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# Cytoplasmic Male Sterility in Barley

## Part 7: Nuclear Genes for Restoration

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**Summary.** Restoration in the *msm1* cytoplasm of barley (*Hordeum vulgare* L. s.l.) was studied from the standpoint of population biology and physiological effects on kernel protein. Restorer genes of 82 accessions of wild barley (ssp. *spontaneum*) from Israel were determined. 38% of the accessions were maintainers of sterility, 48% were partial restorers, and 14% were restorers. Fourteen dominant restorer genes are described, and evidence for three cases of allelism to *Rfm1a* is presented. The restorer accessions and their designated gene symbols are: PI 282636 (*Rfm,,e*), PI 282637 (*Rfm,,f*), PI 282646 (*Rfm,,g*), PI 284742 (*Rfm,,h*), PI 284743 (*Rfm,,i*), PI 284753 (*Rfm,,j*), PI 284755 (*Rfm1d*), PI 296838 (*Rfm,,k*), PI 296850 isolate 16/7 (*Rfm,,l*), PI 296853 (*Rfm,,m*), PI 296856 (*Rfm1b*), PI 296899 (*Rfm,,n*), PI 296919 (*Rfm1c*), PI 296944 (*Rfm,,o*). PI 296850 was found to contain both a restorer and a non-restorer genotype. None of the PI accessions with a restorer gene is a carrier of an *msm1*-type male sterilizing cytoplasm. In the present sample, plants with restoration ability occurred with a higher frequency in the material from the Judean Foothills than that from the other regions of Israel. The greater adaptive value of plants with restoration ability on certain soil associations in semiarid and subhumic climate is suggested. The considerable frequency of restorers and partial restorers in male fertile cytoplasm suggests that the restoration system evolved before the *msm1*-type cytoplasm.

In the nuclear genotype near-isogenic with either 'Adorra' or 'Risφ 1508', *msm1* plants heterozygous for *Rfm1a* produced 98.6 or 98.5% of the protein content in the respective recurrent pollen parent varieties. The amino acid compositions of the derivatives differed little from those of the varieties. In the derivatives, a consistent decrease was found in tryptophan, and consistent increases in isoleucine, phenyalanine, lysine, histidine, and arginine. In relation to glucose consumption, the bioenergetic cost calculated for the amino acid patterns found in the restored *msm1* derivatives was slightly higher than that for the near-isogenic pollen parent varieties. The results suggest that the restorer gene in the heterozygous state normalizes the physiology of *msm1* cytoplasm to a great extent.

**Key words:** *Hordeum vulgare* – Cytoplasmic male sterility – Restorer gene – Polymorphism – Kernel protein and amino acids

## Introduction

Cytoplasmic male sterility in barley (*Hordeum vulgare* L. s.l.) was found as a natural variant in an Israeli strain of wild barley, *H. vulgare* ssp. *spontaneum* (C. Koch) Thellung (Ahokas 1979a). The original strain is a carrier of a dominant restorer gene of fertility, designated *Rfm1a*. No cultivated barley tested has proved to carry a restorer gene, though partial restorers have appeared (Ahokas 1979a, 1979b). It was assumed that complete restorers exist in the wild originator of barley. This has proved to be true (Ahokas 1979b).

In Israel, ssp. *spontaneum* occupies an extraordinarily wide diversity of habitats: from mesic Mediterranean to desert (Harlan and Zohary 1966; Nevo et al. 1979a, 1979b). The Israeli populations of ssp. *spontaneum* have been found to be highly variable in flavonoids (Fröst and Holm 1975), in their resistance to fungal pathogens (Fischbeck et al. 1976; Wahl et al. 1978; Segal et al. 1979; Dr. J.G. Moseman, pers. commun.), in morphology (Kamm 1977), in isozymes by starch gel electrophoresis and in morphology (Brown et al. 1978a; Nevo et al. 1979a, 1979b), in hordein patterns using two electrophoretic systems (Doll and Brown 1979), and in DBC protein content (Ahokas, unpublished). The pattern of isozyme variation has been explained by natural selection (Nevo et al.

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1979a, 1979b). This evidently applies to the resistance to pathogens (Wahl et al. 1978).

The present paper describes the restoration ability of 82 accessions of ssp. *spontaneum* from Israel. The mode of inheritance of the restorer genes found in this and an earlier set (Ahokas 1979b) was studied. The protein content and amino acid composition were studied in two derivatives heterozygous for *Rfm1a*. An increase in protein content was ascribed to *msm1* cytoplasm in unrestored derivatives (Ahokas 1979c).

## Material and Methods

The field conditions (SE Finland, ca. 61°N) and methods of cultivation were the same as described in Ahokas (1979b), the soil being fortified each year with boron. Boron is necessary for male fertility in barley (Löhnis 1940; Simojoki 1972). The early season of 1979 was somewhat warmer (in mean 1.3°C) than that of the previous test season of 1978. The seed sets were tested by bagging at least 10 spikes on several plants at random, preferably on the earliest and next earliest stems. The mean plant density in the test bench was 60 plants per $m^2$ (Table 1). The wild barley pollen donors contain both true wild barleys and types with distinct signs of introgression from cultivated barley. Notwithstanding that, they are all called wild or *spontaneum* barleys in this paper.

