



Soil effects on the plant growth inhibitory activity of S-abscisic acid

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Abstract

The use of natural plant growth regulators (PGRs) as ecofriendly agrochemicals is gaining much attention, but the fate of these compounds once they enter the soil environment is poorly understood. In this work, we compared the plant growth inhibitory activity of the phytohormone S-abscisic acid (S-ABA) in the presence of three soils with that observed in soilless (Petri dish) conditions and related the differences in activity to the sorption and dissipation processes of the phytohormone in the soils. In Petri dishes, S-ABA inhibited the germination of *Eruca sativa*, *Allium porrum*, *Lactuca sativa*, and *Hordeum vulgare* with mean inhibitory concentration values (IC_{50}) in the range of 0.5–8.2 mg/L. *Eruca sativa* was selected for subsequent studies based on its high sensitivity to S-ABA (IC_{50} = 0.5 mg/L). The inhibition of germination of *E. sativa* by S-ABA was fully reversible at a low phytohormone concentration (5 mg/L) and partially reversible at a higher phytohormone concentration (60 mg/L). S-ABA also inhibited the growth of pre-germinated seedlings of *E. sativa*, albeit at higher concentrations than those at which it inhibited germination. The three soils used in the study weakened the inhibitory activity of S-ABA by soil factors in the range of 0.008–0.380. As S-ABA displayed low or even negative sorption in the soils tested, the decrease in the activity of S-ABA was attributed to its biodegradation in the soils, rather than to a decrease in its bioavailability due to sorption. Despite the reduction in the activity of S-ABA observed in the presence of the soils, the phytohormone still expressed its activity at quite low soil concentrations (0.3–20 mg/kg), showing higher activity in soils where the compound degraded more slowly.

Keywords Bioactivity · Persistence · Phytohormones · Plant growth regulators · S-abscisic acid (S-ABA) · Soil

Introduction

Natural plant growth regulators (PGRs) are gaining growing attention in modern agriculture to optimize plant growth, increase crop resilience to biotic and abiotic stresses, and reduce the dependence on other agrochemicals such as pesticides or fertilizers (Das and Das 2018; Madaan et al. 2022; Zahid et al. 2023). In the current global scenario of climate change, the exogenous application of natural PGRs as seed, foliar, or soil treatments, as well as their in situ release by allelopathic plants and microorganisms, are seen

as eco-friendly strategies that can help crops improve nutrient use efficiency and stress tolerance, lessen the risks of crop failure, and ensure the profitability of farmers (Egamberdieva et al. 2017; Hussain et al. 2018; El-Sabagh et al. 2022; Dai et al. 2023).

In expanding the potential of natural PGRs in agricultural production, assessing how their activities are influenced by soil properties can be of primary importance. Despite this, the interaction of PGRs in the soil matrix represents a poorly understood area in natural PGR research. In particular, how sorption and dissipation processes determine the bioavailable concentrations of phytohormones in soils and how such concentrations relate to their activity in receiver plants is largely unknown (Lu et al. 2022). This gap of knowledge probably arises from the fact that traditional uses of PGRs have involved foliar applications, whereby related research has overlooked the role of soil in PGR activity. However, several innovative sustainable crop management strategies based on natural PGRs, such as those that imply their release into the soil by allelopathic plants and microorganisms,

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demand knowledge of their bioavailability and persistence within the rhizospheric soil matrix as essential information for an in-depth understanding of their activity and environmental fate (Kudoyarova et al. 2019; Facenda et al. 2023). Such knowledge will allow the identification of scenarios and strategies under which the direct application of natural PGRs to the soil or their release by allelopathic plants and microorganisms could successfully contribute to sustainable crop management.

S-abscisic acid (S-ABA) is a well-known phytohormone that regulates numerous aspects of plant development and stress responses, including seed and bud dormancy, organ size, and stomatal closure (Milborrow 1974). As a potent natural PGR, exogenous treatments with S-ABA have been investigated mainly in the context of alleviating abiotic stresses that cause important crop losses worldwide (Wani and Kumar 2015; Ibrahim et al. 2018; Yao et al. 2019; Zhang et al. 2022). It has also been proposed that S-ABA could exert allelopathic effects both upon its release from intact plants or microorganisms and through the decomposition of plant residues in soil (Kato-Noguchi et al. 2002; Hirai 2003). Recently, Oliveira et al. (2020) suggested the combination of pre-emergence herbicides with S-ABA as an integrated weed management approach to reduce herbicide selection pressure and/or herbicide failure.

Currently, S-ABA is authorized in many regions, including Australia, the European Union, and the United States, as an active substance for several uses as a PGR. However, little is known about the environmental fate of S-ABA once this compound has reached the soil environment (Hartung et al. 1996; Gámiz et al. 2016, 2017), and even less about how the sorption and dissipation processes S-ABA may suffer in soils could alter the strong activity this phytohormone displays under soilless conditions or upon its direct application to plant tissues (Hiradate et al. 2005).

On the starting hypothesis that knowledge of the sorption and degradation processes S-ABA may undergo in soils should help to a better assessment of its ecological functions and effects and expand its application as an eco-friendly agrochemical, the objectives of this work were: i) to compare the germination and seedling growth inhibitory activity of S-ABA to various model plant species (*Eruca sativa*, *Allium*

porrum, *Lactuca sativa*, and *Hordeum vulgare*) under Petri dish conditions, ii) to characterize the sorption and dissipation of S-ABA in three soils with different physicochemical characteristics, and iii) to assess the effect of the soils on the expression of the germination and seedling growth inhibitory activity of S-ABA compared to that observed under Petri-dish conditions. For the first time, this study addresses how the plant growth inhibitory activity of S-ABA is altered by the presence of different soils, establishing a link between the activity and the sorption and dissipation patterns of the phytohormone in the soils.

Materials and methods

S-ABA and soils

Absciscic acid (ABA) is a weak acid with a pK_a of 4.6, molecular mass of 264 g/mol, and water solubility of ca. 3.2 g/L at 25 °C (BPDB 2023). The abscisic acid used in this work was purchased from Merck (Madrid, Spain, ref. 862169) with a purity of 98%. The specific optical rotation (25 °C) of a 0.1 g/100 mL methanolic solution of the product, $[\alpha]_D$, was measured as $+409 \pm 1^\circ$ (c 0.1, MeOH), close to the value of $+430^\circ$ reported by Milborrow (1974) for pure S-ABA in acidic ethanolic solution. The enantiomeric purity of the chemical was confirmed by chiral high-performance liquid chromatography (HPLC), comparing the chromatogram of a 2 mg/L aqueous solution of the compound with that of a 2 mg/L standard solution of racemic ABA, purchased from Chromadex (USA, ref. ASB-00001014–010, purity > 99%). The results indicated that the enantiomeric purity of the S-ABA used in this work was > 99% (Supplementary Figure S1).

The soils were collected from three agricultural fields located in Southern Spain (soil 1: 37°17'00" N 06°03'58" W, soil 2: 37°16'54" N 06°03'59" W, soil 3: 37°42'50" N 06°31'34" W). Once collected (0–20 cm), they were air-dried, sieved to pass a 2 mm mesh, and characterized at the Analysis Service of IRNAS (Table 1). The sand, silt, and clay content of the soils were determined by the hydrometer method, the carbonate content by the pressure calcimeter

Table 1 Physicochemical properties of the soils used in the experiments

	Texture	Sand (%)	Silt (%)	Clay (%)	CaCO ₃ (%)	OC (%)	N (%)	WHC (%)	pH	Soil respiration (mg CO ₂ kg ⁻¹ wk ⁻¹)
Soil 1	Sandy loam	64	22	14	16.2	1.22	0.12	40	8.2	440
Soil 2	Sandy clay loam	74	5	21	nd	0.40	0.05	35	8.2	374
Soil 3	Sandy loam	75	12	13	nd	0.88	0.14	30	5.3	303

OC: organic carbon; WHC: water holding capacity; N: total nitrogen; nd: not detected

method, the organic C content by dichromate oxidation (Nelson and Sommers 1996), the total nitrogen content by the Kjeldahl method (Bremner 1996), and pH values were measured in 1 g:2.5 mL soil:water slurries (Table 1). Soil respiration values were determined by the alkali trapping method following the methodology described by Zibilske (1994).

