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Barley Autotoxicity as Influenced by Varietal and Seasonal Variation

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With 2 figures and 4 tables

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Abstract

Barley (*Hordeum vulgare* L.) is widely cultivated in the semi-arid region of Tunisia for grain production and grazing, which often occurs during the same season. We previously demonstrated autotoxic effects of barley among varieties. The present study was conducted to test the effects of barley variety and seasonal variation on the expression of autotoxicity by barley. Four barley varieties were grown in a field experiment over three growing seasons (1999–2000, 2000–01, 2001–02). In the laboratory, germination and seedling growth bioassays were used to assess autotoxicity potential of field-harvested barley. Barley autotoxicity was fully expressed based on inhibition of radicle growth detected in seedling bioassays. Stems were often the most allelopathic plant component. Allelopathic activity of the barley varieties differed across growing seasons suggesting the influence of a seasonal effect due to the extent of water deficit during the dry season and monthly rainfall variability. The results suggest that when planning to integrate barley within cropping sequences, barley producers should carefully select appropriate barley varieties to minimize autotoxicity, which can be more severe under drought conditions.

Key words: allelopathy — autotoxicity — barley variety — growing season — plant extracts — seedling bioassays

Introduction

Allelopathy is an interference mechanism based on any direct or indirect effect (primarily inhibitory) by one plant on another through the release of chemicals that escape into the environment (Aldrich and Kremer 1997). Numerous plants possess allelopathic properties, including the crops wheat (*Triticum aestivum* L.) (Wu et al. 2001), sorghum (*Sorghum bicolor* (L.) Moench) (Ben-Hammouda et al. 1995), and rye (*Secale cereale* L.) (Raimbault et al. 1990) and the weed giant foxtail (*Setaria faberii* Herrm.) (Bell and Koeppe 1972), yellow nutsedge (*Cyperus esculentus* L.) Drost and Doll 1980) and *Amaranthus* spp. (Connick et al. 1989). Rye and wheat residues can be managed to suppress weed emergence and seedling growth in the field (Blum et al. 1997). Aqueous extracts of oat (*Avena sativa* L.) and barley plants reduced germination and root growth of the winter annual weeds downy brome (*Bromus tectorum* L.), flixweed (*Descurainia sophia* L. Webb), and stinkweed (*Thlapsi arvense* L.) (Moyer and Huang 1997). Water extracts of oat shoots contain phytotoxic concentrations of L-tryptophan that inhibit germination and radicle and hypocotyl growth of lettuce (*Lactuca sativa* L.) in laboratory bioassays (Kato-Noguchi et al. 1994). The allelopathic potential of maize (*Zea mays* L.) is attributed to the allelochemical, benzoxazolinone, which inhibited root and shoot growth of oat and ryegrass (*Lolium multiflorum*) (Kato-Noguchi et al. 1998).

Allelochemicals produced by barley reduced radicle length and vigour of radicle tips of white mustard (*Sinapis alba* L.) (Liu and Lovett 1993). Read and Jensen (1988) reported that extracts of soil with incorporated barley residues reduced seedling length and dry weight of alfalfa (*Medicago sativa* L.), winter wheat and radish (*Raphanus sativa* L.). Barley residue extracts were also allelopathic towards durum and bread varieties of wheat (Ben-Hammouda et al. 2001).

Barley is an important cereal crop in Tunisia. It is grown for grain and pasture for livestock, frequently for both purposes during the same growing season. Farmers typically produce continuous barley crops under rain-fed or irrigated conditions as long as a net economic gain is realized. We recently reported that some barley varieties grown in Tunisia were allelopathic towards other barley varieties, a condition known as autotoxicity (Ben-Hammouda et al. 2002). Barley autotoxicity may depress grain yields under continuous cropping of barley. Most farmers in Tunisia practise direct drilling into cereal residues, therefore, unexpected grain yield declines due to continuous cropping may be avoided by increasing awareness of potential barley autotoxicity (Tollenaar et al. 1993, Christian et al. 1999). The present work was undertaken to determine the variability in allelopathic potential of barley varieties affected by growing season conditions.

Materials and Methods

Field experiments

Four barley varieties ('Manel', 'Martin', 'Esperance', 'Rihane') were grown at the Experimental Station of the Ecole Supèrieure d'Agriculture du Kef (Tunisia) during three growing seasons (1999–2000, 2000–01, 2001–02). The experimental site was located in the semi-arid zone of northern Tunisia on an alkaline (pH = 8.1) clay soil containing 2 % organic matter and classified as a Calcisol (Dekkers et al. 1998). Barley was sown at the equivalent rate of 120 kg ha^{-1}. Field plots consisted of six 10-m rows at 0.2-m row widths, arranged in a randomized complete block design with four replications per treatment (variety). Field plots were prepared with disc-harrow tillage and planted using a standard grain drill. In general, standard cultural practices for the semi-arid zone were followed that included planting in late November and harvesting in late May. Barley growth was monitored throughout the growing season; when severe wilting was observed, plots were supplied with 40 mm of water. Climatic data relative to the three growing seasons were collected from the neighbouring Boulifa/Kef meteorological station (Table 1).

