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EFFECT OF DIFFERENT BORON LEVELS ON POLLEN GERMINATION OF HICAZ NAR POMEGRANATE (*Punica granatum* L.) CULTIVAR

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Abstract

In this study, it was aimed to determine the effect of different boron doses on pollen germination rates in in vitro conditions of Hicaz Nar pomegranate cultivar at full yield. The A and B flower type of pollens collected separately from the pomegranate trees, were germinated by the 'agar petri' method. Four different boron doses containing 1% agar, 15% sucrose and 0, 10, 20 and 30 mg/l^{-1} boric acid were used as a germination medium. It was determined that the pollen of both types of flower had the highest germination rate at 10 mg/l^{-1} boron application, and these rates were 62.17% in A type flowers and 41.87% in B type flowers. It has been observed that germination rates of flower pollen of both types of flower increased significantly in comparison with control with boron applications.

Keywords: Pomegranate, boron, agar, sucrose, pollen germination,

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INTRODUCTION

Pomegranate (*Punica granatum* L.) was one of the oldest domesticated fruit crops together with; figs, dates, olives and grapes (Kahramanoğlu et al,2018). Pomegranate is mainly confined to the tropics and subtropics and grows well in arid and semi-arid climates (Korkmaz et al, 2016; Okatan et al., 2018). Turkey has different pomegranate types and forms because it is one of the origins of the pomegranate (Derin and Eti, 2001)

Pomegranate (*Punica granatum* L.) is characterized by having two types of flowers on the same tree: Hermaphroditic bisexual flowers and functionally male flowers (Wetzstein et al, 2011). Synonym is for male flowers are A type. Synonym is for female flowers are B type.

Depending on the climatic conditions in pomegranate cultivation, it is seen that the fruit set decreases in some years and the yield is low. It is of importance to be able to increase the fruit set in the years when the product is low. Fruit set depends on pollination and fertilization. In this case, the germination power of the pollen grains is also effective. In the Mediterranean region where pomegranate is intensively grown, the fact that the soil pH is generally high may prevent the intake of boron from the soil.

If the soil pH is higher than 6.3-6.5, a dramatic decrease is usually seen in the availability of boron to plants and in the intake of plants (Havlin et al., 2002). As a result, even if sufficient boron is found in the soil, if the soil pH is high, it cannot be taken by the plant, a decrease occurs in the rate of fruit set due to boron deficiency.

Boron deficiency symptoms first appear at growing points, such as root tips and pollen tube tips. Studies have shown that Boron is promoted pollen germination (Wang et al 2003). In another study conducted, the 1% agar concentration provided a higher germination rate than the 1.5% concentration. Sugar concentration around 10% was found to be appropriate. 48 hours of waiting gave a higher germination percentage compared to 24 hours of waiting. In the same way, the germination rate at 28 °C was higher than at 15 °C (Melgarejo et al., 2008). It is reported that the highest germination rates (over 74%) in pomegranate pollen grains are at 25 and 35 °C, and these rates are lower at lower temperatures. Pomegranate (*Punica granatum*) has two different types of flowers on the same tree. One of them is hermaphrodite bisexual flowers and the other is functional male flowers (Wetzstein et al., 2011).

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In order to determine the germination power of pollen grains, type A flowers were used in a study made on 11 different kinds of pomegranate. In study in which the 'agar-petri' pollen germination test method was used in vitro, 15% and 20% of the sucrose-containing medium at different concentrations gave better results (Engin and Hepaksoy, 2003).

Pomegranate pollen is reported to have a higher germination rate in 15% sugar-containing germination medium compared to 10% sugar-containing medium (Cizmovic at al., 2013).

Knowing the period in which pollen grains gain germination ability in fruit species, is of importance in vitro germination test methods and hybridization studies. In a study conducted on this issue in tobacco by Bürün (2001), it was determined that the tip of the corolla did not turn pink but unopened flower bud pollen gave the best result in both in vitro and in hybridization studies. Tosun and Koyuncu, 2007 reported that Pollen performance testing could be helpful for a fruitful cultivation of genetic progeny for breeding purpose, and especially for selecting which cultivars should be used by growers.

