Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF RELATIVE HUMIDITY ON *IN VITRO* POLLEN GERMINATION AND TUBE GROWTH IN SWEET CHERRIES (*PRUNUS AVIUM* L.)

Sultan Filiz GÜÇLÜ[1], Fatma KOYUNCU[2]

[1]Süleyman Demirel University, Atabey Vocational School, Isparta, Turkey
[2] Süleyman Demirel University, Faculty of Agriculture, Department of Horticulture, Isparta, Turkey

Corresponding author email: sultanguclu@sdu.edu.tr

Abstract

Objective of this study was to examine the effects of global warming on pollen germination in fruit trees. For this purpose Bigarreu Gaucher, Celeste, Lambert, Lapins, Starks Gold, Stella and 0900 Ziraat cherry cultivar's pollens were used. Pollen germination and tube growth rates were observed at 40%, 50%, 60%, 70%, 80% and 90% relative humidity regimes. High humidity regimes were gave best results for both pollen germination and pollen tube growth. 80% was the optimum RH for in vitro germination of Starks Gold and Stella while 90% was optimum for Bigarreu Gaucher. In vitro pollen germination increased with increasing incubation relative and incubation period. 24 hours later was the optimum for all cultivars. The longest pollen tube length was obtained from 90% RH. Set fruitful could be affected by relative humidity and indirectly global warming.

Key words: *relative humidity, pollen germination, Prunus avium* L.

INTRODUCTION

Environmental conditions play a key role for the survival living organisms (Koubourıs et al., 2009). The environment can be an important determinant of pollen performance.

The effects of the environment on pollen germination may be broken down into three categories: (1) prepollination environment, (2) pollination environment, and (3) postpollination environment. The prepollination environment consists of the environmental conditions in which a pollen-donating plant grows. Pollen may also be influenced by the environment experienced during pollen transfer from donor to recipient (Travers, 1999).

Fertilization success in plants is the result of processes that take place during the progamic phase. Recently studies show that environmental conditions affect pollen devolopment and pollen germination as well as pollen tube growth.

Enviromental conditions affect steps such as pollen germination and pollen tube growth as well as development of the female structures (Stephenson et al., 1992; Delph et al., 1997; Hedly et al., 2004).

Among those environmental conditions, temperature is one of the most important factors that affect fruit and seed set. Another factor of pollen germination and tube growth is relative humidity.

Humidity and temperature has been shown to affect the chemical composition of pollen, pollen viability, and/or pollen tube growth and to stimulate the synthesis of heat and shock proteins in pollen grains (Johannson and Stephenson, 1998).

Pollen germination and pollen tube growth are important components of fertilization success in fruit trees (Janıck and Moore, 1996; Tosun (Güçlü) and Koyuncu, 2007).

Few studies, however, have examined both the environmental and the genetic effects of temperature and humidity on pollen performance (Loupassaki et al., 1997).

Temperature and humidity has a clear effect on pollen tube kinetics, expressed as the time required for pollen germination and rate of tube growth. The pollen germination and pollen tube growth are the most important properties in cherry tree fertilization. Living organisms depend on water. Water regulates their biological reactions, serves as a fluid medium and stabilizes the structure of macromolecules.

Although heavy water loss from living organisms may have deleterious effects and may lead to death (Alper and Olıver, 2002; Nepı et al., 2010).
Recent years global warming effects direct pollen germination, pollination timing, and pollen tube growth and set fruitful. We think that it is effected indirectly, reducing relative humidity. Relative humidity is very important for pollen germination stage. Partial dehydration brigs the pollen into equilibrium with the environment without fatal damage to the cytoplasm (Bassani et al., 1994) however uncontrolled water loss induced by high temperature leads to the death of the pollen (Pacini, 1996; Koubouris et al., 2009). We think that humidity rate can be affected by high temperature and indirectly fruit set can be changed.
In this study we try to explain effects of humidity on pollen germination and tube growth in sweet cherries which are grown extensively in Turkey.

