Comparative Effects of Kinetin, Benzyladenine, and Gibberellic Acid on Abscisic Acid Inhibited Seed Germination and Seedling Growth of Red Pine and Arbor Vitae

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Abstract: The effects of kinetin, benzyladenine (BA), and gibberellic acid (GA$_3$) on abscisic acid (ABA) inhibition of seed germination and seedling growth of red pine (Pinus brutia Ten.) and arbor vitae (Thuja orientalis L.) were studied. For this purpose, the seeds of these two species were germinated in Petri dishes containing ABA and the mixtures of its with kinetin, BA, and GA$_3$ alone or in combination at 20ºC for 12 or 15 days for arbor vitae and red pine, respectively. The inhibitory effect of ABA on percentages of seed germination and hypocotyl emergence, the time course of the germination, and the radicle and hypocotyl elongation of the seedlings was overcome very successfully by GA$_3$ alone in comparison with kinetin, BA, and the combinations of these two cytokinins alone with GA$_3$. Kinetin or BA alone did not mostly fail to overcome ABA inhibition on the mentioned parameters. There were the cases that also the combinations of these cytokinins with GA$_3$ were successful or not. GA$_3$ alone was the most successful hormone at all the cases in general.

Key Words: Germination, abscisic acid, gibberellic acid, kinetin, benzyladenine, Pinus brutia Ten., Thuja orientalis L.

Introduction

In relation to the variable responses given by angiospermous seeds to various growth regulator applications, Khan (1) has proposed a model whereby gibberellins, cytokinins, and ABA play primary, permissive, and preventive roles, respectively, in germination. Thus, it appears that germination will occur in the presence of ABA only if both cytokinin and gibberellin are present, but in the presence of gibberellin alone if ABA is absent. On the basis of this model, primary agent is gibberellins, and cytokinins act in the presence of ABA to remove its blockage, and gibberellin-mediated germinative processes cannot occur in the presence of ABA unless there is sufficient cytokinin present to overcome its inhibitory effects. This model has been accepted by most of workers (e.g. 2-4). However, evidence that this model may also apply to conifers, as far as we know, is lacking. In the studies with conifers, it has been mostly utilized either gibberellin or cytokinin alone and these two growth regulators have not been compared in general. Pharis and Kuo (5) cited numerous studies in which exogenously applied gibberellins were found to stimulate germination in a variety of conifer species. Le Page-Degivry (6) also found good evidence for an interaction between gibberellin and ABA in regulation of
seed germination in *Taxus baccata* L. Cytokinins have been less widely tested in conifers than gibberellins, and it has been mostly used BA, but not kinetin and with even more variable results, BA being promotive of germination in *Pinus taeda* L. (7) but not in *P. ponderosa* Dogl. (8) or *Taxodium distichum* (L.) Rich. (7).

The present work was carried out to test Khan’s hypothesis (1) in two species of conifers, red pine (*Pinus brutia* Ten.) and arbor vitae (*Thuja orientalis* L.), and to contribute to studies with conifers on this subject. Moreover, it has been also aimed to participate to very few studies with cytokinins in conifers by using two different cytokinins, BA and kinetin, and to compare these two growth regulators. For this purpose, the seeds of the mentioned species were germinated by adding kinetin, BA, and GA3 to the media of ABA preventing their germinations to a great extent, and the effects of these promoter hormones on ABA inhibition on the seed germination, hypocotyl emergence and the elongations of the radicle and hypocotyl of the seedlings were compared.

**Materials and Methods**

**The Seeds and Hormones**

In this work, seeds of red pine (*Pinus brutia*) and arbor vitae (*Thuja orientalis*) were used. The seeds were surface sterilized with 1.0 % sodium hypochloride.

As test solutions 50 µM kinetin and benzyladenine (BA), and 300 µM gibberellic acid (GA3) were used for both species. Concentrations of abscisic acid (ABA) were 15 and 30 µM for red pine, and also 80 and 100 µM for arbor vitae. Hormone concentrations were determined in a preliminary investigation.

**Germination of Seed**

25 seeds were placed in 10 cm Petri dishes lined by two sheets of Whatman No. 1 filter paper and containing sufficient amount of solutions of ABA at the two different concentrations preventing importantly the germination of the seeds, and of the mixtures of its with kinetin, BA, and GA3 alone or in combination. The seeds were left in an incubator to germinate at 20ºC, in dark for 12 or 15d (days) for arbor vitae and red pine, respectively. Reaching of the radicle a visible length, 1-2 mm, was accepted as a criterion of germination. The germination percentages were determined in a preliminary investigation.

