HYBRIDIZATION AMONG BRAZILIAN OAT (*AVENA SATIVA* L.) CULTIVARS AND THE WILD OAT *A. STERILIS* L.[1]

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ABSTRACT - Hybrids among *Avena sativa* (L.) Brazilian cultivars and *A. sterilis* L. accessions were obtained without *in vitro* embryo culture. Parental samples and their hybrids were investigated by morphological characters of spikelets, meiotic chromosome behaviour and esterase isoenzymes analysis. The F_1 hybrids were intermediate to the parental lines for the morphological spikelet characters. The high esterase zymotype affinity made difficult the diagnostic of the hybrid condition. The hybrids presented more meiotic irregularities than the parental lines. However, predominant bivalent formation shows that the corresponding chromosomes in *A. sativa* and *A. sterilis* are able to pair effectively and should result in the recombination of characters of the two species.

Index terms: interspecific hybrids, meiotic behaviour, esterase isozymes.

HIBRIDAÇÃO ENTRE AVEIA-COMUM E AVEIA-ESTÉRIL

RESUMO - Foram obtidos híbridos entre cultivares brasileiras de aveia-comum (*Avena sativa* L.) e introduções de aveia-estéril (*Avena sterilis* L.) sem a necessidade de cultura de embrião *in vitro*. Amostras parentais e seus híbridos foram analisados quanto a caracteres morfológicos de espigueta, comportamento meiótico cromossômico e análise das isoenzimas esterases. Os híbridos F_1 foram intermediários quando comparados com as linhas parentais referentes aos caracteres morfológicos de espigueta. A alta afinidade dos zimótipos em relação à esterase dificultou o diagnóstico da condição híbrida. Os híbridos, como um grupo, apresentaram mais irregularidades do que as linhas parentais. Entretanto, a formação predominante de bivalentes mostra que os cromossomos de *A. sativa* e *A. sterilis* são capazes de efetivamente parear com a possível ocorrência de recombinação de caracteres entre as duas espécies.

Termos para indexação: híbridos interespecíficos, comportamento meiótico, isoenzimas esterases.

INTRODUCTION

In order to breed qualities of resistance, adaptation and better nutritive value into crops, breeders need sources of genetic diversity to draw upon when required. The genetic variation in the wild relatives of crops presented are a useful resource for plant improvement (Harlan, 1984; Hawkes, 1991).

The hexaploid wild forms of the *Avena genus, A. sterilis* L. and *A. fatua* are the most accessible gene pools for the introgression into cultivated oats (*A. sativa* L.). The genus *Avena* is a typical self-pollinated crop. Although those taxa have been classified in literature as three species, they cross readily and produce fertile F_1's, so they could be considered as genetically divergent subspecies within one species (Ladizinsky, 1989).

A. sterilis L. has contributed genes for crown rust (*Puccinia coronata avenae*) resistance (Fleischmann et al., 1971; Frey & Browning, 1976a, 1976b; Simons et al., 1987), barley yellow dwarf virus (BYDV) resistance (Landry et al., 1984), and increased grain yield (Frey, 1976), groat protein percentage (Briggle et al., 1975; Lyrene & Shands, 1975; Frey, 1977; Cox & Frey, 1985), and groat oil percentage (Frey et al., 1975).

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Recently, the incorporation of genes from *A. sterilis* L. into Brazilian oat cultivars has become a part of the oat improvement program of the Crop Plant Department, Universidade Federal do Rio Grande do Sul.

The objectives of the present study were:

1) to obtain *A. sativa/A. sterilis* hybrids, and

2) to investigate comparative spikelet morphological characters, chromosome pairing and esterase isoenzyme patterns of the parental species and the hybrids.

MATERIALS AND METHODS

Three oat (*Avena sativa* L.) Brazilian cultivars "UFRGS-7", "UFRGS-8", "UPF-7", and seven accessions "I-303", "I-320", "I-325", "I-377", "I-378", "I-428", "I-ARG" of the wild species *A. sterilis* were included in this study. The accessions came from Israel but seeds of I-ARG were obtained from Pergamino Experimental Station - INTA, Argentina. Each of the *A. sterilis* parental lines were derived from a single panicle to minimize heterogeneity within a line. *A. sativa* lines were used as female parents as well as in reciprocal crosses.

