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Abscisic acid is a negative regulator of root gravitropism in *Arabidopsis thaliana*

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ABSTRACT

The plant hormone abscisic acid (ABA) plays a role in root gravitropism and has led to an intense debate over whether ABA acts similar to auxin by translating the gravitational signal into directional root growth. While tremendous advances have been made in the past two decades in establishing the role of auxin in root gravitropism, little progress has been made in characterizing the role of ABA in this response. In fact, roots of plants that have undetectable levels of ABA and that display a normal gravitropic response have raised some serious doubts about whether ABA plays any role in root gravitropism. Here, we show strong evidence that ABA plays a role opposite to that of auxin and that it is a negative regulator of the gravitropic response of *Arabidopsis* roots.

Plants can use environmental signals to direct their organ growth. One excellent illustration of such ability is the growth movement of *Arabidopsis* roots on the surface of agar-solidified media in response to various environmental factors [1,2]. *Arabidopsis* roots grow more or less in a straight downward line towards the center of gravity. In this case, the growth orientation is largely controlled by the gravitropic response. However, when seedlings are grown on a surface inclined at an angle from the gravity vector, roots no longer grow in a straight line; instead, they grow either in 'waves' with alternating left-to-right and right-to-left bends when the angle is less than 90°, or in coil-like loops when the angle equals 90° [1]. Such 'waving' and 'looping' root phenotypes are thought to be the combined effect of three different responses: a positive gravitropic response, referring to movement towards the center of gravity; a negative thigmotropic response, referring to movement away from an obstacle (also called the touch-avoiding response) [1]; and circumnutation, referring to a continuous, circular movement around the growth direction [3].

Root growth movement is affected by the availability of some resources, such as water [4–6]. For example, when *Arabidopsis* seedlings are grown in a closed chamber with a moisture gradient, their roots curve away from the lower water potential region [6]. This hydrotropic response may overcome the gravitropic response. Since water is vital for plant growth, it is not surprising that a plant uses such information to direct the growth movement of roots, the organ responsible for the acquisition of water. Nutrients are important resources for plant growth and are available mainly in the soil. There is considerable evidence that nutritional signals play important regulatory roles in root development [7–9]. Surprisingly, very little information exists about whether plants use specific nutritional signals as directional cues to control the orientation of root growth.

NO_3^- is the major N source available in aerobic soils and is known to play some important regulatory roles in root development [10–12]. In the course of investigating the effects of NO_3^- supplies on lateral roots, we consistently observed that a high concentration of KNO_3 in the medium promoted root waving. This led to a detail investigation of the relationship between high KNO_3 supply and root growth patterns. Here we present data showing that a high concentration of KNO_3 and ABA has a negative effect on root gravitropism and this effect is partly due to the high ionic strength and partly due to high NO_3^- signal.

Materials and methods

Plant materials and growth conditions. Seeds of *Arabidopsis thaliana* ecotype Col-0 and Landsberg were used as experimental controls. To analyze morphology, we used agravitropic mutants such as *aux1-7*, *eir-1*, *axr4-1*, *aba1-1*, *aba2-4*, and *aba3-2*. The basic mineral composition of the growth media used in this study was as previously described [13]. All media contained 5 g/L sucrose and 10 g/L agar-agar (Fisher Chemicals, A/1080/53); they were adjusted to a pH of 5.7 using 1.0 M KOH, autoclaved at 121 °C for 20 minutes, and supplemented with either ABA dissolved in 100% ethanol to a final concentration of 0.5 μM or NPA dissolved in dimethylsulfoxide (DMSO) to final concentrations of 0, 2.5, 5, 7.5,

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10, 12.5, and 15 μM. Seedlings were grown on the surface of these agar media in vertically placed Petri dishes. These dishes were then sealed with Nescofilm, with three openings of 0.3–0.5 cm to allow air exchange during growth, and kept in a growth room at 20 °C with a 16-h/8-h light/dark regimen with overhead lighting. Growth conditions in the dark were the same, except for the absence of light.

Root measures: To measures the amplitude of the root curves, seedlings were photographed using an OLYMPUS C5050 digital camera. The images were then downloaded to a PC and printed out on paper at 4× magnification and measured with a ruler.

