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Soybean Root-Tip-Cell Mitosis under the Influence of Aqueous Extracts of Three Weed Species

Pengaruh Ekstrak Segar Tiga Spesies Gulma terhadap Pembelahan Sel Ujung Akar Kedelai

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ABSTRACT

An experiment aimed to examine the effect of aqueous extracts of three weed species on soybean root-tip-cell mitosis has been carried out at the Laboratory of Plant Physiology and Biotechnology, Department of Agronomy and Soil Science, University of New England, Australia. Aqueous extracts of fresh weed material (*Amaranthus powellii*, *Cyperus rotundus* and *Paspalum dilatatum*) at the concentration of 25% (w/v) were added to the germination medium of soybean seeds cultivar Banjalong, Melrose, and Valiant. The germination was conducted at a growth cabinet with 25 °C and total darkness. All experimental units were arranged in a completely randomized design with 5 replication. Radicles (roots) from the germinating soybeans were harvested when they were about 1 – 1.5 cm long. The soybean root tips were used to observe the mitotic index following a series of hydrolysis and staining process. Results indicate that aqueous extracts of the weeds reduced cell division of the soybean regardless of the cultivars. This contributes to the understanding of the modes of action of the allelopathic effect of the weeds. *Amaranthus powellii* had the most inhibitory effect to the soybean cell division.

Keyword: allelopathy, soybean, mitotic index, amaranthus, cyperus, paspalum

INTRODUCTION

The phenomena of allelopathy have been of interest for over 2000 years. This occurrence was firstly documented by Theophrastus (300 BC) who observed that chickpea (*Cicer arietinum*) negatively affected weeds surrounding it (Rice, 1984). Even though the history of allelopathy was dated back for a long time, the term 'allelopathy' itself was not coined until 1937. It was introduced by Professor Hans Molisch of Vienna (Willis, 1996) to describe any direct or indirect (harmful or beneficial) effect of a plant, including microbes, or another plants through release of chemicals that can escape into the environment (Rice, 1984). In its activity, allelopathy involves a complex chain of chemical communication between plant species (Harborne, 1987).

Hundreds of different compound released from plants and microbes are known to have allelopathic effects on the receiving species. In recent years, many new compounds that have allelopathic properties, known as allelochemicals, have been identified and studied (Einhellig, 1995). They have demonstrated the ability to affect many physiological processes in the receiving plants such as damage to cell membrane integrity in soybean (Baziramakenga *et al.*, 1995) and membrane perturbations may be a starting point for the multiple actions of allelochemicals (Einhellig, 1995), reduction in germination (Wardle *et al.*, 1991; Einhellig and Souza, 1992; Sajjan *et al.*, 1997),

reduction in respiration (Abrahim *et al.*, 2003a; 2003b; Muscolo *et al.*, 2001; Penuelas *et al.*, 1996), and enzyme activity (Baziramakenga *et al.*, 1995; Devi and Prasad, 1996; Maffei *et al.*, 1999; Ng *et al.*, 2003).

Studies have demonstrated that *Amaranthus powellii*, *Cyperus rotundus* and *Paspalum dilatatum* interfere with the growth of different soybean cultivars. These weed species had both competitive and allelopathic effects under *in vitro* and *in vivo* experimental conditions (Chaniago *et al.*, 2003a; 2003b; 2002; 2001). The inhibition in soybean growth as indicated by total biomass decline might be one of the complex physiological responses of soybean towards weed interference.

The work reported here examined the response of soybean growth to amaranth, nutgrass and paspalum as indicated by mitotic index determination of the squashed root tips of germinating soybean. It is anticipated that this experiment would provide a better understanding of the allelopathic mechanisms and a possible mode of action of three weeds on soybean.

