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MOLECULAR AND AGRONOMIC ASSESSMENT OF GENETIC DIVERSITY AND HYBRID BREEDING IN TRITICALE

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- dedicated to my sister Susanne -

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[2] Tams, S. H., A. E. Melchinger, and E. Bauer. 2005a. Plant Breed. 124:154-160.
[3] Oettler, G., S. H. Tams, H. F. Utz, E. Bauer, and A. E. Melchinger. 2005. Crop Sci. 45:1476-1482.
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Abbreviation

AFLP	amplified fragment length polymorphism
ANOVA	analysis of variance
AMOVA	analysis of molecular variance
DNA	deoxyribonucleic acid
EST	expressed sequence tag
f	coefficient of parentage
GCA	general combining ability
GD	genetic distance
GS	genetic similarity
ha	hectare
MI	marker index
MPH	mid-parent heterosis
MPH%	relative mid-parent heterosis
MRD	modified Rogers distance
MRD^2	squared modified Rogers distance
PCoA	principal coordinate analysis
PCR	polymerase chain reaction
PIC	polymorphic information content
RD	Rogers' distance
SCA	specific combining ability
SSR	simple sequence repeat
UPGMA	unweighted pair group method using arithmetic average

General introduction

Plant breeders have a vital interest in the development and release of improved varieties. The two foremost strategies in cereal crops are line and hybrid breeding. In both, assessment of the genetic relationship among genotypes is important for the choice of crossing parents. Genetic diversity largely determines the future prospects of success in breeding programs. In line breeding, a wide genetic distance (GD) between crossing parents results in a broad segregation variance in the offspring and the development of lines with a superior combination of agronomically and economically important characteristics. In all breeding categories except line breeding, heterosis is a major factor (Schnell, 1982). In hybrid breeding, a maximum exploitation of heterosis is possible and, therefore, superior F_1 hybrids can be identified. This strategy becomes attractive if F_1 hybrids outperform their parents and the existing elite line varieties. Therefore, the knowledge of genetic diversity within the breeding material is essential for an effective and successful breeding program.

History of triticale

In the history of cultivated plants, triticale (×*Triticosecale* Wittm.) is a young crop resulting from the hybridization of tetraploid durum wheat (*Triticum turgidum* L.) or hexaploid wheat (*T. aestivum* L.) with diploid rye (*Secale cereale* L.) as male parent. The first report on the intergeneric hybrid was given by Wilson in 1874 about a sterile cross (Wilson, 1876). A fertile hybrid was obtained by Rimpau in 1888 after spontaneous doubling of chromosomes (Rimpau, 1891). The use of colchicine and embryo rescue techniques enabled the extensive production of so-called primary triticale since the 1940s. These newly produced octoploid or hexaploid types were

often agronomically and reproductively unstable but were used as basic breeding material. Commercial triticale programs were initiated in the mid 1950s with secondary triticale being produced by crossing primary triticale or by crossing primary triticale with wheat or rye. Since octoploid types continued to be cytogenetically instable, the work focused predominantly on hexaploid triticale. They combined many of the desirable traits of both of their wheat and rye parents and constituted the commercially grown triticale. The first triticale variety was registered in Germany in 1979 (Bundessortenamt 1979).

Importance of triticale

Triticale is grown worldwide including 24 European countries. Harvest area increased slowly but steadily up to nearly 5% of the total harvest area of small-grain cereals. The importance of triticale is similar to rye in European triticale growing countries (Figure 1).

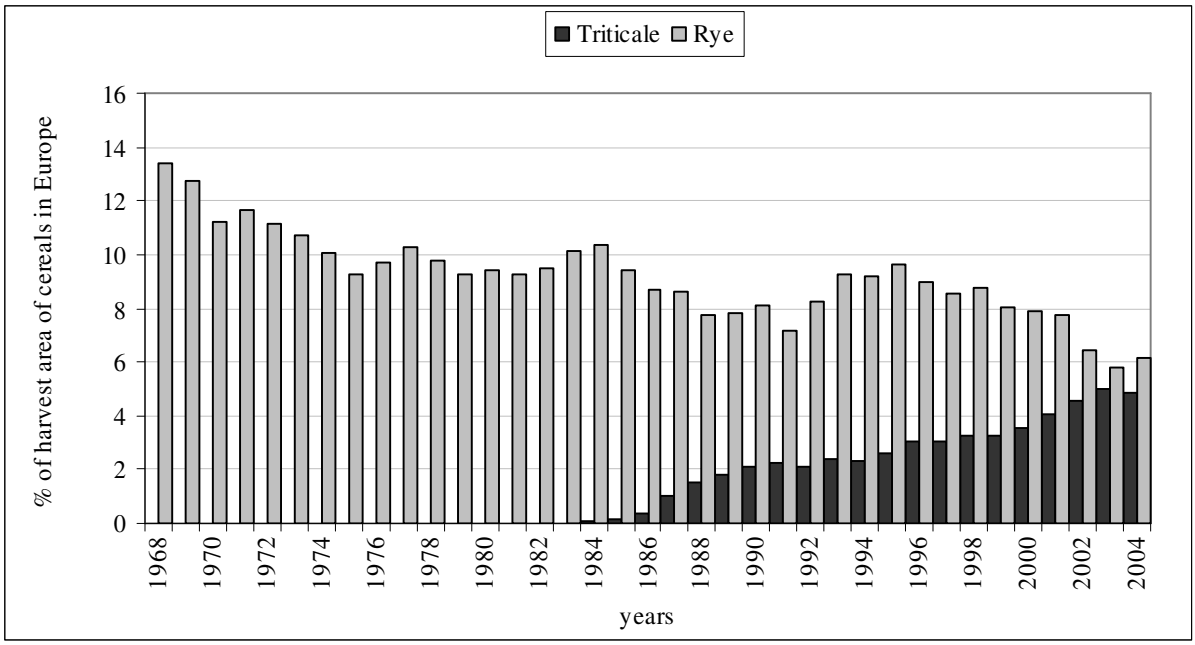

Figure 1: Development of harvest area of triticale and rye in relation to total harvest area of cereals in 25 triticale-growing European countries according to FAO 2005. Triticale-growing countries in Europe are Austria, Belarus, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, France, Germany, Hungary, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

Persistent effort of breeding institutes and breeding companies have led to 199 varieties listed at present (Amtsblatt der Europäischen Union, 2005). In Germany, 13 are protected varieties and a further 22 are listed in 2005. Triticale is mainly bred for the use as is grain feed for pigs and poultry due to its favourable composition of essential amino acids (Cooper and McIntosh, 2001; Horlein and Valentine, 1995). The use as forage crop for cattle is also practiced (Correa et al., 2002). Though triticale is of relatively small importance compared to the major cereals (maize, wheat, barley) in Europe, it claims a permanent market share.

Breeding strategies for triticale

In triticale, methods for self-pollinating species are applied in variety development and line breeding is practised at present, though triticale has an estimated outcrossing rate of about 10% (Oettler, 2005). The exploitation of heterosis in many autogamous crops like wheat has only moderate success (Dreisigacker et al., 2005). Hybrids of allogamous species, however, showed a considerable level of heterosis. Due to the genome constitution with one third of the chromosomes from the allogamous rye ancestor and its floral biology of large extruding anthers and some degree of outcrossing, triticale is expected to have more potential for heterosis and hybrid breeding than wheat. First investigations of a small number of hybrid triticale measured relative mid-parent heterosis (MPH%) for grain yield of 9.5% and 10.1% (Pfeiffer et al., 1998; Oettler et al., 2003). Hitherto, a large-scale and comprehensive study with genetically diverse material was lacking.

Genetic diversity assessment

For both, line and hybrid breeding, information about the genetic diversity is the basis for selection of crossing parents. In triticale such information is scarce even though its breeding history is short. Several direct and indirect genetic diversity measures are applied in crop breeding. Calculation of coancestry coefficient (*f*) as an indirect measure for relative genetic similarity (GS) based on ancestry often fails in breeding material. The assumptions made for calculation of *f* does not always apply as in line breeding of self-pollinating crops selection often takes place towards the elite parent. As a consequence, the presumption that descendants inherit half the genome of each parent is violated. Moreover, the assumptions made regarding genetic drift, selection pressure and relatedness of ancestors with known pedigree can result in a biased estimate of GD (Bohn et al., 1999).

Direct genetic diversity estimates based on molecular marker data are the latest methods, which possess the ability to bypass the assumptions inherent to pedigree analysis. A variety of reliable molecular techniques are available for genome analysis in cereals (Graner et al., 1994; Plaschke et al., 1995; Schut et al., 1997). Even though DNA markers have the advantage that they are not influenced by the environment, the extent of their utility depends on the nature of the markers, their number, the genome coverage and the population under investigation as well as their linkage to traits of interest.

Hybridization-based molecular marker techniques such as restriction fragment length polymorphisms (RFLPs; Botstein et al., 1980; Melchinger, 1993) were replaced by polymerase chain reaction (PCR) based methods. The latter are favoured to obtain information about genetic diversity, because of their reliability and higher throughput. Common techniques are microsatellite markers (or simple sequence repeats, SSRs) and amplified fragment length polymorphisms (AFLPs),

which detect differences in fragment size or DNA sequence directly at the DNA level. Both marker systems have been successfully used to determine genetic distances in cereals such as wheat, barley or rye (Barrett et al., 1998; Huang et al., 2002; Soleimani et al., 2002; Almanza-Pinzon et al., 2003; Ordon et al., 2005; Bolibok et al., 2005). In contrast to AFLPs, SSR markers are codominant, multiallelic and chromosome specific but the development of SSRs for a new species is much more time- and cost-intensive. The advantage of AFLPs is that multiple marker bands are generated in a single assay without prior knowledge of species-specific DNA sequences. Though both marker systems detect polymorphisms directly at the DNA level, the cause of the polymorphisms and the conclusion towards genetic distances related to phenotypic characteristics between individuals differ.

Hybrid performance and heterosis

Prediction of hybrid performance with sufficient accuracy from parental performance could reduce the costs of the most expensive step in hybrid production, namely the production and evaluation of testcrosses in field trials. The breeding strategy could be optimized by concentrating on few but the most promising hybrid combinations. Recent studies assessing the importance of GCA (general combining ability) and SCA (specific combining ability) in triticale are contradictory. In contrast to Grzesik and Węgrzyn (1998), Oettler et al. (2003) conclude that prediction of GCA for grain yield from parental performance was moderate.

Even though the genetic mechanisms that explain heterosis are not fully understood, it is well documented that crosses between unrelated, and consequently genetically distant parents show greater hybrid vigor than crosses between closely related parents (Stuber, 1994; Hallauer, 1999). Therefore, an estimation of parental genetic distance may be another strategy to predict the most promising hybrid combination

and reduce costs and the number of field trials necessary. The relationship between MPH of hybrids and the genetic distance of their parental inbreds, determined with molecular markers, were investigated both in theory (Charcosset and Essioux, 1994) and in numerous experiments with maize and other crops (Brummer, 1999).

The definition of heterotic groups has been a powerful tool in allogamous species to avoid inferior testcrosses and to increase the line *per se* performance of the parents. Successful heterotic groups in maize are Iowa Stiff Stalk *vs.* Non Stiff Stalk in the US Cornbelt and Flint *vs.* Dent in Europe (Duvick et al., 2004) and in rye 'Carsten' *vs.* 'Petkus' (Hepting, 1978). Melchinger, 1999 showed that inter-group hybrids in maize had greater parental GD and MPH than intra-group hybrids. Separate cultivation of maize and rye populations facilitated their classification into heterotic groups according to their evolutionary history and geographic origin. However, in the breeding history of triticale this potential was not exploited. If heterotic groups cannot be discovered, a first step towards their development is the grouping of germplasm based on genetic similarity (Melchinger and Gumber, 1998). Subsequently, crosses could be made among divergent groups to identify promising heterotic patterns.

The objectives of this PhD study were to investigate the basic parameters of hybrid breeding in the European triticale germplasm and the genetic diversity using PCR-based molecular markers. More specifically, the objectives were to

1. investigate the suitability of SSR markers developed from wheat and rye for application in the allopolyploid genome of triticale;
2. assess the genetic diversity within the European winter triticale germplasm pool with the aid of coancestry coefficient, AFLP and SSR markers;

3. compare and correlate the genetic similarity (GS) estimates of AFLP markers (GS_{AFLP}), SSR markers (GS_{SSR}) and the coancestry coefficient (*f*);
4. determine the level of heterosis in 209 winter triticale hybrids for eight agronomic traits;
5. appraise the relative importance of GCA *vs.* SCA effects for triticale hybrids;
6. calculate correlations between GCA and line *per se* performance and between traits in parents and hybrids;
7. examine the association between parental GD and SCA;
8. investigate the existence of genetically distant heterotic groups in elite germplasm; and
9. draw conclusions for future hybrid breeding in winter triticale.

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ORIGINAL PAPER

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Genetic diversity in European winter triticale determined with SSR markers and coancestry coefficient

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Abstract Knowledge of the genetic diversity of a species is important for the choice of crossing parents in line and hybrid breeding. Our objective was to investigate European winter triticale using simple sequence repeat (SSR) markers and the coancestry coefficient (*f*) with regard to genetic diversity and grouping of germplasm. Three to five primer pairs for each of the 42 chromosomes were selected to analyse 128 European winter triticale varieties and breeding lines. SSR analysis resulted in the identification of 657 alleles with an average of 6.8 alleles per primer pair. The average polymorphism information content (PIC) for polymorphic markers was 0.54. Correlation between *f* and genetic similarity (GS) estimates based on Rogers' Distance was low ($r_{f\times GS(ABDR)}$=0.33). The analysis of molecular variance (AMOVA) revealed that 84.7% of the total variation was found within breeding companies, and 15.3% among them. In conclusion, SSR markers from wheat and rye provide a powerful tool for assessing genetic diversity in triticale. Even though no distinct groups within the European winter triticale pool could be detected by principal co-ordinate analysis, this study provides basic information about the genetic relationships for breeding purposes.

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Introduction

Triticale (×*Triticosecale* Wittm.), the intergeneric hybrid between wheat and rye, has gained considerable importance in recent years in Europe as a feed grain, due to its favourable amino acid composition and performance in less productive environments. Triticale is a partially allogamous crop, but for cultivar development it is treated as a self-pollinator and line breeding is practised. The rye genome portion in triticale nurtures the expectation that the crop has a potential for the commercial use of heterosis in hybrids. First experiments with spring and winter triticale showed on average a nearly 10% mid-parent heterosis for grain yield with a wide range among hybrids (Pfeiffer et al. 1998; Oettler et al. 2003). A basic aspect to fully exploit heterosis is the characterisation of crossing parents with regard to the development of heterotic groups.

The search for and establishment of heterotic groups can be based on geographical origin, agronomical traits, pedigree data or on molecular marker data (Melchinger 1999). Up to now, only two studies have investigated the diversity of genetic resources in triticale. Furman et al. (1997) assessed more than 3,000 genotypes from the United States, Canada and Mexico for agronomical traits, but found only differences between 'complete' and 'substituted' types. A study of American and European triticale based on morphological traits revealed the existence of two main groups, winter and spring types, but no grouping according to geographical origin was possible (Royo et al. 1995).

The coancestry coefficient (*f*) is based on pedigree information and provides an indirect measure for the relative genetic similarity of related individuals. If pedigrees are well documented and reliable, as for example in maize, the establishment of groups is possible (Smith et al. 1985). In triticale, however, primary types were synthesised using tetraploid or hexaploid wheat and rye populations. Secondary types were frequently backcrossed to wheat and rye and pedigree data are not well documented or not reliable. Finally, calculation of *f* has

often failed for estimating genetic diversity in breeding material, because assumptions do not always apply (Messmer et al. 1991; Graner et al. 1994). In self-pollinating crops, selection often takes place towards the elite parent. As a consequence, the assumption that the descendants inherit half the parental genome is incorrect.

Molecular markers are the latest and most reliable tools to characterise germplasm and to estimate the relationship between genotypes at the DNA level. A variety of molecular techniques are available for genome analysis in cereals (Graner et al. 1994; Plaschke et al. 1995; Schut et al. 1997). SSRs in particular have been reported to be useful to analyse the structure of germplasm collections, because they are codominant, multiallelic and chromosome-specific (Ahmad 2002; Huang et al. 2002; Parker et al. 2002). Big efforts have been made by several groups to develop SSR markers for wheat and rye (Röder et al. 1995, 1998; Saal et al. 1999; Prasad et al. 2000; Korzun, personal communication). The presumption that genome-specific wheat SSR markers rarely amplify fragments in rye (Röder et al. 1995) gives the opportunity to assess the diversity of the wheat and rye genomes in triticale separately.

The objectives of this study were to investigate the suitability of SSR markers developed from wheat and rye for application in the composite genome of triticale, to estimate the level of diversity of winter triticale using SSR markers and to determine the correlation between the coancestry coefficient and genetic similarities estimated from SSR markers.

Materials and methods

Plant material and pedigree data

A total of 128 winter triticale varieties and breeding lines of middle and east European origin were made available for this study by 13 breeding companies and institutes from seven countries (Table 1). Pedigree information of the genotypes was submitted confidentially. Furthermore, 18 winter wheat (*Triticum aestivum* L.), 2 durum wheat (*T. durum* Desf.) and 8 winter rye genotypes (*Secale cereale* L.) of German origin were also included in this study as references for marker analysis. The Malécot (1948) coancestry coefficient (*f*) was calculated for triticale from pedigree data using the rules of Cox et al. (1985) with the KIN program (Tinker et al. 1993). If available, pedigree information up to the fourth generation was used for calculating *f* values.

SSR marker analysis

From each genotype, DNA was extracted from 40 mg vacuum-dried leaf tissue of a bulk sample of 15–20 individual plants using the sodium bisulfite method (Schweizer et al. 1995). One hundred and ninety-seven publicly available or proprietary primer pairs (Röder et al. 1995; Saal et al. 1999; Prasad et al. 2000; Hackauf et al. 2002; Korzun, personal communication; Röder, personal communication) were screened to characterise loci containing microsatellite sequences among triticale, winter wheat, durum wheat, and rye genotypes. (The list of the SSR markers is included in the electronic supplementary material.) Polymerase chain reaction (PCR) was performed in 10 μl reaction volumes containing the following reagents: 25 ng of template DNA, 0.2 mM of each of the four dNTPs, *Taq* DNA polymerase buffer, 0.3 U *Taq* DNA polymerase (Amersham Pharmacia Biotech, Freiburg), 150 nM of each of the two primers (one was fluorescence-tagged with Cy5). The PCR program consisted of a 3 min initial denaturation step at 96°C, followed by 30–40 cycles with 1 min denaturation at 96°C, 2 min primer annealing at primer-specific temperature (for details see electronic supplementary material) and 1 min primer extension at 72°C. The resulting amplification products were resolved by electrophoresis in polyacrylamide gels. Signals were scored by an ALF Express (Amersham Pharmacia Biotech) automated sequencer and transferred to a 1/0 matrix. For the final analysis, three to five primers were selected for each chromosome according to the quality of banding pattern and location in the genome.

