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A comparison of androgenetic doubled-haploid, and single seed descent lines in Triticale

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Summary. Sixty single seed descent (SSD) lines and about 25 anther-derived doubled-haploid (DH) lines were obtained from two triticale crosses. The frequency distributions of 10 quantitative agronomic traits were compared using parametric and non-parametric tests. A multivariate discriminant analysis was subsequently carried out. Gliadin patterns obtained from each line by polyacrylamide gel electrophoresis were used to calculate intra- and inter-population diversities from relative dissimilarity indices. It was found that DH and SSD lines show significant differences in frequency distributions of 1000 grain weight in both crosses, of heading date for one cross, and of lodging susceptibility for the other cross. The results of intra- and inter-population gliadin diversity indicate that although the SSD method theoretically provides more opportunity for recombination to occur than the DH method, it did not produce a greater range of recombinants. Since there is no significant difference between SSD- and DH-line distributions for grain yield, anther culture appears to be an efficient method for producing high yielding homozygous lines from F_1 hybrids of triticale in a relatively short time.

Key words: X *Triticosecale* – Doubled haploids – Anther culture – Single seed descent – Gliadins

Introduction

Anther culture permits the production of homozygous lines from a segregating generation within a short time. A number of researchers have recently reported on the use of doubled haploid (DH) lines in breeding programs, especially in cereal crops: barley (Ho and Jones 1980; Foroughi-Wehr and Friedt 1984), wheat (Henry and De Buyser 1985), and rye (Friedt et al. 1983). It is of interest to plant breeders to know whether DH lines do show the same potentialities (estimated by means and variances of agronomic characters) as lines obtained by other methods using selfing. In theory, two factors could affect the means and variances of DH lines for a quantitative character: 1) the limited possibilities of genetic recombination through a single meiosis compared with inbred lines in the presence of linkage (Snape 1976; Riggs and Snape 1977); 2) the possible occurrence of a gametophytic selection.

An extensive comparison has been made in barley between DH lines (obtained by the "bulbosum" technique) and F6 lines produced by single seed descent from F1 hybrids (Choo et al. 1981). These authors did not find any significant difference between the distributions of DH and SSD lines for heading date, plant height and yield. In the case of the anther derived DH lines of cereal crops, studies on qualitative characters controlled by a single gene indicate that in most cases, the segregation observed within DH lines fits the expected Mendelian proportions, proving that gametic selection does not occur (Chen et al. 1983 in rice; Chen and Li 1978 in wheat; Foroughi-Wehr and Friedt 1984 in barley). This remains to be demonstrated for quantitative polygenically controlled characters. Chen and Li (1978) compared DH lines and F2 populations of wheat and rice and Friedt and Foroughi-Wehr (1983) tested DH lines derived from F1 hybrids in relation to the parental lines of these hybrids, but no comparison between androgenetic DH lines and homozygous inbred lines derived from hybrids has yet been reported in cereal species.

This paper reports the results of a comparison between triticale androgenetic DH lines and F5 lines derived by single seed descent, for both quantitative agronomic traits and gliadin pattern diversity.

Material and methods

From two F1 hybrids of hexaploid triticale (X *Triticosecale* Wittmack) denoted GE6 × AN2 and GE6 × GE7 (see Charmet

and Bernard 1984), two types of homozygous lines were produced: a) F5 inbred lines derived by single seed descent (SSD lines) under greenhouse conditions, that is with a minimum selection: two seeds were sown from each plant of the preceding generation, one of them being randomly eliminated as a plantlet; b) doubled haploid lines (DH lines) obtained by in vitro anther culture.

From both hybrids, 60 SSD lines were retained, but only 28 and 23 DH lines could be obtained from GE6×AN2 and GE6×GE7 respectively. DH lines were put into a block design field trial with 4 replications at Clermont-Ferrand, France, during November 1983 – August 1984. The 120 SSD lines were sown in this block trial without replication and 6 controls were added in order to estimate the field heterogeneity. Each plot consisted of three 1 m long, 0.20 m spaced rows. The sowing density was 30 seeds per row, that is 150 seeds/m2. The following quantitative characters were observed: 1) heading date: number of days after triticale cv. 'Triton', the earliest control (May 23); 2) plant height (in cm); 3) lodging susceptibility: from 1 = no lodging, to 9; 4) number of spikes per plot; 5) number of spikelets on the main ear: 5 counts per plot; 6) length of the main ear: in cm (5 measurements per plot); 7) grain yield: in grams per plot after drying; 8) 1000 grain weight (weighted on 250 grains); 9) test weight in kg/hl (weighted on 100 ml); 10) mean number of grains per spike: (10) = (7)/(4) × (8).