There are few studies on the reproduction biology of pomegranate. Effective factors to germination, production and viability of pollens are not known enough. Boron is a very important mineral interested with pollen germination. Our study objective was to investigate the effects of different boron levels on the germination of Hicaz variety of pomegranate pollen germinations in vitro conditions.

MATERIALS AND METHODS

In the research, Hicaz pomegranate trees in the full yield period which grow in the area of about 50 decares in Dalyan Neighborhood, Ortaca District, Muğla Province and pollen grains obtained from them were used. Type A (functionally male) and B (hermaphroditic bisexual) flowers were collected separately and examined in the laboratory.

For in vitro studies, the type A (functionally male) and type B (hermaphroditic bisexual) flowers which have not bloomed yet or are about to bloom from the branches of the trees at different directions and heights were brought to the laboratory, the anthers of male organs were extracted and these were spread on paper and enabled to explode at 25 ^{0}C with 12 hours of waiting (Eti, 1991). The 'Agar-petri' method was used to determine the germination rates of pollen grains (Forlani and Rotundo, 1977). As pollen grain germination medium; 1% agar, 15% sucrose and 0, 10, 20 and 30 mg l^{-1} of boric acid were used. The mediums were poured into petri dishes in a thickness of about 2 mm and allowed to cool and pollen cultivation was performed with brush before full solidification. The petri dishes put in a place with light but not direct sunlight. With 24 hours of waiting at 25 °C, the pollen germination rates were determined at the end of this period. Photographs of germinated pollen were taken. The study was carried out in 3 replications, and about 200 pollen were counted for each replication and percentage (%) germination rates were determined under the microscope. Pollen grains with equal to pollen diameter or having grass tube longer than this are considered to be germinated.

IKI (iodized potassium iodide) test was applied to the Pomegranate pollen grains taken from the same garden to determine the flower tobacco viability rate. For this purpose, a drop of IKI was dropped on the slide and then the pollen spread was carried out with brush and covered with a coverslip, and kept in a place with light, but not exposed to direct sunlight for 20 minutes under room conditions. As three replications, 200-300 pollen were counted in each replication and the average was taken. Pollen grains stained brown color were counted as alive, while colorless and yellow-stained ones were classified as dead. The counts were carried out in the third week of May. Soil and leaf samples were taken from the garden in May and boron amounts were determined.

RESULTS and DISCUSSION

Soil and Leaf Analysis Results

pH, salt and boron contents of soil and leaf boron content of Hicaz pomegranate plant leaves are presented in Table 1. According to the analysis results, it was determined that the pH value of the trial soil was 8.2, total amount of salt was 0.026% and boron content was 0.56 mg l^{-1}. The boron content of leaf in the test trees was detected to be 20.0 mg l^{-1}. Accordingly, boron content in soil of the test area is sufficient, but leaf boron content is relatively low. According to the reports of Havlin et al., (2002), the boron intake may be prevented in the case of high soil pH. Also in our study, it is considered that the fact that pH of the test soil is high prevents the boron intake. In a study related to pomegranate leaf analysis, leaf boron content was found to be an average of 20.38 mg l^{-1} on average in Israel by spectrophotometric method (Gimenez et al., 2008). The concentration of boron element generally ranges from 20 to 60 mg l^{-1} in dicots (dicotyledonous). In the mature leaf tissue of most culture plants, the concentration levels of element B, if above 20 mg l^{-1}, are generally considered as adequate (Havlin et al., 2002).

Table 1. Some properties of trial soils and boron (B) content of pomogranate plant leaves

Properties of trials			
pH	Salt (%)	B (mg l^{-1})	Method
8.2 (1:2.5)	0.026 (1:2.5)	0.56	Hot Water-ICP
Boron content of leaves			
B (mg l^{-1})			Method
20.0			ICP

Pollen Viability Rates In Hicaz Pomegranate Cultivars

In the type A (functionally male) flowers, pollen viability rate was determined to be on average of 93.0% whereas in the type B (hermaphroditic bisexual) flowers on average 92.3%. Pollen viability rates in both flower types were determined at fairly high values. In the study conducted by Engin and Gökbayrak (2016), the pollen viability was found to be 78% in the Aşınar variety by the TTC test. Derin and Eti (2001), detected that the pollen viability rate was 75.24% with the TTC test in Hicaz pomegranate male flowers (functionally male) in the studies they conducted. In another study, pollen viability was found between 36.73% and 51.80% by Fluorescent diacetate (FDA) test (Gadže et al., 2011). In a study on pomegranate pollen carried out by Sharma (2003), the pollen viability observed in the acetocarmine solution was found between 91.15% and 97.91%. These studies are considered to be higher when compared with the results obtained. The test method used is thought to give different results.

