PLANT BREEDING AND SEED SCIENCE
Volume 47 (no. 1/2) 2003

P. Wilde, J. Menzel, B. Schmiedchen

Lochow–Petkus GmbH, 29303 Bergen, Germany (email wilde(ä~lochow–petkus.de)

ESTIMATION OF GENERAL AND SPECIFIC COMBINING ABILITY VARIANCES AND THEIR IMPLICATIONS ON HYBRID RYE BREEDING

ABSTRACT

Effects and variances of general (GCA) and specific (SCA) combining ability have been estimated from two experiments. For the first experiment (EXP 1) 19 homozygous lines originating from the Petkus pool were crossed with two *CMS* single cross testers derived from the Petkus pool (intra–pool test crosses) and with two testers from the Carsten–Danko pool (inter–pool test crosses), respectively. In the year 2000 grain yield was measured at 5 locations in Germany. The second experiment (EXP II) comprised a factorial of inter–pool crosses by using 4 homozygous *CMS* Petkus pool lines as seed parents and 6 Carsten–Danko lines as pollen parents. The respective 24 inter–pool crosses were evaluated for grain yield on several locations in 1996 and 1998.

In EXP I GCA–variance of candidate lines (4.7 —5.9 q/ha was estimated to have a similar size as SCA–variance. Possible reasons for an overestimation of SCA–variance are extreme divergence between testers, insufficient removal of inbreeding effects in the intra–pool test crosses and bias due to SCA × year interaction–variance. The use of intra–pool testers should be restricted to breeding material where inbreeding due to consanguinity between testers and candidate lines is either absent or will evenly affect test cross performance of all candidates.

In EXP II GCA–variances for female 9.2 q/ha^2 and male 2.4 q/ha lines were much higher than SCA–variance. Repeatability for GCA–effects over different environments was medium (r=0.67), whereas for SCA–effects it was low (r=0.13). Selection intensity for yield should therefore be moderate, if based on a single year of testing only. Selection of parental components for hybrids and prediction of hybrid performance should mainly base on GCA–effects. Alternative to the use of factorial crosses test crosses with testers representing the opposite pool are recommended. Testing for SCA–effects and for SCA x environment interaction– effects can be a valuable diagnostic tool, to become aware of strengths and weaknesses of the elite material used to build new hybrids. SCA–tests should therefore be performed by using highly divergent environments.

Key words: *CMS* cross testers, general combining ability (GCA), homozygous lines of rye, hybridrye breeding, specific combining ability (SCA)

INTRODUCTION

Hybrid rye breeding aims to detect inbred lines, which excel by a high performance in their crosses. When developing candidate lines their partner lines in the final hybrid are not known in most cases and no direct tests for the hybrid performance are feasible. Therefore selection

---

Communicated by Elisabeth Gunnarsson

must base on indirect selection criteria during line development. By intensive selection on line *per se* performance high heritable traits are improved. By test crosses the breeder aims to identity candidate lines showing excellent general combining ability (GCA) effects for low heritable traits such as yield.

In most breeding programs (Geiger, 1982) lines belonging to the seed parent pool are top crossed by using their near isogenic *CMS* analogues lines. This allows to cross them easily with a male tester, usually a synthetic derived from the pollen parent pool. Lines belonging to the pollen parent pool are outcrossed to *CMS* single cross testers which are regarded to be representative for the seed parent pool. Yield tests of the test crosses grown over several locations and at least one year allow to reduce the number of candidate lines considerably and to focus on the most promising fraction. For final evaluation and for identifying hybrid combinations elite lines from the seed and pollen parent pool can be crossed in a factorial way. This procedure also allows to estimate specific combining ability effects (SCA) of given crosses of elite lines.

In this paper we report about general combing ability effects and their variances when crossing a line population to testers originating from different heterotic pools (EXP I). When developing seed parent lines the breeder may be motivated to replace the normally used opposite pool testers by testers, which have been derived from the seed parent pool, too. After outcrossing S2– or S3– candidate lines on such testers, their **intra–pool test crosses** allow to test for yield in a much earlier stage of line development and to spare time and costs. Thus, they might be a valuable alternative to the traditionally used **inter–pool test crosses** based on more or less isogenic *CMS* analogues of the candidate lines.

Further, we evaluate a factorial cross of seed and pollen parent lines (EXP. II) with regard to their GCA– and SCA–effects and their respective variances.

