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Recurrent selection alternating with haploid steps – a rapid breeding procedure for combining agronomic traits in inbreeders

B. Foroughi-Wehr and G. Wenzel

Federal Biological Research Centre for Agriculture and Forestry, Institute for Resistance Genetics, D-8059 Grünbach, FRG

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Summary. Doubled haploid lines are increasingly used in practical breeding programs. Depending on the nature of the starting material as well as on the breeding aims, one or more haploid steps are recommended, and F_1 hybrids or selfed progenies in later generations serve as starting material for the haploidization. It is demonstrated that one haploid step followed by selection in the greenhouse and in the field during the first androgenetic (A_1) and two subsequent selfed generations (A_2; A_3) is the most efficient procedure, if characters from related varieties are to be combined. For breeding programs that include wild types or unrelated genotypes, recurrent selection alternating with several haploid steps is the most efficient. A prerequisite for successful application of this combination of repeated haploidization and selection steps alternating with backcrosses is an effective selection system. The different approaches are demonstrated on a winter barley breeding program, with the central aim of combining the qualitatively inherited character resistance to barley yellow mosaic virus with quantitatively inherited complexes, particularly yield.

Key words: Barley – Quantitative inheritance – BaYMV – Backcrossing – Haploids

Introduction

Today, doubled haploids are routinely used in practical breeding programs. The main prerequisite for the acceptance of this new procedure has been the improved efficiency in the production of doubled haploids (DH) per desirable genotype or, in other words, the reduction of the costs in obtaining one DH line. Presently, the androgenetic induction of haploids is the most economical way to produce haploids in cereals, rapeseed, and numerous other crops (wheat: Baenzinger et al. 1989; Müller et al. 1989a, b; maize: Pescitelli et al. 1989; rapeseed: Polsoni et al. 1988). In barley, under optimal conditions the regeneration frequency of doubled haploid green plants reaches 5–10% based on the number of anthers cultured (Kuhlmann and Foroughi-Wehr 1989). Such frequencies document a 10- to 15-fold increase in the regeneration capacity during the last 5 years. Similar improvements are true, e.g., with rapeseed (Polsoni et al. 1988).

Since regeneration is no longer the central bottleneck in using DHs, other questions are gaining importance. Snape and Simpson (1981) already discussed the genetic effects when starting from different filial generations. Here, in connection with applied breeding programs, the question is: can DHs be used more economically in an F_1 generation or in later generations, e.g., a preselected F_2 or F_3, or should the haploid step be combined with other breeding techniques?

This paper gives results of the application of some alternative breeding strategies, with the inclusion of the DH technique, for the inbreeder barley. How far the results can be generalized for other inbreeders such as wheat or rice will be discussed. Answers are discussed for four different cases. (1) The recombination of (a) monogenically and (b) polygenically inherited traits between closely related genotypes, and (2) the introduction of (a) monogenic and (b) polygenic traits from distantly related genotypes, such as primitive cultivars or wild species into modern varieties.

Materials and methods

The following varieties and lines were included in the series of experiments: (A) Crosses between related winter barley cultivars: Sonja × Diana and Diana × 591.80 (breeders line), and (B) crosses between distantly related lines or cultivars: yml × Igri, several backcrosses with DH lines, and the varieties Harmonika and Cosima.

All doubled haploid lines were produced as reported previously (Kuhlmann and Foroughi-Wehr 1989). The field experiments followed standard designs.

The DH lines from the two crosses in (A) were grown in the experimental field in Grünbach until the A_3. In the subsequent generations, A_3 to A_5, the investigations were carried out by German breeding companies. The final test was performed at five locations with two to four replications. The yield is stated relative to the standard varieties Danilo or Sonate. The crosses between distantly related varieties (B) were investigated at two locations in Grünbach. The size of the field plots differed from 0.5 to 5.0 m^2, allowing the estimation of quantitatively inherited traits, e.g., the thousand-grain weight (TGW). All experiments concentrated on barley yellow mosaic virus (BaYMV) resistance and on leaf-spot diseases. The BaYMV tests were carried out by mechanical inoculation of the A_1 plants in the greenhouse (Friedt 1983). The ELISA test was used to identify susceptible plants. For resistance to leaf spot, a classification from 1 (resistant) to 9 (heavy infested) was used. The test was scored twice during the growing period by visual examination.

