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Transferring Useful Rye Genes to Wheat, Using Triticale as a Bridge

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Abstract: Rye has already proven to be a good donor of genes for improving important traits and diversity in wheat breeding. The agronomic advantages of wheat-rye translocations, as well as their detrimental pleiotropic effects, were shown to be dependent on the source of the transferred rye chromatin. This justifies continued effort for introgression of rye genes from various sources into various wheat backgrounds. There are still many genes of interest for wheat improvement, not yet transferred, that are available in the rye genome. This paper describes the strategy applied at the National Agricultural Research and Development Institute Fundulea (Romania), to take advantage of the existence of intensive breeding programs in both winter wheat and triticale, and presents some of the results obtained so far by applying this strategy, in obtaining lines with common bunt, barley yellow dwarf (BYDV) and other diseases resistances, as well as improved seedling vigour and crop spectral reflectance.

Keywords: Albedo; bunt; BYDV; markers; vigour

Increased genetic diversity in wheat breeding is desirable for dealing with present and future challenges caused by the need for increased yields, by climate change, and by higher consumer concerns about food safety. Rye has already proved to be a good donor of genes for improving important traits and diversity in wheat breeding (Zeller & Hsam 1983). Many wheat cultivars carrying wheat-rye translocations, particularly involving the short arm of rye chromosome 1R (1RS), have proved successful worldwide (Lukaszewski 1990; Rabinovich 1998; Schneider & Molnár-Láng 2009). Wheat-rye translocations determine a number of useful characteristics such as disease resistance (powdery mildew, stem rust, leaf rust and stripe rust) or tolerance to barley yellow dwarf virus, and insect resistance (Hessian fly, Russian wheat aphid, green bug). They have also been reported to improve yield potential, stress tolerance, and adaptation in bread wheat (Friebe *et al*. 1990, 1995; Carver & Rayburn 1994; McKendry *et al.* 1996; Kim *et al.* 2003). However, they can also have detrimental effects on bread-making quality (Graybosch *et al.* 1993; Graybosch 2001), but these effects, as well the agronomic advantages, proved to be dependent on the specific translocation, on the source of the transferred rye chromatin and on the background (Kumlay *et al.* 2003; Kim *et al.* 2004). This justifies continued effort for the introgression of rye genes from various sources into various wheat backgrounds. There are still many genes of interest for wheat improvement, not yet transferred, that are available in the rye genome.

Introgression of rye chromatin into wheat can be now easily detected using DNA markers, and several markers have been developed to detect specific rye chromosomes or chromosome arms (Lee *et al.* 1993, 1995; Koebner 1995; Brunell *et al*. 1999), or to detect rye specific repetitive DNA sequences present in many chromosome

segments, dispersed throughout all chromosomes (Katto *et al*. 2004). These markers can be useful to associate traits presumably transferred to wheat in crosses involving the rye genome, with specific segments of rye chromatin.

Triticale has long been used as a bridge to facilitate rye gene introgressions into wheat (Sebesta *et al.* 1995; Sethi 1989). To take advantage of the existence at the National Agricultural Research and Development Institute Fundulea – Romania (NARDI) of intensive breeding programs in both winter wheat and triticale, as well as of availability of several rye specific DNA markers, a strategy for introgression of useful genes from the rye genome into wheat, and from wheat to triticale, was developed and has been applied in our program since 1999. This paper describes this strategy and reports some of the results obtained so far.

MATERIALS AND METHODS

The strategy adopted at NARDI Fundulea for introgression of useful rye genes into wheat, using triticale as a bridge includes:

- Large scale crossing of the best hexaploid triticales with the best wheat cultivars;
- Backcrosses with wheat. Grains obtained by open pollination of F_1 plants surrounded by wheat are also harvested and used for planting segregating populations;
- Growing F_2–F_4 generations as bulks. Occasionally outstanding wheat like plants are selected beginning from the F_2;
- Selection of wheat-like plants and progenies with traits potentially controlled by rye genes;
- Detecting potential rye genes introgressions using SSR markers specific to rye chromatin (the universal marker of Katto *et al.* 2004);
- Identification of rye introgressions using rye and/or wheat chromosome specific markers and cytological analysis (FISH and GISH);
- Use of identified lines of interest as parents in further crosses and selection of progenies carrying the desired trait(s), using phenotypic selection and specific markers.

In parallel, triticale × wheat crosses are backcrossed with triticale and are used for selecting triticale-like plants, potentially carrying wheat genes useful for triticale breeding. The best lines are included in the next cycle of crosses for both wheat and triticale breeding, as well as in a next cycle of triticale/wheat crosses. The final objective of these parallel recurrent selection breeding schemes is to cumulate as many useful genes as possible, from both species (Figure 1).

