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Research article

Naringenin inhibits seed germination and seedling root growth through a salicylic acid-independent mechanism in *Arabidopsis thaliana*

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ABSTRACT

Flavonoids fulfill an enormous range of biological functions in plants. In seeds, these compounds play several roles; for instance proanthocyanidins protect them from moisture, pathogen attacks, mechanical stress, UV radiation, etc., and flavonols have been suggested to protect the embryo from oxidative stress. The present study aimed at determining the role of flavonoids in *Arabidopsis thaliana* (L.) seed germination, and the involvement of salicylic acid (SA) and auxin (indole-3-acetic acid), two phytohormones with the same biosynthetic origin as flavonoids, the shikimate pathway, in such a putative role. We show that naringenin, a flavanone, strongly inhibits the germination of *A. thaliana* seeds in a dose-dependent and SA-independent manner. Altered auxin levels do not affect seed germination in Arabidopsis, but impaired auxin transport does, although to a minor extent. Naringenin and *N*-1-naphthylphthalamic acid (NPA) impair auxin transport through the same mechanisms, so the inhibition of germination by naringenin might involve impaired auxin transport among other mechanisms. From the present study it is concluded that naringenin inhibits the germination of Arabidopsis seeds in a dose-dependent and SA-independent manner, and the results also suggest that such effects are exerted, at least to some extent, through impaired auxin transport, although additional mechanisms seem to operate as well.

1. Introduction

Seed germination and seedling establishment are two closely related processes with enormous importance in many aspects of plant biology, from plant ecology to crop productivity [1]. In seeds, flavonoids protect the seed from pathogen attacks and UV radiation, prevent water uptake, and protect the embryo against oxidative stress, among many other functions (for review, see Ref. [2]).

Although seed germination is mainly driven by the opposite action of abscisic acid (ABA) and gibberellins (see Ref. [3] for a recent review), other phytohormones such as ethylene, salicylic acid (SA), auxins and jasmonic acid (JA) are also involved (reviewed by Ref. [4]). Among the hormones that have been reported to influence germination, SA and the auxin indole-3-acetic acid (IAA) share a common biosynthetic origin with flavonoids. The shikimate pathway uses trioses phosphate from the primary metabolism to produce chorismate in plastids. SA is synthesized from isochorismate, which derives from chorismate directly [5]. Chorismate can also yield arogenate, the immediate precursor of phenylalanine and tyrosine. Phenylalanine is the precursor of most plant polyphenols, including flavonoids [6]. SA can be also synthesized from the phenylalanine-derived benzoic acid [5]. Alternatively, chorismate can be transformed, through several precursors, into tryptophan: one —but not the only— IAA precursor [7].

Flavonoids, at least some, have been reported to bind transmembrane auxin efflux carriers thereby impairing their functionality and thus dampening polar auxin transport (see Ref. [8] for review). Although several reports point toward a role of auxins during seed germination [9–12], little is known about such a role, and the possible mechanism(s) underlying it. On the other hand, it is extensively reported that SA inhibits seed germination in a dose-dependent manner in many species [13–15], although the opposite effect has been also reported under salt stress conditions (see, for instance, Ref. [16]).

In the present study we aimed at determining the effect of flavonoids in the process of seed germination in the model species *Arabidopsis thaliana* (L.). We show that naringenin, a flavonoid that is precursor of most flavonoids, in particular of flavonols, in Arabidopsis, strongly inhibits seed germination of seeds. To determine the possible involvement of SA and IAA, two phytohormones

Abbreviations: ABA, abscisic acid; ACC, aminocyclopropane-1-carboxylic acid; IAA, indole-3-acetic acid; JA, jasmonic acid; NPA, *N*-1-naphthylphthalamic acid; SA, salicylic acid.

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biosynthetically-related to flavonoids, in the effects of exogenous naringenin, we used a combined approach of mutant lines and exogenous applications to conclude that the germination inhibition caused by naringenin is independent from SA, and that impaired auxin transport impairs germination, although to a minor extent compared to exogenous naringenin.