Pollen Germination Rates

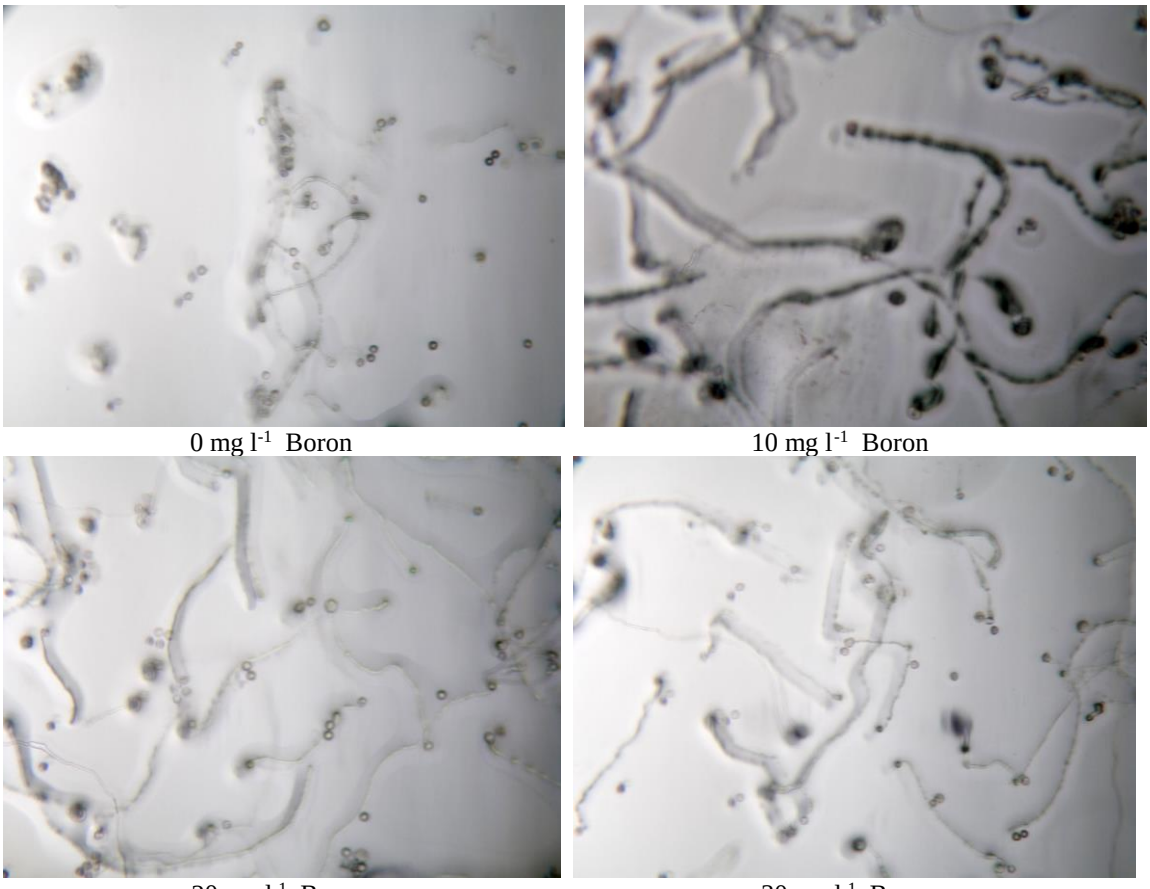
Pollen germination rates in the type A (functionally male) flowers

A statistically significant difference was revealed between the applications in terms of the effects on pollen germination rate in the type A (functionally male) flowers ($p < 0.01$). Pollen germination rates are presented in the Table2.