Measurements of the S-ABA inhibitory activity in Petri dishes

The dose–response curves for the effect of S-ABA on germination of *E. sativa*, *A. porrum*, *L. sativa*, and *H. vulgare* were obtained through bioassays conducted in 9 cm-diameter Petri dishes. Each Petri dish contained one layer of filter paper, 12 seeds of the target plant (Vilmorin, France), and 6 mL of an aqueous solution of S-ABA at concentrations of 0 (control), 0.4, 1, 2, 5, 10, 30, or 60 mg/L (Supplementary Figure S2). The Petri dishes were sealed with Parafilm® and incubated in a germination chamber at 25 ± 2 °C and 16:8-h light:dark photoperiod for 6 (*E. sativa*, *L. sativa*, and *H. vulgare*) or 11 days (*A. porrum*). For each Petri dish, the number of germinated seeds (radicle or hypocotyl/coleoptile > 5 mm) and the average root length and shoot biomass were measured and expressed as a percent of the values obtained for the control. Each concentration treatment was conducted in triplicate Petri dishes. Typically, the percent of germination for the controls exceeded 80% after 3–4 days for *E. sativa*, *L. sativa*, and *H. vulgare* and 7–8 days for *A. porrum*.

For *E. sativa*, the reversibility of the complete inhibitory effect S-ABA displayed on germination at the concentrations of 5 and 60 mg/L was investigated. For this experiment, the (ungerminated) seeds of *E. sativa* that had been in contact with the 5 and 60 mg/L S-ABA solutions for 6 days in the germination test were thoroughly washed with water (until no S-ABA was detected in the leachate), and then re-incubated in Petri dishes containing 6 mL of water for an additional period of 6 days. The number of germinated seeds and their average root length and shoot biomass were measured and expressed as a percent of the values obtained for the controls, consisting of *E. sativa* seeds that had not been pre-treated with S-ABA.

The effect of S-ABA on the growth of pre-germinated seedlings of *E. sativa* was also determined. For this purpose, twelve seeds of *E. sativa* were pre-germinated for three days in Petri dishes containing one layer of filter paper and 6 mL of water. Next, the 6 mL of water were removed and replaced with 6 mL of an aqueous solution of S-ABA at concentrations of 0 (control), 0.4, 1, 2, 5, 10, 30, or 60 mg/L. Triplicate Petri dishes were prepared for each concentration treatment together with an additional triplicate control that was used to determine the average root length and shoot biomass of the seedlings after the 3-day pre-germination period. After incubating the Petri dishes for an additional period

of 6 days, as in the germination test, the increase in root length and shoot biomass of the pre-germinated seedlings were measured ($t=6$ days) and compared to the increase observed for the control.

Sorption and dissipation of S-ABA in the soils

The sorption of S-ABA on the soils selected for this study was determined by the batch equilibrium method at two initial phytohormone concentrations of 5 and 60 mg/L. In triplicate, 4 mL of the initial aqueous solutions of S-ABA (5 or 60 mg/L) were added to 14 mL-glass centrifuge tubes containing 4 g of soil and equilibrated by shaking in an end-over-end shaker at 25 ± 2 °C for 24 h. To mitigate potential experimental artifacts arising from the biodegradation of S-ABA during the sorption measurement, the tubes containing the soil samples were autoclaved 3 times at 121 °C and 200 kPa for 20 min before the addition of the initial S-ABA solutions. Following the 24 h-equilibration period, the tubes were centrifuged (15 min at $2000 \times g$) and an aliquot of the supernatant solution was withdrawn, filtered, and immediately analyzed by HPLC to determine the remaining concentration of S-ABA in the solution (C_{aq}). The chromatographic conditions used for the analysis are indicated in Supplementary Figure S1. Tubes with the initial S-ABA solutions without soil were also shaken for 24 h to serve as controls. The amounts of S-ABA sorbed by the soils (C_s) were calculated from the difference between the initial (controls) and final S-ABA concentration in solution.

The dissipation patterns of S-ABA in the soils were determined through an incubation experiment, which was also conducted in 14 mL-glass centrifuge tubes closed with Teflon® caps. Samples of 3 g of soil were introduced into the tubes and treated with 0.9 mL of an aqueous solution of S-ABA at the two initial concentrations of 5 and 60 mg/L, equivalent to initial concentrations in the soils of 1.5 and 18 mg/kg, respectively, and yielding a soil water content of 30%. For each soil type and application rate tested, independent triplicate tubes were prepared, sealed, and incubated in darkness at 25 ± 1 °C to be withdrawn from the incubator at $t=0, 1, 2, 3, 4$, and 7 days. Immediately after being removed, the tubes were frozen for subsequent extraction with a 70:30 0.01 M H_3PO_4 :acetonitrile mixture (8 mL) by shaking for 24 h in an end-over-end shaker. Next, the tubes were centrifuged (15 min at $2000 \times g$), and the supernatant was filtered and analyzed by HPLC to determine the concentration of S-ABA remaining in the soil at each incubation time. The recovery of this extraction procedure was > 90%, as determined from the comparison of the amount of S-ABA extracted at $t=0$ with the amount initially added to the soils.

Soil bioassays

The soil bioassays were conducted in plastic pots (20 cm² surface), each containing 20 g of soil. Triplicate soil pots were treated with 6 mL of aqueous solutions of S-ABA at concentrations of 0 (control), 1, 5, 10, 30, 60, or 100 mg/L, equivalent to soil application rates in the range 0–30 mg/kg (0–3 kg/ha) and yielding a soil water content of 30%, which corresponded to 75% of the water holding capacity (WHC) of soil 1, 85% of the WHC of soil 2, and 100% of the WHC of soil 3 (Table 1). Immediately after the application of the S-ABA solutions, 12 seeds of *E. sativa* were homogeneously distributed on the soil surface and the pots were incubated in the germination chamber (25 °C, 70% humidity, 16:8-h light:dark photoperiod) for 6 days. Throughout the experiment, the soil moisture content was re-adjusted daily to the value of 30%. Considering that water evaporation between two successive moisture adjustments typically reduced the water content of the soils to 50% of the WHC for soil 1, 55% of the WHC for soil 2, and 65% of the WHC for soil 3, aerobic conditions should have prevailed during the bioassay experiment for all soils. After 6 days, the number of germinated seeds and the average root length and shoot biomass for each pot were measured and expressed as a percentage of the values obtained for the control pots. The experimental conditions used to measure the effects of S-ABA on *E. sativa* in the presence of soil (number of seeds, volume of water, growth chamber conditions) were comparable to those used in the Petri dish bioassays except for the presence of soil (20 g) at a realistic soil water content (30%). For the concentration treatment at 60 mg/L, additional replicate pots were prepared for each soil to determine the concentration of S-ABA remaining in solution in the course of the bioassay ($t=0, 1, 2, 3$, and 4 days). At these times, 10 g of soil were taken from the pots and placed in Macrosep® Advance Centrifugal tubes (Pall Corporation) equipped with a PES membrane (0.45 µm), centrifuged (10 000×g for 15 min), and the filtrate was collected and immediately analyzed by HPLC to determine the concentration of S-ABA.

