CYTOPLASMIC MALE STERILITY IN BARLEY. XI. THE *msm2* CYTOPLASM

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ABSTRACT

A new cytoplasmic male sterility in barley (*Hordeum vulgare* s.l.) is described and designated as *msm2*. The cytoplasm was derived from a selection of the wild progenitor of barley (*H. vulgare* ssp. *spontaneum*). This selection, 79BS14-3, originates from the Southern Coastal Plain of Israel. The selection 79BS14-3 has a normal spike fertility in Finland. When 79BS14-3 was crossed by cv. Adorra, the F_1 displayed partial male fertility and progeny of recurrent backcrosses with cv. Adorra were completely male sterile. Evidently 79BS14-3 is a carrier of a recessive or semidominant restorer gene of fertility. The dominant restorer gene *Rfm1a* for another cytoplasmic male sterility, *msm1*, is also effective in *msm2* cytoplasm. The different partial fertility restoration properties of *msm2* and *msm1* cause these cytoplasms to be regarded as being distinct. Seventy *spontaneum* accessions from Israel have been studied for their capacity to produce F_1 restoration of male fertility both in *msm1* and in *msm2* cytoplasms with a cv. Adorra-like seed parent (nuclear gene) background. The *msm2* cytoplasm shows partial restoration more commonly than *msm1* in these F_1 combinations. The mean restoration percentage per accession for *msm2* is 28, and for *msm1* 4. Most of the F_1 seed set differences of the two cytoplasms are statistically significant. When estimated with partially restored F_1 combinations, *msm2* cytoplasm appeared to be about 50 times more sensitive to the male fertility-promoting genes present in the *spontaneum* accessions. The *spontaneum* sample from Central and Western Negev, which has been found to be devoid of restoration ability in *msm1* cytoplasm, had only low partial restoration ability in *msm2* (mean 0.3%). The female fertility of *msm2* appears normal. The new *msm2* cytoplasm could be useful in producing hybrid barley.

IN the 1980 season the F_1 barley hybrid 79BS14-3 × Adorra displayed partial male fertility. The selection 79BS14-3 is a wild barley (*Hordeum vulgare* ssp. *spontaneum* (C. Koch) Thellung) selected from seeds collected at Berekhya, the Southern Coastal Plain, Israel. The seeds collected were kindly provided by DR. MOSHE FELDMAN. The cultivar Adorra is an Austrian two-rowed domesticated barley. The topmost spikelets of the F_1 hybrid were frequently sterile because of rudimentary anthers in these florets. The emasculated F_1 spikes bore complete seed sets when backcrossed with cv. Adorra. The F_1 sterility was thus different from that caused by translocation heterozygosity. When backcrossed with cv. Adorra, the BC_1 produced a few partially male fertiles, but the major proportion was completely male sterile plants. Male sterility has been maintained by recurrent backcrosses with cv. Adorra, suggesting cytoplasmic transmission of sterility. This article describes the restoration genetics of this cyto-

plasm designated as *msm2*, compared with that of *msm1* cytoplasm, which has been the material described in the previous articles of this series.

MATERIALS AND METHODS

A BC_1 descendant (79BS14-3/2*Adorra) with quite small, degenerated, sterile anthers, and thus evidently without partially restoring genes, was backcrossed with cv. Adorra. This single 79BS14-3/3*Adorra progeny was used for the program of determining the F_1 restoration ability of a number of *spontaneum* barleys. Seventy-one of these *spontaneum* barleys originate from Israel and are called *accessions* (see below). The plant material has been described in earlier papers (AHOKAS 1980, 1981). A single pollen donor plant was also tested from the collection of the weedy barleys in Morocco (MOLINA-CANO and CONDE 1980) obtained from DR. J. L. MOLINA-CANO (Spain).

The method of cultivation of the F_1 plants and testing of seed sets under bags in the 1981 season followed the practice used before with *msm1* (AHOKAS 1980). The mean plant density was 76 plants per m^2.

In pedigrees, the sign * is used to indicate the backcrosses as proposed by PURDY *et al.* (1968). The code 80-414-01 appearing in some pedigrees is the restorer derivative of the following cross: *msm1*/4*Adorra/3/*msm1*/3*Adorra//Sel. 77-1/4/4*Adorra, where Sel.77-1 is the Upper Galilean *spontaneum* selection carrying the *msm1* cytoplasm and the restorer genes *Rfm1a/Rfm1a* (AHOKAS 1979a, 1980).

