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Creating new forms of 4x, 6x and 8x primary triticale associating both complete R and D genomes

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Summary. Strains of *Aegilops squarrosa* L. and *Ae. ventricosa* L. were pollinated either by *Secale cereale* L. or tetraploid triticale. Using in vitro culture of immature F1 embryos, the four corresponding hybrids were obtained. Successful doubling occurred following colchicine treatment, leading to the creation of new amphidiploid structures (C1 plants). These correspond to primary triticale forms involving, at three different levels of ploidy, both R and D full complements. The various combinations were compared for their response at successive steps of the process. Crosses involving *Ae. squarrosa* present a higher fruit setting than those with *Ae. ventricosa*, which in contrast yield colchicine treated-plants with better grain fertility. Experimental data on the cytological behaviour and fertility of colchicine-treated as well as amphidiploid plants are presented. The importance of this material in triticale breeding is discussed.

Key words: *Aegilops* – Rye – Triticale – Intergeneric hybridization – Amphidiploid production – C-banding

Introduction

The D genome of bread wheat, also present in the diploid species *Aegilops squarrosa* (= *Triticum tauschii*), and the tetraploid species *Ae. ventricosa* (= *T. ventricosum*), possesses an interesting genetic information: it is active in determining such characteristics as plant height, seed storage proteins and rheological properties, and susceptibility to diseases (powdery mildew, rusts, eyespot, etc.). On the whole, it comprises an extensive pool of genes to be taken advantage of in breeding programmes. As far as hexaploid triticale is concerned, access to this gene pool is somewhat uncertain. In fact, there are only three practicable approaches: 1) hybridization between 6x-triticale and bread wheat; 2) hybridization between 8x-triticale and tetraploid triticale; 3) direct synthesis of amphiploid plants associating species bearing D and R genomes.

Until now, the second and third approaches have seldom been used, mainly as a result of a non-availability of plant material or a difficulty in obtaining hybrids from crosses, respectively.

The first approach has been widely explored (Bernard and Bernard 1978; Bernard et al. 1985a; Jouve et al. 1985; Lukaszewski et al. 1982, 1985; May and Appels 1982). It has widespread use and already has led to valuable results in triticale breeding programmes. On the other hand, it is not certain that it has allowed an adequate introgression of genes located on the D genome in hexaploid triticale.

Crosses between 8x- and 4x-triticale appear to be a more promising way to introduce D genome chromosomes. It makes it possible to obtain 42 chromosome plants possessing a variable number of D genome chromosomes in addition to the full R genome (Bernard et al. 1985b).

The third approach is dealt with in the present paper which reports on results obtained in the field of direct synthesis of amphiploids, using *Aegilops* germplasm.

Materials and methods

Materials

The parent genotypes used are: 1) the INRA rye synthetic variety 'Beaulieu' (*Secale cereale* L., $2n=2x=14$), and its seven inbred constituants (self-compatible lines); 2) three strains of tetraploid triticale (*X-Triticosecale* Wittmack, $2n=4x=28$) developed in our laboratory since 1977 (genomic structure (*AB*) (*AB*) *RR*, Bernard and Bernard 1985); 3) two strains of *Ae. squarrosa* L. ($2n=2x=14$), and one strain of *Ae. ventricosa* L. ($2n=4x=28$) obtained from the INRA Plant Breeding Station, Rennes. Four different crosses were attempted: 1) *Ae. ventricosa* × rye (hereafter referred to as V×R); 2) *Ae. ventricosa* × 4x triticale (coded V×T4x), and reciprocal; 3) *Ae. squarrosa* × rye (coded Sq×R); 4) *Ae. squarrosa* × 4x triticale (coded Sq×T4x).

The crosses were represented by 8; 2; 3 and 4 different hybrid combinations respectively.

Methods

All materials were grown in spring under greenhouse conditions, with natural daylight and photoperiod. Spikes were hand-emasculated and bagged, then pollinated when stigmas were receptive. The developing caryopses were excised 13 to 20 days following pollination, and the existing embryos were cultivated in vitro on the basic medium developed for triticale anther culture (Bernard 1977) supplemented with 500 mg/l glutamine and 1 mg/l IAA, and solidified with 7 g/l agarose. Developing plants were transferred to soil in pots, and vernalized for 4–8 weeks. With the exception of four V×T4x F1 plants left as checks, all the hybrids obtained were submitted to colchicine treatment by soaking 3–4 tiller-stage plants in a 0.25% solution for 4 h, and then repotted. All treated material was subsequently installed in soil in the greenhouse in late autumn. Chromosome counts were performed using the Feulgen squash method or the Giemsa C-banding procedure reported in Charmet et al. 1986. Meiotic chromosome behaviour was analysed on preparations stained either with acetocarmine or by means of the C-banding technique.

