IN VIVO INCORPORATION OF [^{14}C] LYSINE INTO THE ENDOSPERM PROTEINS OF WILD TYPE AND HIGH-LYSINE BARLEY

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1. Introduction

The Risø mutant no. 1508 in Bomi barley has a 45% increase in the lysine content in the endosperm at maturity [1]. The elevated lysine content is due to a single gene mutation [2] which acts specifically on the endosperm, as normal low lysine endosperms can develop on homozygous mutant plants after fertilization with wild type pollen [3]. The mutant has a low content of hordein [1] and a high content of free amino acids in the endosperm [4]. The mutant thus has similar traits as the high-lysine maize mutant opaque-2 [5,6]. Sodek and Wilson [7] found that lysine supplied to the maize endosperm through the stem was converted more readily to glutamic acid and proline in the wild type than in the opaque-2 endosperm.

This paper reports on the endosperm reserve protein deposition, the amount and partial composition of the free amino acids of the endosperm and the incorporation of ^{14}C into the endosperm proteins following injection of [^{14}C] lysine in the top internode of Bomi barley and its high lysine mutant no. 1508. At advanced stages of endosperm development the mutant did not incorporate glutamic acid and proline derived from conversion of labelled lysine into the hordein fraction while Bomi hordein did reveal such an incorporation.

2. Materials and methods

The wild type Bomi (*Hordeum vulgare* L. cv. Bomi), and its mutant Risø no. 1508 were grown outdoors in pots. 0.3 μCi uniformly labelled [^{14}C] lysine (spec. act.: 287 mCi/mmol) was injected in the top internode below the spikes [8]. The injected lysine was less than 0.2% of the free lysine present in the developing caryopses. The spikes were harvested at different times after the injections, frozen in liquid nitrogen and stored at -21°C.

Free amino acids were extracted and purified as described by Lazarus [9] from endosperms taken from non-labelled spikes. The composition of the fraction was determined by ion-exchange chromatography.

Ten endosperms from the middle of the labelled spikes were extracted successively with 3 × 5 ml H_2O at 4 °C for 30 min (albumin), 3 × 5 ml 0.5 M NaCl at 4°C for 30 min (globulin), 3 × 5 ml 55% (v/v) isopropanol at 20°C for 1 hr, 2 hr and 1/2 hr respectively (hordein) and 3 × 5 ml 0.2 N NaOH at 20°C for 18 hr, 2 hr and 1/2 hr respectively (glutelin). Nitrogen was determined by Nesslerization [10]. Protein was calculated as 6.25 × N.

The proteins were precipitated with TCA and collected on glass fiber discs and counted in a toluene: Triton X-100 scintillator. After counting the filters were hydrolysed and the amino acids separated by ion-exchange chromatography using the columns of a Beckmann 120 C amino acid analyser.

Fractions were collected and the amino acids were located by spot test with ninhydrin and the elution time. The fractions were mixed with the toluene: Triton X-100 scintillator and counted.

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Table 1
Composition of endosperm proteins (mg protein per endosperm) for Bomi barley and its mutant no. 1508 during kernel development

Genotype	Bomi			Mutant 1508		
Days after fertilization	21	27	31	21	27	31
Dry weight mg/endosperm	25.8	41.9	46.5	19.5	35.9	39.8
Total protein mg/endosperm	3.01	4.00	4.44	2.99	3.11	3.62
Albumin	0.86	1.13	0.97	1.37	1.12	0.97
Globulin	0.84	1.18	1.38	0.92	1.04	1.34
Hordein	0.53	0.78	1.02	0.08	0.16	0.35
Glutelin	0.26	0.48	0.78	0.25	0.42	0.61
Residue	0.52	0.43	0.29	0.37	0.37	0.35

3. Results and discussion

Table 1 shows the dry weight and the protein composition of the endosperm of Bomi barley and the mutant no. 1508 during intensive kernel development. The kernels were fully mature 42 days after fertilization. The total protein deposition was smaller in the mutant, but comprised nearly the same proportion of the seed (9%) as in Bomi barley. Reserve protein synthesis comprising globulin, hordein and glutelin proceeded in the two barley genotypes over the period studied, i.e. 21 to 31 days after fertilization. In this period no increase in the albumin fraction, presumably containing the cytoplasmic proteins, took place. Hordein synthesis was distinctly lower in the mutant.

Compared to Bomi three to four times higher amounts of free amino acids were present in the mutant endosperm during the period investigated (table 2). An analysis of the composition of the free amino acids in Bomi and the mutant revealed the same relative amount of lysine, a substantial reduction in the relative amount of glutamic acid at the first two stages and a significant increase in the relative amount of proline in the last two stages of endosperm development in the mutant compared to Bomi. Threonine + serine and alanine were the predominant free amino acids in both endosperm genotypes. Threonine + serine were increased and alanine reduced in relative amount in the mutant compared to Bomi at the last two stages examined.

