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Comparison of single seed descent and anther culture-derived lines of three single crosses of rice

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Summary. Distribution parameters (mean, variance, skewness and kurtosis) of 12 quantitative traits were evaluated for inbred lines generated through single seed descent (SSD) and anther culture (AC) of two japonica × japonica hybrids and one japonica × indica hybrid of rice. For most of the traits the data were normally distributed, and the means and variances were found to be identical for SSD- and AC-derived lines of the given hybrids. However, for some other traits, differences between the two population types were observed, mainly in the lines derived from the intra-japonica crosses. A tentative explanation for these differences is given. Nevertheless, our results suggest that SSD and AC are equally effective breeding techniques for producing agronomically useful lines of rice.

Key words: *Oryza sativa* – Anther culture – Single seed descent – Inbred lines

Introduction

The first doubled-haploid (DH) rice lines were obtained by Niizeki and Oono in 1968. These results proved to be of major interest for the improvement of this autogamous species. The time saved by using doubled haploidy for the fixation of pure lines has encouraged many teams to develop and improve this now widespread and routinely used technique (Zapata et al. 1983; Pulver and Jennings 1986; Guiderdoni et al. 1986; Chung 1987; Huang et al. 1988; Zhang 1989).

The technique is currently well established, and substantial number of doubled-haploid lines have been obtained. Genetic studies can now be carried out to determine whether material produced by doubled haploidy is equivalent to that produced by conventional breeding techniques. In this context, the single seed descent procedure can be used for comparison since it also involves selection only when the lines are fully fixed genetically.

Theoretical studies (Snape 1976; Jinks and Pooni 1981; Snape and Simpson 1981) have provided information on the comparative value of lines obtained by doubled haploidy and single seed descent. These studies have revealed that, in random samples, the potential superiority of either of the two techniques is dependent on the genetic control of the studied trait. Two genetic parameters which can affect the distribution of inbred lines are linkage disequilibrium and additive × additive epistasis between genes controlling a trait. The distributions of DH and SSD lines obtained from a cross are theoretically identical, except when linkage disequilibrium occurs. Epistasis can also modify the shape of phenotypical distributions, – in particular, the ranking of the inbred line means relative to the mid-parental values – but such modifications are identical for the two line sources. Conversely, the means and variances of DH and SSD lines can differ with linkage disequilibrium. The direction of the difference depends on the initial parental configuration: the initial linkage phase (predominance of coupling or repulsion pairs) determines the variance ranking; the dominant type of epistasis (complementary or duplicate) and the predominant linkage phase of these interacting genes determine the ranking of the means.

Field comparisons of DH and SSD lines have been made for different quantitative traits in some species. No differences were observed between the two techniques by Park et al. (1976), Choo et al. (1982) in barley and Henry et al. (1988) in wheat. Various other investigators have observed only a few differences, usually ones concerning the means more than the variances (Turcotte et al. 1980 and Powell et al. 1986, in barley; Mitchell et al. 1988 and Senghor 1990, in wheat; Charmet and Branlard 1985, in

triticale; Jinks et al.1985, in tobacco; Kubba et al. 1989, in Brussels sprouts). Such differences were frequently attributed to linkage disequilibrium between epistatic genes. The observations of Schnell et al. (1980) in tobacco and Morden et al. (1989) in barley are exceptions as they indicate a systematic inferiority of DH lines whose exact origin is currently unknown.

No reports are available on a comparison of DH and SSD lines in rice. The objective of the study reported here was to assess the variability of DH and SSD lines derived from three rice crosses for several quantitative traits.

Material and methods

Plant material

The inbred lines used in this study were obtained in Guadeloupe from three crosses:

– two japonica × japonica F_1 hybrids involving genetically close parental pure lines of different geographical origins (Table 1): IAC × MM ('IAC 47' × 'Mutant of Makouta') and 177 × MM ('IRAT 177' × 'Mutant of Makouta').

– a japonica × indica F_1 hybrid: 177 × AP ('IRAT 177' × 'Apura').

Production and sampling of the inbred lines

The DH lines, which arose by spontaneous doubling, were generated through in vitro anther culture from F_1 donors according to the technique previously described by Guiderdoni et al. (1986). The SSD lines were advanced in isolated plots in the field, up to the F_6 generation for japonica × japonica crosses and the F_7 generation for the japonica × indica cross.

