Barley CMS detected in Finland in 1976 enabled growing of productive winter-barley F_1 hybrids in the European winter-barley zone since 2002

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My wide crossing program of barley (*Hordeum vulgare* s.l.) in 1976 yielded a system which could be used to produce F_1 hybrid seeds. The genotypes were designated as *msm1* (male sterile, maternal), and *Rfm1a* (restorer of fertility in *msm1*). I later found 19 other strains with dominant restorer alleles, which were carriers of a fertile cytoplasm. Hence, the restorer genes probably evolved in advance creating an opportunity for the cytoplasm to mutate to male sterility. Cytological studies revealed an uncontrolled secretion of sporopollenin in the sterile anthers, leading to their starvation and sterility. The *Rfm1a* gene was shown to cause an increase in the cytokinin activity of Fraction 7 in the root sap of barley, regardless of the cytoplasm type. In 1980, I found another male sterile cytoplasm, *msm2*, whose anther can also be restored by the *Rfm1* alleles. The *msm2* strain originally had complementary partial restorer genes and was found to be more responsive to such restorers than *msm1*. In Germany, the *Rfm1* gene was recently translocated to a rye (*Secale cereale*) chromosome to study its response in CMS rye. The *msm2* cytoplasm could be distinguished from *msm1* with electron microscopy at the early stages of the anthers. The *msm1* cytoplasm is not known to be associated with increased disease susceptibility, unlike the T-sterile cytoplasm formerly used to produce hybrid seeds of maize (*Zea mays*). Hybrid cvs: Seeds of *msm1–Rfm1a* were first requested from me by Hilleshög AB in Sweden. Hilleshög later became a part of Syngenta. Hilleshög techniques for sugar-beet hybrids were applied by Syngenta breeders to produce hybrid barley seeds. Syngenta introduced the first commercial winter-barley hybrid in the UK in 2002. Their hybrid cvs were marketed to countries growing winter-barley in Europe. The ha yields of their hybrids exceeded those of conventional cultivars or parental lines by about 1000 kg. In Spain, the winter-barley hybrid yielded 21 percent more than the conventional cultivars in 2015, when grown in the field scale. For the 2016 harvest, hybrids were sown in Germany on more than 140000 ha, which reflects 11.6 percent of the total feed barley area. In 2017, Syngenta launched a cashback scheme for, if their hybrid cultivars did not comfortably out-yield the farmer's conventional counterpart that season. Hybrid seeds must be acquired for each sowing. Unlike conventional monogenic barley cultivars, the hybrids exploit genetic variability and heterozygosity. Hybrid winter-barley is the most competitive of winter cereals with the aggressive weed *Alopecurus myosuroides* in the UK. Thick stems in new hybrids increase lodging resistance. The winter-barley hybrid 'Wootan' gave ha yields up to 6000 kg in Tammisaari, though incompletely winter-hardy in Finland. Maturing a month later than the hybrid 'Hobbit' and with optimal winterhardiness, winter-barley hybrids could exceed ha yields of 10000 kg in Finland. Some other breeding companies seem to work for hybrid barley, too.

Key words: heterosis, *Hordeum vulgare* ssp. *spontaneum*, hybrid winter barley, *msm1*, *msm2*, pleiotropic *Rfm1* restorer

Introduction

The possibility of exploitation of heterosis in the autogamous species, barley (*Hordeum vulgare*) became a scientific topic in the 1960s (Wiebe 1960, Ramage 1965). Hybrids using genic male sterility in association with aneuploidy, the balanced tertiary trisomic were released in the USA, marketed in Arizona, California, and Oregon for some years until 1978, and annually grown on a small area, ranging from 12000 to 20000 ha (Ramage 1983). In Sweden, Louis Lehmann worked on hybrid summer barley before retiring. In 1988, he concluded that "there is still a long way to go in order to produce a hybrid in and for Sweden" (Lehmann 1988). Since 1978, studies and trials of hybrid barley were principally based on cytoplasmic male sterility *msm1* (male sterile maternal 1) and its nuclear restorer gene, *Rfm1a* (restorer of fertility in *msm1*), which made growing hybrid winter barley widespread, commercially beginning in the UK in 2002.