Results

1 Results of crosses

The results of crosses are presented in Table 1. Crosses were only successful when *Aegilops* was used as a female parent, even in the case when both directions were attempted. With this restriction, seeds have been obtained in all 4 types of crosses whatever the parent genotypes tested. Fruit setting, expressed in grains per ear pollinated, is much higher in crosses involving *Ae. squarrosa* (14.2 in Sq×R and 12.8 in Sq×T4x) than those involving *Ae. ventricosa* (3.8 in V×R and 7.0 in V×T4x). At the time of dissection most of the developing caryopses possessed an embryo, as follows: 95.2% in V×R crosses, 76.8% in V×T4x crosses, 81.0% in Sq×R crosses, and 74.5% in Sq×T4x crosses. Not all these embryos were able to develop in vitro: the percentage of viable hybrid plants recovered from embryos ranges from 20.1% in Sq×R crosses to 42.5% in V×R crosses. The final success rate, expressed in number of viable hybrid F1 plants per ear pollinated, is 1.5 in hybrids involving *Ae. ventricosa* germplasm and close to 2.5 in those involving *Ae. squarrosa* germplasm.

2 Results of colchicine treatment

Following colchicine treatment, all the plants treated survived in Sq×T4x and V×T4x combinations, whereas some died before harvesting in combinations involving rye (5 out of 17 in V×R and 6 out of 30 in Sq×R). Table 2 presents the characteristics of hybrid plants harvested following colchicine treatment (Co-plants). On most of these Co plants, ears appeared showing dehiscent anthers, and afterwards, grains, indicating that chromosome doubling had occurred. This was further confirmed by means of chromosome analyses of C1 progenies. In contrast, no seed was produced on non-treated check plants. Depending on the cross considered, the overall percentage of ears with fertile sectors varies from 2.9 to 10.6% of the total number of spikes developed. It is also quite variable between plants within a population of hybrids, with maximum figures per plant reaching 32.5 percent in V×R, 21.7 in V×T4x and 24.3 in Sq×R combinations, and only 10.7 in the Sq×T4x combination. The number of grains recovered in Co plants varies greatly between crosses: colchicine-treated hybrids involving *Ae. ventricosa* germplasm appear much more fertile than those with *Ae. squarrosa*, concerning the average grain number either per plant (120 and 156 vs. 16 and 6.5 grains) or per fertile ear (10 and 19 vs. 4 and 5 grains approximately).

3 Cytology

Samples of these grains were sown to obtain the next generation (amphidiploid C1 plants).

A) In the two combinations involving *Ae. ventricosa*, it was possible to study a small sample of C1 plants. Fifteen V×R plants analysed had either 42

Table 1. Results of crosses

	Ventricosa × rye	*Ventricosa* × T4x	*Squarrosa* × rye	*Squarrosa* × T4x
No. of ears pollinated	11	8	13	4
No. of developing caryopses	42	56	184	51
Developing caryopses per ear	3.8	7.0	14.2	12.8
No. of caryopses with an embryo	40	43	149	38
Embryos/caryopses (%)	95.2	76.8	81.0	74.5
No. of hybrid plants obtained	17	12	30	11
Hybrid plants/embryos (%)	42.5	27.9	20.1	28.9
No. of hybrid plants per ear pollinated	1.55	1.50	2.30	2.75

Table 2. Characteristics of hybrid plants treated with colchicine

	Ventricosa × rye	*Ventricosa* × T4x	*Squarrosa* × rye	*Squarrosa* × T4x
No. of colchicine treated plants (Co plants)	17	8	30	11
No. of Co plants harvested	12	8	24	11
No. of fertile Co plants	8	8	22	6
Total no. of tillers	904	1,021	1,135	272
Mean no. of tillers per plant	75.3	127.6	47.3	24.7
Total no. of fertile ears	96	66	86	8
Mean numbers of fertile ears per plant; (range)	8 (0–67)	8.25 (1–26)	3.58 (0–15)	0.73 (0–3)
Total grain no.	960	1,248	371	39
Grain no. per plant; (range)	80 (0–760)	156 (21–460)	14.8 (0–74)	3.6 (0–22)
Grain no./fertile ear	10	18.9	4.3	4.9

chromosomes (10 plants) or 41 chromosomes (5 plants), the latter all belonging to the same family (V × R 533). The average fertility per ear of the plants with 42 chromosomes was 17 grains, that of the plants with 41 was less than 10 grains. The average meiotic behaviour of the 42 chromosome plants was:

$2_{I} + 19.92_{II}$ (6.7 rods + 13.2 rings) + 0.04_{IV}.

The V × T4x C1 plants had the following chromosome numbers: 56 (4 plants) 55 (7 plants), 54 (7 plants), 53 (1 plant) and 48 (1 plant).

