INTERACTION OF GIBBERELLIC ACID AND FUSICOCCIN IN ABSCISIC ACID-INHIBITED AND THERMO-INHIBITED GERMINATION OF LETTUCE SEEDS

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Abstract - Germination of May Queen lettuce (*Lactuca sativa* L.) seeds is completely prevented either by 10^{-4} M abscisic acid (ABA) or at 31.5 °C. Gibberellic acid (GA_3) and N-substituted phthalimide, AC 94,377, are almost ineffective in overcoming ABA-induced inhibition and thermo-inhibition, but fusicoccin (FC) completely reverts both types of inhibition. An interaction between FC and GA_3 (as well as between FC and AC 94,377) is evident in stimulation of germination under both inhibitory conditions. The extent of interaction, calculated as a ratio of percent of seeds germinated under simultaneous action of stimulators and the sum of their separate effects is 2.25 for ABA- and 1.61 for thermo-inhibited seeds. The obtained data point to the promotive synergism as the type of interaction. The highest interaction occurs if the application of FC is delayed after GA_3 application, the optimal time lag being 36 h for ABA-inhibited, or 24 *h* for thermo-inhibited seeds, respectively.

INTRODUCTION

When a physiological response such as germination is regulated by a combined action of different environmental factors and growth regulators, the mechanisms by which they influence the process are generally poorly understood. It is well known that ABA is involved in maintaining and controlling seed dormancy and that exogenous ABA inhibits non-dormant seed germination in addition. The mode of inhibition seems to be different from dormancy control mechanism(s) (Bewley 1997). Abscisic acid inhibits both dark germination of non-dormant lettuce seeds and isolated embryos (Inoue 1991; Khan 1994), as well as light-induced germination of dormant seeds (Lado *et al.* 1974; Gardner 1983). Nevertheless, it does not induce secondary dormancy (Khan 1994). Well-described light-temperature interrelations are apparently based on membrane phase transitions, which interfere with phytochrome action (Hendricks and Taylorson 1979; VanDerWoude 1985). However, it was shown recently that high temperatures accelerate ABA biosynthesis (Yoshioka *et al.* 1998). The promotive effect of gibberellins (GAs) on lettuce seed germination was shown 40 years ago (Lona 1956), and was

confirmed for a variety of other species since. Exogenous GAs can frequently replace the requirements for environmental stimuli such as light or low temperatures (reviewed in Bewley and Black 1982). Gibberellins are frequently efficient in overcoming inhibition caused by both exogenous (Sondheimer and Galston 1966) and endogenous ABA (Inoue 1991), though in the case of ABA-inhibited *Paulownia tomentosa* seeds or lettuce seeds cv Grand Rapids they are not effective (Gardner 1983; Grubišić *et al.* 1988). Substituted phthalimide AC 94,377, a gibberellin analogue, exhibits gibberellin-like action in plant growth (Los *et al.* 1980) and seed germination (Grubišić and Konjević 1987; Giba *et al.* 1993). Fusicoccin, a phytotoxin isolated from *Fusicoccum amygdali* Del. (Ballio *et al.* 1964) is a well-known growth regulator that affects a number of physiological processes and resembles recognized plant hormones (Muromtsev 1996). Fusicoccin is a highly potent germination stimulator and a dormancy-breaking agent which readily antagonizes ABA-induced inhibition of germination in lettuce (Lado *et al.* 1974), *Haplopappus graci-lis* (Galli *et al.* 1979), *Paulownia tomentosa* (Grubišić *et*

al. 1988) and other seeds. Fusicoccin is also effective in promoting germination at low temperatures (N e l s o n and S h a r p l e s 1980). In this paper we present evidence on the interaction of GA3 and fusicoccin in ABA- and thermo-inhibited germination of lettuce seeds cv. May Queen. The phenomenon was initially reported for *Achillea abrotanoides* seeds (G r u b i š i ć *et al.* 1997) and recently a similar phenomenon was shown for growth retardant and far red light-inhibited lettuce seeds (in press).