The SSD lines were randomly chosen, whereas the DH lines were sorted. A callus step is usually necessary in rice anther culture. Natural fragmentation of some calli may occur, which can lead to the production of identical lines if the fragments are subcultured independently (Mercy and Zapata 1987; Guiderdoni et al. 1989). This phenomenon can bias the genetic analysis. The supposed clones were thus identified using either multivariate techniques (dynamic clusters on quantitative traits measured during the field trials) for japonica × japonica crosses or qualitative data (enzymatic and morphophysiological traits) for the japonica × indica cross, and then discarded (Courtois 1991). The elimination of the clones did not substantially modify the population means and only slightly increased variances. The final population sizes are shown in Table 2.

Field experiments

Three trials, one per cross, were carried out under upland conditions using a block design with two replicates, with the parents, as checks, inserted alternatively every two randomised inbred lines. The basic plot consisted of three 2-m long, 0.3-m spaced rows, with plants spaced 10 cm apart within rows. The observations were recorded on the 19 plants of the central row excluding border plants at each end of the row.

Traits

The 12 quantitative traits studied are listed in Table 3. Most of these traits are of agronomic interest, and some are yield components. Leaf shape was useful in differentiating indica and japonica types.

Table 1. Origin, isozymic group and classification of the parental varieties of the three crosses

Genotype	Geographical origin	Isozyme group[a]	Genetical group
IAC 47 (IAC)	Brazil	VI	Japonica
Mutant of Makouta (MM)	Ivory Coast	VI	Japonica
IRAT 177 (177)	French Guyana	VI	Japonica
Apura (AP)	Surinam	I	Indica

[a] According to Glaszmann (1987)

Table 2. Size of the populations included in this study

Cross	Number of DH lines	Number of SSD lines	SSD generation
IAC × MM[a]	45	60	F_6
177 × MM[b]	66	77	F_6
177 × AP	59	60	F_7

[a] Reduced to 30 DH and 35 SSD lines for 2 traits (NSP, STR), due to incidents during harvesting

[b] Reduced to 61 DH and 72 SSD lines for 3 traits (GLG, GWD, TWD) and 60 DH and 70 SSD for 3 other traits (NSP, STR, YLD)

Table 3. List of the quantitative traits and sample size for these measurements

Traits	Abbreviation	Number of measurements per plot
Heading date (d)	HDT	1
Number of panicles per plant	NPP	10
Plant height (cm)	HGT	10
Second underpanicle leaf length (cm)	LLG	10
Second underpanicle leaf width (cm)	LWD	10
Main panicle length (cm)	PLG	10
Grain length (mm)	GLG	10
Grain width (mm)	GWD	10
Thousand grain weight (g)	TGW	1
Number of spikelets per panicle	NSP	3
Sterility (%)	STR	3
Plot yield (g)	YLD	1

Statistical analysis

Sterility percentages were transformed with the arcsine square root transformation before analysis. The distributions of each quantitative trait were tested for normality by comparison of the Pearson's coefficients of skewness and kurtosis with the critical values defined by Pearson and Hartley (1966).

For traits recorded on an individual plant basis, an analysis of variance was separately performed for both DH and SSD lines based on a mixed model with a fixed block effect and random genotypical, interaction and residual effects. For traits recorded on a whole plot basis, the analysis of variance was limited to the first three effects. The respective genotypical,

interaction and residual variances of the two sets of lines were then compared using three different Fisher tests (ratios of the genotypical, interaction and residual mean squares). The global variance comparison was made at the 5% significance level, which resulted in the three tests being made approximately at the 1% significance level.

For normal distributions and when variances were the same, a two-tailed Student's t test was used to compare the means of DH and SSD lines. When variances differed, a modified Student test (Dagnelie 1975) was applied. For non-Gaussian distributions, the Mann and Whitney non-parametric rank test was performed to compare DH and SSD line distributions. The means of the two sets of lines were compared to the mid-parental value using scaling tests (Mather and Jinks 1971). Significance levels were set at 5% for all of these comparisons.