RESULTS

The backcrosses of 79BS14-3 with cv. Adorra as the recurrent pollen parent retained male sterility both in greenhouse and field environment. Ninety spikes or 1959 florets of 79BS14-3/3*Adorra and 79BS14-3/4*Adorra generations did not set any seed under bags. However, female fertility is evidently complete, since routine hand-pollinations with cv. Adorra for backcrosses resulted in a 95.9% seed set in 1078 florets of 50 spikes.

In spikes under bags, partial fertility in the F_1 hybrid 79BS14-3/Adorra was also found in the 1981 season (Table 1). The F_1 hybrid *msm1*/12*Adorra//79BS14-3 had a significantly ($P < 0.001$) lower seed set than 79BS14-3/Adorra. These two cross combinations are isogenic, differing only in their cytoplasms. These crosses provide the first line of evidence for *msm2* cytoplasm being different from *msm1* cytoplasm. The same nuclear genotype in *Adorra* cytoplasm (Adorra/79BS14-3) had the normal seed set of 98.4%, which is highly significantly different ($P < 0.001$) from those above. When the restorer gene *Rfm1a* was introduced in the F_1 genotype with *msm2* cytoplasm (79BS14-3/80-414-01), the seed set was normal (98.1%) and not significantly different from that of the genotype without *Rfm1a* gene but with *Adorra* cytoplasm (Adorra/79BS14-3), whose seed set was 98.4%. These four F_1 combinations in Table 1 were raised in side-by-side rows, and the seed sets are based on the 21 earliest emerging spikes.

Further evidence for the efficiency of the restorer gene *Rfm1a* in *msm2* is presented in Table 2. Using near isogenic nuclear background with cv. Adorra, *Rfm1a*/+ heterozygotes in *msm2* appeared to have normal fertility: 20 bagged spikes containing 459 florets had a 98.9% seed set. The F_1 segregation of the heterozygous pollen parent (*Rfm1a*/+) and the F_2 segregation fit 1:1 and 3:1 ratios, respectively (Table 2). These data suggest that the restoration results from a single dominant gene, and is evidently caused by *Rfm1a*. A further argument for the effective functioning of *msm1* restoring genes can be found in Table 3. The accessions 79BS15-B and PI 296853 are carriers of the restorer

TABLE 1

Selfing in bagged spikes of isogenic or near-isogenic F_1 combinations in either msml, msm2 *or* Adorra *cytoplasm*

Pedigree	Cytoplasm	Genotype at *Rfm1* locus[a]	Percentage of cv. Adorra nuclear genotype	Percentage of 79BS14-3 nuclear genotype[b]	Period of bagging in days (date of median)[c]	Seed set in 21 bagged spikes			
						Florets			
						With seed	Without seed	%	Significance[d]
msm1/12*Adorra//79BS14-3	*msm1*	+/+	50.0	50.0	8 (July 14)	145	300	32.6	
79BS14-3/Adorra	*msm2*	+/+	50.0	50.0	12 (July 17)	397	46	89.6	
79BS14-3/80-414-01	*msm2*	+/*Rfm1a*	49.0	50.0	12 (July 23)	415	8	98.1	*a*
Adorra/79BS14-3	*Adorra*	+/+	50.0	50.0	10 (July 16)	435	7	98.4	*a*

[a] Sign + refers to the recessive allele of cv. Adorra or that of 79BS14-3.

[b] Carrier of a recessive or semidominant restorer gene and possibly additional minor restorer genes.

[c] The 21 earliest emerging spikes were bagged in each pedigree.

[d] The significance for the total 2×4 table is $P < 0.001$ ($\chi^2 = 813$). Crosses marked with the letter "*a*" are not significantly different, the differences of all the other pairs are significant at $P < 0.001$.

