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Wide hybridization attempts in the tribe *Aveneae* Nees[1])

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Abstract

Gervais C. (1983). Wide hybridization attempts in the tribe *Aveneae* Nees. Bot. Helv. *93:* 195–212. This paper reports the results of 91 different interspecific and intergeneric hybridization attempts involving 30 taxa belonging to genera *Arrhenatherum* Beauv., *Avena* L., *Avenula* Dumort. and *Helictotrichon* Bess. of tribe *Aveneae.*

The objective of the crosses was, on the one hand, to produce valuable hybrids that could be used for the genetic amelioration of cultivated oats and, on the other hand, to throw light on the phylogenetic relationships existing between the studied species and genera.

Seven interspecific crosses within genera *Avenula* and *Helictotrichon* succeeded but no intergeneric hybrids were obtained. However, the occasional presence of degenerated cells, pro-embryos or young embryos of various sizes in the embryo sac of the pollinated ovaries shows that fertilization probably occurs and that it could be possible to obtain some intergeneric hybrids (if the observed embryos do not result from apomictic phenomena).

A "compatibility scale" to evaluate the degree of success of the crosses was devised to single out the groups of taxa with possible phylogenetic links that could probably hybridize. Finally, the most interesting cases where intergeneric hybridizations seem possible (some *Avena* × *Arrhenatherum, Avena* × *Avenula* crosses and reciprocals) are individually discussed.

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Introduction

The results which are reported in this paper refer to 91 different types of interspecific or intergeneric hybridizations which were carried out between various taxa of the following genera of the tribe *Aveneae: Arrhenatherum* Beauv., *Avena* L., *Avenula* Dumort. and *Helictotrichon* Bess.

It would have been instructive and somewhat desirable to test all possible hybrid combinations on a systematic basis with the material at hand, but owing to limited time and resources, the crosses were selected along two principal directions: 1) hybridizations, mostly intergenic, intended to produce valuable hybrids, or haploids, between annual cultivated species of genus *Avena* and various perennial taxa; 2) hybridizations, mostly interspecific, to study the phylogenetic relationships within some perennial taxa of the group.

If the interest of the crosses belonging to the first category is self evident and acknowledged by geneticists (Hanson 1972, Bates and Deyoe 1973, Harlan 1976, Cauderon 1978, etc.), it was thought that the hybridizations of the second type would not be less useful, as they are steps to understand the speciation patterns surrounding the genus *Avena* itself. Common experience shows indeed that a difficult puzzle finally results in a clear picture whatever the corner (usually the easiest) where the pieces begin to be interlocked.

Fortunately, one could say that important sectors of the whole system have been already explored, namely, besides the perennial oats studied by the present author (Gervais 1973), the genus *Avena* that numerous cytologists have thoroughly investigated in recent years (Rajhathy and Thomas 1974).

These previous efforts have evidently brought valuable information and guidelines for the selection of the crosses which are reported in this paper, though some interesting avenues have been left unexplored by the absence of flowers or ill-timed pollen production. It is hoped that the results will contribute to a better understanding of the question.

Previous crosses

Hybridization attempts of the first category mentioned above, between annual representatives of genus *Avena* and perennial taxa of related genera, were tried for the first time by Johnson and McLennan (1939).

These authors fertilized about 3000 emasculated florets from nine annual species *(A. sativa, byzantina, nuda, fatua, sterilis, abyssinica, strigosa, wiestii, brevis)* with pollen from 9 different species of *Avenula, Helictotrichon* and *Arrhenatherum* lumped together as «perennial *Avena*». All these crosses were unsucessful but a certain number of stimulated ovaries were observed when *A. byzantina* «Early Ripe» was pollinated with an *A. pratensis* from accession 1422 or *A. montana.*

More recently, Baum and Rajhathy (1976) have reported unsuccessful hybridization attempts between *Avena macrostachya* Bal. ex Coss. & Dur., a tetraploid perennial species from Algeria, with large drooping spikelets like the annual oats, and three annual species of *Avena,* each possessing a different ploidy level: *A. strigosa* (2n = 14), *A. abyssinica* (2n = 28) and *A. sativa* (2n = 42). It should be noted that the perennial

species were used as male parents in all the crosses reported by Johnson and McLennan as well as in those reported by Baum and Rajhathy. In the present paper, on the contrary, the perennial species have generally served as female parents.

Crosses at the interspecific level within genus *Avenula* were also performed by the present author (Gervais 1973) and have produced 6 different hybrids, some of which have been used as male or female parents in different types of hybridization (see Tables 2 to 4).

Table 1. Names, chromosome numbers and country of origin of the material used in the crosses.

Name of the taxon	Chromosome numbers (2 n)	Origin of the material
Arrhenatherum		
elatius (L.) Beauv.	28	France
Avena		
barbata Pott ex Link	28	Tunisia
byzantina C. Koch	42	Morocco - Algeria
macrostachya Bal. ex Coss. & Dur.	28	Algeria
sativa L.	42	Canada (cultivated)
strigosa Schreb.	14	Spain, Uruguay
Avenula		
albinervis (Boiss.) Lainz	28	Spain
bromoides (Gouan) Scholz	14	Spain, Morocco
bromoides	42	Spain
compressa (Heuffel) Sauer & Chmel.	14	Hungary
planiculmis (Schrad.) Holub	126	Poland
pratensis (L.) Dumort	126	England, Hungary
pratensis s.l.	102–107*	Spain
pratensis s.l.	112	Spain
pratensis s.l.	133	France
pratensis s.l.	146*	France
pubescens (Huds.) Dumort	14	Switzerland, Spain
schelliana (Hack.) Sauer & Chmel.	14	U.S.S.R.
sulcata (Gay) Dumort.	14	Spain, France
vasconica (Senn. ex St-Yves) Gervais	98	Spain, France
Helictotrichon		
cantabricum (Lag.) Gervais	84	Spain
convolutum (Presl.) Henr.	14	Greece, Italy
filifolium (Lag.) Henr.	97–98*	Spain
sarracenorum (Gdgr) Holub	14	Spain
sarracenorum	28	Spain

*Material at least partly aneuploid.

Material and methods

The perennial *Aveneae* which were used as parents in the crosses were selected from a large collection of wild material grown at the experimental garden of the Botanical Institute of Neuchâtel University (Switzerland). These plants came from various countries and were gathered through seed exchange services or by botanical excursions, namely by Dr P. Küpfer. Most of them have been cytologically studied by the present author.

Brought into Canada in 1971, these exotic species were first kept in greenhouse conditions but did not flower unless they were transfered outside (in clay pots buried in the soil) for the winter or at least late autumn. This latter practice was in fact necessary for certain species, as *A. bromoides* and *H. sarracenorum*, which could hardly survive Canadian winter temperatures.