Table 2. Pollen germination rate in the type A (functionally male) flowers

Boron dozes (mg l^{-1})	Pollen germination rate (%)
0	33.83 c
10	62.17 a
20	57.67 a
30	50.13 b
LSD	7.27

Average germination rates varied between 33.83% and 62.17%. The rate of pollen germination increased by 83.8% at 10 mg l^{-1}, 70.5% at 20 mg $l^{-1}$and 48.2% at 30 mg l^{-1} when compared to the control. The best pollen germination rates were obtained at boron application of 10 and 20 mg l^{-1}. The effects of different boron levels on the pollen germination rates in male flower type are shown in Figure 1.



0 mg l^{-1} Boron | 10 mg l^{-1} Boron

20 mg l^{-1} Boron | 30 mg l^{-1} Boron

Figure 1. The effects of different boron levels on the pollen germination rates in the type A (functionally male) flowers

The pollen germination rate in functionally male flower types in the Aşınar variety was found to be 22.3 % (Engin and Gokbayrak, 2016). Derin and Eti (2001) detected that the germination rate was 61.50 % in Hicaz pomegranate male (functionally male) flowers in the studies they conducted. In a study related to the germination power in pomegranate pollen grains, after waiting of 24 hours at 28 ^{0}C in a medium consisting of 1% agar, 10% sucrose, % 0.2 Ca^{+2} and B^{+3} pollen germination rate in male flowers were found to be 15.02 % (Melgarejo et al., 2008). They received a rate close to or lower than our study.

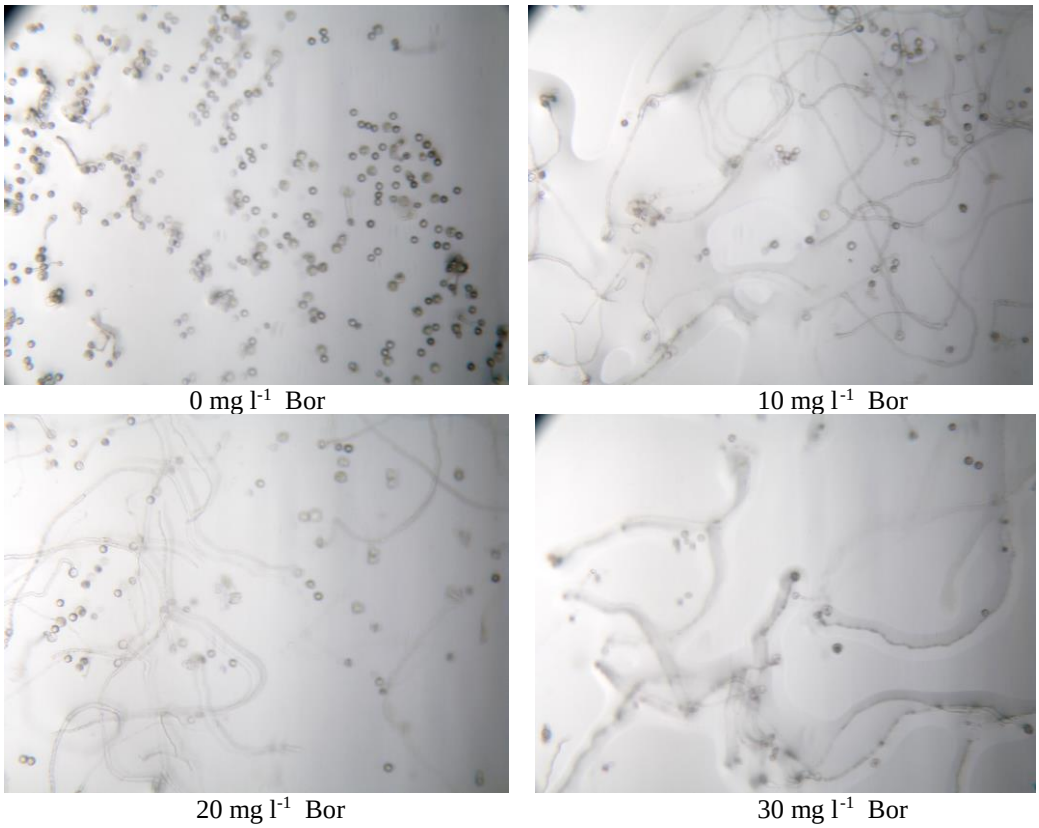
Pollen germination rates in the type B (hermaphroditic bisexual) flowers

A statistically significant difference was revealed between the applications in terms of the effects on the rate of pollen germination in the type B (hermaphroditic bisexual) flowers ($p < 0.01$). LSD Pollen germination rates are presented in the Table 3.

