Some Cytological Observations on Pollen Grains of Wild Poppy Anemone Varieties from Turkey

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Abstract

There are four native varieties of poppy anemone, namely, *Anemone coronaria* var. *coccinea* (red flowered), *Anemone coronaria* var. *alba* (white flowered) *Anemone coronaria* var. *rosea* (pink flowered) and *Anemone coronaria* var. *cyanea* (purple flowered) in Turkey. In this study, flowers of the four varieties were used as plant material in order to determine viability, germination rate and production of the pollen grains.

For the determination of pollen viability, TTC (Triphenyl tetrazolium chloride) and FDA (Fluorescein diacetate) were used. In vitro pollen germination was performed using an agar method and pollen production was counted with the hemacytometric lam method. The maximum pollen viability rate was obtained from the violet-flowered variety in TTC whilst the difference was not found to be statistically important among the varieties when FDA was used. However, viability values of FDA were higher than that of TTC. As far as the pollen germination was concerned, the maximum germination was observed from the red-flowered variety in a medium with 1% agar + 25% sucrose + 150 ppm H_3BO_3. Additionally, the average number of anthers, the pollen production per anther and per flower, morphological homogeneity level and dimensions of pollen were determined for each variety.

INTRODUCTION

Four native varieties of poppy anemone, *Anemone coronaria*, are available in Turkey. These are var. *coccinea* (red flowered), var. *alba* (white flowered), var. *rosea* (pink flowered) and var. *cyanea* (purple flowered) (Davis, 1965).

The floral lifespan of wild *Anemone coronaria* is five to 12 days while the flowering period of a population is 60 days. The flower diameter is between 4 to 8 cm and it has five or six petaloid sepals (Dafni et al., 1990). The flower has between 400 and 800 pistils and almost as many stamens which release a mean of two million pollen grains per flower (Horovitz, 1991). Stamens are arranged in about seven rows of which two or three middle ones develop earlier than the rest. Anther dehiscence begins when filaments have reached their full length about 10 days after stigma maturation. *Anemone coronaria* is largely cross-pollinated by insects (Horovitz et al., 1975). Wind pollination is widespread in the genus and some of the characteristic features of wind pollinated plants are met with in *Anemone coronaria*. The pollen is dry, stores carbohydrates, and has a diameter of less than 40 µm (Horovitz, 1991).

Anemone coronaria is the only species in the genus with intensive breeding efforts. In addition to its commercial value, wild anemones provide clear didactic models of Mendelian and quantitative gene control (Horovitz, 1995). It presents all the classical problems, e.g., preferentially allogamous and autofertile and sensitivity to inbreeding (Meynet, 1993).

In *Anemone coronaria*, polymorphism is widespread for many traits. This situation is shown not only in flower color, but also in anthers, filaments and styles. Anemone flowers are markedly protogynous. Rare cases of spontaneous male sterility have been found and studied for breeding purposes (Horovitz, 1985).

Knowledge about the fertilization biology of a plant and choosing parents are very important for crossing studies in hybrid variety improvement. Obviously, genotypes having pollen grains with high quality and germination rate are preferred in crossing studies. In the studies of fertilization biology, fertilization problems are of interest whether they originate from pollen grains or not.

Anemone coronaria has some problems which are important in regard to breeding studies such as spontaneous male sterility, inbreeding depression, protogyny and seed germination. It is thought that most of the problems are connected with fertilization. Therefore, cytological pollen studies performed for the determination of pollen quality have gained much more importance. Only a few cytological studies (Piskornik et al., 1989; Ari et al., 2003) have been performed for *Anemone coronaria*. In the study of Piskornik et al. (1989), the effects of exogenous ethylene and ACC (1-aminocyclopropane-1-carboxylic acid) on pollen germination of 10 different ornamental species, including *Anemone coronaria* were investigated. It was determined that ethylene or its precursors stimulated pollen germination in 8 of the species studied, including *Anemone coronaria*. It was concluded that the best germination medium consisted of 1% agar + 15% sucrose + 0.3 mM ethephon + 10 M ACC. Ari et al. (2003) examined the viability, germination rate and production of the pollen grains isolated from *Anemone coronaria* var. *coccinea* and var. *alba.* At the end of the study, the maximum pollen viability rate was obtained from var. *coccinea*, whilst the maximum pollen germination was observed from var. *alba* in a medium with 1% agar + 20% sucrose + 150 ppm H_3BO_3.

