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ORIGINAL PAPER

Molecular marker assisted broadening of the Central European heterotic groups in rye with Eastern European germplasm

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Abstract Broadening the genetic base of heterotic pools is a key to ensure continued genetic gains in hybrid breeding and extend hybrid cultivation to new areas. In the present study, two Central European heterotic pools (Carsten and Petkus) and five Eastern European open-pollinated varieties (OPVs, Pop-1 to Pop-5) were studied with the objectives to (1) investigate the genetic diversity in OPVs and the heterotic pools using molecular and field data, (2) evaluate the molecular diversity among OPVs, (3) examine the combining ability for grain yield of the OPVs when crossed with testers in field trials, and (4) develop a strategy for targeted introgression of OPV germplasm into the heterotic pools. In total, 610 S_0 plants, 347 from OPVs and 263 from heterotic pools, were developed. Clones of the S_0 plants of OPVs were crossed with two testers belonging to each heterotic pool, while clones of heterotic pools were crossed with only the opposite tester. Testcrosses were evaluated for grain yield in multi-location trials. In addition, 589 S_0 plants were fingerprinted with 30 SSR markers. The data revealed that the Carsten pool has a narrow genetic base and should be the primary target for broadening the established heterotic pattern. Mean and genetic variance suggested that Pop-2 and Pop-4 are good candidates for introgression in Petkus pool and Pop-5 in Carsten pool. Nevertheless, introgression of Pop-5 in Carsten could reduce the genetic diversity between heterotic pools. Therefore, we suggest that either selected plants of Pop-5 should be introgressed or more Eastern European germplasm should be fingerprinted and field evaluated to identify promising germplasm for broadening the established heterotic pattern.

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Introduction

The University of Hohenheim is the cradle of hybrid rye where research in hybrid breeding was started in 1970 (Geiger and Miedaner 1999). The two heterotic pools Carsten (Pool-C) and Petkus (Pool-P) were soon identified as the most promising heterotic pattern (Hepting 1978). During the first phase of hybrid breeding, parental inbred lines were developed by recurrent selfing of plants from both pools. In the second phase, inbred lines were primarily generated by second-cycle breeding, i.e., from crosses among elite inbreds within heterotic pools. This in combination with selection is expected to reduce genetic

diversity within the heterotic pools (Duvick et al. 2004). The consequences of such a narrowing of germplasm diversity in breeding programs are a decrease in selection gain and an increase in susceptibility to biotic and abiotic stresses coupled with the threat of further genetic erosion (Smith et al. 2004). Consequently, it is important to continuously broaden the genetic base of the established heterotic pools.

In Germany, hybrids are being grown on more than 60% of the area under rye cultivation (Thomas Miedaner, personal communication). In contrast, in Eastern European countries hybrids are just starting to gain importance. To exploit the established Central European heterotic pattern in this region, Pool-C and Pool-P have to be adapted to the specific needs of rye cultivation in Eastern Europe such as increased frost tolerance or snow mold resistance. A promising avenue to achieve this goal is to introgress adapted Eastern European open-pollinated varieties (OPVs) into these heterotic pools. Broadening of the Central European heterotic pools is not only relevant in rye breeding in the original target environment of Central Europe but also for expanding hybrid rye cultivation to new target environments.

The potential usefulness of exotic germplasm to broaden the established heterotic pattern can be assessed on the basis of four criteria: (1) performance of F_1 hybrids, (2) per se performance of the exotic material, (3) heterotic response, and (4) contribution to genetic variability (Melchinger 1999). One difficulty in exploiting genetic resources in hybrid rye breeding is the prevalence of self-incompatibility in OPVs and landraces (Lundqvist 1956). Maintenance of promising gametes requires time- and resource-intensive backcrossing to self-fertile lines. Furthermore, in the absence of prior knowledge of the relationship with heterotic pools, testcrosses with both heterotic pools have to be developed and evaluated.