2. Results

2.1. Germination parameters

Wild type (WT) Arabidopsis seedlings showed a normal germination response, reaching 50% germination (G_{50}) by day 1.13 and a maximum germination percentage (G_{max}) of 98% (Figs. 1 and 2). Exogenous IAA did not affect significantly the G_{50} or the G_{max}, neither at 0.1 μM nor at 0.5 μM (1.07 and 0.97 days; and 99 and 98%, respectively; Figs. 1 and 2). Naringenin inhibited germination: at 0.2 mM WT seeds showed a G_{50} of 2.24 days and a G_{max} of 74% (Fig. 2). At 1 mM, naringenin strongly inhibited seed germination: the first seeds, a 2%, germinated by day 2, reached 50% germination by day 8.63, and showed a G_{max} of 33% (Fig. 2). Exogenous SA (1 mM) also impaired the germination of WT seeds: the first seeds (25%) germinated after 2 days, and reached a G_{max} of 44.74% (Fig. 2). In the presence of 1 mM SA WT seeds do not (and are not predicted to) reach 50% germination (hence this parameter is absent in Fig. 2A).

One mM naringenin strongly delayed the germination in *cyp79B2 cyp79B3*, *eds5-1*, *sid2-1* and NahG seeds, showing G_{50} values of 12.81, 5.12, 8.51 and 7.96 days, respectively (Fig. 2A). A lower dose of naringenin (0.2 mM) also increased the G_{50} values of *cyp79B2 cyp79B3* and *sid2-1* seeds (to 2.45 and 4.01 days, respectively), but not those of *eds5-1* or NahG seeds (1.94 and 1.85 days, respectively) (Fig. 2A). In the presence of 1 mM SA *cyp79B2 cyp79B3*, *eds5-1* and *sid2-1* seeds show G_{50} values of 8.85, 14.40 and 6.65 days, respectively (Fig. 2A). Transgenic NahG seeds, though, showed significantly lower G_{50} values (1.91 days), which are closer to those in plain MS medium (Fig. 2A).

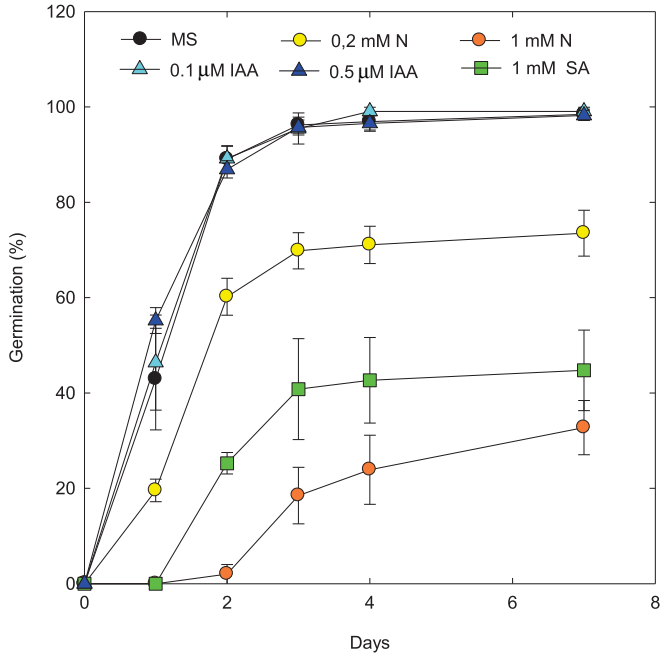

Fig. 1. Germination percentages of *Arabidopsis thaliana* Col 0 wild type seeds in MS medium and MS medium supplemented with 0.2 mM naringenin (N), 1 mM N, 0.1 μM indole-3-acetic acid (IAA), 0.5 μM IAA or 1 mM salicylic acid (SA). The values shown are the mean ± SEM of 6 or 7 replicates of 20–25 seeds each.

