NEW TECHNIQUES IN BACKCROSS BREEDING FOR RICE IMPROVEMENT

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Introduction

In the process of developing new cultivars well adapted to a specific environment, the genetic diversity of our breeding materials has gradually narrowed and some alleles available for future use are likely to be lost. Incorporation of useful alleles from less adapted materials becomes essential for expanding the genetic basis.

The backcross breeding technique has been extensively used to improve a specific attribute of a superior cultivar without spoiling any other aspects of performance, aiming at transferring a useful allele of a less adapted cultivar to a well adapted one. The allele to be transferred is designated as the target allele and its locus as the target locus. The backcross breeding is regarded as a kind of recurrent selection scheme, which involves recurrent cycles of selection for heterozygotes of the target locus accompanied with backcrosses to the recurrent parent.

One of the serious obstacles to overcome in a backcross program is an unfortunate pleiotropic effect of the target locus or the close genetic linkage between the target allele and alleles with unfavourable effects on any quantitative trait.

In the present report, some practical instances of the undesirable linkage around the target allele are presented and a biometrical model is proposed for evaluating the undesirable linkage. Efficient backcross systems are worked out by the use of a male sterility factor. Intercrossing among individual plants in an hybrid population is also suggested to dissipate the undesirable genetic linkage.

Undesirable genetic linkage around the target allele observed in *japonica* x *indica* crosses of rice

Some *indica* rice varieties provide us with useful alleles for blast resistance and have been used as donor parents in backcross programs for improving blast resistance of our cultivars. The undesirable linkage problems often occur in backcross breeding programs aiming at the incorporation of an *indica* allele for blast resistance. A few examples of this kind of linkage are presented here.

First backcross hybrid (B_1F_1) populations derived from *japonica* x *indica* crosses were subjected to inoculation of a blast fungus strain. Resistant and susceptible plants were grown in separate plots in order to observe differences in agronomic traits. As presented in Table 1, close associations were observed between blast resistance and late maturity in three of the seven crosses. Another investigation revealed that promising lines selected from the three crosses carried an identical allele for blast resistance (Fujimaki and Yokoo 1971). The other investigation using a pathogenic mutant of the blast fungus strain showed that various *indica* varieties, such as TKM 1, Co 25, Co 4, ADT 10, Badshabhog (those from India), Morak Sepilai, Kontor (from Malaysia), Leuang Tawng 77-12-5 and Chao Leuang 11 (from Thailand), carried the identical allele for blast resistance (Fujimaki and Yokoo 1971, Nagai *et al* 1973, Fujimaki 1974). The genetic association between blast resistance and late maturity was observed in various crosses with different *indica* varieties (Yokoo and Fujimaki 1971).

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Table 1. Relations between blast resistance and days to heading observed in the first backcross populations of *japonica* x *indica* crosses of rice.

Cross	Resistance to Ken 53–33	Days to heading		Total no. of plants	χ^2-value for uniformity
		Early	Late		
Norin 25 x Co 4	Resist.	20	64	84	
	Suscept.	36	31	67	14.30 **
	Total	56	95	151	
Morak Sepilai x Fujisaka 5	Resist.	19	8	27	
	Suscept.	71	2	73	15.48 **
	Total	90	10	100	
Fujisaka 5 x Kontor	Resist.	14	30	44	
	Suscept.	30	4	34	24.82 **
	Total	44	34	78	
Milek Kuning x Fujisaka 5	Resist.	13	32	45	
	Suscept.	27	38	65	1.84NS
	Total	40	70	110	
Norin 25 x Milek Kuning	Resist.	7	7	14	
	Suscept.	18	18	36	0 NS
	Total	25	25	50	
Norin 25 x Tjina	Resist.	9	8	17	
	Suscept.	13	16	29	0.28NS
	Total	22	24	46	
Norin 8 x Tjahaja	Resist.	17	18	35	
	Suscept.	48	60	108	0.18NS
	Total	65	78	143	

** : Significant at the 1% level, NS : Non-significant.

Taking these facts into account the problem imposed by the undesirable linkage does not seem to be solved by adopting a different donor variety, when a specific allele for resistance is to be transferred by backcrossing.