Data treatment

The sorption distribution coefficients, K_d (L/kg), for S-ABA on the soils were calculated as:

$$K_d = \frac{C_s}{C_{aq}} \quad (1)$$

where C_s (mg/kg) was the amount of S-ABA sorbed on the soils and C_{aq} (mg/L) was the concentration of S-ABA remaining in solution. Final S-ABA concentrations in solution (C_{aq}) lower than that of the initially added solution indicated positive sorption ($K_d > 0$), whereas final concentrations

exceeding that of the initially added solution denoted negative sorption ($K_d < 0$).

Based on the shape of the dissipation patterns obtained for S-ABA in the soils, dissipation data were fit by a sigmoidal three-parameter equation:

$$C = \frac{C_0}{1 + e^{-\frac{t-DT_{50}}{b}}} \quad (2)$$

where C (mg/kg) is the concentration of S-ABA in soil at time t (days), DT_{50} is the time required for the concentration of S-ABA to decrease to half of its initial concentration (C_0), and b defines the shape of the sigmoidal fit (Han et al. 2008).

A log-logistic equation was used to calculate the S-ABA mean inhibitory concentration (IC_{50}) value for the germination and root or shoot growth of the tested plants from the dose–response data:

$$y = \frac{y_0}{1 + \left(\frac{x}{IC_{50}}\right)^b} \quad (3)$$

where y and y_0 represent the average germination, root length, or shoot biomass (% of the control) measured for the treatments at a specific concentration (x) of S-ABA (mg/L) and at $x=0$, respectively, and the parameter b indicates the slope of the log-logistic dose–response decay curve (Seefeldt et al. 1995).

The “soil factor” defined by Hiradate et al. (2010) as:

$$\text{Soil factor} = \frac{IC_{50} \text{ in the absence of soil}}{IC_{50} \text{ in the presence of soil}} \quad (4)$$

was calculated as an indicator of the extent to which the inhibitory activity of S-ABA was weakened by the presence of the soils. A “soil factor” = 0 means that the phytohormone’s activity is completely suppressed by the presence of soil, whereas a value = 1 means that the soil has no effect on the phytohormone’s activity.

All sorption, dissipation, and bioactivity measurements were conducted in triplicate and standard errors were used to express variability among replicates. Curve fits of Eq. 2–4 to experimental data were performed using the regression application of SigmaPlot 14.5 for Windows. Sorption and bioassay data were subjected to one-way ANOVA followed by post-hoc Tukey’s HSD test for pairwise comparison between treatments ($P < 0.05$).

Results

Germination and seedling growth inhibitory activity of S-ABA in Petri dishes

Under Petri-dish conditions, S-ABA inhibited the germination of *E. sativa*, *A. porrum*, *L. sativa*, and *H. vulgare* with IC_{50} values varying in the range 0.5–8.2 mg/L (Fig. 1, Supplementary Table S1). *E. sativa* and *A. porrum* were more sensitive to S-ABA than *L. sativa* and *H. vulgare*, with both the radicle and hypocotyl emergence being similarly affected by the phytohormone (IC_{50} =0.5–0.7 mg/L). For *L. sativa*, S-ABA inhibited the radicle emergence (IC_{50} =1.6 mg/L) at lower concentrations than the hypocotyl emergence

(IC_{50} =3.1 mg/L), whereas for *H. vulgare* the coleoptile emergence (IC_{50} =2.6 mg/L) was inhibited at lower concentrations compared to the radicle emergence (IC_{50} =8.2 mg/L) (Fig. 1, Supplementary Table S1).

The average root length and shoot biomass reached by the seeds at the end of the germination experiment and the parameters resulting from fitting a log-logistic curve to the dose–response data are summarized in Fig. 2 and Supplementary Table S2, respectively. In general, the IC_{50} values were similar to those obtained for the inhibition of germination. This indicated that the phytohormone had little effect on root and shoot growth for the seedlings that germinated during the experiment. *H. vulgare* represented an exception, as the IC_{50} values obtained for the inhibitory effect of S-ABA on the average root length and shoot biomass

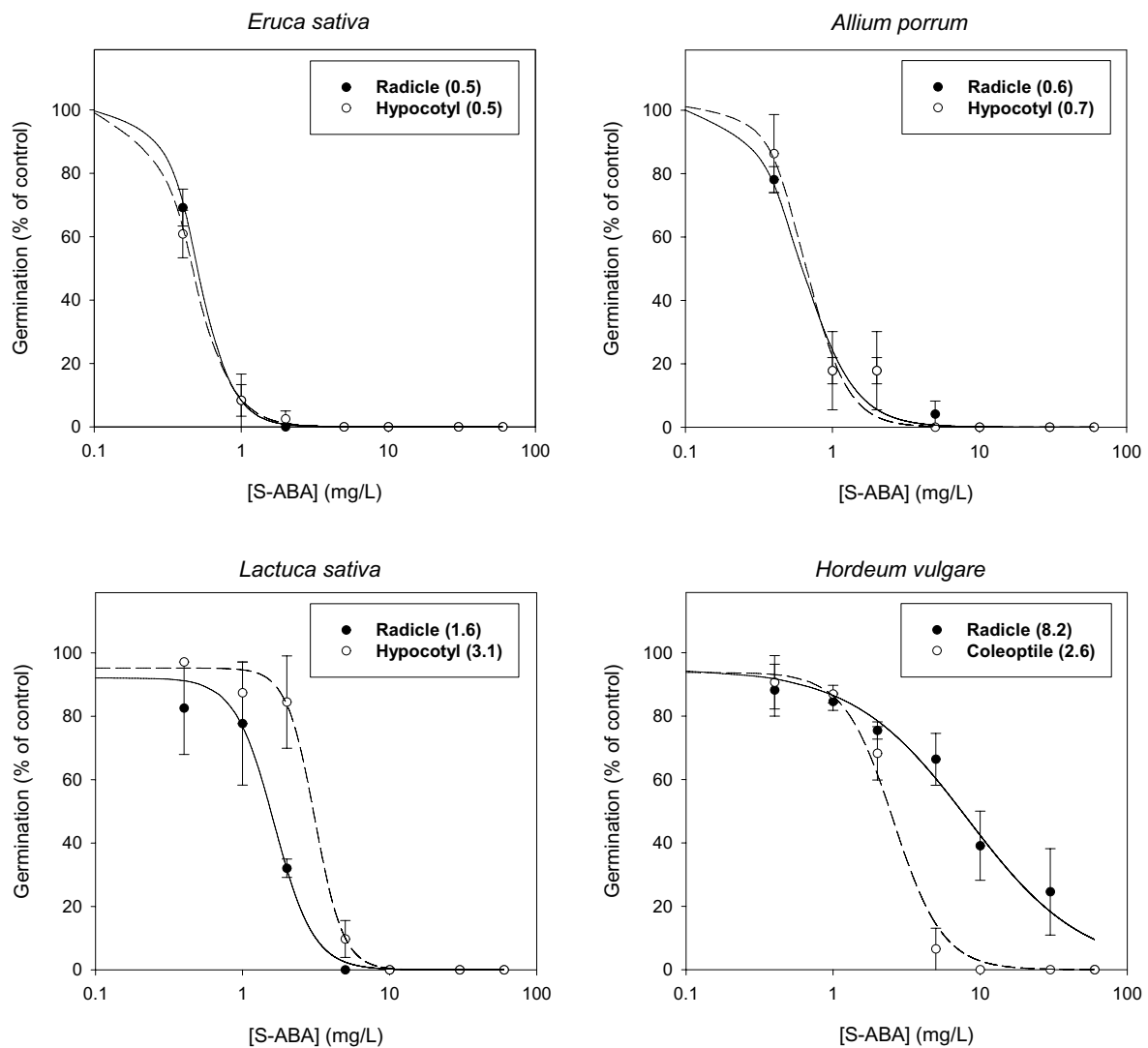


Fig. 1 Dose–response curves for the inhibitory effect of S-ABA on germination (radicle or hypocotyl/coleoptile appearance) of *Eruca sativa*, *Allium porrum*, *Lactuca sativa*, and *Hordeum vulgare* in

experiments conducted in Petri dishes. Numbers in parentheses are the IC_{50} values (mg/L) for the inhibitory effect on germination