The fertility of plants with 56, 55, or 53 was very similar (16–18 grains per ear); that of the plants with 54 was only 10 grains per ear; the plant with 48 was sterile. The meiotic behaviour checked on a plant with 56 was very irregular, as follows:

$7.36_{I} + 24.33_{II}$ (11.98 rods + 12.34 rings).

B) In the Sq × R combination, cytological information on several *DR* hybrids and two amphiploid plants is available.

Hybrids. Out of 100 *DR* PMC'S analysed in C-banding we found the following figures (Fig. 1):

- 54 cells showing 14 univalents,
- 33 cells with one rod bivalent,

of which 20 : R – D
12 : D – D
1 : R – R

- 9 cells with 2 rod bivalents,

of which 6 : R – D R – D
2 : R – D R – R
1 : R – D D – D

(The other possible associations were not observed).

- 2 cells with 3 rod bivalents: R – D R – D D – D
- 2 cells with one trivalent: R – D – D

Amphidiploids. In a sample of 17 C1 plants, 16 exhibited the expected 28 chromosomes, one plant was aneuploid with 29 chromosomes. A C-banded karyotype is presented in Fig. 2, with 14 terminally-banded R chromosomes and 14 light-colored D chromosomes.

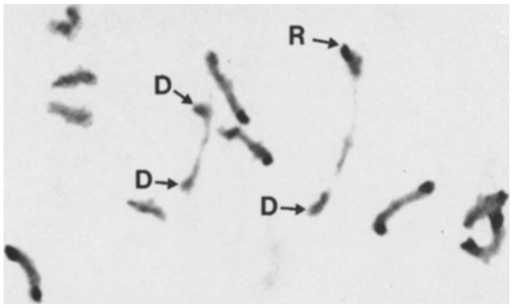

Fig. 1. C-banded metaphase 1 of an *Ae. squarrosa* × rye hybrid

Two *DDRR* plants were analysed (50 cells per plant) and they exhibited the following meiotic behaviour:

$3.04_{I} + 12.48_{II}$,
and $4.5_{I} + 11.7_{II}$.

The C-banding technique made it possible to precise the respective behaviour of R- and D-genome chromosomes in the latter plant:

$R = 3.75_{I} + 5.125_{II}$
$D = 0.85_{I} + 6.575_{II}$.

Figure 3 shows a cell with bivalents only.

C) The following chromosome numbers were found in a sample of 13 C1 Sq × T4x amphidiploids:

2n =	39	40	41	42	43
plant no:	1	1	2	7	2

Meiotic behaviour was analysed with the C-banding technique in two families which differed only by the triticale male parent involved in the initial cross. It was as follows:

$8.43_{I} + 16.57_{II} + 0.12_{III}$

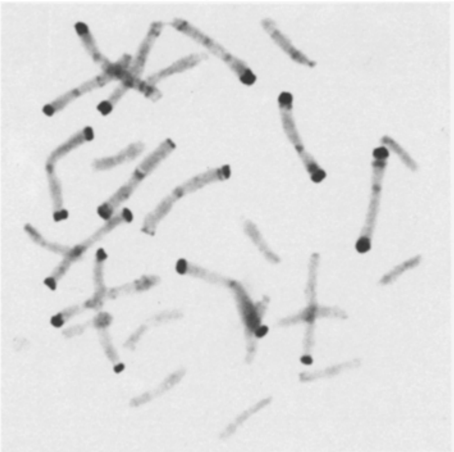

Fig. 2. C-banded karyotype of an *Ae. squarrosa* × rye amphidiploid

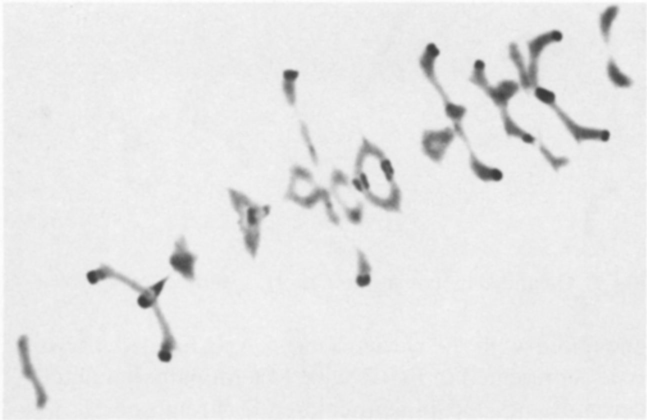

Fig. 3. C-banded metaphase I of the same amphidiploid

of which 3.48 R univalents
and 5.26 R bivalents (40 cells analysed), in one family;
and $5.81_I + 18.10_{II}$
of which 3.81 R univalents
and 5.09 R bivalents (20 cells analysed), in the other family.

