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Allelopathic potential and chemical profile of wheat, rice and barley against the herbicide-resistant weeds *Portulaca oleracea* L. and *Lolium rigidum* Gaud.

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Abstract

Background Weeds cause low crop productivity and increasing costs, and therefore, different solutions, such as manual weeding or synthetic herbicides, have been suggested to solve this problem. These methods involve high efforts and costs, in addition to being harmful to the environment in the case of herbicides, which also result in increasing resistance mechanisms in weeds. Therefore, this work addresses the use of in vivo allelopathic crops to control surrounding weeds. To carry out the experiments, co-cultivation of wheat, rice and barley with the monocot weed annual ryegrass (*Lolium rigidum* Gaud.) and the dicot weed common purslane (*Portulaca oleracea* L.) was conducted without physical contact among crop and weed plants. Germination and growth parameters of weeds, and growth parameters and chemical profile of crops, were analysed after the end of the experiment.

Results The three crops tested caused inhibitory effects on the two target weeds, and significant concentrations of benzoxazinoids were found in the plant tissues and/or root exudates of the different crops in response to the presence of weeds. All the crops showed different responses to the treatments. While the growth of rice was stimulated, barley was not affected, and wheat growth experienced inhibition due to the presence of weeds.

Conclusions This study demonstrates the capacity of wheat, rice and barley to inhibit both growth and germination of *L. rigidum* and *P. oleracea*. The effects observed could be due to the accumulation and/or exudation of benzoxazinoids such as DIMBOA, DIBOA, BOA or HBOA. Barley and rice are able to sustainably manage both target weeds without disrupting their development, while growth of wheat was affected by the presence of weeds. Based on our results, rice would be the most promising crop, since it has the ability to control weeds, while stimulating the development of rice plants. Nevertheless, more research should be carried out to fully confirm this fact, especially under non-controlled conditions.

Keywords Allelopathy, Sustainable weed management, Wheat, Rice, Barley, Agroecology, Annual ryegrass, Common purslane

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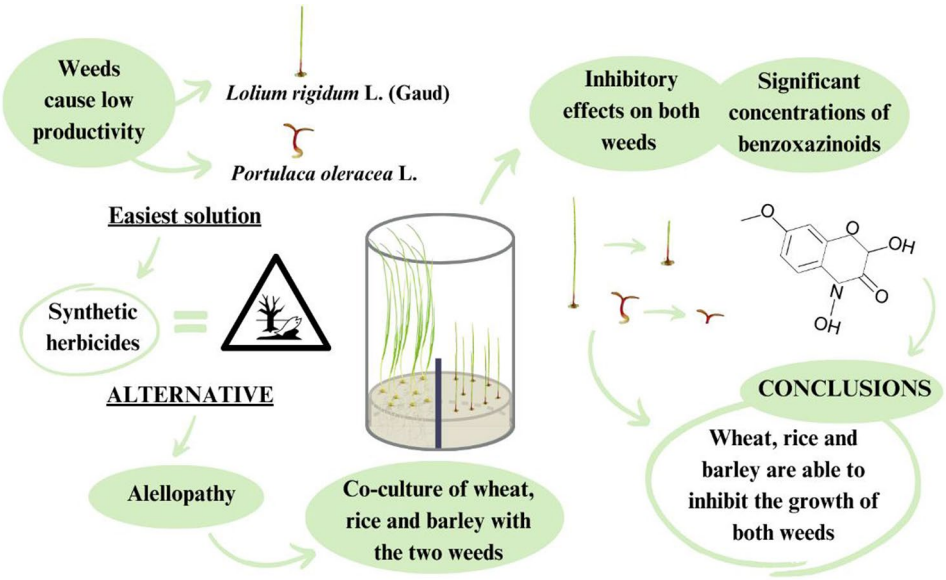
Graphical Abstract



Background

Weeds pose a significant challenge in agriculture, competing with crops for essential resources such as space, light, and nutrients, which in some cases may reduce productivity by up to 34% [1]. Various methods have been developed over the years to address this problem, including manual weeding, mowing, burning, etc. However, the need for continuous repetition, combined with the intensification of cultivation and the resulting increased labour required, has led to the popularization of herbicide use for weed management. In recent decades, the widespread use of chemical inputs has become a quick and effective way to manage weeds [1, 2]. These herbicides are chemical compounds that target specific sites in plant metabolism, effectively preventing weed growth before or after emergence [3]. However, despite their benefits, the uncontrolled use of synthetic herbicides poses significant problems not only for ecosystems and human health but also because weeds have developed resistance to different herbicide modes of action due to their continuous use [4]. For example, according to data from the "International Database of Herbicide Resistant Arable Crops", the monocotyledonous weed *Lolium rigidum* Gaud. (L.) has developed resistance to more than 10 different herbicide modes of action. The first case of herbicide resistance was reported in 1979 in Israel, where the annual grass grew out of control in roadsides and orchards due to resistance to herbicides such as atrazine and simazine, which act by inhibiting photosystem II (Seria 234-Blinders HRAC Group 5 (Legacy C1 C2)). Just three years later, in 1982, populations of annual ryegrass that could overcome 3 herbicides modes of action were reported in Australia. This was the first record of resistance of this species to acetyl CoA inhibitors (Group 1 - Legacy A), acetolactate synthase inhibitors (Group 2 -Legacy B) and microtubule assembly inhibitors (Group 3– Legacy K1). In the same year, resistance to deoxy-D-xylulose phosphate synthase inhibitors (Group 13– Legacy F4), microtubular organization inhibitors (Group 23– Legacy K2) and very long fatty acid synthesis inhibitors (Group 15– Legacy K3 N) were added to this list. In more recent years, it has been reported resistance to herbicides whose mode of action affects the inhibition of the enzyme lycopene cyclase (Group 34– Legacy F3), the inhibition of enolpyruvyl shikimate phosphate synthase (Group 9– Legacy G) and the inhibition of the photosystem I– electron deflection (Group 22– Legacy D) [5]. Similarly, *Portulaca oleracea* L., another target weed, has shown resistance to herbicides. This resistance was first reported in 1991 in the US, where this species affected carrots and vegetables showing resistance to atrazine and linuron, whose mode of action is the inhibition of photosystem II (Serine 264 Binding HRAC Group 5 (Legacy C1 C2). Also, in 1998, it was reported another biotype of linuron-resistant *P. oleracea*. Resistance to this mode of action suggests potential resistance to simazine, chlortoluron or diclofop-methyl, which have the same mode of action [5].

Herbicide resistance is an inherited trait in weeds, and it is the result of genetic mutations that naturally occur due to the prolonged exposure of plants to synthetic chemicals [6]. On the other hand, the continued use of herbicides for weed control is associated with

environmental pollution [2]. Most of the herbicides applied do not reach the desired organ or organism, but spread through the soil, the groundwater and the atmosphere, reaching subterranean water, lakes, rivers, and oceans, that become polluted in many cases in an irreversibly way [3, 7]. The excessive use of synthetic herbicides results in their undesired accumulation in food chains [8], concomitantly affecting non-target organisms. The damage of synthetic chemicals finally results in an imbalance of the entire ecosystem [8].

Due to the problems associated to the methods previously mentioned for weed control, it is mandatory to look for more sustainable alternatives. One of these alternatives could be the use of an intrinsic characteristic that several plant species possess, e.g., allelopathy. Allelopathy is a phenomenon of plant interference, which consists in the production, accumulation and/or release of specialized compounds, known as allelochemicals, either by exudation through the roots, leaching, decomposition of the tissues or volatilization, which can affect the development of surrounding plant species [9]. Of course, this phenomenon is also present in the agroecosystems among crop and weed species [2, 10, 11]. Agroecology and organic agriculture benefit from this phenomenon in different ways. For example, intercropping takes advantage of allelopathy when allelopathic plants are cultivated simultaneously or alternatively in a field for a certain period of time, so that they exude or release natural compounds with allelopathic capacity, allowing the sustainable management of weeds once the crop is rotated to a non-allelopathic one [1]. Mulching can be also another strategy benefited when allelopathic plants are used, which can allow the slow release of different chemical compounds through the complete decomposition of the decay, limiting the development of non-desired spontaneous herbs [12]. In this way, weed development could be managed with a consequent increase in crop productivity [1, 13].

Very important crops from the family Poaceae, which are present worldwide, have been thoroughly investigated for their remarkable allelopathic potential [14, 15]. This group includes, among others, barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), sorghum (*Sorghum* spp.) and oat (*Avena sativa* L.). Related to this, Bouhaouel et al. [16] found inhibitory effects in the root and shoot growth of *Bromus diandrus* Roth and *Stellaria media* L. due to barley root exudates. Germination and growth inhibition of *Echinochloa crus-galli* was observed by Rahaman et al. [17], who studied the allelopathic effect of multiple varieties of rice. Turky-ilmaz Unal and Bayram [18] also demonstrated the allelopathic capacity of wheat root exudates on two weeds, white mustard (*Sinapis alba* L.) and wild mustard (*Sinapis arvensis* L.), observing especially strong effects on the photosynthetic pigment system. Sorghum extracts were used by Tibugari and Chiduza [19] to test the allelopathic potential of this crop against *Eleusine indica* (L.) Gaerth and *Bidens pilosa* L., finding signs of growth inhibition in both weeds after the treatments.

