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Selfcompatibility in Microspore-derived Doubled-haploid Rye Lines and Single Grain Selection for Alkylresorcinol Content*

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Summary. From a total of 138 green androgenetic rye lines, 25 were fertilized and examined in field tests: 7 were heterozygous and 18 were homozygous. Of the homozygotes, 4 turned out to be selfincompatible, while 14 set seed after selfing. Four characters were analyzed in detail: 100 kernel weight, plant height, ear length, and alkylresorcinol content. Here we present the first approach in prescreening selfcompatible androgenetic doubled-haploid rye plants with the single grain procedure. The usefulness of this method was confirmed by quantitative resorcinol determination in the following generation. Furthermore, it was demonstrated that all the homologues of the alkylresorcinol were equally reduced. For all characters the means of the different anther derived lines exceeded the means of the controls in both directions, to the positive as well as to the negative side. The incorporation of such a haploid breeding step into breeding programs is discussed.

Key words: Alkylresorcinol – Doubled-haploids – Rye – *Secale cereale* – Selfcompatibility

Introduction

The use of haploids and, in particular, homozygous doubled-haploids, is progressing from model plants to crops and is slowly expanding into the fields. However, the world's most important crops, the cereals, are very difficult in this respect. The impressive success using doubled androgenetic haploids, for example in China, depends predominantly on enormous labour input. Despite this disadvantage, new rice, wheat and maize lines derived from cultured anthers have been released as varieties in China (Hu Han and Hao Shui 1980). It is claimed that the efficiency of plant breeding could be increased by their use. Using parthenogenetic haploids of barley it has also been demonstrated that variety production could be enhanced (Wienhues cit. Wenzel 1980). We concentrated our efforts in cereal tissue culture on the exploitation of the possibilities opened up by using in vitro derived plants for the improvement of the outbreeder *Secale cereale*.

In previous papers we reported on the production of haploid rye lines via anther culture and stressed that, in addition to homozygosity itself, the major objective of the incorporation of haploids into applied breeding procedures would be the introduction of selfcompatibility (Thomas and Wenzel 1975; Wenzel et al. 1977). Furthermore, we intended to reduce the alkylresorcinol content (Hoffmann and Wenzel 1977). This latter point is of importance as the use of rye in feeding young animals is reported to be limited because of toxic effects of this compound (Wieringa 1967). Hoffmann and Wenzel (1977) described a single grain screening technique which allows the first evaluation of the alkylresorcinol content without destroying the grain, thus enabling the growth of plants from analyzed grains. This is of direct applicability when selfcompatible inbred lines are available which give toxin free progenies by selfing. Such a first approach in screening selfcompatible doubled-haploid rye plants is reported, together with data characterizing androgenetic rye lines in the field.

Materials and Methods

The plant material used for the extraction of haploid rye lines consisted predominantly of F_1 hybrids combined from commercial winter rye varieties and selfcompatible lines descended from mutated progenies of a hybrid between *Secale cereale* and *S. vavilovii* (Kuckuck 1976). The commercial varieties belonged predominantly to the 'Kustro' variety group. In addition to these, a Russian (Moskow dwarf, MZ) and a Bulgarian (M II) short stalk line were incorporated (obtained by H. Kuckuck, Hannover). For hybridizing the

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doubled-haploids, androgenetic selfcompatible and incompatible lines were crossed with a selfcompatible I_7 of the 'Carstens' variety group (L 217; obtained by H.H. Geiger, Stuttgart-Hohenheim).

