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in

Porceddu E. (ed.), Damania A.B. (ed.), Qualset C.O. (ed.).
Proceedings of the International Symposium on Genetics and breeding of durum wheat

Bari : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 110

2014
pages 477-491

Additional genetic factors of resistance to stem rust, leaf rust and powdery mildew from *Dasypyrum villosum*

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Abstract. The gene diversity for rust and powdery mildew disease resistance is very narrow in durum wheat varieties. The chromosome 6V#4 from *D. villosum* contains genes for broad-spectrum resistance to diseases caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) (stem rust), *Puccinia triticina* Eriks. (*Pt*) (leaf rust), *Puccinia striiformis* f. sp. *tritici* Er ks. (*Pst*) (stripe rust), and *Blumeria graminis* f. sp. *tritici (Bgt)* (powdery mildew). Progenies from the cross of a durum wheat F_7 line (derived from 'Cappelli' × 'Peleo') with CS-DA6V#4 (a disomic addition line of chromosome 6V#4 to the *T. aestivum* 'Chinese Spring' genomic background), were backcrossed to durum wheat lines in order to selected plants for resistance to airborne *Bgt* inoculum in the greenhouse as a marker for the presence of chromosme 6V#4. The chromosome number of the progenies of two of those plants, '467-68.1' and '491-50.2', ranged from 28 to 36 with an average of 2n=31, and the presence of 6V#4 was revealed by GISH. The seedlings of the two progenies were tested for response to different races (isolates) of *Pgt* and *Pt* under controlled experiments at CAR-HAS in Hungary, and to *Pgt* and *Bgt* under controlled experiments at CRA-QCE in Italy. All the seedlings from the '467-68.1' and '491-50.2' progenies, were resistant to *Pt* and *Bgt*, and the '467-68.1' progeny displayed resistance to *Pgt.* The NAU/Xibao15_{902} molecular marker linked to *Pm21,* a putative locus in 6V#4 with a gene determining resistance to *Bgt*, was detected in all the seedlings of the two progenies. Plants with chromosome number ranging from 28 to 30 are now field tested and are being prepared for the final round of backcross to the '4.5.1' durum wheat recurrent parent.

Keywords. Gene for resistance – Plant disease – *Triticum turgidum* L. var *durum* – Interspecific hybridization – Gene transfer.

Autres facteurs génétiques de résistance à la rouille noire, la rouille brune et à l'oïdium de Dasypyrum villosum

Résumé. *La diversité génétique pour la résistance aux maladies de la rouille et de l'oïdium est très limitée dans les variétés de blé dur. Le chromosome 6V#4 de D. villosum contient des gènes de résistance à large spectre pour les maladies causées par Puccinia graminis f. sp. tritici (Pgt) (rouille noire), Puccinia triticina Eriks. (Pt) (rouille brune), Puccinia striiformis f. sp. tritici Eriks. (Pst) (rouille jaune), et Blumeria graminis f. sp. tritici (Bgt) (oïdium). Les descendants du croisement d'une lignée F7 de blé dur (issue de «Cappelli 'x' Peleo ') avec CS-DA6V#4 (une lignée d'addition disomique du chromosome 6V#4 au génome de T. aestivum 'Chinese Spring'), ont été rétrocroisés avec des lignées de blé dur pour sélectionner des plantes pour la résistance à l'inoculum aérien de Bgt en serre, en tant que marqueur pour la présence du chromosome 6V#4. Le nombre de chromosomes des descendants de deux de ces plantes, «467-68,1» et «491- 50,2", varie de 28 à 36 avec une moyenne de 2n=31, et la présence de 6V#4 a été révélée par GISH. Les semis des deux descendants ont été testés pour leurs réponses à différentes races (isolats) de Pgt et Pt en conditions expérimentales contrôlées au CAR-HAS en Hongrie, et à Pgt et Bgt en conditions expérimentales contrôlées au CRA-QCE en Italie. Tous les semis des descendants de «467-68,1» et «491-50,2 étaient résistants à Pt et Bgt, et le descendant 467-68,1' affichait une résistance à Pgt. Le marqueur moléculaire NAU/Xibao15902 lié à Pm21, un locus putatif de 6V#4 avec un gène déterminant la résistance à Bgt, a été détecté dans tous les semis des deux descendants. Les plantes avec un nombre de chromosomes compris entre 28 et 30 sont maintenant testées sur le terrain et soumises à la préparation pour la phase finale de rétrocroisement avec le parent récurrent de blé dur "4.5.1".*

Mots-clés. *Gène de résistance – Maladies des plantes – Triticum turgidum L. var durum – Hybridation interspécifique – Transfert de gène.*

I – Introduction

A strong global demand for durum wheat grains is expected until the year 2020. The management issues that are yet to be resolved to consistently sustain production till that time, include those related to phytopathological concerns and climate-related environmental stresses. Rusts and powdery mildew cause major production losses in bread as well as durum wheat.

There is a need for greater genetic diversity for durum wheat improvement in order to face the recent increase in occurrence of virulent and highly aggressive rust strains on all continents (including Europe) (Solh *et al*., 2012; Hodson *et al.,* 2012).

Genes for rust and powdery mildew resistance are numerous in bread wheat but few have been found in durum wheat. Many of the most effective genes have been transferred from wild wheat relatives and species from the secondary genepool, as deduced from the following review.

1. Stem rust

Wheat stem rust is caused by the fungus *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn (*Pg*t). Wheat production is threatened by the spread of a new dangerous race designated as Ug99. Currently, approximately 30 major genes conferring resistance to *Pg*t races from the seedling stage are known, plus five slow-rusting or resistance genes at adult plant stage, are being studied (Pumphrey, 2012). Thirty-eight near-isogenic lines of bread wheat carrying 21 single designated *Sr* genes for resistance to stem rust were produced and tested with nine races of stem rust by Knott (1990). To date, molecular markers have been identified for several stem rust resistance genes (*Sr 2, 6, 9a, 13, 24, 25, 26, 31, 36, 38, 39, 40*) to deploy them in new elite cultivars (Simons *et al*., 2011) and diagnostic DNA markers are being developed for other *Sr* genes (Pumphrey, 2012).