This is a short review about the history of the cytoplasmic male sterility *msm1*, which I identified in Finland in 1976 (Fig. 1), and its use to breed hybrid barley in the recent years. A couple of years earlier, I had applied for and was appointed to a Research Assistant position in the Academy of Finland with the goal of my program to find a system which could be used to produce hybrid barley seeds, unrealistic as it might have seemed. Making a great number of crosses, including interspecific ones, kept me busy, but the program succeeded, even on the natural variation of barley and not using artificial agents like anther sterilants.

A series of events and my wide crossing program led me to detect the *msm1* cytoplasm and its chromosomal restorer gene of fertility, *Rfm1a*, in the wild progenitor (*H. vulgare* ssp. *spontaneum*) of the domesticated barley. I have briefly described the history elsewhere (Ahokas 1998).

Results

The first maternally inherited male sterility with fertility restorer gene in barley was found in 1976

The *msm1* cytoplasm occurred in an Israeli strain of wild barley as the second *msm2* cytoplasm. The original strains carrying *msm1* and *msm2* were fully male fertile. The *msm2* cytoplasm responded more readily to partially restoring genotypes than *msm1* (Ahokas 1982b, Hockett et al. 1989). The strain with *msm2* cytoplasm had a number of complementary nuclear restorer genes. Cytology studied with transmission electron microscopy showed differences between *msm1* and *msm2* anthers (Puska 1985).

In addition to the *Rfm1a* gene, I later found 19 other strains, each with a dominant restorer gene (assigned *Rfm,,b* through *Rfm,,t*), which occurred in fertile cytoplasm of the Israeli strains of wild barley tested in bagged F_1 spikes in standard methods (Fig. 2., Ahokas 1981a, 1982d, 1983). The genes *Rfm1a*, *Rfm1b*, *Rfm1c*, and *Rfm1d* were found to be allelic (Ahokas 1980b). The *Rfm* restorer genes may have evolved in advance, making the evolution of the male sterile cytoplasm possible; the combination of a restorer gene plus male sterile cytoplasm in wild barley is rare and did not exist in the domesticated barley before the combination was introduced by barley breeders.

In 1980, I found a double cytoplasmic mutant, temperature-sensitive albino-lethal in *msm1* (Ahokas 1997a). It has putative use at the selection of transgenic seedlings at the +5 °C lethal temperature (Ahokas 1997b).

Partial restoration (Fig. 4) may respond to the environmental conditions even with steep gradients, as I observed in the F_1 or BC progenies of the early crosses of *msm1*-Adorra with various barleys. At the ends of a 1-m test row (set-up as shown in Fig. 2), the plants could be partially fertile, while in the center of the row, they showed male sterility (Ahokas 1979). Partial restoration was usually higher in the greenhouse than in the field (Ahokas 1979).

Fig. 1. The beginning of the history of the *msm1–Rfm1a* system. The F_2 plants showing first signs of cyto-plasmic male sterility are in the encircled site. There the bagged, test-crossed spikes rescued the sterility, the cytoplasmic inheritance of which was confirmed in the greenhouse in Octo-ber 1976. Experimental plot on the Saarela farm, in Rahikkala, Elimäki (annexed to Kouvola in 2009), Finland. Author's photo in August, 1976.

Fig. 2. F_1 spikes of *msm1*-Adorra as the seed parent crossed with various *Hordeum vulgare* ssp. *spontaneum* accessions as the pollen parents. Possible restoring genotypes in the pollen parents were tested in the spikes bagged in a standard way be-fore the anthesis. Over 300 *spon-taneum* strains were tested in this way and resulted in 19 additional dominant restorer genes designated as *Rfm1b* through *Rfm,,t* (Ahokas 1980b, 1981a, 1982d, 1983b). The Saarela farm. Author's photo, July 3, 1980. I found the *msm2* cytoplasm in the framed area of this plot a few days later, July 11.

An environment permitting an *msm1*-sterile barley to set seeds without recurrent pollination would be helpful for seed suppliers. Towards this, distant test sites (Ahokas and Hockett 1981, Hockett et al. 1989) have been compared as well as temperature manipulation at different plant stages (Bernhard et al. 2017a). High temperature in general was observed to increase seed set and pollen number in CMS (probably *msm1*) barley derivatives (Abdel-Ghani et al. 2013).