MATERIALS AND METHODS

'Bigarreu Gaucher', 'Celeste', 'Lambert', 'Lapins' 'Starks Gold', 'Stella', and '0900 Ziraat' cherry cultivars were used for germination tests. Flowers at balloon stage were collected from plants in early morning. The flowers were transferred to the laboratory quickly. The anthers were removed and placed in dark colored bottles to promote dehiscence at room temperature. The 'agar in plate' method was used to assign pollen germination and pollen tube growth.
Pollen grains were sown in the medium containing 0.5 agar + 15% sucrose + 5 parts per million H_3BO_3 (Boric acid) were put consist of 40%, 50%, 60%, 70%, 80%, 90% humidity cabins at 25°C. Pollen tube long at least as its diameter was considered to be 'germinated' (Tosun (Güçlü) and Koyuncu, 2007).
The percentage of germinating pollen was determined after 2h, 6h, 12h and 24h incubation period. An ocular micrometer was used to measure pollen tube lenght, under a light microscope, at a magnification 40X. Four Petri dishes were used for germination and pollen tube growth experiments. Counts were made from 4 different microscope fields (100-150 pollen grains per field) in each Petri dish. In all experiments, treatments were arranged according to randomized design.
Statistical analyses were performed with GLM models (General Linear Model) using SPSS (V.10; Statistical software, SPPS. Inc., USA). Percentage data were subject to arcsin root square transformation, and analysis of variance was performed. The differences among means were analysed by Duncan's multiple range test at the 0.05 level of significance.

RESULTS AND DISCUSSIONS

Pollen germination rates of seven sweet cherry cultivars are shown in Table 1 after 24 hours at different humidity regimes (40% RH, 50% RH, 60% RH, 70% RH, 80% RH, 90% RH).

Table 1. *In vitro* germination (%) of cherries pollen at different humidity regimes after 24 hours incubation, in a medium containing 0.5 agar + 15% sucrose + 5parts per million H_3BO_3

	Humidity regimes					
Cultivar	40%	50%	60%	70%	80%	90%
Bigarreu Gaucher	4.12a	11.23a	35.30a	54.09b	82.30b	91.69a
Celeste	1.33b	3.19c	14.29d	51.30c	72.46d	81.88b
Lambert	1.09b	2.00d	25.12c	46.76d	71.87d	83.45b
Lapins	1.25b	3.76c	12.30d	54.63b	76.56c	79.83b
Starks Gold	1.79b	6.90b	29.96b	55.88b	88.46a	70.05c
Stella	1.88b	3.01c	23.62c	64.03a	89.76a	71.92c
0900 Ziraat	1.03b	3.00c	8.87e	45.56d	74.36c	66.25d

Different letters in the same column indicate significant differences, according to Duncan's multiple range test (P<0.05).

All cultivars showed highest germination in 90% RH except for 'Bigarreu Gaucher'.'Noble' and '0900 Ziraat'. The lowest pollen germination rates were obtained from 40% RH. There is no difference statistically ($p<0.05$) except from 'Bigarreu Gaucher'.
Pollen germination rates increase with rising relative humidity. 'Bigarreu Gaucher' had the best germination rate both 50% and 60% RH. '0900 Ziraat' have had the lowest value with 8.87 germination rate at 60% RH ($p<0.05$).
The best results obtained from 80% and 90% RH. 80% was the optimum RH for 'Starks

Gold' and 'Stella' while 90% was optimum for 'Bigarreu Gaucher'.

It can be said that '0900 Ziraat' showed the lowest germination rates in general. Germination of cherry pollens reached own maximum percentage in 24 hours for all relative humidity regimes (Figure1).

Pollens of '0900 Ziraat' and 'Lapins' started latest germinated at 40% RH. Pollens of these cultivars germinated 24 hours later. 'Starks Gold' pollens germinated 2 hours later at 40% RH different from other cultivars (0.23). After 12 hours pollen germination rate in Celeste showed a dramatically increase from 13.96 to 69.98.

The pollen tube elongation is showed in Table 2 according to different humidity regimes.

