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GENETICAL ANALYSIS OF RYE (*SECALE CEREALE* L.) III. SELF-FERTILITY OF THE RYE MUTANT *VD* — INHERITANCE AND GENE LOCATION[1]

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Summary. The self-fertility of the rye mutant *vd* (with violet seeds) was analysed, using the trisomic set of rye cv. Esto. Three incomplete self-dominant fertility genes were located on chromosomes 1*R* (*Sf*1), 3*R* (*Sf*2) and 6*R* (*Sf*4). The inheritance of these genes was di- and trigenic-additive. One self-fertility gene was located on chromosome 5*R* (*Sf*3) according to results from literature.

All rye varieties cultivated so far are allogamous. However, there has been a wide interest in factors inducing self-fertility and self-sterility in rye. Lundqvist (1956) examined self-sterility and framed a hypothesis of self-sterility genetics. According to that hypothesis, self-sterility seems to be due to interactions between genes *S* and *Z*. Kuckuck and Peters (1979) reported on an extensive crossing programme with *Secale vavilovii*. They isolated self-fertile lines in progenies of these crosses. It seems very likely that self-fertility was transmitted from *S. vavilovii* to *S. cereale* and that elimination of the *S*-system of *S. cereale* was the reason of self-fertility. This self-fertility was dominant, but Kuckuck and Peters (1979) supposed that more than one gene were involved in the expression of self-fertility. Lundqvist (1968) and Wricke (1969) attributed the occurrence of self-fertility to one dominant extra gene beside genes *S* and *Z*. Ruebenbauer et al. (1983a), however, found several genes that modify genes *S* and *Z* to be the reason of self-fertility. On the other hand, Kuckuck and Peters (1979) explained the occasional occurrence of self-fertility by spontaneous mutations of gene *S*. Derevyanko and Zdrilko (1982) described a case of self-fertility that was due to two genes with dominant additive inheritance.

Locations of self-fertility genes have not been known so far but Wricke and Wehling (1985) found an incompatibility gene on chromosome 1*R*. Surikov (1980), Romanova (1982) and Smirnov and Sosnikhina (1984) observed certain linkages between marker genes and incompatibility genes.

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The development of hybrid breeding has clearly shown that self-fertile lines are necessary to get nonrestorers and restorers of male-sterile lines, and to provide pollen parents with high performance for the production of hybrid seeds. In this context, elucidation of the inheritance and identity of the various sources of self-fertility is becoming an urgent necessity so as to make breeding ever more efficient. For that reason we have started extensive analyses on the genetics of several self-fertility sources. Preliminary results of that work will be outlined in the paper.

MATERIAL AND METHODS

The self-fertility of the Polish mutant *vd* was analysed from 1980 through 1986. The self-sterile primary trisomics of cv. Esto (Sturm 1978, Schlegel et al. in press) were crossed with a heterozygous self-fertile clone of the mutant *vd* and with clones of other self-fertility sources. Only trisomic 7*R* was absent in the set because of its poor vitality and fertility. The selected F_1-trisomics were cloned and selfed, but only the self-fertile F_1-trisomics of the mutant *vd* were used preliminary to test the F_2-segregations. All F_2-plants were then selfed again; for that purpose, in each case three spikes per plant were isolated in Pergamin-bags. The F_2-generations of trisomics 4*R* and 6*R* were tested in the greenhouse and all the other progenies were grown in the field. Self-fertility was determined by calculating the mean number of seeds per floret. The segregations found in the disomic fractions of the F_2-generations were compared by *chi*-square-test (Weber 1978) with the expected segregations. The progenies of the individual F_1-trisomics were kept separate and not mixed. All together seven F_2-populations were analysed: one F_2 each of trisomics 2*R*, 3*R*, 4*R*, 5*R* and 6*R* and two F_2's of trisomic 1*R*.

RESULTS

The distribution of self-fertility values of various F_1- and F_2-generations showed the existence of more than two self-fertility-classes (Tables 1 and 2). Most of the F_1-trisomics had clearly limited self-fertility classes of less than 5%, between 20% - 35% and 35% - 65%, whereas a large scale of self-fertility values was observed in the F_2-progenies. As there had not been reliable experiences regarding the classification of genotypes, group formation had to be done empirically using some assumptions. The following assumptions were accepted:

1. On the basis of the distribution of self-fertility values from F_1-trisomics it was found, that self-sterile plants have a self-fertility level of less than 5%.

