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POLLEN PERFORMANCES OF NATURALLY GROWN BLACKBERRIES IN ISPARTA - TURKEY

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Abstract

Fertilization biology is one of the most important criteria used to identify as candidate genotype and variety. In this research pollens of 6 wild-grown blackberry shrubs, preselected from Isparta - Turkey, were used. In order to determine the pollen performance which is the basis of the studies on fertilization biology, the amount of pollen production, pollen viability degree and the morphological homogeneity rate were determined. In addition, temperature experiments (15^{0}C, 20^{0}C and 25^{0}C) were performed to determine the optimum pollen germination condition. However, the effects of some growth regulators and mineral substances (GA_3, KNO_3, BA) on pollen germination and tube growth were investigated. N6 had the highest values in terms of anther number (71.2) average number of pollens per anther (13151.7), number of pollen per flower (414245.7), and morphological homogeneity (95.8%). 20^{0}C was found optimum temperature for pollen germination and tube growth. Gibberellic acid and Potassium nitrate were determined as promoter while benzyl adenine had inhibitory effect on pollen germination and tube growth. As a result of fertilization biology studies N6 can be thought promising, terms of pollen performance.

Key words*: Rubus canescens, pollen tube growth, TTC, KNO_3.*

INTRODUCTION

Turkey is located in the region of the intersection of three phytogeography regions. While Turkey flora has approximately 11,466 plant taxon, whole Europe continent has approximately 12,000 plant taxon. *Rosaceae* family has some are thorny and trailing that are rarely grassy, bush and tree exist. This family is represented by 115 genus and approximately 3200 species in the world. *Rosaceae* family members are represented by 35 genus and 318 species in Turkey. *Rubus* taxon is an important genus of *Rosaceae* family.

It has about 16 species in Turkey (Güner, 2012). *Rubus canescens* DC. var. *canescens* is low, usually trailing shrub. The red purplish body is angular and often prickly. Thorns are short, flat and sickle-shaped. Leaves are ternate or sometimes pedate with 3 or 5 leaflets, discolorous; leaflets tomentellous, dull green or greyish green, canescent-tomentose with stellate hairs below, bidentate, lateral ones subsessile, terminal one petiolulate, cuneate-obovate to rhombic; stipules linear. Flowering shoots are erect, 15-40 cm, angled, pubescent to shortly tomentose, armed like the turions. Sepals are ovate-oblong, acute, pubescent tomentose, reflexed after flowering. Petals are white (drying pale yellowish), obovate-oblong (rarely broader), 5-8 (-10) mm. Fruits are about 1 cm in size and red purple color. Drupelets are black, small and numerous, glabrous (Davis, 1972).

It is light-half-shadow plant. It is resistant to cold and hot. It likes moisture but can also grow in dry places. It is used of forceps, diuretic and diabetes in folk medicine. The liquid obtained from the fruits is used as a mouthwash against inflammations of the tonsils (Durmuşkahya, 2006).

Fertilization success in plants is the result of processes that take place during the progamic phase (Thompson, 2004; Güçlü and Koyuncu, 2017). Pollen germination and pollen tube growth are important components of fertilization success in fruit trees (Janıck and Moore, 1996; Tosun and Koyuncu, 2007).

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, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed-producing plants.
Pollen performance is clearly affected by the genotype of the pollen (Acar and Kakani, 2010; Hedly et al., 2004).
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, pollen tube growth as well (Johanson and Stephanson, 1998).
Pollen germination and pollen tube growth are important research materials for morphological, physiological, biotechnological, ecological, evolutional, biochemical, systematic and molecular studies. Additionally testing pollen performance could be helpful for a fruit cultivation of genetic progeny for breeding purpose, and especially for selecting which cultivars should be used by researchers and growers.
For this purpose we tried to observe the pollen performances of 6 wild-grown blackberries.