The annual species utilized in the crosses, as well as the perennial *A. macrostachya* (from Algeria), were obtained from the «Wild Oat Gene Pool», a collection maintained by the Canadian Department of Agriculture. The cultivars «Alma» and «Laurent» of *A. sativa,* largely used in the crosses, came from the Faculty of Agriculture of Laval University. A complete list of the species used in the crosses will be found in Table 1, with their chromosome numbers and their origin.

The hybridizations with the plants that had survived the winter outside, under the snow, were performed in the spring, while the others (with the plants brought into the greenhouse in late November) were normally done in January, provided that annual species had been previously sown two to three months earlier.

		Male parent															
		Avena					*Avenula*							*Helict.*			*Ar.*
Female parent	2 n	*strigosa*	*barbata*	*macrostachya*	*byzantina*	*sativa*	*bromoides* (di.)	*compressa*	*sulcata*	*albinervis*	*vasconica*	*pratensis* s.l.	*pratensis*	*convolutum*	*cantabricum*	*filifolium*	*elatius*
		14	28	28	42	42	14	14	14	28	98	102*	126	14	84	98	28
Avena																	
macrostachya	28	–	–	–	–	n	–	–	–	–	–	–	–	–	–	–	–
sativa	42	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	e
Avenula																	
bromoides (di.)	14	e	N	e	–	c	–	–	–	H	–	–	–	n	n	–	–
compressa	14	n	–	–	c	n	H	–	–	–	–	–	–	–	–	–	–
pubescens	14	n	–	–	–	c	–	–	–	–	–	–	–	–	–	–	–
schelliana	14	–	–	–	–	–	–	H	–	–	–	–	–	–	–	–	–
sulcata	14	–	–	–	e	c	H	–	–	–	–	–	n	–	–	–	–
albinervis	28	–	–	–	–	e	–	–	–	–	E	E	–	–	–	–	–
bromoides (hex.)	42	–	–	–	–	c	–	–	–	–	–	–	–	–	–	–	–
vasconica	98	n	–	–	n	c	–	–	–	E	–	–	–	–	–	–	–
pratensis s.l.	102*	–	–	c	–	–	–	–	–	E	–	–	–	–	–	–	–
pratensis s.l.	112	n	–	c	c	c	–	–	–	–	–	–	–	–	–	–	–
pratensis	126	n	–	n	n	c	–	–	n	–	–	–	–	–	–	–	–
pratensis s.l.	133	n	–	–	c	–	–	–	–	–	–	–	–	–	–	–	–
pratensis s.l.	146*	c	–	–	s	c	–	–	–	–	–	–	–	–	–	–	–
Helictotrichon																	
convolutum	14	n	–	–	–	n	–	–	–	–	–	–	–	–	n	–	–
sarracenorum	14	s	–	E	–	S	–	–	–	–	–	–	–	H	E	–	–
sarracenorum	28	n	–	–	–	S	c	–	–	–	–	–	–	n	E	E	–
cantabricum	84	–	–	–	–	n	–	–	–	–	–	–	–	–	–	H	–
filifolium	98	n	–	–	–	N	–	–	–	–	–	–	–	–	–	–	–
Arrhenatherum																	
elatius	28	c	–	–	–	n	–	–	–	–	–	–	–	–	–	–	–

Table 2. Results of 65 interspecific or intergeneric crosses involving 24 taxa of the tribe *Aveneae*. The asterisk (*) indicates aneuploid individuals.

Explanation of symbols

N = negative results (300 flowers and over, pollinated)
n = negative results (less than 300 flowers pollinated)
s = light stimulation of ovaries
S = Stimulation of ovaries
c = unidentified cells present (more or less degenerated)
e = pro-embryo present (less than 0,5 mm long)
E = embryo or pro-embryo present (0,5 mm long and over)
H = viable hybrid

		Male parent								
		Avena				*Avenula*				
Female parent	2 n	*strigosa*	*barbata*	*byzantina*	*sativa*	*sulcata*	*bromoides*	*sulc. × bromoides*	*vasc. × pratensis*	*prat. × planiculmis*
		14	28	42	42	14	14	14	112*	126*
Avena										
sativa	42	–	–	–	–	–	–	–	E	S
Avenula										
bromoides (diploid)	14	–	–	–	–	–	–	n	–	–
sulcata (2 n = 14) × *bromoides* (2 n = 14)	14	n	n	–	N	–	–	–	–	–
bromoides (2 n = 14) × *albinervis* (2 n = 28)	21	–	–	–	–	s	–	–	–	–
bromoides (2 n = 42) × *vasconica* (2 n = 98)	70	–	–	–	n	–	s	–	–	–
vasconica (2 n = 98) × *pratensis* (2 n = 126)	112*	c	–	n	e	–	–	–	–	–
pratensis (2 n = 126) × *planiculmis* (2 n = 126)	126*	c	–	c	e	–	–	–	–	–

Table 3. Results of crosses where one of the parents was a hybrid from previous crosses. The symbols are explained in Table 2. The asterisks (*) indicate fertile hybrids.

		Male parents								
Female parent	2 n	*Avena strigosa* *Avena sativa*	*Avena barbata* *Avena sativa*	*Avena strigosa* *Avena barbata* *Avena sativa*	*Avena sativa* *Avenula vasc. × pratensis***	*Avena sativa* *Avenula pratensis*	*Avena sativa* *Helictotrichon cantabricum*	*Avena sativa* *Helictotrichon sarracenorum* *Helictotrichon filifolium*	*Helictotrichon sarracenorum* *Helictotrichon filifolium*	*Helictotrichon sarracenorum* *Helictotrichon sarracenorum* *Helictotrichon cantabricum*
		14 42	28 42	14 28 42	42 112	42 126	42 84	42 28 98	28 97*	14 28 84
Avenula										
bromoides	14	n	n	c	–	–	–	–	–	–
sulcata	14	n	–	–	E	–	–	–	–	–
bromo. 14 × *albi.* 28	21	–	–	–	–	–	s	–	–	–
albinervis	28	s	–	–	–	E	–	–	–	–
Helictotrichon										
convolutum	14	–	–	–	–	–	–	–	–	n
sarracenorum	14	–	–	–	–	–	–	n	H	–

Table 4. Results of crosses where the female parent was pollinated by two or three male species at the same time. The symbols are explained in Table 2. One asterisk (*) indicates an aneuploid individual, two asteriks (**) a fertile hybrid used as male parent.

The flowers of the perennial female parents were hand emasculated with sharp-pointed brussels and their plumose extruding styles were later pollinated with stamens of dissected annual flowers whose maturity was evaluated *in situ* under a binocular. Crosses between perennials were easier, as mature drooping stamens of the male parent could be gently picked up and shaken over the styles of the emasculated female flowers. In some cases the male parent was simply placed on a support, just over the female plant, so that its pollen could fall by itself onto the styles.

