

Hybrid breeding in autogamous cereals

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Abstract Hybrid breeding in autogamous cereals has a long history of attempts with moderate success. There is a vast amount of literature investigating the potential problems and solutions, but until now, market share of hybrids is still a niche compared to line varieties. Our aim was to summarize the status quo of hybrid breeding efforts for the autogamous cereals wheat, rice, barley, and triticale. Furthermore, the research needs for a successful hybrid breeding in autogamous cereals are intensively discussed. To our opinion, the basic requirements for a successful hybrid breeding in autogamous cereals are fulfilled. Nevertheless, optimization of the existing hybridization systems is urgently required and should be coupled with the development of clear male and female pool concepts. We present a quantitative genetic framework as a first step to compare selection gain of hybrid versus line breeding. The lack of precise empirical estimates of relevant quantitative genetic parameters, however, is currently the major bottleneck for a robust evaluation of the potential of hybrid breeding in autogamous cereals.

Introduction

Hybrid breeding is a remarkable success story in several allogamous species such as maize, sunflower, sorghum, sugar beet, and rye (Coors and Pandey 1999). The main advantages of hybrid versus line varieties are increased trait values due to the exploitation of heterosis (Shull 1908), larger yield stability especially in marginal environments (Hallauer et al. 1988), the ease of stacking dominant major genes (Edwards 2001), and larger return of investment for seed companies due to the built-in plant variety protection by inbreeding depression (Edwards 2001). Hybrid breeding for autogamous cereals was less successful because of the lower amount of heterosis, high seed densities coupled with difficulties to implement a cost-effective system for hybrid seed production, the lack of high yielding heterotic patterns, and the lower selection gain for hybrid compared to line breeding (Edwards 2001; Oettler et al. 2005; Lu and Xu 2010; Singh et al. 2010). Despite these drawbacks, major attempts have been undertaken during the past decades in the public and private domain to develop vital hybrid breeding programs in autogamous cereals. These attempts were recently stimulated by the demand of an increased agricultural productivity per area despite the increasing problems of abiotic stresses caused by the climate change. Moreover, increasing use of farm-saved seed caused a decreasing return in investment of seed companies and led to the need to initiate hybrid breeding programs in autogamous cereals due to the lack of political solutions (Edwards 2001; Rajaram 2001).

In this review, we summarize the status quo of hybrid breeding efforts for the autogamous cereals wheat, rice, barley, and triticale. In addition, research areas to improve the efficiency of hybrid breeding in autogamous cereals are discussed. In particular, we (1) examine the biological

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drawbacks and research needed to improve the hybridization systems, (2) study the current knowledge on the extent of heterosis in these four crops, (3) investigate the implementation of the concept of heterotic groups and patterns for autogamous cereals, and (4) elaborate a quantitative genetic framework as a first step to evaluate the potential selection gain of a hybrid versus a line breeding program.

Status quo of hybrid breeding in autogamous cereals

Hybrid wheat breeding

Efforts to establish hybrid wheat breeding have quite a long history in the public and private domain (Pickett 1993). It can be distinguished into two waves, between 1970s and 1990s and a revival starting in recent years (Keydel 1985; Merfert et al. 1987; Koekemoer et al. 2011). Currently, less than 1 % of the total world wheat area is planted with hybrids. Central Europe plays a major role with about 200,000 ha representing more than half of the world's hybrid wheat production (<http://www.ble-hybride.com>). The main hybrid wheat-growing countries are France (160,000 ha) and Germany (25,000 ha) with the main players in breeding being Saaten Union Recherche and Nordsaat Saatzeitgesellschaft mbH. All hybrids in Europe are currently produced with chemical hybridization agents (CHAs), most of them with Croisor[®]100 (sintofen; former Dupont-Hybrinova, Saaten Union Recherche, France). Until now, Croisor[®]100 has been licensed only for France, but a wider license for several European countries is in preparation (Volker Lein, pers. comm.). Hybrid wheat is cultivated also in China at about 30,000 ha (Prof. Pingzhi Zhang, pers. comm.) and in India at about 35,000 ha, where DWR—Directorate of Wheat Research (<http://www.dwr.in>)—is the main player in hybrid wheat research and breeding. Currently, no wheat hybrid is grown anymore in South Africa and Australia. More than 50 % of hybrid wheat grown in China is produced based on photoperiodic sensitivity, while the remaining ones and those in India are based on cytoplasmic male sterility (CMS) systems from *Triticum timopheevii* (Singh et al. 2010). Recently, new attempts have been made based on novel sources of CMS and complementary fertility restorer genes derived from the wild barley species *Hordeum chilense* (msH1 CMS system; Martin et al. 2009). In durum wheat, no hybrid is currently marketed, but breeding companies have restarted developing hybrids using either CHAs or CMS from *T. timopheevii*.

Hybrid rice breeding

The first studies on the extent of heterosis in rice trace back to work conducted already in the 1920s (Jones 1926).

Extensive research on hybrid rice started in 1964 in China (Yuan 1966) and the first commercial hybrid rice variety was released in 1976 based on a stable CMS system (Yuan and Virmani 1988; Virmani 1994; Yuan et al. 1994). Hybrid rice is currently grown on more than 20 million ha worldwide with a core area in China with an acreage of 17 million ha. Other countries with significant area cultivated with hybrid rice varieties are India with about 1.4 million ha and Vietnam and Bangladesh each with about 0.7 million ha (<http://irri.org/ricetoday>).

Nearly 80–90 % of the commercial hybrid rice in China is produced based on a CMS system mainly originating from *Oryza rufipogon*. The remaining 10–20 % of hybrids are produced based on thermo- or photoperiod-sensitive male sterility system (Si et al. 2011). In other countries, exclusively hybrids based on CMS are marketed. In total, 95 % of the hybrids are based on crosses between *Oryza indica* lines and 5 % are based on crosses between *Oryza japonica* lines, although hybrids between *O. indica* and *O. japonica* show an average higher heterosis compared to crosses within *O. indica* or *O. japonica* (cf. Ikehashi 1991). A high degree of sterility in the F₁ generation is the major obstacle hampering the use of *O. indica* and *O. japonica* crosses (Ikehashi and Araki 1984; Ouyang et al. 2009).

