RESISTANCE OF PLANT CELLS TO CYCLOHEXIMIDE

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Although cycloheximide is an effective inhibitor of protein synthesis in angiosperms (1), little attention has been given to its long term effect on growth. In previous work we showed that the addition of cycloheximide (50 μg/ml) to suspension cultures of Paul's Scarlet rose resulted in an immediate and complete inhibition of protein synthesis (2). Subsequent labeling studies by Davies and Exworth with rose cells indicated that cycloheximide was only a temporary inhibitor of protein synthesis (3). The present study was designed to determine if the apparent short term effect of cycloheximide influenced the growth (dry wt increase) of rose cells.

Suspension cultures of Paul's Scarlet rose were grown on MPR medium as previously described (4). Medium containing cycloheximide concentrations of 0, 0.5, 5.0, 50.0, and 500.0 μg/ml were prepared by adding appropriate amounts of cycloheximide stock solutions through a millipore filter syringe to flasks containing 80 ml of sterile medium. Flasks were inoculated with approximately 0.3 g fresh wt of cells which had been grown on either MPR medium or MPR medium plus cycloheximide for two 14 day growth periods. In the latter case, inoculum cells had been grown in cycloheximide concentrations identical to those to which they were transferred. Cultures were harvested at 7, 14, and 21 days; and dry weights were determined as previously described (5). Three replicate cultures were used for each treatment at each culture age.

Cells grew in all of the cycloheximide concentrations tested (Table 1). A comparison of dry weights on day 7 showed that with one exception the cycloheximide treated cultures weighed less than ½ as much as the controls, but by day 14 the dry weights of the test cultures approximated that of the controls. Thus growth was slowed by cycloheximide between days 0 and 7, but this period of slow growth did not reduce the total amount of growth (maximum dry wt yield observed on day 14). The exception to this was with cells grown in the highest concentration of cycloheximide (500 μg/ml). At this concentration, growth was slow throughout the culture period and by 21 days the dry wt was only 73% of the control at its peak value of 0.719 g which occurred on day 14. A comparison of cells grown for the first time in cycloheximide (Table 1) with cells propagated for 3 growth periods in cycloheximide showed a significant increase in the growth of cells subcultured in medium containing 500.0 μg/ml of the inhibitor.

TABLE 1. *Dry weights (g) of cells harvested during their first or third growth period in cycloheximide.*

Cycloheximide (μg/ml)	Day of First Growth Period 7	14	21
0 (Control)	0.086[a]	0.719	0.579
0.5	0.029	0.631	0.550
5.0	0.046	0.810	0.497
50.0	0.025	0.640	0.531
500.0	0.012[b]	0.118[b]	0.325[b]
	Day of Third Growth Period		
0.5	0.037	0.714	0.559
50.0	0.053	0.755	0.557
500.0	0.037[b]	0.328[b]	0.545[b]

[a] Each value represents the average of three cultures.

[b] Differences in dry wt between cultures grown for 1 versus 3 growth periods in 500.0 μg/ml of cycloheximide were significantly different at the .05 level when compared after 7, 14, and 21 days of growth.

Although low concentrations of cycloheximide inhibited protein synthesis (2) and reduced the growth rate of rose cells, it did not follow that the inhibitor reduced total growth (maximum dry weight yield). Long term studies with other angiosperms may show that growth resistance to cycloheximide is as wide spread among angiosperms as was originally shown for different species of fungi (6). Mechanisms responsible for resistance to cycloheximide were not apparent from our study. There may be an induced change in existing cells as suggested by Davies and Exworth (3). On the other hand, improved growth of cells subcultured in 500 μg/ml of cycloheximide

suggests that certain cell types may be selected during continued propagation in medium possessing a high concentration of cycloheximide.

REFERENCES

1. D. BOULTER. Ann. Rev. Plant Physiol. 21: 91-114. (1970).
2. J. S. FLETCHER and H. BEEVERS. Plant Physiol. 48: 261-264. (1971).
3. M. E. DAVIES and C. P. EXWORTH. Biochem. Biophys. Res. Commun. 50: 1075-1080. (1973).
4. K. K. NESIUS, L. E. UCHYTIL, and J. S. FLETCHER. Planta 106: 173-176. (1972).
5. J. S. FLETCHER and H. BEEVERS. Plant Physiol. 45: 765-772. (1970).
6. A. J. WHIFFEN. J. Bacteriol. 56: 283-291. (1948).