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Abstract

With the advent of hybrid breeding in the last decades of the twentieth century, the image of rye changed from being perceived as a 'forgotten crop' to a pioneer among the cereals. Meanwhile, the genetic basis of the hybrid system with relevant components such as self-fertility, cytoplasmatic male sterility and fertility restoration, heterotic groups and inbreeding tolerance are understood and a sustained reduction for the costs of the hybrid system has advanced. New enabling technologies, including DNA markers, high-throughput phenotyping and gene discovery became rapidly absorbed into the breeding process. Enhancing genetic diversity is a key success factor for long-term genetic gain. Broadening established heterotic patterns and introgressing new QTLs for simple and complex inherited traits are indispensable. With the advent of genomic selection-based breeding schemes, recurrent selection and commercial inbred line development are changing dramatically. The impact on the architecture of a breeding program is illustrated by several practice-orientated examples. Essential preconditions for the inscription of a variety are highlighted with examples of official testing systems and traits relevant for market acceptance. Progress from breeding has been substantial as revealed by the German official tests and can be regarded as a key driver to maintain competitiveness of the crop in Europe but also in other regions of the world. The economic effects of breeding research have been highly profitable from a societal and environmental protection perspective.

2.1 Introduction

Rye (*Secale cereale* L.) was cultivated worldwide on 4.1 million hectares in 2018 (FAOSTAT 2020) and is grown mainly in Northern and Eastern Europe, where about 70% of the world harvest is produced. The top-producing country is Germany, followed by Poland, the Russian Federation, China and Finno-Scandinavian countries. Here, rye is traditionally used for bread making, but can also be utilized as grain feed for animals, for distilling spirits, or for bioenergy production as a substrate for bio-methane or ethanol production. Outside Europe, rye is often used as pasture, hay, or cover crop (Oelke et al. 1990). Rye is outstanding for its early, vigorous growth, high tolerance to abiotic

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and biotic stress factors and adaptation to acid or sandy soils with low water and nutrient availability making it a competitive alternative in regions where growing other crops would not be profitable (Geiger and Miedaner 2009).

The origin of rye is in Southwest Asia, particularly in Turkey, Lebanon, Syria, Iran, Iraq, and Afghanistan (Sencer and Hawkes 1980). Three species are generally recognized in the genus *Secale*: *Secale silvestre* (annual, autogamous), *S. strictum* (perennial, allogamous), and *S. cereale* (annual, allogamous). Within the latter species, cultivated, weedy and wild forms are existing (Fredericksen and Petersen 1998). Cultivated rye is a diploid ($2n = 2x = 14$) species and the only cross-pollinating small-grain cereal of the temperate zone.

2.2 Types of Varieties in Rye

In rye, populations and hybrids are well established as the main types of varieties. Recognizing their characteristics is relevant for diverse applications like breeding methodology, selection purposes, maintenance, seed multiplication, or plant variety protection.

The term 'population' comprises open-pollinated and synthetic varieties as sub-types. In both cases, only self-incompatible germplasm should be used to avoid inbreeding depression from any self-pollination and to enhance random mating. In a population based on many genotypes different from each other, random mating promotes high heterozygosity, which is beneficial for the per se performance of the population. At the same time, a genetic equilibrium (Hardy–Weinberg equilibrium) is maintained, which keeps the phenotypic appearance of the population stable over subsequent generations of seed multiplication. Random-mating or panmictic populations can be regrown by farm-saved seed without performance reduction caused by genetic factors.

To create a new variety, most population rye breeders use half or full sib families and select those showing high performance (Geiger 1982). Because per se performance of a family is correlated with its general combining ability (GCA), an improvement in population performance will be the result. In the case of open-pollinated varieties, there is no methodological difference between breeding for population improvement and for maintenance. In the case of synthetic varieties, families identified as well performing are kept separate and serve as parental components. By targeted intercrossing or random mating, they are regularly used to reconstruct and maintain the final population.

In the case of hybrid varieties, self-fertile inbred lines derived from two genetically distinct populations expressing a heterotic pattern ('heterotic groups') are used as parental components. Lines are selected for per se performance and for GCA effects to the respective opposite pool. Seed parent lines must be carriers of non-restorer genes allowing development of a male fertile and male sterile near-isogenic version. In contrast, pollen parent lines carry restorer genes allowing reconstitution of male fertility in the final hybrid. In practical hybrid breeding, a male sterile single cross of two seed parent lines is crossed with a synthetic built up from 2 to 4 pollen parent lines. Thus, the genetic basis of a typical rye hybrid is comparable to a four-way hybrid or a top cross hybrid in maize. Producing farm-saved seed from hybrids is not advisable due to their narrow genetic basis. Inevitably, resultant partially inbred plants will suffer from inbreeding depression and a correspondingly reduced yield.

2.3 Genetic Basis for Hybrid Breeding

2.3.1 Self-fertility and Self-incompatibility

Rye has a highly effective gametophytic self-incompatibility (SI) system that prevents self-fertilization and secures a high level of heterozygosity in open-pollinated populations (see also Chap. 10). SI is governed by multiple alleles at the two loci S and Z (Lundqvist 1956) that were first localized by isozymes on chromosomes 1R and 2R, respectively (Wricke and Wehling 1985; Gertz and

Wricke 1989). Both loci are gametophytically expressed and cause an SI response when both the S and Z alleles of a haploid pollen grain match the same alleles in the diploid stigma (Hackauf and Wehling 2005). Then, pollen tube growth is inhibited and the pollination is completely restricted. More recently, a third SI locus is known as $S5$ (T) locus on chromosome 5R (Voylokov et al. 1993). Trang et al. (1982) reported 6–7 alleles at the S and 12–13 alleles at the Z locus in the population cultivar Halo. Self-fertility may be caused by a dominant gene but could also follow a more complex pattern of inheritance (Melz et al. 1990). Lundqvist (1956) showed that self-fertility (Sf) mutations occur in both, S and Z genes. The self-fertility used in German hybrid rye material traces back to the early work of Ossent (1938) who selfed 10,000s of rye heads of different populations. Starting with a few self-fertile plants, he reached full self-fertility after a few cycles of recurrent selection due to dominant inheritance.

