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Allelopathic effects of barley extracts on germination and seedlings growth of bread and durum wheats

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Abstract – Phytotoxicity of barley extracts (*Hordeum vulgare* L.) on durum wheat (*Triticum durum* L.) and bread wheat (*Triticum aestivum* L.) was investigated. Water extracts of barley, variety Rihane were bioassayed on germination and seedling growth of both wheat species to: (i) test the heterotoxicity of barley on wheat, (ii) study the dynamics of allelopathic potential over four growth stages and (iii) identify the most allelopathic plant part of barley. Whole barley plants were extracted at growth stage 4 (stems not developed enough), whilst for the following growth stages roots, stems, and leaves were extracted separately. Seedling growth bioassays demonstrated that the two wheat species responded differently to the allelopathic potential of barley with a greater sensitivity shown by the bread wheats. For both wheat species, radicle growth was more depressed than coleoptile growth, though stimulation of seedling growth was observed for durum wheat. The allelopathic potential of barley plant parts was not stable over its life cycle for either bread or durum wheat. It appeared that potential increased near physiological maturity. Leaves and roots were the most phytotoxic barley plant parts for durum and bread wheats, respectively. Results suggested that the response by durum wheat and bread wheat varied depending on the source of allelochemicals (plant part) and the growth stage of the barley plant. Consequently, barley should be considered a depressive prior crop for both durum wheat and bread wheat in a field cropping sequence.

allelopathy / phytotoxicity / barley / durum wheat / bread wheat

Résumé – Effets allélopathiques des extraits d'orge sur la germination et la croissance des jeunes plantes de blé tendre et de blé dur. La phytotoxicité de l'orge (*Hordeum vulgare* L.) sur le blé dur (*Triticum durum* L.) et le blé tendre (*Triticum aestivum* L.) a été étudiée. Les effets des extraits à l'eau d'une variété d'orge (Rihane) ont été évalués en utilisant des tests biologiques de germination et de croissance de plantules. Ceci a été fait afin de : (i) tester l'hétérotoxicité de l'orge, (ii) étudier la dynamique du potentiel allélopathique à travers quatre stades de croissance suivant l'échelle de Feekes et (iii) identifier la composante de la plante-orge la plus allélopathique. L'extraction des résidus de

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la variété «Rihane» a été faite à l'eau distillée pour la totalité de la plante au cours du stade 4 (tiges insuffisamment développées), alors que pour les stades suivants, les racines, les tiges et les feuilles ont été extraites individuellement. Les tests biologiques de croissance de plantules ont montré que le blé dur et le blé tendre répondent différemment au potentiel allélopathique de l'orge, avec une sensibilité plus prononcée chez le blé tendre. Pour les deux espèces, la croissance de la racine a été plus inhibée par les extraits à l'eau de l'orge que celle du coléoptile, bien qu'une stimulation de la croissance de la plantule du blé dur ait été observée. Indépendamment de l'espèce-test, le potentiel allélopathique de l'orge n'est pas stable durant son cycle biologique. Ce potentiel s'intensifie au fur et à mesure que la plante approche de la maturité. Les feuilles et les racines sont les parties de la plante-orge les plus phytotoxiques respectivement pour le blé dur et le blé tendre. Les résultats suggèrent que la réponse du blé dur ou du blé tendre varie en fonction de la source des extraits à l'eau (racines, feuilles, tiges) et du stade de croissance de la plante-orge. En plus, l'expression du potentiel allélopathique de l'orge est relative à l'espèce-test (blé dur vs. blé tendre). Par conséquent, il serait utile de considérer l'orge comme une céréale allélopathique pour le blé dur et le blé tendre quand elle est conduite comme précédent culturel.

allélopathie / phytotoxicité / orge / blé dur / blé tendre

1. Introduction

Allelopathy as a mechanism of plant interference in agroecosystems [11] offers an opportunity to manage weeds in a crop sequence [1], but could also adversely affect crop yields [7] and influence choice of rotation. Previous studies have shown that sorghum (*Sorghum bicolor* L.) vegetation possess a variety of potent inhibitors such as dhurrin, a cyanogenic glycoside [4] and phenolics [8] which are potentially allelopathic to weeds [2, 9] with a maximum of inhibitory activity at harvest [3]. The same results were reported for sudex, a hybrid of sorghum and sudangrass (*Sorghum sudanese*) [16]. This was not the case for all grasses, some exhibited higher toxicity to wheat seedling growth when their residues were still green [7].