The tissue culture derived A_1 plants were grown in greenhouses under semicontroled conditions (18-24°C). After selfing, the A_2 were grown during 1979 and 1980 in the field at Scharnhorst. Selfing and crossing were performed either in isolation chambers or by bagging the spikes; hand pollination was only tried in plants which did not set seeds after the other treatments. Emasculation was performed by cutting. In 1978, the 1979 material was artificially vernalized for 8 weeks at 4°C after some cloning, and was then planted in the field in late April. The material from 1980 was precultured in Jiffy strips and transplanted at the end of October. The test plots had a size of 1 × 2 m. The distance between the plants was 10 cm and between the rows 20 cm, which resulted in 100 plants per plot. Hundred kernel weights were measured using a Numigral M machine (Falling Number, Hamburg); stalk and ear length were measured in the field at seed set. The methods for androgenesis and for single grain screening of the alkylresorcinol content have been published previously (Wenzel et al. 1977; Hoffmann and Wenzel 1977). To make the screening method more efficient, small and shrunken grains were removed as they might have given a misleadingly low alkylresorcinol content. As a blank the alkylresorcinol value of the wheat variety 'Caribo' (alkylresorcinol content 0.4%) was chosen. Quantitative analysis of the total alkylresorcinol was carried out according to Musehold (1973) and separation of homologues on thin-layer-plates according to Musehold (1978).

Results

Description of Selfcompatibility

Under optimal preculture conditions up to 10% of the plated rye anthers from the donor hybrid 6 (Mut 530 × M II; Table 1) developed macroscopic structures on the modified potato extract medium (Wenzel et al. 1977). Other hybrids having this M II as pollinator also yielded regeneration rates above average. This was confirmed in repeated combinations and more recently by Friedt and Foroughi-Wehr (1981). Possible explanations for such a strong influence of the genotype on the androgenetic potential have been discussed in detail for potato (Wenzel and Uhrig 1981). However, even using this high productive pollinator the problem of the high number of albinos among the anther-derived plants could not be solved in rye.

In total we could regenerate 138 green lines of gametophytic origin and 13 lines of sporophytic origin from a total of 21 different responsive anther donor hybrids. From the 138 androgenetic lines, 82 either did not survive heterotrophic conditions, did not produce flowers, or could not successfully be doubled by colchicine. From the 40 plants further examined, 15 were sterile and 25 could be selfed (21) or at least crossed (4). From the selfcompatible plants, 7 turned out to be heterozygous (Wenzel et al. 1976), the others were homozygous. As self-

Table 2. Comparison of plant height, ear length and 100 kernel weight of homozygous androgenetic lines and the F_2 of their anther donor hybrids (control)

A_2 line	Anther donor	Plant height [cm]	Ear length [cm]	100 kernel weight [g]
161	6	120 ± 5	10.1 ± 1.0	3.0 ± 0.8
164	6	120 ± 0	8.2 ± 0.8	3.5 ± 0
176	6	90 ± 5	8.7 ± 0.3	2.0 ± 0
181	6	110 ± 15	8.3 ± 0.7	1.4 ± 4.0
184	6	90 ± 10	9.0 ± 2.0	2.0 ± 0
199	6	100 ± 20	9.2 ± 1.0	3.3 ± 0.2
control F_2	6	90 ± 20	8.4 ± 2.0	2.8 ± 0.8
175	10	65 ± 0	8.2 ± 1.0	1.5 ± 0.2
191	10	120 ± 0	11.2 ± 0.4	2.0 ± 0
201	10	85 ± 5	5.7 ± 0.1	2.0 ± 0
209	10	80 ± 10	7.1 ± 0	1.9 ± 0.5
control F_2	10	90 ± 30	8.8 ± 1.0	2.6 ± 0.3

Table 1. Characterization of regenerated plants from anther culture experiments in rye

F_1 anther donor	Regenerated green lines			Fertility				
No. Parents	total	1x	Spontaneous 2x	No. flowers	Sterile	Incompatible	Selfcompatible	
							Homozygous	Heterozygous
1 Mut 408	1	1	–	–	–	–	1	–
2 Pekuro × MZ	1	–	1	–	–	–	–	1
3 MZ × Mut 511	2	–	2	–	–	–	–	2
4 MZ × Mut 530	4	–	4	–	–	–	–	4
5 MZ × Kustro	3	2	1	2	–	–	1	–
6 Mut 530 × M II	64	14	21	29	11	1	7	–
7 Mut 543 × M II	2	2	–	–	1	1	–	–
8 Mut 530 × Kustro	1	–	1	–	1	–	–	–
9 Mut 543 × Kustro	5	1	4	4	–	–	1	–
10 Perolo × *S. vavilovii*	18	4	8	10	2	2	4	–