MATERIAL AND METHODS

Seeds of *Lactuca sativa* (Asteraceae) cv. May Queen were purchased from seed company "Seme", Belgrade, Yugoslavia. Lots of 100 seeds were sown in 6-cm diameter Petri dishes, containing 2 mL of tested substances that were dissolved in 500 *mg* L^{-1} of nystatin. Seeds were imbibed and germinated in darkness at 25 °C or 31.5± 0.2°C for 3 days. Germination was scored at the end of experiments or with indicated frequency. All manipulations were done under week green safe light. The extent of interaction was calculated as a ratio of percent of seeds germinated under simultaneous action of promoters and additive effect of independent stimulators. In experiments with delayed FC application, the rate of germination was calculated as a function of time lag. Curves representing germination dynamics of samples imbibed in gibberellin and treated with FC at different times were double normalized. Each curve was shifted to the left for the time of specific lag. Vertical normalization was also necessary to eliminate the effect of single GA3 before FC was added. All experiments were repeated two times with 4 replicates. Specific experimental protocols are described in the legends.

Abscisic acid (ABA) and gibberellic acid (GA3) were purchased from Sigma Chemical Co.; AC 94,377 [1-(3-chlorophthalimido) cyclohexane-carboxamide], American Cyanamide Company was obtained as a test substance; fusicoccin (FC) was a gift from Professor Muromtsev, Institute of Agricultural Biotechnology, Russian Academy of Agricultural Sciences, and nystatin from Hemofarm, Vršac, Yugoslavia.

RESULTS

Lettuce seeds cv. May Queen germinate up to about 85% in darkness. Addition of 10^{-4} M ABA (Fig. 1) or rising the temperature above the maximum for germination (31.5 °C, Fig. 2) completely suppress the germination. In both modes of inhibition neither gibbe-
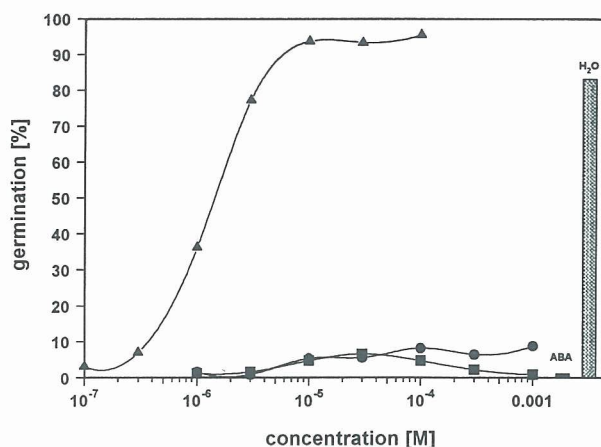

Fig. 1. Effect of stimulators on ABA-inhibited seed germination. Seeds were imbibed for 3 days in darkness at 25 °C in 10^{-4} M ABA (solid bar) or with addition of GA3 (■), AC 94,377 (●) or FC (▲). Shaded bar represents control in water.
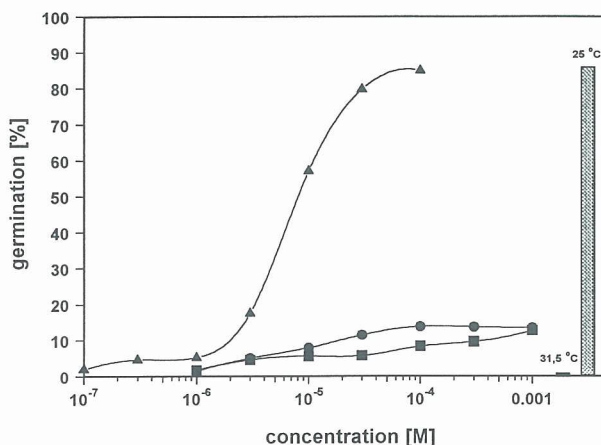

Fig. 2:. Effect of stimulators on thermo-inhibited seed germination. Germination at high temperature (31.5 °C, black bar) in the presence of germination promoters - GA3 (■), AC 94,377 (●) or FC (▲). Shaded bar represents control in water, at 25 °C.

