ACCADEMIA NAZIONALE DELLE SCIENZE
detta dei XL

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

ROMA 2011

© Copyright 2011
ACCADEMIA NAZIONALE DELLE SCIENZE DETTA DEI XL
ROMA

ISSN 03-91-4666

ACCADEMIA NAZIONALE DELLE SCIENZE DETTA DEI XL
00161 Roma - Via L. Spallanzani, 7

Prof. G.T. Scarascia Mugnozza (27 maggio 1925 - 28 febbraio 2011)
Presidente dell'Accademia Nazionale delle Scienze detta dei XL

Prof. G.T. Scarascia Mugnozza (May 27th, 1925 - February 28th 2011)
President of the Italian National Academy of Sciences called "of the Forty"

Dedica

In memoria di uno dei più grandi scienziati dei nostri tempi: Gian Tommaso Scarascia Mugnozza. Le sue conoscenze di biologia, botanica, genetica, mutagenesi, e miglioramento genetico, e la sua vasta cultura gli consentirono di tradurre intuizioni scientifiche ed idee di ricerca, in azioni a favore del prossimo, della società, della sicurezza alimentare dei popoli, e della formazione accademica dei giovani. È stato un acuto osservatore della diversità genetica e biologica sia naturale che indotta sperimentalmente, deducendo principi e metodi operativi per un uso saggio, condiviso, ed efficiente dell'agrobiodiversità al fine di generare le energiche azioni necessarie per contrastare o almeno attenuare la fame e la povertà in diverse parti del globo e creare le condizioni per elevare la qualità della vita delle popolazioni bisognose.

Credeva nella scienza e nella sperimentazione come mezzi per avvicinare l'uomo alla natura, per comprenderne le dinamiche e, potenzialmente, anche per rispettare il creato ed il suo Creatore. La specializzazione insita in ogni disciplina scientifica, doveva quindi contribuire agli approfondimenti e progressi delle conoscenze da mettere a disposizione della collettività. Negli anni, la sua ampia visione e consapevolezza delle problematiche scientifiche hanno consentito la formulazione di obiettivi e la realizzazione di risultati di grande rilievo, nazionale ed internazionale, presso gli istituti di ricerca, di alta formazione, ed accademici da lui fondati o diretti. Le sue doti ammirevoli, il suo entusiasmo, la saggezza, e l'impegno, fuso con le sue innate qualità ed equilibrio, consentivano il rapido passaggio, dal livello del concepimento dell'idea scientifica, fino al livello della realizzazione, ma erano sostenute da lunghe ore di arduo lavoro per concretizzarne i risultati, e ciò è stato fonte di ispirazione per molti giovani scienziati e colleghi in diverse parti del mondo.

L'ampia documentazione scientifica della sua attività di ricerca, sperimentazione e cooperazione, e l'incisiva valorizzazione delle tematiche inerenti la biodiversità e le sue componenti più significative, tra le quali il frumento e le specie selvatiche affini, hanno contribuito sia la formazione di validi modelli di ricerca genetica sperimentale ed applicata, sia la crescita del benessere dell'umanità.

Tutti possiamo guardare avanti verso un futuro ancora rischiarato dal faro di luce scientifica che egli ha acceso, affinché si continui a fornire contributi per il progresso delle conoscenze. Questo libro è solo un piccolo segno che illustra la forza e lungimiranza di alcuni degli obiettivi di ricerca nel campo della genetica agraria proposti dal Prof. G.T. Scarascia Mugnozza.

Dedication

In memory of one of the greatest scientist of our times: Gian Tommaso Scarascia Mugnozza. His breath of knowledge including biology, botany, genetics, mutagenesis, and plant breeding, became tools for generating entirely novel insights about biodiversity and genetic resources for translating ideas into public languages and provide significant contribution to the human society.

He has been a keen observer of the natural and induced plant genetic diversity in his experimental plots and became aware that the wise use of the expressed agrobiodiversity should have been a strong force for mitigating hunger and poverty and provide food security.

He believed in science and experimentation "as an organized method for a meeting between man and nature and, potentially, even between man and his Creator". His vision that all scientific disciplines should contribute to the benefit of the humankind was embodied by the achievements promoted and fulfilled at the institutions for high research and academic studies that he founded or directed.

Through the years, his admirable qualities, his enthusiasm, wisdom, and commitment, were merged with his innate ability, creative thinking to pass from the level of conception to the level of realization, and long hours of arduous work, which have been inspiration to many young scientists, as well as to his peers around the world.

His international contributions through research trials and cooperation, publications, proceedings, and variety release for the advancement of the studies on biological diversity of durum wheat and related wild species as models for genetics and cultivation, is quite remarkable.

We can all look forward to future contributions from his scientific legacy, and this book is just a small token demonstrating afresh the strengths of his scientific objectives and of the way he brought us up through the agricultural genetics research field.

Spighe di *Dasypyrum villosum*
Spikes of *Dasypyrum villosum*

GIAN TOMMASO SCARASCIA MUGNOZZA*

Foreword

A synopsis of Dasypyrum research since 1980 in Italy and California, USA.

The net increase in the grain yield, quality and food safety thereof, are and will remain the primary breeding objectives for bread and *durum* wheat improvement. Among the industry there is a widespread belief that in modern sustainable agriculture, the support to grain yield improvement from the application of the genetics and genomics knowledge and sustainable crop management principles, should continue to play a key role in providing the variety that ensure the achievement of those objectives in harmony with the other biotic components of the acroecosystem[1]. This task is very challenging, especially if the genetic variability available for selection and introduction of new varieties is limited. To this end, there were many attempts and successes, beginning with those of the Nazareno Strampelli in the period 1906-1920, for the transfer through hybridization between cultivated *Triticum* species and other wild species of Triticeae, of the genes for the enlargement of genetic diversity and the improvement of the characters that determine the value of a variety for cultivation. However, only recently have been made available the analytical and methodological tools for interspecific transfer, the necessary connotations of efficiency and precision for the choice of the proper wild species as gene-donor and, in later stages, for the selection and characterization of lines for the future release of new wheat varieties.

In 1999, a virulent fungal strain (Ug99) was detected in Africa, making most commercial wheat varieties around the world vulnerable to stem rust. Also large-scale epidemics caused by new virulent and aggressive strains of *P. striiformis* now

* President of the National Academy of Sciences 'of the Forties', passed away February 28th 2011. The published papers containing results from the Italy-USA research cooperation on *Dasypyrum*, are cited with bracketed numbers, [ ], while other bibliographical citations are shown as footnotes.

[1] Graybosch R., C.J. Peterson, 2010. Genetic improvement in winter wheat yields in the great plains of North America, 1959-2008. *Crop Science* 50: 1882-1890.

pose a severe threat to the world's wheat supply[2]. Since 2000, the United States and Australia have faced severe yellow rust epidemics and recently yellow rust devastated major wheat-producing areas in China, northern and eastern Africa, western and central Asia, and the Middle East. The presence of two virulent and highly aggressive yellow rust strains (PstS1 and PstS2) at high frequencies at epidemic sites on five continents (including Europe) may represent the most rapid and extensive spread of an important crop pathogen. This epidemic trend may continue because the aggressive strains, which can tolerate higher temperatures, are still evolving. Rapid replacement of the wheat cultivars most susceptible to rust with new resistant, or less susceptible varieties is needed to slow down future rust spread. Research covering rust biology and rust-wheat genetic interactions will also be required, and new source of genes within the Triticeae species for introgression breeding into wheat will be necessary. Many dominant genes for adaptation have been lost during cereal crop domestication, but they have been retained in the genome of the wild components of the Triticeae gene-pools. In natural habitats, wild Triticeae[3] species such as *Dasypyrum villosum* (L.) Candargy (syn. *Haynaldia villosa* Schur) (*Dv*), whose genome was exposed to millions of years of climatic and environmental changes, are now expressing increased heading earliness, density stands, and plant biomass. Deploying the dissected *Dv* nuclear genome in the homoeologous wheat genetic background through interspecific hybridization and introgression, is a lower-cost and effective option to help wheat breeders to select the proper germplasm to sustain the needed yearly grain-yield increase.

To limit the negative effects of linkage-drag during the introgression process, it is necessary to dissect the genome of the donor species in several wheat lines, called introgression breeding lines (IBL), to choose those who received only the smallest amount of chromatin of the donor species, sufficient to contain the genes for resistances to fungal pathogens or insect pests.

The introgression procedure of wild Triticeae genes into wheat nuclear genome includes several steps, starting with the wheat × wild species hybridization (usually durum wheat is used as female parent), then backcrossing the hybrid to the wheat parental species to get IBLs or, in alternative, doubling the hybrid chromosome number for the establishment of the AABBVV amphiploid, followed by hybridization between the amphiploid and hexaploid wheat in order to reshuffle the A and B genomes of durum and bread wheat and combine them with the bread wheat genome with a small amount of chromatin from V genome, establishing IBLs with innovative combination of genes.

[2] Hovmøller M.S., S. Walter, A.F. Justesen, 2010. Escalating threat of wheat rusts. *Science* 329: 369.

[3] Löve A., 1984. Conspectus of the Triticeae. Feddes Rep. 95: 425-521.

Dewey D.R., 1984. The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: J.P. Gustafson (ed.) 'Gene Manipulation in Plant Improvement' Proc. 16th Stadler Genet. Symp., Plenum Publishing Corp., New York, USA, pp. 209-279.

The first reported *Triticum*×*Dv* hybridization involved the tetraploid wheat *T. turgidum* var. *durum* cv Frumento nero and *Dv*, and was made by Nazareno Strampelli[4] in 1908. Since then, Strampelli used *Dv* as one parent for 27 other interspecific hybrid combinations. In 1915 the cross *T. aestivum* cv Rieti×*Dv* made by Strampelli gave a fertile F_1 from which (after a putative process of backcrossing to cv Rieti) the 'gigas' winter bread wheat cv Cantore was derived. In 1927, from another hybrid that Strampelli made using *Dv* as one parent (the other parent was *T. aestivum* cv Akagomugi, a very early-heading Japanese cultivar), the bread wheat cv Roma was released[5]. In 20 years (1908-1927) of breeding and selection in progenies derived from wheat×*Dv* hybridization, Strampelli obtained many other interesting types, such as the so called *Triticum giganteum* (which produced large spikes and kernels as big as a coffee seed) or the lines with sweet kernels.

Dv is a widespread species in the ruderal vegetation of disturbed habitats in central and southern Europe [85], is an important ecological component of the rural environment of those areas, and has a very diverse composition of ecotypes due to the remarkable adaptability to ecosystems as different as the semi-arid sandy dunes along the Ionian and Tyrrhenian sea coasts in Puglia and Lazio[6], respectively, to the cold and humid ecosystem at high altitude such as Monte Terminillo at 1100 m above sea level (a.s.l.) in Lazio region, or Mount Armizzone, at 1350 m a.s.l. in Basilicata region in Italy. In the wake of the important results obtained by Strampelli experiments on wheat×*Dv* hybridization for the genetic improvement of cultivated wheats, then in the late seventies of last century, it seemed natural to reassess the role that different ecotypes of *Dv* in Italy might have to further progress wheat cultivation [85, 90].

The interspecific hybridization wheat×*Dv* and the subsequent backcross of the hybrid to the wheat parental species, followed by further self-fertilization, allowed the establishment of homozygous IBLs with *Dv* genes apt to improve: i) the resistance to the fungal pathogens causing powdery mildew, rust, and eyespot, and to mite virus vectors, ii) the earliness of ear emergence, iii) the bread-making quality of the flour, and iv) the concentration of protein, Zn, and Fe [83, 84, 88, 89, 91, 92]. Those IBLs were then used for the further transfer of the *Dv* genes to other wheat commercial varieties. Two IBLs contained a pair of 6V chromosomes. Other wheat limes produced elsewere and containing the chromosome 6V transferred from another ecotype, showed resistance to Ug99[7]. Therefore the two new

[4] Strampelli N., 1932. Origini, sviluppi, lavori e risultati. Istituto Nazionale di Genetica per la Cerealicoltura in Roma. Rome, Italy.

[5] Forlani R., 1954. Il Frumento: aspetti genetici e agronomici del miglioramento della coltura granaria. Monografia di Genetica Agraria. Tipografia del libro, Pavia.

[6] Fanelli G., 1998. *Dasypyrum villosum* vegetation in the territory of Rome. Rend. Fis. Acc. Lincei 9: 155-176.

[7] Pumphrey M., Y. Jin, M. Rouse, L.L. Qi, B. Friebe, B.S. Gill, 2008. Resistance to stem rust race TTKS in wheat relative *Haynaldia villosa*. In: R. Appels, E. Lagudah, P. Langridge, M.

IBLs with the 6V chromosome, when entered in the international circuit of resistance tests to incoming virulent strains of stem rust, will enrich the availability of desired genotypes of wheat with durable resistance genes to the *Pgt* fungal pathogen.

The research carried out at the Universityies of Bari, of Tuscia-Viterbo, and of California, Davis, CA, USA, allowed the establishment and evaluation of the genetic and agronomic properties of the IBLs, and these results were presented and discussed at the conference held in Rome on May 8th, 2009 at the Library of the National Academy of Sciences, 'of the Forties', to highlight the real opportunities for optimization, diversification, and increase of the productivity of wheat by the adoption of suitable IBLs during the registration process of new wheat varieties to achieve the desired yield progress in sustainable agricultural systems.

In the short term, the use of the most fertile areas for the production of wheat could continue to meet, at least temporarily, the worldwide demand for wheat (about +1% per year)[8] despite the reduction, in recent decades, of the genetic progress for grain yield per unit area. However, in the long term, effective genetic strategies to enhance the grain yield progress in the new wheat cultivars, should be pursued. If the breeders will include the IBLs developed from the cooperation of the Italian and U.S.A. universities and research institutions, in the pedigree of future wheat cultivars, then further progress in cereal production meeting the sustainability standards at the national and international level, will be achieved. Through this publication, it is intended to describe the main genetic and phenotypic features of the wheat lines obtained from the introgression process of the *Dv* chromatin and genes into the wheat genomes, and on the suitability of those lines as breeding materials for the trait enhancement of the wheat lines that could be used as base material to release improved wheat cultivars for sustainable agricultural systems.

Summary of the research activities accomplished from 1980 to 2010 to study the genetic contribution of the Dasypyrum species to wheat improvement, through the collaboration of the Italian research team coordinated by Prof. Scarascia Mugnozza at the University of Bari and University of Tuscia (Viterbo), and the group of researchers coordinated by Prof. C.O. Qualset, at the University of California, Davis, California, USA.