Some of those genes have been introgressed in durum wheat, and others are being transferred.

Sr9d is present in the Stakman *et al.* (1962) durum differentials Mindum*,* Arnautka and Spelmar; Many North American durums appear to carry *Sr9e.*

Sr13 is the only studied gene found in durum wheat with moderate resistance and effectiveness against the TTKSK race, one of the three races (the other two being TTKST and TTTSK) within the TTKS lineage originally designated Ug99. *Sr13* localized in the distal region of the long arm of chromosome 6A of several *Triticum turgidum* ssp. *durum* cultivars (McIntosh, 1972; Pumphrey 2012), was mapped within a 1.2–2.8 cM interval (depending on the mapping population) between EST markers CD926040 and BE471213 (Simons *et al*., 2011). The Ethiopian land race ST464 (PI 191365) and the domesticated emmer wheat (*T. turgidum* ssp. *dicoccon* L.) 'Khapli' (CItr 4013) have been the two major sources of *Sr13* in durum (Knott 1962; Klindworth *et al*. 2007) and nowadays *Sr13* is contained in a number of Ug99-complex resistant durum (i.e.,'Kronos', 'Kofa', 'Medora' and 'Sceptre'), in the Canadian durum wheat 'Stewart 63' (together with *Sr7* and *Sr11*) (Knott 1963; Kuznestova, 1980), and cultivated emmer varieties, although its moderate resistance to TTKS races makes it a good candidate for gene pyramiding with other stem rust resistance genes. The *Sr13* resistance gene was transferred, together with *Sr9e*, from 'ST464' to other durum wheat varieties such as 'Leeds' (Luig, 1983).

Sr14 is located very close to the centromere on chromosome 1BL (McIntosh*,* 1980)*. Sr14,* similarly to *Sr13,* was an effective gene for resistance to *Pg*t and was transferred from dicoccum wheat which is called 'Khapli' in India to the hexaploid cv*.* Steinwedel*,* resulting in cv*.* Khapstein (PI 210125) (McIntosh, 1972). However its response to *Pg*t is reduced under high temperature and high light conditions (Knott*,* 1962; Luig*,* 1983*;* Gousseau *et al.,* 1985)*.*

Several effective *Sr* resistance genes had been transferred to wheat from relative species. *Sr21* and *Sr22* were transferred from *T. monococcum*. *Sr24* was originally transferred in 'Agent' bread wheat from *Thinopyrum ponticum* and is present in a translocation involving wheat chromosome 3D and one *T. ponticum* chromosome; *Sr24* is effective against most stem rust races worldwide (Smith *et al*., 1968; Yu *et al*., 2010). The initial TTKSK race was not virulent on the *Sr24* gene but the new variant of TTKS (TTKST) identified in Kenya (Jin *et al*., 2008; Jin *et al*., 2009) was virulent on *Sr24*. *Sr25* is present in 'Agatha' which also has a translocation involving wheat chromosome 7D and an *Agropyron* chromosome; *Sr26* is in a wheat-*Agropyron* translocation derived from 'Agrus" and involving wheat chromosome 6A; and *Sr27* has been found in a wheat-rye (*Secale cereale* L.) translocation line 73.214.3-1 from the University of Sydney. The lines carrying those genes were resistant to all nine *Pgt* races tested by Knott (1990).

Sr31 is a gene located in the short arm of chromosome 1R from 'Petkus' rye and introgressed into hexaploid wheat as a 1RS·1BL translocation, and *Pgt* race TTKSK was the first stem rust race reported to be virulent on this gene (Zhang *et al.,* 2010).

Sr33 gene was discovered from *Ae. tauschii*, the diploid progenitor of the D genome in hexaploid wheat and was introgressed into common wheat (*Triticum aestivum*, genomes AABBDD) (Kerber and Dyck, 1978). It is tightly linked to *Gli-D1* on chromosome arm 1DS (5.6 to 7.6% recombination) and less tightly to the centromere (29.6% rec.) and to *Glu-D1* (39.5% to 40.9 % rec.) (Jones *et al.,* 1991). The *Sr33* gene encodes a coiled-coil, nucleotide-binding, leucine-rich repeat protein and is orthologous to the barley (*Hordeum vulgare*) *Mla* mildew resistance genes that confer resistance to *Blumeria graminis* f. sp. *hordei*. It has been recently cloned (Periyannan *et al*., 2013) and when used for genetic transformation experiments of the 'Fielder' wheat cultivar, which is susceptible to the Australian *Pgt* race 98-1,2,3,5,6, the resulting transgenic lines expressed the *Pgt* resistant phenotype. When introgressed alone into hexaploid wheat, *Sr33* provides a valuable, intermediate level of resistance to diverse *Pgt* races, including the race TTKSK (Rouse *et al.,* 2011) but, preferably, *Sr33* should be deployed together with genes like *Sr2* to maintain its resistance.

Sr35, originally transferred from *Triticum monococcum* to hexaploid wheat (McIntosh *et al*., 1984), is effective against TTKSK (Jin *et al.*, 2007) displaying a strong hypersensitive reaction to that race. Monogenic lines carrying *Sr35* exhibited resistant to moderately resistant infection responses with relatively low disease severity in field nurseries in Kenya in 2005 and 2006 (Jin *et al*., 2007). *Sr35* was first assigned to the long arm of chromosome 3A (McIntosh *et al*., 1984) and later mapped 41.5 cM from the centromere and 1cM from the red grain color gene *R2*. *Sr35* shows also hypersensitive reaction to TRTTF race groups when introgressed into hexaploid wheat but is susceptible to some *Pgt* races and, therefore, should not be deployed alone. The *Sr35* gene has recently been cloned and it was demonstrated (Saintenac *et al*., 2013) that is a coiled-coil, nucleotide-binding, leucine-rich repeat gene lacking in the A-genome diploid donor and in polyploid wheat. The identification and cloning of *Sr33 and Sr35* opens the door to transgenic approaches to control the devastating races of *Pgt* in both durum and bread wheat cultivars.