Data analysis

For each SSR marker, the PIC (polymorphic information content) value was calculated according to Powell et al. (1996) including null-alleles. Genetic similarity (GS) between two triticale cultivars was determined as 1–Rogers' Distance (Rogers 1972) using the statistical software R (Ithaka et al. 1996). As a basis for calculating GS values three different sets of selected markers were used: the whole marker set of 96 loci (GS_{ABDR}), the 68 wheat markers (GS_{ABD}), and the 28 rye markers (GS_R). Genetic distances between groups, defined as breeding companies represented by six or more

Table 1 Country of origin of breeding companies and institutes, their symbol and number of genotypes submitted for the set of triticale varieties

Country of origin	Breeding company/institute	Symbol	No. of genotypes
France	INRA (Institute Nationale de la Recherche Agronomique)	◇	16
Germany	Nordsaat Saatzucht	★	25
Germany	Lochow-Petkus	△	15
Germany	Saatzucht Dr. Hege	✣	16
Germany	SaKa-Ragis Pflanzenzucht	●	10
Germany	W. von Borries-Eckendorf	▲	1
Germany	IG Saatzucht	▼	1
Poland	Danko Breeding	☆	9
Poland	IHAR (Plant Breeding and Acclimatization Institute)	❑	6
Romania	Research Institute for Cereals & Industrial Crops (RICIC)	✚	9
Russia	Agricultural Research Institute of Non-Chernozem Zone (ARINCZ)	■	1
Sweden	Svalöf Weibull	▽	13
Switzerland	RAC (Swiss Federal Research Station for Plant Production)	◆	6

genotypes, were calculated based on Rogers' Distance using the whole marker set (RD_{ABDR}). Correlations of the three estimates based on GS and *f* values were calculated with the computer package PLABSTAT (Utz 2001). Associations among genotypes and companies were revealed by principal co-ordinate analysis (PCoA) based on marker data using the computer package NTSYS-pc-2.11h (Rohlf 1989). To divide the genetic variation into components attributable to the variance within and among triticale genotypes of different breeding companies, an analysis of molecular variance (AMOVA) was performed with the program ARLEQUIN according to Michalakis et al. (1996).

Results

SSR markers which were developed in wheat and rye proved to be suitable for analysing the composite genome of triticale. Altogether, SSR markers for 197 loci were tested. To ensure an even distribution of the markers over the entire triticale genome, we selected 3–5 primers with a clear banding pattern for each chromosome. This set consisted of 93 markers detecting 96 loci (the complete list of the SSR markers is given in the Electronic Supplementary Material). A total of 657 fragments were obtained. In the bulk DNA samples of the triticale genotypes, 10.9% of all loci showed more than one band per SSR marker.

Out of the 39 D-genome-specific markers tested, only three, on chromosomes 2D and 7D, amplified products in some triticale genotypes (Table 2). A D-genome specific primer pair for a repetitive sequence (*Dgas44*, McNeil et al. 1994; data not shown) produced an intense signal in 19 of 128 genotypes and weak signals in further 11 genotypes, but the location of these repetitive sequences is unknown. The number of alleles and PIC values varied in a wide range within the set of 128 triticale genotypes (Table 2). Ten of the 28 rye markers were derived from expressed sequences (ESTs), while the others were from genomic libraries. The average number of alleles for the genomic rye markers was 7.8 with a mean PIC of 0.54, in comparison with an average of 2.7 alleles and a mean PIC of 0.29 for the EST-derived markers.

The number of known ancestors in the pedigree information provided for the 128 triticale genotypes was inconsistent. For several lines only one parent was submitted, but for others the complete pedigree up to the fourth generation was available. For all 128 pairwise comparisons of triticale genotypes the coancestry coefficient varied from 0 to 1, with an average of 0.059

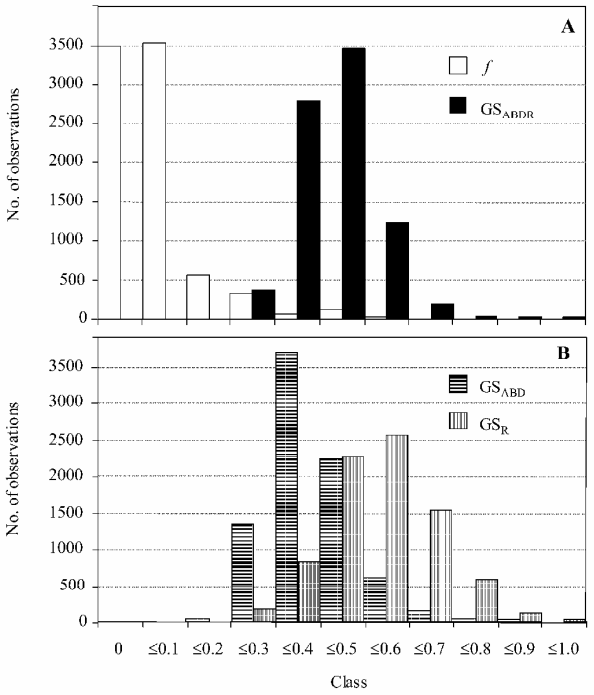

Fig. 1 Distribution of similarity estimates for all pairwise comparisons of 128 triticale genotypes, based on **A** the coancestry coefficient (*f*) and GS_{ABDR}, and **B** GS_{ABD} and GS_R

(Fig. 1A). Of all possible pairwise triticale comparisons, 42% were not related according to the pedigree data. More than 85% had an *f* value smaller than 0.1. Six pairs of genotypes with *f*=1.0 consisted of one genotype and its three mutations. Thus, these four genotypes were regarded as being identical by descent.

For all pairwise comparisons of GS estimates, where the comparison of a genotype with itself was excluded, the GS_{ABDR} was on average 0.43 with a range from 0.16 to 0.94 (Fig. 1A). By comparison, GS_{ABD} averaged 0.38 and ranged from 0.12 to 0.95 and the mean GS_R was 0.54 and ranged from 0.17 to 1.00 (Fig. 1B). Correlations between the coancestry coefficient *f* with GS_{ABDR}, GS_{ABD}, GS_R were low even between related (*f*>0.1) genotypes (Table 3). The moderate correlation between GS_{ABD} and GS_R increased from 0.43 to 0.57 after discarding all unrelated genotypes.

Table 2 Mean and range of number of alleles and the PIC values of SSR markers within triticale, according to their location in the genome

	Location	No. of loci	No. of alleles		PIC	
			Mean	Range	Mean	Range
Wheat genome	A-genome	33	8.9	4–18	0.63	0.28–0.82
	B-genome	32	7.7	3–21	0.57	0.08–0.88
	D-genome	3	2.3	2–3	n.d.[b]	n.d.
Rye genome	R-genome[a]	28	6.0	2–13	0.45	0.03–0.79
Total		96	7.5	2–21	0.54	0.03–0.88

[a] SSR markers from EST and genomic libraries
[b] n.d.: not determined

Table 3 Correlation among estimates of coancestry (*f*) and genetic similarity (GS) based on different marker sets calculated across all triticale combinations (8,128 entries, above diagonal) and across combinations of related genotypes (*f*>0.1, 1,090 entries, below diagonal)

	f	GS_{ABDR}[a]	GS_{ABD}[b]	GS_R[c]
f	–	0.33**	0.34**	0.17**
GS_{ABDR}	0.39**	–	0.93**	0.74**
GS_{ABD}	0.43**	0.96**	–	0.43**
GS_R	0.17**	0.77**	0.57**	–

** Significant at 0.05 level
[a] All markers
[b] Markers from wheat genome
[c] Markers from rye genome

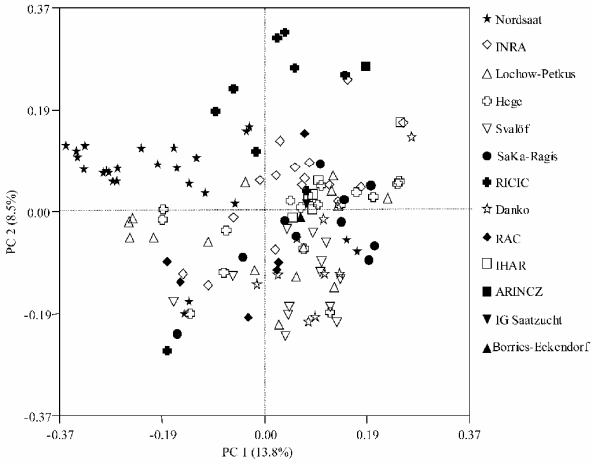

Fig. 2 Two-dimensional principal co-ordinate analysis based on GS_{ABDR} for 128 triticale genotypes. PC1 and PC2 are the first and second principal co-ordinate

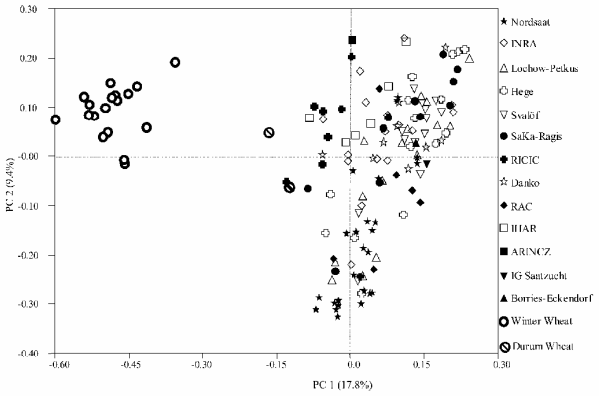

Fig. 3 Two-dimensional principal co-ordinate analysis based on GS_{ABD} with similarity data for 128 triticale, 18 winter wheat and 2 durum wheat genotypes. PC1 and PC2 are the first and second principal co-ordinate

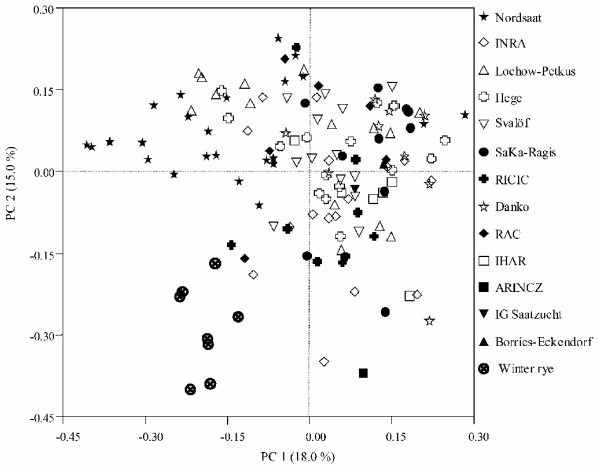

Fig. 4 Two-dimensional principal co-ordinate analysis based on GS_R with similarity data for 128 triticale genotypes and 8 rye genotypes. PC1 and PC2 are the first and second principal co-ordinate

PCoA based on 128 triticale genotypes revealed no distinct groups (Fig. 2). Apart from most of the lines from the breeding company 'Nordsaat' and several genotypes from 'RICIC', there is no clear grouping obvious in the triticale germplasm. The first two principal co-ordinates (PC) together explained 22.3% of the total variation.

To assess the diversity of the wheat genome (ABD) component of triticale, PCoA based on GS_{ABD} was performed and included all triticale and wheat genotypes (Fig. 3). Here, the first two principal co-ordinates together explained 27.2% of the total variation. The two durum wheat genotypes were grouped close to the triticales. The German winter wheat cultivars formed a distinct group. The variation of the wheat genome within triticale was relatively narrow in the first principal co-ordinate (-0.13 to 0.24) in comparison with the second principal co-ordinate (-0.33 to 0.24).

In a separate analysis, PCoA was performed with GS_R values, including triticale and the eight rye genotypes. (Fig. 4). The first two principal co-ordinates explained 33.0% of the total variation. Most of the 'Nordsaat' germplasm formed a distinct group as was also observed for the wheat genome portion (Fig. 3). The genotypes of 'RICIC' were scattered among the other genotypes with regard to the rye genome component.

In the PCoA based on RD_{ABDR} for 10 breeding companies represented by six or more genotypes, the first three principal co-ordinates explained 59.5% of the total variation (Fig. 5). The following groups were clearly separated from other breeding companies by one PC: 'RICIC' (PC1), 'Nordsaat' (PC2), and 'Danko', 'Svalöf', and 'RAC' (PC3). GS_{ABDR} between pairs of companies averaged 0.23 and ranged between 0.18 and 0.40 for those breeding companies represented by six or more genotypes (Table 4). AMOVA based on the whole marker set revealed significant variation of 15.3% among companies

Table 4 Genetic distance based on RD_{ABDR} between companies/institutes (below diagonal) and their standard error (above diagonal)

	Nord-saat	INRA	Lochow-Petkus	Hege	Svalöf	SaKa-Ragis	RICIC	Danko	RAC	IHAR	ARINCZ[a]	IG Saat-zucht[a]	Borries-Eckendorf[a]
Nordsaat	–	0.012	0.015	0.018	0.021	0.018	0.020	0.019	0.019	0.020	0.025	0.028	0.027
INRA	0.30	–	0.014	0.011	0.018	0.012	0.019	0.018	0.014	0.018	0.025	0.026	0.025
Lochow-Petkus	0.27	0.23	–	0.013	0.019	0.014	0.018	0.017	0.017	0.018	0.026	0.026	0.024
Hege	0.29	0.19	0.22	–	0.016	0.010	0.021	0.015	0.016	0.019	0.029	0.029	0.028
Svalöf	0.31	0.27	0.28	0.23	–	0.018	0.023	0.013	0.022	0.022	0.030	0.030	0.029
SaKa-Ragis	0.32	0.21	0.22	0.18	0.27	–	0.020	0.017	0.017	0.020	0.027	0.027	0.026
RICIC	0.38	0.33	0.37	0.35	0.40	0.37	–	0.022	0.023	0.022	0.029	0.032	0.031
Danko	0.33	0.26	0.28	0.23	0.21	0.27	0.38	–	0.020	0.021	0.028	0.030	0.029
RAC	0.30	0.26	0.27	0.25	0.29	0.28	0.39	0.32	–	0.023	0.029	0.032	0.031
IHAR	0.39	0.29	0.33	0.29	0.35	0.30	0.39	0.34	0.36	–	0.030	0.033	0.033
ARINCZ[a]	0.41	0.46	0.43	0.44	0.49	0.45	0.53	0.50	0.42	0.52	–	0.045	0.045
IG Saatzucht[a]	0.51	0.47	0.50	0.45	0.45	0.48	0.55	0.46	0.51	0.53	0.59	–	0.036
Borries-Eckendorf[a]	0.49	0.45	0.47	0.42	0.44	0.45	0.54	0.44	0.47	0.51	0.56	0.13	–

[a] Three groups represented by only one genotype are separated by a dashed line

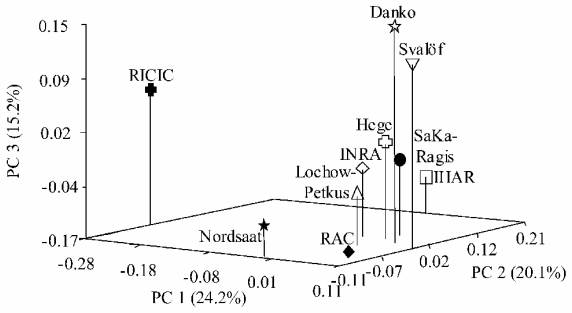

Fig. 5 Principal co-ordinate analysis based on RD_{ABDR} of 10 breeding companies with six or more genotypes. PC1, PC2, PC3 are the first, second and third principal co-ordinate, respectively

in comparison with 84.7% within. Separate computations for the wheat and rye genome portion resulted in similar findings (data not shown).

Discussion

With the objective of selecting and maintaining parental lines to exploit heterosis for a hybrid breeding program in winter triticale, germplasm groups have to be identified and developed. In triticale, the creation of gene pools has not yet received any attention, and pedigree information is scarce and incomplete. For some genotypes used in this study, only information on the female parent was available but for other genotypes the complete pedigree back to the initial wheat×rye cross was submitted by the breeding company. The distribution of *f* values differs clearly from the estimates based on GS_{ABDR} (Fig. 1A), because of the fundamental differences in the concepts underlying both measures (Bohn et al. 1999). Even with detailed and complete pedigree data this would be the case. Hence, the distribution of the *f* values demonstrates the low differentiation power compared with GS estimates. Furthermore, rye as an allogamous species might have transmitted a high degree of heterogeneity to triticale by using population varieties as crossing parents. As ancestor, heterozygous rye in contrast to the strictly autogamous wheat does not comply with the assumption for the calculation of *f* that all ancestors have to be homozygous and homogeneous.

Autogamy limits genetic recombination and allopolyploidy hinders the gene flow from the wild progenitors into the gene pool of the cultivated crop. Therefore, the genetic basis will become narrower during evolution (Spillane et al. 2001). Both mechanisms are absent in rye. Hence, GS_R in triticale should be smaller than GS_{ABD}. However, genetic similarity based on GS_{ABD} with a mean of 0.38 is smaller than that based on GS_R which averaged 0.54 (Fig. 1B). In our study this might be due to the application of 10 EST-derived rye SSR markers instead of genomic markers. The average PIC value of the latter (0.54) was much higher than that of the EST-derived rye SSR markers (0.29) in triticale. The variation of alleles within the expressed regions of DNA is lower but polymorphisms in coding regions might have direct impact on physiology and further on the phenotype. Several groups are working on the isolation of EST-derived SSRs in wheat and rye (Eujayl et al. 2002; Hackauf et al. 2002; Holton et al. 2002), which may improve marker-assisted selection, comparative genetic analysis and exploitation of genetic resources by providing a more direct estimate of functional diversity.