Another example of undesirable linkage was encountered in a breeding program which aimed at transferring an allele for stripe resistance of an *indica* variety to our cultivars. Resistance to stripe disease had been incorporated by a series of backcrosses. As the result of detailed investigations, however, most of the stripe resistant lines selected from *japonica* x *indica* crosses were found to have unsatisfactory grain quality caused by unusually slow ripening of some grains in a single panicle.

We could obtain experimental evidence of possible improvement for the grain quality of stripe resistant lines. An investigation was carried out to compare grain qualities of backcross-derived lines with those of lines which were developed from crosses between the backcross-derived lines and the other commercial cultivars. A remarkable improvement for grain quality was observed in the latter lines. This suggested that effective recombinations around the stripe

resistance locus were promoted in the process of the secondary selection.

A study on genetic linkage provided us with another evidence of possible improvement for grain quality of stripe resistant lines. The genetic relation between stripe resistance and the slow ripening attribute was investigated using F_5 lines randomly selected from the cross between a

Table 2. Slow ripening attribute appearing in stripe resistant lines derived from a *japonica* x *indica* cross.

Chugoku 51 x Nihonmasari

Ripening	Percentage of susceptible plants							Total no. of lines
	0	5	10	15	20	25	30	
Slow	77	13	9	3	7	2	1	112
Normal	14	22	22	34	26	20	4	142

χ^2-value for the uniformity test = 102.71 **

Chugoku 51 x Kankei 623

Ripening	Percentage of susceptible plants							Total no. of lines
	0	5	10	15	20	25	30	
Slow	46	5	0	3	3	0	0	57
Normal	4	94	15	13	24	4	3	72

χ^2-value for the uniformity test = 80.35 **

stripe resistant line and susceptible cultivars. The result was presented in Table 2. The stripe resistance was closely associated with the slow ripening attribute, but some promising (stripe resistant and uniform ripening) recombinants appeared.

The genetic linkage between the target allele and alleles affecting any undesirable agronomic traits limits the success of a backcross breeding program. It is preferable to detect and evaluate the undesirable linkage in as early a generation as possible and take appropriate measures to dissipate the undesirable linkage around the target allele.

Detection and evaluation of undesirable linkage

A biometrical model has been suggested to detect undesirable linkage around the target locus in a backcross program (Fujimaki and Comstock 1977). Normal diploid segregation, complete dominance of the target allele and absence of epistasis among loci are assumed to be present in the model building. A method for analyzing genetic variances among backcross-derived lines is developed based on the proposed model. Practical analysis of genetic variances among backcross-derived lines is conducted to evaluate the linkage effect around the target allele on several agronomic traits.

Recurrent cycles of backcrosses accompanied with selection for heterozygotes of the target locus are repeated t-times to obtain B_tF_1 hybrid plants. One half of B_tF_2 lines is expected to be segregating for the target locus and the other half to be true-bred. The same number of self-pollinated lines (say f) is randomly sampled from the segregating and the true-bred groups. These lines are grown in an appropriate experimental design (e. g. the randomized complete block design) with r replications.

The following linear model may be applied to the analysis of variance.

Table 3. A model of variance analysis for evaluating the linkage effect around the target allele.

Source of variation	Degree of freedom	Sum of squares	Variance	Expectation of variance
Replication	r−1	SSR	V_R	
Between groups	1	SSG	V_G	$\sigma^2 + r\sigma_f^2 + rf\sigma_g^2$
Lines within group	2(f−1)	SSF	V_F	$\sigma^2 + r\sigma_f^2$
Error	(r−1) (2f−1)	SSE	V_E	σ^2

$y_{ijk} = m + g_i + f_{ij} + r_k + e_{ijk}$

where y_{ijk} is the plot mean for the j-th line in the i-th group in the k-th replication, m a general mean effect, g_i the genetic effect of the i-th group, fij the genetic effect of the j-th line in the i-th group, r_k the effect of replication and e_{ijk} represents the error effect. The analysis of variance is made as presented in Table 3.

