THE EFFECT OF CYTOKININS ON FLAX SEED GERMINATION AT LOW TEMPERATURE

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ABSTRACT

Germination of flax seeds (*Linum usitatissimum* L., cv. Szafir) at 5°C was enhanced by continuous white light, gibberellin A_3 (GA_3), kinetin and benzylaminopurine. GA_3 and kinetin at physiological concentrations (10^{-8}-10^{-6} M) improved significantly germination in darkness. Stimulatory effect of benzylaminopurine was visible only in the light. Almost no effect of zeatin and isopentenyladenine (2iP) on germination was observed. Possible causes of this differences were suggested.

KEY WORDS: cytokinins, germination, gibberellin, *Linum usitatissimum* L., seeds, low temperature.

INTRODUCTION

The effects of the phytohormones, especially gibberellins and cytokinins, in stimulation of seed germination at unfavourable conditions have been frequently observed (El-Keblawy et al. 2005; Thomas 1992; Gulzar and Khan 2002). Germination of some seeds under water stress can be improved by treatment with these growth regulators (Heikal et al. 1982; Niedźwiedź-Siegień and Lewak 1992). Similarly, the phytohormones modify the temperature requirements of germination of some seeds (Wang et al. 1996; Thomas and Davies 2002). Moreover, gibberellins and cytokinins can be involved in the light-regulated germination of some seeds (Thomas et al. 1997; Zagórski and Lewak 1983).

The germination-stimulatory effect of light was attributed to light-mediated stimulation of GA synthesis and/or the sensitivity to this hormones (Hilhorst and Karssen 1992; Yamaguchi and Kamiya 2002). The observations, that cytokinins are effective only in the light or in darkness in presence of gibberellins (Thomas 1992) and that the effectiveness of different cytokinins is not the same in different seeds (Poggi-Pellegrin and Bulard 1978) indicate that the interaction between light and cytokinins is more complex.

In many crop plants, seed germination and early seedling growth are the most sensitive stages to environmental stresses (Foolad et al. 2003). Although flax (*Linum usitatissimum* L.) is considered to be a cool season crop, air temperature below 10°C in the spring may inhibit its growth and development, which can delay flowering (Gusta et al. 1997). The germination of wild flax species *Linum catharcticum* L. was temperature- and light-dependent (Van Tooren and Pons 1988). It was also shown (Heikal et al. 1982) that osmotically inhibited germination of *Linum usitatissimum* L. seeds can be improved by a treatment with gibberellin.

Therefore, it seemed of some interest to study the effects of different cytokinins on dark- and light-germination of flax seeds at low, unfavourable temperature. Gibberellin A_3 effects were used as a reference.

MATERIALS AND METHODS

Seeds of oil flax (*Linum usitatissimum* L., cv. Szafir) were obtained from Plant Breeding Station at Borowo (West Poland), and stored dry at 4°C in darkness for 6-12 months before experiments.

Seeds were placed in lots of 30 in 9 cm diameter Petri dishes lined with 2 discs of filter paper moistened with 8 ml of distilled water or an appropriate solution of a growth regulator: gibberellin A_3 (GA_3), kinetin, benzylaminopurine (BA), isopentenyladenine (2iP) and zeatin (Sigma). Dishes were incubated in darkness (in black paper boxes) or under continuous white light (white fluorescent tubes – "Philips", 90 µmol $m^{-2}s^{-1}$) in temperature controlled chamber ("Fitotron", Sanyo, Japan), where the temperature was kept constant within ±1°C. Germination was studied at temperature range from 5 to 30°C at the seed level. For the study of the effects of hormones, seeds were incubated at 5°C only.

Protrusion of radicle through the seed coat was taken as a criterion of germination. The germinated seeds were counted every day under a dim green safety light.

Each experiment was performed in 4 replicates and repeated at least twice. The results are presented as mean values ± SD. The statistical differences were tested using one way analysis of variance (ANOVA), $P<0.05$.

RESULTS AND DISCUSSION

Optimal germination of flax seeds occurred at temperatures of 15-25°C (Fig. 1A). Under these conditions most seeds (above 80%) germinate within four days, both in the light and in darkness (Fig. 1A). Low temperature, however, similar to other seeds (e.g. Gill 2003; Guillermo 2006), causes a slowing of germination of flax seeds. Additionally, at low temperature (5-10°C) their germination was substantially enhanced by light (Figs 1A, B), indicating that the seeds become positively photoblastic. It has been already reported for the seeds of *Calluna vulgaris* (Thomas and Davies 2002), *Trifolium repens* (Niedźwiedź-Siegień and Lewak 1988), *Amaranthus hypochondriacus* (Aufhammer et al. 1998) and *Artemisia* species (Kazuo et al. 2006) that photosensitivity of germination changed with temperature.

