Tolerance of Cultured *Amaranthus retroflexus* Cells to Atrazine

Yoshio Shigematsu*, Sarinee Chaicharoen**, Fumihiko Sato, and Yasuyuki Yamada
Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto 606-01, Japan

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Resistance to *s*-triazine-herbicides in weeds is the most widespread and extensively studied of all intraspecific herbicide-resistance. It is of interest that the resistant biotype appears in some limited genera such as *Amaranthus* spp. and *Chenopodium* spp. much more frequently than in many other significant weeds. We examined the response of cultured *Amaranthus retroflexus* cells to atrazine in comparison with those of several other plant species to understand what causes this differentially inter-specific response. Atrazine scarcely inhibited the cell growth of either atrazine-resistant and susceptible-*Amaranthus* cells. Tobacco cells, however, could not grow as cultured *Amaranthus* cells in high concentrations of atrazine even under heterotrophic culture conditions. Atrazine-resistant tobacco cells were also sensitive to high concentrations of atrazine. The inhibition of cell growth by this secondary effect of atrazine was also observed in cultured wheat and rice cells. Atrazine-sensitive *Chenopodium* cells are relatively more resistant to high concentrations of atrazine. The importance of potential tolerance to the secondary effects of atrazine is discussed with respect to the frequent occurrence of triazine-resistant biotypes in limited plant species.

Introduction

Resistance to *s*-triazine-herbicides in weeds is the most widespread and extensively studied of all intraspecific herbicide-resistances. Subsequent to Ryan's first report [1] that *Senecio vulgaris* could no longer be controlled by triazine in a western Washington nursery after application of simazine for several years, triazine resistant biotypes of many weeds have been reported in North America as well as in Europe (see ref. in [2] and [3]). In both areas, *Amaranthus* spp. and *Chenopodium* spp. are the major resistant genera. These genera are certainly important weeds, however, it is of interest that the resistant biotype appears in these limited genera much more frequently than in many other significant weeds. What causes this differential interspecific response is not understood, even though the resistance within several weed species has been traced to the same mutation of a triazine-target protein [4–7]. Cultured cells provide a simple system well for an investigation of the physiological differences associated with triazine resistance among plant species. We examined the response of cultured cells of *Amaranthus retroflexus* in comparison with those of tobacco, wheat, rice, maize and *Chenopodium album* L. and discuss the relationship between potential tolerance in *Amaranthus* and *Chenopodium* to atrazine and the frequent appearance of resistant mutants.

* *Present address:* Agricultural Chemicals Research Laboratories, Sankyo Co. Ltd., Yasu, Shiga 520-23, Japan
** on leave from: Department of Botany, University of Mahidol, Rama VI Road, Bangkok, Thailand.

Reprint requests to F. Sato.

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Materials and Methods

Cultured cells

Calli of atrazine-resistant and susceptible *Amaranthus retroflexus* L. were induced from stems on a solidified Murashige-Skoog (M.S.) medium [8] with 1 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 μM benzylaminopurine (BA) and green *Amaranthus* cells were selected in Linsmaier-Skoog (L.S.) medium [9] with 1 μM naphthaleneacetic acid (NAA) and 1 μM BA. Cultured green cells of tobacco (*Nicotiana tabacum cv.* Samsun NN) were maintained in modified L.S. medium with twice the original strength of vitamin concentration, 10 μM NAA and 1 μM kinetin as described before [10]. Pale green calli of *Chenopodium album* L. were induced from stems on a solidified Murashige-Skoog medium containing 1 μM 2,4-D. Non-green calli of rice (*Oryza sativa* L. *cv.* Nihonbare)

were induced from seed-embryo on a modified M.S. medium containing twice the vitamin concentration of B5 medium [11], 10 μM 2,4-D, 1.14 mM asparagine, 100 mg/l casamino acids and 12 mM proline. Non-green cultured cells of wheat (*Triticum aestivum* var. Chinese Spring) were maintained in L.S. medium with 10 μM 2,4-D as described previously [12]. Non-green calli of maize (*Zea mays cv.* Goldencross Bantham) were induced from shoots on a L.S. medium containing 10 μM 2,4-D. Seeds of atrazine-resistant and susceptible biotypes of *Amaranthus retroflexus* L. and *Chenopodium album* L. [13] were kind gifts from Dr. R. S. Radosevich (Oregon State University). All cells except heterotrophically cultured tobacco cells were cultured in liquid medium with 3% sucrose with 100 rpm shaking in the light (*ca.* 3000 lux). Heterotrophically cultured tobacco cells were grown in the dark.

