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Number of Alleles at the Incompatibility Loci in *Secale cereale* L.

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Summary. Two experiments were performed to estimate the number of alleles at the two incompatibility loci of rye in the variety 'Halo'. In one experiment I_1 progenies from enforced selfing under controlled conditions were isolated. In the other experiment a genotype, homozygous at both incompatibility loci, was used as pollinator for a sample of the 'Halo' population, which was regarded as an equilibrium population. Genotypes, which are homozygous at both incompatibility loci, can be found after selfing. The estimate for the number of alleles was 6 to 7 at one locus and 12 to 13 at the other locus.

Key words: Rye – Inbreeding – Fertility in compatibility – Genotypic frequencies

Introduction

Rye possesses a gametophytic two-locus multiallelic incompatibility system (Lundqvist 1956). The same system is found in several other species of the Gramineae (Weimarck 1967). The incompatibility specificities are formed by some kind of complementary interaction between the two loci, *S* and *Z*, where each specific pair of an *S* and a *Z* allele carried by the pollen must be matched by an identical allele in the pistil to prevent fertilization. The total number of specificities therefore is $n_1 n_2$, if n_1 is the number of alleles at locus *S* and n_2 at locus *Z*. The population consists of two types of genotypes: heterozygous at both loci like $S_{ij}Z_{kl}$ and heterozygous at only one locus like $S_{ii}Z_{kl}$ or $S_{ij}Z_{kk}$.

This effective outbreeding mechanism is still fully active in cultivars of rye, which has a great implication on breeding methodology (Wricke 1979). In this connection the question arises how many alleles are present at the two loci of widely used cultivars in crop production.

Till now an estimation of the number of alleles in rye has not been reported. Lundqvist (1962) gave an estimate of the number of alleles for a breeding population of Meadow Fescue (*Festuca pratensis*), assuming that this population was at equilibrium. For an equal number of alleles at both loci his estimate was 12 to 13. Charlesworth (1979) reinvestigated the genotypic frequencies in such populations for a given number of alleles and the case $n_1 = n_2$. Weber et al. (1982) gave formulas for the same problem with a slightly different approach. These formulas also cover the case of different allele numbers and are used in this paper. In later publications Lundqvist used more direct methods to estimate the allele numbers in Meadow Fescue, resulting in numbers of about 7 to 8 alleles in the S series and 17 to 18 alleles at Z (Lundqvist 1964) and 14 alleles at S and 13 alleles at Z (Lundqvist 1969). As Lundqvist stated, the last are the more reliable ones since in this investigation the strict identification of the individual members within the two allelic series was performed.

The present paper gives data on the estimation of the number of alleles at the two incompatibility loci in the German cultivar 'Halo'.

Materials and Methods

By application of high temperatures (30 °C) day and night a few days before flowering and during flowering it is possible to get seed set after selfing (Wricke 1978). By this method a sample of 90 plants (I_0) from the German cultivar 'Halo' was selfed. In the next year each selfed progeny (I_1) was isolated in a cabinet. A sample of 16 to 36 kernels (I_1G_1) from each I_1-family again was isolated. Depending on the genotype-heterozygosity at one or both loci- the number of plants setting little or no seed differs. All plants are recorded as fully incompatible with other members of the family and itself when they had less than 5 kernels per ear (hereafter called sterile).

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Table 1. Genotypic frequencies in self progenies of rye

Generation	I_0 = ho het			I_0 = het het		
	Genotype	Frequency	Sterile under isolation	Genotype	Frequency	Sterile under isolation
I_1	ho ho	1/2	no	ho ho	1/4	no
	ho het	1/2	yes	ho het	1/2	no
	het het	0	–	het het	1/4	yes
$I_1 G_1$	ho ho	0	–	ho ho	0	–
	ho het	1	yes	ho het	5/9	no
	het het	0	–	het het	4/9	yes

Table 1 gives the expected number of genotypes homozygous at both loci (ho ho), heterozygous at one locus (ho het) and heterozygous at both loci (het het), for the I_1 and I_1G_1 generation. If the I_0 plant is ho het, half of the I_1 and the total I_1G_1 is sterile. Otherwise, if the I_0 plant is het het, a quarter of the I_1 and four ninths of the I_1G_1 is sterile. By this experiment the proportion of ho het and het het plants in the original population can be estimated. This proportion depends on the number of alleles at both loci.

In a second experiment ho ho plants were used. To find out such ho ho plants, a clonal part of I_1 plants, derived from ho het I_0 plants, was pollinated by the corresponding ho het I_1G_1 plants. Only ho ho plants can give full seed set.

