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SCRITTI E DOCUMENTI
XLIV

Trasferimento di geni dalla specie selvatica *D. villosum* al frumento per aumentarne l'adattamento ai sistemi agrari sostenibili

Risultati ottenuti dalla collaborazione scientifica tra Università italiane e l'Università della California

Transferring genes from the wild species *D. villosum* to wheat for increasing adaptation to sustainable agricultural systems

Results from the research collaboration between Italian Universities and the University of California

a cura di
CIRO DE PACE

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Convegno per la presentazione dei risultati del progetto:

Workshop for the presentation and disussion of the results obtained from the MiPAAF Project:

F R U M I G E N*

Ottimizzazione, diversificazione ed incremento della produttività del frumento tenero: costituzione di anfiploidi e linee derivate da ibridazione intergenerica *Triticum sp. x Dasypyrum villosum*

Diversification and optimization of bread wheat productivity through the synthesis of amphiploids and introgression inbred lines from Triticum sp. x Dasypyrum villosum intergeneric hybridization.

La biodiversità del *Dasypyrum villosum* per il miglioramento genetico del frumento e per la tutela dell'ambiente rurale

Role of Dasypyrum villosum in bread wheat genetic improvement and rural landscape ecology

Roma, 8 maggio 2009

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Development of valuable wheat inbred lines through the introduction of *Dasypyrum villosum* germplasm in their pedigree*

Summary – Selection of improved stable wheat inbred lines for low input production systems was achieved through the relevant combination of wheat and *Dasypyrum villosum* (*Dv*) germplasm using two approaches: (1) a re-evaluation of a tri-parental species breeding scheme, and (2) a modified bulk-breeding method. Both methods optimized selection outcome. The first breeding method was based on the synthesis of a *T. turgidum* var *durum* × *Dv* hexaploid amphiploid, and crossing of that amphiploid to a bread wheat cultivar. The second breeding method was developed by bulk harvesting seeds from F_1 and F_2 from each biparental combination involving commercial wheat cultivars and wheat introgression breeding lines (IBLs) containing various amount of chromatin from *Dv*. In the first case, the M × V amphiploid displayed typical adaptive traits of *Dv* such as high resistance to diseases and heading earliness, and the further crossing of the amphiploid to *Triticum aestivum* cv Chinese Spring (CS) provided wheat breeeding lines showing additional enhancements for grain size and grain end-use quality. GISH analyses revealed that *Dv* chromatin introgression occurred in some of them, coupled to chromosome-arm exchanges. Interestingly, three of the selected lines, after two years of genetic analyses and low-input field tests, showed dominance and genetic stability of the adaptive traits. In the second case, the F_1s expressed the

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'useful' *Dv* genes inherited from the IBLs, providing strong evidence that the *Dv* genes for trait enhancements are expressed not only in CS but also in 'elite' wheat genetic background. The F_2 progenies displayed wide segregation offering ample opportunity for selecting the desired combination of the 'elite' traits with the traits expressed by the 'useful' *Dv* genes inherited through the IBLs.

Key words: Triparental hybridization, bulk breeding, gene transfer, wheat improvement.

1. Introduction

Permanent challenging key questions for the development of new varieties for low input farming systems, relate to both the gene-pools to be used for gene transfer and selection of new breeding materials, and the criteria to identify suitable genotypes and predict their future yield performance and stability.

The likely outcome from breeding major cereal crops such as wheat, using intraspecific genetic variation and quantitative traits as selection criteria, is one of small increase in genetic progress despite considerable research investment. Transfer of genes between wheat species, will be more promising, and may occur by: (i) crossing the tetraploid durum wheat *Triticum turgidum* ssp. *durum* (*Td*) with the hexaploid wheat *T. aestivum* ssp. *aestivum* (*Ta*) [5], or (ii) crossing synthetic hexaploid wheat lines (developed from *Td*×*Aegilops tauschii* D genome donor) to elite hexaploid wheat cultivars. The first procedure provide favorable genetic recombination and segregants for ploidy levels without the need for extensive backcrossing to elite cultivars (cv) for deriving commercial varieties, while the second require repeated backcrossing to the elite cv and several generations of prebreeding before new cv can be released. We have re-evaluated a breeding scheme which merges the methodological simplicity of approach (i) with the pedigree complexity of approach (ii). It is based on the use of *Td*×*Dasypyrum villosum (Dv)* amphiplod in a bridge crossing to *Ta*, for transferring, through recombination, *Td* and *Dv* genes to hexaploid wheat inbred breeding lines (IBLs) that display trait enhancement for grain yield, yield stability and grain quality under low-input field trials.

In 1908, Strampelli (1932) [19], obtained what he called "Frumento nero×*Triticum villosum*", which was the first amphiploid from the hybridization *Triticum turgidum* var *durum* cv "Frumento nero"×*Dasypyrum villosum* (L.) Candargy (abbrev. *Td*×*Dv*). Several authors have demonstrated that unreduced gametes are formed at meiosis of the *Td*×*Dv* F_1 and upon their union, F_2 embryos were produced from which fertile plants or new amphiploids were rised (see review in De Pace *et al.*, 2011 [4]). Sears (1953, 1976) [16, 17] carried out the triparental hybridization (*T. dicoccum*×*Dv*)×*T. aestivum* cv Chinese Spring hybrid to get the first set ever produced of the disomic addition lines of V chromosomes to CS chromosome complement.

