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Research paper

Quercetin and gamma-aminobutyric acid content and in vitro germination in pollen grains of *Citrus* species at different temperatures

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Abstract
Environmental factors such as temperature have negative effects on the reproductive phase and can cause yield loss in *Citrus* species. Pollen grains play a key and essential role in the fertility of plants. Quercetin and gamma-aminobutyric acid (GABA) are involved in certain biochemical functions to regulate growth and increase tolerance to biotic and abiotic stresses. The purpose of this study was to evaluate the effect of different temperatures on pollen germination and also on the levels of quercetin and GABA. The branches containing flowers of *Cirus reticulata, C. sinensis, and C. paradisi* were exposed to 5, 10, 15, 20, and 25 °C for six hours and then pollen grains were collected. Levels of quercetin and GABA were quantified by HPLC. The amount of quercetin was highest at 5 °C and then decreased by increasing the temperature in all species. Also, with increasing temperature, the amount of GABA decreased in all species. The highest and lowest pollen germination was observed at 25 °C and 5 °C, respectively.

Keywords: germination; low temperature; phenolic compounds; pollen; secondary metabolites

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Introduction

The process of pollen grain germination and subsequent pollen tube growth plays a significant role in the reproduction and distribution of plants. Investigating the efficiency of pollen grains and their tolerance to temperature on the performance of fruit trees, especially *Citrus* fruits, is important. The genotype of the pollen grain, male and female mixture, as well as the interaction of genotype by temperature in the reproductive stage are very important in determining the role of the pollen grains (Distefano *et al*. 2012).

The surface of the stigma provides a suitable environment under natural conditions for the germination and growth of the pollen tube. Differences in germination percentage have been reported among different plants (Irenaeus and Mitra 2014).

Flowers are the organs that develop into fruits and any changes in the temperature result in flower abortion, and reduction of seed set and yield (Porter and Semenov 2005; Kumar *et al.* 2011). Low temperature can cause the reduction of anther opening, reduction of pollen grains on the stigma, and failure of pollen tube growth (Kumar *et al*. 2011). Also, low temperature leads to pollen sterility via disturbance of sugar metabolism and finally produces starch in the pollen grains (Oliver

et al. 2007). Pollen grain responds to environmental fluctuations (Rezanejad 2009). Some researchers have shown that pollen germination is stimulated or reduced by flavonoids (Izadi *et al*. 2008; Distefano *et al*. 2012). Gamma-aminobutyric acid (GABA) is a derivative of glutamate and is a non-proteinogenic amino acid involves in pH regulation, plant development, and resistance to stress factors in plants (Michaeli and Fromm 2015). GABA as an antioxidant, causes defense responses against abiotic (Michaeli and Fromm 2015) and biotic stresses (Forlani *et al*. 2014).

The effect of GABA treatment on pollen tube development was first found in *Arabidopsis* (Palanivelu *et al*. 2003; Yu *et al*. 2014). Although no studies have been conducted on the accumulation of GABA during germination of the *Citrus* pollen grains, studies have shown the accumulation of GABA during seed germination in the germinated mung bean and soybean (Tiansawang *et al*. 2016).

Secondary metabolites, including phenolic and flavonoid compounds, play a significant role in resistance to adverse environmental conditions in plants (Rhodes 1994). They are usually accumulated in the flower parts including petals, pistils, ovaries, and anthers of plants (Ylstra *et al*. 1994).

Phenolic compounds are known as factors involved in stress conditions and accumulate in large amounts in response to these conditions. Phenolic compounds are involved in pollination, pollen germination, and pollen tube growth in plants (Rezanejad 2012). These compounds increase the level of factors that stimulate germination in the stigma. Quercetin and other flavonoids control auxin transport by combining with phytotropin or N-1-naphthylphthalamic acid and increase pollen tube growth by preventing the oxidative degradation of indol botiric acid (Jacobs and Rubery 1988). In the kiwifruit plant, quercetin, rutin, and other flavonoid compounds increase the growth of the pollen tube (Antognoni *et al*. 2004).

This research was conducted to investigate the effect of different temperatures on the amount of quercetin, GABA, and pollen germination in *Citrus* species.