Table 3. Comparison of plant height, ear length and 100 kernel weight of hybrids between homozygous androgenetic lines or the F_2 of their anther donors (control) and an I_7 of the Carstens variety group

F 1 ($A \times I_7$)	A line			Plant height [cm]	Ear length [cm]	100 kernel weight [g]
	No.	Fertility	donor			
a	161	selfcomp.	6	110 ± 10	8.4 ± 1.4	3.2 ± 0.3
b	168	incomp.	6	95 ± 20	10.0 ± 0	3.4 ± 0.1
c	175	selfcomp.	10	130 ± 0	10.3 ± 0.2	2.5 ± 0.5
d	177	incomp.	10	120 ± 5	10.3 ± 0.1	3.1 ± 0.1
e	188	incomp.	10	125 ± 0	10.0 ± 1.0	3.4 ± 0.4
f	191	selfcomp.	10	110 ± 10	12.8 ± 0.8	3.4 ± 0.2
control ($F_2 \times I_7$)			6	95 ± 20	8.4 ± 2.0	3.0 ± 1.0
control ($F_2 \times I_7$)			10	85 ± 5	7.6 ± 0.6	2.4 ± 2.0

compatibility is dominant, no sterile heterozygotes were found (Table 1). In the homozygous lines and in some of their hybrids with an I_7 of the 'Carsten' variety group, 100 kernel weights, stalk and ear length were estimated (Tables 2 + 3). As can be seen in Table 2, the variability of

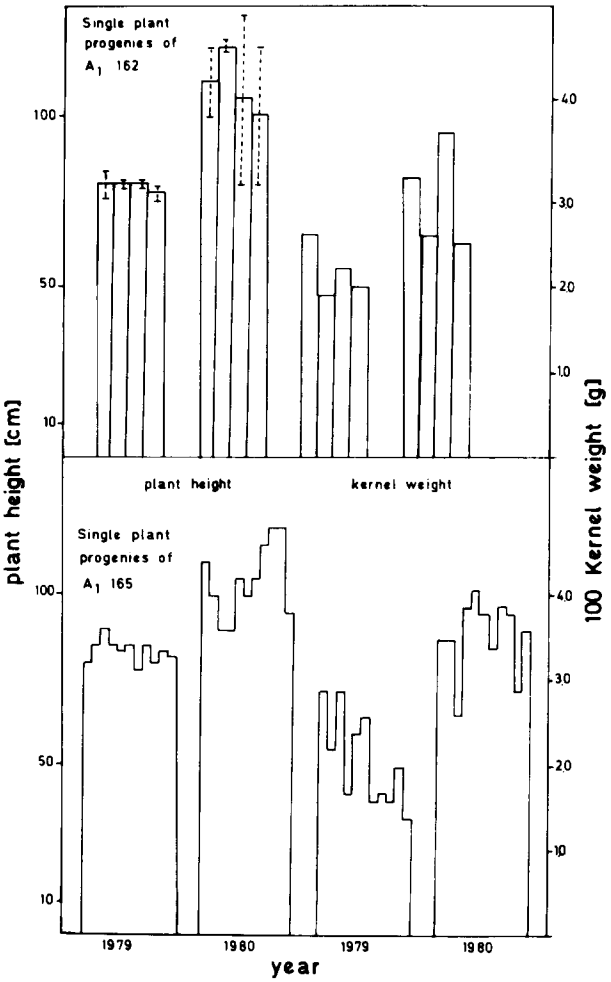

Fig. 1. Variation in plant height and 100 kernel weight of single plant progenies from 2 selffertilized doubled-haploid androgenetic rye plants