In the triparental hybrid *T. durum* cv. CI 1320×*Dv* with *T. aestivum*, Liu *et al.* (1986) [11] showed that the F_1s (AABBDV) had 14 bivalents and 14 univalents per

PMC and 3% self fertility, which increased to 7 to 38% after backcrossing. This study suggested that the production of aneuploid wheat lines with added V chromosomes was possible when *Dv* was used as a bridge in crosses involving durum and bread wheat parents.

Von Bothmer and Claesson (1998) [21] made the triparental hybridization (*T. turgidum* ssp. *dicoccum*×*Dv*) F_1×*T. aestivum* cv. CS which were backcrossed to either cv. Kadett or CS. Fertile BC_2 plants were obtained whose progenies were tested for agronomic performance, some of them expressed resistance to *Puccinia striiformis*, and several progenies segregated for rachis fragility and weak straw.

Triparental crosses were also performend at the Chinese Agricultural Academy of Sciences (CAAS), Beijing, China, by crossing the TH1, TH2, and TH3 aphiploids obtained by Chen *et al.* (1996) [2] to several *T. aestivum* cultivats and lines. Homozygous materials 94G32-1,94G33-1, 94G22-1 and 94G25-1, with the powdery mildew resistant gene from *Dv* were developed by backcrossing the amphiploids to *T. aestivum* followed by immature embryo culture or anther culture [2]. Shang *et al.* (1997) [18] evidenced that the lines 94G22-1, and 94G33-1 were DS 6V(6D) that is: disomic substitution lines in which the 6V chromosome pair substitute the 6D chromosome pair. The 6V in those lines was designated 6V#5 (Table 11 in De Pace *et al.*, 2011 [4]). After crossing the TH1 and TH1W amphiploids to several bread wheat lines ("CS", "Luzi 357", "84Jia-7911515", "NPFP", and "91E27") followed by immature embryo *in vitro* culture of the resulting hybrids with genome AABBDV, Li *et al.* (2000a,b) [8, 9] selected translocation lines involving V and D chromosomes near the centromere. The translocation lines 97R377 and 97R282 were selected from the cross TH1W×"CS", and the translocation line 97R447 was selected from the TH1×"NPFP" cross. Because the root-tip cells of F_1 seedlings from immature embryos did not show translocations, it was concluded that they were induced from tissue culture during regeneration of the lines [8, 9]. The young embryo and anther culture of the F_1 from crossing the TH3 amphiploid with the bread wheat cv "Wan7107" (developed in Nanyang Inst Agric Sci, Henan Prov, China, and susceptible to powdery mildew), followed by three backcrosses to the bread wheat parent, allowed the selection (using biochemical markers and GISH) of the disomic T 6DL·6VS lines Pm97033, Pm97034 and Pm97035 with 2n=42 [6] resistant to *Blumeria graminis* f. sp. *tritici*. RW15 is a 6V(6D) disomic substitution line derived from TH3/Wan7107*2//Jimai30*3/// Shanxi859 [2]. The 6V#5S chromosome arm transferred in those lines at CAAS from a *Dv* ecotype from former USSR, have genes for powdery mildew resistance [7] but lack the alleles for WCM resistance found in T6AL·6V#4S developed at the Cytogenetic Institute, Nanjing Agricultural University, China (CINAU) or DS 6V#6(6A) in lines GN21 and GN22 [12] developed at Guizhou Agric Univ, Guiyang, China, from the hybridization *T. durum* cv "Sauwne20" with a *Dv* ecotype (different from the ex-USSR *Dv* ecotype used as sources of 6V#5) collected by CAAS researchers [8].

The triparental hybrid derived from direct and reciprocal crossing the [*T. turgidum* var. *durum* cv 'Modoc' (M) (2n = 28; genomes AABB) × *Dv*] amphiploid (= M × Vnb) with CS was used by Minelli *et al*. (2003, 2005) [13, 14] to study the behavior and interactions of parental genomes and the changes that parental genomes and chromosomes may undergo in the hybrids.

The above experiments showed that triparental hybridization involving *Dv*, *Td*, and *Ta* followed by several backcrosses provide 2n = 42 plants with one or very few translocations involving a V chromosome selected purposefully using either GISH or molecular markers. No attempt have been made so far to evidence the potentiality of the triparental hybridization to provide breeding lines with 2n = 42 and promising agronomic features since the earlier generations after the tri-parental hybridization.

The (*Td* × *Dv*) × *Ta* hybridization associated with the production of IBL may be a good model for breeding because it is faster than other bi- and triparental hybridization schemes for the rapid combination of genes from different gene pools to produce enhanced wheat breeding lines necessary for success in sustainable farming systems.

Another method for enlarging the endowement of trait enhancing genes in lines purposefully selected to become future cv, is based on the biparental cross combination involving 'elite' wheat cultivars and IBLs containing various amount of *Dv* chromatin and already tested for the presence of useful major genes. Bulk harvesting of plants from the F_1 through the F_3 generation and a further replicated trial of single plant progenies expressing the major gene in the F_3 generation, rapidly lead to promising selected lines. In this paper the results of (a) triparental hybridization and (b) bulk breeding, are reported.

