AMINO ACID ANALOG RESISTANCE IN THE SPECIES OF SOYBEAN AND TOBACCO

P.K. Das* and J.M. Widholm

Department of Agronomy
University of Illinois
Urbana, IL
USA

* Present address:

Department of Genetics and Plant Breeding
B.C.K.V.V.
Kalyani
West Bengal
INDIA

INTRODUCTION

Interest for isolating plant cell culture mutants has been increasing. Many cell lines with resistance to growth inhibition by amino acid analogs are now reported. Often they produce higher than normal amounts of the corresponding natural amino acids, and, hence, growth in specific amino acid analog is used as a selection tool (1). Several mechanisms can give rise to analog resistance, but most recovered variants appear to possess altered amino acid levels caused by changes in regulatory enzymes resulting in relaxed feed back control (1, 2). We attempted to select several amino acid analog resistant cell lines from plant suspension cultures and a few of them derived from soybean and tobacco cell suspension are characterized. We present in this communication a brief report of our study.

MATERIALS AND METHODS

The cell lines used in the experiment were DX (Datura innoxia M.), TXD (Nicotiana tabacum L.), SB (Glycine max L.), CCh (Daucus carota L.) and ALF (Medicago sativa L.). They were maintained as
batch culture in liquid basal Murashige and Skoog (MS) medium (3) with 0.4 mg/l 2,4-dichlorophenoxy acetic acid. Five amino acid analogs were used. They were A2C (Azetidine-2-carboxylic acid) a proline analog, AEC [S-(2-Aminoethyl)-cysteine] a lysine analog, 5MT (DL-5-methyl tryptophan) a tryptophan analog, and Eth (S-ethylhomocystein) a methionine analog. The concentrations used for A2C and 5MT were 10, 100 and 500 µM, for AEC and Eth were 20, 200 and 1000 µM. Amino acid analogs of different concentrations were dissolved in culture media, pH 5.8 and autoclaved. Growth studies were done by inoculating 0.5 g fresh weight cells into 50 ml of analog-culture media in 125 ml Erlenmeyer flasks. The cultures were incubated on reciprocating shakers (105-110 rpm) at 27°C ± 1°C for 7-12 days, collected by vacuum filtration on Miracloth and weighed. Following determination of complete growth inhibitory concentration of amino acid analogs, 1.0 g fresh weight of cells were inoculated in 100 ml culture medium containing inhibitory concentration of amino acid analogs in 250 ml Erlenmeyer flasks for screening resistant lines. The flasks were incubated in reciprocating shakers for up to 10 weeks since the growth of one resistant cell should be detectable within that time, assuming a doubling of 2-3 days. The resistant lines were further reinoculated in different concentrations of analogs and their growth was compared with corresponding normal cell lines by measuring fresh weight (g) after a certain period of time. The resistant lines were also grown away from analogs for 25 cell generations and again reinoculated in the media containing analogs to examine whether resistant cell lines continued to maintain resistance. Free amino acids of resistant cell lines and of corresponding normal cell lines were extracted from exponentially growing cells which had been cultured away from inhibitory analogs for at least 10 cell generations, using methanol:chloroform:water (12:5:3) as described by Singh et al. (4). The aqueous extract was taken to dry mass under vacuum at 45°C. The residue was analyzed by physiological fluid methodology with Beckman 119Cl amino acid analyzer.

RESULTS AND DISCUSSION

Of the amino acid analogs the growth inhibitory effect of AEC was most striking. Cell lines of ALF (Alfalfa), SB (Soybean), CCh (Carrot) and DX (Datura) needed higher concentration of AEC for 50% growth inhibition than TXD (Tobacco) and the corresponding lysine level in these species in general was high. For ALF, the 50% growth inhibition concentration of 5 MT was low and so was the corresponding amino acid tryptophan level. The correlation study, however, indicated no relationship between amino acid analog concentration for 50% growth inhibition and the corresponding free amino acid level (Table 1).

Following screening in liquid medium, no cell lines of DX