MATERIALS AND METHODS

Experiment I:

As candidate line population we used 19 homozygous lines from the Petkus pool. From these lines the male fertile version in the Normal–cytoplasm (N–L) and their respective *CMS* analogues (P–L) were available. The latter were obtained by backcrossing the male fertile version to a donor plant for the Pampa cytoplasm over at least five generations. In 1998 the N–L were outcrossed to two *CMS* single cross testers (Ti and T2) developed also from the Petkus pool. Ti and T2 were chosen to be unrelated to each other. Both testers were selected by pedigree analysis to have either no or only distant ancestors in common with the candidate lines. Thus, biases due to inbreeding effects in the **intra–pool test crosses** should be kept as low as possible. In case candidate lines and testers had ancestors in common, adjusted yield (Y_{adj})

was calculated from observed yield data (Y_{obs}) and from the inbreeding coefficient (F) (Falconer and Mackay, 1996) of the test crosses as derived from pedigree data. The formula:

$$Y_{adj} = \frac{Y_{obs}}{1-0.7\times F}$$

assumes an inbreeding minimum of 30 % of the heterozygote performance (Wricke, 1973) and a linear relationship between degree of heterozygosity and performance level. In test crosses with Ti inbreeding coefficients ranged between 0 ⟨ F ⟩ 0.03125 for 3 candidate lines, whereas in test crosses with T2 F was calculated to range between 0.0625 ⟨ F ⟩ 0.0859 for 7 candidate lines and for the rest of the lines 0 ⟨ F ⟩ 0.0469. In 1997 and 1999 the P–L were used as seed parents to produce **inter–pool test crosses** with two other testers (T3 and T4). Both testers were derived from Carsten–Danko breeding populations, which are used in our hybrid program as pollen parent pool. T3 was an inbred line in S2–L generation and T4 is a synthetic developed of two inbred lines in S3–L generation. T3 and T4 are unrelated to each other.

In the year 2000 yield plots (5 m^2) of the test crosses were grown at five locations in Northern and Eastern Germany with two replications/location. The experiment was laid out as a split plot design with lines as main units and tester × line–combinations as sub–units.

Experiment II:

Experiment II comprised 24 factorial inter–pool crosses between the *CMS* analogues of four homozygous inbred lines from the Petkus pool and six S2–L from the Carsten–Danko pool. The lines represent actual breeding material and most of them are parental components in registered hybrids. The inter–pool crosses were grown in seven environments (i.e. 3 and 4 locations in Northern and Eastern Germany in the years 1996 (Erfurt, 1997) and 1998, respectively) with two replications/location.

For brevity, in the following only grain yield data (q/ha) are presented.

RESULTS AND DISCUSSION EXPERIMENT I:

Means of the **inter–pool test crosses** (71.3 q/ha) surpass those of the **intra–pool test crosses** (62.8 q/ha) for about 8 q/ha. Because all testers have been comparably preselected for combining ability, superiority of **inter–pool test crosses** can be attributed to heterotic increase due to combining divergent gene pools. With regard to their ability to differentiate between candidate lines a slight advantage for the Carsten testers compared to the Petkus testers can be stated. On the other hand intra–pool test cross results are less impeded by masking effects such as genotype × location interaction and error effects. This finding can be explained by two reasons:

First, sawing in autumn 1999 suffered from unfavourable weather and soil conditions and seed quality severely influenced germination rate at the different locations. **Intra–pool test cross** seedlings could benefit from excellent seeds grown on their heterozygous seed parents (Ti and T2). In contrast, **inter–pool test cross** grains suffered from bad seed quality caused by the homozygous *CMS* analogues serving as seed parents (Table 1).

Table 1

Estimates of means, variance components and heritability (h^2) for yield (q/ha) in the test cross series with 19 Petkus pool lines (EXP. 1)

Parameter	Origin of testers							
	Petkus				Carsten–Danko			
	T1		T2		T3		T4	
Mean	62.8		62.8		73.3		69.4	
σ^2_t	18.8**	±4.6§	6.9**	±3.4*	14.0**	±6.7	8.2**	±4.1
σ^2_{tp}	14.3**	±3.1	11.6**	±3.1	27.5**	±5.8	27.5**	±3.7
σ^2_e	8.3	±1.2	12.6	±1.9	13.4	±2.1	13.4	±1.4
h^2 [%]	74.5		65.7		67.2		64.6	

σ^2_t – genetic component
σ^2_{tp} – genotype × location interaction
σ^2_e – error variance component
§ – standard error of the respective variance component estimate
*, ** – estimates are significant at probability level P=0.05 and P=0.01 respectively

Secondly, the exceptionally high σ^2_{tp}, estimate for T3 test crosses is in accordance with other experiments showing a rather low environmental stability of T3. Moreover, because T3 is an inbred line, the respective test crosses are genetically more narrow based than those of the three other testers. Genetic structure however is known to influence buffering capacity of experimental hybrids (Becker and Leon, 1988).

