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THEORETICAL AND APPLIED GENETICS

Isolation of a Recessive Barley Mutant Resistant to S-(2-Aminoethyl)L-cysteine

S.W.J. Bright, P.B. Norbury and B.J. Miflin
Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts (UK)

Summary. S-(2-aminoethyl)L-cysteine (AEC) inhibits the growth of mature barley (*Hordeum vulgare* L vars. 'Bomi' and 'Maris Mink') embryos grown on sterile medium. This inhibition is relieved by lysine and, to a lesser extent, arginine and ornithine. In order to try and select plants which accumulate lysine, 8200 M2 embryos of sodium azide mutagenised barley were screened for growth in the presence of 0.25 mM AEC. One line, R906 was selected for further characterisation. Progeny of the originally selected plant after selfing were all resistant to AEC. In a reciprocal cross with a sensitive barley the resistant trait was inherited as a single recessive nuclear gene which we designate *aec*-1.

Key words: Selection – Barley – Mutant – S-(2-aminoethyl)L-cysteine

Abbreviation

AEC S-(2-aminoethyl)L-cysteine

Introduction

For monogastric animals lysine is the nutritionally limiting amino acid in the proteins of cereal seeds. High-lysine mutants of barley have been selected (Doll et al. 1974). They are all mutants with decreased synthesis of the major storage proteins and also decreased yield (Doll and Kφie 1975). Mutants which overproduce soluble amino acids are another possible route to increasing the lysine content of barley grains. Tissue cultures with higher levels of free tryptophan, proline, methionine and lysine have been isolated by selection for resistance to the growth-inhibitory effects of a corresponding amino acid analogue (Widholm 1972, 1976; Chaleff and Carlson, 1975). We have selected for analogue-resistance in barley in a system where the genetics and seed biochemistry of any mutant can be investigated. We report here the isolation and genetic characterisation of a mutant of barley resistant to the lysine analogue S-(2-aminoethyl)L-cysteine (AEC) selected by screening mature barley embryos.

Materials and Methods

Embryos were hand dissected from mature barley (*Hordeum vulgare* L. Var. 'Bomi' or 'Maris Mink') seed, surface sterilised and grown in petri dishes under sterile conditions for 7 days at 25 ± 2°C with a 16 hr day as previously described (Bright et al. 1978). The agar medium was that of Murashige and Skoog (1962) with the omission of indole acetic acid and kinetin. Sucrose was present at 5 or 30 g/l. Filter-sterilised amino acids were added to the medium after autoclaving. AEC was autoclaved after control experiments had shown that this had no adverse effect. For selection of mutants embryos were taken from M2 seed of the variety 'Bomi' where M1 seed had been treated with the mutagen sodium azide (M2 seed, of 30% chlorophyll mutations on M1 spike basis, was a generous gift of Dr. V. Haahr, Agricultural Research Department, Risφ, Denmark). After growth for 7 days the shoot length of plantlets was measured and selected plants transferred to soil in small peat pots in a humid environment and then to pots in a glasshouse or controlled environment cabinet and grown to maturity.

Results

The lysine analogue AEC at concentrations of 0.1-0.5 mM inhibits the growth of barley embryos grown in sterile culture (Figs. 1,3). Root growth is particularly sensitive to inhibition but for quantitative measurement of growth we have used shoot length or fresh weight. Inhibition due to AEC can be relieved by lysine (Fig. 1). Other amino acids also partially relieve the inhibition by AEC, particularly arginine and ornithine which are also basic amino acids (Table 1). Not only do lysine, arginine and ornithine re-

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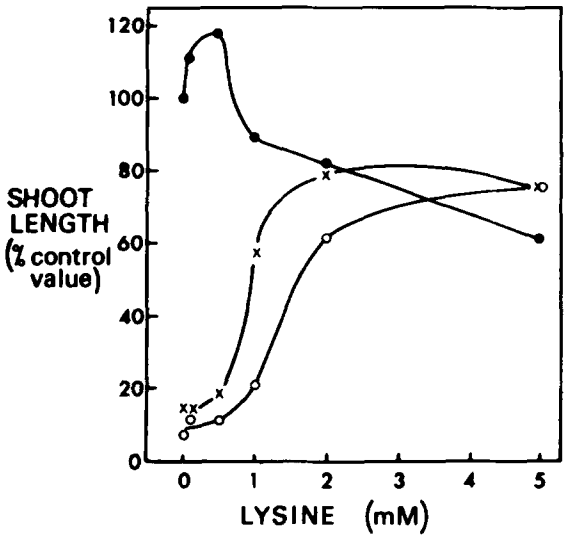

