Theor. Appl. Genet. 55, 269-272 (1979)

Cytoplasmic Male Sterility in Barley

Part 4: Effect of *msm1* Cytoplasm and Partial Fertility on Kernel Protein and Lysine

H. Ahokas
Department of Genetics, University of Helsinki, Helsinki (Finland)

Summary. The effect of the *msm1* cytoplasm of barley (*Hordeum vulgare* L.) on kernel protein and lysine was studied using the near-isogenic, unrestored derivatives of seven barley varieties. With normal lysine varieties, 'Adorra', 'Bomi', 'CI 4362', and 'Hankkija's Eero', the *msm1* cytoplasm produced an average of one percentage point more protein than did the normal cytoplasm of the same varieties. There was no difference between the two cytoplasms with respect to their effect on the lysine content. With high lysine varieties, 'Bomi Risφ mutant 13', 'Bomi Risφ mutant 1508', and 'CI 3947', *msm1* produced almost one percentage point more protein but protein with a somewhat decreased lysine content.

Induced partial spike fertility in normal 'Adorra' was found to be associated with lysine in meal (r = –0.999), with protein in meal (r = –0.984), and with lysine in protein (r = 0.941). Removal of the spikes on the secondary tillers affected both the protein and its lysine content. It is suggested that good spike fertility is an important pre-requisite when selecting high lysine and/or high protein segregants or mutants.

Key words: Barley – Protein and lysine content – Cytoplasmic male sterility – Maternal effect – Partial spike fertility

Introduction

Cytoplasmic male sterile (*msm1*) barley has recently been described (Ahokas 1978a, 1978b, 1979a, 1979b). The appearance of the starchy endosperm under the scanning electron microscope (SEM) was not found to be affected by this cytoplasm (Ahokas 1978b). In an early test with incompletely isogenic material, *msm1* cytoplasm was found to produce a significantly higher kernel protein content (Ahokas 1979b). The present study was carried out in order to confirm the result with more isogenic material, and to examine the protein quality in terms of the most limiting amino acid, lysine. A maternal effect on protein and lysine in barley has been reported by Ullrich and Eslick (1978).

The production of kernel material with *msm1* is carried out via hand pollination. Since hand pollination does not usually result in full seed sets, the effect of partial spike fertility on protein in normal barley was also studied. With partial fertile (desynaptic) mutants a negative correlation has been shown between head fertility and N percentage (Ahokas 1977a).

Material and Methods

Plants, Growing Conditions, and Seed Production

The *msm1* derivatives of the seven varieties or mutants were obtained by back-crossing. The levels of isogeny are presented in Table 1. The plants were grown in 1 m rows, at intervals of 15 cm. The seeds were planted by hand. The resulting stand was somewhat less sparse than usual for barley in Finland. The two cytoplasmic types were in alternate rows, i.e. *msm1* – normal – *msm1* – normal[1]. Blocks of this kind were separated from the adjoining ones by border rows containing the normal variety of the block.

The first spike on each plant was treated. On the day of bagging the awns were cut at the top of the spike. On the day of pollination the awns were cut at the base without damaging the lemmas. The success of the hand pollination of *msm1* spikes was checked two to three days later, and repeated if necessary. The spikes of the other tillers were cut at the base of the spike at emergence. On the control plants with normal cytoplasm the awns were removed in two phases; the spikes bagged and the later tillers decapitated. The seeds of at least five plants were pooled for analysis.

To induce partial seed sets in normal 'Adorra', the pistils were extracted with forceps at anthesis or a little later. One quarter, a

