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Attempts to Produce Alien Addition Lines in *Triticum durum*

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ABSTRACT

Aneuploid stocks in durum wheat are few, and alien additions are particularly rare. The present contribution describes the results of a program aimed at the addition of alien chromosomes to tetraploid wheat. *Aegilops caudata*, *Ae. longissima* and *Dasyphyrum villosum* were used as chromosome donors. The crossing program involved the production of amphidiploids with the wheat cytoplasm and the recurrent crossing with durum wheat pollen. 15 monosomic addition ($2n=29$) plants were obtained and partly selfed and/or backcrossed to wheat. Two disomic addition ($2n=30$) plants, possessing two different *Ae. caudata* chromosomes, were obtained; unfortunately they were very weak and highly sterile, thus preventing their further propagation. The difficulties experienced during the development of the crossing program are discussed in detail.

INTRODUCTION

In Italy, and especially in the Southern regions, *Triticum durum*, macaroni wheat, is a cereal crop of relevant interest. In addition to a long lasting cultural tradition, the cultivation of this crop is maintained because of its importance in the pasta making industry. In the latest years some legislation is being developed which introduces a "premium" for pasta types made out of Italian durum wheat varieties; as a consequence there is a renewed breeders interest in improving this species.

Moreover, there is a growing consumer's interest in "natural" products, that is in food produced from plants grown without any chemical treatment. Consequently there is a need for plant types particularly fitted to be "biologically" grown. Several interesting traits can be found in wild species which could be introduced in improved varieties fitted for the above aims, such as genes coding for storage proteins able to improve the technological quality of the semolina or genes conferring resistance to biotic or abiotic stress.

Most of the outstanding cytogenetic work in wheat has

been carried out on *T. aestivum*. In durum wheat, conversely, aneuploid stocks are few, possibly because of two reasons: minor economic importance of this crop as compared to common wheat, and more intrinsic technical difficulties due to a lower level of genetic tolerance to the aneuploid condition.

Nevertheless, in the cv. 'Langdon', some aneuploid series have been developed, namely double-ditelosomics, dimonotelosomics, D-genome disomic substitutions, and some other well balanced aneuploids, such as intervarietal substitutions (Joppa 1993); in the cv. 'Senatore Cappelli' also primary trisomics were established (Blanco *et al.* 1982). Reports of alien disomic or monosomic additions are very few and concern some $2n=29$ aneuploid lines carrying chromosomes from *Dasyphyrum villosum* (Blanco *et al.* 1987).

In 1988 at the Germplasm Institute of Bari we started a project aimed to produce monosomic or, better, disomic alien additions of *Aegilops* or *Dasyphyrum* chromosomes to *Triticum durum*. The present contribution reports on the results obtained from this project.

MATERIALS AND METHODS

All the *Triticum* and *Aegilops* accessions used in the present study are part of the collection held at the Germplasm Institute. $2n=42$ amphidiploids involving *Triticum durum*, *Aegilops caudata*, *Ae. longissima* and *Dasyphyrum villosum*, respectively, with wheat cytoplasm obtained earlier. Five different wheat accessions were used as the female parent in the production of the amphidiploids and as recurrent male parent in later crosses. A list of the parent material is given in Table 1.

BC₂ seeds were scored for chromosome number: root tips from plantlets showing $2n=29$ were banded in order to try cytologically to identify the extra chromosome. $2n=29$ BC₂ plants were selfed in order to try the obtention of disomic additions or crossed with the wheat parent in order to maintain them.

Table 1. Origin of the amphidiploids used in the present study: female parent was *durum* wheat (TTnn)

Amphidiploid	Origin
GXX04	TT01 x <i>Ae. caudata</i>
GXX05	TT05 x <i>D. villosum</i>
GXX06	TT48 x <i>Ae. longissima</i>
GXX07	TT48 x <i>Ae. longissima</i>
GXX09	TT01 x <i>Ae. caudata</i>
GXX12	TT01 x <i>Ae. longissima</i>
GXX13	TT24 x <i>Ae. caudata</i>
GXX15	TT08 x <i>Ae. caudata</i>

TT01: cv 'Senatore Cappelli', TT05: landrace 'Bidi', TT07: landrace 'Sciacca', TT48: landrace 'Capinera', TT24: cv 'Langdon'

Crosses were made in the field in different years; spikes of the female parent were hand emasculated and pollinated. Chromosome counts were made on the root tips of the hybrid seeds, after pretreatment with ice-water for 18-24 hours and overnight fixation in Farmer's fluid, using the Feulgen squash method. Chromosome banding was performed using the technique of Giraldez *et al.* (1979).