Results

1. The transfer of monogenically inherited traits among closely related genotypes

The aim of these experiments was to transfer the monogenic trait, BaYMV resistance, from one adapted winter barley line to another. For this purpose, theoretically, only one round of recombination is necessary and desirable, as it is required that most of the genome be kept together. Consequently, it is preferable to start haploid production in the F_1 generation, following the scheme shown in Fig. 1. Fifty percent of the homozygous DH plants (A_1) carry the desired character and can be selected in this generation. The essential number of DH individuals for a special breeding aim depends on the degree of relationship of the starting material. The more similar the parents are in morphological and physiological characteristics, the smaller the random sample of DH lines can be.

The combining ability will not be known for most of the parents, so one should produce less than 60–80 DH lines but broaden the genetic basis, thus transferring the desired characteristic into different genetic backgrounds. Poor as well as superior crosses can easily and definitely be determined in the A_1 offspring. Table 1 gives an example for the introduction of the BaYMV resistance from the variety Diana into the variety Sonja or into a breeders line, which was performed with the DH method using anther culture. The A_1 plants that regenerated from F_2 microspores were tested for virus resistance at the three- to four-leaf stage. The resistance segregated in the expected 1:1 ratio. From A_3 onwards, field tests of the resistant lines were performed by breeding companies.

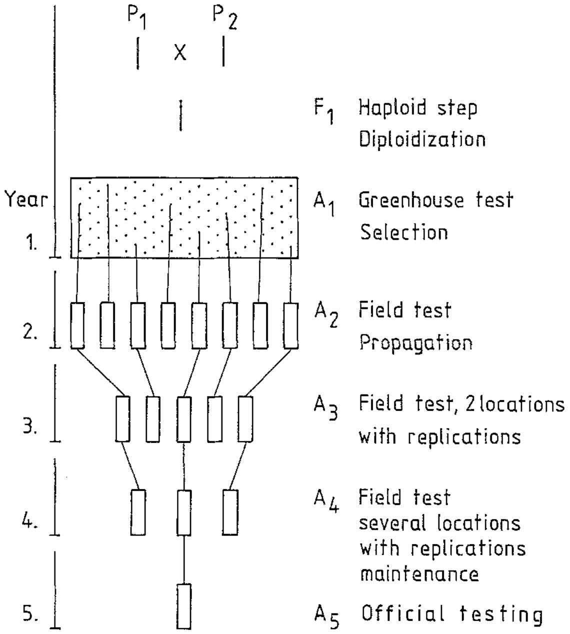

Fig. 1. Breeding scheme for the introduction of mono- or polygenically inherited traits from related varieties in inbreeders using a haploid step

2. The transfer of polygenically inherited traits from closely related genotypes

The aim of most breeding programs is to recombine complex characters, e.g., yield or different quantitatively inherited resistance, without drastic changes in the genetic background. To fulfill this requirement, the same breeding scheme shown in Fig. 1 was followed. The selection was started in the A_1, where it is more reliable and effective, due to the complete homozygosity. Table 1 shows, parallel to the data on the incorporation of the monogenic BaYMV resistance, the results of field tests for the expression of the complex, quantitatively inherited trait yield, where selection was performed in 3 consecutive years. The yield is stated in relation to standard varieties and, as expected, there is significant variation between the DH progenies. One and two lines, respectively, were selected as resistant against BaYMV and superior in yield and other agronomic characteristics. These lines are now in the official seed board test.