MATERIALS AND METHODS

Pollens were collected from flowers of 6 wild-grown blackberry shrubs (N1, N2, N3, N4, N5, N6) at balloon stage.
The 50 flowers at balloon stage were picked up. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into the dark-colored bottle to promote dehiscence at room temperature. For the pollen performance, pollen production capacity, morphological homogeneity, pollen viability rates were investigated.
In addition, optimum germination temperature and optimum germination medium consist of some growth regulators and mineral substances were determined. The pollen production capacity and morphological homogeneity percentages of pollens were assessed with the hemocytometer (Marienfeld, Germany) slide (Eti, 1990). Imperfectly shaped pollen grains were considered as aborted pollen.
The final percentage of morphological homogeneity (MH) was defined as:

$$MH = \frac{Ns - Na}{A} x100$$

Ns: Number of shaped pollen per area;
Na: Number of aborted pollen per area;
A: Total number of pollen area.

Pollen viability was determined by using TTC (2, 3, 5-triphenyl tetrazolium chloride) staining test. A few drops of 1% TTC (0.2 g. TTC and 12 g. sucrose were dissolved in 20 ml distilled water) were dropped by Pasteur pipettes on microscope slides and pollen were shaked with a slim brush (each brush used only one plant type) covered with a coverslip of used four microscope slides with three replication were counted after 2 hours.
To determine the pollen viability, pollens of each cultivar (of four different areas) were observed onto two slides under a light microscope (×100 magnification). The stained pollen was considered as 'viable' in the test. The 'agar in plate' method was used to establish pollen germination and pollen tube growth (Koyuncu and Tosun, 2009).
Pollen tube long at least as its diameter was considered to be 'germinated'. For temperature experiments; pollen grains were sowed in the medium containing 15% sucrose+0.5% agar-agar+5 ppm (H_3BO_3) boric acid at 15^0C, 20^0C and 25^0C in the dark.
For the growth regulators and mineral substances experiments GA_3, KNO_3 and BA were added at the determined doses as a preliminary experiment (50 ppm GA_3, 50 ppm KNO_3, and 50 ppm BA) (Tosun and Koyuncu, 2007b).
An ocular micrometer was used to measure pollen tube length, under a light microscope, at a 40x magnification. Four Petri dishes were used for germination and pollen tube growth experiments. For each assay, 2 mL of medium was placed into Petri dishes. Counts were made from 4 different microscope fields (100-150 pollen grains per field for each Petri) (Hedly et al., 2004; Koyuncu, 2006).
Statistical analysis was conducted using Duncan's multiple range test within the general linear model procedure of SPSS 16.0.

RESULTS AND DISCUSSIONS

Pollen production capacity and pollen viability

Pollen production amount and viability results were shown in Table 1. The difference in the amount of pollen production of genotypes was statistically significant ($p<0.05$). The highest results were taken from N6, in terms of numbers of anthers in a flower (71.2), mean pollen number in an anther (13,151.74) and pollen number in a flower (414,245.07). However, the difference between pollen viability and morphological homogeneity were not statistically significant ($p<0.05$). The pollen viability rate ranged from 79.88% (N2) to 83.22% (N6). Morphological homogeneity rates were found upper than 92%. Türemiş and Derin (1999), reported that pollen viability levels varied from 79.75% to 91.94% some blackberry cultivars. Koyuncu (2006) studied strawberry pollens using TTC viability test and reported that pollen viability ratios reached to 82% for cvs. 'Allstar' and 'Elvira' and 86.5% for cv. 'Chandler'. Koyuncu and Tosun (2009) used TTC, FDA and IKI stain tests for the sweet cherry cultivars. They reported that the pollen viability differed according to stain methods and cultivars. The viability and morphological homogeneity related to pollen quality. They are the most important properties in fruit trees. These properties are useful for plant breeders, geneticists and growers. However, an easy method for determining pollen viability is required to increase the efficiency of the breeding program and the selection of a suitable pollinizer while the orchard is being established (Ercisli, 2007).

Table 1. Pollen production capacity, morphological homogeneity and pollen viability test

	n	m	pn	pv (%)	MH (%)
N1	67.4b*	12919.14b	391742.11b	83.10x	95.12x
N2	65.5b	12431.10b	371130.07b	79.88	93.5
N3	66.3b	12782.19b	383435.14b	81.16	92.2
N4	66.9b	12830.71b	392424.16b	80.23	92.9
N5	65.8b	12754.09b	374126.09b	81.63	94.0
N6	71.2a	13151.74a	414245.07a	83.22	95.8

n: numbers of anthers in a flower; m: mean pollen number in an anther, pn: pollen number in a flower; pv: pollen viability, MH : morphological homogeneity.