Hybrid barley breeding

With the description of the first recessive nuclear male sterility gene, Suneson (1940) awoke the interest in hybrid barley. Then, public institutions conducted several surveys on heterosis and seed production systems, which resulted in a balanced tertiary trisomic hybridization system (Ramage 1965) and first hybrid cultivars. The hybrid varieties outyielded the best line cultivars by 15–20 % and were released and grown commercially in Arizona on 12,000–20,000 ha per year (Ramage 1983). With the introduction of short-strawed lodging-resistant line cultivars from 1978 onward, hybrids lost their yield advantage and disappeared from the market. In addition to the balanced tertiary trisomic hybridization, a CMS system with a reliable single dominant restorer gene was described in 1979 (Ahokas 1979). Despite the availability of a CMS system, public institutions did not further emphasize on developing hybrid cultivars in barley for the next few decades. In 1994, Paul Bury, barley breeder at New Farm Crops, Ltd. (now Syngenta Seeds) started to develop hybrid barley based on the above-mentioned CMS system. In the year 2002, the first commercial hybrid variety ‘Colossus’ was released in the UK. Since then, Syngenta released more than ten hybrid varieties, which are all six-row winter barley varieties. The hybrids are currently grown on more than 200,000 ha. The main growing countries are Germany, France, and the UK (Gunther Stiewe, pers. comm.). Barley hybrids for use in malting are not yet

available. Owing to the use of the harvested F_2 generation, segregation might influence the malting quality of germinated seeds. No study was published, however, investigating this issue in more detail.

Hybrid triticales breeding

Fundamental research on hybrid triticales breeding was initiated in several public institutes in the 1980s (Nalepa 1990). First large-scale studies on the potential of triticales hybrids relied on CHAs (e.g., Oettler et al. 2003, 2005). Despite the ease of using CHAs, their toxicity hampered until now their successful registration (Oettler et al. 2005). Alternatively, CMS systems based on *T. timopheevii* have been evaluated and under huge efforts thousands of lines have been tested for their suitability to serve as maintainer or restorer line (Ralf Schachschneider, pers. comm.). A limited set of restorer and maintainer lines have been identified and are currently exploited in commercial and public hybrid triticales breeding programs in Europe. In 2012, two hybrid varieties have been registered in France and Germany by Saatucht Dr. Hege GbR, a winter type ‘HYT Prime’ and a spring type ‘Kulula’. Further triticales hybrids are in the official registration trials in Germany.

Constraints in hybridization systems for autogamous cereals

The biological constraints of autogamous cereals hamper the implementation of a cost-efficient hybrid seed production (Pickett 1993). Selfing must be prevented by the use of an easy and secure sterility system. Sterile females must open their flowers at the time when the males release plenty of viable pollen with good aerodynamics outside the floret. For autogamous cereals, several sterility systems exist with different pros and cons. The details have been discussed recently (for review, see Oettler et al. 2005; Li et al. 2007; Singh et al. 2010; Kempe and Gils 2011). Briefly, the most promising hybridization systems for autogamous cereals are sterility by a CHA or CMS, sterility caused by differences in day length and/or temperature, or genetically modified hybridization systems. For all of the four crops considered in our review, at least one hybridization system has already been applied as summarized in the previous crop-specific chapters.

For wheat and barley and less pronounced for triticales and rice, a major problem is the limited amount and spread of pollen (Omarov 1976; Pickett 1993; Virmani 1994). Therefore, large ratios of male versus female lines are required for hybrid seed production resulting in high seed costs (Pickett 1993; Kempe and Gils 2011). Genetic variation for amount and spread of pollen has been described in

the primary and secondary gene pool, and pollination traits seem to have moderate to high heritabilities (e.g., Virmani and Athwal 1972; Scholz and Künzel 1982; Pickett 1993). An increase in the amount and spread of pollen would enable the reduction of male lines in the hybrid seed production fields and, thus, blends with small amount of males like in allogamous rye can be managed (Geiger and Miedaner 2009). This mixed planting, which is feasible for all hybridization systems except CHAs (Pickett 1993; Edwards 2001), is an effective strategy to tremendously improve the economy of hybrid seed production for autogamous cereals (Maruyama et al. 1991; Kempe and Gils 2011).

Hybrid seed production in autogamous cereals is not only limited by the amount and spread of pollen, but also by reduced pollen viability (D’Souza 1970; Hammer 1977; Yan et al. 2009). For instance, wheat pollen is viable for about 0.5–3 h and rice pollen is viable for less than 5 h compared with about 72 h for rye (D’Souza 1970; Yan and Li 1987; Yan et al. 2009). Furthermore, the duration of receptivity of female flowers is quite short. For instance in wheat, opening of the female flower lasts only 2–3 days (Pickett 1993). Consequently, a crucial step in hybrid breeding is an optimized nick of parents for hybrid seed production (Pickett 1993; Edwards 2001; Koekemoer et al. 2011) requiring very precise phenotyping on date of pollen shedding of the males and date of floret opening of sterile females. Knowledge on the genetic basis of pollen viability and duration of female receptivity within crops are very limited, but comparison across species revealed large genetic variation (D’Souza 1970).

In conclusion, although research efforts have been made in the last 50 years, male and female traits relevant for a successful hybridization system have been improved only with moderate success (e.g., D’Souza 1970; Keydel 1972, 1977; Merfert et al. 1987). Thus, substantial research is needed to better understand and manipulate the genetic architecture of pollen traits (amount, spread, viability) and female traits (opening of the glume, time of stigma receptivity) in autogamous cereals. New precision phenotyping techniques (Montes et al. 2007) for traits related to flowering biology coupled with advanced high-density genomic tools offer promising possibilities to widen this bottleneck.

Heterosis and its consequences for hybrid breeding in autogamous cereals

If not stated otherwise, we will define heterosis as the difference between the hybrid and the mean of its two homozygous parents (Schnell 1961). Experimental results on the extent of heterosis in the literature differ largely for

autogamous cereals. Due to constraints in hybrid seed production, most published studies were based on a low number of environments (<3 environments) with small plot size and low seed density and must be handled with care. We summarized experimental results of the last 20 years focusing on studies with trials based on precise phenotyping in yield plot (>5 m²) on at least three environments and comparable agronomic conditions for lines and hybrids.

For wheat, durum, triticale, and barley, scientific literature fitting our phenotyping criteria was found with a comparable magnitude of average heterosis for grain yield being around 10 %, for plant height 7 %, and for heading time and quality traits around 0 % (Table 1; Supplementary Table S1). For rice, we have not found any published study on the extent of heterosis based on phenotypic data in more than two environments, although we intensively studied the literature. Several studies were published on less than three environments reporting extremely high variation in the magnitude of heterosis (e.g., Virmani 1996). These findings point to the lack of precise phenotyping of heterosis in rice. Consequently, profound phenotypic evaluation of the extent of heterosis in rice is needed.