The distributions of each of these quantitative traits were tested for their normality according to Pearson and Hartley (1954) by calculating skewness- and kurtosis indices (Snedecor and Cochran 1956). Student's-*t*-test for means and Fisher's-*F*-test for variances being only available for Gaussian distributions, two nonparametric tests were used to compare DH and SSD lines frequency distributions, namely the Mann-Whitney-*U*-test and Kolmogorov-Smirnov-*two-samples*-test.

Tests of significance were conducted according to the procedures outlined in Siegel (1956).

A multivariate "factorial discriminant" analysis was then carried out for each cross using a FORTRAN programme (Bachacou et al. 1981). This analysis allows the visualisation of the cloud of individual plots corresponding to ten variables by projection on a principal plan. The new main axes are calculated in order to maximize the dispersion between DH and SSD lines.

Electrophoretic diagrams of gliadins on polyacrylamid gels were obtained from a single grain of each DH or SSD line following the method of Bushuk and Zillman (1978) modified by Branlard (1980) and Courvoisier (1984). Gliadin patterns were used to estimate the intra- and inter-population diversities by calculating relative dissimilarity indices (RDI) (Autran and Bourdet 1975; Branlard and Mabault 1984): when comparing two individual diagrams i and j, the relative dissimilarity index is given by RDIij = (D/N) × 100, where D is the number of bands differing from one diagram (i) to another (j) and N is the number of bands with different mobility present on both diagrams. The possible differences in concentration for bands of the same mobility have not been taken into account, only the presence/absence character. For a given population of n analysed grains, the diagrams are compared two by two. Thus n (n−1)/2 RDI are calculated with a computer. The intra population diversity is defined as the mean of these n (n−1)/2 RDI. For two different populations, for which the numbers of diagrams are respectively n1 and n2, each of the n1 diagrams from the first population is compared with each of the n2 diagrams from the second population, and n1 × n2 RDI are calculated. The inter population diversity is given by the average value of these n1 × n2 RDI.

Six populations with comparable line numbers were considered in this study: the two populations of DH lines (28 and 23 diagrams respectively for GE6×AN2 and GE6×GE7), and four populations of 30 SSD lines: within the 60 SSD lines from each cross, two groups of 30 lines were taken randomly, in order to test the effect of sampling on the diversity values.

Results

Quantitative characters

Table 1 shows the results of the variance analysis made from the block trial data. Genotypic effects are highly significant for all the characters studied and the precision of the trial can be appreciated through the coefficient of variation. It is generally satisfactory except for lodging and grain yield. The block effect is non significant in most cases, so that we can consider the homogeneity of the field as quite good.

The results of monofactorial comparisons between the distributions of SSD and DH lines are given in Tables 2 and 3 for the progenies of GE6×GE7 and GE6×AN2, respectively. Between SSD and DH lines from GE6×GE7, highly significant differences are found concerning lodging susceptibility, spike density and 1000 grain weight. DH lines have on average a lower 1000 grain weight but a higher spike density and lodging susceptibility. SSD and DH lines from GE6×AN2 differ highly significantly for heading date and 1,000 grain weight: DH lines are later than inbred lines and show a lower grain weight. DH and SSD lines from GE6×AN2 show differences for two other characters but these are only significant at the 5% level for the Kolmogorov – Smirnov-*D*-test: lodging (DH lines being more susceptible) and grain yield (DH being less productive).

The comparative frequency distributions of heading date, plant height, yield and 1,000 grain weight are illustrated in Figs. 1–4.

Both DH and SSD lines seem to be equivalent in their proportion of high yielding lines: within the progeny of GE6×GE7, 5 DH lines out of 23 (22%) and 10 SSD lines out of 60 (17%) have a yield which does not significantly differ from that of the 6 control lines. For GE6×AN2, 2 DH lines out of 28 (7.1%) and 4 SSD lines (6.7%) do not differ in yield from the control lines, i.e. yield more than 850 g per plot.

