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Approaches to Evaluate the Abortion of Hot Pepper Floral Structures Induced by Low Night Temperature

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Summary

The abortion of buds and flower buds induced by low night temperature stress is an important constraint to the production of pepper (*Capsicum annuum* L.) grown in unheated greenhouses during winter. The abortion of floral structures was investigated in two local hot pepper varieties ('Beldi' and 'Baklouti') grown in a growth chamber at a day/night temperature regime of either low night temperature (25/10 °C, LNT) or optimum night temperature (25/20 °C, ONT as a control). The negative effect of LNT on the differentiation of floral structures, expressed by the percentage abortion, was recorded for both varieties. LNT affected pepper floral structures early: buds and flower bud stages were more sensitive than flowers at the anthesis stage. The percentage abortion of these structures was less pronounced in 'Beldi' (41.5 %) than in 'Baklouti' (49.6 %) after 5 d of the treatment.

Key words. *Capsicum annuum* – abortion – flower buds – low temperature regime

Introduction

In Tunisia, hot peppers (*Capsicum annuum* L.), which are culinary highly appreciated, are grown in unheated greenhouses for early harvest and are frequently exposed to low night temperature, which often prevails during winter. These conditions have a considerable negative effect on pepper flower development and limit crop yield. Flower retention and fruit set are highly sensitive to environmental factors, particularly temperature, in many species (Van Doorn and Stead 1997).

Low temperature stress is one of the most significant abiotic stresses affecting plant growth, flowering and yield of many cold-sensitive vegetable species such as pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.) and melon (*Cucumis melo* L.) (Hansen et al. 1994; Tarchoun et al. 2003; Schwarz et al. 2010). Cold stress during the reproductive phase causes structural and functional abnormalities in reproductive organs, leading to failure of fertilization or premature abortion of flower buds or fruits. During reproductive development low temperatures induce flower abortion, pollen sterility, pollen tube distortion, ovule abortion and reduces fruit set (Thakur et al. 2010).

Several studies have indicated that abortion of plant reproductive organs depends on the stage of differentiation and on the type of stress. Under 60 % shading nets, Wien et al. (1989) noted that open flowers of sweet pepper (also *Capsicum annuum*) were the organs most susceptible to abortion, while Aloni et al. (1991b), applying heat stress to sweet pepper, with a minimum of 28 °C, concluded that immature flower buds were more susceptible to abortion than the anthesis stages. Applying shade (60 %) and heat stress (> 30 °C) at different stages of flower differentiation, Marcelis et al. (2004) noted that sweet pepper flowers were susceptible to abortion a few days before anthesis.

The effects of low temperature on the reproductive stages of various other plant species, including rice (Mamun et al. 2006), barley (Koike et al. 2003), and chickpea (Nayyar et al. 2005a), have been reported. From the viewpoint of a plant's physiological response to low temperature, it would be helpful to ascertain how low temperature affects plant reproductive stages, to identify the stage(s) in which the reproductive organ is most sensitive to low temperature, and to delineate the commonalities or differences in sensitivity among crop species (Ohnishi et al. 2010).

Investigations on the effect of low night temperature on the abortion of vegetable floral structures are scarce. The objective of this study was to determine whether hot pepper flowers would be adversely affected by low night temperature (LNT) during different stages of development.