The distribution of label in the endosperm proteins following injection of [^{14}C] lysine at two different times after fertilization is given in table 3. Label was incorporated into all endosperm protein fractions. At an early stage of endosperm development (13 days after fertilization) preferential labelling of the albumin fraction followed by globulin and glutelin was observed. The label did not appear to move between the fractions in the 18 day period studied and only small

Table 2
Amounts of total free amino acids and free lysine, glutamic acid, proline, threonine + serine and alanine at various stages of endosperm development in Bomi barley and its mutant no. 1508.

Days after fertilization		16		24		32	
Genotype		Bomi	1508	Bomi	1508	Bomi	1508
μmol free amino acids per endosperm		1.053	3.624	0.989	2.408	0.700	3.286
Lysine	mol %	4	2	2	2	2	2
Glutamic acid	mol %	17	5	30	8	27	20
Proline	mol %	6	6	7	13	5	10
Thr + Ser	mol %	25	30	18	35	21	30
Ala	mol %	22	35	24	16	24	8

Table 3
Per cent distribution of ^{14}C-label among endosperm proteins for Bomi barley and its mutant 1508 during kernel development. U-[^{14}C] lysine was injected into the top internode 13 and 21 days after fertilization and proteins of the developing endosperm analysed different times thereafter.

Injection-days after fertilization	13		13		13		21	
Analyses-days after fertilization	21		27		31		29	
Genotype	Bomi	1508	Bomi	1508	Bomi	1508	Bomi	1508
	per cent							
Albumin	43	49	47	46	45	46	23	24
Globulin	23	24	22	28	20	29	31	29
Hordein	9	5	12	2	16	6	13	8
Glutelin	20	19	16	21	16	17	30	31
Residue	6	3	3	3	2	4	3	3
Total cpm/endosperm	800	1050	700	1700	2340	2000	1800	1040

differences were observed in spite of the intensive protein synthesis in the period. It is thus assumed that all the recovered label was translocated and incorporated into the endosperm proteins within the first week after the injections were made. The total activity per endosperm varied substantially due to the irreproducibility of the injection procedure. Injections at an advanced stage of endosperm development (21 days after fertilization) resulted in preferential labelling of the globulin and glutelin fractions. The albumin fraction incorporated at this stage only half as much label as at the early stage of endosperm development. This indicates a less intensive synthesis of the lysine rich components of the albumin fraction at late stages of endosperm development. The reversed pattern was observed in the glutelin fraction. The mutant hordein was less labelled than the Bomi hordein in both the early and late injection experiment.

Analysis of the constituent amino acids of the labelled endosperm proteins revealed only activity in lysine, glutamic acid and proline (table 4). Early supply of lysine (13 days after fertilization) showed no significant difference in the labelling pattern of the albumin, globulin and the glutelin fractions of the two endosperm genotypes. One third of the Bomi hordein label was located in glutamic acid and proline whereas these two amino acids contained one fifth of the mutant hordein label. Supply of lysine at the advanced stage of endosperm development (21 days after fertilization) resulted in the same label distribution among the amino acids of the albumin and the globulin fractions from both genotypes as at

Table 4
Per cent distribution of ^{14}C-label among amino acids of labelled endosperm proteins at an early and late stage of kernel development in Bomi barley and its mutant 1508. U-[^{14}C] lysine was injected 13 or 21 days after fertilization and the endosperm analysed after 18 and 8 days, respectively.

Injection-Days after fertilization	13				21			
Analyses-Days after fertilization	31				29			
Genotype	Bomi		1508		Bomi		1508	
	Lys	Glu + Pro	Lys	Glu + Pro	Lys	Glu + Pro	Lys	Glu + Pro
Albumin	92	8	95	5	92	8	96	4
Globulin	94	6	96	4	91	9	100	0
Hordein	63	37	80	20	68	32	100	0
Glutelin	91	9	92	8	81	19	89	11
Residue	60	32	66	33	65	35	74	16

the early stage of endosperm development. A greater part of the label in the Bomi glutelin was located in glutamic acid and proline than in the glutelin of the mutant and those from the early injection experiment. One third of the label incorporated in the Bomi hordein at this advanced stage of endosperm development was again found in glutamic acid. The mutant endosperm, on the other hand, did not incorporate labelled glutamic acid or proline into hordein. That the conversion of lysine to glutamic acid and proline is specifically inhibited in the hordein synthesising compartement and not generally blocked at this stage of endosperm development is revealed by the presence of labelled glutamic acid and proline in the mutant glutelin and residual protein. On a whole endosperm basis, 13% of the recovered activity in Bomi and 8% in the mutant endosperm were present in glutamic acid and proline following injection of [^{14}C] lysine at the early stage of endosperm development. The figures were 15% and 6% respectively with injections at the advanced stage of endosperm development.

Conversion of lysine to glutamic acid and proline has been demonstrated for the developing endosperm of wheat [11] and maize [7]. The hordeins of barley are known to be a heterogenous group of proteins rich in glutamic acid and proline but low in lysine [12]. The high lysine content of the mutant endosperm is due to the reduction in the lysine poor hordeins with a compensating increase in lysine rich reserve proteins and the increased amount of free lysine. It is likely that the restriction in the conversion of lysine to glutamic acid and proline is related to the reduced ability of the mutant endosperm to synthesise hordein. To what extent this is the cause for the elevated amount of free amino acids will have to be elucidated in further studies.

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