2.3.2 Cytoplasmic-Male Sterility

Several sources of cytoplasmic-male sterility (CMS) have been detected in rye (Table 2.1, see also Chap. 10). Of these, only the Pampa (P) cytoplasm from an Argentinean landrace of rye ("Waldstaudenroggen") gained importance for hybrid production. It is environmentally very stable and easy to maintain, but hard to restore. In an experiment across 10 European locations in two years, no pollen fertility occurred in maintainer lines and their crosses (Geiger et al. 1995). In European material, maintainer genotypes dominate and only 3–5% of the gametes are (partial) restorers. All other mentioned cytoplasms belong to the V (Vavilov) type that is, in contrast, hard to maintain and easy to restore due to a high frequency of gametes with partial or full restoration. Łapiński and Stojałowski (2001) reported that the majority of male sterility sources from 50 rye populations belonged to the Vavilov type. In a follow-up study, Stojałowski et al. (2008) detected P cytoplasm only in two Iranian sources ('IRAN I', 'IRAN IX') that have been reported earlier to contain CMS (Geiger and Morgenstern 1975) and in Argentinean populations ('Pico Massaux', 'San Jose', 'Trenelense') similar to those where the P cytoplasm was detected. Further, PCR-based markers can be used to identify CMS phenotypes, namely the differentiation of the normal cytoplasm from the P and V type cytoplasm is possible (Stojałowski et al. 2004).

2.3.3 Restoration of Male Fertility

Pollen-fertility restoration is of crucial importance for hybrid rye growing. Incomplete restoration might result in poor seed set, but definitely increases the infection by the ergot fungus (*Claviceps purpurea*) that produces toxic alkaloids in the purple-black sclerotia growing on rye heads after infection at flowering. The less pollen available, the higher is the incidence of ergot infection (Miedaner and Geiger 2015). Low tolerance limits for ergot sclerotia in commercial grain lots for food and feed (<0.05% and <0.1% per 500 g grain, respectively) exist in the European Union and are expected to tighten in the coming years. European restorer sources for the P cytoplasm are scarce, highly dependent on environment and the seed parent genotype, often leading to incomplete restoration (Geiger et al. 1995). Even worse, they show oligogenic inheritance, often with a major gene that requires several minor genes for full restoration (Miedaner et al. 2000). Restorer of fertility (Rf) genes are nuclear encoded and should display full dominance to be useful in hybrid breeding. In those germplasms where CMS has been found, effective Rf genes were detected, notably in Iranian primitive rye (e.g., 'IRAN IX', 'Altevogt 14161') and Argentinean landraces (e.g., 'Pico Gentario'). Male-fertility restoration from these sources is mainly monogenically inherited (Miedaner et al. 2000) and shows much lower environmental and seed parent effects than the European sources (Miedaner et al. 2005). The Rf gene of 'IRAN IX' was first introduced into the commercial hybrid cultivar 'Pollino' released in 2005. Hybrid cultivars carrying this gene have a much lower ergot incidence after inoculation by

Table 2.1 Different sources of CMS cytoplasm (Schlegel 2016, adjusted)

Type	Description	References
P	Pampa cytoplasm	Geiger and Schnell (1970)
C	Cytoplasm of wild rye, *S. montanum*	Łapiński (1972)
R	Russian cytoplasm	Kobyljanski and Katerova (1973)
S	Mutated cytoplasm of Kärtner rye	Warzecha and Salak-Warzecha (2003)
G	Mutated cytoplasm of Schlägler Alt, Norddeutscher Champagner	Melz et al. (2003)

Claviceps purpurea than those without this restorer gene (Miedaner et al. 2005; Miedaner and Geiger 2015). However, non-adapted *Rf* genes display negative side effects such as lower grain yield, lower 1000-grain weight and taller plant stature, even in testcross progenies (Miedaner et al. 2017). Due to a large range in yield penalty between lines derived from the same non-adapted source, the opportunity exists to select for better adaptation to the elite gene pool.

whereas the pollen parent (Carsten) pool exhibits large spikes and a good seed set, but a high lodging susceptibility, a high level of pre-harvest sprouting and a low stand density contributing to a considerably lower per se performance than the Petkus derived populations 'Nomaro' and 'Kustro' (Hepting 1978). This contributed, together with its wide adaptability and high yield performance, to a preponderance of the Petkus population in world-wide rye breeding.

