

Continuous Light Enhances Chilling Tolerance in Chilling-Sensitive Plants

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Abstract— It has been demonstrated in three chilling-sensitive species (*Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Solanum melongena* L.) that improvement of chilling tolerance may be induced by continuous lighting (CL) without low temperature treatment and the acquisition of this chilling tolerance was related to proline. The results suggest that induction of plant tolerance to unfavourable temperature by absorption of excess light by plants can occur not only under high-intensity light as shown in several studies, but also due to increasing of the daily light integral by extending the photoperiod. The improvement of chilling tolerance induced by CL without cold treatment occurs to a lesser degree than when induced by low temperature (2 h temperature drop to 10°C in the end of the night).

Index Terms— *Solanum melongena*, *Lycopersicon esculentum*, *Capsicum annuum*, chilling tolerance, continuous light.

I. INTRODUCTION

It has long been known that light is essential for enhanced freezing tolerance induced by cold temperature in herbaceous winter annuals and biennials, and the development of freezing tolerance in the light is more effective than hardening of plants in the dark [1, 2]. Also it was reported that the higher total number of photons received by plants during cold acclimation rather than the duration of photoperiod or higher light intensities result in more rapid and/or greater freezing tolerance within the limits imposed by photoinhibition at high light intensities [3]. The most obvious role of light is photosynthetic carbon fixation, which necessary for the accumulation of soluble sugars exerting their positive effects to protect plant cells from damage caused by cold stress through many ways including serving as osmoprotectants, substrates that can easily be used for energy and synthesis processes as well as interacting with the phospholipid bilayer and functioning as signaling molecules [4]. Light, through the process of photosynthesis, provides

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The reported study was partially supported by Russian Foundation for Basic Research, research project № 14-04-00840 and Federal Agency for Scientific Organizations (№ 0221-2014-0002). Experimental facilities for this study were offered by the Shared Equipment Center of the Institute of Biology, Karelian Research Center of RAS.

the energy required to attain the cold-acclimated state [5]. Recent studies show that not only parameters closely linked to the photosynthesis affected by light during hardening at low temperature, but light may also have an influence on several cold-acclimation processes including lipid metabolism, antioxidant activity, polyamine synthesis and hormonal signaling [6]. The important role of light in the development of freezing tolerance was indicated by the enhancement of freezing tolerance under elevated light conditions without cold treatment [2, 7]. All these studies evidence a complex cross-talk between light and low temperature signals in the regulation of cold acclimation [6, 8].

Unlike in cold-resistant plants, the possible role of light in the development of chilling tolerance in chilling-sensitive plants has been only poorly studied [9-11]. In our research there has been demonstrated in three chilling-sensitive species (*Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Solanum melongena* L.) that improvement of chilling tolerance may be induced by continuous lighting (CL) without low temperature treatment.

II. MATERIALS AND METHODS

Eggplant (*Solanum melongena* L., cv. Almaz), pepper (*Capsicum annuum* L., cv. Nezhnost) and tomato (*Lycopersicon esculentum* Mill., cv. Verlioka F1) plants were grown during two weeks after emergence under controlled conditions (growth chamber Vötsch, Vötsch Industrietechnik GmbH, Germany) in 7 cm pots containing a growth media based on peat. One plant per pot were used. Plants were watered daily and fertilized once a week with a complete nutrient solution (pH 6.2-6.4). The first week after emergence all plants were grown under a photoperiod of 16 h and temperature of 26°C.

Four treatments combining photoperiods of 16 and 24 h and constant temperature of 26°C or daily temperature drop to 10°C for 2 h (DROP) in the end of the night were applied during the second week: 16 h + constant temperature (16 h), 16 h + DROP (16 h + DROP), 24 h + constant temperature (24 h), 24 h + DROP (24 h + DROP).

Photosynthetic photon flux density (PPFD) was 155 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by high-pressure mercury lamps (daily light integral (DLI) was 8.64 and 12.96 $\text{mol m}^{-2} \text{day}^{-1}$ for 16 h and 24 h photoperiod, correspondingly). Treatments with equal DLI of 12,96 $\text{mol m}^{-2} \text{day}^{-1}$ were also used (similar data were obtained, data not shown).

Leaf chilling tolerance was assessed at 15 DAE (days after emergence) by testing the vitality (LT_{50}) of cells after 5-min freezing (temperature from -10 to -5°C) of leaf discs in a micro refrigerator [12]. The difference between

temperatures that caused death in treated and control plant cells was accepted as plant tolerance increment.

Free proline content in leaf tissues was determined at 15 DAE according to Bates et al. [13].

The results are presented as mean values and standard errors determined from two independent experiments with at least six replicates per experiment. Significant differences between the means were determined at $P < 0.05$ using the Statistica software (v. 8.0.550.0, StatSoft Inc.).