Results and discussion

Means and standard deviations per trait for the DH and SSD inbred lines as well as mid-parental values for the three crosses are given in Table 4. Distributions were found to be normal, as determined according to Pearson's coefficients of skewness and kurtosis, except for a few traits, notably sterility, even after data transformation. However, a complementary analysis of the frequency distribution histograms indicated a tendency to bimodality for some traits such as plant height, leaf length and width, specifically concerning the two crosses with 'Mutant of Makouta'.

The analyses of variance, performed separately for DH and SSD lines revealed a highly significant genotypical variability for all but 2 traits: the tillering ability and yield in the DH lines of the IAC × MM cross. However, a clear genetic variance was noted for these 2 traits in the SSD lines. Thus, there were probably allelic differences between the parents for all traits, even when the phenotypes seemed identical.

Comparisons of the genotypical, interaction and residual mean squares for the DH and SSD lines revealed some rare significant differences (Table 5). This concerned 4 traits, but genotypic variance differences were involved in only 2 i.e. leaf width and grain width of the 177 × MM cross. The other differences concerned residual variances with, in most cases, higher variances in the SSD lines than in the DH lines.

The means of the two groups of inbred lines were mostly identical (Table 6). However, there were some cases of differences between the means of the DH and SSD lines or between one of them and the mid-parental value.

The most plausible explanation of these results concerns the genetic control of the trait under consideration. According to the theories developed by Snape (1976), Snape and Simpson (1981) and Jinks and Pooni (1981), the different categories of the situations numbered in Table 6 can be explained as follows:

Table 4. Mean values for mid-parent, DH and SSD samples for the three crosses

Trait	IAC × MM			177 × MM			177 × AP		
	Mid-parental value	DH Mean ± SD	SSD Mean ± SD	Mid-parental value	DH Mean ± SD	SSD Mean ± SD	Mid-parental vaue	DH Mean ± SD	SSD Mean ± SD
HDT	125	125 ± 4	123 ± 4	124	125 ± 3	125 ± 3	145	140 ± 8	141 ± 7
NPP	4.9	4.8 ± 0.6	4.8 ± 0.6	5.6	5.8 ± 0.7	5.6 ± 0.8	6.8	6.5 ± 1.2	7.0 ± 1.3
HGT	110	108 ±13	111 ±12	115	112 ±15	118 ±13	105	102 ±11	103 ±10
LLG	55.4	54.1 ± 5.4	55.3 ± 5.5	57.2	57.7 ± 5.1	58.5 ± 5.6	57.5	56.4 ± 8.0	56.3 ± 7.5
LWD	16.3	16.4 ± 1.1	16.9 ± 1.2	16.3	16.7 ± 1.1	16.8 ± 0.8	11.8	12.5 ± 2.0	11.9 ± 1.8
PLG	23.0	22.6 ± 1.5	23.4 ± 1.2	23.6	23.5 ± 1.7	24.4 ± 1.3	25.5	24.1 ± 2.2	23.8 ± 1.9
GLG	9.76	9.60 ± 0.37	9.81 ± 0.33	10.06	9.94 ± 0.35	10.13 ± 0.32	10.95	10.40 ± 0.59	10.53 ± 0.51
GWD	3.36	3.52 ± 0.24	3.41 ± 0.22	3.45	3.40 ± 0.18	3.40 ± 0.12	2.86	2.87 ± 0.18	2.86 ± 0.19
TGW	37.0	38.5 ± 2.4	37.9 ± 2.3	43.5	42.2 ± 2.5	43.1 ± 2.3	33.0	32.6 ± 3.3	33.0 ± 3.3
NSP	105	113 ±15	128 ±24	122	121 ±24	121 ±22	111	129 ±37	122 ±30
STR	14	13 ± 9	13 ± 9	8	8 ± 8	9 ± 9	19	29 ±17	25 ±13
YLD	107	102 ±20	119 ±18	155	156 ±38	158 ±37	109	96 ±34	103 ±27

