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AUTOTOXICITY OF BARLEY

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ABSTRACT

Allelopathic potential of a crop species varies depending on stage of growth. Because allelopathy of barley (*Hordeum vulgare* L.), an important cereal grain adapted to semi-arid conditions of northern Tunisia, has not been widely reported, a study was conducted to determine i) the potential autotoxicity of barley and ii) the differential allelopathic potential of barley plant components over four phenological stages. The study involved experiments with barley seed germination and seedling growth bioassay techniques for detection of allelopathic activity. Plant parts of field-grown 'Rihane' barley were extracted with distilled water. At growth stage 4 (stems not well developed), whole plants were extracted. Thereafter, roots, stems, and leaves were extracted separately. Water extracts of 'Rihane' barley plant parts were bioassayed on four varieties of barley. Seedling growth bioassays revealed autotoxicity of barley, which appeared

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to be more pronounced on radicle growth than coleoptile growth, especially when plants near physiological maturity were extracted. Autotoxicity was not significant when 'Rihane' barley was simultaneously the donor and recipient of water extracts. Leaves were the most important source of allelopathic substances. Root extracts were least inhibitory toward both radicle and coleoptile growth. Results suggest qualitative and quantitative changes in allelopathic substances in barley plant parts during plant development.

INTRODUCTION

Allelopathy is the detrimental effect of one plant species on germination, growth, or development of a plant of another species (1). Allelopathy between plants within the same species is referred to as autotoxicity, exemplified by alfalfa (*Medicago sativa* L.) (2). Autotoxicity causes poor seedling establishment and reduces dry matter yield when alfalfa is re-seeded after a previous alfalfa crop (3). Alfalfa plants contain water-soluble toxins that can be released into the environment from both fresh and dry plant components (4). This cropping constraint can partially be resolved by screening alfalfa varieties with low allelopathic potential or with tolerance to phytotoxins (5). For example, some alfalfa cultivars can be ranked based on their relative sensitivity to extracts of alfalfa tissues (6).

Cereal species known to exhibit an interspecific allelopathic potential include durum wheat (*Triticum durum* L.) and barley (*Hordeum vulgare* L.), which release toxins that inhibit root growth of winter wheat (*Triticum aestivum* L.), especially when conditions are favorable for microbial activity (7). The allelopathic potential depends on crop species (8), cultivar within a species (9), and plant component (10). Sorghum as an allelopathic crop produces varying concentrations of phenolic compounds over different growth stages (11).

Because little is known about the autotoxicity of cereal crops, the present work was undertaken to determine i) the potential autotoxicity of barley and ii) the differential allelopathic potential of barley plant components over four phenological stages.

MATERIALS AND METHODS

Collection of Barley Plant Material

Barley cultivar 'Rihane' was seeded November 20, 1996, at the experimental station of Ecole Superieure d'Agriculture du Kef, Tunisia, on a

sandy clay soil. The soil (Calcisol) is alkaline with a pH of 7.9 and 1.6% organic matter. From soil preparation to harvest, standard cultural practices for the semi-arid zone were applied. Plants were irrigated when severe wilting was observed.

Following the Feekes scale (12), intact plants were removed from the field at four growth stages (stage 4 = leaf sheaths lengthen, stage 8 = last leaf just visible, stage 10 = in boot, and stage 11 = grain development). For stage 11, plants were sampled late June 1997.

Preparation of Water Extracts

Plants were gently washed with distilled water, blotted 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. A fresh portion (2.5 g) of each plant component was extracted in 50 mL distilled water. Each sample was placed in a 500-mL flask on an horizontal shaker for 24 h at 200 rpm. Extracts were passed through cheesecloth and stored at 5°C until bioassayed. For stage 4 plants, stems were not well developed, thus, the whole plant was extracted as one unit following the same technique as described for plant components.

Bioassay of Barley Plant Extracts

Water extracts of whole plants at stage 4 and roots, stems, and leaves at stages 8, 10, and 11 were tested for phytotoxicity toward seed germination, radicle growth, and coleoptile growth of four varieties of barley ('Rihane', 'Manel', 'Martin', and 'Esperance'). For bioassays, molten agar was amended with 20 mL of each plant part extract (stages 8, 10, and 11) and of the whole plant (stage 4) to make a water-extract-agar (1.2%) as a medium for barley germination and barley seedling growth. The medium of 1.2% water-agar alone was considered as a control.

For germination bioassays, seeds of barley 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 and incubated for 35 h at 25°C. Seeds were considered germinated when radicles protruded 2 mm from the seed coat.

Seedling growth bioassays were determined with a test tube (TT) technique using pre-germinated surface-sterilized seeds (10). Tubes, plugged with cotton, contained agar amended with extract slanted at a 45° angle. Seedlings with 3-mm long radicles were transplanted into tubes. After 60 h incubation at 25°C, lengths

of both the coleoptile and central radicle of each barley seedling were measured. Radicle growth inhibition was expressed according to the following equation: [Inhibition = (Control − Treatment)/Control × 100].