For the protein determinations, the variety used as recurrent pollen parent and its near-isogenic derivative were grown in alternate rows as described in Ahokas (1979c). The derivative rows displayed a 1 : 1 segregation of restored and male sterile plants. The same number of the near-isogenic fertile derivatives and of the variety itself were harvested at random in the inner part of the rows. Grinding was carried out as previously (Ahokas 1979c). The protein was estimated with the Kjeldahl method. The N values were corrected for reagent control (sucrose) and for recovery of N (alanine). Tryptophan was determined by spectrophotofluorometry (Aminco Bowman) as described in Ahokas (1978a), the other amino acids by automatic analysis (Jeol 6 AH). The meals were hydrolyzed with 6N HCl at 110°C for 22 h. Elution was carried out according to Jeppsson and Karlsson (1972). The automatic analysis were purchased from the Technical Research Centre of Finland, and were performed by Drs. M. Kiesvaara and T. Hattula. All protein and amino acid values are means of two determinations.

## Results

Of the 82 pollen parent accessions of wild barley tested in the 1979 season 31.5 (38%) were maintainers of sterility, 39 (48%) were partial restorers with a detected restoration ability ranging from 0.5 to 52.3%, and 11.5 (14%) were restorers of fertility, with a detected restoration ability ranging from 90.6 to 97.2% (Table 1). The reasons for the decrease from complete seed sets in the bagged restored $F_1$ spikes are presented elsewhere (Ahokas and Hockett 1980). One non-restorer and another restorer plant were found in the accession PI 296850. In this paper, these pollen parent plants are designated PI 296850-11/7 and PI 296850-16/7, the former being the non-restorer and the latter the restorer. These two isolates of PI 296850 are difficult to distinguish by their morphology. The two genotypes may, however, represent a spontaneous cross in the past, and the segregation therefrom. The distribution of the accessions in different fertility classes is presented in Table 2, along with the 31 crosses tested in the season of 1978 (Ahokas 1979b). All these crosses have an 'Adorra'-type genetic background in the *msm1* seed parent. Not all different seed parent backgrounds are comparable (Ahokas 1979b). Sterility due to chromosomal interchanges is negligible: 99 of these *spontaneum* accessions have been crossed with normal 'Adorra', and evidently only three accessions (collected in Sde Hemed) had partial $F_1$ fertility when selfed or backcrossed, suggesting heterozygosity for the rearrangements. These three accessions were classified as maintainers of sterility.

The restorer accessions comprise both true wild or weedy specimens and a single accession with signs of introgression from cultivated, probably six-rowed barley (PI 284755), accessions with relatively short anthers (e.g. PI 296899) and rather long anthers. However, the longest anthers the author has ever seen in *H. vulgare* s.l. were found in an accession maintaining male sterility, PI 296796. These robust plants bore anthers with an anthesis length of up to 8 mm.

The original collection regions in Israel could be traced for most of the accessions, and for some the collection locality was also available (Anonymous 1968; Wahl 1972; Drs. Nava Eshed and J.G. Moseman, pers. commun.). The distribution of the accessions in different restoration classes is presented in Table 2. The sample is concentrated in the region of the Judean Foothills. The collection area in Judean Foothills, as limited by the 1948 boundary, is almost entirely covered by 'areas 1' of Dan (1977: 'thick Nari cover on chalks and marls'), or the intervening alluvial valleys. In the other ten regions, this type of lime crust occurs sporadically or not at all. Significantly more restoration ability was found in the accessions from Judean Foothills than in those from the other regions (Table 2; significance for two-tailed test, $P < 0.025$. In general, the restoration ability is assumed to be more common in the accessions from the central inland regions. Wild barley occurs in more southern areas in the Negev. These southernmost, strikingly arid regions should perhaps be considered a separate, major region.

Since the segregation of the winter growth habit hampers the recording of the $F_2$ generation, backcrossed $F_1$ generations were scored to determine the mode of inheritance of the restorer genes. The results suggest that all the restorer accessions are carriers of a single dominant restorer gene (Table 3).

The restorer accessions were screened for the presence of any *msm1*-type sterilizing cytoplasm. As carriers of a

**Table 1.** Maintainers of sterility, restorers and partial restorers of fertility among the tested PI accessions of wild barley (ssp. *spontaneum*) from Israel in the season of 1979