SD, Standard deviation

Table 5. F values for variances for DH and SSD lines

Trait	IAC × MM			177 × MM			177 × AP		
	GMS	IMS	EMS	GMS	IMS	EMS	GMS	IMS	EMS
HDT	1.14	1.23	–	1.19	1.30	–	1.42	1.25	–
NPP	1.04	1.06	1.06	1.29	1.26	1.00	1.29	1.38	1.17**
HGT	1.10	1.71	1.15	1.22	1.08	1.33**	1.28	1.65	1.18**
LLG	1.02	1.85	1.06	1.21	1.26	1.10	1.13	1.39	1.05
LWD	1.25	1.31	1.09	1.82**	1.17	1.02	1.28	1.08	1.14
PLG	1.58	1.04	1.09	1.65	1.08	1.11	1.36	1.02	1.24**
GLG	1.26	1.38	1.02	1.19	1.33	1.85**	1.37	1.07	1.01
GWD	1.24	1.62	1.09	2.06**	1.77	1.48**	1.13	1.60	1.01
TGW	1.04	1.81	–	1.12	1.79	–	1.03	1.58	–
NSP	2.32	1.07	1.00	1.25	1.46	1.30	1.53	1.06	1.16
STR[a]	1.02	1.02	1.32	1.26	1.11	1.13	1.59	1.00	1.22
YLD	1.00	1.18	–	1.02	1.44	–	1.56	1.45	–

*,** P significant at 5% or 1% level, respectively
GMS, ratio of the DH versus SSD genotypic mean squares; IMS, ratio of the DH versus SSD interaction mean squares; EMS, ratio of the DH versus SSD error mean squares
[a] Tests on angular transformed data

Table 6. Mean comparisons. Summary of the different situations observed

	IAC × MM	177 × MM	177 × AP
$+\overline{DH}=\overline{SSD}$			
1) $\overline{DH}=\overline{SSD}=$mpv with σ^2_G DH $=\sigma^2_G$ SSD	NPP, HGT, LLG, STR	NPP, LLG, NSP, STR, YLD	HGT, LLG, LWD, PLG, GLG, GWD, TGW, NSP, YLD
2) $\overline{DH}=\overline{SSD}=$mpv with σ^2_G DH $\neq\sigma^2_G$ SSD	–	LWD, GWD	
3) $\overline{DH}=\overline{SSD}\neq$mpv with σ^2_G DH $=\sigma^2_G$ SSD	TGW	HDT	HDT
$+\overline{DH}\neq\overline{SSD}$			
4) $\overline{DH}=$mpv$>\overline{SSD}$	HDT	–	–
or $\overline{DH}=$mpv$<\overline{SSD}$	LWD, NSP, YLD	–	–
5) $\overline{DH}>$mpv$=\overline{SSD}$	GWD	–	–
or $\overline{DH}<$mpv$=\overline{SSD}$	–	TGW	–
6) $\overline{DH}\leqq$mpv$\leqq\overline{SSD}$	PLG, GLG	HGT, PLG, GLG	NPP

mpv, Mid-parental value; $\overline{DH}$, DH mean; $\overline{SSD}$, SSD mean; σ^2_G, genotypic variance
=, Difference not significant at 5% level; >, difference significant at 5% level; $\leqq$, not significantly different from the last in rank, but significantly different from the last but one

1) There were no significant differences in the means and genotypic variances between the two sets of inbred lines, and the means were equal to the mid-parental value: this corresponds to the simplest case of no detectable epistasis and linkage equilibrium in genes controlling the trait.

2) The situation was identical to (1) for the means, but the genotypic variances of the two groups of lines differed: this could be evidence of a linkage disequilibrium in the contributions of coupling and repulsion pairs in the absence of non-allelic interactions. For the two traits concerned, variance in the DH lines was higher than in the SSD lines, which suggests a preponderence of genes in the coupling phase. The extraction of DH lines from F_1 hybrids, which results in maximizing the genetic variance while saving the greatest amount of time, appears to be justified for this specific genetic configuration.

3) The means of the DH and SSD lines, themselves identical, differed from the mid-parental value without any variance differences: this could be due to epistasis without linkage disequilibrium.

4, 5 and 6 pooled) The means of the DH and SSD lines were significantly different, with the mean of one of these groups of lines differing from the mid-parental val-

ue: this may indicate a linkage disequilibrium between epistatic genes. The only unambiguous situation (where the means of the DH and SSD lines and the mid-parental value were all significantly different) was not observed. In this group of situations, the means of the DH lines were, in most cases, lower than the means of the SSD lines. According to Jinks and Pooni (1981), this supposedly occurs if an excess of coupling pairs is associated with duplicate interactions or if an excess of repulsion pairs is associated with complementary interactions. With respect to traits for which the phenotypes of the two parents are markedly different, which is generally the case for traits under selection, the hypothesis of an excess of coupling pairs, and therefore duplicate interactions, would be the most plausible. Thus the extraction of DH lines from F_1 hybrids would be justifiable.

