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# Effects of Heat Stress on Pollen Viability and Pollen Tube Growth in Pepper

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**Abstract:** The effects of heat on pollen grains was studied in pepper (*Capsicum annuum* L.) under greenhouse conditions using a completely randomized design. Seeds of the California Wander variety were grown in a growth chamber and flowers plucked and placed in 38°C (treatment) and 25°C (check) for a period of 8 h and consequently pollen viability, pollen tube growth and the growth of pollen tube inside the style was studied. Results indicate that pollen viability and pollen tube growth was considerably reduced at 38°C as compared with the check. In addition, pollen tube growth inside the style at 38°C was twisted and they grew in spiral and helical forms.

**Key words:** Heat, pollen grain, pollen tube, pollen viability

## INTRODUCTION

High and low temperatures are considered as limiting factors in the distribution, adaptation and productivity of wild and domestic plants. Minor increase and decrease of temperature even for a short period has an effect on the biochemical and physiological processes of plants (Prasad, 1997). High temperatures exert its effect on the vegetative and reproductive growth, nutrient absorption, protoplasmic movement, transport of materials, photosynthesis, respiration, metabolism, flower growth, fertilization, fruit maturation and the quality of seeds (Chen *et al*., 1982; Prasad, 1997; Fowden *et al*., 1993). The formation of flower and fruit is a sensitive stage to heat and its response depends on the stage of flower growth and plant genotype. Although, different genotypes do not respond similarly to high temperatures but reports indicate the lack of viable pollen as the main factor for lack of fruit formation (Abdul-Baki, 1992; Atherton and Rudish, 1986). Although both the male and female gametophytes are sensitive to high temperatures, but, the male gametophyte shows a higher vulnerability such that under higher temperatures, pollen germination and the degree of pollen tube growth is reduced significantly (Weaver, 1989; Kakani *et al*., 2005). Comparison between pollen grains and embryo sac also indicate that the embryo sac is more resistant to heat. Hence, reduced pollen production and reduced embryo sac potential are among the major and basic factors in incomplete pollination (Abdolla and Verkerk, 1968; Shelby *et al*., 1978). As there is a direct link between plant yield and fruit tissue, therefore, research in the field of fruit growth is essential and in the meantime all steps from pollination to fruit maturity has its effect on the quality and quantity of yield (Aneja *et al*., 1992; Cerovic and Ruzic, 1992).

## MATERIALS AND METHODS

Pepper (*Capsicum annuum* L.) seeds of California Wander variety, obtained from the Karaj Research Center for Seed and Plant Improvement was planted in pots containing soil, plant compost and sand with a ratio of 2:1:1. Pots were placed in growth chamber with a photoperiod of 16 h day at 25°C and 8 h night at 20°C. Light intensity at plant height was 13000 Lux with a humidity of 60%. Following, the completion of the vegetative period, all experiments were performed at the reproductive stage. Flowers were picked daily from 8 to 10 A.M. and were divided into 2 groups. One group was placed in an incubator with a high temperature of 38°C for 8 h and the other placed in another incubator as a check with a lower temperature of 25°C for 8 h.

**Pollen viability experiment:** The Mercado *et al*. (1994) growth medium for pollen viability having 10% sucrose, 0.1 mmol boric acid and one mmol calcium chloride was used. For gelling of the medium, 1% agar was used. Pollen grains obtained from heat treated and check groups of flowers were grown in petri dishes at 25°C. Each treatment was replicated 3 times and analysis of variance was performed and means were subjected to Duncan test at 95% confidence limits.

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**Observation of pollen tubes inside the style:** Specimens of style and ovary tissues were passed through the following solutions: Fixation for 24 h in a fixative containing formalin, 80% ethanol and glacial acetic acid (1:8:1). Rinsing for 24 h with tap water. Transfer to 8N sodium hydroxide solution for 8-24 h. Rinsing for 24 h with tap water. Staining with aniline blue dye solution for 4 h (Martin, 1986).

## RESULTS AND DISCUSSION

Results obtained from this experiment indicates that pollen germination in pepper is drastically reduced when plants are grown in 38°C as compared with 25°C (Fig. 1). In addition, average length of pollen tube after 1, 2 and 3 h of their growth in the growth medium also indicates their significant difference at 38 and 25°C such that the average pollen tube length at 38°C is much less than the length when flowers were placed at 25°C (Fig. 2 and 3). There are a number of reports indicating the effects of high temperature on the male reproductive organ resulting in flower drop and yield reduction. Under heat stress, the amount of pollen grain production, pollen viability, rate of pollen tube growth, anther and pollen sac structure and the process of pollination is affected (Norman *et al*., 1997; Leah and Aloni, 2002). Temperatures above 34°C during pollen germination results in a reduction in percent germination and the amount of pollen tube growth and this damage is significant when pollen grains are obtained from flowers that have grown under high temperature conditions. It has been shown in tomato that temperatures above 28°C for 12 h in the flowering period has reduced pollen germination (Kakani *et al*., 2005). It appears that the reason for the observed reduction in percent pollen germination under heat stress is the effect of high temperature in reducing pollen moisture. Under the normal amount of Relative Humidity (RH) pollen grains are able to perform their normal metabolic activities but reduction of RH due to increased temperature dessicates the pollen grains which has an unfavorable effect on the metabolic activities and membrane integrity. In effect, high temperature results in the derangement of molecular structure of proteins and enzymes (Shelby *et al*.,1978; Yates and Sparko, 1989).