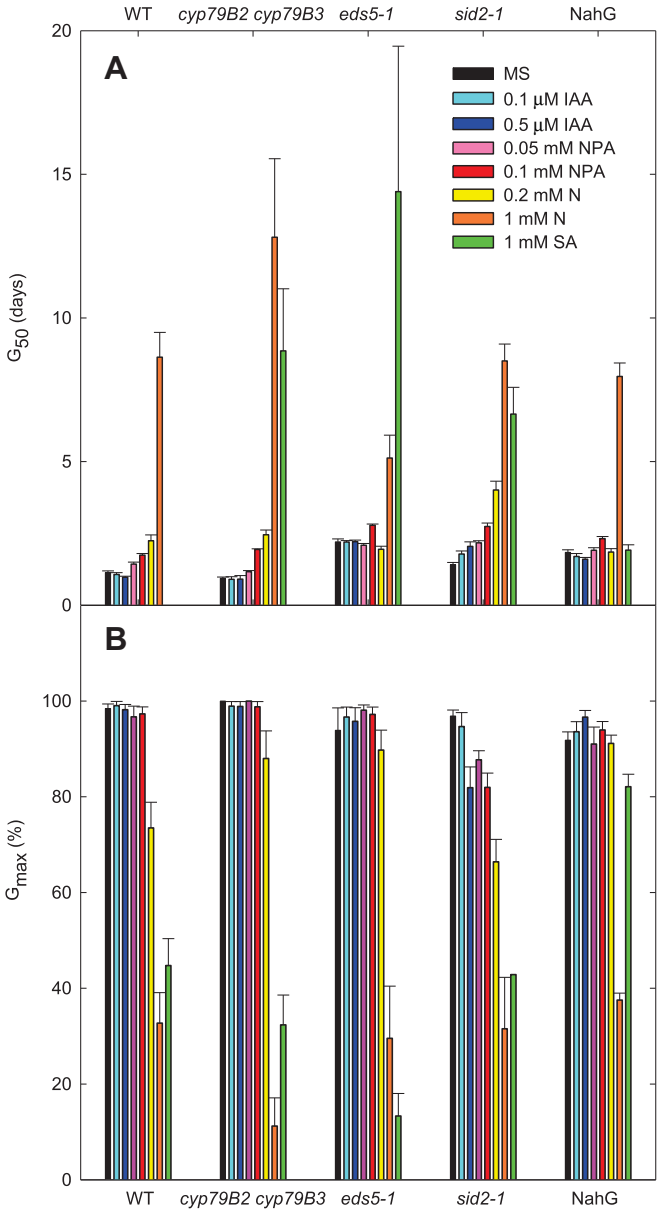

Fig. 2. Time required to reach a 50% germination (G_{50}; panel A), and maximum germination percentages (G_{max}; panel B), in *Arabidopsis thaliana* seeds of different genotypes in MS medium, or MS medium supplemented with 0.1 μM indole-3-acetic acid (IAA), 0.5 μM IAA, 0.05 mM *N*-1-naphthylphthalamic acid (NPA), 0.1 mM NPA, 0.2 mM naringenin (N), 1 mM N, or 1 mM salicylic acid (SA). The values shown are the mean ± SEM of 6–7 replicates of 20–25 seeds each. The G_{50} value calculated for SA-treated Col 0 wild type (WT) seeds is omitted since it does not reach 50% germination in the 7 days and, according to the nonlinear regression (see Materials and methods), it is not expected to reach such percentage. ANOVA tests show that differences in G_{max} and G_{50} among lines, treatments, and the interaction between both factors, are statistically significant ($P < 0.01$).

In WT seeds, the presence of 0.1 mM *N*-1-naphthyl phthalamic acid (NPA) increased G_{50} significantly from 1.13 to 1.74 days (Fig. 2A). This increment was also observed in *cyp79B2 cyp79B3*, *eds5-1*, *sid2-1* and NahG seeds (1.94, 2.78, 2.74 and 2.32 days, respectively). Exogenous IAA hardly affected the G_{50} in any of the lines tested, which was around 1 day in all cases (Fig. 2A and B).

All lines reached G_{max} values over 90% in MS medium. IAA and NPA treatments hardly affected G_{max} in any of the lines tested (Fig. 2B). 1 mM SA reduced the G_{max} to 32, 13 and 43% in *cyp79B2*