Their allelochemical profiles include compounds such as the benzoxazinoids DIMBOA, DIBOA, MBOA, BOA, HMBOA, HBOA, and their derivatives, phenolic acids, and terpenoids [20–22]. Research on these specialized compounds, especially on benzoxazinoids, is thriving, as they appear to be able to sustainably manage different surrounding weeds [21, 23, 24]. Benzoxazinoids are classified into two big groups, benzoxazinones (i.e., DIBOA, DIBOA, HBOA, and HMBOA), and benzoxazolinones (i.e., BOA and MBOA). Besides, three subcategories can be found within benzoxazinones: e.g., hydroxamic acids (i.e., DIBOA and DIMBOA), lactams (i.e., HBOA and HMBOA), and methyl derivates [21]. This study will be mainly focused on the allelopathic role of the hydroxamic acids, the lactams and the benzoxazolinones, all of them named with the general term of benzoxazinoids (BZXs), which will be the term mainly used along this paper. BZXs have very relevant functions, such as defence against microscopic pathogens, herbivorous insects, and other competing plants [25–27]. The most phytotoxic BZXs are the hydroxamic acids followed by the benzoxazolinones, and the lactams [28]. The decrease in plant growth caused by these compounds may be due to multiple alterations into the plant metabolism of target plants, such as the interruption of mitosis, the rupture of key cellular organelles (mitochondria, nuclei and chloroplasts), or the induction of oxidative stress and induced senescence, among others [23, 29–31].

Since these substances have a short lifetime in the donor and the receptor plants [32], their phytotoxic potential under controlled conditions is crucial to elucidate how the amount and distribution of these specialized metabolites can affect the surrounding plants. Moreover, in vitro bioassays allow researchers to carefully manipulate key variables such as temperature, humidity and light to simulate ideal or specific growth conditions facilitating early detection of potential issues and optimization of growing conditions to maximize crop yield and quality, and to provide precise and detailed information on crop performance and characteristics in a controlled and reproducible environment.

However, although a lot of research has been done on the study of the allelopathic potential of extracts, residues or isolated compounds from wheat, barley and rice to control weeds [33–35], very few research has been done testing the in vivo ability of these crop plants to control the germination and/or development of surrounding weeds, and even less on the control of herbicide-resistant weeds. Therefore, is in this context that the present work

study the in vivo allelopathic potential of rice (commercial variety, Illa de Riu), barley (local commercial variety, Alto do Trigo Agrícola), and wheat (the variety Annie, provided by the European ECOBREED project, GA: 771367), with the aim of sustainably manage herbicide-resistant weeds, such as the dicot *P. oleracea* and the monocot *L. rigidum* without external inputs.

Methods

Germination and growth bioassays

Three crop species (wheat, rice, and barley) were selected to test their allelopathic potential against two weed species, the monocot *Lolium rigidum* Gaud. (L.) and the dicot *P. oleracea* L., by using, with slight modifications, the method of agar with equal compartments developed by Wu et al. [33].

Wheat seeds (*Triticum aestivum* L. var. Annie) were obtained from European project ECOBREED (771367; ecobreed.eu) in 2023. These seeds were previously selected due to their potential favourable properties for organic agriculture. Barley seeds (*Hordeum vulgare*) and rice seeds (*Oryza sativa* L.), previously selected for their high rates of germination, were obtained commercially in Alto do Trigo S.L. (Valladares, Spain) in 2023. Seeds of annual ryegrass (*L. rigidum* Gaud.) were commercially obtained from Herbiseed (Reading, UK) in 2021, and seeds of common purslane (*P. oleracea* L.) were purchased to Semillas Canthueso S. L. (Córdoba, Spain) in 2021.

Firstly, the seeds of the three crops were sterilized with 70% EtOH for 5 min, 4% bleach for 15 min, and finally washing twice with distilled water for 8 min each. Subsequently, seeds of each crop were germinated under different methods and conditions depending on the species. Wheat was germinated in 0.3% agar (pH 6.0) on a tray for 1–2 days, while rice and barley were germinated on petri dishes with filter paper and distilled water (4 mL each) for 2–3 days in the case of rice and 1–2 days for barley. The conditions for wheat were 22 °C with 16 h darkness and 8 h light, while rice and barley were germinated in darkness, with a temperature of 25/28°C for rice and 15 °C for barley. Once germinated (0.5 cm of radicle), eight seeds of each crop were transferred to one half of a 2 L glass beaker with 250 mL of 0.3% agar (nutrient-free and pH 6.0) under sterile conditions. The different crops were grown in plant cultivation chambers with different conditions and for a different period (i.e., wheat and barley were grown for 7 days, while rice was grown for 10 days). Regarding the conditions of the growth chambers, wheat was grown with 22 °C and 8/16 h light/darkness, while rice and barley were grown at the same temperature but with 16/8 h light/darkness photoperiod. After the established time, eight seeds of *L. rigidum* or *P. oleracea* were added to the other half of the beaker for the bioassays of non-pre-germinated seeds, while eight weed seedlings were added for the pre-germinated seed bioassays. Weed seeds were pre-germinated on petri dishes with filter paper and distilled water (4 mL each) for 1–2 days, in cultivation chambers with the same conditions as the crop they were going to be grown with. Crops and weeds were co-cultured for 7 days, although they were physically separated through a piece of plastic that allowed the diffusion of compounds throughout the agar medium but avoided physical contact of the different species [33]. The control of both weeds and crops consisted of eight seeds of each crop or weed placed in beakers alone and left to grow for the same time and under the same conditions than the treatments.

Three experiments were performed, one with each crop and all three of them were repeated five times with five replicates per treatment (Fig. 1):

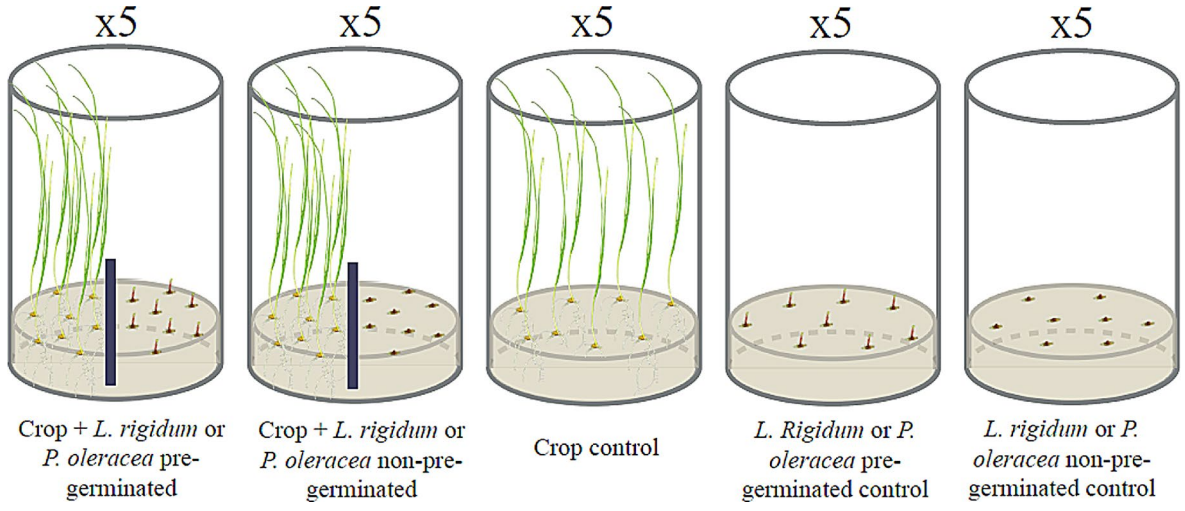


Fig. 1 Example of how the experiments were carried out during the study

1) Crop growing alone (control).
2) Crop seedlings + non-pre-germinated seeds of *L. rigidum* or *P. oleracea* (germination bioassay).
3) Crop seedlings + pre-germinated *L. rigidum* or *P. oleracea* (growth bioassay).
4) Pre-germinated *L. rigidum* or *P. oleracea* growing alone (growth bioassay control).
5) Non-pre-germinated *L. rigidum* or *P. oleracea* growing alone (germination bioassay control).

After harvesting, the length of shoots and roots of each plant (crops and weeds) was measured. Also, in the case of crops, the number of root tips was counted. After this, shoots and roots of the plants were freshly weighed, except for *P. oleracea*, where the whole plant was freshly weighed without separation of shoots and roots. Samples were then left at 70 °C (Gallenkamp, Hot Box Oven, Size 2) for 3 days, and weighed again to obtain the dry weight (DW)/fresh weight (FW) ratio.

The bioassay with non-germinated weed seeds was used to evaluate how the presence of each crop affects the germination process (G), or the invasiveness potential of each weed (SIC: shoot invasive capacity; RIC: root invasive capacity), which allows us to know the ability of each weed to colonize and occupy the surrounding space. To obtain these parameters all weed seeds were included in the equation (ungerminated seed after 7 days treatment = 0 cm for shoot or root length).

$$SIC = \sum shoot\ length\ of\ all\ treated - seeds/total\ EquationNumber\ of\ treated\ seeds \quad (1)$$

$$RIC = \sum root\ length\ of\ all\ treated - seeds/total\ EquationNumber\ of\ treated\ seeds \quad (2)$$

Bioassays with pre-germinated *L. rigidum* or *P. oleracea* seeds were used to evaluate how the growth of the weeds (SL: shoot length; RL: root length), and the weeds' weight (PW: plant weight) were affected in the presence of the different crops. As well, the seedling vigour index (SVI) of the weeds, which provides information on the viability of *L. rigidum* and *P. oleracea* seedlings to reproduce and establish under adverse conditions, was calculated according to the equation:

$$Seedling\ Vigour\ Index\ (SVI) = \%\ germination\ x\ (average\ shoot\ length + root\ length) \quad (3)$$

The data obtained in the bioassays were given as percentage compared to the control. For pre-germinated weeds bioassays, the values calculated were RL (root length), SL (shoot length), PW (plant weight), and SVI (seedling vigour index), while SIC (shoot invasive capacity), RIC (root invasive capacity), and G (germination) were calculated for non-pre-germinated weeds bioassays.