MATERIALS AND METHODS

Soybean cv. Banjalong, Melrose and Valiant were used in this experiment. Fifty g of fresh weed materials, both aerial and underground parts at the ratio of 1:1 (w w^{-1}), were cut into 3-cm portions and soaked in 200 mL of distilled H_2O in a glass beaker for 24 hours at room temperature. To avoid the effects of light interference that might breakdown the compounds, the glass was wrapped with aluminium foil. The extracts were then filtered through cheesecloth before further filtration through No. 1 Whatmann filter paper

Soybean seeds were surface-sterilised by immersion in 70% aqueous ethanol for 1 minute and were then soaked in 1% (w v^{-1}) of sodium hypochloride (NaOCl) solution for 15 minutes. The seeds were then rinsed with sterilized distilled water 4 times.

Ten seeds of each soybean cultivar were placed in a 10-cm diameter Petri dish on 2 discs of No. 1 Whatmann filter papers supplied with 10 mL of the amaranth, nutgrass or paspalum extracts. The same amount of distilled water was added to the treatment without extracts (control group). The treatments were replicated 5 times and all Petri dishes were arranged in a completely randomized design (CRD). The experiment was conducted in a thermostatically controlled growth cabinet at 25 °C and in total darkness. Data were analysed using two-way analysis of variances (ANOVAs).

Radicles were harvested when they were about 1 – 1,5 cm long and were soaked in 0,1% colchicines for 4 hours. The radicles were then kept in 70% ethanol solution (for preservation purposes) before hydrolisation with 1N HCl for 5 minutes at 60 °C. The radicles were then stained to contrast chromosome by soaking in 1% orcein + 1 N HCl for 10 minutes at 60 °C.

The root tips were cut into 0.5-mm portions and one piece of the root tip was placed on a slide glass. One drop of 1% orcein solution was added to and the root tip was finely squashed with the tip of a scalpel blade. The suspension was then covered for microscopic examination and the edge of the glass slide cover was sealed using a fast-drying nail gloss to prevent the evaporation of the stain solution. Each treatment had 3 replicates.

Every glass slide was considered as a replicate of each treatment combinations. Ten fields of view were examined under a light microscope for each glass slide with 400X magnification. The number of cells that were at the mitotic stage, regardless of which mitotic phase, were recorded. This was conducted by counting the number of cells showing their chromosomes. The mitotic index was calculated as percentage of the mitotic cells to the number of total cells under one field.

RESULTS AND DISCUSSION

The interaction effect of soybean cultivars and weed-species extract on the mitotic index values is presented in Figure 1. The interaction between soybean cultivar and weed-species extract had a marked effect on the mitosis of soybean root tips ($P<0.001$). Valiant showed the lowest mitotic index (33,9% of control) under the influence of Powell amaranth extract and had the

smallest mitotic index in response to nutgrass extract and distilled water. However, the extract of paspalum affected this cultivar less than Melrose in terms of its mitotic index. All soybean cultivars tested responded negatively to the extracts as indicated by reductions in mitotic index.

Each soybean cultivar had a different mean of mitotic index ($P<0,001$) and the index in Melrose was higher than that of the other cultivars. However, the presence of either Powell amaranth or nutgrass extracts had similar effects on the mitotic index in cultivars Banjalong and Melrose. In contrast, the effects of paspalum resulted in marked difference in mitotic index among all cultivars and Melrose was affected most. The data indicate that cultivars and weed extracts interact to give different levels of reduction in cell division in soybean cultivars.

When focusing on the weed species effect on cell division of soybean root tips, regardless of its cultivars, all weed species markedly reduced the mitotic index ($P<0,001$) (Table 1). Powell amaranth had a distinct ability to inhibit the mitosis of the soybean root tips. This weed species caused the greatest reduction in mitotic index in all cultivars. The reductions were 62, 68, and 66% of control treatment in Banjalong, Melrose, and Valiant, respectively.

The aqueous extract of weed species resulted in a decline in the mitosis of soybean root tips. In order of magnitude, Powell amaranth, paspalum and nutgrass showed potential as a mitotic disrupter to soybean seedling growth. Powell amaranth had a distinct ability to inhibit the mitosis of the soybean root tips. However, the result in the present experiment suggest that all three weed species studied resulted in a decline in growth as a result of a level low mitotic index.