100 kernel weight, plant height and ear length is relatively low in comparison to the F_2 of the anther donor material which served as controls. The means of the different anther derived lines exceeded the means of the controls on both the positive and the negative side. The behaviour of anther donor line 10, which was still closely related to the wild variety, was particularly striking. The A_2 191 gave relatively high values, while line 209 already showed phenotypically the appearance of *S. vavilovii*. The 100 kernel weight was still much higher than *S. vavilovii* which has a 100 kernel weight of 0.7 g. In Table 3 the results from 6 of the hybrids are summarized: the controls were combinations of the F_2 of the anther donor plant with the same selfcompatible 'Carstens' I_7 (L217). In comparison to the selfed A_2 lines, most hybrids did express hybrid vigour, measured in stalk and ear length as well as in kernel weight. But there was only a small tendency in F_1 b (A_1 168 $\times$ I_7 L 217) of a change in the correlation long stalk: long ear. The phenotypic uniformity of the F_1's was striking and was higher than expressed in Table 3 due to the different culture conditions in 1978/79 and 1979/80 (in the first year artificial vernalization made hand planting in spring necessary while in the next year the plants were sown in autumn). Figure 1 demonstrates a result which underlines the findings in selfed double-haploid lines of wheat (Picard 1981) that such progenies may vary if single plant descends are separated. From the two A_1 lines 162 and 165 of rye in 1978 different single seed descends were harvested. Six lines descended from the originally haploid A_1 162, 13 lines from the spontaneously doubled A_1 line 165. Four and 11 progenies respectively were grown separately in 1979 and 1980. In 1979 all lines of 162 were rather uniform within the plot and also amongst the plots. However, A_1 165 already showed slight differences in the first year amongst the plots and these differences became more evident after selfing and regrowing in 1980. The general increase from 1979 to 1980 in the characters measured again depended partially on the different growing conditions in the two years.

Alkylresorcinol Estimations

An androgenetic line of A_1 6 (Mut 530 X M II) was predominantly selected for screening as it was the most abundant. In the A_2 2000 seeds were screened: 27 grains were found with a very low content and 10 kernels with a very high content. The A_2 plants were grown and selfed. From these selfed A_2 plants the total alkylresorcinol content of the A_3 seeds were estimated. In Figure 2 the results of this final test are represented. The alkylresorcinol content of the anther donor plant in the F_2 and of the microspore derived A_1 plants equaled 1%; that of the single grain method preselected material showed values between 0.4 and 1.5%. The difference between the group preselected as alkylresorcinol rich (+ in Fig. 2) and as poor (– in Fig. 2) is highly significant (t_{35} = 5.4; t_{35} for P 0.001 = 3.6). The distribution of the values further demonstrates that we did not involuntarily select for the kernel weight via the 'physiological marker' alkylresorcinol content (Fig. 2). Two lines gave an extreme low value. Furthermore it was tested if the reduction was due to a uniform decrease of the different resorcinols or if single compounds were reduced or eliminated. Figure 3 demonstrates that the homologues were reduced uniformly. This might be of importance for the toxidity behaviour. In further tests the other breeding characters of these alkylresorcinol-poor and selfcompatible homodiploid lines will be estimated.