Extraction of benzoxazinoids (BZXs)

The extraction of the BZXs was done according to Hussain et al. [36] with slight modification, as follows:

Shoot and root profiles for wheat, rice and barley

Five replicates for shoots and roots of 300 mg each were cut into very small pieces and crushed with a pestle in a porcelain mortar with liquid nitrogen to get a powder, which was suspended in 10 mL of 1 mM HCl to macerate the plant samples. The final volume was collected into falcon tubes, cold sonicated (Branson Ultrasonic Corporation, Woonsocket, Rhode Island, USA) for 10 min, and cold centrifuged (10°) at 20,000 rpm (Sorvall RC 5B Plus; DuPont, Dalton, Georgia, USA) for 15 min. Then, the supernatant was extracted with 10 mL diethyl ether to obtain the aqueous and the organic layers after vigorous mixings. The organic phase was collected in a new falcon tube, and the process was repeated two more times with the rest of the sample to obtain approximately 30 mL of organic extract. Then, the solvent was evaporated with a multivapor (P-12; Buchi, Switzerland) under reduced pressure (456 mbar). The organic solvent was evaporated and condensed in an attached crystal balloon. Final volume of residual solution was approximately 1 mL and this solution was further dried with N_2. Once the solvent methanol was added to the residual powder, the samples were ready to be injected for LC-MS analysis.

Root exudates profiles for wheat, rice and barley

The samples were adjusted to pH 3.0 and cold sonicated for 15 min. Five replicates of 25 mL agar each were poured into separating funnels and 15 mL of diethyl ether was added to each sample, repeating the process once more. The agar samples were then treated in a similar way to those of roots and shoots samples.

Preparation of the samples for LC-MS analysis

The HPLC-MS was performed with a compact mass detector (TRIPLE QUAD 3500; AB SCIEX Instruments). Benzoxazinoids present in the samples were separated using a C18 column (Phenomenex Luna, 150 mm x 2 mm, 3 µm particle size; Phenomenex, Torrance, CA, USA) at 30 °C and with a flow rate of 300 µL min^{-1}. The column was equilibrated for 6 min between runs and 10 µL of sample was injected. A mixture of two solvents was used for gradient elution, first (A) being 78% acetonitrile in water and 20 mmol L^{-1} acetic acid, second (B) 3% acetonitrile in water and 20 mmol L^{-1} acetic acid. At the

beginning of elution, the conditions were 10% A and 90% B, which were maintained for 2 min and A was reduced to 50% at 9 min. Then A was modified and set up to 100% at 9.5 min until 14 min, returning to initial conditions after this time.

Statistical analysis of the results

The experiments were carried out using a completely randomized design with five replicates. The data were analysed using IBM SPSS software (SPSS Inc., Chicago, Illinois, version 25.0). An exploratory analysis of the data was performed to detect outlier values. The Kolmogorov-Smirnov test was used to check the deviation from normality and the Levene test to check the homogeneity. Depending on the homoscedasticity of the samples, ANOVA or Kruskal Wallis tests were performed to stablish the significant effect ($p \leq 0.05$) of the treatments (different crops).

Results

Germination and growth bioassays

As shown in Fig. 2, the three tested crops showed ability to impact the growth and development of the two target weeds, although, in general, root parameters were more statistically affected than shoot parameters for *L. rigidum* and *P. oleracea* in the presence of the three different crops tested (wheat, rice and barley). By contrary, barley was the only crop able to inhibit germination of at least

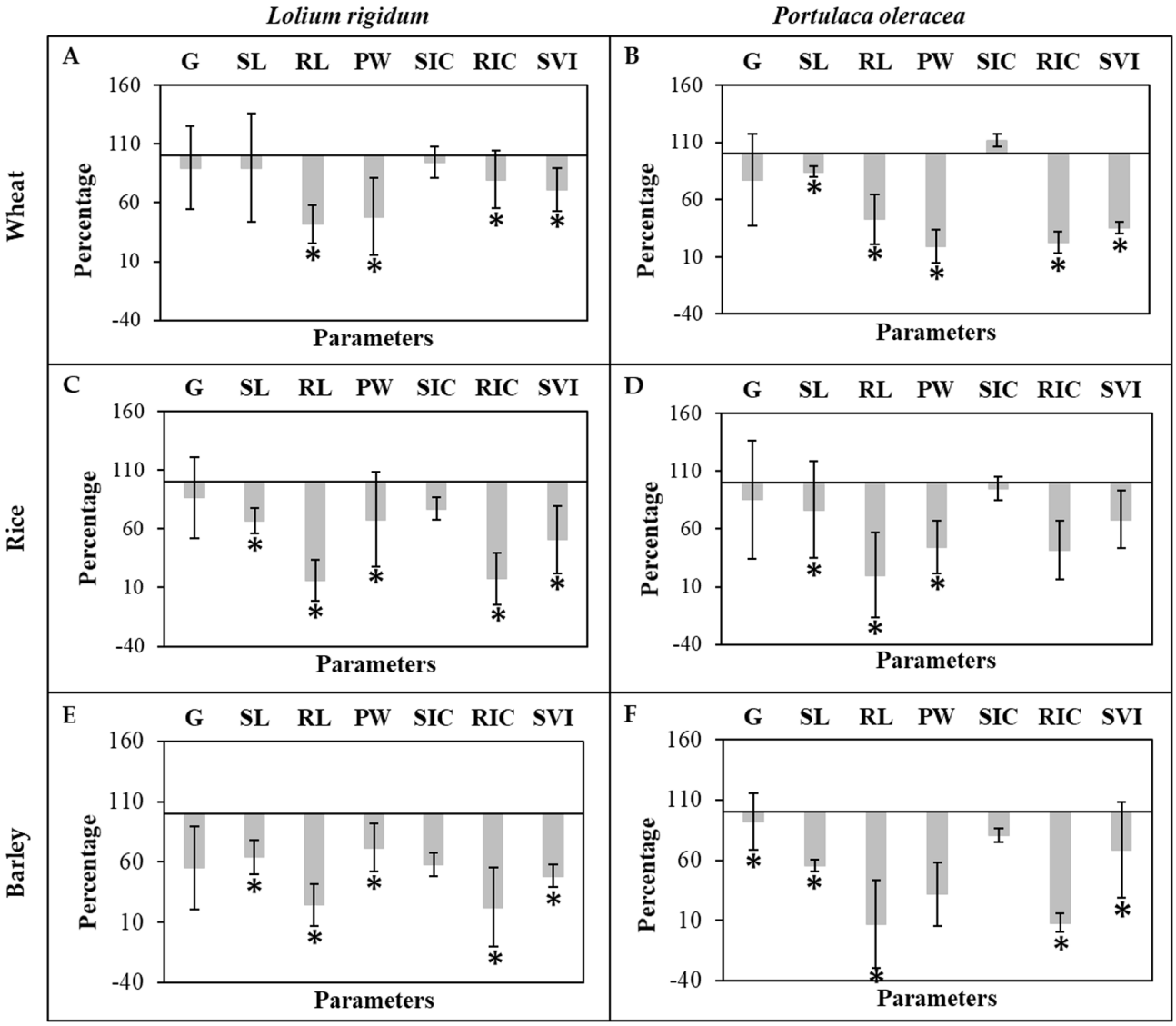


Fig. 2 Results represented as percentage of the control for each parameter from the co-cultivation of crops with weeds. (**A**) Wheat with *L. rigidum*; (**B**) wheat with *P. oleracea*; (**C**) Rice with *L. rigidum*; (**D**) Rice with *P. oleracea*; (**E**) Barley with *L. rigidum* and (**F**) Barley with *P. oleracea*. Parameters: germination rate (**G**), shoot length (SL), root length (RL), plant weight (PW), shoot invasive capacity (SIC), root invasive capacity (RIC), and seedling vigour index (SVI). Significant differences between control and treatments were determined by Kruskal-Wallis test (* $p \leq 0.05$). $N = 5$

one of the weeds tested (*P. oleracea*), as this parameter remained unaltered when the weeds were germinated in the presence of the other crops (Fig. 2).

In particular, co-cultivation of wheat with both weeds showed more parameters affected in *P. oleracea* (Fig. 2B) than in *L. rigidum* (Fig. 2A). Wheat adversely affected five parameters of *P. oleracea* while significantly reduced four parameters of *L. rigidum*. Statistically significant decreases were detected especially for PW and RIC of *P. oleracea*, with values of just 19.3% and 22.7% of the control. As well, SVI of *P. oleracea* was 35.2% of the control followed by RL (42.9%), and SL (84.4%). Nevertheless, the most significant reductions in *L. rigidum* were found for RL (41.8%), PW (48.0%), SVI (71.3%) and RIC (79.7%).

By contrary, after co-cultivation with rice, RL, RIC, SVI and PW were significantly lower than the control in *L. rigidum* with values of just 16.1%, 17.6%, 50.7% and 68.0% of the control, respectively (Fig. 2C), while only SL, RL and PW were significantly inhibited in *P. oleracea*, although the inhibitions found for these parameters were much stronger than in *L. rigidum*, especially for RL and PW, where values of just 20% and 44.5% of the control were found for RL and PW, respectively (Fig. 2D).

Finally, after barley cocultivation, both weeds, *L. rigidum* (Fig. 2E) and *P. oleracea* (Fig. 2F), were strongly inhibited. While SVI (68.8% of the control), SL (55.6%), PW (31.7%), RIC (8.0%), and RL (7.2%) of *L. rigidum* were significantly lower than the control (Fig. 2E), *P. oleracea* (Fig. 2F) showed strong significant decreases for SL (64.0% of the control), SVI (48.5%), G (55.36%), RL (24.5%), and RIC (22.3%).