Experimental Design and Statistical Analysis

Barley 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 barley radicle or coleoptile bioassays, an average across a cluster of 10 growth TT with one pregerminated seed each was used as a single observation for each treatment. All experiments were conducted two times. Analyses of variance were conducted using SAS (13) and means were separated using Fisher's protected LSD at the 0.05 level of probability (14).

RESULTS AND DISCUSSION

Germination Bioassays

Water extracts of whole plants of 'Rihane' barley at stage 4 did not significantly affect seed germination of the tested barley varieties ('Rihane', 'Manel', 'Espérance', and 'Martin'). Similar results were observed for water extracts from plant components (roots, stems, and leaves) of 'Rihane' barley at stages 8, 10, and 11 when bioassayed on 'Manel', the most sensitive barley variety tested. The germination bioassay was not a sensitive test for determining allelopathic potential, similar to results of other studies (2,10).

Seedling Growth Bioassays

As was the case for seed germination, coleoptile growth of the four tested barley varieties was not significantly depressed by whole plant extracts of 'Rihane' barley as at stage 4. However, extracts significantly inhibited radicle growth of three barley varieties (Table 1). The highest radicle growth inhibition was 38% by barley variety 'Manel' (Table 1). Consequently, 'Manel' was used as the test variety for bioassays of extracts of 'Rihane' barley at stages 8, 10, and 11.

Table 1. Radicle Growth of Four Barley Varieties Treated with Water Extract of Intact Plants (Feekes Stage 4) of 'Rihane' Barley

	Radicle Length (mm)			
Treatment	'Rihane'	'Manel'	'Espérance'	'Martin'
Control	5.3	6.1	6.6	6.7
Extract	4.7	3.8	4.2	4.3
LSD (0.05)	1.0	0.5	0.8	0.5

In contrast to observations at stage 4 (Table 1), extracts of plant components from stage 8 'Rihane' barley did not inhibit radicle growth. However, coleoptile growth of 'Manel' was significantly reduced by root and leaf extracts (Table 2). At stage 10, the radicle growth of 'Manel' was similarly depressed by root, stem, and leaf extracts of 'Rihane' barley; however, coleoptile growth was slightly enhanced (Table 2). At plant maturity (stage 11), extracts of all plant components (roots, stems, and leaves) of 'Rihane' inhibited both radicle and coleoptile growth, with the leaf extract having the greatest inhibitory activity (Table 2). Previous work with sorghum also demonstrated that allelopathic potential was greatest for plant components sampled near physiological maturity (10,15).

Allelopathic potential of plant parts of 'Rihane' barley was not constant over the life cycle of the plant. A similar pattern was reported for allelopathic effects of barley toward several wheat varieties (16). As 'Rihane' plants matured, autotoxicity appeared to be more pronounced on radicle than coleoptile growth, with leaf extract as the most phytotoxic (Table 2, Fig. 1).

Table 2. Radicle and Coleoptile Growth Response of 'Manel' Barley Treated with Water Extracts of Plant Components from 'Rihane' Barley at Different Developmental Stages[†]

	Radicle Growth (mm)			Coleoptile Growth (mm)		
Treatment	Stage 8	Stage 10	Stage 11	Stage 8	Stage 10	Stage 11
Control	1.6	3.2	3.6	7.2	5.6	7.7
Root extract	1.5	1.6	1.8	5.9	5.5	6.7
Leaf extract	1.5	1.3	1.2	5.6	5.7	3.5
Stem extract	1.6	1.5	1.5	7.0	5.9	5.9
LSD (0.05)	0.4	0.4	0.7	1.0	1.3	1.0

[†]Based on Feekes scale: stage 4 = leaf sheaths lengthen, stage 8 = last leaf just visible, stage 10 = in boot, stage 11 = grain development.