In the present study, viability, germination rate and production of the pollen grains isolated from four wild *Anemone coronaria* varieties, var. *cyanea*, var. *rosea*, var. *coccinea* and var. *alba* were investigated.

MATERIALS AND METHODS

Plant Material

Pollen grains isolated from the flowers of four native *Anemone coronaria* varieties, var. *alba*, var. *coccinea*, var. *cyanea*, and var. *rosea* grown in Adana, which is located in the Mediterranean region of Turkey, were used in this study. In order to obtain pollen grains, the flowers at the stage of pre-anthesis were picked and brought to Cytology Laboratory of Horticulture Department of Cukurova University in Adana. The flowers were opened and anthers were allowed to burst under room conditions. The pollen grains obtained, then, were used for viability and germination tests.

Pollen Viability

Viability of pollen grains was determined by the methods of 2,3,5-triphenyl tetrazolium chloride (TTC, Merck) and Fluorescein diacetate (FDA, Merck) (Norton, 1966; Heslop-Harrison and Heslop-Harrison, 1970; Eti, 1991).

In TTC test, the pollen grains staining to dark red were evaluated as “viable”, while grains staining to light pink or red were counted as “semi-viable”. Nonstained pollen grains were counted as “unviable”. 50% of the semi-viable pollen grains were assumed to be viable. Therefore, half of the number of semi-viable pollen grains was added to the number of viable pollen grains.

In the FDA test, the pollen grains showing strong fluorescent-radiation were evaluated as “viable”, while the pollen grains showing pale fluorescent-radiation or no fluorescent-radiation were counted as unviable.

Pollen Germination

The agar method (Stanley and Linkens, 1985) was used to determine the most suitable pollen germination medium for the *Anemone coronaria* varieties tested. For this purpose, different concentrations (50 ppm, 100 ppm, 150 ppm and 200 ppm) of boric acid (H_3BO_3, Sigma) and sucrose (15%, 20% and 25%) were examined in the media with 1% agar (Merck). In total, 15 different in vitro germination media were investigated for the pollen grains of each variety.

Pollen Production

The Hemacytometric lam method (Eti, 1990) was used for the determination of pollen production.

Data Analysis

The results of the experiments were analyzed in a randomized split-plot design with three replications. LSD multiple range test was applied at a level of 5%.

RESULTS AND DISCUSSION

The results of TTC and FDA viability tests are shown in Table 1. In the TTC test, differences in pollen viability between the varieties were found to be statistically significant. The highest pollen viability was obtained from var. *cyanea* with a rate of 72.3%.

In the FDA test, germination differences were not statistically significant between the varieties. However, the highest pollen viability was determined in var. *rosea* (89.3%). Comparison of the results shows that the viability levels of FDA test are higher than that of TTC test for all varieties.

The pollen germination results, which were obtained from 15 different in vitro germination media for obtaining the best medium, are given in Table 2. As can be seen from the table, the maximum pollen germination was observed for var. *alba* (30.5%) and var. *cyanea* (18.0%) in the medium with 1% agar + 25% sucrose + 100 ppm H_3BO_3, while the medium containing 1% agar + 25% sucrose + 150 ppm H_3BO_3 was found to be the best pollen germination medium for var. *coccinea* (31.3%) and var. *rosea* (25.7%).