TABLE 2

Segregation of the Rfm1a *restorer gene in* msm2 *cytoplasm with Adorra-like genetic background*

Pedigree	Restorer genes in the latter pollen parent	Generation	Segregation		Significance
			Male fertile	Male sterile	
79BS14-3/2*Adorra//80-414-01	*Rfm1a/Rfm1a*	F_1	118	0	—
79BS14-3/3*Adorra//80-414-01	*Rfm1a/Rfm1a*	F_1	61	0	—
79BS14-3/3*Adorra/3/*msm1*/11*Adorra//80-414-01	*Rfm1a/+*	F_1	57	55	$\chi^2_{1:1} = 0.036$; $P > 0.80$
79BS14-3/2*Adorra//80-414-01	*Rfm1a/Rfm1a*	F_2	173	53	$\chi^2_{3:1} = 0.289$; $P > 0.50$

genes *Rfm,,r* and *Rfm,,m*, respectively (AHOKAS 1980, 1981). Both the F_1 of these accessions in *msm2* cytoplasm resulted in restored fertility (Table 3). The F_2 segregation of the cross 79BS14-3/Adorra was 130 male sterile or partially male fertile to 39 fertile. This fits a 3:1 ratio ($\chi^2 = 0.33$; $P > 0.50$), suggesting that 79BS14-3 is a carrier of a recessive or semidominant restorer gene. The F_2 seed was produced in bagged F_1 spikes.

Seventy-one accessions of *spontaneum* barleys have been tested for restoration both in *msm1* and *msm2* cytoplasms with an Adorra-like seed parent background produced by repeated backcrosses using cv. Adorra as the pollen parent. A single accession has been tested only in *msm2*. The results appear in Table 3 and in Figure 1. With the exception of the Moroccan weedy barley, each sample originated from one of the six geobotanical regions of Israel. The mean restoration percentage in *msm2* and *msm1* were 28.4% and 4.1%, respectively (Table 3). Although the *msm1* material was tested in other seasons than the F_1s in *msm2* cytoplasm, the result provides a second argument for the genetic difference between *msm2* and *msm1*. An Algerian domesticated barley CI 3694 has been found to be one of the most potent partially restoring cultivars (AHOKAS 1979b). A single pollen donor plant of CI 3694 was crossed with each of the cytoplasms. The F_1s were grown in side-by-side rows in the 1981 season. The F_1 selfings were 21.2% in the 259 florets or 15 bagged spikes of *msm1*/12*Adorra//CI 3694, and 57.5% in the 240 florets or 16 bagged spikes of *msm2*/3*Adorra//CI 3694 ($\chi^2 = 68$; $P < 0.001$). In addition to the results in Table 1, the crosses with CI 3694 also indicate that the observed difference with *spontaneum* material is not caused by any seasonal difference.

With the partial restorers, one can estimate the relative sensitivity of the two cytoplasms to the partially restoring genes appearing in the F_1s with an Adorra-like seed parent background. The *spontaneum* crosses with 0.0% restoration in either or both of the cytoplasms, and the crosses with 79BS15-B and PI 296853 carrying the complete restorer genes were excluded. The partial restoration efficiency was 51.2 ± 11.7 ($\bar{x} \pm s_{\bar{x}}$) times higher in *msm2* than in *msm1* cytoplasm.

When selecting the *spontaneum* sample for the test crosses in *msm2*, nothing was known about the mode of restoration in this cytoplasm. 79BS14-3 originates from the Southern Coastal Plain of Israel. It was for this reason that the accessions from this region were selected. The Central Coastal Plain included in the sample does not differ ecologically to any great extent from the Southern Coastal Plain. The sample from Central and Western Negev was found to be devoid of restoration ability in *msm1* (AHOKAS 1981). The accessions from the

TABLE 3

Seed sets in bagged spikes of F_1 hybrids in msm2 *cytoplasm with an Adorra-like seed parent background and various* spontaneum *barleys as the pollen parent*

