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Cytoplasmic male sterility in barley

12. Associations between disease resistance and restoration of *msm1* fertility in the wild progenitor of barley

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Summary. The associations between seedling reactions to three fungal pathogens (*Puccinia hordei, Pyrenophora teres,* and *Rhynchosporium secalis*) or between adult plant reaction to *Rh. secalis* and the male fertility restoration ability of *msml* cytoplasm were studied in about 100 accessions of *Hordeum spontaneum.* Significant differences in the severity of infection between classes of restoration ability were observed with two cultures of *P. hordei* (751 and 7,649) and with *Rh. secalis* on adult plants in the field. The cultures 7,432, 751 and 7,649 of *P. hordei* showed significant positive correlations between infection severity and restoration percentage. The culture 7,620 of *P. hordei* displayed a significant negative correlation. *Rh. secalis* (cultures 492A and 531 combined) on seedlings and the natural field infection in the 1978 season showed significant positive correlations. The accession class with the partial restoration ability of 0.1 to 5.0% of the four arbitrary classes (0.0, 0.1–5.0, 5.1–55.0, and 90.1–100%), displayed the lowest mean severity of infection in six of the eleven tests. Some frequently appearing races of these pathogens may operate as selective agents in the maintenance of restoration ability in the original *spontaneum* populations.

Key words: *Hordeum vulgare* s.l. – Restoration of CMS fertility – Fungal pathogens – Disease resistance – Polymorphism

Introduction

The wild progenitor of barley, *Hordeum vulgare* ssp. *spontaneum* (C. Koch) Thellung, occupies a wide diversity of habitats in Israel, from mesic Mediterranean to desert (Harlan and Zohary 1966; Nevo et al. 1979). The Israeli populations of *spontaneum* barley have been found to be highly variable in flavonoids (Fröst and Holm 1975), in their resistance to fungal pathogens (Fischbeck et al. 1976; Moseman and Craddock 1976; Wahl et al. 1978; Moseman et al. 1979; Moseman 1980; Moseman et al. 1980), in morphology (Kamm 1977; Brown et al. 1978; Nevo et al. 1979), in electromorphs by starch gel electrophoresis (Brown et al. 1978; Nevo et al. 1979; Kahler and Allard 1981), in hordein patterns by gel electrophoresis (Doll and Brown 1979), in restoration of anther fertility in *msml* and *msm2* cytoplasms (Ahokas 1980b, 1981, 1982a), and in kernel protein and lysine content (Ahokas 1982b). The pattern of electromorph variation (Nevo et al. 1979) and evidently the resistance variation (Moseman 1971; Anikster et al. 1976; Wahl et al. 1978) can be explained by natural selection.

In this paper, the relationship between the infection severity by the pathogenic fungi *Puccinia hordei* Otth. (which causes leaf rust), *Pyrenophora teres* Drechs. (which causes net blotch), and *Rhynchosporium secalis* (Oud.) J.J. Davis (which causes scald) and the restoration ability of male fertility of *msml* cytoplasm is reported. The *spontaneum* accessions originate in Israel, which is also the source of the *msml* cytoplasm (Ahokas 1979a).

Materials and methods

The 112 *spontaneum* accessions used in this study study were collected at many sites in Israel and maintained as PI accessions by the U.S. Department of Agriculture. Most of the acessions had been found resistant to culture CR3 of *Erysiphe graminis* DC. ex Merat f. sp. *hordei* em. Marchal and culture 57-19 of *P. hordei* (Moseman et al. 1980). The accessions can be regarded as a random sample with respect to resistance to the studied races of the pathogens and to the restoration ability. None of the accessions are carriers of an *msm1*-like cytoplasm (Ahokas 1980b).

The seedling resistances were investigated by Dr. B.H. Tan (at Max-Planck-Institut für Züchtungsforschung, FRG), and Dr. J.G. Moseman (at USDA Beltsville Agricultural Research Center, Md, USA). The reactions were observed on seedlings which had been inoculated with the pathogen when they were in the first or second leaf stage. The reactions were read 11 to 13 days, and 13 to 15 days following inoculation with *P. hordei* and *Rh. secalis*, respectively. The severity to infection was read on a 0 to 9 scale: 0 = no visible sign of infection; 1–3 = highly resistant to resistant reactions; 4–6 = moderately resistant to moderately susceptible reactions; and 7–9 = susceptible to highly susceptible reactions. The *P. teres* reactions were quoted from Anonymous (1968), originally obtained from Dr. I. Wahl (Tel-Aviv University, Israel) (Moseman, pers. commun.). The *P. teres* reaction scores were scaled as follows: resistant = 0; medium resistant = 3; medium susceptible = 6; susceptible = 9. When more than one reaction was observed the average of the reactions was used in the calculations. These multiple scores occurred with the *P. teres* tests, but only rarely or never with the other diseases. The severity of the natural infection by *Rh. secalis* under field conditions was observed in SE Finland at about the anthesis in July of 1978, when there was a moderately severe scald in cv. 'Paavo', the major adjacent cultivar. The 0 to 9 scale was used.