3. Introduction of monogenically inherited traits from exotic sources

There are alternative strategies for introducing a monogenically inherited character from an exotic source or an unrelated variety into an important cultivar using haploid techniques. One possibility is a conventional selection program until the F_3 or F_4. Here, several rounds of recombination can take place and plants carrying unde-

Table 1. Breeding of high-yielding, BaYMV-resistant winter barley lines using the DH technique

F_1 Cross	A_1		A_2 No.	A_3		A_4		A_5	
	No.	BaYMV res.		No.	Range of yields (%)	No.	Range of yields (%)	No.	Rel. yield (%)
Sonja x Diana	74	36	36	36	77–100[a]	6	92–102	1	**102**
Diana x 591.80	473	191	164	98	77–115[b]	15	91–109		
						2[c]	108 / 102		**OT**

[a] to Danilo (Lochow Petkus)
[b] to Sonate (Saatzucht Carsten)
[c] the two best lines from the 15 above
OT = official seed board test

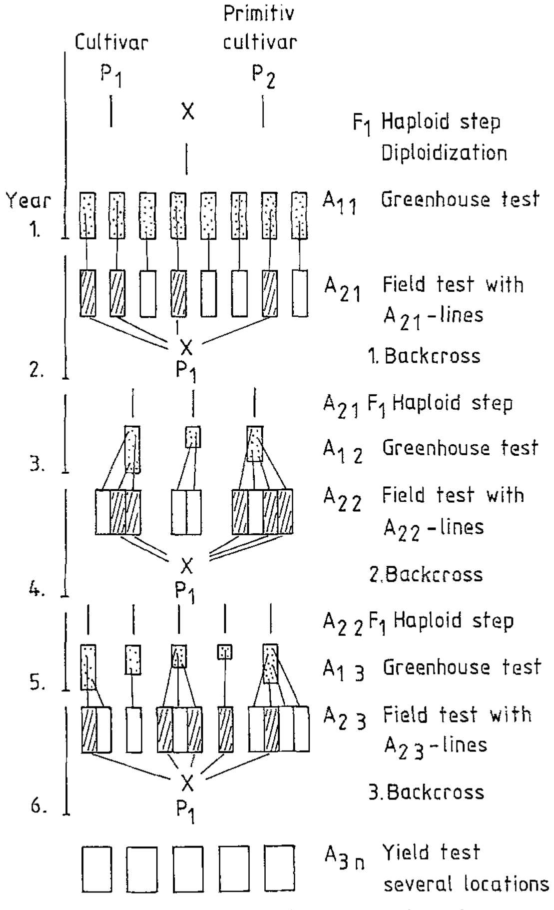

Fig. 2. Breeding scheme for the introduction of mono- or polygenically inherited traits from distantly related genotypes: recurrent selection alternating with haploid steps

sirable characters can be eliminated, thus increasing the chances of obtaining more valuable DH lines. On the other hand, such a preselection lasts at least 2 years and selection, even for qualitatively inherited characters, is often uncertain. Consequently, with wide crosses, it was thought advantageous to start the haploidization from F_1 hybrids.

Selection was made immediately in the A_1 or, at the latest, in the A_2. Since further androgenetic generations followed in this approach, these first DH generations were marked with the additional subscript 1, e.g., A_{11} or A_{21}. The selected A_{21} were backcrossed to an optimized new variety, resulting in a new F_1 from the cross $A_{21} \times P$, and this F_1 was used as donor material for a second DH step, giving the A_{12} which was selfed to the A_{22}. As Fig. 2 demonstrates these cycles have been repeated, to date, three times. Depending on the starting material, further cycles might be necessary. Since this backcrossing procedure combines recurrent selection with haploid cycles, it is named "recurrent selection alternating with haploid steps".

In the first DH generation (A_{11} or A_{21}), only a few lines resembled the cultivated type; consequently, strong selection pressure was applied. The first backcross was carried out with these best DH lines either immediately or after additional propagation and selection in the subsequent year. The selection pressure in the second cycle was not as strong as in the first one.

