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# Effect of Chlorsulfuron on Morphogenetic and Disordered Cell Division in Cultures of *Passiflora edulis*

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**Abstract.** We examined the effects of a sulfonylurea herbicide, chlorsulfuron, which is known as a potent inhibitor of plant cell division, on morphogenetic cell division and disorganized cell division using the culture system of multiple shoot primordia and callus of *Passiflora edulis.* The multiple shoot primordia tissue treated with chlorsulfuron failed to achieve shoot morphogenesis, and a large part of the tissue was necrotized during the posttreatment culture, even when it was washed and transferred to chlorsulfuron-free medium. The inhibition of *Passiflora* shoot morphogenesis by chlorsulfuron was not reversed by the simultaneous addition of branched amino acids, which are known to reverse the inhibitory effect of chlorsulfuron. In contrast, the same treatment of chlorsulfuron on the callus did not kill the cells, although the growth resumption was retarded by a prolonged lag period. The addition of branched amino acids enhanced the recovery growth of the chlorsulfuron-treated callus. These results suggest that the inhibition of disorganized cell division (callus growth) by chlorsulfuron is reversible, whereas morphogenetic cell division (shoot morphogenesis), which is under complex regulation, is inhibited irreversibly by chlorsulfuron. Qualitative differences between morphogenetic cell division and disordered simple proliferative cell division are discussed.

**Key Words.** Cell cycle—Cell division—Chlorsulfuron—Morphogenesis—*Passiflora edulis*—Sulfonylurea herbicide

**Abbreviations:** BA, 6-benzylaminopurine; 2,4-D, 2,4-dichlorophenoxyacetic acid; IBA, indole-3-butyric acid; 5-FdUrd, 5-fluorodeoxyuridine; MS medium, Murashige and Skoog medium; MSP, multiple shoot primordium(-dia).

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The sulfonylurea herbicide chlorsulfuron (2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]-benzene sulfonamide) arrests cell division by blocking the cell cycle progression in both the $G_1$ and $G_2$ phases (Rost 1984, Rost and Reynolds 1985). This arrest of cell division is reversible because chlorsulfuron-treated cells begin to divide after the removal of chlorsulfuron from the culture medium (Ray 1984, Reynolds 1986, Rost 1984, Rost and Reynolds 1985).

At the cytodifferentiation level, chlorsulfuron was shown to inhibit neither growth regulator-induced cell elongation (Ray 1982a, 1982b) nor tracheary element differentiation (Reynolds 1986). Fukuda and Komamine (1980, 1981) used a number of chemical inhibitors in cultures of *Zinnia elegans* mesophyll cells and showed that tracheary element differentiation occurs in the $G_1$ phase and does not require replication of the whole DNA in the S phase. Therefore, it is not unreasonable that cytodifferentiation occurs in chlorsulfuron-treated cells.

At the biochemical level, this herbicide has been shown to inhibit the activity of acetolactate synthase, the enzyme that catalyzes the first step in the biosynthesis of the branched amino acids (Chaleff and Mauvais 1984, Hawkes et al. 1989, Kishore and Shah 1988, Ray 1984). Because the addition of valine and isoleucine resulted in the recovery of cell division in the root tip of *Pisum sativum* (Ray 1984, Robbins and Rost 1987, Rost 1984, Rost and Reynolds 1985), these branched amino acids were implicated in the regulation of cell cycle activity (Rhodes et al. 1987). In addition, in suspension cultures of *Solanum carolinense* the effect of chlorsulfuron on cell division could be reversed by the exogenous application of branched amino acids to the medium (Reynolds 1986), suggesting that cell growth without morphogenetic cell division, such as in suspension cell or callus growth, is not prevented by chlorsulfuron when branched amino acids are added to the culture medium.

The question thus arises as to how chlorsulfuron functions at the morphogenetic level. Morphogenesis such as the shoot formation of higher plants is the integration of cell division and cytodifferentiation. Moreover, the cell division underlying morphogenesis is highly organized both spatially and temporally by complex regulation, whereas callus or suspension cells propagate via disorganized cell division. It would be of interest to know how chlorsulfuron acts on higher plant morphogenesis. However, there has been no such study, to our knowledge.