Restoration of fertility was evaluated in SE Finland on F_1 plants with the *spontaneum* accessions as the pollen parents. The *msm1* seed parent had an Adorra-like nuclear gene background. The method appears elsewhere (Ahokas 1979b, 1980b).

The coefficients of correlations were determined to see if there is any trend among data, notwithstanding the possible non-significance of the F values of the ANOVA of the classified material. The hypothesis is that the restoration ability causes changes in the plant's physiology, which might modify the reaction of the plant to some pathogens. Therefore, an inner factor or factors would be the explaining attribute, and correlation between restoration ability and disease reaction is possible to be tested statistically.

The restoration of fertility was determined in F_1 plants, i.e. heterozygotes, but the diseases were scored on homozygotes, or almost homozygotes. This may be a source of error, because the semidominant or the additive recessive genes of partial restoration are expected to cause a higher level of restoration when homozygous. In most cases, this discrepancy appeared to be relieved by the logarithmic transformation of the partial and complete restoration percentages presented separately in Table 1 for the material showing a restoration of $\geqq 0.1\%$.

Non-significance and significance at $P < 0.10$, $P < 0.05$, $P < 0.01$, and $P < 0.001$ are indicated with ns, *, **, ***, and ****, respectively.

Results

The number of accessions in the samples, F values of the differences between arbitrary classes of restoration ability, hereafter called 'restoration classes' (0.0, 0.1–5.0, 5.1–55.0, and 90.1–100%) and estimates of the coefficients of correlation are presented in Table 1. There is no observation of restoration of 55.1–90.0% in this material. Leaf rust cultures 7,639 and 7,657 did not

Table 1. F test estimates and significances of the differences between the severity indices of the four restoration classes, and estimates of coefficients of correlation between indices and restoration percentages

Pathogen	Total sample				Partial and complete restorers	
	Sample size[a]	F	Restoration class with the lowest mean severity index (%)	r	Sample size	r (logarithmic transformation of the restoration percentages)
P. hordei						
7432	103	2.476*	0.1 – 5.0	0.212**	67	0.299**
7437	75	1.132ns	0.1 – 5.0	0.180ns	45	0.282*
751	108	4.434***	0.1 – 5.0	0.266***	68	0.354**
7527	106	1.658ns	0.1 – 5.0	0.088ns	68	0.211*
7620	103	1.135ns	90.1 – 100	– 0.620****	66	– 0.073ns
7639	78	0.654ns	0.0	0.123ns	50	0.078ns
7649	98	3.673**	0.1 – 5.0	0.322***	61	0.333***
7657	105	0.164ns	90.1 – 100	– 0.073ns	65	– 0.040ns
P. teres						
Composite	52	1.867ns	0.0	– 0.063ns	35	– 0.253ns
Rh. secalis						
492A + 531	107	2.593*	0.1 – 5.0	0.228**	69	0.229*
Natural field infection of adult plants	106	5.593***	5.1 – 55.0	0.313***	69	0.281**

[a] Fractions of the set of 112 accessions of *spontaneum* barley

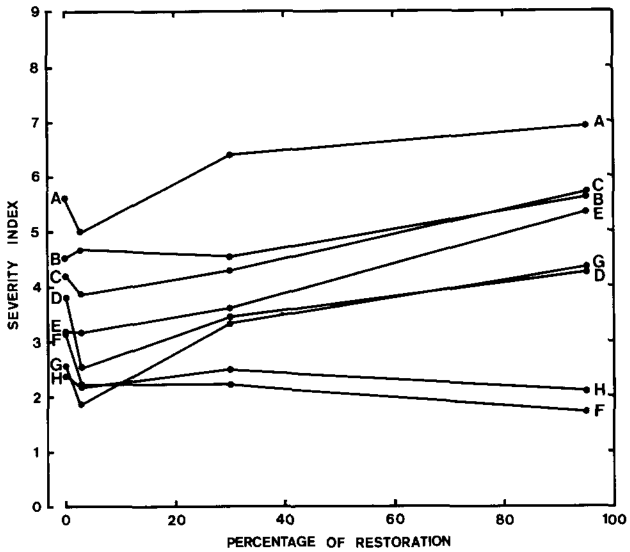

Fig. 1. The diagram depicts mean severity indices of infection in the four restoration classes tested with eight different cultures of *P. hordei.* The numbers of the observations varied from 75 to 108 among 110 *spontaneum* accessions (Table 1). The letters indicate the diagrams of the different cultures as follows: *A* = 7,432; *B* = 7,639; *C* = 7,437; *D* = 7,527; *E* = 7,649; *F* = 7,620; *G* = 751; *H* = 7,657