Sr36 is an additional wild-relative-derived stem rust resistance gene frequently used by wheat breeders (Olson *et al*., 2010a). *Sr36* was transferred from *Triticum timopheevii* (Allard and Shands, 1954) and is present in several commercial wheat varieties (Olson *et al*., 2010a; Yu *et al.*, 2010). The initial TTKSK race was not virulent on that gene. Unfortunately, the new variant of TTKS (TTTSK) identified in Kenya (Jin *et al*., 2007; Jin *et al*., 2009) was virulent on plants carrying *Sr36* gene.

Sr44 maps on the short arm of the *Th. intermedium* 7J#1S short chromosome arm. Liu *et al*. (2013) produced a line with a homozygous compensating wheat-*Th. intermedium* T7DL•7J#1S Robertsonian translocation which carries *Sr44* on the 7J#1S fragment. *Sr44* confers resistance the Ug99 race complex including races TTKSK, TTSKT, and TTTSK.

Sr52 was transferred into wheat from *Dasypyrum villosum*. A set of whole arm Robertsonian translocations involving chromosomes 6A of wheat and 6V#3 of *D. villosum* was produced through centric breakage-fusion (Qi *et al*., 2011). *Sr52* was mapped to the long chromosome arm 6V#3L of *D. villosum*, and when it was transferred to wheat it translocated with chromosome arm 6AL. *Sr52* shows a temperature-sensitive resistance pattern to stem rust race Ug99 (TTKSK): it is most effective at 16°C, partially effective at 24°C and ineffective at 28°C. *Sr15* becomes also less effective at higher temperatures (Roelfs, 1988). The variation of resistance related to the temperature could hinder field deployment, since the fungal pathogen is more active at warmer temperatures.

Significant stem rust resistance quantitative trait locus (QTL) were detected on chromosome 4B of the durum wheat cv Sachem (Singh *et al*., 2013).

2. Leaf rust

Leaf rust caused by *Puccinia triticina* Eriks. (*Pt*) is an important disease that causes significant wheat production losses worldwide. At present over 50 genes controlling wheat leaf resistance are known (McIntosh *et al.* 1995) and only two of them, *Lr14a* and *Lr23,* originated from tetraploid wheat (Herrera-Foessel *et al.*, 2005). Survey studies based on tests of seedlings with different rust isolates and molecular genotyping have shown the presence of *Lr1*, *Lr3*, *Lr10*, *Lr14a*, *Lr16*, *Lr17a*, *Lr19*, *Lr23*, *Lr25*, *Lr33*, *Lr61* and *Lr64* in the elite durum wheat germplasm (Terracciano *et al*., 2013).

Race-specific genes for leaf rust resistance frequently undergo “boom-and-bust” cycles. Examples of this are given by genes *LrAlt* in ‘Altar 84’ released in 1984 which was broken down in 2001 by race BBG/BN; and genes *LrAlt, 27+31 in* ‘Jupare’ released in 2001 which broke down in 2007 by race BBG/BP (Singh, 2012). The novel virulent leaf rust race BBG/BN and its variant BBG/BP overcame the resistance of widely adapted durum cultivars in North-western Mexico which had been effective and stable for more than 25 years (Huerta-Espino *et al.,* 2009 a, b).

Lr14a is a dominant leaf rust resistance gene originally transferred from emmer wheat ‘Yaroslav’ to the hexaploid wheat lines Hope and H-44 by McFadden (1930). It has been found in the Chilean durum cv. ‘Llareta INIA’ and in CIMMYT-derived durum ‘Somateria’. The *Lr14a*-resistance gene was also present in the durum wheat cv. ‘Creso’ and its derivative cv. ‘Colosseo’ is one of the best characterized leaf-rust resistance sources deployed in durum wheat breeding. It was mapped to chromosome arm 7BL through bulked segregant analysis using the amplified fragment length polymorphism (AFLP) technique. Several simple sequence repeat (SSR) markers, including *Xgwm344-7B* and *Xgwm146-7B*, were associated with the *Lr14a* resistance gene in both common and durum wheat (Herrera-Foessel *et al*., 2008) in the distal portion of the chromosome arm 7BL, a gene-dense region (Terracciano *et al*., 2013). Gene *Lr14a* is linked to stem rust and powdery mildew resistance genes *Sr17* and *Pm5*, respectively. However, the original ‘Yaroslav’ accession that carried the relevant genes (i.e., *Sr17, Lr14a*, and *Pm5*) and the slow-rusting stem rust resistance gene *Sr2* (chromosome 3B) has been lost (McIntosh *et al.,* 1995.).

Lr19 was a highly effective gene against five different *Pt* pathotypes (TKF/H, SKF/G, PHT/B, THT/F, and KHP/C) and was identified in ‘Dur’ and ‘Valdur’ varieties (Shynbolat and Arakeyat, 2010).

Lr23 was shown to be an effective resistance gene against the five mentioned *Pt* pathotypes avirulent on *Lr19* and was found in the durum wheat varieties ‘Albatross’, ‘Cocorit71’, ‘VZ-187’ and ‘Nauryz6’ (Shynbolat and Arakeyat, 2010). *Lr23* was transferred to common wheat from durum wheat cv. ‘Gaza’ and cytogenetically mapped to chromosome 2BS (McIntosh and Dyck, 1975).

The wild emmer wheat *T. turgidum* ssp. *dicoccoides* was the source of many genes for resistance to *Pt* such as *Lr53,* located in chromosome 6BS (Marais *et al*., 2005) and another genes expressing the same infection types as *Lr33* (Dyck, 1994).

Evidence have been provided that resistance to *Pt* in the F_2 and F_3 progenies of ‘Atil C2000’ (susceptible durum parent) × ‘Hualita’ (resistant durum parent) was due to complementary leaf rust resistance genes (Herrera-Foessel *et al*., 2005). Previously identified and designated complementary leaf rust resistance genes were *Lr27* and *Lr31* in bread wheat (Singh and McIntosh, 1984 a, b) which were located on chromosomes 3BS and 4BL, respectively (Singh and McIntosh, 1984b). Gene *Lr31* is either completely linked or the same as *Lr12* (Singh *et al.,* 1999).