| Pollen parent | Maternal background[a] | Number of pollen donor plants tested | Number of $F_1$ plants | Period of bagging (days) | Number of bagged spikes | Seed set in bagged spikes | | Classification[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Florets with seed/without seed | % | |
| PI 282572 | 1 | 1 | 17 | 11 | 12 | 19/168 | 10.2 | S-PF |
| PI 282575 | 2 | 1 | 17 | 7 | 11 | 0/191 | 0.0 | S |
| PI 282583 | 2 | 1 | 18 | 5 | 14 | 0/232 | 0.0 | S |
| PI 282586 | 1 | 1 | 19 | 7 | 10 | 0/160 | 0.0 | S |
| PI 282591 | 2 | 1 | 18 | 8 | 14 | 0/231 | 0.0 | S |
| PI 282599 | 1 | 1 | 17 | 10 | 13 | 0/209 | 0.0 | S |
| PI 282608 | 1 | 1 | 19 | 5 | 13 | 85/115 | 42.5 | S-PF |
| PI 282609 | 2 | 1 | 15 | 5 | 12 | 97/ 93 | 51.1 | S-PF |
| PI 282611 | 1,2 | 2 | 36 | 6 | 14 | 0/200 | 0.0 | S |
| PI 282611, atypical[c] | 1 | 1 | 16 | 8 | 12 | 0/154 | 0.0 | S |
| PI 282613 | 2 | 1 | 14 | 5 | 11 | 0/186 | 0.0 | S |
| PI 282616 | 1 | 1 | 15 | 15 | 12 | 1/198 | 0.5 | S-PF |
| PI 282620 | 1 | 1 | 12 | 16 | 12 | 0/219 | 0.0 | S |
| PI 282621 | 2 | 1 | 15 | 7 | 15 | 0/250 | 0.0 | S |
| PI 282631 | 2 | 1 | 32 | 6 | 16 | 5/254 | 1.9 | S-PF |
| PI 282635 | 1 | 1 | 24 | 11 | 24 | 0/391 | 0.0 | S |
| PI 282636 | 1,3 | 1 | 21 | 15 | 18 | 297/ 20 | 93.7 | Restorer[d] |
| PI 282637 | 2 | 1 | 9 | 15 | 16 | 260/ 11 | 95.9 | Restorer[d] |
| PI 282638 | 2 | 1 | 14 | 4 | 11 | 81/119 | 40.5 | S-PF |
| PI 282640 | 1 | 1 | 19 | 9 | 11 | 11/141 | 7.2 | S-PF |
| PI 282642 | 2 | 1 | 12 | 16 | 16 | 137/125 | 52.3 | S-PF |
| PI 282644 | 2 | 1 | 15 | 8 | 13 | 15/174 | 7.9 | S-PF |
| PI 282645 | 1 | 1 | 34 | 9 | 24 | 7/334 | 2.1 | S-PF |
| PI 282646 | 1,3 | 1 | 29 | 13 | 19 | 272/ 10 | 96.5 | Restorer[d] |
| PI 282663 | 1 | 1 | 17 | 8 | 11 | 0/172 | 0.0 | S |
| PI 282665 | 1 | 1 | 17 | 4 | 10 | 34/124 | 21.5 | S-PF |
| PI 282666 | 2 | 1 | 17 | 7 | 14 | 18/222 | 7.5 | S-PF |
| PI 282669 | 1 | 1 | 16 | 8 | 13 | 46/188 | 19.7 | S-PF |
| PI 282670 | 1 | 1 | 11 | 35 | 14 | 0/253 | 0.0 | S |
| PI 282672 | 1 | 1 | 17 | 9 | 13 | 17/211 | 7.5 | S-PF |
| PI 282674 | 2 | 1 | 14 | 11 | 15 | 0/251 | 0.0 | S |
| PI 282679 | 1 | 1 | 19 | 5 | 12 | 3/193 | 1.5 | S-PF |
| PI 284742 | 1,3 | 1 | 24 | 15 | 21 | 309/ 32 | 90.6 | Restorer[d] |
| PI 284743 | 1,3 | 1 | 22 | 11 | 21 | 334/ 15 | 95.7 | Restorer[d] |
| PI 284753 | 2 | 1 | 22 | 12 | 22 | 332/ 32 | 91.2 | Restorer[d] |
| PI 284754 | 1,2 | 1 | 31 | 5 | 12 | 0/169 | 0.0 | S |
| PI 284755 | 2,3 | 1 | 30 | 15 | 38 | 590/ 17 | 97.2 | Restorer[d] |
| PI 296796 | 1 | 1 | 17 | 8 | 11 | 0/200 | 0.0 | S |
| PI 296812 | 1 | 1 | 16 | 14 | 12 | 0/202 | 0.0 | S |
| PI 296813 | 2 | 1 | 18 | 4 | 13 | 0/217 | 0.0 | S |
| PI 296815 | 2 | 1 | 19 | 9 | 11 | 1/172 | 0.6 | S-PF |
| PI 296827 | 2 | 1 | 13 | 6 | 11 | 0/192 | 0.0 | S |
| PI 296834 | 1 | 1 | 16 | 5 | 12 | 92/100 | 47.9 | S-PF |
| PI 296838 | 2 | 1 | 20 | 15 | 26 | 406/ 17 | 96.0 | Restorer[d] |
| PI 296839 | 1 | 1 | 17 | 5 | 11 | 0/185 | 0.0 | S |
| PI 296850[e] | 1 | 1 | 13 | 17 | 12 | 0/192 | 0.0 | S |
| PI 296850[f] | 2 | 1 | 2 | 52 | 10 | 175/ 12 | 93.6 | Restorer |
| PI 296853 | 2,3 | 1 | 24 | 12 | 29 | 409/ 22 | 94.9 | Restorer[d] |
| PI 296854 | 1,2 | 2 | 31 | 3 | 11 | 0/188 | 0.0 | S |
| PI 296861 | 1 | 1 | 15 | 6 | 11 | 0/172 | 0.0 | S |
| PI 296863 | 1 | 2 | 38 | 7 | 20 | 3/308 | 1.0 | S-PF |
| PI 296864 | 1 | 1 | 18 | 13 | 10 | 27/152 | 15.1 | S-PF |
| PI 296865 | 2 | 2 | 25 | 16 | 15 | 9/227 | 3.8 | S-PF |
| PI 296873 | 1,2 | 2 | 34 | 7 | 18 | 0/271 | 0.0 | S |
| PI 296875 | 2 | 1 | 17 | 14 | 11 | 1/183 | 0.5 | S-PF |