rellin nor its analogue were effective in reverting inhibition, as they produced about 8% (Fig.1) or 13% of germination respectively (Fig. 2). If the stimulation of ABA-inhibited seeds was prolonged to 7 days, GA3 and AC 94.277 were slightly more effective, inducing about 30% of seeds to germinate but only if applied in 10^{-5} - 10^{-4} M concentration (data now shown). Fusicoccin could fully overcome ABA-induced inhibition, and in higher concentrations even induce a response above that of the control without ABA (Fig. 1). Fusicoccin was also several orders of magnitude more potent than GA3 in thermo-inhibited seeds (Fig. 2). In both modes of inhibition, an interaction in stimulation of germination between fusicoccin and either GA3 or its analogue was evident. The combined action of these compounds was
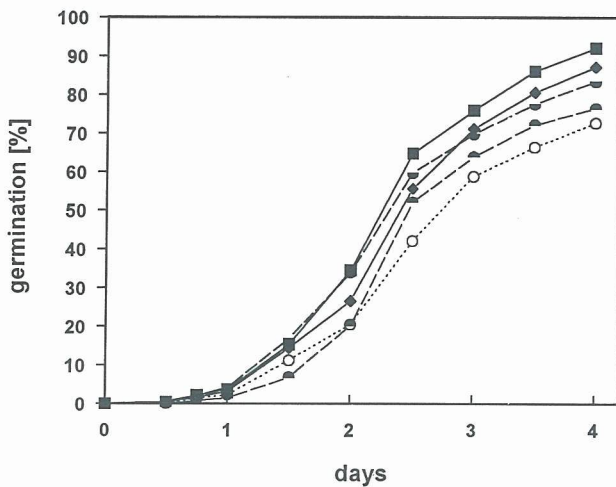
Fig. 3. Effect of combining stimulators on ABA-inhibited seed germination. Seeds were imbibed in darkness in 10^{-4} M solution of ABA, at 25°C, with addition of stimulators in concentrations of 10^{-3} M for GA$_3$ and AC 94,377 and 10^{-6} M for FC. Open symbols represent effect of FC alone (dotted line   ) added at the beginning of imbibition. Results of GA$_3$ and AC 94,377 action, applied alone and added at the beginning of imbibition, as well as GA$_3$+AC applied simultaneously, are not included as their effect was negligible. Solid symbols represent various combinations of stimulators, added at the beginning of imbibition (full lines: GA$_3$+FC -■;AC+FC - ◆), or application of second stimulator was delayed for 12h (dashed lines: GA$_3$+FC after 12h - FC+GA$_3$ after 12h-▲; FC+GA$_3$ after 12h -●). Control without stimulators did not germinate. Germination was scored every 12 h during 4 days.

Fig. 5. Extent of the interaction between GA$_3$ and FC in ABA-inhibited seeds. Seeds were imbibed in darkness, at 25 °C, in solutions of 100 μM ABA and indicated concentrations of GA$_3$ and FC. Germination was scored after 3 days.The extent of interaction was calculated as a ratio of percent of seeds germinated under simultaneous action of promoters and additive effect of independent stimulators.
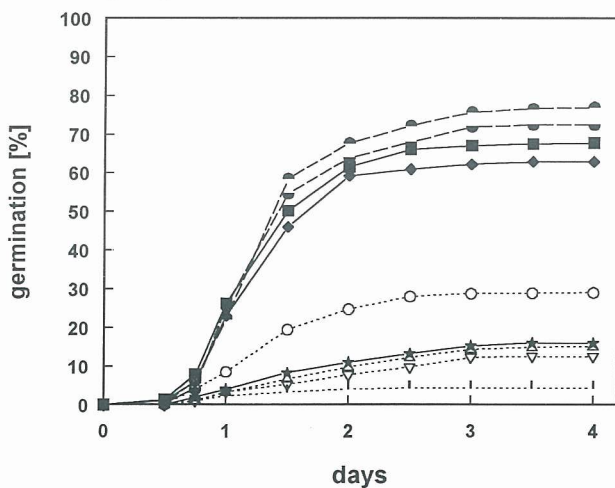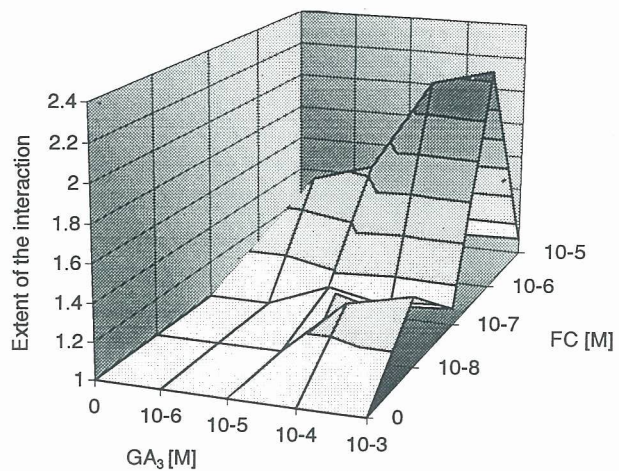