Even though we tested only a limited number of D-genome specific SSR primers, the lack of amplification products in most triticale genotypes (Table 2) agrees with the presumption that winter triticale varieties are 'complete', i.e. without substitutions of D/R chromosomes (Mergoum et al. 1998). We suppose that the observed banding patterns of D-genome specific primers are the result of translocations instead of D/R substitutions, because of the lack of null alleles for the tested R-genome specific primers.

The low but significant correlation between coancestry and DNA-based similarity measures (Table 3) corresponds to findings in barley and wheat (Graner et al.

1994; Bohn et al. 1999; Corbellini et al. 2002). Tighter associations were found in maize (Lübberstedt et al. 2000; Lu and Bernardo 2001; Enoki et al. 2002), where pedigrees are more reliable and the simplifying assumptions more appropriate. In our study, the lowest correlation exists between *f* and GS_R, which corresponds with the uncertainties of calculating the coancestry coefficient for heterozygous ancestors.

PCoA showed no distinct groups within triticale (Fig. 2) except for two companies, i.e. most genotypes from 'Nordsaat' and 'RICIC' were situated apart from the remaining triticale varieties and breeding lines. In contrast, Sun et al. (2001) found a strong grouping according to breeding companies in maize when assessing the genetic diversity of commercial maize hybrids with SSR and RAPD markers. The finding in triticale corresponds with the free exchange of breeding material in self-pollinating crops. A further reason for the lack of distinct groups might be the exclusive use of triticale in Europe for one end-use purpose, namely grain feed. Hitherto, no management of germplasm with regard to hybrid breeding has taken place, which requires the division of the germplasm pool into several sections.

The limited number of wheat and rye genotypes included in the study as references for marker analysis gives a first impression on the relationship between the triticale AB(D)R genome portions and the winter wheat ABD and rye R genome. Clear clustering of the German wheat cultivars (Fig. 3) suggests a low influence on the wheat genome portion of European triticale. To illustrate the impact of *T. aestivum* or *T. durum* on triticale, a broader range of wheat genotypes has to be investigated. Our study, analysing only two durum wheats, might suggest that the AB genome portion of triticale may descend from durum wheat (Fig. 3). German wheat and rye genotypes differ clearly from German triticale with regard to the wheat and rye genome respectively (data not shown). To broaden the genetic diversity of triticale, information on the relationships between a wider range of winter wheat and triticale genotypes is required for the choice of crossing parents.

The AMOVA revealed lower but significant variation among breeding companies (15.3%) than within (84.7%) for RD_{ABDR}. The amount of molecular variance due to breeding programs in a comparable study for sugar beet (DeRiek et al. 2001) was much smaller (2.6%). Another study with seven tropical maize populations revealed only 10.2% between-population variation (Reif et al. 2003). Li et al. (2001) assessed soybean landraces from Korea, Japan and China and found 12.4% variation attributed to variation between countries of origin.

The widest genetic distance was 0.40 between 'Svalöf' and the 'RICIC' and may be attributed to the widest differences in our study for climatic and environmental conditions (Northern Europe vs Southeast Europe). It may also have historical reasons due to the initiation of the breeding programs in different parts of Europe. However, large RD values (0.39) were also found between companies from more similar regions ('IHAR'×'RICIC', 'IHAR'×'Nordsaat', 'RAC'×'RICIC').

Our study shows that wheat and rye SSR markers are suitable for triticale genome analysis. The application of these SSR markers leads to basic information for the development of germplasm pools. The genotypes of breeding companies with the widest differences may be a first basis for establishing heterotic pools in a hybrid breeding program. Parents from putative gene pools have to be selected for testcrosses to evaluate heterosis and hybrid performance. First results will be published in a companion study, where F_1 hybrids have been tested in field trials to assess hybrid performance and heterosis.

Acknowledgements This paper is dedicated to Prof. Dr. agr. H.H. Geiger on the occasion of his 65th birthday. The present study was supported by grants from Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft (BMVEL) and Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP, grant G88/00HS). The authors gratefully acknowledge the skilled technical assistance of Angela Harmsen. We are indebted to the breeding companies for making available seeds and supplying confidential pedigree information. We thank Dr. Marion Röder, Dr. Bernd Hackauf and Dr. Viktor Korzun for providing confidential primer information.

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Genetic similarity among European winter triticale elite germplasms assessed with AFLP and comparisons with SSR and pedigree data

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Abstract

Genetic similarities (GS) based on molecular markers are well suited for direct exploration of relationships within a germplasm pool. The objectives of this study were to: (i) assess the genetic diversity in the European winter triticale germplasm by using AFLP markers, and (ii) compare the GS estimates of AFLP markers, simple sequence repeat (SSR) markers and MALÉCOT's coancestry coefficient (*f*). A representative set of 127 European winter triticale varieties and breeding lines, previously investigated with SSR, was assessed with 10 *Pst*I/*Taq*I primer combinations (PC). AFLP analysis identified 344 polymorphic fragments with an average polymorphic information content per PC of 0.25 and a marker index of 8.56. GS-values between genotypes (calculated after DICE) averaged 0.61 for AFLP and 0.43 for SSR. The mean *f*-value was 0.06. Dendrograms based on 'unweighted pair-group method and arithmetic average' showed no clear groupings within the triticale germplasm pool, but smaller clusters were consistently found. Both molecular marker systems were superior to the coancestry coefficient for genetic diversity assessment within the elite triticale germplasm.

Key words: x*Triticosecale* — cluster analysis — coancestry coefficient — genetic diversity — molecular markers

Triticale (×*Triticosecale* Wittm.) is an intergeneric hybrid between wheat and rye and a partially allogamous crop. For cultivar development it is treated as a self-pollinator and line breeding is practised at present. However, hybrid breeding has come into focus recently (Oettler et al. 2003). For both line and hybrid breeding, information about the genetic diversity within a germplasm pool is the basis for the selection of crossing parents and establishing heterotic groups. In triticale such information is scanty.

Genetic diversity can be determined via agronomic and biochemical characters, which are, however, strongly influenced by the environment. The coancestry coefficient is another indirect measure and based on the probability that alleles at a certain locus are identical by descent. Underlying pedigree data frequently contain erroneous or incomplete information. For this reason, and owing to unrealistic assumptions in calculating *f*, estimations often fail to describe the correct relationship (Graner et al. 1994). Molecular marker techniques detect differences directly at the DNA level and are not influenced by the environment. However, the extent of their utility in a crop species may depend on the nature of the markers, their number, genome coverage and the population under investigation as well as their linkage to traits of interest.

Polymerase chain reaction (PCR)-based marker systems such as simple sequence repeats (SSR) and amplified fragment length polymorphisms (AFLP) have been successfully used to determine genetic distances in cereals (Barrett et al. 1998, Huang et al. 2002, Soleimani et al. 2002, Almanza-Pinzon et al. 2003). Codominant SSR are highly polymorphic, but their development is very time- and cost-intensive. In a companion study, genetic diversity assessment of the composite genome of triticale was accomplished with the application of SSR markers previously developed in wheat or rye (Tams et al. 2004). The major advantage of AFLP compared with SSR is the generation of multiple marker bands in a single assay without prior knowledge of DNA sequences. In triticale, the AFLP technique has not yet been applied.

The objectives of this study were to (i) assess the genetic diversity within the European winter triticale germplasm pool with the aid of AFLP markers and (ii) compare the genetic similarity (GS) estimates of AFLP markers (GS_{AFLP}), SSR markers (GS_{SSR}) and the coancestry coefficients.

Materials and Methods

Genetic material and pedigree data: A set of 128 genotypes of winter triticale (varieties and breeding lines) of central and east European origin from 13 breeding companies and research institutes was analysed. In dendrograms, the names of released cultivars were given, but breeding lines were coded (Table 1). For one genotype two seed sources were assayed (cultivar 'FOCUS' and seed of the corresponding breeding line 'NoSaF'). The coancestry coefficient *f* (Malécot 1948) was calculated from pedigree data using the rules of Cox et al. (1985) with the KIN-program (Tinker and Mather 1993).

AFLP and SSR marker analysis: For AFLP analysis, DNA was extracted from a bulk of 15–20 individual plants from each genotype. AFLP markers were generated by Keygene N.V. (Wageningen, the Netherlands) using the procedure of Vos et al. (1995). The design of *Pst*I and *Taq*I primers was described by Reijans et al. (2003). Ten of 15 prescreened *Pst*I/*Taq*I PC were selected for analysis. Banding patterns were transferred to a binary data matrix. Monomorphic AFLP markers were excluded from the analysis of genetic diversity. When, in some genotypes, a given AFLP fragment had an intermediate band intensity, the fragment was scored 'uncertain' and treated as a missing value for the calculation of GS. For computation of MI, these fragments were regarded as present.

In a companion study (Tams et al. 2004), 93 publicly available or proprietary primer pairs (Röder et al. 1995, Saal and Wricke 1999,

Table 1: Country of origin of breeding companies or research institutes, their codes and number of winter triticale genotypes

Country of origin	Breeding company/Institute	Code for breeding lines	No. of genotypes
France	Institut Nationale de la Recherche Agronomique (INRA)	INRA	16
Germany	Nordsaat Saatzucht GmbH	NoSa	25
Germany	Lochow-Petkus GmbH	LoPe	16
Germany	Saatzucht Dr Hege GbRmbH	Hege	14
Germany	SaKa-Ragis Pflanzenzucht GbR	SaKa	10
Germany	W. von Borries-Eckendorf GmbH & Co.	–[1]	1
Germany	IG Saatzucht GmbH & Co. KG	–	1
Poland	Danko Breeding Co. Ltd.	–	9
Poland	Plant Breeding and Acclimatization Institute (IHAR)	IHAR	6
Romania	Research Institute for Cereals & Industrial Crops (RICIC)	–	9
Russia	Agricultural Research Institute of Non-Chernozem Zone (ARINCZ)	–	1
Sweden	Svalöf Weibull BV	Sva	13
Switzerland	Swiss Federal Research Station for Plant Production (RAC)	–	6

[1] Only cultivars were submitted and names are given in the dendrograms.

Prasad et al. 2000, Hackauf and Wehling 2002, V. Korzun, pers. comm., M. Röder, pers. comm.) detecting 96 SSR loci in triticale were analysed. Evenly distributed SSR markers from available wheat and rye genetic maps were chosen to warrant a uniform genome coverage. Only three SSR on chromosomes 2D and 7D amplified products in some triticale genotypes, all other markers detected loci on the A, B or R genomes. For technical details of SSR analysis and a list of SSR markers see Tams et al. (2004).

Data analysis The polymorphic information content (PIC)-value and marker index (MI) were calculated for both molecular marker systems according to Powell et al. (1996) using the formula:

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2$$

where p_i is the frequency of the ith allele. For AFLP markers, an average PIC-value was calculated for each PC from the average of all polymorphic bands. MI was calculated as $MI = \overline{PIC} \times n\beta$, where $\overline{PIC}$ is the average PIC-value, n is the number of loci detected and β is the proportion of polymorphic bands.

For both matrices, genetic similarity estimates (GS_{AFLP}, GS_{SSR}) were calculated using the DICE coefficient of similarity (Dice 1945). Standard deviations were obtained by a bootstrap procedure with resampling over markers (Weir 1996). Analyses were performed with the Plabsim software (Frisch et al. 2000), which is implemented as an extension of the statistical software R (Ithaka and Gentleman 1996). Clusters were generated by the unweighted pair-group method using arithmetic averages (UPGMA). Cophenetic correlation was calculated to test for the goodness-of-fit between GS-values obtained from the cluster and the original GS matrix. A Mantel Z-test reveals the correspondence of two matrices. The significance of Z was determined by comparing the observed Z-value with a critical Z-value after 1000 permutations. Computations were performed with appropriate procedures of the software NTSYSpc 2.11 h (Rohlf 2000). Correlations of GS and f-values were calculated with the computer package PLABSTAT (Utz 2001). Support for each dendrogram was determined by a bootstrap procedure (400 replications) using the computer package WINBOOT (Yap and Nelson 1996).

Results

A total of 344 polymorphic bands was detected by AFLP analysis using 10 PC (Table 2). The number of polymorphic bands per PC ranged from 27 to 55 with a mean of 34.4. Average PIC-values ranged from 0.14 to 0.28, with a mean of 0.25 when uncertain bands were considered to be present (only for the PIC and MI calculation). The MI ranged from 4.1 to 13.2 and averaged 8.6 for AFLP. Analysis of 93 SSR markers representing 96 loci resulted in 657 polymorphic bands (Tams et al. 2004). The percentage of rare alleles with a frequency of < 5% was lower for AFLP (29.1%) than SSR (52.5%).

Table 2: Degree of polymorphism and mean PIC and MI for 10 AFLP primer combinations applied to 128 European winter triticale genotypes

PC	Selective bases	Polymorphic bands: No.	Polymorphic bands: Proportion (%)	$\overline{PIC}$	MI
P32/T61	AAG/CTG	29	0.50	0.14	4.06
P39/T54	AGA/CCT	31	0.53	0.28	8.68
P40/T48	AGC/CAC	34	0.62	0.24	8.16
P40/T49	AGC/CAG	27	0.44	0.27	7.29
P40/T54	AGC/CCT	36	0.51	0.28	10.08
P41/T48	AGG/CAC	29	0.45	0.23	6.67
P41/T49	AGG/CAG	55	0.63	0.24	13.20
P41/T56	AGG/CGC	43	0.60	0.28	12.04
P41/T59	AGG/CTA	30	0.50	0.26	7.80
P42/T50	AGT/CAT	30	0.45	0.25	7.50
Mean		34.4	0.53	0.25	8.56

MI, marker index; PIC, polymorphism information content.
PC, primer combination; $\overline{PIC}$, average PIC-value for each primer combination; P, *Pst*I; T, *Taq*I.

For all pairwise comparisons of GS estimates, GS_{AFLP} ranged from 0.38 to 0.99 with an average of 0.61 (±0.043). GS_{SSR} ranged from 0.13 to 0.94 with an average of 0.43 (±0.050). f-values varied from 0 to 1 with an average of 0.06. The distributions of the 8128 pairwise comparisons based on molecular marker data (GS_{AFLP} and GS_{SSR}) and f-values differed clearly, with f-values being skewed towards 0. More than 85% of the pairwise comparisons had an f-value < 0.1 (Fig. 1).

Correlations of GS_{AFLP} and GS_{SSR} with f were low, even between related ($f > 0.1$) genotypes (Table 3). The significant correlation between GS_{AFLP} and GS_{SSR} increased from 0.70 to 0.81 after discarding all unrelated genotypes. In addition, the Mantel Z-test revealed a moderate but significant correspondence of GS_{AFLP} and GS_{SSR} matrices ($r = 0.71$).

For GS_{AFLP} and GS_{SSR}, cluster analysis generated dendrograms with no clearly separated clusters (Fig. 2a,b). The cophenetic correlations were moderate for GS_{AFLP} ($r_{coph} = 0.72$) and GS_{SSR} ($r_{coph} = 0.77$). For f (dendrogram not shown) the cophenetic correlation was higher (Table 3, diagonal). All branches with bootstrap values above 70% are highlighted

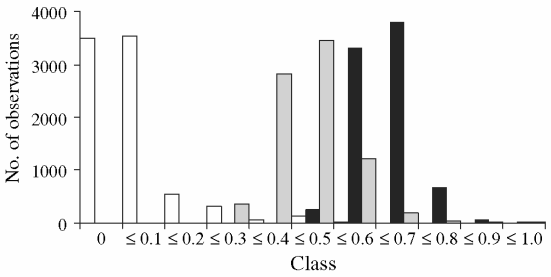

Fig. 1: Distribution of similarity estimates for all 8128 pairwise comparisons based on *f*-value (white), GS_{SSR} (grey), and GS_{AFLP} (black)

Table 3: Correlation between *f*, GS_{AFLP} and GS_{SSR}

	f	GS_{AFLP}	GS_{SSR}
f	*0.89*	0.33**	0.32**
GS_{AFLP}	0.40**	*0.72*	0.70**
GS_{SSR}	0.39*	0.81**	*0.77*

Above diagonal, correlations for all 8128 pairwise comparisons.
Below diagonal, for related lines with $f > 0.1$ (1090 entries).
Cophenetic correlations for UPGMA dendrograms are shown in italics on the diagonal.
Significant at *,** $P = 0.05$ and $P = 0.05$, respectively.

with bold lines in the dendrograms. Only two groups of genotypes clustered together in both dendrograms with high bootstrap values (circle or triangle). The group marked with a circle was mainly composed of Nordsaat (NoSa) germplasm, while cultivars marked with a triangle originated from the Romanian Research Institute for Cereals & Industrial Crops (RICIC). Genetic similarity between the pair having different seed sources ('FOCUS' and 'NoSaF') was 0.98 (±0.010) for AFLP and 0.92 (±0.027) for SSR. These values are in the same range as for the most similar pair of cultivars, 'TRINIDAD' and 'SANTOP', with $GS_{AFLP} = 0.99$ (±0.008) and $GS_{SSR} = 0.93$ (±0.026). The genetic similarity between the pairs of cultivars ('TRINIDAD', 'SANTOP') and seed sources ('FOCUS', 'NoSaF') did not differ significantly, based on either GS_{AFLP} or GS_{SSR}.

Discussion

A companion study demonstrated that SSR markers are a suitable tool for assessing the genetic diversity in triticale, especially when pedigree information is sparse or questionable (Tams et al. 2004). AFLP markers have been recommended as the most efficient marker system in several crops, because a higher number of loci per assay can be detected than can be achieved with other marker systems (Powell et al. 1996, Lübberstedt et al. 2000, Belaj et al. 2003). Accordingly, the MI of AFLP (average 8.6) was much higher than that of SSR (average 0.55, data not shown) although only preselected polymorphic SSR markers were included in the analysis with only three SSR detecting more than one locus. Therefore, MI is biased in comparison with a random sample of SSR markers, but it is still smaller than for AFLP.

In the AFLP analysis, bands with an intermediate intensity in some DNA samples could occur because of heterozygosity or heterogeneity of the seed lot in the bulked sample. However, AFLP analyses have been reported to be insensitive to DNA mixtures up to 10% admixed DNA from another genetically distinct accession (Zhu et al. 1998), which decreases the risk of erroneous scoring as a result of sample contamination. Triticale cultivars are not as homogenous as, for example, the strictly autogamous wheat ancestor, and therefore it is possible that within one bulked seed sample more than 10% of the seeds could be off-types. In particular, the advanced breeding lines might be even more heterogeneous than registered cultivars, which could lead to bands with intermediate intensity. To address this question further, 15–20 single seeds would have to be analysed separately from each accession. A substantial degree of heterogeneity and/or heterozygosity in the germplasm assayed was also observed with the codominant SSR markers (10% of all loci). However, banding patterns were easier to assign to presence or absence than patterns generated by AFLP markers, because SSR markers have been tested and preselected for a clear banding pattern in triticale (Tams et al. 2004). The prescreening of SSR is time-consuming, but interpretation of SSR banding patterns in triticale is easier and information is provided for further investigations such as allelic diversity and the analysis of graphical genotypes.