**Table 1.** (continued)

| Pollen parent | Maternal background[a] | Number of pollen donor plants tested | Number of $F_1$ plants | Period of bagging (days) | Number of bagged spikes | Seed set in bagged spikes | | Classification[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Florets with seed/without seed | % | |
| PI 296877 | 2 | 1 | 10 | 16 | 12 | 1/220 | 0.5 | S-PF |
| PI 296881 | 2 | 1 | 16 | 15 | 12 | 1/185 | 0.5 | S-PF |
| PI 296882 | 2 | 2 | 27 | 7 | 18 | 43/238 | 15.3 | S-PF |
| PI 296884 | 1 | 1 | 16 | 11 | 15 | 44/185 | 19.2 | S-PF |
| PI 296886 | 2 | 1 | 16 | 15 | 16 | 59/204 | 22.4 | S-PF |
| PI 296892 | 1 | 1 | 22 | 13 | 14 | 13/242 | 5.1 | S-PF |
| PI 296899 | 1,3 | 2 | 29 | 10 | 33 | 503/ 30 | 94.4 | Restorer[d] |
| PI 296904 | 2 | 1 | 16 | 9 | 13 | 48/152 | 24.0 | S-PF |
| PI 296915 | 2 | 1 | 20 | 9 | 14 | 16/220 | 6.8 | S-PF |
| PI 296932 | 2 | 1 | 17 | 16 | 12 | 0/187 | 0.0 | S |
| PI 296933 | 2 | 1 | 18 | 7 | 12 | 0/189 | 0.0 | S |
| PI 296934 | 1 | 1 | 24 | 11 | 16 | 0/254 | 0.0 | S |
| PI 296944 | 2,3 | 1 | 26 | 15 | 25 | 391/ 17 | 95.8 | Restorer[d] |
| PI 296945 | 2 | 1 | 15 | 11 | 16 | 3/252 | 1.2 | S-PF |
| PI 296952 | 1 | 1 | 20 | 7 | 10 | 66/106 | 38.4 | S-PF |
| PI 296956 | 2 | 1 | 18 | 7 | 14 | 76/144 | 34.5 | S-PF |
| PI 349807 | 2 | 1 | 17 | 4 | 10 | 44/121 | 26.7 | S-PF |
| PI 349808 | 1,2 | 2 | 20 | 11 | 13 | 20/199 | 9.1 | S-PF |
| PI 349809 | 1 | 1 | 18 | 15 | 10 | 6/154 | 3.8 | S-PF |
| PI 354926 | 1 | 1 | 22 | 5 | 12 | 0/189 | 0.0 | S |
| PI 354927 | 1 | 1 | 32 | 4 | 12 | 0/183 | 0.0 | S |
| PI 354928 | 2 | 1 | 14 | 11 | 11 | 49/114 | 30.1 | S-PF |
| PI 354929 | 1 | 1 | 17 | 16 | 12 | 4/178 | 2.2 | S-PF |
| PI 354936 | 2 | 1 | 17 | 8 | 12 | 16/195 | 7.6 | S-PF |
| PI 354942 | 2 | 1 | 33 | 11 | 17 | 0/256 | 0.0 | S |
| PI 354944 | 1 | 1 | 18 | 10 | 11 | 1/165 | 0.6 | S-PF |
| PI 354947 | 1 | 1 | 20 | 10 | 12 | 0/204 | 0.0 | S |
| PI 354949 | 1 | 1 | 12 | 11 | 11 | 0/177 | 0.0 | S |
| Control, *msm1*/6*Adorra or *msm1*/8*Adorra | | | | 47 | 87 | 0/1875 | 0.0 | S |

[a] 1 = *msm1*/5*Adorra; 2 = *msm1*/6*Adorra; 3 = *msm1*/7*Adorra
[b] S = male sterile; S-PF = male sterile to partially male fertile
[c] Plant organs larger than in the typical plants of the accession
[d] Additional six plants classified as male fertile in the greenhouse in winter 1978-1979
[e] Hereafter called PI 296850-11/7
[f] Hereafter called PI 296850-16/7

single dominant restorer gene, the $BC_1$-$F_1$ generation with 'Adorra' (a non-restorer) as the recurrent pollen parent will reveal the tentative presence of such a cytoplasm. No sterile segregant was found, and the hypothesis of the *msm1*-type cytoplasm can be rejected for all the accessions ($P < 0.001$) (Table 4). It is also improbable that such a cytoplasm is present in any of the partial restorers.