The collaborators of Prof. G.T. Scarascia Mugnozza at the University of Bari and University of Tuscia (Viterbo), who contributed to the development of the

Mackay (eds.). Proceedings of the 11th International Wheat Genetics Symposium. University Press, Sydney, Australia (http://hdl.handle.net/2123/3367); Xu S.S., I.S. Dundas, M.O. Pumphrey, Y. Jin, J.D. Faris, X. Cai, L.L. Qi, B.R. Friebe, B.S. Gill, 2008. Chromosome engineering to enhance utility of alien-derived stem rust resistance. In: Appels R., Eastwood R., Lagudah E., Langridge P., Mackay M., McIntyre L., Sharp. P. (eds.). Proceedings of the 11th International Wheat Genetics Symposium. University Press, Sydney, Australia (http://hdl.handle.net/2123/3483).

[8] Graybosch R.A. and C.J. Peterson, (2010). Genetic Improvement in Winter Wheat Yields in the Great Plains of North America, 1959-2008. *Crop Science* 50: 1882-1890.

research activities on the diploid (*Dv*) and tetraploid (*D. breviaristatum*, syn. *D. hordeaceum*) [*Db*(4x)] *Dasypyrum* species, were: A. Blanco, C. De Pace, O.A. Tanzarella, R. Simeone, A. Cenci, D. Vittori, V. Del Re and A. Ciofo. The research group supervised more than a dozen students in the preparation and realization of their theses project to get the University degree on topics related to population genetics, molecular genetics and cytogenetics, genome dissection, and introgression breeding of *Dv* and *Db*(4x) chromatin. Collaborations have been established on those scientific topics also with P.G. Cionini, M. Ceccarelli, and M.E. Caceres at the University of Perugia (Perugia, Italy), with G. Boggini, M. Corbellini and P. Vaccino at the Research Unit for the selection of cereals and the development of plant varieties (S. Angelo Lodigiano, LO, Italy) (CRA-SCV), with M. Pasquini at the Research Unit for the cereal grain quality (Rome) (CRA-QCE), and G. Vida and M. Molnar-Lang at the Agricultural Research Institute, Hungarian Academy of Sciences, Martonvàsàr, Hungary.

The collaborators of Prof. C.O. Qualset at the University of California, Davis, USA, who contributed to the development of the research activities on *Dv*, were P. McGuire (former director of the Genetic Resources Conservation Program, University of Califirnia, Davis, CA, USA up to 2008), C.C. Jan (1980 to 1987, now Research Geneticist, USDA, ARS, Northern Crop Science Laboratory, Fargo, ND, USA), and G.-Y. Zhong (1989 to 1992, now Research Leader, USDA-ARS, Grape Genetics Research Unit, Geneva, NY, USA).

The results achieved by the scientific cooperation between the mentioned researchers, have been published in more than seventy papers and can be grouped according to four research periods, which will be described in the following paragraphs.

Research period: 1980 to 1985

After the proposal of Prof. Qualset and Prof. Scarascia Mugnozza to re-evaluate *Dv* as gene-donor species for the genetic improvement of wheat [1, 2], various coordinated experiments were started concerning: (i) collection, evaluation and conservation of accessions of *Dv* populations from the territories of Tuscia, Puglia and Calabria [2]; (ii) karyotyping of the *Dv* nuclear genome [3]; (iii) establishment of an effective interspecific hybridization method to produce wheat × *Dv* hybrids and amphiploids; and (iv) the description of the morphological and cytogenetical features of the hexaploid amphiploid 'M × V' obtained after hybridization *T. turgidum* var. *durum* cv Modoc (M) × *Dv*. The adopted hybridization method was based on embryo rescue, *in vitro* embryo culture, and hybrid chromosome doubling using colchicine treatment. The 14- to 17-day-old hybrid embryos were rescued from the developing kernel and then placed in test-tube containing B5-*in vitro* culture medium. At the two-leaf stage, the hybrid plants were transferred to potted soil in the greenhouse. Root-tip cells of the hybrid seedlings were used for

chromosome counting. The hybrid cells contained 21 chromosome pairs (14 pairs from *T. turgidum* var. *durum* and 7 pairs from *Dv*). During the tillering stage, the hybrid plants were treated with colchicine, which allowed the development of fertile culms with spikes that, upon self-fertilization, formed caryopses. Germination of those caryopses provided seedlings with 2n = 42 chromosomes, and fertile plants which were the founders of the M × V amphiploid with AABBVV genomes [7, 10]. Applying the same procedure after *T. aestivum* cv Chinese Spring (CS) × *Dv* hybridization, the octoploid amphiploid CS × V-86 was produced [7, 8, 9, 10]. When *T. turgidum* var. *durum* cv Creso (Cr) × *Dv* hybridization was performed, five 12- to 15-day-old hybrid embryos were rescued from the developing caryopses and were placed in the *in vitro* culture medium. Those embryos provided three vigorous seedlings, two of which reached to adulthood stage without colchicine treatment [4]. The ears of the hybrid plants presented high sterility, but in two of them some grains were formed by the union of unreduced gametes. The embryos from those caryopses developed plants with 2n = 42 chromosomes, which gave rise to the Cr × V hexaploid amphiploid [5]. The electrophoretic pattern of the seed-storage proteins of the Cr × V amphiploid reflected the genetic contribution of the parental species to the synthesis of the prolamin polypeptides [6]. The morphology of the M × V and Cr × V amphiploid plants was similar to that of the wheat parent, but specific *Dv* traits such as brittle rachis, bicarenate glumes, long-beaked and bristled glumes, and the presence of bristles bordering the auricles and leaf-lamina, were inherited by the two amphiploids. Pollen mother cells at meiosisI evidenced chromome pairing to form at least 20 bivalents, indicating a slight tendency of the amphiploids to chromosome-number instability [10, 39, 59].

Research period: 1986 to 1995

The research carried out from 1986 to 1995 addressed questions related to (i) the somatic dimorphism of caryopses within the *Dv* spikelet, (ii) the reproductive system within *Dv* populations, (iii) the biochemical and molecular properties of anphiploids and their parental species, (iv) the production of aneuploid wheat lines containing individual *Dv* chromosomes, (v) the formation of durum wheat × *Db*(4x) hybrids, and (vi) the similarities between the genomes of wheat and *Dv* and between the genomes of *Dv* and *Db*(4x).

Preliminary evaluations indicated that abnormal concentrations of antioxidants such as ascorbic acid, affected *Dv* pollen viability [39]. For quantitative traits (length of the developmental stages, height and number of culms, size and fertility of the ear, leaf size and pubescence of the ear), the differentiation among populations was higher than the within populations diversity, and this pattern of variation was related to differences in climatic conditions among sites where the *Dv* populations were collected [24]. The genetic variation found for 5 of 12 traits (spike length, plant height, days to flag-leaf emergence, heading time, and anthesis time)

studied in 43 natural populations (31 from Italy and 12 from Croatia and Montenegro in the former Yugoslavia where *Dv* was widely distributed) grown in a common garden in California [48], followed also the pattern of higher differentiation among populations compared to the within populations variability. However, 7 of the 12 traits followed a reverse trend, that is a larger proportion of genetic variation was distributed within populations than among populations. As few as five populations, each with five or more half-sib seeds taken randomly from *5* plants, was expected to capture more than *95%* of the total genetic variation encountered in all the sampled populations, but sampling a much larger number of seeds per population (>1000) was suggested for long-term storage to facilitate the supply of seeds for several decades to researcher and plant breeders [48].

Also the genetic variability between populations for biochemical characters (isozymes and seed storage proteins) was lower than that within populations, especially if the accessions were from sites less than 50 km apart and/or with similar habitats [7, 8, 13, 23]. In fact, only 10% of the variability for the allele frequencies at loci coding for the isoenzyme systems glutamate-oxaloacetate transaminase (GOT-2, GOT-3) and esterase, was accounted by the allelic diversity between populations, while the rest of the variation depended on differences within populations [13].

A similar pattern of diversity was observed for the high-molecular-weight (HMW) glutenin subunits of the seed storage proteins in populations sampled in their natural habitats in Italy and former Yugoslavia [42]. The results of progeny testing confirmed that the genetic variation for HMW-glutenin subunits in *Dv* was controlled by allelic variation at a single locus (*Glu-V1*). Fourteen alleles at *Glu-V1* were found among 982 individuals representing the 12 populations from Italy and two from the former Yugoslavian territory, with a mean of seven alleles per population. Among the 14 *Glu-V1* alleles, one was null, 10 coded for a single subunit, and three for two subunits. The mobilities of all the subunits in SDS-polyacrylamide gels were greater than that of the reference subunit 7 of CS. Eight of the alleles were relatively abundant (mean frequency over all populations ranged from 0.08 to 0.17) and distributed largely among 8 to 14 populations, but five other alleles were rare (0.003 to 0.021) and found in only 1 to 5 populations [42].

The within *Dv* population allele frequencies for genes encoding isoenzyme systems were compatible with a reproductive system with a prevalence of outcrossing [47]. The within-family variation for isozyme allelic frequencies (i.e. the variation detected in seedlings raised from kernels harvested from the same plant) was generally greater than the variation between families (i.e., the variation among seedlings raised from kernels harvested from different plants of the same population). One population, labeled I-84-16, collected from a site near the 'Monster Park' entrance at Bomarzo, Italy, showed unique features [13]. It showed a high outcrossing rate and the highest number of alleles per locus (up to 4) but a low within-family variance component for number of culms per plant, spike length, and flag leaf size. The genetic and ecological situation encountered in population I-84-

16 suggested that, most likely, a small number of founder plants led to the establishment of that population which maintained high frequencies for few alleles at each locus, coupled to inbreeding effect due to the small size and isolation of the population despite the high observed outcrossing rate. A natural soil-depression in the surface of the land placed the population site at about 60 m below the town of Bomarzo which is about 270 m from the population site. This caused isolation from the ancestral population growing around the town, leading to perturbation of the population genetic structure due to the accumulation of the inbreeding effects, random changes in allele frequencies due to genetic drift, near fixation of one allele at each locus and other alleles maintained at low frequencies, large between-family variation despite the high outcrossing rate, and phenotypic differentiation from the ancestral population due to genetic drift and selection of alleles for adaptive morphological and phenological traits such as heading earliness. In common garden studies carried out at Viterbo (Italy) and Davis (CA, USA), plant-progenies from population I-84-16 headed 1 month earlier then plant-progenies from other populations collected at Bomarzo and near Viterbo which is about 30 km far from Bomarzo.

Caryopses developed in florets of the same *Dv* spikelet, expressed differences for size, color, and germination ability. This phenomenon was termed 'caryopses somatic dimorphism'. It was found [13] that about 55% of the caryopses formed in the spike under open pollination in natural populations had amber color, while the other caryopses (45%) were red-brown colored. In addition, it was observed that when both the proximal florets of each spikelet formed caryopses, the kernel in the first floret (the most basal one) was reddish-brown in at least 66% of the cases, while in the floret immediately above (or second floret) the kernel formed were red-brown only in 0-20% of the cases. Therefore the probability that a kernel would have amber color was the highest for those formed in the second floret and their weight was 10 to 60% higher than those expressing the red-brown color. When released from the glumes, the caryopses with red-brown color germinated 24-hours later than the caryopses with amber color [44], while if they were left in the glumes, the amber-colored caryopses germinated in 100% of cases, while the red-brown-colored caryopses germinate in 0-2% of the cases. The plants that grew from polymorphic kernels showed no significant differences for morphological characters (plant height, leaf and ear size, etc.) or for the allele frequencies at loci coding for seed storage proteins and isoenzymes [13, 43, 44]. On the other hand, significant differences were found between kernels of different color for both the DNA content and the number of tandem-repeated sequences of 380bp residing at the chromosome ends (telomeric sequences). As matter of fact, cells in seedlings from red-brown caryopses had a lower DNA content and fewer 380bp-repeat sequences compared to seedlings derived from amber colored kernels [35, 36, 45].

The electrophoretic analysis of the restriction fragments from the ribosomal genes or rDNA (that is, the nuclear DNA that includes genes encoding the 18S and 26S ribosomal RNA which, together with protein subunits, build the ribosomes

necessary for protein synthesis) showed significant differences in rDNA fragment sizes between *Dv* populations [20]. The availability of lines with monosomic and disomic addition of *Dv* chromosomes to the genome of bread wheat [7, 8, 14, 15, 22, 25], and durum wheat [11, 12, 16, 27, 37], enabled the chromosomal location of *Dv* genes for the synthesis of seed storage proteins and isoenzymes, and for other genetic markers [21, 31, 36, 41, 54]. It was shown for the first time that genes encoding a-gliadins-like proteins were located in chromosome 4V as well as in chromosomes 1V and 6V by Montebove *et al.* (1987) [15]. These results were confirmed by Blanco *et al.* (1991) [34].

The zymogram phenotypes of glucosephosphate isomerase (GPI), alcohol dehydrogenase-l (ADH-1), glutamate-oxaloacetate-transaminase (GOT), and superoxide dismutase (SOD) isoenzyme systems were determined for five disomic addition lines each containing a single *Dv* chromosome added to the CS nuclear genome. The genes *Gpi-V1*, *Adh-V1*, *Got-V2*, and *Sod-V2* coding for GPI-1, ADH-1, GOT-2, and SOD-2 isozymes were located in *Dv* chromosomes 1V, 4V, 6V, and 7V, respectively [15]. The presence/absence of isoenzyme bands in the electrophoretic patterns of M × V amphiploid plants from shriveled caryopses, was proposed as a procedure to identify aneuploids for single V chromosome and to assign genes to the specific V chromosome for which the amphiploid plant was nullisomic. Six different nullisomic amphiploid plants involving different V chromosomes were isolated by the use of the presence/absence phenotype for isoenzyme (ADH-1, GOT-2, SOD-2) bands, and also by determining which phenotype occurred for the rachis (brittle/tough), glume (hairy/glabrous), and seed storage proteins (gliadin) [32].

In May 1986 introgression lines of *Dv* chromosomes in wheat genome were produced at the University of Tuscia (VT). Eleven IBLs were selected from a population of 150 aneuploid lines developed through (CS × *Dv*, F_1) × CS backcross, followed by three generations of selfing (BC_1 F_1 S_1 through S_3), five generations of single-spike descent (from S_4 through S_8), and four generations of seed increase (S_{12} IBLs). The *Dv* parent used in the hybridization to CS was a progeny from the I-84-16 ecotype collected at Bomarzo [13].

An euhaploid durum wheat line 2n = 2x = 14, with 7 chromosomes for each of the A and B genomes including the 5B chromosome with the *Ph-1* allele preventing the homoeologous chromosome pairing, showed 0.37 chiasmata per cell at meiosis I. Cytological studies on 11 durum × *Dv* F_1 hybrids (each being 2n = 3x = 21, ABV), showed that in some of them the presence of the V genome promoted pairing between homoeologous chromosomes overcoming the effect of the *Ph-1* allele. In fact, the number of bivalents per cell in those ABV hybrids, ranged from 0.38 to 3.97. In the ABV hybrids with 3.97 bivalents, clearly the V-genome favored homoeologous-pairing despite the presence of the *Ph-1* allele [18]. The homoeologous-pairing-promoting effect exerted by the V-genome was similar to that observed in lines where the *Ph-1* locus was deleted or the recessive *Ph-1* mutant allele was pres-

ent. As matter of fact, in those lines devoid of the *Ph-1* allele, 3.88 chiasmata per cell were observed. The average number of bivalents per cell in ABV hybrids has been 2.5, with 12.5% of them forming A-V or B-V pairs. This meant that in 40 microspore mother cells, 100 bivalents were observed, 12 of which were of the A-V or B-V types. Considering that other authors observed a frequency close to zero for the pairing between chromosomes R and V chromosomes in AABBVR hybrids, it was inferred that the V genome was more similar to A or B genome of wheat than to the R genome of rye.