The heterosis observed for the studied autogamous species (Supplementary Table S1) is much lower than in allogamous species. This can be explained by a lower degree of dominance and/or a low genetic distance among parental lines considering alleles at QTL underlying the trait of interest (Falconer and Mackay 1996). A further explanation of the low amount of heterosis is the presence of epistasis. This also includes favorable interactions between genes on homoeologous loci—a phenomenon often referred to as fixed heterosis (Abel et al. 2005). Considering only digenic epistasis and using the F₂ metric (Cockerham 1954; Schnell and Geiger 1970; Yang 2004), Melchinger et al. (2007a) derived the following quantitative genetic formula for heterosis:

$$H = \sum_{i \in Q} \left[d_i - \frac{1}{2} \left(\sum_{j \in Q_i} aa_{ij} \right) \right]$$

with Q_j denoting the loci set Q segregating among the two parents under consideration and underlying the agronomic trait of interest excluding element i , d_i being the dominance effect of locus i , and aa_{ij} referring to the additive × additive epistatic effect between locus i and j . Therefore, a low amount of heterosis can not only be explained by low magnitude of dominance effects and genetic relatedness, but can also be caused by positive additive × additive epistatic effects.

The lack of quantitative genetic theory to investigate heterosis using genomic tools hampered profound studies on the role of dominance versus additive × additive epistasis in crops (Melchinger et al. 2008). In the past few

years, huge efforts have been made to bridge this gap, and the necessary quantitative genetic theory and biometrical models have been elaborated to analyze the genetic basis of heterosis in more detail (e.g., Melchinger et al. 2007a, 2008; Garcia et al. 2008; He and Zhang 2011). Studies on the autogamous model plant *Arabidopsis thaliana* suggested the presence of positive additive × additive epistatic effects (Kusterer et al. 2007; Melchinger et al. 2007b; Reif et al. 2009). This is in agreement with a survey based on the North Carolina design III in rice, pointing also to the impact of epistatic effects contributing to heterosis (Garcia et al. 2008). Consequently, the low level of heterosis observed in experimental studies in wheat, rice, barley, and triticale (Table 1) can also be explained by favorable additive × additive epistatic effects and does not necessarily preclude the presence of dominance effects substantially larger than zero.

An economically successful implementation of hybrid breeding in autogamous cereals depends besides heterosis also on other factors such as costs of hybrid seed production and expected selection gain of hybrid versus line breeding (cf. Edwards 2001; Oettler et al. 2005). Therefore, it is difficult to determine the amount of heterosis required for an economically successful hybrid breeding program. Often, commercial heterosis (Dhillon and Singh 1977), the difference between the hybrids with the best available commercial line, is considered to judge the prospects of hybrid breeding (Pickett 1993; Edwards 2001). For instance, Perenzin et al. (1998) estimated commercial heterosis of wheat hybrids produced from the best available line cultivars across 10 years. In the fourth cycle of selection, >60 % of the produced hybrids had positive commercial heterosis. In addition, a recent experimental study based on germplasm from the current market leader in hybrid wheat breeding showed up to 20 % commercial heterosis (Gowda et al. 2012a). The authors highlighted that commercial heterosis is often overestimated due to the comparison of experimental hybrids with already registered line varieties. To solve this problem, Gowda et al. (2012a) corrected the line values with the average annual selection gain in registration trials and showed that even under this strict scenario, positive commercial heterosis was observed. These results suggest that successful hybrid breeding in autogamous cereals seems feasible under the constraint that selection gain per time unit is competitive. The expected selection gain in hybrid versus line breeding will be discussed in the next section.

Heterotic groups and patterns in autogamous cereals

Melchinger and Gumber (1998) defined a heterotic group “as a group of related or unrelated genotypes from the same or different populations, which display similar

Table 1 Summary of most important facts for hybrid breeding in autogamous cereals

	Wheat	Rice	Barley	Triticale
World hybrid production area (in Mha)	0.2–0.3	17.0–20.0	0.2	~0
Main countries	France	China	Germany, France, UK	Germany, France, Poland
Average yield heterosis (%)	3.5–15	? ^a	11.4	2–10
Heterotic groups	Not existing	Not existing	No published study available	Not existing
Main problems	Pollen amount, spread, and viability for seed production	Rare CMS sources, rare sources of restorers with good heterosis	No CHA for experimental hybrids	High selection intensity for maintainer lines
Mostly used seed production system	CHA	CMS	CMS	CMS
Main CMS source	<i>T. timopheevii</i>	<i>O. rufipogon</i> (CMS-WA)	<i>H. spontaneum</i>	<i>T. timopheevii</i>
Limitation to use CMS	Efficient MAS for introgression of CMS and restorers	Lack of good restorers	–	Many restorer genes
Potential alternative CMS sources	<i>Ae. Kotschyii</i> ; <i>Ae. Variabilis</i> ; <i>H. chilense</i>	cms-G; cms-ID; cms-DA; cms-D; cms-HL; cms-K; cms-BT	–	<i>Ae. ovata</i> , <i>Ae. Juvanalis</i> , <i>Ae. heidreichl</i> , <i>Ae. Sharonensis</i> , <i>T. dicoccoides</i>
Other sterile systems	Photoperiod and thermosensitive male sterility, used only in China	Photoperiod and thermosensitive male sterility, used in 20 % of China's hybrids	–	CHA
Biotech approach for seed production	Project stopped	–	–	–

^a No scientific study on mid-parent heterosis in rice was found on investigating parents and hybrids in a minimum of three different environments and plot sizes of >5 m²

combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups.” Heterotic patterns refer to a specific pair of two heterotic groups, which express high heterosis and consequently high hybrid performance in their cross. Current surveys on heterosis and hybrid performance based on diallel crosses are rare for autogamous cereals. The limited number of reported studies often failed to identify groups of genotypes with similar heterotic response or combining ability (e.g., Fischer et al. 2010). This finding is not surprising considering the intensive plant material exchange for elite lines and the lack of long-term recurrent reciprocal selection programs in the four crops.