The graphical results of multivariate discriminant analyses are presented in Figs. 5 and 6 for GE6×GE7 and GE6×AN2, respectively. For DH and SSD lines from GE6×GE7 (Fig. 5), only the first principal component is highly significant (F = 70). This axis 1 is positively correlated with the initial variables 3 (lodging susceptibility) and 4 (number of ears per plot), and negatively correlated with the 1,000 grain weight. The

Table 1. Variance analysis (mean squares) for 51 DH lines and 6 control lines in a block design with 4 replications

Source of variation (DF) Character	Genotypes (56)	Replication (3)	Error	Coefficient of variation (%)
Heading date	61.0**	1.7	1.0	7.6
Plant height	839.9**	26.7	16.6	3.4
Lodging susceptibility	5.9**	0.7	0.4	18.4
No. of spikes per plot	6,694.7**	1,036.0	548.8	8.5
No. of spikelets	15.8**	0.9	0.6	2.4
Length of spike	4.2**	1.0	0.7	6.0
No. of grains per spike	423.7**	44.0	19.7	9.0
1,000 grain weight	91.8**	9.4	4.2	4.5
Test weight	23.9**	4.1	3.1	2.5
Grain yield	284,880**	27,882*	5,101	10.7

*,** Significant at the 5% or 1% level, respectively

Table 2. Tests of comparison between DH lines and SSD lines from the cross GE6 × GE7

Character	SSD lines			DH lines			Test of comparison			
	Mean	SD	Normality	Mean	SD	Normality	*F*	*t*	*U*	*D*
Heading date	12.45	3.29	Yes	13.20	3.44	Yes	1.09	0.90	770	0.159
Plant height	131.5	14.75	Yes	129.2	15.27	Yes	1.07	0.63	620	0.145
Lodging susc.	3.11	2.23	Yes	4.29	1.43	No	2.43*	2.85*	939*	0.456**
No. spikes	228.0	35.5	No	268.5	35.3	Yes	1.01	4.66**	1,048**	0.540**
No. spikelets	32.11	1.88	Yes	32.59	2.32	Yes	1.51	0.89	793	0.187
Length of spike	14.16	1.48	Yes	14.28	1.78	Yes	1.45	0.30	697	0.195
No. grains spike	49.28	17.20	Yes	51.82	14.87	Yes	1.33	0.66	780	0.192
1,000 grain weight	55.79	5.63	Yes	48.07	4.76	Yes	1.40	6.25**	211**	0.530**
Test weight	71.57	3.98	No	70.91	3.88	No	1.05	0.69	570	0.221
Grain yield	614.1	233.2	Yes	653.5	225.7	Yes	1.07	0.70	752	0.211

*,** Significant at the 5% or 1% level, respectively

Table 3. Tests of comparison between DH lines and SSD lines from the cross GE6 × AN2

Character	SSD lines			DH lines			Test of comparison			
	Mean	SD	Normality	Mean	SD	Normality	*F*	*t*	*U*	*D*
Heading date	13.75	2.76	Yes	16.52	3.28	Yes	1.42	3.87**	1,211**	0.331**
Plant height	112.7	9.85	Yes	112.4	8.18	Yes	1.45	0.19	820	0.138
Lodging susc.	2.15	2.04	Yes	2.94	1.82	Yes	1.25	1.81	1,053	0.259*
No. spikes	272.2	56.10	No	273.3	79.90	Yes	2.02*	0.02	840	0.185
No. spikelets	33.24	1.82	Yes	32.85	3.06	Yes	2.82**	0.66	808	0.145
Length of spike	14.72	1.33	Yes	14.70	1.38	Yes	1.08	0.06	860	0.131
No. grains spike	45.72	13.91	No	46.88	15.46	Yes	1.23	0.34	888	0.131
1,000 grain weight	49.22	7.52	Yes	40.50	5.81	Yes	1.67	5.94**	313**	0.452**
Test weight	69.83	2.82	Yes	69.97	4.25	No	2.27*	0.18	792	0.140
Grain yield	607.0	188.0	No	513.1	240.6	Yes	1.64	1.82	665	0.278*