Table 2. The effect of relative humidity on pollen tube growth (µm) cherry cultivars *in vitro* after 24 hours

	Humidity regimes					
Cultivar	40%	50%	60%	70%	80%	90%
Bigarreu Gaucher	17.96a	35.58a	52.29a	82.35a	116.33a	119.22a
Celeste	6.98c	16.23c	39.96c	69.73b	80.11c	89.74d
Lambert	5.11c	13.29d	26.34de	58.96c	71.23d	92.33d
Lapins	6.74c	15.71c	30.33d	63.72bc	88.9c	100.01c
Starks Gold	10.12b	21.79b	43.25b	66.12b	93.7b	110.23b
Stella	6.03c	14.1c	29.91d	56.73c	71.32d	90.83d
0900 Ziraat	4.79cd	10.11e	21.03e	30.54d	62.23e	80.09e

Different letters in the same column indicate significant differences, according to Duncan's multiple range test ($p<0.05$).

Results were found statistically significant ($p<0.05$). Pollen tube growth increased with increased humidity. All cultivars showed longest pollen tube growth at 90% relative humidity and 24 hours incubation period.

The longest pollen tubes were obtained from 'Bigarreu Gaucher' at all temperatures while the shortest were found for '0900 Ziraat'. 'Lambert' showed the lowest pollen tube elongation from 40% RH to 90% RH.

All cultivars showed different response for relative humidity. Oyiga et al. (2010), reported that pollen germination is species depend on environmental factors. Another study which was carried out in olive cultivars showed extreme temperature and relative humidity incidents, even for a short period, reduced pollen germination and growth capacity in 'Koroneiki', 'Kalamata', 'Mastoidis' and 'Amigdalolia' olive and may affect fruit set and yield (Koubourıs et al., 2009). *Petunia hybrida* and *Cucurbita pepo* pollens were exposed to 30 and 75% relative humidity (RH).

Water content, viability and carbohydrate content (glucose, fructose, sucrose and starch) were measured at fixed intervals over 6 h. Water content of *C. pepo* pollen decreased drastically at both RH, while *P. hybrida* pollen dehydrated slightly at RH 30% and hydrated at RH 75% (NEPI et al., 2010). 24 hours later pollen germination reached maximum.

These results of incubation duration experiments were closely parallel to findings of Koyuncu (2006), who reported that germination of strawberry pollens began within 1h at 24°C. Similarly, the pollen of 'Tsanoki' pear started to germinate after 1h incubation (Vasilikakis and Porlingis 1985). Koyuncu and Tosun (Güçlü) (2008) reported that the germinating rates increased with length of incubation period. Leech et al. (2002), investigated the responses of a period of up to 72 hours on pollen germination of different strawberry cultivars. The growth of the pollen tube in flowering plants is exceedingly rapid and its requirements, in general, seem quite unimpressive, i.e., water, oxygen and a suitable osmotic environment. Despite extensive attempts to hasten this growth process with the conventional host of growth factors, few have met with convincing success (Brewbaker and Kwack, 1963).

In vivo pollen germination and tube growth are highly sensitive to climatic factors, particularly, temperature and relative humidity (Sıngh at al., 2009). While temperature and other abiotic stresses are clearly limiting factors for crops cultivated on marginal lands, crop productivity everywhere is often at the mercy of random environmental fluctuations. Current speculation about global climate change is that most agricultural regions will experience more extreme environmental fluctuations (Solomon et al., 2007; Zinn et al., 2010). On our opinion climatic change can affect relative humidity, pollen germination, bee activity and fruitset.

