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SUMMARY – As a result of the application of tools from both molecular genetics and cytogenetics, wheat-alien gene introgression projects carried out by means of chromosome engineering approaches are now benefiting by a substantial gain in efficiency with respect to the past. We have recently exploited such tools in producing durum wheat recombinant lines carrying separately the *Lr19+Yp* genes from *Agropyron elongatum*, the *Pm13* gene from *Aegilops longissima* and the *Gli-D1/Glu-D3* and *Glu-D1* genes from common wheat. Fluorescence *in situ* hybridization (FISH), with total genomic DNA (GISH) of the alien donor or specific DNA sequences as probes, was mostly useful for isolation and characterization of desired products, i.e. lines containing alien chromosomal segments of sufficiently reduced size to be well tolerated in a tetraploid background. Preliminary field and quality tests indicated the validity of such recombinant lines as promising candidates for durum wheat genetic improvement and varietal development.

Key words: Wheat-alien recombinants, marker-assisted selection, physical mapping, *Lr19+Yp* genes, *Pm13* gene, 1D storage protein genes.

RESUME – "Blé dur modifié génétiquement : Le potentiel des introgressions de gènes étrangers affectant la résistance aux maladies et la qualité". À l'aide de l'application des instruments de la génétique et de la cytogénétique moléculaire, les projets d'introgression de gènes entre le blé et les espèces apparentées, à travers des manipulations chromosomiques, bénéficient maintenant d'une remarquable augmentation d'efficacité par rapport au passé. Récemment nous avons exploité ces instruments pour la production de lignées recombinées de blé dur contenant séparément les gènes Lr19+Yp *de l'*Agropyron elongatum, *le gène* Pm13 *d'*Aegilops longissima *et les gènes* Gli-D1/Glu-D3 *et* Glu-D1 *du blé tendre. L'hybridation* in situ *en fluorescence (FISH), utilisant comme sonde l'ADN génomique total (GISH) de l'espèce apparentée ou des séquences spécifiques d'ADN, a été particulièrement utile pour l'identification et la caractérisation des produits désirés. Ces derniers sont des lignées contenant des segments chromosomiques étrangers de dimension suffisamment réduite pour être bien tolérés dans un génome tétraploïde. Des essais préliminaires dans les expériences au champ et concernant la qualité du gluten, ont révélé la validité de ces lignées recombinées comme candidats prometteurs pour l'amélioration génétique et la création variétale de blé dur.*

Mots-clés : Recombinants blé-espèces apparentées, sélection assistée par marqueurs, cartographie physique, gènes Lr19+Yp, *gène* Pm13, *gènes de protéines de réserve du 1D.*

The need for new genetic diversity in a changing agricultural scenery

Consistent crop improvement has been achieved in this century by manipulating genetic variation existing within the cultivated gene pools. For wheat, the most significant advances are associated with the innovative breeding approaches that characterized the work of N. Borlaug and his colleagues at CIMMYT (Borlaug, 1968) and, even earlier, between 1910 and 1950, that of Nazareno Strampelli and other pioneering Italian breeders (Worland, 1999). The success of these so called "Green Revolutions" was largely based on a few key crosses that introduced novel genetic variation into breeding programmes, from which high yielding, early flowering, disease resistant, semi-dwarf wheat varieties were selected. The contribution of such germplasm in improving wheat yields worldwide is universally recognized. On the other hand, it is also apparent that remarkable yield increases of modern varieties become mostly evident when much higher input conditions are applied than those adopted in the past to grow older cultivars or even local populations.

However, for several aspects the present agricultural scenery appears to be profoundly different from that of earlier times, with concerns on natural resources increasingly acquiring a global dimension

(Porceddu, 1999). Thus, as recalled by Tanksley and McCouch (1997), while in the past we have met the demand for increased agricultural productivity by combining genetic improvements with greater farming inputs (fertilizers, pesticides and water) and cultivation of more land, today, due to progressive shortage of available farmland, water and energy reserves, as well as to increased problems concerning the environment's capacity to assimilate the multiple forms of pollution generated by the economic growth, different avenues have to be considered. These will have to keep pace not only with the anticipated growth of the human population and the consequent request for higher food supply (Braun *et al.,* 1998), but also with an array of newly arisen needs of environmental and socioeconomic relevance.