2. The limits of self-fertility classes have to be the same in all investigated F_2-progenies and in all tested inheritance types simultaneously.

Against that background we tested various monogenic, digenic and trigenic inheritance types. Since a part of the F_1-trisomics were self-fertile, dominant inheritance was taken for granted. The interpretation of the observed F_2-segregations using

Table 1. Distribution of self-fertility values in F_1-trisomics from crosses between primary trisomics and various self-fertile mutants

Self-fertility classes %	Number of observed plants in self-fertility classes in F_1-trisomics					
	1*R*	2*R*	3*R*	4*R*	5*R*	6*R*
0 - 4.9	1	4	1	4	2	2
5 - 9.9	–	–	–	–	–	–
10 - 14.9	1	–	–	–	–	–
15 - 19.9	2	–	–	–	–	2
20 - 24.9	–	1	3	–	1	–
25 - 29.9	4	–	1	2	2	3
30 - 34.9	1	2	–	2	1	3
35 - 39.9	–	–	1	1	2	1
40 - 44.9	4	1	1	–	1	2
45 - 49.9	2	–	4	–	1	1
50 - 54.9	–	1	–	–	–	2
55 - 59.9	–	–	–	–	1	–
60 - 64.9	–	–	–	–	–	–
65 - 69.9	–	–	–	–	1	–
n	15	9	11	9	12	16

a monogenic model was not possible because of the occurrence of less self-sterile plants than expected. The following tests showed clearly that there were two distinct segregation types, because most of the F_2-plants of the trisomics 1*R*-II, 4*R* and 6*R* had self-fertility values less than 50%, whereas self-fertility values of more than 50% were observed in most of the F_2-plants of the trisomics 1*R*-I, 2*R*, 3*R* and 5*R*. On the basis of the mentioned assumptions and general segregations of di- and trigenic inheritance (Table 3) further limits were introduced. All plants

Table 2. Distribution of self-fertility values in F_2's from crosses between primary trisomics and the self-fertile mutant *vd*

Self-fertility classes %	Number of observed plants in self-fertility classes in F_2's of trisomic							
	Control	1*R*-I	1*R*-II	2*R*	3*R*	4*R*	5*R*	6*R*
0 - 4.9	3	1	11	3	4	8	1	3
5 - 9.9	2	5	10	0	8	3	3	2
10 - 14.9	3	5	11	4	6	4	3	7
15 - 19.9	5	4	15	5	4	8	5	3
20 - 24.9	5	5	12	5	11	3	4	4
25 - 29.9	8	10	12	7	14	10	5	1
30 - 34.9	16	10	10	9	18	10	9	1
35 - 39.9	19	11	7	9	11	15	4	1
40 - 44.9	23	17	7	9	13	16	16	3
45 - 49.9	11	8	3	9	11	7	8	3
50 - 54.9	29	20	4	11	15	12	13	2
55 - 59.9	27	13	1	14	17	10	16	1
60 - 64.9	27	10	0	16	10	7	10	0
65 - 69.9	21	9	2	17	10	7	14	0
70 - 74.9	21	4	0	23	11	5	8	0
75 - 79.9	8	2	0	12	6	0	12	0
80 - 84.9	8	1	0	7	6	1	1	0
85 - 89.9	2	0	0	4	0	0	4	0
90 - 94.9	1	0	0	1	0	0	1	0
95 - 100	0	0	0	0	0	0	0	0
n	239	135	105	165	175	126	137	31

with self-fertility values between 5% and 29,9% were effected by one dominant gene, whereas all the plants between 30% and 54,9% were effected by two heterozygous dominant genes and all the plants between 55% and 100% by two homozygous or three dominant genes (Table 4).