Materials and Methods

Plant material and growth conditions

Seeds of two local hot pepper varieties ('Beldi' and 'Baklouti'), obtained from INRAT, Tunisia, were sown in alveolated trays in a soil-less medium containing fertilized peat (NPK, 12-14-24 %) and allowed to germinate in a growth chamber at 25 ± 2 °C. Seedlings at the 6–8 leaf stage were transplanted into 0.5-L pots, one plant per pot, containing the same substrate and were grown in a greenhouse in day/night temperatures of 25/20 ± 2 °C, under a natural photoperiod. Plants were watered when needed and fertigated weekly with fertilizer (NPK, 22-5-11 %) at 1 g L^{-1}. Plants at the first-node stage were repotted into 12-L pots, one plant per pot, containing fertilized peat (NPK, 12-14-24 %) with a ~ 2 cm layer of clay (Argex 4/10) and maintained until 8–10 reproductive nodes developed. For all plants, buds, open flowers and fruitlets were pinched prior to transfer to a growth chamber with day/night temperatures of either a LNT regime of 25 °C/10 °C or an optimum night temperature (ONT) regime of 25 °C/20 °C; these temperature regimes were chosen according to previous reports showing the limit of pepper flower tolerance to LNT and the optimal temperature for flower development (Mercado et al. 1997; Tarchoun 2003). Plants were grown in a 16-h photoperiod with 250 ± 5 µmol m^{-2} s^{-1} photosynthetic active radiation (Mercado et al. 1997). The relative humidity was maintained at about 70 ± 5 %. Ten plants per variety were randomly placed in each chamber. After the temperature treatment started, plants were watered daily and fertilized bi-weekly with the same fertilize (NPK, 22-5-11 %) at 2 g L^{-1}.

Sensitivity of developmental stage of flower structures to LNT

The sensitivity of floral structures to LNT was evaluated by transferring 5 plants, having 8 to 10 bifurcations, of each variety from an ONT to an LNT and back, with at least 7 d at any temperature regime according to a previous study (Tarchoun 2003).

Four developmental stages of floral structures were considered based on the size (length) and morphology of flowers: stage A (bud); stage B (buds 2.5–3.5 mm in length with green petals fused to sepals, 4–5 d before anthesis); stage C (buds 4–6 mm in length with white petals lightly fused to sepals, 1–2 d before anthesis); stage D (anthesis) (Fig. 1).

A minimum of 10 floral structures from each of the five plants for each temperature treatment were tagged at each developmental stage before transferring to LNT or to ONT. Each treatment was repeated five times.

The sensitivity of floral structures was estimated after plants were transferred to LNT or ONT by evaluating the percentage abortion of flower structures that developed at different stages.

For the control, five plants were maintained at constant ONT. Plants were re-randomized weekly within chambers to reduce variation in temperature and irradiation caused by chamber effects.

Statistics

The experiment was arranged as a randomized split-split-plot design with temperature regime as the main plot factor and varieties as the second factor (sub-plot). The developmental stages of floral structures represent the smallest experimental unit (sub-sub-plot). Data analysis was performed using SAS v. 6.0; One-way analysis of variance (ANOVA) was used to separate the means which were compared by Duncan's multiple range test (DMRT) at P = 0.05.

Results and Discussion

Sensitive buds stages to low temperature

Fig. 2 shows the development of buds to flower buds for 'Beldi' and 'Baklouti' plants transferred from ONT to LNT and back within 7 d. The effect of temperature was significant from the third day of treatment and stabilized on the fifth day for both temperatures regimes and varieties. The transfer from ONT to LNT caused an increase in bud abortion, especially on 'Baklouti'; 50 % of buds aborted after three days and this value reached 82 % and 88 % by the fifth day for 'Beldi' and 'Baklouti', respectively (Fig. 2 A). The reciprocal transfer (i.e. from LNT to ONT) shows a significantly decrease in bud abortion that