Taking the above into account, the effects of hormonal treatments on flax seeds germination were checked at the lowest studied temperature (5°C) only. Dark germination was improved by low concentrations of GA_3 (10^{-8}-10^{-7} M, Fig. 2). However, similarly to other seeds (Miyoshi and Mii 1995; Wang et al. 1996) higher levels of this hormone had an inhibitory effect, both on light and in darkness. It can be assumed, that low temperature, that slowed down the germination, increased simultaneously the sensitivity of flax seeds to GA_3. The similar substitution by GA treatment the effect of light, indicating its influence on GA biosynthesis, and/or on the increased sensitivity to the hormone (Casal and Sanchez 1998; Yamaguchi and Kamiya 2002) was observed earlier (Derkx et al. 1994).

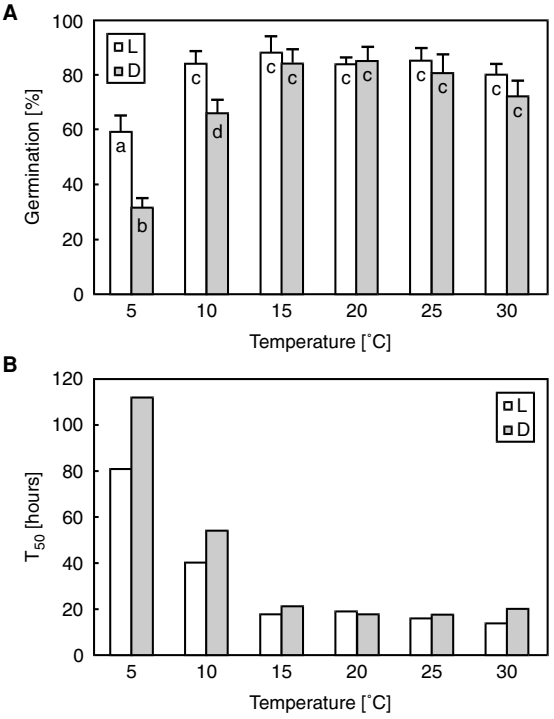

Fig. 1. A – effect of different temperatures on the final percent of germination of flax seeds after 4 days in darkness (D), or under continuous white light (L). Vertical bars indicate ± SD. Means marked with the same letters are not significantly different ($P<0.05$) according to ANOVA analysis; B – effect of continuous white light on flax seed germination at different temperatures. Times (h) necessary for 50% germination on the light (L) and in darkness (D) were calculated from extrapolated germination curves. SD did not exceed 10% for germination tests (examples in figures 1A, 2 and 3).

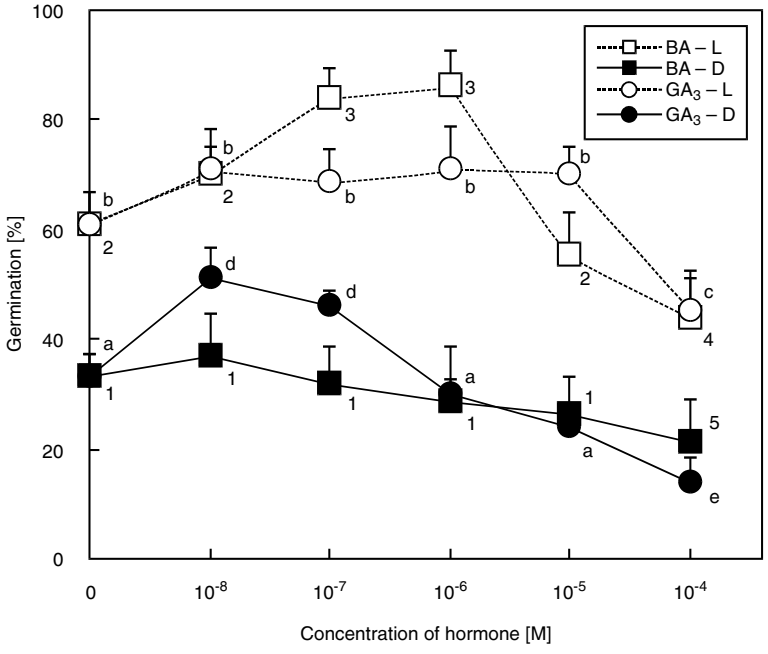

Fig. 2. Effect of GA_3 and benzylaminopurine (BA) on germination of flax seeds for 4 days at 5°C in darkness (D) and after continuous illumination with white light (L). Vertical bars indicate ± SD. Means marked with the same letters (for GA_3), or numbers (for BA) are not significantly different ($P<0.05$) according to ANOVA analysis.