Materials and Methods

Plant material

Branches containing flowers of different species of *Citrus* such as *C. reticulata* var Kara, *C. sinensis* var Valencia, and *C. paradisi* var Redblash were collected and transferred to the laboratory. Then, the shoots were exposed to different temperatures of 5, 10, 15, 20, and 25°C for six hours (Mohammadrezakhnai *et al*. 2017). For the temperature experiment, flowers were collected at the anther dehiscence stage. For the other analyses, pollen grains were air-dried and stored at − 20 °C until use.

Analysis of quercetin by HPLC

Half a gram of pollen grains of each species was weighed and 5 ml of methanol was added to them. After vortexing, the resulting mixture was centrifuged at 12,000 g for 15 min at room temperature, and the supernatant was used for high-pressure liquid chromatography (HPLC) analysis (Agilent model 1100). Elution was carried out using a flow rate of 0.8 mL min^{-1} at 24 °C.

The injection content was 20 µl. Quercetin was assayed by a C18 column and UV–visible detector. The solvents were adjusted to pH 2.5 with orthophosphoric acid (A) and acetonitrile (B). A linear gradient was used according to the following: starting with 100% A, decreasing to 91% over the next 12 min, to 87% over the next 8 min, and to 67% over the next 10 min. The solvent was held in this composition for 2 min, phase A decreased to 57% over the next 10 min., and then it was kept at this level till the end of the 60-min analysis. The column effluent was monitored at 340 nm (Campos *et al*. 1997; Rezanejad 2012).

Determination of GABA by HPLC

The extracts of 0.2 g of pollen grains were prepared and homogenized with 1 ml water: choloform: methanol (3:5:12 v/v/v) solution and centrifuged for 2 min at 10000g at 4 °C. Supernatants were dried and re-dissolved in 100µl water. 150 µl Borax buffer (PH 8) and 250 µl of 2-hydroxynaphthaldehyde (3% w\v in methanol) was added to these solvents. The resulting mixture was heated to 80°C in a water bath for 20 minutes and cooled to room temperature. Before the injection, the final volume was adjusted to 1 ml with methanol and then the extracts were filtered with a 0.45 µm filter. The mobile phase was composed of methanol: water (62: 38 v\v) and was used at a flow rate of 0.5 ml/min. The retention time was 10 min for GABA and detection was monitored at 330 nm (Baum *et al*. 1996; Bor *et al*. 2009).

Determination of in vitro pollen germination and pollen tube growth

The culture media for pollen germination was Brewbaker (1963) solid culture medium in Petri dishes. The medium composition contained 15% sucrose, 200 mg/l $MgSO_4$, 100 mg/l KNO_3, 100 mg/l H_3BO_3, and 1% agar. The pollen germination percentage (%) was determined using light microscopy after 30 min. About 100 pollen grains from 4-5 fields of view were used to measure the germination percentage for each replication. Pollen tube length (μm) was measured using an ocular micrometer fitted to the microscope. The pollen tube length was measured by calculating the average length of 20 pollen tubes after 3 h (Sahar and Spiegel-Roy 1980; Rezanejad 2009).

Statistical analysis

The present research was conducted in the form of a completely randomized design with three replications. Data were compared using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test ($p \leq 0.05$).

Results

Quercetin content

The highest quercetin concentration was observed at 5 ° C in all three *Citrus* species. In all species, quercetin decreased with the temperature increases (Table 1). However, there was no significant difference in the amount of this compound at 5, 10, and 15 °C. Also, no significant difference was observed between 20 °C and 25 °C. The amount of Quercetin was higher in *C. reticulata* compared to other species (Table 1).

HPLC chromatogram of GABA

The results showed that the amount of GABA in the three species studied did not show any obvious

Table 1. Content of quercetin (µg/g) in three *Citrus* species under different temperatures

Species	Temperature (°C)				
	5	10	15	20	25
C. reticulata	0.50[a]	0.47[a]	0.45[a]	0.40[ab]	0.40[ab]
C. sinensis	0.34[b]	0.30[b]	0.30[b]	0.25[c]	0.24[c]
C. paradisi	0.30[b]	0.30[b]	0.30[b]	0.23[c]	0.23[c]

Values with different letters are significantly different at $p \leq 0.05$ (Duncan's multiple range test).

difference in temperatures 5-25 °C in *C. reticulata.* But in *C. sinensis* its amount decreased with increasing temperature to 20 °C and 25 °C compared to 5 °C. Thus, the highest levels were in the range of 5-15 °C. The content of GABA in *C. reticulata* was higher than the other two species (Table 2).