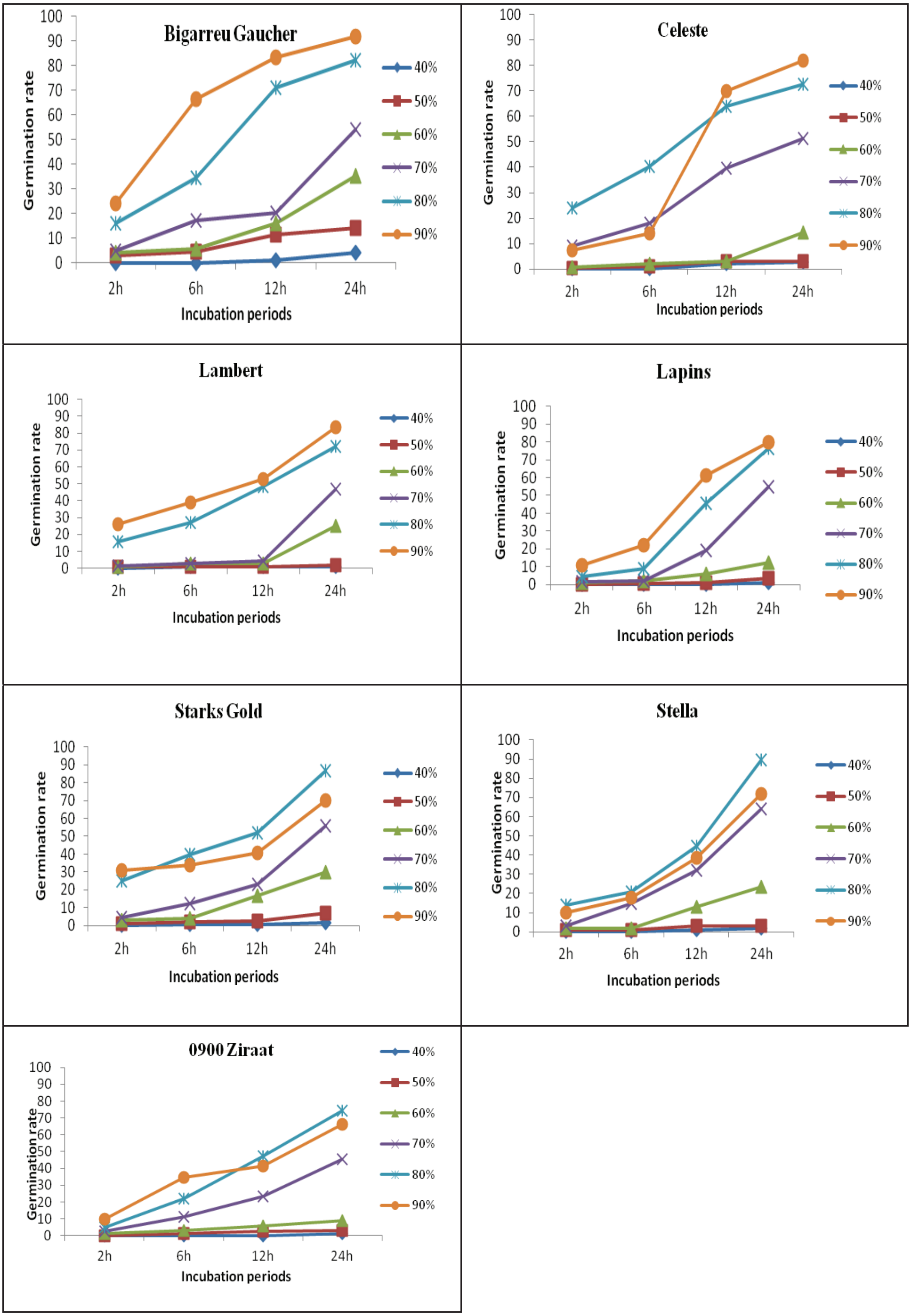

Figure 1. Germination of different cherry varieties pollens

CONCLUSIONS

Indirect effects of global warming on pollen germination and fruit set has examined in this study.

Pollen germination and pollen tube growth reached own maximum percentage at high humidity regimes.

'Starks Gold' and 'Stella' gave best resutls at 80% humidity.

90% humidity was optimum for 'Bigearreu Gaucher'.

Highest percentage was taken at 24 hours later after incubation for all cultivars.

Pollen germination and tube growth increased by incubation period.

On our opinion climatic change can affect relative humidity, pollen germination, bee activity and fruitset.

REFERENCES

Alper P., Oliver M.L(2002). Drying without dying. In: Black M, Pritchard HW (eds) Desiccation tolerance in plants. CAB International, Wallingford, pp 3–43.Assessment Report of the Intergovernmental Panel on Climate Change. New York: Cambridge University Press.