Biological interpretations of genetic variance components (σ_g^2 and σ_f^2) presented in the table are made clear by investigating their theoretically expected values. An allele *a* affecting a certain agronomic trait is assumed to be linked with the target allele *B* and their counterparts to be *A* and *b*. The genotypes of the donor and the recurrent parent are *aB/aB* and *Ab/Ab* respectively. The heterozygotes for the target locus (*B/b*) are selected and successively backcrossed to the recurrent parent. Genetic effects of the three genotypes for the *A* locus (*A/A, A/a, a/a*) on a quantitative agronomic trait are supposed to be 2u, u + au and 0, where "u" is an additive effect and "a" is a degree of dominance for the locus, and the recombination value between *A* and *B* be c. The frequencies of four genotypes appearing in the B_tF_1 generation and the mean genotypic values of B_tF_2 lines are presented in Table 4.

σ_g^2 represents a variance component due to genetic differences between groups and σ_f^2 is a component due to the genetic variability among lines in a group. The expected values of these components of genetic variance can be computed using the figures presented in Table 4.

$$\sigma_g^2 = \sum_{i=1}^{n} \left[\frac{1}{2}(1-2c_i)(1-c_i)^{t-1} \right]^2 (u_i - \frac{1}{2}a_i u_i)^2$$

$$\sigma_f^2 = \sum_{i=1}^{n} (1-c_i)^{t-1}[1-(1-c_i)^{t-1}(1-2c_i+2c_i^2)](u_i - \frac{1}{2}a_i u_i)^2$$

The total genetic variance of the B_tF_2 lines (σ_h^2) is easily shown to be equal to the sum of the above two genetic variance components. That is $\sigma_h^2 = \sigma_g^2 + \sigma_f^2$ It is clear from their expected values that σ_g^2 includes the pleiotropic effect of the target allele ($c_i = 0$) and the effects of alleles linked with it ($c_i < ½$). The effects of alleles independent of the target allele are included in σ_f^2 It is possible by the analysis of variance in Table 3 to separate and evaluate the two components of genetic variance.

This method was practically applied to the analysis of genetic variance of backcross lines derived from a hybrid population segregating for smoothness of rice plants. The attribute of smoothness is known to be advantageous for reducing dust in harvesting and threshing operations. The smoothness character is found in some cultivars from the Philippines and the U. S. A.

Table 4. Genotypic frequencies in B_tF_1 population and mean genotypic values of B_tF_2 lines.

Genotype	$\frac{aB}{Ab}$	$\frac{AB}{Ab}$	$\frac{ab}{Ab}$	$\frac{Ab}{Ab}$
Genotypic frequency in $B_{t-1}F_1$ for the target allele	$(1-c)^{t-1}$	$1-(1-c)^{t-1}$	0	0
Change of genotypic frequencies by backcrossing to the recurrent parent	$\frac{1}{2}(1-c)$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}(1-c)$
			$\frac{1}{2}c$	$\frac{1}{2}c$
Genotypic frequency in B_tF_1 population	$\frac{1}{2}(1-c)^t$	$\frac{1}{2}[1-(1-c)^t]$	$\frac{1}{2}c(1-c)^{t-1}$	$\frac{1}{2}[1-c(1-c)^{t-1}]$
Genotypic contribution of the A locus to B_tF_2 lines	$u+\frac{1}{2}au$	$2u$	$u+\frac{1}{2}au$	$2u$

c: Recombination value, t : No. of backcrosses, u and a : Additive effect and a degree of dominance of the A locus.

In order to incorporate an allele for smoothness of a U. S. cultivar into a Japanese one, the first backcross (B_1F_1) population was developed using the U. S. cultivar as a donor and the Japanese as a recurrent parent. Fifty plants were sampled at random from heterozygous and homozygous genotypes for the smoothness locus. These hybrid plants were self-pollinated to obtain B_1F_2 lines. The hundred backcross-derived lines were cultivated in the randomized complete block design with two replications. The observed traits were days to heading, culm length, panicle length, width of flag leaf, thickness of panicle neck and number of panicles.