Four restorer genes were tested for allelism. The test consisted of crossing a heterozygous and a homozygous restorer in *msm1* cytoplasm, and recording segregating and non-segregating $F_2$ families (Table 5). In effect, this method cannot distinguish between linked genes and allelism, but is readily able to distinguish between non-linkage versus linkage or allelism. The restorer gene in PI 284755, PI 296856, or PI 296919 is evidently allelic to the *Rfm1a* allele in Sel.77-1. Further tests on allelism can be carried out when the genes have been extracted into a spring growth habit and cultivated background.

The segregation of the restorer gene *Rfm1a* in highly 'Adorra'-like backgrounds was recorded in the season of 1979 (Table 6). All the results fit the monohybrid dominant mode and confirm the earlier findings (Ahokas 1979a).

Some quantitative properties of the heterozygous, restored, near-isogenic *msm1* derivative of 'Adorra' were compared with those of normal 'Adorra' (Tables 7 and 8), and those of a corresponding less isogenic derivative of 'Risø 1508' with those of 'Risø 1508' (Table 8). These derivatives are visually indistinguishable in appearance

**Table 2.** Ranked distribution of restoration ability in the accessions of wild barley from Israel. Selfing determined in bagged $F_1$ spikes with an 'Adorra'-type seed parent in *msm1* cytoplasm in the season of 1978 and 1979

| Geobotanical region | Seed set percentage[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | 0.1-1.0 | 1.1-5.0 | 5.1-10.0 | 10.1-20.0 | 20.1-30.0 | 30.1-40.0 | 40.1-50.0 | 50.1-60.0 | 60.1-90.0 | 90.1-100.0 | Total |
| Judean Foothills | 17 | 3 | 10 | 5 | 5 | 3 | 3 | 2 | 2 | – | 9 | 59 |
| Ten regions[b] | 17.5[c] | 9 | 2 | 2 | 1 | 3 | – | 1 | – | – | 4.5[c] | 40 |
| Region unknown and atypical accessions | 7 | 3 | 1 | 1 | – | 1 | 1 | – | – | – | – | 14 |
| Number of accessions tested in the season of 1978[d] + 1979 | 10 + 31.5 | 8 + 7 | 6 + 7 | 0 + 8 | 1 + 5 | 3 + 4 | 1 + 3 | 0 + 3 | 0 + 2 | 0 + 0 | 2 + 11.5 | 31 + 82 |
| Total | 41.5 | 15 | 13 | 8 | 6 | 7 | 4 | 3 | 2 | 0 | 13.5 | 113 |
| % | 36.7 | 13.3 | 11.5 | 7.1 | 5.3 | 6.2 | 3.5 | 2.7 | 1.8 | 0.0 | 11.9 | 100 |

[a] Kolmogorov-Smirnov test (Siegel 1956) between the distributions of 'Judean Foothills' and 'Ten regions': D = 0.324
[b] Western, Upper and Lower Galilee, Zevulon Plain, Mt. Carmel, Jordan and Bet Shean Valley, Coastal Plain, Judean (Jerusalem) Mountains, Negev
[c] PI 296850 is scored half to the class 0.0 and half to the class 90.1-100.0, see text
[d] Data presented in Ahokas (1979b)

**Table 3.** Backcross $F_1$ segregation of the restorer genes of the fourteen accessions

| Restorer accession | Transmission of restoration through pollen: fertile/male sterile plants[a] | Transmission of restoration through egg cells: fertile/male sterile plants[b] | Total: fertile/male sterile plants | $\chi^2$ for total, expected 1 : 1 | P | Suggested gene symbol |
|---|---|---|---|---|---|---|
| PI 282636 | 47/47 | 18/17 | 65/ 64 | 0.008 | >0.90 | *Rfm,,e* |
| PI 282637 | 49/52 | 35/25 | 84/ 77 | 0.304 | >0.50 | *Rfm,,f* |
| PI 282646 | 55/63 | 31/33 | 86/ 96 | 0.549 | >0.30 | *Rfm,,g* |
| PI 284742 | 32/38 | 24/15 | 56/ 53 | 0.083 | >0.70 | *Rfm,,h* |
| PI 284743 | 43/51 | 18/33 | 61/ 84 | 3.648 | >0.05 | *Rfm,,i* |
| PI 284753 | 66/60 | 43/51 | 109/111 | 0.018 | >0.80 | *Rfm,,j* |
| PI 284755 | 75/62 | 29/28 | 104/ 90 | 1.010 | >0.20 | *Rfm1d* |
| PI 296838 | 47/53 | 24/53 | 71/ 78 | 0.329 | >0.50 | *Rfm,,k* |
| PI 296850-16/7 | 54/46 | NT | 54/ 46 | 0.640 | >0.30 | *Rfm,,l* |
| PI 296853 | 59/54 | 22/15 | 81/ 69 | 0.960 | >0.30 | *Rfm,,m* |
| PI 296856 | 54/34 | 40/52 | 94/ 86 | 0.356 | >0.50 | *Rfm1b* |
| PI 296899 | 42/39 | NT | 42/ 39 | 0.111 | >0.70 | *Rfm,,n* |
| PI 296919 | 47/64 | 29/30 | 76/ 94 | 1.906 | >0.10 | *Rfm1c* |
| PI 296944 | 17/27 | 29/26 | 46/ 53 | 0.495 | >0.30 | *Rfm,,o* |