*,** Significant at the 5% or 1% level, respectively

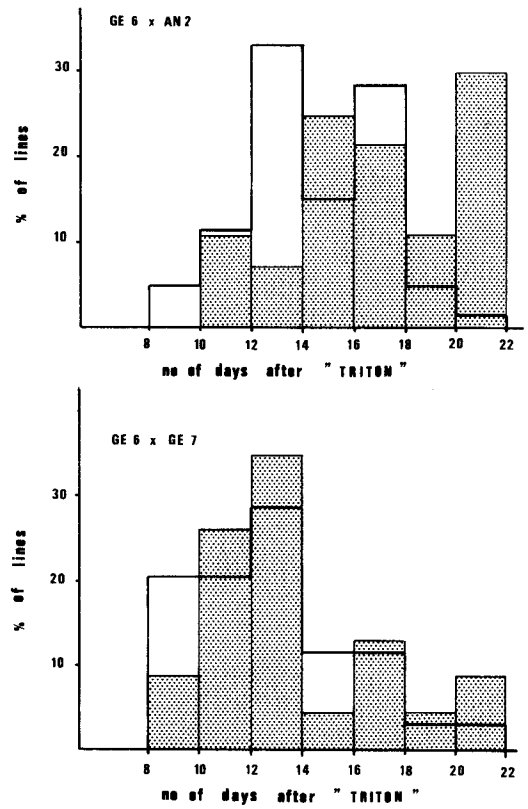

Fig. 1

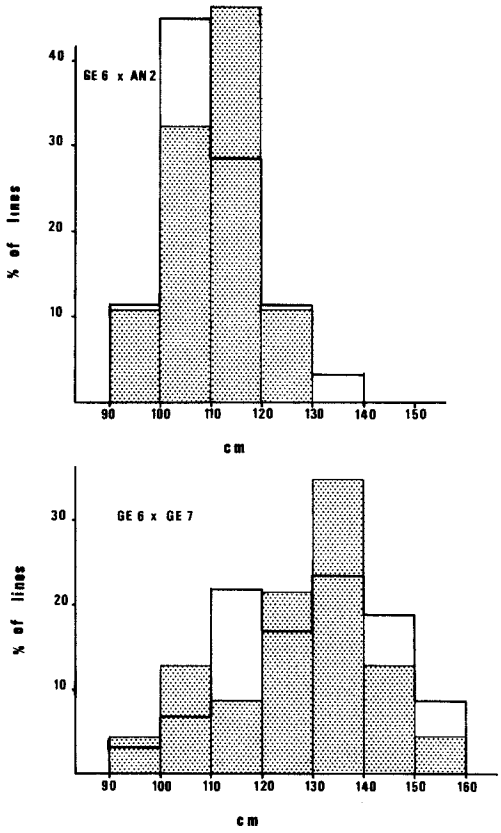

Fig. 2

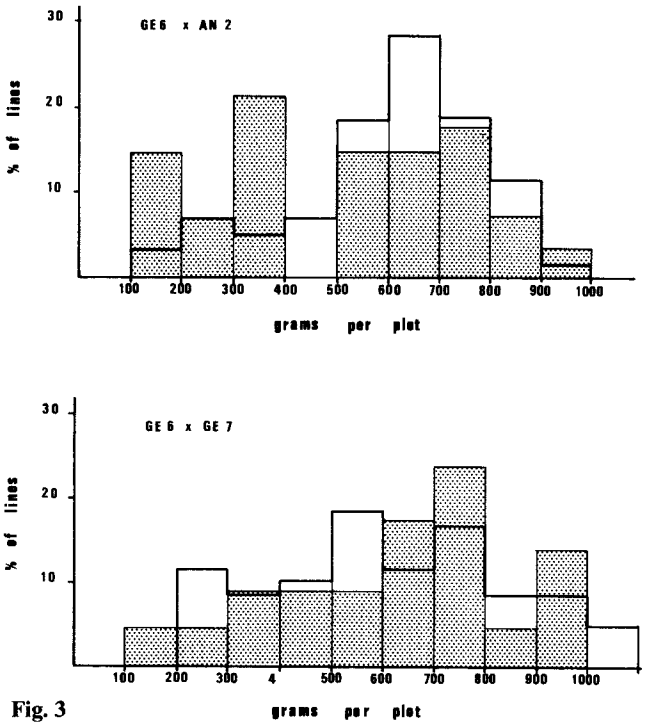

Fig. 3

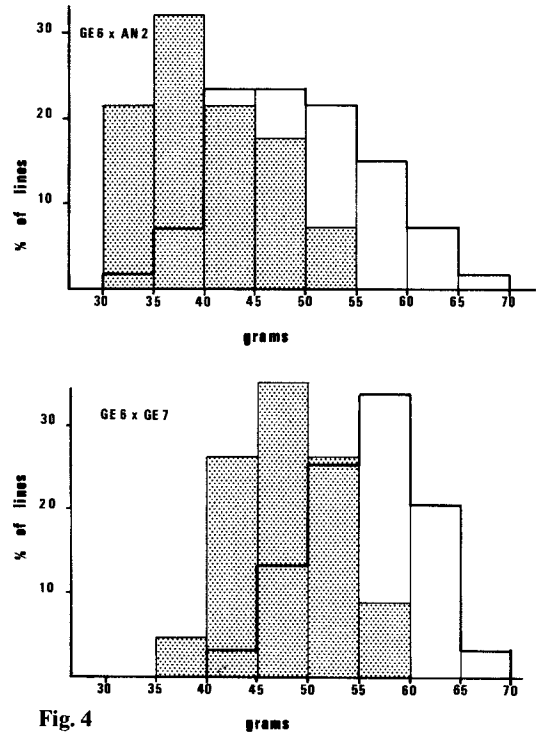

Fig. 4

Figs. 1–4. Frequency distributions of doubled haploid (*shaded distribution*) and single seed descent (*unshaded distribution*) lines produced from GE6×AN2 (*upper figure*) and GE6×GE7 (*lower figure*) for the following characters: **1** heading date; **2** plant height; **3** grain yield; **4** 1,000 grain weight