Some seed storage proteins such as the low molecular weight prolamin aggregates and the α-type prolamins were synthesized in wheat and *Dv* caryopses but not in rye caryopses, indicating that, contrary to the results from morphological analysis, the *Dv* nucleotide gene sequences for seed storage proteins strongly differed from the R homoeologous genes and less from the expression of the wheat homoeologous genes. The analysis of about 15 seed storage polypeptides and leaf isoenzymes in *Dv*, evidenced that 60% of the alleles encoding them, were orthologous to genes located in chromosomes of the A and B genomes of hexaploid wheat [26].

The homoeologous pairing observed between *Dv* and wheat chromosomes by Blanco *et al.* (1988) [18], was immediately seen as a very promising biological mechanism for the transfer of genes from *Dv* to wheat chromosomes by crossing-over [19]. The short arm of chromosome 6V transferred to *durum* wheat, conferred resistance to powdery mildew [17]. The biology of the *Dv* and its importance for the genetic improvement of wheat were reviewed by Qualset *et al.* (1993) [40].

New allele combinations in amphiploids derived from wheat × Dv hybridization, enlarged the number of polymorphic enzymatic proteins and increased the biochemical complexity within the cell, giving to the amphiploid the molecular endowment for a greater resilience to environmental stresses compared to the parental species [9, 22, 28]. The hexaploid amphiploid AABBVV combined the durum wheat plant morphology with either water stress resistance [30, 51], or resistance to powdery mildew and good competitive ability in root nutrient uptake [46] expressed by *Dv.* The amphiploid accumulated biomass and protein in the leaves and grain at a pace and proportion higher than that occurring in the same plant parts of the durum wheats 'Modoc' and 'Creso' or of the amphiploids synthesized by crossing Modoc to rye [29]. The nitrogen content accumulated in the amphiploid plants sampled at regular intervals from tillering to the milk-stage of the caryopses, was 30% and 50% higher than the accumulation observed in the parental wheat and *Dv* species, respectively. The amount of total dry matter in the amphiploid plant at maturity was similar to that produced by the barley cv Barberousse, the best winter cereal crop for forage. These data suggested an in-depth assessment of the wheat × *Dv* amphiploids as a new self-seeding (due to their brittle-rachis trait) species for the production of high-protein dry biomass for animal feeding.

Karyotype analysis of *Db*(4x) [33] showed 28 chromosomes and formation of quadrivalents at meiosis I, indicating for this species the genomic formula

$2n = 4x = 28$, $V^bV^bV^bV^b$. The hybridization durum wheat × *Db*(4x) enabled the study of the homoeologous pairing of the *Db*(4x) chromosomes with those of the A and B genomes [38]. It was agreed that the presence or absence of the *Ph-1* gene did not affect the proportion of homoeologous pairing events at meiosis-I of the ABV^bV^b hybrid, and in both cases about 7.6 bivalents per cell were formed. The pairing involved only the 14 chromosomes inherited from *Db*(4x), indicating that they were composed of 7 pairs of homologous V^b chromosomes. This confirmed the origin of *Db*(4x) by autopolyploidy. The durum wheat × *Db*(4x) hybrid was perennial as was the *Db*(4x) parental species, and the backcross to the cv Creso gave caryopses whose embryos contained the chromosome number ranging from 40 to 43 and formed partially fertile plants.

Research period: 1996 to 2005

Research undertaken in the period 1996-2005 was aimed at the elucidation of: (i) the genomic affinity between *Dv* and *Db*(4x) and the organization of heterochromatin in the *Db*(4x) chromosomes; (ii) the variability for the size of the nuclear genome in different *Dv* populations; (iii) the ecological properties of new *Dv* ecotypes; (iv) the location of further genetic markers in the *Dv* chromosomes; (v) the *Dv* genes that improve the bread-making quality of the wheat flour and the resistance to powdery mildew and to drought in the wheat IBL; (vi) the significance of a spontaneous mutation determining the 'tough rachis' phenotype in the otherwise 'brittle-rachis' M × V amphiploid; (vii) the genomic constitution of new wheat × *Dv* hybrids; and (viii) the evolutionary relationships of the *Dv* chromosomes with those of the A, B and D genomes.

Molecular cytogenetic methods were used to study the controversial phylogenetic relationships between the *Dv* and *Db*(4x) species. Using labeled and blocking total genomic DNA from the two species as probes for *in situ* hybridization, it was found that the pericentromeric regions of the chromosome arms of both species were similar, while distal regions showed substantial differences [52, 58]. Two dispersed repetitive DNA sequences (pDbKB45 and pDbKB49) isolated and cloned from *Db*(4x), were assessed to be distributed along the chromosomes and were abundant in the subtelomeric regions of *Db*(4x) chromosomes (pDbKB45), or were less amplified in terminal regions of both species (pDbKB49). Southern transfers of size-separated restriction enzyme digests of *Db*(4x) and *Dv* nuclear DNA, showed several fragments in common when probed with labeled *Db*(4x) DNA and *Dv* DNA used as blocking, while few fragments where in common between the two species when probed with labeled *Dv* nuclear DNA and *Db*(4x) DNA as blocking; in this case substantial hybridization patterns were observed on DNA fragments from *Dv*. Genomic *in situ* hybridization to meiotic metaphases from an interspecific hybrid showed seven bivalents of *Db*(4x) origin and seven univalents from *Dv*. Probably the extensive differences for repeated DNA sequences between *Dv* and

Db(4x) impeded meiotic pairing between *Dv* and *Db*(4x) chromosomes. Because the pairing was observed only between *Db*(4x) chromosomes, an autotetraploid origin for *Db*(4x) was hypothesized, but its genome may have diverged from the *Dv* genome either before or after the polyploidization event. In the first case it was supposed that geographical and altitudinal barriers may have caused differentiation of the sibling *Db*(2x) taxa from *Dv* followed by the union of unreduced gametes (whose formation could have been favoured by the low temperature during anthesis at high altitude) and formation of autopolyploid embryos to originate *Db*(4x). In the second case autopolyploidy might have occurred as an adaptation of *Dv* to strive in the cold environments at high altitude followed by genetic divergence and reproductive isolation from *Dv*. The rise of the perenniality trait was thought as an adaptation of the *Dv* genome to the cold winters at high altitude. The autotetraploid origin of *Db*(4*x*) from *Dv* suggested by Blanco *et al.* (1996) [49], did not find consensus on the basis of the lack of pairing of the V^v and V^b chromosomes in hybrids containing the two V^v and V^b genomes. Nonetheless, the homology between the *Dv* and *Db* species for the peculiar spike morphology, the shared unilocus molecular and biochemical markers [49], as well as the few signals detected on *Db*(4x) chromosomes but not on other Triticeae species (except *Th. bessarabicum*) using the pHv62 *Dv*-species-specific repeated sequence[9], were indicative of *Dv-Db* common ancestry. The genetic distance between *Dv* and *Db* for 320 RAPD loci was lower than their distance from *Secale* species[10]. Despite the very low average pairing between the chromosomes of *Dv* and *Db* (2x) in their V^vV^b F_1 hybrid, 1-2 bivalents were formed which indicated a residual homology existing between their genomes. Therefore, rather then remote ancestry between *Dv* and *Db*(2x), the differentiation between *Dv* and *Db* might be due to recent adaptability events to the diverse ecogeographic area occupied by the *Dv-Db* ecotypes. The formation of the *Dv-Db*(2x)-*Db*(4x) species complex and their biological and taxonomical status may be explained by a sequence of ecological adaptive events after the main Triticeae clade diverged several million years ago. The main adaptive trend might have involved a progressive habitat shift from the lowland and sandy-dune *Dv* habitats towards the high altitude (>1100 m a.s.l.) habitats in Italy, Morocco, and Greece. In Italy, *Dv* populations from high altitude sites such as Monte Armizzone at 1350 m a.s.l. in Basilicata region or Monte Terminillo at 1100 m a.s.l. in Lazio, when transferred in common garden at low altitude, expressed very late heading and early sign of vegetative reproduction. As matter of fact**,** dormant axillary buds at the basal nodes of mature culms of those plants,

[9] Uslu E., S.M. Reader, T.E. Miller, 1999. Characterization of *Dasypyrum villosum* (L.) Candargy chromosomes by fluorescent *in situ* hybridization. Hereditas 131:129-134.

[10] Yang Z.J., C. Liu, J. Feng, G.R. Li, J.P. Zhou, K.J. Deng, Z.L. Ren, 2006. Studies on genome relationship and species-specific PCR marker for *Dasypyrum breviaristatum* in Triticeae. Hereditas 143: 47-54.

during summer sprouted and epicotyles terminating with a rooted shoot was formed, which in turn produced further epicotyles forming a stolon-like structure eventually persisting up to April-May in the next year, when an heading shoot developed. Mesocotyls were formed from dormant buds on basal nodes or from middle or top nodes of old and dry culms [85]. The *Dv-Db* transition has been stronger on the Atlas mountains of Morocco where *Dv* disappeared and it was replaced by the *Db*(2x) and *Db*(4x) cytotypes.

The reproductive isolation of the high-altitude *Db*(2x) prototype[11] from the lower-altitude *Dv* ecotypes, most likely paralleled the rapid evolution of different sets of repeated sequences in the chromosomes of the different evolving ecotypes. Then, the low temperatures during anthesis at high altitude favoured the formation of unreduced gametes and their union determined the autopolyploidization event leading to the *Db*(2x)→*Db*(4x) transition and incipient reproductive isolation of *Db*(4x) from *Db*(2x). In the *Dv-Db*(2x)-*Db*(4x) evolutionary trend, the *Dv-Db*(2x) step determined the strongest divergence between *Dv* and *Db*, while the *Db*(2x)-*Db*(4x) step has not been accompanied by high genomic divergence yet, probably because temporary more recent compared to the previous evolutionary step. The number of trivalents in the *Db*(2x)×*Db*(4x) hybrid is higher than the number of bivalents in the *Dv*×*Db*(2x) hybrid. Very limited V^v vs V^b genome divergence has been deduced for the gene-rich DNA segments, as suggested by (i) the strong similarity between *Dv* and *Db* genomes for the restriction fragment patterns of genomic DNA, (ii) the phenotypes for some isozyme systems, and (iii) the hybridization signals of the labeled *Db*(4x) DNA on *Dv* DNA fragments found by Galasso *et al.* (1997) [52], as well as the similarity in nucleotide sequence and chromosomal location of the gliadin genes [49]. The *Db*(2x)→*Db*(4x) autopolyploidization event favored the extensive *Db*(4x) colonization of the high-altitude habitat in the north African Atlas up to the Mt. Taygetos in the Peleponnesus mountains of Greece. Chromosomal divergence between the Moroccan and Greek *Db*(4x) ecotypes is not evident yet.

Cytophotometric DNA measurements in root-tip meristematic cells prepared with the nuclear-specific Feulgen staining method, were carried out in embryos sampled from *Dv* populations collected at sites with large differences in geographic coordinates. Absorbance of the 550 nm radiation by the Feulgen-stained nuclei of root-tip cells showed a substantial quantitative variation between populations indicating significant variation for their average nuclear DNA content. In particular, there was a positive correlation between altitude of origin of the populations and the amount of radiation absorbed from the Feulgen stained root-tip meristematic cells prepared from embryos sampled in those populations; this, in turn, deter-

[11] Ohta S., M. Koto, T. Osada, A. Matsuyama, Y. Furuta, 2002. Rediscovery of a diploid cytotype of *Dasypyrum breviaristatum* in Morocco. Genet. Resour. Crop. Evol. 49: 305-312.

mined the positive correlation between altitude and 1C DNA content estimated by the cytophotometric values [50, 53]. The average 1C DNA content estimated in a population collected at 350 m a.s.l. in the Lazio region, was 7.01 pg, while the 1C DNA content in root tip cells from a population collected at 1000 m a.s.l. also in Lazio region, was 8.12 pg. The population IV-1 from a collection site at 500 m a.s.l. in Basilicata (S. Martino d'Agri) contained 6.8 pg of 1C DNA, while for the population IV-6 collected in Basilicata at 1350 m on Mount Armizzone, the 1C DNA content was 7.63 pg. In 15 populations analyzed, the 1C DNA content was positively correlated with both the total length of the 14 chromosomes and the long arm / short arm length ratio of chromosome 7V. These data indicate that the change in genome size (= amount of nuclear DNA) is one of the adaptive mechanisms enacted by *Dv* populations while progressing towards higher altitude colonization sites. The increase in genome size paralleled the increase in copy number of repeated DNA sequences [35, 45].

One way to test this hypothesis of adaptation due to natural selection at different altitudes, is the assessment of ecotypic differentiation for traits with adaptive significance such as glume pubescence, heading time, awn length, and plant height. Therefore, *Dv* plant populations were sampled along six altitudinal transects (from 200 to 1350 m a.s.l.) identified in Italian regions of Lazio, Basilicata and Calabria. The sampled populations were grown in common garden at 350 m a.s.l., in the Experimental Farm of University of Tuscia, Viterbo, Italy. The populations collected from sites at high altitude (1000-1350 m a.s.l.), had 60-100% of plants with pubescent glumes, while the populations collected from sites at medium altitude (350-800 m a.s.l.) the proportion of plant with pubescent glumes was between 5 and 45%. The populations collected from sites below 350 m a.s.l. did not contain plants with pubescent glumes, or the proportion of such plants was at most 5% of the entire plant community [57]. Significant differences were also detected for date of ear emergence, culm height, awn length, and spikelet fertility. The mean values for those traits recorded in populations from altitudes higher than 580 m a.s.l. showed values significantly lower than the mean values for the same traits scored in populations from altitudes below 580 m a.s.l. Only progenies from plants with pubescent glumes expressed this trait, while the progenies from glabrous glumes were also devoid of pubescence, which confirmed the high inheritance of the glume pubescence trait state.

The parent-offspring analysis was adopted also to estimate the inheritance for the other morphological traits evaluated in the common garden study [57]. A high heritability coefficient was found for culm height, leaf size and number of days to ear emergence. The parental plants collected at higher altitudes sites (1100 and 1350 m a.s.l.) in Calabria, Basilicata and Lazio and their offspring, expressed significantly lower values for the height of the culm, leaf size and awn length, and higher values for the number of days to ear emergence, compared to the parental plants and offspring from populations collected at altitudes of 900-1000 m a.s.l. of

the respective transects. Collectively, those results other than indicating good heritability for the examined traits, confirmed the ecotypic status of the populations collected at different altitudes along a transect, which provided support to the hypothesis that natural selection shaped the genetic structure of the *Dv* ecotypes at the different altitudes. In addition, the height of the main culm and the awn length could be considered significant indicators of the ecotipic differentiation for planning or decide which *Dv* population gather during germplasm collecting missions.