Table 3. Expected segregations in the disomic part of F_2-generations from crosses between primary trisomics and a mutant in the case of mono-, di- and trigenic inheritance of the mutant

Types of inheritance	Expected segregations
Monogenic-disomic	
– recessive	3*A.* : 1*aa*
– intermediate	1*AA* : 2*Aa* : 1*aa*
– dominant	3*A.* : 1*aa*
Monogenic-trisomic	
– recessive	8*A.* : 1*aa*
– intermediate	1*AA* : 4*Aa* : 4*aa* / 4*AA* : 4*Aa* : 1*aa*
– dominant	5*A.* : 4*aa*
Digenic-disomic	
– general	9*A.B.* : 3*A.bb* : 3*aaB.* : 1*aabb*
Digenic-trisomic	
– dominant-general	15*A.B.* : 5*A.bb* : 12*aaB.* : 4*aabb* / 15*A.B.* : 12*A.bb* : 5*aaB.* : 4*aabb*
– recessive-general	24*A.B.* : 8*A.bb* : 3*aaB.* : 1*aabb* / 24*A.B.* : 3*A.bb* : 8*aaB.* : 1*aabb*
Trigenic-disomic	
– general (F_1 = *AaBbCc*)	27*A.B.C.* : 9*A.B.cc* : 9*A.bbC.* : 9*aaB.C.* : 3*A.bbcc* : 3*aaB.cc* : 3*aabbC.* : 1*aabbcc*
Trigenic-trisomic	
– dominant-general (F_1 = *AaaBbCc*)*	45*A.B.C.* : 15*A.B.cc* : 15*A.bbC.* : 36*aaB.C.* : 5*A.bbcc* : 12*aaB.cc* : 12*aabbC.* : 4*aabbcc*
– recessive-general (F_1 = *AAaBbCc*)*	72*A.B.C.* : 24*A.B.cc* : 24*A.bbC.* : 9*aaB.C.* : 8*A.bbcc* : 3*aaB.cc* : 3*aabbC.* : 1*aabbcc*

* = General segregations for F_1-trisomics *AaBbbCc*, *AaBBbCc*, *AaBbCcc* and *AaBbCCc* can be adapted.

A gene dosage effect was observed in F_1-trisomics 1*R* and 6*R*, which means the occurrence of an additional self-fertility class between 10% and 20%. This effect may be attributed to trisomy of one self-fertility gene and incomplete dominance. But, it is very difficult to identify single homozygous or heterozygous plants in the F_2-progenies because of variation of self-fertility values and little experience. Therefore homo- and heterozygous plants were distinguished in Table 4 in the presented way.

The F_2-segregations of the trisomics 1*R*-II and 6*R* followed the trisomic segregation of the digenic-dominant inheritance type (Table 5). This means that one dominant self-fertility gene is located on each of these two chromosomes (1*R* and 6*R*). Besides that, two self-fertility genes were located, it was an obvious conclusion to test the progenies of the other trisomics for trigenic inheritance. The F_2-segre-

Table 4. Classification of self-fertility types adequate to genotypes

Self-fertility classes	Frequency of genotypes adequate to the inheritance type			
	Disomic digenic	Trisomic digenic	Disomic trigenic	Trisomic trigenic
0 - 4.9% (self-sterile)	1 *aabb*	4 *aabb*	1*aabbcc*	4*aabbcc*
5 - 29.9%	3 *A.bb*	5 *A.bb*	3*A.bbcc*	5*A.bbcc*
	3 *aaB.*	12 *aaB.*	3*aaB.cc*	12*aaB.cc*
			3*aabbC.*	12*aabbC.*
30 - 54.9%	4 *AaBb*	8 *AaBb*	4*AaBbcc*	8*AaBbcc*
	2 *AaBB*	4 *AaBB*	4*AabbCc*	8*AabbCc*
	2 *AABb*	2 *AABb*	4*aaBbCc*	16*aaBbCc*
			2*AabbCC*	4*AabbCC*
			2*AaBBcc*	4*AaBBcc*
			2*AABbcc*	2*AABbcc*
			2*AAbbCc*	2*AAbbCc*
			2*aaBbCC*	8*aaBbCC*
			2*aaBBCc*	8*aaBBCc*
55 - 100%	1 *AABB*	1 *AABB*	1*AABBcc*	1*AABBcc*
			1*AAbbCC*	1*AAbbCC*
			1*aaBBCC*	4*aaBBCC*
			27 *A.B.C.*	45 *A.B.C.*
Expected segregation	1 : 6 : 8 : 1	4 : 17 : 14 : 1	1 : 9 : 24 : 30	4 : 29 : 60 : 51

gations of trisomics 2*R* and 5*R* fitted the expected disomic 1 : 9 : 24 : 30 segregation, but clearly trisomic segregations were found in the F_2-progenies of trisomics 1*R*-I and 3*R* (Table 5). While the location of one self-fertility gene on chromosome 1*R* has thus been proved twice and other self-fertility genes were located on chromosomes 3*R* and 6*R*, the segregation of trisomic 4*R* in the F_2-generation has not been