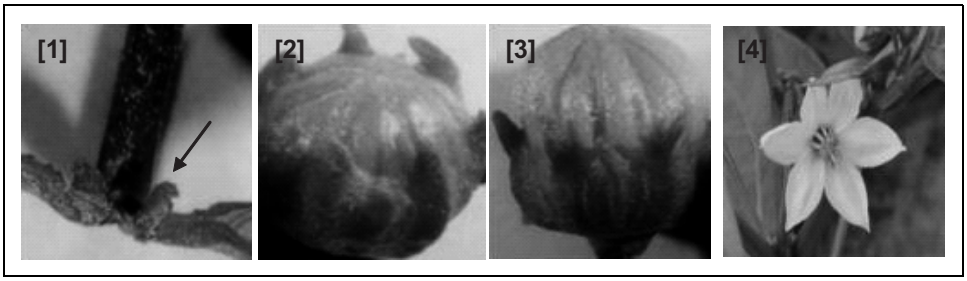


Fig. 1. Floral growth stages based on dimensional and morphological criteria: bud (stage A) [1], flower bud (stage B) [2], flower bud (stage C) [3] and flower at anthesis (stage D) [4]. Arrow indicates flower bud. N = 50.

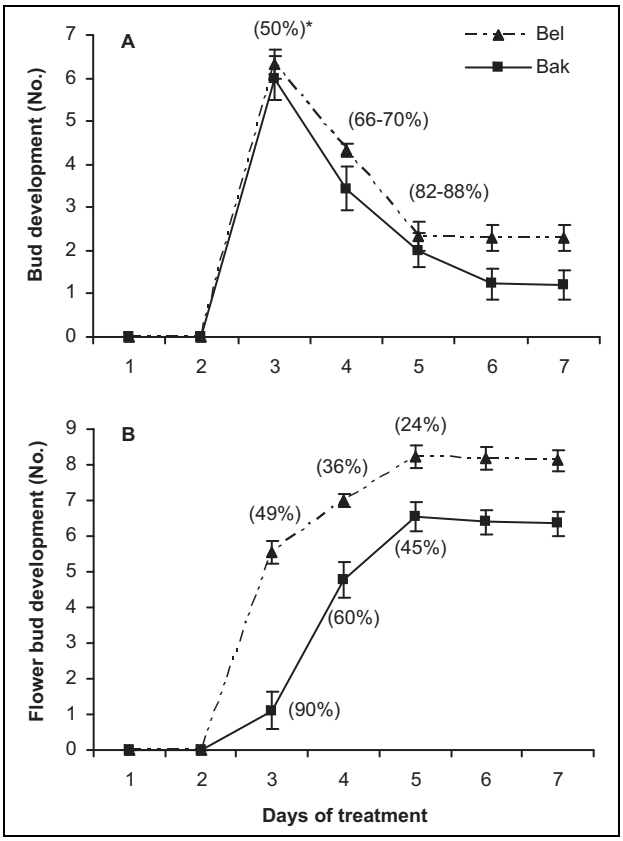


Fig. 2. Development evolution of buds to flower buds on 'Beldi' (Bel) and 'Baklouti' (Bak) varieties following plant transfer from 25/20 °C to 25/10 °C (A) and back (B). * Numbers in brackets represent the percentage of bud abortion after 3, 4 and 5 days of treatment. N = 50.

depended on the variety. From the third day to the fifth day of treatment the percentage abortion of buds decreased to 24 % from 49 % for 'Beldi' and to 45 % from 90 % for 'Baklouti' (Fig. 2 B).

Sensitive flower buds stages to low temperature

The development of flower buds of plants transferred from an ONT to an LNT regime showed an increase in abortion 3 d after treatment, 56 and 69 % for 'Beldi' and 'Baklouti', respectively. Abortion was more pronounced on the fifth day of treatment and reached 86 % for 'Beldi' and 91 % for 'Baklouti' (Fig. 3 A). Although at the end of the treatment period, flower buds at stage C aborted less than those at stage B, both varieties reacted differently. The flower buds at stage C seem to be more tolerant to LNT than those at stage B (Fig. 3 B). These results are in agreement with those of Ohnishi et al. (2010), who noted a decrease in pod set and fertilization in soybean plants when flowers were exposed to low temperature (15/10 °C, day/night) about 12.5 d before anthesis (i.e., early flower development) and 3–4 d before anthesis; low temperature sensitivity after anthesis was not detected.