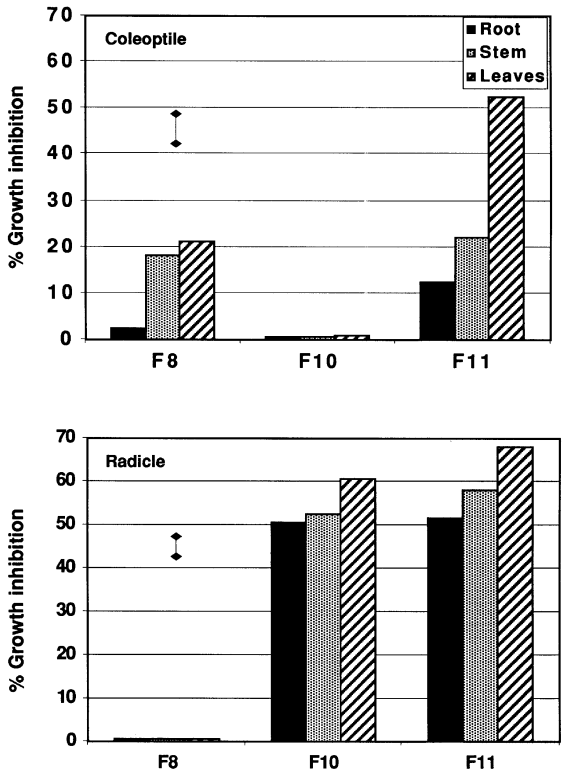

Figure 1. The growth response of coleoptile and radicle of 'Manel' barley seedlings to water extracts of plant components from 'Rihane' barley. Vertical bars indicate least significant difference ($p = 0.05$) between extracts across all growth stages (Feekes scale).

CONCLUSIONS

Our study confirms that barley is an allelopathic crop as reported previously (7). However, we demonstrated for the first time that barley exhibits autotoxicity with a differential response among varieties. The allelopathic potential varies i) among plant components of barley plant, as shown for sorghum (10), and ii) with growth stage, as reported for alfalfa (17). Using a barley variety as the indicator species, radicle growth was more sensitive than coleoptile growth, especially as the donor variety ('Rihane') approached maturity. Consequently, we suggest that barley should be considered as a "high-risk crop" for potential allelopathic effects in a barley-barley cropping sequence, especially if above-ground plant residues remain in the field after harvest.

REFERENCES

1. Putnam, A.R.; Duke, W.B. Allelopathy in Agroecosystems. Annu. Rev. Phytopathol. **1978**, *16*, 431–451.
2. Hegde, R.S.; Miller, D.A. Allelopathy and Autotoxicity in Alfalfa: Characterization and Effects of Preceding Crops and Residue Incorporation. Crop Sci. **1990**, *30*, 1255–1259.
3. Miller, D.A. Allelopathy in Forage Crop System. Agron. J. **1996**, *88*, 854–859.
4. Hall, M.H.; Henderlong, P.R. Alfalfa Autotoxic Fraction Characterization and Initial Separation. Crop Sci. **1989**, *29*, 425–428.
5. Read, J.J.; Jensen, E.H. Phytotoxicity of Water-Soluble Substances from Alfalfa and Barley Soil Extracts on Four Crop Species. J. Chem. Ecol. **1989**, *15*, 619–628.
6. Chung, I.M.; Miller, D.A. Differences in Autotoxicity among Seven Alfalfa Cultivars. Agron. J. **1995**, *87*, 596–600.
7. Cochran, V.L.; Elliott, L.F.; Papendick, R.I. The Production of Phytotoxins from Surface Crop Residues. Soil Sci. Soc. Am. J. **1977**, *41*, 903–908.
8. Bowmick, P.C.; Doll, J.D. Corn and Soybean Response to Allelopathic Effects of Weed and Crop Residues. Agron. J. **1982**, *74*, 601–606.
9. Hicks, S.K.; Wendt, C.W.; Gannaway, J.R.; Baker, R.B. Allelopathic Effects of Wheat Straw on Cotton Germination, Emergence and Yield. Crop Sci. **1989**, *29*, 1057–1061.
10. Ben-Hammouda, M.; Kremer, R.J.; Minor, H.C. Phytotoxicity of Extracts from Sorghum Plant Components on Wheat Seedling. Crop Sci. **1995**, *35*, 1652–1656.
11. Waniska, R.D.; Ring, A.S.; Doherty, C.A.; Poe, J.H.; Rooney, L.W. Inhibitors in Sorghum Biomass during Growth and Processing into Fuel. Biomass **1988**, *15*, 155–164.
12. Robert, K.M.; Walker, A.J. *An Introduction to the Physiology of Crop Yield*; John Wiley & Sons: New York, 1989.
13. SAS Institute. *SAS User's Guide: Statistics*, Version 6.0; SAS Inst. Inc.: Cary, NC, 1985.
14. Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistics*, 2nd Ed.; McGraw-Hill: New York, 1980.
15. Guenzi, W.D.; McCalla, T.M.; Norstad, F.A. Presence and Persistence of Phytotoxic Substances in Wheat, Oat, Corn and Sorghum Residues. Agron. J. **1967**, *59*, 163–165.
16. Ben-Hammouda, M.; Ghorbal, H.; Kremer, R.J.; Oueslati, O. Allelopathic Effects of Barley Extracts on Germination and Seedling Growth of Bread and Durham Wheats. Agronomie **2001**, *21*, 65–71.
17. Guenzi, W.D.; Kher, W.R.; McCalla, T.M. Water-Soluble Phytotoxic Substances in Alfalfa Forage: Variation with Variety, Cutting, Year and Stage of Growth. Agron. J. **1964**, *56*, 499–500.