Bioassays of germination, radicle growth and coleoptile growth are used to test the allelopathic potential of a crop species [7, 13, 16]. The allelopathic potential can be observed in the form of autotoxicity as in the case of alfalfa (*Medicago sativa* L.) [5, 6] or heterotoxicity as in the case of tall fescue (*Festuca arundinacea* L.) [10].

Since the allelopathy of small grain cereals has been little studied, the present work aimed to: (i) test the heterotoxicity of barley on durum wheat and bread wheat varieties, (ii) study changes in allelopathic potential over four growth stages on both durum wheat and bread wheat and (iii) identify the most allelopathic plant part.

2. Materials and methods

2.1. Growth of barley plants

Barley, variety "Rihane" was sown on November 20, 1996 at the experimental station of École Supérieure d'Agriculture du Kef (Tunisia). The sandy-clay soil was alkaline with a pH of 7.9 and 1.6% of organic matter. From soil preparation to harvest, standard cultural practices for the semi-arid zone were applied. Plants were watered whenever severe wilting was observed. Whole barley plants were pulled out of the field at four growth stages (stage 4 = leaf sheaths lengthening; stage 8 = last leaf just visible; stage 10 = in boot; stage 11 = grain development) following Feekes scale [11]. For the stage 11, plants were sampled in late June 1997.

2.2. Preparation of water extracts

Barley plants were gently washed with distilled water, dried between two paper towels and then separated into roots, stems and leaves. All plant components were chopped into 1-cm long pieces and dried at 50 °C for 24 h. An unground 2.5 g dried portion of each plant component was extracted in 50 ml cold distilled water. Plants were extracted in a 500-mL flask on an horizontal shaker for 24 h at 200 rpm. Extracts were passed

through cheese cloth and stored at less than 5 °C until bioassayed.

At stage 4, barley stems were not developed enough. So, whole plants were extracted as one unit (including roots) following the same technique described for plant components.

2.3. Growth medium for bioassays of barley extracts

Water extracts of the whole plant of barley at stage 4 and roots, stems and leaves for stages 8, 10 and 11 were tested for phytotoxicity to seed germination, radicle growth and coleoptile growth of 4 varieties of durum wheat (“Karim”, “Razzek”, “Khiar”, “Chili”) and 4 varieties of bread wheat (“Ariana”, “Vaga”, “Salambo” “Douga”). For the bioassays, molten agar was amended with 20 ml extract of each plant part to make a water-extract-agar medium (1.2%). The medium of 1.2% distilled water-agar was used as a control.

2.4. Germination bioassays

For germination bioassays, seeds of wheat were surface sterilized with a 5% aqueous solution of sodium hypochlorite for 1 min, rinsed 5 times with distilled water and dried between two paper towels. Surface sterilized seeds were placed in a 10 × 150-mm Petri Dish (PD) containing 15 ml of water-extract-agar as growth medium and incubated for 35 h at 25 °C. Seeds were classed as germinated when the radicle extended 2 mm out of the seed coat.

2.5. Radicle and coleoptile growth bioassays

Radicle and coleoptile growth bioassays were determined using a Test Tube (TT) technique and pre-germinated seeds. Surface sterile seeds were pre-germinated on a 10 × 150-mm PD between two filter papers moistened with 1.2 ml of distilled water. Test tubes were covered with cotton, slanted at 45° allowing 15 ml agar medium to solidify. Then, seedlings with radicle 3-mm long were

transplanted into tubes. After 60 h incubation at 25 °C, lengths of both the coleoptile and central radicle of each wheat seedling were measured.