Pollen parent		Tests in *msm2* cytoplasm in the 1981 season			Seed set in *msm1* cytoplasm[b] (%)	Significance between seed sets in *msm1* and *msm2* cytoplasms[c] (P)
Identification	Geobotanical region[a]	No. of bagged spikes	No. of bagged florets	Seed set under bags (%)		
79BS05-A	CWN	17	279	0.0	0.0	NS[d]
79BS05-B	CWN	17	274	0.0	0.0	NS
79BS06-A	CWN	19	292	0.0	0.0	NS
79BS07-A	CWN	17	295	0.3	0.0	NS
79BS08-A	CWN	18	268	0.0	0.0	NS
79BS08-B	CWN	19	301	0.7	0.0	NS
79BS09-A	CWN	19	272	0.0	0.0	NS
79BS10-A	CWN	16	274	0.0	0.0	NS
79BS11-A	CWN	19	314	0.0	0.0	NS
79BS12-A	CWN	18	325	0.3	0.0	NS
79BS12-B	CWN	21	329	0.9	0.0	NS
79BS12-C	CWN	18	308	1.6	0.0	NS
79BS12-D	CWN	17	294	0.0	0.0	NS
79BS12-E	CWN	20	352	0.0	0.0	NS
79BS13-A	CWN	17	284	0.0	0.0	NS
79BS13-B	CWN	18	283	0.0	0.0	NS
79BS13-C	CWN	17	304	0.3	0.0	NS
79BS15-A	SCP	21	332	11.7	0.0	<0.001
79BS15-B	SCP	20	305	91.8	98.3	<0.001
79BS16-A	CCP	15	244	0.0	0.0	NS
79BS16-B	CCP	16	283	0.0	—	—
79BS28-A	UJV	16	301	40.9	0.8	<0.001
79BS29-A	UJV	15	245	7.3	2.9	NS
79BS29-B	UJV	15	239	87.0	11.2	<0.001
79BS29-C	UJV	17	285	26.0	0.0	<0.001
79BS30-A	G	17	297	46.1	1.1	<0.001
79BS30-B	G	16	265	77.4	10.4	<0.001
79BS30-C	G	16	222	76.1	14.3	<0.001
79BS31-A	G	17	256	0.0	0.0	NS
79BS31-B	G	16	286	5.9	0.0	<0.01
79BS32-A	G	16	234	1.3	0.0	NS
79BS34-A	G	16	270	0.0	0.0	NS
M79-1159-A	BSV	16	307	16.0	0.0	<0.001
M79-1159-C	BSV	32	631	3.2		<0.02
M79-1159-D	BSV	16	330	51.2	0.8	<0.001
R79-1161	CWN	17	237	1.7	0.0	NS
R79-1162	CWN	18	288	0.0	0.0	NS
R79-1163	CWN	18	246	0.4	0.0	NS
PI 296796	JF	18	287	30.3	0.0	<0.001
PI 296800	UJV	16	240	44.6	0.0	<0.001
PI 296801	SCP	15	210	0.0	0.0	NS
PI 296821	UJV	21	318	27.7	0.2	<0.001
PI 296829	UJV	17	260	61.5	0.8	<0.001
PI 296830	UJV	17	237	56.5	0.6	<0.001
PI 296831	SCP	18	265	87.9	23.6	<0.001
PI 296853	CCP	20	302	97.4	94.9	NS
PI 296854	CCP	16	254	0.0	0.0	NS
PI 296874	CCP	17	322	14.9	1.0	<0.001
PI 296904	UJV	18	299	96.0	24.0	<0.001

TABLE 3—*Continued*

Pollen parent		Tests in *msm2* cytoplasm in the 1981 season			Seed set in *msm1* cytoplasm[b] (%)	Significance between seed sets in *msm1* and *msm2* cytoplasms[c] (P)
Identification	Geobotanical region[a]	No. of bagged spikes	No. of bagged florets	Seed set under bags (%)		
PI 296932	CCP	15	227	9.3	0.0	<0.001
PI 349803	CCP	16	252	0.0	0.0	NS
PI 391096	BSV	18	264	35.6	4.1	<0.001
PI 391097	BSV	17	274	47.4	0.8	<0.001
PI 391098	CCP	15	250	60.4	0.0	<0.001
PI 391099	CCP	17	262	55.0	0.0	<0.001
PI 391100	CCP	19	301	57.1	0.0	<0.001
PI 391101	CCP	17	242	41.7	0.0	<0.001
PI 391102	CCP	22	304	53.3	0.0	<0.001
PI 391103	CCP	18	304	56.3	0.0	<0.001
PI 391104	CCP	18	264	28.0	0.0	<0.001
PI 391105	CCP	17	236	78.4	0.0	<0.001
PI 391107	SCP	23	312	31.1	1.6	<0.001
PI 391108	SCP	14	236	54.2	0.5	<0.001
PI 391109	SCP	19	313	63.6	0.0	<0.001
PI 391110	SCP	16	248	74.6	0.4	<0.001
PI 391111	SCP	17	272	75.0	0.0	<0.001
PI 391112	SCP	16	254	39.4	0.5	<0.001
PI 391113	SCP	16	259	52.1	0.0	<0.001
PI 391114	SCP	17	290	39.0	0.0	<0.001
PI 391134	CWN	18	280	0.4	0.0	NS
PI 391137	CWN	16	240	0.0	0.0	NS
Moroccan	—	15	233	0.0	0.0[e]	NS