On the other hand, **intra–pool test crosses** can be sensitive to another source for masking genotype effects: In contrast to **inter–pool test crosses** they are male sterile and seed set therefore depends on a sufficient pollen cloud provided by neighbouring plots. This may become a bottleneck if grown at the border of the experimental field or in case of unfavourable weather conditions during flowering period. Heritability is one of the components and key factors of the expected genetic gain (Falconer and Mackay, 1996). With regard to this parameter first choice would be to use Ti (h^2=74.5%) for test crosses.

As already mentioned intra–pool test crosses were adjusted for inbreeding effects. (Fig. 1). If combining ability to the mean of the two opposite pool testers T3 and T4 is regarded as target criterion, effectiveness of adjustment can be studied. The phenotypic correlation of test cross means with Ti to the mean of test crosses with T3 and T4

($r_{adj.}$=0.68) is scarcely influenced by inbreeding effects, because there were only three candidate lines showing slight consanguinity to the tester. In contrast, correlation of test crosses with T2 (r_{unadg}=0.46) could be improved considerably by adjustment ($r_{adj.}$=0.61). The adjustment above described is simple to calculate, but it's efficiency depends on:

(i) correct pedigree data which are not always available under practical conditions,
(ii) the size of sampling effects with regard to the contribution of the ancestors genome to the candidate and tester genome,
(iii) a linear relation between hetero– and homozygous performance.

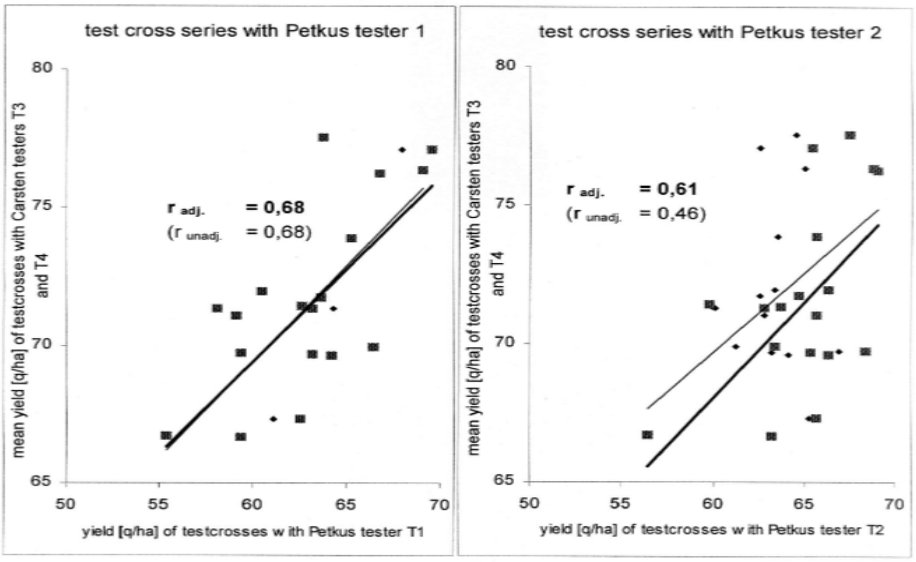

Fig. 1 Phenotipic correlation of test crosses with intra–pool testers T1 and T2 to the mean of test crosses withopposite pool testers T3 and T4 (Experiment I). Squares and diamonds – adjusted and unadjusted values respectively

The present experiment offers no possibility to examine in how far a better adapted adjustment would influence the association between ultra and inter–pool test cross series. The phenotypic correlation of test cross means with Ti to the mean of test crosses with T3 and T4 ($r_{adj.}$=0.68) is comparable in size to the corresponding correlation between the test cross series with the opposite pool testers T3 and T4 (r=0.63). This results demonstrates, that test crosses with unrelated **intra–pool testers** can be useful to test candidate lines for GCA to the opposite pool. On the other hand, results with related testers such as T2 clearly show, that **intra–pool testers** may become a pitfall leading to a rather inaccurate gca estimation of the candidate lines, if no adequate adjustment for inbreeding effects is possible.