2.3.4 Heterotic Groups

Information about heterotic groups is fundamental for the maximum exploitation of heterosis (Melchinger and Gumber 1998). The seed and pollen parents should be derived from genetically unrelated germplasm in order to maximize heterosis. According to a 7×7 diallel of European rye populations, two germplasm groups from Germany showed the highest panmictic mid-parent heterosis relative to the mean with 18.7% for Petkus and 10.8% for Carsten (Hepting 1978). They were bred by two German rye breeders, Ferdinand von Lochow from the small village Petkus near Berlin and Dr. h.c. R. Carsten from Bad Schwartau, starting with different open-pollinated landraces. Since then, all hybrids released in Germany belong to the Petkus $\times$ Carsten type. This pattern was confirmed by analysis with about 180,000 SNPs clearly showing two separate clusters and strong differentiation between the seed and pollen parent pool ($F_{ST} = 0.229$, Bauer et al. 2017). The seed parent (Petkus) pool shows high yield performance, high kernel weight and lodging resistance,

2.3.5 Inbreeding Tolerance

In rye, as an outcrossing crop, performance greatly depends on the degree of heterozygosity. During subsequent selfing, the performance drops dramatically due to inbreeding depression. Crossing inbred lines, however, results in substantial heterosis (H) defined as H = F1-MP with F1 and MP indicating the F1- and the mid-parent performance of a pair of inbred lines, respectively. Inbreeding depression is highest for traits showing high heterosis. In maize, Schnell (1974) showed that with extended breeding cycles, the relative amount of heterosis (expressed as a percent of MP performance) drops because the inbred lines and subsequently, the hybrid performance, increase. This could also be shown for rye, where first-cycle lines yielded relative heterosis for grain yield of 192% (Geiger and Wahle 1978), whereas 22 years later the relative heterosis was estimated as 139% (Geiger et al. 2001) caused by strong selection for inbreeding tolerance that raised the performance of inbred lines considerably.

2.3.6 GCA/SCA Variance Relationship

The general (GCA) and specific combining ability (SCA) variances are important parameters for predicting hybrid performance (Melchinger et al. 1987) and for designing hybrid-breeding programs. The higher the GCA relative to the SCA variance, the better superior hybrids can be predicted from their GCA effects and the more effective early testing procedures become. Producing hybrids between heterotic groups generally decreases the relative importance of SCA as shown in maize (Melchinger and Gumber 1998). Estimations of GCA and SCA variance in rye showed a high preponderance of GCA variance for grain yield and plant height and, consequently high importance of additive gene action (Tomerius et al. 1997). However, in crosses with inbred lines pre-selected for high GCA effects, the importance of SCA variance might increase considerably (Geiger 1982). For practical breeding, the proportion of GCA/SCA variances greatly determines the number of testers needed for combining ability tests. Based on model calculations one tester was optimal for the first stage of GCA selection and three testers for the 2nd stage (Tomerius et al. 2008).

2.3.7 Correlation Inbred Lines: Testcrosses

The correlation between inbred lines and their testcrosses determines which traits can be reliably selected based on per se performance among inbred line populations or must be selected on a testcross basis. Selection on per se performance in early generations is highly advantageous for the breeder because it saves time, seed production costs and a larger proportion of additive genetic variance can be exploited in lines compared to hybrids. The genotypic correlation depends on the complexity of the trait under consideration as well as on the predominant type of gene action (Hallauer et al. 2010; Mihaljevic et al. 2005). In elite breeding populations, the genotypic correlations were highest ($r_g \geq 0.7$) for plant height, test weight, thousand-kernel weight, falling number and starch content (Miedaner et al. 2014). In contrast, for grain yield a much lower genotypic correlation (Table 2.2) has been found, providing the main reason for the need to perform testcrosses in early generations.

2.4 Enabling Technologies

2.4.1 Marker Technology

For a long time, rye lagged behind other cereals in developing new marker technologies because of its low international importance. With the advent of next generation sequencing (NGS) it became feasible for the first time to develop a public single nucleotide polymorphism (SNP) Illumina® Infinium iSelect HD Custom BeadChip comprising more than 5000 markers (Haseneyer et al. 2011). Based on this array, an extended custom 16 k Illumina BeadChip was produced (Auinger et al. 2016) and recently even a high-density Affymetrix Axiom® Rye600k genotyping array was designed (Bauer et al. 2017). Alternatively, the application of NGS technology for genotyping-by-sequencing (GBS) is well established and is offered as DArTseq by Diversity Arrays Technology Pty Ltd., Australia. A medium-density 16 k chip was successfully used for mapping quantitative traits (Miedaner et al. 2012; Hackauf et al. 2017a). However, although some quantitative trait loci (QTL) with high effects were detected in adapted populations, they have not been used in practical rye breeding, mainly caused by missing validation experiments. In contrast, molecular markers are a perfect tool for marker-assisted selection (MAS) of monogenic traits in breeding populations.

A good example is the introgression of non-adapted Rf genes by molecular markers. In inbred lines developed from the accessions 'IRAN IX' and 'Pico Gentario', pollen-fertility restoration of the P cytoplasm was assigned to two genes, *Rfp1* and *Rfp2*, respectively, that were both mapped on chromosome 4RL; one of these genes is sufficient to reach full pollen fertility (Stracke et al. 2003). Studies with newly developed co-

Table 2.2 Estimates of genotypic correlation between inbred lines and testcrosses

Reference	Grain yield	Plant height	1000-grain weight	Falling number
Köhler (1986)	0.51	0.87	0.81	–
Wilde (1987)	0.56	0.83	0.73	–
Hartmann (1997)	0.56	0.81	0.76	0.90
Miedaner et al. (2014)[a]	–	0.48/0.80	0.68/0.67	0.94/0.73