Initial experimental studies in maize indicated the potential of molecular markers to assign new germplasm to heterotic pools (Reif et al. 2003). In rye, Wilde et al. (2006) evaluated the use of simple sequence repeat (SSR) markers to guide the introgression of a Russian population for broadening the existing heterotic pools. The results suggested that molecular markers are a promising tool for a systematic and efficient introgression. Nevertheless, to draw a general conclusion on the applicability of this approach, more information is needed.

The main goal of this study was to evaluate the utility of molecular marker data for a systematic broadening of the Central European heterotic pattern in rye with Eastern European OPVs. In particular, our objectives were to (1) investigate the genetic diversity in five Eastern European OPVs and the two Central European heterotic pools with molecular and field data, (2) evaluate the molecular diversity among the populations, (3) examine the combining ability of the five Eastern European OPVs for grain yield when crossed with testers of both heterotic pools in field trials, and (4) develop a strategy for targeted introgression of Eastern European OPV germplasm into the Central European heterotic pattern.

Materials and methods

Plant materials

The basic material comprised five Eastern European OPVs namely PR2733/92/1 (Pop-1), Agricolo/1 (Pop-2), SMH2502/1 (Pop-3), ROM103/1 (Pop-4), and LAD302/1 (Pop-5), and the two Central European heterotic pools (Pool-C and Pool-P). Pop-1 originated from Belarus and the other four populations from Poland. A total of 610 heterozygous S_0 plants in five OPVs and the heterotic pools were selected at random. Of these, 347 S_0 plants belonged to the five OPVs. The S_0 plants of each population were split randomly into two sub-groups of similar size (Table 1). Each plant was multiplied through clonal propagation to obtain 16 plants (further denoted as S_0 clones). The clones in one sub-group were crossed with Tester-C and those in the other with Tester-P. The testers were synthetics developed from Pool-C and Pool-P. Furthermore, 263 S_0 plants within full-sib families were developed from Pool-C and Pool-P, and clones of these S_0 plants in a pool were crossed with only the opposite tester.

Table 1 Number of parental S_0 plants sampled from five Eastern European rye populations (Pop-1 to Pop-5) and the two Central European heterotic pools (Pool-C and Pool-P) crossed with two testers (Tester-P and Tester-C) as well as gene diversity, number of alleles per locus, and unique alleles identified in five populations and two heterotic pools

	Pop-1	Pop-2	Pop-3	Pop-4	Pop-5	Pool-C	Pool-P
Number of S_0 plants							
Tester-P	33	37	40	32	16	142	–
Tester-C	33	45	42	49	20	–	121
Total	66	82	82	81	36	142	121
Genotyped	65	79	81	81	31	139	113
Genetic diversity and number of alleles							
Gene diversity	0.56	0.55	0.56	0.56	0.55	0.43	0.53
Alleles per locus	4.80	4.77	4.63	4.87	4.40	3.00	3.83
Unique alleles	6	1	1	5	2	0	1

Field experiments and statistical analyses

The testcrosses of the 610 S_0 plants along with eight checks were evaluated for grain yield (Mg ha^{-1}, determined at 140 g kg^{-1} moisture) in field trials separately for both testcross series in 2007. Testcrosses with Tester-P were grown at Bernburg, Blönsdorf, Dohnsen, and Klausheide; and those with Tester-C, at Bekedorf, Lohne, Petkus, and Prislich. Each testcross series was divided into sub-experiments laid out in 9 × 9 or 8 × 9 alpha-lattice designs with two replications. Plant density was 230 plants m^{-2} and plot size ranged from 5 to 6 m^2.

We used a mixed model with fixed and random effects to estimate variance components and means of the testcrosses from each population or pool. All values were estimated separately for both testcross series by the following model:

$$\begin{aligned} &\text{C} : \text{L} + \text{C} \cdot \text{L} + \text{L} \cdot \text{SUB} + \text{L} \cdot \text{SUB} \cdot \text{R} + \text{L} \cdot \text{SUB} \cdot \text{R} \cdot \text{B} + \text{Within-FSF-Pool} + \text{Among-FSF-Pool} \\ &\quad + \text{Pop-1} + \text{Pop-2} + \text{Pop-3} + \text{Pop-4} + \text{Pop-5} + \text{Within-FSF-Pool} \cdot \text{L} + \text{Among-FSF-Pool} \cdot \text{L} \\ &\quad + \text{Pop-1} \cdot \text{L} + \text{Pop-2} \cdot \text{L} + \text{Pop-3} \cdot \text{L} + \text{Pop-4} \cdot \text{L} + \text{Pop-5} \cdot \text{L}, \end{aligned}$$