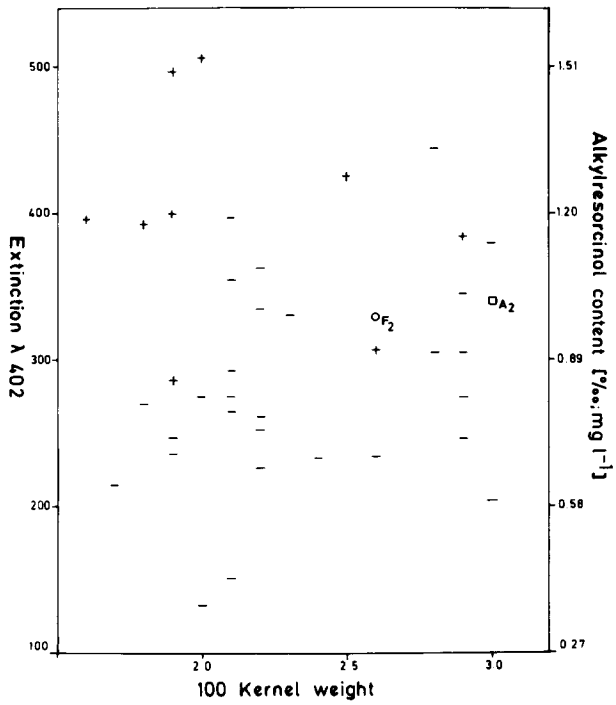

Fig. 2. Alkylresorcinol content of single plant progenies from a doubled-haploid androgenetic rye plant after previous single grain screening (+ = progeny from a high resorcinol grain; – = progeny from a low resorcinol grain). To avoid misinterpretations the corresponding 100 kernel weights are given

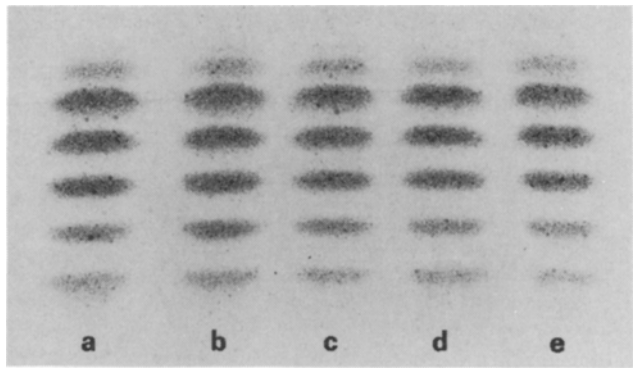

Fig. 3a-e. Chromatogram of acetone extracts of rye grains from lines with different contents of alkylresorcinols. By applying equal amounts of substance it can be seen that the reduction of the toxin in 'low lines' d, e or the increase in 'high lines' a is due to a uniform alteration and not to an increase or reduction/elimination of single homologues (b and c = A_2 and A_3 of the anther donor)

Discussion

The results of the alkylresorcinol content of the selected plants demonstrate that, by using the single grain screening technique, alkylresorcinol-poor lines can be quickly enriched within a large bulk of grains. Furthermore, it should be noted that all measurable compounds were reduced. This is in addition to the effect on the toxicity which is important for resistance breeding programs. Resistance, e.g. to fungi, might demand a specific spectrum of different alkylresorcinol derivatives, or might be closely linked to such compounds. The stability of the pattern found reduces the danger of destroying specific resistances and this should be true also for forthcoming breeding programs. Regarding the analysis of the androgenetic lines we have shown that the anther culture passage delivered selfcompatible inbred lines, when initiated from heterozygous selfcompatible F_1 anther donor hybrids. The quality of most of these hybrids was not very impressive; in our opinion this was not due to their inbred nature but because of their close relation to the wild variety *S. vavilovii*. If it would have been possible to produce larger numbers this question could have been clarified, since valuable combinations should appear amongst a larger population. However, even then it might not be ideal to extract the haploids from such early crosses, as for example the F_1 *S. cereale* X *S. vavilovii*. The extraction should possibly be performed after some backcrossing and preselection. As long as the preselection has gone in the right direction and a positive selection pressure can be applied – supposition of which is a precise breeding aim, e.g. alkylresorci-

nol content – this would not be too critical. However, since only negative selection procedures are applicable in most cases, the advantage of not missing anything by starting from an early cross will be replaced by the disadvantage of being forced to screen a large population of rather still expensive in vitro-born plants. A further problem of a direct application is the spontaneous formation of diploid plants. This should not be too great a problem, as fertile homozygous diploids are desired. However, as heterozygotes appear spontaneous diploids have to be screened again.

In the experiments reported only a few characters were studied in relatively small trials. A further, more intensive, testing is in progress with special attention to the combining ability of the selfcompatible lines (H.H. Geiger). In conclusion, we have shown that androgenetic haploids are useful, particularly for the rapid incorporation of selfcompatibility and alkylresorcinol poorness. For real applications however, a still more efficient yield of haploids is necessary.

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