In this context, that of genetic improvement becomes a key role. For the genetic approach to be successful, a continuing infusion of new genetic diversity represents a necessary requisite. However, in spite of its notable achievements recalled above, modern plant breeding has seriously threatened the genetic base on which future progress depends.

Similarly to all other crop plants, the currently cultivated wheat germplasm has a rather narrow genetic base, essentially derived from intraspecific selection and fixation of a relatively limited set of favourable genes and allelic variants of them. However, whereas variability for some traits is limited or even exhausted within the primary wheat gene pool, secondary and even tertiary gene pools represent a wide and yet little exploited reservoir of desirable alien genes that can be incorporated into cultivated genotypes.

Chromosome engineering: New tools for a higher efficiency

The reason why until now we have been only modestly successful in utilizing such resources for wheat improvement does not reside in the lack of proper transfer strategies. Whereas genetic transformation is still far from being a feasible strategy for the majority of traits of agronomic relevance, already more than twenty years ago Sears (1972) described the main avenues of the "chromosome engineering" approach, through which the "transferring of segments of alien chromosomes carrying particular desired genes to wheat chromosomes" can be accomplished. He particularly highlighted and proved with several successful examples (Sears, 1973, 1981, 1983) the advantages associated with the strategy based on manipulations of the wheat pairing control system. In fact, with respect to other methods (Sears, 1972), following wheat-alien homoeologous pairing and recombination mediated by use of wheat mutations for the main homoeologous pairing suppressor gene *Ph1* (Sears, 1977; Giorgi, 1978), transfers can be obtained which present a minimum disturbance of the recipient chromosome and genotype. This is due not only to the cytogenetic affinity which relates the recipient to the donor chromosome, but also to the possibility to limit the amount of alien chromatin flanking the desired gene(s) (see, e.g., Sears, 1983), and thus break possible unfavourable linkages of the donor species.

Whereas Sears' procedures are essentially those followed by wheat cytogeneticists working nowadays on chromosome engineering, in the last years a substantial improvement has occurred in the way the introgression of alien chromatin can be monitored. In this, both molecular marker technology (e.g. RFLPs, RAPDs and other PCR-based approaches), as well as molecular cytogenetic techniques, such as non radioactive *in situ* hybridization (e.g. FISH and GISH), can effectively complement classical diagnostic and selection tools and make more efficient and accurate detection and characterization of desired products.

Physical mapping: An important aid in alien introgression projects

The substantial gain in efficiency of alien introgression projects has contributed considerably to the successful results that we could achieve in engineering durum wheat with chromosomal segments of different origin and gene content (Fig. 1). Through this approach, lines possessing the *Lr19+Yp* genes from *Agropyron elongatum* (Ceoloni *et al.,* 1998, 1999)*,* the *Pm13* gene from *Aegilops longissima* (Ceoloni *et al.,* 1988, 1992) and the *Gli-D1/Glu-D3* and *Glu-D1* genes from common wheat (Ceoloni *et al.,* 1995, 1996; Vitellozzi *et al.,* 1997) have been produced.

In all such cases identification and precise analysis of the wheat-alien recombination events was obtained by genetic and physical mapping strategies. Genetic maps can be useful to establish a relative "ranking" among a series of recombinant products (see, e.g., Donini *et al.,* 1995), but they represent poor indicators of physical distances along chromosomes. To assess the physical amount of exchanged material, which is a critical parameter in evaluating the potential impact of an alien transfer on the recipient