Fig. 4. Effect of combining stimulators on thermo-inhibited seed germination. Seeds were imbibed in water (dotted line   ) or in solutions of tested substances, at 31.5 °C, in darkness. Stimulators were applied in concentration of 10^{-3} M for GA$_3$ and AC 94,377 and 10^{-6} M for FC. Open symbols represent effects of single stimulators (dotted lines: GA$_3$ -■ AC 94,377 - ● FC▲) added at the beginning of imbibition. Solid symbols represent various combinations of stimulators, added at the beginning of imbibition (full lines: GA$_3$+FC -■; AC+FC - ◆GA$_3$+AC ✳, or application of second stimulator was delayed for 12 h (dashed lines: GA$_3$+FC after 12h -▲; FC+GA$_3$ after 12h -●). Germination was scored every 12 h during 4 days.

Fig. 6. Extent of the interaction between GA$_3$ and FC in thermo-inhibited seeds. Seeds were imbibed in darkness, at 31.5 °C, in solutions of defined concentrations of GA$_3$ and FC. Germination was scored after 3 days. The extent of interaction was calculated as a ratio of percent of seeds germinated under simultaneous action of promoters and additive effect of independent stimulators.
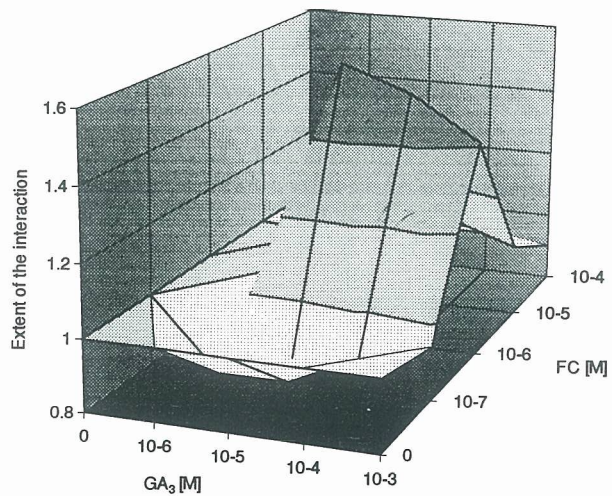
more effective than when each one was used separately.Figs 3 and 4 show the net results of a combined action of FC and the two other substances. In both cases GA$_3$ was shown to be slightly more effective than AC 94,377. In the case of ABA-inhibited seeds the extent of interaction was the largest (2.25) when GA$_3$ was applied in 10^{-3} M and FC in 10^{-6} M concentration (Fig. 5). In a similar experiment when AC 94,377 and FC were com-