Table 3. Pollen germination rate in the type B (hermaphroditic bisexual) flowers

Boron dozes (mg l^{-1})	Pollen germination rate (%)
0	17.97 b
10	41.87 a
20	35.43 a
30	40.03 a
LSD	10.45

Average germination rates varied between 17.97 % and 41.87 %. Pollen germination rate increased by 133.0% at 10 mg l^{-1}, 97.2% at 20 mg l^{-1}' and 122.8 % at 30 mg l^{-1} when compared to the control. Germination rates in type B (hermaphroditic bisexual) flowers appear to be higher than those of type A (functionally male) flowers, when the effects of the flowers pertaining to different flower types of boron applications on the pollen germination are compared. In Aşınar variety, in hermaphroditic bisexual flower types, pollen germination rate was found to be 43.5% (Engin and Gokbayrak, 2016). In a study related to the germination power in pomegranate pollen grains, after waiting of 24 hours at 28 ^{0}C in a medium consisting of 1% agar, 10% sucrose, % 0.2 Ca^{+2} and B^{+3} pollen germination rate in hermaphroditic flowers was found to be 5.46 % (Melgarejo et. al., 2008). The germination rates of pollen grains belonging to different pomegranate cultivars in 15% sucrose and 1% agar medium were detected between 7.70% and 48.53% (Engin and Hepaksoy, 2003). The results obtained gave higher results than the results of the studies carried out by Melgarejo et al. (2008), Engin and Gokbayrak, (2016) and closer results with the study of Engin and Hepaksoy, (2003). The effects of different boron levels on the pollen germination rates in bisexual flower type are presented in Figure 2.



0 mg l^{-1} Bor | 10 mg l^{-1} Bor

20 mg l^{-1} Bor | 30 mg l^{-1} Bor

Figure 2. The effects of different boron levels on the pollen germination rates in the type B (hermaphroditic bisexual) flowers

In a study related to pomegranate pollen grains by Sharma (2003), the rate of pollen germination in the medium containing 12.5% sucrose and 10 mg l^{-1} of boric acid and carried out with the hanging drop method was determined as 72.05%.

In a study in which 5 mg boric acid was added to the pollen germination medium (Eshghi et al., 2010), unlike our study, in Rabab variety of pomegranate the rate of pollen germination was determined higher in type B (hermaphroditic bisexual) flower (60.07%) than in the type A (functionally male) flowers (45.73%). Pollen germination rates also gave the same result when compared to our study. This situation is thought to be caused by the difference between the varieties.

Similarly, to our study, it was pointed out that pollen germination in boric acid-treated medium gave better results (Gadže et al., 2011; Eshghi et al., 2010).

CONCLUSIONS

Pollen viability rate was found at high rates in Hicaz variety of pomegranate. In vitro conditions, type A (functionally male) flowers (33.83% in Control) were detected to indicate higher rate of pollen germination compared to the type B (hermaphroditic bisexual) flowers (control 17.97%). Boron applications have been observed to significantly increase the pollen germination rates in vitro. However, this increase in type B flowers was higher compared to the type A (functionally male) flowers.

Recent researches indicate that in the areas where boron deficiency is seen, spraying boron from leaf to trees increases fruit yield. The study conducted suggests that boron applications from leaves in pomegranate gardens, especially in the areas where boron deficiency is seen may provide beneficial results in terms of increasing the pollen germination rate.

REFERENCES

Bürün, B. 2001. Determination of the fertilization ability of pollen with Hybridization and in vitro techniques in Tobacco. Anadolu University Journal of Science and Technology. Vol:2 No:2,357-361

Cizmovic, M., Popovic, R., Lazovic, B., Adakalıc, M., Dzubur, A., 2013. Pollen Germination of Some Pomegranate (*Punica Granatum* L.) Varieties Grown in Montenegro. IV International Symposium Agrosym.