The weeds were also scanned to evaluate visual effects after co-cultivation with the three different crops (Fig. 3). Control seedlings of *L. rigidum* (Fig. 3A) and *P. oleracea* (Fig. 3E) were compared to the same weed after growing with wheat (Fig. 3B and F, respectively), rice (Fig. 3C and G, respectively), or barley (Fig. 3D and H, respectively). In the case of *L. rigidum*, evident reductions of root length, but also of the number of roots were clearly seen in the co-cultured seedlings. Regarding *P. oleracea*, the results showed a strong reduction in root development but also an increased curliness of the plant.

At the end of the bioassays, growth parameters of crop plants were also measured to evaluate whether the presence of these two weeds could affect the shoot length (SL), root length (RL), number of roots (NR), shoot weight (SW) or root weight (RW) of wheat, rice, and barley plants.

Wheat was the most affected crop after growing with both weeds (Table 1). A significant decrease of RL, SW, and RW was observed after growing with *P. oleracea* seedlings, as can be seen in Fig. 4B. Wheat roots and shoots were shorter and narrower than the control (Fig. 4A) When co-cultured with *L. rigidum* seedlings (pre-germinated weed bioassays), significant reductions in RL were detected (Fig. 4C). After growing with *L. rigidum* seeds (non-pre-germinated weeds bioassay), root length did not significantly change, but the number of roots decreased (Fig. 4D). However, the results also showed an increase in the NR of this crop when co-grown with pre-germinated weeds. When wheat was grown with *P. oleracea* seeds, total length (shoot and root) and total weight (shoot and roots) of wheat plants decreased

Fig. 3 Scanned weedy seedlings. (**A**) *L. rigidum* control; (**B**) *L. rigidum* after growing with wheat, (**C**) *L. rigidum* after growing with rice; (**D**) *L. rigidum* after growing with barley; (**E**) *P. oleracea* control; (**F**) *P. oleracea* after growing with wheat, (**G**) *P. oleracea* after growing with rice; (**H**) *P. oleracea* after growing with barley

Table 1 Growth data of the different crops after co-cultivation with pre-germinated (P) or non-pre-germinated (NP) *Lolium rigidum* or *Portulaca oleracea* seeds. Different letters indicate significant differences between treatments for each crop. Asterisks represent significant reductions, while bold letters indicate significant increases when compared to the control ($p \leq 0.05$). $N = 5$. Shoot length (SL, cm), root length (RL, cm), number of roots (NR), shoot weight (SW, g) and root weight (RW, g)

Crop	Treatment	SL	RL	NR	SW	RW
Wheat	Crop alone	26.7 ± 0.2 a	18.5 ± 0.3 a	5.1 ± 0.3 b	1.10 ± 0.19 a	0.94 ± 0.33 a
	+*L. rigidum* P	26.6 ± 0.5 a	16.9 ± 1.0 b*	**5.7 ± 0.2 a**	0.98 ± 0.12 ab	0.67 ± 0.15 ab
	+*L. rigidum* NP	25.7 ± 0.8 a	18.1 ± 0.4 a	4.9 ± 0.2 c*	1.03 ± 0.17 ab	0.77 ± 0.22 a
	+*P. oleracea* P	24.4 ± 1.8 ab	15.6 ± 0.5 b*	5.4 ± 0.1 ab	0.89 ± 0.11 b*	0.50 ± 0.12 bc*
	+*P. oleracea* NP	21.9 ± 1.3 b*	12.9 ± 0.9 c*	**5.6 ± 0.3 a**	0.84 ± 0.14 b*	0.37 ± 0.09 c*
Rice	Crop alone	11.4 ± 0.5 b	3.9 ± 0.2 b	6.7 ± 0.5 b	0.31 ± 0.02 b	0.41 ± 0.05 a
	+*L. rigidum* P	**12.4 ± 0.3 a**	**4.1 ± 0.2 a**	7.1 ± 0.6 b	0.36 ± 0.06 ab	0.43 ± 0.07 a
	+*L. rigidum* NP	11.1 ± 0.7 b	4.7 ± 0.2 a	7.2 ± 0.4 b	0.33 ± 0.09 ab	0.43 ± 0.11 a
	+*P. oleracea* P	11.2 ± 0.4 b	3.8 ± 0.4 b	**7.9 ± 0.5 a**	**0.38 ± 0.03 a**	0.46 ± 0.04 a
	+*P. oleracea* NP.	**12.9 ± 0.6 a**	4.2 ± 0.2 b	**7.8 ± 0.1 a**	0.33 ± 0.02 b	0.41 ± 0.09 a
Barley	Crop alone	13.7 ± 0.4 a	4.1 ± 0.5 a	6.1 ± 0.1 a	0.92 ± 0.10 a	0.52 ± 0.07 a
	+*L. rigidum* P.	13.7 ± 0.3 a	3.7 ± 0.2 ab	5.7 ± 0.1 b*	0.86 ± 0.08 a	0.47 ± 0.07 a
	+*L. rigidum* NP.	13.3 ± 0.6 a	3.5 ± 0.5 b*	5.8 ± 0.2 ab	0.85 ± 0.09 a	0.44 ± 0.07 a
	+*P. oleracea* P.	13.5 ± 0.2 a	3.4 ± 0.4 ab	6.5 ± 0.2 ac	0.85 ± 0.08 a	0.46 ± 0.11 a
	+*P. oleracea* NP.	13.1 ± 1.1 a	3.6 ± 0.5 ab	6.0 ± 0.2 abc	0.87 ± 0.12 a	0.48 ± 0.05 a

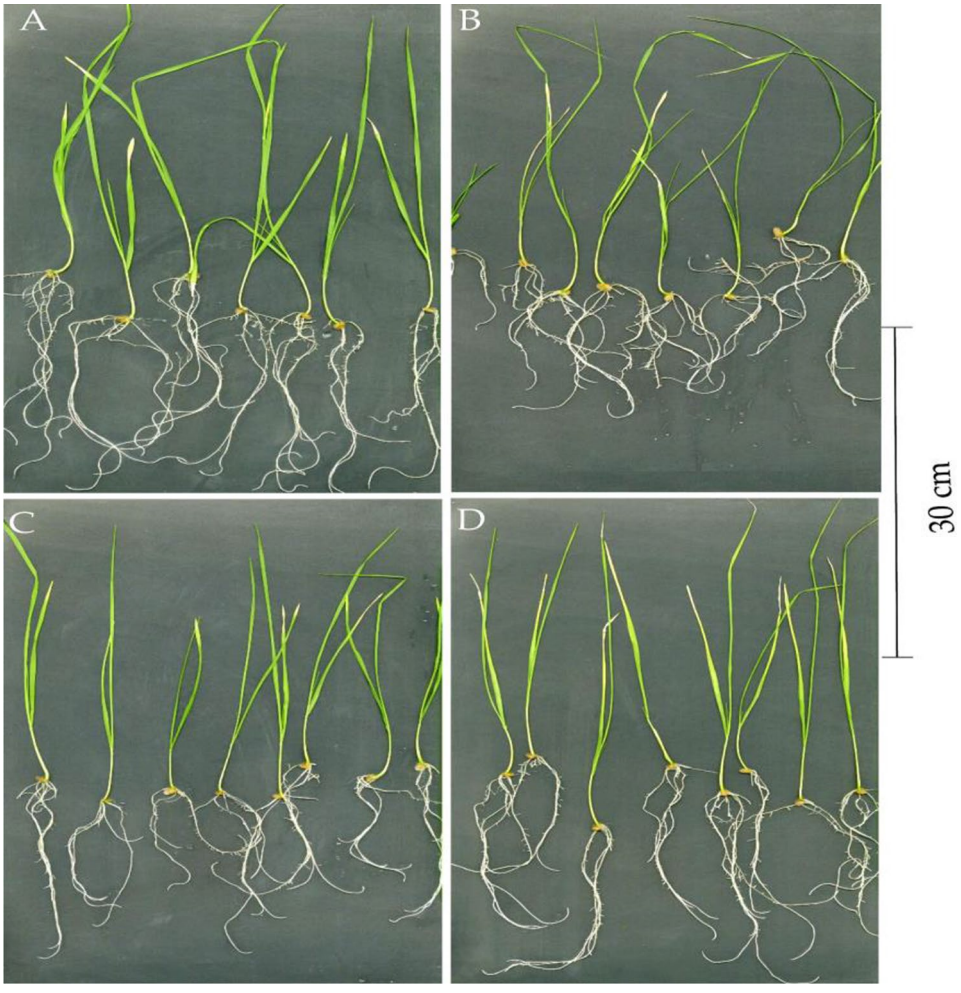


Fig. 4 Effects of the different treatments on wheat growth. (**A**) Wheat growing alone; (**B**) Wheat after growing with pre-germinated *P. oleracea*; (**C**) Wheat after growing with pre-germinated *L. rigidum*; (**D**) Wheat after growing with non-pre-germinated *L. rigidum*

significantly, while the number of roots was significantly higher than when growing alone.

Barley (Table 1) was however slightly affected by the presence of *L. rigidum*, as the inhibitions were only detected in NR with *L. rigidum* seedlings and in RL with *L. rigidum seeds*. As shown in Fig. 5, the effects on barley could be visually observed. When compared barley growing alone (Fig. 5A) with barley after growing with non-pre-germinated *L. rigidum* seedlings (Fig. 5B), a reduction in root length was observed, while root number did not decrease significantly (Fig. 5C). Finally, no significant effects were observed after growing with any *P. oleracea* treatment (Fig. 5D).