Fig. 1. Lysine relief of AEC-inhibition. Each point represents the mean of 40-50 embryos of 'Maris Mink' grown in 2 dishes for 7 days in the presence of 0 (•), 0.3 (x) or 0.5 mM (o) AEC (sucrose 5 g/l) and varying lysine concentrations

lieve inhibition of shoot length and fresh weight increase, but they allow root growth in the agar medium.

Any mutant which accumulates lysine should be able to grow better than normal plantlets in the presence of AEC. We have screened 8200 M2 embryos for ability to grow on medium (sucrose 5 g/l) containing 0.25 mM AEC. Seed (M3 generation) was obtained from 26 selfed plants selected as growing well in 0.25 mM AEC. M3 embryos were tested on 0.25 mM AEC and one line, designated R906, was selected for further study.

The originally selected M2 plant and the M3 and M4 progeny of R906 all had enhanced shoot growth and long roots which penetrated the agar in the presence of 0.25

Table 1. Relief of AEC inhibition by amino acids

Amino acid additions	Fresh weight (mg/plant)	%	Shoot length ± std dev.	%
None	70.8	100	51 ± 14	100
AEC	22.6	32	11 ± 5	22
AEC + lysine	56.4	80	36 ± 14	71
AEC + ornithine	43.8	62	28 ± 9	56
AEC + arginine	40.0	57	29 ± 9	58
AEC + methionine	34.9	49	20 ± 4	40
AEC + citrulline	31.8	45	19 ± 7	38
AEC + glutamine	29.3	41	18 ± 7	36
AEC + histidine	28.0	39	19 ± 7	37
AEC + homoserine	27.4	39	18 ± 7	36
AEC + threonine	25.8	36	13 ± 5	27

AEC at 0.3 mM; other amino acids at 1.0 mM. 13-15 Bomi embryos were plated in one dish on 25 ml medium (sucrose 30 g/l). Results are pooled from at least two dishes

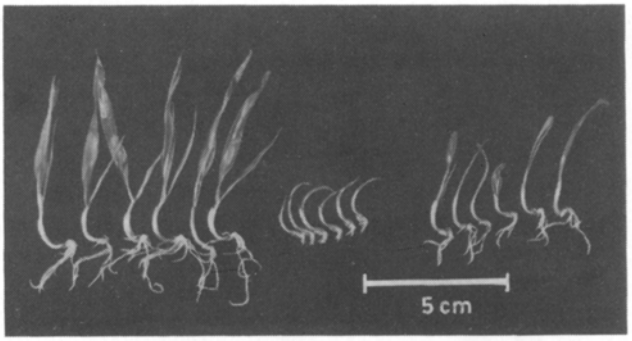

Fig. 2. Growth of R906 and 'Bomi' embryos on AEC medium. Left: 'Bomi', AEC 0; middle: 'Bomi', AEC 0.25 mM; right: R906, AEC 0.25. Embryos grown for 7 days (sucrose 30 g/l)

mM AEC (Fig. 2). Embryos of R906 were still inhibited by AEC but particularly at the selective concentration they were clearly more resistant than the parent variety 'Bomi' (Fig. 3). R906 embryos are inhibited by lysine plus threonine (2 mM each) to the same extent as the parent (data not shown). R906 plants have normal morphology, growth and seed set.

