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Effects of Heat and Cold Stresses on Pollen Grain Germination of Three Male Genotypes of Pistachio Trees

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Abstract

The present study aims to determine the germination percentage of pollen grains in three male genotypes of pistachio trees (early, mid and late flowering) under temperature stresses (heat and cold) in *in vitro* condition. After transferring male flower clusters to the laboratory for 1, 2 or 3 hours they were under temperature stress in the incubator. For cold stress 0, -2, -4 and -6°C temperatures and for heat stress 25, 30, 35 and 40°C temperatures were considered and 0 and 25°C temperatures were set as control under cold and heat stresses respectively. At the end of each period, pollen grains were cultured in a medium contained 150g sucrose, 10g Agar and 0.01g boric acid, and after 5 hours pollen grain germination percentage was calculated. The experiment was conducted as factorial in a completely randomized design with 3 factors (genotype, temperature and time) in 3 replications. According to the results, temperatures showed significantly difference on pollen grain germination percentage and by increasing stress duration, germination percentage of pollen grain was reduced. Under low temperatures, the highest germination percentage (65.66%) was found in mid-flowering genotype at 0°C after one hour cold treatment and the lowest germination percentage (13.26%) was recorded in late-flowering genotype at -6°C after 3 hours cold treatment. As for heat stress, the highest germination percentage (80.15%) was found in mid-flowering genotype at 25°C after one hour heat treatment and the lowest germination percentage (3.84%) was recorded in late-flowering genotype at 40°C after 3 hours heat treatment. According to the results, pollen germination percentage is affected by temperature, genotype, stress duration as well as their interaction.

Keywords: *Genotype; Germination in vitro; Pistachio; Pollen grain; Stress*

Introduction

Pistachio (*Pistciavera* L.) is one of the deciduous wooden trees from Anacardiaceous family [6]. Pistachio trees have male and female flowers which are born on distinct trees and for this, cross pollination is necessary [6]. Pistachio's flowers do not have petal so, they are not attractive for insects. Therefore, pollination, is took place with wind. The flowering period of male and female trees does not overlap and female flowers usually can't receive enough pollen grain [23]. For the time difference between blooming of male and female trees, their pollination yield is low. Other reasons may be because of male trees are not planted in the suitable ratio and in appropriate direction in orchards. Moreover, in the most orchards pollen quality is low. Yield reduction and blank fruits increasing is the results of this situation. By decreasing the yield, economic problems increase [34].

Pollination has a vital role to survive the plants species. Various factors such as heat, moisture, plant nutrition and pollinators effect on pollination [6]. Plants response to environmental stress is different. Optimum temperature for growth, development, and reproduction is depending on plant species as well as genotypes of a given species. In many plant species, high temperatures can induce sterility before or during anthesis [30]. It is reported that reproductive phase is more sensitive to high temperature stress than vegetative phase, and the maturation of pollen is the most sensitive process in the entire life cycle of plants, but the sensitivity is depend to plant species [25]. Heat stress has negative effects on pollen meiosis and germination, ovule development and viability and development of the embryo [24]. High temperatures also intervene with floral bud development due to flower abortion. Subjecting wheat plants (*Triticumaestivum* L.) to high temperatures for eight days during the double-ridge stage (25ºC) and/or during anthesis (35ºC) showed that grain yield was only significantly reduced when treatments were applied at both stages or at anthesis alone [32]. Exposing strawberries to high temperatures reduced pollen viability, germination percentage and pollen tube growth, which could led to low fruit set specially in 'Toyonoka' cultivar [13]. The degeneration of tapetum cells was also found in common bean (*Phaseolus vulgaris* L.) at 33/29°C [28] that caused premature pollen development within the anther during early development.