[a]Two inbred line populations and their testcrosses ($N = 220$ per population)

dominant markers permitted to delimit *Rfp1* within a 0.7 cM genetic interval and allowed prediction of *Rfp1* genotypes with high precision (Hackauf et al. 2012). A third restorer gene, *Rfp3*, was detected in the Iranian primitive rye 'Altevogt 14161' (Falke et al. 2009) that could also be mapped to the same interval as *Rfp1* and *Rfp2* (Hackauf et al. 2017b). This subgenomic region is syntenic to the *Rfm1* locus of barley on chromosome 6HS. Interestingly, *Rf* genes for the G (Börner et al. 1998) and C (Stojałowski et al. 2005) cytoplasms in rye, which both are functionally different from P (Geiger et al. 1995), have been mapped to the same segment of chromosome 4RL (Hackauf et al. 2009). Likewise, the rye gene *Rfc4*, which restores male fertility in hexaploid wheat with *Triticum timophevii* sterility-inducing cytoplasm was mapped on chromosome 4RL (Curtis and Lukaszewski 1993). For *Rfp1* from 'IRAN IX', the marker interval could be reduced to 0.2 cM, equivalent to about 120 kb, by establishing new markers and mapping a population of about 5,000 plants of a backcross population (Wilde et al. 2017). Two tightly linked and equivalent, but independently acting *Rf* genes were detected in the respective interval (*Rfp1a*, *Rfp1b*). Compared to earlier studies, the introgression segment from Iranian primitive rye was shortened and the yield penalty considerably lowered but, not totally suspended, yet. This example illustrates the enormous advantage of applying molecular markers when the breeder is targeting background selection. Moreover, markers tremendously shorten the selection procedure for *Rf* genes in foreground selection, because testcrossing (1st generation) of genotypes putatively carrying the target gene and phenotyping

of the testcross progenies for pollen-fertility restoration (2nd generation), is no longer necessary or can be restricted to the final step confirming the success of the backcross procedure.

The potential of genomic selection (GS) for rye breeding has been investigated recently by the use of SNP arrays (Auinger et al. 2016; Bernal-Vasquez et al. 2017; Wang et al. 2014, 2015). In spite of the fast decline of linkage disequilibrium (LD) detected in rye candidate genes (Li et al. 2011), the use of medium-sized arrays (e.g., 16 k Infinium iSelect HD BeadChip, Auinger et al 2016) suffices. GS uses information from the whole genome, i.e., on all polymorphic markers, that are available and thus can be expected to take into account also small effect gene loci for a given trait that cannot be captured by QTL analyzes (Jannink et al. 2010). The ultimate goal for the rye breeder would be to predict the genetic value of non-phenotyped entries to reduce cycle length and costs. This is especially useful for hybrid rye breeding, where every line has to be testcrossed before phenotyping for grain yield, thus needing two years for one stage of yield selection. GS improved prediction accuracy compared to MAS in all tested quantitatively inherited traits (grain yield, plant height, starch and total pentosane content), especially in cases where only a low proportion of genotypic variation could be explained by MAS (Wang et al. 2014). Accuracy of prediction, which is defined as the correlation (r_{MG}) of the effect as estimated by *M*arker to the true *G*enetic effect, highly decreased when an estimation set from one biparental population was compared to a test set from another population, although both populations shared one parental line (Wang et al. 2014). Even for analyzing the

very resource-demanding parameter phenotypic stability, a GS approach could detect stable QTLs for quality traits (test weight, soluble pentosane content and falling number), but not for yield-related traits (Wang et al. 2015), indicating that quality traits have a simpler genetic architecture. Another application for exploiting the potential of GS in breeding programs is the prediction of breeding values across selection cycles. Pedigree, genomic and phenotypic data of four consecutive breeding cycles from a commercial rye breeding program were used for a detailed cross-validation analysis. It could be demonstrated that GS for grain yield, plant height and TKW yielded improved prediction accuracies when data across cycles were accumulated (Auinger et al. 2016). For the three traits mentioned, prediction accuracies obtained from cross-validation with large calibration sets ($N = 832$) derived from all four available cycles were around 0.70, which looks rather promising. However, prediction accuracies usually yielded much lower means and showed considerable variance of estimates when lines from a given cycle had to be predicted across-cycle from calibration sets where the given cycle is missing. The authors concluded that uncertainty of prediction is an important factor, which should not be neglected when discussing the potential of GS. Among other factors, connectivity over breeding cycles via common ancestors is of particular importance to ensure persistency of prediction accuracy.

The authors suggest a number of opportunities of GS specifically for hybrid rye: (1) reduction of cycle length, (2) applying indices combining genomic and phenotypic information and (3) better exploitation of segregation variance within families. Some more applied examples on how to exploit these opportunities are illustrated in Sect. 2.6 of this chapter. Implementing GS for practical breeders is challenging. For example, a sophisticated management and design of crosses and knowledge of the respective familial structures is necessary when the breeder strives to maintain genetic variance and to maximize or balance short- but also long-term gain from selection.

2.4.2   High-Throughput Phenotyping

Phenotyping is a bottleneck for practical selection in plant breeding (Würschum 2019). To identify superior genotypes 10,000s of candidates have to be tested in field trials for mainly complex inherited quantitative traits. Although technical achievements for improved machinery equipment enable today for high power field-work, it is still a labor- and time-intensive procedure. Given the decreasing costs for marker assays, phenotyping is an even higher restriction for exploiting the genetic architecture of important traits. Using sensor technology to assess multiple traits might be the first step to high-throughput phenotyping.