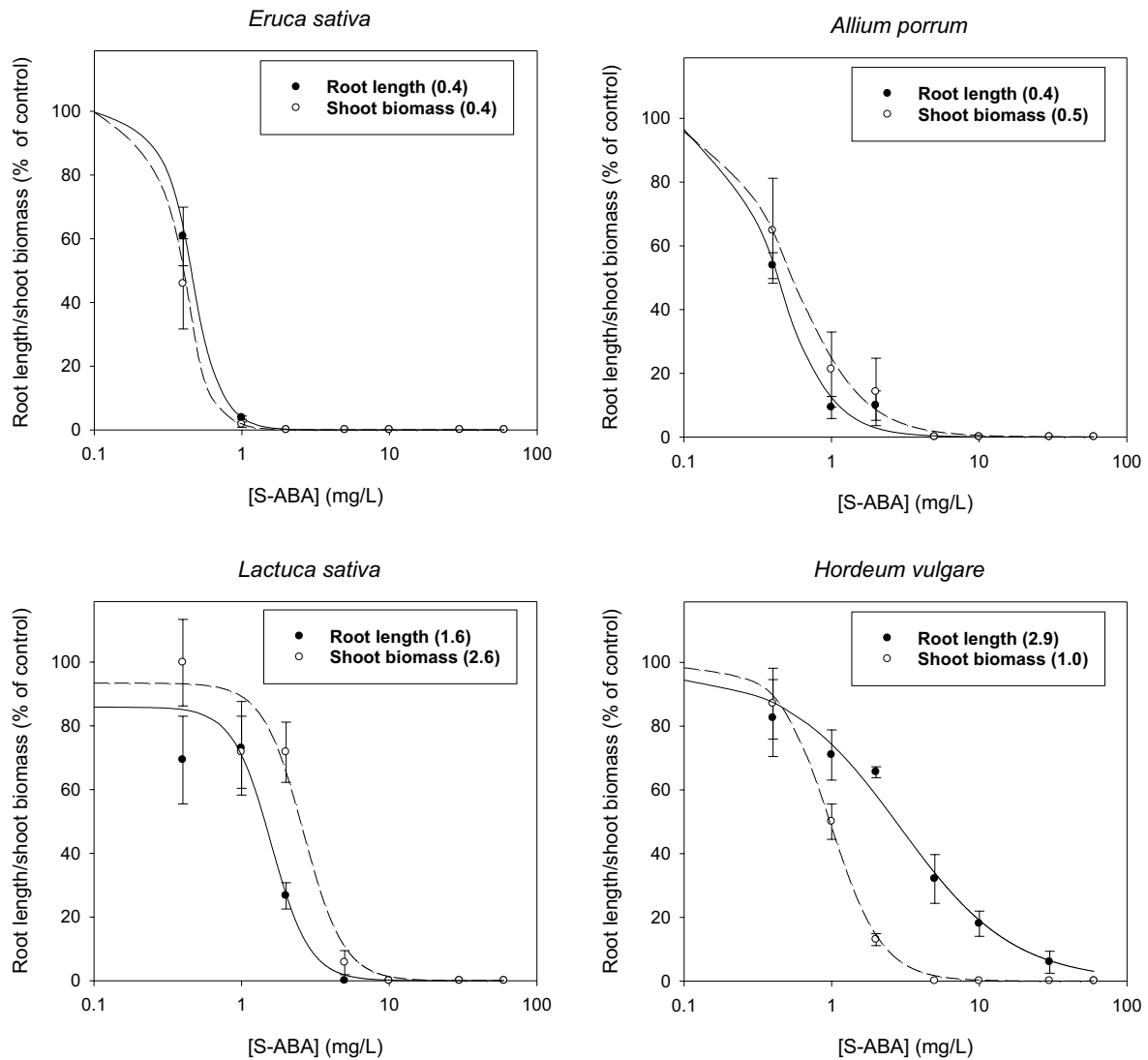


Fig. 2 Dose–response curves for the inhibitory effect of S-ABA on root length and shoot biomass of *Eruca sativa*, *Allium porrum*, *Lactuca sativa*, and *Hordeum vulgare* in experiments conducted in Petri

dishes. Numbers in parentheses are the IC_{50} values (mg/L) for the inhibitory effect on root length and shoot biomass

were 2.6–2.8 times lower than those obtained for germination (Supplementary Tables S1 and S2). This reflected that S-ABA significantly reduced the root and shoot growth at sub-inhibitory concentrations for germination.

For the most sensitive plant species (*E. sativa*), the reversibility of the complete inhibition S-ABA produced on seed germination at the concentrations of 5 and 60 mg/L was studied by thoroughly rinsing the treated seeds with water and then re-incubating the washed seeds for an additional period of 6 days. The results showed that the anti-germinative effect of S-ABA was completely reversible for the seeds treated with S-ABA at the concentration of 5 mg/L, and partially reversible for the seeds treated at the concentration of 60 mg/L (Fig. 3). It appears that some of the seeds treated with the higher concentration of S-ABA maintained

internal (or surface-adsorbed) levels of phytohormone that were sufficient to inhibit germination during the subsequent incubation period. Interestingly, the S-ABA pre-treated seeds that were able to germinate upon washing reached root lengths and shoot biomasses comparable to those measured for untreated seed controls (Fig. 3).

Finally, the root and shoot growth inhibitory activity of S-ABA on pre-germinated seedlings of *E. sativa* was determined. Both the root and shoot growth of the pre-germinated seedlings decreased with increasing concentrations of S-ABA in the solution (Supplementary Figure S3). The inhibitory effect on root growth was more pronounced than that on shoot growth, but in either case, it was significant only at phytohormone concentrations > 2 mg/L, i.e., greater

