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IN VITRO POLLEN VIABILITY AND GERMINATION OF BISEXUAL AND FUNCTIONAL MALE FLOWERS OF SOME TURKISH POMEGRANATE CULTIVARS

SUMMARY

The evaluation of pollen viability and germination are two essential criteria for characterization of pollinating cultivars in pomegranate which is an andromonoecious species. With this point of view, pollens were collected from both bisexual and functional male flowers of 'Aşınar' and 'Caner II' cultivars to determine the pollen viability and germination. Colorimetric test of 2,3,5-triphenyl tetrazolium chloride (TTC) was used to estimate the pollen viability. Pollen germination was tested in vitro on a medium containing 1% agar and 20% sucrose. Results showed that pollen viability was not dependent on the type of the flowers. The highest pollen viability ratio (78%) was obtained in the pollen collected from functional male flowers of 'Aşınar'. Pollen germination ratio, however, varied with regard to the flower type in the same cultivar. Bisexual and male flowers in 'Aşınar', respectively, had 43.5% vs 22.3% pollen grains germinated. Pollens from two sexual morphs of pomegranates show different germination and viability ratios. Investigation on viability and germination capability of pollen grains coming from both bisexual and functional male flowers in pomegranate can enable growers and breeders to better select best crossing pollinators.

Keywords: *Punica granatum* L., Pollen, Viability, Germination.

INTRODUCTION

Pomegranate (*Punica granatum L.)* is characterized by having two types of flowers, bisexual flowers and functional male flowers, developing on the same tree. Each type of flowers can affect productivity and yield separately. Functional male flowers drop and generally fail to set. Fruits develop exclusively from bisexual flowers. Nevertheless, functional male flowers can be a way to spread genes, because pollen spread is more efficient.

Fertility of the pollen depends on various factors such as environmental factors, nutrition, varieties and plant regulators (Gökbayrak and Engin, 2015). Pollen viability and germination are important parameters in the analysis of gene flow in plant studies, highlighting the male reproductive potentiality of the

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species and contributing to taxonomic, ecological, genetic and palynological studies (Gadze et al., 2011; Piccinini et al., 2012). To investigate pollination potential, estimates should be made on pollen quantity and viability, as well as on pollen germination capability. The previous studies on pomegranates showed that in vitro pollen germination ratio was affected by genotypes or methods used and chemical concentration in the medium (Gözlekçi and Kaynak, 2000, Melgarejo et al., 2000; Engin and Hepaksoy, 2003). Studies on in vitro pollen viability show that it is also possible to differentiate the aborted pollen grains from non-aborted ones. Aborted pollen grains do not have a nucleus, thus staining only the cellulose in the wall (Biondo and Battistin, 2001; Gökbayrak and Engin, 2015).

A comparison of the form and function of flower parts in the two sexual morphs, including pollen viability and germination, would provide information useful to enhance fruit production. This study was conducted to determine viability and in vitro germination ability of pollens derived from functional male and bisexual flowers of some Turkish pomegranate cultivars.

MATERIAL AND METHODS

Both functional male and bisexual flowers of the pomegranate cultivars, 'Aşınar and Caner II' were collected from the collection orchard located at the Dardanos campus of Çanakkale Onsekiz Mart University, Çanakkale, Turkey. Flowers of different sexes were hand-picked and taken to the laboratory. Their anthers were detached slowly using a forceps and then put on a paper at room temperature of 22°C for about 12-18 hours to waste some of their moisture, split and release pollens.

Colorimetric test of 2,3,5-triphenyl tetrazolium chloride (TTC, 1%) were used to estimate pollen viability. The analysis was immediately performed after two hours under a light microscope, considering pollen grains viable with dark-red tonality and non-viable with light-red tonality, lacking protoplasm. Ratio calculations (%) were made with viable pollens divided to non-viable ones.

Pollen grains obtained from the two sexual morphs of the pomegranate cultivars, 'Aşınar' and 'Caner II', were cultured in a medium containing 20% sucrose and 1% agar at 26±1°C under 8 hours dark and 16 hours daylight conditions. Twenty-four hours later, germinated and ungerminated pollens as a percentage were counted using a light microscopy (Olympus CX-41) at 10X magnification from a random selection of four-field views.

The statistical analysis was performed using MINITAB statisctical package software (Minitab Inc., ver.16), and the significant means were compared using Tukey's test.

RESULTS AND DISCUSSION

Pollen viability and *in vitro* germination of flowers with different sexes were tested on Turkish pomegranate varieties 'Aşınar and Caner II' (Table 1).

Table 1. Comparison of pollen sources (bisexual and functional male flowers) in the pomegranate cultivars ‘Aşinar’ and ‘Caner II’ for their pollen viability and in vitro germination ratios (%).

Cultivars	Pollen viability (%)			Pollen germination (%)		
	Bisexual	Functional male	*p* value	Bisexual	Functional male	*p* value
Aşınar	71.49	78.41	*0.476*	43.48 a	22.25 b	*0.002*
Caner II	50.36	64.18	*0.221*	30.53	20.46	*0.097*

TTC test of pollen viability of the bisexual and functional male flowers did not exhibit any statistically significant differences, although male flowers had slightly higher viable pollens. As opposed to comparatively higher pollen viability, germination was markedly low in the cultivars. These results corroborate those of derin and eti (2001) who reported that percentage of viable pollens in Turkish pomegranates ranged from 75.2 to 82.4% by TTC test and fluorescein diacetate (FDA) test, respectively. Thus, TTC can be recommended for testing pollen viability in pomegranates.