In the few cases where annual species were used as female parents, the upper flower was eliminated and the other one was emasculated with brussels after its tip has been cut with scissors.

As it was suspected that unreceptive stigmas could be responsible for hybridization failure, the following procedure was tried in some of the crosses: young stamens of an individual belonging to the same species as the female parent were collected and finely crushed in a drop of water, on a glass slide, and the resulting suspension was deposited on the stigmas after their pollination by an alien species. It was hoped that released substances could trick the stigmas into accepting the pollen.

The chances that a female plant could be accidentally fecundated by airborne pollen being remote in the greenhouse where they were kept, the panicles were not bagged. When such a risk was possible, the plants were isolated and accidents could always be detected by the chromosome number of the progeny.

The pollinated flowers were left on the panicles until maturation but, in disarticulating species, they were collected just before they could fall. They were then dissected and their caryopsis (or dried ovaries) carefully extracted, examined, measured if necessary, divided into categories and counted. If the hybrid caryopsis appeared normal or nearly so, they were germinated in Petri dishes and transfered later to soil. The abnormal, undersized, flattened or shriveled seeds were grown in test tubes on Orchid Agar after sterilization with alcohol (60%, 2 minutes), mercuric chloride (0,1%, 2 minutes) and thorough washing in 5 Petri dishes with sterile water. If they did germinate, the young plantlets were carefully extracted from the tubes and transfered to soil. When the results of some hybridizations seemed very poor (undeveloped or slightly stimulated ovaries), a small number of the best ovaries were selected and placed in agar tubes, as a test. In a few cases, in later crosses, some abnormal seeds were dissected to remove the hybrid embryo and try to germinate it on various culture media. In fact, no embryos were visible but a small portion of the seed was sectioned, assuming that an embryo might be present. The results were negative.

Most of the caryopsis or ovaries that did not germinate on agar after one year or more were removed and stored in an emollient solution (water, alcohol, glycerine, ⅓ each) so that they could be later dissected or embedded in paraffin. The paraffin sections were 10 to 15 microns thick, colored with crystal violet and mounted in Canada balsam.

Results and discussion

With the exception of 7 crosses where viable offspring were obtained and proved to be true hybrids after cytological examination, the remaining results were difficult to interpret and classify. In some cases the seeds looked somewhat normal in length but more or less flattened, an indication of probable endosperm failure; in other instances the crosses did not produce seeds but the ovaries stayed apparently unfertilized, slightly stimulated or not, or, more rarely, definitely stimulated.

It would have been easy, of course, to stay with two simple categories of results: positive, if a hybrid plant was obtained, negative if not. As it was however very desirable to collect the most information possible from the crosses and eventually determine the type of sterility barrier responsible for the failures, 7 classes of negative results (and one for positive) were tentatively devised. They were based on the number

of pollinated flowers, the apparent development of the seeds and the study of their inner structure by dissection or slide preparation, a step that proved to be very useful as it revealed the presence of embryos or pro-embryos in apparently unfertilized «seeds». The resulting classification is as follows:

1- Very negative results (N), when a large number of flowers (300 and over) were pollinated without any apparent result or stimulation.

2- Negative results (n), when a small number of flowers (or less than 300, to set a limit) were pollinated without any apparent result or stimulation.

3- Light stimulation (s), when at least some ovaries seem to be a little more developed than unfertilized ovaries of the same plant or species.

4- Stimulation (S), when at least one of the ovaries shows a definite elongation if compared to unfertilized ovaries of the same plant or species.

5- Presence of unidentified and more or less degenerated cells (c), in the embryo sac. This special category, discussed below, had to be introduced because of specific problems with the present hybridizations.

6- Presence of embryonnary cells (e), at least two, outlining a structure (pro-embryo or embryo) less than 0,5 mm long. Possible traces of endosperm cells.

7- Presence of an embryo (E) 0,5 mm long and over. Some endosperm cells usually present.

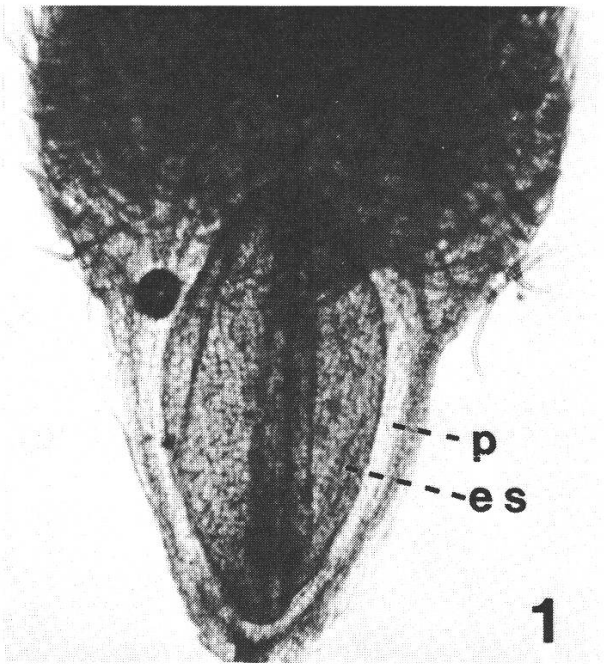
8- Viable hybrids (H).

Another category, unviable hybrids (h), could have been introduced but was not necessary for the current crosses.

The "compatibility scale" which is given above has led to the elaboration of Tables 2 to 4 that reflect, hopefully, the real or approximate situation of hybridization capability and also the degree of phylogenetic relationship. Some practical problems, however, were met in the utilization of the scale and they must be taken into account.

Besides the inconvenients of the two classes system used to evaluate the negative results (N,n) or the embryo development (E,e), that could always be corrected by giving the real data, the most difficult problem was the appreciation of the degree of ovary stimulation. If it was sometimes very clear that ovaries had been stimulated, judging from their seed-like appearance, the common situation was different. In many crosses, one part of the collected ovaries looked slightly larger than the rest, but it remained difficult to attribute such an apparent stimulation to the presence and action of pollen. Natural size variations in unpollinated ovaries do occur indeed between individuals, panicles and even flowers; the ovaries of the basal flowers of a spikelet, for example, seem to be larger than those of the upper flowers. The question could be statistically studied but its importance is probably not sufficient to justify such a research. In the present Tables, the degree of stimulation (S or s) was visually appreciated and a few ovaries were measured when necessary.