For quality traits, like water (dry matter) and protein content, the application of near-infrared spectroscopy (NIRS) provided a breakthrough in the 1990s. More recently, water content can be measured very precisely on-field by specially equipped harvesters. This made obvious the advantages of a fast, precise, non-destructive measurement for plant breeding and boosted research into high-throughput phenotyping platforms (review by Würschum 2019). Multiple sensors are available with either morphological or spectral measurements and they can be applied either as field-based mobile platforms or as unmanned aerial vehicles, like drones (Haghighattalab et al. 2016). Hyperspectral imaging is able to measure water content, abiotic stress and disease severity, composition of plants and ingredients of plant parts. Vegetation Indices (VIs) summarize canopy reflectance information in simple algorithms used for qualitative and quantitative assessment of a wide range of plant parameters, like water, chlorophyll, or carotenoid content (Xue and Su 2017). Prediction of biomass yield via hyperspectral data is an interesting application when candidate lines are routinely phenotyped for grain yield. Thus, factorial experiments with two harvest dates, at milk ripening for biomass yield and at full ripening for grain yield, can be made superfluous.

The VIs alone reached a prediction ability (PA) of maximal 0.42 when both flights were combined (Table 2.3).

In a second attempt, the most informative bands from all 400 assessed hyperspectral wavelengths (410–993 nm) were chosen by variable selection methods, a procedure that increased PA (Galán et al. 2020). Even higher values were achieved by combining the hyperspectral information with the routinely assessed plant height. An additional increase could be achieved by combining hyperspectral and genomic data with plant height. The high information value of plant height is due to a positive correlation between plant height and biomass ($r = 0.57$, $P < 0.001$). For most parameters, the late flight had higher PA values. Other applications for high-throughput phenotyping are the identification of drought-tolerant genotypes by growing them on sandy soils under rainfed conditions or the quantification of disease severities by either hyperspectral or thermal imaging (Mahlein et al. 2012).

2.4.3 Gene Discovery

In practice, breeding populations do not contain the full genetic diversity needed to fast-forward genetic gain or to supply specific traits of interest, which are mandatory to enter new market areas. To compensate for these shortcomings, the breeder aims to make genes or major QTL available that are explaining a major part of the genotypic variance of the respective trait. Germplasm collections from gene banks or indigenous populations adapted to unfavorable climatic or edaphic conditions, for example, can be valuable sources of genetic variation. A high

amount of time and resources are required to (1) phenotype accessions for the trait of interest, (2) identify the most relevant genes/QTL controlling the trait and their mode of inheritance and finally (3) transfer them into adapted germplasm. Current technologies help to increase the chance to mine new and valuable QTL by narrowing the number of accessions to be analyzed in-depth down to the most promising ones, for example by combining genomic (Yu et al. 2016) or passport data (Crossa et al. 2017) with environmental data, which indicate the desired tolerance to biotic or abiotic stresses. Current experimental approaches targeting the identification of QTL and candidate genes in crops can be divided into natural or experimental populations (Cavanagh et al. 2008).

Gene discovery in rye is a specifically difficult task due to self-incompatibility, low agronomic performance, low inbreeding tolerance and the unknown heterotic group of genetic resources (Haussmann et al. 2004). Frost tolerance of high yielding European breeding populations is, for example, insufficient for the Canadian and Russian cropping areas. In a paradigmatic and successful case study, new alleles for frost tolerance (Erath et al. 2017) could be explored in a biparental QTL mapping population from a cross between a European inbred line and a gamete from the Canadian cultivar Puma. Phenotypic variance has been estimated for several traits recorded in field experiments and controlled test environments at −20 and −23 °C. A QTL at the Frost resistance locus 2 *(Fr-R2)* on chromosome 5R, explained a high proportion of the phenotypic variance in recombinant inbred lines and

Table 2.3 Prediction ability for biomass yield assessed by hyperspectral information collected on an early (after flowering) and late (yellow ripening) drone flight and by plant height for 274 three-way hybrids across 4 locations and 2 years. Data from Galán et al. (2019, 2020)

Parameter	Early flight	Late flight	Both flights
All vegetation indices (VI$_{all}$, $N = 20$)	0.35	0.42	0.42
Hyperspectral bands (HYP, $N = 32$ selected wavelengths)	0.54	0.52	0.59
VI$_{all}$ + Plant height (PH)	0.55	0.62	0.58
HYP + PH	0.55	0.50	0.62
HYP + PH + genomic data	0.72	0.75	0.75

their testcrosses. This QTL was mapped close to the well-known Vernalization-response (*Vrn-R1*) locus. Other consistent QTLs were found on chromosomes 4R and 7R. As a selection strategy, the results suggest a three-stage procedure with (1) MAS based on markers from the *Fr-R2* locus, (2) applying a genomic prediction model capturing also effects of smaller QTLs and (3) a final verification in a multi-environmental field and lab phenotyping platform. From an enabling technology point of view the study demonstrates some crucial lessons to be considered in further gene discovery studies: (1) Gaining insight into the architecture of frost tolerance in winter rye should make use of pre-existing knowledge (Pasquariello et al. 2014) from homoeologous groups in other related species, i.e., the *Triticeae*, (2) development of genomic tools in the target species itself (Bauer et al. 2017) is of utmost importance, (3) mapping populations tracing back to parents that are highly diverse for the trait of interest should be built up and last but not least, (4) it pays also for all subsequent breeding steps to invest in a solid phenotyping platform under controlled and field environments.

2.5 Enhancing Genetic Diversity

2.5.1 Broadening of Central European Pools

Continuous selection within heterotic pools is expected to reduce genetic diversity as pointed out by Duvick et al. (2004). Due to selection on GCA to the opposite pool, the decrease in genetic diversity within pools will correspond with an increase in difference between them. In rye, genome-wide selection signals (Bauer et al. 2017) could be identified and the genetic differentiation between breeding pools revealed. Candidate genes identified to be under selection affect morphological traits such as plant height and traits connected with the hybrid system, such as restoration of male fertility.