The French durum wheat cultivar Sachem was resistant, while Strongfield, the predominant cultivar grown on the Canadian prairies, was moderately susceptible to stripe rust, BBG/BN leaf rust race and Ug99 stem rust races. A major leaf rust QTL was identified on chromosome 7B at *Xgwm146* in Sachem. In the same region on 7B, a stripe rust QTL was identified in Strongfield. A significant leaf rust QTL was detected on chromosome 2B where a *Yr* gene derived from Sachem conferred resistance (Singh *et al*., 2013).

Adult-plant resistance genes *Lr13* and *Lr34* singly and together have provided the most durable resistance to leaf rust in bread wheat throughout the world (Kolmer, 1996). *Lr34* has been found in Strampelli varieties ‘Ardito’ and ‘Mentana’ (Salvi *et al*., 2013) and in ‘Chinese Spring’ bread wheat in which the *Lr12* gene is also present (Dyck, 1991). Previous studies have located the codominant gene *Lr34* on the short arm of chromosome 7D. This location hindered the transfer of *Lr34* in durum wheat to support durable resistance. *Lr34* is linked to *Yr18* and co-segregate with other traits such as leaf tip necrosis (*Ltn1*), *Pm38* for powdery mildew resistance and *Bdv1* for tolerance to Barley yellow dwarf virus (Kolmer *et al,* 2008). *Lr34* has been cloned (Krattinger *et al.* 2009) and when deployed with other adult plant resistance genes, near-immunity can be achieved (Singh and Trethowan, 2007).

It would be extremely useful if an *Lr34*-like gene associated to other multiple disease resistance could be found in diploid relatives, because it will provide breeders with diverse genes for pyramiding and increase the durability of resistance in durum wheat.

3. Stripe rust

Stripe rust (or yellow rust) of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), has become more severe in eastern United States, Australia, and elsewhere since 2000. Markell and Milus (2008) observed that isolates collected before 2000 had diverse virulence phenotypes, were usually virulent only on a few of the differential lines, and were always avirulent on resistance genes *Yr8* and *Yr9*. On the other hand, isolates collected since 2000 had similar virulence phenotypes, were usually virulent on approximately 12 of the differential lines, and were always virulent on differentials carrying *Yr8* and *Yr9*. Those results indicated that isolates causing severe epidemics in the United States since 2000 did not arise by mutation from the existing population and were most likely from an exotic introduction adapted to warmer temperatures (Milus *et al.,* 2009).

About 52 permanently named and more than 40 temporarily designated genes or quantitative trait loci (QTL) for stripe rust resistance have been reported (Chen *et al.*, 2002; Chen 2005; Ren et al. 2012). Among the permanently named resistance genes, *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr16*, *Yr18*, *Yr29*, *Yr30*, *Yr34*, *Yr36*, *Yr39*, *Yr46*, *Yr48* and *Yr52* confer adult plant or high-temperature adult plant (HTAP) resistance, which is expressed when plants grow old and weather becomes warm, whereas the others confer all-stage resistance (Park *et al*., 1992; Xu *et al.*, 2013). Of the permanently named *Yr* genes, 14 were transferred from common wheat relatives, such as *T. aestivum* ssp. *spelta* var. *album*, *T. dicoccoides*, *T. tauschii*, *T. turgidum*, T. *turgidum* var. *durum*, *T. ventricosum*, *Aegilops* (*Ae*.) *comosa*, *Ae. geniculata*, *Ae*. *kotschyi*, *Ae*. *neglecta*, *Ae*. *sharonensis*, *Dasypyrum villosum*, and *Secale cereale* (Chen 2005; Xu *et al*., 2013). At least one gene for resistance to *Pst* was located on the short arm of chromosome 6V of *D. villosum* in the

6VS/6AL-translocation line from cv. Yangmai-5 (obtained by Chen PD, CAAS, China); this gene was named *Yr26* (Yildirim *et al*., 2000).

Resistance genes *Yr7*, *Yr15*, *Yr24*/*Yr26* and *Yr36* originated from tetraploid wheat accessions (Xu *et al*., 2013). *Yr36* was first discovered in wild emmer wheat (*T. turgidum* ssp. *dicoccoides* accession FA15-3. In controlled environments, plants with *Yr36* are resistant at relatively high temperatures (25° to 35°C) but susceptible at lower temperatures (e.g., 15°C) (Fu *et al*., 2009). The *Yr36* gene has been cloned but it has not yet been transferred in modern durum and bread wheat varieties (Fu *et al*., 2009). The durum wheat PI 480148 from Ethiopia possessed the gene *Yr53,* was resistant to *Pst* races under controlled greenhouse conditions at the seedling stage, and was resistant also at multiple USA locations subjected to natural infection of *Pst* for several years (Xu *et al*., 2013). The gene was mapped to the long arm of chromosome 2B and is flanked by the SSR marker *Xwmc441* (5.6 cM) and RGAP marker *XLRRrev/NLRRrev350* (2.7 cM). Xu *et al*, 2013, found that the gene is different from *Yr5*, which is also located on 2BL, 21 cM away from the centromere (Law, 1976). The *Yr5* gene confers resistance to all *Pst* races identified so far in the United States.

4. Powdery mildew

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most severe diseases of wheat worldwide. Up to now, 41 loci (*Pm1 to Pm45*, *Pm18*=*Pm1c*, *Pm22*=*Pm1e*, *Pm23*=*Pm4c*, *Pm31*=*Pm21*) with more than 60 genes/alleles for resistance to *Bgt* isolates have been identified and located on various chromosomes in bread wheat and its relatives (Alam *et al*., 2011). Thirteen *Pm* genes were found in tetraploid wheats but only one gene, *Pm3h*, might have originated from a cultivated *T. durum* Ethiopian line (Hsam and Zeller, 2002). Several genes were identified and transferred from other domesticated as well as wild relatives, such as *T. timopheevii* (Zhuk.), *T. monococcum* (L.), *T. tauschii* (Schmalh), *Aegilops speltoides* (Tausch), *Thinopyrum intermedium (Pm43), Secale cereale* (L.) and *Dasypyrum villosum*. In this last species, a putative serine/ threonine protein kinase gene (*Stpk-V*) in the *Pm21* locus (Cao *et al*. 2011) was characterized as conferring durable resistance and was located on the short arm of chromosome 6V (Chen et al., 1995). Pm21 provide a broad-spectrum resistance to *Bgt* which cannot easily be overcome by newly developed *Bgt* races and is correlated with durability of resistance; *Pm21* was transferred to wheat as a 6VS·6AL translocation (Cao *et al*., 2011).