genotype, molecular cytogenetic techniques of non radioactive *in situ* hybridization, particularly fluorescence *in situ* hybridization (FISH), represent a very efficient tool. In fact, using FISH with specific DNA sequences or total genomic DNA (GISH) of the alien species as probe, we could precisely estimate the size of chromosomal segments incorporated into durum wheat and, in some cases (see ahead), directly select on this basis promising lines for practical utilization.
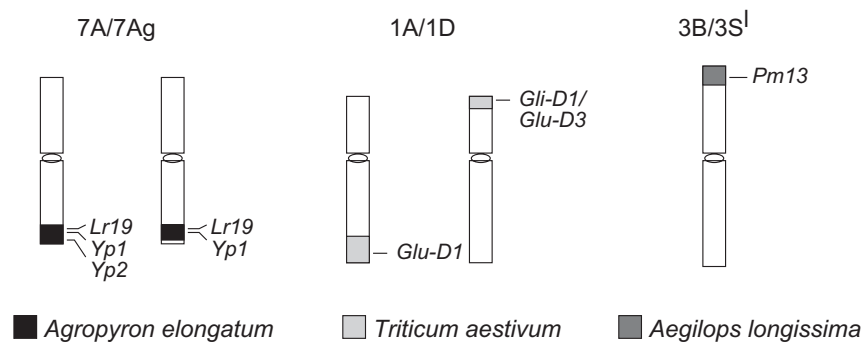

Fig. 1. Physical maps of recombinant chromosomes of selected durum wheat-alien transfers carrying chromosomal segments of different origin and gene content: the recombinant arms contain 23% and 13%, respectively, of 7AgL (black), around 25% and 20% of 1DL and 1DS, respectively (light grey), and about 20% of 3S^{l}S (dark grey).

Delivering to the breeder a transfer chromosome that includes the shortest possible alien segment represents a goal of general validity, for both common and durum wheat. As a matter of fact, in several instances common wheat transfers harbouring even sizable alien introgressions have been commercially exploited (see, e.g., Friebe *et al.,* 1996). Among them, the 6AL/6AgL transfer, with a nearly complete arm of the *Agropyron elongatum* donor, extensively used in breeding in Australia as carrier of the highly effective stem rust resistance gene *Sr26*, and the 1RS whole arm translocation, with genes on the *Secale cereale* chromosome arm able to confer resistance to several diseases and even a heterotic effect on yield (Villareal *et al.,* 1995). However, in the latter case the presence of the 1RS arm turned out to be associated with poor dough quality. Interestingly, a partial recovery in quality was detected in wheat/rye recombinants in which part of the originally missing wheat arm, and so the *Gli-1/Glu-3* storage protein genes there located, had been restored (Koebner and Shepherd, 1988). Even for the 6AL/6AgL transfer, lines with reduced size of the *Ag. elongatum* chromatin were recently produced in an attempt to improve the yielding capacity of cultivars carrying *Sr26* (Dundas and Shepherd, 1998).

On the other hand, the tetraploid condition of durum wheat is inherently associated with a reduced "buffering" capacity of its genome toward genetic and chromosomal imbalances as compared to that of the hexaploid common wheat (see, e.g., Rao, 1978; Ceoloni *et al.,* 1996). As a consequence, incorporation of alien segments of minimal size becomes for a durum wheat transfer an essential requisite in view of its use in breeding. This can in fact reduce the likelihood that either the presence of unselected alien genes and/or the loss of corresponding wheat genes can result in effects detrimental to the development of the gametophyte and sporophyte, besides that to any other agronomically important trait.

Isolation of durum wheat lines carrying reduced portions of *Agropyron elongatum* chromatin with the *Lr19+Yp* genes

The above considerations are well exemplified by the transfer project we recently carried out to incorporate into durum wheat the *Agropyron elongatum Lr19* (resistance to leaf rust) and *Yp* (yellow pigmentation) genes, known to be closely associated on the long arm of the alien 7Ag chromosome. Such a tight linkage, which results unfavourable for common wheat breeding, was instead considered of great interest for durum wheat improvement. A single transfer could have in fact produced beneficial effects for both the leaf rust resistance trait and the yellow colour of semolina and pasta products.

As a first attempt, a 7A/7Ag common wheat translocation line (Sears, 1973; Eizenga, 1987) was employed to move the 7Ag segment from the hexaploid into the tetraploid background by homologous

recombination. However, almost no male transmission of the carrier chromosome was observed in the progeny of tetraploid heterozygous resistant plants. A plausible explanation was provided by the results of a GISH analysis (Ceoloni *et al.,* 1996). This revealed the alien portion to span the whole long arm and about half of the short arm of the primary recombinant chromosome, thus being probably too large to be tolerated in a tetraploid background.

