Journal of Life Science 2009 Vol. 19. No. 5. 581~586 ©JLS / ISSN 1225-9918

Effect of Low Night Temperature on Reproductive Organ Development in Relation to Pollen Viability of Bell Pepper

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Received April 17, 2009 / Accepted May 6, 2009

Bell pepper plants (*Capsicum annuum* cv. Plenty) were grown under low night temperatures (LNT: day/night temperature of 28/13°C) and optimum night temperatures (ONT: day/night temperature of 28/20°C) in growth chambers. Pollen grains were collected from plants in the growth chamber and incubated at 10, 15, 20, 25, and 30°C. After 24 hr incubation, in both ONT and LNT, the highest percent pollen germinations were observed at 25°C followed by germinations at 30°C. Percent pollen germination at 25°C was 42% in ONT - two times higher than in LNT at 21%. Pollen tube length was much longer at ONT than at LNT, regardless of incubation temperature. Compared with other treatments, earlier and quicker pollen tube elongation was observed in ONT pollen grains incubated at 25°C. To find pollen viability in plant growing conditions, pollen grains were incubated in LNT (28/13°C) and ONT (28/20°C) growth chambers for 24 hr. Petri-dishes with pollen grains were put in the growth chambers at the beginning of the night condition. Pollen grains in the LNT growth chamber did not germinate at night (13°C), but began to germinate when the day condition (28°C) started. Pollen grains in the ONT condition, however, started germinating from the early night (20°C) and germination continued during the day (28°C). Plants in LNT showed increased flower stalk length, ovary diameter, stamen length, flower weight, and fruit length. LNT conditions did not impair seed set. There were no differences in seed sets between fruits at LNT and ONT. Normal seed sets in LNT show that fertilization may be completed during daytime. However, further investigation is needed to find what extent of temperature stress causes malformed and/or parthenocarpic fruits in this bell pepper.

Key words : *Capsicum annuum*, pollen germination, pollen tube length, seed set

Introduction

Paprika (*Capsicum annuum* L.) cultivation area in Korea has been increased as one of the profitable crops since first introduction from The Netherlands in 1996. Almost paprika fruits produced in Korea are exported to Japan and take 70% of Japanese paprika market. Japanese consumers prefer good shaped and middle sized fruits but malformed Korean paprika fruits in the Japanese market lose consumers preference resulting in decrease in the growers' income.

Temperature has a considerable effect on flower morphology and function, development of pollen, which in turn can impinge on fruit set and seed development [6,14]. Bell pepper is grown in two different types in Korea as highland cultivation from March to November and lowland cultivation from September to July. From highland cultivation in Hamyang (600 m of altitude) and Hapcheon (800 m of altitude) regions, pumpkin-like malformed fruits are one of the main problems at the period of first harvest in May (average air temp. 12°C), but not from the lowland cultivation at the period of first harvest in November (average temp. 17°C). This implies the low temperature condition during May have triggered the increase in malformed fruits in the highland cultivation compared with the lowland cultivation.

Low temperature causes reduction in both the number pollen grain and the pollen viability [11]. Larger flowers, more swollen ovaries, and shorter styles were observed under lower temperature [10]. Non-extreme temperature condition (18°C day and 15°C night) did not hinder fruit set, but diminished pollination causes small, flattened, and parthenocarpic fruits [13]. However, sensitivity of pollination and fruit shape of bell pepper to low temperature are cultivar-dependent. Some cultivar of bell pepper in low night temperature (10°C) showed normal fruit shape and similar number of seeds to bell pepper in normal night temperature (20°C) [14].

It is not clear to what extent of night temperature regime and of exposure duration to low night temperature will af-

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fect seed set in bell pepper. It is important to find out the optimum temperatures for pollen viability and relationship between pollen viability and fruit characteristic. This study was conducted to determine the influence of low night temperature during flowering initiation on pollen viability and the characteristics of flower and fruit.

Materials and Methods

Plant materials

Twelve paprika (cv. Plenty) plants were used for each treatment. Uniform sized seedlings of 50 days old after sowing were selected and transplanted to rockwool slabs in a growth chamber (GC-1000TLH, Jeio Tech, Korea). Nutrition solution was irrigated 5 times per day and each time 120 ml per plant to ensure 20% draining of total amount of irrigation. Day/night temperatures for low night temperature (LNT) and optimum night temperature (ONT) treatments were 28/13°C and 28/20°C, respectively. Light condition in the growth chamber was 80,000 lux at day and dark at night. Plants were trained to a double stem.