As with other studies (Bohn et al. 1999, Corbellini et al. 2002), GS_{AFLP} and GS_{SSR} were loosely correlated (Table 3) with the coancestry coefficient. In breeding hybrid maize, where pedigrees are more reliable and the simplifying assumptions are more appropriate, tighter associations were found (Lübberstedt et al. 2000, Lu and Bernardo 2001, Enoki et al. 2002). In the case of line cultivars, selection often favours the elite parent. Consequently, homozygous progenies do not always inherit exactly half of the parental genome, as is assumed for the calculation of *f*. Furthermore, low differentiation power of the *f*-values is revealed by their skewed distribution compared with GS estimates (Fig. 1) and is a result of the incompleteness of pedigrees for a number of genotypes.

The correlation between AFLP and SSR genetic similarity estimates in other studies varies widely. In winter wheat, the low correlation between similarity estimates of SSR and AFLP markers was attributed to disproportion of marker loci and the possibility of clustered AFLP markers (Bohn et al. 1999). The moderate and significant correlation here between GS_{AFLP} and GS_{SSR} ($r = 0.70$) is comparable with the findings in maize ($r = 0.67$) of Pejic et al. (1998), who conclude in their study that SSR and AFLP markers provide consistent information for germplasm identification, because the main clusters in the dendrograms were consistent for all marker systems. The Mantel *Z*-test also revealed a moderate and significant correlation ($r = 0.71$) for the matrices of GS_{AFLP} and GS_{SSR}. Powell et al. (1996) suggested that the correlation of GS_{SSR} with GS of other marker types may decline in any comparisons of either very closely related or highly unrelated genotypes (e.g. interspecific comparisons).

In contrast to the SSR markers used in the current study, for which the chromosomal location is known from mapping studies in wheat and rye, the distribution of the AFLP markers across the triticale genome is unknown. In maize, *Eco*RI/*Mse*I AFLP were not randomly distributed over the genome but over-represented in the centromeric regions while the distribution of *Pst*I/*Mse*I AFLP was more uniform (Vuylsteke et al. 1999). Menz et al. (2004) reported similar results for *Sorghum*

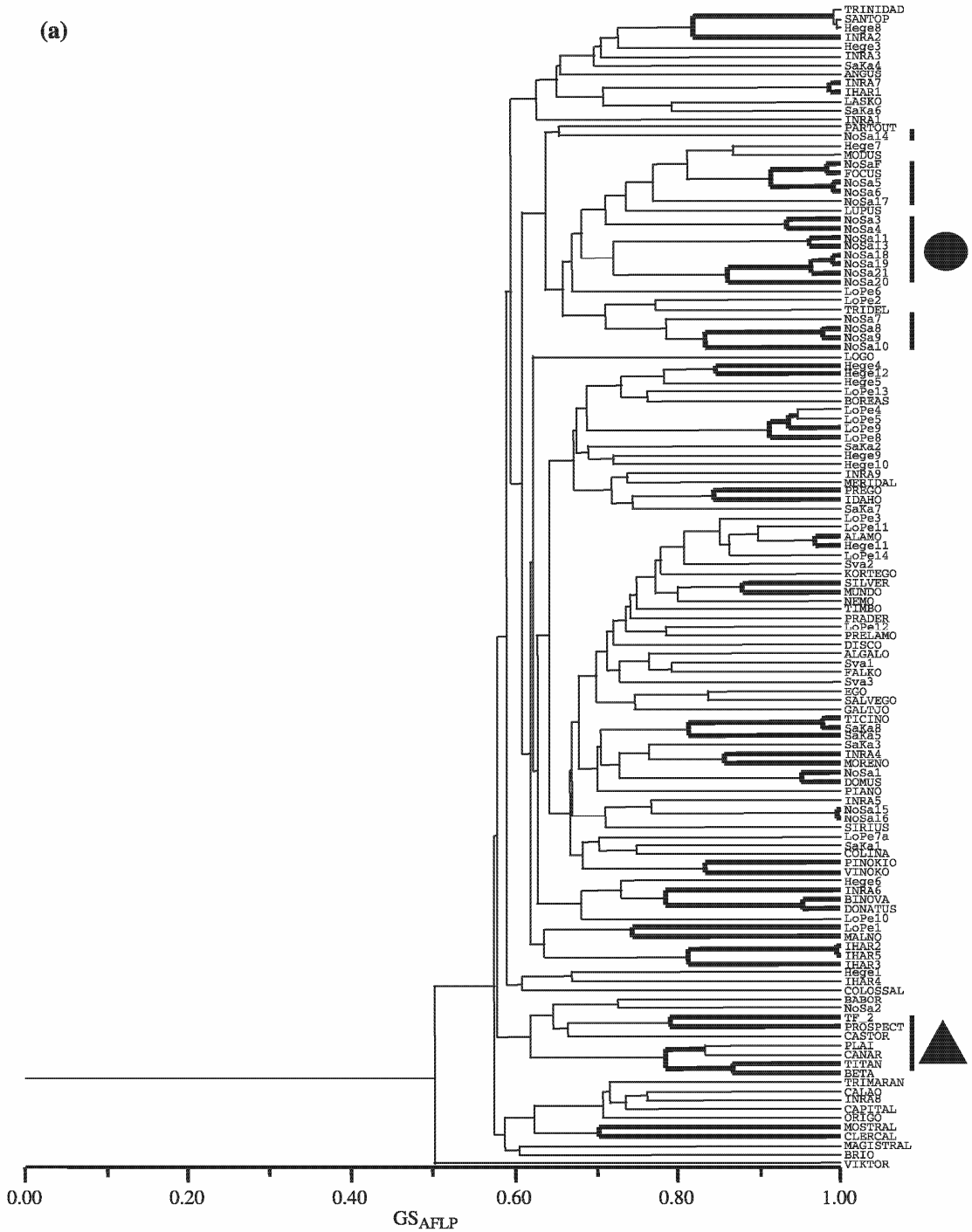

Fig. 2: Cluster with all 128 genotypes based on (a) GS_{AFLP} and (b) GS_{SSR}. ● and ▲ are subclusters with relatively high bootstrap values. The bold lines are branches with bootstrap values above 70%

and emphasized the use of *Pst*I enzyme combinations for AFLP markers if saturated genetic maps do not exist. Therefore, in the present study with *Pst*I as the rare cutter, an adequate genome coverage is assumed, although mapping information is lacking so far. In the case of AFLP, clustering will produce redundant information. Thus, the reduction in

Fig. 2: Continued.

informative haplotypes would lead to an overestimation of genetic similarity, which may explain the higher average GS for AFLP in this study.

Cluster analysis based on GS_{AFLP} or GS_{SSR} showed no clear grouping within the triticale germplasm. Likewise, principal coordinate analysis based on SSR markers in the companion

study could not separate distinct groups (Tams et al. 2004). As concluded from the cophenetic correlations (0.72 and 0.77), dendrograms provide only a poor representation of the information in the original similarity matrices of AFLP and SSR data. This corresponds with the bootstrap analyses, where only a small number of knots appeared in more than 70% of the bootstrap steps (Fig. 2a,b). This can be explained by a large number of pairwise comparisons with intermediate values for pairwise genetic similarity (Fig. 1), which allow a number of similar variants for dendrogram branching. A better fit of the matrix of *f*-values and cluster analysis (dendrogram not shown) was confirmed by a high cophenetic correlation ($r = 0.89$). The lack of relations between pairs of genotypes is exhibited by many pairwise comparisons with a coancestry coefficient of zero (43%), which leads to an unambiguous clustering in the dendrogram.

In the GS_{SSR}-based dendrogram, only two groups of genotypes (black circle and triangle) formed subclusters in >70% of the bootstrap samples. These genotypes belong mainly to two breeding companies (circle, Nordsaat; triangle, RICIC) and also clustered in the AFLP dendrogram, but with lower bootstrap values. Both groups also formed subgroups in the principle coordinate analysis (Tams et al. 2004).

In AFLP analysis of sugar beet varieties, differences between seed samples were as important as differences among varieties or breeding programme (de Riek et al. 2001). The set of triticale genotypes used included two seed samples of one genotype ('FOCUS' and 'NoSaF'), which showed a comparably high genetic similarity estimate to that of a pair of closely related cultivars ('TRINIDAD' and 'SANTOP') with both marker systems. Heckenberger et al. (2002, 2003) reported that the standard deviation of genetic distance measures between accessions of maize inbred lines varied up to 0.07 based on SSR marker analysis, and up to 0.022 based on AFLP marker analysis. For the identification of essentially derived varieties (EDV) especially, it is necessary to obtain genetic similarity estimates with low standard deviations to distinguish between independent varieties, EDV or identical genotypes. A threshold at a genetic distance of 0.1 was suggested, where the genotypes are indisputably essentially derived. From 0.1 up to 0.2, a zone of uncertainty was defined, where genotypes can be independent or essentially derived depending on the underlying errors in the investigation (Heckenberger et al. 2002). In the study reported here, the two cultivars 'TRINIDAD' and 'SANTOP' were released by the same breeding company. They are of common ancestry ($f = 0.656$) and, thus, may be regarded as EDV despite some differences in agronomic characters (e.g. heading date, plant height, powdery mildew resistance and yield) as described by the German National Variety List (Bundessortenamt 2003). A further pair of varieties ('BINOVA' and 'DONATUS') exceeded the first threshold (GS > 0.8) in both marker systems, although similarity based on pedigree data was only $f = 0.25$. The linkage between genes or quantitative trait loci of interest and markers is unknown in the present study and so genetic similarity might not represent diversity in terms of morphological or agronomic traits, such as growth habit or yield, which are important for cultivar registration.

Pejic et al. (1998) suggested the use of codominant SSR markers for heterozygous material because of the possibility of distinguishing between hetero- and homozygous individuals. In contrast, for inbred lines, AFLP marker assessements would be preferred to exploit the full potential of each system. Parker et al. (2002) recommended SSR markers if the ancestry is the focus of the investigation. For genetic identification of cultivars, they suggested a broad sampling of the genome with a high-throughput marker system like AFLP, but this ignores the problem of non-uniform chromosome coverage. For the purpose of EDV identification, Heckenberger et al. (2003) recommended using both molecular systems in a complementary way.

In triticale, both marker techniques are suitable and have been shown to be more informative than pedigrees. For detecting EDV, the set of AFLP and SSR markers used here may need to be enlarged for better differentiation between highly related genotypes. Genetic similarity based on AFLP analysis had a smaller standard error than GS_{SSR} and a large number of data points can be generated in a short time. However, more complications were observed in the evaluation of AFLP banding patterns. In this study, SSR seem to be preferable because of their clear banding pattern and the possibility of distinguishing between homozygous and the significant portion of heterozygous or heterogenous genotypes. None of the two marker systems could detect a clear grouping of germplasm, which is not unexpected because so far no germplasm management with respect to the pool establishment of clearly separated germplasm pools has taken place, as would be desirable for hybrid breeding (Tams et al. 2004).

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Prospects for Hybrid Breeding in Winter Triticale: I. Heterosis and Combining Ability for Agronomic Traits in European Elite Germplasm

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ABSTRACT

Triticale (×*Triticosecale* Wittmack) (genomes AABBRR, 2*n* = 6*x* = 42) hybrid breeding and heterosis have received increased attention in recent years, but a comprehensive study is lacking. We investigated (i) the level of heterosis, (ii) the relative importance of general combining ability (GCA) vs. specific combining ability (SCA), (iii) correlations between GCA and line per se performance, (iv) trait correlations in parents and hybrids, and (v) prospects for hybrid breeding. Two hundred nine F_1 hybrids of winter triticale, produced by a chemical hybridizing agent, together with their 57 female parents and five tester (male) lines were evaluated in six environments in Germany during the season 2001–2002. Midparent heterosis for grain yield averaged 10.3% and varied from −11.4 to 22.4%, whereas better-parent heterosis averaged 5.0% and varied from −16.8 to 17.4%. Midparent heterosis was also positive for 1000-kernel weight, number of kernels per spike, test weight, and plant height but negative for number of spikes per square meter, falling number, and protein concentration. GCA variance ($\hat{\sigma}^2_{GCA}$) was more important than SCA variance ($\hat{\sigma}^2_{SCA}$) for all traits except grain yield and protein concentration. For most traits, GCA × location and SCA × location interaction variances were small relative to $\hat{\sigma}^2_{GCA}$ and $\hat{\sigma}^2_{SCA}$, respectively. Genetic correlations between midparent and hybrid performance and between GCA effects and line per se performance showed similar trends, being moderate for grain yield and protein concentration and higher for the other traits. We concluded that grain yield heterosis in winter triticale crosses from parents in the current European germplasm pool is adequate to justify continuing research on hybrid breeding. By selecting parents for combining ability and establishing genetically diverse heterotic groups, a midparent grain yield heterosis of 20% could presumably be surpassed. Further information is needed on F_1 seed production and the cytoplasmic male sterility system.

Commercial exploitation of heterosis in hybrids from parents of genetically divergent germplasm is practiced worldwide in allogamous crops such as rye (*Secale cereale* L.), maize (*Zea mays* L.), and pearl millet [*Pennisetum americanum* (L.) K. Schum]. In contrast, exploitation of heterosis by hybrid breeding in many autogamous crops like wheat (*Triticum aestivum* L.) has had only moderate success (Pickett and Galwey, 1997; Jordaan et al., 1999). In hexaploid triticale, the interspecific cross between wheat (*Triticum* spp.) and rye, hybrid breeding and heterosis have been investigated in recent years. In spring triticale, Pfeiffer et al. (1998) measured a midparent grain yield heterosis of 9.5% in 31 hybrids. Oettler et al. (2003) investigated 24 winter triticale hybrids and estimated 10.1% midparent grain yield heterosis. Weißmann and Weißmann (2002) discussed triticale hybrid breeding from a plant breeder's point of view and also considered economic aspects.

Owing to its genome constitution with one third of the chromosomes from the allogamous rye and its floral biology of large extruding anthers and some degree of outcrossing (Yeung and Larter, 1972; Sowa and Krysiak, 1996), triticale is expected to have more potential for heterosis and hybrid breeding than wheat. Modern rye hybrids displayed substantial midparent heterosis for grain yield (92%) and are widely cultivated in several European countries (Geiger and Miedaner, 1999).

An important prerequisite for establishing a hybrid breeding program is a sufficiently high level of heterosis. Previous reports based on single plants or small plot experiments tended to overestimate heterosis. Trethowan and Darvey (1994) estimated an average of 17% midparent grain yield heterosis in hill-plots. In small plots of 2.5 to 3 m^2, Oettler et al. (2001) measured 10.5% midparent heterosis. A recent study used larger plots seeded at normal rates and reported an average of 10.1% midparent grain yield heterosis, but this estimate was based only on 24 hybrids from six female and four male parent lines (Oettler et al., 2003). Hitherto, a large-scale and comprehensive study with genetically diverse material was lacking.

A fundamental issue in hybrid breeding is the choice of parents and identification of superior hybrid combinations. Grzesik and Węgrzyn (1998) found GCA effects to be more important than SCA effects in winter triticale. In contrast, Oettler et al. (2003) reported predominance of SCA effects for grain yield and concluded that prediction of GCA from parental performance was moderate.

The objectives of this study were to (i) determine the level of heterosis for eight agronomic traits in 209 winter triticale hybrids, (ii) assess the relative importance of GCA vs. SCA effects, (iii) calculate correlations between GCA and line per se performance, (iv) estimate trait correlations in parents and hybrids, and (v) discuss the prospects for hybrid breeding in winter triticale.

MATERIALS AND METHODS

Genetic Materials and Field Trials

Two hundred nine F_1 hybrids of winter triticale produced with the aid of the chemical hybridizing agent (CHA) Genesis

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Abbreviations: BPH%, relative better-parent heterosis; CHA, chemical hybridizing agent; GCA, general combining ability; HYB, hybrid performance; LP, line per se performance; MP, midparent value; MPH, absolute midparent heterosis; MPH%, relative midparent heterosis; SCA, specific combining ability.

(Monsanto Co., St. Louis, MO, USA) were evaluated. The parental material comprised 57 female parents and five tester (male) lines. Effectiveness of the CHA was checked by isolating spikes of the female parents with glassine bags in various parts of the plot, and complete male sterility was observed in all CHA-treated plants. Parents were elite breeding lines or cultivars from six countries: France (2), Germany (46), Poland (2), Romania (3), Sweden (3), and Switzerland (6). The hybrids were subdivided in three trials grown side by side at each of six climatically and ecologically diverse locations in Germany: Ranzin (Mecklenburg-West Pomerania), Klausheide and Hildesheim (Lower Saxony), Petkus (Brandenburg), Hohenheim, and Oberer Lindenhof (Baden-Württemberg). Technical difficulties resulted in insufficient hybrid seed supplies of some entries, necessitating a subdivision of the entries into three experiments. Experiment I comprised 95 hybrids from 19 female parents and all five testers (Babor, Disco, Domus, Lupus, Partout). Experiments II and III each comprised 57 hybrids from two sets of 19 different females and the same three testers (Babor, Disco, Partout). Hybrids and female parents were included as single entries in all trials, whereas testers were grown as duplicate entries. Three cultivars (Lamberto, Modus, Trinidad), used as checks in official trials for plant variety registration in Germany, were also included as duplicate entries. The experimental designs were 13 × 10 (Experiment I) and 10 × 9 generalized lattices (Experiment II and III) with two replications at each location. The experiments were planted in September–October 2001 and harvested in July–August 2002. Depending on local practices, plots consisted of six to 10 rows with interrow distances between 15.6 and 20.8 cm, and were drill-seeded at 220 viable kernels m^{-2}. Standard production practices for application of fertilizer, growth regulator, herbicides, and fungicides were used at each location (Karpenstein-Machan et al., 1994). Plot size at harvest was between 5 and 6 m^2. Data were recorded for the following traits: grain yield (Mg ha^{-1} determined at 140 g kg^{-1} moisture), number of spikes per square meter (counted in two 1-m rows), 1000-kernel weight (g), number of kernels per spike (calculated as grain yield m^{-2} × 1000)/(1000-kernel weight × no. spikes m^{-2}), test weight (kg hL^{-1}), falling number(s) based on two subsamples of a 9-g wholemeal sample following the IACC (1968) protocol, protein concentration (g kg^{-1}) determined by the Perstorp near infrared spectroscopy analyzer system 6500 (Foss GmbH, Rodgau, Germany) following the protocol of Tillmann (1996), and plant height (cm). Because of limited resources, falling number and protein concentration could only be determined at two locations.