where C, L, SUB, R, B, and FSF denote effects of genotypic groups (checks, Pool-C, Pool-P, Pop1–Pop5), locations, sub-experiments, replications, blocks, and full-sib families, respectively. We used the syntax suggested by Patterson (1997), where fixed effects appear before the colon and random effects after the colon. Dummy variables were used to separate genotype groups and estimate genetic effects and variances for each group following Piepho et al. (2006), but for sake of simplicity we suppressed dummies in the model stated above.

Best linear unbiased estimators (BLUE), variance components, and best linear unbiased prediction (BLUP) values together with their standard errors were determined by the restricted maximum likelihood (REML) method. All analyses were performed with software ASReml (Gilmour et al. 2002). Variance components were assumed to be significantly larger than zero when their estimate was at least 1.96 ($P < 0.05$) or 2.58 ($P < 0.01$) times as large as their standard error according to Lynch and Walsh (1997). This test is approximate and conservative (Stram and Lee 1994). Heritability on an entry-mean basis was calculated as the ratio of genotypic to phenotypic variance according to Melchinger et al. (1998). The approximate nature of this definition of heritability in incomplete block designs and with correlated genetic effects is discussed by Piepho and Möhring (2007). Significance tests for the difference in the average performance of the populations were conducted with t tests using PROC Mixed in SAS (SAS Institute 1999) according to Piepho (2004).

The usefulness criterion (Schnell 1983) of each population was predicted as

$$U_{ij} = c_{ij} + i_{\alpha} h_{ij} \sigma_{g_{ij}},$$

where c_{ij} denotes the mean of population i when crossed with tester j. The parameters i_{α}, h_{ij}, and σ_{gij}, refer to selection intensity, square root of the heritability, and genotypic standard deviation of the population i when crossed with tester j. The h_{ij} was estimated for the five Eastern European OPVs as:

$$h_{ij} = \sqrt{\frac{\sigma^2_{g_{ij}}}{\sigma^2_{g_{ij}} + \frac{\sigma^2_{gl_{ij}}}{l} + \frac{\sigma^2_{e_{ij}}}{l \cdot r}}},$$

where σ^2_{gl} is the genotype × location variance (Pop-1 · L, Pop-2 · L, Pop-3 · L, Pop-4 · L, or Pop-5 · L), $\sigma^2_e$is the error variance of the above-mentioned model, l is the number of locations, and r the number of replicates per location. For heterotic pools, twice the estimate of genotypic variance within full-sib families was used to consider the expected genetic variance. We calculated U_{ij} assuming selection intensities of $i_{0.05} = 2.063$ and $i_{0.40} = 0.966$.

Molecular data analyses

A total of 589 S_0 plants were fingerprinted by KWS LOCHOW GMBH following standard laboratory protocols (for details see Stich et al. 2008) with 30 SSR markers (Supplementary Table 1). The 30 marker loci were uniformly distributed across the entire genome. Genetic relationships among the 589 S_0 plants were analyzed with a model-based clustering approach using software package STRUCTURE (Pritchard et al. 2000). The number of clusters k varied from two to seven. Runs of STRUCTURE were performed by setting the burn-in time to 50,000 and the replication number to 100,000. The optimal number of clusters k was determined based on the ad hoc statistic described by Evanno et al. (2005). In addition, modified Rogers' distances (MRD) were calculated according to Wright (1978) (i) among S_0 plants and (ii) among

populations. Principal coordinate analysis (PCoA) was performed following Gower (1966) based on the matrix of MRD values between populations. Gene diversity (Nei 1987, p. 164) was calculated with PowerMarker (Liu 2002). All other molecular analyses were performed using software package Plabsoft (Maurer et al. 2008).