Pollen viability

Germinating medium for pollen grains was made with a slight modification of Pressman's methods [11]. Germinating medium solution consisted of 20% sucrose ($C_{12}H_{22}O_{11}$), 2mM boric acid (H_3BO_3), 2mM calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$], 2 mM magnesium sulfate ($MgSO_4$), and 1 mM potassium nitrate (KNO_3). Ten percent of agar was added to the solution and kept at 80°C hot bath for 30 min. After completing dissolution of agar, 5 ml of germinating medium was filled into 10 ml Petri dishes and cooled for 30 min to solidify the agar. The plant of thirty-day old after transplanting was used for this experiment. Five fully-opened flowers from 5 plants of each OLT and NLT were tapped with a finger to collect pollen grains into a Petri dish with germinating media and three Petri dishes were used for replication. Five fields in each Petri dish were measured.

For examining the pollen viability responding to temperature, the Petri dishes with pollen grains were incubated at 10, 15, 20, 25, and 30°C for 24 hr in the dark.

For studying pollen germinability under field condition, the Petri dishes with pollen grains were kept at the LNT (day/night, 28/13°C) and ONT (day/night, 28/20°C) growth chambers for 24 hr.

Germination and tube length of pollen grains were measured under a light microscope (Eclipse E200, Nikon Instech Co. Ltd., Kanagawa, Japan). Germination was considered when pollen tube length was twice longer than the grain diameter. Percentage pollen germination was calculated by counting the total number of pollen grains. For each replication, fifty pollen grains were used for pollen tube length measurement and the tube length was measured with captured photos by an imaging solution program (i-Solution Lite, IMT i-Solution Inc., Vancouver, Canada).

Flowers and fruits characteristic

The effect of the temperature regime on morphology of flowers and fruits were recorded. Flower stalk length, flower diameter, ovary diameter, flower weight, number of petal, number of stamen, stamen length, anther length, and style length were measured for flower characteristic when flowers were fully opened. Fruit stalk diameter, fruit diameter, fruit length, fruit weight, and calyx diameter were measured for fruit characteristic with 14-day-fruits after fruit set. Thirty flowers and 30 fruits were used for this experiment.

Results and Discussion

Pollen grains from flowers in growth chambers were incubated for 24 hr at 10, 15, 20, 25, and 30°C. Percent pollen germination was higher in ONT than LNT, regardless of incubation temperature (Fig. 1). In both ONT and LNT, the highest percent pollen germinations were observed at 25°C followed by at 30°C after 24 hr incubation. Compared with pollen in LNT, earlier pollen germination was observed in ONT throughout all incubation temperatures. Duration of plant exposure to high/low temperature stress may affect pollen viability at optimum temperature. Herrero and

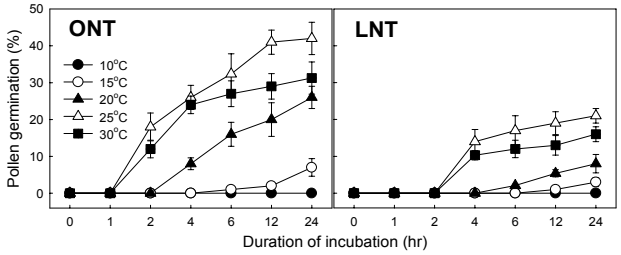

Fig. 1. Effect of temperatures on pollen germination of bell pepper *in vitro*. Bell pepper plants (cv. Plenty) were grown under ONT (day/night, 28/20°C) and LNT (day/night, 28/13°C). Collected pollen grains from flowers in a growth chamber were incubated for 24 hr at 10, 15, 20, 25, and 30°C. Vertical bars represent SE (n=15).