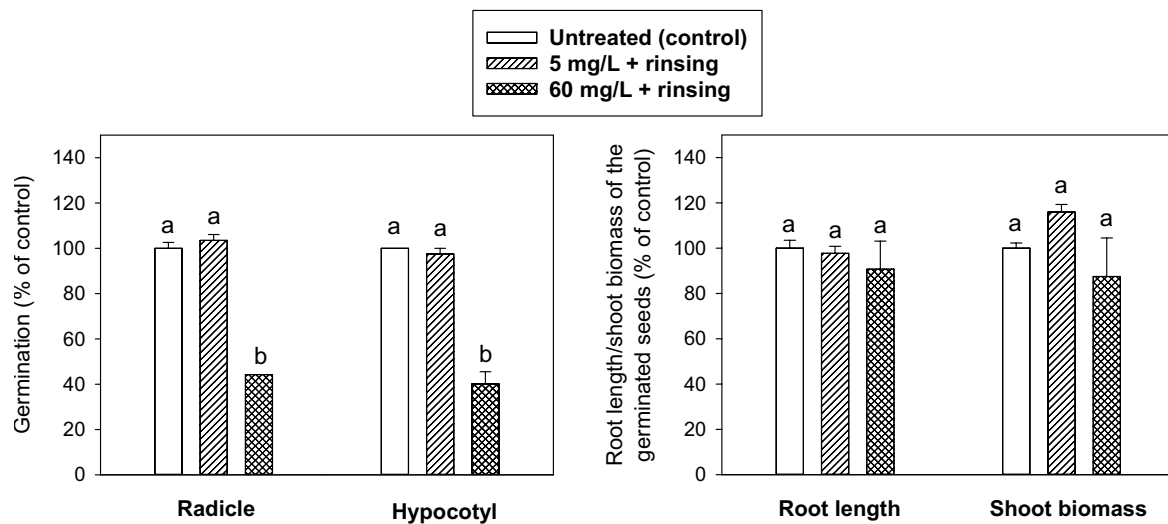


Fig. 3 Reversibility of the inhibitory effect of S-ABA on germination (radicle/hypocotyl appearance) and seedling growth (root length/shoot biomass) of *Eruca sativa* in bioassays conducted in Petri dishes. The seeds were soaked into 5 or 60 mg/L S-ABA solutions

for 6 days and then thoroughly rinsed with water before initiating the germination test ($t=6$ days). Different letters above the bars denote statistically significant differences

than those at which S-ABA completely inhibited the germination of the seeds.

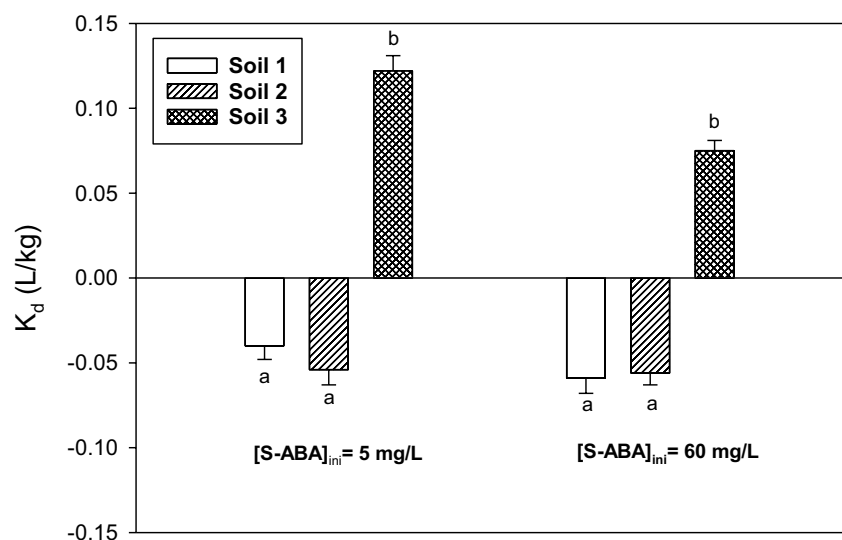
Sorption and dissipation of S-ABA in the soils

Figure 4 shows the sorption distribution coefficients for S-ABA on the three soils selected for this study measured at two initial phytohormone concentrations of 5 and 60 mg/L. S-ABA did not sorb on soils 1 or 2. In fact, the solution concentrations upon equilibration with these soils exceeded those of the initial solutions added, yielding negative sorption coefficients, K_d , in the range of -0.04 to -0.06 L/kg. In contrast, a low but measurable positive sorption

of S-ABA occurred in soil 3 ($K_d=0.07$ – 0.12 L/kg, Fig. 4). The decrease observed in the K_d value measured on soil 3 upon increasing the initial concentration of S-ABA from 5 to 60 mg/L (Fig. 4) reflected non-linear sorption that approaches saturation at high sorbate concentrations (Wauchope et al. 2002).

Figure 5 shows the dissipation patterns of S-ABA in the soils at a water content of 30% and two initial S-ABA concentrations in solution of 5 and 60 mg/L, corresponding to 1.5 and 18 mg S-ABA/kg soil, respectively. The dissipation data were well described by sigmoidal curves with determination coefficients $R^2 > 0.98$ (Table 2). DT_{50} values ranged between 0.7 and 8.4 days depending on the soil and the

Fig. 4 Sorption distribution coefficients for S-ABA on soils measured at two initial phytohormone concentrations (5 and 60 mg/L). For each initial concentration, different letters above the bars denote statistically significant differences between soils



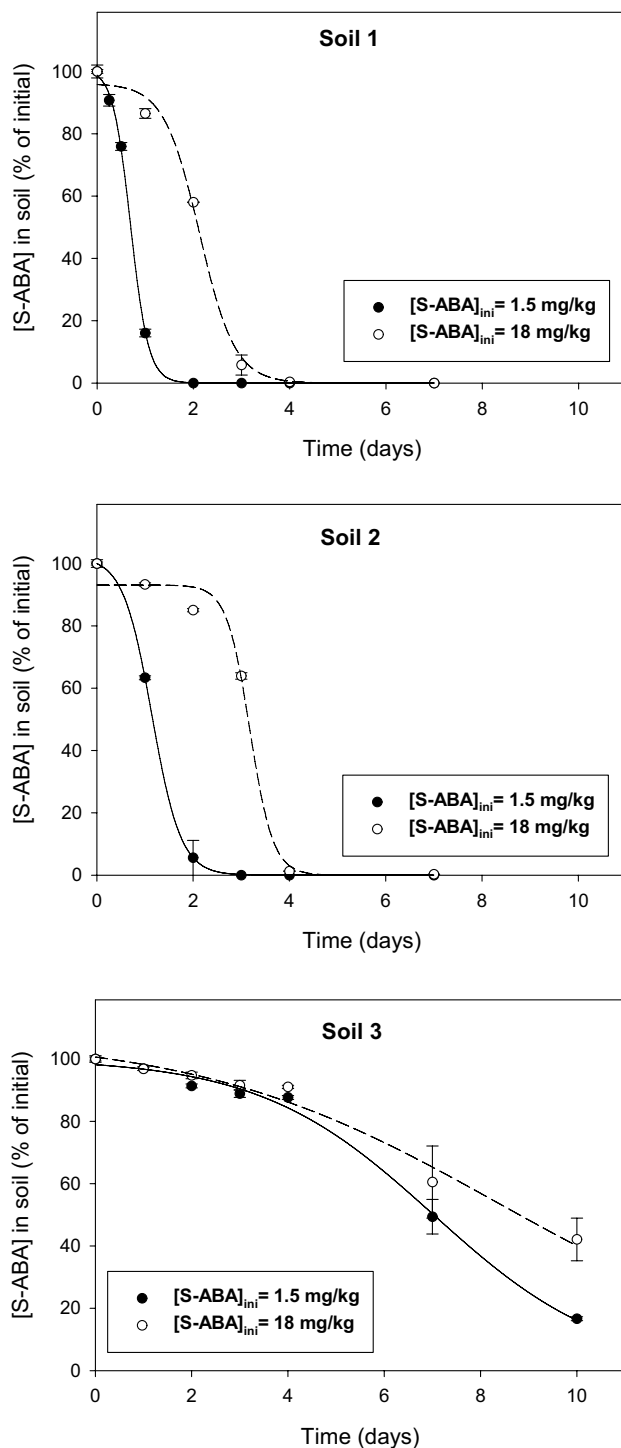


Fig. 5 Dissipation curves for S-ABA in soils at two initial phytohormone concentrations (1.5 and 18 mg/kg)

initial concentration of S-ABA. The persistence of S-ABA in the soils increased in the order: soil 1 < soil 2 < soil 3, and for all three soils, dissipation was faster at a lower initial concentration of the phytohormone.