Figure 4 depicts the presence of haploid tissue inside the diploid tissue of the style. The observed haploid tissue is the pollen tube itself that can be traced with their callose plaques. These plaques which are stained with aniline blue dye are observed with their pale green and yellow crystals under ultraviolet light. Judging from the amount and distribution of these plaques along the pollen tube it is possible to detect the presence or absence of the

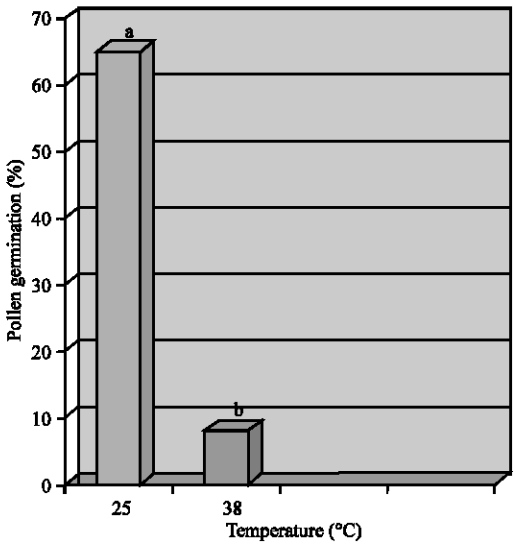


Fig. 1: Percent pollen germination for plants grown at 25°C (a) as compared with plants grown at 38°C (b)

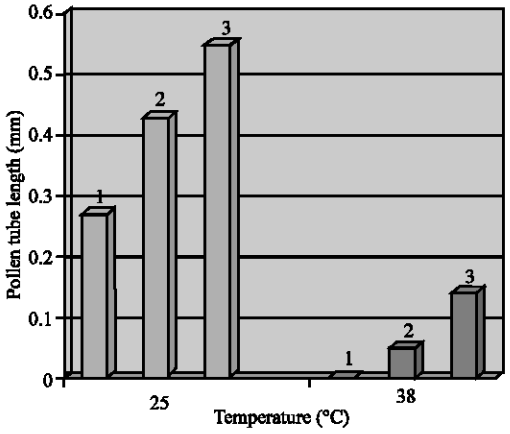


Fig. 2: Average length of pollen tube in plants grown at 25°C as compared with 38°C. Measurements were performed 1, 2 and 3 h following the placement of pollen specimens on the growth medium

pollen tubes in the stylar tissue (Smith *et al*.,1995). While, Fig. 4a shows the normal growth of pollen tubes, Fig. 4b clearly shows their abnormal growth having a twisted and contorted form. In fact the composition and cellular environment of the style exerts its specific effect on the growing process of pollen tubes. In this respect, the distribution of several ions namely calcium, potassium and magnesium varies along the length of the style and the occurred changes in the environmental temperature has its effect on this distribution. The directed growth of pollen tube inside the stylar tissue indicates the role of ATPase and calcium ions in cell membranes. In this respect, calcium has a basic role such that its absence

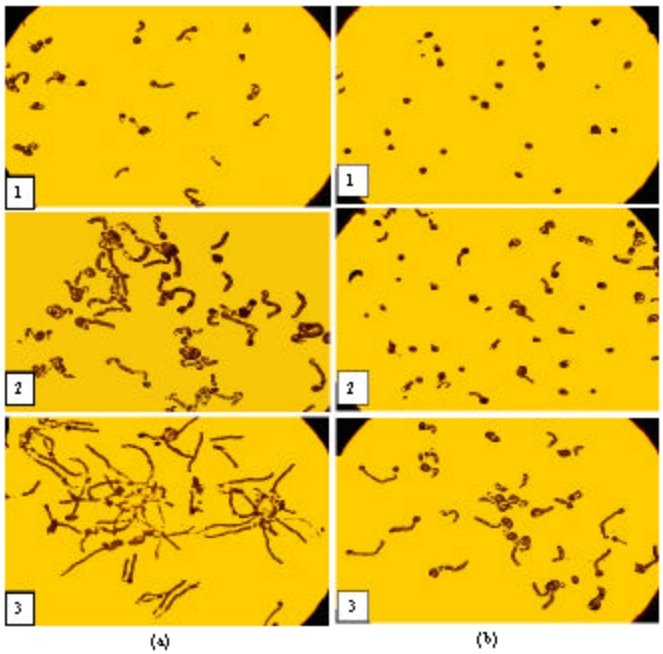


Fig. 3: Progressive pollen tube growth 1, 2 and 3 h after placement of pollen grains on Mercado growth medium. (A) Flowers kept at 25°C and (B) flowers kept at 38°C for 8 h