2. Materials and Methods

2.1. *Genotypes*

2.1.1. Tri-parental hybridization.

An F_2-like breeding population with broad genetic diversity, was obtained from selfing the F_1 plants obtained after crossing the non-brittle rachis mutant of the hexaploid amphiploid (M × Vnb; 2n = 6x = 42, genomes AABBVV) to the hexaploid bread wheat CS (2n = 6x = 42; genome A'A'B'B'DD) (Fig. 1). In the 2007/2008 growing season, three of those IBLs, named "VT 8-1", "VT 41-3", and "VT Mut 3-04", were tested in the field at two sites: S. Angelo Lodigiano, SAL, near Lodi in northern Italy, 45° 14' N, 9° 24' E, 74 m a.s.l., and Tolentino, TOL, near Macerata in central Italy, 43° 12', 13° 17' E, 238 m a.s.l.) in a randomized block design with three replications at each site. The blocks were managed using low-input criteria. The hexaploid wheat cultivars "Bologna" and "PR22R58" were used as checks. HT, heading time (days from April 1st) and yield components were

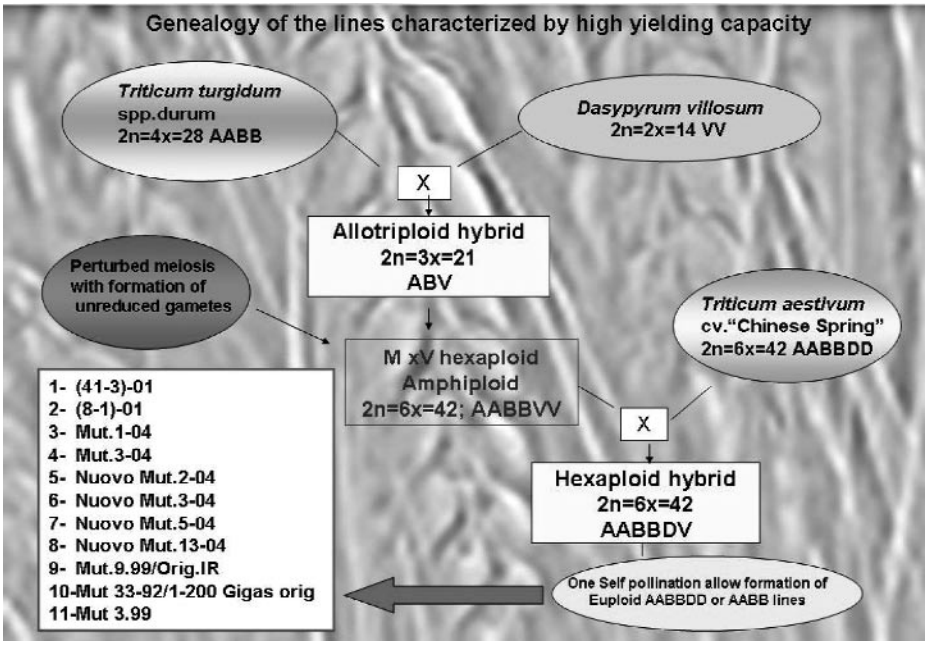

Fig. 1. Triparental hybridization scheme.

evaluated at both sites. Small- (PC, protein content as % of dry matter; SSV, SDS-sedimentation volume, mL; GI, Gluten index as % of strong gluten over the total gluten), as well as large-scale (DS, Farinograph degree of softness in BU; W, Alveograph W value × 10-4J; BV, bread olume, mL) bread making quality tests, were performed only at SAL. Data from the 2009 trials were included in this paper after the presentation at the meeting in order to evidence the traits for which the performance pattern was repeatable compared to the control entries. Further details are reported in the paper of Corbellini *et al.* (2011) in these proceedings.

2.1.2. Modified bulk-breeding method.

This breeding method was developed by bulk harvesting seeds from F_1, F_2, and F_3 from each biparental combination involving the commercial wheat cultivars 'Blasco', 'Isengrain', 'Provinciale', 'Sagittario', and 'Salgemma' used as female parents, and the introgression wheat breeding lines (IBLs) 'CS × V32', 'CS × V59', 'CS × V60', 'CS × V63', and 'CS 1BL/1V' expressing major genes for earliness, powdery mildew and rust resistance, and grain quality, were used as male parent. Details on this set of IBLs are reported in the papers of Vaccino *et al.*, 2011 [20] and Caceres *et al.* (2011), [1] in these proceedings.

2.2. *DNA extraction and labelling*

DNA was extracted from leaves and purified according to Dellaporta *et al.* (1983). Labelling was performed with digoxigenin-11-dUTP or biotin-16-dUTP by nick translation after having mechanically sheared genomic DNA to 2-10-kb fragments.

2.3. *Chromosome counting and GISH*

Root apices were treated with a saturated aqueous solution of alpha-bromonaphtalene for 4 h at room temperature and fixed in ethanol-acetic acid 3:1 (v/v), macerated in a solution of pectinase (4%; Sigma) and cellulase (3%; Calbiochem) in citrate buffer pH 4.6 for 1 h at 37 °C, and squashed under a coverslip in a drop of 45% acetic acid. The coverslips were removed as described above and the preparations were air-dried. In situ hybridization was performed according to Schwarzacher *et al.* (1989) [15] with minor modifications. The DNA pool from nuclei and chromosomes was denatured in a thermal cycler for 6 min at 70 °C and the preparations were incubated overnight at 37 °C with 2 ng/mL of labelled, heat-denatured genomic DNA probes. Unlabelled, fragmented genomic DNA was added to the hybridization mixtures in a 15-fold excess as blocking DNA. The digoxigenin or biotin at the hybridization sites was detected by using sheep anti-digoxigenin-fluorescein or streptavidin-Cy3, respectively. The preparations were then counterstained with a 2% solution of DAPI (4,6-diamidino-2-phenylindole) in McIlvaine buffer pH 7.0 and mounted in antifade solution (AFI; Citifluor). Interphase nuclei and metaphase chromosomes were examined with a fluorescence microscope equipped with a Leica Q500MC image analyzer, and images were recorded on Fuji 800 film.