It is often claimed that for successful hybrid breeding in autogamous cereals, heterosis must be enhanced by increasing genetic divergence of the parents (Coors and Pandey 1999). One approach to increase genetic diversity is making hybrids between adapted and non-adapted lines, e.g., winter by spring types (Koekemoer et al. 2011). This leads to high mid-parent heterosis because of the low performance of the non-adapted parent and in some cases also to positive values of better-parent heterosis. However, problems might arise due to different requirements

regarding vernalization, photoperiodic reaction, and frost tolerance.

Genetically divergent heterotic groups are important as they lead to a low ratio of variance due to specific (σ_{SCA}^2) versus general combining ability (σ_{GCA}^2) effects (Fischer et al. 2008). The advantages of predominance of σ_{GCA}^2 are high recurrent selection gain and the ease in identifying promising hybrids based on GCA prediction (Reif et al. 2007). Our literature review revealed that σ_{SCA}^2 was less pronounced compared to σ_{GCA}^2 (Supplementary Table S1). Nevertheless, several studies reported σ_{SCA}^2 values substantially larger than zero (e.g., Gowda et al. 2012a). Consequently, it seems beneficial to develop genetically distinct heterotic patterns with the aim of increasing the relevance of σ_{GCA}^2 through reciprocal recurrent selection as it was done in commercial hybrid breeding programs of the US corn belt (Duvick et al. 2004). Obviously, the selection of suitable heterotic patterns through recurrent reciprocal selection is a resource- and time-intensive work. A joint effort guided by public breeding programs seems to be a valuable approach to tackle this tedious task.

A clear two-pool concept possesses the further advantage of an easy handling of the sterility system (e.g., introgression of CMS-plasma and restorers only in one pool) and simplifies the handling of flowering biology of females versus male lines. For instance, an optimized nick requires taller males flowering later than females, properties which can be fixed through a two-pool concept. Furthermore, a bottleneck due to selection for increased pollination capability is then only a concern in the male pool. Thus, for this pool, a long-term selection strategy could aim at intensively selecting pollination capability at the beginning of hybrid breeding in order to rapidly fix good pollination capability facilitating future selection.

Finally, a clear two-pool concept also simplifies handling of major dominant QTL. While in line breeding all these major QTL have to be fixed in the new potential line, complementary stacking of genes in hybrid breeding requires fixation of the major QTL in only one out of two parental lines. This point further underlines the advantages to apply hybrid breeding with a two-pool concept. Nevertheless, this would require a shift from common breeding practices to consider hybrid breeding in autogamous cereals simply as a spin-off from line breeding programs toward the implementation of interpopulation selection programs.

Quantitative genetic framework for the comparison of hybrid versus line breeding

The effectiveness of hybrid versus line breeding can be characterized based on the realized selection gain per year assuming the same total budget. Selection gain is defined as $\Delta G = (ih\sigma_G)/y$, where i is the selection intensity, h the square root of the heritability, σ_G the square root of the genetic variance, and y the number of years required to finish one breeding cycle (Cochran 1951). Among these four parameters, the length of a selection cycle is very crucial (Gordillo and Geiger 2008). Sticking to genetic systems such as CMS to produce hybrids often causes an increase in y , and, therefore, results in a severe loss of the competitiveness of hybrid versus line breeding. In contrast, the use of CHAs coupled with doubled haploid (DH) technology leads to comparable length of selection cycles for hybrid and line breeding (Pickett 1993; Oettler et al. 2005). For the following considerations, we assumed that CHAs are available resulting in similar cycle length for hybrid and line breeding. One further important question is the relative magnitude of the genetic variance in line versus hybrid breeding.

Assume two different gene-orthogonal base populations π_1 and π_2 , absence of epistasis, and two alleles per locus with frequency of the favorable allele of $p_{\pi_1} \in [0, 1]$ and $p_{\pi_2} \in [0, 1]$. For investigating the available genetic

variance, we concentrate on a one-locus model and assume the use of fully inbreds in line and hybrid breeding. For hybrid breeding, the total genetic variance is then defined as the sum of

$$\sigma_{GCA'}^2 = \sigma_{A'}^2 = p_{\pi_1}(1 - p_{\pi_1})[a - (2p_{\pi_2} - 1)d]^2,$$

$$\sigma_{GCA''}^2 = \sigma_{A''}^2 = p_{\pi_2}(1 - p_{\pi_2})[a - (2p_{\pi_1} - 1)d]^2,$$