The male sterile cytoplasm was not found to be subject to increased disease susceptibility. The normal *adorra* and *msm1* cytoplasm responded in a similar way to toxin preparation from *Helminthosporium* (*Bipolaris*) *maydis* race T, which distinguished *T* male sterile and normal cytoplasm of the maize (*Zea mays*) inbred line A632 in root inhibition assay (Ahokas 1978b). Maize with *T* and *P* cytoplasm were more susceptible to *Helminthosporium maydis* than *S* or *C* male steriles and non-sterile cytoplasm (Hooker et al. 1970). The male-sterile cytoplasm *msm1* and *msm2* have no effect on resistance to the BaYM virus (Matsui et al. 2002b). Reaction to the *Fusarium* head blight was decreased by the male sterile *msm1* cytoplasm in 'Adorra' genetic background (Matsui et al. 2002b).

There may be an increased ratio of nuclear genes to cytoplasmic genome in tetraploidy. I induced tetraploid *msm1*/4*Villa with colchicine and backcrossed it with tetraploid 'Villa'. The tetraploid *msm1*/5*Villa was male sterile, but had a seed set similar to that in the tetraploid euplasmic 'Villa', when pollinated with tetraploids, 'Villa' or 'Nota' (Ahokas 1981b). If they were otherwise feasible, barley hybrids at the tetraploid level might provide thick stems and an opportunity to increase genetic variation. Ellerström and Hagberg (1967) showed monogenic heterosis in tetraploid barley.

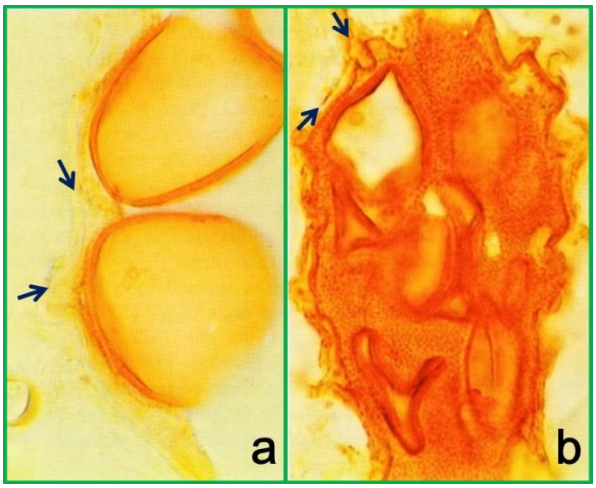

Fig. 3. Light microscopic views of microspores with the sporopollenin stained brownish-red with Fast Blue B, which I invented as a staining method specific for sporopollenin (Ahokas 1975). Cryostat sections. – (a) Euplasmic cv. 'Adorra' with normal sporopollenin staining on the sectioned microspores. The arrows show the thin sporopollenin layer on the outer surface of the tapetum. Anther length 2.2 mm. – (b) Sporo-pollenin staining in the loculus of a male sterile, *msm1*-Adorra anther. The microspores have thick exines, otherwise the loculus is filled with sporo-pollenin globules. Cell surfaces of the tapetum display considerable sporopollenin deposits, the sporopollenin layer on the outer surface being much thicker (arrows) than in euplasmic 'Adorra' (a). The loculus is at the onset of collapse after starvation. Anther length 2.1 mm. – Author's microphotos in the same scale.

