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Stimulatory potential of rapeseed residues for barley seedling growth

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SUMMARY – A rapeseed/canola (*Brassica napus* L.) variety 'Drakkar' was grown and collected at the green silique stage. The allelopathic potential of water-extracts of rapeseed plant parts was studied using radicle growth bioassays with 3 local barley test-varieties ('Manel', 'Martin' and 'Rihane'). To investigate the chemical basis of the rapeseed allelopathic potential, total-phenolics (TP) and 5 phenolic acids (PA) [p-hydroxybenzoic (POH), vanillic (VAN), syringic (SYR), p-coumaric (PCO), ferulic (FER)] within rapeseed plant parts were quantified by analytical methods (spectrophotometry, HPLC). Except for roots-water-extracts, the remaining water-extracts had an effect switching from stimulation to inhibition of barley radicle growth, depending on the barley test-variety. Roots-extracts had a steady stimulatory effect on barley radicle growth, with 'Rihane' being the most sensitive variety. TP analysis of plant parts revealed that rapeseed accumulates phenolics in increasing order: roots ‹ stems ‹ siliques ‹ leaves. For root, leaf and stem water-extracts, all studied PA were present. However, POH and VAN were absent in siliques-water-extracts. FER was the most abundant PA in all rapeseed plant parts with roots-water-extracts having the lowest concentration. Results of bioassays and phenolics suggest a combined threshold concentration [TP & FER], under which the allelopathic effect of rapeseed is strictly stimulatory for barley radicle growth. The potential stimulatory effect of rapeseed root residues on barley makes the rapeseed/barley sequence of great interest for farmers practising conventional drilling. However, a better understanding of the allelopathic potential of the whole rapeseed plant residues will help developing appropriate rapeseed residue management to practice direct drilling and grow barley or other small grains.

Keywords: Allelopathic potential, rapeseed, barley, total-phenolics, phenolic acid.

RESUME – *"Potentiel des résidus du colza pour stimuler la croissance des plantes d'orge". Des plantes entières de la variété 'Drakkar' de colza (Brassica napus L.) ont été collectées au stade silique verte, pour étudier le potentiel allélopathique de toutes leurs composantes. La technique de bio-essais de la croissance de la radicule a été utilisée pour 3 variétés-test ('Manel', 'Martin', 'Rihane') d'orge. En utilisant les techniques de spectrophotométrie et de chromatographie (HPLC), une étude chimique du potentiel allélopathique du colza a porté sur des analyses quantitatives du total-phénols (TP) et de 5 acides phénoliques (AP) [p- hydroxybenzoïque (POH), vanillique (VAN), syringique (SYR), p-coumarique (PCO), férulique (FER)]. L'analyse du TP a montré que le colza accumule les phénols par ordre croissant: racines ‹ tiges ‹ siliques ‹ feuilles. Tous les AP, objet d'étude, étaient présents dans les racines, feuilles et tiges. Cependant, POH et VAN ne faisaient pas partie des extraits-eau des siliques. Vu que: (i) les racines-extraits-eau ont manifesté une stimulation de la croissance de la radicule de toutes les variétés-test d'orge et (ii) ces mêmes extraits-eau ont les concentrations les plus faibles en TP et en FER, il paraît qu'il y a un certain seuil combiné de concentrations [TP & FER] pour que le potentiel allélopathique du colza soit strictement stimulateur. Un tel potentiel des résidus-racines du colza serait d'un grand intérêt pour les céréaliers qui pratiquent le semis conventionnel. Cependant, l'étude du potentiel allélopathique de toutes les parties (racines, feuilles, tiges), qui pourraient faire l'objet d'une couverture végétale pour un semis direct, aide mieux à développer le management le plus approprié des résidus du colza pour la production de l'orge ou d'une autre céréale.*

Mots-clés : *Potentiel allélopathique, Colza, Orge, Phénols, Acide phénolique.*

Introduction

Several crucifer species were reported to have a phyto-toxic potential, such as the case of black mustard [*Brassica nigra* (L.) Koch.], which reduced wild barley (*Hordeum spontaneum* Koch.) growth (Tawaha and Turk, 2003). Four phenolic acids (ferulic, p-hydroxybenzoic, p-coumaric, m-coumaric), extracted from rice hull, reduced seed germination and seedling dry weight of barnyard grass [*Echinochloa crus-galli* (L.) Beauv.], with an increasing effect as the concentration of phenolic acids gets higher (Chung *et al.*, 2002).