By contrary, growth and development of rice (Table 1) was especially favored by the presence of *L. rigidum* and *P. oleracea*, as many of the parameters measured were significantly stimulated in the presence of both weeds. As can be observed in Fig. 6, both root and shoot length of rice was stimulated after growing with pre-germinated *L. rigidum* (Fig. 6B). After co-culture of rice with non-pre-germinated *P. oleracea* seeds, rice had longer shoot and increased number of roots, while root length did not statistically change (Fig. 6C). Number of roots was also higher after growing with pre-germinated *P. oleracea* (Fig. 6D).

There were also notable statistically significant differences between crop's growth during the whole experiment. Wheat showed the greatest shoot length (26.7 cm), followed by barley (13.7 cm), and rice (11.4 cm) at the end of the experiment (Table 1). However, while wheat showed also the greatest root length (18.5 cm), rice and barley showed much lower values, which were similar for both crops (3.9 and 4.1 cm, respectively). By contrary, rice (6.7) was the crop which showed the highest statistically significant number of roots, followed by barley (6.1) and wheat (5.1). Regarding the weight of the aerial part, the values were similar for wheat (1.10 g) and barley (0.92 g), being statistically higher than rice (0.31 g). Finally, root weight was similar for all the crops, despite all the above-mentioned differences in root length and root number among wheat, barley and rice.

Chromatograms of chemical characterization of phytotoxic compounds

The benzoxazinoids DIMBOA, BOA and MBOA were chromatographed, their peaks and their relative retention

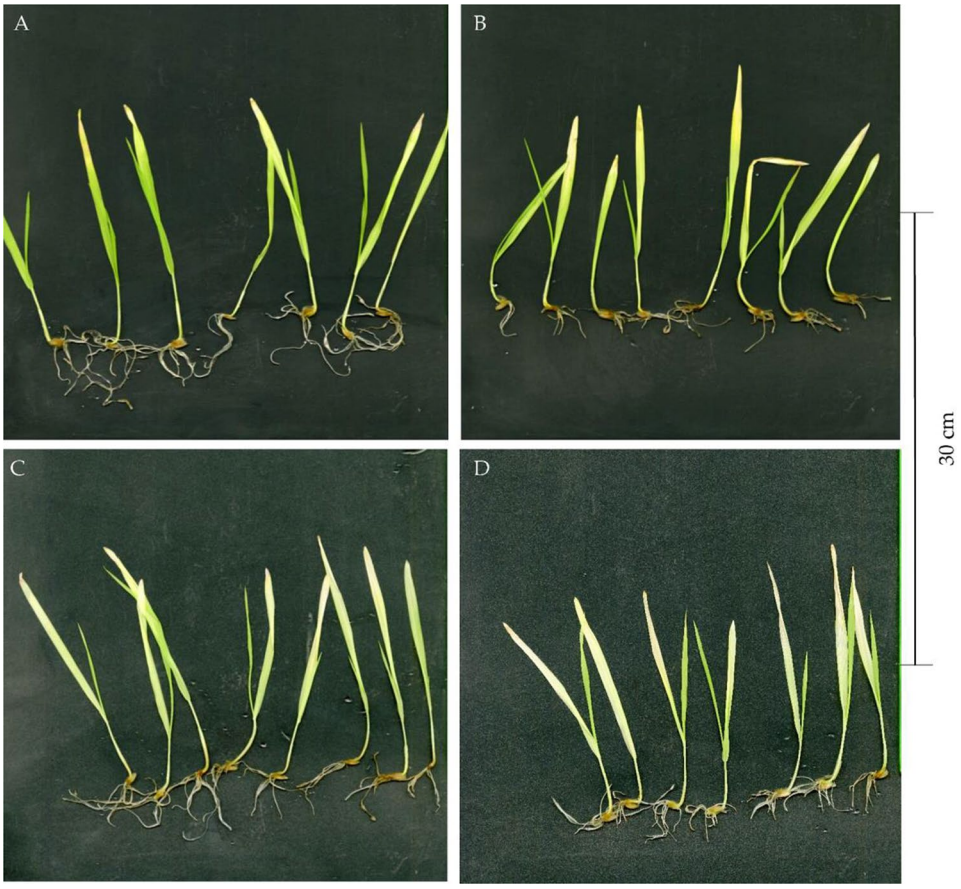


Fig. 5 Effects of the different treatments on barley growth. (**A**) Barley alone; (**B**) Barley after growing with non-pre-germinated *L. rigidum*; (**C**) Barley after growing with pre-germinated *L. rigidum*; (**D**) Barley after growing with pre-germinated *P. oleracea*

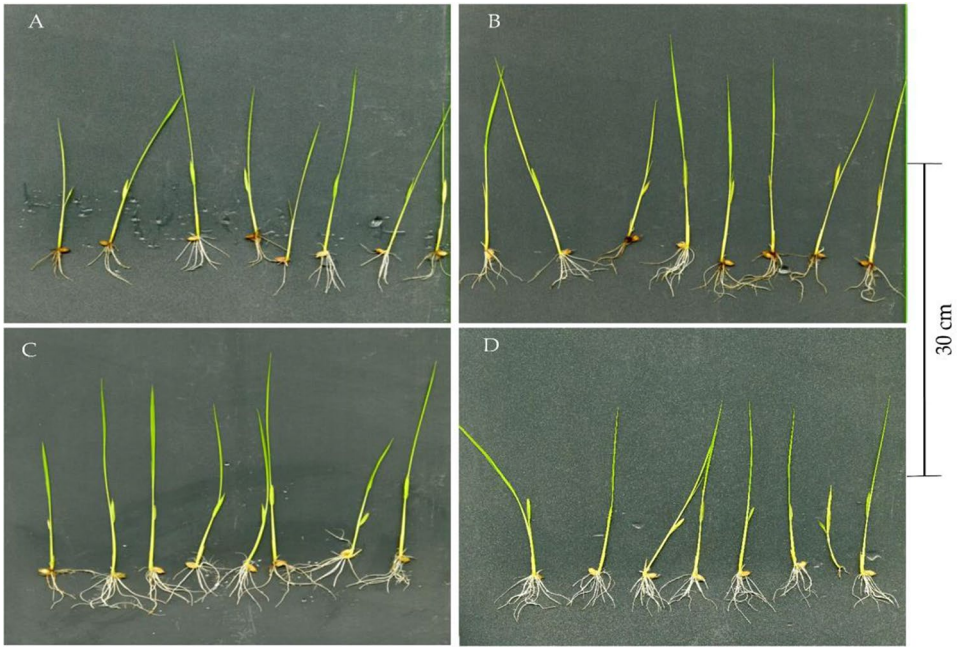
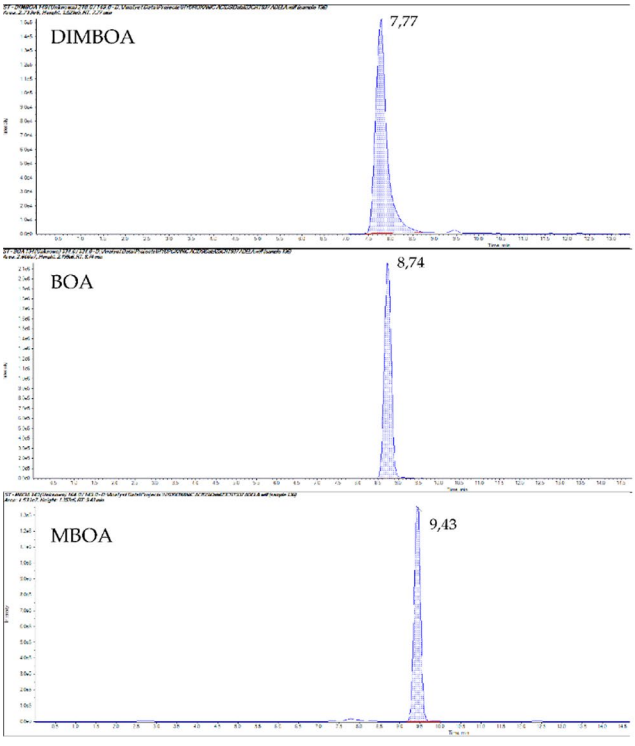


Fig. 6 Effects of the different treatments on rice growth. (**A**) Rice growing alone; (**B**) Rice after growing with pre-germinated *L. rigidum*; (**C**) Rice after growing with non-pre-germinated *P. oleracea*; (**D**) Rice after growing with pre-germinated *P. oleracea*



BZXs	RT min
DIBOA	6,8
HMBOA	7,5
DIMBOA	7,7
BOA	8,7
MBOA	9,4
HBOA	9,6

Fig. 7 Chromatogram of the benzoxazinoids DIMBOA, BOA and MBOA, and their relative retention times

times (7.77, 8.74, and 9.43, respectively) obtained, and the values compared to the DIMBOA, MBOA and BOA standards (Fig. 7). DIBOA, HMBOA and HBOA were identified by co-chromatography with the reference compound BOA and their retention times obtained (6.8, 7.5 and 9.6, respectively).