As can be seen (Table 2) from the high variance between testers ($\acute{o}^{2}_{T}$=21.4 q/ha) use of all testers allows to test candidate lines on an ex–

tremely divergent genetic background. This variance is mainly due to origin of testers, as becomes obvious when testers are grouped according to their pools. Compared to estimates in the literature (Kolasińska and Wegrzyn, 1998; for review see also Tomerius, 2001) the size of SCA–variance relative to GCA–variance is rather high. In contrast to the 1:1 relation between SCA– and GCA–variance found in our experiment most estimates in the literature attribute much lower values to SCA–variance. Possible reasons for our deviating results are: extreme divergence of testers (in case of all four testers), insufficient removal of inbreeding effects (in case of Petkus testers) and narrow versus broad based tester (in case of Carsten testers). Anticipating results from Experiment II, it should be realised, that in a one– year–experiment estimators of SCA–variance can be severely biased upwards by SCA × year interaction variance.

Table 2

Estimates for variance components and heritability (h^2) for yield (q/ha) with the test crosses grouped according to the origin of the tester

Parameter	Origin of testers					
	PET + CAR		PET		CAR	
σ^2_{T}	21.4**	±14.1§	62.8	±1.5	73.3	±6.1
σ^2_{GCA}	4.7*	±3.2	6.9**	±3.1	14.0**	±4.6
σ^2_{SCA}	5.0**	±1.2	11.6**	±1.8	27.5**	±2.1
$\sigma^2_{GCA \times E}$	15.9**	±3.2	12.6	±2.8	13.4	±4.5
$\sigma^2_{SCA \times E}$	1.9**	±0.6	65.7	±0.9	67.2	±1.2
h^2	47.5		39.4		42.4	

σ^2_{T} – tester component
σ^2_{GCA} – general combining ability component
σ^2_{SCA} – specific combining ability componrent
$\sigma^2_{GCA \times E}$ – interaction of general combining ability with environment
$\sigma^2_{SCA \times E}$ – interaction of specific combining ability with environment
§ – standard error of the respective variance component estimate
*, ** – estimates are significant at probability level P=0.05 and P=0.01 respectively

For practical breeding purposes the following conclusions can be drawn:

(i) The use of intra–pool testers for testing candidate lines from the seed parent pool should be restricted to recurrent selection programmes or to **breeding populations**, which have been derived from self incompatible base populations. In all these cases consanguinity between testers and candidate lines will be either absent or will evenly affect test cross performance of all candidates.

(ii) In contrast, with **second cycle material** from the seed parent pool it will be hard to avoid distortion caused by considerable variance of inbreeding effects due to consanguinity between candidate lines and testers. Therefore, in this case use of testers from the opposite pool is crucial for an unbiased GCA–estimation.

(iii) If **early testing procedures** are intended with second cycle material from the seed parent pool *CMS* testers have to be developed from the pollen parent pool. This however can become a tedious work, because restorer genes have to be removed completely for the sake of a reliable male sterility of the *CMS* testers.

Experiment II

Regarding GCA–effeets on the basis of seven environments (Table 3) the variability among seed parent lines is mainly caused by line F3 and F4 showing highly significant positive and negative effects, respectively. In comparison, variability on the pollen parent side was smaller and mainly due to line M4 and M5. SCA–effects were generally found to be less important than GCA–effects and showed higher standard errors.

Table 3

General combining ability(GCA) and specific combaining ability (SCA) for yield (q/ha) of a factorial cross of 4 seed parent (F1–F4) and 6 pollen parent lines (M1–M6) estimated over 7 environments (EXP. II)

Line	M1	M2	M3	M4	M5	M6	GCA$_F$
	SCA						
F1	1.8	1.1	–1.5	–0.7	–1.3	0.7	–0.6
F2	–1.1	0.9	–0.6	–0.6	0.3	1.0	1.1
F3	–0.2	–1.2	0.2	0.5	–0.1	0.9	3.4
F4	–0.5	–0.9	2.0	0.8	1.1	–2.6	–4.0
GCA$_M$	–0.5	–0.8	1.8	–2.4	2.7	–0.8	0.6

Corresponding standard errors (SE):
GCA–effects: 0.78 q/ha and 1.09 q/ha for seed and pollen parent lines respectively
SCA–effect: 1.24 q/ha

Testing environments in the years 1996 and 1998 were rather different, with a high yield average (77.4 q/ha) in the first year and a rather poor yield level 55.9 q/ha) in the second year, which was due to unfavourable weather conditions. Thus, dependence of GCA– and SCA–estimators on different environmental conditions can be studied.