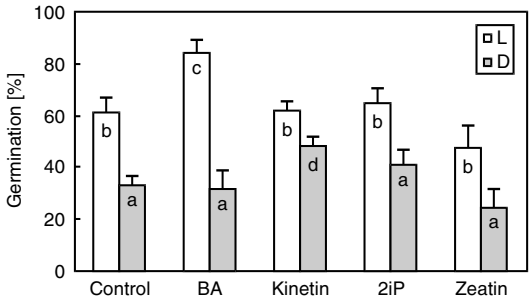

Fig. 3. Effect of different cytokinins (10^{-7} M) on flax seeds germination for 4 days at 5°C in darkness (D) and after continuous illumination with white light (L). Vertical bars indicate ± SD. Means marked with the same letters are not significantly different ($P<0.05$) according to ANOVA analysis.

Germination of flax seeds was enhanced significantly (by 40% of control) by BA (10^{-7}-10^{-6} M), but only on light (Fig. 3). This observation could indicate, that for stimulatory effect of BA a certain level of P_{fr} is necessary as it has been suggested for other seeds (Thomas 1992; Thomas et al. 1997). According to some hypotheses, cytokinins could be the "mediators" of light, and may be aiding in overcoming the embryonic block in germination (Thomas et al. 1997; Brault and Maldiney 1999). However, for better understanding the interaction of light and cytokinin in the regulation of flax seed germination at low temperature the results of experiments with monochromatic light would be helpful.

Other cytokinins (kinetin, 2iP, zeatin) were applied at the same concentrations range (10^{-8}-10^{-4} M) as it was shown for BA in figure 2 (results not shown). To display the different cytokinins influence on germination of flax seeds, the effects of one (10^{-7} M) the most effective concentration of these phytohormones were shown (Fig. 3). Kinetin stimulated the germination of the flax seeds in darkness only, but the degree of stimulation (by 50%) was similar to that of BA in light. On the other hand there was almost no effect of isoprenoid cytokinins (zeatin and 2iP) on flax seeds germination. Similarly, stimulatory effects of BA and kinetin and no effects of 2iP and zeatin on lettuce seed germination was earlier observed (Poggi-Pellegrin and Bulard 1976).

The significant effect of aromatic cytokinins (BA and kinetin) on germination and almost no effects of the aliphatic ones (zeatin and 2iP, Fig. 3) can result from the rapid destruction of the last ones by native plant enzymes (Poggi-Pellegrin and Bulard 1976). Another explanation of these results may be, that the rate of penetration of different cytokinins into the seeds is different (Thomas 1992). Kinetin and BA would reach the embryonic tissues faster than aliphatic cytokinins, thus their effect would be higher. It is not excluded that both these mechanisms are operating simultaneously.

The stimulatory effect of GA_3, BA and kinetin on germination of flax seeds at low temperature have been also observed for corn (*Zea mays*) and soybean (*Glycine max*) seeds germinated under the same stress (Wang et al. 1996). These hormones are also involved in alleviation of salinity effects during seed germination of *Prosopis juliflora* (El-Keblawy 2005), *Suaeda salsa* (Li et al. 2005), and also water stress during germination of *Cicer arietinum,* seeds (Kaur et al. 1998). There are some data, that all these stresses do affect water status of the cell and delay seed germination by causing water stress (Liptay and Schopfer 1983). Moreover, there is a suggestion that same genes might control the rate of seed germination under cold, salt and drought stress (Foolad et al. 2003). All stress conditions usually cause the imbalance of growth regulators, resulting in increased levels of inhibitors, and a decrease in endogenous growth promoters (Bewley and Black 1994; Hare et al. 1997). So, the increase in flax seed germination under temperature stress, after exogenous application of GA_3, kinetin or BA may be attributed to the ability of these growth regulators to reduce the moisture requirement or to enhance water uptake during germination (El-Keblawy et al. 2005). It cannot also be excluded, that these regulators may enhance the activities of some enzymes involved in sugars' metabolism during germination of flax seeds, as it was suggested for germination of other seeds under water stress conditions (Kaur et al. 1998, 2000).

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