The above information indicate that durum wheat has a narrow genetic basis for rust and powdery mildew resistance, and only few well characterized disease resistance genes are known in that species, which have been prevalently transferred from Ethiopian accessions or its wild relative *T. dicoccoides*. Transfer of disease resistance genes from wheat relatives to bread wheat occurred directly neglecting the role of durum wheat as a bridge species especially in the transfer of disease resistance genes from diploid wheat relatives. Most designated *Sr*, *Lr*, *Yr*, and *Pm* genes which are effective in the wheat genetic background have been transferred from wild relatives. Some of those genes provide broad-spectrum resistance such as the stem rust resistance *Sr33* from *Ae. tauschii*, the leaf rust resistance gene *Lr34* from Chinese bread wheat landraces, the stripe rust resistance gene Yr36 from T. turgidum ssp. dicoccoides, and the powdery mildew resistance gene *Pm21* in *D. villosum.*

Those genes are scattered in different chromosomes of diverse varieties and are difficult to pyramid in one wheat variety. However, the above review indicated that chromosome 6V from the diploid wild species *D. villosum* of the secondary gene-pool of wheat (De Pace *et al*., 2011), contains genes at the *Sr52* locus for resistance to *Pg*-Ug99 races, and at the *Yr26* and *Pm21* loci for resistance to *Pst* an *Bgt* races, respectively. Other observations indicated that 6V contain stronger genes then *Lr34* for resistance to *Pt* (Bizzarri *et al*., 2009). Therefore, 6V is a rich source of genes for broad-spectrum resistance to *Pg*, *Pt*, *Pst*, and *Bgt*, which can simultaneously be transferred to wheat in one round of hybridization. This has been achieved, and the 6V#4 chromosome has been added to the ‘Chinese Spring’ (‘CS’) genomic background as disomic addition (IBL CS×V63, 2n=44) or as disomic 6V#4(6B) substitution (IBL CS×V32, 2n=42). Those IBLs have repeatedly expressed adult plant resistance to *Pgt*, *Pt*, *Pst*, and *Bgt* under controlled greenhouse conditions and at two locations subjected to natural infection for several years, while ‘CS’, used as control, expressed susceptibility. Therefore, 6V#4 is a good candidate for simultaneously transferring multiple genes for rusts and powdery mildew resistance to durum wheat. Here we report the first attempts in such endeavor.

II – Material and methods

1. Plant material

The lines used in this study included: (a) the durum wheat line ‘4.5.1’; (b) the durum wheat cvs ‘Cappelli’ (used as control for the infection experiments in the greenhouse) and ‘Creso’ (used as control for the PCR experiments); (c) the introgression breeding lines (IBL) obtained after crossing *T. aestivum* cv ‘Chinese Spring’ (‘CS’) to *Dasypyrum villosum*, followed by backcrossing to ‘CS’ and several generations of selfing; the IBLs contained chromosome 6V#4 in ‘CS’ genomic background under the configuration of a disomic additon CS-DA6V#4 in line ‘CSxV63’ and as a disomic substitution CS-DS6V#4(6B) in line ‘CSxV32’; and (d) two progenies from the plants ‘467’ (progeny 68.1) and ‘491’ (progeny 50.2) whose pedigree is depicted in Fig. 1. After the initial cross between a durum wheat F_7 line (derived from crossing the durum wheat cvs ‘Cappelli’ × ‘Peleo’) and ‘CSxV63’, the hybrid progenies were composed by the plants labeled ‘481’, ‘488’, and ‘494’. Those hybrid plants were crossed to ‘4.5.1’ (selected from the progeny of ‘Peleo’ × ‘Trinakria’) and the resulting F_3 progenies were backcrossed to ‘4.5.1’ to produce the progeny from which the plant ‘491’ was selected. The hybrid plants were also crossed to the line ‘498’ (from ‘Cappelli × Peleo’), and the resulting F_3 progeny was crossed to ‘4.5.1’ obtaining the progeny from which the plant ‘467’ was selected. Plants ‘467’ and ‘491’ were selected for their resistance to air-born *Bgt* inoculum in greenhouse (Fig. 2). Caryopses of the ‘467-68.1’ and ‘491-50.2’ progenies were germinated and the root-tips were used for chromosome counting; the seedlings were tested for response to different races (isolates) of *Pgt* and *Pt* under controlled experiments at CAR-HAS, Martonvásár, Hungary, and to two isolates of *Pg*t and one isolate of *Bgt* under controlled experiments at CRA-QCE, Rome, Italy.

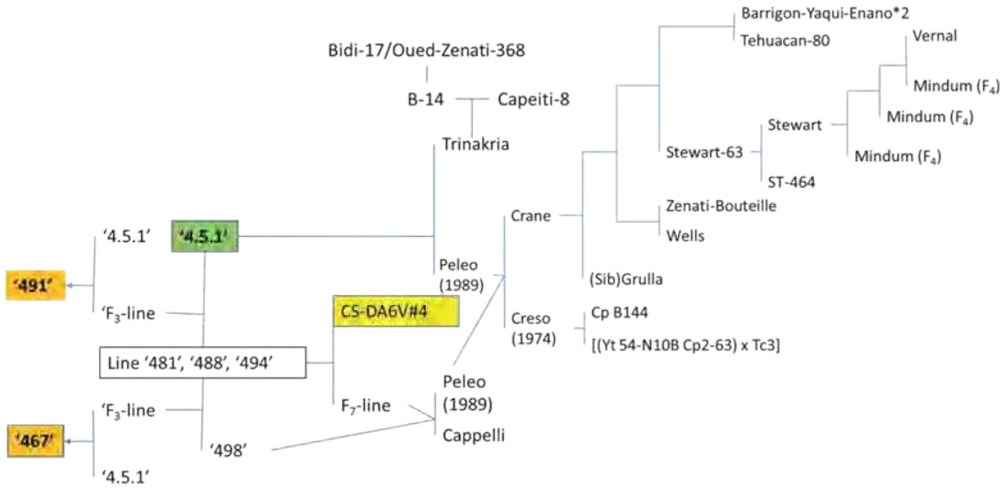

Figure 1. Pedigree of the ‘467’ and ‘491’ plants resistant to *Bgt* infection in the greenhouse.