We obtained multiple shoot primordia (MSP) from an excised leaf segment of *Passiflora edulis* (Kawata et al. 1995). These MSP have been subcultured repeatedly for more than 4 years on a subculture medium, maintaining their morphogenetic ability. When the MSP are transferred to a medium for shoot development, shoots do develop from them. This culture system is suitable for the examination of how a herbicide acts on morphogenetic ability. We have been subculturing the callus of this plant species, which is another material proliferating by disorganized cell division.

In the present investigation, experiments were performed with the MSP and callus of *P. edulis* to answer the following three questions: When MSP tissue is treated with chlorsulfuron, how is the shoot morphogenesis influenced in posttreatment culture? If normal morphogenesis is disrupted by chlorsulfuron treatment, does the addition of branched amino acids neutralize this inhibitory effect of chlorsulfuron? How does chlorsulfuron act on the callus growth of *P. edulis*, which occurs by disorganized cell division?

## Materials and Methods

### *Plants and Growth Conditions*

Multiple shoot primordia of *P. edulis* Sims var. maracuja peroba were induced primarily from an excised leaf segment and have been subcultured for more than 4 years as described in detail (Kawata et al. 1995). Young mature-type leaves (within 2 weeks after leaf expansion) were surface sterilized with 70% (v/v) ethanol for 1 min and 1% sodium hypochlorite solution for 10 min followed by rinsing with sterilized water. The sterilized leaves were cut into small segments (2 mm × 3 mm) with a razor blade. Leaf segments were first cultured for 1 month on Murashige and Skoog (1962) medium supplemented with 3% (w/v) sucrose (MS medium), 1 μM BA, and 1 μM IBA, solidified with 0.2% (w/v) Gellan Gum (Wako-junyaku, Osaka, Japan) for the induction of MSP. Induced shoot primordia were subcultured on MS medium supplemented with 10 μM BA at 3-week intervals. For shoot formation, the MSP mass subcultured for 7 days was cut into small aggregates (2.5–3.5 mm, 0.03–0.04 g) and transferred to MS medium containing a lower concentration of BA (0.1 μM). Callus was induced by the culture of a leaf segment on MS medium supplemented with 0.1 μM BA and 10 μM 2,4-D and then subcultured on the same medium at an interval of 3 weeks. All media were adjusted to pH 5.7–5.8 before the addition of Gellan Gum and autoclaved at 121°C for 15 min. All cultures were incubated at 26°C under continuous fluorescent light at 30 $\mu mol \cdot m^{-2} s^{-1}$.

### *Chemical Treatment*

Chlorsulfuron (stock solution dissolved in acetone), aphidicolin (stock solution dissolved in methanol), or 5-FdUrd (stock solution dissolved in distilled-deionized water) was mixed into liquid culture medium to give an objective concentration before the pH adjustment, followed by sterilization by filtration. Each chemical was applied to MSP tissue or callus that had been subcultured for 7 days at 26°C for various periods and was then removed by washing the tissues with liquid medium four times. The washed tissues were transferred to solidified medium and cultured for 3 weeks.

To know whether branched amino acids reverse the effect of chlorsulfuron, various concentrations of isoleucine and valine were added simultaneously to chlorsulfuron solution and applied to MSP tissue or callus cultures.

### *Evaluation of Shoot Formation and Growth of MSP Tissue and Callus*

The shoots developed from MSP tissue were evaluated with a stereoscopic microscope at 3 weeks after the commencement of the posttreatment culture. Shoots more than 5 mm in length were considered developed shoots. Growth of MSP tissue and callus was evaluated by measuring the fresh mass at 3 weeks after the commencement of the posttreatment culture.

## Results

Fig. 1 shows the effect of chlorsulfuron on shoot morphogenesis in *Passiflora.* Pieces (2.5–3.5 mm, 0.03–0.04 g) of MSP tissue were treated with chlorsulfuron at 26°C for 0–3 days, washed with liquid medium, and then cultured on the medium for shoot development. The number of shoots that developed to more than 5 mm in length was measured after 3 weeks of culture. As shown in Fig. 1, the chlorsulfuron treatment for only 1 day inhibited shoot morphogenesis to 27% of the control level, and 2 or more days of treatment reduced the shoot morphogenesis level to less than 10% of the control. Together with the inhibition of shoot morphogenesis, whole growth of MSP tissue was also inhibited severely (Fig. 2), and a large part of the MSP tissue was necrotized (Fig. 3). These results indicate that the cells constituting the MSP tissue could not resume cell division even when chlorsulfuron was removed from the medium.