New molecular and biochemical genetic markers for assessing the grain quality of the IBLs

As mentioned earlier, CS-IBLs containing substitution, addition or recombination of V chromosomes from the *Dv* ecotype I-84-16 were obtained in May 1986 at the University of Tuscia (VT) by CS × *Dv* interspecific hybridization. Since then, the IBLs underwent more than 12 generations of selfing. About 150 aneuploid IBLs were available, and a sample composed by the IBLs CS × V11, CS × V32, CS × V58, CS × V59, CS × V60, CS × V63, CS × V79, and CS-1BL-1VS (this last one was bred by E.R. Sears at University of Missouri, Columbia, MO, USA), was selected for further evaluation. It was assessed that those IBLs were significantly different from CS for heading time (CS × V58, CS × V59, CS × V60), resistance to powdery mildew (CS × V32 and CS × V63) and the sedimentation volume of the flour in a solution of SDS (CS × V32, CS × V11 and CS-1BL-1VS) [55, 56, 60]. The gluten quality of CS × V63 was not lower that of CS but the grain protein content was about 30% higher than in the CS kernels, and in general, aneuploid wheat lines containing chromosomes 1V, 4V and 6V showed grain protein content 13% higher than in the CS caryopses [56]. The flour from caryopses of the IBLs containing chromosome 1V had a significantly higher gluten strength than the flour obtained from CS caryopses [55, 56, 60].

These data, suggested that chromosome 6V can be transferred into wheat to introgress genes conferring resistance to fungal pathogens causing foliar diseses in wheat without any adverse effects on the quality of flour, and chromosome 1V can be introgressed to transfer genes for improving end-use grain quality, providing a better performance than the introgression of 1R chromatin into wheat genome. Other lines, such as CS × V58, expressing alleles for ear-emergence earliness will be useful to improve this trait when transferred in other wheat varieties. These IBLs have received particular attention during the research period 2006-2010 in which the FRUMIGEN project was carried out.

The IBLs were further evaluated for a set of 28 complex genetic trait scored during four consecutive growth stages (1-germination, 2-seedling elongation, 3-tillering, 4-stem elongation-maturity) in greenhouse. The evaluated IBLs (and the corresponding V segment added) were: CS1V (1V), CS 1BL-1VS, CS2V (2V), A18-2 (3V), CS4V (4V), CS5V (5V), CS6V (6V#1), CS × V63 (6V#4), and CS7V (7V).

For each IBL, groups of thirty plants replicated twice were evaluated in the greenhouse. The following explanatory model was adopted: (a) for a given quantitative trait, the pattern of phenotypic differences between individual disomic aneuploid lines and CS provide: (a.1) an estimate of the 'composite additive effects' (CE) of the QTL alleles summed over the *n* loci determining the trait and located in the V chromosome of that IBL, and (a.2) suggestive evidence on the location of those QTL alleles in that V chromosome; (b) correlation is expected among 'CE' for a subset of traits expressed in the same growing stage, largely due to pleiotropy; (c) each V chromosome exerts a specific CE on the same subset of traits; (d) growth stages serve as developmental landmarks and triggers for the expression of CEs for independent subsets of traits [62, 65]. Factor analysis of the genetic correlation matrix showed four uncorrelated 'Factors' accounting for about 70% of the total variation for the additive effects. The number (four) of detected Factors had biological meaning and indicated that a minimum of four independent CE on each V chromosome accounted for the additive variation and covariation observed for the panel of disomic aneuploid lines. Enhancing additive effects were expressed by QTLs in chomosomes 1V and 7V (earliness at tillering stage), 4V (rapid germination), and 5V (seedling robustness)].

Constitution of new 'durum wheat × Dv' amphiploids and identification of a spontaneous 'tough rachis' mutant in the M × V amphiploid

An important result obtained in this period was the establishment of new durum × *Dv* hybrids to gather further information on the chance occurrence of amphiploids through sponaneous hybridization and chromosome doubling. A total of 11 interspecific hybridization experiments had already been carried out in late April 1994 and March 1995, but the caryopses with hybrid embryo were planted in the greenhouse in spring 1996 (29 caryopses) and 1999 (19 caryopses). The mating design involved the *T. turgidum* var. *durum* cv Modoc and cv Creso as female parent and three different populations collected at Bomarzo (VT), Ferento (VT) and Mottola (TA), as male parents. The chromosome number of the hybrid embryos was equal to the sum of the haploid chromosome number of the parents, that is 14 chromosomes from *T. turgidum* var. *durum* (7 chromosomes of genome A and 7 chromosom of genome B) and 7 chromosomes from *Dv*, for a total of 21 chromosomes. Each of the 21 chromosome were in monosomc condition, so it was expected that at meiosis the mother cells of micro- and macro-spores should have formed tetrads with an abnormal chromosome number and in rare cases, by chance, meiocytes with unreduced chromosome number, n = 21, could have formed. As a consequence, the hybrids were expected to be higly sterile, and very rare caryopses should have formed by the union of the unreduced gametes. As matter of fact, only two caryopses were formed in the spikes of 48 hybrid plants. From these caryopses, fertile plants were obtained, confirming that each have been

formed by the union of unreduced gametes, which had restored the bivalent condition for each of the 21 chromosomes [63, 69].

Crossability was about 11%; the spikelets of the F_1 had hulled glumes; unreduced gametes at the frequency of 1.24% and 3.7% in the male and female germ line, respectively; wedge-disarticulation at maturity (brittle-rachis); and fertile F_2 progeny at the frequency of 0.046%, derived from selfing F_1 plants [63]. Those data on crossability between parental species, and the occurrence and frequency of viable unreduced gametes for the rise of the first fertile hybrid plant, were relevant data to indirectly quantify early events in wheat domestication [61, 63]. Also relevant are the data on the elapsed time span for the appearance of important domestication-syndrome related traits in the fertile progenies of the wild durum wheat types that entered the first cultivation systems used by humans in the Fertile Crescent area about 10,000 years ago. Information on this aspect have been obtained from a tough-rachis mutant plant appeared in the progeny of the brittle-rachis M × V amphiploid.

The brittle-rachis M × V, amphiploid gave phenotypically stable 2n = 6x = 42, AABBVV progeny up to the F_8 when a nonbrittle-rachis mutant plant appeared at a rate of 4.4×10^{-5} per individual per generation [63]. Because of the paucity of archaeobotanical data documenting the early stages of hexaploid wheat domestication, the estimated mutation frequency provide a recapitulation of population size and time lapse required for the transition from a hard-to-harvest tetraploid wheat crop because of rachis brittleness to a whole-harvestable spike of the domesticated tough-rachis wheats. The appearance and identification, by the earlier farmers, of the nonbrittle-rachis mutant, might have occurred in just few decades after the beginning of cultivation of the brittle-rachis tetraploid (such as *T. dicoccoides*) and hexaploid spelt wheats].

The use of the M × V amphiploid as a bridge for shuffling the durum and bread wheat chromosomes of the A and B genome, and further data on the homoeology of the V chromosomes and those of the A, B, and D genome.

The chromosome number instability of the amphiploid M × V, was evidenced by Zhong and Qualset (1990) [32] and Contento *et al.* (2001) [59]. However, the chromosome instability involved only the V genome. This phenomenon was exacerbated when M × V (AABBVV) was crossed with CS (A'A'B'B'DD). The hybrid should have had 42 chromosomes and genome constitution AA'BB'DV, but in most root-tip meristematic cells of different hybrid embryos, less than 42 chromosomes were counted. In all sampled cells, the 14 chromosome pairs of the AA' and BB' genomes were always present, in addition to 3 to 7 D monosomes and fewer or absent V monosomes. After one generation of selfing the V chromosomes were lost and lines with either 28 chromosome and putative genome composition AA'BB' or 42 chromosomes with putative genome composition AA'BB'DD were recovered in

the majority of the progenies examined [81]. Several A-B chromosomal exchanges were detected.

The set of lines having the disomic addition of single V chromosomes prepared by E.R. Sears, were cytologically re-examined after about ten generation of selfing at University of Viterbo. The GISH (*Genomic In Situ Hybridization*) analyses confirmed the expected chromosomal constitution, with the exception of the line 'CS1V', which proved to be a line of substitution rather than a disomic addition, and the line 'CS5V', which was remarkably unstable for the presence/absence of 5V [66].

The results obtained (have shown good ability to distinguish hybrids, through the use of genetic markers and the chromosomes of the parent species and between those who are carriers of the different genomes. Despite the often observed polymorphism of the complement of *Dv*, the individual chromosome pairs have proved to be recognizable by banding patterns produced by the RFLP molecular markers, and GISH and DAPI (4',6-diamidino-2-phenylindole fluorochrome that binds strongly to DNA) DNA labeling methods [54, 64, 66]. Polymorphism was observed for the satellited V cromosome and for the size of the telomeric bands of the metacentric V chromosomes, when they were compared in cytological preparations of *Dv* and the disomc addition lines. Similar analyses carried out on the IBLs produced at Viterbo, allowed the identification of the disomic addition for 3V in CS genome complement, which was not available in the E.R. Sears' disomic addition set. This line expressed the 'red aleurone' trait that [27] had already attributed to a gene in chromosome 3V of the corrsponding monosomic addition in durum wheat genomic complement. The 3V is the longest submetacentric V chromosome pair, and had a stained band on the telomeric region of the short arm and an intersitial band on the long arm [64, 66]. The line with the shorter submetacentric V chromosome pair, which was identified as 6V because of the powdery mildew resistance trait showed by the plants of that line and it had a faint band on the telomeric region of the long arm. Because all the IBLs produced at Viterbo, although harboring different portions of the *Dv* chromatin and had awned spikes, it was deduced the awn-promotion genes should have been scattered in different V chromosomes.

The IBLs CSV58, CSV59, and CS × V60 expressed the early heading phenotype of *Dv* but no GISH detectable *Dv* chromatin, perhaps because a very small segment was incorporated in the hexaplaid background [66]. However, amplicons specific for *Vrn* genes in *Dv* have also been evidenced in those IBLs suggesting that cryptic introgressions of *Dv* chromatin has occurred in those lines [82]. The pair of satellite *Dv* chromosomes, was structurally similar to the satellite chromosome pair of the A and B genome indicating a closer ancestry of V genome to the A and B genomes. The V chromosomes showed only slight structural similarity with those of the D genome of wheat. From those observation it was inferred that *D. villosum* belongs to the same phyletic lineage of *T. urartu* (donor of the A genome of wheat) and *Aegilops speltoides* (B genome), and that *Ae. squarrosa* (D genome) diverged earlier.

Research period: 2006 to 2010

The research carried out in this period was organized according to the outlines on which the project FRUMIGEN was based, thanks to the financial support given by MiPAAF (Ministero delle Politiche Agricole Alimentari e Forestali or Ministry for Agriculture, Food and Forestry Policy).

The assumptions behind the project objectives and the main results were illustrated at the 2nd Int. Symp. on Genomics of Plant Genetic Resources, held in Bologna 24-27 April 2010 [84], and are summarized henceforth.

The rate of yield increase for the global cereal grain production should be maintained at about 1% every year in order to meet predicted demands for the next forty years. Cereal breeding may occur through advanced mutagenesis, interspecific introgression, and genetic engineering methods. These approaches could provide varieties expressing trait-enhancing genes and adaptations suitable for yield progress, especially when supported by genomic (MAS) and precision phenotyping selection tools. Resilience of gene expression is a requirement when the selected varieties will experience year-to-year fluctuating climatic conditions and implementation of sustainable use of environmental resources in farming systems. Many dominant genes for adaptation have been lost during cereal crop domestication, but they have been retained in the genomes of the wild components of the Triticeae gene-pools. In natural habitats, wild Triticeae species such as *Dv,* whose genome was exposed to over hundred million years of climatic and environmental changes, are now expressing adaptations to the latest climatic pattern and as consequences the new ecotypes are expressing increased earliness in time of flowering, high density stands, and large plant biomass [85]. These new ecotypes need to be preserved and studied [92]. Deploying whole and dissected *Dv* nuclear genome in the homoeologous wheat genetic background through interspecific hybridization and introgression, is a lower-cost and effective option to help wheat breeders to select the proper germplasm to sustain the needed yearly grain-yield increase. As we have seen in the previous paragraphs, several hexaploid amphiploids have been produced by combining the *Dv* and *T. turgidum* var *durum* nuclear genomes. These plants display the typical adaptive traits of *Dv* such as: (i) high resistance to diseases affecting caryopses (such as smut caused by *Tilletia tritici)*, leaves and stems such as those initiated by air-borne spore inoculum of *Blumeria graminis* f. sp. *tritici* (*Bgt*) *(*causing powdery mildew), *Puccinia triticina* (*Pt*) (causing leaf or brown rust), *P. graminis* f. sp. *tritici* (*Pgt*) (causing stem or black rust), and *P. striiformis* (*Ps*) (causing stripe or yellow rust); (ii) fortified caryopses (high protein and micronutrient contents); (iv) heading earliness, low genotype-by-environment (G × E) interaction, good grain yield potential; and (v) promising water and nitrogen-use efficiency. The dissection of the *Dv* genome by '(CS × *Dv*) × CS' hybridization and backcrossing, has provided IBLs showing one or more of the *Dv* adaptive traits [85]. The multi-environment testing of the amphiploids and IBLs, is the way to gauge the agronomical and technological features of those materials to become

future registered varieties or parental lines to transfer adaptive traits to other wheat commercial varieties. The expression in some IBLs of genes for resistance to biotic stresses, for improved nutritional value and end-use quality, are the main benefits expected from *Dv* chromatin introgressions into wheat].

The IBLs, especially the CS × V60, showed good stability and phenotypic expression of ear emergence earliness in those environmental conditions in which CS and other control lines showed late ear emergence and significant G × E interaction. The CS × V60 is, therefore, of particular significance for breeding new resilient varieties for the organic aricultura systems, where the currently used varietie express high G × E interaction. Yield stability becomes an important criterion for vaiety acceptance by the organic farmers [68, 69, 71]. Genetic innovations which may enable stability inthe phenotypic expression of yield related traits, may be provided by the wild gene-pools adapted to million of years of environmental changes especially under conditions of reduced chemical inputs (fertilizers, pesticides, etc.). The introgression of genes for resistance to pathogens and good water and nitrogen use efficiency in terms of protein concentration and quality for bread-making will be less demanding in soil fertility and cultural practices, and more productive per unit area, thereby reducing the cost of cultivation, the impact of agricultural activities on the environment, and demand for new fertile lands to cultivate. The preparation of segregating progeny for novel genes after introgression of the wild *Dv* gene pool into wheat has the potential to fulfill the criteria of the new genotypes for sustainable agriculture as depicted in the FRUMIGEM project [68, 70]. The working units (WU) involved in the project were: *WU VT*, Department of Agrobiology and Agrochemistry, University of Tuscia, Viterbo (Staff involved: C. De Pace, D. Vittori, A. Ciofo, M. Bizzarri). *WU CRA-SCV* (Research unit for the selection of cereals and the development of plant varieties, S. Angelo Lodigiano, LO; Staff involved: G. Boggini (1st year only), J. Bianchi (1st year only), M. Corbellini, P. Vaccino, R. Banfi, T. Coppa) and *CRA-QCE* (Research unit for the grain quality of cereals, Rome; Staff involved: M. Pasquini, A. Matere, F. Nocera, L. Sereni). *WU PG*, Department of Cellular and Environmental Biology, University of Perugia (Staff involved: P.G. Cionini, M. Ceccarelli, M.E. Caceres, V. Sarri, E. Polizzi).