1 'Normal' cytoplasm refers to the original cytoplasm of a variety

0040-5752/79/0055/0269/$ 1.00

Table 1. Effects of cytoplasm on kernel proteine and lysine

Cytoplasm	Nuclear genotype in embryo/in endosperm	Homozygous for special mutant gene	Spike and kernel type	Spike fertility %[a]	Mean kernel weight mg (P)[b]	Protein %[c]	Lysine[d]	
							In meal %	In protein %
Adorra	Adorra, 100%/100%	–	Two-rowed, covered	95.5	43.2 ± 1.06 (> 0.20)	11.07	0.412	3.72
msm1	Adorra, 99.2%/99.0%	–	Two-rowed, covered	95.5	44.5 ± 0.72	12.39	0.418	3.38
Bomi	Bomi, 100%/100%	–	Two-rowed, covered	97.2	50.6 ± 1.53 (> 0.40)	10.79	0.343	3.17
msm1	Bomi, 96.9%/95.8%	–	Two-rowed, covered	95.8	49.2 ± 0.91	11.00	0.358	3.25
Bomi, Risφ 13	Risφ 13, 100%/100%	*sex4f*	Two-rowed, covered	98.9	40.1 ± 1.03 (< 0.01)	10.41	0.509	4.89
msm1	Risφ 13, 96.9%/95.8%	*sex4f*	Two-rowed, covered	98.7	36.5 ± 0.68	11.96	0.516	4.31
Bomi, Risφ 1508	Risφ 1508, 100%/100%	*sex3c*	Two-rowed, covered	98.3	41.3 ± 1.09 (> 0.20)	8.13	0.652	8.03
msm1	Risφ 1508, 96.9%/95.8%	*sex3c*	Two-rowed, covered	97.4	39.6 ± 0.81	8.57	0.609	7.10
CI 3947, Hiproly	CI3947, 100%/100%	*lys1*	Two-rowed, naked	79.3	21.0 ± 0.71 (> 0.50)	15.56	0.689	4.43
msm1	CI3947, 96.9%/95.8%	*lys1*	Two-rowed, naked	70.3	21.8 ± 0.95	15.88	0.636	4.00
CI 4362, Hiproly Normal	CI4362, 100%/100%	–	Two-rowed, naked	87.6	36.8 ± 1.44 (< 0.05)	15.30	0.453	2.96
msm1	CI4362, > 93,8%/> 91.7%	–	Two-rowed, naked	83.7	33.4 ± 0.83	17.11	0.502	2.93
Hankkija's Eero	H's Eero, 100%/100%	–	Six-rowed, covered	73.0	34.9 ± 1.42 (< 0.05)	11.17	0.435	3.90
msm1	H's Eero, 96.9%/95.8%	–	Six-rowed, covered	75.7	30.0 ± 1.32	11.86	0.464	3.91

[a] None of the pairs 'Normal – *msm1*' differ significantly with 2 X 2 contingency test, 0.75 > P > 0.20
[b] Determined from 50 kernels, accuracy 0.1 mg. Probability, in parentheses, determined with t-test
[c] Mean of three determinations
[d] Mean of two determinations

half, or three quarters of the florets were emptied in this way for each spike on all the tillers in order to induce 75, 50, or 25% spike fertility respectively. The decapitation of all the secondary tillers was also tested in normal 'Adorra'. On average, 3.2 spikes per plant were removed. The remaining spikes displayed a 100% seed set.

Before planting, 400 kg/ha of a 15-20-15 fertilizer was drilled into the test field in a direction crosswise to the plant rows.

Grinding and Determination of Protein and Lysine

The kernel weight was determined from a random sample of 50 measurements. The grinding procedure has been described earlier (Ahokas 1977b). Protein was determined from 21-25 mg samples of meal using the UV method described earlier (Ahokas 1978c). The percentage protein content was calculated using an equation based on 6.25 X N. The UV determination is based on peptide linkages rather than on nitrogen content. The lysine content of the meal was determined using the 2,4,6-trinitrobenzene-sulfonic acid method by Kakade and Liener (1969), slightly modified (Ahokas 1977b).

Results

Effects of Partial Fertility and Decapitation of Secondary Tillers

Kernel weight was not found to be strongly correlated with the induced sterility (Fig. 1). In general, the kernels were found to be heavier as a result of sterilization while decapitation was found to increase kernel weight considerably (Fig. 1).

The lysine content of meal displays an almost linear negative correlation with fertility ($r = -0.999^{***}$). The protein content, too, is almost linear and is also negatively correlated ($r = -0.984^{*}$). The lysine content of protein displays a positive correlation with some departure from linearity at the higher fertility levels ($r = 0.941^{\circ}$). These features are illustrated in Figure 1.