Further, genetic drift also leads to a loss of genetic diversity that is hard to avoid when generating inbred lines by second cycle breeding,

because only a limited number of elite lines is used for intercrossing. Actually, as revealed from the long-term experiment in corn, investigating reciprocal recurrent selection over 18 cycles between the Iowa Stiff Stalk Synthetic (BSSS) and the Iowa Corn Borer Synthetic No1 (BSCB1) (Gerke et al. 2015), most of the observed reduction in genetic diversity could be attributed to genetic drift.

The established Central European heterotic pools trace back to the Petkus (Pool P) and Carsten pools (Pool C). Benefits from broadening the genetic diversity of these pools could potentially arise from; (1) enhancing genetic gain from selection, which is proportional to the genetic standard deviation and (2) from expanding the traditional Central European target environment to new markets, such as Eastern Europe, Canada or Asia, which require specific traits such as overwintering capability.

The potential of Eastern European populations to broaden established heterotic pools has been investigated (Wilde et al. 2006; Fischer et al. 2010) by exploiting both phenotypic and genomic data. In the latter study (Table 2.4) around 30 S_0-plants randomly sampled from the candidate populations had been cloned in each case, outcrossed to pool P and C and genotyped with 30 SSR markers evenly distributed on the seven rye chromosomes. In total, P and C were represented with 121 and 142 S_0-plants respectively. Testcrosses with P and C were grown in separate yield trials at 4 locations and 2 replications in Germany in 2007.

For grain yield, parameters, such as means, genetic variances and usefulness, were estimated. The usefulness criterion (Schnell 1983) with U (α) = mean + i h σ_G combines population mean and the expected genetic gain, which is proportional to the genetic standard deviation (σ_G) of a candidate population, the selection intensity (i) and the square root of the heritability (h). Thus, the parameter reflects the performance of the selected fraction from the candidate population. The lower selection intensity ($i \sim 1$) might consider that after discarding for non-yield traits only a limited number of S_0-plants are available for selection on grain yield. The higher selection

Table 2.4 Parameter estimates for grain yield (dt ha^{-1}) of testcrosses from S_0-clones sampled from 5 Eastern European populations (POP 1–5) and the Central European Pool P and C. Means, genetic variances (σ^2_G) and usefulness values (U, explanation see text) given for a low and a high selection intensity (i)

Parameter	Population						
	POP 1	POP 2	POP 3	POP 4	POP 5	Pool P	Pool C
	Tester: Pool P						
Mean	55.1	56.0	54.0	53.8	57.8		58.2
σ^2_G	3	7	9	21	9		3
U($i_{0.05=2.063}$)	58.1	60.8	59.7	62.5	63.8		62.6
U($i_{0.40=0.966}$)	56.5	58.3	56.6	57.9	60.6		60.3
	Tester: Pool C						
Mean	58.0	59.6	58.2	55.8	58.6	61.1	
σ^2_G	14	13	17	35	11	13	
U($i_{0.05}$ = $_{2.063}$)	64.9	66.6	66.0	67.4	64.8	70.8	
U($i_{0.40}$ = $_{0.966}$)	61.3	62.9	61.9	61.2	61.5	65.6	

intensity ($i \sim 2$) might be appropriate if the breeder can reduce the number of candidate populations beforehand, which will then allow more investment of capacity into the most promising populations. Within the testcross series with Pool P, S_0-plants sampled from Pool C showed the highest mean for grain yield as expected which, was closely followed by the mean of POP 5. Genetic variances were smallest for Pool C underlying the need for broadening this pool. Usefulness was highest for POP 5 benefitting from both an above average mean and genetic variance. Thus, this population can be regarded as the first choice to enhance genetic diversity in Pool C. Within the testcross series with C as a tester, the mean of P ranked first followed by POP 2. Except for POP 4, genetic variances were similar to those of P. Usefulness of the POP 1–5 did not surpass the Pool P value.

Gene diversity found in the Eastern European populations (Table 2.5) support the high genetic variances estimated from phenotyping. In addition, molecular analyzes suggested that there is "new" variance because a number of SSR alleles present in POP 1–5 were found to be absent in Pool P and C. As expected, the highest genetic distance between populations was found between Pool P and C. Conversely, this means

that including Eastern European populations into one of the pools will reduce diversity among pools and possibly also lead to a reduction of heterosis.

Phenotyping candidate populations and estimating the relevant parameters is cost and time consuming. Therefore, a multi-stage approach to select between candidates and to focus on the most promising individuals within selected populations is highly meaningful:

1. Populations can be screened by analyzes of genotypic data for criteria such as gene diversity and genetic distance to established pools and to identify other candidate populations with little cost.

2. An evaluation of population per se performance will provide valuable information on non-yield traits such as disease resistance, plant height, quality.

3. A sample of 30–50 S_0-plants or S_0-clones from the pre-selected populations should be outcrossed to the established heterotic pools for testcross seed production. Due to self-incompatibility, the gametic array of the S_0-plants cannot be maintained by producing S_1-lines but their genetic content can be conserved by crossing them to elite lines derived from the established pools.