The inheritance of resistance to AEC was followed by crossing R906 plants (M3 generation) with a normal AEC-sensitive barley variety, 'Maris Mink'. The cross was confirmed by analysis of the hordein polypeptides of endosperm portions of the F1 seeds in which both parental types could be distinguished (Shewry et al. 1978). R906 seed proteins were indistinguishable from the parental pattern. An F1 seed of each of the reciprocal crosses was grown through to seed and at least 100 F3 embryos from each plant tested for growth on 0.25 mM AEC. Root penetrance and shoot length were scored after 7 days

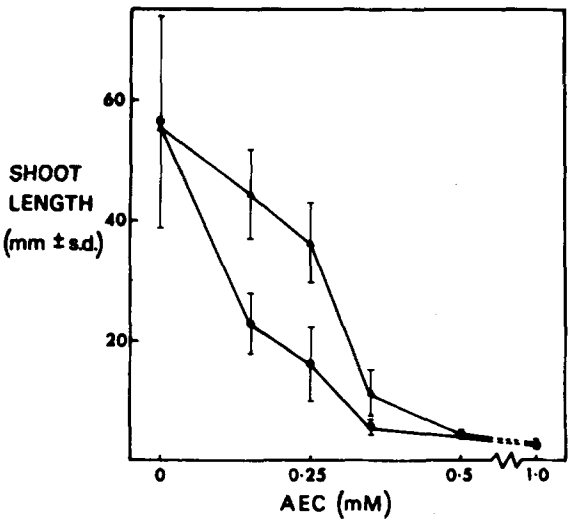

Fig. 3. AEC inhibition of 'Bomi' and R906 embryos. 28-32 'Bomi' (•) and R906 (▲) embryos were grown in two separate dishes on medium (sucrose 30 g/l) with varying AEC

Table 2. Inheritance of root penetrance in F2 of reciprocal crosses of R906 X 'Maris Mink'. Embryos grown 7 days on medium (sucrose 30 g/l) + 0.25 mM AEC

	R906 X 'Maris Mink'		'Maris Mink' X R906	
	♀	♂	♀	♂
Roots penetrant	21		20	
Roots non-penetrant	94		85	

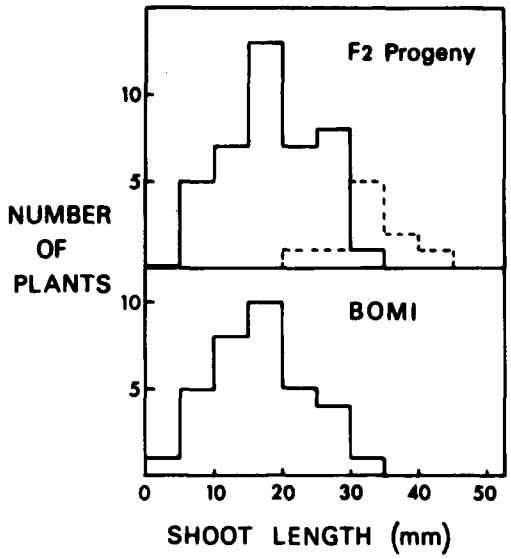

Fig. 4. Segregation of shoot length and root penetrance in F2 progeny of R906 X 'Maris Mink'. Upper F2 embryos were grown on medium (sucrose 30 g/l) with 0.25 mM AEC and plantlets with roots penetrating the agar (- - -) and those without penetrating roots (——) measured and results plotted in 5 mm classes. Lower: sensitive parental embryos grown as above

(Table 2, Fig. 4). The gene involved we call *aec*-1 (normal = +, resistant = *aec*−1). Six plants from the F2 of R906♀ X 'Maris Mink'♂ were taken from each of the three classes of growth response on 0.25 mM AEC: (1) Shoot greater than 35 mm, penetrant roots (2) Shoot greater than 25 mm, non-penetrant roots (3) Shoot less than 10 mm, non-penetrant roots. These 18 F2 plants were grown through to seed and the progeny embryos tested on 0.25 mM AEC and scored as above. The F2 plants could then be classified as either *aec*−1/*aec*−1, *aec*−1/+ or +/+ (Table 3).