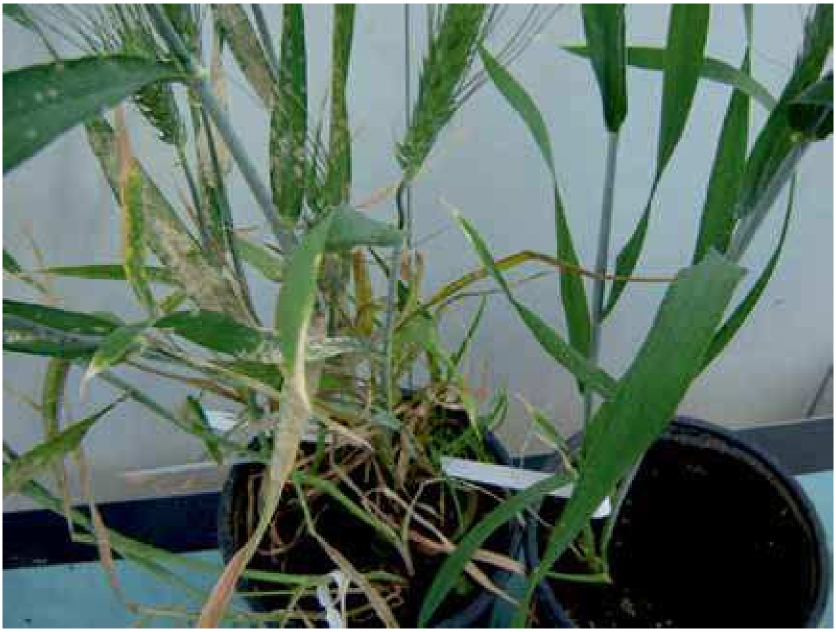

Figure 2. ***Bgt*-resistant plant '491' (right) and the *Bgt*-susceptible parental durum wheat line '4.5.1' (left), grown side-by-side: clear qualitative differences in their response to the natural mildew population in the greenhouse are displayed.**

A. Chromosome counting and Genomic *in situ hybridization (GISH).*

For chromosome counting, the root apical meristems of seedlings from the '467-68.1' and '491-50.2' progenies were pretreated with a 0.05% aqueous solution of colchicine (Sigma) for 4 h at room temperature, fixed in ethanol-acetic acid 3:1 (v/v), and Feulgen-stained after hydrolysis in 1N HCl at 60°C for 8 min. The apices were treated with a 5% aqueous solution of pectinase (Sigma) for 30 min at 37°C and squashed under a coverslip in a drop of 60% acetic acid. The coverslips were removed by the solid CO_2 method. After air-drying, the slides were subjected to three 10-min washes in SO_2 water prior to dehydration and mounting in DPX (BDH).

B. Controlled infection at Martonvásár using Pg*t and* Pt *isolates*

The plants were inoculated in the seedling stage with a mixture of *Pt* or *Pgt* uredospores collected from varieties with various genetic backgrounds and multiplied in the greenhouse. The *Pt* pathogen population used was avirulent on the 'Thatcher'-based near-isogenic lines (NILs) with *Lr9*, *Lr19* or *Lr29* and the severity was less than 10% on the NILs carrying *Lr24*, *Lr25* or *Lr28* resistance genes in the adult plant stage. The pathotypes in the *Pgt* population were avirulent on the *Sr36* 'LMPG'-based NIL, and the severity was 20% with moderately susceptible response for NILs with *Sr9d* and *Sr31* genes. Seedlings were inoculated with uredospore suspension of *Pt* or *Pgt* by brush at GS11 and the symptoms were evaluated according to the 0-4 scale (0 = immune, 4 = susceptible; Stakman *et al*., 1962) ten days after inoculation.

C. Controlled infection CRA-QCE Rome using Pgt and Bgt races.

The material was tested at 10-day-old seedling stage in the greenhouse using the *Bgt* isolate O2 and the *Pgt* isolates 16716-2 and 16713-5-2, identified within the Italian pathogen populations of the respective pathogens. These isolates, collected from experimental nurseries located in Central Italy, were chosen because of their virulence characteristics with respect to known resistance genes. The *Bgt* isolate O2 was virulent to many known mildew resistance genes, including *Pm1*, *Pm2*, *Pm3c*, *Pm4a*, *Pm4b*, *Pm5*, Pm6 and *Mli*, but it was avirulent to *Pm3a*, *Pm3b* and *Pm3d*. The two *Pgt* isolates showed low infection types (ITs 0; to 2) on differential lines with resistance genes *Sr17, Sr24, Sr26, Sr27, Sr31, Sr35*, *Sr38* and high infection types (ITs 3 to 4) on lines with *Sr5*, *Sr6*, *Sr7b*, *Sr8a, Sr8b, Sr9a, Sr9b*, *Sr9e, Sr9d, Sr9g, Sr10, Sr15, Sr22, Sr36, Sr38* and *SrTmp.* Seedlings, with the first leaf fully expanded, were inoculated and incubated at 100% relative humidity for 24h at 20°C in the dark and then placed on greenhouse benches covered with clear plastic chambers, at 22 ± 2°C with a photoperiod of 14 h.

For what concerns powdery mildew infection types at the seedling stage were recorded 10-12 days after inoculations, following the 0-4 infection type (IT) scoring system, in which ITs from 0 (no micelia) to 2 (small micelia spots) were considered the expression of resistance and ITs from 3 to 4 (dense and large micelia spots) were considered as host susceptibility (Pasquini and Delogu, 2003). Regarding stem rust, infection types (ITs) on the basis of a 0-4 scale according to Stakman *et al*. (1962) were assessed 12 and 15 days post inoculation. Also in this case infection types from 0 to 2 were considered as a low response, indicating a resistant or moderately resistant host. Infection types from 3 to 4 were considered as a high response, indicating a moderately susceptible or susceptible host.