The other clonal part of an ho ho genotype was selfed in the growth chamber under high temperatures of 30 °C which gives good quantities of seed of ho ho plants of identical genotype.

Such ho ho plants can generate only one incompatibility specificity, say S_1Z_3. With plants of this identical genotype a sample of 120 plants of the original population was pollinated. The proportion of sterile combinations gives the proportion of plants $S_{1x}Z_{3y}$ in the population carrying S_1Z_3. This proportion depends on the number of alleles at both loci. Pollinations were done in the field and in the laboratory. In the laboratory the pollen tube growth on pistils was investigated as described by Lundqvist (1961).

The allele numbers n_1 and n_2 were calculated with the formulas given by Weber et al. (1982). The same notation is used here. x_1 and x_2 are the frequencies of ho het plants $S_{ii}Z_{kl}$ and $S_{ij}Z_{kk}$ at equilibrium. The frequency of het het plants is x_3. Then

$$x_1 = (n_1n_2-2)\,(n_1n_2-n_1-2)\,(n_2-1)/W,$$
$$x_2 = (n_1n_2-2)\,(n_1n_2-n_2-2)\,(n_1-1)/W$$

and

$$x_3 = (n_1n_2-1)\,(n_1n_2-4)\,(n_1-1)\,(n_2-1)/W$$

with

$$W = (n_1n_2-2)\,(n_1n_2^2+n_1^2\,n_2-4n_1n_2-n_1-n_2+4) + (n_1-1)\,(n_2-1)\,(n_1n_2-1)\,(n_1n_2-4).$$

The proportion of ho het genotypes in the original population, estimated by the first experiment, is

$$H_1 = x_1 + x_2. \quad (1)$$

In the second experiment the proportion of plants carrying a specific allele combination say S_1Z_3 is

$$H_2 = (2(x_1+x_2)+4\,x_3)/(n_1n_2). \quad (2)$$

This relation can be derived as follows. There are $n_1n_2\,(n_2-1)/2$ different types $S_{ii}Z_{kl}$. Only n_2-1 of them carry S_1 and Z_3, giving a proportion of $2\,x_1/(n_1n_2)$. In a similar way the two other proportions can be found. If H_1 and H_2 are estimated experimentally, Eqs. (1) and (2) can be solved for n_1 and n_2. Since the equations are nonlinear, the solution is found graphically with n_2 as a function of n_1. Besides the fact that n_1 and n_2 can be exchanged, only one solution for positive values of n_1 and n_2 exists.

Results

Table 2 shows the result of the first experiment. 14 isolated I_1G_1 progenies could not be evaluated since the plastic covers were damaged by the wind and isolation was not complete. 13 of the remaining 76 progenies contained only sterile plants. The corresponding I_0 plants therefore were regarded as ho het. 55 isolations showed no significant deviation from a ratio of 4 sterile to 5 fertile plants, as it is expected, if the I_0 plant is het het. 8 isolations showed a segregation ratio deviating significantly from 4 to 5. These isolations were excluded. No explanation can be given for this deviation. As a control 10 self fertile lines additionally were tested. These lines showed the expected full seed set. The proportion of ho het types in the

Table 2. Fertility in the I_1 G_1 progenies

Degree of fertility	Number of lines	Type of I_0 plant
Completely sterile	13	ho het
5 fertile : 4 sterile	55	het het
Other ratios of fertile : sterile	8	?
No results	14	?
Completely fertile (control)	10	self fertile

original population is estimated by

$H_1 = 13/68 = 0.1912.$

The results of the second experiment are given in Table 3. 5 plants of the population were sterile in the field and in the laboratory test after pollination with one ho ho type. The remaining 110 plants, tested in the laboratory, were self fertile. Ten of them were sterile in the field test. Only the results of the laboratory were used. An explanation is given in the discussion. The proportion of plants carrying a specific allele combination, therefore, is estimated by

$H_2 = 5/115 = 0.0435.$

The solution of Eqs. (1) and (2) found graphically is given in Fig. 1. The estimated allele numbers are

$n_1 = 6.8$ and $n_2 = 12.2$.