2.6. Experimental design and statistical analysis

Germination and seedling growth bioassays were conducted in a Complete Randomized Design (CRD) with four replications. A non-amended treatment was included as a control. For germination bioassays, 25 seeds were placed in a PD. Each experimental unit consisted of two PD. For radicle or coleoptile bioassays, an average across a cluster of 10 growth TT with one pre-germinated seed each was used as a single observation for each treatment. Analysis of variance was conducted using SAS [14] and Fisher’s protected LSD at the 0.05 level of probability [15].

3. Results

3.1. Germination bioassays

Extracts of barley plants at stage 4 did not significantly affect seed germination of either durum or bread wheat varieties. When plant components (roots, stems, leaves) of barley were extracted separately at stages 8, 10 and 11 and bioassayed on “Chili” (Durum) and “Ariana” (Bread), both characterized by sensitive radicle growth (Tab. I), germination bioassays again did not appear to be a sensitive test for allelopathic effects. Therefore no data is presented from the germination bioassays.

3.2. Seedling growth bioassays

Extracts of whole barley plants at stage 4 significantly affected radicle growth of just one durum wheat variety (“Chili”). However, with bread wheat, three varieties (“Ariana”, “Vaga”, “Douga”) had reduced radicle growth (Tab. I).

“Ariana” was the most sensitive bread wheat variety, with radicle growth inhibited [Inhibition = (Control - Treatment)/Control × 100] by 46%.

Table I. Radicle growth (mm) of four varieties of Durum wheat and four varieties of Bread wheat, treated with water extracts* of barley.

Treatment	Radicle growth (mm)							
	Durum wheat				Bread wheat			
	“Karim”	“Razzek”	“Khar”	“Chili”	“Ariana”	“Vaga”	“Salambo”	“Douga”
Control	2.6 a	3.5 a	3.3 a	3.8 a	5.4 a	5.8 a	2.3 a	5.3 a
Extract	2.3 a	2.9 a	3.2 a	2.3 b	2.9 b	3.4 b	2.1 a	3.1 b
LSD (0.05)	0.9	1.6	1.0	0.7	1.4	1.8	1.0	1.9

* A whole plant of var. “Rihane” was extracted at stage 4 (stage 4 = leaf sheaths lengthening).

“Chili” was the most sensitive variety of durum wheat with radicle growth inhibited by 40% (Tab. I).

For coleoptile growth bioassays, none of the durum wheat varieties was sensitive to barley extracts. However, one variety of bread wheat “Salambo”, which had a tolerant radicle (Tab. I), showed a sensitive coleoptile (Tab. II).

Based on the radicle bioassays, “Chili” (Durum) and “Ariana” (Bread) were selected as test-varieties for further bioassays at stages 8, 10 and 11.

Water extracts of plant components (roots, stems, leaves) of “Rihane” at stages 8, 10 and 11 showed a significant inhibitory activity on radicle growth of “Ariana” (Tab. III). The inhibitory activity was not stable over the life cycle regardless of the source of extract.

The response of “Chili” was very different from the response of “Ariana” to the extent that radicle growth of “Chili” was stimulated when treated with stem extracts at stage 8 (Tab. III). Overall, inhibitory activity was greater in “Ariana” than in “Chili” (Figs. 1 vs. 2).

Coleoptile growth of “Chili” was unaffected at stage 8, increased at stage 10 and reduced at stage 11 (Tab. IV). “Ariana” was highly sensitive to water extracts with growth being reduced at all stages.

As it happened to radicle growth, coleoptile growth of “Chili” was significantly enhanced with all types of water extracts (roots, stems, leaves) at

Table II. Coleoptile growth (mm) of four bread wheat varieties treated with water extracts* of barley.