D. DNA extraction and Marker Analysis

Seedlings from the controlled infection experiment carried-out at CRA-QCE, Rome, were sprayed with fungicide after scoring the response to *Bgt*, and moved to the glasshouse of University of Tuscia in Viterbo for growing until the grain ripening stage. The tips from newly emerged leaves were used for DNA extraction applying the DNeasy Plant Mini kit (Qiagen) according to the manufacturer instructions.

Polymerase chain reaction (PCR) amplification using the NAU/Xibao15$_{902}$ foreward and reverse primers flanking the coding sequence of *Pm21* gene located in the short arm of chromosome 6V#4 (Cao *et al*., 2006) took place in 25-µL volume, running in a GeneAmp PCR System 9700 (Applied Biosystems) thermocycler. The PCR mixture consisted of 1x PCR buffer, 0.2 mM of each dNTPs, 5 pmol of each primer, 1 unit of *Taq* DNA polymerase, and an amount of 20 ng of DNA template. Reagents were obtained from Applied Biosystems (Foster City, CA). Temperature profiles consisted of an initial DNA denaturation at 94° C for 3 min, and then 32 amplification cycles according to the following programme: 94° C for 30 s, 55° C for 30 s, and 72° C for 2 min. A final 8-min extension at 72° C was also employed.

The amplification products were separated on 1.5% (w/V) agarose gel in TBE buffer (1×), stained with ethidium bromide; the gels were visualized under UV light and pictured using the Kodak Gel Logic 100 Imaging System.

III – Results and discussion

The average chromosome number in the progenies ‘467-68.1’ and ‘491-50.2’ was 2n=31, and the highest proportion of metaphase plates contained 2n=32 chromosomes (Table 1). The homologous pair of 6V#4 was present among the 31 chromosomes of ‘467’-68.1, together to 14 A, 14 B, and 3 D chromosomes (Fig. 3). The NAU/Xibao15$_{902}$ molecular marker linked to the *Pm21* locus in 6V#4 which carry the putative gene determining resistance to *Bgt,* was detected in all the seedlings of the ‘467-68.1’ and ‘491-50.2’ progenies and in ‘CSxV63 (Fig. 4) but was absent from the ‘4.5.1’ and ‘Creso’ durums.

The parental lines ‘CSxV63’ and ‘4.5.1’ when tested at Martonvásár with *Pg*t isolates during the seedling stage, expressed infection types that denoted host susceptibility. When tested at CRA-

QCE-Rome, a similar response was observed for ‘4.5.1’ but the ‘CSxV63’ line was resistant. This result might be explained by assuming different effects of the pathogen-genotype x host-genotype interaction exerted by the *Pg*t isolates used in Rome experiments compared to the *Pg*t isolates used in the Martonvásár experiments. The resistance to *Pgt* and *Pt* expressed at the seedling stage by ‘CSxV63’ is an unexpected observation, because in previous infection experiments, the genes for resistance to leaf rust were fully expressed at adult stage rather than at the seedling stage in the ‘CSxV63’ parental line (Bizzarri *et al*., 2009).

Table 1. Chromosome number counted in metaphase plates prepared from root-tips of seedlings of the progenies ‘ 491-50.2’ and ‘467-68.1’. The progenies were obtained from the plants ‘467’ and ‘491’ whose pedigree is drawn in Fig. 1.

Chromosome	Metaphase plates (%)	
No.	491-50.2 progeny	467-68.1 progeny
26	9.4	3.2
27	0	4.3
28	3.1	10.6
29	6.3	1.7
30	14.6	21.3
31	6.3	8.5
32	18.8	28.7
33	15.6	3.2
34	15.6	8.6
35	9.3	0
Average	31.5	30.5

All the seedlings from the ‘467-68.1’ progeny were consistently resistant to the *Pg*t and *Pt*, isolates used in the controlled infection experiments (Table 2). The seedlings of the ‘491-50.2’ progeny expressed susceptibility symptoms when infected with *Pg*t isolates at Martonvásár (no data were available from the experiment in Rome due to poor seedling growth), but displayed resistance when infected with *Pt* isolates (Table 2). It is not known whether the rust resistance genes in 6V#4 interact with other genes in the chromosomes of the ‘467-68.1’ line to produce improved resistance. However, the two lines had both an average chromosome number of 31, and the extra chromosomes over the euploid 2n=28 number, might be different between the two lines, providing opportunities for differential interaction. In other instances, it has been found that rust genes such as *Lr34* can interact with other genes to give enhanced levels of resistance (Dyck and Samborski, 1982; Dyck, 1991).

Table 2. Tested materials at the seedling stage for response to isolates of stem rust (*Pg*t), leaf rust (*Pt*) and powdery mildew (*Bgt*) in controlled infection experiments carried-out at CAR-HAS, Martonvásár (Hungary) and CRA-QCE, Rome (Italy).

Tested entry	Response(1) to			
	Pgt Martonvasar	***Pgt*** Rome	***Pt*** Martonvasar	***Bg*** Rome
467-68.1	0/N	1-	X	1=
391-50.2	3	°	2	0
CSxV63	3	0	4	0 to 1
CSx32	4	3+	X	0 1
4.5.1	3	3-	3	3-
Cappelli	4	4	3	3

(1) Infection types 0, N, X, 1, 2 indicate a resistant host response; Infection types 3-, 3, 3+ and 4 represent susceptible reactions.