Results

Field evaluations

Average grain yield of testcrosses with Tester-P ranged from 5.38 (Pop-4 × Tester-P) to 5.82 Mg ha^{-1} (Pool-C × Tester-P) (Table 2). Among all Eastern European OPVs, Pop-5 × Tester-P showed yield potential similar to Pool-C × Tester-P with testcrosses of several S_0 plants of Pop-5 outperforming the best testcross of Pool-C. Estimates of genotypic variances were significantly different from zero ($P < 0.01$). Close agreement was observed for ranks of usefulness criterion for two selection intensities. Testcrosses of Pop-5 showed the largest usefulness value among the five OPVs as well as in comparison with Pool-C.

For the testcross series with Tester-C, average grain yield of testcrosses ranged from 5.58 (Pop-4 × Tester-C) to 6.11 Mg ha^{-1} (Pool-P × Tester-C) (Table 3). Among the five Eastern European OPVs, testcrosses of Pop-2 showed the highest yield potential on average, whereas the testcross of one S_0 plant of Pop-4 outperformed the best testcrosses of Pool-P. Estimates of genotypic variances were significantly different from zero ($P < 0.01$). The rank of usefulness criterion for both selection intensities showed wide fluctuation which was most pronounced for the testcrosses of Pop-4. The highest values of usefulness criterion were obtained for Pool-P × Tester-C for both selection intensities. Among the five Eastern European OPVs the highest value was obtained for testcrosses of Pop-4 under strong selection intensity ($i_{0.05} = 2.063$) and for testcrosses of Pop-2 under moderate selection intensity ($i_{0.40} = 0.966$).

Molecular genetic diversity within populations

The total number of alleles detected for the 30 loci was 174, with the number of alleles per locus ranging from 2 to 21. In total, 54 alleles were present in the five OPVs, but absent in the two heterotic pools. The number of unique alleles per population for the five Eastern European OPVs and the two heterotic pools ranged from 0 (Pool-C) to six (Pop-1) (Table 1). A comparison of triplets of Pool-C, Pool-P, and one Eastern European OPV revealed a large number of unique alleles for each OPV with an average of 28 (Fig. 1). The number of common alleles in all five Eastern European OPVs was higher with Pool-P than Pool-C. Pool-C and Pool-P shared 72% of the alleles. Average gene diversity of the five OPVs and the two heterotic pools was 0.53 with a minimum of 0.43 (Pool-C) and a maximum of 0.56 (Pop-1, Pop-3, and Pop-4) (Table 1).

Molecular genetic diversity among populations

The genetic distances between all five Eastern European OPVs and Pool-C were larger than between the OPVs and Pool-P (Table 4). In the PCoA based on MRD estimates of

Table 2 Mean, minimum, and maximum grain yield (Mg ha^{-1}) of testcrosses of 300 S_0 plants from five Eastern European populations (Pop-1 to Pop-5) and the Carsten pool (Pool-C) with a tester of the Petkus pool (Tester-P), variances of the testcrosses for genotypes (G) and genotype × environment (G × E) and their standard errors, heritability (h^2), and usefulness values (U) with two different selection intensities i_α

	Pop-1	Pop-2	Pop-3	Pop-4	Pop-5	Pool-C
Mean	5.51^{ab}	5.60^{bc}	5.40^{a}	5.38^{a}	5.78^{cd}	5.82^{d}
Minimum	5.08	5.02	4.60	3.94	5.29	5.40
Maximum	5.79	6.02	5.93	6.05	6.24	6.12
Variances						
σ^2_G	0.03 ± 0.01*	0.07 ± 0.02**	0.09 ± 0.03**	0.21 ± 0.06**	0.09 ± 0.04*	0.03† ± 0.01**
$\sigma^2_{G\times E}$	0.06 ± 0.01**	0.05 ± 0.01**	0.06 ± 0.01**	0.10 ± 0.02**	0.02 ± 0.01*	0.02 ± 0.00**
h^2	0.62	0.80	0.83	0.87	0.89	0.73
$U(i_{0.05} = 2.063)$	5.81	6.08	5.97	6.25	6.38	6.26‡
$U(i_{0.40} = 0.966)$	5.65	5.83	5.66	5.79	6.06	6.03‡