Cold stress can cause pollen sterility, which may be due to disruption of sugar metabolism in the tapetum, finally suspending starch accumulation in the pollen grains [22]. Cold temperatures, caused the pollen germination rate and seed production of *Arabidopsis* significantly reduced [14]. The growth of pollen tubes is required for fertilization in flowering plants. Because of this crucial function in reproductive cycle of plants, pollen has been the object of considerable molecular biological research. Many studies showed that cellular membranes are the primary site of freezing injury in plants [26, 29]. Cell membrane is damaged by disruption of protein lipid structure and protein denaturation. Low temperature stress causes the fatty acids become unsaturated and the lipid protein ratios of the membrane become altered which ultimately affect the membrane fluidity and structure as well [31]. Low temperature of the field environment restricted the vegetative growth and delayed all the phenological stages of chickpea in comparison to control plants [12]. Abscission of juvenile buds and flowers and abortion of pods caused by low temperatures in chickpea at reproductive phase. Generally, pollen development at young microspore stage appeared to be severely affected by stressed conditions compared to the control conditions. Pollen viability was decreased during stressed conditions (60%) compared to normal plants (95%) [12]. Flower buds of *Simmondsia chinensis* can be damaged or killed by temperatures of −2°C to −5.5°C [21]. In canola low, but nonfreezing temperatures prior to flowering slowed the rate of plant development, delayed flowering, slower the rate of flower opening and reduces the amount of pollen shed and in severe frost the pod abortion is evident in many species [16]. The effect of low temperature at the early stages of plant growth is sometimes associated with the premature flowering in Chinese cabbage [10].

The aim of the current studies was to investigate the effects of high and low temperature stress on pollen grains viability and germination.

Material and Methods

This experiment was carried out in the Rafsanjan Pistachio Research Institute of Iran in 2015. The site is situated on the cross point of 55˚ 59' E and 30˚ 23' N, 1469 m above sea level. At first, some 20 years old male trees (early, mid and late flowering) selected for pollen germination test. Flower clusters of male trees that their colors have changed from red to light green and almost 10% of their flowers were opened, collected. Inflorescences of early flowering genotype from 5th to 11^{th} of March 2015 and mid-flowering genotype from 13^{th} to 17^{th} of March and late flowering genotype from 20^{th} to 22^{nd} of March 2015 collected and their pollen germination percentage under temperature stress were investigated. Male flower clusters in the morning at 7 am harvested from the middle and almost above branches of trees and immediately transferred to the laboratory and then placed in disposable cups water, then for 1, 2 or 3 hours they kept under temperature stresses in the incubator. Anthers of open flowers on the day of sampling were inserted in to 50 ml cups and the flowers were vibrated by tooth brush for 10 seconds. For cold stress 0, -2, -4 and -6°C temperatures and for heat stress 25, 30, 35 and 40°C temperatures were considered and 0 and 25°C temperatures were set as control under cold and heat stresses respectively. 3 replications for each treatment used.

In vitro Germination of Released Pollen

The pollen grains were cultured on germination culture medium including 150 gram sucrose and 0.01% Boric acid and solidified with 10 gram agar [11]. Pollen grains were spread uniformly on the germination medium in 6 cm diameter petri dishes. They were incubated for 5 hours at 25°C and then fixed by acetic acid [9]. Germinated pollen grains were counted when the pollen tube length exceeded twice the pollen diameter. Four random microscopic fields were counted in a petri dish and three petri dish were counted per genotype. By dino-lite digital microscope am4113zt model with a resolution of 3.1 megapixels and 20 to 50 times magnification connected to the computer randomly from 4 parts of petri dish took photos and saved in computer. Then total number of pollen grains per field and the number of germinated and ungerminated pollen grains were counted. Pollen germination percentage calculated by dividing the germinated pollen grains to total number of pollen grains in each Petri dish then multiplied in 100.

Statistical analysis

The experiment was conducted as factorial in a completely randomized design with 3 replications. All statistical analysis (e.g., analysis of variance and LSD mean comparisons) was performed using SPSS software. Data was subjected to statistical analysis and level of significance measured at $P < 0.05$.