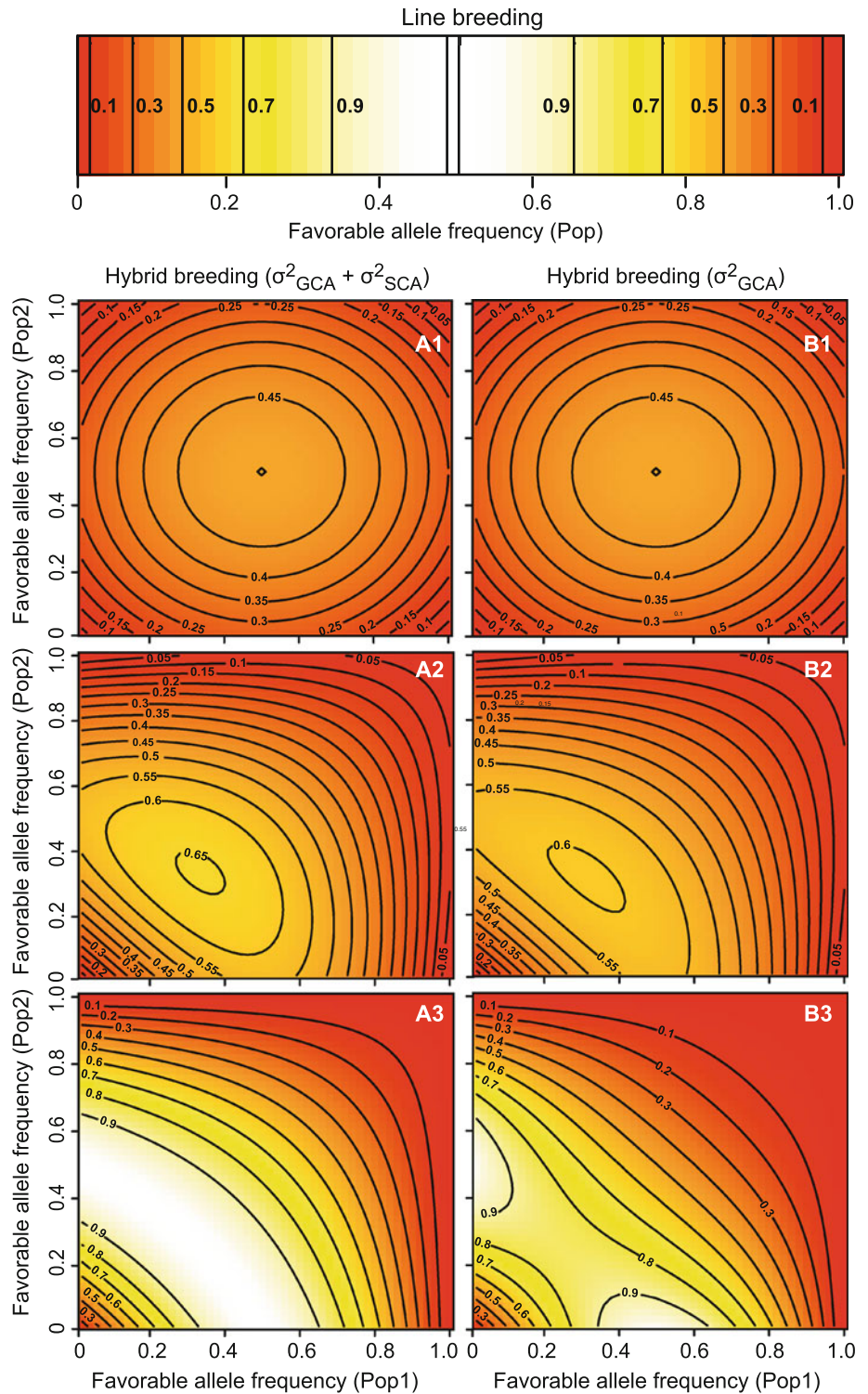
and

$$\sigma_{SCA}^2 = \sigma_D^2 = 4d^2 p_{\pi_1} p_{\pi_2} (1 - p_{\pi_1})(1 - p_{\pi_2}),$$

where a and d denote the additive and dominance effect at the locus under consideration, $\sigma_{A'}$ and $\sigma_{A''}$ refer to the additive variance of populations π_1 and π_2 , respectively, and σ_D^2 is the dominance variance (Schnell 1965). For line breeding, dominance variance cannot be exploited and the total genetic variance is defined as $\sigma_G^2(\text{line}) = \sigma_A^2 = 4p(1 - p)a^2$. (Bernardo 2002). For absence of dominance ($d = 0$), $\sigma_G^2(\text{line})$ is twice as large as $\sigma_G^2(\text{hybrid})$ (Fig. 1). Most important agronomic traits, however, are quantitative traits and, consequently, the assumption of d equal to zero is unrealistic. This is reflected by significant σ_{SCA}^2 observed in experimental studies (Supplementary Table S1). Assuming values of d larger than zero leads to increased values of $\sigma_G^2(\text{hybrid})$ surpassing $\sigma_G^2(\text{line})$ when the frequency of the favorable allele is rare in population π_1 and close to 0.5 in population π_2 , or vice versa (Fig. 1). Experimental studies comparing $\sigma_G^2(\text{hybrid})$ versus $\sigma_G^2(\text{line})$ are rare and often based on small sets of parents. The few experimental studies with sample sizes larger than 50 individuals, however, clearly suggested that the assumption of $\sigma_G^2(\text{hybrid}) = 0.5\sigma_G^2(\text{line})$ is unrealistic for complex traits such as grain yield (e.g., Oettler et al. 2005).

In order to determine the recurrent selection gain of hybrid breeding, precise estimates of σ_{SCA}^2 and σ_{GCA}^2 are needed (Longin et al. 2007; Gordillo and Geiger 2008). In addition, it has been hypothesized that hybrids possess higher yield stability than lines and consequently also lower variance due to genotype \times environment interactions. Experimental studies comparing hybrids and lines showed higher (Jordaan 1996; Koekemoer et al. 2011) or similar yield stability (Bruns and Peterson 1998; Koemel et al. 2004). However, different statistical measures were used and only very limited number of varieties were compared. Furthermore, a precise evaluation of yield stability requires a very large number of environments (Piepho 1998). Thus, more research efforts are required to investigate the variance due to genotypes and genotypes \times environment interactions for the four studied crops based on current elite germplasm. Consequently, there is an urgent need to determine relevant variance components for hybrid breeding in extensive experimental studies.

Fig. 1 Genetic variance in dependence of the frequency of the favorable allele for line breeding and hybrid breeding regarding hybrids with GCA + SCA (a), or recurrent selection with GCA, where SCA cannot be exploited (b), for $d = 0$ (A1, B1), $d = 0.5$ (A2, B2), and $d = 1$ (A3, B3). It is important to note that in hybrid breeding there are two base populations, while in line breeding there is only one population

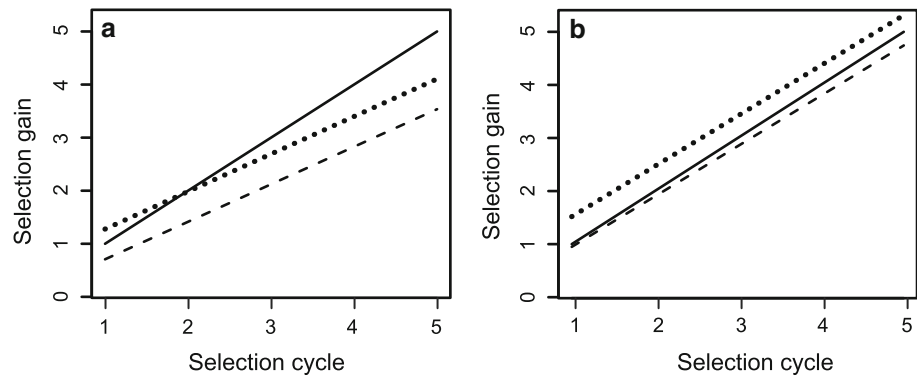


Selection gain in hybrid versus line breeding

Because of the lack of precise estimates of the above outlined relevant parameters to judge the prospects of hybrid versus line breeding, we investigated the worst case scenario for hybrid breeding assuming $\sigma_G^2(\text{hybrid}) =$

$0.5\sigma_G^2(\text{line})$, but also a favorable scenario assuming $\sigma_G^2(\text{hybrid}) = 0.9\sigma_G^2(\text{line})$. In addition, we assumed that the variance due to genotype \times environment interactions and the variance of the residuals were the same for hybrid compared to line breeding (for further details, see “Appendix”).