3. Results

3.1. *IBLs from the tri-parental hybridization*

Chromosome painting (GISH) analysis, as expected, evidenced in the parental M × V amphiploid the presence of 14 V chromosomes, 14 A chromosomes and 14 B chromosomes.

3.1.1. F_1 generation.

When CS was pollinated by M × V mature microspores, 80% of the pollinated florets produced caryopses with hybrid embryo (considered F_1). Homologous pairing and recombination between the A and between the B-genomes of M and CS and the random assortment of the chromosomes of the D and V genomes, occurred at meiosis of the F_1 plants. This favoured the arrangement of aneuploid and euploid AB, ABV, or ABD gamete configurations and various assortments of

Fig. 2. Spike morphology of the parents, and a sample of the F_1 and F_2 progenies obtained after the '(M×V)×CS' hybridization scheme.

genetic-blocks from the A and B parental genomes. The selfed F_1 plants were partially fertile and the surviving F_2 embryos tended to be euploid due to the lack of viability of the aneuploid gametes. In Fig. 2 the spike morphology of a sample of F_1 and F_2 plants is displayed.

3.1.2. F_2 generation.

About 42% of the F_2 seeds produced complete fertile plants. In fact, F_2 plants having 14 A chromosomes, 14 B chromosomes, 7 D chromosomes, and 7 V chromosomes were rather rare (4.5%). Many progeny plants (45.5%) had the hexaploid wheat complement with 42 chromosomes and no V chromosome or chromatin was detected. Fifty percent of the plants had fewer than 42 chromosomes and about 30% of them had two to four V chromosomes. In most cases, D chromosomes were retained at higher frequency than V chromosomes (Minelli *et al.*, 2005) [14].

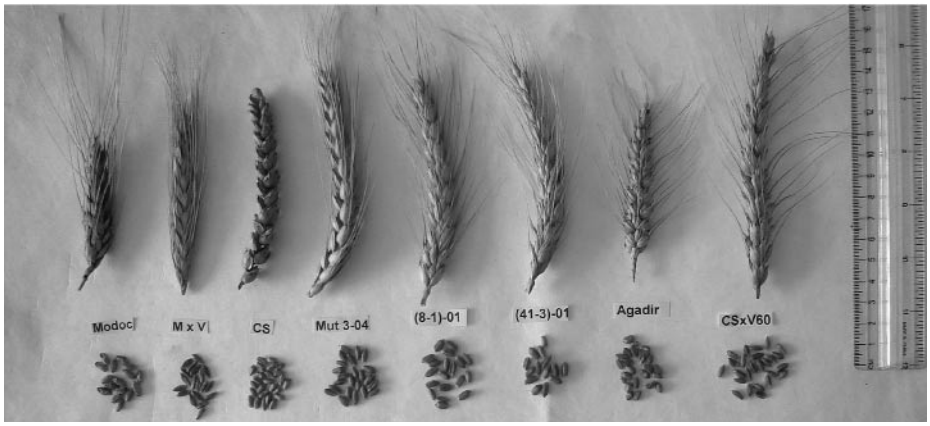

Fig. 3. Spike and caryopses of a sample of a F_3 offspring from fertile F_2 plants. The spikes of the 'M × V' amphiploid, the bread wheat cv 'CS' and 'Agadir', the IBL 'CS × V60' and the *durum* 'Modoc', were included for comparison.

TABLE 1 – Mean per plot of plant traits measured on three inbred breeding lines derived from hybridization [*(T. turgidum* var *durum* cv Modoc × *Dv)* × *T. aestivum* cv Chinese Spring], and four controls, grown at two sites (SAL and TOL) for two years (2008 and 2009).

Entry	Plant height (cm)		Heading time (Days from April 1st)		1000 kernel weight (g)		Grain yield (t ha^{-1})		Test weight (Kg hL^{-1})	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
IBL										
VT 41-3	89	72	32	34	44.3	44.3	6.8	5.0	78.6	79.8
VT Mut 3-04	87	72	31	35	44.2	44.5	6.7	4.9	78.4	80.0
VT 8-1	90	72	31	35	43.2	43.7	6.7	5.3	77.4	79.7
Controls										
Artico	85	73	35	40	37.0	32.0	7.2	5.9	73.5	73.8
Bologna	83	71	38	40	32.0	29.7	6.8	5.3	79.4	80.4
Blasco	84	71	36	40	35.1	33.3	6.6	5.4	80.6	81.8
PR22R58	79	68	38	41	39.4	33.1	7.9	5.7	76.3	76.4
MEAN	84	71	34	38	38.5	36.5	6.4	5.5	76.3	78.0
LSD	4.4	ns	0.4	4.5	2.10	8.86	0.13	ns	1.08	ns
SED	9.2	9.8	0.9	1.4	4.38	2.72	0.26	1.29	2.26	1.93
CV	5.0	19.5	2.2	5.1	6.47	10.50	5.76	33.60	2.33	3.50

3.1.3. F_3 generation.

Root-tip chromosome counting of the resulting F_3 seeds confirmed the prevalence of 14A, 14B, and 14D chromosome configurations. Using GISH, F_3-seedlings with one to seven V-chromosomes were recognized [14]. Selfing, occurring from F_3 to F_4 generations, coupled to: (a) chromosome counting for selecting $2n = 42$ plants, (b) field-plot trials managed using low-input criteria, and (c) selection for spike fertility and plant yield components, allowed the identification of several euploid IBLs with interesting plant and grain quality features [20].