Means in a row followed by a common letter are not significantly different according to a t test ($P < 0.05$)

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively

† Estimates are based on genetic variance within full-sib families

‡ Usefulness criteria are based on expected response to selection calculated by standardizing expected genetic variance components

Table 3 Mean, minimum, and maximum grain yield (Mg ha^{-1}) of testcrosses of 310 S_0 plants from five Eastern European populations (Pop-1 to Pop-5) and the Petkus pool (Pool-P) with a tester of the Carsten pool (Tester-C), variances of the testcrosses for genotypes (G) and genotype × environment (G × E) and their standard errors, heritability (h^2), and usefulness values (U) with two different selection intensities i_α

	Pop-1	Pop-2	Pop-3	Pop-4	Pop-5	Pool-P
Mean	5.80^{ab}	5.96^{bc}	5.82^{ab}	5.58^{a}	5.86^{ab}	6.11^{c}
Minimum	5.08	5.32	4.78	3.87	4.91	5.51
Maximum	6.55	6.52	6.60	7.00	6.38	6.92
Variances						
σ^2_G	0.14 ± 0.04**	0.13 ± 0.03**	0.17 ± 0.04**	0.35 ± 0.08**	0.11 ± 0.05*	0.13† ± 0.02**
$\sigma^2_{G\times E}$	0.07 ± 0.02**	0.05 ± 0.01**	0.05 ± 0.01**	0.09 ± 0.02**	0.06 ± 0.02**	0.05 ± 0.01**
h^2	0.82	0.85	0.87	0.91	0.81	0.85
$U(i_{0.05} = 2.063)$	6.49	6.66	6.60	6.74	6.48	7.08‡
$U(i_{0.40} = 0.966)$	6.13	6.29	6.19	6.12	6.15	6.56‡

Means in a row followed by a common letter are not significantly different according to a t test ($P < 0.05$)

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively

† Estimates are based on genetic variance within full-sib families

‡ Usefulness criteria are based on expected response to selection calculated by standardizing expected genetic variance components

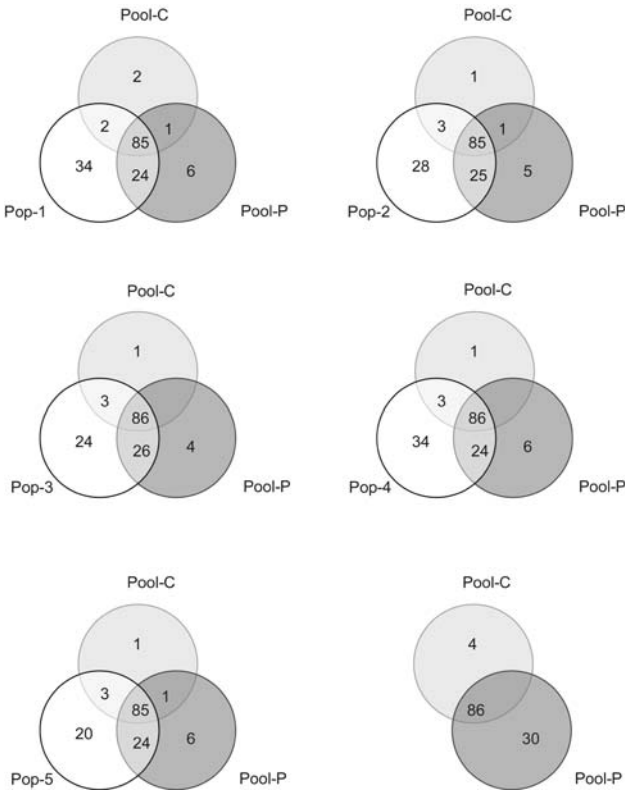

Fig. 1 Venn diagrams each showing the proportion of common alleles among the Central European heterotic pools (Pool-C and Pool-P), and one of the five Eastern European rye populations (Pop-1 to Pop-5)

the five Eastern European OPVs and the two heterotic pools, the first three principal coordinates explained 39.5, 24.8, and 16.3% of the molecular variance (Fig. 2). Pool-C was separated from the five OPVs and Pool-P with respect