The incorporation of resistance to BaYMV from Japanese spring barley material into German winter barley cultivars demonstrates the use of recurrent selection alternating with haploid steps. Table 2 summarizes the results. First, only a few DH lines were selected from the F_1 (yml x Igri), because most of them carried negative characteristics from the nonadapted Japanese parent, such as lacking chilling resistance, high lodging, and high susceptibility to leaf diseases. The five best A_{21} lines were backcrossed with ten leading German varieties. From the F_1s about 1,500 DHs were regenerated as A_{12} lines and selected in the greenhouse. After selfing, the A_{22} lines were additionally screened in the field. The second backcross was carried out with 56 DH lines, followed by the third haploid step resulting in the A_{13} generation. Field tests in 1990, in four replications, will give the answer as to whether the developed resistant lines are already close enough to German cultivars or whether an additional cycle is necessary to achieve acceptable lines.

4. The introduction of polygenically inherited traits from less related genotypes

In this series of experiments the leaf diseases and TGW were recorded and evaluated in DH lines of a first backcross (A_{22}). The starting material was a cross between the high-yielding, BaYMV-susceptible, German winter barley variety Igri and the Japanese spring barley line ym1, resistant to BaYMV. From the first F_1, one DH line (W122 35.2) was selected in A_{21} with chilling resistance and virus resistance. This line was backcrossed to the two leading German varieties, Cosima and Harmonika. The BaYMV-resistant DH lines (A_{22}) from the F_1 hybrids of the crosses $A_{21} \times$ Cosima and $A_{21} \times$ Harmonika were grown in the field and investigated together with the starting material. The BaYMV-resistant line W122 35.2 was higher yielding than the parent ym1, but resistance against leaf diseases was relatively low. The varieties Cosima and Harmonika were more resistant and their TGW was higher. The DH progeny of the two crosses expressed a tremendous variability for both characters. There were lines with a combination of all the negative characters of the parents, low TGW, and high susceptibility. Some lines still carried the negative characters from ym1. But there were also DH lines that responded better for both characters than the best parent (Fig. 3). From these results it can be concluded that even only one opportunity for genetic recombination, leading to recombinant microspores in each F_1, is sufficient for a broad spectrum of variability.

Table 2. The introduction of BaYMV resistance in German winter barley lines using the method of recurrent selection alternating with haploid steps

Year	Generation	F_1 cross	DH lines	Breeding step
1984	$F_1{}^a$	1		Haploid step
1985	A_{11}		56	Selection in greenhouse
1986	A_{21}		20	Field-test
	$A_{21}F_1$	15	5	**1. Backcross**
1986/87				Haploid step
1987/88	A_{12}		1,565	Selection in greenhouse
1989	A_{22}		679	Field test
	$A_{22}F_1$	112	56	**2. Backcross**
1989/90				Haploid step Selection in greenhouse

[a] ym1 × Igri

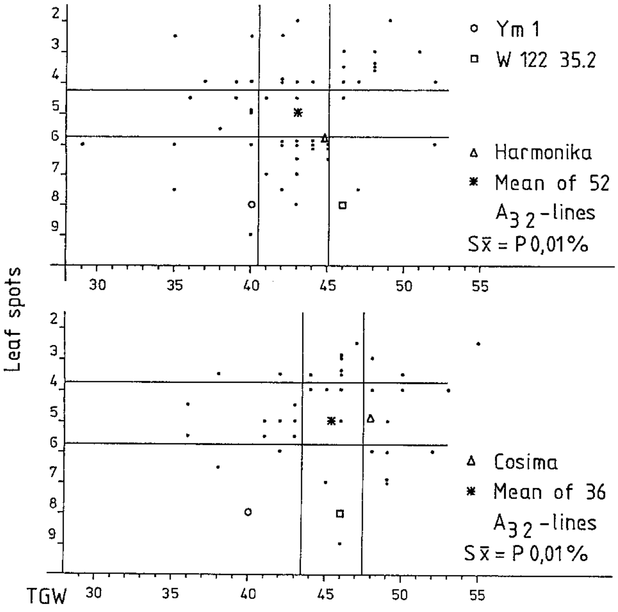

Fig. 3. Performance in a field trial of DH lines derived from recurrent selection alternating with haploid steps in comparison to the parents (leaf diseases and thousand-grain weight)