Fig. 2 Selection gain for line breeding (*solid line*), reciprocal recurrent selection (exploitation of GCA in hybrid breeding; *dashed line*), and hybrid breeding exploiting GCA + SCA from factorials (*dotted line*) across the time for $\sigma_G^2(\text{hybrid}) = 0.5\sigma_G^2(\text{line})$ (a), and $\sigma_G^2(\text{hybrid}) = 0.9\sigma_G^2(\text{line})$ (b)



In reciprocal recurrent selection programs, we expect a steady increase in the mean of the hybrid population due to the exploitation of $\sigma_{A'}^2 + \sigma_{A''}^2$. In contrast, selection gain due to σ_D^2 cannot be exploited in a recurrent sense. It can be exploited, however, during the selection among the total set of factorial crosses. Thus, to determine the selection gain per time unit, we have to add a constant increase due to σ_D^2 (Fig. 2) as long as the magnitude of σ_D^2 does not change across different cycles of selection.

Assuming $\sigma_G^2(\text{hybrid}) = 0.5\sigma_G^2(\text{line})$, recurrent selection gain is much higher for line than hybrid breeding across years (Fig. 2a). This higher recurrent selection gain, which is reflected by the slope of the regression line, cannot be counterbalanced by the additional exploitation of σ_{SCA}^2 , which is reflected by a higher intercept. In contrast, assuming $\sigma_G^2(\text{hybrid}) = 0.9\sigma_G^2(\text{line})$ yields comparable recurrent selection gain for hybrid and line breeding resulting in larger selection gain in hybrid breeding due to the additional exploitation of σ_{SCA}^2 in the factorials.

Besides these theoretical considerations, one study compared hybrid versus line breeding using experimental data of official variety tests in hard winter wheat across 20 years in the USA (Koemel et al. 2004). The authors observed a higher selection gain across time for hybrids compared to line breeding. A further experimental study based on germplasm from the current market leader in hybrid wheat breeding, revealed that most of the hybrids showed positive commercial heterosis pointing toward a competitive selection gain of hybrid versus line breeding (Gowda et al. 2012a). These findings are very stimulating, but further experimental data are needed to substantiate the long-term selection gain for hybrid versus line breeding in autogamous cereals.

Conclusions

The basic requirements for hybrid breeding in autogamous cereals are fulfilled and hybrid varieties are an attractive

niche market for wheat, barley, and triticale. Rice hybrids show the highest market penetration. Further research to optimize the existing hybridization systems is urgently required and should be coupled with the development of a clear male and female pool concept. In order to judge whether hybrid varieties in autogamous cereals can gain a larger market share than currently, further experimental studies are urgently needed to investigate relevant quantitative genetic parameters such as variance components to calculate the expected selection gain of hybrid versus line breeding.

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Appendix

Selection gain was calculated based on the formula of Cochran (1951). There is a lack of robust information on variance components, correlations, and other parameters relevant to the calculation of selection gain. As our aim was to roughly investigate trends in hybrid and line breeding, we assumed a selection intensity (i) and square root of heritability (h) of $i = h = 1$ and modified only the genetic variance by assuming $\sigma_G^2(\text{line}) = 1$, while $\sigma_G^2(\text{hybrid})$ was either $0.5\sigma_G^2(\text{line})$ or $0.9\sigma_G^2(\text{line})$. For the factorial crosses, we assumed that the budget was 20 % of that of a line breeding program (Longin et al. 2007) and

that σ_{SCA}^2 amounted to 20 % of the total genetic variance and calculated it as an additional one-stage selection gain. Selection gain across time was calculated as the sum of the single selection cycles, assuming that all variances were constant across years. The selection gain of the factorials, however, was only added once after each selection cycle.