It is worth mentioning that the Fundulea triticale breeding program has been based on diverse rye

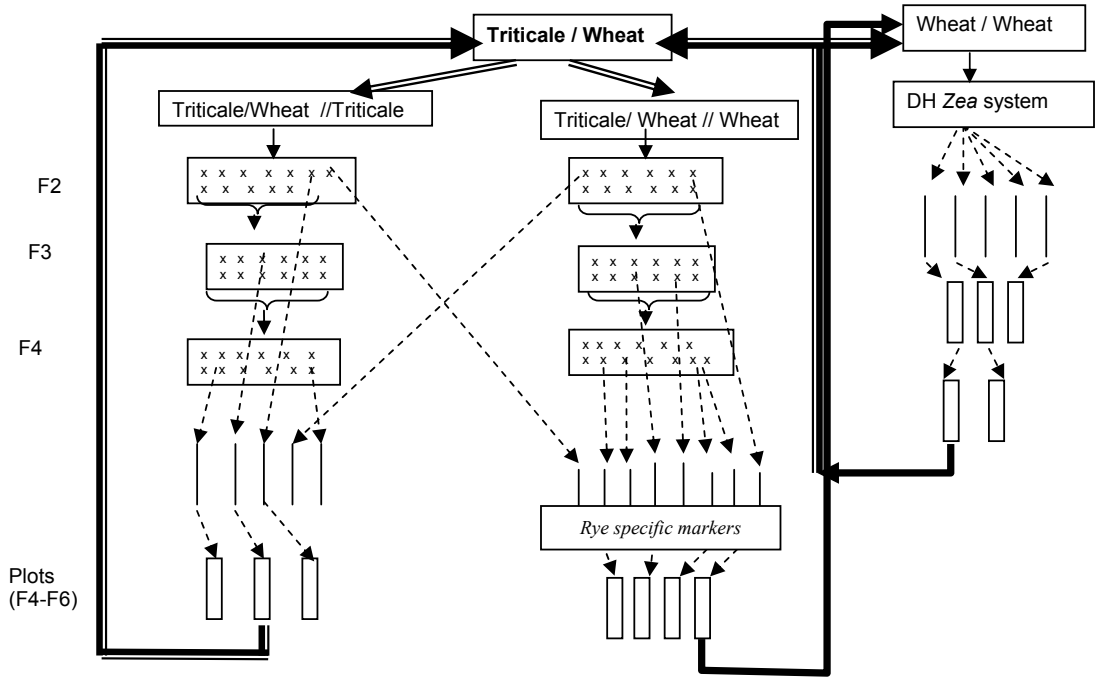

Figure 1. Simplified diagram of the strategy used at NARDI Fundulea for transferring useful rye genes to wheat

germplasm, including the Russian cultivars Saratovskaia krupnaia zerna, Malyshin and short straw mutant EM1, Polish variety Dankowskie Zlote and Romanian variety Gloria, lines from the cross Snoopy-Danae and several inbred lines from the Romanian rye breeding program at Suceava.

Phenotypic selection was performed for traits potentially controlled by rye genome genes, as follows: common bunt resistance under artificial inoculation, Barley Yellow Dwarf Virus in special nurseries, planted about one month earlier than the normal planting date, to favour the attack of aphids, the vectors for the virus; powdery mildew resistance under natural infection, leaf rust resistance under artificial inoculation, albedo (crop reflectance) measured using a portable albedometer (Cotfas *et al.* 2008), seedling vigour estimated visually and by measuring leaf width of the second leaf (as recommended by Richards *et al.* 2001).

To trace rye chromatin introgression in the selected lines, total DNA was isolated from leaves and purified following the protocol proposed by Saghai-Maroof *et al.* (1984), using 2% CTAB. Amplification was performed in a 25 µl final volume using Applied Biosystem 9600 thermal cycler, with Go Taq Polymerase – Promega. To detect rye chromatin, the set of primers F3/R3, described by Katto *et al.* (2004) as a universal marker for rye chromatin was used, using their protocol, i.e. 5 min at 94°C, 30 cycles, each consisting of 1 min at 94, 55 and 72°C, and a final extension of 10 min at 72°C. For some of the lines, where we detected introgression of rye chromatin, additional markers were used to identify the translocation involved. Primers for SCM9, specific to the 1RS rye chromosome (Saal & Wricke 1999), were used according to the protocols described at http://maswheat.ucdavis.edu.protocols/Drought/index.htm, i.e. 2 min at 94°C, 40 cycles, each consisting of: 60 s at 94°C, 60 s at 60°C, 60 s at 72°C and a final extension of 5 min at 72°C. PCR products were evaluated by electrophoresis on 1.2% agarose (for routine use-Sigma) for F3/R3 primers and 2% agarose (High resolution-Sigma) gels for SCM9; with 0.5× TBE buffer. The bands were visualized by ethidium bromide staining. Images were taken with the help of a BioPrint documentation system.