Discussion

The classical F_n generation corresponds to the A_1 in haploid production, and therefore selection in A_1 uncovers both the genotypic and phenotypic value even of additive genes, as long as they are not interacting with the environment. The necessary offspring size of DH populations for quantitative characters depends on the degree of linkage and on the number of genes involved. It was shown by Schwarzbach (1981) that using a single-seed descent (SSD) breeding procedure with 200 recombinant genotypes, one homozygous line will be found, with a probability of 68% for a characteristic coded by 48 independent genes. The SSD method is comparable to the haploid method, but in the SSD method efficient selection starts in the F_6, whereas with DH progeny, one can successfully select at A_2. Repeated rounds of recombination, e.g., in the F_2 or in the F_3, result in too much recombination between desired linked genes (Snape 1988). This is a great disadvantage of classical backcrossing, where possibly important characteristics are lost because the differences in the progeny are too small for effective selection.

Since in cereals such as barley, rice, and wheat, and also in a number of other crops like *Lolium* and *Brassica* the production of homozygous lines via haploids has advanced, DHs can be used in "combination breeding programs". For the incorporation of monogenic traits in breeding lines, the advantages of using haploids have already been discussed extensively. Here it has been demonstrated that haploids can increase the efficiency also for the most complex breeding problem: the transfer of quantitatively inherited traits from distant genotypes to cultivars. For such complex breeding programs a procedure has been developed combining recurrent selection, which guarantees variability, and haploid selection, which guarantees secure selection. This technique is called recurrent selection alternating with haploid steps.

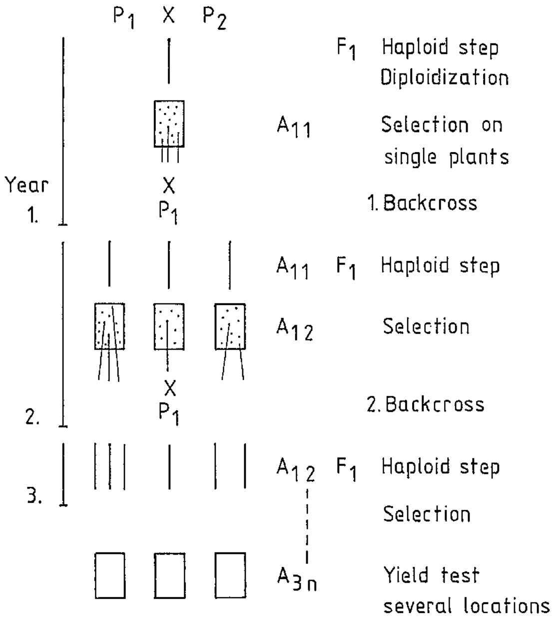

Fig. 4. Scheme for recurrent selection alternating with haploid steps using an early selection in the A_1, e.g., molecular markers

The degree of relationship of the two parents determines the number of backcrosses needed. Recurrent selection alternating with haploid steps leads to new homozygous lines, which can be tested for agronomic value in replicated field experiments. If positive selection pressure is possible in the A_1, e.g., with molecular markers for defined characters, the backcross can be done in this generation. The earlier an effective selection can be obtained, the shorter is the breeding time. If the selection can be done on a single plant of the A_1 without destroying this plant, then it will be possible to shorten the recurrent selection alternating with haploid steps as shown in Fig. 4. Such a strategy would speed up the breeding process even more than demonstrated with the present examples. This will be true also for other inbreeders such as wheat and rice, particularly since the production frequencies for haploids are still increasing.

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