Seeds were germinated on moist filter paper. After 24 hours at room temperature they were refrigerated for two weeks to break dormancy, to promote tillering and flowering, as previously described (Lagos et al., 1982). Germinated seeds were grown in plastic bags filled with soil, in a net house under natural conditions of light and temperature.

Immature panicles were emasculated the day before anthesis and immediately pollinated with pollen from newly opened flowers. Since most of the seeds developed normal embryos and endosperm, embryo rescue and culture was not required, and seeds were harvested at maturity.

Parents and progeny seeds were sowed in 1989 and the plants were grown under the same conditions as above described.

The hybrid identity of the F_1 plants was investigated by: a) morphological characters of spikelets; b) cytogenetics analysis and c) isoenzyme patterns.

The studies of meiotic chromosomes behaviour were carried out in young panicles previously fixed in 3:1 alcohol - acetic acid solution and the pollen mother cells stained with propionic carmin. Chromosome pairing was analized in at least 10 cells per plant during diakinesis and metaphase I. Meiotic index (MI = percentage of normal pollen quartets; Love, 1951) was calculated from at least 100 microspore quartets per plant.

Biochemical investigations investigated esterases isoenzyme to α and ß in patterns of extracts in young leaves from parental forms and their hybrids. Nine percent horizontal polyacrylamide gels and Scandalios (1969) buffers were used. The zymograms were done based on the mobility of bands. Comparing band-by-band, the zymograms of each population were combined to represent each species.

RESULTS AND DISCUSSION

Hybridization of *A. sativa* with *A. sterilis* resulted in 140 seeds out of 1220 florets pollinated.

Crossability barrier was not observed in hybrids because all putative crossed seeds matured in the plants. Hybrid seeds germinated normally and F_1 plants were highly fertile and had vigorous vegetative growth.

The morphology of *A. sativa* spikelet was very distinct from that of *A. sterilis* since the latter has the characteristic shattering spikelets of wild oats. In *A. sterilis* the lemma was covered with a dense growth of hairs, and both primary and secundary florets of the spikelet had a long bore, strong and geniculate awns. In *A. sativa* all the florets were awnless, glabrous and were retained on the plant at maturity. The F_1 hybrids were intermediate to their parents in their morphological spikelet traits. They didn't have shattering spikelets but showed pubescence and awn only on the lemma of the primary floret (Fig. 1).

The presence of the awn on the primary floret is a characteristic feature of hybrids between wild and cultivated oats (Thomas et al., 1980). Dillenburg (1984) reported the same features in *A. sativa/A. sterilis* F_1 hybrids.

Jensen cited by Coffmann (1961) has reviewed the literature on the genetics of these spikelet characters. Shattering versus non-shattering is controlled by a single gene and the non-shattering cultivated base is dominant. The presence of awns is a pleiotropic effect of the base-type gene or is controlled by a tightly linked gene, since in shattering base genotypes a strong geniculate awn is present on the primary and secondary florets, while in the F_1 hybrids the awn is only formed on the primary floret.

Data on esterase activity were obtained from 27 plants (3 cultivars) in *A. sativa* (5 to 13 plants in each cultivar), 29 in *A. sterilis* (7 accessions - 3 to 10 in

FIG. 1. The picture shows the difference among morphological traits of spikelets: *Avena sterilis* (left), F_1 hybrid (central) and *Avena sativa* L. (right).

each accession) and 26 putative hybrids, which presented intermediate spikelet morphology.

The zymograms of the two species were similar in their patterns and most of their bands were homologous (Fig. 2). Nine bands were present in *A. sativa* and ten in *A. sterilis*. Their overall patterns differed uniquely in the presence of a slow band (RM 0,33) in *A. sterilis*. These results corroborate those of Craig et al. (1972), who found a high esterase zymotype affinity between *A. sativa* and *A. sterilis*.

However, the cultivars of *A. sativa* group and the accessions of *A. sterilis* had different isoenzyme patterns. Thus, the three cultivars of *A. sativa* could be identified by different bands. Two bands in UFRGS-7 (RM 0,70 and RM 0,52) and one in UFRGS-8 (RM 0,56) occurred only in these two cultivated genotypes. Moreover, in *A. sterilis* group I-377 and I-ARG had bands (RM 0,70 and RM 0,52; RM 0,33respectivelly) that were unique to their zymotypes. On the other hand, the same pattern was shared by different genotypes of the two species. Examples are UFRGS-8 and I-325; UPF-7 and I-320.