Table 3. Number of fertile and sterile plants after pollination with pollen from a single ho ho genotype in the field and in the laboratorium

Number of plant	Field	Laboratorium
5	sterile	sterile
10	sterile	fertile
95	fertile	fertile
5	–	fertile
5	fertile	–

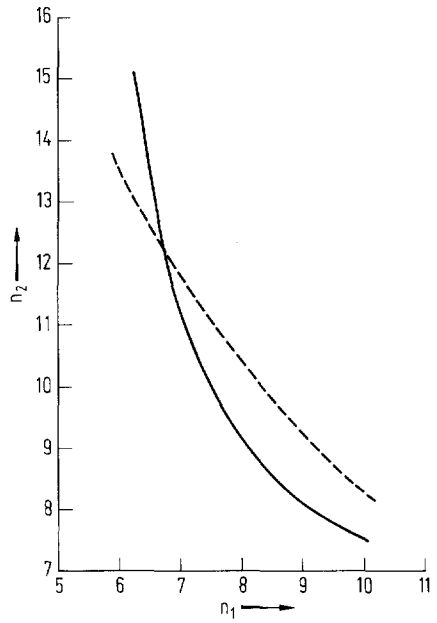

Fig. 1. Graphical solution for n_1 and n_2. —— $= H_1 = x_1 + x_2$ (Eq. 1); ---- $= H_2 = \frac{2(x_1 + x_2) + 4x_3}{n_1 n_2}$ (Eq. 2)

Discussion

Self incompatibility prohibits selfing under natural conditions, since pollen cannot grow on pistils carrying the same alleles as the pollen. Under natural conditions therefore no ho ho genotypes $S_{ii}Z_{kk}$ exist. Under controlled conditions selfing can be achieved, using high temperatures during the flowering period (Wricke 1978). Selfing under controlled conditions thus opens the way to finding ho ho genotypes. If in the next generation I_1 plants are open pollinated, the incompatibility system is effective, and no selfing occurs.

Under isolation one half of the plants of an I_1 progeny is sterile if the I_0 plant was ho het (Table 1). If the I_0 plant was het het, only a quarter of the plants of an isolated I_1 progeny is sterile. Therefore I_1 progenies can be used to estimate the type of the I_0 plant. Since the number of plants of the I_1 progenies was small, the test was not done with I_1 progenies but with I_1G_1 progenies. The isolated I_1G_1 progeny of an ho het I_0 plant is completely sterile and therefore can be recognized easily.

The pollination of some plants by pollen of an ho ho type did not give the same result in the field as in the laboratory (Table 3). No plant was found which was sterile in the laboratory and fertile in the field. But 10 out of 110 plants tested under both conditions were sterile in the field and fertile in the laboratory. This result can be explained by the fact that the ho ho plants used as pollinators were inbred (inbreeding coefficient $F = 0.75$) and were remarkably reduced in plant height. Therefore plant height may be the reason why the pollination was not successful in the field for these 10 plants.

The expected proportions H_1 and H_2 are reduced with increasing values of n_1 and n_2. Since H_1 and H_2 always are small, the precision of the estimates for n_1 and n_2 is low. From the binomial distribution the confidence interval for H_1 and H_2 can be derived. For this material the 95 per cent confidence intervals are given by $0.0981 \leqq H_1 \leqq 0.3160$ and $0.0156 \leqq H_2 \leqq 0.0919$. To get more precise results, the number of plants must be increased. Table 4 shows what happens if in the first experiment 12 or 14 instead of 13 ho het I_0 plants were found and in the second experiment the number of sterile plants was 4 or 6 instead of 5. While the estimate of the allele number for one locus is nearly the same (n_1 varies between 5.8 and 7.2), n_2 varies from 10.7 to 17.5. No solution can be found if $H_1 = 12/68$ and $H_2 = 5/115$ or $6/115$ or if $H_1 = 13/68$ and $H_2 = 6/115$.

Lundqvist (1964) could estimate the allele numbers of both loci in Meadow Fescue in another way, since he had 4 specified ho ho types $S_{11}Z_{33}$, $S_{11}Z_{44}$, $S_{22}Z_{33}$ and $S_{22}Z_{44}$. The values he obtained ($n_1 = 7.6$ and $n_2 = 17.3$) were of the same order as the results for rye in our

Table 4. Allele number n_1 and n_2 at the incompatibility loci depending on the estimates of H_1 and H_2 (–: no solution)

H_2 \ H_1 =	12/68	13/68	14/68
6/115	–	–	$n_1 = 6.3$ $n_2 = 10.9$
5/115	–	$n_1 = 6.8$ $n_2 = 12.2$	$n_1 = 5.8$ $n_2 = 14.2$
4/115	$n_1 = 7.3$ $n_2 = 14.3$	$n_1 = 6.2$ $n_2 = 16.5$	$n_1 = 5.8$ $n_2 = 17.5$

material. However, in a later publication Lundqvist (1969) could find $n_1 = 14$ alleles at the S locus and $n_2 = 13$ alleles at the Z locus by diallel crossings.

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