Table 5. *Chi*-square-tests of the observed F_2-segregations from crosses between primary trisomics and the mutant *vd* ($\chi_{0.05;3}=7.81$)

Trisomic	*n*	Number of observed plants in self-fertility types				χ^2-values of segregations			
		<5%	<30%	<55%	<100%	1 : 6 : 8 : 1	4 : 17 : 14 : 1	1 : 9 : 24 : 30	4 : 29 : 60 : 51
1*R* - I	135	1	29	66	39	127.2	361.6	19.9	5.5*
1*R* - II	105	11	60	31	3	24.5	4.6*	238.4	129.5
2*R*	165	3	21	47	94	711.5	1758.8	7.5*	33.6
3*R*	175	4	43	68	60	237.7	650.8	20.3	2.3*
4*R*	126	8	28	60	30	81.5	222.4	42.2	11.9
5*R*	137	1	20	50	66	401,8	1030.6	0.7*	11.4
6*R*	31	3	17	10	1	5.5*	2.5*	61.4	33.4
7*R*	–	–	–	–	–	–	–	–	–
Control	239	3	23	98	115	735.4	1873.1	4.3*	24.4

clarified yet. As seen from Table 5, the observed segregation does not fit any of the expected segregations. The functions of genes on chromosome 7*R* is still unknown since that chromosome was absent in the trisomic set. The located genes are named *Sf*1 on chromosome 1*R*, *Sf*2 — on chromosome 3*R* and *Sf*4 — on chromosome 6*R*.

DISCUSSION

The analyses revealed surprisingly well-defined segregations. Therefore, the supposed limits of self-fertility seem to nearly correspond to the genetic ratios. Further classifications would be possible, but they do not seem to be sensible since a relatively high variation of self-fertility in the individual plants would result in some overlapping of new classes. In this connection it should be pointed out that the observed self-fertility values do not permit the absolutely definite classification of individual plants to various self-fertility types because of potential overlapping of the applied limits again. Moreover, factors not directly related to self-fertility genes may also influence the expression of self-fertility. Therefore, the used limits should rather be regarded as limits in the statistical sense.

It seems, that a non-genetic (environmental) factor was effective in the F_2-generations of trisomics 4*R* and 6*R*. Contrary to the other progenies the F_2-generations trisomics 4*R* and 6*R* were tested in the greenhouse. Hence, it is possible that the more optimal environment as compared with outdoor tests had a positive effect on seed set. This means that pseudo-self-fertility as described by Wricke (1978) and Grau and Herden (1983) may have shifted the limits of self-fertility as compared with those in the field. But, the distribution of values and the amount of self-fertile plants clearly indicated disomic digenic-dominant segregation in the F_2-progeny of trisomic 4*R* and trisomic digenic-dominant segregation in the F_2-progeny of trisomic 6*R*. This environmental effect demonstrated, that it will be necessary to test all F_2-generations of one trisomic analysis in the same environment.

Smirnov and Sosnikhina (1984) described a self-fertility gene linked with some marker genes, but there are some difficulties because some single segregations were disturbed. Surikov (1980) found a linkage between gene *an* (anthocyanless) and a self-fertility gene, but meanwhile *an*-genes were located on chromosomes 2*R*, 4*R* and 7*R* (Schlegel et al., in print). Therefore, it seems better to clarify the results of Smirnov and Sosnikhina (1984) and Smirnov (1980) by additional analyses.

Romanova (1982) observed a linkage between a self-fertility gene and gene *b* (brittle stem). This self-fertility gene is located on chromosome 5*R*, because gene *b* had been detected by translocation analysis, on that very chromosome 5*R* (De Vries, Sybenga, 1984). The gene is designated *Sf*3.