In terms of the frequency of each case, situations with no detectable gene interaction or disequilibrium were the most common. Situations solely involving allelic interactions seemed to occur rarely and only concerned specific traits (heading date and thousand grain weight), as did situations involving only linkage disequilibrium. However, linkage disequilibrium between interacting genes occurred more frequently. There is no way to bear out such a partition in the present data, but a confirmation of the genetic effects involved could be obtained by comparing the means and variances of DH lines derived from two different generations, as demonstrated by Snape and Simpson (1986). The method developed by Choo and Reinbergs (1982), based on the analysis of skewness and kurtosis coefficients, could be another way of detecting the presence of additive × additive epistasis, but a large sample size and an absence of genotype × environment interaction (Pooni et al. 1977) are required.

Quantitative genetic models were therefore probably the most appropriate for explaining the observed situations. Nevertheless, to apply these models certain underlying assumptions must be checked because their failure could also affect distributions. These assumptions include: absence of selection (gametic or genotypic), and thus randomness of the sample, and identical effects of all genes.

First, the observed differences could have arisen from sampling problems within DH lines that resulted either from limited sample sizes due to poor anther culture yields or from cloning problems caused by calli fragmentation that could have been responsible for a residual bias in the sample despite our efforts to get rid of the clones. This eventual bias concerns only the japonica × japonica crosses, since the identification techniques using qualitative data enabled the elimination of all of the clones in the distant cross.

With respect to gametic selection, experiments with rice carried out by Chen et al. (1982), Chen et al. (1983), Siva Reddy (1987), Chung (1987) and Courtois (1991) using morphological markers indicated that gametic selection during the anther culture process either did not occur or was undetectable in all of the varietal groups studied. Conversely, using molecular markers, Guiderdoni et al. (1989) and Guiderdoni (1991) detected gametic selection, independent of the distortions generated by distant hybridization, in DH lines derived from indica × japonica hybrids; this gametic selection had an apparent neutral effect with regards to the indica/japonica differentiation. However, the japonica × indica cross showed the smallest number of differences in this study.

The hypothesis of identical additive effects for all genes is not necessarily justified. The expression of a trait could be the result of the combined action of a large number of minor genes with weak effects and a few major genes with strong effects. For instance, the observed differences in means for plant height and panicle length, traits which were probably pleiotropically linked in these crosses (Courtois 1991), could be due to the presence of a major recessive gene controlling the average height of 'Mutant of Makouta' and 'IRAT 177' (Clément and Poisson 1987). This would explain why height distributions, although continuous, were not perfectly unimodal in the IAC × MM and 177 × MM crosses. Undetected major genes might also interfere with the distributions of other traits.

Although the material was obtained after several selfing rounds (F_6 generation for close crosses, F_7 for the remote cross), residual heterozygosity might also occur in SSD lines in the 177 × AP cross. This hypothesis could be supported by the heterogeneity sometimes observed with morphological and enzymatic qualitative traits in this cross (Courtois 1991) and by the residual variances, which were slightly higher for some traits in SSD lines than in DH lines. The persistence of a significantly higher heterozygosity than should occur at this stage of breeding could be explained by cross-pollinations that could have taken place during selfing of the SSD lines. These cross-pollinations were possibly facilitated by the important sterility phenomena; these were observed up to the late generations and were partly due to the genetic distance between parents. However, distribution differences were minimal in the 177 × AP cross, which puts the importance of this factor in true perspective. The rapidity of the fixation of the two japonica × japonica crosses and their normal fertility demonstrated that such a hypothesis could not apply to these crosses.

Other factors might potentially contribute to differences between DH and SSD lines, such as the occurrence of genetic variations during the rice anther culture process where a de-differentiated callus step increases risks (Oono 1983; Schaeffer 1982; Schaeffer et al. 1984; Zakri 1986). However, this hypothesis is unverifiable in our study since variations were not separable from the overall hybridization-induced variability.