Discussion

1 Obtaining hybrids and amphiploid plants

If we compare the four different crosses, we can notice their different behaviour at each step of the process leading to established amphidiploids: crosses involving *Ae. squarrosa* show a much higher seed set than those involving *Ae. ventricosa*, which is in agreement with Pagniez and Hours' results (1986). Concerning the success rate of the colchicine treatment, the particular reaction to colchicine of hybrids involving *Ae. ventricosa* germplasm should be mentioned: most of the time, ears either remained fully sterile, or became highly fertile, with nice-looking regular grains all along the ear. This differs from what was obtained with *Ae. squarrosa* (a few seeds scattered along the ear), or with doubled haploid triticale material, in which sectorial doubling appears to be the rule (Bernard, unpublished). As regards the fertility of the colchicine-treated hybrids, the advantage goes to crosses involving *Ae. ventricosa.* Thus, in the experiments presented, there is no relationship between the success rate of the cross and the fertility observed in the Co plants.

The literature reports numerous attempts aimed at obtaining hybrids and amphiploids from crosses between various *Triticum, Aegilops* and *Secale* species:

a) Crosses with rye. From *Ae. squarrosa* × rye, Melnyk and Unrau (1959) and Krolow (1970) each obtained one F1 plant. Abirached-Darmency et al. (1984) produced such a hybrid with rye cv. 'Petkus' as a pollen parent, which was used along with other *Triticum* × *Secale* hybrids for chromosome pairing studies. Pagniez and Hours (1986) mention high rates of fruit setting but could not achieve hybrid production. The corresponding amphiploid was reported only once in the literature by Fedak and Sampson (1983), following in vitro culture of immature hybrid spikes. It was also obtained by Y. Cauderon (pers. commun.).

The cross *Ae. ventricosa* × rye was performed by Chomsai and Jones (unpublished), who obtained an amphiploid in 1975. It was supplied to Dosba and Jahier who used it as female parent in crosses with bread wheat (1981). From the some cross, Pagniez and Hours (1986) again obtained seeds with embryos, but no hybrid plant.

In the field of such *Aegilops sp.* × *Secale* crosses, our results do not differ significantly from previous reports with regard to seed set or the proportion of caryopses with an embryo. In contrast, they greatly differ as regards in vitro embryo viability (number of plants recovered from embryos cultivated in vitro), as well as chromosome doubling following colchicine treatment. This suggests that careful attention should be paid to the in vitro culture step, which appears critical in such wide-cross programmes. Further, in addition to physical conditions (temperature and lightening) available during the colchicine treatment itself, optimal growing conditions should be devised before and after the treatment for successful chromosome doubling of hybrid materials. Our conditions, which determine slow growing and allow profuse tillering in the colchicined hybrids, yield a high proportion of surviving plants and of fertile amphidiploids among them: 22 out of 24, from 30 *Ae. squarrosa* × rye F1 hybrids; and 8 out of 12, from 17 *Ae. ventricosa* × rye F1 hybrids produced.

b) Crosses with tetraploid triticale. With regard to *Aegilops* sp. × tetraploid triticale crosses, the results presented here constitute the first report of obtaining both hybrid plants and the corresponding amphiploids, with gametic genome constitution $(AB)\ DR$ or $(AB)\ DM^vR$.

Regarding the genomic constitution the closest materials earlier obtained were trigeneric hybrids made by Siddiqui (1971) from crosses between existing *Aegilops* × *Triticum* amphiploids as female parents and *Secale cereale* as the male parent. However, amphiploids were not obtained.

2 *Cytology of amphiploid triticales*

From a cytological point of view the following remarks can be made regarding the newly created amphiploid triticales.

In the combinations studied, it appears that:

1) The amphiploid plants have a diploid-like behaviour (almost no multivalent associations). This may be explained by two different mechanisms: a) lack of affinity between D and R chromosomes; b) presence of the 5B chromosome in amphiploids involving tetraploid triticale.

2) The level of asyndesis is variable, probably depending on ploidy level, but not very high in 28 and 42 chromosome plants.

3) In *DD RR* plants, 80% of asyndesis can be attributable to rye chromosomes.

4) The genotype of the parents seems to play an important rôle in the cytological behaviour, the fertility and the morphological features of the amphiploid obtained. We plan to study this aspect in more detail in a certain number of combinations.

Further studies will be undertaken to evaluate the importance of this new group of triticales with regard to its biochemical grain characteristics and its behaviour with respect to various diseases.

It would appear that this approach of direct synthesis of amphiploids associating R and D genomes is quite practicable. This opens possibilities of directly using genetic variability available in the D genome, and of creating numerous new genomic structures. In particular, the *Ae. squarrosa* × tetraploid triticale amphidiploid can be regarded as the first hexaploid triticale with both R and D full complements. This approach will also make available a cytoplasmic variability which should be investigated for triticale improvement.

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