The explored research topics were the following:

<u>Research topic 1</u> - *Evaluation of the agronomical and phenotypical stability features of the IBLs with 'new' genes or chromatin transferred from Dv.*

The studied target genes were considered 'new' because they were transferred from accessions of *D. villosum*, *T. kicharae*, and *T. timopheevi* were used before for gene introgression into wheat genomes. The WU CRA-SCV provided the following materials: 26 hexaploid lines derived from crossing *T. aestivum* × *T. kicharae*, 9 lines derived from crossing *T. aestivum* × *T. timopheevii*, and 19 lines derived from crossing *T. durum* × *Ae. squarrosa*.

The WU VT contributed the following materials:

(A) 10 IBLs from [('M × V') × CS] including 'VT-8-1', 'VT-41-03', and 'VT-Mut 3-04'. The control varieties were Bologna and Blasco.

(B) A set of 11 S_{12} IBLs selected from a population of 150 aneuploid lines obtained through the ('CS' × *Dv*, F_1) × CS breeding scheme inplemented in 1986. The S_{12} breeding lines traced to the same S_4 plant were considered 'sister lines'. Genetic uniformity within lines and differentiation among lines have been tested using AFLP (*Amplified Fragment Length Polymorphism*) and GISH analyses [74, 81]. On average sister lines differed for 7% of AFLP fragments and nonsister lines differed for >20% of AFLP fragments. The pairs of IBLs that were thorougly investigated were: CS × V32a and CS × V32b; CS × V58a and CS × V58b; CS × V59a and CS × V59b; CS × V60a and CS × V60b; CS × V63a and CS × V63b; and CS 1BL-1VS (a single line).

(C) Two hexaploid amphiploid ('M × V' non-brittle rachis, and 'Mut 7-04') and two lines selected for large grain-size from the amphiploids with tough glumes ('Mut-12' and 'Mut-16') to be evaluated as new types of 'farro' compared with conventional small-diploid (*T. monococcum*), medium-tetraploid (*T. dicocoides*) and large-hexaploid (*T. spelta*) 'farro'.

The materials (A), (B) and (C) were analyzed by WU CRA-SCV using AFLP method for 'DNA typing', and by WU PG using 'chromosome painting' for chromosomal identification and chromosome counting.

The preliminary agronomical analysis was carried-out using IBL CS × V58, CS × V59, CS × V60 to evaluate their heading earliness as response to vernalization temperatures in replicated plots prepared in the experimental fields of WU CRA-SCV and WU VT. The materials were sown in three periods which shared similar duration of the photoperiod but were different for the average temperature during the tillering phase that preceded the differentiation of the ear. The three sowing dates were: (1) autumn (November) to have the tillering stage in February-March when the photoperiod was increasing to 12 hours; (2) early March to have tillering to March-early April when the length of the day was just over 12 hours; and (3) late August to have the tillering phase in September when the length of the day was just over 12 hours. It was expected that the lines insensitive to thermoperiod would be heading at least 7 days earlier when the autumn sowing was performed and tillering occurred during the cold months of January and February, or 40 days earlier when sowing was done in March and August and tillering occurred in the months with average temperatures above 12 °C, compared to the cold-sensitive controls. The observed results conformed to those expected [83]. During the third year (2008) and subsequent tests (due to the extension of six months of the project, until June 30, 2009), replicated agronomic trials were conducted for the evaluation of the materials chosen in the first and second years.

The phenotypic stability of the IBLs was evaluated in terms of the amount of their G × E interaction because that imposes additional efforts for selection of supe-

rior breeding lines. An understanding of the type of G × E interaction is important in all stages of plant breeding especially for pursuing sustainable low-input agriculture. Crop production in low-input agriculture requires cultivated varieties showing resilience. The most important G × E interaction patterns in breeding materials can be divided in two categories: one of low genotypic variance, significant crossover interaction and genotypes endowed with environmental specialization, and the other that leads few genotypes to perform better than others in several environments ('universal' genotype) [71]. This type of G × E interaction was assessed in IBLs.

In a uniform (greenhouse) environment, three pairs of sister lines (CS × V58a and CS × V58b, CS × V59a and CS × V59b, and CS × V60a and CS × V60b) initiate anthesis about 10 days earlier than CS and had 2n = 42 chromosomes; two pairs of sister lines (CS × V32a and CS × V32b and CS × V63a and CS × V63b) are immune to powdery mildew and have the additional comosome 6V from *Dv*; and one line (CS 1BL/1VS) showed high gluten strength. The 11 IBLs and CS have been tested in 5 different environments: 3 years in one locality as representative of unpredictable environmental variation features, and one additional year in two localities to include features of predictable environmental variation. In each cropping environment, off-farm inputs were minimized [69].

The main G × E interaction pattern displayed by the breeding lines is of the category 'higher proportion of genotypic variance compared to the G × E interaction variance component' [71]. Multiplicative model and biplot analyses invariably grouped similar genotypes (sister lines) in the same cluster and indicated the line CS × V60 as the 'ideal breeding line' in terms of genotypic main effects and stability for yield in the tested environments. Good stability has been detected for time of anthesis and gluten strength. The observed results are compatible with the hypothesis that the main genotypic effects observed over environments are traceable to the direct and indirect effects of chromatin from *Dv*. These lines are ideal starting points for studying individual QTLs with a pretested environmental stability and for transferring those QTLs to commercial varieties.

Research topic 2 - Assessment of the resistance to the causal agents of foliar wheat diseases conferred by Dv genes in IBLs.

To assess the incidence of major foliar diseases (powdery mildew, rusts, leaf blotch, tan spot) in the field, the IBLs with chromosome 6V were included in the national epidemiological nurseries to evaluate their response to the foliar pathogens in different environments and enforce their assessment as useful breeding materials to constitute new wheat varieties endowed with durable resistance to foliar diseses [88, 89].

Crop varieties derived from IBLs with durable genetic resistance to major pests and diseases may reduce the use of fungicides and input resources. Therefore, in a DPSIR (Driving forces-Pressure-State-Impact-Response) conceptual frame-

work, the number of genes controlling the productivity resilience of stress resistant crop varieties become a reliable driving force indicator of the reduction of disease severity symptoms due to genetic field resistance, which in turn is linked to a reduced pressure of agriculture on environmental resources. Evidence was provided that indicators of the driving forces promoting sustainability can be standardized from genetic information on: (a) *Triticum aestivum* (bread wheat) response to powdery mildew caused by *Bgt*, and (b) *T. turgidum* var. *durum* (durum wheat) field response to brown rust disease caused by *Pt* [68]. Alien gene transfer from the wild species (such as *Dv*) included in wheat secondary gene-pool (or GP-2) to replenish the primary wheat gene-pool (or GP-1) with disease resistance genes to oncoming virulent fungal pathogen strains, is the most effective way to overcome the narrow genetic basis of wheat due its domestication bottleneck and long selection pressure for yield performance over the centuries [85].

Powdery mildew is an important wheat disease that cause grain yield losses in Europe and in other areas where wheat is grown under cool, temperate conditions. The use of resistance genes is the most effective way to combat the spread of this disease. One such gene was reported to be located at locus *Pm21* on the short arm of chromosome 6V introgressed in *T. aestivum* from *Dv* [67, 72, 78, 88, 89]. Previous attempts to study the genetic basis of the resistance were unsuccessful and prevented the preparation of a genetic map of 6V and identification of molecular markers closely linked to the *Pm21* locus. Only a deletion map for four groups of RFLP markers and one SCAR marker were available for 6V#2. The CS×V63 disomic addition (DA) line of chromosome 6V#4 introgressed into the CS chromosome complement from a *Dv* population collected in Lazio-Italy showed immunity to powdery mildew. This line has been crossed to the susceptible CS-DA6V#1 obtained by E.R. Sears and an F_2 population has been produced for assessing the genetic basis of the immunity to powdery mildew and preparing materials (F_3 lines) to be used in conventional genetic mapping procedures to locate and mark the *Pm21* locus [67, 72]. The DA state for 6V was checked in both parents and randomly selected F_2 seedlings using GISH. The caryopses from parents and individual F_1 plants (kernels with F_2) embryo were cut in half and the embryonic side was used to obtain the two-leaf seedlings that were infected with a powdery mildew isolate in the greenhouse, while the endosperm end was used for seed storage protein electrophoretic analyses. Three rounds of infections on seedlings from different subsamples of about 120 F_2 caryopses were performed to test segregation for powdery mildew resistance. The level of powdery mildew symptom expression was estimated visually by examining the number and size of mycelia spots using a 0-to-4 scale, 0 = no mycelia, 4 = dense and large mycelia spots. All F_2 plants were transplanted to produce $F_{2:3}$ seed (F_2-derived F_3 lines) for progeny testing and molecular genotyping. Segregation for resistance to powdery mildew fit a 3:1 monogenic dominant inheritance pattern [67, 72]. The segregation was assumed occurring at the *PmVt* locus (most likely homologous to the *Pm21* locus) and the resistance

allele on 6V#4 was designated *PmVt-R* and the susceptible allele on 6V#1 as *PmVt-S* [78]. Segregation for blocks of gliadin protein subunits encoded at the *Gli-V2* locus on 6VS segregated according to a 1:2:1 monogenic codominant inheritance pattern. Co-segregation of the phenotypes determined by the genotypes at the *PmVt* and *Gli-V2* loci [88] indicated a map distance of 14 cM between them, giving the first information on the genetic map for the region surrounding the *PmVt* locus and confirming their location on 6VS [72]. Molecular analyses using both the marker $OPH17_{1900}$ and *NAU/Xibao15902* (reported as linked to *Pm21*) were carried out to confirm the location of *'PmVt'* on the 6VS. In our 6V materials, $OPH17_{1900}$ was not linked to *PmVt* [72]. $NAU/Xibao15_{902}$ was detected by PCR in both parental lines, amplicons displayed the same molecular weight but with significant difference in band intensity between the two parental lines, the CS-DA6V#1 expressing the fainter band. $NAU/Xibao15_{902}$ primers targeted a DNA sequence encoding for a serine-threonine kinase enzyme that might be involved in the resistance response [78]. The amplified $NAU/Xibao15_{902}$ DNA fragments showed differences in the nucleotide sequences at the exons 3 and 4 which include the regions complementary to the primers. A nucleotide mutation in one of the primer pairing sites in CS-DA6V#1 might explain the fainter band [80].

Puccinia triticina, the fungal pathogen causing leaf rust (LR) of wheat, occurs throughout the wheat growing regions of the world and the resistance to this disease represents a strategic aspect of many wheat breeding programmes. Adult plant resistance (APR) to leaf rust has been recognized as a major component of durable rust resistance. The disomic addition lines 'CS6V' (CS-DA6V#1; 2n = 44) and 'CS × V63' (CS-DA6V#4, 2n = 44) and the disomic substitution line 'CS × V32' (CS-DS6V#4, 2n = 42; in this line a pair of chromosome 6V#4 substituted the 6B chromosomes as evidenced by Vaccino *et al.*, 2010a [91]), expressing different introgression events of chromosome 6V of *Dv* in the genome of the bread wheat cultivar CS, showed susceptibility to several selected pathotypes of LR when inoculated at the seedling plant stage. When controlled inoculations on the flag-leaf lamina of these lines were performed with a mixture of leaf rust strains, the CS-DA6V#4 and CS-DS6V#4 lines evidenced strong APR (0 and 0-10 pustules respectively), while CS resulted highly susceptible (40-80 pustules per flag-leaf lamina). The APR of CS-DA6V#4 and the susceptibility of CS with respect to natural LR infections were confirmed in multilocation epidemiological trials carried out in Italy during 2007 and 2008 (National Phytopathological Surveys [88]). The disomic addition line (DA) 'CS × V63' was chromosomally stable while the disomic substitution line (DS) 'CS × V32', showed chromosomal number instability. Two cycles of selection within the 'CS × V32' disomic substitution line allowed the development of two sister lines. After genomic *in situ* hybridization, it was evidenced that one of the sister line was a monosomic substitution line for chromosome 6V#4 (CS-MS6V#4; 2n = 41); this line (CS × V32-R) displayed APR to LR in the field in Italy (Rome and Viterbo) and at the Agricultural Research Institute, Martonvásár, Hungary (ARI-

MH), when exposed to natural pathogen infections. In the same fields, the second sister line, lacking the 6V#4 chromosome, resulted susceptible to LR (CS×V32-S) [78, 88, 89]. These observations confirm the hypothesis that 6V#4 chromosome carries gene/s controlling APR to LR [76] in addition to resistance to *Bgt*. The genetic map distance between *Gli-V2* and the locus encoding genetic resistance to LR was 70 cM, suggesting that the LR resistance gene was in 6VL. The genetic basis of APR to LR conferred by the 6V#4 chromosome, was studied in the $F_{2:3}$ progenies grown in Viterbo, derived from the cross between the DA6V#4 and DA6V#1 lines. Symptoms caused by air-borne LR infections in the field were scored by counting the number of uredia on the flag leaf and the leaf below it for each plant. DA6V#4 showed an average of 4.4 small uredia (min. 0; max 20; StEr 6.43) and was considered resistant (R); DA6V#1 evidenced an average of 80 uredia (min. 30; St Er. 30.9) and was considered susceptible (S). About 55% of the $F_{2:3}$ progenies showed no more that 20 uredia on the upper leaves and about 25% of the $F_{2:3}$ progenies showed a significant higher number (>50) of pustules. In total 236 F_3 plants were scored, 150 of which showed less than 20 pustules per plant (R) and 86 expressed over 20 and up to 350 pustules per plant (S); CS showed an average of 94,7 pustules. The null hypothesis of 10R:6S ratio for the $F_{2:3}$ plants was not rejected when chi-square test was adopted; that ratio was compatible with a 3R:1S segregation ratio among the F_2 mother-plants from which the $F_{2:3}$ progenies were derived [76]. Further analyses are in progress in order to confirm the hypothesis that the adult plant resistance to *Pt* surveyed in the 6V#4-introgression lines could be controlled by a single resistance gene that is different from those already present in CS (*Lr12*, *Lr34*). Resistance to *Pgt* was observed in CS-DA6V#4 line by controlled infection at the seedling stage at CRA-QCE. Therefore 6V#4 is extremely important to deploy genes for multiple resistance to new virulent races of fungal pathogens in wheat germplasm. Therefore 6V#4 seems to be a very interesting source of genes for resistance to wheat powdery mildew, leaf rust, and stem rust. It is expected that the resistance to *Pgt* expressed by gene/s on 6V#4, will be similar to that expressed against many North American *Pgt* races and TTKSK (Ug99) by a gene on the 6V introgressed (by researcher in China) in wheat from a different *Dv* ecotype [80, 85].