Figure 8 shows the comparison of the chromatograms of the standards (Fig. 8A) with the presence of these compounds in the agar after co-cultivation of wheat

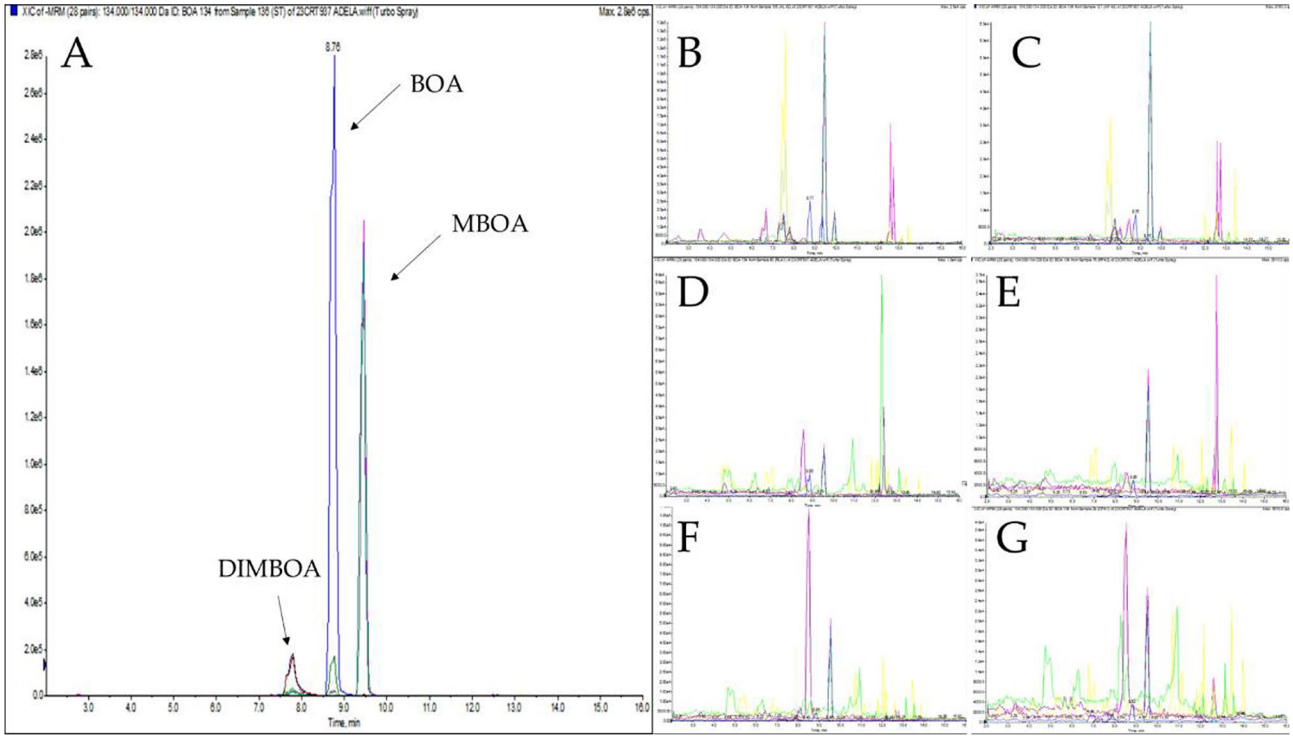


Fig. 8 HPLC profiles of the identification of benzoxazinoids (DIMBOA, BOA and MBOA) in (**A**) Standard; (**B**), agar of the co-culture of wheat + *L. rigidum* (**C**) agar of the co-culture of wheat + *P. oleracea* (**D**) agar of the co-culture of rice + *L. rigidum* (**E**) agar of the co-culture of rice + *P. oleracea*, (**F**) agar of the co-culture of barley + *L. rigidum* and (**G**) agar of the co-culture of barley + *P. oleracea*

with *L. rigidum* (Fig. 8B) or *P. oleracea* (Fig. 8C), of rice with *L. rigidum* (Fig. 8D) or *P. oleracea* (Fig. 8E), and co-cultivation of barley with *L. rigidum* (Fig. 8F) or *P. oleracea* (Fig. 8G). The results showed more clear differences depending on the crop than depending on the surrounding weed. The most similar chromatograms were observed in wheat, with the same peaks of compounds. For example, a larger peak of MBOA was seen after both co-cultures, as the exudation of this BZX was significantly increased. For rice or barley, slight differences were observed when comparing the differences after co-cultivation with the different weeds, being the most striking the large number of peaks observed after co-cropping barley with *P. oleracea*.

Characterization and quantification of benzoxazinoids (BZXs)

The results obtained from the allelochemical profile of wheat against weeds (Table 2) showed an increased accumulation of BZXs in the roots of 6-times for BOA, almost 4-times for MBOA and HBOA, more than 3-times for HMBOA and DIMBOA, after co-cultivation with *L. rigidum*, while DIBOA just increased 1.5 times when compared to the control. As well, BZXs accumulated also in wheat roots when co-cultured with *P. oleracea*, showing increases of 5-times for HMBOA, MBOA, and HBOA, almost 4-times for BOA, and 2.5-times for DIMBOA when compared to the control. However, almost no statistical differences were observed in the shoots of wheat when co-cultured with any of the weeds, showing just a 2-times increase of HBOA after co-culture with *P. oleracea*. In contrast, only root exudation of BOA was significantly inhibited (53%) in the presence of *P. oleracea*. On the other hand, when accumulation or exudation were compared between treatments, significant differences were observed between the co-cultivation with *L. rigidum* and with *P. oleracea*. Regarding the accumulation of compounds in the roots, higher concentrations of BOA, DIMBOA and DIBOA were found in wheat roots after growing with *L. rigidum* than after co-culture with *P. oleracea*. However, MBOA, HMBOA and HBOA presented higher values in wheat co-cultivated with *P. oleracea*. Concerning the presence of BZXs in the shoots, significant differences in HBOA were found in wheat depending on the co-cultured weed, with higher concentrations when wheat was co-cultured with *P. oleracea*.

By contrary, the allelochemical profile of rice (Table 3) was not so strongly altered after co-cultivation with weeds, although significant increases in root exudation could be detected in the presence of *L. rigidum* (around 3-times for BOA and 1.5-times for DIBOA). As well, significant increases in DIMBOA (125%) and HMBOA (113%) were also observed in the roots after co-cultivation with *L. rigidum*. However, a decrease in DIMBOA

Table 2 Allelochemical profile of benzoxazinoids in wheat plants when grown alone or in co-cultivation with *Lolium rigidum* or *Portulaca oleracea*. Different letters indicate significant differences between treatments for each crop. Asterisks represent significant reductions, while bold letters indicate significant increases when compared to the control ($p \leq 0.05$). $N = 5$. Shoot and root values are given in μg kg^{-1} and agar values in μg L^{-1}

Sample	Treatment	DIBOA	DIMBOA	BOA
Shoot	Crop alone	2.10 ± 0.46 a	589.3 ± 173.1 a	1.73 ± 0.49 a
	+*L. rigidum*	2.61 ± 1.08 a	898.3 ± 988.6 a	2.46 ± 1.43 a
	+*P. oleracea*	2.63 ± 0.32 a	446.3 ± 294.3 a	2.24 ± 0.45 a
Root	Crop alone	1.78 ± 0.02 b	463.7 ± 322.8 b	1.84 ± 0.60 b
	+*L. rigidum*	**2.94 ± 1.19 a**	**1478 ± 709.7 a**	**11.05 ± 5.7 a**
	+*P. oleracea*	2.07 ± 0.38 b	**1135 ± 276.0 a**	**7.02 ± 1.72 a**
Agar	Crop alone	1.06 ± 0.03 a	10.15 ± 6.350 a	3.88 ± 1.42 a
	+*L. rigidum*	1.19 ± 0.24 a	8.860 ± 4.160 a	4.15 ± 1.90 a
	+*P. oleracea*	1.06 ± 0.03 a	14.47 ± 9.180 a	2.43 ± 1.45 b*
Sample	**Treatment**	**MBOA**	**HBOA**	**HMBOA**
Shoot	Crop alone	295.0 ± 125.3 b	179.1 ± 81.25 b	83.81 ± 5.500 a
	+*L. rigidum*	405.6 ± 295.8 ab	222.1 ± 172.2 b	68.15 ± 46.29 a
	+*P. oleracea*	595.5 ± 157.2 a	**364.3 ± 98.11 a**	77.65 ± 27.84 a
Root	Crop alone	187.8 ± 147.84 b	117.3 ± 95.16 b	25.59 ± 17.73 b
	+*L. rigidum*	**704.1 ± 338.9 a**	**437.9 ± 214.3 a**	**89.49 ± 39.69 a**
	+*P. oleracea*	**925.0 ± 123.4 a**	**577.1 ± 80.89 a**	**129.5 ± 15.40 a**
Agar	Crop alone	17.40 ± 13.09 a	10.75 ± 7.750 a	6.42 ± 3.300 a
	+*L. rigidum*	15.94 ± 11.53 a	10.34 ± 7.270 a	5.86 ± 5.080 a
	+*P. oleracea*	11.88 ± 4.600 a	7.650 ± 2.650 a	4.33 ± 1.260 a

2,4-dihydroxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (DIBOA), 2,4-dihydroxy-7-methoxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (DIMBOA), benzo[d]oxazol-2(3 H)-one (BOA), 6-methoxybenzoxazolin-2-one (MBOA), 2-hydroxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (HBOA), 2-hydroxy-7-methoxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (HMBOA)

Table 3 Allelochemical profile of benzoxazinoids in rice plants when grown alone or in co-cultivation with *Lolium rigidum* or *Portulaca oleracea*. Different letters indicate significant differences between treatments for each crop. Asterisks represent significant reductions, while bold letters indicates significant increases when compared to the control ($p \leq 0.05$). $N = 5$. Shoot and root values are given in μg kg^{-1} and agar values in μg L^{-1}