Several F_3 progenies of the fertile F_2 plants were remarkably uniform, and provided enough kernels for seed multiplication and replicated trials in the F_4 (2008) and F_5 (2009) generations.

3.1.4. F_4 and F_5 generation in replicated trials.

Three IBLs were tested: 'VT 8-1', 'VT 41-3', and 'VT Mut 3-04'. The IBLs expressed repeatable earlier heading time and larger grains compared to the controls, and for grain yield their difference from the controls were significant only for one year (2008) (Table 1). The gluten quality and bread-making properties were much higher than the parental bread wheat variety (CS) and comparable to the best checks (Table 2).

GISH analyses allowed the detailed description of both the chromosomal configuration of the wheat introgression lines and the size (chromosome arm or whole chromosome) of the introgressed V chromatin.

The best performing lines from '(M × V) × CS' had stabilized euploid $2n = 42$ chromosome number, with few chromosome arm exchanges, and no GISH detectable chromatin. The IBLs had extensive A-B recombination events (Fig. 4).

3.2. *Modified bulk-breeding method*

A sample of the F_1 caryopses of the cross-combinations involving the 'CS × V59' and 'CS × V63' parental IBLs were planted on August 3rd 2008 in the greenhouse in non-vernalizing condition (the daily temperature ranged between 30 and 34 °C) and unlimited water supply. These conditions favoured heading only for the genotypes that received the cryptic chromatin with the earliness *Dv* genes from 'CS × V59' (Fig. 5), and facilitated development of the powdery mildew disease symptoms in plants that did not have the 6V chromosome.

'Isengrain', 'Salgemma', and 'Provinciale' reached heading time after experiencing the winter cold temperatures and occurred about 230-250 days after sowing. On the other hand, the F_1 progeny from crossing those cv to 'CS × V59' started heading 50-60 days from sowing (Fig. 5 and Fig. 7), right at the end of summer, when the temperatures were still above 22 °C. 'Blasco' and 'Sagittario' behaved as spring types; nonetheless, their F_1 progeny from crossing to 'CS × V59' headed about 10 days before the parental 'elite' wheat cultivar. The F_1 progenies of the

TABLE 2 – Grain quality traits of the three Introgression Breeding Lines (IBLs) belonging to Group A of materials, grown at S. Angelo Lodigiano (LO) and Tolentino in 2008 and 2009.

IBL	Year	TW (kg/hL)	PC (%dm)	SSV (mL)	SSSV	Gluten index	Brabender farinograph			Chopin alveogaph				Bread test	
							Stab. (min)	Degree of soft. (BU)	Water abs (%)	P (mm)	L (mm)	P/L	W (x10^{-4}J)	Vol (cm^3)	Height (mm)
Mut 3-04	2008	77.0	12.6	91	7.2	99	13.1	33	58.1	93	136	0.68	375	720	105
	2009	80.0	13.8	88.5	6.4	76	17.8	11.5	62.9	80.5	119	0.68	266	790	110
41-03	2008	76.4	12.3	89	7.2	98	14.6	25	57.7	92	119	0.77	334	685	101
	2009	79.8	14.5	87.5	6.1	80	17.8	21	62.6	72	119	0.61	234	770	105
8-1	2008	74.9	12.6	91	7.2	99	18.2	23	57.8	104	128	0.81	400	710	100
	2009	79.6	13.8	88.5	6.4	84	17.4	25	63.0	80	130	0.62	286	735	103
CS	2008	64.7	12.2	48	3.9	32	3.9	93	56.4	54	61	0.89	90	560	84
Bologna	2009	80.4	13.9	86.5	6.2	97	18.5	22	58.5	78	140	0.57	396	720	105
PR22R58	2009	76.4	12.1	87.5	7.2	97	19.0	21	54.5	56	115	0.49	219	695	98
Best check	2009													785	109