III. RESULTS AND DISCUSSION

The study showed that 24 h photoperiod at normal temperature of 26°C induced leaf chilling tolerance enhancement in eggplant, pepper and tomato compared to 16 h photoperiod (Fig.1). The increment in leaf chilling tolerance was 0.5°C in eggplants and 0.8-0.9°C in tomato and pepper plants. The daily short-term temperature drop enhanced leaf chilling tolerance in all three species irrespective of photoperiod (16 or 24 h) (Fig. 1a). The changes in chilling tolerance occurred in plants affected by a temperature drop were much greater than in plants which were kept under CL at normal temperature. It has previously been reported for wheat plants that excess light energy (not utilized in *photosynthesis*) causes a number of non-specific adaptive changes in plants that occur also as a result of other environmental factors effects, such as heat shock, cold, salt and osmotic stress [14]. Excess light has been shown to activate defense-related genes, induce synthesis of stress proteins, affect antioxidant enzyme activity and the activity of some fatty acid desaturases, which may result in increased membrane phospholipid unsaturation [15, 16]. Changes in the photosynthetic apparatus in response to low temperatures are similar to those observed in plants exposed to high light and vice versa, the photosynthetic response of unhardened plants similar to that of plants under low light. Thus, we suggest that continuous light of moderate intensity in a way similar to high intensity light creates conditions of excess light for the plant, which may lead to the induction of a number of non-specific adaptive mechanisms.

In our experiments neither photoperiod, no daily light integral modified the effect of DROP on chilling tolerance of plants. It evidences that there is physiological limit to which chilling-sensitive plants can acclimate in response to CL or excess light along.

In all cases chilling tolerance increase was accompanied by endogenous proline accumulation in leaves (Fig. 1b). The maximum accumulation of proline was observed in leaves of 24 h + DROP eggplants and tomato with lesser content in leaves of 24 h and 16 h + DROP though exceeding the proline content in leaves of 16 h plants. In pepper plants proline content in leaves of 24 h, 24 + DROP and 16 h + DROP plants was equally higher compared to leaves of 16 h plants. Proline accumulates in many plant species in response to environmental stresses, it has multiple functions such as osmotic adjustment, ROS-scavenging, redox-buffering, energy status, as well as acts as small molecular chaperone and plant development signal [17]. During exposure to low temperatures, proline accumulation could be detected only under light conditions, as reported in various plant species [8, 18]. Accumulation of endogenous proline may improve chilling tolerance [19]. This probably took place in our experiments when CL caused accumulation

of proline, which induced the improvement of plant chilling tolerance.

However, low-temperature induced acquisition of chilling tolerance in our experiments is obviously not related to proline accumulation only. Thus, DROP-induced increments of chilling tolerance in eggplant and tomato under 16 h and 24 h photoperiod do not differ, but proline content is much higher under CL. This can be explained by the fact that stress-induced proline synthesis in leaves is much faster in light than in darkness [20] and in our work low temperature treatments were applied in the end of the night under 16 h photoperiod, and at the same time, but in the light under 24 h photoperiod as CL implies no dark period. Induction of chilling tolerance development by low temperature is obviously not light-dependent.

In summary, continuous light (24 h photoperiod) with relatively low PPFd could improve leaf chilling tolerance in eggplant, pepper and tomato plants at normal temperature and the acquisition of this chilling tolerance was related to proline. The results suggest that induction of plant tolerance to unfavorable temperature by absorption of excess light by plants can occur not only under high-intensity light as shown in several studies [6, 14], but also due to increasing of the daily light integral by extending the photoperiod. The improvement of chilling tolerance induced by CL without cold treatment occurs to a lesser degree than when induced by low temperature.

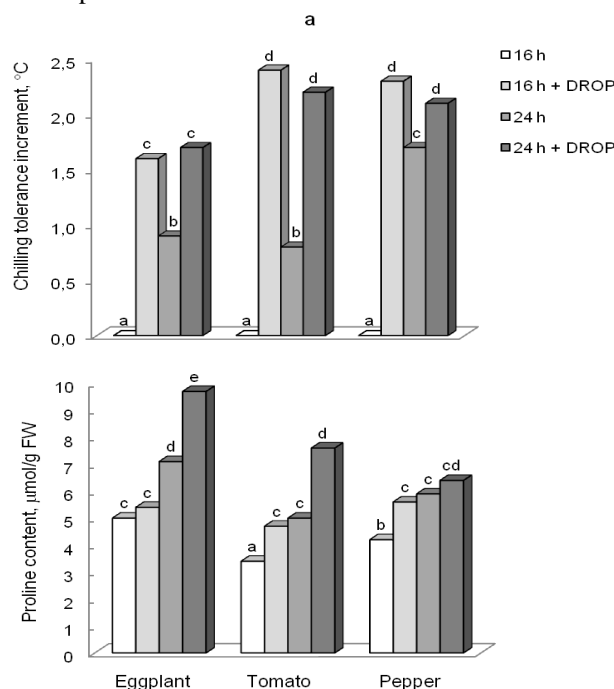


Fig. 1 Leaf chilling tolerance increment (a) and free proline content (b) in eggplant, tomato and pepper plants under 16 h or 24 photoperiod combined with constant temperature of 26°C or daily temperature drop to 10°C for 2 h (DROP). Different letters indicate statistical differences at $P < 0.05$.

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