Derin, K., Eti, S. 2001. Determination of pollen quality, quantity and effect of cross pollination on the fruit set and quality in the pomegranate.Turkish Journal of Agriculture and Forestry. 25 : 169-173.

Engin, H, Gokbayrak, Z. 2016. In Vitro Pollen Viability and Germination of Hermaphroditic bisexual and Functionally Male Flowers of Some Turkish Pomegranate Cultivars. Agriculture and Forestry, Podgorica, 62: 91-94.

Engin, H., Hepaksoy, S. 2003. Determination of Pollen Germination of Some Pomegranate Cultivars. Ege Üniv. Ziraat Fak. Derg., 40 (3): 9-16. ISSN 1018-8851.

Eshghi, S., Da Silva, J. A. T., Ranjbar, R. 2010. Molybdenum and Boron Affect Pollen Germination of Strawbertty and Ferile and Infertile Flowers of Pomegranate. Fruit, Vegetable and Cereal Science and Biotechnology. 4:148-150.

Eti, S. 1991. Bazı meyve tür ve çeşitlerinde değişik *in vitro* testler yardımıyla çiçektozu canlılık ve çimlenme yeteneklerinin belirlenmesi. Ç.Ü.Z.F. Dergisi, 6, (1) :69-80

Forlani, M., Rotundo, A., 1977. Flowering biology of apricot I. A study of pollen germination. Annali della Facolta di Scienze Agrarie Della Univ., p: 70-79.

Gadže, J., Radunic, 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: 297-305.

Gimenez, M., Martinez, J., Oltra, M.A., Martinez, J.J., Ferrandez, M. 2008. Pomegranate (*Punica granatum* L.) leaf analysis: Correlation with harvest. http:// ressources.ciheam.org/om/pdf/a42/00600270.pdf.

Havlin, J.L., Beaton, J.D., Tisdale, S.L., Nelson, W.L. 2002. Soil Fertility and Fertilizers, (Çev. N. Güzel, K.Y. Gülüt, G. Büyük), Ç.Ü. Ziraat Fakültesi Genel Yayın No:246, Ders Kitapları Yayın No:A-80. s.373-381.

Kahramanoğlu, İ., Usanmaz, S., Alas, T., Helvacı, M., Aşkın, M.A. 2018. Fungicides Effect on The Heart Rot Infestations at Pomegranate Fruit. Int J Agric For Life Sci, 2(2) 2018,1-5.

Korkmaz, N., Aşkın, M.A., Ercişli, S., Okatan,V. 2016. Foliar Application of calcium nitrate , boric acid and giberellic acid affects yield and quality of pomegranate (*Punica granatum* L.). Acta Scientiarum Polonorum Cultus, 15 (3) 2016,105-112.

Okatan, V., Çolak, A. M., Güçlü, S. F., & Gündoğdu, M. (2018). The comparison of antioxidant compounds and mineral content in some pomegranate (Punica granatum L.) genotypes grown in the east of turkey. Acta Scientiarum Polonorum. Hortorum Cultus, 17(4).

Melgarejo, P., Martinez, J.J., Hernandez, F. 2008. A study of different culture media for pomegranate (Punica granatum L.) pollen. http://ressources.ciheam.org/om/pdf/a42/00600253.pdf.

Sharma, N., 2003. Studies on polen characters and pollination behaviour of pomegranate (*Punica granatum* L.) cultivars in mid-hills of Himachal Pradesh. Hayrana Journal of Horticultural Sciences. 32:11-14.

Tosun, F., Koyuncu, F. 2007. Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations .Hort. Sci. (Prague), *34*, 2007 (2): 47–53

Wang, Q., Lu, L., Wu, X., Li, Y., Lin, J. 2003. Boron influences pollen germination and pollen tube groth in Picea meyeri.

Tree Physiology.23,345-351
Wetzstein, H.Y., Ravid, N., Wilkins, E., Martinelli, A. P. 2011. A Morphological and Histological Characterization of Bisexual and Male Flower Types in Pomegranate. J. Amer. Soc. Hort. Sci. 136: 83-92.