GCA–effects revealed a medium correlation (Fig. 2) over years (r=0.67), whereas SCA–effects proved to be rather inconsistent (r=0.13).

If estimated over both years genetic variation among inter–pool crosses (Table 4) could almost exclusively be attributed to GCA–variance. Large differences, however, were found for this parameter between female and male lines (9.2 vs. 2.4 q/ha).

Beside limited precision in parameter estimation there are two genetic reasons to explain this discrepancy.

(i) The elite female and male lines used for the experiment do not reflect variability in the unselected seed and pollen parent pool, respectively, but represent the remaining variability after two stages of selection on GCA–effects. Thus, differences in intensity of selection will also influence parameter size.

(ii) Variability between female lines is expected to be higher, because they are almost completely homozygous, whereas male lines have been inbred only up to 52–L generation.

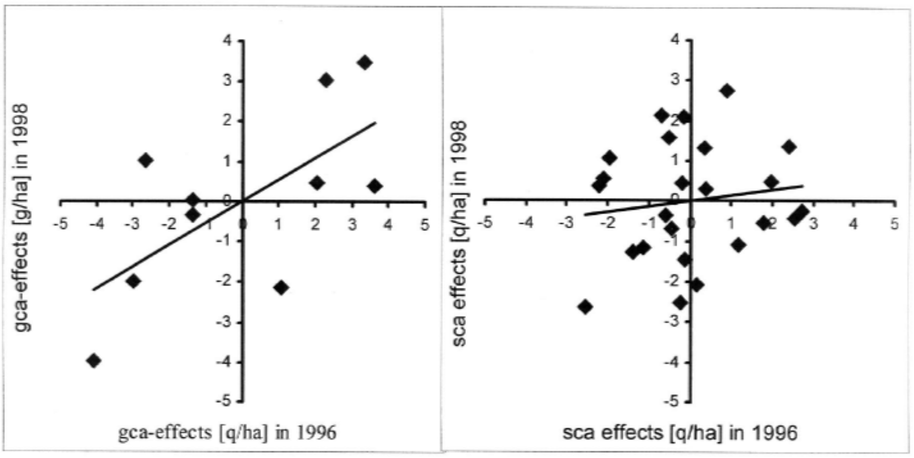

Fig. 2 Relationship between GCA–effects and SCA–effects [q/ha] estimated in 1996 (3 locations) and 1998 (4 locations) – Experiment II

Table 4

Variance components and heritability (h^2) for yield (q/ha) estimated over seven environments (Experiment II)

Parameter	Female		⇐Lines⇒		Male	
σ^2_{GCA}	9.2**	±6.2§			2.4*	±1.9
σ^2_{SCA}			0.44	±0.77		
$\sigma^2_{GCA \times E}$	2.0*	±1.2			4.9**	±1.9
$\sigma^2_{SCA \times E}$			5.34**	±1.76		
h^2_{GCA}	93.8				66,4	

Abreviations – see the legend of the Table 2

In comparison with GCA–varianee SCA–variance was of almost negligible size and showed a high standard error. As already pointed out in Fig. 2 interaction with environments played an important role in our experiment leading to highly significant $\sigma^2_{GCA \times E}$ (male lines) and $\sigma^2_{GCA \times E}$ variances. Consequently, heritability estimators were found to be medium to high for GCAeffects and very low for SCA–effects (data not shown). Jenkins (1934) developed methods to predict hybrid performance depending on estimation of GCA– and SCA– effects. An application of his method B could be to use the performance of the single crosses measured in the first environment for the prediction of their performance in a second environment. In this case prediction bases on GCA– and SCA–ef–

fects. Method C is differing from method B by using only GCA–effects for prediction. In Fig. 3 both methods are compared. In the first case single cross performance in 1998 is predicted by using single cross performance in 1996. In the second ease forecast of hybrid performance in 1998 bases exclusively on GCA–effects as estimated in 1996. Both methods can be regarded as equivalent with regard to their ability to predict single cross performance. Due to rather precise estimation of GCA–effects method C can compete with method B, which should be superior, if SCA–effects are important and could be estimated with high accuracy.