Bassanı M.,. Pacın E., Franchı G.G., 1994. Humidity stress responses in pollen of anemophilous species. Grana, 33, 146-150.

Brewbaker, J.L. and Kwack H.B 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am J Bot, 50 (9), 747-858.

Delph L., Johannson F. and Stephenson A.G., 1997. How environmental factors affect pollen performance, ecological and evolutionary perspectives. Ecology. 78, 1632-1639.

Hedly A.. Hormaza J.I and Herrero M., 2004. Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (*Rosaceae*). Am J Bot 91 (4), 558-564.

Janıck J., Moore N.J., 1996. Fruit Breeding, Tree and Tropical Fruits Vol. 1. New York, John Wiley and Sons Inc

Johannson L.F. and Stephenson A.G., 1997. How enviromental factors affect pollen performance; ecological and evolutionary perspectives. Ecology 78, 1632-1639.

Johannson H.M. and Stephenson G.A. 1998. Effect of temperature during microsporogenesis on pollen performance in *Cucurbita pepo* L. (*Cucurbitacea*). Int J Plant Scı, 159 (4), 616-626.

Koubourıs C.G., Metzıdakıs T.I. and Vasılakakıs D.M., 2009. Impact of temperature on olive (*Olea europaea* L.) pollen performance in relation to relative humidity and genotype. Envıron Exp Bot 67, 209–214.

Koyuncu F. and. Tosun (Guclu) F 2008. Evaluation of pollen viability and germinating capacity of some sweet cherry cultivars grown in Isparta, Turkey. Acta Hort.795 ISHS, (1), 71-75.

Koyuncu F., 2006. Response of *in vitro* pollen germination and pollen tube growth of strawberry cultivars to temperature. Europ J. Hor. Sci. 71(3), 125-128.

Leech L.D,, Simpson W. and Whıtehouse A.B., 2002. Effect of temperature and relative humidity on pollen germination in four strawberry cultivars. Acta Hort. 567 ISHS, 261-263

Loupassakı M., Vasılakakıs M., and Androulakıs, I. 1997. Effect of Pre-incubation Humidity and Temperature Treatment on the Germination of Avocado Pollen Grains. Euphytica, 94, 247-251.

Nepı M., Cresti. N.. Guarnıerı M. and Pacını E., 2010. Effect of relative humidity on water content, viability and carbohydrate profile of *Petunia hybrida* and *Cucurbita pepo* pollen. Plant System Evolution, 284, 57–64.

Oyiga B.C., Uguru M.I. and Aruah C.B., 2010. Pollen behaviour and fertililization impairment in Bambara groundnut (*Vigna subterrenea* [L.] Verdc.). JPBCS 2(1), 12-23.

Pacını E., 1996. Types and meaning of pollen carbonhydrate reserves. Sex Plant Reprod, 9, 362-366.

Sıngh S., Rana A. and Chauan S.V.S. 2009. Impact of environmental changes on the reproductive biology in *Pyrostegia venusta* Presl. J Environ Biol, 30 (2), 271-273.

Solomon S., Qın D, Mannıng M., Marquıs M., Averyt K., Tignor M.M.B., Mıller H.L and Cheng Z. 2007. Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth

Stephenson A., Lau T.C., Quesada M. and Wınsor J.A. 1992. Factors that influence pollen performance In: Ecology and Evolution of Plant Reproduction (R. Wyatt, ed,) pp.119-134

Tosun (Güçlü), F. and 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. HortSci(Prague), 34, (2), 47-53

Travers S.E., 1999. Environmental effects on components of pollen performance in *Faramea occidentalis* (L.) A. Rich. (*Rubiaceae*). Biotropica, 31 (1), 159-166.

Vasılakakıs M. and. Porlıngıs I.C, 1985. Effect of temperature on pollen germination, pollen tube growth, effective pollination period, and fruit set of pear. Hortsci., 20, 733-735.

Zınn E.K., Tunç-Özdemır M., and Harper F.J., 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. J Exp Bot 61 (7), 1959–1968.

EX·TERRA·AVRVM