The thymidine analog 5-FdUrd is known as an inhibitor of DNA synthesis by blocking thymidylate synthase (Hartmann and Heidelberger 1961). Aphidicolin is a specific inhibitor of replicative nuclear α- and α-like DNA polymerase in animal and plant cells, respectively, and prevents the initiation of DNA synthesis (Ikegami et al. 1978, Sala et al. 1983). Both 5-FdUrd and aphidicolin

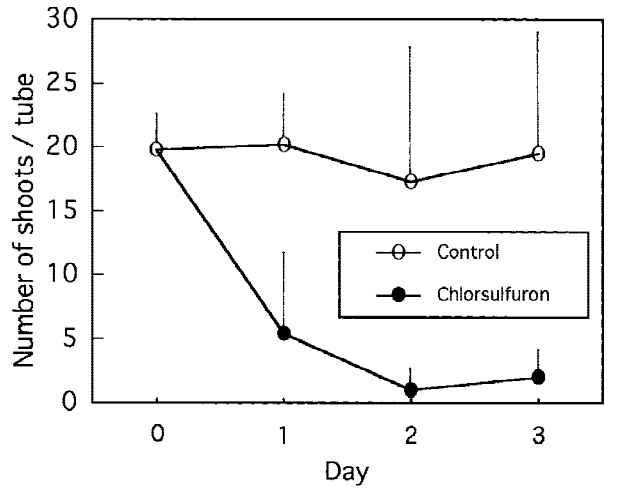


**Fig. 1.** Effect of chlorsulfuron on shoot morphogenesis of *P. edulis*. MSP tissues were treated with MS medium supplemented with (●) or without (○) 1 μM chlorsulfuron for various periods and washed with liquid medium. The tissues were then cultured on the medium for shoot formation. The number of shoots was measured after 3 weeks of culture. Data are the mean ± S.D. of five measurements.

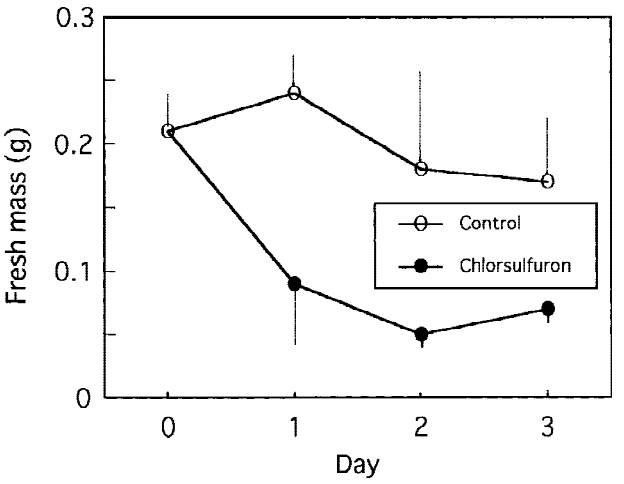


**Fig. 2.** Effect of chlorsulfuron on morphogenetic growth of *P. edulis*. MSP tissues (0.03–0.04 g) were treated with MS medium supplemented with (●) or without (○) 1 μM chlorsulfuron for various periods and washed with liquid medium. The tissues were then cultured on the medium for shoot formation. Fresh mass was measured after 3 weeks of culture. Data are the mean ± S.D. of five measurements.

block cell cycle progression at the $G_1$/S transition, whereas chlorsulfuron blocks it at the $G_1$ or $G_2$ phase. Table 1 shows the effect of these drugs on *Passiflora* shoot morphogenesis. The average number of shoots developed from the 5-FdUrd- or aphidicolin-treated MSP was the same as the control, showing no effect of the two drugs on shoot morphogenesis. This result suggests that reversible arrest at the $G_1$/S transition by treatment with 5-FdUrd or aphidicolin does not affect the shoot morphogenesis of *Passiflora.*

At the biochemical level, chlorsulfuron inhibits acetolactate synthase, the key enzyme in the biosynthesis of branched amino acids (Chaleff and Mauvais 1984, Kishore and Shah 1988, Ray 1984). This action is neutralized by the simultaneous application of valine and isoleucine (Ray 1984, Reynolds 1986, Robbins and Rost 1987, Rost 1984, Rost and Reynolds 1985). Table 2 shows the effect of the simultaneous application of branched amino acids on shoot morphogenesis in the culture of MSP treated with chlorsulfuron. No significant recovery of shoot morphogenesis by the simultaneous application of branched amino acids was observed. In addition, in the culture of MSP tissue treated with chlorsulfuron plus branched amino acids, a large portion of the tissue was necrotized during the posttreatment culture. This result indicates that the lethal effect of chlorsulfuron was not reversed by the addition of branched amino acids.