**Results**

**Effects of Kinetin, BA, and GA3 on ABA Inhibition of Seed Germination**

The retardative and preventive effect of ABA on the germination of red pine (Table 1) and arbor vitae (Table 2) seeds was enormously overcome by GA3 alone. Neither BA nor kinetin were able to show a success at all the cases, except relative success of BA on 15 µM ABA inhibition of the final germination of red pine. BA, although it could not overcome ABA inhibition, was mostly statistically superior to kinetin in arbor vitae. However, while the combinations of BA and kinetin with GA3, even though they were not so successful as GA3 alone, exhibited success to varying
Effects of Kinetin, BA, and GA₃ on ABA Inhibition of the Hypocotyl Emergence

The inhibitory effect of ABA on the emergence of hypocotyl was importantly overcome by GA₃ alone in both species (Table 2). Kinetin and BA were unsuccessful at all the cases. The combinations of these two promoters with GA₃ overcame ABA inhibition on the hypocotyl emergence of red pine seedlings at the same degree in statistical, whereas in arbor vitae kinetin + GA₃ showed no success, but BA + GA₃ was rather successful in this species too. However, GA₃ alone was the most effective hormone at all the cases, except the successes of the combinations of BA and kinetin with GA₃ on the hypocotyl emergence of red pine seedlings in 30 µM ABA.

Effects of Kinetin, BA, and GA₃ on ABA Inhibition of the Radical and Hypocotyl Elongation

GA₃ played a primary role in overcoming ABA inhibition of the radical elongation except one case, 100 µM ABA in arbor vitae (Table 1,2). BA + GA₃ combination became more successful on the elongation of arbor vitae radicles in 100 µM ABA than GA₃ alone. GA₃, here, was secondarily important. Kinetin and BA, separately or in combinations with GA₃, exhibited success to varying extents. On the other hand, they had also failures at some cases, e.g., BA + GA₃ and kinetin in the cases of 15 and 30 µM ABA, respectively, in red pine, and also in arbor vitae kinetin at the both levels of ABA, and kinetin + GA₃ in 100 µM ABA. BA alone had statistically more effect in arbor vitae than kinetin.

The circumstance in the elongation of hypocotyl was not different from the previous findings. Here also, ABA inhibition was primarily broken by GA₃. Kinetin, except its success on the elongation of red pine hypocotyl in 30 µM ABA, and kinetin + GA₃ were unsuccessful at all the cases. BA + GA₃ showed an equal success to that of GA₃ in arbor vitae, even if it exhibited no success in red pine.

Discussion

In this work, it was observed that GA₃ alone, in contrast to expected, alleviated mostly excellently ABA inhibition on the germination and hypocotyl emergence, the radicle and hypocotyl elongations of seeds of red pine and arbor vitae, compared to kinetin, BA and the combinations of these two with GA₃. Kinetin and BA alone did not generally show a success on the inhibition of the germination and hypocotyl emergence. However, while kinetin became unsuccessful on the inhibition of the radicle elongation particularly in arbor vitae, BA was rather successful on this parameter in both species. The response of the hypocotyl elongation to both cytokinins was mostly negative, especially in arbor vitae. The combinations of these two cytokinins with GA₃ showed performances varying from failure to success. Though the germination and the radicle elongation of arbor vitae responded better to BA + GA₃ than kinetin + GA₃, the hypocotyl gave no response to kinetin + GA₃. In the case of red pine, the performances of these two combinations were about at the same degree at all the cases in general.
The findings obtained suggest that ABA and gibberellin interaction may have a competitive nature in conifers, at least in red pine and arbor vitae investigated. Le Page-Degivry (6) also demonstrated, indeed, this interaction in germination of Taxus baccata. Exogenously applied gibberellin counteracted the inhibitory effect of ABA in this species. In addition, it was reported that dormancy-breaking treatments in conifers usually cause a marked increase in levels of endogenous gibberellin (5, 6, 10). Cytokinin activity was also found to increase for seed of Pinus lambertiana Dogl. (10), P. sylvestris and Picea sitchensis (Bong.) Carr. (7), but not that of Pseudotsuga menziesii (Mirb.) Franco (10). On the other hand, Kabar (11) and Kabar and Baltepe (12) reported that exogenous application of kinetin was more successful in dicots, but GA3 in Gramineae in removing secondary dormancy imposed by environmental stresses such as high temperature and salinity.