The obtained data were analyzed by the method of variance analysis mentioned before. Regarding days to heading, culm length, panicle length and flag leaf width, the between group variance (V_G) was statistically significant at the 1% level. The within-group variance (V_F)

Fig. 1. Plant types of a self-fertile (left) and a male-sterile line (center) resulting from segregation for male sterility induced by chemical treatment and parental plant "Nihonmasari" (right).

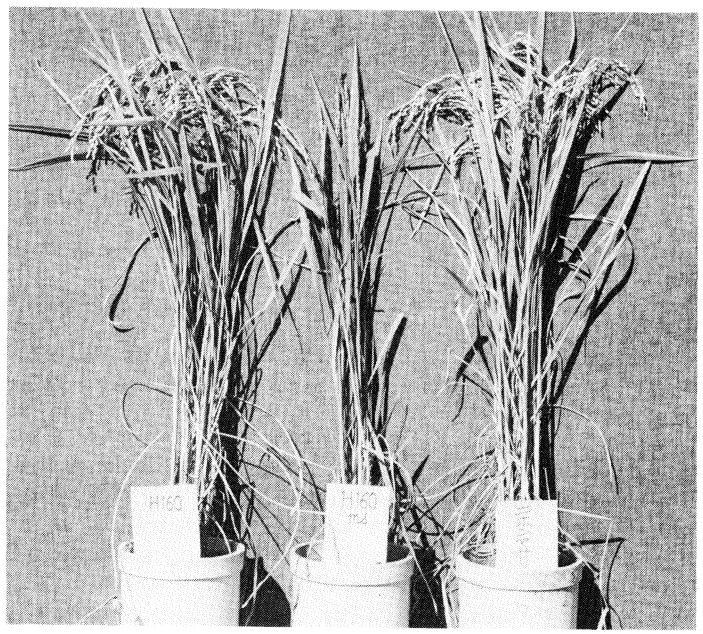

Fig. 2. Plant types of a self-fertile (left) and a male-sterile line (center) resulting from segregation for male sterility induced by irradiation and parent plant "Nihonmasari" (right).

Table 5. Estimated values of genetic variance components and heritabilities

Character	Variance component		Heritability		
	σ_g^2	σ_f^2	h^2	H	σ_g^2 / σ_f^2
Days to heading	1.6600**	33.110**	0.821	0.814	0.0477
Culm length	3.0173**	30.807**	0.708	0.688	0.0892
Panicle length	0.0581**	0.734**	0.681	0.665	0.0733
Flag leaf width	0.0464**	0.641**	0.739	0.725	0.0675
Panicle neck thickness	–	0.836**	0.630	0.630	–
No. of panicles	–	0.944**	0.450	0.450	–

Notes: $\sigma_h^2 = \sigma_g^2 + \sigma_f^2$, $h^2 = \sigma_h^2 / (\sigma^2 + \sigma_h^2)$, $H = \sigma_f^2 / \sigma_h^2$

was 1% significant for all characters. Estimated values of genetic variance components were computed and presented in Table 5. The components of genetic variance associated with the target allele were far smaller than those independent of it. Most parts of the genetic variance of B_1F_2 lines seemed to be due to the alleles independent of the allele for smoothness. The proportions of σ_g^2 to the total genetic variance σ_h^2 for the four characters, in which the between-group variances were significant, ranged from 4.77% to 8.92%. These values were very close to the theoretical values of genetic contribution of a single pair of linkage group, 4.16% to 8.33%, which were computed assuming that each pair of 12 linkage groups would equally affect the development of the character concerned.

The following conclusions would be drawn from the present results. Some alleles affecting the agronomic traits such as days to heading, culm length, panicle length and flag leaf width are considered to be genetically associated with the allele for smoothness. But the alleles affecting these traits will not be concentrated on the linkage group involving the smoothness locus. Therefore undesirable linkage will not adversely affect the progression of the backcross breeding program for incorporating the smoothness allele.

