Valine-Induced Inhibition of Growth of Haploid Tobacco Protoplasts and Its Reversal by Isoleucine

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L-valine inhibits the division of haploid tobacco mesophyll protoplasts. This inhibition is reversed by simultaneous addition of L-isoleucine to the culture medium.

The efficiency of a mutagenic treatment of microorganisms has frequently been determined by calculating the number of induced mutant colonies resistant to a toxic drug. Valine, an analogue of isoleucine, inhibits the growth of *Escherichia coli* K12 [1] and in their studies of the optimal conditions for the use of N-methyl-N′-nitro-N-nitrosoguanidine on *Escherichia coli* K12, Adelberg *et al.* [2] chose valine-resistance as such a criterion "because this phenotype is produced by mutation at any one of many mutational sites within several loci" and "is generally dominant" [2–4]. In order to determine wether this relatively easy technique could be adapted to *in vitro* cultured plant cells we tested the action of L-valine on cultured haploid tobacco protoplasts.

Protoplasts were prepared from leaves of haploid tobacco *(Nicotiana tabacum L*, c. v. *Xanthi)* and cultivated in a semisolid modified Murashige and Skoog's medium [5] as previously described [6, 7] at an initial cellular density of 6.5×10^4 protoplasts per ml. Media with L-amino acids at 10^{-4} M were prepared by adding to sterile culture medium appropriate amounts of filter-sterilized 10^{-2} M amino acid solutions. Plating efficiency after 12 days of culture was calculated as the percentage of initial protoplasts grown to colonies.

As shown by the results presented in Table I, during a 12 days growing period valine at 10^{-4} M strongly inhibited the division and subsequent cell colony regeneration of the protoplasts; this inhibition was reversed by isoleucine but not by either arginine or lysine used at the same molarity.

Valine is among the amino acids found by Filner [8] to inhibit the growth of tobacco cell suspension cultures when added to a liquid medium containing nitrate as the sole nitrogen source. As the same amino acids were shown to inhibit nitrate uptake and nitrate reductase activity in tobacco cell cultures [8–10], Heimer and Filner [9] concluded that growth inhibition stems from a specific inhibition of nitrate assimilation. Behrend and Mateles [11] recently confirmed these findings but they observed similar inhibition by single amino acids of tobacco cells grown on urea and thus suggested that amino acids inhibit assimilation of intracellular ammonium into amino acids.

Table I. Effects of L-valine and of L-valine plus L-arginine, L-isoleucine or L-lysine on growth of haploid tobacco protoplasts.

Medium	Plating efficiency: percentage of protoplasts grown to colonies after 12 days of culture (initial cellular density : 6.5×10^4/ml)	
	1st exp.	2nd exp.
Basal medium BM	20	31
BM + 10^{-4} M valine	2	6
BM + 10^{-4} M valine + 10^{-4} M arginine	4	5
BM + 10^{-4} M valine + 10^{-4} M isoleucine	29	40
BM + 10^{-4} M valine + 10^{-4} M lysine	3	3

Filner [8] and Behrend and Mateles [11] found that in their respective experimental conditions the inhibition of growth by amino acids such as valine is prevented by the addition of certain other amino acids; isoleucine which specifically reverses the inhibitory effect of its analogue valine on the growth of *E. coli* K12 [1] falls in this class of polyvalent antagonist amino acids. Thus we tested the effectiveness as possible antagonists of valine of two other amino acids belonging to this class according to Filner [8], arginine and lysine. Among the three amino acids tested only isoleucine reversed the effects of valine. Therefore I suggest that the valine-induced growth inhibition observed in our experimental conditions should be ascribed to a specific inhibition of one or more steps in isoleucine metabolism [1, 4] rather than to an inhibition of nitrate or ammonium assimilation.

Thus it should be feasible to test the efficiency of a mutagenic treatment of haploid protoplasts of tobacco, and possibly of other species, by looking for induced mutant colonies resistant to valine. On the other hand this selection scheme could possibly provide mutants derepressed for isoleucine synthesis as it is the case for certain valine-resistant strains of *E. coli* S12 [4].

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