Treatment	Coleoptile growth (mm)			
	“Ariana”	“Vaga”	“Salambo”	“Douga”
Control	5.7 a	5.6 a	5.4 a	5.7 a
Extract	6.2 a	5.2 a	4.7 b	5.1 a
LSD (0.05)	0.7	0.6	0.6	0.7

* A whole plant of var. “Rihane” was extracted at stage 4 (stage 4 = leaf sheaths lengthening).

stage 10 (Fig. 1). At stage 11, extracts of leaves and roots were the most phytotoxic to the growth of “Chili” and “Ariana” coleoptiles, respectively.

In contrast to “Chili”, no stimulation was observed for radicle or coleoptile growth of “Ariana”. Radicle and coleoptile growths were always inhibited by water extracts of plant parts of “Rihane” at all stages 8, 10 and 11, with the radicle being more sensitive than the coleoptile (Fig. 2).

4. Discussion and conclusion

Germination bioassays of barley at four different phenological stages were not sensitive enough to detect the heterotoxicity potential of any plant

Table III. Radicle growth (mm) of “Chili” (Durum) and “Ariana” (Bread) treated with water extracts of plant parts prepared from barley var. Rihane at stages 8*, 10* and 11*.

Treatment	Radicle growth (mm)					
	Stage 8		Stage 10		Stage 11	
	“Chili”	“Ariana”	“Chili”	“Ariana”	“Chili”	“Ariana”
Control	3.4 c	4.8 a	1.7 ab	5.5 a	2.9 a	5.3 a
Root extract	2.6 b	1.7 c	2.1 a	2.5 b	2.5 a	2.0 b
Leaf extract	2.6 b	1.8 bc	1.5 b	1.5 c	1.5 b	2.7 b
Stem extract	4.8 a	2.3 b	1.7 ab	1.3 c	1.5 b	2.7 b
LSD (0.05)	0.7	0.6	0.5	0.6	0.6	0.8

* Stage 8 = last leaf just visible; stage 10 = in boot; stage 11 = grain development.

Table IV. Coleoptile growth (mm) of “Chili” (Durum) and “Ariana” ((Bread) wheats treated with water extracts of plant parts prepared from barley var. Rihane at stages 8*, 10* and 11*.

Treatment	Coleoptile growth (mm)					
	Stage 8		Stage 10		Stage 11	
	“Chili”	“Ariana”	“Chili”	“Ariana”	“Chili”	“Ariana”
Control	7.9 a	8.2 a	8.9 b	6.2 a	6.8 a	4.7 a
Root extract	7.8 a	5.8 b	11.8 a	4.7 b	6.4 a	3.3 c
Leaf extract	6.7 a	5.1 b	13.3 a	3.8 c	1.6 c	3.9 b
Stem extract	7.0 a	6.0 b	10.8 ab	3.3 c	3.5 b	4.3 ab
LSD (0.05)	1.4	1.1	2.5	0.7	0.8	0.5

*Stage 8 = last leaf just visible; stage 10 = in boot; stage 11 = grain development.

component of barley. However, seedling growth bioassays were sensitive to allelopathic effects with the radicle being relatively more sensitive than the coleoptile (Figs. 1, 2). Results of both types of bioassay are in agreement with the results reported by Hedge and Miller [5] and Kimber [7], respectively.

Irrespective of the wheat species, radicle growth was generally reduced by barley extracts (Figs. 1, 2), except for stem extracts at stage 8 and root extracts at stage 10 which stimulated radicle growth of the durum wheat “Chili” (Fig. 1). The allelopathic potential of a barley plant on wheat species varied according to the source of extracts

as was found with sorghum [3, 8]. The sensitivity of the radicle was higher for bread wheat than durum wheat (Figs. 1 vs. 2). This could be the case among varieties within the same species as reported by Kimber [7] and Rose et al. [13]. In addition, the allelopathic potential of barley was unstable over the life cycle of the barley plant. This potential was at maximum near physiological maturity (Figs. 1, 2) as was for sorghum plant [3].

These results support the use of seedling bioassays as a tool to screen for tolerance or sensitivity of a crop species to the allelopathic potential of another crop species.