To reduce the size of the alien segment, a chromosome engineering strategy was adopted. To develop suitable genotypes for homoeologous pairing and recombination to occur between the 7A and 7Ag portions of the critical chromosome pair, tetraploid plants bearing the primary 7A/7Ag chromosome were crossed and backcrossed to the *ph1c* mutant of the durum wheat cv. Creso. The necessary *ph1c* homozygous condition was selected by applying FISH with the pSc119.2 highly repeated DNA sequence as probe (Gill *et al.,* 1993). Out of a progeny of more than 500 plants, ten secondary recombinants were isolated in which exchanges involved the 7AL and 7AgL critical arms (around 2.5% recombination frequency). As revealed by GISH, in all cases recombination occurred in the distal half of the arms, and gave rise to six recombinants with a 7A chromosome harbouring a distal 7AgL segment (spanning from 22% to 40% of the 7AL/7AgL arm), and to four with a 7A/7Ag chromosome containing terminal 7AL segments (from 10% to 44% of the recombinant arm length).

The correlation between the GISH-based physical maps of the recombinant chromosomes and the *Lr19* and *Yp* phenotypes of the corresponding lines indicates that the alien genes, of which *Lr19* is more proximally located, are included in the most distal quarter of 7AgL. *Lr19*, in particular, can be precisely located to the 1% fraction differentiating the two recombinants with the smallest distal alien segments, one resistant to leaf rust and possessing a 23% of 7AgL (Fig. 1), the other susceptible, with a 22% of 7AgL. Comparison of the pigment content (β-carotene) of different recombinants suggests the existence of two *Yp* genes, tentatively named *Yp1* and *Yp2*, of which *Yp1* is possibly more closely linked to *Lr19* than *Yp2*. When both genes are present, as in all recombinants with distal 7AgL segments containing *Lr19* (Fig. 1), an about 70% increase in yellow pigment is observed (8-9 p.p.m. of β-carotene) with respect to the controls (around 5 p.p.m. of β-carotene), whereas in lines where probably either one is included, about half of such an increment is noticed.

Employment of the GISH technique was also useful in evaluating the gametic competitive ability of the different recombinant chromosomes. GISH screening of selfed heterozygous recombinants revealed a clear-cut, inverse correlation between transmission ability of the 7A/7Ag chromosomes and their relative alien chromatin content, and indicated 7AgL distal segments spanning a 28% of the arm length to represent the uppermost limit for normal transmission of the recombinant chromosome through both germlines.

The wide spectrum of secondary 7AL/7AgL recombinants available not only represents a valuable material for mapping studies, but also includes promising candidates for breeding. This is particularly the case for the line containing all the desired *Agropyron* genes in a distal 7AgL 23% long segment (Fig. 1, Table 2). Moreover, some of the types isolated provide the opportunity for further manipulations. In fact, plants with a 13% subterminal, interstitial 7AgL segment have been recently obtained as a result of homologous recombination in the region shared by two secondary 7A/7Ag chromosomes with complementary patterns of 7AL and 7AgL chromatin (one with a 23% distal 7AgL and the other with a 10% distal 7AL). Such a tertiary recombinant type, which contains *Lr19* and, most probably, *Yp1* only (Fig. 1), appears as an additional interesting line for practical utilization.

Fine physical mapping of additional durum wheat-alien recombinant lines

Also in the case of the other transfers we recently brought to completion in durum wheat, i.e. that of the *Pm13* gene from *Aegilops longissima* and of the *Gli-D1/Glu-D3* and *Glu-D1* genes from common wheat, the exchanged segments turned out to be of relatively small size, representing in all cases no more than a 25% of the recombinant CINT arm.