Breeding systems for backcrosses by the use of genetic male sterility

Jain and Suneson (1963) originally pointed out the utility of a male sterility factor for promoting outcrosses in a hybrid population of a self-pollinated crop. Breeding systems for recurrent selection of self-pollinated crops such as sorghum and soybean were proposed using a male sterility factor (Gilmore 1964, Doggett and Eberhart 1968, Brim and Stuber 1973). Models of backcross systems by the use of a male sterility factor were also suggested in relation to rice breeding (Fujimaki 1975). Genetic male sterility was artificially induced by gamma ray irradiation and a chemical mutagen ethyleneimine and shown to be useful for promoting backcrossing or intercrossing among plants in rice hybrid populations (Fujimaki *et al* 1977). Morphological appearances of these male sterile mutants are presented in Fig. 1 and Fig. 2. The male sterile plants induced by the chemical mutagen are as much vigorous as parental plants (Fig. 1) but those induced by irradiation are slightly less vigorous than their parents (Fig. 2).

In the present investigation three models for backcross systems were worked out by the use of a recessive allele for male sterility. A model for intercrossing is also suggested in relation to promoting genetic recombinations.

Backcross-breeding system Model I (Fig. 3)

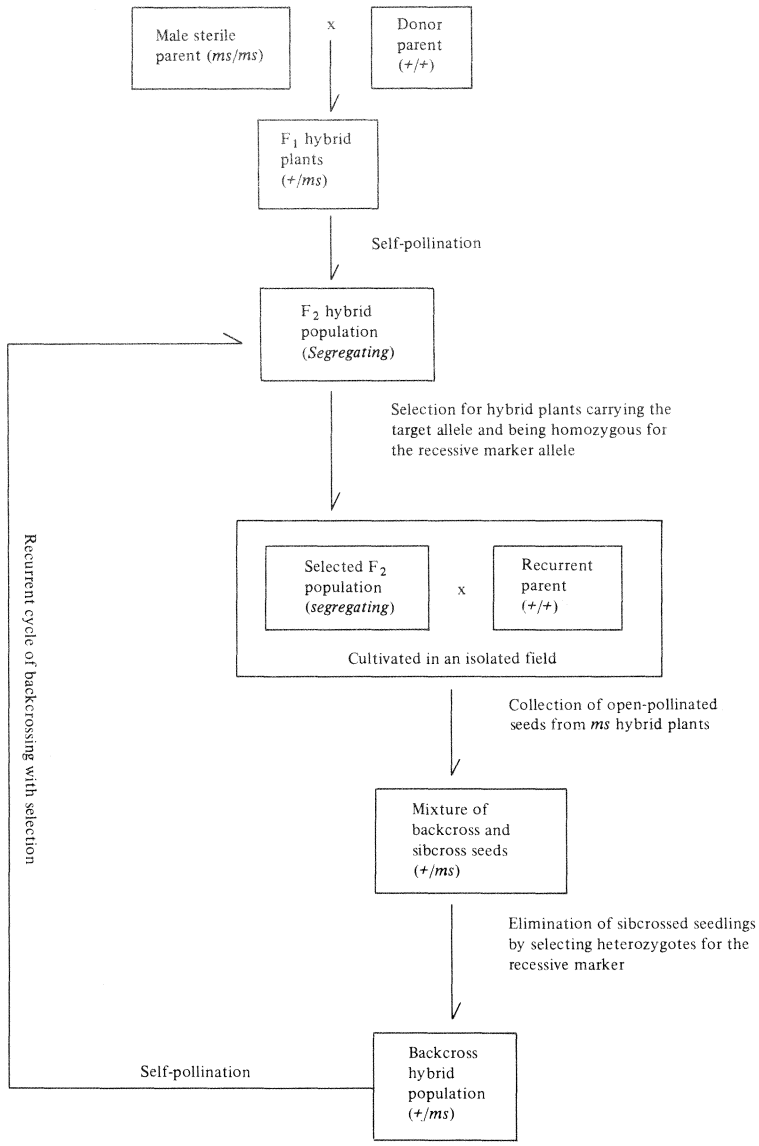

Fig. 3. Backcross breeding system Model I.