Another problem which was difficult in the appreciation of the present results was the observation of degenerated and hardly identifiable cells (c) in the embryo sac of a relatively large number of ovaries. The source of this problem lies apparently in the adopted procedure: the seeds, or ovaries, were collected at maturity, stored in envelopes or placed on sterile agar for long periods before they were dissected or sectioned. No fresh material, collected soon after pollination, was studied and some internal structures were probably altered.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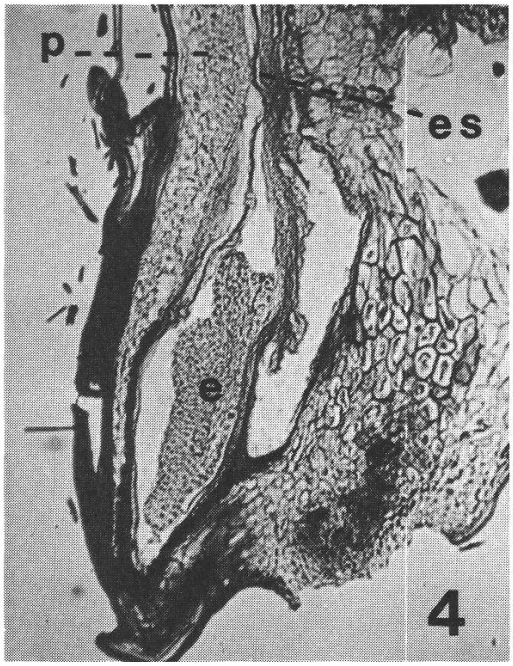
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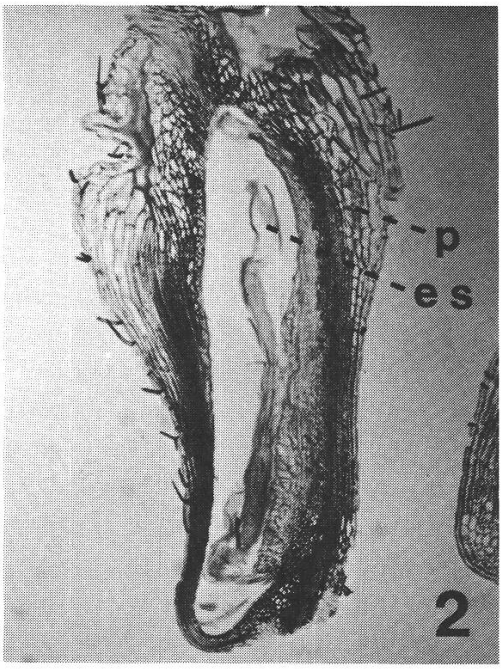
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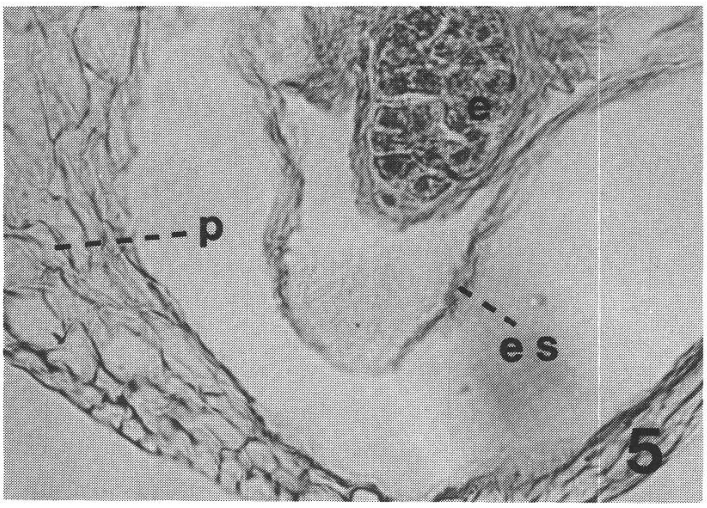
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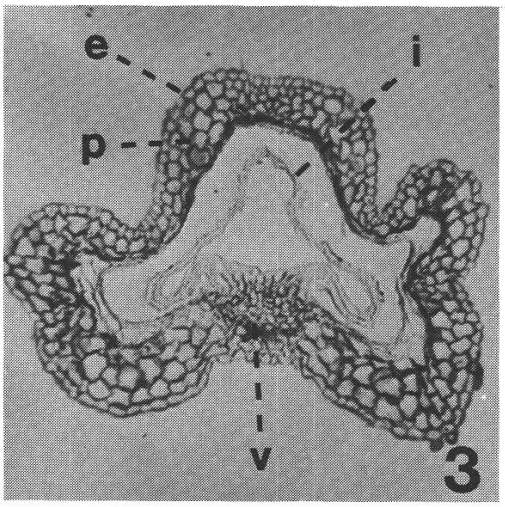
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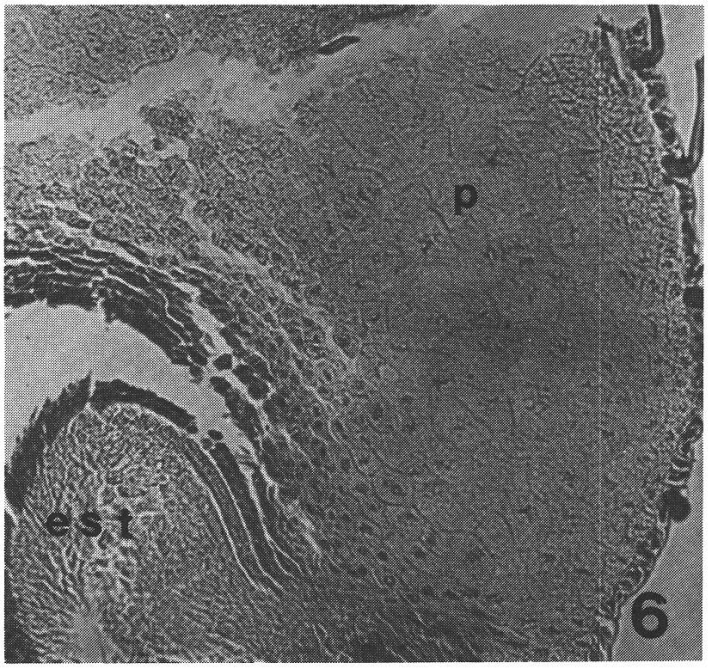
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The general aspect of an undeveloped or slightly developed ovary in genus *Avenula, Helictotrichon* or *Arrhenatherum* is a small size (0,5–2,5 mm) roughly conical structure covered with hairs and with style remnants at the top (Fig. 1). The embryo sac in such ovaries looks like an apparently empty vesicle usually occupying ½ to ⅔ of an inside cavity surrounded by the pericarp (Fig. 1 to 3).