Statistical Analyses

Ordinary lattice analyses of variance were performed with the data from each experiment in each location. Since the means of the three experiments were not significantly different and the ranges of parent lines were similar, we pooled data across experiments to present the results most parsimoniously. Thus, we assumed three random samples of triticale lines and presented combined analyses with the following model for the adjusted hybrid means from the lattices (Cochran and Cox, 1957):

$$x_{ijklm} = \mu + l_m + a_i + (al)_{im} + t_k + (tl)_{km} + (at)_{ik} + (atl)_{ikm} + g_{ij} + s_{ijk} + (gl)_{ijm} + (sl)_{ijkm} + \bar{e}_{ijkm},$$

where μ = general mean, l_m = effect of the mth location, a_i = effect of the ith experiment, t_k = effect of the kth tester, g_{ij} = gca effect of Line j in Experiment i, s_{ijk} = sca effect of Line j in Experiment i with Tester k, and corresponding interaction effects with locations, and $\bar{e}_{ijkm}$ = averaged plot residual. A fully random model was assumed. The combined analysis was separated for hybrids and parents because common variances for the two groups could not be assumed. Heritabilities were estimated on an entry-mean basis and their 95% confidence intervals were calculated after Knapp and Bridges (1987). Variance components of female GCA (σ^2_{GCA}) and SCA (σ^2_{SCA}) were estimated for all traits by standard methods (Bernardo, 2002). Genotypic correlations (r_g) were calculated between midparent and hybrid performance and between female GCA effects and their line per se performance (LP). Empirical 95% confidence intervals for these correlations were determined by 2000 bootstrap MANOVA samples after Liu et al. (1997).

For each cross combination (P1 × P2), hybrid performance (HYB), midparent value (MP), absolute midparent heterosis (MPH), relative midparent heterosis (MPH%), and relative better-parent heterosis (BPH%) were calculated as follows: MP = (P1 + P2)/2; MPH = HYB–MP; MPH% = (MPH/MP) × 100; BPH% = (HYB–Pmax)/Pmax × 100, where Pmax refers to the higher performing or taller parent. The significance of MPH was tested by a t test using the pooled interaction variances of hybrids × locations and parents × locations as the error term. The minimum and maximum values of MPH were tested by Scheffé's test (Steel and Torrie, 1980).

RESULTS

Compared with their midparent values, hybrids averaged significantly ($P < 0.01$) higher grain yield (corresponding to relative midparent heterosis of 10.3%), higher 1000-kernel weight (9.3%), higher test weight (2.1%), and more kernels per spike (4.4%), but fewer spikes per square meter (−3.3%), lower falling number (−10.6%), lower protein concentration (−3.4%), and taller plants (5.7%, Table 1). Midparent heterosis for grain yield ranged from −11.4 to 22.4% (absolute values from −1.08 to 1.81 Mg ha^{-1}). The widest range (71.2%) in midparent heterosis (absolute values from −42.5 to 28.7 s) was observed for falling number. Falling number is an indirect measure for preharvest sprouting. Lower falling numbers and negative heterosis indicate a higher sprouting risk of the hybrids. Relative better-parent heterosis was positive only for grain yield (5.0%), 1000-kernel weight (4.2%), and plant height (2.2%) and about half the size of relative midparent heterosis. Average hybrid grain yield surpassed the average yield of the three checks by 0.5 Mg ha^{-1} (5.4%; data not shown).

Estimates of genotypic variance ($\hat{\sigma}^2_G$) were significant ($P < 0.01$) for parents and hybrids and exceeded twice their respective standard errors for all traits (Table 2). Both groups displayed similar $\hat{\sigma}^2_G$ for grain yield. For the remaining traits, $\hat{\sigma}^2_G$ for the parents was about twice as large as for the hybrids. For parents, estimates of genotype × location interaction variances ($\hat{\sigma}^2_{G\times L}$) were smaller than $\hat{\sigma}^2_G$ for all traits and most of the differences were significant ($P < 0.01$). Estimates of σ^2_{GCA} were significant ($P < 0.01$) for all traits except protein concentration and were larger than $\hat{\sigma}^2_{SCA}$ for most traits. For grain yield and protein concentration, however, $\hat{\sigma}^2_{SCA}$ surpassed $\hat{\sigma}^2_{GCA}$. GCA × location and SCA × location interaction variances ($\hat{\sigma}^2_{GCA\times L}$, $\hat{\sigma}^2_{SCA\times L}$) were significant ($P < 0.01$) in most cases but small relative to $\hat{\sigma}^2_{GCA}$ and $\hat{\sigma}^2_{SCA}$, except for number of kernels per spike and protein concentration. For most traits, $\hat{\sigma}^2_{GCA\times L}$ was larger than $\hat{\sigma}^2_{SCA\times L}$, but

Table 1. Mean, minimum and maximum of hybrid performance, midparent value, absolute and relative midparent heterosis, and relative better-parent heterosis for eight traits of 209 hybrids and their 62 parent lines of European winter triticale grown at six locations.

	Grain yield	No. of spikes	1000-Kernel weight	No. of kernels	Test weight	Falling number†	Protein conc.†	Plant height
	Mg ha^{-1}	no. m^{-2}	g	no. spike^{-1}	kg hL^{-1}	s	g kg^{-1}	cm
Hybrid performance								
Mean	9.22	331	47.3	61.8	68.5	91	134	123.5
Minimum	7.33	281	41.1	48.3	64.5	62	67	108.3
Maximum	10.49	396	53.7	76.1	73.4	189	148	134.1
$LSD_{5\%}$	0.53	39	1.8	8.5	1.3	31	8	3.7
Midparent value								
Mean	8.38	344	43.3	59.4	67.1	103	139	116.8
Minimum	7.46	290	38.2	50.4	62.3	62	124	109.3
Maximum	9.06	416	47.9	72.0	72.4	191	150	124.8
$LSD_{5\%}$	0.38	30	1.4	5.4	1.4	30	7	2.7
Absolute midparent heterosis								
Mean	0.84**	−13**	4.0**	2.4**	1.4**	−13**	−4**	6.7**
Minimum	−1.08	−60	0.3	−8.8	1.6	−52	−76	−6.8
Maximum	1.81**	36	8.0	11.3	4.5	20	67	13.0
Relative midparent heterosis (%)								
Mean	10.3	−3.3	9.3	4.4	2.1	−10.6	−3.4	5.7
Minimum	−11.4	−15.8	1.0	−12.5	−2.4	−42.5	−53.8	−5.7
Maximum	22.4	12.0	18.4	20.2	7.1	28.7	7.2	11.3
Relative better-parent heterosis (%)								
Mean	5.0	−11.7	4.2	−4.5	−0.3	−24.0	−6.1	2.2‡
Minimum	−16.8	−34.0	−11.0	−23.0	−5.0	−60.0	−54.5	−11.2
Maximum	17.4	4.0	13.7	11.9	4.2	20.9	7.3	8.0

** Significantly different from zero at the 0.01 level of probability.
† Falling number and protein concentration determined only in two locations.
‡ Relative taller-parent heterosis.

both interaction variances were of the same order for grain yield. Heritability estimates ranged from 58.9 to 95.5% among parents and from 50.1 to 91.8% among hybrids.

Midparent performance was significantly ($P < 0.01$) correlated with hybrid performance for all traits (Table 2). The lowest correlation was for grain yield (r_g = 0.48) and the highest for number of spikes per square meter (r_g = 0.98). Correlations were higher for yield components than for grain yield. Genotypic correlations between GCA and line per se performance were also significant ($P < 0.01$) for all traits, except protein concentration, and ranged from r_g = 0.60 for grain yield to r_g = 0.98 for number of spikes per square meter (Table 2). Genotypic correlations were generally weaker between midparent and hybrid performance than between GCA and line per se performance, especially for grain yield.

Genotypic trait correlations were moderate to low and similar for parents and hybrids (Table 3). The highest association (r_g = –0.6, $P < 0.01$) was observed between grain yield and protein concentration in both

Table 2. Estimates of variance components ($\hat{\sigma}$) and their standard errors and entry-mean heritabilities (h^2) and their confidence intervals for eight traits estimated from 62 parental lines and their 209 hybrids grown in six locations, and genotypic correlation coefficients between midparent values and hybrid performance [r_g (MP, HYB)] and between general combining ability (GCA) and line per se performance [r_g (GCA,LP)] of 57 female parent lines.

Source	Grain yield	No. of spikes	1000-Kernel weight	No. of kernels	Test weight	Falling number†	Protein conc.†	Plant height
	Mg ha^{-1}	no. m^{-2}	g	no. spike^{-1}	kg hL^{-1}	s	g kg^{-1}	cm
Parents‡								
$\hat{\sigma}^2_G$	0.218 ± 0.044**	1224 ± 253**	10.92 ± 1.98**	35.31 ± 7.46**	6.69 ± 1.24**	1165 ± 242**	18.53 ± 5.86**	38.89 ± 7.04**
$\hat{\sigma}^2_{G\times L}$	0.144 ± 0.018**	368 ± 116**	2.05 ± 0.26**	−1.50 ± 4.01	2.59 ± 0.23**	327 ± 76**	12.31 ± 4.38**	5.59 ± 0.88**
$\hat{\sigma}^2_e$	0.172 ± 0.006	2064 ± 76	2.38 ± 0.09	95.61 ± 3.45	0.59 ± 0.02	224 ± 14	24.84 ± 1.64	10.86 ± 0.40
$h^2_{Females}$§ (CI)¶	85.1 (77.6; 89.5)	84.0 (76.0; 88.8)	95.3 (92.9; 96.7)	82.1 (73.2; 87.4)	93.3 (90.0; 95.3)	84.2 (74.1; 90.3)	58.9 (33.0; 74.8)	95.5 (93.3; 96.8)
Hybrids‡								
$\hat{\sigma}^2_G$	0.193 ± 0.023**	517 ± 71**	4.59 ± 0.49**	17.96 ± 2.72**	2.34 ± 0.25**	457 ± 59**	9.36 ± 2.09**	14.95 ± 1.64**
$\hat{\sigma}^2_{GCA}$	0.081 ± 0.024**	134 ± 37**	3.83 ± 0.79**	8.72 ± 2.38**	1.66 ± 0.35**	181 ± 51**	2.51 ± 1.64	12.32 ± 2.63**
$\hat{\sigma}^2_{SCA}$	0.100 ± 0.015**	18 ± 67	0.68 ± 0.11**	2.35 ± 1.35*	0.32 ± 0.05**	73 ± 19**	3.25 ± 1.47*	3.02 ± 0.50**
$\hat{\sigma}^2_{GCA\times L}$	0.054 ± 0.008**	22 ± 30	3.60 ± 0.59**	4.02 ± 1.71**	0.43 ± 0.05**	86 ± 24**	4.69 ± 1.53**	2.36 ± 0.39**
$\hat{\sigma}^2_{SCA\times L}$	0.059 ± 0.008**	18 ± 67	0.25 ± 0.09**	3.56 ± 3.21	0.16 ± 0.03**	28 ± 18*	−1.45 ± 1.54	2.03 ± 0.44**
$h^2_{Hybrids}$ (CI)¶	83.8 (79.8; 86.8)	72.3 (65.5; 77.4)	91.8 (89.8; 93.3)	65.6 (57.2; 72.0)	91.4 (89.3; 93.0)	78.0 (71.1; 83.3)	50.1 (31.2; 64.4)	89.6 (87.0; 91.5)
r_g (MP,HYB)	0.48 ± 0.08**	0.98 ± 0.03**	0.82 ± 0.02**	0.89 ± 0.04**	0.88 ± 0.02**	0.90 ± 0.02**	0.59 ± 0.11**	0.76 ± 0.05**
r_g (GCA,LP)	0.60 ± 0.18**	0.98 ± 0.11**	0.90 ± 0.04**	0.89 ± 0.10**	0.95 ± 0.03**	0.93 ± 0.08**	n.d.††	0.88 ± 0.06**

* Significant at the 0.05 level of probability using bootstrap confidence intervals for estimation of $\hat{\sigma}^2$ and r_g.
** Significant at the 0.01 level of probability using bootstrap confidence intervals for estimation of $\hat{\sigma}^2$ and r_g.
† Falling number and protein concentration determined only in two locations.
‡ G = genotype; G×L = genotype × location interaction; e = error valid for parents and hybrids; GCA = general combining ability; SCA = specific combining ability; GCA×L = general combining ability × location interactions; SCA×L = specific combining ability × location interactions.
§ h^2 for 57 female parent lines *per se*.
¶ 95% Confidence interval.
†† Not determined.

Table 3. Genotypic correlation coefficients between eight agronomic traits of 62 parental lines (above diagonal) and their 209 hybrids (below diagonal) grown in six locations.

	Grain yield	No. of spikes	1000-Kernel weight	No. of kernels	Test weight	Falling number†	Protein conc.†	Plant height
Grain yield		−0.16	0.27*	0.56**	0.22	0.05	−0.60**	0.32**
Number of spikes	0.21**		−0.54**	−0.58**	0.03	0.44	−0.03	−0.25*
1000-Kernel weight	0.02	−0.51**		−0.14	−0.20*	−0.42**	−0.20	0.34**
Number of kernels	0.53**	−0.49**	−0.17*		0.30**	0.15	−0.21	0.17
Test weight	0.30**	0.08	−0.32**	0.33**		0.03	−0.07	0.17
Falling number†	0.19*	0.45*	−0.55**	0.04	−0.14*		−0.13	0.07
Protein concentration†	−0.62**	−0.31**	0.15	−0.25*	−0.36**	−0.05		−0.23
Plant height	0.37**	0.10	0.23**	−0.01	0.19**	0.09	−0.27**	

* Significant at the 0.05 level of probability using bootstrap confidence intervals.
** Significant at the 0.01 level of probability using bootstrap confidence intervals.
† Falling number and protein concentration determined only in two locations.

parents and hybrids. In parents, protein concentration was negatively associated with all other traits.

DISCUSSION

Heterosis and Hybrid Performance

The level of midparent heterosis observed for all traits in the present study with 209 hybrids evaluated in six locations substantiated an earlier estimate of significant heterosis in a study with 24 winter triticale hybrids tested in two locations (Oettler et al., 2003). The mean 10.3% grain yield heterosis of our study corresponded with the 9.5% measured in 31 spring triticale hybrids by Pfeiffer et al. (1998). These estimates of heterosis in triticale compared favorably with those reported in wheat. For example, Martin et al. (1995) measured an average midparent heterosis of 9.2% for grain yield in 21 wheat hybrids, but this was based on single-plant measurements, which tend to overestimate heterosis. Oury et al. (2000) tested 299 hybrids in drill-seeded plots of 6 to 7.5 m^2 and observed heterosis of the same magnitude. In contrast, an average of zero grain yield heterosis was found in 108 hybrids of spring wheat, also in plots of normal sowing density (Dreisigacker, pers. commun.).

The mean relative better-parent grain yield heterosis of 5.0% in the present experiment, which amounts to half the mean midparent heterosis, was in agreement with the results of the earlier study (Oettler et al., 2003) and with the 5.2% measured in spring triticale (Pfeiffer et al., 1998). Information from wheat varies greatly and appears to be influenced by the material tested. Oury et al. (2000) reported 6.5% better-parent heterosis, whereas Dreisigacker (pers. commun.) observed a negative better-parent heterosis of −9.3% in wheat.

In view of the allopolyploid nature of triticale (AABBRR), one might expect a considerable influence on heterosis from the R-genome chromosomes of the cross-pollinating rye. In recent rye hybrids, a relative midparent grain yield heterosis of 92% was observed (Geiger and Miedaner, 1999). The average heterosis of hybrids from parent lines of the current triticale germplasm pool is, however, closer to wheat than to rye. One reason might be that in the allopolyploid triticale there is already "fixed" heterosis in lines caused by epistatic interactions between genes from different genomes, which results only in a moderate level of additional heterosis (Mac Key, 1970). Furthermore, because no systematic hybrid breeding has been conducted hitherto and no distinct heterotic groups exist in triticale, a considerably higher level of heterosis than estimated in our study can be expected with long-term systematic hybrid breeding. The maximum midparent grain yield heterosis of 22.4%, although probably overestimated due to genotype × environment interactions, is an indication of this potential. According to Jordaan (1996), the biggest limitation in wheat for breeding hybrids has been to neglect the basic requirement of developing heterotic groups. A recent study in wheat underlines the benefits of heterotic groups for hybrid performance in an autogamous crop. The maximum midparent grain yield heterosis of 8% in intragroup hybrids was less than half the value (19%) measured in intergroup hybrids (Liu et al., 1999).

The largest heterosis in yield components was observed for 1000-kernel weight. A low or even negative heterosis for number of spikes per square meter is well established in hybrids of small-grain cereals and has been documented earlier for winter triticale (Oettler et al., 2003), spring triticale (Pfeiffer et al., 1998), wheat (Borghi et al., 1988), and for the early rye hybrids (Geiger and Miedaner, 1999). However, the wide range with a maximum of 12% midparent heterosis for this trait indicated potential for considerable improvement, as found in modern rye hybrids with a positive heterosis of 7% (Geiger and Miedaner, 1999).

Preharvest sprouting, indirectly measured by falling number, still remains one of the most serious problems of triticale. Current cultivars show poor sprouting resistance (Oettler, 2002). In hybrids, this defect was exacerbated as revealed by the significant negative heterosis for falling number. Deterioration of preharvest sprouting will hamper farmers' acceptance of hybrid over pure line cultivars in triticale. The significant genetic variation in parents and hybrids, as well as the relative maximum midparent heterosis of 28.7%, showed potential for improvement by systematic breeding for this trait.