In this system a recurrent cycle for backcrossing requires two generations. Each backcross is followed by self-pollination and backcrosses advance every other generation. A recessive allele for a distinctive seedling marker character is effectively utilized to discriminate backcrossed seedlings from sibcrossed ones in the hybrids obtained by open-pollination. The recurrent procedures are advanced in the following steps.

(1) A male sterile line is crossed to a donor parent carrying the target allele.
(2) F_1 hybrid plants are self-pollinated to obtain a F_2 hybrid population.
(3) Hybrid plants carrying the target allele (homozygous or heterozygous) and being homozygous for the recessive marker allele are selected from the F_2 population.
(4) Selected F_2 plants are interplanted with the recurrent parent plants in an isolated field.

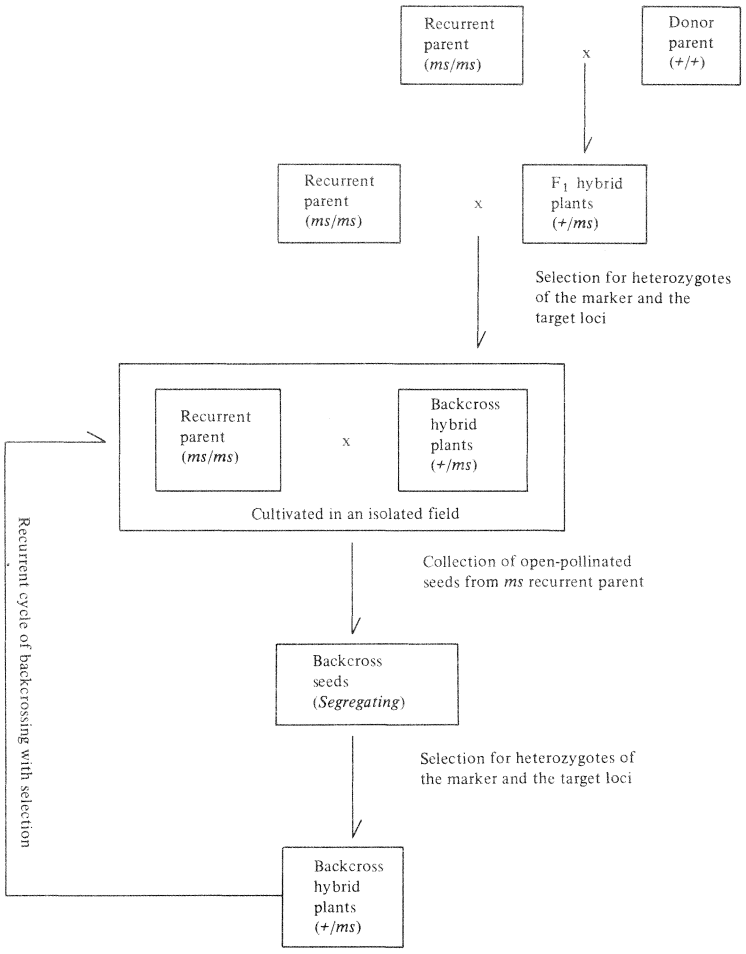

Fig. 4. Backcross breeding system Model II.

(5) Open pollinated seeds are collected from male sterile plants in the hybrid swarm. They are a mixture of backcross and sibcross products.
(6) Backcross seedlings are selected by eliminating plants homozygous for the recessive marker allele.
(7) Selected backcross plants are again self-pollinated to obtain another F_2 population.
(8) The recurrent cycle of selection and backcross followed by self-pollination is repeated until the genetic variation of the hybrid population gets sufficiently small.

Backcross-breeding system Model II (Fig. 4)

This model requires a recessive allele for a distinctive seedling marker character which is closely linked with the recessive allele for male sterility. Backcrosses proceed in every generation.
(1) A donor parent is hybridized with a recurrent parent which contains the male sterility allele and a closely linked recessive allele for the seedling marker character.
(2) The male sterile recurrent parent is interplanted between hybrid plant rows in an isolated field.
(3) Open-pollinated seeds are harvested from male sterile plants of the recurrent parent.

(4) Seedlings heterozygous for the male sterile allele are selected by eliminating homozygotes for the recessive marker and the selection is simultaneously applied for the target allele.
(5) Selected hybrid plants are cultivated with the male sterile recurrent parent and the cycles are repeated.