Occasionally, FISH and GISH analysis was performed to confirm the location of the translocation.

We have observed large variation in end-use quality in progenies carrying rye chromatin, therefore testing quality parameters is an essential part of the program for introgressing rye genes into wheat.

RESULTS AND DISCUSSION

Using the above mentioned strategy and methods, transfers of rye genes was quite efficient, as 37% of the wheat like plants selected phenotypically in the F_2 were found to carry rye chromatin, of which 31% were 1R carriers. The percentage of rye chromatin carriers increased slightly in the selected F_3 head-rows (Table 1). In subsequent generations, several lines were selected, which are potentially useful in breeding wheat for the following traits.

Common bunt resistance

Despite progress in developing effective systemic fungicides, bunt resistance continues to be of interest, especially for organic farming, where chemical treatments are forbidden, but also for small farmers, which sometimes do not use chemical treatments or do not apply them correctly. Starting from the observation that most rye and triticale cultivars are resistant to wheat bunt (*Tilletia* sp.), we tested many lines derived from triticale/wheat crosses, by artificially inoculating the seed with bunt spores.

Table 1. Results of molecular analysis with rye specific markers

	Individuals (%)	
	in plants selected in F_2	in headrows selected in F_3
With rye chromatin, of which	37.0	42.0
– with 1R	31.0	34.0
– with other rye chromosomes	6.0	8.0
Without rye chromatin	63.0	58.0

Several lines from the cross F00628G, showed resistance to common bunt races prevalent in Romania (Ittu *et al.* 2006; Oncica & Saulescu 2008). They also showed resistance in the European *Tilletia* Ring test, coordinated by Fabio Mascher-Frutschi, ACW, Swiss (Table 2).

Molecular analysis using Katto's universal marker for detection of rye chromatin showed that F00628G34-1 carry rye chromatin, and analysis with SCM9 proved that rye chromosome 1R was present. To further clarify the nature of the rye chromatin transfer present in line F00628G34-1, FISH analysis using probes pSc119 marked with flouorogreen and AFA family marked with fluorored, as well as GISH analysis were used, with the assistance of the wheat genetics team of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary. Results showed that the rye introgression in line F00628G34-1 is a 1A/1R translocation.

Studies are underway to clarify the genetic control of bunt resistance in this line and the association of this resistance with the 1A/1R translocation.

Line F00628G34-1 also showed resistance to powdery mildew (*Erysiphe graminis* f.sp. *tritici*), leaf rust (*Puccinia recondita*) and stem rust (*Puccinia graminis*), both at Fundulea and according to the results of the 16th Facultative Winter Wheat Observation Nursery for Irrigated conditions (16th FAWWON-IR), 2008–2009 season, (http://

Table 2. Results of testing the bunt resistance of line F00628G34-1 in Romania and other European countries in the European *Tilletia* Ring test

Location	Contributor	Year	*Tilletia* source	Bunted spikes (%)	
				susceptible check	F00628G
Fundulea, RO	Ittu M.	2005	mix of spores	45.4	0
Fundulea, RO	Ittu M.	2007	mix of spores	46.8	0
Fundulea, RO	Ittu M.	2007	FUN	66.7	0
Fundulea, RO	Ittu M.	2007	SIM	83.5	0
Fundulea, RO	Ittu M.	2007	SUA	80.3	0
Fundulea, RO	Ittu M.	2008	mix of spores	59.9	0
Fundulea, RO	Ittu M.	2009	mix of spores	97.6	0
Nyon, CH	Mascher Frutschi F.	2007	mix-CH	83.7	2.7
Nyon, CH	Mascher Frutschi F.	2007	Wilchingen-CH	90.9	0.5
Dottenfelderhof, DE	Spieß H.	2007	local	48.6	0
Tulln, AT	Buerstmayr H.	2007	mix-AT	50.4	11.5
Slagelse, DK	Nielsen B	2007	mix-DK	24.8	1.5
Odessa, UA	Babayants O.	2007	Race T7	100.0	7.4
UA	Babayants O.	2007	Race T9	91.5	4.2
UA	Babayants O.	2007	Race T17	98.3	9.3
UA	Babayants O.	2007	Race T02	95.6	4.7
Darzau, DE	Timmerman M.	2007	mix-DE	8.3	0
Tulln, AT	Buerstmayr H.	2008	mix-AT	64.0	0
Le Subdray, FR	Du Cheyron P.	2008	mix-FR	24.8	0
Darzau, DE	Timmerman M.	2008	mix-DE	91.6	15.5
Nyon, CH	Mascher Frutschi F.	2008	mix-CH	29.7	2.6
Nyon, CH	Mascher Frutschi F.	2008	Wilchingen-CH	29.7	3.0

RO – Romania, CH – Switzerland, DE – Germany, AT – Austria, UA – Ukraine, DK – Denmark, FR – France

www.iwwip.org/). However, we do not have yet any data that would associate these traits with the rye introgression.