Histochemical staining of GUS activity. Histochemical staining for GUS reporter activity was carried out as previously described by Beeckman and Engler [14]. Briefly, seedlings were incubated in

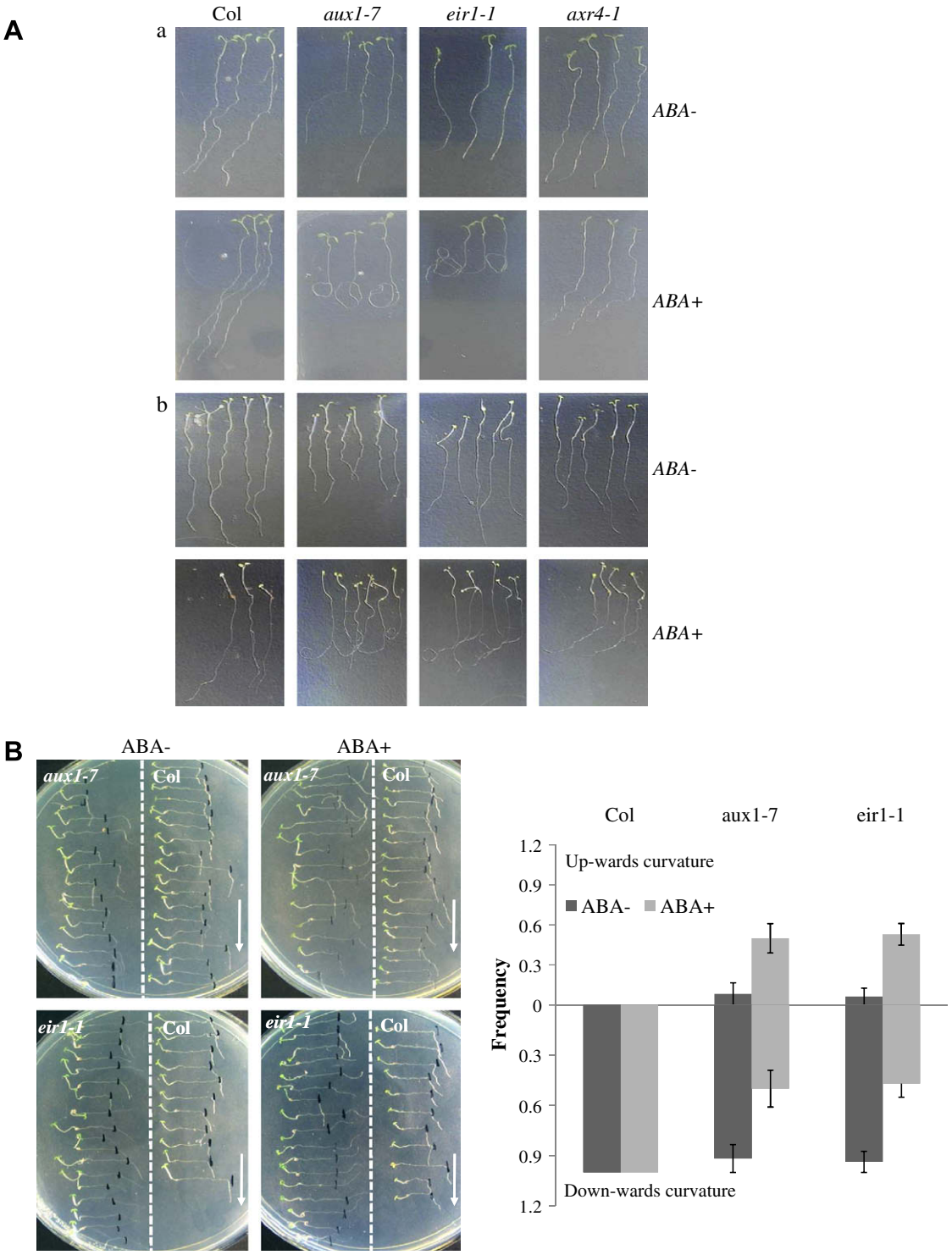

Fig. 1. (A) ABA dramatically affects the direction of root growth in wild-type and agravitropic mutants. (a) Growth under normal conditions. (b) Growth in the dark. Seedlings were first germinated and grown for five days on low KNO_3 medium, and were then transferred to ABA− and ABA+ media. All seedlings were 10 days old. (B) ABA affects the frequency with which horizontally placed roots of agravitropic mutants curve upwards or downwards on ABA− and ABA+ medium. Arrows indicate the direction of gravity. Seedlings were first germinated and grown for 5 days on low KNO_3 medium, and were then transferred to ABA− and ABA+ medium. Photographs were taken two days after the transfer.