Table 3. Inheritance of root penetrance in F3 progeny of the cross R906 X 'Maris Mink'. Embryos grown 7 days on medium (sucrose 30 g/l) + AEC 0.25 mM

F2 plantlet		F3 plantlet roots		
		Penetrant	Non penetrant	Genotype of F2 plantlet
Long shoot and penetrant roots	1	18	4	*aec*-1/*aec*-1
	2	24	0	*aec*-1/*aec*-1
	3	21	2	*aec*-1/*aec*-1
	4	24	0	*aec*-1/*aec*-1
	5	23	0	*aec*-1/*aec*-1
	6	23	0	*aec*-1/*aec*-1
Long shoot and non-penetrant roots	1	20	4	*aec*-1/*aec*-1
	2	6	16	*aec*-1/+
	3	4	20	*aec*-1/+
	4	5	19	*aec*-1/+
	5	11	13	*aec*-1/+
	6	24	0	*aec*-1/*aec*-1
Short shoot and non-penetrant roots	1	5	18	*aec*-1/+
	2	9	15	*aec*-1/+
	3	4	17	*aec*-1/+
	4	0	22	+/+
	5	3	20	*aec*-1/+
	6	0	21	+/+
Total for *aec-1*/+ genotype		47	138	

Discussion

AEC has been used to select for lysine accumulation in bacteria (Sano and Shiio 1970) and plant tissue cultures (Widholm 1976; Chaleff and Carlson 1975). We have selected a system in which fertile plants can be readily obtained in order to study the genetics and seed composition of any mutants obtained. This is an advantage over present barley tissue culture systems. The main disadvantage is in the small numbers of plants which can be tested. Highly mutagenised starting material is required.

In the barley embryo system inhibition by AEC is relieved most effectively by lysine, but also be arginine and ornithine. This is in agreement with results on oat seedling roots (Green, C.E., personal communication). The other amino acids tested were less able to relieve the inhibition due to AEC. It is probable that any plant which accumulated free lysine would be resistant to the inhibitory effect of AEC. Conversely, resistance to AEC could arise from accumulation of arginine or ornithine or by other mechanisms.

AEC can act as a false-feedback inhibitor of two plant enzymes involved in lysine synthesis, aspartate kinase (Shewry and Miflin 1977) and dihydrodipicolinate synthase (Mazelis, M.M. personal communication). However, to relieve the inhibition by AEC, lysine is required at greater than equimolar amounts (Fig. 1) suggesting that the lysine is competing with AEC for some site of action rather than acting to relieve a starvation for lysine.

The mutant line R906 was selected from a screened population of less the 10^4 M2 seeds. All the progeny of the originally selected plant were also resistant to AEC so the resistant trait is transmitted without segregation upon selfing. The data on inheritance of long penetrant roots in

a cross with a normal barley (Table 2) do not differ significantly from the 3 : 1 ratio expected for a single recessive nuclear gene ($p = 0.1$ for each of the reciprocal crosses). We call this gene *aec*-1. The slight shortfall in the numbers of progeny with penetrant roots can be explained by the data on the F3 progeny (Table 3) which show that some *aec*-1/*aec*-1 plants do not produce penetrant roots. This is also true of plants grown on control medium. The values for shoot length of F2 embryos grown on 0.25 mM AEC (Fig. 4) suggested that there might be some effect of the *aec*-1 gene in the heterozygous form. However, from Table 3 it can be seen that plants with the shortest shoots were 4 *aec*-1/+ and 2 +/+ as would be expected from a completely recessive gene.

The biochemical basis for resistance to AEC is being investigated. The fact that R906 embryos are not resistant to the inhibitory effect of lysine plus threonine suggests that the aspartate kinase in the mutant has unaltered sensitivity to lysine (Bright et al. 1978). The *aec*-1 gene may serve a useful function as a marker which can be scored very soon after germination even if it has no effect upon free lysine levels.

As barley is a naturally self-fertilising species it is not surprising that the originally selected resistant plant should carry a homozygous recessive mutation since there has been segregation after the mutagenic treatment of the M1 seed. The system described here thus has additional advantages over a diploid tissue culture system where only dominant or co-dominant mutations can be recovered.

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Dr. Simon W.J. Bright
Dr. P.B. Norbury
Dr. Benjamin J. Miflin
Biochemistry Department
Rothamsted Experimental Station
Harpenden, Herts AL 5 23Q (UK)