In-vitro pollen germination

Different temperature treatments significantly differed for the pollen germination in the studied species. The pollen germination increased with increasing the temperature. The lowest germination percentage was observed at 5 °C and the highest at 25 °C. The comparison of pollen germination between species showed that the highest level of pollen germination was observed in *C. reticulata* while *C. paradisi* recorded the lowest percentage. Pollen germination increased from 7.5 to 19% (*C. reticulata*), 5.5 to 17% (*C. sinensis*), and 3.5 to 9% (*C. paradisi*) with increasing temperature from 5 to 25 °C (Figure 1).

Discussion

During flower bud development, temperature stress causes fluctuations in flowering time and asynchronous growth of male and female organs (Zinn *et al*. 2010). During reproduction in the *Citrus* fruits, temperature is one of the most important environmental factors affecting performance (Distefano *et al*. 2012).

Citrus fruits are sensitive to cold stress (Charrier *et al*. 2015). In this study, GABA decreased with increasing temperature in all species. GABA accumulates in plants in response to various stresses (Kinnersley and Lin 2000). In the cowpea plant, the amount of GABA increased during heat stress. Also, GABA increased in soybean under cold stress and mechanical damage (Mayer *et al*. 1990). At the cold stress conditions, GABA stimulated the antioxidant defense system to eliminate reactive oxygen species and prevents oxidative damage in the peach fruits (Yang et al. 2011). In general, the amount of endogenous GABA within the plant is low, but it

Table 2. Content of GABA (µg/g) in three *Citrus* species at different temperatures

Species	Temperature (°C)				
	5	10	15	20	25
C. reticulata	0.35[a]	0.30[a]	0.24[ab]	0.25[ab]	0.25[ab]
C. sinensis	0.30[a]	0.25[ab]	0.25[ab]	0.20[b]	0.20[b]
C. paradisi	0.25[ab]	0.22[ab]	0.20[b]	0.18[b]	0.15[b]

Values with different letters are significantly different at $p \leq 0.05$ (Duncan's multiple range test).

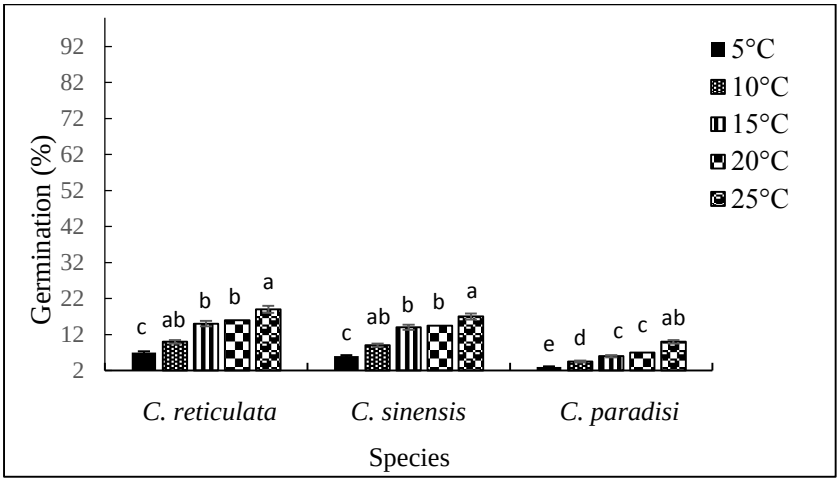

Figure 1. In vitro pollen germination percentage at different temperature treatments in three *Citrus* species. Different letters above the columns indicate significant differences between temperature ($p \leq 0.05$; based on Duncan's multiple range test).

accumulates quickly under stress and causes resistance (Kinnersley and Lin 2000). GABA is a nitrogen absorber in plants when in adverse environmental conditions. GABA also plays a role in nitrogen metabolism and C:N fluxes (Li *et al*. 2016).