The decapitation of tillers in 'Adorra' had practically no effect on the lysine content of meal (corresponding to

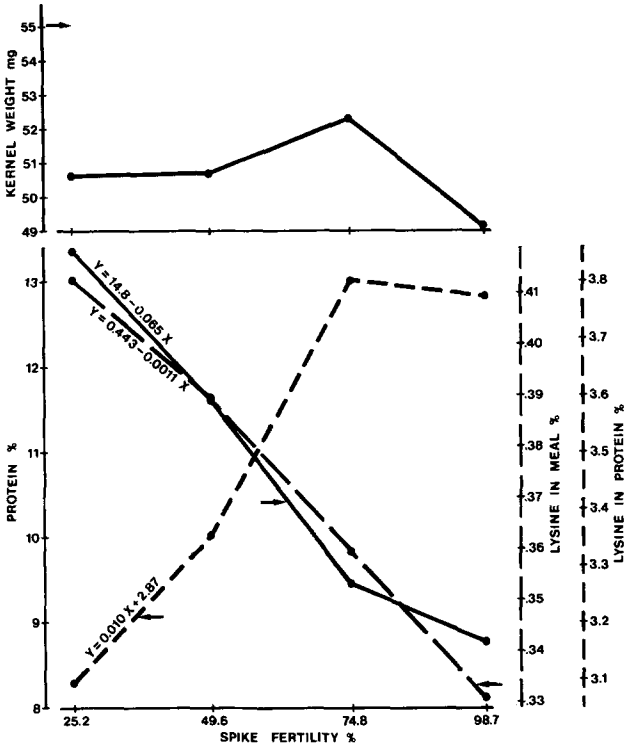

Fig. 1. Effects of induced partial spike fertility and decapitation of secondary tillers in cv. 'Adorra'. Kernel weight (top plot), protein, lysine, and lysine in protein plotted against spike fertility. The arrows indicate the ordinates of the results obtained with the decapitated material. Identical ordinate axis and curve line correspond to each other. Equations of linear approximation are presented

97% spike fertility when determined with the linear equation presented in Fig. 1). The decapitation increased the protein content to some extent (corresponding to 68% spike fertility) and consequently considerably reduced the lysine content of protein (corresponding to 33% spike fertility, Fig. 1.).

Effects of msm1 Cytoplasm

The isogenics (or near-isogenics) of each pair in the two cytoplasms displayed little or no difference between their kernel weights. The kernels produced on male sterile spikes tend to be slightly lighter (Table 1). This may be due to mechanical damage to floret parts caused by the forceps at pollination, or else to the 1-6 days delay in pollination. The variation in kernel size is usually smaller in *msm1* spikes but the reasons for this are not known. The spike fertility of normal barley fell slightly as a result of bagging the spikes and cutting the awns. The isogenic pairs did not differ significantly from each other in terms of their seed sets. In the isogenics of 'CI 3947' and 'Hankkija's Eero' the decreased spike fertility could affect the results (see preceding chapter), while the rest may be regarded as normally fertile for the purpose of the protein analyses.

Table 2. Grouped data of normal and high lysine barleys

Cytoplasm	Varieties	Protein %	Lysine %	
			In meal	In protein
Normal	Adorra, Bomi, CI 4362, Hankkija's Eero	12.09 (100)	0.410 (100)	3.44 (100)
msm1	Adorra, Bomi, CI 4362, Hankkija's Eero	13.09 (108)	0.436 (106)	3.37 (98)
Normal	Risφ 13, Risφ 1508, CI 3947	11.37 *(100)*	0.617 *(100)*	5.78 *(100)*
msm1	Risφ 13, Risφ 1508, CI 3947	12.14 *(107)*	0.587 *(95)*	5.14 *(89)*

The results of protein studies are presented in Tables 1 and 2. With normal lysine barleys, *msm1* cytoplasm appears to produce 1.08 times the protein content of normal cytoplasms with an approximately equal lysine content. With the three high lysine varieties, *msm1* cytoplasm produces 1.07 times the protein content of normal cytoplasms. This protein is somewhat inferior in its lysine content to that produced by the mutants in normal cytoplasm.

Discussion

The slight difference in kernel weight does not explain the higher protein content of *msm1*. For instance, both members of the 'Adorra' pair, with the highest isogeny in the material, have the same fertility and a similar kernel weight, and yet the protein content of *msm1*-'Adorra' is higher (Table 1). The vegetative yield components (tiller number and size, and floret number per spike) have not been found to be affected by *msm1* cytoplasm (Ahokas 1979b and unpublished). The effects of the restorer gene of fertility, *Rfm„a*, on protein content can be studied as soon as isogenic material is available.