References

- Abel S, Mollers C, Becker HC (2005) Development of synthetic *Brassica napus* lines for the analysis of “fixed heterosis” in allopolyploid plants. *Euphytica* 146:157–163
- Ahokas H (1979) Cytoplasmic male sterility in barley. *Acta Agric Scand* 29:219–224
- Barbosa-Neto JF, Sorrels ME, Cisar G (1996) Prediction of heterosis in wheat using coefficient of parentage and RFLP-based estimates of genetic relationship. *Genome* 39:1142–1149
- Bernardo R (2002) Breeding for quantitative traits in plants. Stemma Press, Woodbury
- Borghini B, Perenzin M (1994) Diallel analysis to predict heterosis and combining ability for grain yield, yield components and bread-making quality in bread wheat (*T. aestivum*). *Theor Appl Genet* 89:975–981
- Bruns R, Peterson CJ (1998) Yield and stability factors associated with hybrid wheat. *Euphytica* 100:1–5
- Cochran WG (1951) Improvement by means of selection. In: *Proceedings 2nd Berkeley Symposium Math Stat Prob*, pp 449–470
- Cockerham CC (1954) An extension of the concept of partitioning hereditary variance for analysis of covariance's among relatives when epistasis is present. *Genetics* 39:859–882
- Coors JG, Pandey S (1999) The genetics and exploitation of heterosis in crops. ASA, CSSA, and SSSA, Madison
- Corbellini M, Perenzin M, Accerbi M, Vaccino P, Borghini B (2002) Genetic diversity in bread wheat, as revealed by coefficient of parentage and molecular markers, and its relationship to hybrid performance. *Euphytica* 123:273–285
- D'Souza VL (1970) Investigations concerning the suitability of wheat as a pollen-donor for cross pollination by wind as compared to rye, Triticale and Secalotricum. *Zeitschrift für Pflanzenzüchtung* 63:246–269
- Dhillon BS, Singh J (1977) Combining ability and heterosis in diallel crosses of maize. *Theor Appl Genet* 49:117–122
- Duvick DN, Smith JSC, Cooper M (2004) Long-term selection in a commercial hybrid maize breeding program. In: Janick J (ed) *Plant breeding reviews, long term selection: crops, animals, and bacteria, part 2, vol 24*. Wiley, New York, pp 109–151
- Edwards IB (2001) Origin of cultivated wheat. In: Bonjean AP, Angus WJ (eds) *The world wheat book—a history of wheat breeding, vol 1*. Lavoisier publishing, Paris, pp 1019–1045
- Falconer DS, Mackay TF (1996) *Introduction to quantitative genetics*, 4th edn. Longmans Green, Harlow
- Fischer S, Möhring J, Schön CC, Piepho H-P, Klein D, Schipprack W, Utz HF, Melchinger AE, Reif JC (2008) Trends in genetic variance components during 30 years of hybrid maize breeding at the University of Hohenheim. *Plant Breeding* 127:446–451
- Fischer S, Maurer HP, Würschum T, Möhring J, Piepho H-P, Schön CC, Thiemt E-M, Dhillon BS, Weissmann EA, Melchinger AE, Reif JC (2010) Development of heterotic groups in triticale. *Crop Sci* 50:584–590
- Garcia AAF, Wang S, Melchinger AE, Zeng Z-B (2008) Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. *Genetics* 180:1707–1724
- Geiger HH, Miedaner T (2009) Rye breeding. In: Carena MJ (ed) *Cereals (Handbook of Plant Breeding)*, vol 3. Springer, New York, pp 157–181
- Gordillo AG, Geiger HH (2008) Alternative recurrent selection strategies using doubled haploid lines in hybrid maize breeding. *Crop Sci* 48:911–922
- Gowda M, Kling CK, Würschum T, Liu W, Maurer HP, Hahn V, Reif JC (2010) Hybrid breeding in durum wheat: heterosis and combining ability. *Crop Sci* 50:2224–2230
- Gowda M, Longin CFH, Lein V, Reif JC (2012a) Relevance of specific versus general combining ability effects in wheat. *Crop Sci* (In press)
- Gowda M, Zhao Y, Maurer HP, Weissmann EA, Würschum T, Reif JC (2012b) Best linear unbiased prediction of triticale hybrid performance. *Euphytica* (in review)
- Hallauer AR, Russell WA, Lamkey KR (1988) Corn breeding. In: Sprague GF, Dudley JW (eds) *Corn and corn improvement*, 3rd edn. Agron Monogr 18 ASA, CSSA, SSSA, Madison, pp 469–565
- Hammer K (1977) Fragen zur Eignung des Pollens der Kulturgerste (*Hordeum vulgare* L. s.l.) für die Windbestäubung. *Kulturpflanze* 25:13–23 (in German)
- He X-H, Zhang Y-M (2011) A complete solution for dissecting pure main and epistatic effects of QTL in triple testcross design. *PLoS ONE* 6:e24575
- Herrmann M (2007) A diallel analysis of various traits in winter triticale. *Plant Breed* 126:19–23
- Ikehashi H (1991) Genetics of hybrid sterility in wide hybridization in rice. In: Bajaj YPS (ed) *Biotechnology in agriculture and forestry*. Springer, Berlin, pp 113–127
- Ikehashi H, Araki H (1984) Varietal screening for compatibility types revealed in F1 fertility of distant crosses in rice. *Jpn J Breed* 34:304–312
- Jones JW (1926) Hybrid vigor in rice. *J Am Soc Agron* 18:423–428
- Jordaan JP (1996) Hybrid wheat: advances and challenges. In: Reynolds MP et al (eds) *Increasing yield potential in wheat: breaking the barriers*. CIMMYT, Mexico, pp 66–75
- Kempe K, Gils M (2011) Pollination control technologies for hybrid breeding. *Mol Breed* 27:417–437
- Keydel F (1972) Blütenbiologische Untersuchungen. 1. Offenblütigkeit verschiedener Winterweizensorten. *Bayer Landw Jb* 49:688–701 (in German)
- Keydel F (1977) Offenblütigkeit und Fremdbefruchtung bei selbstbefruchtenden Getreidenarten am Beispiel Winterweizen. Bericht über die Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter in Gumpenstein S. 22–24 (in German)
- Keydel F (1985) Die Entwicklung von Hybridsorten bei Weizen mit Hilfe von Gametoziden. 1. Erfahrungen. In: Bericht über die Arbeitstagung 1985 der Arbeitsgemeinschaft der Saatzuchtleiter in Gumpenstein S. 13–23 (in German)
- Koekemoer FP, van Eeden E, Bonjean AP (2011) An overview of hybrid wheat production in South Africa and review of current worldwide wheat hybrid developments. In: Bonjean AP, Angus WJ (eds) *The world wheat book—a history of wheat breeding, vol 2*. Lavoisier publishing, Paris, pp 907–950
- Koemel JE Jr, Guenzi AC, Carver BF, Payton ME, Morgan GH, Smith EL (2004) Hybrid and pure line hard winter wheat yield and stability. *Crop Sci* 44:107–113
- Kusterer B, Piepho H-P, Utz HF, Schön CC, Muminovic J, Meyer RC, Altmann T, Melchinger AE (2007) Heterosis for biomass-related traits in *Arabidopsis* investigated by a novel QTL analysis of the triple test cross design with recombinant inbred lines. *Genetics* 177:1839–1850
- Li SQ, Yang DC, Zhu YG (2007) Characterization and use of male sterility in rice breeding. *J Integr Plant Biol* 49:791–804
- Longin CFH, Utz HF, Melchinger AE, Reif JC (2007) Hybrid maize breeding with doubled haploids. II. Optimum type and number of