Results and Discussion

1- Heat stress

According to the results, different temperature treatments showed significant difference on pollen germination and by the time germination percentage of pollen grains was reduced. Under high temperatures, the highest and lowest pollen grain germination percentage was found in 25°C and 40°C, respectively (Figure 1) (Figure 9 b, c). Najafi and Nadour (2006) investigated the effects of heat stress on pollen germination and tube growth in maize (*In vitro* Condition) and reported same results. Daneshmand and Manouchehri Kalantari (2008) showed heat stress on pepper plant (*Capsicum annuum* L.) caused flower abscission. By temperature increasing from optimum, pollen grain germination decreased. It is thought that reducing the rate of pollen germination at high temperatures is due to the proteins and enzymes structure denaturation. There is little information about molecular mechanisms of pollen response to heat stress. Some plant species by specific proteins called heat shock proteins (HSP), respond to high temperatures and in this way protect cells against thermal stresses. HSPs synthesis in various stages of pollen grain development [7].

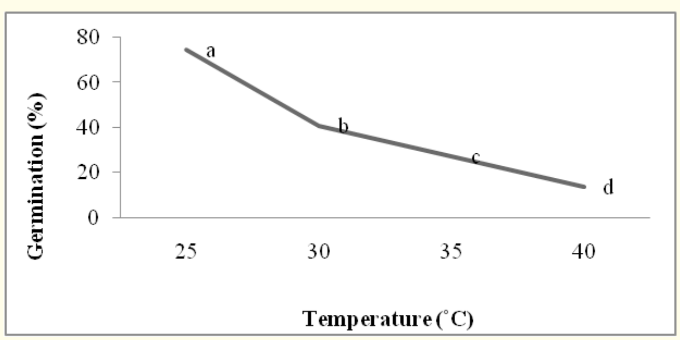

***Figure 1:** The effect of temperature (heat stress) on germination percentage of pollen grains.*

Among three genotypes, the highest and lowest pollen grain germination obtained in early and late flowering genotypes respectively (Figure 2). Abu-Zahra and Al-Abbadi (2007) reported same results in pistachio. Genotype and rootstock can effect on pollen germination under heat stress [1]. Malgorzata., *et al.* (2008) showed that physiological response of some tomato (Lycopersicon esculemtum L.) to high temperature stress varies among genotypes.

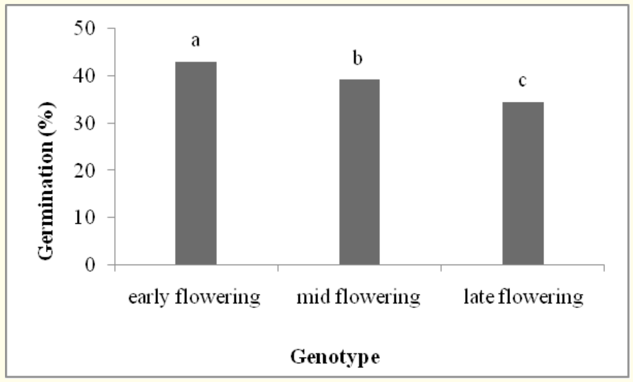

Figure 2: *The effect of genotype on germination percentage of pollen grains.*

According to the results, germinated pollen grain percentage of mid-flowering genotype at 25°C was higher than the others. However, early and mid-flowering genotypes didn't show significant difference at 25°C on pollen grain germination. The lowest germinated pollen grain percentage (6.95%) obtained in late-flowering genotype at 40°C (Figure 3). Results showed that the released pollen grain from anthers, are so sensitive to high temperatures. In fact, when pollen is released from the anthers, directly exposed to temperature changes and this situation affected on pollen structures and finally decreased pollen viability, germination and growth of pollen tubes. The effects of high temperatures were varies among different genotypes and mid-flowering genotypes showed higher pollen germination percentage in this study. It is probably due to genetic factors and stress resistance of these genotypes to high temperatures. Morrison and Stewart (2002) believed that different pollen germination percentage of summer Brassica was due to different genotypes. Suranyi (1995) showed that pollen germination percentage of apricot varieties at high temperatures was varying.