Preparation of water extracts

Whole barley plants were randomly collected from field plots when grain reached physiological maturity. Plants were gently washed with distilled water, dried between two paper towels and separated into roots, leaves, stems and seeds. Except for seeds, plant components were chopped into 1-cm segments and dried at 50 °C for 24 h. The extraction followed the procedure reported by Ben-Hammouda et al. (1995). Briefly, a 2.5-g sample of each plant component was suspended in 50 ml distilled water in a 500-ml flask, which was placed on a rotary shaker for 24 h at 200 rpm. Extracts were passed through cheesecloth, centrifuged at 2000 × ***g*** for 20 min, and filter-sterilized through a 0.2-μm membrane. Extracts were stored in sterile containers at 5 °C prior to bioassay.

Bioassays of barley extracts

Extracts of barley were tested for phytotoxicity on seed germination and seedling growth using 'Manel' barley, a variety previously identified as highly sensitive to allelochemicals (Ben-Hammouda et al. 2002). Molten agar (1.2 %) was amended with plant extract (20 ml 1 l^{-1} agar); controls were non-amended agar. Germination and seedling growth bioassays followed procedures developed by Ben-Hammouda et al. (2001, 2002). Seeds were surface-sterilized with 5 % sodium hypochlorite followed by rinsing in sterile water, placed on agar plates, and incuba-

Table 1: Climatic data for three successive growing seasons (1999–2000, 2000–01, 2001–02) during barley production

	Rainfall (mm)			ETP (mm)			Water balance (mm)		
Month	1999–2000	2000–01	2001–02	1999–2000	2000–01	2001–02	1999–2000	2000–01	2001–02
November	124.6	13.5	37.0	214.0	46.1	141.8	−89.4	−32.6	−104.8
December	80.9	34.1	16.8	88.6	32.9	93.6	−7.7	1.2	−76.8
January	8.6	71.0	18.2	14.6	32.0	95.8	−6.0	39.0	−77.6
February	22.0	48.1	15.8	27.3	37.7	136.3	−5.3	10.4	−120.5
March	7.4	35.9	13.2	46.5	86.6	250.2	−39.1	−50.7	−237.0
April	33.4	28.4	35.1	73.4	96.7	193.4	−40.0	−68.3	−158.3
May	160.0	51.9	50.2	87.4	156.9	264.1	72.6	−105.0	−213.9
Total	436.9	282.9	186.3	551.8	488.9	1175.2	−114.9	−206.0	−988.9
Mean/month	62.4	40.4	26.6	78.8	69.8	167.9	−16.4	−29.4	−141.3
CV (%)	97.3	45.8	53.0	84.0	66.6	41.5	−301.2	−169.4	−45.4

Source: Meteorological Station of Boulifa/Kef, adjacent to the experimental site.
ETP, Evapotranspiration potential.

ted at 25 °C for 35 h at which time germination was determined. For seedling bioassays, pre-germinated 'Manel' barley seedlings (3 mm radicles) were placed on agar slants in test tubes and incubated at 25 °C for 60 h. After incubation, the coleoptile and central radicle of each barley seedling were measured.

Data analysis

Germination and seedling growth bioassays were arranged as a completely randomized design with four repetitions. Data were subjected to analysis of variance using SAS (SAS Institute 1985). Treatments with a significant main effect were separated using Fisher's protected LSD ($P < 0.05$) (Steel and Torrie 1980). Using the average of individual plant component effects as a depression amplitude (DA), (DA = control−treatment/Control × 100), for making a single observation relative to one variety, it was possible to conduct a combined analysis of variety effects on barley autotoxicity across three growing seasons.

Results

Germination bioassays

Of the four barley varieties ('Manel', 'Martin', 'Esperance', 'Rihane') tested during the first growing season (1999–2000), plant component extracts of 'Manel' and 'Esperance' significantly affected barley seed germination (Table 2). However, seed germination was not significantly affected by extracts of any tested variety sampled in the two subsequent growing seasons. Therefore, as shown previously (Ben-Hammouda et al. 2001, 2002), the germination bioassay did not appear to be a sensitive test for detecting autotoxic effects of barley plant extracts.

Seedling growth bioassays

Extracts of barley plant components significantly affected coleoptile growth, but the magnitude of the effects was inconsistent (Table 2). In contrast, plant extract effects on radicle growth were very highly significant ($P < 0.001$) over all test barley varieties and all growing seasons. Therefore, the present report emphasizes seedling growth response to water extracts based on seedling radicle length.