- testers in two-stage selection for general combining ability. *Theor Appl Genet* 114:393–402
- Lu Z-M, Xu B-Q (2010) On significance of heterotic group theory in hybrid rice breeding. *Rice Sci* 17:94–98
- Martin AC, Atienza SG, Ramirez MC, Barro F, Martin A (2009) Chromosome engineering in wheat to restore male fertility in the msH1 CMS system. *Mol Breed* 24:397–408
- Maruyama K, Kato H, Araki H (1991) Mechanized production of F1 seeds in rice [*Oryza sativa*] by mixed planting. *JARQ* 24:243–252
- Melchinger AE, Gumber RK (1998) Overview of heterosis and heterotic groups in agronomic crops. In: Lamkey KR, Staub JE (eds) Concepts and breeding of heterosis in crop plants. ASA-CSSA-SSSA Publication, Madison, pp 29–44
- Melchinger AE, Piepho HP, Utz HF, Muminović J, Wegenast T, Törjék O, Altmann T, Kusterer B, Zeng ZB, Schön CC (2007a) Genetic basis of heterosis for growth-related traits in Arabidopsis investigated by testcross progenies of near-isogenic lines reveals a significant role of epistasis. *Genetics* 177:1827–1837
- Melchinger AE, Utz HF, Piepho HP, Zeng ZB, Schön CC (2007b) The role of epistasis in the manifestation of heterosis—a systems-oriented approach. *Genetics* 177:1815–1825
- Melchinger AE, Utz HF, Schon CC (2008) Genetic expectations of quantitative trait loci main and interaction effects obtained with the triple testcross design and their relevance for the analysis of heterosis. *Genetics* 178:2265–2274
- Merfert W, Faustmann M, Täubert K (1987) Höhe und Verbreitung von Hybrideffekten bei Weizen. *Arch. Züchtungsforsch* 17:63–72 (in German)
- Montes JM, Melchinger AE, Reif JC (2007) Novel throughput phenotyping platforms in plant genetics studies. *Trends Plant Sci* 12:433–436
- Mühleisen J, Maurer HP, Stiewe G, Bury P, Reif JC (2012) Hybrid breeding in barley. *Crop Science* In review
- Nalepa S (1990) Hybrid triticale: present and future In: Proceedings 2nd International Triticale Symposium, Passo Fundo, Brazil, pp 402–407
- Oettler G, Burger H, Melchinger AE (2003) Heterosis and combining ability for grain yield and other agronomic traits in winter triticale. *Plant Breed* 122:318–321
- Oettler G, Heinrich N, Miedaner T (2004) Estimates of additive and dominance effects for Fusarium head blight resistance of winter triticale. *Plant Breed* 123:525–530
- Oettler G, Tams SH, Utz HF, Bauer E, Melchinger AE (2005) Prospects for hybrid breeding in winter triticale: I. Heterosis and combining ability for agronomic traits in European elite germplasm. *Crop Sci* 45:1476–1482
- Omarov DS (1976) Some selective genetic aspects of the biology of flowering and fertility in barley. In: Proceedings 3rd International Barley Genet Symposium Munich, pp 802–804
- Oury F-X, Brabant P, Berard P, Pluchard P (2000) Predicting hybrid value in bread wheat: biometric modeling based on a top-cross design. *Theor Appl Genet* 100:96–104
- Ouyang Y, Chen J, Ding J, Zhang Q (2009) Advances in the understanding of inter-subspecific hybrid sterility and wide-compatibility in rice. *Chinese Sci Bull* 54:2332–2341
- Perenzin M, Corbellini M, Accerbi M, Vaccion P, Borghi B (1998) Bread wheat: F1 hybrid performance and parental diversity estimates using molecular markers. *Euphytica* 100:273–279
- Pickett AA (1993) Hybrid wheat—results and problems. In: Advances in Plant Breeding 15, Paul Parey Scientific Publishers, Berlin
- Piepho HP (1998) Methods for comparing the yield stability of cropping systems: a review. *J Agron Crop Sci* 180:193–213
- Rajaram S (2001) Prospects and promise of wheat breeding in the 21st century. *Euphytica* 119:3–15
- Ramage RT (1965) Balanced tertiary trisomics for use in hybrid seed production. *Crop Sci* 5:177–178
- Ramage RT (1983) Heterosis and hybrid seed production in barley. In: Frankel R (ed) Heterosis: reappraisal of theory and practice. (Monogr Theor Appl Genet vol 6). Springer, Berlin Heidelberg New York, pp 71–93
- Reif JC, Gumpert F, Fischer S, Melchinger AE (2007) Impact of genetic divergence on additive and dominance variance in hybrid populations. *Genetics* 176:1931–1934
- Reif JC, Kusterer B, Piepho H-P, Meyer RC, Altmann T, Schon CC, Melchinger AE (2009) Unraveling epistasis with triple testcross progenies of near-isogenic lines. *Genetics* 181:247–257
- Schnell FW (1961) Heterosis and inbreeding effect. *Schriftenreihe des Max-Planck-Instituts für Tierzucht und Tierernährung*, pp 251–272 (in German)
- Schnell FW (1965) Die Kovarianz zwischen Verwandten in einer genorthogonalen Population. I. Allgemeine Theorie. *Biometrische Z* 7:1–49 (in German)
- Schnell FW, Geiger HH (1970) Zur Beeinflussung der Heterosis durch Epistasie. *Die Naturwissenschaften* 9:461–462 (in German)
- Scholz F, Künzel G (1982) Progress and problems with hybrid barley. In: Barley Genetics IV, Proc 4th Barley Genet Symp, Edinburgh, UK, pp 758–765
- Shull GH (1908) The composition of a field of maize. *Am Breeders Assoc Rep* 4:296–301
- Si HM, Liu WZ, Fu YP, Sun ZX, Hu GC (2011) Current situation and suggestions for development of two-line hybrid rice in china. *Chinese J Rice Sci* 25:522–544 (in Chinese)
- Singh SK, Chatrath R, Mishra B (2010) Perspective of hybrid wheat research: a review. *Indian J Agric Sci* 80:1013–1027
- Suneson CA (1940) A male sterile character in barley. *J Hered* 31:213–214
- Virmani SS (1994) Prospects of hybrid rice in the tropics and subtropics. In: Hybrid rice technology: new developments and future prospects. Selected papers from the Int Rice Res Conf, Manila, pp 7–20
- Virmani SS (1996) Hybrid rice. *Adv Agronomy* 57:377–462
- Virmani SS, Athwal DS (1972) Genetic variability in floral characteristics influencing outcrossing in *Oryza sativa* L. *Crop Sci* 13:66–67
- Yan WG, Li SF (1987) Study on out-crossing characteristics among male sterile lines containing same nucleus in rice. *Hybrid Rice* 4:8–11 (in Chinese)
- Yan W, Li Y, Agrama H, Luo D, Gao F, Lu X, Ren G (2009) Association mapping of stigma and spikelet characteristics in rice. *Mol Breed* 24:277–292
- Yang RC (2004) Epistasis of quantitative trait loci under different gene action models. *Genetics* 167:1493–1505
- Yuan LP (1966) A preliminary report on male sterility in rice. *Chinese Sci Bull* 4:32–34 (in Chinese)
- Yuan LP, Virmani SS (1988) Status of hybrid rice research and development. In: Hybrid rice: Proc Int Symp Hybrid Rice in Changsha, Hunan, China, 1986. International Rice Research Institute, Manila, Philippines, pp 7–24
- Yuan LP, Yang ZY, Yang JB (1994) Hybrid rice research in China. In: Hybrid rice technology: New developments and future prospects. Selected papers from the International Rice Research Conference, Manila, pp 143–148