Table 2.5 Parameter estimates from 30 genome-wide distributed SSR loci for gene diversity (Nei 1987) and Modified Rogers distances (MRD, Wright 1978)

Parameter	Population						
	POP1	Pop2	POP3	POP4	POP5	Pool P	Pool C
Gene diversity	0.56	0.55	0.56	0.56	0.55	0.53	0.43
Pool P	0.21	0.25	0.2	0.22	0.2		0.29
Pool C	0.28	0.29	0.26	0.25	0.27	0.29	

4. Testcrosses grown in multi-environmental trials provide estimates for most relevant parameters such as mean, genetic variance and usefulness.
5. Superior S_0-plants from finally selected populations may enter with their progeny from elite line crosses into a synthetic.
6. The synthetic has to undergo several cycles of recurrent selection by using a selection index based on phenotypic and genomic-estimated GCA effects.

Occasionally and quite rightly, genetic diversity in landraces or exotic populations is regarded as "gold reserve" (Böhm et al. 2017) for plant breeding. Converting the "gold" into productive "working capital" needs to close the performance gap between resources and elite material. This is a demanding task because not only performance for individual traits, but a minimum threshold for all relevant traits should be reached when used, for example, in intercrossing a genotype derived from exotic resources.

Apart from rare lucky punches and from the authors' experience, usually two to three breeding cycles must be completed before benefitting from exotic resources in commercial lines. In consequence and in order to prevent raising false or unrealistic expectations, exotic resources should be integrated into a breeding program as a mid- to long-term approach.

Breeding designs should carefully be analyzed to reduce loss of genetic diversity due to genetic drift. Deficiencies made in this field cannot be compensated by using exotic genetic resources which can be a costly and cumbersome process and can negatively affect the competitiveness of a breeding program.

2.5.2 Use of Non-adapted Material for Introgressing Single Genes

In contrast to the tedious work of broadening gene pools for quantitative traits, the introgression of monogenic traits into elite germplasm is more straightforward. An example is the improvement of leaf rust (*Puccinia recondita*) resistance. In the 1990s, all first-cycle inbred lines were highly susceptible to this important disease. Due to the introduction of Eastern European resistance sources, this has changed. Similarly, the introgression of non-adapted pollen-fertility genes (see Sect. 2.4.1) or stem rust (*Puccinia graminis* f.sp. *secalis*) resistance genes are future challenges. The necessary steps are: (1) detection of the desired trait expression in donor genotypes, (2) establishing segregating populations by backcross and selfing steps, (3) discovery of linked molecular markers by either linkage or association mapping, (4) introgression into elite pools by marker-assisted backcrossing. In rye, hundreds of self-incompatible plant genetic resources (PGR) are stored in gene banks. They comprise wild species, weedy rye populations, old European landraces and elite populations from different rye-growing countries. PGR is usually a rich source of monogenically inherited resistance (*R*) genes. Musa et al. (1984), for example, detected ten *R* genes for leaf-rust resistance in only six inbred lines. Further, four *R* genes were found in the Russian rye cultivars 'Sanim' (from 'Sangaste' × 'Immunaya1'), 'Immunaya 1' (origin *Secale strictum),* 'Chulpan', and 'Novozybkovskaya 4', respectively (Solodukhina, 1994; Kobylanski and Solodukhina, 1996). In

Germany, Wehling et al. (2003) and Roux et al. (2004) identified in total five dominant *R* genes for leaf-rust resistance, either derived from local inbred lines (*Pr1, Pr2*) or from rye genetic resources: 'Jaroslavna'/Russia (*Pr3*), 'Turkey'/Canada (*Pr4*), 'WSR'/Germany (*Pr5*). Molecular markers for these sources are available, so the target genes can be easily introgressed into elite material (Wehling et al. 2003; Roux et al. 2004). Similarly, for stem rust, resistance sources were found in Austrian landraces, Russian populations and the US forage rye (Miedaner et al. 2016). The proportions of fully resistant plants within the populations varied from 2 to 70%. Indeed, a gene for qualitative resistance was detected in two Russian populations on chromosome 7RL and a quantitative resistance with at least three QTL in another population (Gruner et al. 2020). Association mapping revealed additional *R* genes on chromosomes 1R, 2R and 6R in two Austrian landraces, 'Oberkärntner' and 'Tiroler'. Other candidates for MAS of monogenic traits are dwarfing genes, like the dominant *Ddw1* on chromosome 5R (Kalih et al. 2014), that was recently used in practical breeding of rye and triticale (X. *Triticosecale* Wittmack).

Depending on their origin, target genes might be linked with negative agronomic traits. An example is the pollen-fertility restorer genes on chromosome 4RL, derived from Iranian and Argentinean sources (Fig. 2.1). The *Rfp3* gene

significantly enhanced the restorer index by 80% on average, as expected, but also led to taller plants and decreased grain yield. The yield penalty ranged from 0.95 to 10.0 dt ha^{-1} among seven introgression lines and illustrates a potential for selecting lines with shorter introgression intervals with markers. Similar yield penalties were observed for *Rfp* genes from the primitive ryes 'IRAN III', 'IRAN IX' and the Argentinean landraces 'Pico Gentario' and 'Trenelense' (Wilde et al. 2017). In the latter study, the yield penalty ranged from 3.05 to 7.00 dt ha^{-1} depending on the marker haplotype.