Table 2 Constants obtained after fitting a 3-parameter sigmoidal curve to the S-ABA soil dissipation data

	C_0	b	DT_{50}	R^2
	(%)		(days)	
[S-ABA] _{ini} = 1.5 mg/kg				
Soil 1	101.1 ± 0.1	-0.185 ± 0.008	0.7 ± 0.1	1.000
Soil 2	102.2 ± 0.1	-0.299 ± 0.002	1.1 ± 0.1	1.000
Soil 3	100.2 ± 2.4	-1.807 ± 0.176	7.0 ± 0.2	0.995
[S-ABA] _{ini} = 18 mg/kg				
Soil 1	96.1 ± 3.7	-0.369 ± 0.076	2.1 ± 0.1	0.995
Soil 2	93.1 ± 3.5	-0.240 ± 0.118	3.2 ± 0.1	0.990
Soil 3	107.6 ± 6.8	-3.134 ± 0.626	8.4 ± 0.5	0.982

Value ± standard error of the calculated parameter

Soil bioassays

The dose–response curves and log-logistic 3-parameter constants (Eq. 3) for the inhibitory effect of S-ABA on germination and root and shoot growth of *E. sativa* under soil pot conditions (t = 6 days) can be compared in Fig. 6 and Table 3 with those obtained under soilless (Petri dish) conditions. In the presence of the soils, S-ABA inhibited the germination of *E. sativa* at higher concentrations than in the Petri dish experiment (Fig. 6 and Table 3). The soils weakened the anti-germinative activity of S-ABA yielding the following activity order: soil 1 < soil 2 < soil 3. In terms of soil factors (Eq. 4), values in the range 0.008–0.043 (Table 3) implied that IC_{50} for the inhibition of germination in the presence of the soils became between 125 (soil 1) and 25 (soil 3) times greater than those measured under soilless (Petri dish) conditions. Analyzing the germination data in terms of radicle or hypocotyl emergence led to similar conclusions (Table 3).

A relevant observation in the soil study was that, in contrast to what was found in Petri dishes, the IC_{50} values for the inhibitory effect of S-ABA on the average root length and shoot biomass in the presence of the soils were markedly lower than the values obtained for germination (Table 3). In soil 3, for example, the IC_{50} for the inhibition of radicle emergence was 11.6 ± 0.9 mg/L, whereas the IC_{50} for the inhibition of the average root length was 1.47 ± 0.28 mg/L (Table 3). This reflected that S-ABA provoked a reduction in the root and shoot growth of the seeds that germinated during the soil experiment. Again, this effect was soil-dependent, increasing in the order: soil 1 < soil 2 < soil 3 (Fig. 6 and Table 3).

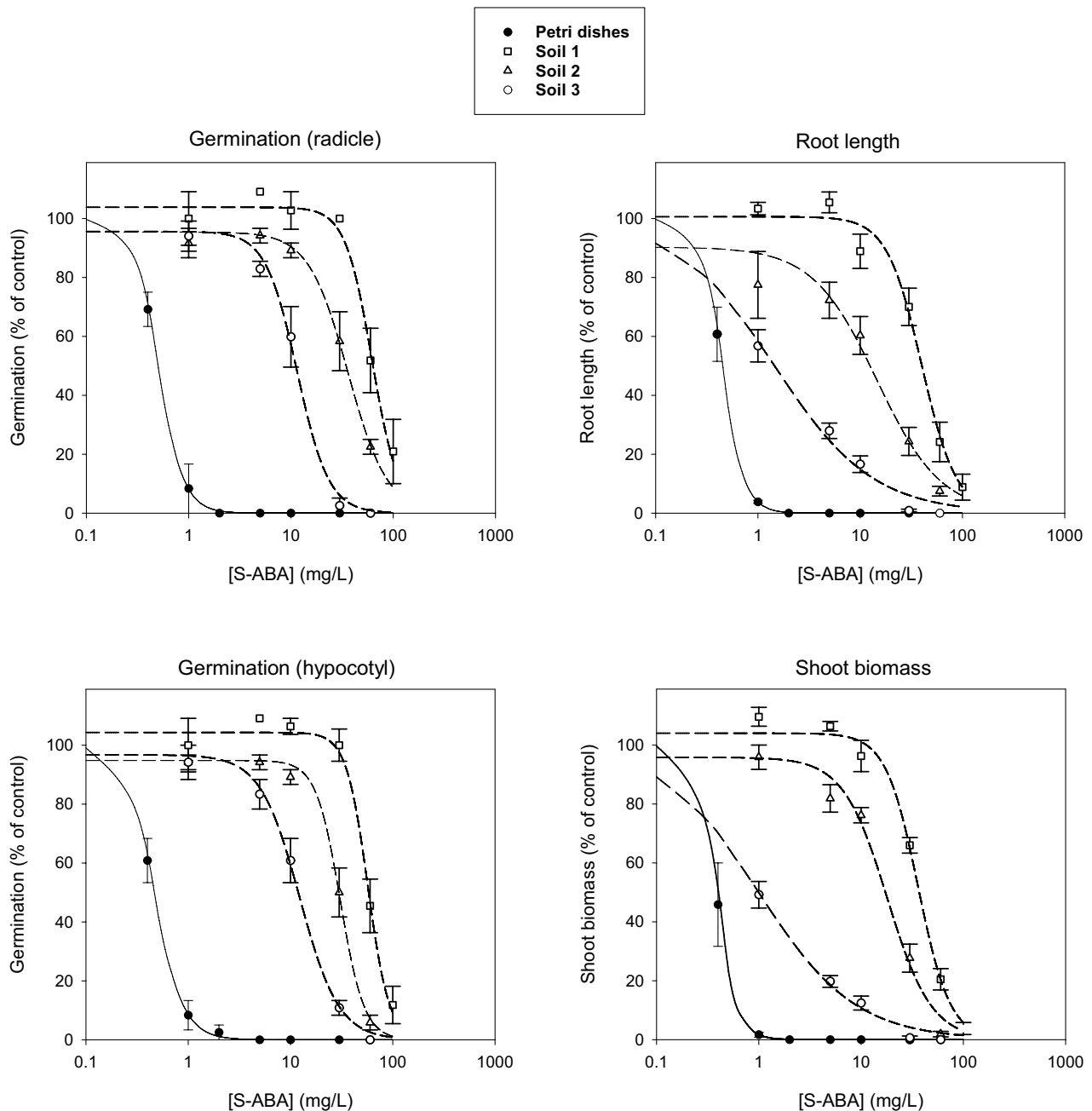


Fig. 6 Dose–response curves for the inhibitory effect of S-ABA on germination (radicle/hypocotyl appearance) and growth (root length/shoot biomass) of *Eruca sativa* under Petri dish conditions and in the presence of three soils ($t=6$ days)