Rapeseed is used frequently as a prior crop to small grains in conventional agriculture, based on soil preparation (Guy, 1999). However, in conservation agriculture based on direct drilling, residues of a prior crop could be advantageous whenever they release any stimulatory substance into the risosphere of an economic crop.

Experimental work on conservation agriculture, based on direct sowing, started in Tunisia in 2000/01 (M'hedhbi *et al*., 2003). The present preliminary work aims to study: (i) the form of rapeseed allelopathy; (ii) the differential allelopathic potential of rapeseed plant parts; (iii) differential sensitivity of barley varieties to an eventual allelopathic potential of rapeseed; and (iv) the role of total-phenolics and individual phenolic acids in the expression of a such potential.

Materials and methods

Rapeseed plant preparation

Plant material of 'Drakkar', a rapeseed variety, was collected in June/2001 from the Experimental Station of Ecole Supérieure d'Agriculture du Kef (ES/ESAK), located in the semi-arid zone of Tunisia, while plants were still at the green silique stage.

Bioassays

Three local barley varieties ('Manel', 'Martin', 'Rihane') were tested for the allelopathic potential of rapeseed. Water-extracts of rapeseed plant parts (roots, leaves, stems, siliques) were prepared for radicle growth bioassays, following the procedure described by Ben-Hammouda *et al*. (1995a, 2001, 2002). Since radicle growth bioassay is known to be the most sensitive test to water-extracts of allelopathic crops (Hegde and Miller 1990; Ben Hammouda *et al*., 2001; Ben Hammouda *et al*., 2002), only radicle growth (cm) is reported in this present work. Radicle growth inhibition of barley test-varieties was calculated as follows: [(Control – Treatment)/(Control) × 100].

Determination of total-phenolics

For total-phenolics (TP) analysis, Folin-Denis method was applied (AOAC, 1990), with tannic acid (TA) as a standard, following the procedure described by Makkar (2000).

HPLC analysis of phenolic acids

Rapeseed water-extracts were analyzed for 5 phenolic acids (PA) [p-hydroxybenzoic (POH), vanillic (VAN), syringic (SYR), p-coumaric (PCO), ferulic (FER)], following the procedure reported by Ben-Hammouda *et al*. (1995b).

Statistical analysis

The experimental design for radicle growth bioassays was a Complete Randomized Design (CRD) with 4 repetitions. Data were subjected to an analysis of variance, using SAS package (SAS Institute, 1985). Mean separation for significant main effect was conducted using the least significant difference test (LSD), at the 5% level of probability.

Results and discussion

Radicle growth bioassay

Water-extracts, having a significant effect on radicle growth, showed a stimulatory or inhibitory effect depending on the test-variety. Except stems-water-extracts, the remaining water-extracts stimulated 'Manel' radicle growth, with leaves-water-extracts being the most effective (26%). 'Martin' radicle growth was significantly stimulated (16%) by roots-water-extracts but significantly inhibited (24%) by siliques-water-extracts. For 'Rihane', leaves-water-extracts were inhibitory (38%) and the remaining water-extracts had a stimulatory effect, with the highest effect (48%) for siliques-water-extracts (Table 1).