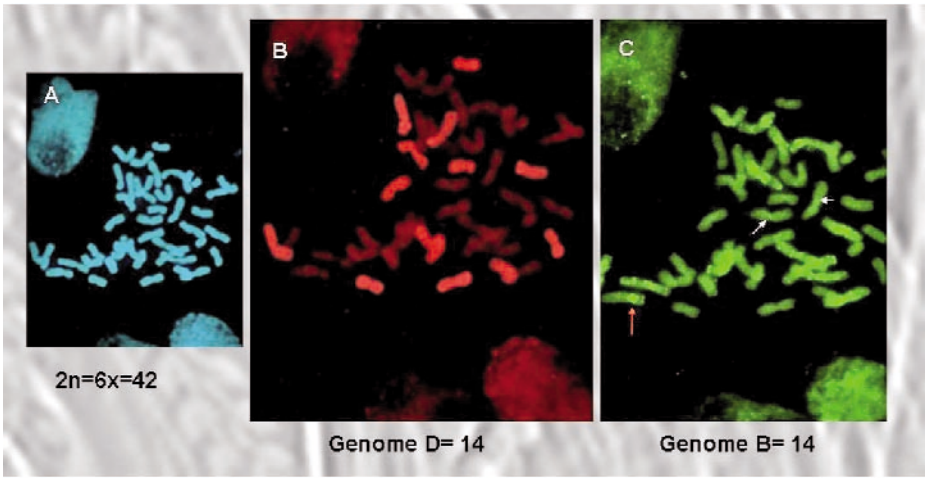

Fig. 4. Metaphase plate prepared from root tip of 'VT 41-3' seedlings. DAPI staining (A) and GISH using labelled DNA from genome D (*Ae. squarrosa*) and blocking DNA from *T. durum* (B). GISH using labelled DNA from genome B (*Ae. speltoides*) and blocking DNA from genome D and A (*T. urartu*; C). Arrows in frame C indicate chromosomes involved in A-B exchange events.

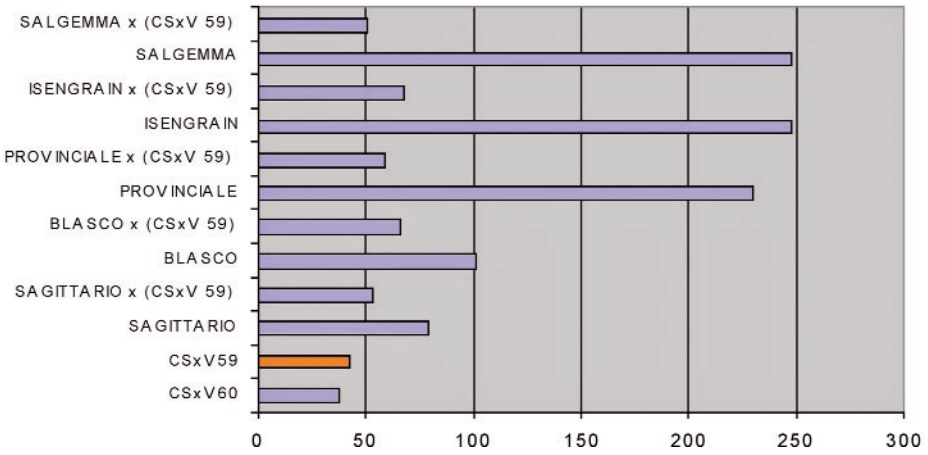

Fig. 5. Heading time for the F_1 plant from crossing elite winter-type cultivar (as mother plant) to the spring-wheat inbred breeding line 'CS × V59' (paternal plant) taken in a period of 250 days from sowing date (August 3rd, 2007) in the greenhouse at Univ. of Tuscia, Viterbo, Italy.

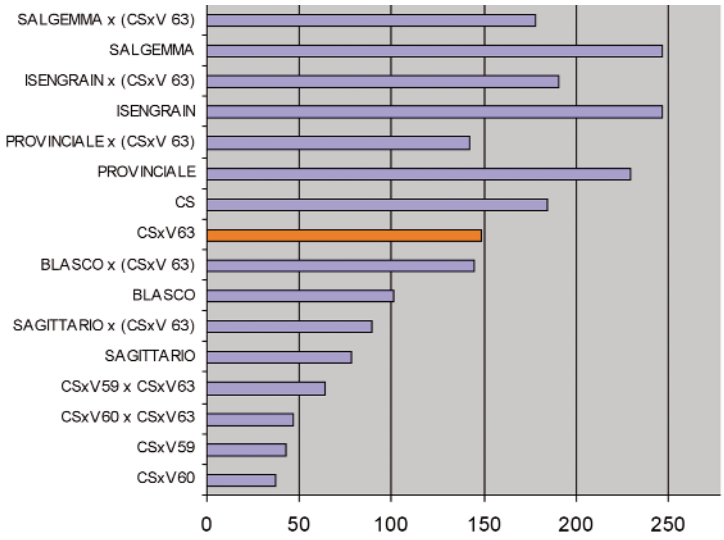

Fig. 6. Heading time for the F_1 plant from crossing elite winter-type cultivar (as mother plant) to the spring-wheat inbred breeding line 'CS × V63' (paternal plant) taken in a period of 250 days from sowing (August 3rd, 2007) in the greenhouse of Univ. of Tuscia at Viterbo.

same cultivars crossed to 'CS × V63' were as late as the respective 'elite' parental wheat cv and no positive effect of 'CS × V63' on the earliness of the offspring was observed (Fig. 6 and Fig. 7). When 'CS × V63' was crossed to either 'CS × V59' or 'CS × V60', the offspring was as early as the earliest parent (Fig. 6). All the F_1 having 'CS × V63' as one parent, were resistant to powdery mildew. Among the 'elite' cv, only 'Isengrain' expressed powdery mildew resistance.

In April 2008, a sample of the F_1 were sown from all the combinations and by July 2008 F_2 caryopses were available for all cross combinations. The bulked F_2 seeds from each combination was sown in November 2008 and notes were taken on several morphological and phenological traits. A sample of graphs illustrating the segregation observed for the main culm height, is reported in Fig. 8. Ample segregation was evident in all the cross combinations examined, attesting that the F_1 caryopses were true hybrids and not the result of selfing.