The line F00628G34-1 has had a good performance in yield tests, both in Romania and in the 16th FAWWON in 2008–2009. Its bread-making quality is rather low, but this can be attributable not only to the presence of 1R chromatin, but also to the 2 + 12 HMW glutenins.

Resistance to BYDV

Warm and long autumns favour infestation of the wheat crop by aphids, which are vectors for the BYDV virus. We have seen in the last few years higher autumn temperatures, making BYDV a serious problem for wheat. Climate change scenarios forecast conditions that would make BYDV one of the most dangerous wheat diseases. BYDV resistance was transferred to wheat from *Thinopyrum* (*Agropyron*) *intermedium*, but reliance on only one resistance gene may be unsafe. Rye was already cited as a possible source of tolerance to BYDV in a 2R translocation line (Nkongolo & Comeau 1998).

Good BYDV resistance was previously identified in the Fundulea triticale breeding program, in several hexaploid triticale cultivars, with resistance most probably coming from rye, because the genomes A and B are not known to carry BYDV resistance genes.

Several lines, derived from crosses between BYDV resistant triticale lines and wheat, were tested in a nursery planted very early for favouring virus transmission by aphid vectors. Lines presented in Table 3, showed good virus resistance, at the level of best wheat lines carrying the resistance gene from *Thinopyrum intermedium.*

Improved seedling vigour

Improved seedling vigour can improve stand establishment and increase water use efficiency by reducing water waste through evaporation (Rebetzke & Richards 1999). There is little genetic variation for this characteristic among semi-dwarf wheat cultivars (Richards *et al.* 2001), but triticale has a much better seedling vigour than most wheat cultivars.

We identified variation for first and second leaf width among lines derived from triticale × wheat crosses. The best line identified so far, line F06659G1-1, selected from the cross triticale

Table 3. Wheat lines obtained from triticale/2 × wheat crosses, selected for resistance to BYDV

Line	Genealogy	BYDV scores	
		score 1	score 2
F04294T1-2	triticale 93161T2-2201/2 × wheat	2	3
F06659G4	triticale 94896T2-1011/2 × wheat	3	2
F05901G3-3	*Thinopyrum intermedium* derivative check	3	3
Wheat	susceptible check	6	6

Table 4. Width of first and second leaves in line F06659G1-1, derived from triticale/2 × wheat crosses, as compared with triticale and wheat cultivars (in mm)

	First leaf		Second leaf	
	average width	SD	average width	SD
Triticale cv. Cascador	0.780	0.078	0.895	0.089
Wheat cv. Glosa	0.450	0.064	0.621	0.081
Wheat cv. Miranda	0.550	0.100	0.680	0.057
Line F06659G1-1	0.629	0.039	0.771	0.039

SD – standard deviation

94896T2-1011/Izvor//wheat, has significantly larger seedling leaves than wheat, though not as large as triticale (Table 4). The F3/R3 universal marker identified rye chromatin in this line.

Improved albedo (crop spectral reflectance)

The radiative energy load on the canopy (net radiation), of which only a fraction is used for photosynthesis, is dissipated mainly by transpiration. A reduction in transpiration can be achieved by reducing net radiation by way of reflection, namely increasing crop albedo (Blum 2005). The cropland albedo is also of primary importance for determining the magnitude of the global temperature change (Matthews *et al*. 2003). Ridgwell *et al*. (2009) calculated that increasing canopy albedo by 20% drives a > 1°C reduction in summertime surface air temperatures in a wide latitudinal band spanning North America and Eurasia.

Rye and triticale generally have higher albedo than most wheat cultivars. We visually identified several lines, derived from triticale/wheat crosses, which have higher glaucousness than the wheat parents. Estimates of albedo using the portable albedometer of Cotfas *et al.* (2008) showed that the line F06622G2-1, derived from a triticale/2 × wheat cross had a 16% higher albedo than the wheat cultivar Boema.

CONCLUSIONS

Using hexaploid triticale as a bridge in transferring useful rye genes to wheat has several advantages such as:

– taking advantage of the large genetic progress made in triticale breeding,
– relatively easy crossing of triticale with wheat,
– selection of potentially useful rye genes based on their expression in the presence of two wheat genomes.

Large scale triticale × wheat crossing and phenotypic selection for traits that might be controlled by rye genes, combined with identification of rye introgressions using rye chromatin specific markers, allowed selection of several lines with good bunt or BYDV resistance, improved seedling vigour or higher albedo.

Parallel recurrent selection and convergent breeding in both wheat and triticale could increase diversity and accelerate genetic progress in both crops.

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