The reciprocal transfer (from 25/10 °C to 25/20 °C) appeared to stabilize abortion at the fifth day of treatment as well as for the different stages of flower buds with only a slight difference between both varieties (Fig. 3 C and 3 D).

Analysis of the sensitivity of floral structures after 3 and 5 d of treatment shows that plants grown under ONT showed the lowest percentage abortion after either 3 or 5 d of treatment while the highest percentage was recorded for plants transferred from 25/20 °C to 25/10 °C (Table 1; Fig. 4 and 5).

After 3 d of treatment, transfer to LNT enhanced the abortion of floral structures from 33.3 to 47.0 %, within the range of values of flowers at a constant temperature regime. However, the transfer of plants from LNT to ONT decreased abortion by 5 % during the same period. These results suggest an early effect of LNT on pepper floral structures. A similar response was reported by Aloni et al. (1991b), who observed that the development of buds was affected by high temperatures (> 30 °C) during the first 6 h post treatment while growth completely ceased after 24 h of heat stress. Studying the negative effect of high temperature on bell pepper flower development, Erickson and Markhart (2002) noted two sensitive stages of these flowers: a) when exposed to high temperature (33 °C) for 48 or 120 h and b) a decrease in fruit set when flowers were exposed during early and late flower development. In this experiment, an increase (up to 74.5 %) in abortion was observed after 5 d of LNT treatment, while the inverse treatment, i.e., transfer to ONT, reduced abortion to 40 % (Table 1). The sensitivity of floral structures to LNT

Table 1. The average effect of temperature on pepper floral structures sensitivity expressed by the abortion percent after 3 and 5 days of the treatment, constant 25/20 °C, transfer from 25/20 °C to 25/10 °C (ONT to LNT) and inversely (LNT to ONT).

Treatment	3 days	5 days
Constant 25/20 °C	13.9 ± 3.94 b*	25.9 ± 2.31 c*
Transfer from 25/20 °C to 25/10 °C	47.2 ± 5.91 a	74.5 ± 6.73 a
Transfer from 25/10 °C to 25/20 °C	42.2 ± 4.83 a	33.9 ± 5.62 b

* Means in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to DMRT. N = 50.