Plant component extracts of all four varieties inhibited 'Manel' barley radicle growth (Table 2); extracts of stems were most allelopathic in 75 % of the bioassays (Table 3). The level of radicle growth inhibition by plant extracts was similar during both 1999–2000 and 2000–01 growing seasons. The severe water deficit during the 2001–02 growing season (Table 1) seemed to decrease allelopathic activity; all barley plant components significantly inhibited radicle growth but to a lesser extent compared with previous seasons (Table 4). Water deficits were 114.9, 206.0 and 988.9 mm, respectively, for the 1999–2000, 2000–01 and 2001–02 growing seasons (Table 1). In semi-arid zones, barley growth and grain yield is influenced by a consistent, monthly rainfall pattern. The relative even distribution of rainfall during the 2000–01 growing season appears to have contributed to the greater barley autotoxicity compared with the other growing seasons (Fig. 1).

The main effect due to variety was significant (Table 4), however, only 'Rihane' significantly inhibited radicle growth of 'Manel' independently of growing season (Fig. 2). The depressive effect of 'Rihane' on seedling growth was not stable because a significant interaction between growing season and variety was detected (Table 4).

Discussion

Bioassays based on radicle growth were more sensitive in detecting allelopathic inhibition than were bioassays of coleoptile growth (Table 3),

Table 2: Treatment mean squares for germination, radicle and coleoptile growth of 'Manel' barley seedlings assayed against plant components of four barley varieties collected over three growing seasons

Growing season	Barley variety											
	'Manel'			'Martin'			'Esperance'			'Rihane'		
	G	RL	CL	G	RL	CL	G	RL	CL	G	RL	CL
1999–2000	3.80*	7.41***	0.09*	1.33	7.25***	0.02	8.68***	4.99***	0.11*	5.83	1.07**	0.03
2000–01	0.20	11.26***	0.09***	0.55	6.61***	0.20**	1.58	8.64***	0.31**	0.93	10.57***	0.17*
2001–02	0.25	8.87***	0.30*	7.6	6.77***	0.09***	1.87	7.48***	0.11*	2.50	9.19***	0.73***

Significantly different from control at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (remaining values are not significant).

G, germination; RL, radicle length; CL, coleoptile length.

Table 3: Effects of water extracts of plant components from four barley varieties collected over three growing seasons on radicle growth of 'Manel' barley seedlings

	Radicle growth (mm)											
	'Manel'			'Martin'			'Esperance'			'Rihane'		
Treatment	1999–2000	2000–01	2001–02	1999–2000	2000–01	2001–02	1999–2000	2000–01	2001–02	1999–2000	2000–01	2001–02
Control	4.58 a	5.05 a	9.98 a	4.13 a	3.85 a	5.70 a	3.95 a	4.60 a	5.03 a	3.88 a	4.68 a	4.98 a
Root extract	1.83 b	1.28 c	2.83 c	1.08 b	0.80 c	3.28 bc	1.63 b	1.80 b	3.70 b	3.50 a	0.90 c	2.20 c
Leaf extract	1.68 bc	1.25 c	2.83 c	1.03 b	1.25 b	2.90 cd	1.58 b	1.58 bc	2.95 b	2.83 b	0.90 c	1.63 cd
Stem extract	1.18 c	1.03 c	2.20 c	1.20 b	1.05 bc	2.28 d	1.23 b	0.95 d	2.10 c	2.65 b	0.98 c	1.18 d
Seed extract	1.65 bc	2.03 b	3.90 b	1.18 b	0.90 c	3.73 b	1.48 b	1.25 c	1.55 c	2.90 b	2.03 b	3.25 b
LSD ($P < 0.05$)	0.615	0.49	0.88	0.51	0.26	0.66	0.68	0.46	0.78	0.58	0.50	0.64

Values followed by lower-case letters in a column represent a significant difference at $P < 0.05$.

Table 4: ANOVA of growing season and barley variety effects on radicle growth depression of 'Manel' barley seedlings

SV	df	SS	MS	F-value	P > F*
Total	47	1.2116			
Growing season	2	0.4384	0.2192	58.37	0.0001
Variety	3	0.1078	0.0359	9.57	0.0001
Growing season × variety	6	0.5301	0.0883	23.53	0.0001
Error	36	0.1352	0.0038		

*Significantly different at $P < 0.001$.