The unidentified and more or less degenerated cells which were observed (c in the tables) were frequently intensely colored by crystal violet, or yellowish; they could be found from the micropylar end of the sac to its median portion (Fig. 7) and their shape was more or less regular to amiboid. They could perhaps be traces of antipodal cells or of endosperm cells but if they are found completely at the micropylar end of the sac and have more regular forms, they suggest degenerated pro-embryos. Whatever it may be, a conservative attitude was adopted in the assertion of embryo presence.

It must be noted finally that a negative result (N or n) in the Tables does not necessarily indicate complete cross-incompatibility between two taxa, as only a small number of ovaries were usually dissected or sectioned. It could happen that the presence of some pro-embryos in a small proportion of the ovaries remained undetected. However, as the chances of finding pro-embryos or degenerated cells are statistically higher in crosses where they are more frequent, the Tables probably reflect the real situation.

A first glance examination of Tables 2 and 4 shows that viable hybrids (H) resulted from 7 interspecific crosses, including the hybrid of Table 4 where *H. filifolium* proved to be the male pollinator. In addition, the Tables reveal that large (E) or small (e) embryos were also detected in many crosses at the interspecific as well as the intergeneric levels. It should be noted that some of these embryos could have perhaps given rise to hybrids if special embryo culture technics had been applied instead of the whole seeds (or ovaries) culture which was used. The Tables do not show the limits and barriers to hybrid production but the results that were obtained with relatively simple methods.

Fig. 1. Ovary of *Avenula pubescens* (pollinated with *Avena sativa*). The empty embryo sac (es) is visible through the transparent pericarp (p).

Fig. 2. Longitudinal section of an ovary of *Avena macrostachya* (pollinated with *Avena sativa*). Remnants of embryo sac teguments (es) are visible in a central cavity surrounded by the pericarp (p).

Fig. 3. Transverse section of an ovary of *Avenula pratensis* s.str. (pollinated with *Avena sativa*). Epidermis (e), pericarp (p), integuments (i) of the embryo sac, vascular bundle (v).

Fig. 4. Transverse section of a seed from a cross between *Avena sativa* and *Avenula vasconica-pratensis* (see text). The embryo (e) is 0.67 mm long and shows a beginning of differentiation in scutellum and coleoptile. The thick pericarp (p) is compressing the otherwise empty embryo sac (es).

Fig. 5. Longitudinal section of a seed from a cross between *Avenula albinervis* and *Avena sativa.* A pro-embryo (e), 0.46 mm long, is visible at the bottom of the embryo sac (es) surrounded by parenchymatous tissue of the pericarp (p).

Fig. 6. Longitudinal section of a "hard" seed obtained from a cross between *Avena sativa* and the fertile hybrid *Avenula pratensis-planiculmis.* A thick sclerified pericarp (p) surrounds an embryoid structure (e st) possibly originating from the nucellus.

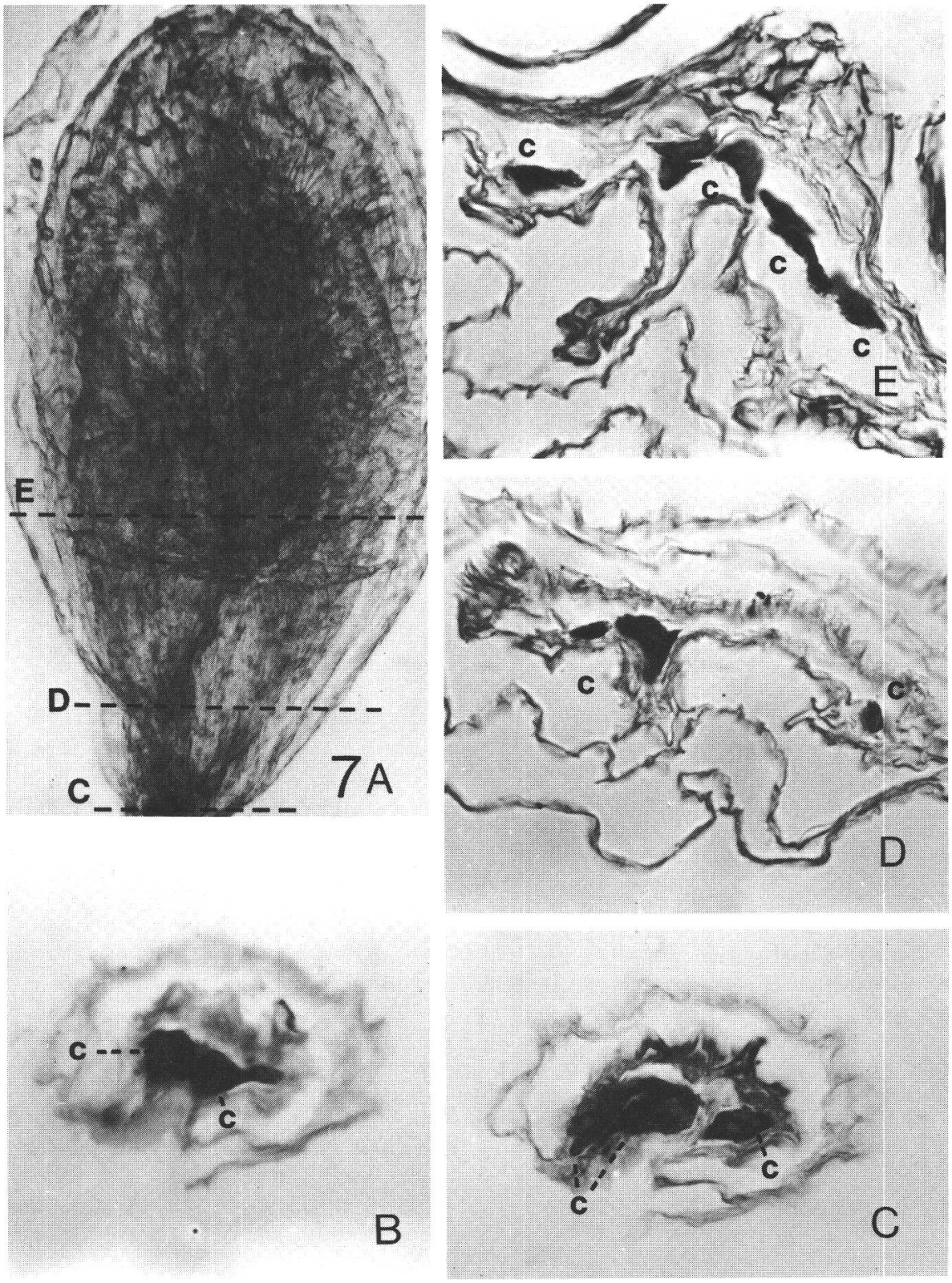


Fig. 7. Embryo sac of *Avenula pratensis*, 2n = 146, (pollinated with *Avena sativa*) and four of its transverse sections. A) The embryo sac (c. 1 mm) after dissection from the seed and before sectioning. The dotted lines indicate the approximate levels where sections C, D and E were taken. Section B comes from the bottom of the sac, not visible on the photography. B–E) Transverse sections of the embryo sac at four different levels, showing the presence of degenerated dark coloured cells (c).