The average number of F_1 plants per cross was 18, ranging from 10 to 41. The corresponding seed set percentages in *msm1* cytoplasm are presented for comparison.

[a] Keys for the abbreviations: BSV = Bet Shean Valley; CCP = Central Coastal Plain; CWN = Central and Western Negev; G = Golan; JF, Judean Foothills; SCP = Southern Coastal Plain; UJV = Upper Jordan Valley.

[b] Accurate seed sets presented in AHOKAS 1979b, 1980, 1981 Appendix.

[c] Significance between the pooled seed sets of the two cytoplasms: $\chi^2 = 3531$; $P < 0.001$.

[d] Not significant.

[e] Four additional pollen parents resulted in 0.0% F_1 seed sets in *msm1*-Adorra.

Bet Shean Valley, the Upper Jordan Valley, and Golan are interesting, because they are geographically distinct from the Southern Coastal Plain populations in the *spontaneum* material previously tested in *msm1* cytoplasm. The distribution of restoration in the Southern Coastal Plain sample taken as a standard, differs significantly ($P < 0.025$) from the pooled remainder of the sample (Table 4). However, of the regional samples only that from the Negev differ significantly ($P < 0.001$) from that of the Southern Coastal Plain (Table 4).

The sample from the Southern Coastal Plain, that from the Central Coastal Plain, and that from the Bet Shean Valley and the Upper Jordan Valley had significantly different ($P < 0.005$) restoration distributions in *msm2* and *msm1* cytoplasms, whereas the Negev and the small Golan sample did not differ significantly (Table 4). The pooled seed sets of the cytoplasms differ very significantly ($P < 0.001$, Table 3 footnote *c*). The single accession PI 296796 from the Judean Foothills region was selected for the test because of the large anthers in the PI 296796 plants (AHOKAS 1980), which may be used to recombine wind pollinators in barley. Although a maintainer of sterility in *msm1*, PI 296796 was