Discussion

The strong inhibitory effect S-ABA exerted on the germination of *E. sativa*, *A. porrum*, *L. sativa*, and *H. vulgare* under Petri-dish conditions (Fig. 1) agrees with the well-known plant growth regulatory activity of this phytohormone. Nakano et al. (1995), for example, reported an IC_{50} for the inhibitory effect of S-ABA on the germination of *L. sativa* of 0.9 mg/L, and Finkelstein and Lynch (2000) observed that

adding ABA (mixed isomers) at a concentration of 0.8 mg/L was sufficient to limit the germination of *Arabidopsis* seeds to less than 10%. Hiradate et al. (2005) categorized S-ABA as one of the most active plant growth inhibitors among natural products. In this work, we observed that the inhibitory concentrations of exogenous S-ABA ranged from 0.5 mg/L for the radicle and hypocotyl emergence of *E. sativa* to 8.2 mg/L for the radicle emergence of *H. vulgare* (Fig. 1). Depending on the considered plant species, the radicle and

Table 3 Log-logistic 3-parameter constants for the effect of S-ABA on germination (hypocotyl appearance) and growth (root length/shoot biomass) of *Eruca sativa* in bioassays conducted in the absence and the presence of soil

	y_0	b	IC_{50}	R^2	Soil factor
	(%)		(mg/L)		
<i>Germination (radicle appearance)</i>					
Soilless (Petri dishes)	100 ± 1	3.520 ± 0.054	0.50 ± 0.01	1.000	-
Soil 1	104 ± 2	3.339 ± 0.587	62.2 ± 3.3	0.985	0.008
Soil 2	95 ± 2	2.259 ± 0.331	36.2 ± 2.2	0.992	0.014
Soil 3	96 ± 3	2.817 ± 0.575	11.6 ± 0.9	0.994	0.043
<i>Root length</i>					
Soilless (Petri dishes)	100 ± 1	4.003 ± 0.033	0.45 ± 0.01	1.000	-
Soil 1	104 ± 3	2.552 ± 0.452	40.0 ± 3.2	0.987	0.011
Soil 2	90 ± 7	1.384 ± 0.386	14.6 ± 3.6	0.961	0.031
Soil 3	100 ± 4	0.906 ± 0.122	1.47 ± 0.28	0.993	0.306
<i>Germination (hypocotyl appearance)</i>					
Soilless (Petri dishes)	100 ± 1	3.408 ± 0.089	0.46 ± 0.01	1.000	-
Soil 1	104 ± 2	4.148 ± 0.758	57.1 ± 2.4	0.991	0.008
Soil 2	95 ± 2	3.700 ± 0.851	30.7 ± 1.4	0.993	0.015
Soil 3	97 ± 2	2.266 ± 0.247	12.3 ± 0.7	0.997	0.037
<i>Shoot biomass</i>					
Soilless (Petri dishes)	100 ± 1	4.273 ± 0.026	0.38 ± 0.01	1.000	-
Soil 1	104 ± 2	2.771 ± 0.414	36.1 ± 2.2	0.993	0.010
Soil 2	96 ± 4	2.013 ± 0.386	18.1 ± 2.5	0.985	0.021
Soil 3	100 ± 3	0.919 ± 0.093	1.00 ± 0.13	0.997	0.380

Value ± standard error of the calculated parameter

hypocotyl/coleoptile emergence and growth were either equally or differently affected by the phytohormone (Figs. 1 and 2). Based on the particularly high sensitivity to S-ABA shown by *E. sativa*, this species was selected to characterize its response to the phytohormone in subsequent experiments under soilless and soil conditions.

Schopfer et al. (1979) showed that mustard seeds that had been kept dormant in a 5 mg/L solution of (±)-ABA for 3 days rapidly started the germination process after removal of the hormone. We observed a similar effect on *E. sativa* exposed to an external concentration of S-ABA of 5 mg/L for 6 days, but not when the concentration of S-ABA was increased to 60 mg/L. At this concentration, a significant fraction of the treated seeds remained ungerminated up to 6 days after removal of the hormone (Fig. 3), showing that the reversibility of the germination inhibitory activity of S-ABA was concentration-dependent. The seeds that germinated reached root lengths and shoot biomasses similar to the control (Fig. 3). This was in accordance with the observation that the concentrations of S-ABA needed to inhibit the root and shoot growth of pre-germinated seedlings of *E. sativa* were higher than those to inhibit the germination of the seeds (Supplementary Figure S3).

S-ABA displayed different sorption and dissipation behaviors in the three soils selected for this study (Figs. 4 and 5). The alkaline soils (soils 1 and 2) yielded negative sorption, while for the acid soil (soil 3) a measurable,

although low, positive sorption was recorded (Fig. 4). Negative sorption is a common observation in the interaction of anionic species with negatively charged surfaces, particularly when certain mechanisms, such as the formation of multivalent cation bridges, do not significantly contribute to the interaction. The sorbate is repelled from the sorbent surface, which in turn sorbs water in preference to the sorbate producing an increase in its solution concentration upon equilibration (Celis et al. 1999; Polubesova and Borisover 2009). At the alkaline pH of soils 1 and 2 (pH = 8.2, Table 1), S-ABA ($pK_a = 4.6$) should have predominated as anionic species (> 99.9%) and been repelled by the negatively charged surfaces of soil mineral and organic constituents, resulting in negative sorption (Celis et al. 1999, 2002; López-Cabeza et al. 2017; Galán-Pérez et al. 2021). The negative sorption of S-ABA measured in this work represented an apparent anion exclusion volume of ca. 0.06 mL per g of soil (Bolt and de Haan 1979; Polubesova and Borisover 2009).

At the acidic pH of soil 3 (pH = 5.3, Table 1), S-ABA should have existed partially (ca. 15%) as protonated species, and the neutral forms of organic acids usually have a higher affinity for soil particles than the ionized (anionic) ones (Real et al. 2019; Galán-Pérez et al. 2021). In addition, at acidic pH levels, some positively charged sorption sites may also arise on variable charge-mineral soil constituents, such as clay minerals and metal oxides, providing sorption

sites for anionic species. These mechanisms may have driven the positive, albeit low, sorption of S-ABA observed in soil 3. It should be noted that the relatively low organic carbon content of the soils used in this study (Table 1) may have contributed to the low or even negative sorption observed for S-ABA. It is likely that soils with higher organic carbon contents, especially when accompanied by low pH values, could exhibit increased sorption of the phytohormone due to interactions involving hydrophobic or hydrophilic regions of the soil organic matter.

To mitigate potential experimental artifacts arising from the biodegradation of S-ABA during the sorption measurements, the sorption experiments of this study were conducted with autoclaved soils. It is known that autoclaving can lead to alterations of soil properties and, consequently, of the sorption capacity of soils (Wolf et al. 1989). To address this issue, we conducted a 24-h experiment with soil 3, which revealed that the sorption of S-ABA on autoclaved soil ($K_d = 0.12 \pm 0.01$ L/kg) was not significantly different from that on non-autoclaved soil ($K_d = 0.13 \pm 0.01$ L/kg). Because soil 3 was the only soil exhibiting positive sorption of S-ABA (Fig. 4), along with negligible biodegradation of the phytohormone within 24 h (Fig. 5), it was considered the most suitable to conduct such a comparison. Furthermore, the negative sorption displayed by S-ABA on autoclaved soil 2 (Fig. 4) was reproduced in a short-term (3 h) sorption experiment with non-autoclaved soil, the duration of which prevented the degradation of the phytohormone. Based on these results, we concluded that potential alterations caused by autoclaving did not significantly impact the low-to-negative original sorption capacity of the soils used in this study for S-ABA.