*Values within a column followed by different letters are significantly different ($p<0.05$).[x]The difference between the values is not statistically significant. SUB TABEL 1!

Pollen germination and pollen tube growth tests

Three constant temperature regimes (15^0C, 20^0C and 25^0C) were evaluated on the pollen germination and expressed as the percentage of germinated pollen (Table 2). As seen as Table 2, the effects of different constant temperature on pollen germination were statistically significant ($p<0.05$). N6 is the best genotype with highest value (52.25) in terms of pollen germination rate, at all temperature. This is followed by N1 (32.40) and N5 (23.39), respectively. The lowest pollen germination rate was obtained from N3 (7.29). 20^0C was determined as the optimum germination temperature for all genotypes. When the temperature rose up from 15^0C to 20^0C, the pollen germination rate also increased dramatically, but when it reached 25^0C, germination began to fall down. This shows us of high temperatures had the negative effect on the pollen germination.

Table 2. *In vitro* pollen germination (%) of blackberries pollen at different temperatures after 24 hours incubation, an a medium containing 15% sucrose + 0.5% agar-agar + 5 ppm (H_3BO_3)

	Incubation temperature (°C)			
	15	20	25	Mean
N1	9	48.21	40	**32.40b**[x]
N2	8.56	12.9	12	**11.15d**
N3	2.96	10.5	8.41	**7.29d**
N4	5.78	13.24	10.67	**9.90d**
N5	7.25	33.59	29.34	**23.39c**
N6	16.64	75.87	64.23	**52.25a**
Mean	**8.37c**[y]	**32.39a**	**27.44b**	

[x]Values within a column followed by different letters are significantly different ($p<0.05$).
[y]Values within same row followed by different letters are significantly different ($p<0.05$).

The *in vitr*o elongation of pollen tubes was affected by incubation temperature (Table 3). Responses of tested cultivars to different temperatures were statistically significant ($p<0.05$). As seen as Table 3, the longest pollen tubes for all varieties were measured at 20^0C when the shortest ones at 15^0C. The most augmentation pollen tube elongation was obtained from N6 at 15 to 20^0C (95.88 µm-154.36 µm). Pollen tube length of N6 was above the average (Figure 1).

Table 3. The effect of incubation temperature on pollen tube growth (μm) after 24 hours

	Incubation temperature (°C)			
	15 °C	20 °C	25 °C	Mean
N1	67.86	89.74	82.51	**80.04d**[x]
N2	91.26	101.36	95.9	**96.17b**
N3	72.25	98.25	84.63	**85.04d**
N4	84.26	97.65	92.54	**91.48c**
N5	92.33	106.67	99.51	**99.50b**
N6	95.88	154.36	148.69	**132.98a**
Mean	**83.97c**[y]	**108.01a**	**100.63b**	

[x]Values within a column followed by different letters are significantly different (p<0.05).
[y]Values within same row followed by different letters are significantly different (p<0.05).