As to the *Ae. longissima* transfer, a distal segment of the short arm of the 3S^l chromosome, containing the powdery mildew resistance gene *Pm13*, was homologously introduced into durum from a primary common wheat 3BS/3S^{l}S homoeologous transfer (Ceoloni *et al.,* 1988, 1992, 1996). Based on genetic and cytogenetic estimates (Donini *et al.,* 1995), one 3BS/3S^{l}S recombinant line (R1B) was selected as the most suitable donor of the *Pm13* gene to durum wheat. Normal transmission of the recombinant

chromosome through both germlines in its tetraploid derivatives (Ceoloni *et al.,* 1996), indicated that in this case a primary recombination product, firstly obtained at the 6x level, was also well tolerated at the 4x level. The size of the R1B and of other 3S^{I}S transferred segments was measured by means of FISH with a highly repeated (pSc119.2) and a low-copy (PSR907) sequence as probes (Biagetti *et al.,* 1999). This strategy allowed the precise positioning of the 3BS/3S^{I}S R1B breakpoint distal to the 3BS *Xpsr907* locus, in the adjacent, subtelomeric euchromatic interval separating the two most distal pSc119.2 sites of 3BS. Interestingly, *Xpsr907*, known to be located at less than 25 cM from the 3BS centromere (Devos *et al.,* 1992), turned out to be separated from the telomere by a segment spanning no more than 20% of the physical arm length. Thus, of even smaller size must evidently be the 3BS segment replaced by 3S^{I}S chromatin in line R1B (Fig. 1).

Another work of chromosome engineering in which FISH turned out to be a powerful tool for identification and selection of exchange products concerns the transfer into durum wheat of common wheat 1D chromosomal segments containing the *Glu-D1* (1DL) and *Gli-D1/Glu-D3* (1DS) storage protein genes (Ceoloni *et al.,* 1995, 1996). From this work two lines of unequivocal 1A/1D recombinational origin were isolated. While the recombinant nature of the *Gli-D1/Glu-D3* carrier was proved by the solely use of endosperm protein markers (Ceoloni *et al.,* 1996), for the line expressing the HMW-glutenin subunits "5+10" coded by the *Glu-D1d* locus, this indication could be obtained resorting to FISH with the pAs1 highly repeated DNA sequence as probe. This same approach allowed quantification of 1D chromatin introgressed in each of the two lines. pAs1 shows characteristic hybridization sites in the distal portions of 1DL and 1DS, no site on 1AL and a minor, distal one on 1AS (Vitellozzi *et al.,* 1997).

When probed with this sequence, the "5+10" durum wheat line exhibited a normal 1AS and a clearly recombined 1AL/1DL chromosome, in which only the minor and more distal of the two pAs1 bands typical of 1DL, located in the distal third of the arm, was retained. The 1DL portion could thus be estimated to represent a nearly 25% of the recombinant 1AL (Fig. 1 and Vitellozzi *et al.,* 1997).

On the other hand, the recombinant 1AS arm showed a prominent doublet of pAs1 sites at its telomere which appeared of clear 1DS origin and occupied about 20% of the arm (Fig. 1). Combining the FISH result with information obtained from RFLP mapping, it has been possible to locate the 1AS/1DS breakpoint in a position just proximal to the pAs1 1DS sites, with the adjacent 5S rDNA locus being already included in the 1AS portion.

Both the 1AL/1DL and the 1AS/1DS recombinant chromosomes are transmitted normally through both germlines (Ceoloni *et al.,* 1996; Vitellozzi *et al.,* 1997).

Evaluation of agronomic and grain quality characteristics of durum wheat-alien recombinant lines

Quality tests on lines containing the *Gli-D1/Glu-D3* or *Glu-D1* genes

For the *Gli-D1/Glu-D3* recombinants, three lines of each 1AL.1AS/1DS genotype (homozygous for the 1DS segment and for either the *Glu-B1* "7+8" or *Glu-B1* "20" locus), as well as of non-1DS sibs (1AL.1AS), together with the durum wheat cultivars Simeto and Cappelli and the bread wheat cultivar Pandas used as controls, were grown in the season 1998-98 in a randomized complete block design containing two replicates.