The availability of isozyme labels could provide useful tools to distinguish sexual hybrids from self (Arus et al., 1982; Byrne & Littleton, 1989). However, the high affinity of the zymotypes difficulted the diagnostic since inbred plants could not be distinguished from hybrid plants when the esterase patterns of the two parental lines were the same. Actually, only two out of the 26 putative hybrids had their hybrid nature confirmed by the electrophoretic analysis. They were obtained from crosses between I-377 x UFRGS-7 and UPF-7 x I-ARG. If other enzyme systems were studied, additional differences in isozyme patterns might be detected providing more reliable diagnostics.

The meiosis analysis (Table 1) showed that *A. sativa* and *A. sterilis* parental lines used in this study presented the expected 21 bivalents. The occasional

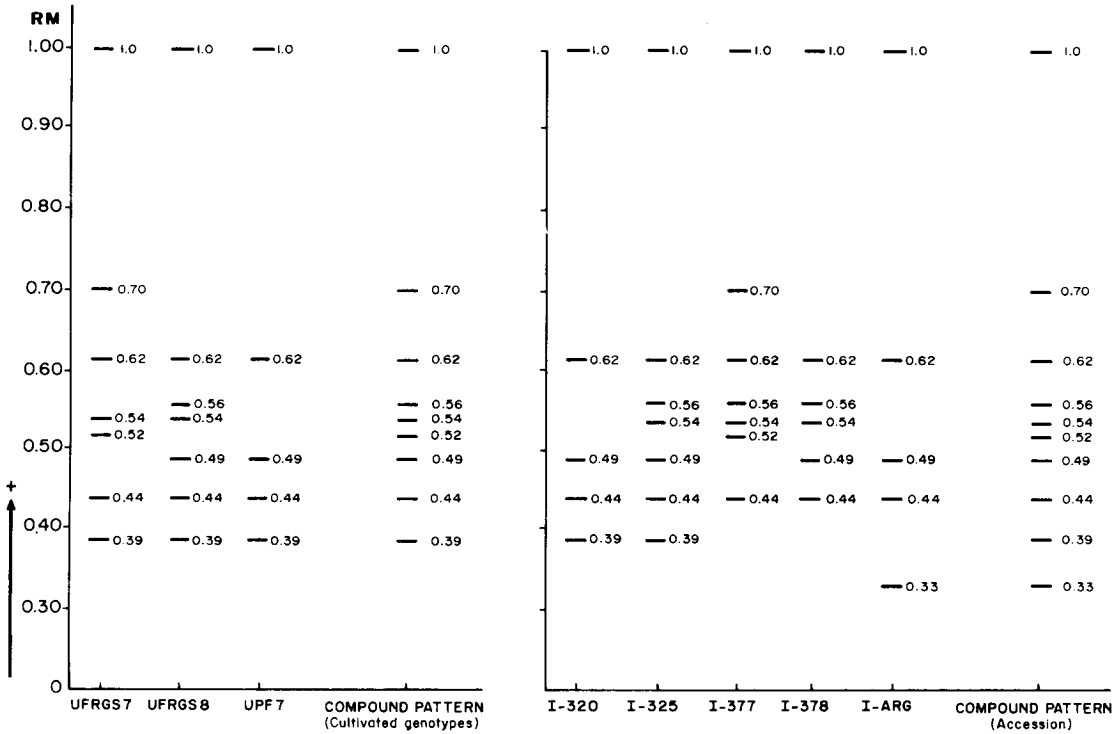

FIG. 2. The diagrams show zymograms of two species of oats: cultivated genotypes on the left and wild accession on the right.
RM = relative mobility

abnormalities were related to chromosome pairing at diakinesis and metaphase I. Unexpectedly a multivalent (IV) was observed in one PMC of UFRGS-7 cultivar. The univalent number ranged from 0.03 to 0.18 per PMC. However, these values are higher than those obtained by McMullen et al. (1982), that recorded less than 0.01 univalents per cell in nine *A. sativa* and seven *A. sterilis* genotypes.