Measurement of atrazine tolerance of cultured cells

A half gram of cultured cells was inoculated in media containing various concentrations of atrazine (medium volume: 12.5 ml in 50 ml flask). Atrazine-resistance of these cell lines was evaluated by the inhibition of cell growth after 2 weeks (for *Amaranthus, Chenopodium* and tobacco), or 3 weeks (for maize, rice and wheat). All experiments were done in duplicate.

Results and Discussion

The responses of atrazine-resistant and susceptible-*Amaranthus* cells to atrazine are shown in Fig. 1 A. Atrazine scarcely inhibited the cell growth of either cell type in the medium with 3% sucrose. The susceptible line was only partially inhibited in 300 μM atrazine-containing medium with 0.5% sucrose, in which chloroplasts are more developed. This low sensitivity of susceptible cells to high concentrations of atrazine indicates that cultured *Amaranthus* cells grow heterotrophically even in the light, and, as a result, are resistant to inhibition by atrazine.

Tobacco cells, however, could not grow as cultured *Amaranthus* cells in high concentrations of atrazine even under heterotrophic culture conditions (Fig. 1 B [10]). This suggests that the heterotrophic growth of tobacco cells is inhibited by a secondary effect of atrazine. This was confirmed by the result that atrazine-resistant tobacco cells were also sensitive to high concentrations of atrazine (Fig. 1 B [14]); resistant cells showed only 15% of normal growth in the medium containing 200 μM atrazine, in which concentration the Hill

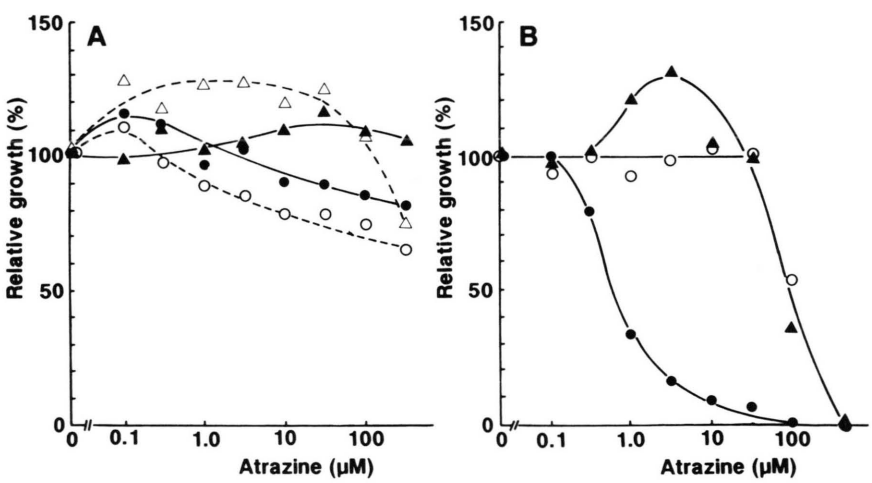

Fig. 1. A: The effect of atrazine on the growth of atrazine-resistant (▲, △) and susceptible (●, ○) cultured *Amaranthus retroflexus* cells. One half gram (F.W.) of cells was inoculated and cultured for two weeks. The average increase of fresh weight in the medium containing 3% (▲, ●) or 0.5% (△, ○) sucrose was 3.5 g and 0.65 g, respectively. Relative growth = (increase of cells treated with atrazine)/(increase of control cells without atrazine treatment).
B: The effect of atrazine on the growth of atrazine-resistant (▲), susceptible (●) cultured tobacco cells under photomixotrophic condition as well as on that of heterotrophic cultured cells (○) in the medium containing 3% sucrose. The average increase of fresh weight was 3.0 g. The data were taken from the ref. [10, 14].