As the positive results of the crosses at the interspecific level were obtained earlier, involved exclusively perennial species and formed a category of their own, they were discussed separately in two previous papers (Gervais 1977, 1981) which included somatic chromosome counts and descriptions, studies of meiotic behaviour when possible, morphological comparisons with the parents and reflexions on the phylogenetic aspects of the crosses. Consequently, the following annotations will be mainly devoted to hybridizations where one of the parents belongs to genus *Avena.* The results of the interspecific crosses are however reported in the Tables for comparison with the others and general information.

Avena sativa × *Avenula vasconica-pratensis* hybrid, and reciprocal. Table 3.

The first of these crosses, where the male parent is a fertile hybrid created in 1969 (Gervais 1973), produced the most developed embryo to be observed in the present intergeneric hybridizations. Its length is 0.67 mm and it shows a beginning of differentiation in scutellum and coleoptile (Fig. 4). There is apparently no endosperm and the development of the pericarp has appressed the walls of the embryo sac one against the other.

The small size of the hybrid seed (5 mm), its wrinkled aspect and the absence of endosperm are indications that it really results from a cross (or induced apomixis?) and not from an accidental auto-fecundation. A pro-embryo of about 10 cells was also observed in the reciprocal cross, *Avenula vasconica-pratensis* × *Avena sativa.*

Crosses involving *Avenula sulcata* or *albinervis* and *Avena sativa, byzantina* or mixtures of pollen. Tables 2, 4.

These crosses are discussed together because the annual parents, *Avena sativa* and *A. byzantina,* are considered as the same species by some taxonomists (Baum 1977), while the perennial parents, *Avenula albinervis* and *A. sulcata,* are close relatives though their chromosome numbers are different. This has been shown by previous hybridizations by the present author (Gervais 1973).

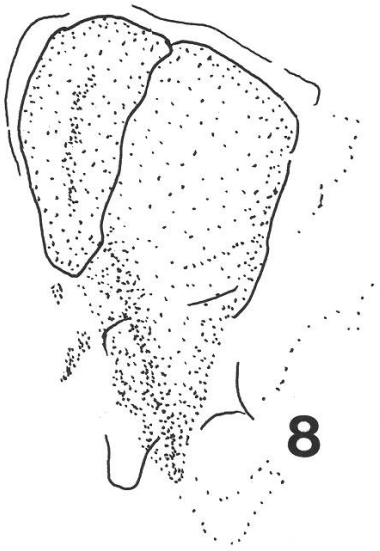
The most promising result, among the various hybridizations of this category, was the finding of a pro-embryo (0,46 mm long, Fig. 5) in an ovary from *albinervis-sativa* crosses. The attempts involving *Avenula sulcata* were also interesting as some cells, probably belonging to altered pro-embryos, were observed at the bottom of embryo sacs in a cross with *A. byzantina,* while some hard «seeds», with embryoid structures (see next crosses), were found in *sativa-sulcata* hybridizations.

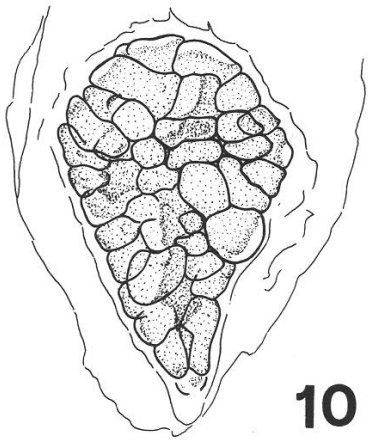
It is interesting to note that the results of the crosses where *A. albinervis* ($2n = 28$) was a parent, were better, on the whole, than those where this role was played by *A. sulcata* ($2n = 14$); it was hoped that the results would have been even better with *A. occidentalis* (Gervais) Holub which is closely related to *A. albinervis* and *A. sulcata* and is hexaploid ($2n = 42$) like *Avena sativa.* Unfortunately, the flowering of *A. occidentalis* could not be induced.

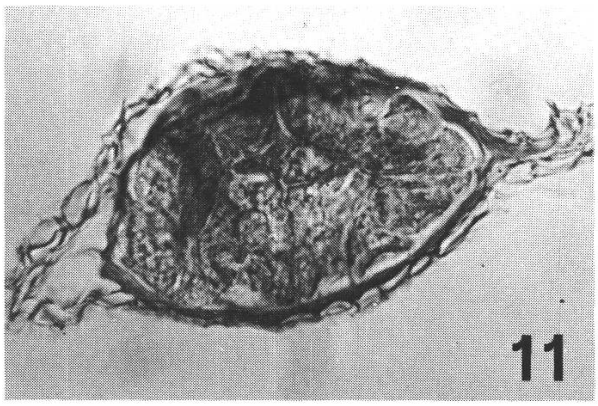
Avenula pratensis-planiculmis hybrid × *Avena sativa* and reciprocal,
Avenula pratensis-planiculmis hybrid × *Avena strigosa* or *A. byzantina.* Table 3.

The perennial parent in these crosses was a highly fertile hybrid obtained in previous cytological researches (Gervais 1973). In the first attempt (perennial hybrid ×

sativa), over 460 flowers were pollinated and the results appeared very negative until the examination of ovary sections revealed the presence of probable embryonnary cells in otherwise empty sacs. The reciprocal cross has produced some clearly stimulated ovaries or some short conical hard "seeds" whose anatomy (Fig. 6) has been interpreted







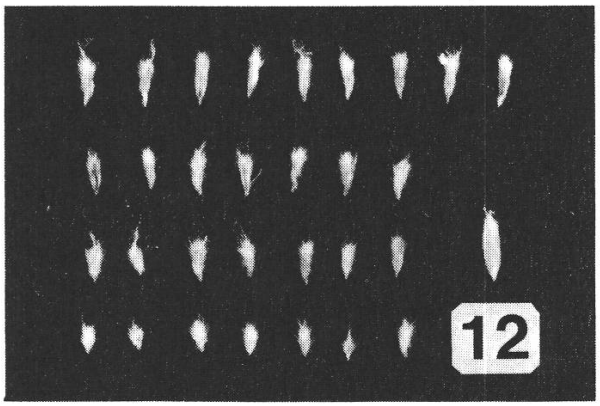


Fig. 8. Camera lucida drawing of a pro-embryo (3 cells?) found in an ovary of *Avenula bromoides* pollinated with *Avena strigosa.*

Fig. 9. Small embryo (0.21 mm long) observed at the bottom of a collapsed embryo sac dissected from a hybrid seed: *Avena sativa* × *Arrhenatherum elatius.* A protuberance on the right side of the embryo simulated a second lateral embryo.