Discussion

The interesting performance for heading earliness, and both grain yield and grain quality of the selected IBLs compared to the best control cultivars, indicated that the tested triparental hybridization scheme allowed the identification of superior lines for end-use grain quality. GISH revealed the conservation of the wheat chromosomal number and new chromosome-arm arrangements in the selected lines

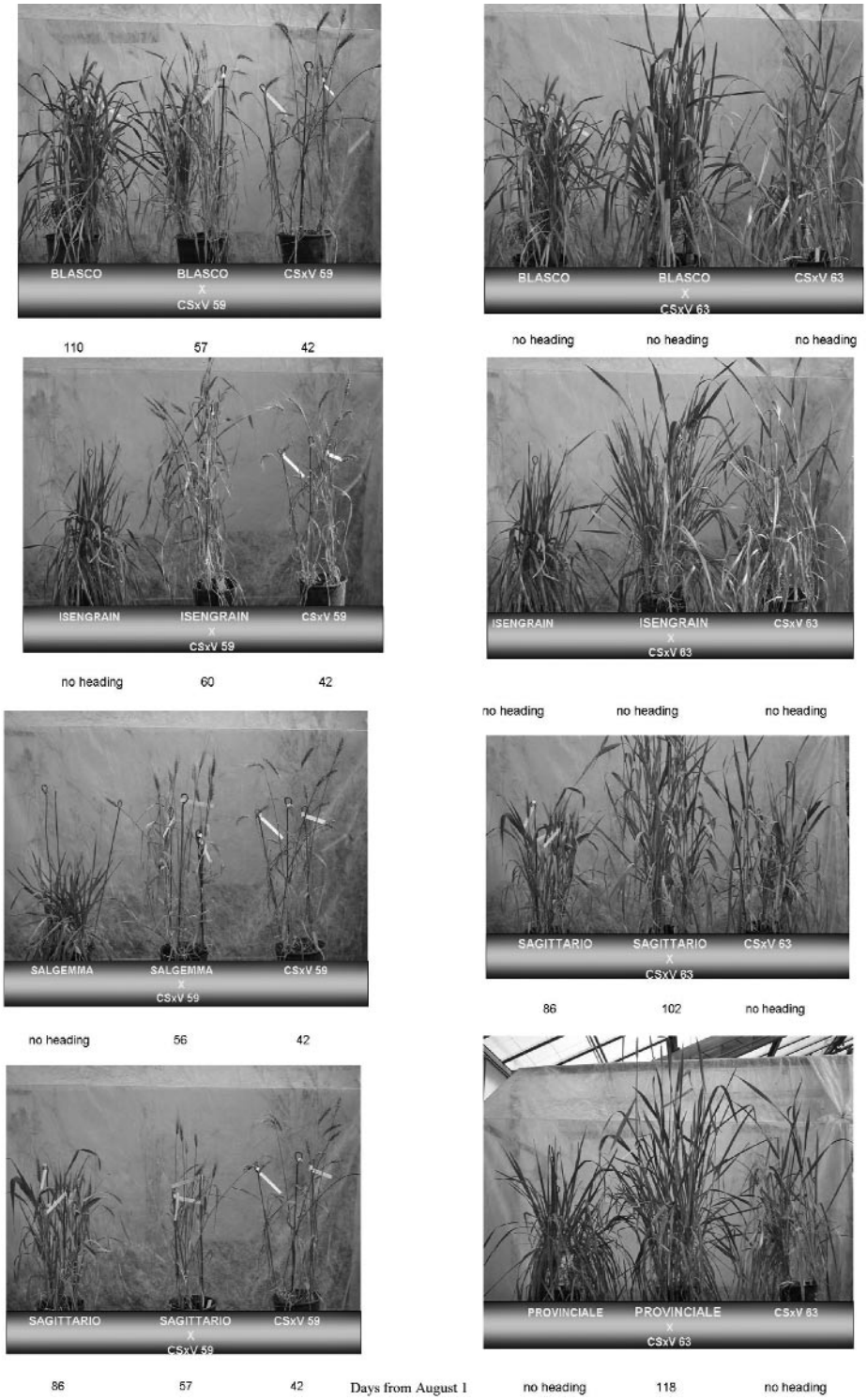

Fig. 7. Expression of the phenological traits in the parents and F_1 offspring of selected cross-combinations between 'elite' cultivars and IBLs.