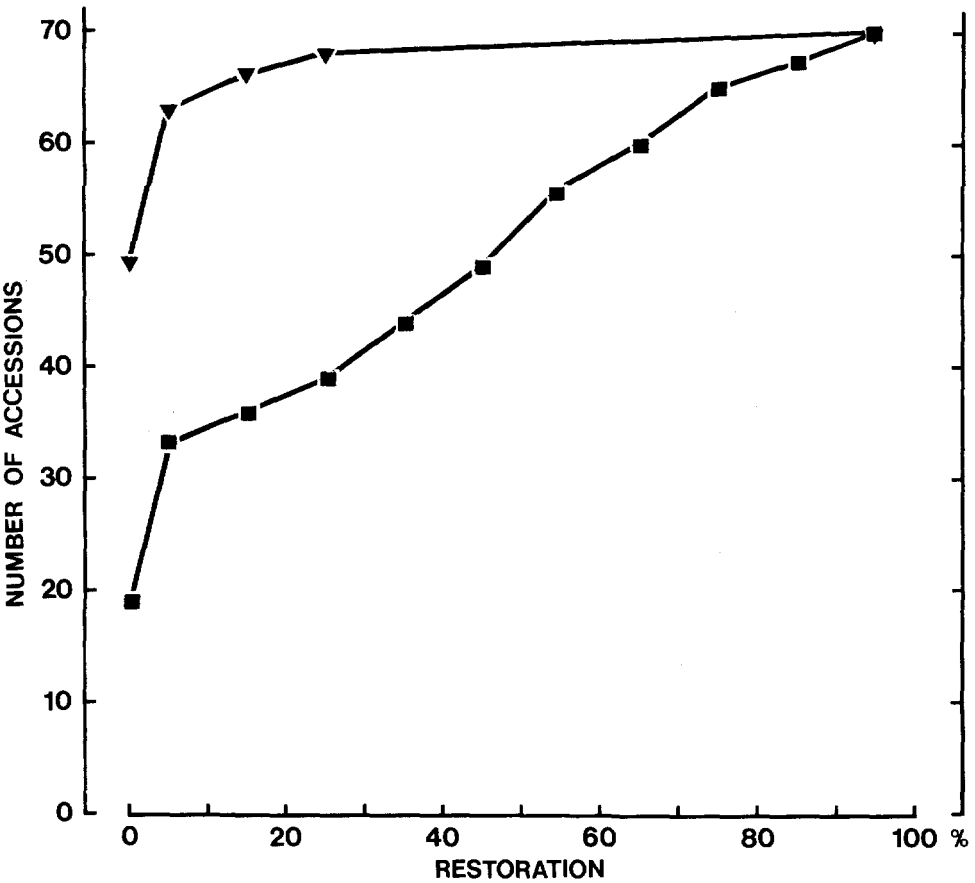

FIGURE 1.—Cumulative sum frequency distribution of *msm1* and *msm2* restorers among the 70 *spontaneum* accessions tested in each cytoplasm. ▼ = *msm1*; ■ = *msm2*.

found to be a distinct partial restorer in *msm2* (Table 3). In partially restored *msm2* spikes, the fertile florets appear in the basal parts of the heads, which is also the behavior of partially restored *msm1* spikes (AHOKAS 1979b).

DISCUSSION

The present results confirm that the cytoplasm of 79BS14-3 induces male sterility with a suitable nuclear gene background, e.g., that of cv. Adorra and many *spontaneum* barleys, especially those originating from the desert of Negev. The dominant *msm1* restorer gene *Rfm1a* is also capable of restoring male fertility in *msm2* cytoplasm. The higher and highly significant different F_1 fertility restoration abilities of CI 3694 and many *spontaneum* barleys in *msm2* than in *msm1* and the results presented in Table 1 (items 1 and 2) are, however, strong evidence that these cytoplasms, *msm1* and *msm2*, are different from each other. Electrophoretic separation of the plastidial and mitochondrial DNA fragments after digestion with restriction endonucleases may be helpful in distinguishing these cytoplasms from each other.

Only some cultivars of domesticated barley have been tested in *msm2* so far.

TABLE 4

Comparisons of regional distributions of F_1 restoration ability of spontaneum *accessions in* msm2 *and* msm1 *cytoplasms*

Geobotanical region[a]	No. of accessions studied	Percentage of accessions maintaining F_1 sterility		Mean percentage of restoration $\bar{x} \pm s_{\bar{x}}$		Significance between each regional *msm2* restoration distribution and that of Southern Coastal Plain[b,c]	Significance between restoration distributions in *msm2* and *msm1* cytoplasms[b,d]
		In *msm2*	In *msm1*	In *msm2*	In *msm1*		
SCP	12	8	50	51.7 ± 8.0	10.4 ± 7.9	—	D = 0.750; P < 0.005
BSV + UJV	14	0	29	42.9 ± 7.0	3.3 ± 1.7	D = 0.226; NS	D = 0.786; P < 0.001
CCP	14 or 15[e]	27	86	36.8 ± 8.0	6.9 ± 6.5	D = 0.250; NS	D = 0.662; P < 0.005
CWN	22	59	100	0.3 ± 0.1	0.0 ± 0.0	D = 0.917; P < 0.001	D = 0.409; P > 0.05
G	7	29	57	29.5 ± 12.6	3.7 ± 2.1	D = 0.488; NS	D = 0.429; NS

Determined with an Adorra-like seed parent background.

[a] For the abbreviations, see footnote a of Table 3.

[b] Significance determined according to the Kolmogorov-Smirnov two-sample test. The samples were classified into 13 classes of restoration: 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, 60.1–70.0, 70.1–80.0, 80.1–90.0, 90.1–100%.

[c] Significance between the SCP sample distribution and that of the pooled sample of the other regions: D = 0.489; P < 0.025.

[d] Significance between the restoration distributions of pooled samples in the two cytoplasms: D = 0.464, P < 0.001.