The quantitative and qualitative responses of plants to different cytokinins may differ considerably. Thomas et al. (13) and Biddington and Thomas (14) demonstrated that BA is more active than any other cytokinin in germination and in breaking dormancy of celery and lettuce seeds. The seeds of red pine and arbor vitae used in this work gave generally a positive response to either BA nor kinetin singly in overcoming the inhibitory effect of ABA on their germination. In the germination and radicle elongation of arbor vitae in the medium of ABA, however, BA, although it was not successful on the germination, made more its presence felt than kinetin, and also BA + GA3 combination than kinetin + GA3. In addition, while arbor vitae hypocotyl gave no response to kinetin + GA3 combination, BA + GA3 became rather effective on this parameter. Red pine responded generally equally to these two combinations at all the cases. The reasons for these differences in activity are not clear. BA and kinetin singly may not probably be effective in red pine and arbor vitae. The different degree of effectiveness of different cytokinins and also different gibberellins as it will be indicated below may be due to variations in metabolism or to active forms or to differences in primary mechanisms of action. Alternatively, responses of plants to cytokinins and gibberellins and their different types may also change according to species, ecotype (e.g., halophytes, 15-17), and even presumably location of plants in systematic. It seems, therefore, more appropriate not to make a generalization including all plants about roles of these two promoters in germination and breaking dormancy of seeds.

On the other hand, gibberellins have the unique ability among plant hormones to promote extensibility of intact plants of many species. But several species of Pinaceae, e.g., Abies grandis Lindl., Pinus radiata Dor., Pseudotsuga menziesii, show few or no elongation responses to GA4 (5). However, they respond well to a mixture of GA4 and GA7 (18), e.g., Pinus elliottii. P. radiata. There are also conifers showing positive response to GA3, e.g., Pinus pungens Lam., P. strobus L., P. siberica L., Thuja occidentalis L., (7). It has not been encountered and report about the response of P. brutia and T. orientales to GA3. In this investigation, the seeds of these species responded very well to GA3 in removing both germination and seedling growth inhibition of ABA.

It has been emphasized before (1, 10) that seeds of the same or different species may contain different levels of gibberellins, cytokinins and inhibitors leading to various depths of dormancy, from no apparent dormancy to absolute dormancy. The seeds, therefore, should not be expected to give the same response to application of a gibberellin or cytokinin. There is evidence from plant tissues that during primary (4) or secondary dormancy (19-20) ABA is at a supra-optimal level and cytokinins and/or gibberellins at a sub-optimal level. Under this situation, addition of a favorable promoter hormone, cytokinin or gibberellin, might be expected to release the dormancy by restoring the promoter/inhibitor ratio to an effective level for removal of dormancy.

The most common response of cells to ABA is, as known, growth inhibition. Numerous studies concerning the possible importance of ABA in causing seed dormancy have been conducted (4, 21). Exogenous ABA is also a potent inhibitor of seed germination in many species. ABA can inhibit nucleic acid (22) and protein synthesis (23). Reversal of ABA inhibition of germination by cytokinin (24) or gibberellin (25, 26) may be accompanied by and increase in RNA synthesis. Moreover, both cytokinins (27, 28) and gibberellins (23, 29, 30) may also remove ABA-induced dormancy by elevating polyribosome level and protein synthesis. The function of ABA in controlling dormancy and inhibition of germination may be to prevent these basic metabolic processes which start very soon after the beginning of imbibition. Thus, germination will be inhibited in its early stages and dormancy will then be
marked by lowered metabolic activity. ABA, depending on its concentration, may have delayed and/or prevented the germination of red pine and arbor vitae seeds for the reasons mentioned, and in addition, may have blocked growth of the seedlings. GA3 may have also overcome these inhibitions by counteracting ABA through its mentioned and other actions.

There is also supporting evidence for a number of alternative mechanisms of cytokinins and gibberellins. For example, it was reported that both cytokinins (31) and gibberellins (32) may stimulate the growth and development by promoting cell division. Gibberellins can also be effective on the growth and development by causing and increase in hydrolytic activity (33) and in cell wall plasticity (34).

That gibberellins do also perform some functions such as stimulation of cell division and polysome formation, the effects at the level of transcription and translation of cytokinins put forward to have a permissive role under situations of endogenous and exogenous inhibition is interesting. The similarity of some functions of cytokinins and gibberellins seems like and insurance for life of plant so that if which of them is more effective in a plant, it will succeed these functions. As seen, the explanation of the problem is difficult and far from clear.

The obtained data in the present work and the others (11, 12) may serve to provide a new conceptual tool for designing hypotheses of seed dormancy and germination and for reinterpreting old concepts and findings.

References

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