In terms of germination, there was a significant difference in the functional male flowers compared to the bisexual flowers in the cultivar ‘Aşinar’. Pollens of the cultivar ‘Caner II’ showed no differences. In general, two-thirds of the pollens of the both cultivars did not germinate in the male flowers. On the other hand, at least 60% of the pollens from the female flowers germinated.

Based on the observations made under light microscope, it was noted that pollen germination ratios for the different pomegranate cultivars were not synchronous, but heterogeneous. Pollen germination assays conducted at different temperatures showed that pollen germination is markedly influenced by temperature (Gaaliche et al., 2013). Yang et al. (2013) did not find significant differences in the sucrose ratio in the basal medium in ‘Taishanhang’ pomegranate. Malgarejo et al. (2000) showed that the male flowers of me15 clone of pomegranate gave a higher germination in vitro than the bisexuals when low sucrose concentrations were used. In the present study, germination capability of pollen grains from functional male flowers was not higher in each cultivar.

This means that genotype is a factor regulating this effect. In pomegranate, Derin and eti (2001) reported that there was a significant difference in germination ratio between bisexual and functional male flowers the cultivar of ‘33 n 26’, but not in the cultivar of ‘Hicaz’.

Gözlekçi and Kaynak (2000), in support of our study, in cultivar of ‘Caner II’ found that there was no difference in the germination ratios of the pollens derived from the different types of flowers. In general, there is a linear relation between pollen viability and germination capability in many fruit species (Stanley and Linskens, 1974), but in our study there is no linear relation between pollen viability and germination capability in both functional male and bisexual flowers of the pomegranate cultivars.

CONCLUSIONS

Pollens from two sexual morphs of pomegranates show different germination and viability ratios. Investigation on viability and germination capability of pollen grains coming from both bisexual and functional male flowers in pomegranate can enable growers and breeders to better select best crossing pollinators.

REFERENCES

Biondo, E., Battistin, A. (2001). Comparison of the efficiency of different staining on the estimate of the viability of pollen grains in the species of genus Eriosema (DC.) G. Don and Rhynchosia Lour. (Leguminosae- Faboideae) native to southern Brazil. Bioikos, PUC-Campinas, 15(1), 39-44 (English abstract)

Derin, K., Eti, S. (2001). Determination of pollen quality, quantity and effect of cross pollination on the fruit set and quality in the pomegranate. Turk. J. Agric. For. 25, 169-173.

Engin, H., Hepaksoy, S. (2003). Determination of pollen germination of some pomegranate cultivars. E.U. Faculty of Agriculture Journal, 40 (3), 9-16.

Gaaliche, B., Majdoub, A., Trad, M., Mars, M. (2013). Assessment of pollen viability, germination, and tube growth in eight Tunisian caprifig (Ficus carica L.) cultivars. ISRN Agronomy.

Gadže, J., Radunić, M., Petric, I.V., Ercisli, S. (2011). In vitro pollen viability germination and pollen tube growth in some pomegranate (Punica granatum L.) cultivars from Croatia and Bosnia and Herzegovina. Acta Sci. Pol. Hortorum Cultus, 10(3), 297-305.

Gökbayrak, Z., Engin, H. (2015). Effect of plant growth regulators on enhancing in vitro pollen germination in grapevine cultivars. 3rd Balkan Symposium on Fruit Growing, September 16-18, Belgrade, Serbia

Gözlekci, S., Kaynak, L. (2000). Investigations on pollen production and quality in some standard pomegranate (Punica granatum L.) cultivars. In : Melgarejo P. (ed.), Martínez-Nicolás J.J. (ed.), Martínez-Tomé J. (ed.). Production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology. Zaragoza: CIHEAM, p. 71-77 (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 42)

Melgarejo, P., Martínez, J.J., Hernández, F. (2000). A study of different culture media for pomegranate (Punica granatum L.) pollen. In: Melgarejo P. (ed.), Martínez-Nicolás J.J. (ed.), Martínez-Tomé J. (ed.). Production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology. Zaragoza: CIHEAM, pp. 63-69 (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 42)

Piccinini, F. Frescura, V.D., Perez, N.B., Tedesco, S.B. (2012). Pollen viability of Eragrostis plana genotypes from different geographic populations in Rio Grande do Sul. 1. Graduando em Agronomia da Universidade Federal de Santa Maria Centro Científico Conhecer, Goiânia, 8(15), 1316-1324.

Stanley, R.G., Linskens, H.F. (1974). Pollen: Biology, Biochemistry, Management. Springer, New York, NY, USA.

Yang, S., Yuan, Z., Li, Y., Li, Q., Yin, Y., Feng, L., Zhao, X. (2013). Biological characteristics of pollen germination of 'Taishanhong' pomegranate. Scientia Silvae Sinicae 49, 48-53.