Backcross-breeding system Model II′ (Fig. 5)

Male sterile plants of the recurrent parent should be vegetatively propagated in the present model. Backcrosses can be repeated in successive generations.
(1) A donor parent is crossed with male sterile plants of a recurrent parent.
(2) Vegetatively propagated male sterile plants of the recurrent parent are interplanted between rows of hybrid plants in an isolated field.
(3) Open-pollinated seeds are collected from male sterile plants of the recurrent parent.
(4) Male sterile plants of the recurrent parent are interplanted in the backcross hybrid population under the isolated environment.

Backcrosses advance in alternate generations by the Model I but in successive generations by the Model II and II′. In this respect, the last two models are of higher efficiency than the first one in terms of time required for breeding.

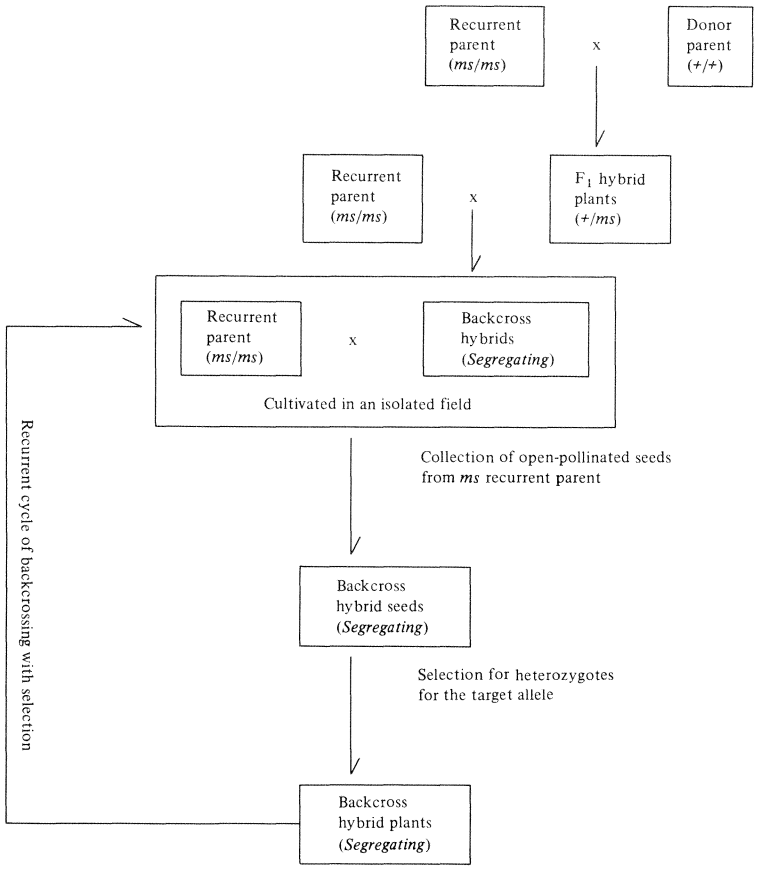

Fig. 5. Backcross breeding system Model II'

A remarkable merit of the Model I is that the target allele of a specific donor parent can be transferred to any arbitrary recipient cultivar because a male sterility factor involved in the non-parental materials is incorporated into the hybrid population and used for promoting backcrosses. This can not be attained by the Model II or II', in which the target allele is able to be transferred to a specific recipient cultivar carrying a factor for male sterility.

In any model of backcrossing the extent of outcrossing of male sterile plants is seriously affected by differences in heading time. It is essential for enhancing the efficiency of backcross to synchronize the heading time of hybrid plants with that of the recurrent parent concerned.

The selection for hybrid plants with the heading time of the recurrent parent will be very effective in the early segregating generations (e.g. B_1F_1 or B_2F_1). Once hybrid plants with the heading time of the recurrent parent are strictly selected for further backcrosses, the heading time of the hybrids will remarkably approach that of the recurrent parent. This selection seems to be one of the most promising ways to recover the heading time of the recurrent parent in the hybrid population.