Johnson [4] found that prolonged temperature stress during growth of Maize completely inhibited pollen germination in vitro. In this experiment, when plants were grown at LNT and ONT condition during pollen development, percent pollen germination in vitro was much lower at LNT than at ONT. For instance, percent pollen germination in vitro at 25°C was twice higher in ONT with 42%, compared with that in LNT with 21%. Pepper pollen was sensitive to low temperature stress. Both ONT and LNT pollen grains in vitro did not germinate at 10°C and only 10% germination was observed after 24-hr incubation at 15°C (Fig. 1). Pollen grains may be impaired their functioning and completely lose their viability below 10°C.

Pollen tube lengths were much longer in ONT than LNT, regardless of incubation temperatures (Fig. 2). In both ONT and LNT, earlier and quicker pollen tube growth was shown at higher incubation temperature. Pollen tube growth speed was quicker in pollen grains from ONT than from LNT throughout all incubation temperatures. Pollen tube growth at optimum temperature appeared to reach about more than 80% of their final length in first few hrs after beginning of germination. For instance, ONT pollen tube at 25°C elongated 705 μm in 1 hr after germination and it was 82% of 24 hr total pollen tube length. Pollen tube lengths of pepper were significantly different by species and genotypes in response to temperature and a mean of maximum pollen tube length of 7 pepper cultivars was 737 μm [12]. In this study, pollen tube length of 'Plenty' bell pepper was 850 μm after 24 hr incubation at 25°C.

Compared with ONT pollen grains, lower tube length and percent germination in LNT pollen may be attributed to more decreased contents of carbohydrates and soluble sugar in the mature pollen grains by low temperature. Carbohydrates are the most important nutrient for pollen development [8] and a deficiency in carbohydrates in tomato showed abnormal pollen development [3]. Pollen uses sugars during germination as principal metabolic substances [15] and starch and sugar accumulations in pollen grains were decreased under LNT condition [14].

Pollen grains from LNT and ONT were kept at the same plant growing conditions to compare pollen germinability in a field-like condition. During warmer night (20°C), percent germination of pollen grains in ONT increased steadily from 4 hr after incubation with 7% and it was 20% after 12 hr-incubation (Fig. 3). After day condition treatment with 28°C, pollen germination increased gradually till 18 hr-incubation and afterward remained constant in ONT. Pollen grain in LNT, however, did not germinate during 12 hr colder night condition (13°C) but afterward pollen grain began to germinate immediately after treatment of day condition with 28°C. Pollen grains produced in ONT condition showed much higher pollen germination than those in LNT.

There were no differences in flower diameter and petal number between ONT and LNT (Table 1). Flower stalk length, ovary diameter, flower weight and stamen length were higher in LNT than in ONT, whereas number of stamen and style length was lower in LNT than in ONT. Polwick and Sawhney [10] observed that low temperature condition caused larger ovary in diameter and longer style

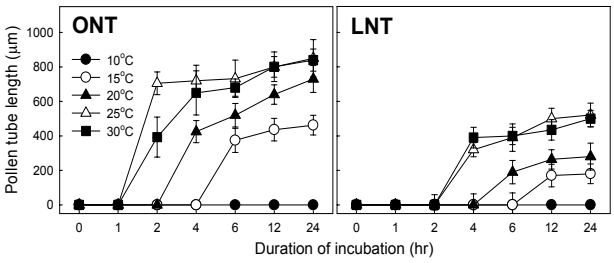

Fig. 2. Effect of temperatures on pollen tube length of bell pepper *in vitro*. Bell pepper plants (cv Plenty) were grown under optimum night temperature (ONT: day/night, 28/20°C) and low night temperature (LNT: day/night, 28/13°C) conditions. Collected pollen grains from flowers in a growth chamber were incubated for 24 hr at 10, 15, 20, 25, and 30°C. Vertical bars represent SE (n=15).