Table I. Responses of cultured rice, wheat, maize and *Chenopodium album* cells to atrazine.

Atrazine Conc. [μM]	Relative growth				
	Rice	Wheat	Maize (1)	Maize (2)	*Chenopodium*
Control	100	100	100	100	100
10	109	87	83	85	110
30	81	100	95	93	110
100	21	58	112	88	109
300	<0	15	112	43	64

The average increase of fresh weight of control cells of rice, wheat, maize (cell line 1), maize (cell line 2) and *Chenopodium* cells was 1.27 g, 2.0 g, 1.6 g, 2.0 g and 2.2 g, respectively. Relative growth = (increase of cells treated with atrazine)/(increase of control cells without atrazine treatment).

reaction of atrazine-resistant thylakoid membranes, the primary target site of atrazine, of tobacco was only inhibited by 50%.

The inhibition of cell growth by this secondary effect of atrazine was also observed in cultured wheat and rice cells when grown heterotrophically (Table I). Different cell lines of maize showed different responses to atrazine. Maize intact plants are not killed by low concentration of atrazine due to its production of detoxifying enzymes (*e.g.*, glutathione-S-transferase [15]). Different responses of cultured maize cells suggests that maize cells were potentially sensitive to the secondary effect of atrazine and the detoxifying enzyme might express differently among cell lines.

Atrazine-sensitive *Chenopodium* cells are relatively more resistant to high concentrations of atrazine as was observed for *Amaranthus* cells. It is worthwhile to note that atrazine-resistant biotypes have been isolated from both *Amaranthus* and *Chenopodium*. If the growth of cells were inhibited by the secondary effects of high concentrations of atrazine, it is unlikely that resistant biotypes could survive, even though their photosystem would work normally as a result of a mutation in the primary target protein for atrazine. This implies that potential tolerance to the secondary effects of atrazine is important for the appearance of resistant weeds.

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[1] C. F. Ryan, Weed Sci. **18,** 614–616 (1970).
[2] J. D. Bandeen, G. R. Stephenson, and E. R. Cowett, in: Herbicide Resistance in Plants (H. M. LeBaron and J. Gressel, eds.), pp. 9–30, John Wiley and Sons, New York 1982.
[3] J. Gressel, H. U. Ammon, H. Fogelfors, J. Gasqueq, Q. O. N. Kay, and H. Kees, in: Herbicide resistance in Plants (H. M. LeBaron and J. Gressel, eds.), pp. 31–55, John Wiley and Sons, New York 1982.
[4] J. Hirschberg and L. McIntosh, Science **222,** 1346–1349 (1983).
[5] J. Hirschberg, A. Bleecker, D. J. Kyle, L. McIntosh, and C. J. Arntzen, Z. Naturforsch. **39c,** 412–420 (1984).
[6] P. Goloubinoff, M. Edelman, and R. B. Hallick, Nucleic Acid Res. **12,** 9489–9496 (1984).
[7] S. McNally, P. Bettini, M. Sevignac, H. Darmency, J. Gasquez, and M. Dron, Plant Physiol. **83,** 248–250 (1987).
[8] T. Murashige and F. Skoog, Physiol. Plant. **15,** 473–497 (1962).
[9] E. M. Linsmaier and F. Skoog, Physiol. Plant. **18,** 100–127 (1965).
[10] F. Sato, S. Takeda, and Y. Yamada, Plant Cell Reports **6,** 401–404 (1987).
[11] O. L. Gamborg, R. A. Miller, and K. Ojima, Exp. Cell. Res. **50,** 151–158 (1968).
[12] Y. Kinoshita, N. Ohnishi, Y. Yamada, T. Kunisada, and H. Yamagishi, Plant Cell Physiol. **26,** 1401–1409 (1985).
[13] S. R. Radosevich, Weed Sci. **25,** 316–318 (1977).
[14] F. Sato, Y. Shigematsu, and Y. Yamada, Mol. Gen. Genet. **214,** 358–360 (1988).
[15] D. S. Frear and H. R. Swanson, Phytochemistry **9,** 2123–2132 (1970).