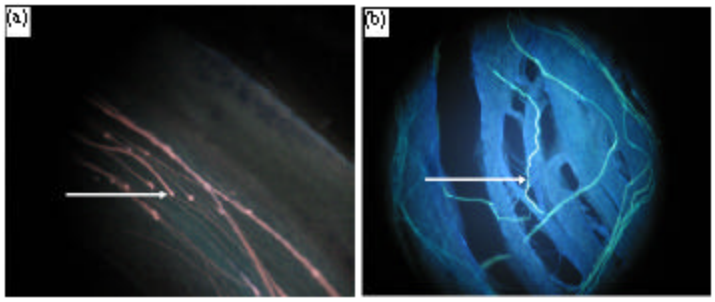


Fig. 4: Observation of the growing pollen tubes inside the style using ultraviolet light microscopy. (A) normal tube growth at 25°C and (B) Abnormally thick and wavy tube growth under heat stress of 38°C. Callose plaques are indicated by arrows

causes their abnormal and twisted growth (Fernandez-Escobar *et al*., 1983; Abdolla and Verkerk, 1968; Tangmitcharoen and Owens, 1997). Studies in cherry and sour cherry has shown that with increasing temperature, ovule longevity is reduced and the process of aging is enhanced. As the normal growth of pollen tubes inside the stylar tissue requires the receipt of signals from the ovule or embryo sac, older or dead ovules would cause a change in these signals and make the pollen tubes to grow abnormally in the style and would have difficulty in finding its direction towards the ovary and the ovule (Cerovic and Ruzic, 1992; Muleahy, 1985).

## REFERENCES

Abdolla, A.A. and K. Verkerk, 1968. Growth, flowering and fruit set of the tomato at high temperature. Neth. J. Agric. Sci., 16: 71-76.

Abdul-Baki, A.A., 1992. Determination of pollen viability in tomatoes. J. Am. Hort. Sci., 117: 473-489.

Aneja, M., T. Gian Fagva, E. Ng and I. Badilla. 1992. Carbon dioxide and temperature influence on pollen germination and fruit set in cocoa. Hort. Sci., 27: 1038-1040.

Atherton, J.C. and J. Rudish, 1986. The tomato crop. Chapman and Hall, pp: 67-170 and 201-215.

Cerovic, R. and D. Ruzic, 1992. Pollen tube growth in sour cherry (*Prunus cerasuo* L.) at different temperatures. Hort. Sci., 62 (3): 333-340.

Chen, H.H., Z.Y. Shen and P.H. Li, 1982. Adaptability of crop plants to high temperature stress. Crop Sci., 22: 719-725.

Fernandez-Escobar, R., G. Gamez-Valledor and L. Rallo, 1983. Influence of pistil extract and temperature on in vitro pollen germination and pollen tube growth of olive cultivars. J. Hort. Sci., 58: 216-227.

Fowden, L., T. Manstield and J. Stoddart, 1993. Plant adaptation to environmental stress. Landan, pp: 109-132.

Kakani, V.G., R.K. Reddy, S. Koti, T.P. Wallace, P.V.V. Prasad, V.R. Reddy and D. Zhao, 2005. Differences in *in vitro* pollen germination and pollen tube growth of cotton cultivars in response to high temperatures. Ann. Bot., 96: 607-612.

Leah, K. and B. Aloni, 2002. Fructokinase and hexokinase from pollen grains of bell pepper (*Capsicum annuum* L.): possible role in pollen germination under conditions of high temperature and $CO_2$ enrichment. Ann. Bot., 90: 607-612.

Martin, F.W., 1986. Staining and observation of pollen tubes in the style by means of fluorescence. Stain Technol., 34: 125-127.

Mercado, J.A., R. Fernandez-Munoz and M.A. Quesada, 1994. *In vitro* germination of pepper pollen in liquid medium. Scientia Horticul., 57: 273-281.

Muleahy, G.L., 1985. Ovarian influence on pollen tube growth. Am. J. Bot., 72: 1078-1080.

Norman, J., S.J. Weller and A.K. Sak, 1997. Pollination biology outcrossing rates in hermaphroditic schiedealydgatei (caryophylaceae). Am. J. Bot., 85 (5): 641-648.

Prasad, M.N.V., 1997. Plant ecophysiology. John Wiley and Sons Inc., pp: 101-126.

Shelby, R.A., W.H. Greenleah and C. Peterson, 1978. Comparative fertility in heat tolerant and heat sensitive tomatoes. J. Am. Hort. Sci., 103: 778-780.

Smith, W.H.M., J.K. Jones, A.R. Sebastia and W. Pillai, 1995. Pollen storage of Fragaria and Potentilla. Euphytica, 41: 65-69.

Tangmitcharoen, S. and J.N. Owens, 1997. Floral biology, pollination, pistil receptivity and pollen tube growth of teak. Ann. Bot., 79: 227-241.

Weaver, M.L., 1989. Screening tomato for high temperature tolerance through pollen viability tests. Hort. Sci., 24: 493-495.

Yates, I.E. and D. Sparko, 1989. Hydration and temperature influence in *in vitro* germination of pecan pollen. J. Am. Soc. Hort. Sci., 114: 599-605.