Research topic 3 - *Assessment of the end-use grain quality of the IBLs.*

The IBLs obtained from '(CS×*Dv*, F_1)×CS' backcross were evaluated for agronomic qualitative, and biochemical traits (gliadins and glutenins). The results were tested for correlations with the flour quality performance, which was assessed by both small-scale and large-scale analyses. Prolamin genes from *Dv* and wheat were coexpressed in the IBLs and differentially affected bread-making quality.

In particular, the kernels produced in the agronomic trials have been subjected to physical and chemical analyses (test weight, moisture and protein content, kernel

hardness), technological tests (SDS sedimentation volume, gluten index, alveograph and farinograph dough test), biochemical analysis of storage proteins and puroindoline, and bread-making (leavened bread volume and height).

The ascertained effects did not depend on the size of the introgressed *Dv* chromatin and were not confounded by the cryptic variation of the genetic background. The analyses of gliadins and glutenins pattern of the IBLs and CS allowed the identification of the blocks of prolamins encoded at the homoeologous *Gli-V1*, *Gli-V2*, *Glu-V1* and *Glu-V3* loci of *Dv* introgressed in the IBLs, and to establish their effect on the bread-making properties of the flour prepared from the IBLs caryopses [91, 92].

The high protein content (>17%) of lines CS×V32 and CS×V63 was also associated with a higher content of Fe and Zn in the grain compared to CS, and the presence in these lines of chromosome 6V. A similar association between high protein and kernel micronutrient content was attributed to the pleiotropic effects of the *Gpc-B1* gene in chromosome 6B of *Triticum dicoccoides* introgressed into the *durum* wheat cultivar Langdon[12]. Therefore it was suggested that in 6V#4 there was a gene, named *Gpc-V1*, homoeologous to *Gpc-B1*.

The *Dv* chromatin introgressed in the IBL 'CS1BL-1VS', included genes at the *Glu-V1* locus encoding for a high Molecular weight glutenin subunit (labeled '1v' in Fig. S3 of Vaccino *et al*., 2010a [91]), which significantly improved the flour bread-making quality of that IBL (Fig. 5 and Tab. 2 in Vaccino *et al*., 2010a [91]). As matter of fact, an isogenic line of 'CS 1BL-1VS' selected after backcrossing of 'CS 1BL-1VS' to CS, restored the 1B chromosome in place of 1BL-1VS chromosome, and its bread-making quality values were not significantly different from those of CS (see Fig. 3 in Vaccino *et al*., 2010a [91]). The *Gli-D1g*-like mutation at locus *Gli-D1* in chromosome 1D of CS, occurred right after the CS×*Dv* hybridization in 1986, was transferred in the IBL 'CS×V32', and conferred a strong enhancement of the bread-making quality of the flour from that line compared to CS [91].

These positive effects allow breeders to consider *Dv* as an important direct and indirect source of genes for grain quality improvement in wheat, also in conjunction with the introgression of *Dv* chromatin into wheat for enhancing expression of other traits such as disease resistance.

The IBLs 'Mut 12' and 'Mut 16', derived from whole V-genome transfer into durum wheat genome, produced grains with >20% grain size compared to 'M×V' and were considered promising lines for enlarging the 'farro' germplasm resources available to farmers adopting sustainable agricultural systems.

[12] Joppa L.R., C. Du, G.E. Hart, and G.A. Hareland, 1997. Mapping gene(s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. *Crop Sci*. 37: 1586-1589; Uauy C., A. Distelfeld, T. Fahima, A. Blechl, and J. Dubcovsky, 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314: 1298-1301.

Research topic 4 - Assessment of the molecular and cytogenetical features of the IBLs compared to the wheat and Dv parental species.

GISH and molecular analysis of the nuclear genome of the IBLs, has allowed: (i) the detailed description of the cytogenetic events that occurred during the selection of these lines [74, 81], (ii) the identification of the chromosomes of *Dv* and wheat genomes involved in those events, and (iii) the 'DNA typing' methods for the recognition of the occurred cytogenetical events. For example, it has been possible, by the GISH method to examine the progenies of the 'CS × V58', 'CS × V59', and 'CS × V60' IBLs obtained in consecutive selfing generations, to detect in earlier selfing generation, the presence in 'CS × V58' of an acrocentric chromosome, probably derived by centromeric breackage of a V chromosome. That piece of chromatin was lost two selfing generations later, and the lines maintained a stable 42 chromosome number and absence of GISH-detectable V chromatin. However, other genetic markers such as the presence of awns on the lemma, the heading earliness and the additional AFLP bands compared to 'CS', pointed to the occurrence of cryptic V chromatin introgressions in those lines [73, 82]. As matter of fact, further molecular analyses revealed that in CS × V58, CS × V59 and CS × V60 had occurred also the transfer of nucleotide regions of two different *Dv* promoters homologous to the promoters of the *Vrn-A1* and *Vrn-B3* genes of wheat. These lines are now considered fundamental to the development of early heading wheat cultivars with high productivity because they will be able to fill and mature grains before water stress effects occur due to reduced rainfall in late spring.

The CS × V58, CS × V59 and CS × V60 IBLs when compared to CS showed differences for about 16% of the 166 AFLP fragments detected in replicated runs of amplification and gel electrophoresis [73]. This indicated that substantial cryptic chromosome mutations or recombinations or gene mutations have occurred during the earlier generations following the hybridization event and that these rearrangements were transmitted to the plants from which the IBLs derived. One of these mutations might have affected a flowering-promoting gene at a locus different from *Vrn-1*, causing (under nonvernalizing condition and 13-14-hour daylength) the IBLs, Salgemma × CS × V59 and Isengrain × CS × V59 F_1 plants to start anthesis in less than 50 days from sowing compared to 'CS' and to the two winter bread wheat cultivars Salgemma and Isengrain, which flowered about 150 days later [73, 86].

Flow karyotyping and sorting of *Dv* chromosomes can promote the physical mapping and cloning of useful *Dv* genes and their direct transfer into the wheat genome. Grosso *et al.* (2009) [72] started attempts to obtain the first flow karyotyping and chromosome sorting in *Dv*, and developed a complete system for cell cycle synchronization and chromosome isolation in suspension from fixed root tips. Theoretical flow karyotype modeling obtained from measured chromosome metaphases was challenged to real flow karyotypes obtained from chromosomes in suspension isolated from double synchronized root tip cultures.

It was shown that only the large V chromosome forms a single peak while all the other V chromosomes group in two large peaks. In order to assign the chromosomes content of each peak FISH (*Fluorescent In Situ Hybridization*) was applied using *Dv* specific sequence (pHv62) and with large repetitive sequences (GAA_5 and pSc119.2) on metaphase and sorted chromosomes to compare FISH discrimination capabilities [87]. The preliminary results showed that $(GAA)_5$ is the probe with stronger and most reproducible signal pattern.

Research topic 5 - Preparation of breeding materials combining the useful Dv genes of the IBLs with the elite genetic background of the current bread wheat commercial varieties.

The most challenging key questions for the development of new varieties for low input farming systems relate to both the genetic resources to be used for selecting new genotypes and the selection criteria suitable to predict the future performance and stability of those genotypes. Conventional wheat breeding is now limited by its exhausted narrow gene pool and reshuffling the same genetic variation slows the pace of the improvement rate. The likely outcome from wheat breeding using intraspecific genetic variation and quantitative traits as selection criteria, is one of small increase in genetic progress despite considerable research investment. Natural biodiversity of the secondary and tertiary gene pools of wheat, can enrich the genetic basis of the cultivated wheat primary gene pool by interspecific gene transfer. Transfer of genes between wheat species may occur by: (i) crossing the tetraploid durum wheat *Triticum turgidum* ssp. *durum* (*Td*) with the hexaploid wheat *T. aestivum* ssp. *aestivum* (*Ta*), 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 for deriving commercial varieties, while the second requires repeated backcrossing to the elite cultivars and several generations of prebreeding. A new breeding scheme has been developed which merges the methodological simplicity of approach (i) with the pedigree complexity of approach (ii) [75, 86]. It is based on the use of *Td*×*Dasypyrum villosum (Dv)* amphiplod (='M×V') in bridge-crossing to *Ta* (CS), for transferring, through recombination, *Td* and *Dv* genes to hexaploid wheat. IBLs that. Eighty percent of the M×V florets fertilized using CS pollen, produced F_1 caryopses and the selfed F_1 plants were partially fertile; about 42% of the F_2 seeds produced completely fertile plants. Root-tip chromosome counts of the resulting F_3 seeds showed 14A, 14B and 14D chromosomes, and no apparent trace of V chromosomes. Hence, *Dv* genome acted as a genetic bridge to transfer genes from tetraploid to hexaploid wheat. After 5 years of selfing and seed multiplication, two lines from the fully fertile F_2 plants display trait enhancement for grain yield, yield stability and grain quality under low-input field trials and were better than the highest yielding check in a four-environment trial [79, 83].

To test the expression of the valuable *Dv* genes in the IBLs when transferred to different wheat genotypes, a biparental mating design between a set of commercial wheat varieties (Blasco, Isengrain, Provinciale, Sagittario and Salgemma) and a set of IBLs (CS × V32, CS × V63, CS × V59, and CS.1BL/1VS) was planned and implemented [86]. Furthermore, hybridizations were also planned between the early heading and disease susceptible IBL CS × V60 and both the late heading and disease resistant IBL CS × V63 and the late heading good bread-making quality IBL CS-1BL/1VS. The purpose common to these last two hybridizations was the selection in the F_2 progenies of those plants that express the combination of the parental useful genes to achieve the 'gene pyramiding' for earliness and resistance to rust and powdery mildew on one side, and heading and good bread-making quality on the other. After the F_2 plants with those gene combinations are available, further crossing between them will be planned to convey in one genotype all the useful genes identified so far in the IBLs. The phenotype of all the F_1 plants from the biparental crosses, indicated that the *Dv* genes for earliness and powdery mildew resistance are dominant in all hybrid combinations. The observed segregation in the F_2 progenies made it possible to confirm the simple inheritance of earliness, the resistance to powdery mildew, and the presence of the '1v' HMW-glutenin subunit encoded at locus *Glu-V1* transferred with the recombinant chromosome 1BL-1Vs transferred by the CS.1BL-1VS parent [86]. Most of the F_3 progenies obtained from the selected F_2 plants containing the desired gene combinations, confirmed the F_2 phenotype.

In synthesis, the results from the FRUMIGEN project were the following:

– Genetics and cytogenetical characterization of the CS × V32 and CS × V63 IBLs carrying the 6V#4 chromosome encoding dominant alleles for resistance to powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*), leaf rust (caused by *Puccinia tritici*) and stem rust (caused by *P. graminis* f. sp. *tritici*), and for increased grain protein concentration and micronutrien content;

– Cytogenetical and technological characterization of the CS 1V/1B and CS × V32 IBLs which carry genes for enhancing end-use grain quality;

– Molecular characterization of the CS × V58, CS × V59 and CS × V60 carrying genes for heading earliness;

– Agronomic evaluation of two selections (Mut 12 and Mut 16) from the 'M × V' hexaploid amphiploid to enlarge the genetic resources of 'farro' for farmers practicing sustainable agricultural systems;

– Selection and agronomic evaluation of the IBLs VT 8-1, VT 41-03 and VT Mut 3-04, for the eligibility to be included in the National Register of the new wheat commercial varieties;

– Preparation of breeding materials combining the useful *Dv* genes of the IBLs with the elite genetic background of the current bread wheat commercial varieties.

Saluto di benvenuto prima dell'avvio dei lavori del Convegno. Da sinistra: Dr. G. Vida (Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary), Prof. De Pace (Università degli Studi della Tuscia, Viterbo), Prof. G.T. Scarascia Mugnozza (Presidente dell'Accademia Nazionale delle Scienze detta dei XL, Roma), e Prof. C.O. Qualset (University of California, Davis, CA, USA).

Pubblicazioni che hanno consentito di divulgare i risultati illustrati nella Prefazione

Publications cited in the Foreword

1981

[1] Qualset C.O., C. De Pace, C.C. Jan, G.T. Scarascia Mugnozza, O.A. Tanzarella, 1981. *Haynaldia villosa*: a species with potential use in wheat breeding. In: *Proc. of the 1981 annual Meetings of the American Society of Agronomy, CSSA, and SSSA*, Nov. 29 - Dec. 4, 1981, Atlanta, Georgia (U.S.A.), Agronomy Abstracts: 70.

1982

[2] Scarascia Mugnozza G.T., C. De Pace, O.A. Tanzarella, 1982. *Haynaldia villosa* (L.) Schur.: una specie di potenziale valore per il miglioramento genetico del frumento. I. Analisi di alcuni caratteri morfologici. In: *Proc. of the 25th Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, 28-30 May, 1981 Gardone Riviera (BS). Genetica Agraria, 36: 191. (Abstract).

1983

[3] Bisignano V., R. Simeone, A. Blanco, 1983. Analisi cariotipica di *Haynaldia villosa* (L.) Schur. *An. Fac. Agr. Univ. Bari*, 33: 65-70.

[4] Blanco A., R. Simeone, O.A. Tanzarella, B. Greco, 1983. Morphology and chromosome pairing of hybrid between *Triticum durum* Desf. and *Haynaldia villosa* (L.) Schur. *Theor. Appl. Genet*., 64: 333-337.

1984

[5] Blanco A., R. Simeone, C. Orecchia, 1984. Cytology, morphology and fertility of the amphiploid *Triticum durum* Desf. ×*Haynaldia villosa* (L.) Schur. *Proc. 6th Int. Wheat Genet. Symposium*, Kyoto (Japan), 1983: 171-177.

[6] Della Gatta C., O.A. Tanzarella, P. Resta, A. Blanco, 1984. Protein content in a population of *Haynaldia villosa* and electrophoretic pattern of the amphiploid *T. durum*×*H. villosa*. *Proc. Workshop on* '*Breeding methodologies on durum wheat and Triticale*', Viterbo, Nov. 17-19, 1983: 49-54.

1985

[7] De Pace C., C.C. Jan, V. Delre, G.T. Scarascia Mugnozza, C.O. Qualset, L. Montebove, 1985. Morphological and biochemical description of a new hexaploid Tritinaldia (*Triticum durum* cv 'Modoc'×*Dasypyrum villosum*). *Proc. of the 28th Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Bracciano 3-5 ottobre, 1984. *Genetica Agraria*, 39(3): 321. (Abstract).