bined, the extent of interaction was 2.34 (data not shown). In the case of thermo-inhibited seeds, GA3 interacted with FC to the extent of 1.61, while the optimal concentrations were 10^{-5} M for GA3 and 10^{-6} M for FC (Fig. 6). Combination of AC 94,377 and FC in thermo-inhibited seeds caused an interaction in stimulating germination of 2.3 (data not shown).
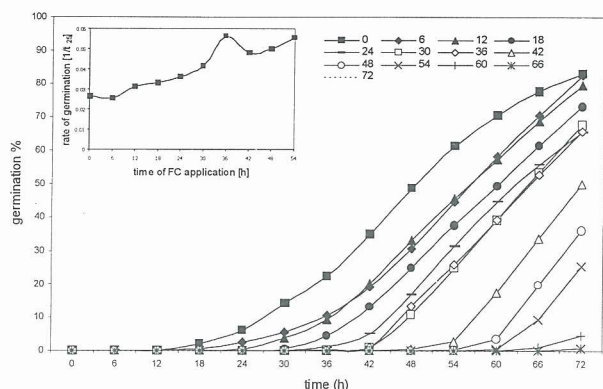

Fig. 7. Effect of delaying FC application in ABA-inhibited seeds. Seeds were imbibed in solution of 10^{-4} M ABA and 1 mMGA3 in darkness, at 25°C. To the first sample of 400 seeds FC (10^{-6} M) was added at the beginning of imbibition, and to the rest with the time lag indicated in the legend. Germination was scored every 6 *h*, and date were plotted cumulatively. Insert: rate of germination calculated from normalized curves.
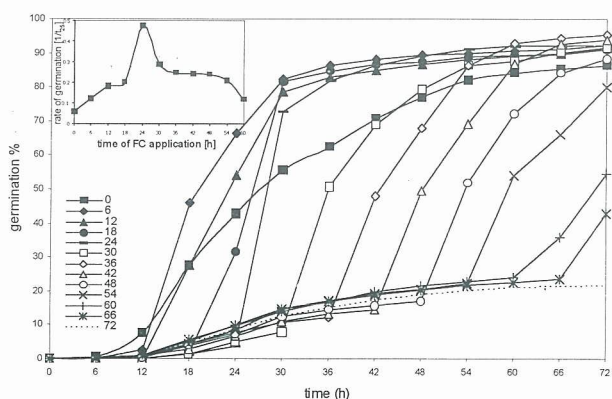

Fig. 8. Effect of delaying FC application in thermo-inhibited seeds. Seeds were imbibed in darkeness, in solutio of 1 mM GA3 at 31.5 °C. Fusicoccin (1 µM) was added at the time indicated in legend. Exprerimental protocol was the same as indicated for Fig. 7. Insert: rate of germination calculated for normalized curves.

The promoting effect of delayed FC application, previously described for tetcyclacis- and FR light inhibited May Queen seeds (Simonović *et al.* in press) was also obvious for ABA- and thermo-inhibited seeds (Figs. 7 and 8). In ABA-inhibited seeds the rate of germination rapidly increases with increasing time lag between application of GA3 and FC reaching a maximum at 36 *h* (insert in Fig. 7). In thermo-inhibited seeds, curve for the rate of germination displays a sharp peak if the FC application is delayed for 24 *h* (insert in Fig. 8).

DISCUSSION

The data presented here strongly point to the specific type of interaction between gibberellic acid and fusicoccin in removing inhibition of germination, caused by abscisic acid and high temperature. However, the nature of that interaction is not known. Abscisic acid is known to prevent the premature germination of seeds, to induce and maintain seed dormancy and to inhibit germination of non-dormant seeds (reviewed in Bewley 1997). In lettuce seeds exogenous ABA acts in terminal stages of germination process, affecting elongation of the radicle (Bex 1972). Inhibition of germination of May Queen seeds by 10^{-4} M ABA (Fig. 1) is in good correlation with literature data for other lettuce cultivars (Lado *et al.* 1974; Gardner 1983; Khan 1994). However, in lettuce seeds, gibberellins are partially effective (Lado *et al.* 1974) or ineffective (Gardner 1983) in overcoming ABA-induced inhibition, unless the seeds are punctured (Inoue 1991). In addition, in photoblastic *Paulownia tomentosa* seeds, GAs are also ineffective in overcoming ABA-induced inhibition (Grubišić *et al.* 1988). Therefore, low efficiency of GA3 in restoring ABA-inhibited germination of May Queen seeds (Fig. 1) fits well with these findings. Ability of FC to antagonize ABA inhibition of germination is well described in literature both for lettuce (Lado *et al.* 1974) and other species (Ballarin-Denti and Cocucci 1979; Grubišić *et al.* 1988).