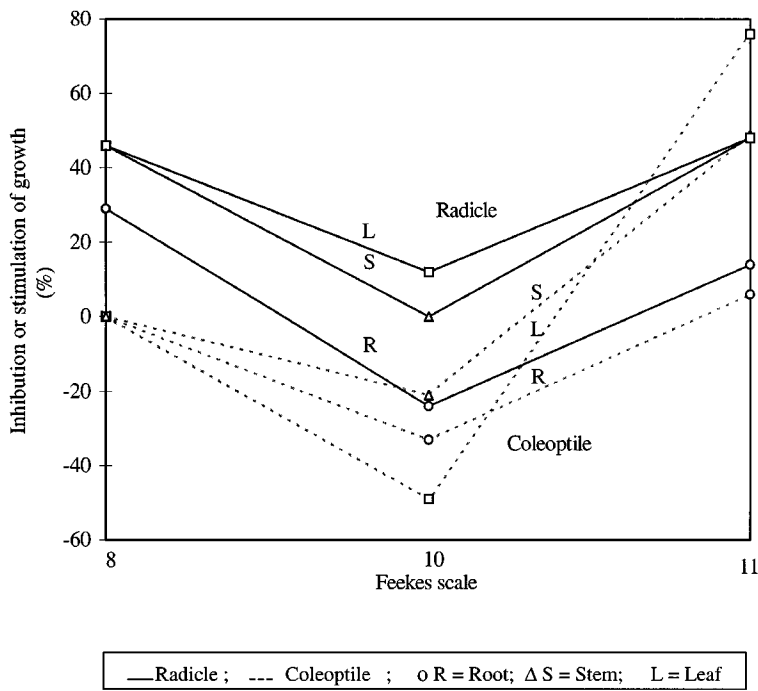


Figure 1. Response of radicle and coleoptile of "Chili" Durum wheat to water extracts of plant parts prepared from barley var. "Rihane" at stages 8, 10 and 11.

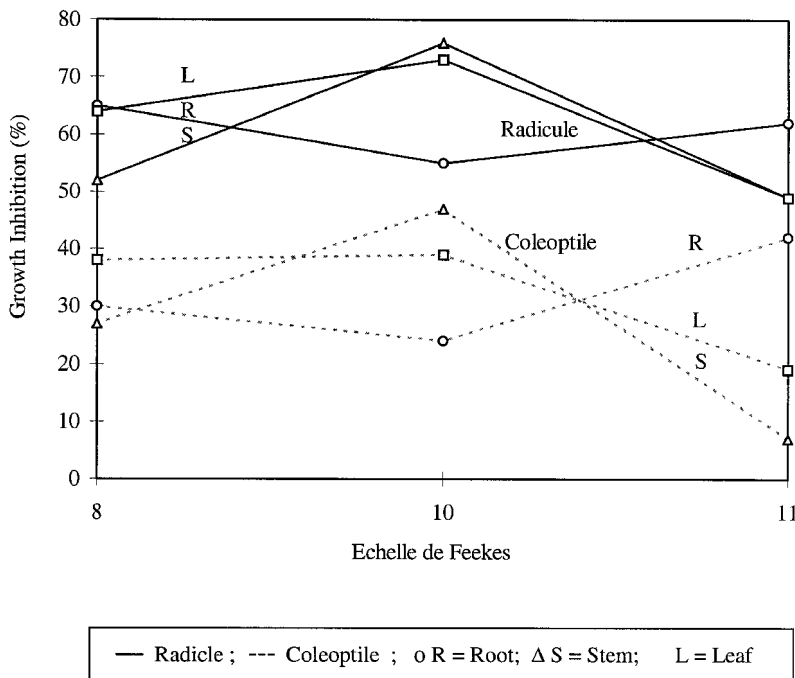


Figure 2. Response of radicle and coleoptile of "Ariana" Bread wheat to water extracts of plants prepared from barley var. "Rihane" at stages 8, 10 and 11.

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