RESULTS AND DISCUSSION

We decided to follow a crossing scheme starting from some $2n = 42$ amphidiploids (*T. durum* x alien diploid species) which had been previously obtained in crossing programs. The main advantage of this choice was essentially the reproducibility of the results, since it was possible to restart the backcrossing program at any stage, which would not be possible starting with hybrids (amphihaploids). Moreover the amphidiploids have better female fertility than amphihaploids and consequently the BC_1 seed set is higher. Amphidiploids with the wheat

cytoplasm were chosen in order to avoid cytoplasmic influence on gametogenesis. The female gametes of amphihaploids show high levels of meiotic non reduction, thus yielding gametes which are genetically indistinguishable from those of the relative amphidiploid.

In Table 2 the data from the backcrossing (BC_2) program are reported on the basis of the amphidiploids involved. 509 BC_2 seeds were obtained out of 5155 pollinated spikelets on BC_1 plants (0.099 seeds/spikelet); the low seed set depends on the reduced fertility of the $2n = 35$ BC_1 plants. The backcrosses of some amphidiploids, were much more fertile than the average, and particularly high values were observed in BC_2 from XX 05 (0.363 seeds/spikelet) and XX 15 (0.381 seeds/spikelet); also backcrosses of XX 09 showed high fertility (0.171 seeds/spikelet) although not as high as the previous hybrids. These amphidiploids involved *D. villosum* (XX 05) and *Ae. caudata* (XX 09 and XX 15). Most of our work concentrated on these BC_2 seeds. The backcrosses of other amphidiploids set fewer seeds.

Table 2. Seed set of each amphidiploid when backcrossed to wheat

Amphidiploid	Seeds	Spikelets	Ratio
XX04	31	965	0.03
XX05	182	501	0.36
XX06	1	357	0.0
XX07	63	1652	0.04
XX09	104	607	0.17
XX12	0	24	0.0
XX13	59	868	0.07
XX15	69	181	0.38

Table 3. Chromosome constitution of BC₂ plants

2n =	Plants
28	342
29	15
30	20
31	23
32	27
33	4
34	12
35	31

The big difference observed in seed set in the backcrosses of different amphidiploids might be the influence of the alien genome in the amphidiploids alone or the interaction resulting from the genetic combination in the hybrid. In fact, although possessing the same genome, the accessions used in the crossing program were rather genetically different: TT 01 was the old Italian cv 'Senatore Cappelli' (in XX 09); TT 08 was an old Sicilian landrace (in XX 15); in the program we also used an accession from Ethiopia which strongly differed from the others (TT 48). The influence of the genotypes on the crossability in interspecific hybridization had been already evidenced during the course of the crossing program (Pignone and Cifarelli 1990).

The BC₂ seeds were analysed for chromosome numbers; out of 509 seeds 37 failed to germinate; other few plants were too weak and did not reach maturity. Most of the analysed seeds (342) showed an euploid chromosome complement. Only 15 seeds showed a 2n= 29 genotype; in all these plants the added chromosome belonged to *Ae. caudata*. The results of chromosome number analysis are shown in Table 3.

No 2n= 29 BC₂ plants with *D. villosum* addition could be detected. On the basis of previous results (Blanco *et al.* 1987), we expected to obtain some *D. villosum* additions; possibly this failure depends on the different wheat and *Dasyphyrum* genotypes used in our crossing program.

The 2n= 29 plants derived from the same *Ae. caudata* line and from two different wheat accessions, TT 01 and TT 08, the most successful accession in producing addition lines was TT 08. Nine out of 15 plantlets had its genetic background.

The chromosomes of these plants were stained with the C-banding technique in order to identify the added chromosome. It is interesting to note that from a cytological point of view the additions only showed 4 different chromosomes in similar relative proportions. Plants showing the same added chromosome also possessed very similar morphological traits, thus confirming the cytological assessment.

The maintenance of these addition lines was tried following two paths: selfing or crossing with the wheat parent.

In general 2n= 29 BC₂ plants were not fully vigorous:

tillering was poor, culms developed late and spikes were rather small. Anthers were shrivelled and did not deliver much pollen. Selfing was, therefore, quite difficult, and often self fecundation was manually assisted, breaking mature anthers within the same flower. Nevertheless 157 selfed seeds were obtained. Only 6 of them showed 2n= 29 chromosomes (4%), and 2 were 2n= 30 (1%). Few plants showed some rearrangement of the added chromosome which appeared as a telo or isochromosome (Figure 1). The number of seeds obtained is low, inferring any evaluation of the transmission rate of the added chromosome and, moreover, data from additions involving different chromosomes are grouped; nevertheless it appears that this value is rather low and possibly even lower than the one observed in *D. villosum* additions (Blanco *et al.* 1987).