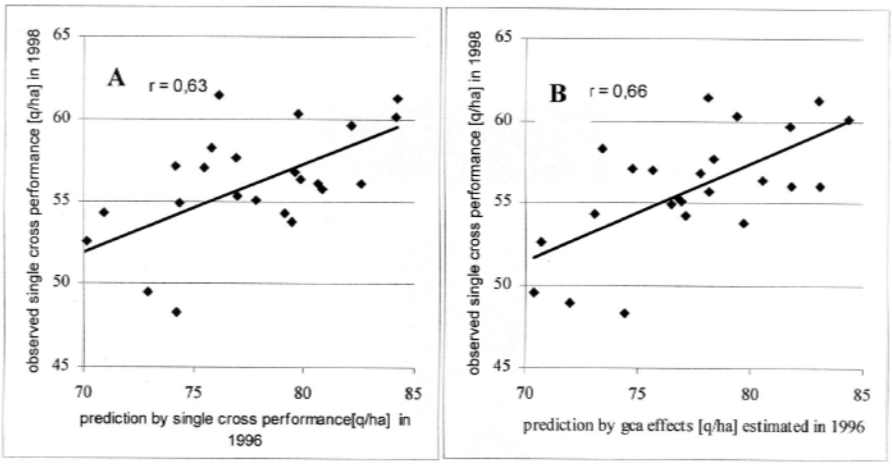

Fig. 3 Prediction of single cross performance in 1998 by using single cross performance (A) and parental GCA–effects (B) in 1996, respectively (Experiment II)

CONCLUSIONS

The following conclusions can be drawn for practical breeding programs:

(i) Selection of parental components for hybrids and prediction of hybrid performance should mainly base on GCA–effects, because precision of prediction is at least equivalent to other methods taking also SCA–effects into account.

(ii) For GCA–tests 2–3 testers chosen for a good representation the opposite pool should be used. Compared to factorial crosses of inbred lines costs for seed production will be lower and testing efficiency will be superior.

(iii) Selection intensity for yield should be moderate if based on a single year of testing, only. Reliability of results can considerably be improved by applying multi–stage selection procedures (Wilde, 1996, Tomerius, 2001) over subsequent years.

(iv) Estimating SCA–effects and their variances will be justified only at the end of the breeding process, where a very small number of line crosses has to be tested.

(v) Testing for SCA–effects and for SCA × environment interaction–effects can be a valuable diagnostic tool, to become aware of strengths and weaknesses of the elite material used to build new hybrids. SCA–tests should therefore be performed by using highly divergent environments.

REFERENCES

Becker H., C. Leon J. 1988. Stability analysis in plant breeding. Plant Breeding, 101, 1–23.

Erfurt M. C. 1997. Schatzung der Linieneigenleistung, Kombinationsfahigkeit und Heterosis fifr quantitative Merkmale bei Winterroggen. Diplomarbeit, Univ. Hohenheim, Stuttgart.

Faleoner D. S., Mackay T. F. C. 1996. Introduction to quantitative genetics. 4th Ed., Longman Sci and Tech, Harlow, UK.

Geiger H. H. 1982. Breeding methods in diploid rye *(Secale cereale* L.). Tag. Ber., Akad. Landwirtsch.–Wiss. DDR, Berlin, 198, 305–332.

Jenkins M. T. 1934. Methods of estimating the performance of double crosses in corn. J. Amer.Soc. Agron., 37, 143–145.

Kolasińska I., Wegrzyn S.. 1998.Combining ability for four characters of rye restorer lines. Biuletyn IHAR, 205/206, 15–22. [in Polish]

Tomerius, A. M. 2001. Optimizing the development of seed parent lines in hybrid rye breeding. Diss. Univ. Hohenheim, Stuttgart.

Wilde P. 1996. Multi–stage selection for combining ability among pollen parents lines in hybrid rye breeding. Vort. PflanzenzUehtg. 35, 15–25

Wricke, G. 1973. Inzuehtdepression und Genwirkung beim Roggen (*Secale cereale* L.). Theoret. Applied Genet. 43, 83–87.