To examine the effect of cell cycle blockers on the disorganized cell growth of *Passiflora*, calli were treated with chlorsulfuron, 5-FdUrd, or aphidicolin for 2 days and cultured for 3 weeks after removal of the drug. As shown in Table 3, only chlorsulfuron inhibited the callus growth, whereas the calli treated with 5-FdUrd or aphidicolin grew as much as did the controls. However, the growth inhibition of the chlorsulfuron-treated callus was different from the inhibition of shoot morphogenesis, in which the chlorsulfuron-treated MSP hardly grew, and a large part of the MSP tissue was necrotized. By contrast, the chlorsulfuron-treated calli grew vigorously, although the growth resumption was retarded at the initial stage in culture. The chlorsulfuron-treated calli grew from 0.02 g to 0.1 g in fresh mass in the 3 weeks of culture. Therefore, it could be said that the decrease in fresh mass was caused by the lag period prolonged by chlorsulfuron treatment. This result suggests that chlorsulfuron treatment merely retarded the cell proliferation and that its toxicity is not lethal. Vital staining (Gaff and Okong'O-ogola 1971) showed no significant difference of the alive cell percentage between chlorsulfuron treatment and controls, although a slight decrease in the triphenyltetrazolium chloride reduction rate (Steponkus and Lanphear 1967) was recognized in chlorsulfuron-treated cells compared with the control cells (data not shown).

To determine whether or not the application of branched amino acids neutralizes the inhibitory effect of chlorsulfuron on growth, calli were treated with chlorsulfuron alone or with chlorsulfuron plus various concentrations of isoleucine and valine, washed in fresh medium, and then cultured on the solidified medium for 3

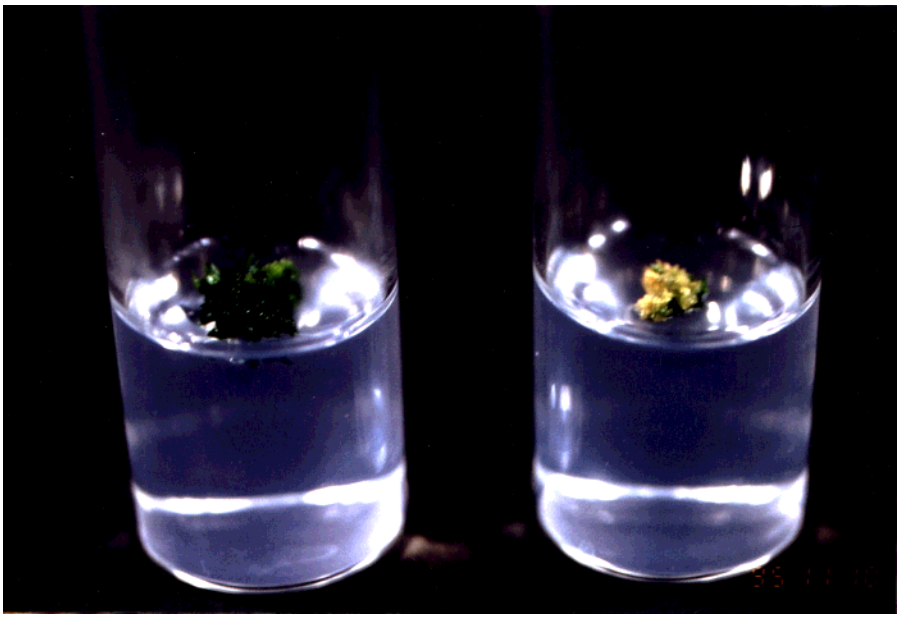

**Fig. 3.** Effect of chlorsulfuron on shoot morphogenesis of *P. edulis*. MSP tissues were treated with MS medium supplemented with (*right*) and without (*left*) 1 μM chlorsulfuron for 2 days and washed with liquid medium. The tissues were then cultured on the medium for shoot formation. Photographs were taken after 3 weeks of culture.