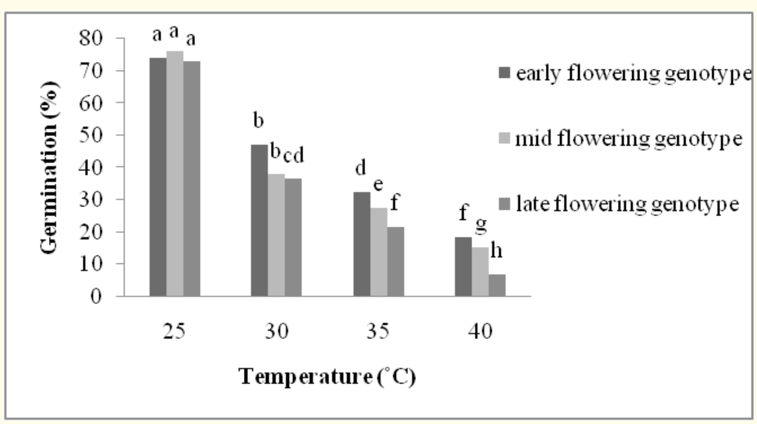

Figure 3: *The effect of temperature and genotype on pollen grains germination percentage of three male genotypes of pistachio. (Means with different letter are significant in LSD test at 5% level).*

The highest pollen germination percentage obtained at 25°C and duration of heat treatment (1, 2 or 3 h) didn't show significant difference [Figure 4], but pollen were kept at 25°C for 2 hours showed the highest germination percentage (75.73%) [Figure 4]. The lowest pollen germination percentage (12.04%) observed in pollens that saved at 40°C for 3 hours [Figure 4]. This situation can decrease fruit set, which may be due to disruption of sugar metabolism in the tapetum, finally suspending starch accumulation in the pollen grains [22].

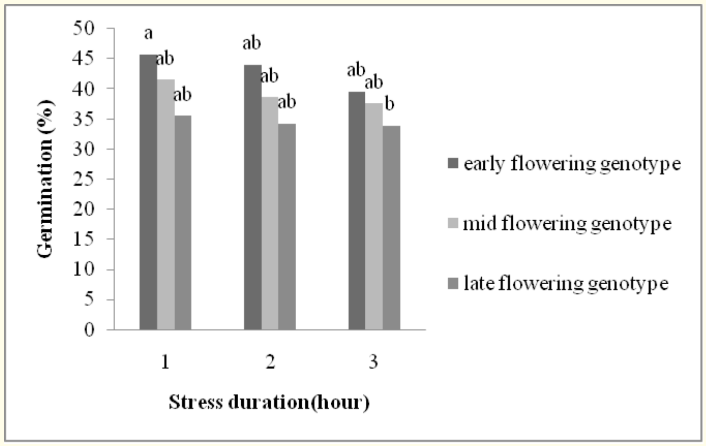

***Figure 4:** The effect of genotype and stress duration on pollen grains germination percentage of three male genotypes of pistachio. (Means with different letter are significant in LSD test at 5% level).*

2- Cold temperatures

The dimension less concentrations of the species A, R and S, u_A, u_R and u_S as a function of dimensionless time, $\tau = k_1t$ is shown in Figure 4. The step size used in the computer simulations was h = 0.01. The distribution of products R and S as a function of conversion of reactants A, $X_A = 1 - u_A$ is shown in Figure 5. Figure 4 and Figure 5 were obtained for reaction rate constant ratio values of $\kappa = \varepsilon = \omega = 0.2$. It can be seen that after 70% conversion of reactant A the concentration of product species R formation decreases. At about 88% conversion of reactant A the concentration of species S also decreases. At higher conversions the reactant a species concentration is lower and hence the rate of the reaction decreases.