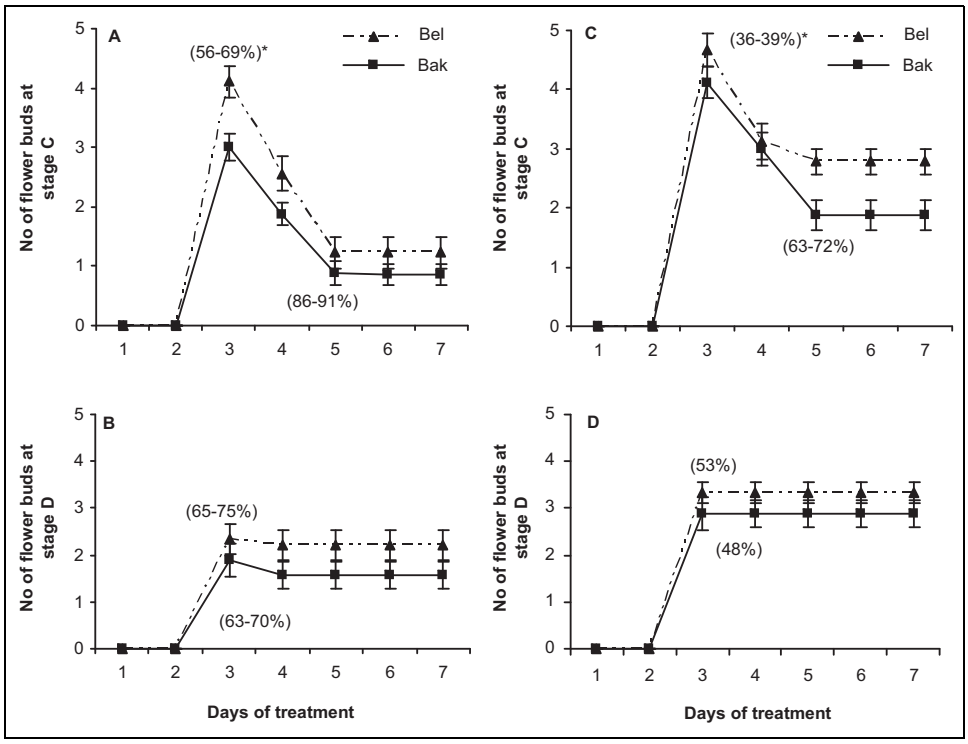


Fig. 3. Development evolution of flower buds, on 'Beldi' (Bel) and 'Baklouti' (Bak) varieties, at stage C and at stage (D) following plant transfer from 25/20 °C to 25/10 °C (A and B) and back (C and D). * Numbers in brackets represent the percentage of flower bud abortion after 3 and 5 days of treatment. N = 50.

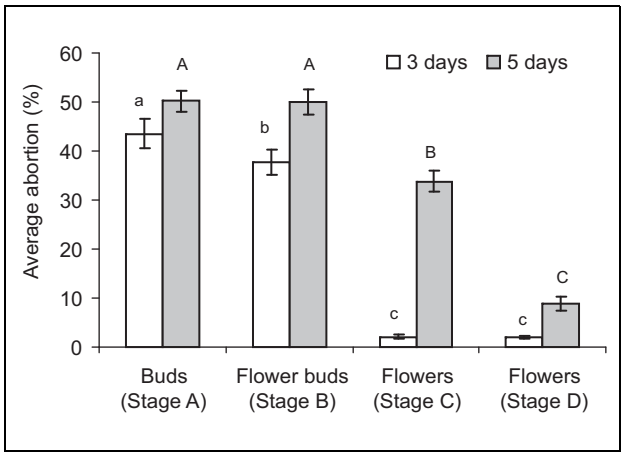


Fig. 4. The average abortion of hot pepper flower structures after 3 and 5 days of transfer from optimal temperature regime (25/20 °C) to low night temperature (25/10 °C). N = 50.

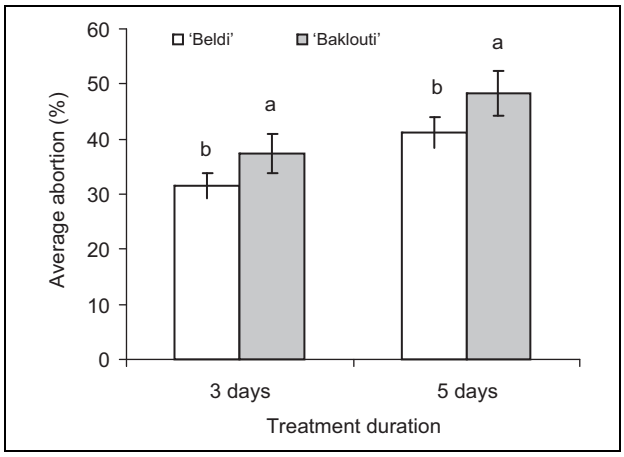


Fig. 5. The average abortion of hot pepper (var. 'Beldi' and 'Baklouti') flower structures after 3 and 5 days of transfer of the plants from optimal temperature regime (25/20 °C) to low night temperature (25/10 °C). N = 50.

depended strongly on the developmental stage (Fig. 4). The most pronounced negative effect of LNT (expressed as a percentage abortion) was shown in stages A and B (i.e. 3 or 5 d after transfer from ONT to LNT), while the lowest percentage abortion was noted at later flowers stages (stages C and D, corresponding to pre-anthesis and anthesis stages, respectively). Although all flower structures on plants grown 5 d after transfer to LNT from ONT demonstrated an increasing level of abortion compared to day 3. Wubs et al. (2005) reported that the levels of abortion of reproductive organs (i.e., buds, flowers, and young fruits) in sweet pepper plants are high; stages susceptible to abortion are very young buds (< 2.5 mm), buds close to anthesis, and flowers and fruits up to 14 d after anthesis. Many factors and processes are involved in flower abortion in sweet peppers: more light, higher CO_2 concentrations and temperature stress (Erickson and Markhart 2002; Tarchoun et al. 2003).