A low frequency of micronuclei, probably produced as a direct consequence of univalent formation, were observed in quartets of microspores. The meiotic index varied from 95 to 99. Based on these data the two *A. sativa* cultivars and the two *A. sterilis* accessions may be considered cytologically stable, since, according to Love (1951), all plants with a meiotic index exceding 90 are included in this category.

The most remarkable cytological feature of the hybrids, as in previous reports, is the extensive chromosome pairing, which was clearly dominated by bivalent formation (more than 20 II/PMC in average). Most of the PMC had no irregularities; the most the hybrids had more univalents, more multivalents and lower Meiotic Index than the parental lines.

Univalents presented, at diakinesis and metaphase I, ranges of 0.0 to 0.47 per PMC in the studied hybrids. McMullen et al. (1982) found a range of 0.02 to 0.27 univalents per cell at diakinesis in *A. sativa/A. sterilis* hybrids. The highest values obtained in this study could be account by the addition of diakinesis and metaphase I data. The mean frequency of univalents/cell in the hybrids increased from diakinesis to metaphase I (McMullen et al., 1982). According to the author this increasement suggests that desynapsis of homologues occurs as meiosis progresses.

Cells containing multivalents were also recorded in all the nine examined hybrid combinations. The multivalent number ranged from 0.02 to 0.10 per PMC (Table 1). Our values are near to the lower border of

TABLE 1. Chromosome pairing at diakinesis and metaphase I of some plants of *Avena sativa* cultivars *A. sterilis* wild accession and interspecific hybrids.

	Number of plants	PMC* observed	Chromosome configuration mean nº /PMC			Microspore quarts observed	MI**
			bivalent	univalent	multivalent		
Cultivars							
UFRGS-7	9	288	20.98	0.03	0.003	1831	99
UFRGS-8	8	281	20.91	0.18	0.00	2180	95
Accession							
I-325	9	198	20.86	0.14	0.00	2428	99
I-378	7	213	20.97	0.06	0.00	1397	99
F_1 Hibrid							
I-320xUPF-7	2	42	20.90	0.00	0.05	40	95
I-325xUPF-7	3	173	20.91	0.09	0.02	491	83
I-377xUPF-7	2	143	20.69	0.31	0.08	650	91
I-378xUPF-7	3	188	20.81	0.11	0.07	562	86
I-ARGxUPF-7	1	45	20.87	0.02	0.04	450	90
I-325xUFRGS-7	3	191	20.83	0.09	0.06	543	89
I-377xUFRGS-7	2	53	20.83	0.15	0.04	424	94
I-377xUFRGS-8	3	268	20.60	0.47	0.06	671	74
I-378xUFRGS-8	5	707	20.59	0.42	0.10	1101	65

* PMC = pollen mother cells

** MI = Meiotic Index

the range recorded by McMullen et al. (1982): 0.08 to 0.55 multivalents (IV and VI) per cell.

A. sterilis has shown frequent occurrence of complex associations if crossed with other hexaploid species (Nishiyama et al., 1989). The multivalent configuration in the hybrids could be a result of: a) homologous pairing due to partial breakdown of the diploidisation mechanism, and b) chromosome rearrangements in the different lines. The fact that different lines of hexaploid oats (*A. sterilis* - *A. sativa* group) were found to differ from each other by chromosome rearrangements (Ladizinsky, 1970; McMullen et al., 1982) indicates that at least part of the multivalent formation is of the latter cause.

There is therefore substantial evidence that *A. sterilis* chromosomes are able to pair effectively with their corresponding chromosomes in *A. sativa* and should result in the recombination of characters of the two species. However, since the frequencies of meiotic irregularities varied among crosses, it could be assumed that the amount of recombination might also vary. If crossing-over is less frequent in hybrids with increased frequencies of meiotic abnormalities, the selection of parental combinations whose hybrid progeny produce, present the most normal meiosis which may increase recovery of desirable recombinants.

CONCLUSIONS

1. The high chromosomal homology detected on cytological and electrophoretical level support for successful in the directed genes transference from *A. sterilis* to cultivated oat species.

2. Among three technologies used, the morphologic analised shown to be the most important on the selection the hybrid plants; although, this proceeding system is based on the plant development until the heading.

3. The present work shows the importance of studies about agronomic and morphological traits, and meiotic and isozymes analyses; also they can give high important support on the plant breeding programmes.

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