Some of these factors (residual heterozygosity within the SSD lines, variations) should be expressed by an apparent lower vigour in DH lines. When differences were observed, the DH line means were generally inferior to the SSD line means. However, no clear conclusions could be drawn on this trend due to the limited number of these cases.

The observed differences between the two sets of inbred lines seemed to be associated with the type of cross, with the most frequent abnormalities resulting from japonica × japonica crosses. This could have resulted from a sampling problem since the number of crosses studied was low. Hence, further studies should now be carried out with a greater number of intra- and inter-subspecific crosses.

Conclusion

We observed very few differences between rice lines produced by the two techniques. When differences were observed, several elements indicated that they probably resulted from the genetic control of the traits, from the presence of major genes and from residual heterozygosity (for the distant cross). This confirms previous results in other species. Assuming that these results are representative and can be further extended to a greater number of crosses, we consider that doubled haploidy and single seed descent are equally effective techniques in rice to obtain high-performance lines from single crosses.

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References

Charmet G, Branlard G (1985) A comparison of androgenetic doubled-haploid and single seed descent in triticale. Theor Appl Genet 71:193–200

Chen CC, Chiu WL, Yu LJ, Ren SS, Yu WJ (1983) Genetic analysis of anther-derived plants of rice: independent assortment of unlinked genes. Can J Genet Cytol 25:324–328

Chen CM, Chen CC, Lin MH (1982) Genetic analysis of anther derived plants of rice. J Hered 73:49–52

Choo TM, Reinbergs E (1982) Analyses of skewness and kurtosis for detecting gene interaction in a doubled-haploid population. Crop Sci 22:231–234

Choo TM, Reinbergs E, Park SJ (1982) Comparison of frequency distribution of doubled-haploid and single seed descent lines in barley. Theor Appl Genet 61:215–218

Chung GS (1987) Application of anther culture techniques for rice improvement. In: Proc Korea-China Plant Tissue Cult Symp. Korean Soc Plant Tissue Cult, Agric Sci Inst, Inst Bot, Acad Sin, Suweon, Korea, pp 36–55

Clement G, Poisson C (1987) Etude du déterminisme génétique de la «taille moyenne» de six mutants induits de riz. Doc interne CIRAD-IRAT

Courtois B (1991) Variabilité des descendances de trois croisements de riz (*Oryza sativa* L.) fixées par haplodiploïdisation et par filiation unipare. Thèse de Docteur Ingénieur, Université de Montpellier, France

Dagnelie P (1975) Théories et méthodes statistiques. Applications agronomiques, vol 2. Presses Agronomiques de Gembloux, Belgique

Glaszmann JC (1987) Isozymes and classification of Asian rice varieties. Theor Appl Genet 74:21–30

Guiderdoni E (1991) Gametic selection in anther cultures of rice (*Oryza sativa* L.). Theor Appl Genet 81:406–412

Guiderdoni E, Courtois B, Dechanet R, Feldmann P (1986) La production de lignées haploïdes doublées de riz par culture d'anthères in vitro. L'Agron Trop 41:250–258

Guiderdoni E, Glaszmann JC, Courtois B (1989) Segregation of 12 isozymes genes among doubled haploid lines derived from a japonica × indica cross of rice. Euphytica 42:45–55

Henry Y, De Buyser J, Agache S, Parker BB, Snape JW (1988) Comparisons of methods of haploid production and performance of wheat lines produced by doubled haploidy and single seed descent. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Cambridge, pp 1087–1092

Huang CS, Tsay HS, Chern CG, Chen CC, Yeh CC, Tseng TH (1988) Japonica rice breeding using anther culture. J Agric Res China 37:1–8

Jinks JL, Chowdury MKU, Pooni HS (1985) Comparison of the inbred lines derived from a hybrid of tobacco (burley × flue cured) by dihaploidy and single seed descent. Heredity 55:125–133

Jinks JL, Pooni HS (1981) Properties of pure breeding lines produced by dihaploidy, single seed descent and pedigree breeding. Heredity 46:391–395

Kubba J, Smith BM, Ockendon DJ, Setter AP, Werner CP, Kearsey MJ (1989) A comparison of anther culture-derived material with single seed descent in Brussel sprouts (*Brassica oleracea,* var 'gemmifera'). Heredity 63:89–95