Pepper flowers have been shown to have sink activity at early developmental stages (Aloni et al. 1997); the dif-

ferential sensitivity of these floral structures to LNT (Fig. 4) would be attributed to sucrose availability since the alteration of source-sink relationships in the plant under low temperature stress can affect carbohydrate concentration and growth rate (EDWARDS et al. 2010; THAKUR et al. 2010). In fact, TAIZ and ZEIGER (2006) indicated that assimilate partitioning, taking place via apoplastic and symplastic pathways, has significant effects on transport and transfer processes in plants. Studying the relationship between carbohydrate and sweet pepper abortion, ALONI et al. (1999) concluded that carbohydrate concentration, especially of reducing sugars and starch, is an important factor in the determination of pepper flower retention or abortion. Low abortion percentage of the late flower stages (C and D) would be explained by the translocated sucrose that inhibits their abscission and thus ensures their continuous growth.

A low sink strength, evaluated by sucrose synthase and invertase activities, for melon (SCHAFFER et al. 1987), for tomato (EDWARDS et al. 2010) and for sweet pepper (ALONI et al. 1996, 1997; MARCELIS et al. 2004) compared to other sinks (young leaves, roots, shoots) suggested that abortion under LNT is governed by an endogenous metabolic control, particularly the distribution of assimilates.

BERTIN (1995), however, suggested that the abortion of tomato inflorescences before anthesis could be due to competition for assimilates between young vegetative organs and inflorescences or to the competition for assimilates between fruits and flowers of the same bunch or a superior bunch.

In this study, the abortion of floral structures might be attributed to the poor translocation capacity of assimilates related to their size or developmental stage since the percentage abortion was higher in flower buds stages A and B than in stages C and D (Fig. 4), although the later floral stages (C and D) also showed high levels of abortion after 5 d, suggesting the possible competition between these flowers and other sink organs. The competition for assimilate translocation between young leaves and flowers could be also a reason for flower abortion in tomato (SATO and PEET 2005) and pepper (MARCELIS et al. 2004) since these young leaves constitute a more powerful sink than the adjacent flowers (ALONI et al. 1991a, b). However, in sweet pepper plants, TURNER and WIEN (1994) did not report such an effect, since the removal of leaves did not improve flower retention. This contradictory finding assumes that the reason for pepper floral structure abortion is assigned to competition between all floral structures at different stages of their differentiation.

The abortion of floral structures also seems to depend on variety; 'Beldi' showed a lower percentage abortion than 'Baklouti', 32 and 38 % at 3 d, respectively, and 41 and 50 % at 5 d, respectively (Fig. 5). These two local varieties are usually grown in winter under unheated greenhouses in Tunisia; the delay in fruit production, resulting in the abortion of flower buds, was more noticeable in 'Baklouti' than in 'Beldi'.

These results also suggest that, in addition to the temperature effect, other factors, e.g., genetic, might regulate metabolic activity. Evaluating seven *Capsicum* accessions (four species and three genotypes) in response to night temperature, RAJA REDDY and KAKANI (2007) indicated that high percentage pollen germination and fruit set represent real species variability which can be used to screen *Capsicum* species and genotypes within the species for high temperature tolerance. Under low temperature of 24/10 °C (day/night), SHAKED et al. (2004) studied the pollen sensitivity of four pepper cultivars and found that the temperature × cultivar interaction was significant, indicating different susceptibilities to low night temperatures of these cultivars.

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