Flavonoids are one of the most important secondary metabolites, which include pigments (chalcones, anthocyanins) and colorless compounds (flavanones, flavones, flavonols) (Kuhn *et al*. 1984). Flavonoids are important in plant growth and development, defense against biotic and abiotic stresses, pollen germination, pollen tube growth, and polar auxin transport (Loughnan *et al*. 2014). The lack of flavonoids in the mutant *Petunia juss* causes the sterility of the male organ and prevents the germination of pollen grains (Woo *et al*. 2005). Quercetin increases in stressed plants due to its defense and antioxidant role against environmental stresses (Dixon and Paiva 1995; Rezanejad 2009). Mohammadrezakhani *et al*. (2017) reported the increased levels of flavonoids in the pollen grains of *Citrus* species under cold stress It has been reported that flavonoids have higher antioxidant properties than vitamins C and E, and carotenoids (Rahman 2007).

Fertility in plants decreases under various stresses due to direct and indirect effects on the reproductive system. Pollen germination may be used as a sensitive biological indicator, so studies of pollen germination can show the harmful and destructive effects of different stresses on plants. In herbaceous plants, the effects of temperature stress on the growth of the male structure have been well-defined (Hedhly 2011). Heat and cold stresses play a role in pollen maturation and germination. (Zinn *et al*. 2012). Low temperature stimulates pollen sterility in lemon (Soost *et al*. 1975). High or low temperatures have adverse effects on pollen germination in apricot (Egea *et al*. 1992), *Pistacia* spp. (Acar amd Kakani 2010), and mango (Sukhvibul *et al*. 2000).

In this study, we evaluated the pollen germination response to temperatures from 5 to 25 ºC. In-vitro pollen germination of all *Citrus* species

was accelerated with increasing the temperature. Cell division at meiosis, microtubules, and cytoskeleton change in response to temperature fluctuations (Muller and Rieu 2016).

The highest pollen germination was observed at 25 ºC. The highest and lowest levels of pollen germination were obtained in *C. reticulata* and *C. paradise*, respectively. It seems that *C. paradisi* has lower resistance against biotic and abiotic conditions. *Citrus* is very sensitive to the low-temperature stress in the reproductive stage (Crifo *et al*. 2011). Studies on pollen germination of *C. medica* L., *C. reticulata* Blanco, and *C. maxima* showed that the amount of germination enhance by increasing the temperature from 10 to 30 ºC and the optimum temperature was 25 ºC (Distefano *et al*. 2012). The maximum percentage of germination at 25 °C in *C. reticulata* Blanco and *C. maxima* has been reported in the range of 28 and 45% (Distefano *et al.* 2012).

Temperature changes in plants such as apple (Yoder *et al*. 2009), pear (Lombard *et al*. 1972), plum (Jefferies *et al*. 1982), sweet cherry (Guerrero-Prieto *et al*. 1985), litchi (Stern and Gazit 1998), olive (Vuletin Selak *et al.* 2013), *Pistacia* spp (Acar and Kakani 2010), and date palm (Slavkovic *et al*. 2016) affects germination and pollen tube growth (Irenaeus and Mitra 2014). Temperature is an important factor in the reproductive phase for pollen germination and pollen tube growth (Alburquerque *et al*. 2007). Fruit formation and fruit growth are done by hormones produced in the ovary after pollination (Botu *et al*. 2002).

In conclusion, different temperatures affected the pollen germination and the amount of quercetin and GABA. The highest and lowest pollen germination was obtained at 25 °C and 5 °C, respectively. The amount of quercetin decreased by increasing the temperature in all species. Also, as temperature increased, the amount of GABA decreased in all species.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

References

Acar I and Kakani VG. 2010. The effects of temperature on *in vitro* pollen germination and pollen tube growth of *Pistacia* spp. Scientia Horticulturae 125: 569-572.

Alburquerque N, Garcia MF, and Burgos L, 2007. Influence of storage temperature on the viability of sweet cherry pollen. Spanish Journal of Agricultural Research 5(1): 86-90.