Cytological examination allowed us to determine that two different disomic additions had been obtained; 2n= 30 chromosome plants were extremely weak, had a chlorotic aspect and were very late. They produced few small spikes which were completely male sterile, showing undeveloped anthers. Fecundation with wheat pollen was also attempted without success.

Backcrossing of the 2n= 29 plants was attempted in order to maintain the obtained additions. The seed set was poor, since most of the seeds resulted 2n= 28 tetraploids (Figure 2). Only three 2n= 29 true addition plants were recovered while in other cases the added chromosome appeared rearranged. In four plants the extra chromosome had a C-banding pattern different from the donor chromosomes, thus indicating a more complicated rearrangement than centromere misdivision alone.

The low level of fertility of 2n= 29 plants poses a big limitation to the maintenance of these cytogenetic stocks. It is likely that hybrids with different genetic combinations might have a more favourable transmission. In fact, some unreported data might indicate that changing the wheat parent accession at each generation improves the proportion of healthy aneuploid plants; this method has the disadvantage of introducing undesired variation in the wheat genome which could spoil any use of the aneuploid stocks. This restriction also poses a reservation for the utilization of this aneuploid material, because under these conditions any possible use is hampered by the possibility of losing the lines.

Chromosome constitution

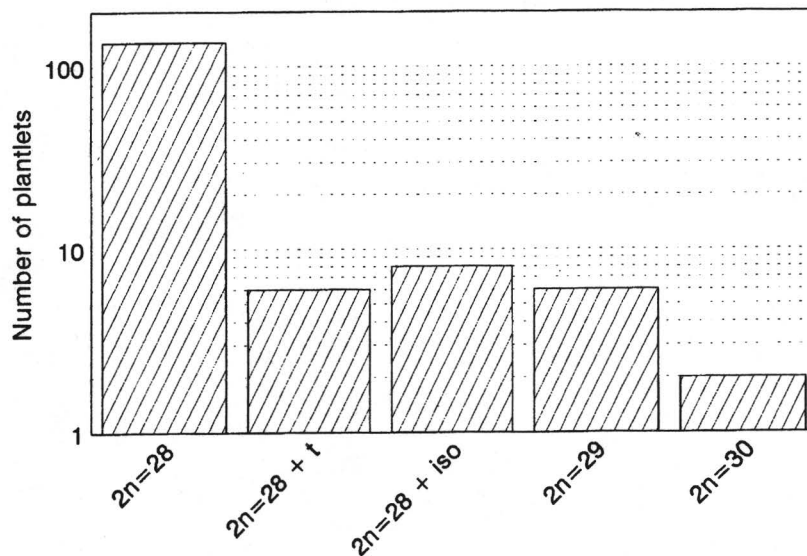


Figure 1. Seed set of selfed 2n= 29 plants

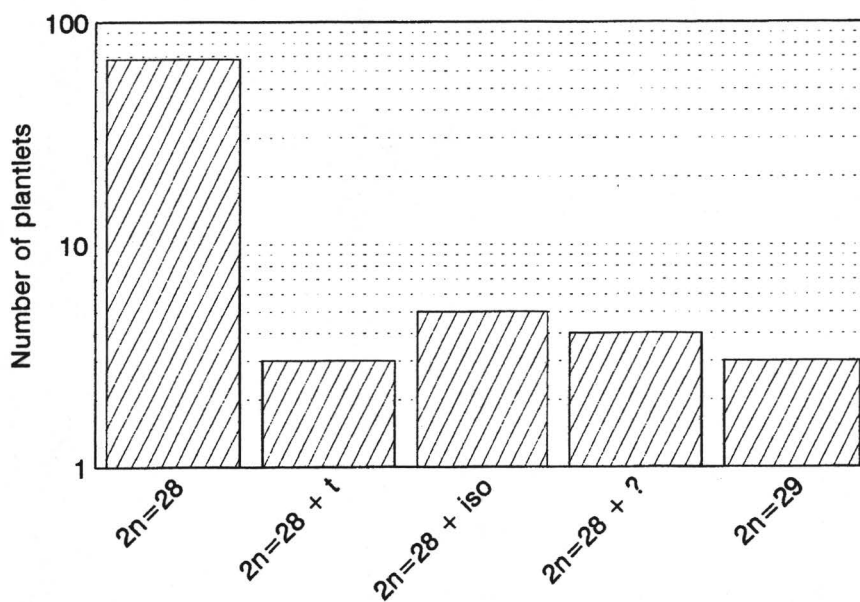


Figure 2. Seed set of 2n= 29 plants crossed to the wheat parent

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