Fig. 10. Camera lucida drawing of the same embryo.

Fig. 11. Transverse section of the embryo shown on Figs 9 and 10. There is apparently no trace of the protuberance.

Fig. 12. Stimulated and unstimulated (lowest row) ovaries from a cross between *Helictotrichon sarracenorum* (2n = 28) and *Avena sativa.* A normal seed (tetraploid *sarracenorum*) is on the right side.

as some kind of adventitious embryo, or embryoid structure, surrounded by a thick sclerified pericarp.

In the other crosses, with *A. strigosa* or *A. byzantina,* degenerated cells possibly belonging to denatured embryos were observed. The probability of success of intergeneric hybridizations with the perennial taxon *"pratensis-planiculmis"* does not look very promising though it was hoped that this vigorous plant could contribute interesting characters to *A. sativa.*

Avenula pratensis (diverse chromosomal races) × *Avena sativa* or other cultivated species. Table 2.

Though these hybridizations have involved high polyploid taxa as female parents (2n = 102 to 146), they have nevertheless resulted in ovaries where remnants of cells, possibly belonging to embryos, were frequently observed (6 cases, or 7 if *A. vasconica,* closely related to *A. pratensis,* is included). In particular, some hybridizations between an *A. pratensis* s.str. from England (Holy Island, *legit* C.E. Hubbard) and *A. sativa* or *A. byzantina* produced some remarkably stimulated ovaries. This is not indicated in Table 2 as the character "unidentified cells present" (c) was judged to be predominant over "stimulation of ovaries" (S). The results are consistent with those reported by Johnson and McLennan (1939).

Avenula bromoides (2n = 14) × *Avena strigosa* (or other annuals),
Avenula bromoides (2n = 42) × *Avena sativa.* Tables 2, 4.

A small pro-embryo (Fig. 8) which appears to have 3 cells was found after the dissection of some *bromoides* × *strigosa* ovaries, while unidentified cells were observed in other crosses, with *A. sativa* or with a mixture of pollen. These results are rather disappointing since a considerable effort (nearly 1500 flowers pollinated) was furnished as regards this species.

Serious attention was also paid to promising crosses between the hexaploid race of *A. bromoides* and *A. sativa* (513 flowers presumably pollinated) but the only result was the observation of deteriorated cells in a sectioned ovary. More crosses would have been carried out with this rare taxon but the available plants died.

Avena sativa × *Arrhenatherum elatius* and reciprocal,
Arrhenatherum elatius × *Avena strigosa.* Table 2.

Though a very small number of flowers (14) were pollinated in the cross with *A. sativa* as female parent, most of the ovaries were strongly stimulated and a pro-embryo of about 0.21 mm (Fig. 9 to 11) was found in one of the "seeds". This embryo appeared to be double (Fig. 9–10) with a small one on its side (from a synergid?) but this interpretation became less evident after the embryo was sectioned and examined (Fig. 11). A malformation could be responsible of a lateral lobe. The reciprocal cross (76 flowers pollinated) was negative.

In the second cross, with *A. strigosa,* some unidentified cells were found in a series of sections. A repetition of the crosses of the first group *(A. sativa* × *Arrhenatherum),* on a larger scale, would be interesting.

Other *Avenula* × *Avena* crosses. Tables 2, 4.

A few crosses are to be mentioned in this category. On the one hand, *Avenula compressa* × *Avena byzantina* and *Avenula pubescens* × *Avena sativa* gave some ovaries with degenerated cells present. On the other hand, Table 4 indicates the observation of large embryos in crosses where the female parents, *Avenula sulcata* and *A. albinervis*, were pollinated with a mixture of pollen. As these embryos did not germinate, it is impossible to know which male species was responsible for their formation though *Avenula pratensis* or the fertile hybrid *vasconica-pratensis* can be suspected. These latter taxa, indeed, also induced the formation of large embryos in crosses with *A. albinervis* as female or male parent (see Table 2).

Intergeneric crosses between some *Helictotrichon* and *Avena*. Table 2.

With the exception of the crosses involving *Avena macrostachya*, the results of which will be discussed below, the 9 hybridization attempts conducted between different species of the genera *Helictotrichon* and *Avena* were rather negative, on the whole, though nearly 1000 flowers were emasculated and presumably pollinated. Noteworthy stimulation, however (Fig. 12), was observed in some *H. sarracenorum* × *A. sativa* crosses and this result is reminding some analogous observations of Johnson and McLennan (1939) with *H. montanum* (Vill.) Henr. (*Avena montana* Vill., in their terminology).

Crosses with *Avena macrostachya* as male or female parent. Table 2.

Avena macrostachya is an endemic species from Algeria whose systematic position is rather ambiguous as it possesses large drooping spikelets like the annuals but is perennial. This taxon, whose precise description can be found in St-Yves (1931), Maire (1953) or Baum (1977), has been classified in a special section of genus *Helictotrichon* by Holub (1958) but is seen as an *Avena* by Baum (1968, 1977). Because of its apparently intermediate position, *A. macrostachya* was selected as a promising material for annual × perennial crosses.

According to the results of Table 2, *A. macrostachya* seems more closely related to genera *Helictotrichon* and *Avenula* than to genus *Avena*. A 0.675 mm long embryo was indeed obtained after a few flowers (21!) of a diploid *H. sarracenorum* were pollinated with *A. macrostachya*, while a small embryo, or denatured cells, were seen in crosses conducted with diverse species of *Avenula* on a relatively large number of flowers (over 600). On the contrary, some hybridization attempts of *A. macrostachya*, as female parent, by *A. sativa* (167 flowers), were unsuccessful, this outcome being similar to the results of Baum and Rajhathy (1976) who have used *A. macrostachya* as a pistillate parent in crosses with *A. strigosa*, *A. sativa* and the tetraploid *A. abyssinica* Hochst.

The interesting results found with the diploid *H. sarracenorum* (or some *Avenula*) and the failure of the crosses with annual *Avena* are in keeping with the classification of Holub (1958) and may indicate that the efforts to use *A. macrostachya* in crosses with cultivated *Avena* are in a direction where serious difficulties will be met. This could be readily confirmed by more hybridization attempts of *macrostachya* with *H. sarracenorum* (its tetraploid race if possible!) or with the tetraploid *H. montanum* (Vill.) Henr. ssp. *planifolium* (Willk. & Lange) Gervais which is also a taxon that could be close to *A. macrostachya*.

Intergeneric crosses between some *Helictotrichon* and *Avenula.* Table 2.