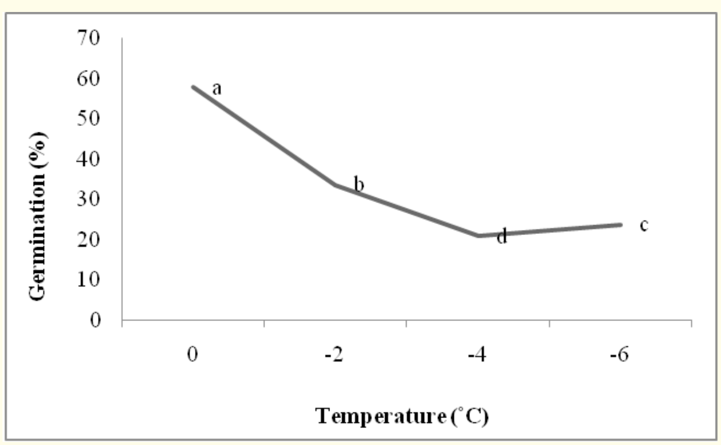

***Figure 5:** The effect of temperature on pollen grains germination.*

The highest and lowest pollen germination percentage (37.15% and 29.78%) obtained in early and late flowering genotypes, respectively (Figure 6). Bassi., et al. (1995) reported that tolerance of apricot to spring frost was not correlated to late-flowering genotype that was according to our results. It seems that mid-flowering genotype better than others can tolerate cold stress and showed higher pollen viability and germination.

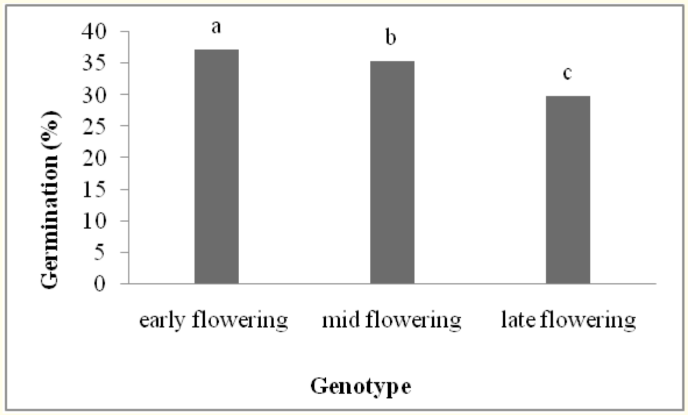

Figure 6: *The effect of genotype on pollen grains germination of pistachio under cold stress.*

The lowest pollen grain germination percentage was recorded in late-flowering genotype at -4 and -6°C (Figure 7). In figure 9 a germinated pollen grains of mid-flowering genotype at -6°C is shown. Mousavi Nejad (2010) showed significant difference between genotypes on germination and pollen tube growth in several almond cultivars under different temperatures. Yamada., *et al.* (2004) reported higher ion leakage in susceptible genotypes of ryegrass to cold temperatures. Under cold stress, cell membrane damaged and resulting in intracellular contents leak out of the cell and electrical conductivity of solution in susceptible genotypes increased [33]. Potassium content of cells is one of the factors contributing to increasing plant tolerance to adverse environmental conditions such as cold. This is probably due to the higher accumulation of potassium in mid-flowering genotypes than others.

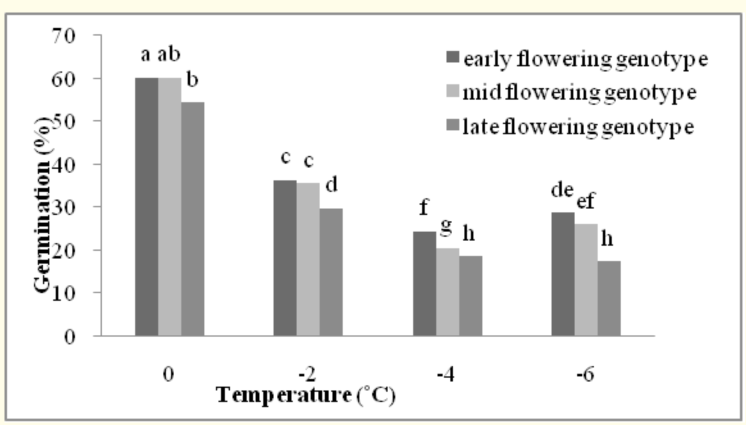

Figure 7: *The effect of temperature and genotype on pollen grains germination percentage of three male genotypes of pistachio. (Means with different letter are significant in LSD test at 5% level).*