They were evaluated for certain grain parameters, such as protein content and SDS-sedimentation test, and for gluten rheological properties as measured by the alveograph test (Table 1a). Variation in quality characteristics of lines and cultivars was not significantly related to that in grain protein content, indicating that gluten properties can be ascribed to factors other than protein content, i.e. protein composition. All *Gli-D1/Glu-D3* recombinants, irrespective of the variation at the *Glu-B1* locus, showed SDS-sedimentation values significantly higher than those of non-1DS sister lines and of the durum wheat controls Simeto and Cappelli, though significantly lower than that of the bread wheat Pandas. Gluten rheological properties appeared to be strongly affected by the presence of the 1AS/1DS translocation and by the allelic variation at the *Glu-B1* locus. Lines possessing *Gli-D1/Glu-D3* encoded proteins and *Glu-B1* "7+8" subunits showed the best alveographic performances, with W values significantly higher than those of all control cultivars. Although P/L values of all durum lines analysed generally corresponded to the tenacious gluten type, lines carrying the 1AS/1DS translocation and the *Glu-B1* "20" allele had

acceptable values for the W index, and a more equilibrated ratio between tenacity and elasticity than Simeto and the recombinant lines with a *Glu-B1* "7+8" locus.

Table 1. Mean values of quality parameters of durum wheat recombinants carrying 1D storage protein genes as well as of control sister lines and cultivated varieties

Rec. lines and controls	Genotype			Protein[†] content %	SDS[†] (mm)	Alveograph values[††]	
	Gli-D1/ Glu-D3	*Glu-D1*	*Glu-B1*			W	P/L
a) *Short arm recombinants*							
1AL.1AS/1DS	+	–	7+8	16.1 ab	66.7 b	439.8 a	3.00 b
1AL.1AS	–	–	7+8	16.9 a	55.7 c	345.0 b	2.92 b
1AL.1AS/1DS	+	–	20	17.2 a	69.5 b	296.7 bc	1.85 c
1AL.1AS	–	–	20	17.0 a	46.5 d	150.0 e	1.29 d
Simeto				15.6 ab	57.0 c	320.0 bc	4.22 a
Cappelli				18.1 a	46.0 d	192.5 de	0.86 d
Pandas				14.3 b	96.0 a	245.0 cd	0.61 d
Total mean				16.7	61.0	296.8	2.19
Tukey (P ≤ 0.05)				2.6	9.1	90.0	0.54
b) *Long arm recombinants*							
1AL/1DL.1AS	–	5+10	7+8	16.6 b	91.9 a		
1AL.1AS	–	–	7+8	16.9 ab	47.9 c		
1AL/1DL.1AS	–	5+10	20	17.4 ab	90.0 a		
1AL.1AS	–	–	20	16.9 ab	26.2 d		
Simeto				16.0 bc	49.0 c		
Cappelli				18.5 a	24.0 d		
Pandas				15.9 bc	92.5 a		
Total mean				16.9	61.0		
Tukey (P ≤ 0.05)				1.7	9.1		

[a,b,c,d]Values with the same letter do not differ significantly.

[†]Data on protein content and SDS test for the short arm and long arm recombinants derive from different field trials.

[††]Alveograph indexes: W = strength, P = tenacity, L = extensibility.

Regarding the *Glu-D1* "5+10" recombinants, six plants of each 1AL/1DL.1AS genotype (homozygous for the 1DL segment and for either the *Glu-B1* "7+8" or *Glu-B1* "20" locus), as well as of non-1DL sibs (1AL.1AS), together with the same durum and bread wheat cultivars mentioned above, were tested for protein content and for SDS-sedimentation values (Table 1b). Results indicated that gluten strength, as measured by the SDS-sedimentation test, independently from variation at the *Glu-B1* locus and from protein content, was strongly affected by the presence of the "5+10" subunits. In fact, all 1AL/1DL recombinant lines had SDS-sedimentation values exceptionally high for durum wheats and comparable to those exhibited by bread wheat.

Preliminary assessement of field performance

To have a preliminary evaluation of the effects of different alien introductions on agronomic traits, $BC_{2:3}F_3$ progeny of durum wheat recombinant lines, together with check varieties, were field-tested during the season 1998-99, under naturally occurring disease-free conditions and with normal nutrient application management. Seeds were sown at 15 cm distance in 1.2 m long rows, with 25 cm between rows. Results on fertility and other agronomically significant plant traits, deriving from 20 randomly selected individuals of each recombinant progeny and of control varieties, are presented in Table 2.

