudiados, difirieron de modo significativo en función de las concentraciones de sacarosa del medio. El mejor índice de germinación del polen se halló en el genotipo 554-14 (35.80 %). La mejor concentración de sacarosa apta a inducir un elevado índice de germinación del polen y de crecimiento del tubo de polen fue del 10%. Para un mismo genotipo, los resultados variaron en razón de la localización diferente de las zonas de estudio (Terme y Fatsa). Para la mayoría de los genotipos, la relación entre las concentraciones de sacarosa y la longitud de tubo de polen resultó ser significantemente linear. El tubo de polen más largo se encontró en el genotipo 554-14 (74 µm).

Turquía / *Castanea sativa* / germinación del polen / tubos polínicos / crecimiento