Table 4 Estimates of modified Rogers' distances among five Eastern European rye populations (Pop-1 to Pop-5) and the two Central European heterotic pools (Pool-C and Pool-P)

	Pop-1	Pop-2	Pop-3	Pop-4	Pop-5	Pool-C
Pop-2	0.20					
Pop-3	0.15	0.16				
Pop-4	0.15	0.18	0.12			
Pop-5	0.17	0.20	0.14	0.09		
Pool-C	0.28	0.29	0.26	0.25	0.27	
Pool-P	0.21	0.25	0.20	0.22	0.20	0.29

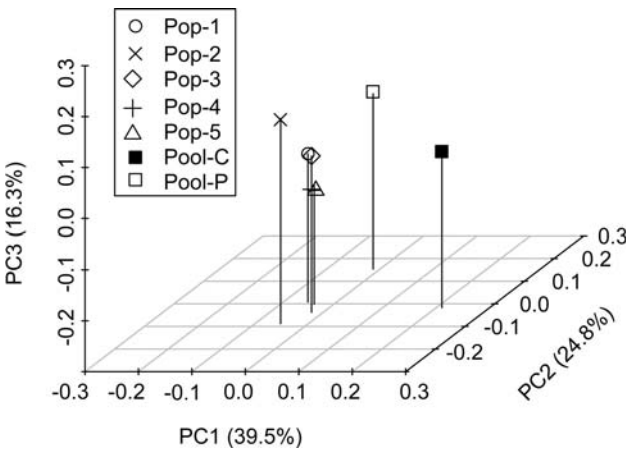

Fig. 2 Principal coordinate analysis based on modified Rogers' distances among five Eastern European rye populations (Pop-1 to Pop-5) and the Central European heterotic pools (Pool-C and Pool-P). PC1, PC2, and PC3 are the first, second, and third principal coordinates, respectively

to the first principle coordinate. Pool-P was separated from the five Eastern European OPVs and Pool-C with respect to the second principal coordinate.

The ad hoc statistic described by Evanno et al. (2005) revealed an optimum of two subpopulations. The first subpopulation was composed of all individuals of Pool-C and one individual each of Pop-3 and Pop-4. All individuals of Pool-P, Pop-1, Pop-2, and Pop-5, and the remaining genotypes of Pop-3 and Pop-4 formed the second subpopulation.

Discussion

Hybrid rye breeding in Germany is currently based on the Petkus × Carsten heterotic pattern (Geiger and Miedaner 1999). The Petkus population was originally developed by Ferdinand von Lochow starting in 1880 by improving a cross between the landraces Pirnauer Roggen and Probsteiner Landroggen (Miedaner 1997). Main target traits besides grain yield were tolerance to abiotic stresses, performance under high plant density, and good kernel development. The original selection environments were characterized by sandy soils and continental climate. The steadily improved Petkus population played a dominant role in the twentieth century in rye cultivation worldwide and it was a parental source of many OPVs (Miedaner 1997). The Carsten population was developed by Rudolf Carsten based on the landrace Heinrich Roggen (Erich Knopf, rye breeder, personal communication). In contrast to other rye breeding programs, the breeding philosophy of Carsten was to maintain his germplasm pool strictly isolated avoiding introgression from other germplasm. Main target traits besides grain yield were large spikes and good seed setting. The original selection environments were characterized by sandy loam soils and maritime climate.

Molecular and phenotypic diversity within populations

We observed substantially lower gene diversity and smaller number of alleles for Pool-C compared with Pool-P (Table 1; Fig. 1). The analyses of phenotypic data for grain yield also gave similar results: genetic variance component was significantly higher for Pool-P compared with Pool-C (Tables 2, 3). Thus, both molecular and phenotypic data suggested that Pool-C is less diverse than Pool-P, which was in accordance with the expectations based on the genealogy of heterotic pools. Therefore, Pool-C should be the primary target for broadening the established Central European heterotic pattern.