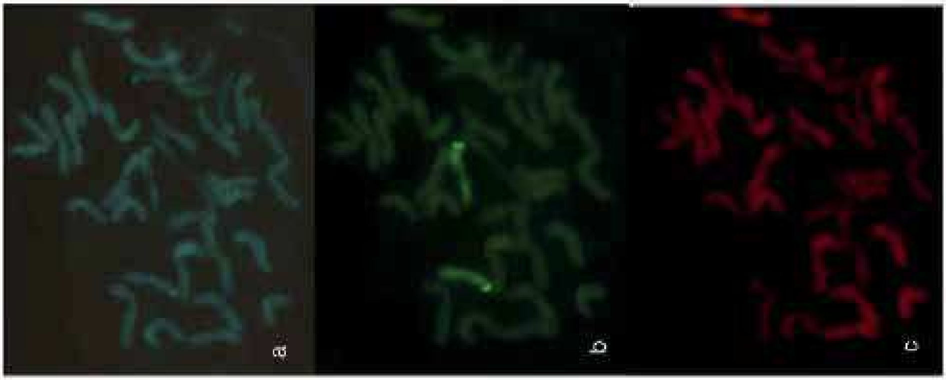

Figure 3. Metaphase plate in a root-tip meristem cell of the line '467-68.1' containing 33 chromosomes (14 'A', 14 'B', 3 'D', and 2 '6V'). (a) DAPI staining; (b) GISH using labeled DNA of *D. villosum* (FITC) and *Ae. speltoides* (wheat B genome) blocking DNA; (c) GISH using labeled DNA of *Triticum urartu* (A genome; Cy3) and *Ae. speltoides* blocking DNA. The 6V chromosome pair can be seen in b, and 14 chromosomes of wheat A genome can be seen in c. ×1,500.

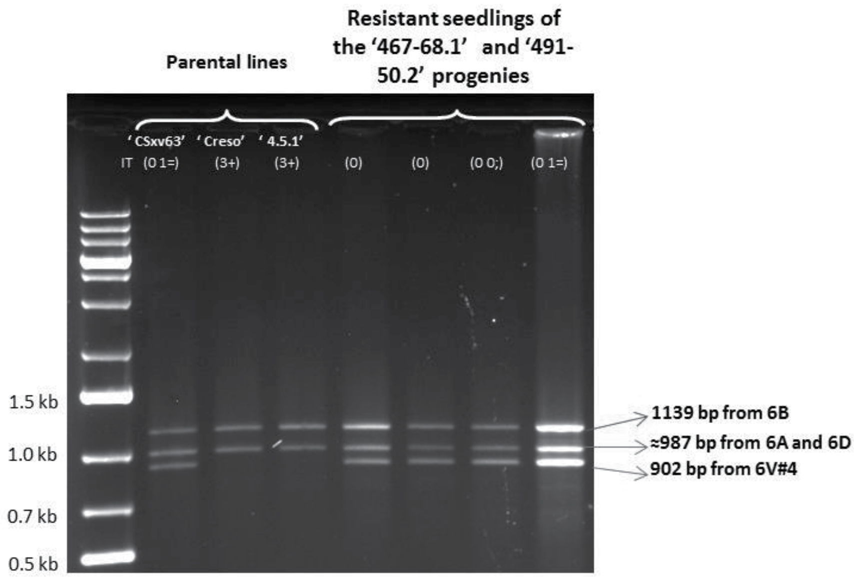

Figure 4. Amplicon of 902 bp obtained from the PCR using NAU/Xibao15 primers flanking the locus *Pm21* on the short-arm of 6V#4 containing a gene encoding a serine/ threonine protein kinase gene (*Stpk-V*) conferring broad-spectrum resistance to powdery mildew caused by Bgt. The primers amplify also an orthologous amplicon of 1.139 kbp from 6B, and another orthologous amplicon of about 0.987 kbp from 6A and 6D chromosomes. The 902 bp amplicon was absent in the pattern of the '4.5.1' parental line and in the 'Creso' durum wheat, but was present in 'CSxV63' parental line, and was detected in all the plants of the '467-68.1' and '491-50.2' progenies expressing infection type (IT) denoting host resistance to *Bgt*.

All the seedlings from the '467-68.1' progeny were consistently resistant to the *Pg*t and *Pt*, isolates used in the controlled infection experiments (Table 2). The seedlings of the '491-50.2' progeny expressed susceptibility symptoms when infected with *Pg*t isolates at Martonvásár (no data were available from the experiment in Rome due to poor seedling growth), but displayed resistance when infected with *Pt* isolates (Table 2). It is not known whether the rust resistance genes in 6V#4 interact with other genes in the chromosomes of the '467-68.1' line to produce improved resistance. However, the two lines had both an average chromosome number of 31, and the extra chromosomes over the euploid 2n=28 number, might be different between the two lines, providing opportunities for differential interaction. In other instances, it has been found that rust genes such as *Lr34* can interact with other genes to give enhanced levels of resistance (Dyck and Samborski, 1982; Dyck, 1991).

Infection with *Pt* isolates demonstrated that both parental lines were susceptible at the seedling stage while the 'CSxV32' control line carrying also 6V#4 and both '467-68.1' and '491-50.2' progenies, displayed a resistant infection type. Since the 'CSxV63' and 'CSxV32' IBLs contain the same 6V#4 but in a different genomic background (6B is missing in 'CSxV32), the different reaction of the two IBLs to *Pt* infection at Martonvásár ('CSxV32' is more resistant than 'CSxV63) might reflect the possibility that the resistance genes to *Pt* in 6V#4 interact with genes in 6B of the 'CSxV63' line resulting in higher susceptibility rating. Such possibility of interactions in the tested lines needs further investigation.

All the entries with chromosome 6V#4 (the progenies '467-68.1' and '491-50.2', 'CSxV63' and 'CSxV32') were highly resistant to *Bgt*, while the durum wheat entries '4.5.1' and 'Cappelli' were susceptible, confirming that 6V#4 carry the allele for resistance to *Bgt* at the *Pm21* locus.

IV – Conclusions

All the seedlings from the '467-68.1' progenies were consistently resistant to virulent strain of the *Pg*t, *Pt*, and *Bgt* pathogens because they inherited, from the 'CSxV63' parental line, the chromosome 6V#4 with the genes for resistance to races of these pathogens. The resistance to *Pg*t and *Pt* expressed at the seedling stage was an unexpected observation, because in other experiments it was shown that the rust resistance genes were expressed at the adult stage. Selected plants from the '467-68.1' progeny with chromosome number ranging from 28 to 30 and expressing resistance to rusts and powdery mildew under controlled experiments, are the best candidates for: (a) scoring their response to airborne inoculum of *Pg*t, *Pt*, *Pst*, and *Bgt* at the adult stage and (b) completing the transfer of chromosome 6V#4 in the euploid 2n=28 durum wheat genome by a final round of backcross to the '4.5.1' durum wheat recurrent parent.

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