[a] General pedigree: *msm1*-Adorra//*msm1*-Adorra/PI Accession, with a 73.8 or 74.2% countable genetic background of 'Adorra'
[b] General pedigree: *msm1*-Adorra/PI Accession//Adorra, with a 74.2 or 74.6% countable genetic background of 'Adorra'. NT = not tested

**Table 4.** Test crosses for the tentative existence of an *msm1*-type sterilizing cytoplasm in the restorer accessions. $BC_1$-$F_1$ segregation of the crosses PI Accession/2*Adorra

| Restorer accession (The donor of the cytoplasm in the cross) | $BC_1$-$F_1$ segregation[a] | |
|---|---|---|
| | Male fertile | Male sterile |
| PI 282636 | 96 | 0 |
| PI 282637 | 124 | 0 |
| PI 282646 | 96 | 0 |
| PI 284742 | 97 | 0 |
| PI 284743 | 144 | 0 |
| PI 284753 | 126 | 0 |
| PI 284755 | 87[b] | 0 |
| PI 296838 | 94 | 0 |
| PI 296850-16/7 | 38 | 0 |
| PI 296853 | 74 | 0 |
| PI 296856 | 150 | 0 |
| PI 296899 | 109 | 0 |
| PI 296919 | 182 | 0 |
| PI 296944 | 199 | 0 |

[a] Expected segregation 1 : 1, if a carrier of an *msm1*-type cytoplasm and a single locus restoration of fertility. Probability for each cross $P < 0.001$

[b] Involves a single partially fertile plant

from the corresponding normal varieties. Little or no significant difference could be shown by measurements (Table 7). The mean spike fertility did not differ in this pair, though the *msm1* derivative had a significantly ($P < 0.05$) different seed set on the two highest stems. In the nuclear genetic background of 'Adorra' and 'Risφ 1508', *msm1* cytoplasm plus restorer gene decreased the protein (N x 6.25) content by 1.4 or 1.5%, consistently decreased the tryptophan content of the protein, and consistently increased the isoleucine, phenylalanine, lysine, histidine, and arginine content of the protein (Table 8). The derivatives produced a higher total basic amino acid content than the normal varieties. The increase was 0.60 and 0.34 g/16 g N in the 'Adorra' and the 'Risφ 1508' derivative, respectively. The relative energy required with reference to glucose (Mitra et al. 1979) to synthesize the amino acid composition of the four barley stocks is presented in Table 8. The amino acids of the derivatives involve more energy than those of the normal varieties. A small surplus of 0.2 or 0.3% remains when the lower protein content in the derivatives is taken into account (Table 8).

**Table 5.** Ratios of segregating to non-segregating $F_2$ families in crosses testing the allelism of four *Rfm* genes

| Cross[a] | Number of $F_2$ families[b] | | P (Expected 1 : 1)[c] | Tentative gene symbol (and accession of the gene) |
|---|---|---|---|---|
| | Segregating | Non-segregating | | |
| *Rfm,,b*/+ × *Rfm1a/Rfm1a* | 7 | 13 | 0.264 | *Rfm1b* (PI 296856) |
| *Rfm,,c*/+ × *Rfm1a/Rfm1a* | 6 | 7 | 1.000 | *Rfm1c* (PI 296919) |
| *Rfm,,b*/+ × *Rfm,,c/Rfm,,c* | 5 | 7 | 0.774 | See above |
| *Rfm1a*/+ × *Rfm,,d/Rfm,,d* | 12 | 7 | 0.360 | *Rfm1d* (PI 284755) |

[a] Sign + refers to the recessive allele of 'Adorra'

[b] Families containing less than 16 plants were excluded

[c] Probability determined using the binomial test (Siegel 1956)

**Table 6.** Segregation of the restorer gene *Rfm1a* in 'Adorra'-type genetic background with *msm1* cytoplasm. Data from the 1979 season

| Test | Countable genetic background of 'Adorra' | Plants fertile/male sterile | Expected ratio | $\chi^2$ | P |
|---|---|---|---|---|---|
| Transmission through pollen, $F_1$ plants | 97.7% | 39/44 | 1 : 1 | 0.301 | >0.50 |
| Transmission through egg cells, $F_1$ plants | 96.1 or 98.0% | 99/75 | 1 : 1 | 3.310 | >0.05 |
| $F_2$ segregation | 96.1% | 203/66 | 3 : 1 | 0.031 | >0.80 |
| Heterogeneity between transmissions through the gametes | | | | 1.936 | >0.10 |

Table 7. Effects of the restorer gene in *msm1* cytoplasm with an 'Adorra'-type genetic background. Sample sizes 20 plants

| Cytoplasm | *Rfm* gene[a] | Countable nuclear genotype of cv. 'Adorra' | Plant density per m² | Plant height (cm) | Spikes per plant | Total number of florets per spike | Seed set percentage | | | Mean kernel weight on total sample (mg) | Yield per plant (g) | Protein % N × 6.25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | On two highest tillers | On the other tillers | On all tillers together | | | |
| Adorra | + / + | 100.0% | 135 | 91.1 ± 0.80 | 4.45 ± 0.35 | 17.89 ± 0.47 | 98.6% | 91.7% | 95.3% | 38.3 | 2.90 ± 0.21 | 7.61 |
| *msm1* | *Rfm1a*/ + | 98.8% | 135 | 92.3 ± 1.05 | 4.55 ± 0.33 | 17.73 ± 0.43 | 97.0% | 94.1% | 95.6% | 39.5 | 3.04 ± 0.24 | 7.50 |
| t ($\chi^2$) | – | – | – | 0.945 | 0.209 | 0.256 | (4.010) | (3.065) | (0.056) | Not deter- | 0.446 | Not deter- |
| P | | | | >0.30 | >0.80 | >0.70 | <0.05 | >0.05 | >0.80 | mined | >0.60 | mined |