Antognoni F, Ovidi E, Taddei AR, Gambellini G, and Speranza A, 2004. In vitro pollen tube growth reveals the cytotoxic potential of the flavonols, quercetin and rutin. Alternatives to Laboratory Animals 32: 79-90.

Baum G, Lev-Yadun S, Fridmann Y, Arazi T, Katsnelson H, Zik M, and Fromm H, 1996. Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development of plants. The EMBO Journal 15: 2988-2996.

Bor M, Seckin B, Ozgur R, Yılmaz O, Ozdemir F, and Turkan I, 2009. Comparative effects of drought, salt, heavy metal and heat stresses on gamma-aminobutryric acid levels of sesame (*Sesamum indicum* L.). Acta Physiologiae Plantarum 31: 655-659.

Botu M, Sarpe C, Cosmulescus S, and Botu I, 2002. The genetic control of pollen fertility, pollinating and fruit set for the *Prunus domestica* L. plum cultivars. Acta Horticulturae 577: 139-145

Campos M, Markham KR, Mitchell KA, and Proenca da Cunha A, 1997. An approach to the characterization of bee pollens via their flavonoid/phenolic profiles. Phytochemical Analysis 8: 181-185.

Charrier G, Ngao J, Saudreau M, and Ameglio T, 2015. Effects of environmental factors and management practices on microclimate, winter physiology, and frost resistance in trees. Frontiers in Plant Science 6: 259.

Crifo T, Puglisi I, Petrone G, and Recupero GR, 2011. Angela Roberta Lo Piero expression analysis in response to low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. Gene 476: 1-9.

Distefano G, Hedhly A, Casas GL, Malfa SL, Herrero M, and Gentile A, 2012. Male-female interaction and temperature variation affect pollen performance in *Citrus*. Scientia Horticulturae 140: 1-7.

Dixon RA and Paiva NL. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7: 1085-1097.

Egea J, Burgos L, Zoroa N, and Egea L, 1992. Influence of temperature on the in vitro germination of pollen of apricot (*Prunus armeniaca* L.). Journal of Horticultural Science 67: 247-250.

Forlani G, Bertazzini M, and Giberti, S, 2014. Differential accumulation of γ–aminobutyric acid in elicited cells of two rice cultivars showing contrasting sensitivity to the blast pathogen. Plant Biology 16: 1127-1132.

Guerrero-Prieto VM, Vasilakakis MD, and Lombard PB, 1985. Factors controlling fruit set of Napoleon sweet cherry in western Oregon. Horticulture Science 20: 913-914.

Hedhly A, 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. Environmental and Experimental Botany 74(1): 9-16.

Irenaeus TKS and Mitra SK, 2014. Understanding the pollen and ovule characters and fruit set of fruit crops in relation to temperature and genotype. Journal of Applied Botany and Food Quality 87: 157-167.

Izadi Khaleghabadi M, Rezanezhad F, and Manouchehr Kalantari K, 2008. Effects of some environmental and chemical factors on germination and pollen tube growth in petunia hybrid. Iranian Journal of Biology 21(4): 667-675 (In Persian with English abstract).

Jacobs M and Rubery PH, 1988. Naturally occurring auxin transport regulators. Science 241: 346-349.

Jefferies CJ, Brain P, Stott KG, and Belcher AR. 1982. Experimental systems and mathematical models for studying temperature effects on pollen-tube growth and fertilization in plum. Plant, Cell and Environment 5: 231-236.

Kinnersley AM and Lin F, 2000. Receptor modifiers indicate that 4-aminobutyric acid (GABA) is a potential modulator of ion transport in plants. Plant Growth Regulation 32: 65-76.

Kuhn DN, Chapall J, Boudet A, and Hahlbrock K, 1984. Induction of phenylalanine ammonia-lyase and 4-coumarate: CoA ligase mRNAs in cultured plant cells by UV induction or fungal elicitor. Proceedings of the National Academy of Sciences of the United States of America 81: 1102-1106.