**Table 1.** Effects of chlorsulfuron, 5-FdUrd, and aphidicolin on shoot morphogenesis of *P. edulis*. MSP tissues were treated with 1 μM chlorsulfuron, 5-FdUrd, or aphidicolin for 2 days, washed with liquid medium, and then cultured on the medium for shoot formation. The number of shoots was measured after 3 weeks of culture. Data are the mean ± S.D. of five measurements.

| Treatment | Average no. of shoots/tube |
|---|---|
| Control | 18.2 ± 5.6 |
| Chlorsulfuron | 1.4 ± 1.5 |
| 5-FdUrd | 14.8 ± 10.0 |
| Aphidicolin | 16.0 ± 11.9 |

**Table 2.** Effects of chlorsulfuron and branched amino acids (isoleucine and valine) on shoot morphogenesis in *P. edulis*. MSP tissues were treated with 1 μM chlorsulfuron alone or chlorsulfuron plus branched amino acids for 2 days and washed with liquid medium; they were then cultured on the medium for shoot formation. The number of shoots was measured after 3 weeks of culture. Data are the mean ± S.D. of five measurements.

| Treatment | Average no. of shoots/tube |
|---|---|
| Control | 16.0 ± 4.2 |
| Chlorsulfuron | 2.6 ± 2.9 |
| Chlorsulfuron + isoleucine and valine | |
| Each at 0.1 mM | 4.6 ± 5.7 |
| Each at 1 mM | 3.0 ± 2.6 |
| Each at 10 mM | 3.8 ± 3.8 |

weeks. As shown in Table 4, the addition of branched amino acids enhanced the proliferation of callus cells, although the growth rate was not recovered completely to the level of the control. This enhancement was caused by the shortening of the lag period at the initial period of the posttreatment culture by the branched amino acids.

## Discussion

In this study we observed a difference in the effects of chlorsulfuron and the other two drugs (5-FdUrd and aphidicolin). Although chlorsulfuron treatment strongly inhibited the shoot morphogenesis of *Passiflora* and retarded the regrowth of callus, the other two drugs inhibited neither shoot morphogenesis nor callus growth. This difference is perhaps caused by a difference in the mode of action between chlorsulfuron and the other drugs. A second major finding was the different response to chlorsulfuron treatment between shoot morphogenesis and callus growth. Chlorsulfuron inhibited both shoot morphogenesis and callus growth. However, the two inhibitory effects were different in quality and quantity. Together with the inhibition of shoot morphogenesis, a large part of the MSP tissue was necrotized during the posttreatment culture, even when the tissue was washed and transferred to fresh medium. By contrast, chlorsulfuron did not show a lethal effect on the callus cells immediately after the treatment, according to the vital staining test and triphenyltetrazolium chloride reduction assay. In addition, although the chlorsulfuron treatment retarded regrowth of the calli, showing growth inhibition, the calli resumed proliferation after a prolonged lag period. These two different responses to chlorsulfuron are perhaps the result of differences in the qualities of morphogenetic cell division and simple proliferative cell division.

It was reported that chlorsulfuron strongly inhibits

**Table 3.** Effects of chlorsulfuron, 5-FdUrd, and aphidicolin on callus growth of *P. edulis*. Calli were treated with 1 μM chlorsulfuron, 5-FdUrd, or aphidicolin for 2 days, washed with liquid medium, and then cultured on the medium for callus growth. Fresh mass was measured after 3 weeks of culture. Data are the mean ± S.D. of five measurements.

| Treatment | Fresh mass (g) |
|---|---|
| Control | 0.29 ± 0.03 |
| Chlorsulfuron | 0.13 ± 0.04 |
| 5-FdUrd | 0.33 ± 0.08 |
| Aphidicolin | 0.30 ± 0.02 |

**Table 4.** Effects of chlorsulfuron and branched amino acids (isoleucine and valine) on callus growth of *P. edulis*. Calli were treated with 1 μM chlorsulfuron alone or chlorsulfuron plus branched amino acids for 2 days and washed with liquid medium. The calli were then cultured on the medium for callus growth. Fresh mass was measured after 3 weeks of culture. Data are the mean ± S.D. of five measurements.