A significant midparent heterosis for plant height of 6.7 cm (5.7%) was in conflict with the general breeding objective of reducing the straw length in cereals. However, most hybrids fell within the range of their parents and were only 3.0 cm taller than the check cultivars. Therefore, plant height should have little impact on the potential to commercialize triticale hybrids.

Combining Ability and Prediction of Hybrid Performance

Optimum allocation of resources in hybrid breeding depends on efficient methods for choosing parents and identifying superior hybrid combinations. The analysis of combining ability provided information about the relative importance of GCA vs. SCA effects and gave an indication of the gene action involved in the inheritance of the traits. Estimates of $\hat{\sigma}^2_{GCA}$ were greater than $\hat{\sigma}^2_{SCA}$ for all traits except grain yield and protein concentration, indicating that additive effects were more important than non-additive effects. For grain yield, however, non-additive effects predominated. This was in accordance with our earlier study (Oettler et al., 2003) and with the study in wheat by Oury et al. (2000). Genetic variance of the hybrids, which was only half the size of $\hat{\sigma}^2_G$ of the parents for most traits, supports the assumption of only additive gene action. The greater $\hat{\sigma}^2_G$ of parents than of hybrids for grain yield, which was also reported for wheat by Borghi et al. (1988), might be the result of dominance effects, a relationship of a tester with some hybrids, or meiotic instability. Even in elite triticale breeding lines and cultivars, cytological disturbances such as univalents are still present and may be more serious in some F_1 hybrids (Lelley, 1996).

The close correlation between GCA effects and female line per se performance for all traits except grain yield indicated predominance of additive over dominance effects. Selection of potential parents based on per se performance was effective when traits other than grain yield were considered. In contrast, $\hat{\sigma}^2_{SCA}$ was greater than $\hat{\sigma}^2_{GCA}$, and r_g (GCA, LP) was only moderate for grain yield, indicating that selection for per se performance of parents or GCA was of little predictive value for hybrid performance. Furthermore, r_g (MP, HYB) was low for grain yield and only 23% of the variation in hybrid performance was explained by the midparent value. In addition, future experimental testcrosses should be evaluated in multi-environment trials, because $\hat{\sigma}^2_{GCA\times L}$ and $\hat{\sigma}^2_{SCA\times L}$ were important for grain yield and most other traits. It is conceivable, however, that with the establishment of heterotic groups in triticale, the ratio of $\hat{\sigma}^2_{SCA}$ to $\hat{\sigma}^2_{GCA}$ might be reduced for grain yield and the other traits.

Implications for Hybrid Breeding

Pure lines are currently the predominant type of cultivar in commercial triticale production, and released cultivars are nearly homozygous and homogeneous lines. Breeders have frequently used lines from other programs as parents when developing breeding populations that resulted in a leveling out of the genetic diversity in the European winter triticale germplasm pool (Tams et al., 2004). If hybrid breeding in triticale becomes a long-term goal, diversity between parent lines must again be promoted and heterotic pools should be established. A recent study on hybrid maize from the U.S. Corn Belt demonstrates how two genetically distant heterotic groups evolved from a rather mixed germplasm pool that lacked distinct subgroups (Duvick et al., 2004). This process is, however, a long-term task.

For private plant breeding companies, hybrid seed business will be highly attractive because of the built-in plant variety protection of hybrids. Farmers have to buy new seed every growing season. Schachschneider (2000) estimated that from 1996 to 2000 in Germany 30 to 35% of the triticale area was planted with farm-saved seed.

The decision to embark on a hybrid breeding program and commercialization of hybrids should depend on a number of factors, including fertility, heterosis, trait correlations, and F_1 seed production costs. Fertility, which had been a problem in the earlier phases of cultivar development in triticale, has been improved by intensive breeding (Bundessortenamt, 1988, 2004). None of the parents and hybrids in our experiment showed poor fertility.

The amount of midparent grain yield heterosis necessary to make hybrids commercially viable was estimated to range between 6 to 17% for wheat in the UK (Pickett and Galwey, 1997). Schachschneider (1997) regarded a 0.6 to 1.0 Mg ha^{-1} yield advantage of hybrid wheat over pure lines sufficient for commercial production in Germany. For hybrid triticale, Weißmann and Weißmann (2002) considered a 0.9 to 1.0 Mg ha^{-1} higher yield enough to justify the production of triticale hybrids for European market conditions. Therefore, the average midparent heterosis of 0.84 Mg ha^{-1} (10.3%) observed in the present study is encouraging, although relative better-parent heterosis was only half as much. By grouping germplasm into genetically diverse heterotic groups, as is currently under way in our laboratory with the aid of molecular markers (Tams et al., 2004), and by the production of inter-pool hybrids, it appears feasible to reach or even surpass the present maximum midparent heterosis of 1.81 Mg ha^{-1} (22.4%). Furthermore, because triticale is mainly grown under marginal and stress conditions such as sandy soils, water stress or mineral toxicity (Varughese et al., 1996; Banaszak and Marciniak, 2002), the relative yield advantage of hybrids over pure line cultivars is expected to be even higher. Such a relative yield advantage in stress conditions has been demonstrated in wheat (Jordaan, 1996) and rye (Geiger and Miedaner, 1999). Therefore, with regard to the amount of heterosis, our results are more promising for developing hybrids in triticale than in wheat.

The commercially exploitable yield advantage of hybrids will also depend on the rate of progress in line breeding and the time-scale for the development of hybrid vs. line cultivars. At present, the level of hybrid grain yield is on a par with that of the check cultivars. Forty-six of our hybrids outyielded the mean of the check cultivars by 10% or more. Considering the breeding efforts in line cultivar development for triticale in recent years in Europe (Arseniuk and Oleksiak, 2002; FAOSTAT), substantial progress can also be expected in the future. On the assumption that a CHA is used for inducing male sterility in the seed parent, Pickett and Galwey (1997) estimated that a wheat hybrid could be released 1 yr after its line parents, if the latter had proved suitable as line cultivars. However, if cytoplasmic male sterility had to be introgressed into the female

parent lines by recurrent backcrossing, the developmental time lag for hybrids would be considerably longer.

Trait correlations in parents and hybrids were generally similar. All three yield components contributed in the same way to grain yield in both groups. The well-established negative relationship between grain yield and protein concentration was also found in parents and hybrids. Likewise, the breeder must be aware that an increase in grain yield might be in conflict with the breeding objective of reduced plant height. Furthermore, an increase in kernel size might result in a lower falling number that makes genotypes more susceptible to preharvest sprouting.

One of the critical issues for a hybrid program is the cost-effective production of high quality F_1 seed. Several factors, including the need to devote large areas to the male parent (which does not produce marketable F_1 seed) in seed production fields, difficulties in synchronizing the flowering times of female and male parents, and the production of limited quantities of hybrid seed on the male-sterile female parent, contributed to the failure of hybrid wheat production (Pickett and Galwey, 1997). These authors consider a 6% heterosis to be required for wheat to counterbalance a two-fold cost of hybrid seed relative to pure-line seed at a performance level of 6 Mg ha^{-1}. At four-fold hybrid seed costs, a 17% heterosis would be required to meet the extra costs. The floral biology and a tendency to outcrossing in triticale could help to keep seed costs down. Whereas a male to female ratio from 1:1 to 1:3 is required for adequate F_1 seed production of wheat (Pickett and Galwey, 1997; Schachschneider, 1997), a wider ratio can probably be used in triticale. The reduced levels of inputs generally used for triticale, lower seeding rates, and utilization of female parents selected for improved seed set, could also help to reduce seed costs.

Finally, the future of hybrid breeding in triticale depends crucially on a reliable hybridizing system. The CHAs of high sterilizing power, as used in this study, are not licensed for commercial production in the European Union because they are regarded as hazardous to the environment. The cytoplasmic male sterility system from the tetraploid wheat *Triticum timopheevi* (Zhuk.) Zhuk. with suitable restorers, which is used successfully in some countries for hybrid wheat production (Jordaan, 1996; Duvick, 1999), is currently considered the most promising biological hybridizing system for triticale. Warzecha and Salak-Warzecha (2002) presented the first report on male sterile lines in winter triticale. Commercial release in Australia of a spring triticale hybrid based on the *T. timopheevi* cytoplasm is expected for 2005 (Darvey and Roake, 2002). However, a final decision on the usefulness of the *T. timopheevi* cytoplasm for triticale cannot yet be reached. Research in the area of hybridizing systems, including other cytoplasmic male sterility sources, should be given high priority.

In conclusion, grain yield heterosis in winter triticale crosses from parents of the current European germplasm pool appears sufficient to justify continuing work on hybrid breeding. By selecting parents for high combining ability and establishing heterotic groups, this should result in the production of intergroup hybrids with higher levels of heterosis than presently obtained. Further research and more substantial information are needed on F_1 seed production, the sterilizing system and also on the growing regimes suitable for hybrids before the prospects for commercial hybrid triticale can be reliably evaluated. Hybrids are not expected to replace line cultivars on a large scale, but they may be good options for more marginal environments.

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Prospects for hybrid breeding in winter triticale: II. Relationship between parental genetic distance and specific combining ability

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With 1 figure and 5 tables

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Abstract

Significant relative midparent heterosis (MPH%) for grain yield in triticale (×*Triticosecale* Wittm.) has generated interest in the development of hybrid cultivars. The objectives of this study were to (i) examine the association between parental genetic distance (GD) and specific combining ability (SCA), (ii) investigate the existence of genetically distant heterotic groups in elite germplasm, and (iii) draw conclusions for future hybrid breeding in winter triticale. Genetic distance between 61 lines was estimated, based on 93 polymorphic simple sequence repeat (SSR) marker loci and 10 AFLP (amplified fragment length polymorphism) primer-enzyme combinations (PEC). Agronomic data of 206 F_1 crosses and their 61 parental lines grown in six German environments were published recently in a companion study. Correlations were made between SCA for grain yield, number of spikes/m^2, 1000-kernel weight and number of kernels per spike with GD estimates of the 56 female and five male parents (testers). Principal co-ordinate analyses (PCoA) based on SSR data revealed no distinct subgroups in the germplasm. Correlations between GD and SCA were low for all traits ($|r| \leq 0.31$), which hampers the prediction of SCA from molecular data. A multi-stage procedure is recommended for future hybrid breeding in triticale by applying a pragmatic approach for the grouping of germplasm following the early history of hybrid breeding of maize.

Key words: ×*Triticosecale* — genetic distance — heterosis — heterotic groups — molecular markers — specific combining ability

In autogamous crops such as triticale (×*Triticosecale* Wittm.), hybrid breeding becomes attractive if F_1 crosses significantly outperform their parents and the existing elite inbred cultivars. Compared with line cultivars, hybrids have the breeder's advantage of built-in plant variety protection, i.e. they show substantial yield reduction when farm-saved seed is used (Duvick 1999). In many allogamous crops such as maize, sorghum, sugar beet and rye, hybrids have been successfully introduced into commercial production and prevailed against alternative types of variety. Hybrid breeding has also been successfully established in autogamous species such as rice (Zhang et al. 1995), with a 15–20% yield increase in commercial hybrid cultivars compared with line cultivars (Xu et al. 2002). In a companion study (Oettler et al. 2005), winter triticale hybrids showed, on average, 10.3% relative midparent heterosis (MPH%) for grain yield with a wide range (−11.4 to 22.4%) among hybrids. Relative better parent heterosis (BPH%) for grain yield reached a maximum of 17.4% with an average of 5%, and nearly one-third of the hybrids outyielded the mean of the check cultivars by 10% or more. Therefore, hybrid breeding appears to be a promising alternative to line breeding for triticale. Pilot production of commercial triticale hybrids has been successful in Europe (Fossati et al. 1998).

One of the most expensive steps in hybrid cultivar development is the identification of parental combinations that produce hybrids with superior yield. Prediction of hybrid performance with sufficient accuracy from parental performance or molecular data could reduce costs for producing and evaluating testcrosses in field trials, and would optimize breeding strategies by concentrating on fewer but more promising hybrid combinations. A companion paper (Oettler et al. 2005) on prediction of triticale F_1 hybrid performance (HYB) from parental line *per se* performance by quantitative-genetic parameters indicated that specific combining ability (SCA) effects were more important than general combining ability (GCA) effects for grain yield. Neither parental performance *per se* nor GCA had a predictive value for grain yield of F_1 crosses, which is the main agronomic trait for successful hybrid breeding. Under the conditions of linkage disequilibrium between markers and the loci involved in heterotic response, SCA expressed by a hybrid is related to heterozygosity at the marker loci and thus to genetic distance (GD) between its parental lines (Charcosset and Essioux 1994). Therefore, measuring parental GD may be another strategy to predict SCA and reduce field trials and costs.

In allogamous crops, heterotic groups have been established to optimize hybrid performance by choosing promising parental combinations and thus avoiding inferior testcrosses. Examples of heterotic groups in maize are Iowa Stiff Stalk *vs.* Non-Stiff Stalk in the US Cornbelt and Flint *vs.* Dent in Europe (Duvick et al. 2004), and in rye Carsten *vs.* Petkus pool (Hepting 1978). Melchinger (1999) showed that inter-group hybrids in maize had greater parental GD and midparent heterosis (MPH) when compared with intra-group hybrids. If heterotic groups and patterns are not available, as in the mainly autogamous triticale, a first step towards their development is the grouping of germplasm based on genetic similarity (Melchinger and Gumber 1998). Subsequently, crosses could be made among divergent groups to identify promising heterotic patterns.

In two further companion studies (Tams et al. 2004, 2005), SSR and AFLP markers were used to describe GD in European winter triticale elite germplasm. This provided a way of grouping the materials based on molecular information. Heterosis and combining ability estimates of 62 parents and 209 hybrids were presented by Oettler et al. (2005). The

objectives of the present study were to (i) examine the association between parental genetic distance and SCA, (ii) investigate the existence of genetically distant heterotic groups in the elite germplasm, and (iii) draw conclusions for future hybrid breeding in winter triticale.

Materials and Methods

Plant materials: The field data used for this investigation were taken from a companion study of European winter triticale ×*Triticosecale* lines and hybrids, described in detail by Oettler et al. (2005). Briefly, agronomic data (grain yield, number of spikes/m^2, 1000-kernel weight, number of kernels per spike) of 62 parents and 209 hybrids were evaluated in field trials in six environments with two replications in Germany. Each trial was subdivided into three experiments, each consisting of 19 different females crossed with five ('Babor', 'Disco', 'Domus', 'Lupus', 'Partout'; experiment 1) or three testers ('Babor', 'Disco', 'Partout'; experiments 2 and 3). The parents were a subset of 128 European winter triticale lines and cultivars analyzed with 90 SSR primer pairs at 93 polymorphic loci described in a first paper (Tams et al. 2004) and 10 *Pst*I/*Taq*I AFLP primer–enzyme combinations (PEC) described in a second paper (Tams et al. 2005). The SSR markers were evenly distributed over the triticale genome. For technical reasons, one female parental line had to be excluded. Consequently, the present study was based on 61 parents and 206 hybrids (Table 1). The 61 parental lines represent the genetic variability of the European winter triticale germplasm pool.

Statistical analyses: The estimates of the polymorphic information content (PIC) and DICE genetic similarity (GS) were calculated for both molecular marker systems, as described by Tams et al. (2005), and converted in the present study to genetic distances (GD = 1-GS). The DICE criterion is a suitable estimate for the allelic non-informative AFLPs and enables a comparison with SSRs. Standard errors were obtained by a bootstrap procedure with resampling over markers for SSRs and fragments for AFLPs (Weir 1996). To use the combined information of both, GD_{AFLP} and GD_{SSR}, the mean was calculated (GD_{MM}) by weighting with the inverse variances, as described by Cochran and Caroll (1953). For SSR markers, GDs were additionally calculated based on modified Rogers' distance (MRD, Rogers 1972) and squared modified Rogers' distance (MRD^2, Goodman and Stuber 1983) because Euclidian distances are a prerequisite for most multivariate methods. Genetic distance estimates and standard errors were performed with the PLABSIM software (Frisch et al. 2000), which is implemented as an extension of the statistical software R (Ithaka and Gentleman 1996). A principal coordinate analysis (PCoA) based on MRD was calculated with the software NTSYSpc2.11h (Rohlf 2000).

To reveal heterotic founder groups, a multi-stage procedure was used based on grain yield. Using the SCA effects of the five testers with 19 females (experiment 1), correlations between testers were calculated to identify tester pairs with comparable SCA effects. For each female, HYB and MPH% were averaged across both testers for each of the two tester pairs 'Domus'–'Partout' and 'Disco'–'Lupus', which had the highest correlations for SCA estimates (Table 4). Then, a *t*-test based on the averaged HYB and MPH was calculated to identify least significant differences. Finally, females were subdivided into groups based on $LSD_{5\%}$ for HYB and MPH with both tester pairs. The computations were performed with the software package PLABSTAT (Utz 2001).

Results

The 90 SSR markers detected 93 polymorphic loci and generated a total of 595 fragments in the 61 parental lines and PIC per primer pair ranged from 0.03 to 0.84 with a mean of 0.53 (Table 2). In total, 302 polymorphic bands were amplified by AFLP analyses with ten PECs. The number of polymorphic bands per PEC ranged from 24 to 50 with a mean of 30.2. Average PIC values for each PEC ranged from 0.20 to 0.29 with a mean of 0.25. Genetic distance estimates among SSRs ranged from 0.38 to 0.73 and averaged 0.57 ± 0.051 (Table 3). GD_{AFLP} had a narrower range (0.24–0.49) and a smaller mean (0.38 ± 0.043). The weighted mean genetic distance GD_{MM} ranged from 0.33 to 0.60 with an average of 0.49. The Euclidian distance MRD^2 had a similar range (0.28–0.66) and mean (0.45). The correlation between GD estimates and SCA were generally low ($|r| \leq 0.31$) and not significant for grain yield and the yield components, irrespective of the marker systems or GD estimates considered (Table 3).

