Assessment of possible allelopathic interactions between soybean (*Glycine max*) and *Amaranthus powellii* and *Cyperus rotundus* using *in vitro* systems.

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Abstract

We studied the allelopathic activities of aqueous extracts of Powell's amaranth (*Amaranthus powellii*) and nutgrass (*Cyperus rotundus*) on growth of soybean cvs. Banjalong, Melrose and Valiant. Weed extracts at 0, 1 or10% (v/v) was added to Murashige and Skoog (MS) basal medium. At 4 weeks of age plant fresh weight was reduced by 71% when Melrose was grown in media supplemented with 10% of amaranth extract. Regardless of the soybean cultivars, 1% of Powell's amaranth or nutgrass reduced callus fresh weight of the hypocotyls by 28 and 16%, respectively. The superior growth in Melrose grown on medium without weed extract was supported by its high mitotic index of 0.87 compared to 0.27 when germinated in 25% (w/v) of Powell's amaranth extract.

Key words

allelopathy, Powell's amaranth, nutgrass, tissue culture, mitotic index

Introduction

In common with other crops, soybean, may interfere with the growth of its neighboring plants either directly, through resource competition or via chemical inhibition. Research has shown that soybean growth and yield can be reduced through allelopathic mechanisms (1,2,4). However, adequate *in vitro* studies of allelopathy on this crop have not been reported. We studied the response of soybean grown *in vitro* to aqueous extract of nutgrass and Powell's amaranth as part of a series of studies on the allelopathic mechanisms and mode of action of weeds on soybean, both *in vitro* and *in vivo*.

Methods

In vitro culture

Whole fresh weed material was cut into 3 cm portions and soaked in distilled water for 24 hours at room temperature, filtered through No. 1 Whatmann filter paper, the pH was adjusted to 5.8 then aseptically sterilized through 20-µm filters. Weed extract was added at 0% (control), 1% or 10% (v/v) to MS basal medium (3) containing 3% sucrose and 8 g L^{-1} BiTek agar at pH 5.8. Soybean seeds were germinated on $1/10^{th}$ strength MS medium for one week. Shoot tips were grown for shoot growth examination and the hypocotyl sections, 5 mm, were grown in Petri dishes at 25??2?C with a 16 hour-photoperiod and 30 µmol m^{-2} s^{-1} of light. Factorial experiments using a completely randomized design (CRD) and 5 replicates were used. After 4 weeks, plantlets fresh weight and fresh weight of callus, including hypocotyls, were recorded.

Mitotic index determination

Ten seeds of each soybean cultivar were germinated in 10-cm Petri dishes with 10 mL of either 25% (w/v) weed extract or distilled water (control). This experiment was conducted at 25?C in total darkness with 5 replicates using a CRD design. Radicles, 1-1.5 cm long, were harvested and soaked for 4 hours in 0.1%

colchicines, hydrolised with 1N HCl for 5 minutes at 60?C and stained in 1% orcein + 1 N HCl for 10 minutes at 60?C. The root tip, 0.5 mm, was placed on a glass slide with one drop of orcein solution and finely squashed. The suspension was covered for microscopic examination with 400X magnification. Ten fields of view were examined for each glass slide and the number of cells at the mitotic stage was recorded and the percentage of mitotic cells compared to the number of total cells under one field was assessed as the mitotic index.

Results

The interaction of soybean cultivars, weed species and concentration of weed extract caused marked effects on the callusing of soybean hypocotyls ($P<0.001$) and plantlet fresh weight ($P<0.005$). Weed extracts reduced callus weight and the more concentrated the extract, the larger the reduction (Table 1). All hypocotyls sections remained dark green and enlarged from their original size. Callus formed in this experiment was light green in color.

The higher concentration of weed extract markedly reduced soybean fresh weight. The 10% Powell's amaranth extract treatment reduced plantlet fresh weight by 71% in Melrose compared to the control treatment. A similar trend occurred for the other cultivars with the exception of Melrose in the nutgrass extract treatment. An increase in plantlet fresh weight of approximately 18% was observed when the concentration of nutgrass extract was increased from 1% to 10%, although the increase was not significant. There was no effect of the extract treatments on leaf chlorophyll content (data not shown).

Interactions of soybean cultivars and weed extract reduced mitosis of soybean root tips ($P<0.001$). Overall, Powell's amaranth caused the lowest mitotic index (0.26) and reduced mitosis in all cultivars (Table 1). All weeds minimised mitotic index in Valiant indicating varietal differences among the cultivars tested.

Table 1. Growth response of soybean cultivars to different levels of weed extract

Cultivars	Weed extract	Mitotic index[1]	Callus weight[2]	Plant weight
			(mg)	
Banjalong	Control	0.76 $^{A\,c}$	141.0 $^{A\,a}$	918.0 $^{A\,a}$
	Amaranth 1%	0.29 $^{A\,a}$	100.1 $^{A\,a}$	643.2 $^{A\,ab}$
	Amaranth 10%		93.9 $^{A\,a}$	512.7 $^{A\,b}$
	Nutgrass 1%	0.57 $^{A\,b}$	128.7 $^{A\,a}$	413.5 $^{A\,bc}$
	Nutgrass 10%		110.3 $^{A\,a}$	220.8 $^{A\,c}$
Melrose	Control	0.87 $^{B\,c}$	118.2 $^{A\,a}$	1220.3 $^{B\,a}$

	Amaranth 1%	0.27 $^{A\ a}$	86.9 $^{A\ a}$	380.6 $^{A\ b}$
	Amaranth 10%		83.9 $^{A\ a}$	353.8 $^{B\ b}$
	Nutgrass 1%	0.63 $^{B\ b}$	115.9 $^{A\ a}$	180.9 $^{A\ b}$
	Nutgrass 10%		91.6 $^{A\ a}$	213.2 $^{A\ b}$
Valiant	Control	0.61 $^{C\ c}$	126.3 $^{A\ a}$	1013.2 $^{AB\ a}$
	Amaranth 1%	0.21 $^{B\ a}$	93.4 $^{A\ ab}$	698.1 $^{B\ b}$
	Amaranth 10%		80.8 $^{A\ ab}$	501.6 $^{A\ bc}$
	Nutgrass 1%	0.27 $^{C\ b}$	121.3 $^{A\ ab}$	398.4 $^{A\ c}$
	Nutgrass 10%		60.0 $^{A\ b}$	314.2 $^{A\ c}$
		P<0.001	P<0.001	P<0.005
		(*n*=30)	(*n*=30)	(*n*=5)

Values within the same column followed by the same small letters are not significant within the same soybean cultivar. Values sharing the same capital letters are not significant between soybean cultivars for the corresponding treatment at 5% LSD.
[1]Both amaranth and nutgrass at 25% (w/v)
[2]Callus from hypocotyl culture

Conclusions

Aqueous extracts of both amaranth and nutgrass caused allelopathic effects on soybean cell division and soybean growth *in vitro* as demonstrated by plant weight and mitotic index reduction. Amaranth had the most inhibitory effect on soybean grown *in vitro* and on cell division. These changes in plant growth may in part explain some of the negative effects that weed species have on soybean growth.

References

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