| Treatment | Fresh mass (g) |
|---|---|
| Control | 0.40 ± 0.09 |
| Chlorsulfuron | 0.13 ± 0.07 |
| Chlorsulfuron + isoleucine and valine | |
| Each at 0.1 mM | 0.27 ± 0.04 |
| Each at 1 mM | 0.28 ± 0.07 |
| Each at 10 mM | 0.28 ± 0.05 |

plant cell division (Rost 1984) but has no effect on DNA, RNA, or protein synthesis or on respiration (Ray 1982a, 1982b). We had therefore expected that the continuous treatment of chlorsulfuron would affect the shoot morphogenesis of *Passiflora* to some degree. However, we did not expect that shoot morphogenesis would be inhibited strongly even when the MSP tissues were washed and transferred to chlorsulfuron-free medium. Earlier studies showed that cell division arrested by chlorsulfuron could be reversed by the removal of the drug (Reynolds 1986, Rost and Reynolds 1985). We suspect that the unexpected response of MSP tissue after chlorsulfuron treatment is caused by a difference in the mode of cell division between morphogenesis and callus growth.

The morphogenetic process in higher plants includes mitosis, polarization, the formation of primordia, and the development of meristems, and it involves a series of complicated physiological and biochemical events (Reinert et al. 1997, Thorpe 1980), whereas callus or suspension cells proliferate by disorganized cell division. If chlorsulfuron induces biochemical or physiological alterations in the cells of MSP, the morphogenetic cell division may be difficult to continue because it is highly regulated both spatially and temporally. In contrast, callus cells divide under more simplified regulation, similar to the growth of bacteria or single cell algae. Therefore, if physiological alteration occurs in callus cells, individual cells may repair the physiological alteration and resume division after the removal of chlorsulfuron.

At the biochemical level, chlorsulfuron inhibits the activity of acetolactate synthase, the key enzyme in the biosynthesis of the branched amino acids (Chaleff and Mauvais 1984). However, the starvation of branched amino acids does not appear to be the cause of the morphogenetic inhibition because the simultaneous application of branched amino acids did not neutralize the effect of chlorsulfuron. It has been suggested that sulfonylurea herbicide toxicity is caused by additional effects and the accumulation of a toxic metabolite in bacteria (LaRossa et al. 1987, LaRossa and VanDyk 1989), *Chlorella* (Landstein et al. 1990, 1995), and in a higher plant (Clayton and Reynolds 1991, Rost et al. 1990). If the accumulation of a toxic metabolite induces a further physiological alteration of MSP cells, their highly integrated cell division might be disordered, terminating the normal morphogenetic process.

Another speculation is as follows. 5-FdUrd, aphidicolin, and chlorsulfuron are all inhibitors of plant cell division, but the former two drugs arrest cell division at the $G_1$/S transition (Hartmann and Heidelberger 1961, Ikegami et al. 1978, Sala et al. 1983), whereas chlorsulfuron blocks it at the $G_1$ and $G_2$ phases (Rost 1984, Rost and Reynolds 1985). From the present study, we cannot assert the relationship between the cell cycle stage at which chlorsulfuron acts and disorganization of morphogenetic cell division of *Passiflora* occurs. But there is an interesting report that is helpful in discussing this problem. Oliev (1994) has reported that in cultures of the unistratose meristem of *Riella helicophylla*, the response of cells treated with 5-FdUrd or aphidicolin differed from that of cells treated with chlorsulfuron. His result suggests that the phase of the cell cycle differs with regard to auxin synthesis and competence to respond to auxin. This difference is of interest for us to speculate the cause of the morphogenetic inhibition because hormone balance, especially between auxin and cytokinin, strongly affects plant morphogenesis. If the hormone balance is disrupted easily at a cell cycle stage at which a drug acts, for example at the $G_1$ phase, compared with the other stages, it can be considered that the morphogenesis of the tissue arrested at $G_1$ phase is disrupted easily.

## References

Chaleff RS, Mauvais CJ (1984) Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. Science 224:1443–1445

Clayton DS, Reynolds T (1991) Chlorsulfuron effects on protein synthesis and accumulation in cultured root tips of *Pisum sativum* L. J Plant Physiol 137:337–341

Fukuda H, Komamine A (1980) Direct evidence for cytodifferentiation of tracheary elements without intervening mitosis in a culture of

single cells isolated from the mesophyll of *Zinnia elegans.* Plant Physiol 65:61–64

Fukuda H, Komamine A (1981) Relationship between tracheary element differentiation and DNA synthesis in single cells isolated from the mesophyll of *Zinnia elegans:* analysis of inhibitors of DNA synthesis. Plant Cell Physiol 22:41–49