Table 1: Sixty-one European cultivars and breeding lines of winter triticale parents grouped according to their country of origin and breeding companies or institutes

Country Cultivar/breeding line	Breeding company/institute
Germany	
'Babor', Hege3, Hege4, Hege5, Hege6, Hege7, Hege8, Hege9, 'Partout', 'Trinidad'	Saatzucht Dr Hege GbRmbH
'Domus', 'Lupus', Nosa1, Nosa6, Nosa7, Nosa11, Nosa13, Nosa15, 'Modus'	Nordsaat Saatzucht GmbH
Lope2, Lope5, Lope6, Lope10, Lope11, Lope12, Lope13, 'Trimaran'	Lochow-Petkus GmbH
'Boreas', Saka2, Saka4, Saka5, 'Ticino'	Pflanzenzucht SaKa GbR
'Binova'	IG Saatzucht GmbH and Co. KG
'Donatus'	W. von Borries-Eckendorf GmbH and Co.
Poland	
'Disco', 'Nemo', 'Moreno', 'Mundo', 'Piano', 'Prego'	Danko Hodowla Roślin Spółka z o.o.
Ihar5, 'Malno'	Plant Breeding and Acclimatization Institute (IHAR)
France	
'Angus', 'Colossal', Inra2, Inra3, Inra6, Inra7, Inra9	Institut National de la Recherche Agronomique (INRA)
Switzerland	
'Brio', 'Prader', 'Meridal', 'Sirius', 'Timbo', 'Tridel'	Swiss Federal Research Station for Plant Production (RAC)
Sweden	
'Algalo', 'Prelamo', Sva3	Svalöf Weibull AB
Romania	
'Colina', 'Prospect', 'Silver'	Research Institute for Cereals and Industrial Crops (RICIC)

Table 2: Mean and range of number of AFLP fragments and SSR alleles as well as polymorphic information content (PIC) values for markers analyzed in 61 triticale parental lines

		No. of alleles/ fragments		PIC[1]	
Marker set	No. of loci	Mean	Range	Mean	Range
AFLP markers	302	30.2	24–50	0.25	0.20–0.29
SSR markers[2]	93	6.4	2–15	0.53	0.03–0.84

[1]SSR markers: PIC per locus, AFLP markers: PIC per primer–enzyme combination.
[2]SSR markers from EST-derived and genomic libraries.

Table 3: Mean and range of parental genetic distance (GD) estimates of 206 triticale hybrids (GD_{AFLP}, GD_{SSR}, GD_{MM}, MRD^2) and correlation coefficients (r) of GD with specific combining ability (SCA) for four agronomic traits (experiments 1, 2 and 3)

			r (GD; SCA)			
Estimate	Mean	Range	Grain yield	No. of spikes/m^2	1000-kernel weight	No. of kernels/ spike
GD_{AFLP}	0.38	0.24–0.49	0.18	0.00	0.21	−0.01
GD_{SSR}	0.57	0.38–0.73	−0.03	0.06	0.31	−0.29
GD_{MM}	0.49	0.33–0.60	0.02	0.05	0.31	−0.24
MRD^2	0.45	0.28–0.66	−0.03	0.01	0.31	−0.24

Table 4: Correlation among SCA estimates for grain yield (experiment 1) between five testers (above diagonal) and genetic distance between the testers based on GD_{MM} (below diagonal)

Tester	'Babor'	'Disco'	'Domus'	'Lupus'	'Partout'
'Babor'		0.80**	0.76**	0.78**	0.89**
'Disco'	0.55		0.90**	0.96**	0.92**
'Domus'	0.47	0.44		0.89**	0.94**
'Lupus'	0.57	0.57	0.50		0.92**
'Partout'	0.51	0.52	0.45	0.53	

**Significant at $P = 0.01$.

Genetic distances between all parental lines were illustrated in a two-dimensional PCoA, which revealed no distinct groups (Fig. 1). However, in each quadrant, genotypes from one breeding company or institute were predominant (I: Hege, II: Danko, III: RAC, IV: Nordsaat). The five testers ('Babor', 'Disco', 'Domus', 'Lupus', 'Partout') were distributed over all four quadrants. The first two principal co-ordinates (PC) together explained 13.7% of the total molecular variance.

The correlations between all possible tester pairs for SCA of grain yield were significant ($P < 0.01$) and ranged from 0.76 to 0.96 (Table 4). Genetic distances between testers were moderate and not significant. The tester pairs 'Domus'–'Partout' and 'Disco'–'Lupus' showed the highest correlations ($r_{Domus;\ Partout} = 0.94$ and $r_{Disco;\ Lupus} = 0.96$). The fifth tester 'Babor' had the lowest correlations with the other testers. A suggested grouping of the 19 females according to significantly better HYB and MPH% with the two tester pairs ('Domus'–'Partout' and 'Disco'–'Lupus') resulted in three groups (Table 5). Assignment of females to group A was due to superior HYB, while females assigned to group B had a higher MPH%. Group C consisted of females, which could not be assigned to either of the two tester pairs.

Discussion

A considerable amount of heterosis for grain yield in winter triticale was reported by Oettler et al. (2005). Therefore, hybrid breeding appears to be a promising alternative to line breeding. It is lucrative for breeding companies, because it restricts the use of farm-saved seed. For commercial purposes, however, several problems remain to be solved: (i) the establishment of an efficient hybrid mechanism for seed production, (ii) inception of genetically divergent groups, and (iii) an efficient method for the identification of superior hybrid combinations. The latter two aspects were investigated.

Relationship between parental genetic distances and SCA

As illustrated by PCoA, females as well as testers were genetically distant and evenly distributed over all quadrants (Fig. 1 and Table 3). All five testers were modern cultivars, selected according to their pedigree information. Their low relatedness (coancestry coefficient $f \leq 0.04$) was confirmed by genetic similarity analyses (Tams et al. 2004). In PCoA, tester pair 'Disco'–'Lupus' was separated from the tester pair 'Domus'–'Partout' and tester 'Babor' by PC1 (Fig. 1).

Quantitative genetic theory suggests a linear correlation between MPH and MRD^2 under simplifying assumptions, such as biallelism and absence of epistasis (Falconer and Mackay 1996). This relationship has been reported for intra-pool crosses in maize, rapeseed and rice, as reviewed by Melchinger (1999). Charcosset et al. (1998) reported tighter correlations between GD and MPH for intra-group than for inter-group crosses in maize, with the presence of the same linkage phase between QTL and marker loci in the maternal and paternal gametic array, which results in a positive covariance between GD and MPH. Significant correlations have also been reported between parental GD and hybrid yield potential in rice, rapeseed and tropical maize (Diers et al. 1996, Xiao et al. 1996, Reif et al. 2003a). In a theoretical study, Charcosset and Essioux (1994) partitioned hybrid performance into GCA and SCA and showed the latter to be the more important component concerning the relationship with parental GD. Further, Ajmone Marsan et al. (1998) illustrated that the correlation between specific genetic distance and SCA in maize was largely due to heterotic group effects. In the present study, none of the GD estimates was significantly correlated with SCA for yield or yield components and none exceeded the value of $r = 0.31$ for 1000-kernel weight. Correlations between GD and HYB or MPH% for these traits were of the same magnitude as for r(GD; SCA). Thus, prediction of hybrid performance to exclude inferior crosses before field testing was not feasible with the aid of the set of molecular markers used in this study, irrespective of the marker system or genetic distance estimate used.

Grouping of germplasm

In commercial hybrid breeding programmes, heterotic groups enable the efficient use of heterosis by selecting crossing parents from divergent pools. The superiority of inter-group over intra-group crosses in terms of mean hybrid performance and heterosis is well documented (for review see Melchinger and Gumber 1998). The expectation of increasing heterosis by optimizing genetic distance between the heterotic groups emphasizes the need for their development. Knowledge about

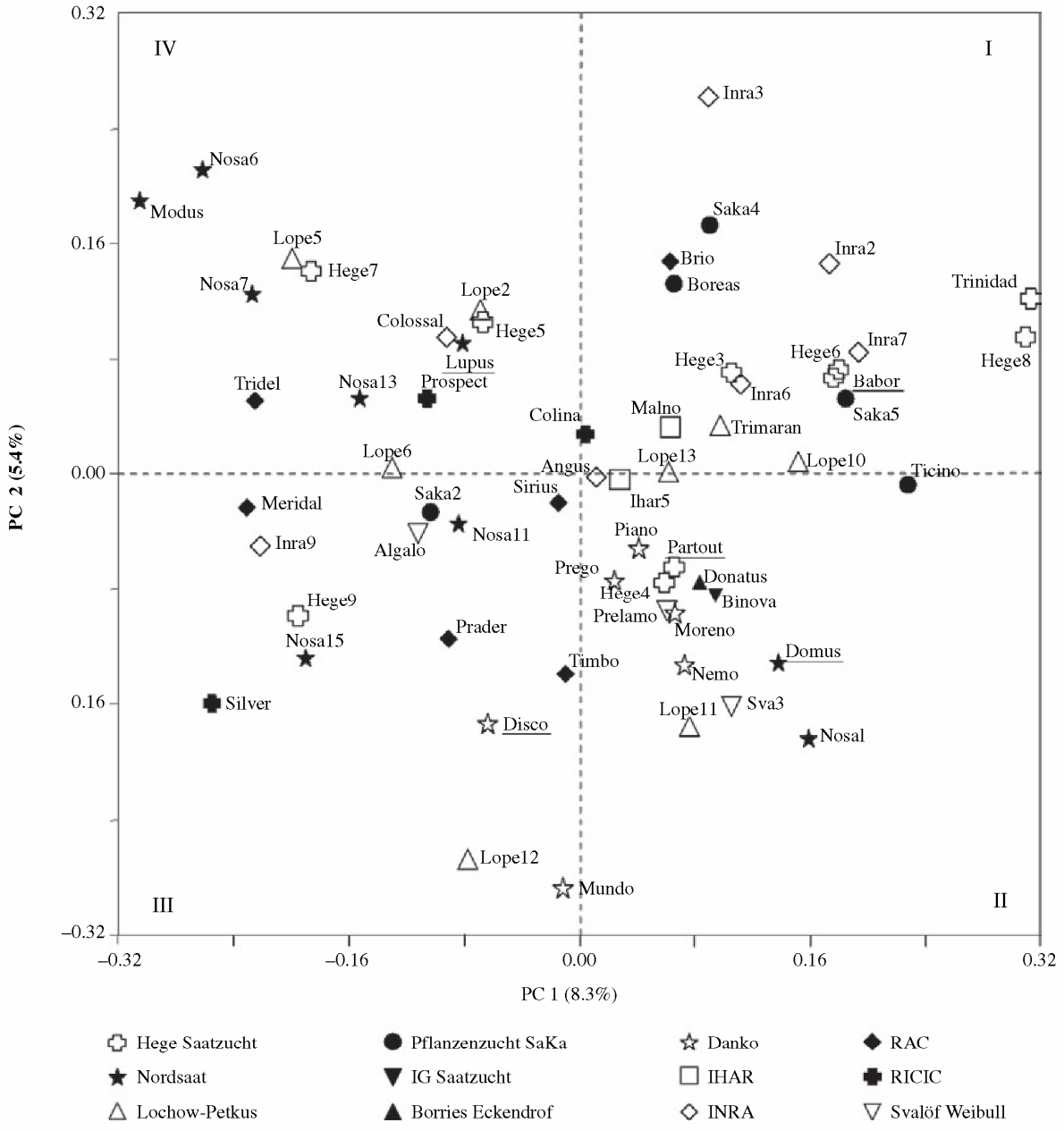

Fig. 1: Principal co-ordinate analysis based on MRD for 61 European winter triticale parental lines. PC1 and PC2 are the first and second principal coordinate. Breeding companies/institutes are characterized by symbols. The five testers are underlined

existing groups allows the focus to be only on promising crosses between the groups and thus reduces costs for producing and evaluating testcrosses. In addition to the prediction of SCA from molecular markers, the establishment of heterotic groups is another possibility for identifying hybrids with superior performance. Hybrid breeding programmes in allogamous or partially allogamous crops can be supported by marker analyses to reveal genetic relationships among latent germplasm pools. Reif et al. (2003a) proposed additional new heterotic groups in CIMMYT tropical maize with the aid of SSR markers. In subtropical maize, SSRs have been used to classify exotic germplasm as a first step for its introgression into existing heterotic groups (Reif et al. 2003b).

In triticale, PCoA based on MRD did not allow grouping of parental lines (Fig. 1). This is also supported by the results of *k*-means cluster algorithms using the software packages STRUCTURE and k-MEANS (MacQueen 1967, Pritchard et al. 2000) with different marker systems and diversity indices, which did not reveal a clear grouping (data not shown). The results were inconsistent for runs with varying parameters, such as number of suggested groups, marker system and diversity indices, and were therefore not useful for a clear

Table 5: Hybrid performance (HYB) and relative midparent heterosis (MPH%) for each female triticale line averaged across both testers of the two tester pairs ('Domus'–'Partout' and 'Disco'–'Lupus') for grain yield. Least significant differences ($LSD_{5\%}$) were calculated based on the averaged HYB and MPH% (experiment 1)

	HYB (t/ha)		MPH%	
Female	'Domus'–'Partout'	'Disco'–'Lupus'	'Domus'–'Partout'	'Disco'–'Lupus'
Group A[1]				
'Brio'	9.2*	8.7	11.4*	7.9
Hege5	9.3*	9.0	9.6	9.1
Lope11	9.5*	9.2	9.0	8.7
Lope13	9.5*	9.2	11.1*	9.6
'Mundo'	9.0*	8.6	3.9	2.4
Nosa15	9.2*	8.6	10.0*	6.1
'Prego'	9.3*	9.0	11.9	10.8
Group B				
Inra2	9.3	9.4	10.8	13.6*
Lope10	10.0	10.1	10.7	14.4*
'Meridal'	8.0	8.8*	−0.7	9.4*
Saka4	9.6	9.6	8.7	10.4*
'Tridel'	9.6	9.7	14.0	17.0*
'Trinidad'	9.5	9.9*	6.4	12.5*
Group C				
'Binova'	9.8	9.7	11.4	12.4
Ihar5	8.8	8.6	6.2	6.2
Lope2	8.9	8.9	2.2	4.0
Lope6	9.7	9.6	11.6	12.4
'Moreno'	9.3	9.2	7.7	8.8
Nosa11	9.0	9.0	11.9	13.9

[1]Grouping of females according to significant values for HYB or MPH% with one of the tester pairs.
*Significantly higher average performance of a female with the respective tester pair at $LSD_{5\%} = 0.3$ t/ha for HYB or $LSD_{5\%} = 2.5\%$ for MPH%.

grouping based on genetic similarity estimates. The lack of grouping is most probably a result of line breeding, where divergent crossing parents are used for obtaining a broad genetic variance in segregating populations. The exchange of germplasm between breeders and regions may have led to a reduction in the genetic distance between genotypes, if at all present at the beginning. This tendency could have been increased by the fact that triticale has a short breeding history and is bred only as grain feed and for adaptability to a wide range of climatic conditions. From the fact that neither breeding history nor marker analyses imply divergent groups in triticale, one may conclude that all the hybrids were produced from parents within a common germplasm pool in Europe.

Charcosset and Essioux (1994) stressed that heterozygosity at marker loci would be predictive only if markers are linked to genes affecting heterosis in the groups of interest. The authors conclude that if two lines belonging to the same heterotic group are closely related at the DNA level, they should display similar SCA with testers of the complementary groups. Although it can be assumed that the suggested intra-group situation is true for European triticale (Tams et al. 2004), the genomic markers might not be linked closely enough to QTLs influencing heterosis of the target traits. So far, no information about linkage disequilibrium is available in European triticale. Markers from expressed sequence tags (EST) or candidate genes from wheat and rye associated with important QTLs could be more suitable for exposing a relationship between GD and heterosis or hybrid performance.

Implications for the establishment of heterotic groups

Prospects for the successful development of genetically diverse and heterotic germplasm pools by long-term systematic breeding in triticale are encouraging. In a recent study with SSRs, Duvick et al. (2004) illustrated, with the maize breeding material at Pioneer Hi-Bred®, that in the 1950s, the ancestors of the Stiff Stalk *vs.* Non-Stiff Stalk heterotic groups formed one large unstructured collection. Thus, heterotic groups were not derived from geographically or genetically distant material and were initially based on traits such as pollination and combining abilities. After 50 years of intensive hybrid breeding using a clear pool concept, the lines in the Stiff Stalk *vs.* Non-Stiff Stalk heterotic groups have diverged. The development of these pools relied on widespread performance tests of hybrids and on a pragmatic use of methods or sources of germplasm. Such a long-term strategy should also be applied in triticale.

For the identification of heterotic groups, Melchinger and Gumber (1998) suggested the grouping of germplasm based on GS as a first step of a multi-stage procedure. In triticale, however, parental GS proved to have low predictive value for SCA and hybrid performance. Hence, in a first step some females could be sub-grouped in this study (Table 5, groups A and B) based on their heterotic response and SCA for grain yield with two tester pairs ('Disco'–'Lupus'; 'Domus'–'Partout', Table 4). The proposed use of these first two heterotic founder groups would be similar to the history of early hybrid breeding in maize (Duvick et al. 2004).

As a second step in the multi-stage procedure proposed by Melchinger and Gumber (1998), representative triticale genotypes from each subgroup should be selected for producing diallel crosses. The third step involves the evaluation of diallel crosses among the subgroups together with their parental lines in replicated field trials. In addition, Charcosset et al. (1998) showed that the introduction of marker distances as a predictor of SCA is efficient for improving the prediction of hybrid performance in situations where coancestry is unknown or only suspected. Hence, for all triticale females which were assigned to subgroups A and B, as well as for additional but yet unassigned genotypes, further performance trials of hybrids with additional testers are necessary to expand the suggested grouping. Later, heterosis among groups could be enhanced by recycling and selecting superior lines within groups based on HYB when crossed with lines from other groups and on line *per se* performance. Trait-associated molecular markers could assist these procedures by complementing field trials to allow more efficient planning of testcrosses. Thus, further research on marker-trait associations in triticale is necessary, both for marker-assisted selection and grouping of germplasm.

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General discussion

Knowledge about the genetic diversity of crop species enables well-directed strategies for breeding purposes and influencing the future genetic variability. Among the estimators for genetic distance, those based on DNA showed to be superior to pedigree-based estimators if information of ancestors is scarce. In cereals, molecular markers have been developed initially in economically more important crops like maize and wheat (Hoisington and Lander, 1987; Melchinger et al., 1991; Röder et al., 1995). These results suggest the application of molecular markers for genetic diversity assessment also in triticale. Further, investigation of heterosis and hybrid performance will supply information about the prospects of hybrid breeding in this allopolyploid crop. In addition, knowledge about genetic diversity and agronomic parameters enables the search for methods to predict the performance of triticale hybrids.