Based on quantitative genetic theory, we expect that the genetic variance among testcrosses of S_0 plants within Eastern European OPVs is ½ σ^2_A, where σ^2_A refers to the additive variance among the testcross progenies as defined by Schnell (1965). In contrast, expected genetic variance among testcrosses of S_0 plants within full-sib families of Pool-C and Pool-P is ¼ σ^2_A. This has to be considered while comparing genetic variances of the Eastern European OPVs and the heterotic pools. The magnitude of the estimates of genetic variance of the five OPVs was similar to Pool-P, but substantially higher than for Pool-C (Tables 2, 3). Especially Pop-4, which had up to a sixfold higher genetic variance than Pool-C, showed high genetic variance. The large genetic variance of the five Eastern European OPVs is in agreement with the results of the molecular diversity analyses with higher gene diversity values and average number of alleles per locus compared to Pool-C (Table 1). In addition, molecular analyses indicated that Eastern European OPVs are rich reservoirs of untapped allelic variation, because 31% of the total number of alleles are present in the OPVs but absent in the heterotic pools Pool-C and Pool-P. Consequently, the five Eastern European OPVs are promising source germplasm for broadening the genetic base of both established Central European heterotic pools.

Gene diversity, number of alleles, and number of unique alleles per population did not vary substantially among the five Eastern European OPVs (Table 1). Therefore, the molecular data did not provide much information on differentiation of which OPV is of particular interest to broaden the genetic base of the Central European heterotic pattern. In contrast, the estimated genetic variance components for grain yield revealed large differences among the five populations with high values for Pop-3 and Pop-4 in crosses with both testers and for Pop-5 when crossed with Tester-P (Tables 2, 3). This discrepancy between the molecular and field data can be explained by the use of neutral markers. Summarizing, phenotypic data lead to the hypothesis that Pop-3, Pop-4, and Pop-5 are promising candidates as potential donors on a long term for novel untapped alleles for grain yield.

Genetic diversity among populations

A decrease in genetic distance can lead to a reduction in the magnitude of heterosis (Falconer and Mackay 1996) and predominance of variance due to specific (SCA) versus general combining ability (GCA) effects (Reif et al. 2007). Larger variation due to SCA reduces accuracy in predicting hybrid performance on the basis of GCA effects. Therefore, the success of hybrid breeding programs depends on the genetic distance among heterotic pools (Melchinger and Gumber 1998).

The maximum genetic distance was observed between Pool-P and Pool-C (Table 4), which is expected and explainable by their genealogy and the selection for

interpopulation improvement of both pools. The five Eastern European OPVs formed a homogeneous cluster separated from both heterotic groups at the population level but were more closely related to the Petkus rather than to the Carsten pool (Fig. 2). The close relationships among the five OPVs point to common ancestors and/or gene flow among them.

The results of the model-based clustering based on individual genotypes indicated that the Eastern European OPVs are related to Petkus (Fig. 3). This is expected as Petkus is a dominant parental component of many OPVs (Miedaner 1997). Introgression of the five Eastern European OPVs in the Petkus pool would not affect the molecular diversity between the heterotic groups, but introgression of the OPVs in the Carsten pool would obviously lead to a substantial loss of molecular diversity between them.

Combining ability of the five Eastern European OPVs

Grain yield of the testcrosses of five Eastern European OPVs did not surpass the average testcross performance of the established heterotic pools (Tables 2, 3). This is expected as the Petkus × Carsten pattern has been improved via intra- and interpopulation selection. A criterion better suited than the mean of a population is the usefulness criterion (Schnell 1983), which considers the mean as well as the expected genetic gain of a population. Thus, the disadvantage of a lower mean can be compensated by a larger genetic variance. For crosses with Tester-P, values for the usefulness criterion for grain yield revealed that Pop-5 of the Eastern European OPVs—having the highest value at both selection intensities—is among the tested population the best source germplasm for introgression into the opposite heterotic partner, Pool-C (Table 2).