Kumar S, Kaur R, Kaur N, Bhandhari K, Kaushal N, Gupta K, Bains TS, and Nayyar H, 2011. Heat-stress induced inhibition in growth and chlorosis in mungbean (*Phaseolus aureus Roxb*.) is partly mitigated by ascorbic acid application and is related to reductionin oxidative stress. Acta Physiologiae Plantarum 33: 2091-2101.

Lembi CA, Morre DJ, Thomson KS, and Hertel R, 1971. N-1- naphthylphthalamic-acid-binding activity of a plasma membrane-rich fraction from maize coleoptiles. Planta 99: 37-45.

Li W, Liu J, Ashraf U, Li G, Li Y, Lu W, Gao L, Han F, and Hu J, 2016. Exogenous γ-aminobutyric acid (GABA) application improved early growth, net photosynthesis, and associated physio-biochemical events in maize .Frontiers in Plant Science 7: 919.

Lombard PB, Williams RR, Scott KG, and Jefferies CJ, 1972. Temperature effects on pollen tube growth in styles of Williams' pear with a note on pollination deficiencies of comice pear. Compte Rendue du Symposium 'Culture du Poirier', 4-8 Septembre, Station de Recherche d'Anger, INRA, France. Simadess, Anger, France, pp. 265-279.

Loughnan D, Thomson J, Ogilvie J, and Gilbert, B, 2014, *Taraxacum officinale* pollen depresses seed set of montane wildflowers through pollen allelopathy. Journal of Pollination Ecology 13: 146-150.

Mayer R, Cherry J, and Rhodes D, 1990. Effect of heat shock on amino acid metabolism of cowpea cells. Plant Physiology 94(2): 796-810.

Michaeli S and Fromm, H, 2015. Closing the loop on the GABA shunt in plants: are GABA metabolism and signaling entwined? Frontiers in Plant Science 6: 419.

Mohammadrezakhani S, Hajilou J, and Rezanejad F, 2017. Evaluation of phenolic and flavonoid compounds in pollen grains of three *Citrus* species in response to low temperature. Grana 57: 214-222.

Muller F and Rieu I, 2016. Acclimation to high temperature during pollen development. Plant Reproduction 29: 107-118.

Oliver SN, Dennis ES, and Dolferus, R, 2007. ABA regulates apoplastic sugar transport and is a potential signal for cold-induced pollen sterility in rice. Plant and Cell Physiology 48: 1319-1330.

Palanivelu R, Brass L, Edlund AF, and Preuss D, 2003. Pollen tube growth and guidance is regulated by POP_2, an Arabidopsis gene that controls GABA levels. Cell 114: 47-59.

Porter JR and Semenov MA, 2005. Crop responses to climatic variation. Philosophical Transactions of the Royal Society of London. Series B, Biological 360: 2021-2035.

Rahman K, 2007. Studies on free radicals, antioxidants, and co-factors. Clinical Interventions in Aging 2(2): 219-36.

Rezanejad F, 2009. Air pollution effects on structure, proteins and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae). Grana 48(3): 205-213.

Rezanejad F, 2012. Air pollution effects on flavonoids in pollen grains of some ornamental plants. Turkish Journal of Botany 36: 49-54.

Rhodes MJ, 1994. Physical role for secondary metabolites in plants: some progress, many outstanding problems. Plant Molecular Biology 24: 1-20.

Sahar N and Spiegel-Roy P, 1980. *Citrus* pollen storage. Horticulture Science 15: 81-82.

Slavkovic F, Greenberg A, Sadowsky A, Zemach H, Ish-Shaloma M, Kamenetskya R, and Cohena Y, 2016. Effects of applying variable temperature conditions around inflorescences on fertilization and fruit set in date palms. Scientia Horticulturae 202: 83-90.

Soost RK and Cameron JW, 1975: *Citrus*. In: Janick J and Moore JM (eds.). Advances in Fruit Breeding. Purdue University Press, Indiana, USA.

Stern RA and Gazit S, 1998. Pollen viability in lychee. Journal of American Society for Horticultural Science 123: 41-46.

Sukhvibul N, Whiley AW, Vithanage V, Smith MK, Doogan VJ, and Hetherington SE, 2000. Effect of temperature on pollen germination and pollen tube growth of four cultivars of mango (*Mangifera indica* L.). The Journal of Horticultural Science and Biotechnology 75(2): 214-222.