Three tests only, involving about 200 flowers, were done in this category of crosses and were negative except for the hybridization of a tetraploid *H. sarracenorum* with a diploid *A. bromoides* where degenerated cells were observed in one ovary. The results would have been better, perhaps, if a diploid *sarracenorum* had been used in the cross.

Conclusions

The following comments were added, in the form of conclusions, as general information and possible guidelines that could be useful in future research.

Regarding the technical aspect of the hybridizations, the method which was used was efficient enough to produce some interesting interspecific hybrids between apparently distant taxa with, in many cases, divergent chromosome numbers. The same approach, at the intergeneric level, has not given the same results and this suggests the interference of naturel barriers to pollen germination, penetration or to embryo development. As at least a certain number of small embryos were obtained in some of the intergeneric crosses (Tables 2–4), it may be assumed that the critical stage in hybrid formation is not pollen penetration and egg fertilization (unless the observed embryos result from apomixis) but early embryo breakdown, as probable result of failure of endosperm formation. It is nevertheless possible that the pollen germination was weak and should be enhanced to obtain more embryos.

In this respect, it seems that the deposition of extracts of crushed conspecific pollen on the styles of a plant, together with alien pollen, did not have a decisive effect on the success of the crosses. Likewise, addition of giberellic acid (75 ppm) or fertilization attempts with different types of pollen deposited together, or at short intervals (Table 4), remained inconclusive, as the percentage of success was not higher with these methods than with classic crosses (Table 2). If additional intergeneric hybridizations are to be pursued, some technical improvements to favour pollen penetration are not to be excluded but it seems that more or less early embryo removal and culture would be the most important points.

Another delicate question, related to the crosses, was the problem of their direction. This was due to the fact that the material to hybridize was heterogenous (diploids and polyploids, annuals and perennials) and that it was not usually possible to repeat each cross by its reciprocal. As aforesaid, the perennial taxa were usually selected as female parents because the opposite direction had been tested without much success in previous research. Looking at the results, the selection of the annual taxon as female or male parent does not seem to have played a special role in the appearance of embryos, though the most developed one came from a cross where the annual *A. sativa* was the female parent.

In the interspecific crosses, within genus *Avenula* or genus *Helictotrichon,* the species with the lower chromosome number was preferably selected as female parent and this practice has been successful until now, even when the chromosome numbers are quite different (diploid *H. sarracenorum* × 14-ploid *H. filifolium,* Table 4, for exemple). In 7 out of 13 successful interspecific hybridizations (Tables 2, 4 and Gervais 1973), the female parent had the lower chromosome number while in the 6 others the degree of ploidy of the parents was the same.

In the intergeneric crosses, no attention was paid to the chromosome numbers and their position but when a species was represented by two or more chromosomal races, the preference was given, if possible, to the taxon that could improve the chromosomal balance between the parents. The biggest effort, in terms of the number of pollinated flowers, was generally directed towards the crosses where the balance of the chromosome numbers appeared favourable. It seems on the other hand that the importance of the direction of the crosses, in relation to the chromosome numbers, was less important at the intergeneric level. According to the present results, 3 of the 8 embryos which were found resulted from combinations where the female parent was a higher polyploid and 4 came from crosses where the opposite situation prevailed (the chromosome numbers were the same in one case).

The phylogenetic implications of the success or the failure of the hybridizations are a more controversial subject as it is not certain 1) that the Tables accurately measure the real cross-compatibilities 2) that these compatibilities reflect the real degree of the phylogenetic relationships.

Taking these restrictions into account, it may be inferred from the Tables, at the intergeneric level, that genus *Avena* is more closely related to genus *Avenula* than to genus *Helictotrichon.* Genus *Arrhenatherum (A. elatius* at least) seems also relatively close to *Avena* but its relationships to *Avenula* and *Helictotrichon* have not been tested. It appears also, as already said, that the systematic position of *Avena macrostachya* could be closer to genus *Helictotrichon* than to genus *Avena* where this species was generally inserted. The presence of large drooping spikelets in *A. macrostachya* could come from an evolutive trend that has repeated itself later when the "annual" phylum appeared.

Summing up the information that this study has brought out, it could be advanced that if efforts are to be done to cross *A. sativa,* or *A. strigosa,* with perennial species, the most promising avenues would be with the *Avenula sulcata-albinervis-occidentalis* complex or with the *A. pratensis* complex. *Arrhenatherum elatius* could be also an interesting material and possibly *H. sarracenorum,* though the genus *Helictotrichon* seems less related to genus *Avena* than genus *Avenula* does.

As the perennial oats are forming a very large complex whose African, Asiatic and even Mediterranean reprensentatives are not yet fully understood, it is possible that other taxa, not included in the crosses, would be more readily hybridized with the annual cultivated species. One could mention, for exemple, *Avenula breviaristata* (Barratte apud Battand. et Trabut) Holub, a rare (if not extinct) Algerian species which combines, according to St-Yves (1931), some taxonomic characters of *A. pratensis, A. bromoides* and of the true *Avena* (i.e. *Genuinae*). Obviously, very much work lies ahead before a clear picture of the species relationships for this interesting section of the tribe *Aveneae* becomes visible.

Résumé

Ce travail réunit les résultats de 91 essais d'hybridation interspécifiques et intergénériques différents impliquant 30 taxons appartenant aux genres *Arrhenatherum* Beauv., *Avena* L., *Avenula* Dumort. et *Helictotrichon* Bess. de la tribu des *Aveneae.*

Le but des croisements était d'obtenir, d'une part, des hybrides utiles à l'amélioration génétique des avoines cultivées et de mettre en évidence, d'autre part, les relations phylogénétiques reliant les espèces et les genres étudiés.

Sept croisements interspécifiques ont été réussis à l'intérieur des genres *Avenula* et *Helictotrichon* mais aucun hybride intergénérique n'a été obtenu. Toutefois, la présence occasionnelle de cellules dégénérées, de préembryons ou de jeunes embryons de diverses tailles dans le sac embryonnaire des ovaires pollinisés démontre que la fécondation a probablement eu lieu et qu'il serait possible d'obtenir certains hybrides intergénériques (si les embryons observés ne résultent pas de phénomènes d'apomixie).

Une «échelle de compatibilité» permettant d'évaluer le degré de succès des croisements a finalement été établie pour mettre en évidence les groupes de taxons phylogénétiquement voisins et susceptibles de se croiser. Les cas les plus intéressants où des hybridations intergénériques semblent possibles (certains croisements *Avena* × *Arrhenatherum*, *Avena* × *Avenula* et réciproques) sont finalement discutés individuellement.

Ce travail est dédié à M. le Professeur Claude Favarger, directeur de l'Institut de botanique de l'Université de Neuchâtel. Nous voudrions le remercier très sincèrement de nous avoir initié à la recherche cytologique et toujours encouragé par la suite.

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