[8] Montebove L., 1985. *Studio delle caratteristiche genetiche e morfologiche di anfiploidi Triticum*×*Dasypyrum.* Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

1986

[9] Delre V., C. De Pace, G.T. Scarascia Mugnozza, C.O. Qualset, C.C. Jan, L. Montebove, 1986. Genetical basis for some biochemical characters in amphiploids obtained from inter-

generic hybridization *Triticum×Dasypyrum*. *Proc. of the 29th Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Pontecagnano (SA), 9-11 Ottobre 1985. *Genetica Agraria*, 40: 446 (Abstract).

[10] Jan C.C., C. De Pace, P.E. Mcguire, C.O. Qualset, 1986. Hybrids and amphiploids of *Triticum aestivum* (L.) and *T. turgidum* (L.) with *Dasypyrum villosum* (L.) Candargy. *Zeit. für Pflanzenzüchtg*., 96: 97-106.

[11] Blanco A., R. Simeone, 1986. Isolation of *durum* wheat - *Haynaldia villosa* chromosome addition lines. *EWAC Newsletter*: 22-23.

1987

[12] Blanco A., R. Simeone, P. Resta, 1987. The addition of *Dasypyrum villosum* (L.) Candargy chromosome to *durum* wheat (*Triticum durum* Desf.). *Theor. Appl. Genet.*, 74: 328-333.

[13] De Pace C., 1987. *Genetic variability in natural popolations of Dasypyrum villosum* (L.) *Candargy.* Ph. D. Dissertation, University of California, Davis, California, U.S.A.

[14] Mini P., 1987. Ottenimento di nuovi anfiploidi CS×V (*Triticum aestivum* cv CS×*Dasypyrum villosum*, AABBDDVV, 2n=8x=56) e studio della diversità genetica di linee derivate da CS×V81. Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

[15] Montebove L., C. De Pace, C.C. Jan, G.T. Scarascia Mugnozza, C.O. Qualset, 1987. Chromosomal location of isozyme and seed storage protein genes in *Dasypyrum villosum* (L.) Candargy. *Theor. Appl. Genet.*, 73: 836-845.

[16] Resta P., D. Lafiandra, A. Blanco, 1987. *Dasypyrum villosum* (L.) Candargy chromosomes affecting *durum* wheat proteins. *Proc. 3rd Int. Workshop on Gluten Proteins*, Budapest, May 6-9, 1987: 299-311.

1988

[17] Blanco A., V. Perrone, P. Resta, R. Simeone, M. Urbano, 1988a. The incorporation of powdery mildew resistance from *Dasypyrum villosum* (L.) Candargy to *durum* wheat. *Genetica Agraria* 42: 62.

[18] Blanco A., V. Perrone, R. Simeone, 1988b. Chromosome pairing variation in *durum* wheat ×*Dasypyrum villosum* (L.) Candargy hybrids and genome affinities. *Proc. 7th Int. Wheat Genet. Symp.*, Cambridge (England): 63-67.

[19] Blanco A., R. Simeone, 1988. Use of *Dasypyrum villosum* (L.) Candargy in durum wheat improvement. *Proc. of the 3rd Int. Symp. on durum wheat:* '*The future of cereals for human feeding and development of biotechnological research*' (G. Wittmer, ed.). Chanber of Commerce of Foggia, Italy. pp. 215-228.

[20] Delre V., C. De Pace, F. Maggini, G.T. Scarascia Mugnozza, C.O. Qualset, 1988. Ribosomal DNA spacer length polymorphism in *Dasypyrum villosum* populations. In: '*Population Genetics and Germplasm Resources in Crop Improvement*', an Int. Symposium held at Davis, California, U.S.A., August 11-13, 1988. Report No. 3, Genet. Res. Cons. Progr., Univ. California, Davis, CA, USA. 45.

[21] De Pace C., S. Benedettelli, C.O. Qualset, G.E. Hart, G.T. Scarascia Mugnozza, V. Delre, D. Vittori, 1988a. Biochemical markers in *Triticum×Dasypyrum* amphiploids and derived disomic addition lines. In: '*Proc. 7th Int. Wheat Genetics Symposium*', (T.E. Miller and R.M.D. Koebner, eds.), held in Cambridge, England, 13-19 July 1988. Inst. Plant Sci. Res., Cambridge Lab., Trumpington, Cambridge CB22JB England. 503-509.

[22] De Pace C., L. Montebove, V. Delre, C.C. Jan, C.O. Qualset, G.T. Scarascia Mugnozza, 1988b. Biochemical versatility of amphiploids derived from crossing *Dasypyrum villosum* Candargy and wheat: genetic control and phenotypical aspects. Theor. Appl. Gen., 76: 513-529.

[23] De Pace C., C.O. Qualset, G.T. Scarascia Mugnozza, V. Delre, D. Vittori, 1988c. Isozyme and seed storage protein polymorphisms in *Dasypyrum villosum* populations. In: '*Population*

Genetics and Germplasm Resources in Crop Improvement', an Int. Symposium held at Davis, California, U.S.A., August 11-13, 1988. Report No. 3, Genet. Res. Cons. Progr., Univ. California, Davis, CA, USA. 45.

[24] Iapichino G., 1988. *Studio del polimorfismo fenotipico e genetico di popolazioni di Dasypyrum villosum* (L.) *Candargy*. Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

[25] Mini P., C. De Pace, G.T. Scarascia Mugnozza, V. Delre, D. Vittori, 1988. Morphological and isozyme divergence in CS × V amphiploids (*Triticum aestivum* cv Chinese Spring × *Dasypyrum villosum*, AABBDDVV, 2n = 8x = 56) and derived lines from CS × V. *Proc. of the 31st Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Como 30/9-2/10, 1987. *Genetica Agraria*, 42: 84-85.

[26] Montebove L., C. De Pace, 1988. Comparative analysis at the morphological, biochemical and genetic level to develop a strategy for studying biological similarities in *Triticinae*. In '*Semi-dwarf cereal mutants and their use in cross-breeding III*'. IAEA, Vienna. IAEA-TECDOC-455. pp. 219-229.

[27] Urbano M., P. Resta, S. Benedettelli, A. Blanco, 1988. A *Dasypyrum villosum* (L.) Candargy chromosome related to the homoeologous group 3 of wheat. *Proc. 7th Int. Wheat Genet. Symp.*, Cambridge (England): 169-173.

1989

[28] Vitti C., 1989. *Studio delle basi genetiche e dei meccanismi di resistenza a Erysiphe graminis in anfiploidi Triticum × Dasypyrum e specie parentali.* Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

1990

[29] De Pace C., R. Paolini, G.T. Scarascia Mugnozza, C.O. Qualset, V. Delre, 1990. Evaluation and utilization of *Dasypyrum villosum* as a genetic resource for wheat improvement. In: *Wheat genetic resources: meeting diverse needs* (J.P. Srivastava and A.B. Damania, eds.). John Wiley & Sons. 279-289.

[30] Ricciardi L., G. Fanizza, A. Blanco, 1990. Influenza dello stress idrico sull'apparato vegetativo e radicale di genotipi di frumento duro (*Triticum durum* Desf.) e *Dasypyrum villosum* L. Candargy. *Proc. of the 34th Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Lecce. Abstract 57, p. 132.

[31] Simeone R., A. Blanco, M.D. Gale, 1990. Uso di RFLP nell'identificazione di linee di addizione interspecifica di frumento. *Proc. of the 34th Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Lecce. Abstract 82, p. 154.

[32] Zhong G.Y., C.O. Qualset, 1990. An alternative method for assigning genes to specific chromosomes of *Dasypyrum villosum* (L.) Candargy. In: Kimber G. (ed.) Proc. 2nd Int. Symp. on Chromosome Engineering in Plants. Univ. Extn., Univ. of Missouri-Columbia, pp. 302-30.

1991

[33] Blanco A., M. Antonacci, P. Resta, M.C. Simonetti, R. Simeone, 1991. Karyotype and genome analyses of *Dasypyrum hordeaceum* (Cos. et Dur.) Candargy. *EWAC Newsletter*: 63-65.

[34] Blanco A., P. Resta, R. Simeone, S. Parmar, P.R. Shewry, P. Sabelli, D. Lafiandra, 1991. Chromosomal location of seeds storage protein genes in the genome of *Dasypvrum villosum* (L.) Candargy. *Theor. Appl. Genet.* 82: 358-362.

[35] Frediani M., R. Cremonini, C. De Pace, V. Delre, G.T. Scarascia Mugnozza, P.G. Cionini, 1991. Localizzazione cromosomica di una sequenza di DNA ripetuta di *Dasypyrum villosum* e sua instabilità quantitativa nel genoma. (Comunicazione presentata al 86° Congresso Sociale S.B.I.). *Giornale Botanico Italiano*, 125(3): 212.

1992

[36] De Pace C., V. Delre, G.T. Scarascia Mugnozza, C.O. Qualset, R. Cremonini, M. Frediani, P.G. Cionini, 1992. Molecular and chromosomal characterization of repeated and single-copy DNA sequences in the genome of *Dasypyrum villosum*. *Hereditas*, 116: 55-65.

1993

[37] Antonacci M., M. Urbano, A. Blanco, 1993. Isolamento e caratterizzazione di una linea di addizione monosomica *T. turgidum* (L.) - *Dasypyrum villosum* (L.). *Ann. Fac. Agraria, Univ. Bari*, vol. 34: 95-99.

[38] Blanco A., R. Simeone, 1993. Chromosome pairing in hybrids and amphyploids between *durum* wheat and the tetraploid *Dasypyrum hordeaceum*. *Proc. 8th Int. Wheat Genet. Symp.*, Beijing (China), pp. 305-309.

[39] De Gara L., C. Paciolla, R. Liso, A. Stefani, A. Blanco, O. Arrigoni, 1993. Ascorbate metabolism in mature pollen grains of *Dasypyrum villosum* (L.) Borb. during imbibition. *J. Plant Physiology*, 141: 405-409.

[40] Qualset C.O., G.-Y. Zhong, C. De Pace, P.E. Mcguire, 1993. Population biology and evaluation of genetic resources of *Dasypyrum villosum*. In: *Biodiversity and wheat improvement* (A.B. Damania, ed.). John Wiley & Sons, Chichester. 227-233.

[41] Simonetti M.C., R. Simeone, A. Blanco, 1993. Analisi genomiva in frumento e *Dasypyrum villosum*. *Proc. of the 37th Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Orvieto. Abstract 61, p. 111.

[42] Zhong G.Y., C.O. Qualset, 1993. Allelic diversity of high-molecular-weight glutenin protein subunits in natural populations of *Dasypyrum villosum* (L.) Candargy. Theor. Appl. Genet. 86: 851-858.

1994

[43] De Pace C., S. Geng, C.O. Qualset, V. Delre, R. Caccia, 1994. Polymorphism of monomeric prolamins in *Dasypyrum villosum* (L.) Candargy. In: *Proceedings of the 2nd Int. Triticeae Symp.* (R.R.-C. Wang, K.B. Jensen, and C. Jussi, eds.), June 20-24, 1994, Logan, Utah, U.S.A. 173-178.

[44] De Pace C., C.O. Qualset, and G.T. Scarascia Mugnozza, 1994. Somatic dimorphism of caryopsis color in *Dasypyrum villosum* (L.) Candargy: some reproductive and ecological relationships. In: *Proceedings of the 2nd Int. Triticeae Symp.* (R.R.-C. Wang, K.B. Jensen, and C. Jussi, eds.), June 20-24, 1994, Logan, Utah, U.S.A. pp. 234-245.

[45] Frediani M., N. Colonna, R. Cremonini, C. De Pace, V. Delre, R. Caccia, P.G. Cionini, 1994. Redundancy modulation of nuclear DNA sequences in *Dasypyrum villosum*. *Theor. Appl. Gen.*, 88: 167-174.

[46] Zuccari D., 1994. *Competizione a livello aereo e radicale fra l'anfiploide 'M×V' (Triticum turgidum var. durum cv Modoc×Dasypyrum villosum Candargy) ed i genotipi parentali.* Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

1995

[47] De Pace C., C.O. Qualset, 1995. Mating system and genetic differentiation in *Dasypyrum villosum* (Poaceae) in Italy. *Pl. Syst. Evol.*, 197: 123-147.

[48] Zhong G.Y., C.O. Qualset, 1995. Quantitative genetic diversity and conservation strategies for an allogamous annual species, *Dasypyrum villosum* (L.) Candargy (Poaceae). Theor.. Appl. Genet. 91: 1064-1073.

1996

[49] Blanco A., R. Simeone, P. Resta, C. De Pace, V. Delre, R. Caccia, G.T. Scarascia Mugnozza, M. Frediani, R. Cremonini, P.G. Cionini, 1996. Genomic relationships between *Dasypyrum villosum* (L.) Candargy and *D. hordeaceum* (Cossonet Durieu) Candargy. Genome, 38: 83-92.

[50] Caceres M.E., C. De Pace, G.T. Scarascia Mugnozza, P. Kotsonis, P.G. Cionini, 1996. Size variations in the nuclear genome of *Dasypyrum villosum*. *Giornale Botanico Italiano*, 130 (4-5-6): 915-918.

[51] Ricciardi L., G. Fanizza, A. Blanco, 1996. Breeding for drought stress tolerance in durum wheat and *Dasypyrum villosum* (L.) Candargy. *Agr. Med.*, 126: 396-404.

1997

[52] Galasso I., A. Blanco, A. Katsiotis, D. Pignone, J.S. Heslop-Harrison, 1997. Genomic organization and philogenetic relationships in the genus *Dasypyrum* analysed by Southern and in situ hybridization of total genomic and cloned DNA. *Chromosoma*, 85: 41-49.

1998

[53] Caceres M.E., C. De Pace, G.T. Scarascia Mugnozza, P. Kotsonis, M. Ceccarelli, P.G. Cionini, 1998. Genome size variations within *Dasypyrum villosum*: correlations with chromosomal traits, environmental factors and plant phenotypic characteristics and behaviour in reproduction. *Theor. Appl. Genet*., 96: 559-567.

[54] Cenci A., D. Snidaro, O.A. Tanzarella, M. Ciaffi, D. Vittori, C.O. Qualset, G.T. Scarascia Mugnozza, C. De Pace, 1998. Assignment of genetic markers to *Dasypyrum villosum* (L.) Candargy chromosomes and their introgression into hexaploid wheat. In: *Proc. of the 9th Int. Wheat Genet. Symp*., Vol. 2, sect. 2: *Germplasm development and breeding* (compiled by A.E. Slinkard). 2-7 August 1998, Saskatoon, Saskatchewan, Canada. pp. 176-178.