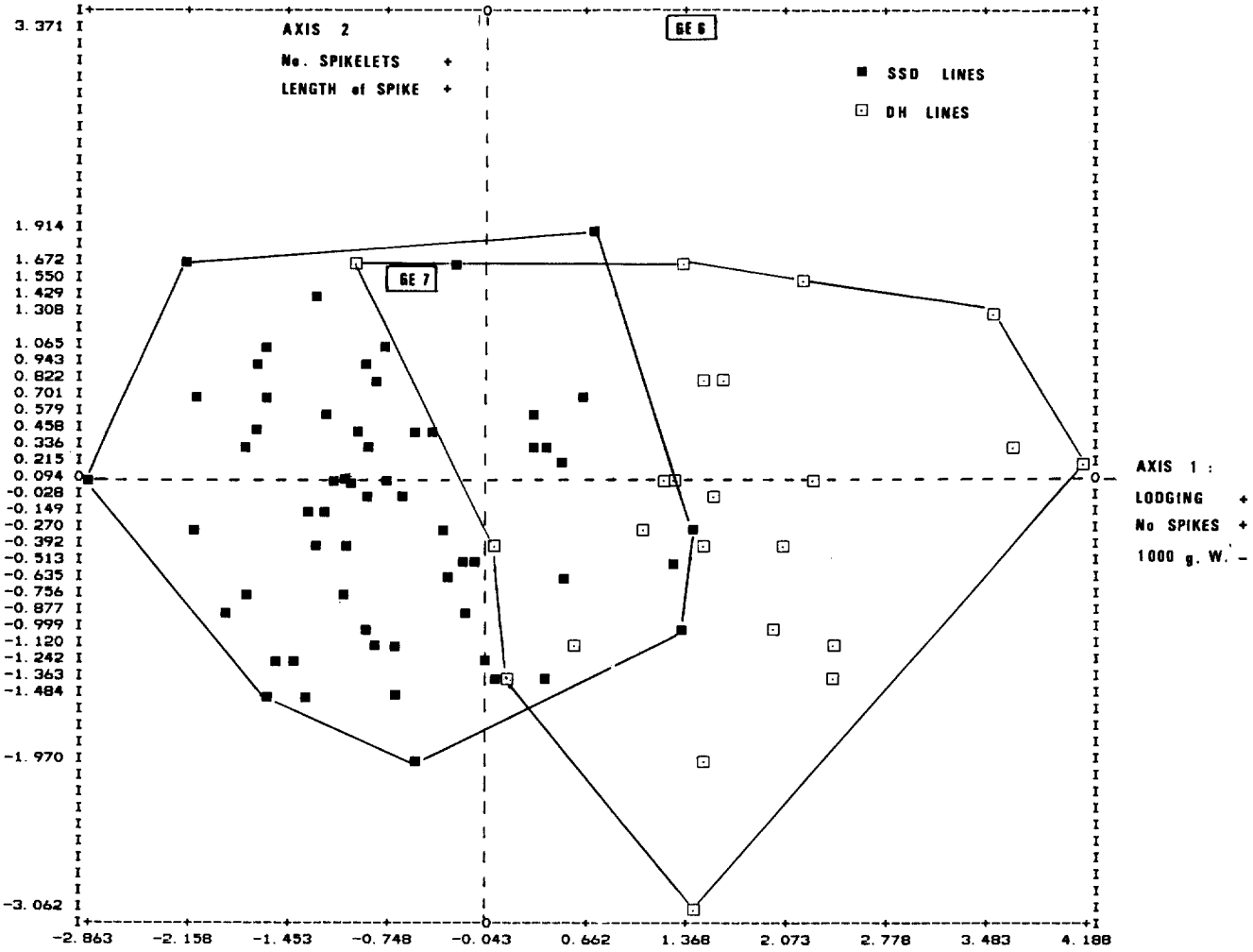

Fig. 5. Multivariate discriminant analysis of DH and SSD lines from GE6 × GE7

graphics of plan 1–2 clearly shows the segregation between DH lines and SSD lines along the first discriminant axis. The common area for both DH and SSD populations only contains 7 DH lines (out of 23) and 16 SSD lines (out of 60).

As for the second cross, the first component is still the only discriminant ($F = 56.7$) and is positively correlated with 1,000 grain weight and grain yield, and negatively correlated with heading date and lodging susceptibility. We found again the same characters, for which monofactorial comparisons previously showed significant differences. The two groups of lines here (Fig. 6) present a more extended common area, including 16 DH and 25 SSD lines.

2 Gliadin diversity

An example of gliadin diversity within the DH lines from GE6 × GE7 is given in Fig. 7. In all the diagrams obtained, 35 gliadin bands of different mobilities were observed.

"Intra population" diversities are presented in Table 4 for the 6 groups of lines considered. The different populations of lines obtained from a single cross do not differ significantly from each other with regards to their gliadin diversity. Differences in average values of RDI between DH and SSD lines from a given cross are similar to the differences observed between two groups of SSD lines and can thus be considered as resulting from random sampling.

The matrix of "inter-populations" diversities is shown in Table 5. It is to be noted that the diversities between two populations of lines from the same cross are very similar to each other and to the values of the "interpopulations" diversity previously observed. On the other hand, the values of inter-populations diversity for two groups of lines obtained from different crosses are significantly higher.