Tiansawang K, Luangpituksa P, Varanyanond W, and Hansawasd C, 2016. GABA (γ-aminobutyric acid) production, antioxidant activity in some germinated dietary seeds and the effect of cooking on their GABA content. Food Science and Technology. doi: http://dx.doi.org/10.1590/1678-457X.0080

Yang A, Cao S, Yang Z, Cai Y, and Zheng Y, 2011. γ-aminobutyric acid treatment reduces chilling injury and activates the defence response of peach fruit. Food Chemistry 129: 1619-1622.

Ylstra B, Busscher J, Franken J, Hollman PCH, Mol JNM, and van Tunen AJ, 1994. Flavonols and fertilization in *Petunia hybrida*: localization and mode of action during pollen tube growth. Plant Journal 6: 201-212.

Yoder K, Yuan RC, Combs L, Byers R, McFerson J, and Schmidt T, 2009. Effects of temperature and the combination of liquid lime sulfur and fish oil on pollen germination, pollen tube growth, and fruit set in apples. Horticulture Science 44: 1277-1283.

Yu GH, Zou J, Feng J, Peng XB, Wu JY, Wu YL, Palanivelu R, and Sun MX, 2014. Exogenous γ-aminobutyric acid affects pollen tube growth via modulating putative Ca^{2+} permeable membrane channels and is coupled to negative regulation on glutamate decarboxylase. Journal of Experimental Botany 12: 3235-3248.

Vuletin Selak G, Perica S, Goreta Ban S, and Poljak M, 2013. The effect of temperature and genotype on pollen performance in olive (*Olea europaea* L.). Scientia Horticulture 156: 38-46.

Woo HH, Jeong BR, and Hawes MC, 2005. Flavonoids: from cell cycle to biotechnology. Biotechnology Letters 27: 365-374.

Zinn KE, Tunc-Ozdemir M, and Harper JF, 2012. Temperature stress and plant sexual reproduction: uncovering the weakest links. Journal of Experimental Botany 61(7): 1959-1968.

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چکیده

عوامل محیطی مانند دما دارای اثرات منفی بر فاز زایشی هستند و می‌توانند باعث کاهش عملکرد در گونه‌های مرکبات شوند. دانه‌های گرده نقش کلیدی و اساسی در باروری گیاهان دارند. کوئرستین و گاما آمینوبوتیریک در کارکردهای بیوشیمیایی خاصی برای تنظیم رشد و افزایش تحمل به تنش‌های زیستی و غیرزنده دخالت دارند. هدف از این مطالعه ارزیابی دماهای مختلف بر جوانه زنی گرده و نیز میزان کوئرستین و گابا بود. شاخه‌های حاوی گل‌های پرتقال والنسیا (*Citrus sinensis* var Valencia)، نارنگی کارا (*C. reticulata* var. Kara) و گریپ فروت ردبلاش (*C. paradisi* var. Red blash) در معرض دماهای مختلف (۵، ۱۰، ۱۵، ۲۰ و ۲۵ درجه سانتی‌گراد) برای مدت شش ساعت قرار گرفتند و سپس دانه‌های گرده جمع آوری شدند. میزان کوئرسیتین و گابا با استفاده از HPLC سنجیده شد. بیشترین و کمترین جوانه زنی گرده به ترتیب در دماهای ۲۵ درجه سانتی‌گراد و ۵ درجه سانتی‌گراد مشاهده شد. بیشترین مقدار کوئرستین در دمای ۵ درجه سانتی‌گراد به دست آمد و سپس با افزایش دما میزان آن در تمامی گونه‌ها کاهش یافت. همچنین با افزایش دما میزان GABA در هر سه گونه کاهش یافت. بیشترین و کمترین جوانه زنی گرده به ترتیب در دماهای ۲۵ درجه سانتی‌گراد و ۵ درجه سانتی‌گراد مشاهده شد.

واژه‌های کلیدی: ترکیبات فنولی؛ جوانه زنی؛ دانه گرده؛ دمای پایین؛ متابولیت‌های ثانویه