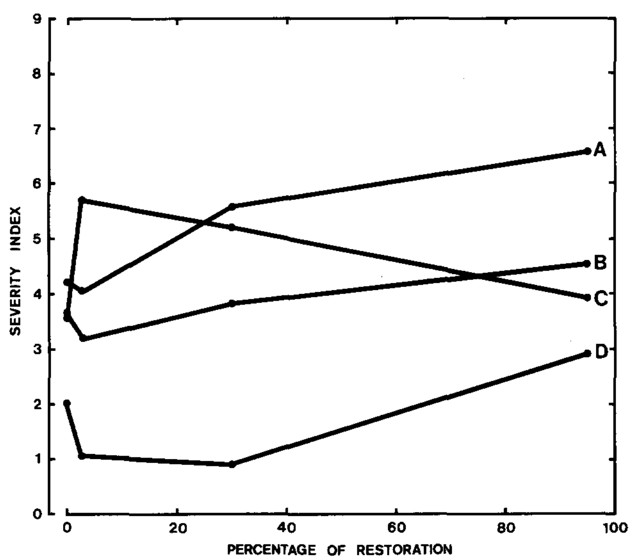

Fig. 2. Mean severity indices of infection in the four restoration classes. Diagram *A* = *Rh. secalis* at seedling stage; *B* = *P. hordei* (mean of means of the eight cultures); *C* = *P. teres;* *D* = *Rh. secalis* at the anthesis stage in the field

display either a significant difference between the scores of the four restoration classes, or any significant correlations between individual scores and fertility percentages. Cultures 7,432, 751, and 7,649 displayed a significant positive correlation between restoration ability or the logarithm of that of the accessions showing any restoration ability and the disease score at $P < 0.05$. Culture 7,620 had a significant negative correlation between the two variables when the whole material was considered ($P < 0.001$). Cultures 7,437 and 7,527 displayed almost significant correlations ($P < 0.10$) after the logarithmic transformation (Table 1). Significant differences between the restoration classes appeared in severity with races 751 and 7,649. The relationships between the two variables are depicted in Fig. 1.

At the seedling stage, the material was moderately susceptible to *Rh. secalis.* The variation among accessions was wide, and the F estimate is almost significant ($P = 0.057$). The coefficient of correlation between restoration percentages and disease severity indices is significant ($P < 0.05$), and almost significant between the logarithms of fertility percentages and indices ($P < 0.10$). Under field conditions at the anthesis, the unknown race(s) of *Rh. secalis* infected most severely the complete restorers; both the F estimate and both the r's being significant (Table 1).

The limited number of accessions infected with *P. teres* did not give statistical significance (Table 1). However, the form of the distribution (Fig. 2) may be close to the real one, and would be interesting in that it is rather a mirror image of the others. Furthermore, the races were evidently isolated in Israel, which is the country of origin of the *spontaneum* barleys, too.

Discussion

Some of the differences in disease reactions between the arbitrary restoration classes must be sufficiently high to be subject to natural selection, though more specialized resistance genes existing in the material may interfere considerably. The significant correlations also support this concept.

Leaf rust is probably the most important leaf disease on barley in Israel. The coexistence of leaf rust cultures with virulences similar to those studied may explain the relatively high frequency of the low partial restorers observed in the Israeli *spontaneum* barleys having restoration ability (Ahokas 1981). The studied races of *P. hordei* and *Ph. secalis* as selective agents would not explain the high frequency of the maintainers of sterility. The Israeli races of *P. teres* might favour the maintainers of sterility and complete restorers. Additional biotic or nonbiotic factors must exist. For instance, accessions collected in the Negev desert, in whose arid and short season the diseases are usually avoided, were found to lack restoration ability in *msm1* cytoplasm (Ahokas 1981). On the other hand, the lack of the restorer genes in the Negev desert populations might mean that the restoration ability is either evolved or maintained only under the multiplicity of diseases. Even within a pathogen species, there may appear races which can exert opposite selection, as e.g. *P. hordei* cultures 7,620 and 7,649.

All the cultures do not seem to be sensitive to the restoration ability, which would suggest that the restoration ability exerts some race specificity. On the other hand, at least two pathogens of the three studied reacted significantly suggesting that the influence is not necessarily even species-specific.

The pathogens *P. teres* (Bach et al. 1979) and *Rh. secalis* (Auriol et al. 1978) are known to produce toxins. A chlorophyll retaining factor causing the green islands around the pustules may result from the infection with *P. hordei.* Cytokinins have similar chlorophyll retaining properties. Many physiological effects of the complete restorer gene in *msm1* can be explained as functions of cytokinins (Ahokas 1980a, 1982c). Recent results also support the importance of cytokinins in restoration of fertility (Ahokas 1982c). In creased endo- or exogenous cytokinin levels have been found to make different host plants more sensitive to pests (Kirkham and Hignett 1970; Van Staden and Dimalla 1977; Haberlach et al. 1978), or more resistant to various pathogens (Nakagaki 1971; Lloyd 1972; Sridhar et al. 1978; Tavantzis et al. 1979), or cytokinins appear in the host pathogen interaction (Van Andel and Fuchs 1972; Sziraki et al. 1976; Mandahar and Arora 1978; Vizárová 1979; Dekhuijzen 1980, Murai et al. 1980; Weiler and Spanier 1981).

All these accessions of *H. vulgare* ssp. *spontaneum* including those which are complete restorers, are evidently carriers of a male fertile cytoplasm. The physiological interaction of a homozygous restorer gene plus a male fertile cytoplasm is expected to be different from that of a heterozygous restorer gene plus *msm1* cytoplasm, which condition would appear in a hybrid barley produced with this cytoplasm.

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