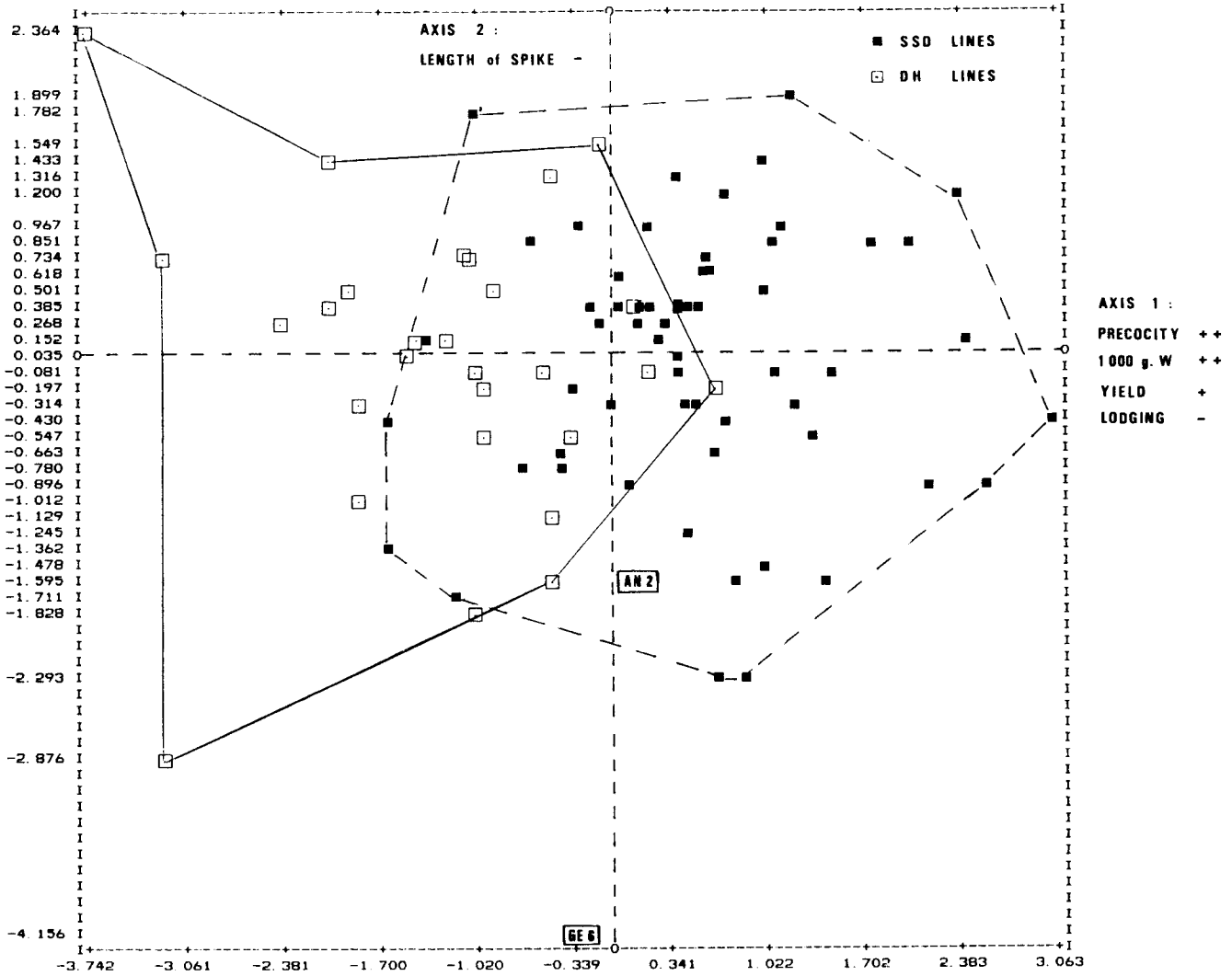

Fig. 6. Multivariate discriminant analysis of DH and SSD lines from GE6×AN2

Table 4. "Intra-population" diversities of gliadins for 6 groups of DH or SSD lines

Population of lines	No. of RDI calculated	Intra-population diversity		Student's-*t*-test
		Mean of RDIs	SD	
GE6×GE7–SSD 1	435	40.706	12.716	0.65 NS
GE6×GE7–SSD 2	435	40.199	13.354	
GE6×GE7–DH	253	40.748	12.662	0.52 NS
GE6×AN2–SSD 1	435	48.574	20.533	0.66 NS
GE6×AN2–SSD 2	435	47.448	18.012	
GE6×AN2–DH	378	47.407	16.445	0.03 NS

Table 5. "Inter-population" diversities of gliadins between 6 groups of DH or SSD lines

Populations of lines	GE6×GE7 SSD 1	GE6×GE7 SSD 2	GE6×GE7 DH	GE6×AN2 SSD 1	GE6×AN2 SSD 2	GE6×AN2 DH