Most of the cells were under metaphase stage when the chromosomes are lined at the equator of a spindle, forming a metaphase plate. Soybean root tips in this experiment were soaked in colchicine solution to prevent the alignment and separation of the chromosomes which in turn produced so-called arrested metaphase. The inclusion of colchicines in this experiment was to contrast the chromosomes and made them possible to be counted. Vaughan and Vaughn (1988) also reported that onion root cells treated with caffeine affected the completion of cell division following nuclear division. Caffeine is an alkaloid isolated from the fruit of coffee. This work has now suggested that allelochemicals interfered with cell division in soybeans.

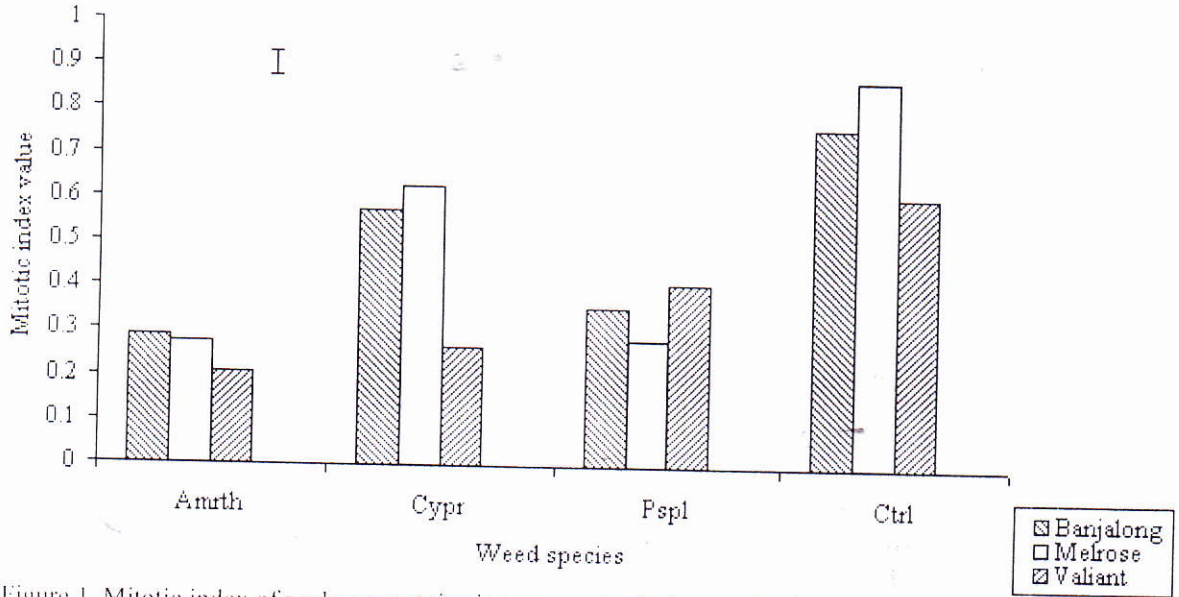

Figure 1. Mitotic index of soybean root tips in response to the interaction between soybean cultivars and weed species. Error bar indicates standard error of means ($P<0,001$). Each bar is a mean of 30 fields of view. Amrt=amaranth, Cypr=nutgrass, Pspl=paspalum, Ctrl=no weed

Table 1. Mean values of mitotic index of soybean root tips in response to different weed species extracts

Weed species	Mean of mitotic index*
Control	0,75
Amaranthus powellii	0,26
Cyperus rotundus	0,49
Paspalum dilatatum	0,35

Mean value of each treatment is an average of 30 fields of view. ($P<0.001$)