The highest pollen grain germination percentage (31.31%) was obtained in mid-flowering genotype after one hour cold stress treatment (Figure 8). However, significant difference didn't observe between them and mid-flowering genotype after 1, 2 or 3 hours of cold treatment. Also, the lowest pollen grain germination percentage (27.46%) was recorded in late-flowering genotype after three hours cold stress treatment (Figure 8). Nejatiyan and Ebadi (2006) studied pollen germination and pollen tube growth of eleven apricot cultivars on *in vitro* Condition and reported significant difference between cultivars.

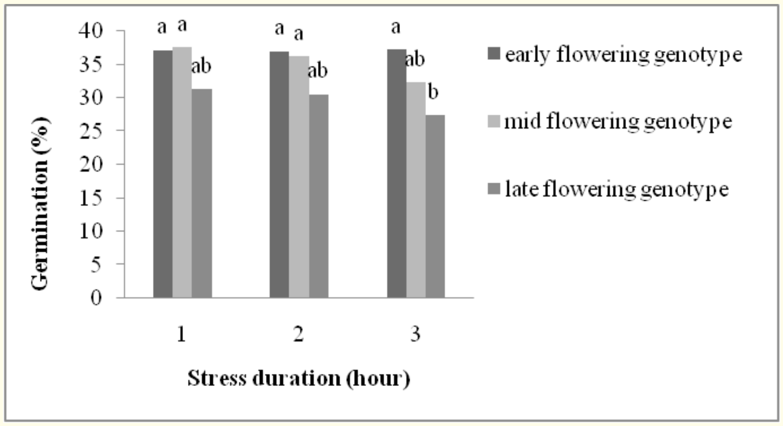

Figure 8: The effect of genotype and stress duration on pollen grains germination percentage of three male genotypes of pistachio. (Means with different letter are significant in LSD test at 5% level).

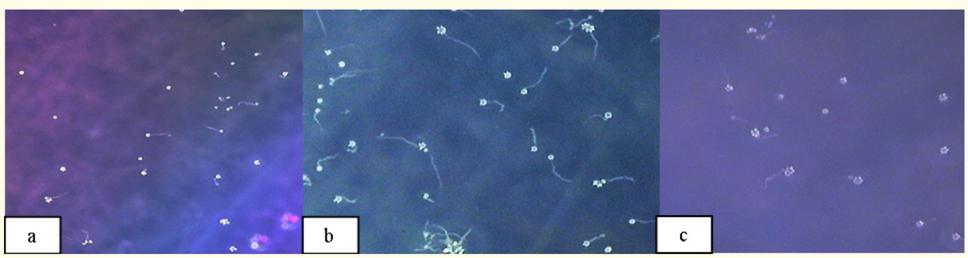

Figure 9: The effect of cold (-6°C) (a) and heat stresses [(25°C) (b) & 40°C (c)] on pollen germination of mid-flowering genotype.

Conclusion

Pollination has an important role in the fruit set. Temperature especially in pollination period can affect on pollen viability and finally fruit set. According to the results, pollen germination percentage is affected by temperature, genotype, stress duration as well as their interaction. Pollen exposed to extreme temperatures (Low and high) showed low germination in compare to the control. However, by increasing stress duration, pollen viability decreased. Obtained results showed that mid-flowering genotype, is the best tolerant genotype in response to the extreme temperatures and we can propose this genotype to the farmers in Rafsanjan region.

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Volume 3 Issue 1 February 2016