Sample	Treatment	DIBOA	DIMBOA	BOA
Shoot	Crop alone	2.69 ± 0.70 a	5.88 ± 1.89 a	1.52 ± 0.15 a
	+*L. rigidum*	3.03 ± 0.47 a	4.79 ± 1.2 ab	1.51 ± 0.11 a
	+*P. oleracea*	2.60 ± 0.21 a	3.93 ± 0.49 b*	1.53 ± 0.12 a
Root	Crop alone	1.99 ± 0.30 a	4.80 ± 0.60 b	1.55 ± 0.09 a
	+*L. rigidum*	1.73 ± 0.05 a	**6.03 ± 0.37 a**	1.56 ± 0.35 a
	+*P. oleracea*	1.81 ± 0.07 a	4.16 ± 0.55 b	1.46 ± 0.12 a
Agar	Crop alone	1.03 ± 0.03 b	2.53 ± 1.03 a	1.10 ± 0.03 b
	+*L. rigidum*	**1.50 ± 0.24 a**	3.12 ± 0.46 a	**3.07 ± 2.22 a**
	+*P. oleracea*	**1.08 ± 0.02 a**	3.12 ± 0.83 a	1.17 ± 0.19 b*
Sample	**Treatment**	**MBOA**	**HBOA**	**HMBOA**
Shoot	Crop alone	5.75 ± 4.33 a	4.44 ± 2.74 a	1.09 ± 0.11 a
	+*L. rigidum*	5.93 ± 3.11 a	4.71 ± 2.34 a	1.11 ± 0.14 a
	+*P. oleracea*	3.27 ± 2.15 a	2.16 ± 0.72 a	1.01 ± 0.06 a
Root	Crop alone	4.70 ± 0.97 a	4.14 ± 0.20 a	1.04 ± 0.02 b
	+*L. rigidum*	6.90 ± 2.98 a	5.07 ± 1.98 a	**1.18 ± 0.01 a**
	+*P. oleracea*	5.27 ± 0.86 a	4.56 ± 1.31 a	1.06 ± 0.08 b
Agar	Crop alone	3.68 ± 3.00 a	2.68 ± 1.91 a	0.62 ± 0.04 a
	+*L. rigidum*	35.1 ± 57.4 a	23.6 ± 38.5 a	1.01 ± 0.53 a
	+*P. oleracea*	4.31 ± 1.08 a	2.99 ± 0.63 a	0.69 ± 0.01 a

2,4-dihydroxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (DIBOA), 2,4-dihydroxy-7-methoxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (DIMBOA), benzo[d]oxazol-2(3 H)-one (BOA), 6-methoxybenzoxazolin-2-one (MBOA), 2-hydroxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (HBOA), 2-hydroxy-7-methoxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (HMBOA)

Table 4 Allelochemical profile of benzoxazinoids in barley plants when grown alone or in co-cultivation with *Lolium rigidum* or *Portulaca oleracea*. Different letters indicate significant differences between treatments for each crop. Asterisks represent significant reductions, while bold letters indicates significant increases when compared to the control ($p \leq 0.05$). $N = 5$. Shoot and root values are given in Mg kg^{-1} and agar values in Mg L^{-1}

Sample	Treatment	DIBOA	DIMBOA	BOA
Shoot	Crop alone	12.4 ± 2.01 a	7.53 ± 4.8 ab	1.44 ± 0.18 b
	+*L. rigidum*	8.76 ± 2.02 b*	11.31 ± 2.3 a	**2.39 ± 0.82 a**
	+*P. oleracea*	5.87 ± 1.55 b*	3.84 ± 0.72 b	1.39 ± 0.10 b
Root	Crop alone	1.83 ± 0.12 b	7.57 ± 1.70 a	1.61 ± 0.15 a
	+*L. rigidum*	2.07 ± 0.2 ab	6.21 ± 2.55 a	1.44 ± 0.18 a
	+*P. oleracea*	**2.00 ± 0.09 a**	4.81 ± 0.63 a	1.38 ± 0.17 a
Agar	Crop alone	1.12 ± 0.08 a	3.09 ± 0.15 b	1.29 ± 0.15 a
	+*L. rigidum*	1.18 ± 0.10 a	**3.72 ± 0.29 a**	1.28 ± 0.19 a
	+*P. oleracea*	1.27 ± 0.12 a	2.45 ± 0.79 b	1.31 ± 0.08 a
Sample	**Treatment**	**MBOA**	**HBOA**	**HMBOA**
Shoot	Crop alone	14.2 ± 13.9 ab	10.05 ± 9.4 ab	1.32 ± 0.36 a
	+*L. rigidum*	27.97 ± 2.31 a	21.88 ± 5.24 a	1.64 ± 0.14 a
	+*P. oleracea*	3.69 ± 2.620 b	2.89 ± 1.800 b	1.05 ± 0.11 a
Root	Crop alone	13.99 ± 6.39 a	10.08 ± 4.51 a	1.29 ± 0.17 a
	+*L. rigidum*	19.20 ± 18.5 a	13.18 ± 12.2 a	1.36 ± 0.28 a
	+*P. oleracea*	14.79 ± 11.4 a	10.29 ± 7.68 a	1.22 ± 0.18 a
Agar	Crop alone	5.22 ± 0.78 b	3.65 ± 0.42 b	0.68 ± 0.09 b
	+*L. rigidum*	**8.35 ± 5.30 a**	**5.70 ± 3.43 a**	**0.90 ± 0.07 a**
	+*P. oleracea*	6.34 ± 0.78 b	**4.47 ± 0.60 a**	0.79 ± 0.12 b

2,4-dihydroxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one(DIBOA), 2,4-dihydroxy-7-methoxy-2 H-benzo[b] [1,4] oxazin-3(4 H)-one (DIMBOA), benzo[d]oxazol-2(3 H)-one (BOA), 6-methoxybenzoxazolin-2-one (MBOA), 2-hydroxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (HBOA), 2-hydroxy-7-methoxy-2 H-benzo[b] [1, 4] oxazin-3(4 H)-one (HMBOA)

(66% of the control) was observed in rice shoots after co-cultivation with *P. oleracea*. Finally, it is important to highlight that significant differences were observed in the accumulation of compounds in roots and root exudation when comparing the treatments with *L. rigidum* and *P. oleracea*. Higher concentrations of DIMBOA and HMBOA were found in rice roots after co-culture with *L. rigidum*, and, in the same way, BOA values also increased in root exudates after *L. rigidum* co-culture.

Finally, the allelochemical profile of barley (Table 4) was different after co-cultivation with both weeds, especially for root BZXs. Significant increases in root exudations for DIMBOA, MBOA, HMBOA and HBOA were especially relevant in the presence of *L. rigidum*, while just HBOA was significantly more exuded in the presence of *P. oleracea*. As well, significant increases in BOA (166%) and HBOA (218%) were observed in barley shoots just after growing with *L. rigidum*. In the case of the roots, a significant increase in DIBOA was noted (109%) after co-cultivation with *P. oleracea*. Finally, only DIBOA values were significantly lower than the control after co-cultivation with both weeds, with values of 70% of the control with *L. rigidum* and 47% with *P. oleracea*.

Discussion

In organic farming systems, crop genotypes with strong competitive traits can effectively use light, nutrients, and water, thereby restraining the proliferation and development of nearby weeds. This emphasizes the significance of selecting crops that not only thrive themselves, but actively suppress also weed growth, contributing to sustainable and efficient agricultural practices. Our results show that crops not only differ in their potential for weed management but also in the way they use different growth strategies.

Summarizing, the three tested crops showed potential to sustainably manage growth and development of the target weeds *L. rigidum* and *P. oleracea* in a similar way, although wheat growth was inhibited by the presence of *P. oleracea*, while rice was stimulated by both weeds, and barley was practically unaffected. In general, although both weeds were inhibited by the presence of these three Poaceae species, root parameters, and more particularly root length (RL) and root invasive capacity (RIC), were more affected than shoot parameters. Finally, the chemical profile of crops showed strong differences in terms of accumulation and exudation of BZXs, being wheat the crop with the highest accumulation and exudation of BZXs, followed by barley, and finally rice.

Elucidating the processes by which each crop can manage the surrounding weeds, while regulating or preserving its own growth, is a fundamental step in understanding and improving integrated weed management strategies [37]. This knowledge is crucial for developing sustainable and efficient agricultural strategies to maximizing crop productivity while minimizing weed competition. By understanding how crops interact with their environment and how they can be selected or modified to optimize their competitiveness against weeds, we can move towards more resilient agricultural systems that are less dependent on herbicides [1, 2, 37].

More in detail, wheat showed the highest accumulation and exudation of most of the BZXs, resulting in the significant inhibition of PW, RL and SVI of *L. rigidum*, and SL, RL, PW, RIC and SVI of *P. oleracea*. The observed effects on weeds are probably due to the presence of these compounds in the medium, as wheat has been repeatedly reported as an allelopathic crop [38–40]. DIMBOA, the main hydroxamic acid present in wheat [11, 41] has previously found to suppress the growth of different weeds. For example, Li et al. [42] found a correlation between an increase of DIMBOA in the medium and the inhibition of several weeds as *Abutilon theophrasti* Medik, *Aegilops tauschii* Coss, *Amaranthus retroflexus* L., *Avena fatua* L., and *Digitaria sanguinalis* (L.) Scop. Something similar was found for the weed *Alopecurus myosuroides* Hudson, which, after growing in the presence of DIMBOA, showed shorter roots [43]. Moreover, the study

of Vieites-Álvarez et al. [31] confirmed that the wheat accession "Maurizio" induced strong root inhibition in *L. rigidum* and *P. oleracea*, probably due to the effects of DIMBOA and MBOA that were present in the agar medium. MBOA, a BZX resulting from DIMBOA transformation, was not only reported as phytotoxic [27, 44, 45] but also as more stable in aqueous solutions [46], which could explain the higher amounts found in wheat agar in this study. Despite these inhibitory effects on weeds, no significant increases in root exudation of BZXs were found when wheat was co-cultured with any of both weeds, but a strong significant increase in root accumulation was found after growing with *L. rigidum* or *P. oleracea*. In fact, after growing with *L. rigidum*, wheat roots accumulated all BZXs, which suggests a huge investment of energy in the production and accumulation of these compounds, and correlates with wheat growth parameters, which showed shorter but thicker roots of wheat plants after co-growing with this monocot weed. This accumulation behavior occurred in young wheat seedlings when competing with other plants during early development [46]. The accumulation of DIMBOA and HMBOA observed in the roots was also detected in another wheat co-cultivation experiment with *L. rigidum* performed by Hussain et al. [36]. They suggested that wheat could accumulate a large amount of benzoxazinoids in root tissues, as these specialized metabolites could be relocated to other tissues or eventually released to the medium, if necessary, resulting in an energy cost to the plant.