Both nutgrass and amaranth spp. have been shown to affect germination and root growth in a range of species (Setyowati dan Suprijono, 2000; French and Leather, 1979; Friedman and Horowitz, 1971; Reynold, 1977; Connock *et al.*, 1987). This reduced growth might be due to the disruption in cell division at an early stage of vegetative growth. This mitotic disruption suggested interference with a fundamental regulatory mechanism in cell division. Friedman and Horowitz (1971) also reported that the reduction in germination and root elongation of some crop species occurred when 25% (w v^{-1}) extracts was undiluted meanwhile the diluted aqueous extract at 100 to 1000 times stimulated root elongation. This finding suggested that nutgrass extracts causes effects that are species and concentration-specific. The experiment reported here used 25% (w v^{-1}) undiluted extracts of weeds and all weed species resulted in a reduction of mitotic index relative to distilled water.

Rice (1984) noted that allelochemicals can affect the growth or aspects of function of the receiving species. These effects may be manifested through a wide variety of metabolic activities including cell division and elongation, action of inherent growth regulators, mineral uptake, photosynthesis, respiration, stomatal opening, protein synthesis, lipid and organic acid metabolism, membrane permeability and the action of specific enzymes.

The dramatic reductions in mitotic index in all soybean cultivars by all weed extracts indicated the presence of a very important factor in allelopathic interactions from the weed species tested under the present study. The potent inhibiting compounds from the weed extracts could well explain the decline in soybean growth and biomass production both *in vitro* and *in vivo*.

CONCLUSION

Aqueous extracts of Powell amaranth, nutgrass and paspalum reduced mitotic index in all soybean cultivars under the experimental conditions. Powell amaranth caused the most inhibitory effects on cell division and Melrose was affected most by paspalum. This result provides a better understanding of the mechanisms of allelochemicals in the target plants.

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REFERENCES

Abrahim, D., A. C. Francischini, E. M. Pergo, A. M. Kelmer-Bracht and E. L. Ishii-Iwamoto. 2003a. Effects of alpha-pinene on the mitochondrial respiration of maize seedlings. Plant Physiology and Biochemistry, 44: 985-991.

Abrahim, D., L. Takahashi, A. M. Kelmer-Bracht and E. L. Ishii-Iwamoto. 2003b. Effects of phenolic acids and monoterpenes on the mitochondrial respiration of soybean hypocotyl axes. Allelopathy Journal, 11: 21-30.

Abrahim, D., W. L. Braguini, A. M. Kelmer-Bracht and E. L. Ishii-Iwamoto. 2000. Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. Journal of Chemical Ecology, 26: 611-624.

Baziramakenga, R., G. D. Leroux and R. R. Simard. 1995. Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. Journal of Chemical Ecology, 21: 1271-1285.

Chaniago, I., A. Taji and R. S. Jessop. 2001. Interactions of soybean varieties and weed species: Effects of weed density. *In*

Proceedings: The 4th International Legume Conference, 2-6 July 2001, "Legumes Down Under", Australian National University, Canberra.

Chaniago, I., A. Taji and R. S. Jessop. 2002. In vitro study of weed alelopathy on soybean (*Glycine max*). *In*. A. Taji and R. Williams, (eds.) *p.* 255-262. Proceedings: The 7th meeting of the International Association for Plant Tissue Culture and Biotechnology (The Australia Region), 20-23 Jan 2002, The importance of plant tissue culture and biotechnology in plant sciences, University of New England, Armidale, University of New England Publications Unit, Australia.

Chaniago, I., A. Taji and R. S. Jessop. 2003a. Assessment of possible allelopathic interactions between soybean (*Glycine max*) and *Amarantus powellii* and *Cyperus rotundus* using *in vitro* system. *In*. M. Unkovich and G. O'Leary, (eds.) Proceedings: The 11th Australian Agronomy Conference, 2-6 Feb. 2003, Solutions for a better environment, Geelong, Victoria, Australian Society of Agronomy,Available:*http://www.regional.org.au/au/asa/2003/p/3/chaniago.htm.*

Chaniago, I., A. Taji and R. S. Jessop. 2003b. Weed interference in soybean (*Glycine max*). *In*. M. Unkovich and G. O'Leary, (eds.) *Proceedings: The* 11th Australian Agronomy Conference, 2-6 Feb. 2003, Solutions for a better environment, Geelong, Victoria, Australian Society of Agronomy,Available:*http://www.regional org.au/au/asa/2003/c/18/chaniago.htm.*

Connock, Jr., W. J., J. M. Bradow, M. G. Legendre, S. L. Vail and R. M. Menges. 1987. Identification of volatile allelochemicals from *Amaranthus palmeri* S. Wats. J. Chem. Ecol., 13:463-472.