Pollen production per anther and per flower, morphological homogeneity level and dimensions of pollen grains and average number of anthers are shown in Table 3. As seen from the table, var. *coccinea* had the greatest number of anthers. The highest pollen productions per anther and per flower were seen in var. *rosea* and var. *cyanea*, respectively. As far as the morphological homogeneity level and the dimensions of pollen grains were considered, the difference among the varieties was found to be statistically insignificant.

The present study was carried out to determine pollen quality of four varieties of *Anemone coronaria*, pollen viability levels were found to be between 61.0 and 89.3% from both TTC and FDA tests. Addition of H_3BO_3 to the medium with agar and different concentrations of sucrose increased pollen germination rate and a medium containing 1% agar + 25% sucrose + 150 ppm H_3BO_3 was found to be the best pollen germination medium among the other media tested.

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Tables

Table 1. Pollen viability rates of four *Anemone coronaria* varieties.

Variety	Viability rate in TTC (%)	Viability rate in FDA (%)
	Viable	Viable
var. *alba*	61.0 b	88.6
var. *coccinea*	64.1 ab	87.9
var. *cyanea*	**72.3 a**	87.3
var. *rosea*	66.6 ab	**89.3**
LSD (5%)	6.22	NS

Table 2. Pollen germination rates of four *Anemone coronaria* varieties.

Germination medium (1% agar + ...)	*Anemone coronaria* varieties			
	var. *alba*	var. *coccinea*	var. *cyanea*	var. *rosea*
15% suc.	7.3 d	7.2 d	8.8 bc	4.8 g
20% suc.	19.8 abc	11.8 cd	10.6 bc	7.8 efg
25% suc.	11.2 cd	18.0 abcd	11.6 abc	14.7 cde
15% suc + 50 ppm H_3BO_3	16.4 bcd	15.6 bcd	15.4 ab	14.3 de
15% suc + 100 ppm H_3BO_3	11.8 cd	15.7 bcd	7.2 c	7.3 fg
15% suc + 150 ppm H_3BO_3	13.4 cd	15.6 bcd	6.2 c	13.9 def
15% suc + 200 ppm H_3BO_3	12.6 cd	16.9 abcd	6.3 c	12.2 def
20% suc + 50 ppm H_3BO_3	22.9 abc	14.5 bcd	12.8 abc	9.2 efg
20% suc + 100 ppm H_3BO_3	19.2 abc	13.9 bcd	11.2 abc	11.8 def
20% suc + 150 ppm H_3BO_3	20.1 abc	21.4 abcd	14.4 ab	17.9 bcd
20% suc + 200 ppm H_3BO_3	16.0 bcd	20.4 abcd	13.1 abc	10.5 efg
25% suc + 50 ppm H_3BO_3	22.5 abc	21.8 abcd	15.3 ab	18.0 bcd
25% suc + 100 ppm H_3BO_3	**30.5 a**	28.2 ab	**18.0 a**	24.8 ab
25% suc + 150 ppm H_3BO_3	21.3 abc	**31.3 a**	14.6 ab	**25.7 a**
25% suc + 200 ppm H_3BO_3	25.8 ab	23.0 abc	14.5 ab	21.5 abc
LSD (5%)	6.27	8.15	3.76	3.68

Table 3. Average number of anthers, number of pollen grains per anther and flower, morphological homogeneity level and dimension of pollen grains for four *Anemone coronaria* varieties.

Variety	Average number of anthers	Amount of pollen per anther	Amount of pollen per flower	Morphologic homogenity level (%)	Dimension of Pollen (µm)		
					General	Biggest	Smallest
var. *alba*	118 ab	12,925	1,640,772 ab	95.55	27.5	32.3	27.2
var. *coccinea*	147 a	11,118	1,628,329 ab	94.72	29.4	32.2	25.7
var. *cyanea*	132 a	13,571	**1,791,431 a**	94.42	28.9	34.4	22.8
var. *rosea*	84 b	17,274	1,428,437 b	94.63	29.6	32.3	25.4
LSD (5%)	23.8	NS	233,031	NS	NS	NS	NS