Gaff DF, Okong'O-ogola O (1971) The use of nonpermeating pigments for testing the survival of cells. J Exp Bot 22:756–758

Hartmann KU, Heidelberger C (1961) Studies of fluorinated pyrimidines. XIII. Inhibitor of thymidylate synthetase. J Biol Chem 236:3006–3013

Hawkes TR, Howard JL, Pontis SE (1989) Herbicides that inhibit the biosynthesis of branched chain amino acids. In: Dodge A (ed) Herbicides and plant metabolism (seminar series). Cambridge University Press, Cambridge, pp 113–137

Ikegami S, Taguchi T, Ohashi M (1978) Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase α. Nature 275:458–459

Kawata K, Ushida C, Kawai F, Kanamori M, Kuriyama A (1995) Micropropagation of passion fruit from subcultured multiple shoot primordia. J Plant Physiol 147:281–284

Kishore GM, Shah DM (1988) Amino acid biosynthesis inhibitors as herbicides. Annu Rev Biochem 57:627–663

Landstein D, Chipman DM, Arad S, Barak Z (1990) Acetohydroxyacid synthase activity in *Chlorella emersonii* under auto- and heterotrophic growth conditions. Plant Physiol 94:614–620

Landstein D, Epelbaum S, Arad S, Barak Z, Chipman DM (1995) Metabolic response of *Chlorella emersonii* to the herbicide sulfometuron methyl. Planta 197:219–224

LaRossa RA, VanDyk TK (1989) Leaky pantothenate and thiamin mutations of *Salmonella typhimurium* conferring sulphometuron methyl sensitivity. J Gen Microbiol 135:2209–2222

LaRossa RA, VanDyk TK, Smulsky DR (1987) Toxic accumulation of 2KB caused by inhibition of the branched chain amino acid biosynthetic enzyme acetolactate synthase in *Salmonella typhimurium.* J Bacteriol 169:1372–1378

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–493

Oliev R (1994) Response to auxin by cells of *Riella helicophylla* during reversible arrest in different cell cycle phase. Planta 194:510–515

Ray TB (1982a) The mode of action of chlorsulfuron: a new herbicide for cereals. Pestic Biochem Physiol 17:10–17

Ray TB (1982b) The mode of action of chlorsulfuron: the lack of direct inhibition of plant DNA synthesis. Pestic Biochem Physiol 18: 282–266

Ray TB (1984) The site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol 75:827–831

Reinert J, Bajaj YPS, Zbell B (1977) Aspects of organization: organogenesis, embryogenesis, cytodifferentiation. In: Street HE (ed) Plant tissue and cell culture. 2nd ed. Blackwell Scientific Publications, Cambridge, MA, pp 389–427

Reynolds TL (1986) Effects of chlorsulfuron, valine and isoleucine, on division and tracheary element differentiation in cell suspension cultures of *Solanum carolinense* L. J Plant Physiol 125:179–184

Rhodes D, Hogen AL, Deal L, Jamieson GC, Hawoth P (1987) Amino acid metabolism of *Lemna minor* L. II. Responses to chlorsulfuron. Plant Physiol 84:775–780

Robbins J, Rost TL (1987) Chlorsulfuron inhibition of cell cycle progression and the recovery of $G_1$ arrested cells by Ile and Val. J Plant Growth Regul 6:67–74

Rost TL (1984) The comparative cell cycle and metabolic effects of chemical treatments on root tip meristems. III. Chlorsulfuron. J Plant Growth Regul 3:51–63

Rost TL, Gladish D, Steffen J, Robbins J (1990) Is there a relationship between branched amino acid pool size and cell cycle inhibition in roots treated with imidazolinone herbicides? J Plant Growth Regul 9:227–232

Rost TL, Reynolds T (1985) Reversal of chlorsulfuron-induced inhibition of mitotic entry by isoleucine and valine. Plant Physiol 77:481–482

Sala F, Galli MG, Nielsen E, Magnien E, Devreux M, Pedrali-Noy G, Spadari S (1983) Synchronization of nuclear DNA synthesis in cultured *Daucus carota* L. by aphidicolin. FEBS Lett 153:204–208

Steponkus PJ, Lanphear O (1967) Refinement of the triphenyltetrazolium chloride method of determining cold injury. Plant Physiol 42:1423–1426

Thorpe TA (1980) Organogenesis in vitro: structural, physiological, and biochemical aspects. Int Rev Cytol 11A:71–112