[e] The *msm1* and *msm2* samples contain 14 and 15 accessions, respectively.

These cultivars have proved to be maintainers of sterility or displayed low partial restoration. The domesticated barley cultivars are also usually maintainers of sterility in *msm1* (AHOKAS 1979a, 1979b). Thus, with respect to the restoration ability in these cytoplasms, the *spontaneum* barleys from Central and Western Negev resemble the domesticated barley more than the *spontaneum* barleys from several other less xeric geobotanical regions of Israel. At present, it cannot be explained why the desert *spontaneum* and the domesticated barley resemble each other in the rarity of restoration ability.

The *spontaneum* accessions tested suggest that these wild barley populations, with the exception of the desert sample, contain much genetic variation in terms of capacity to restore fertility in *msm2*. The nuclear gene variation revealed by *msm2* cytoplasm is actually more pronounced than that by *msm1* cytoplasm (Figure 1). The Israeli populations of *spontaneum* barleys have been found to be highly variable or polymorphic in several respects: in flavonoids (FRÖST and HOLM 1975), in their resistance to pathogenic fungi (FISCHBECK *et al.* 1976; MOSEMAN and CRADDOCK 1976; WAHL *et al.* 1978), in morphology (KAMM 1977; NEVO *et al.* 1979), in electromorphs or protein patterns by gel electrophoresis (DOLL and BROWN 1979; NEVO *et al.* 1979; KAHLER and ALLARD 1981), in kernel protein and lysine contents (AHOKAS 1982), and in restoration of fertility in *msm1* cytoplasm (AHOKAS 1979b, 1980, 1981). The patterns of electromorph variation (NEVO *et al.* 1979) and the existence of resistance to pathogenic fungi (ANIKSTER, MOSEMAN and WAHL 1976; WAHL *et al.* 1978) were explained by natural selection.

Because the Israeli *spontaneum* barleys are principally self-pollinators (BROWN, ZOHARY and NEVO 1978), the restorer gene(s) may have persisted in a given cytoplasm over long periods, especially in environments favoring self-pollination. The possibility exists that evolution has led to a relatively mild cytoplasmic mutant like *msm2* under a recessive or semidominant restorer gene or genes, and to a more stringent cytoplasmic mutant like *msm1* under dominant restorers like *Rfm1a/Rfm1a*. Restoring genes appear in a pre-adaptive manner in respect to cytoplasmic male sterility in these *spontaneum* barley populations. All the 19 restorer accessions for *msm1* carry a dominant restorer in a fertile cytoplasm (AHOKAS 1980, 1981, unpublished). Many of the partially restoring genotypes (Table 3) could be expected to cause a complete restoration when in double doses in *msm2* cytoplasm. Of the 49 partial restorer accessions with more than 0.1% F_1 seed set (Table 3), 39 cytoplasms have been studied by crossing at least twice with cv. Adorra as the recurrent pollen parent. These 39 accessions have a fertile cytoplasm in comparison with *msm2* and *msm1*.

The discovery of these cytoplasmic variants make it probable that other types of cytoplasmic variations also exist in these populations. Chloroplast DNA variation has been found in *Nicotiana debneyi* populations in Australia (SCOWCROFT 1979) and mitochondrial DNA variation in indigenous maize races of Latin America (WEISSINGER *et al.* 1982).

The application of *msm1* cytoplasm in producing hybrid barley is under investigation. If there are difficulties in complete restoration with the *Rfm1a* gene in some environments or parental combinations, the present *msm2* cytoplasm may overcome such problems since it is evidently more sensitive to restorer genes. On the other hand, with *msm2* the maintenance of complete

male sterility may fail with some pollen parents. Such pollen parents might be used as the R lines when recombined with a restorer gene.

The restoration of fertility by a suitable chemical treatment of the vegetative plants is more likely to be successful with *msm2* than with *msm1* because of the milder nature of *msm2*. Chemical restoration of fertility might facilitate the maintenance and multiplying of the male sterile stocks. It should be noted, however, that no chemical is presently known that will restore fertility in cytoplasmically male sterile plants. There is some hope to find such chemical restorers for *msm2* or *msm1* because an increase in a particular cytokinin fraction has recently been shown in the *Rfm1a* restorer gene carriers (AHOKAS unpublished).

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