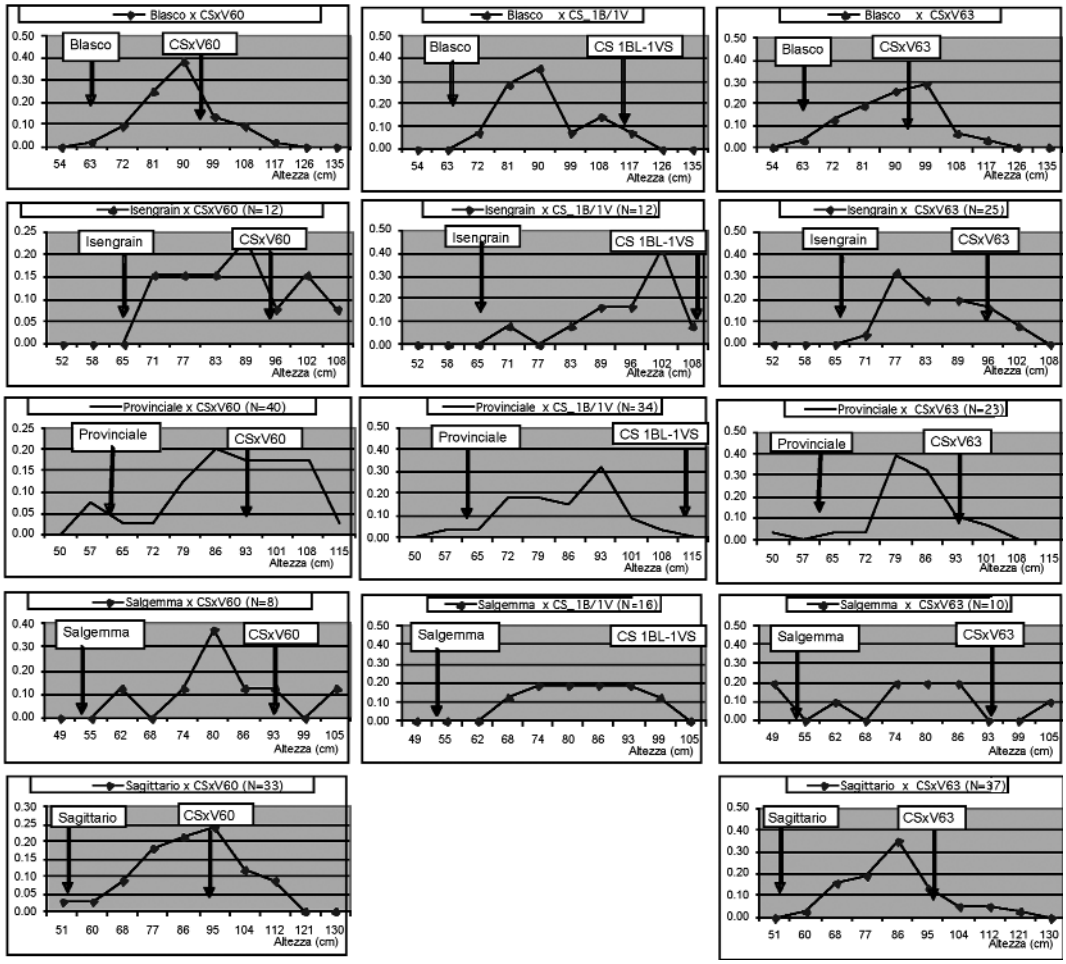

Fig. 8. Frequency distribution for main culm height of the F_2 plants obtained after crossing 'elite' commercial wheat cv with wheat IBLs possessing different *Dv* useful major genes.

(Fig. 4), and microintrogressions shaping the phenotypic performance were not excluded. Selfing from the F_1 generation through F_4 and F_5 helped in maintaining the new chromosomal arrangements and the cryptic introgressions that otherwhise should have been lost if repeated backcrosses to the wheat parent was adopted. Despite the expected AA'BB'DD genome constitution of the F_3 embryos, no further segregation was observed, which is an unexpected outcome. Although this observation pose several basic questions about the mechanisms that prevented further segregation, there is the practical advantage of having rapidly produced stable new IBLs with improved traits compared to the parents, and with the features needed for being included in the variety registration process.

The modified bulk-breeding method has expressed the expected results in terms of efficiency in assessing the breeding value of the IBLs, and has given the important evidence that the 'useful' *Dv* major genes introgressed in the parental IBLs are also expressed in the genetic background of the five different 'elite' wheat cultivars.

Riassunto – Nuove linee di frumento con buona precocità di spigatura, capacità produttiva, qualità della farina, e adattamento a sistemi di coltivazione a bassi apporti di fertilizzanti, sono state selezionate adottando due diverse procedure che hanno un aspetto in comune: la formazione di linee che presentano nuove combinazioni tra geni di *Dasypyrum villosum* (*Dv*) e geni di frumento. Il primo approccio consiste nell'applicazione di uno schema di ibridazione tra tre specie (*Dv*, frumento duro e frumento tenero), mentre il secondo metodo è una modificazione del sistema "bulk" di preparazione di progenie da selezionare. L'approccio triparentale inizia con la costituzione di un anfiploide dell'ibrido *T. turgidum* var. *durum* x *Dv*, la successiva ibridazione dell'anfiploide esaploide al frumento tenero 'Chinese Spring' (CS), e l'autofecondazione per due generazioni della progenie ottenuta. L'anfiploide manifesta i tipici caratteri adattativi di *Dv* (resistenza a ruggine ed oidio e precocità di spigatura e maturazione delle cariossidi), che vengono evidenziati anche in diverse progenie ottenute dopo ibridazione a CS. Alcune delle progenie hanno espresso anche un elevato peso delle cariossidi e un'ottima qualità panificatoria. In tali progenie, l'analisi GISH ha evidenziato la presenza di cromosomi che verosimilmente erano derivati da ricombinazione omeologa. Il metodo bulk modificato, consiste nell'ibridazione tra una cultivar commerciale di frumento tenero e una linea di frumento tenero (IBL) contenente cromatina trasferita da *Dv* in CS, nell'autofecondazione della progenie F_1, e nella raccolta in bulk delle cariossidi delle progenie F_2 ed F_3. Queste ultime hanno espresso diverse combinazioni dei caratteri presenti nei parentali compresa la contemporanea manifestazione della resistenza ad oidio e ruggine riconducibile ai geni che risiedevano nella cromatina di *Dv* della IBL parentale, e la bassa taglia e ottima produttività della cultivar commerciale. Questo risultato indica che i geni trasferiti in frumento da *Dv* esprimono dominanza non soltanto in CS, ma anche in linee che contengono molti cromosomi della cultivar commerciale di frumento.

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