[a] Sign + refers to the recessive allele of 'Adorra'

## Discussion

The two genotypes in the accession PI 296850 suggest that nonisogeny occurs within a few of the accessions and that the 1-2 plant sample per accession probably did not reveal all the variation within the accessions. One accession was observed to segregate male sterility due to a tentative male sterile gene mutant.

Among the partial restorers, there may be semidominant single gene restorers. In some instances, if not in all, partial restoration depends on more than one allele or gene (Ahokas 1979b). The fertility class 90.1-100.0%, widely separated from partial restorers (Table 2), as well as the four allelism cases found, suggest that the restorer genes are likely to be in a single locus. If the criterion for allelism (Table 5) is accepted, the central inland population, and probably some other populations as well, can be regarded as at least dimorphic, and perhaps polymorphic for the restorer gene. Since the 14 carriers of the restorer gene are not in an *msm1*-type cytoplasm, we are faced with three distinct groups of phenomena in nature: 1) the ecophysiology and genetics of restorer gene in fertile cytoplasm, 2) the ecophysiology and genetics of *msm1* cytoplasm, and 3) the interaction of restorer gene and *msm1* cytoplasm. Which one is dealt with in further discussions, may be seen in the context. The physiological effects of the partial restorer genotypes may be similar to, but milder than those of the restorer gene.

The restorer gene appears independently of the *msm1* cytoplasm with considerable frequency. Furthermore, a wide range of partial restorers occurs with high frequency in these areas. These observations suggest that the restorer gene evolved before the *msm1* cytoplasm, whose physiological effects are quite well normalized by the gene *Rfm1a*, at least in the heterozygous state (Tables 7 and 8; Ahokas 1978b, 1980).

A striking part of the genetic variation found in 28 populations of wild barley in Israel (from a wider area than the present material) was correlated with some environmental factors, chiefly with combinations of temperature and humidity, and also with soil types and vegetation (Nevo et al. 1979b). It may be noted that these factors are interrelated. Nevo et al. (1979b) found the highest polymorphism in the regions with an annual rainfall of 200-600 mm in the curvilinear association. The pattern of variation was concluded to suggest the operation of natural selection in *spontaneum* barley in Israel.

Unfortunately, the accurate collection locality is known only for part of the present accessions, and no direct information about the soil type in the habitats is available. On the other hand, changes in the accessions since the collection have been assessed as small: outcrossing is minimal in the greenhouse maintenance of the stocks (Dr. J.G. Moseman, pers. commun.). Five atypical

**Table 8.** Amino acids in kernel protein (g/16 g N) and quantitative comparisons between heterozygous, restored *msm1* derivatives of 'Adorra' and 'Risφ 1508' and the original varieties

| Amino acids | Variety and *msm1* derivative (countable nuclear isogeny in parenthesis) | | | |
|---|---|---|---|---|
| | 'Adorra' | | 'Risφ 1508' | |
| | In *msm1*, *Rfm1a*/+ (98.8%) | Normal 'Adorra', +/+ (100.0%) | In *msm1*, *Rfm1a*/+ (87.5%) | Normal 'Risφ 1508', +/+ (100.0%) |
| Tryptophan | 0.82 | 0.86 | 0.69 | 0.76 |
| Aspartic acid | 6.15 | 6.09 | 7.38 | 7.36 |
| Threonine | 3.66 | 3.59 | 4.12 | 4.11 |
| Serine | 4.23 | 4.22 | 4.51 | 4.45 |
| Glutamic acid | 19.77 | 19.36 | 14.41 | 14.71 |
| Proline | 9.57 | 9.63 | 6.13 | 5.92 |
| Glycine | 4.01 | 4.05 | 5.09 | 5.02 |
| Alanine | 4.31 | 4.28 | 4.99 | 4.91 |
| Cysteine | 1.51 | 1.37 | 1.31 | 1.37 |
| Valine | 4.84 | 4.86 | 5.06 | 5.09 |
| Methionine | 1.05 | 1.19 | 1.17 | 1.17 |
| Isoleucine | 3.60 | 3.21 | 3.16 | 2.42 |
| Leucine | 6.24 | 6.19 | 5.92 | 5.94 |
| Tyrosine | 2.97 | 3.14 | 2.98 | 2.97 |
| Phenylalanine | 4.67 | 4.55 | 3.84 | 3.79 |
| Lysine | 3.64 | 3.47 | 5.11 | 4.85 |
| Histidine | 2.22 | 2.18 | 2.60 | 2.56 |
| Arginine | 5.19 | 4.79 | 6.26 | 6.23 |
| Protein N X 6.25 (Relative) | 7.50 (98.6 ) | 7.61 (100.0 ) | 8.67 (98.5 ) | 8.80 (100.0 ) |
| Relative glucose requirement for the amino acids[a] | 12057 (101.8 ) | 11845 (100.0 ) | 11698 (101.7 ) | 11501 (100.0 ) |
| Relative glucose requirement for the amino acids per unit of meal | 100.3 | 100.0 | 100.2 | 100.0 |
| Spike fertility | 95.6% | 95.3% | 96.3% | 97.2% |
| Number of plants in sample | 20 | 20 | 15 | 15 |
| Plant density in the plot, plants per $m^2$ | 135 | 135 | 84 | 84 |