It is reasonable to assume that decapitation had the same type of effect and to the same extent in both cytoplasms. It is unlikely that any mechanical irritation caused by forceps at pollination would increase the protein content.

The protein content of the mutant 'Risφ 1508', which

has a low prolamine content (Ingversen et al. 1973), is likely to be somewhat underestimated with the extractive method used.

As in the desynaptic mutants of cv. 'Betzes' with partial spike fertility (Ahokas 1977a), the induced partial spike fertility was found to increase the protein content. Since there is a high correlation between spike fertility and lysine content, it is of great importance that in screening high lysine plants or mutants the spike fertility is good on all vigorous tillers. Segregants in the breeder's population with a high vegetative tiller mass and relatively small head (low kernel number and size) resemble, in effect, partial fertility. These can, therefore, be expected to have a higher protein content than the reverse types. The increase in protein content caused by partial fertility, combined with the absence of an increase in kernel size, provides evidence that the amino acid pool of tillers are exhausted to a constant level while the accumulation of carbohydrates in kernels varies. The decapitation experiment on 'Adorra' barley provides evidence that considerable translocation of amino acids from tiller to tiller is possible.

The *msm1* cytoplasm has been suggested to be a plastid mutant (Ahokas 1978b). This hypothesis is not rejected by the difference in protein contents. At the very least nitrite reductase, one of the enzymes for nitrate reduction, is located in plastids, and its synthesis has been found to involve plastid ribosomes in some plant species (Hewitt 1975). A functional model could also be based on a difference in the efficency of membrane transport of nitrite into plastids. In the further metabolism of nitrogen, chloroplastidic glutamine synthetase and glutamate synthase (Lea and Miflin 1974; Keys et al. 1978) and membrane transport of substrates and products could also constitute the control sites in chloroplasts.

Acknowledgement

The work was done under the auspices of the Academy of Finland.

Literature

Ahokas, H.: Increase in protein by partial fertility. Barley Genet. Newslett. **7**, 6-8 (1977a)

Ahokas, H.: Lysine and tryptophan content of some high-protein stocks and species of *Hordeum*. Hereditas **86**, 143-145 (1977b)

Ahokas, H.: Cytoplasmic male sterility in barley. I. Anther and pollen characteristics of *msm1*, restored and partially restored *msm1* genotypes. Z. Pflanzenzücht. **81**, 327-332 (1978a)

Ahokas, H.: Cytoplasmic male sterility in barley. II. Physiology and anther cytology of *msm1*. Hereditas **89**, 7-21 (1978b)

Ahokas, H.: A simple and rapid screening method for the determination of protein and tryptophan in kernel halves and small samples of barley meal. J. Sci. Food Agric. **29**, 47-52 (1978c)

Ahokas, H.: Cytoplasmic male sterility in barley. III. Maintenance of sterility and restoration of fertility in the *msm1* cytoplasm. Euphytica **28** (1979a, in press)

Ahokas, H.: Cytoplasmic male sterility in barley. Acta Agric. Scand. **29** (1979b, in press)

Hewitt, E.J.: Assimilatory nitrate-nitrite reduction. Ann. Rev. Plant Physiol. **26**, 73-100 (1975)

Ingversen, J.; Køie, B.; Doll, H.: Induced seed protein mutant of barley. Experientia **29**, 1151-1152 (1973)

Kakade, M.L.; Liener, I.E.: Determination of available lysine in protein. Anal. Biochem. **27**, 273-280 (1969)

Keys, A.J.; Bird, I.F.; Cornelius, M.J.; Lea, P.J.; Wallsgrove, R.M.; Miflin, B.J.: Photorespiratory nitrogen cycle. Nature **275**, 741-743 (1978)

Lea, P.J.; Miflin, B.J.: Alternative route for nitrogen assimilation in higher plants. Nature **251**, 614-616 (1974)

Ullrich, S.E.; Eslick, R.F.: Protein and lysine maternal inheritance effects in high protein and high lysine barley mutant Hiproly. Barley Genet. Newslett. **8**, 108-109 (1978)

Received April 12, 1979
Communicated by R. Hagemann

Dr. H. Ahokas
Department of Genetics
University of Helsinki
P. Rautatiekatu 13
00100 Helsinki 10 (Finland)