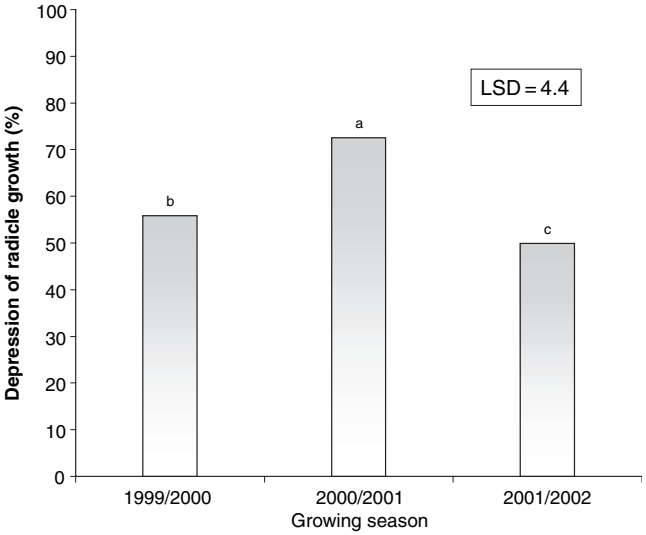

Fig. 1: Effect of growing season on radicle growth (% depression) of 'Manel' barley. Bars having the same letter are not significantly different at $P < 0.05$

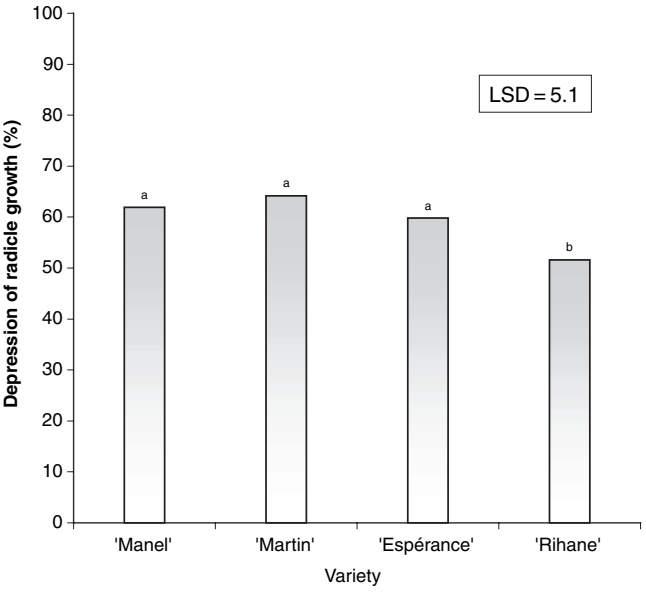

Fig. 2: Effect of variety on radicle growth (% depression) of 'Manel' barley. Bars having the same letter are not significantly different at $P < 0.05$

which agree with previous reports (Hedge and Miller 1990, An et al. 1996, Ben-Hammouda et al. 2002). Radicle growth bioassays detected differential allelopathic potentials of barley plant components similar to effects of sorghum plant components on wheat (Ben-Hammouda et al. 1995). In general, stem extracts were most inhibitory to radicle growth of barley. Similar results were reported for high allelopathic activity of stem extracts of alfalfa (Guenzi et al. 1964) and sorghum (Ben-Hammouda et al. 1995).

Based on the overall allelopathic potential of plant components, barley varieties differentially inhibited radicle growth of 'Manel' barley seedlings. Varietal differences in allelopathic activity of other crops have been documented (Guenzi et al. 1967, Ebana et al. 2001). The present study demonstrates that the allelopathic potential of barley varieties not only differed by source of extract but also by growing season conditions (Fig. 1). The laboratory assays of field-collected barley plants indicated potential allelopathic activity under field conditions in Tunisia and suggested that different types and/or concentrations of allelochemicals may accumulate within the different varieties. Supporting information on effects of water availability during the growing season on allelopathic activity is limited. However, increased allelochemical content of *Calluna vulgaris* due to increasing seasonal water deficit has been reported (Brachet and Mousseau 1974). The extent of decreased cereal grain yields as a result of drought depends on the growth stage when water stress begins and the duration of water stress during the growing season (Ozturk and Aydin 2004). Allelopathic activity combined with water stress at critical plant growth stages may magnify yield decreases in barley grain yields.

Conclusions

This study demonstrated that the autotoxic potential of barley grown under rain-fed conditions in a semi-arid zone varied among varieties and between plant components, and across growing seasons, suggesting that the allelopathic potential of barley as a physiological trait is controlled by the growth environment. Laboratory assays using 'Manel' barley as the indicator species effectively detected potential autotoxic activity among barley varieties, which may be valuable in predicting whether a particular variety will affect the growth of other barley varieties in subsequent seasons in the same field. Consequently, barley producers who rely on a barley–barley cropping sequence must sow the least autotoxic variety prior to the most tolerant one, if market prices favour a profitable economic return. Barley should be considered a high allelopathic risk in a barley–barley cropping sequence, especially in the semi-arid zone, which is generally characterized by periods of severe water deficits during the growing season.

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