By contrary, rice, the most stimulated crop by the presence of surrounding weeds, exhibited a pronounced impact on *L. rigidum* (RL, PW, RIC, and SVI) and *P. oleracea* (SL, RL, and PW), especially on root parameters. This weed inhibition and crop stimulation of rice was correlated to increased amounts of BZXs exuded by this crop when co-cultured with both weeds, especially with *L. rigidum*. The exudation of DIBOA and its degradation product BOA were significantly higher when compared to rice growing alone and, in fact, the concentration of BOA in the root exudates of rice co-cultivated with *L. rigidum* was double than the concentration of DIBOA. This could be explained by the fact that DIBOA is an unstable chemical in aqueous solutions, so it could have been transformed to BOA, which is more stable and has a longer half-life in the growth medium [28, 46, 47]. Both benzoxazinoids are molecules with demonstrated strong phytotoxic potential [28–30, 48]. BOA was found to inhibit the synthesis of ATP in mitochondria [49], and Sánchez-Moreiras et al. [30] demonstrated its capacity to induce early senescence by reducing photosynthesis, while degrading pigments and proteins, and increasing hydrogen peroxide content and lipid peroxidation. In addition, Li [50] observed that the emergence of cotton was inhibited when BOA was applied to the soil, suggesting the potential of BOA to inhibit plant germination. DIBOA was shown to have inhibitory effects on the growth and germination of *L. rigidum*, but also on *Echinochloa crus-galli* and *Megathyrsus maximus* [48]. The co-culture of rice with *P. oleracea* resulted in a decrease of SL, RL and SVI, inhibiting the normal development of this dicot weed. This could be correlated with the increased exudation of DIBOA by rice plants, as found when growing in the presence of *L. rigidum* plants. In this regard, Hussain et al. [36] reported that a higher root exudation of BZXs like DIBOA could induce a reduction in germination, weight, and root length of this dicot weed. Furthermore, in another experiment, Tabaglio et al. [51] observed the phytotoxic potential of rye (*Secale cereale* (L.) M. Bieb.) against *P. oleracea*. In this particular case, the rye mulching induced negative effects on the development of different weeds such as *P. oleracea* and *A. retroflexus*, probably due to the allelopathic effect of BZXs released by the dead rye tissues. Regarding the allelochemical profile of rice, significant differences were found between co-cultivation with *L. rigidum* or *P. oleracea* supporting the idea of different growth mechanisms of rice depending on the surrounding environment. For example, higher accumulation of DIMBOA and HMBOA, and enhanced exudation of BOA, was found in rice roots after co-culture with *L. rigidum*. By contrary, just DIBOA exudation was significantly higher compared to the control after growing with *P. oleracea*, which could also explain the strong root reduction observed in this dicot weed. Plants possess the remarkable ability to detect nearby vegetation through a process known as "plant-plant signaling", communication which occurs through the release of volatile organic compounds (VOCs) into the atmosphere, effectively signaling the presence of neighboring plants [52]. This induced defense could be more pronounced after physical damage [53], as the significant reduction observed in *L. rigidum* roots and *P. oleracea* shoots and roots. In fact, Fiorenza et al. [54, 55] found six VOCs emitted by *Lolium multiflora* after leaf damage. In response to these signals, neighboring plants may adjust their growth patterns [56], which would be in concordance with the stimulated shoot and root length observed in rice after growing with *L. rigidum*, and the increased number of roots after growing with *P. oleracea*. Recently, Almeida et al. [57] observed that volatile compounds promote growth and induce metabolic changes in rice. Moreover, Ramesh et al. [58] reported that rice with enhanced traits demonstrated higher weed competitive ability against weeds. The contrasting effects of rice on *L. rigidum* and *P. oleracea* underscore dynamic allelochemical responses influenced by surrounding conditions. These findings not only highlight the potential role of chemical signaling in weed management strategies but

also suggest that rice growth may be modulated through such interactions, emphasizing the complexity of plant-weed dynamics in agricultural ecosystems.

Regarding barley, the results of this experiment revealed the significant inhibitory impact of barley on the growth and germination of both weed species, indicating the broad-spectrum of allelopathic activity of barley against different weed species. The observed inhibition of weed growth by barley aligns with previous studies demonstrating the allelopathic effects of barley on weed species [1, 16]. In this study, *L. rigidum* showed significant decreases in SL, RL, RIC and SVI when co-cultivated with barley, which were correlated with significantly higher root exudations of BZXs in barley, especially DIMBOA, MBOA, HBOA and HMBOA. Interestingly, barley exhibited differential responses to the two weed species. It effectively inhibited the germination of *P. oleracea*, while its growth was not affected by the presence of this weed. Only slight reductions in barley root length and root number were shown with the presence of *L. rigidum*. Co-cultivation of barley with *P. oleracea* resulted in decreased values of G, SL, RL, RIC and SVI in *P. oleracea*, being the only crop with potential to inhibit germination, while barley plants remained unaffected. Even there was an increase in the exudation of HBOA, considered a phytotoxic compound [23], the mechanisms for managing this dicot weed are still unknown as no strong exudations or stimulated growth were observed. However, more than 50 different types of allelochemicals such as phenolic acids, flavonoids, and derivatives have been detected in barley [59], and although they were not quantified in this study, have been reported to be very effective on *P. oleracea* germination [60], which is in agreement with the observed results.

The study highlights the importance of inherent crop competitiveness against herbicide-resistant weeds, particularly in agroecological and organic farming systems. The findings demonstrate that different crops exhibit different allelopathic potential against different weed species, with barley, wheat, and rice plants showing varying responses too. Elucidating the precise mechanisms underlying allelopathic effects, is crucial for further optimizing crop selection and the modification of strategies. Additionally, exploring other agroecological strategies for weed management, such as cover cropping with barley based on its ability to suppress germination and manage weed development without reducing crop yield, mulching with wheat based on the high accumulation of BZXs in wheat shoots, or intercropping with rice based on the enhanced competitiveness by stimulating crop plants growth while inhibiting weed development, could enhance weed suppression while promoting crop growth and soil health. Investigating the role of chemical signaling in plant-plant interactions and weed management, particularly in rice, where volatile organic compounds may play a significant role in modulating crop growth and weed competitiveness, is also essential.

Overall, in this research, we undergo trough different chemical and physical processes of these three main crops. Understanding crop-weed interactions is paramount for developing sustainable and efficient agricultural practices that can minimize reliance on herbicides and promote agroecosystem resilience trough the correct selection of crops with strong inherent potential to manage surrounding weeds [1, 2]. This alternative would not involve any input, either synthetic or economic, and could be used in organic agriculture or agroecology [1, 2, 13, 61]. However, it is important to note that the conditions used in our study were deliberately chosen for specific scientific objectives. Our main goal was to examine potential chemical interactions under controlled conditions, which would aid in our understanding of how bioactive compounds behave. However, it is crucial to acknowledge that these concentrations may not accurately reflect exposure levels in natural environments due to various factors like dilution, microbial activity, physico-chemical transformations, or degradation processes. These natural variables can significantly alter the actual concentrations of bioactive compounds. Therefore, the role of these factors is pivotal in influencing compound concentrations in natural settings. Future research should prioritize thorough investigations of these compounds' presence and effects under field conditions. This approach will help overcoming current research limitations and deeping our understanding of the mechanisms by which these compounds operate in natural ecosystems.

Conclusions

Our results show that crops such wheat, barley, and rice exhibit varying degrees of allelopathic activity against the weed species *L. rigidum* and *P. oleracea*. Wheat induced significant inhibition of both target weeds, particularly through the accumulation and exudation of BZXs, while barley effectively suppressed the germination of *P. oleracea*, and rice, which was interestingly stimulated by the presence of both weeds, showed enhanced root exudation of allelochemicals like DIBOA and BOA, that inhibited weed growth. Based on our results, rice would be the most promising crop, since it has the ability to control weeds, while stimulating the development of rice plants. These findings underscore the potential of using these crops for other agroecological strategies. For example, cover cropping with barley can suppress weed germination and manage weed development without reducing crop yield. Mulching with wheat, due to its high accumulation of BZXs in shoots, can further inhibit weed growth. Intercropping with rice, which enhances

competitiveness by stimulating crop plant growth while inhibiting weed development, can also be a viable strategy. Nevertheless, more research should be carried out, especially under field conditions.

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Author contributions
Conceptualization: Y.V-Á. and A.M.S.-M., methodology: Y.V.-Á., A.M.S.-M. and E.G.-G., data cu-ration: E.G.-G. and Y.V.-Á., formal analysis: E.G.-G. and Y.V.-Á., funding acquisition: A.M.S.-M., investigation: E.G.-G. and Y.V.-Á., project administration: A.M.S.-M., supervision: Y.V-Á. and A.M.S.-M., visualization: Y.V-Á. and A.M.S.-M., writing-review and editing: A.M.S.-M., Y.V.-Á. and E.G.-G. All authors have read and agreed to the published version of the manuscript.

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Data availability
Data is provided within the manuscript and raw data will be provided upon request.

Declarations

Ethics approval
Not applicable.

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Competing interests
The authors declare no competing interests.

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