A range of diversity measurements are available which are based on the relatedness of pairs of genotypes due to (i) common ancestors or (ii) genetic diversity. For all triticale genotypes, confidential pedigree information has been supplied by the breeding institutes or companies. The number of known ancestors varied from the knowledge back to the initial wheat x rye cross to the female parent only. Therefore, the calculation of the coancestry coefficient (*f*) was not based on a well-balanced data stock to be truly informative. Further, the assumptions regarding relatedness of ancestors, parental contribution to the offspring, absence of selection and genetic drift are mostly not applicable to modern breeding material (Cox et al., 1985). Pedigree analyses of triticale resulted in a high amount of *f* values < 0.1 (85%) and

the distribution of *f* demonstrated low differentiation power. Therefore, *f* is not useful for prediction purposes in triticale.

Molecular marker assessment

The development of SSR markers for a new species is a time-consuming procedure. Due to the allopolyploid genome constitution of triticale with chromosomes from wheat and rye, SSR markers developed in both of the ancestor species were available for application (Röder et al., 1995; Röder et al., 1998; Saal and Wricke, 1999; Prasad et al., 2000; Korzun pers. communication). Their utilization in triticale showed that the quality and amount of banding patterns were reliable and informative for most of the markers. It was presumed that the genome specific wheat or rye markers rarely amplify fragments in the opposite genome (Röder et al., 1995), which could be confirmed in the present study. Therefore, the diversity of the triticale genome was assessed in total as well as separately for the wheat and the rye genome. Distribution of genetic similarity estimates (GS) based on SSRs showed that diversity within the wheat genome is wider than in rye. This result was unexpected for the rye genome portion as allogamy promotes genetic recombination and gene flow should not be limited in successive generations. In wheat, autogamy can lead to a narrower genetic basis during evolution. In the present study, in addition to genomic wheat and rye SSR markers, SSRs derived from rye expressed sequence tag (EST) were applied. EST-derived SSRs generally have lower polymorphic information content and in the present dataset they may be the reason for higher similarity within the rye genome than within the wheat genome, where only genomic SSRs were used. Nevertheless, SSR markers proved informative to assess triticale genetic diversity. Principle coordinate analysis (PCoA) based on GS_{SSR} showed no distinct groups within triticale. Only genotypes of two companies ('Nordsaat' and 'RICIC') were separated

from the remaining varieties and breeding lines. In contrast, a strong grouping according to breeding companies has been reported in commercial maize hybrids (Sun et al., 2001). An analysis of molecular variance (AMOVA) confirmed the lack of grouping in triticale by revealing lower variation of the genotypes among the companies than within. In European triticale, the free exchange of breeding material and the exclusive use for one end-use purpose, namely grain feed, may have resulted in the absence of clear groups.

AFLP markers have been recommended as the most efficient marker system in crops, because of the highest number of loci per assay detected compared to other systems (Powell et al., 1996; Lübberstedt et al., 2000; Belaj et al., 2003). In triticale, these findings were confirmed by comparing AFLPs to SSRs. Regarding the quality of the AFLP banding patterns, the occurrence of bands with intermediate intensity in some DNA samples raised the question whether it is necessary to analyse several single seeds of each genotype instead of bulked samples. In triticale, off-types or heterozygosity are more probable than in strictly autogamous species, which may lead to an intermediate intensity. Nevertheless, an admixture up to 10% off-type DNA should not disrupt correct amplification and identification (Zhu et al., 1998). Further, a substantial degree of heterogeneity and/or heterozygosity was also observed with codominant SSRs. Testing and preselecting SSR markers for clear banding patterns is an advantage to define the threshold for abscence presence of banding patterns. Both molecular marker systems generated reliable results but they differ in their advantages regarding time or information content of banding pattern.

None of the dendrograms generated by the UPGMA (unweighted pair-group method, arithmetic average) cluster algorithm resulted in a clear grouping of triticale genotypes. The correlation between GS estimates and *f* were low due to the sparse information content of pedigree data. GS_{SSR} and GS_{AFLP} were moderately

correlated, which is in concordance with the findings of the Mantel Z test. Studies in other crops showed a wide variation for correlation coefficients (Pejic et al., 1998; Bohn et al., 1999). Powell et al. (1996) suggested that correlations of GS based on different marker systems are highly influenced by the relationship of the genotypes assessed and may decline if the individuals are either very closely related or highly unrelated. In the present study, genotypes from extremely differing environmental conditions as well as related genotypes from the same breeding company were included. The unstructured variation within the triticale germplasm may hinder higher correlations in the present study.

Additionally, cophenetic correlations were also moderate, which implies that the dendrograms based on GS_{AFLP} and GS_{SSR} provide only a poor representation of the information in the original similarity matrices. This is confirmed by the results of the bootstrap analysis, discovering only small groups of genotypes conserved in both dendrograms. The genotypes in the accumulations belong mainly to two breeding companies, and they clustered also in the PCoA based on SSRs. As a conclusion, SSRs and AFLPs seem to have a comparable discrimination power even though the results differ due to the differences in the nature of the marker systems and in the location of the markers distributed within the genome.

Hybrid performance and heterosis

The levels of average midparent heterosis in triticale were more similar to wheat than to rye. In recent rye hybrids, relative MPH for grain yield of 92% was observed in comparison with 10.3% in triticale (Geiger and Miedaner, 1999). In wheat, heterosis seems to be influenced by the material tested. Several studies reported MPH and better-parent heterosis (BPH) of the same magnitude as in triticale

(Martin et al., 1995; Oury et al., 2000). However, others discovered zero MPH and -9.3% BPH grain yield in spring wheat (Dreisigacker et al., 2005).

The results of an earlier investigation of heterosis in triticale with a smaller number of hybrids (Oettler et al., 2003) was confirmed in the present study for all traits by the evaluation of 209 hybrids in six locations. Further, the mean of 10.3% grain yield heterosis corresponds with the findings in spring triticale (Pfeiffer et al., 1998) even though the results were based only on small plot measurements. Trait correlations showed that the yield component 1000-kernel weight made the largest contribution to grain yield heterosis. In correspondence with hybrids of other small-grain cereals, heterosis for spikes per square meter is often low or negative. Even for this trait, variation of heterosis is wide with a maximum of 12% based on mid-parent value. A crucial issue for the acceptance of any triticale varieties by farmers is the tolerance to pre-harvest sprouting. The present study revealed significant genetic variation within parental lines and hybrids. The maximum value of 28.7% MPH showed potential for improving falling number even though the average heterosis for this trait was low (-10.6%). For successful future hybrid breeding, triticale shows sufficient heterosis and variation for all traits. Further, one third of the hybrids outyielded modern triticale line varieties, which were included as checks. This is also encouraging in the present study, where no pre-selection of parental lines took place. With the benefit of developed heterotic groups, higher heterosis can be expected for inter-group crosses as reported in wheat (Liu et al., 1999).

General and specific combining ability

The relation between GCA and SCA effects is important for the successful prediction of hybrid performance (Melchinger et al., 1987). When GCA predominates SCA, superior hybrids can be identified and selected mainly based on their prediction

from GCA effects. In addition, GCA effects were more important than SCA in inter-group crosses than in intra-group crosses. The superiority of the former in terms of mean performance and heterosis for grain yield is well documented (for review see Melchinger and Gumber, 1998). In triticale, the analysis of combining ability resulted in higher estimates for $\hat{\sigma}^2_{GCA}$ than $\hat{\sigma}^2_{SCA}$ for all traits except for grain yield and protein concentration. Further, correlation of GCA and line *per se* performance of parents was only moderate. Both parameters indicate low predictive value of GCA or parental line *per se* performance for superior hybrid performance with regard to grain yield and protein content. This emphasizes the need of developing heterotic groups or prediction methods based on parental GD. Considering the relationship in the European triticale germplasm pool as an intra-group situation, predominance of SCA over GCA for grain yield was expected. In contrast, for most of the agronomic traits GCA is more important, which is an indication of inter-group tendencies, although a clear grouping was not possible yet.

Time- and cost-reducing methods for pre-selection of hybrid parents

Since SCA effects are more important than GCA effects for the most important agronomic trait grain yield, the association between GD and SCA was examined. Charcosset and Essioux, 1994) recommended in theory that the most important component concerning correlation with GD is SCA. In triticale, none of the GD estimates were significantly correlated with SCA of any trait. Hence, hybrid performance could not be predicted reliably with the aid of genetic distance estimates or line *per se* performance. Information based on GD or on agronomic traits of parents or hybrids was not helpful to define heterotic groups in the European triticale germplasm pool. Consequently, the development of heterotic

groups is necessary for a successful future hybrid breeding program. The long-term progress from a large unstructured cluster of maize varieties in the 1950s to distinct pools of Stiff Stalk *vs.* Non Stiff Stalk heterotic groups was illustrated by Duvick et al. (2004). Hence, two heterotic founder groups with female triticale parents have been proposed based on the concept of divergence in the breeding history of maize. The females have been sub-grouped according to their heterotic response and SCA for grain yield with two tester pairs. As a strategy to develop future heterotic groups, a long term multi-stage procedure is recommended. Evaluation of the suggested groups is essential by producing further intra-group hybrids in diallel crosses as recommended by Melchinger and Gumber (1998). Crossing with additional testers will supply information to expand the grouping. The heterotic effect among the groups can be enhanced by recycling and selecting superior lines within the groups. Future research on trait-associated markers will offer new possibilities for a successful marker-assisted selection and grouping of germplasm.

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Summary

Knowledge of the genetic diversity of a species is of paramount importance for the choice of crossing parents in line and hybrid breeding. Genetic distance (GD) estimates based on molecular markers proved to be well suited for direct exploration of the relationship within a germplasm pool. Triticale hybrid breeding and heterosis have received increasing attention in recent years. Hybrid seed production is highly attractive for autogamous species because of the built-in variety protection of hybrids in comparison to line varieties.

The main objective was to appraise the prospect of hybrid breeding in European winter triticale and develop time- and cost-reducing strategies. In particular, the main objectives were to (i) assess and compare genetic diversity estimates in European winter triticale elite germplasm based on molecular markers and pedigree data, (ii) determine hybrid performance and heterosis in multiple environments, and (iii) evaluate prediction methods for hybrid performance and heterosis to support future hybrid breeding programs.

Average coancestry coefficient between all pairs of the 128 European elite genotypes was low (f = 0.059) due to scanty information available for the majority of the varieties and breeding lines. Better estimates of genetic distance of triticale genotypes were obtained by molecular marker assessment with 93 simple sequence repeat (SSR) markers and 10 *Pst*I/*Taq*I primer combinations of amplified fragment length polymorphism (AFLP) markers. While SSR markers have been developed in wheat and rye and are mapped in the genome, the location and distribution of AFLP markers is unknown. Both marker systems resulted in reliable genetic diversity estimates. The moderate correlation between genetic distance estimate (GD) of SSR

and AFLP marker analyses (GD_{SSR}; GD_{AFLP}) corresponded with other studies. Cluster analysis and principle coordinate analysis revealed no clear separation of germplasm groups. Supported by a bootstrap analysis, it was concluded that both marker systems provide consistent information for germplasm identification. The lack of grouping is in concordance with the breeding history of triticale as a self-pollinator, the wide adaptation of the inter-generic species and the single end-use purpose.

Simultaneously to the marker assessment, 209 F_1 hybrids were produced by a chemical hybridizing agent. The hybrids and their parents (57 females and five testers) were evaluated in field trials in six environments in Germany during the season 2001-2002. A combined analysis revealed significant heterosis for all eight traits. The level of mid-parent heterosis was positive for grain yield, 1000-kernel weight, number of kernels per spike, test weight and plant height and negative for number of spikes per m^2, falling number and protein concentration. Forty-six of the hybrids outyielded modern varieties, which were included as checks, by 10% and more. This aspect is important for the success of hybrids on the market for commercial production. Results regarding hybrid performance, heterosis, GCA/SCA relationship, trait correlation in hybrids and parents and aspects regarding cost-effective high quality F_1 seed production appear to be sufficiently positive to encourage further work on hybrid breeding. Approaches to reduce time and costs for the identification of superior parental combinations and the prediction of hybrid performance revealed no reliable method yet. Correlations between SCA and GD of parents based on the different marker systems were low for all traits, which hampers prediction. Grouping of germplasm based on GD estimates or on heterotic response of the hybrids could not be discovered in triticale. As a consequence, a first step for an optimum allocation of resources in commercial hybrid breeding programs is the development of heterotic groups. In the present study, several females have been

sub-grouped according to their heterotic response and SCA for grain yield with two tester pairs. Following the early history of hybrid breeding in maize, a multi-stage procedure was suggested for triticale to evaluate and expand the sub-grouping and enhance heterosis among groups.

Zusammenfassung

Die Kenntnis der genetischen Diversität innerhalb einer Art ist sowohl in der Linienzüchtung als auch in der Hybridzüchtung für die Wahl der Kreuzungspartner von größter Bedeutung. Auf molekularen Markern basierende genetische Distanzen eignen sich besonders, um die Verwandtschaft direkt im genetischen Hintergrund aufzudecken. Hybridzüchtung und Heterosis bei Triticale haben in den letzten Jahren wachsende Aufmerksamkeit erfahren. Die Produktion und der Vertrieb von Hybridsaatgut sind aufgrund des implizierten Sortenschutzes besonders für selbstbefruchtende Arten attraktiv.

Im Rahmen der vorliegenden Arbeit sollten vor allem die Perspektiven für Hybridzüchtung in europäischem Wintertriticale abgeschätzt und zeit- und kostenminimierende Strategien dazu entwickelt werden. Im Einzelnen sollten (i) die Schätzwerte für genetische Distanzen des europäischen Elitezuchtmaterials mit Hilfe von molekularen Markern und Abstammungsdaten beurteilt und miteinander verglichen, (ii) das Ausmaß von Hybridleistung und Heterosis in mehrortigen Leistungsprüfungen festgestellt, und (iii) Vorhersagemethoden für Hybridleistung und Heterosis zur Unterstützung zukünftiger Hybridzüchtungsprogramme bewertet werden.

Der durchschnittliche Abstammungskoeffzient zwischen allen Paaren der 128 europäischen Elitegenotypen war aufgrund eingeschränkter Angaben für eine Vielzahl der Sorten und Zuchtstämme niedrig (f = 0,059). Die genetische Diversität in Triticale wurden durch Untersuchungen mit 93 ‚simple sequence repeat' (SSR-) Markern und 10 *Pst*I/*Taq*I Primerkombinationen von ‚amplified fragment length polymorphism' (AFLP-) Markern besser abgebildet. Die SSR-Marker dieser Studie wurden im Weizen- und Roggengenom entwickelt und kartiert. Im Gegensatz dazu

war die Lokalisation und Verteilung der AFLP-Marker im Genom unbekannt. Beide Markersysteme resultierten in zuverlässigen Schätzwerten für die genetische Diversität. Die moderate Korrelation zwischen genetischer Distanz (GD) der SSR und AFLP Markeranalysen ($GD_{SSR;}$ GD_{AFLP}) wurde auch in Studien anderer Arten beobachtet. Cluster- und Hauptkoordinatenanalysen zeigten keine klar abgegrenzten Gruppen. Unterstützt durch eine ‚Bootstrap'-Analyse konnte der Schluss gezogen werden, dass die Informationen beider Markersysteme von ähnlicher Qualität und Aussagekraft für die Erfassung der genetischen Diversität sind. Die fehlende Gruppierung stimmt mit den Schlussfolgerungen aus der Züchtungshistorie von Triticale als Selbstbefruchter, seiner breiten Anpassungsfähigkeit an Umweltbedingungen und dem Fehlen unterschiedlicher Nutzungsrichtungen überein.

Zeitgleich zu den Markeranalysen wurden 209 F_1 Hybriden unter Verwendung eines chemischen Hybridizierungsmittels produziert. Die Hybriden wurden zusammen mit ihren 57 Müttern und fünf väterlichen Testern in sechsortigen Leistungsprüfungen in Deutschland während der Vegetationsperiode 2001-2002 geprüft. Eine kombinierte statistische Auswertung ergab signifikante Heterosis für alle acht Merkmale, wobei die Ergebnisse vergleichbar mit anderen Studien bei Weizen waren. Das Ausmaß der Heterosis im weiteren Sinn (Heterosis zum Elternmittel) war für Kornertrag, 1000-Korn Gewicht, Anzahl der Körner, Hektolitergewicht und Pflanzenhöhe positiv und für Ährenzahl pro Quadratmeter, Fallzahl und Proteinkonzentration negativ. Vierundsechzig Hybriden übertrafen auch moderne Liniensorten, die als Standards mitgeprüft wurden, im Ertrag um mehr als 10%. Diese Überlegenheit ist als ein kommerziell nutzbarer Ertragsvorteil für ein erfolgreiches Hybridzüchtungsprogramm bedeutend.

Die Ergebnisse zu den verschiedenen Aspekten der kosteneffizienten Produktion von hochwertigem F_1 Saatgut von Triticale lassen es als gerechtfertigt erscheinen, weiteren Aufwand für die Etablierung der Hybridzüchtung bei Triticale zu betreiben. Methoden, die Zeit und Kosten bei der Identifikation der besten Elternkombinationen reduzieren oder die sich zur Vorhersage von Hybridleistung eignen, müssen für europäischen Wintertriticale allerdings erst entwickelt werden. Die Korrelationen zwischen spezifischer Kombinationseignung und genetischer Distanz der Eltern waren für alle Merkmale niedrig. Die Triticalegenotypen konnten weder aufgrund der genetischen Distanzen in Gruppen unterteilt werden, noch konnten mit Hilfe der agronomischen Daten heterotische Gruppen definiert werden. In kommerziellen Hybridzuchtprogrammen ist als ein erster Schritt zur optimalen Nutzung der Ressourcen die Entwicklung solcher Gruppen notwendig. In dieser Studie wurden einige Hybridmütter aufgrund ihrer heterotischen Reaktion im Kornertrag gegenüber zwei Testerpaaren in Untergruppen eingeteilt, wobei auch die Ergebnisse bezüglich SCA herangezogen wurden. In Anlehnung an den Beginn der Hybridzüchtung von Mais konnte eine mehrstufige Vorgehensweise für Triticale vorgeschlagen werden, um die Untergruppen zu evaluieren, zu vergrößern und letztendlich die Heterosis zwischen den neu definierten Gruppen zu erhöhen.

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Erklärung

Hiermit versichere ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und unter Zuhilfenahme von angegebenen Quellen und Hilfsmitteln angefertigt wurde. Diese Arbeit wurde von noch keiner anderen Prüfungsbehörde vorgelegt.

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den 9. April 2006

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