For crosses with Tester-C, testcrosses of none of the five Eastern European OPVs surpassed the usefulness value of Pool-P × Tester-C. One plant of Pop-4 outperformed the best plant of Pool-P (Table 3). This can be interpreted as an indicator that Pop-4 represents a promising germplasm resource to broaden Pool-P by introgressing selected S_0 plants with high-yielding testcross performance. Among the five Eastern European OPVs, Pop-2 showed high usefulness values across both selection intensities. This suggests that Pop-2 is another source candidate to broaden Pool-P.

Strategy for broadening the Central European heterotic pattern

The analyses of molecular and phenotypic data underlined the importance to broaden the genetic base of the Carsten pool (Tables 1, 2, 4). The phenotypic data on testcross performance revealed that Pop-5 is exceptionally suited to broaden the Carsten pool. Among the remaining OPVs, Pop-2 and selected plants of Pop-4 are promising source for introgression into the Petkus pool. This strategy provides an avenue to exploit new genes of Eastern European OPVs in both heterotic pools.

The high degree of relatedness among the five Eastern European OPVs and the Petkus pool (Figs. 2, 3; Table 4), however, indicated that introgression of Pop-5 into the Carsten pool would adversely affect the genetic distance between the two established heterotic pools. A reduced genetic distance between the heterotic pools can decrease inter-pool heterosis and also could result in an unfavorable ratio of variances due to SCA versus GCA effects. The effect of the introgression of Pop-5 into the Carsten pool on the ratio of variances due to SCA versus GCA effects will depend, among other factors, on the shift in allele frequencies. The introgression can be efficiently steered by using a limited number of highly promising plants from Pop-5 into the Carsten pool.

The dominant role of Petkus as an ancestor of many elite OPVs worldwide reduces the probability of finding populations that are genetically diverse from the Petkus pool. Therefore, we suggest as an alternative strategy to introgress Pop-2 and selected plant(s) of Pop-4 in the Petkus

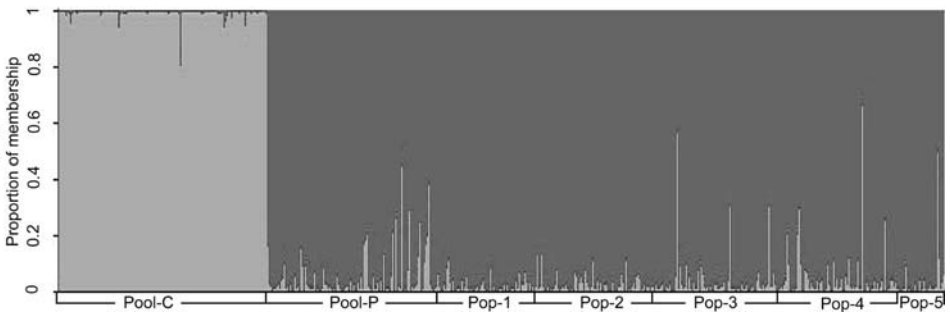

Fig. 3 Estimated population structure of 589 rye S_0 plants belonging to five Eastern European populations (Pop-1 to Pop-5) and the two Central European heterotic pools (Pool-C and Pool-P). Each individual plant is represented by a thin vertical line, which is partitioned into *k* segments that represent the individual estimated membership to the two clusters

pool and save Pop-5 as potential candidate to broaden the Carsten pool. In addition, fingerprinting of more exotic OPVs should be conducted with the aim to detect germplasm sources which are unrelated at the molecular level to the Petkus pool and, consequently are potential source germplasm to broaden the Carsten pool. SSR bulk analyses are a promising technique to carry out this screening in a very cost effective manner (Reif et al. 2005). Eastern European elite OPVs were crossed with each other in the past and Petkus has a large contribution in all of them. Therefore, they are not promising sources for broadening the Carsten pool. Populations with a genetic distance to the Petkus pool comparable or higher to that between the two established heterotic pools may be identified as candidates to be evaluated in a second step in extensive field trials on performance of testcrosses. If no appropriate candidate(s) are detected, Pop-5 can be used to broaden the Carsten pool.

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