Discussion

Genetic linkage is a serious problem relative to success in a backcross breeding program when alleles with unfavourable effects on any quantitative trait are closely associated with the target allele. Less adapted cultivars are often employed as donor parents. Such cultivars are supposed to have a number of undesirable alleles, some of which are probably linked with the target allele.

Backcrossing of hybrid plants to a recurrent parent holding the target locus heterozygous or intercrossing among individual plants in a hybrid population is theoretically shown to be highly effective to break up the initial linkage block around the target locus (Hanson 1959). Several experimental evidences have been also obtained to indicate the effectiveness of intercrossing to dissipate the undesirable linkage (Miller and Rawlings 1968, Meredith and Bridge 1971).

Intercrossing is accomplished in the following manner when a recessive male sterility factor is incorporated into a hybrid population.

(1) Any hybrid population segregating for male sterility is raised under an isolated environment.
(2) Open-pollinated seeds are collected from male sterile plants.
(3) The recurrent procedures are repeated in every generation.

In earlier backcross generations, the heading time of hybrid plants largely deviates from that of the recurrent parent. Therefore backcrosses should be made by hand pollination. As backcrosses proceed, however, the heading time of the recurrent parent is recovered in the hybrids. When the heading time of the hybrids is synchronized with that of the recurrent parent, the population is ready to be subjected to backcrossing or intercrossing by the use of male sterility.

Backcrossing enhances effective genetic recombinations especially around the target locus held heterozygous, but intercrossing induces effective recombinations in all the heterozygous linkage blocks as well as around the target locus. Backcrossing is preferable to intercrossing in the earlier generations when a lot of loci independent of the target locus are still heterozygous. Intercrossing becomes more effective in the later generations when most of independent loci get homozygous for the recurrent parent alleles. It should be noticed that intercrossing is advanced without selection and less labourious than backcrossing.

When undesirable linkage around the target locus is supposed to be a serious barrier to success in a backcross program, first several generations should be advanced through backcrossing. An appropriate number of intercrossing generations should follow in order to increase genetic recombination.

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Discussion

F. Kikuchi, Japan: What generation is most efficient for estimating the genetic linkage?

Answer: From the standpoint of practical breeding, the detection and the evaluation of genetic linkage should be done in as early a generation as possible. B_1F_1 which is the first backcross generation is the most desirable. However, the efficiency of the test for linkage detection increases in the later generations when the genetic variation due to alleles independent of the target allele is reduced by repeated backcrosses.

K Sakai, Japan: You have mentioned the effect of linkage between major genes, namely disease resistance genes and genes governing late maturity. Aren't you interested in linkage between polygenic characters and major gene characters?

Answer: At the beginning of the investigation, I was interested in the genetic linkage between the target allele with major effect and the alleles affecting the other quantitative agronomic traits. The variance analysis model presented here is mainly concerned with the evaluation of the undesirable linkage between a major gene and so-called polygenes. As my work proceeded, I became more interested in dissipating the undesirable traits.

J. T. Rao, India: Do you think you can effectively use irradiation methods for breaking the undesirable linkage?

Answer: In relation to the improvement of self-pollinated crops, genetic linkage will be more effectively broken up by manipulating the breeding system of a hybrid population than by using irradiation, because most of the effective recombinations are supposed to be restricted by inbreeding through self-pollination.

S. Sakaguchi, Japan:

1. How about the seed-set percentage of male sterile plants under field conditions?
2. Is the seed-set ability of male sterile lines sensitive to the environment, for example to the weather conditions?

Answer: As far as my male sterile mutants are concerned, the seed-set percentages were around several percent in the ordinary spacing (30 x 18 cm). The seed-set ability seemed to fluctuate depending on various environmental factors. However, I have no idea of the extent of environmental fluctuations of seed fertility, at the present time.

M. Kobayashi, Japan: Can we expect any epistatic effect by this backcrossing procedure?

Answer: Yes, we can expect epistatic gene actions. However, for convenience, I have ruled out the epistatic effect of gene actions when I designed the biometrical model for evaluating the undesirable linkage effect.