[55] De Pace C., D. Snidaro, M. Ciaffi, D. Vittori, A. Ciofo, A. Cenci, O.A. Tanzarella, C.O. Qualset, G.T. Scarascia Mugnozza, 1998. The introgression in hexaploid wheat of *Dasypyrum villosum* chromatin of chromosome 1V and 6V affect the grain quality and disease resistance of wheat. *XV EUCARPIA General Congress on* '*Genetics and breeding for crop quality and resistance*', Sept. 21-25th 1998, Viterbo, Italy, (Poster Abstract).

[56] Snidaro D., 1998. *Utilizzazione del Dasypyrum villosum per il miglioramento genetico del frumento tenero ai fini dell'agricoltura sostenibile.* Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

1999

[57] Kotsonis P., 1999. *Dasypyrum villosum: aspetti di biologia riproduttiva e di biologia delle popolazioni utili per l'attitudine foraggera della specie.* Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

2000

[58] Pignone D., I. Galasso, A. Blanco, R. Cremonini, 2000. Heterochromatin organization in metaphase chromosomes and interphase nuclei of *Dasypyrum breviaristatum* (Lindb.) Frederiksen. *Acta Soc. Bot. Pol*., 69: 5-9.

2001

[59] Contento A., S. Minelli, G. Caputi, C. De Pace, P.G. Cionini, 2001. Chromosome instability in: *Triticum turgidum* var. *durum*×*Dasypyrum villosum* anphiploids. *Chromosome Res.*, 9. Suppl. 1: 88-89. (http://periodici.caspur.it:3131/pdflinks/08091400183412914.pdf)

[60] De Pace C., D. Snidaro, M. Ciaffi, D. Vittori, A. Ciofo, A. Cenci, O.A. Tanzarella, C.O. Qualset, G.T. Scarascia Mugnozza, 2001. Introgression of *Dasypyrum villosum* chromatin into common wheat improves grain protein quality. *Euphytica*, 117: 67-75.

2002

[61] Caputi G., 2002. Valutazione genetica ed agronomica di nuovi anfiploidi *Triticum turgidum* var *durum*×*Dasypyrum villosum* da coltivare in sistemi agricoli sostenibili. Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

[62] Mariani M., 2002. *Analisi di caratteri a base genetica semplice e complessa in linee di frumento tenero che presentano addizione, sostituzione e ricombinazione di cromosomi trasferiti*

da Dasypyrum villosum. Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo, Italia.

2003

[63] De Pace C., C.C. Jan, G. Caputi, G.T. Scarascia Mugnozza, 2003. Genetical events occurring during and after *Triticum turgidum* var. *durum* × *Dasypyrum villosum* hybridization recapitulate the population size and time span required for the transition from tetraploid to hexaploid wheat domestication. In: *Proc. 10th Int. Wheat Genet. Symp.* Istituto Sperimentale per la Cerealicoltura, Rome, Italy, Vol. 2: 472-474.

[64] Minelli S., M. Ceccarelli, F. Galeotti, M. Mariani, C. De Pace, P.G. Cionini, 2003. Localization of genes in the chromosomes of *Dasypyrum villosum* and phylogenetic implication of their homeology to the A, B, and D chromosomes of wheat. In: *Proc. 10th Int. Wheat Genet. Symp.*, Istituto Sperimentale per la Cerealicoltura, Rome, Italy, Vol. 2: 494-496.

[65] Mariani M., S. Minelli, M. Ceccarelli, P.G. Cionini, C.O. Qualset, C. De Pace, 2003. *Dasypyrum villosum* chromosome segments introgressed in hexaploid wheat provide opportunities for prebreeding and preparing primary mapping populations for analyzing complex genetic traits. In: *Proc. 10th Int. Wheat Genet. Symp*., Istituto Sperimentale per la Cerealicoltura, Rome, Italy, Vol. 2: 613-615.

2005

[66] Minelli S., M. Ceccarelli, M. Mariani, C. De Pace, P.G. Cionini, 2005. Cytogenetics of *Triticum* × *Dasypyrum* hybrids and derived lines. *Cytogenetic and Genome Research* 109: 385-392.

2007

[67] Bizzarri M., P. Vaccino, M. Pasquini, F. Nocente, J. Bianchi, P.G. Cionini, E. Caceres, C. De Pace, 2007. The genetics of wheat powdery mildew resistance expressed after the introgression of chromosome 6V in: *T. aestivum*. *Proc. of the 51st Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Palazzo dei Congressi, Riva del Garda (TN), 23/26 Settembre 2007.

[68] De Pace C., M. Pasquini, A. Matere, 2007a. Genetic information on crop varieties provide high quality indicators to address agri-environmental issues. In: *Farming Systems Design 2007, Int. Symposium on Methodologies on Integrated Analysis on Farm Production Systems*, (M. Donatelli, J. Hatfield, A. Rizzoli, eds.), Catania (Italy), 10-12 September 2007, book 2-Field-farm scale design and improvement, pp. 155-156.

[69] De Pace C., P. Vaccino, M. Corbellini, G. Boggini, M. Pasquini, P.G. Cionini, E. Caceres, D. Vittori, A. Ciofo, 2007b. Wheat Breeding Lines for Low-input Agriculture. EUCARPIA Symposium on '*Plant breeding for organic and sustainable, low-input agriculture: dealing with genotype-environment interactions*'. Wageningen, 7–9 November 2007. (http://orgprints.org/13110/01/lammerts-vanbueren-etal-2007-eucarpia.pdf)

[70] Vaccino P., M. Corbellini, J. Bianchi, M. Pasquini, L. Sereni, P.G. Cionini, E. Caceres, D. Vittori, M. Bizzarri, C. De Pace, 2007a. Nuovi frumenti per un'agricoltura che cambia. *L'Informatore Agrario*, 32/2007: 58-59.

[71] Vaccino P., M. Corbellini, M. Cattaneo, S. Negri, M. Pasquini, P.G. Cionini, E. Caceres, D. Vittori, A. Ciofo, C. De Pace, 2007b. Analysis of Genotype-by-Environment interaction in wheat using aneuploid lines with chromatin introgressed from *Dasypyrum villosum*. *Proc. of the 51st Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Palazzo dei Congressi, Riva del Garda (TN), 23/26 Settembre 2007.

2008

[72] Bizzarri M., 2008. *Espressione di nuovi geni che conferiscono resistenza ad oidio, ruggine bruna e carie in linee di frumento tenero derivate da ibridazione Triticum aestivum* (L.) × *Dasypyrum villosum* (L.) *P. Candargy*. Tesi di Laurea, Facoltà di Agraria, Università degli Studi della Tuscia, Viterbo.

[73] Caceres M.E., P. Vaccino, M. Corbellini, P.G. Cionini, V. Sarri, E. Polizzi, D. Vittori, C. De Pace, 2008a. Flowering earliness in wheat inbred breeding lines derived from *T. aestivum* 'Chinese Spring' × *Dasypyrum villosum* hybridization is not related to allelic variation at the vernalization loci *VRN-A1*, *VRN-B1*, and *VRN-D1*. In: '*Modern Variety Breeding for Present and Future Needs*' (J. Prohens and M.L. Badenes, eds.). Proceedings of the 18th EUCARPIA General Congress, 9-12 September 2008, Valencia, Spain. Publisher: Editorial Universidad Politécnica de Valencia, Valencia, Spain, pp. 329-334.

[74] Caceres M.E., V. Sarri, M. Ceccarelli, P. Vaccino, M. Corbellini, C. De Pace, 2008b. Molecular and cytogenetic analyses of *Triticum* × *Dasypyrum* hybrids and derived breeding lines. *10th National Biotechnology Congress*, 17-19 Sept. 2008, Perugia, Italy, p. 223.

[75] Vaccino P., R. Banfi, M. Corbellini, E. Caceres, P.G. Cionini, M. Pasquini, M. Bizzarri, C. De Pace, 2008. Wheat breeding for responding to environmental changes: enhancement of modern varieties using a wild relative for introgression of adapted genes and genetic bridge. *52nd Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, 14-17 September, 2008, Padova, Italy, Abstract 4.05.

2009

[76] Bizzarri M., M. Pasquini, A. Matere, L. Sereni, G. Vida, A. Sepsi, M. Molnar-Lang, C. De Pace, 2009. *Dasypyrum villosum* 6V chromosome as source of adult plant resistance to *Puccinia triticina* in wheat. *Proc. of the 53rd Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, 16-19 Sept. 2009, Torino, Italy.
http://www.siga.unina.it/SIGA2009/SIGA_2009/2_21.pdf

[77] Grosso V., D. Giorgi, L. Nardi, C. De Pace, S. Lucretti, 2009. Flow karyotyping and sorting of *Dasypyrum villosum* chromosomes in suspension. *Proc. of the 53rd Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, 16-19 Sept. 2009, Torino, Italy.
http://www.siga.unina.it/SIGA2009/SIGA_2009/7_41.pdf

[78] Pasquini M., M. Bizzarri, F. Nocente, L. Sereni, A. Matere, P. Vaccino, M. Corbellini, C. De Pace, 2009. *Dasypyrum Villosum* as source of resistance to leaf rust and powdery mildew for wheat improvement. *EASAC Conference on Plant Genet. Res. for Food*, Acc. Lincei, 5-6 Nov. 2009, Roma, Italy (Abstract).

[79] Vaccino P., M. Corbellini, C. De Pace, 2009. Optimizing low input production systems using improved and stable wheat inbred lines arising from a new breeding scheme. In: '*Farming Systems Design 2009: an international symposium on Methodologies for Integrated Analysis of Farm Production Systems*', August 23-26 2009, Monterey, CA, USA.
http://www.iemss.org/farmsys09/uploads/FSD2009_program_v08052009.pdf

2010-2011

[80] Bizzarri M., M. Pasquini, F. Nocente, L. Sereni, A. Matere, D. Vittori, G. Vida, C. De Pace, 2010. Multiple resistance to powdery mildew, leaf rust and stem rust in wheat conferred by genes on chromosome 6V introgressed from *Dasypyrum villosum. Proc. of the 54th Annual Congress of the Italian Society of Agricultural Genetics* (SIGA), Session IV-Plant-Environment Interactions. 27-30 Sept. 2010, Matera, Italy, Poster 4.43.

[81] Caceres M.E., M. Ceccarelli, C. De Pace, P.G. Cionini, 2011a. Citogenetica di ibridi fra *Triticum aestivum* e *Dasypyrum villosum* e di linee da essi derivate. In: '*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*'. Collana 'Scritti e Documenti' XLIV, pp. 189-199, Accademia Nazionale delle Scienze detta dei XL, Roma.

[82] Caceres M.E., P. Vaccino, M. Ceccarelli, V. Sarri, E. Polizzi, C. De Pace, P.G. Cionini, 2011b. Introgressione criptica di DNA parentale in linee di frumento derivate da ibridazione intergenerica fra *Triticum aestivum* e *Dasypyrum villosum.* In: '*Trasferimento di geni dalla specie selvatica D. villosum al frumento per aumentarne l'adattamento ai sistemi agrari soste-*

nibili: Risultati ottenuti dalla collaborazione scientifica tra Università italiane e l'Università della California'. Collana 'Scritti e Documenti' XLIV, pp. 201-211, Accademia Nazionale delle Scienze detta dei XL, Roma.

[83] Corbellini M., P. Vaccino, R. Banfi, T. Coppa, C. De Pace, 2011. Stabilità e capacità produttiva di linee inbred di frumento contenenti geni o cromosomi di *D. villosum.* In '*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*'. Collana 'Scritti e Documenti' XLIV, pp. 137-148, Accademia Nazionale delle Scienze detta dei XL, Roma.

[84] De Pace C., M. Pasquini, P. Vaccino, M. Bizzarri, F. Nocente, M. Corbellini, M.E. Caceres, P.G. Cionini, D. Vittori, G. Vida, 2011a. Deployment of either a whole or dissected wild nuclear genome into the wheat gene pool meets the breeding challenges posed by the sustainable farming systems. 2nd Int. Symposium on Genomics of Plant Genetic Resources. Bologna 24-27 April 2010. *Plant Genetic Resources*, doi: 10.1017/S1479262111000141.

[85] De Pace C., P. Vaccino, P.G. Cionini, M. Pasquini, M. Bizzarri, C.O. Qualset, 2011b. *Dasypyrum*. In: *Wild Crop Relatives: Genomic and Breeding Resources*. Vol 1: *Wild Relatives of Cereals* (C. Kole, ed.). Springer, Heidelberg, Berlin, New York, pp. 185-292.

[86] De Pace C., P. Vaccino, M. Corbellini, E. Caceres, D. Vittori, S. Bizzarri, 2011c. Development of valuable wheat inbred lines through the introduction of *Dasypyrum villosum* germplasm in their pedigree. In '*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*'. Collana 'Scritti e Documenti' XLIV, pp. 121-136, Accademia Nazionale delle Scienze detta dei XL, Roma.

[87] Grosso V., A. Farina, D. Giorgi, L. Nardi, C. De Pace, S. Lucretti, 2010. Flow cytogenetics of *Dasypyrum villosum* chromosomes. In: *Proc. of the 54th Annual Congress of the Italian Society of Agricultural Genetics (SIGA)*, Session I, Biodiversity and Genetic Resources, 27-30 Sept. 2010, Matera, Italy, Poster 1.39.

[88] Pasquini M., M. Bizzarri, F. Nocente, A. Matere, L. Sereni, F. Casini, A. L'Aurora, P. Vaccino, M. Corbellini, T. Coppa, D. Vittori, C. De Pace, 2011a. *Dasypyrum villosum* come fonte di geni di resistenza a oidio e ruggine bruna per il miglioramento dei frumenti coltivati. In '*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*'. Collana 'Scritti e Documenti' XLIV, pp. 171-188, Accademia Nazionale delle Scienze detta dei XL, Roma.

[89] Pasquini M., F. Nocente, M. Bizzarri, L. Sereni, A. Matere, D. Vittori, G. Vida, C. De Pace, 2011b. Resistance to *Blumeria graminis tritici* and *Puccinia triticina* in aneuploid wheat lines with chromatin introgressed from *Dasypyrum villosum*. *Annual Wheat Newsletter* 56: 97-99.

[90] Qualset C.O., 2010. Genetics and Conservation of *Dasypyrum villosum* populations. In: '*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*'. Collana 'Scritti e Documenti' XLIV, pp. 95-103, Accademia Nazionale delle Scienze detta dei XL, Roma.

[91] Vaccino P., R. Banfi, M. Corbellini, C. De Pace, 2010. Improving the wheat genetic diversity for end-use grain quality by chromatin introgression from the wheat wild relative *Dasypyrum villosum. Crop Science* 50: 528-540

[92] Vaccino P., T. Coppa, M. Corbellini, C. De Pace, 2011. Aspetti genetico-molecolari e tecnologici del trasferimento al frumento di geni del *Dasypyrum villosum.* In: '*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*'. Collana 'Scritti e Documenti' XLIV, pp. 151-169, Accademia Nazionale delle Scienze detta dei XL, Roma.

Il volume è stato pubblicato grazie al contributo concesso dalla «Fondazione Carivit», Viterbo

This volume has been printed thanks to the financial contribution of the «Fondazione Carivit», Viterbo