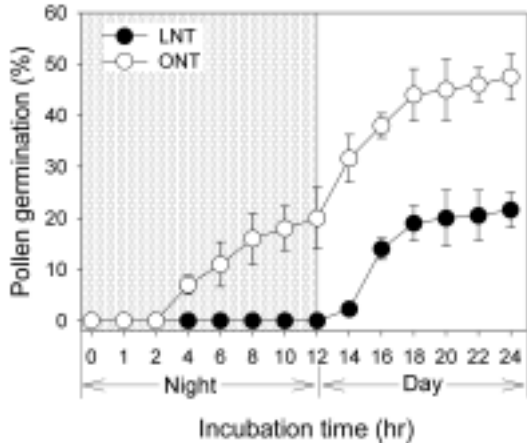

Fig. 3. Pollen germination of bell pepper in field-like conditions. Bell pepper plants (cv Plenty) were grown under optimum night temperature (ONT: day/night, 28/20°C) and low night temperature (LNT: day/night, 28/13°C) conditions. Collected pollen grains from flowers in a growth chamber were incubated for 24 hr at the ONT and LNT condition. Vertical bars represent SE (n=15).

Table 1. The effect of low night temperature (LNT: day/night, 28/13°C) on the flower features in bell pepper 'Plenty', compared with optimum night temperature (ONT: day/night, 28/20°C). Flowers at anthesis were used for the measurement.

Parameter	ONT	LNT
Flower stalk length (mm)	10.1±0.13	11.5±0.09
Flower diameter (mm)	22.8±0.22	23.2±0.30
Ovary diameter (mm)	5.4±0.08	6.1±0.06
Flower weight (g)	0.5±0.01	0.6±0.01
Number of petal	6.2±0.10	6.2±0.10
Number of stamen	6.5±0.07	6.2±0.14
Stamen length (mm)	2.9±0.02	3.1±0.04
Anther length (mm)	0.9±0.01	0.8±0.02
Style length (mm)	3.3±0.17	2.7±0.12

in length, while we found that style length was shorter in LNT than in ONT. As Myster and Moe [7] found greater day/night temperature differentials contributed to increased stem elongation, we observed flower stalk and stamen length were more increased in LNT than in ONT.

Fruits stalk diameter was a little bigger in ONT than in LNT and longer fruit length was observed in LNT (Table 2). There were no differences in fruit characteristics between treatments.

LNT (24°C day/10°C night) condition decreased pollen functioning in pepper by decreasing the concentration of soluble sugar in mature pollen grains and resulted in smaller, seedless, and misshapen fruits [14]. Interestingly, these authors found that cv. Devilla had normal seed set and fruits under LNT condition. We also found that fruits of both ONT and LNT plants (cv. Plenty) grown in the chamber had normal seed set and there were no differences in number of seed between treatments (Table 2 and Fig. 4). Even if, no germination was observed at 10°C *in vitro* (Fig. 1), this normal seed set at 13°C night in the chamber indicates that pollination was possibly done during day time (Fig. 3). After 2-hr incubation, pollen tube growth *in vitro* was 85% at 25°C and 77% at 30°C (Fig. 2). Increased temperature from 13°C to 28°C triggered immediate germination of pollen grain in LNT condition (Fig. 3). These results imply that fertilization *in vivo* may be completed in a few hrs after pollen tube formation on a stigma under optimum temperature. In the field condition, pollen tube elongated to beneath the junction of the bract within 7 hr after pollination [5]. In maize, pollen tubes elongated a style very quickly by tip extension within the intercellular matrix and pollen tubes grew about 1 cm/hr [2].

Table 2. The effect of low night temperature (LNT: day/night, 28/13°C) on the fruit features in bell pepper 'Plenty', compared with optimum night temperature (OT day/night, 28/20°C). Fruits 2 weeks after anthesis were used for the measurement.

Parameter	ONT	LNT
Fruit stalk diameter (mm)	28.7±0.02	26.6±0.11
Fruit diameter (mm)	25.4±0.25	26.4±0.30
Fruit length (mm)	31.5±0.84	34.4±0.13
Fruit weight (g)	12.2±0.28	12.9±0.12
Calyx diameter (mm)	19.4±0.23	21.0±0.41
Locule per fruit	3.5±0.14	3.4±0.12
Seed per fruit	163.0±10.60	150.7±4.98