Table 2. Mean values and ranges (in brackets) of agronomic traits of durum wheat recombinant lines homozygous for different alien segments and of some recurrent parents

Rec. lines and controls	Tillers per plant	Seeds per spike	Spikelets per spike	Seeds per spikelet	Plant height (cm)	Heading time (days from April 1st)	1000 kernel weight (g)
7AL/7AgL (*Lr19+Yp*)	11.8 a (3-25)	63.1 a (40.7-87.7)	17.7 ab (11.7-20.5)	3.5 ab (2.7-4.5)	72.3 c (64-85)	42.9 a (38-48)	43.7 b (32.0-54.6)
1AL/1DL (*Glu-D1*)	9.7 a (4-17)	63.2 a (32.8-82.7)	17.4 b (10.5-20.5)	3.5 ab (2.3-4.2)	85.0 ab (59-110)	40.5 ab (34-49)	45.6 b (37.4-54.4)
3BS/3S^{l}S (*Pm13*)	11.6 a (2-23)	61.1 a (41.4-89.5)	19.1 ab (14.7-22.3)	3.1 b (2.1-4.4)	87.2 a (74-100)	40.2 ab (37-47)	43.6 b (33.3-56.5)
Simeto	9.2 a (4-12)	69.0 a (53.5-89.7)	17.8 ab (15.2-20.7)	3.8 a (3.3-4.3)	77.2 abc (73-88)	37.1 b (30-42)	51.7 a (46.0-60.6)
Creso	8.7 a (7-16)	68.9 a (59.2-81.2)	19.4 a (17.1-21.8)	3.6 ab (2.9-3.9)	75.4 bc (69-83)	44.5 a (42-49)	46.0 ab (43.5-56.1)
Total mean	10.2	65.1	18.3	3.5	79.4	41.1	46.1
Tukey (P ≤ 0.05)	3.7	14.3	1.7	0.5	11.4	4.7	5.7

[a,b,c]Values with the same letter do not differ significantly.

For data evaluation, one has to consider that while two (*Lr19+Yp* and *Glu-D1* recombinants) or three (*Pm13* recombinant) backcrosses to the cultivar Simeto as the recurrent parent were performed with all tetraploid recombinants, these, however, not only differed for their particular alien segment but also for their background genotype. The *Pm13* durum recombinant was in fact obtained from an initial cross with the hexaploid cv. Chinese Spring R1B recombinant. Also the *Lr19+Yp* recombinant (to be field-tested was the one with 23% distal 7AgL chromatin, see Fig. 1) had Chinese Spring in its background, as well as the durum cv. Creso, whose *ph1c* mutant was crossed and backcrossed to the primary tetraploid recombinant (see above). The *Glu-D1* recombinant, then, derived from an original hexaploid cv. Torim 73 x Chinese Spring *ph1b* mutant cross, followed by crosses to the *ph1c* mutant of the durum cv. Cappelli (Ceoloni *et al.,* 1995; Vitellozzi *et al.,* 1997).

Altogether, the observed results look quite promising (Table 1), with no particularly detrimental effect being apparently due to the presence of any of the alien segments. In fact, although some undesirable characteristics, most probably ascribable to some parental lines (e.g. excessive height from cvs. Chinese Spring and Cappelli, or lateness from cv. Creso), could still be observed in individual plants, several others of each of the field-tested progeny performed at an equal or even better level than the control varieties. Further backcrosses and/or selection of the available materials are then expected to result in isolation of fully desirable genotypes.

Parallely, the best selections of each different recombinant progeny are being intercrossed in an attempt to combine in a single genotype several useful genes not present in the cultivated germplasm. The effect of the presence of multiple alien translocations on agronomic performance was recently assessed for the 7DL.7AgL + 1BL.1RS combination in hexaploid common wheat (Singh *et al.,* 1998). No case history is available to our knowledge in durum wheat. Thus, also considering the above highlighted greater difficulty in carrying out successful chromosome manipulations at the 4x level, it will be particularly interesting to verify also in the latter species the feasibility and the eventual outcome of such a strategy.

Acknowledgements

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