Although it is common to classify seeds of lettuce cultivars to dormant (photoblastic) and non-dormant, all of them actually exhibit relative dormancy (Bewley and Black 1982) dependent on temperature. Below some critical temperature (e.g. 15 °C for Grand Rapids, Borthwick *et al.* 1954) lettuce seeds germinate readily in darkness, but at high temperatures the germination is suppressed and the maximum temperature depends on the cultivar and light conditions (Bewley and Black 1982). Differences among lettuce cultivars are sharp and not only quantitative (Kristie *et al.* 1981). In May Queen seeds, prolonged imbibition at high temperature can induce thermo-dormancy (Bewley and Black 1982). Almost 20 years ago it was suggested that the induction of thermo-dormancy

in lettuce seeds is based on ABA accumulation (K r i s - t i e *et al.* 1981). Recently it was confirmed that high temperature affects the rate of ABA biosynthesis since inhibitors of its synthesis (fluridone) enable germination of lettuce and other species at inhibitory temperatures (Y o s h i o k a *et al.* 1998). Some authors, however, doubt about the specificity of fluridone action (K h a n 1994).

In both modes of inhibition an interaction between GA₃ (or its analogue) and FC in stimulating germination was obvious (Figs. 3 - 6). The first report on the phenomenon was the case of *Achillea abrotanoides* seeds inhibited with tetcyclacis (G r u b i š i ć *et al.* 1997). A similar type of interaction was seen in May Queen seeds inhibited with growth retardants or far-red light in our recent report (S i m o n o v i ć *et al.* in press). The interaction was apparent in all instances where FC was effective in overcoming inhibition, irrespective of GAs efficiency. Hence the mechanism(s) of overcoming various types of inhibitions by GAs probably differ from their ability to enhance FC action. In all types of inhibition the rate of germination was the greatest if FC was applied with a certain delay after GA₃ treatment (Figs. 7-8). It is well established that FC action in majority of physiological responses is based on its specific binding to Fusicoccin Binding Protein, FCBP, a member of 14-3-3 protein family (K o r t h o u t and d e B o e r 1994; de B o e r and K o r t h o u t 1996). It was reported that density, and/or affinity of FCBP can be modulated by several factors such as pH of the cytosol (T r o f i m o v a *et al.* 1997), auxins (A d d u c i *et al.* 1986), maturity of the tissue, and fusicoccin itself (B a s e l *et al.* 1994). Our results suggest that gibberellins may also affect FCBP properties, although this view would need further research and confirmation.

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ИНТЕРАКЦИЈА ГИБЕРЕЛНЕ КИСЕЛИНА И ФУЗИКОКЦИНА У КЛИЈАЊУ СЕМЕНА САЛАТЕ ИНХИБИРАНИХ АБСЦИСИНСКОМ КИСЕЛИНОМ И ВИСОКОМ ТЕМПЕРАТУРОМ

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Клијање семена салате "Мајска Краљица" се потпуно спречава применом било 10^{-4} М АBA било високом температуром (31.5 °C). GA$_3$ и N-субституисани фталимид AC 94,377 су скоро потпуно неефикасни у превазилажењу инхибиције индуковане абсцисинском киселином (АBA) и високом температуром, док фузикокцин (FC) потпуно превазилази инхибицију у оба случаја. Код оба типа инхибиције је уочена интеракција између FC и GA$_3$ (као и између FC и AC 94,377) у стимулацији клијања. Величина интеракције, израчуната као однос процента семена проклијалих под симултаним дејством стимулатора и њиховог адитивног ефекта је 2.25 код семена инхибираних абсцисинском киселином и 1.61 код термоинхибираних семена. Закључено је да је интеракција типа промотивног синергизма. Интеракција је највећа ако се FC примени са извесним одлагањем у односу на време примене GA$_3$, при чему је оптимално време одлагања 36 сати код клијања инхибираног абсцисинском киселином, односно 24 сата код термоинхибираних семена.