Table 1. Effects of water-extracts from different plant parts of rapeseed on radicle growth (cm) of 'Manel', 'Martin' and 'Rihane' barley varieties

Treatment	Radicle growth (cm) of test varieties		
	'Manel'	'Martin'	'Rihane'
Control	1.94 bc†	4.52 bc	3.36 c
Roots-extract	1.96 bc	5.24 a	4.41 b
Leaves-extract	2.44 a	4.79 ab	2.08 d
Stems-extract	1.68 c	4.20 c	4.23 b
Siliques-extract	2.33 ab	3.46 d	4.98 a
LSD (5%)	0.47	0.56	0.44

†Means within a column followed by different letters are significantly different at 5% level of probability

Since roots-water-extracts were steady stimulatory to radicle growth of all test-varieties and the fact that roots of rapeseed as a prior crop to barley are to be left in the soil whether farmer is practicing conventional or direct sowing, differential sensitivity of test-varieties radicle growth was based on the stimulatory effect of rapeseed roots-water-extract. 'Rihane' had its radicle growth 30 and 2 times more stimulated than 'Manel' and 'Martin', respectively (Table 1). Differential sensitivity of barley varieties to rapeseed allelopathic potential indicates their genetic differences. Similar results were reported, concerning the differential response of both durum and bread wheat varieties (Ben-Hammouda *et al.*, 2001) and barley varieties (Ben-Hammouda *et al.,* 2002) to barley allelopathy.

Total-phenolics and phenolic acids

Analysis of TP content of rapeseed plant parts revealed that roots-water-extracts which were exclusively stimulatory to barley radicle growth, had the lowest concentration (53 μg equivalent-TA/g-tissue),whereas leaves-water-extracts had the highest (452 μg equivalent-TA/g-tissue) concentration (Table 2).

Leaves had the highest TP content in 3 grain-sorghum (*Sorghum bicolor* L.) hybrids when compared to seeds, glumes, culms and roots (Ben-Hammouda *et al.*, 1995b). For a concentration over 53 μg equivalent-TA/g-tissue, inhibitory effect may appear for leaves (452 μg equivalent-TA/g-tissue), stems (223 μg equivalent-TA/g-tissue) and siliques (314 μg equivalent-TA/g-tissue) water-extracts, depending on test-variety. These results suggest that there's a threshold concentration for TP inhibition to take it over a stimulation. Therefore, the relationship between TP and inhibition is not always positive. This is a sign for a quality role of phenolics in the expression of rapeseed allelopathy. Rapeseed plant tissues accumulate preferentially phenolics in stems, siliques and leaves with 4, 6 and 8 times more than roots, respectively (Table 2). Similar results were obtained for phenolics partitioning within grain-sorghum plant parts (Sène *et al.*, 2001).

All investigated PA were present in rapeseed plant parts, except POH and VAN which were absent in siliques. Leaves and stems water-extracts had the same content of the 5 investigated PA, with FER having the highest concentration as was the case for all plant parts. Roots had the lowest concentration of FER when compared to leaves, stems and siliques. The exclusive stimulatory effect of roots-water-extract could be probably due to a threshold concentration of FER or to its manner of combining with other implicated PA to make a TP effect (Table 2).

Table 2. Total-phenolics (TP) and phenolic acids (PA) contents in plant parts of rapeseed

Source of extract	TP content[†]	PA	PA Content[††]
Roots	53.0	POH	0.008
		VAN	0.230
		SYR	0.142
		PCO	0.010
		FER	0.316
Leaves	451.7	POH	0.207
		VAN	1.676
		SYR	0.113
		PCO	0.027
		FER	3.291
Stems	223.2	POH	0.207
		VAN	1.676
		SYR	0.113
		PCO	0.027
		FER	3.291
Siliques	313.9	POH	0.000
		VAN	0.000
		SYR	0.006
		PCO	0.001
		FER	0.384

[†]μg equivalent-TA/g-tissue
[††]μg/g-tissue

Conclusions

The stimulatory form of rapeseed allelopathy, originated by roots or other plant parts, gives an opportunity to cereal producers to use rapeseed residues as a cover crop in a barley/rapeseed sequence. Farmers practicing conventional drilling had better ask for a roots-water-extract bioassay to make the appropriate choice of a subsequent barley variety to rapeseed. However, for farmers practicing direct drilling, results of this present work showed that the aerial rapeseed plant parts (leaves, stems) could exhibit an inhibitory activity on a barley variety. A better understanding, with a future laboratory work is highly recommended, taking into consideration the effect of the whole plant water-extract and not water-extracts of plant parts taken separately. It would be more useful to run the laboratory work jointly with a field experiment to evaluate the allelopathic potential of rapeseed residues on barley.

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