Devi, S. R. and M. N. V. Prasad. 1996. Ferulic acid mediated changes in oxidative enzymes of maize seedlings: Implications in growth, Biologia Plantarum, 38:387-395.

Einhellig, F. A. 1995. Allelopathy: current status and future goal, *In* Allelopahy: organisms, processes, and applications, (eds.) Inderjit, K. M. M. Dakshini and F. A. Einhellig. ACS Symposium Series 582, American Chemical Society, Washington DC.

Einhellig, F. A. and I. F. Souza. 1992. Phytotoxicity of sorgoleone found in grain sorghum root exudates, Journal of Chemical Ecology, 18: 1-11.

French, R. C. and G. R. Leather. 1979. Screening of nonanal and related volatile flavor compounds on the germination of 18 species of weed seed, J. Agric. Food Chem., 27:828-832.

Friedmann, T. and M. Horowitz. 1971. Biologically active substances in subterranean parts of purple nutsedge. Weed Sci., 19:398-401.

Harborne, J. B. 1987. Chemical signals in the ecosystem, Annals of Botany, 60: 39-57.

Maffei, M., C. M. Bertea, F. Garneri and S. Scannerini. 1999. Effect of benzoic acid hydroxy- and methoxy- ring substituents during cucumber (*Cucumis sativus* L) germination. I. Isocitrate lyase and catalase activity, Plant Science, 141: 139-147.

Muscolo, A., M. R. Panuccio and M. Sidari. 2001. The effect of phenols on respiratory enzymes in seed germination - Respiratory enzyme activities during germination of *Pinus laricio* seeds treated with phenols extracted from different forest soils, Plant Growth Regulation, 35: 31-35.

Ng, P. L. L., M. L. L. Ferrarese, D. A. Huber, A. L. S. Ravagnani and O. Ferrarese-Filho. 2003. Canola (*Brassica napus* L.) seed germination influenced by cinnamic and benzoic acids and derivatives: effects on peroxidase, Seed Science & Technology, 31: 39-46.

Penuelas, J., M. RibasCarbo and L. Giles. 1996. Effects of allelochemicals on plant respiration and oxygen isotope fractionation by the alternative oxidase, Journal of Chemical Ecology, 22: 801-805.

Reynold, T. 1977. Comparative effects of alipathic compounds on inhibition of lettuce fruit germination. Ann. Bot. 41:637-648.

Rice, E. L. 1984. Alelopathy, 2nd edition, Academic Press, Orlando, Florida.

Sajjan, A. S., S. M. Hiremath, B. D. Biradar and G. G. Koppa. 1997. Allelopathic effects of aqueous extracts of *Parthenium hysterophorus* plant parts on germination and seedling growth of some crops, Crop Research Hisar, 14: 37-43.

Setyowati, N dan E. Suprijono. 2000. Efikasi alelopati teki formulasi cairan terhadap gulma *Mimosa invisa* dan *Melochia corchorifolia*. J. Ilmu-Ilmu Pertanian Indonesia, 2:75-82.

Wardle, D. A., M. Ahmed and K. S. Nicholson. 1991. Allelopathic influence of nodding thistle (*Carduus nutans* L.) seeds on germination and radicle growth of pasture plants. New Zealand Journal of Agricultural Research, 34: 185-191.

Willis, R. J. 1996. The history of alelopathy 1. The first phase 1785 - 1845: the era of A. P. de Candolle. Allelopathy Journal, 3: 165-184.