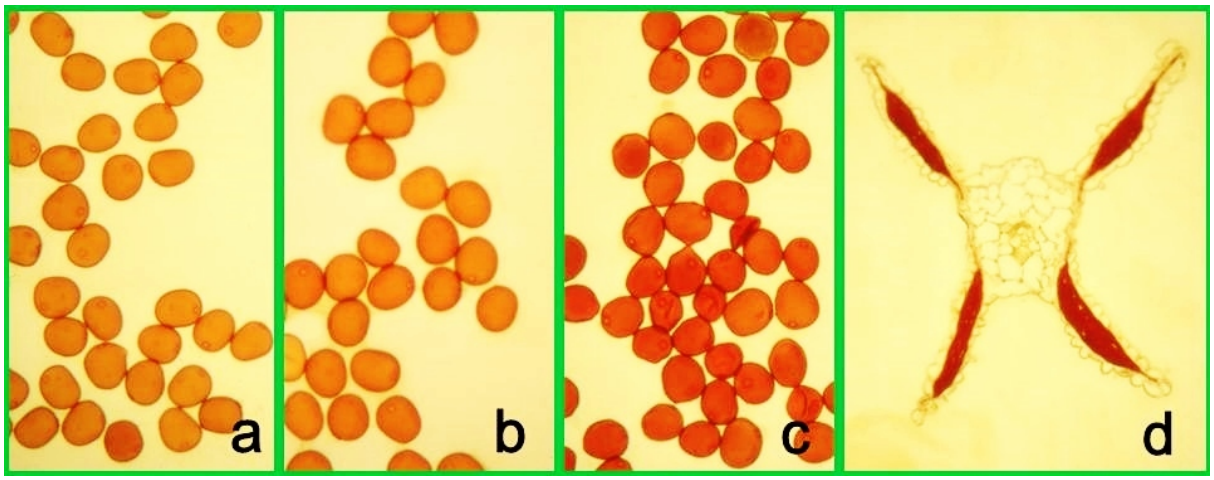

Fig. 4. Light microscopic views of pollen grains (a, b, c) and an anther section (d) stained with Fast Blue B to show sporopollenin (Ahokas 1975). – (a-d) Author's microphotos in the same scale. – (a) Euplasmic cv. 'Adorra'. – (b) Restored *msm1*-Adorra in F_1 of the cross of *Hordeum vulgare* ssp. *spontaneum*, Sel.77-1. – (c) Partially restored *msm1*-Adorra in F_1 of the cross of *H. vulgare* ssp. *spontaneum*, CI 4142. Intense staining of sporopollenin with Fast Blue B and partly deformed pollen grains. – (d) The anther section of male sterile *msm1*-Adorra, with collapsed locules, the underdeveloped microspores forming a deeply staining mass with sporo-pollenin (Ahokas 1978b). – Pollen and anthers in Scanning EM photos of these genotypes were described in Ahokas (1978a).

The restorer gene Rfm1a has pleiotropic effects

The *Rfm* restorer genes are hardy neutral in their effects in the Israeli environment. The dominant nuclear restorer gene affects many qualitative or quantitative properties of its carrier plant, which were detected in different studies: *Rfm1a* blocked the production of sporopollenin in the *msm1* anther locule (Ahokas 1978b), accomplished the occurrence of a late polypeptide, and prevented the drastic proteolysis present in the late stage of the *msm1* anthers (Ahokas 1980a), normalized the lipoxygenase activity towards β-carotene in the *msm1* anthers, guaranteed amino nitrogen pool in the anthers (Ahokas 1982a), and controlled senescence of chlorophyll in leaf pieces subjected to salt stress (Ahokas 1980a). The *Rfm1a* gene increased the cytokinin activity in the root sap of *msm1* barley, especially the cytokinin activity of Fraction 7 in the root sap of barley, regardless of the cytoplasm type (Ahokas 1982c). The severity scores of some fungal leaf diseases at seedling stage or full-sized plants showed dependence on the restorer or partial restorer genotypes of the different tested *Hordeum vulgare* ssp. *spontaneum*

accessions (Ahokas 1983a). Distribution of partial plus complete restorer genotypes among 304 ssp. *spontaneum* accessions from Israel showed statistically significant differences between biological territories, suggesting adaptive value of the restoring genes (Ahokas 1981a).

The *Rfm1* locus was allocated to chromosome 6H (Matsui et al. 2001) and its short arm 6HS (Ui et al. 2015). Based on DNA sequence data recently obtained for the restorer locus, it is thought to encode a PLS class protein with repeats (Rizzolatti et al. 2017). Such proteins have RNA-binding activity and are involved in the editing of RNA (Ui et al. 2015, Rizzolatti et al. 2017). Since the effects of the restorer gene *Rfm1a* appear pleiotropic, the edition of several RNAs probably occurs in various tissues of barley.

The *Rfm1a* gene was not found to significantly the affect studied malting qualities in cv. 'Adorra' genetic background. Neither *adorra*, *msm1* nor *msm2* cytoplasm caused significant differences in malting quality (Matsui et al. 2002a). In the grain of cv. 'Adorra' and 'Bomi' Risø mutant 1508, the *msm1* cytoplasm was found to somewhat change the amino acid proportions (Ahokas 1980b).