GE6×GE7–SSD 1	(40.706)	40.306	40.553	58.447	55.644	55.651
GE6×GE7–SSD 2		(40.199)	40.478	57.522	54.712	54.972
GE6×GE7–DH			(40.748)	59.161	55.787	56.159
GE6×AN2–SSD 1				(48.754)	48.569	48.983
GE6×AN2–SSD 2					(47.448)	46.829
GE6×AN2–DH						(47.407)

(in brackets): intra-population diversity

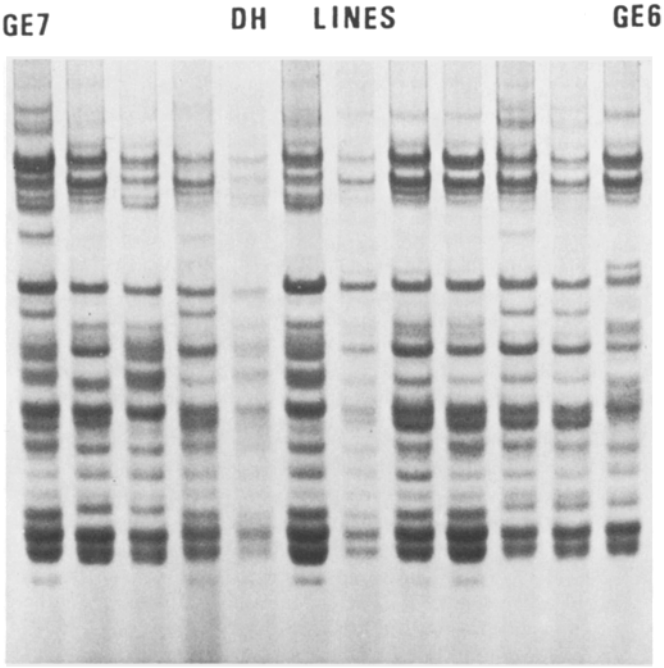

Fig. 7. An example of the gliadin band diversitiy of 10 DH lines from GE6×GE7

It can thus be concluded that DH lines and SSD lines from a single cross are very similar with regards to their gliadin diversity and can hence be considered as a unique population of homozygous lines derived from a F1 hybrid.