Ruebenbauer et al. (1983b) described the inheritance of the violet seed colour mutant *vd*, but they did not examine self-fertility in particular. Material from Radzików and several inbred lines (I_{22}) were used to analyse seed colour. However, it was not possible to trace the exact parents of the line mutant *vd* described in this paper. As the inbred lines are likely to have been integrated in the mutant, their self-fertility had also been transmitted. Although further self-fertility in the mutant cannot be definitely excluded, the described self-fertility seems to be the result of inbreeding.

Apart from that self-fertility induced by inbreeding, other sources have been described, too (Wricke 1969, Kuckuck, Peters 1979, Derevyanko, Zdrilko

1982). Meanwhile the first analyses of Zdrilko's self-fertility were carried out using trisomics. It was observed, that this source showed the same disomic trigenic-dominant inheritance as the mutant *vd*. The self-fertility of the mutant *M* 123 (Ruebenbauer et al. 1983c) appeared to be of the same inheritance type as *vd*.

Wricke and Wehling (1985) localized a self-incompatibility gene on chromosome 1*R*. This gene is sensible to temperature and produces pseudo-self-fertility. Now, the chromosome 1*R* was found to have the gene *Sf*1 in the mutant *vd*. Therefore, it is an obvious conclusion to suppose that gene *Sf*1 is possible to be a mutant of the gene localized by Wricke and Wehling (1985). Moreover, selfing in various populations produced self-fertile lines already after few inbred generations. This suggests a relatively frequent occurrence of self-fertility, which together with a pseudo-self-fertility effect, in turn, would indicate some lability to exist in the incompatibility system of rye. Spontaneous self-fertility may be due to mutations or recombination processes and may result in minimum numbers of potential self-fertile gametes, which can produce self-fertile plants relatively easy because of dominant inheritance of mutation, the enormous number of male gametes and the enforced selfing. Consequently, it seems that self-fertility genes are mutations of the genes *S* and *Z*, as supposed by Kuckuck and Peters (1979), or additional modifying genes.

The authors believe that the results presented in this paper will contribute to a better understanding of self-fertility genetics. Nevertheless, many questions are still waiting for solution. It still remains in the dark whether more than three genes may induce self-fertility at the same time.

Other open questions concern genes involved in the expression of digenic and trigenic inheritance, the existence or not of differences in the expression of single genes and confirmation of interactions between specific genes.

Therefore, further trisomic analyses and test crosses will have to answer these questions and at the same time to define other self-fertility sources as to their genetics and identity.

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GENETYCZNA ANALIZA ŻYTA (*SECALE CEREALE* L.) III. SAMOPŁODNOŚĆ MUTANTA *VD* — DZIEDZICZENIE I LOKALIZACJA GENÓW

Streszczenie

Analizowano samopłodność mutanta żyta *vd* (z fioletowymi nasionami) po skrzyżowaniu z pierwotnie samopłodnym trisomikiem cv. Esto. Trzy geny samopłodności o niepełnej dominacji zlokalizowano na chromosomach 1*R* (*Sf*1), 3*R* (*Sf*2) i 6*R* (*Sf*4). Dziedziczenie tych genów było monogenicznie pośrednie lub digenicznie addytywne. Zgodnie z tym co podają inni autorzy zaobserwowano, że jeden z genów warunkujących samopłodność występuje na chromosomie 5*R*.

ГЕНЕТИЧЕСКИЙ АНАЛИЗ РЖИ (*SECALE CEREALE* L.) III. САМООПЛОДОТВОРЕНИЕ МУТАНТА РЖИ *VD* — НАСЛЕДОВАНИЕ И РАСПОЛОЖЕНИЕ ГЕНОВ

Резюме

С помощью трисомиков ржи сорта Эсто анализировалось самооплодотворение мутанта ржи *vd* (фиолетовые семена). Три неполностью доминирующих гена находилось на хромосомах $1R(Sf1)$, $3R(Sf2)$ и $6R(Sf4)$. Наследование этих генов было моногенно-промежуточным или двугенно-аддитивным. Один ген самооплодотворения находился на хромосоме $5R(Sf3)$ согласно данным литературы.