Mather K, Jinks JL (1971) Biometrical genetics, 3rd edn. Chapman and Hall, London

Mercy ST, Zapata FJ (1987) Initiation of androgenesis in rice (*Oryza sativa* var. 'Taipeï 309'). Proc Indian Natl Acad B53:253–258

Mitchell MJ, Busch RH, Rines HW (1988) A comparison of anther-derived doubled-haploid lines and single seed descent lines in spring wheat. Agron Abstr 1988 Annu Meet Am Soc Agron, p 8

Morden LP, Rossnagel BG, Kao KN (1989) Performance of anther culture-derived breeding lines of barley versus lines developed by pedigree, single seed descent and the *Hordeum bulbosum* techniques – field comparisons. Can J Plant Sci 69:546

Niizeki H, Oono K (1968) Induction of haploid rice plants from anther culture. Proc Jpn Acad 44:554–557

Oono K (1983) Genetic variability in rice plants regenerated from cell culture. In: Institute of Genetics, Acad Sin and IRRI (eds) Proc Symp Tissue Culture Tech Cereal Crops Improv. Beijing, China, pp 95–105

Park SJ, Walsh E, Reinbergs E, Song SP, Kasha KJ (1976) Field performance of doubled-haploid barley lines in comparison with lines developed by the pedigree and single seed descent methods. Can J Plant Sci 56:467–474

Pearson ES, Hartley HO (1966) Biometrika tables for statisticians (vol 1). University Press, Cambridge

Pooni HS, Jinks JL, Cornish MA (1977) The causes and consequences of non-normality in predicting the properties of recombinant inbred lines. Heredity 38:329–338

Powell W, Caligari PSD, Thomas WTB (1986) Comparison of spring barley lines produced by single seed descent, pedigree inbreeding and doubled haploidy. Plant Breed 97:138–146

Pulver EL, Jennings PR (1986) Application of anther culture to high volume rice breeding. In: Rice genetics. IRRI, Manila, pp 811–820

Schaeffer GW (1982) Recovery of heritable variability in anther-derived doubled-haploid rice. Crop Sci 22:1160–1164

Schaeffer GW, Sharpe FT, Cregan PB (1984) Variation for improved protein and yield from rice anther culture. Theor Appl Genet 67:383–389

Schnell RJ, Wernsman EA, Burk LG (1980) Efficiency of single seed descent vs anther-derived dihaploid breeding methods in tobacco. Crop Sci 20:619–622

Senghor PT (1990) Etude de méthodes d'obtention de lignées homozygotes. I. Androgenèse in vitro sur le riz: amélioration du rendement par traitement au gamétocide. II. Analyse de la variabilité génétique de deux populations de blé tendre dérivées par haplodiploïdisation in vitro et filiation unipare. Thèse de Docteur ès Sciences, Université d'Orsay, France

Siva Reddy V, Leelavathi S, Sen SK (1987) In vitro segregation of marker genes in anther culture of rice. In Vitro Cell Dev Biol 23:515–518

Snape JW (1976) A theoretical comparison of diploidised haploid and single seed descent populations. Heredity 36:275–277

Snape JW, Simpson E (1981) The genetical expectations of doubled-haploid lines derived from different filial generations. Theor Appl Genet 60:123–128

Turcotte P, St-Pierre CA, Ho KM (1980) Comparaison entre des lignées pedigree et des lignées haploïdes doublées chez l'orge (*Hordeum vulgare* L.). Can J Plant Sci 60:79–85

Zakri AH (1986) Somaclonal variation in rice. In: Rice genetics. IRRI, Philippines, pp 753–762

Zapata FJ, Kush GS, Crill JP, Neu MH, Romero RO, Torrizo LB, Alejar M (1983) Rice anther culture at IRRI. In: Institute of Genetics, Acad Sin and IRRI (eds) Cell and tissue culture techniques for cereal crop improvement. Peking Sci Press, Gordon and Breach Science Publ, pp 27–46

Zhang ZH (1989) The practicability of anther culture breeding in rice. In: Mujeeb-Kazi A, Sitch LA (eds) Proc 2nd Int Symp Genet Manipulation Crops. CIMMYT, Mexico and IRRI, Philippines, pp 31–42