Discussion

The differences observed between frequency distributions of DH and SSD lines for certain quantitative characters must be considered with caution. Yield components are not independant of each other: differences in one of them between the DH and SSD lines could lead to differences in another yield component. It is therefore difficult to determine whether genotypic differences involve the control of 1,000 grain weight itself, or act on other physiological factors such as tillering on ear fertility.

However, the most significant differences between DH and SSD lines observed in both crosses, concern 1,000 grain weight. It should then be questioned whether such differences are really caused by negative gametophytic selection during in vitro anther culture. Other hypotheses can be put forward to explain the differences observed: a better seed quality in SSD lines (although both types of lines were harvested under the same conditions), or an unconscious undesirable selection for big grains during the single seed descent process, although care was taken to avoid any selection pressure. Such occurrences are more unlikely for heading date, for which differences were observed between DH and SSD lines from GE6×AN2, because all plants were harvested each year, from the earliest to the latest without exception.

On the other hand, the possibility for gametophytic selection to occur during the four successive generations of sexual reproduction cannot be completely excluded. It is well known that, in cereal species, only one pollen grain out of more than 6,000 participates in the fertilization process. We could consider a gametic selection by pollen competition for fertilization rather than a selection for in vitro development and shoot differentiation.

Further studies are needed to verify whether a gametophytic selection does occur or not during in vitro culture or during the single seed descent process. This is more difficult to establish for a quantitative character than for a monogenically controlled feature. To date, only in barley has it been shown that DH lines obtained by the bulbosum method (Johns 1974) as well as SSD lines (Choo et al. 1982) are a random sample of the potential genotypes. If it is true for triticale SSD lines, our results would indicate that a gametophytic selection may be responsible for the differences observed between SSD and anther derived DH lines.

The information resulting from gliadin diagram analyses is of primary importance for plant breeders. The similarity found between DH and SSD lines from a single cross as regards the gliadin diversity indicate that DH lines offer the same opportunities for genetic recombination as F5 inbred lines. Riggs

and Snape (1977) and Snape and Simpson (1981) showed that in the presence of linkage, a population of inbred lines theoretically has a greater chance of recombination than DH lines derived from F1 hybrids which are the result of only one meiosis. This should be the case for gliadin bands, which are controlled by several genes located only on the short arms of chromosomes 1 and 6 of each group of homeology (see Brown and Flavell 1981). The present results are thus in contradiction with such theoretical considerations. Since the frequency of recombinant gliadin diagrams is similar for both DH lines and SSD lines, it would mean that haploid production from F1 hybrids is as efficient for providing recombination opportunities as several generations of selfing. This could be explained by the relative importance of inter-chromosomic recombination (by random distribution of the chromosomes at meiosis), especially in an allopolyploid species like triticale, and/or by the fact that the first meiosis (in F1 plants) provides half of the total efficient crossing-over between the parental genomes.

It can thus be suggested, in agreement with Choo et al. (1982), that the production of anther-derived DH lines of triticale from F1 plants provides the same range of recombinant genotypes as inbred lines obtained by several generations of selfing, even for tight-linked genes such as those controlling gliadin bands. It does not therefore appear necessary to delay the production of haploids until the F2 generations as proposed by Snape and Simpson (1981).

Frequency distributions of DH lines and SSD lines differ significantly, especially for 1,000 grain weight in both crosses, and also for earliness and tillering in one cross. Since recombination opportunities cannot be an explanation (the differences observed involving the means rather than the variances when distributions are Gaussian), it seems that DH lines often present different developmental pathways than selfed inbred lines. Nevertheless, on average the two types of homozygous lines do not differ from each other for grain yield. This is especially true when considering the frequency of high yielding lines.

In spite of the differences found between DH lines and SSD lines for some agronomic characters, it seems nevertheless possible to create doubled haploid androgenetic lines which present interesting agronomic characteristics.

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