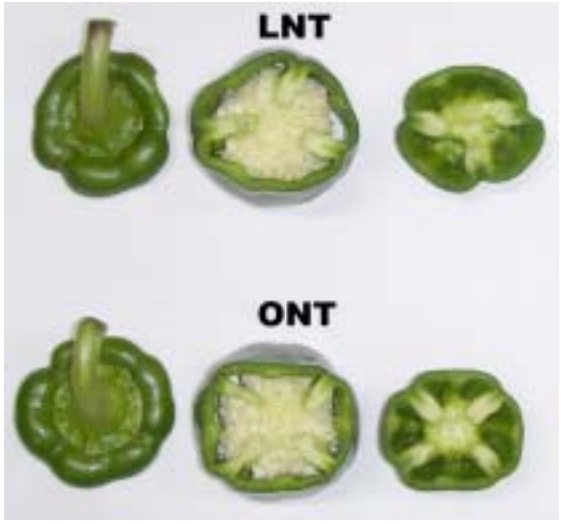

Fig. 4. Seed development of bell pepper (cv. Plenty) in response to growing temperatures. Plant (cv. Plenty) were grown under optimum night temperature (ONT: day/night, 28/20°C) and low night temperature (LNT: day/night, 28/13°C) conditions. Fruits 2 weeks after anthesis were used for the measurement.

Another possible explanation for no differences in seed set between ONT and LNT fruits in the present study is that day and night temperatures do not influence bell pepper seed set independently. It was reported that plants are sensitive to mean temperature and/or accumulated diurnal heat units [9]. There may be a threshold of mean temperature and/or accumulated diurnal heat units to hinder pollination of pepper in the present study.

In conclusion, LNT condition hindered pollen germinability and tube growth *in vitro*, regardless of incubation temperature. In both LNT and ONT, earlier and better pollen germination was observed at 25°C and higher pollen tube elongation was found at range of 25~30°C. Plant in LNT

showed increased flower stalk length, ovary diameter, stamen length, flower weight, and fruit length. Duration for completing fertilization of bell pepper in the field condition may be less than 8 hr (day time), because pollen tube in vitro elongated to almost their potential length in the first few hrs after incubation and LNT condition did not impair seed set. These results possibly indicate that continuous low temperature condition throughout day and night may have caused the fruit-malformation in the highland cultivation in Korea.

Acknowledgments

I gratefully acknowledge the financial support provided by Inst. of Agri. & Life. Sci. GNU, Rural Development Administration, and Agricultural R&D Promotion Center in Korea.

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초록 : 야간 저온조건이 파프리카 화분 활력 및 생식기관 발달에 미치는 영향

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고랭지 파프리카 재배 초기에 기형과 및 단위결과 과실이 많이 발생하는데 야간 저온이 화분 활력 및 생식기관 발달에 미치는 영향을 알아보고자 본 연구를 수행하였다. 주간 28℃ 동일 조건에서 야간온도를 13℃ (LNT)와 20℃ (ONT)로 달리 처리하여 생장상에서 파프리카(*Capsicum annuum* cv. Plenty)를 재배하였다. ONT와 LNT에서 화분을 채취하여 10, 15, 20, 25, 30℃에서 발아시켰다. 24시간 후 화분의 활력을 살펴 본 결과, 처리에 관계 없이25℃에서 화분 활력이 가장 좋았고 다음이 30℃였다. 25℃에서 발아율은 ONT화분이 42%, LNT 화분이 21%였다. 화분관 신장길이는 화분발아 온도에 관계 없이 ONT화분이 가장 길었다. 화분관 신장 속도는 25℃ 에서 발아시킨ONT화분이 가장 빨랐다. 파프리카가 재배된 ONT와 LNT 조건에서 화분 활력의 차이를 알아보기 위해 배지에 화분을 치상 후 ONT와 LNT에서 화분발아 시험을 실시하였다. LNT에서는 야간(13℃) 동안 화분이 발아되지 않았지만 주간(28℃)조건으로 전환되자 화분이 발아되기 시작하였다. 하지만 ONT에서는 야간(20℃) 초기부터 화분이 발아되기 시작하여 주간(28℃)까지 계속 발아가 진행되었다. LNT에서는 과병길이, 자방지름, 수술길이, 화중 및 과중이 증가하였다. 종자수는 처리간 차이가 없었다. LNT처리구에서도 종자가 잘 형성되었던 것은 주간에 수분수정이 이루어졌기 때문인 것으로 보여지지만 기형과나 단위결과 발생 방지를 위해서는 화분발아를 저해하는 저온범위에 대한 정밀한 연구가 수행되어야 할 것으로 보여진다.