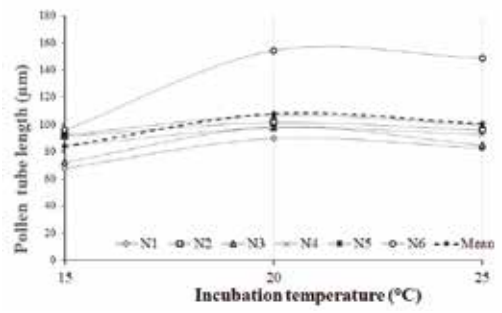

Figure 1. Pollen tube elongation at different incubation temperature

Temperature is among the most important environmental factors affecting plant reproductive processes such as pollen germination pollen tube growth and fruit set (Kakani et al., 2005). Temperature ranges and optimum temperature values for pollen germination and tube growth were studied for different fruit species, including strawberry (Koyuncu, 2006), pears (Vasilakasis and Porlingis, 1985), cherries (Tosun and Koyuncu, 2007a), pistachio species (Acar and Kakani, 2010), pomegranate (Gökbayrak and Engin, 2018) The optimum temperature required for pollen germination and tube growth was about 20^0C for apricot, cherry and sour cherry (Austin et al., 1998; Koyuncu and Tosun, 2009). These results parallel to our findings. Another study which was conducted at apricots as our study, pollen germination and tube growth above 25°C reduced (Egea et al., 1992). Low or high temperatures have negative effects on pollen germination and tube growth. The optimum temperature for pollen germination varies among species and cultivars of the same species (Mert, 2009; Kuroki et al., 2017).

The effects of chemicals on pollen germination and tube growth were statistically different, ($p<0.05$). Potassium nitrate and gibberellic acid were determined as promoter while benzyl adenine effects as inhibitory pollen germination and tube growth (Tables 4, 5). Pollen germination rate decreased to 24.82 in BA supplemented medium and increased to 30.72% in GA_3 added medium by 41.16%. The study which was conducted at blackberries it is reported that the effects of minerals on pollen germination were found to vary according to cultivars and doses (Türemiş and Derin, 1999). Kumar et al. (2016) found GA_3 as a promoter for ornamental tropical tree species pollens.

Table 4. The effect of different plant growth regulator on pollen germination (%)

	Control	**BA**	**KNO_3**	**GA_3**	Mean
N1	48.21	36.2	52.7	55.3	**48.10b**[x]
N2	12.9	8.2	14.71	19.34	**13.78d**
N3	10.5	7.7	18.6	24.5	**15.32d**
N4	13.24	10.2	18.22	26.4	**17.01d**
N5	33.59	27.7	39.79	45.61	**36.67c**
N6	65.87	58.9	68.71	75.8	**67.32a**
Mean	**30.72c**[y]	**24.82d**	**35.46b**	**41.16a**	

Table 5. The effect of different plant regulators on pollen tube length (μm) after 24 hours

	Control	**BA**	**KNO3**	**GA3**	Mean
N1	98.22	94.65	114.26	128.11	**108.81c**[x]
N2	103.3	82.33	109.55	117.09	**103.06c**
N3	94.68	103.26	113.6	120.39	**107.98c**
N4	145.63	127.58	139.41	154.69	**141.82b**
N5	130.65	107.89	156.86	165.99	**140.34b**
N6	151.36	144.85	161.48	174.25	**157.98a**
Mean	**120.64c**[y]	**110.09d**	**132.52b**	**143.42a**	

[x]Values within a column followed by different letters are significantly different (p<0.05).
[y]Values within same row followed by different letters are significantly different (p<0.05).

Pollen germination regulated by water, amino acids, sugars, calcium and growth regulators such as gibberellins, auxins and kinetin. Gibberellins have been traced in developing pollen grain after anthesis (Singh et al., 2002). Our results have been supported these findings. Pollen performance criteria (pollen vitality,

germination and pollen tube growth rate) are critical for discharging male gametes in the embryo sac and are a prerequisite for fertilization and fruit set. *In vitro* germination studies are powerful tools for genetic, physiological, biochemical and cytochemical studies for a wide range of plant species belonging to different families (Radıčević et al., 2013). These studies are also a good predictor of *in vivo* pollen behavior but only for autotrophic phase of pollen growth where the initial steps of pollen germination and pollen tube growth are independent of style nutrients, sugars and plant growth regulators. They help with selections for breeding programmers, *in vitro* assessments can also help to predict possible problems of sterility of that particular genotype in commercial orchards (Fotirić Akšić et al., 2017)

CONCLUSIONS

Pollen performances of wild-grown black-berries were determined. N6 can be thought promising type in for pollen performances. 20^0C was found optimum incubation temperature for pollen germination and pollen tube growth.

Potassium nitrate and gibberellic acid were determined as promoter while benzyl adenine effects as inhibitory pollen germination and tube growth

Fertilization biology studies should be continued at *in vivo* conditions.

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