The *Rfm1* gene has been recombined to rye (*Secale cereale*) in the syntenic 4RL/6HS segment. The 4RL chromosome arm of rye is the carrier of the rye restorer gene *Rfp3* (Hackauf et al. 2017). The efficiency of the barley restorer in any cytoplasmic male sterility of rye was not shown in the article.

Commercial winter-barley hybrid produced with msm1–Rfm1 was released in 2002

In 1994, Paul Bury, barley breeder at New Farm Crops Ltd. (later Syngenta Seeds Ltd.) converted the cytoplasmic male sterility system into European breeding lines and started systematic hybrid breeding in barley (Longin et al. 2012, Mühleisen et al. 2013). The Swiss company Syngenta released the first winter-barley hybrid in the UK in 2002 after five seasons of trials (Karlberg 2003, Phillips). The released hybrids in barley are three-way crosses (Philipp et al. 2016, Li et al. 2017). In Germany, hybrid winter barley was sown on more than 140000 ha in 2015, which reflects 11.6% of the total feed barley area (G. Stiewe in Philipp et al. 2016). Hybrid winter barley also out-yielded conventional cultivars in 2017 (Meredith 2017). The activities of Syngenta have recently been taken over by the Chinese Governmental company ChemChina.

Finland is north of the winter-barley zone. Some winter-barley hybrids of Syngenta have been tested in South Finland. The winter-barley hybrid 'Wootan' gave yields up to 6000 kg ha^{-1} in Tammisaari though inadequately winter-hardy in Finland (Markkula 2017). The wild barley species, *Hordeum jubatum* might have genes of winterhardiness useful for barley breeding. Some populations of this imported species tolerate winters in harsh roadside sites in South Finland.

The selection towards anemophily is on-going in the hybrid-barley programs. The largest anthers I have seen in any barley were those in *H. vulgare* ssp. *spontaneum* PI 296796 with length of up to 8 mm (Ahokas 1980b), which is comparable with the anthers of the domesticated rye, an anemophilous species.

Hybrid winter barley suppresses the aggressive weed, blackgrass, and shows heterosis for biomass

In Lincolnshire, UK, wheat crop badly infested by blackgrass (*Alopecurus myosuroides*) can produce 4.5 t ha^{-1} of blackgrass seed, while winter-barley produces 2 t ha^{-1}, and hybrid winter barley 0.66 t ha^{-1} of blackgrass seed (J. Cussans from NIAB in Jones 2015). Independent two-year trials at the Hertfordshire/Essex border, UK, confirmed that the hybrid winter barley is the most competitive of winter cereals against blackgrass (Impey 2016).

Soil covered with winter barley is less leached during the winter and could be followed by a second crop in the more southern latitudes. The use of whole-plant winter-barley silage for bioenergy production with hybrids was suggested and studied (Bernhard et al. 2017b). In hybrids grown in trials in Germany and France, the average heterosis over the best-parent in grain yield was 7.7% and 9.1%. of dry matter

harvested at late milk ripeness. Bernhard et al. (2017b) found genetic distance of the parents to correlate with the dry matter yield of the hybrids.

Conventional monogenic cultivars versus heterozygous and polymorphic hybrid barley

Unlike conventional monogenic barley cultivars, the hybrids exploit genetic variability and heterozygosity. Landraces of cereals were normally genetic mixtures with some heterozygosity. A hybrid barley cultivar can exploit different disease- and pest-resistance alleles, and dominant resistance genes can be heterozygous to lower their possible higher genetic load when homozygous. So, only the hybrid cvs 'Hobbit' and 'Wootan' had segregating genetic resistance to powdery mildew among barley cultivars registered from 2011 to 2015 in the Czech Republic (Dreiseitl 2017). The environment varies temporally, spatially, and in the terms biotic factors. Root life is also controlled by plant genes and their interactions with the heterogeneous and changing soil environment and biota. The root ability of different barley genotypes to mobilize phosphorus from different sources varies (Ahokas and Manninen 2001, Ahokas et al. 2007): complementation in phosphate acquisition might occur between the different genotypes of a hybrid cultivar. Genetic variability of the plants in cultivar like hybrids would be natural to meet the varying and changing environment.

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