The dissipation of S-ABA in the soils followed sigmoidal curves (Fig. 5), which are typical of the biodegradation of compounds that sustain the growth of the degrader organisms (Johnsen et al. 2013). Hartung et al. (1996) found that ABA was readily metabolized in soils with formation of the metabolites phaseic acid and dihydrophaseic acid, as observed in plant tissues. Another relevant (photo)-transformation product of ABA is *trans*-ABA, but it was not expected to be formed in our incubation experiments as these were conducted in the absence of light (Gámiz et al. 2016). The soil decomposition times observed in this work for S-ABA (Table 2) are in line with those previously reported for other allelochemicals, such as umbelliferone (0.2–2.1 days), coumarin (0.4–2.0 days), pulegone (0.8–2.0 days), or scopoletin (0.1–3.2 days) (Gámiz et al. 2021; Galán-Perez et al. 2021, 2022; Facenda et al. 2023).

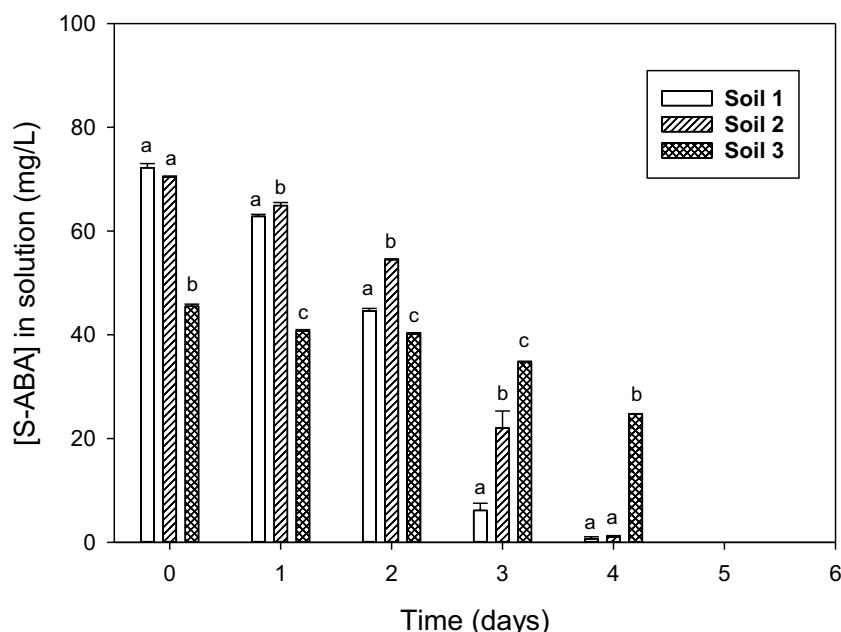
The dissipation rates of S-ABA in the soils used in this study followed the sequence: soil 1 > soil 2 > soil 3 and decreased when the initial concentration of phytohormone was increased from 1.5 to 18 mg/kg (Fig. 5 and Table 2). The faster dissipation of S-ABA in soil

1 and soil 2 ($DT_{50} = 0.7$ – 3.2 days) compared to soil 3 ($DT_{50} = 7.0$ – 8.4 days) probably reflects a combination of a high bioavailability of the phytohormone, as a result of its negative sorption (Fig. 4), and high microbial activity, as indicated by the soil respiration values compiled in Table 1. At a high concentration, S-ABA was more slowly consumed by soil microorganisms than at a low concentration (Fig. 5 and Table 2) most likely as a result of the existence a limited initial number of S-ABA degraders and the higher need for bacterial growth to complete the degradation of the compound (Johnsen et al. 2013; Wirsching et al. 2020).

The dose–response curves shown in Fig. 6 illustrate how the soils used in this study weakened the inhibitory activity of S-ABA compared to that observed in the Petri-dish study. The effect was soil-dependent, with soil 1 reducing the activity of S-ABA to a greater extent than soil 2 and this provoking a greater reduction than soil 3. According to the K_d values obtained in the batch sorption study (Fig. 4), S-ABA was expected to remain highly available in all three soils during the bioassays, with a fraction of phytohormone in the soil solution of 100% for soils 1 and 2, and 70–80% for soil 3. As, due to negative sorption, the fraction of S-ABA in an immediately available (non-sorbed) form was higher in soils 1 and 2 than in soil 3, we attributed the lower activity of S-ABA observed in soils 1 and 2 to a more rapid biodegradation of the phytohormone in these soils (Fig. 5).

That biodegradation processes rather than sorption determined the activity of S-ABA in the soils was supported by monitorization of the solution concentrations of S-ABA during the soil bioassays, conducted for the treatment at 60 mg/L (Fig. 7). Although the negative sorption in soils 1 and 2 implied a higher concentration of phytohormone in solution at the beginning of the experiment, the level of phytohormone in soils 1 and 2 decreased more rapidly with time than in soil 3. At $t = 4$ d, for example, the solution concentration of S-ABA had already been reduced to < 1 mg/L in soils 1 and 2, but remained as high as 25 mg/L in soil 3 (Fig. 7). This difference in the time-dependent concentration pattern among soils was expected to be exacerbated at lower S-ABA concentrations, for which the dissipation in soils 1 and 2 was particularly fast (Fig. 5). Finally, the fact that, in the presence of the soils, the reduction in average root length and shoot biomass of *E. sativa* exceeded that expected from the sole effect of the phytohormone on the extent of germination at the end of the bioassay (Fig. 6 and Table 3) reflected a reduced growth of the germinated seedlings, which can be explained by a delay in their germination when S-ABA was applied at low concentrations (Supplementary Figure S4), as germination did not start until S-ABA had been degraded to non-active concentrations in the soil.

Fig. 7 Changes in the solution concentration of S-ABA with time during the bioassay experiment with *Eruca sativa* in three soils. The concentration of the phytohormone solution added to the soils was 60 mg/L. For each time, different letters above the bars denote statistically significant differences between soils



Conclusions

The results of this study provide quantitative data on how the plant growth inhibitory activity of S-ABA was weakened by the presence of different soils and relate this to the sorption and dissipation patterns of the phytohormone in the soils. As S-ABA displayed negative to low sorption in the soils tested, its persistence, rather than its sorption, determined its activity in the presence of the soils. This conclusion, however, might not necessarily apply to soils with substantially higher organic carbon contents, in which the phytohormone could exhibit increased sorption as a result of its interaction with hydrophobic or hydrophilic regions of the soil organic matter. A general observation was that S-ABA delayed the germination of *E. sativa* until biodegradation reduced its concentration to sub-inhibitory levels in the soils. High application rates and low microbial activities delayed the dissipation of S-ABA in the soils favoring the expression of its activity. Despite the observed reduction in the activity of S-ABA produced by the presence of the soils, the IC_{50} values for the inhibition of germination of *E. sativa* in the soils tested in this study ranged between 11.6 and 62.2 mg/L, whereas those for the reduction of the average root length and shoot biomass at 6 days after treatment ranged between 1.0 and 40 mg/L. Under the experimental conditions used in the study, these values represented soil application rates between 0.03 and 2 kg/ha and soil concentrations between 0.3 and 20 mg/kg, which are significantly lower than those reported for many other inhibitory allelochemicals (Gámiz et al. 2021; Galán-Pérez et al. 2021, 2022). The fact that S-ABA could express its plant growth inhibitory activity at

such low concentrations in soils may be relevant in assessing its allelopathic functions and in expanding its application as an eco-friendly agrochemical. Soil treatments or delivery systems that protect S-ABA from rapid biodegradation processes could further enhance the residual bioactivity of the compound and its potential as a PGR agrochemical. Furthermore, elucidating the impact of the soil microbiome on S-ABA dissipation and discerning potential differences between the dissipation patterns in the proximity of germinating seeds or growing seedlings (rhizosphere) and the bulk soil are proposed as future research lines that may aid in advancing our understanding of the activity of this phytohormone in soil environments.

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Data Availability Data are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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