[a] Based on calculations by Mitra et al. (1979)

constant plants separated from 110 accessions probably removed the rest of the material from seed mixings. The selection since collection is low because wild barley is principally homozygous (Brown et al. 1978b). There is no conscious selection with respect to restoration among the sample.

In the present material, there is no apparent association between the distribution of restorers or partial restorers and the two climatic region systems, according to either Köppen or Thornthwaite (Rosenan and Mané 1970). To determine whether or not salt plays a role in the distribution of restoration ability, further material must be studied. Tests on material with pollen parents from two regions of Negev rather reject the association between restoration and salty habitats. These $F_1$ plants in *msm1* cytoplasm have so far been tested only in the greenhouse environment.

The dominant restorer gene is evidently long-term in

activity, i.e. constitutive or nearly constitutive, and affects many plant organs, or even the whole plant (Ahokas 1978b, 1980). This combined with its dominance, makes the restorer gene well subjected to natural selection. A constitutive gene is expected to be correlated with a selective factor(s), which is likely to operate for long periods or even for the entire life of the plant. Many soil factors are persistent or change little during an annual's life. Evidence for the involvement of soil has been received from two unpublished observations. Partial restoration responds to the level of soil nitrogen. $KNO_3$ fertilizer, which is the beneficial ion pair for nitrate uptake in barley (Blevins et al. 1978), was found to raise partial restoration up to a certain level of fertilization. Secondly, there was a decrease in partial restoration due to a change in the soil type (from a mixture of clay, sandy loam, and Finnpeat B2 to Finnpeat B2 alone) in the greenhouse when other conditions were kept constant.

One restorer accession from Judean Foothills has recently been found in addition to those described here. Thus, 15 out of 16 restorer plants originate from hilly or mountainous regions. Such regions with slopes, valleys and top plateaux offer a great variety of soil environments. The catenary soil relationships on a hill and the adjoining valley in Judean Foothills were accurately described by Dan et al. (1972). These soils have pH values from 7.1 to 8.1, and may be highly calcerous. Clay and silt are the major particle fractions (Dan et al. 1972). 10-15 out of 16 restorer plants originate from the eroded areas 'characterized by thick Nari cover on chalks and marls' (Dan 1977: 'areas 1') or the intervening valleys. Similar marly slopes and alluvial valley soils also appear in En Kerem (for PI 296850) and in Moza (for PI 296919) in the environs of Jerusalem (Arkin 1973). The collection site for PI 296856 is not unambiguous (Anonymous 1968), but this accession is likely to originate from 'areas 1' between Beer Sheva and Arad. Therefore, the number is likely 13 or more out of 16 restorers. Nari areas are found in the inland on soft chalky and marly parent material, where the climate is subhumic or semiarid down to the 200 mm isohyet. The 'areas 1' also appear sporadically in Galilee (Dan 1977), from where one restorer besides Sel.77-1 originates.

Most, if not all, of the restorer accessions originate from the wide areas between the 200-600 mm isohyets of annual rainfall (Rosenan 1970). The highest genetic polymorphism is expected to occur under these precipitation conditions (Nevo et al. 1979b). Most, if not all, of the restorers also appear inside the areas with 30-50 rainy days per year (Rosenan 1970). It is plausible that the semiarid conditions reinforce the hypothetical effective soil factor or participate in the soil formation, e.g. affecting its carbonate content (Gal et al. 1974).

The performance of the heterozygotes for the restorer gene is of interest from the standpoint of the possible use of this system in hybrid barley production. An increase in protein was attributed to the unrestored *msm1* cytoplasm (Ahokas 1979c). In this cytoplasm, the heterozygotes for *Rfm1a* have a protein content and amino acid composition very similar to that of the normal varieties, 'Adorra' and 'Risø 1508' (Tables 7 and 8). Thus, in the heterozygous state, the restorer gene to a great extent also normalizes the kernel composition, as it does many other features in the plant (Table 7; Ahokas 1978b, 1980). The small differences in spike fertility are expected to have negligible effect on the protein content (see Ahokas 1979c). Interestingly, the small surplus in the calculated glucose consumption for the amino acids was of the same magnitude in both the derivatives, though the varieties have a widely different amino acid composition and a different protein content. This suggests that the surplus is probably not due to a chance, and that the bioenergetic values determined by Mitra et al. (1979) are close to the real situation in barley. This observation of the bioenergetic contents raises the question of the slight superiority of *Rfm1a* heterozygotes in normal cytoplasm.

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