2.5.3 Enhancing Quantitative FHB Resistance

Genetic diversity for quantitative traits is a key objective for high selection gain in breeding. Because of the well-known challenges for introgressing quantitative traits from exotic plant genetic resources (Haussmann et al. 2004), the breeders firstly search within their elite gene pools. When the observed trait level is too low, recurrent selection (RS) is a method for improving the population mean without decreasing genetic variance. In hybrid breeding, S_1 line selection is commonly used (Hallauer and Carena 2009). Resistance to Fusarium head blight (FHB) is an example of a quantitative trait

Fig. 2.1 Deviation of restorer index, plant height and grain yield for seven introgression lines with the pollen-fertility restorer gene *Rfp3* compared to near-isogenic lines without this gene. Data from Miedaner et al. (2017)
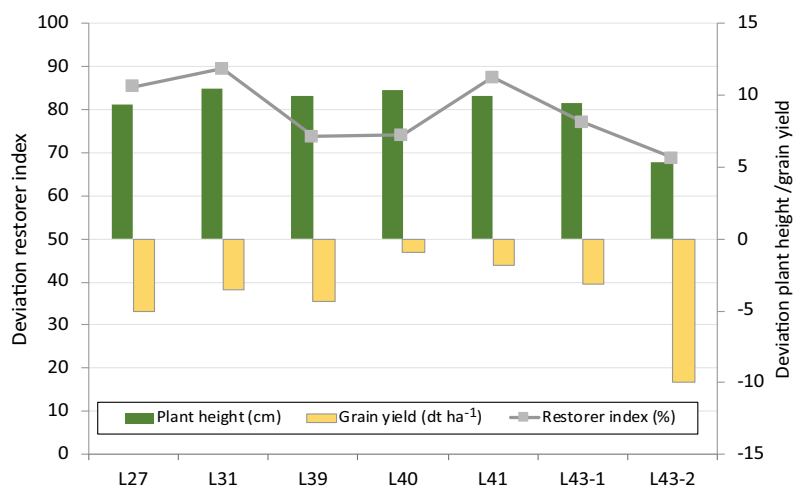
that is not easy to handle. FHB is caused by an array of *Fusarium* species, reducing grain yield and quality and contaminating the harvest with mycotoxins, of which deoxynivalenol (DON) is one of the most frequently found. For FHB resistance in rye, large genotypic variation in breeding populations has been reported. However, resistant genotypes are scarce (Miedaner et al. 2003b). Making the situation even more difficult, the correlation of line per se to testcross performance has been regarded to be low for this trait (Miedaner et al. 2003b). A re-evaluation of a RS program showed that indeed within the materials selected in previous cycles, the correlation was absent ($r = 0.09$), while after adding a sample of 111 unselected S1-L tested in the same experiment, the correlation changed to moderate ($r = 0.68$). The S_1 lines have been evaluated after the fifth cycle of an RS where each cycle selection was based on an index of line and testcross performance. The effect of recurrent selection can be tremendous (Fig. 2.2).
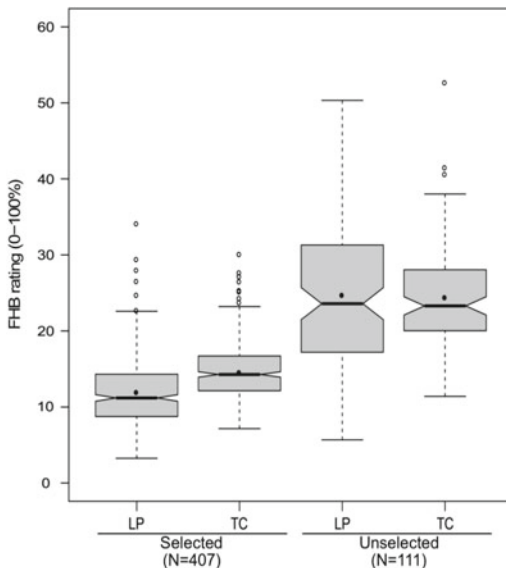
Fig. 2.2 Boxplots of Fusarium head blight (FHB) ratings (0–100%) of 407 S_1 lines from the fifth cycle of a recurrent selection program and 111 unselected S_1 lines for line performance (LP) and their corresponding testcross performance (TC) after inoculation by *Fusarium culmorum* in two locations. Data from Miedaner and Wilde (2019)

Low FHB infection generally leads to a lower DON content in grain (Miedaner et al. 2003a, b). High genotypic correlation coefficients between both traits (0.8–0.9) promote indirect selection for reduced DON content by assessing FHB resistance. Genotype × environment interaction played a major role for both traits (Miedaner et al. 2003b) illustrating the necessity of selecting in several environments (location × year combinations). Resistance QTL on rye chromosomes 1R, 4R, 5R and 7R were reported in a mapping study with four triticale populations (Kalih et al. 2015). The dominant rye-dwarfing gene *Ddw1* had a significantly negative impact on FHB resistance in triticale (Kalih et al. 2014). Additionally, it reduced plant height as expected and delayed heading.

In a recent genome-wide association study (GWAS), the lines from the RS program mentioned above were genotyped by a 15 k SNP assay (Gaikpa et al. 2020). Data were corrected for population structure by the genomic kinship matrix (K) and the first principal component. In total, 15 QTLs for FHB resistance on all rye chromosomes, except chromosome 7, were identified that jointly explained 74% of the genotypic variance. Among them, two major QTLs were detected on chromosomes 1R and 5R explaining 33% and 14% of the genotypic variance, respectively. Genome-wide prediction resulted in 44% higher cross-validated prediction abilities than marker-assisted selection revealing the quantitative nature of FHB resistance also in rye. Genomic approaches, thus, may accelerate breeding for complex traits.

2.6 Breeding Schemes

2.6.1 Recurrent Selection to Improve Base Populations

Recurrent selection (RS) is a cyclic process of selection and recombination targeted at improving genetically broad-based populations by (1) increasing the frequency of all favorable alleles and (2) maintaining genetic variability (Hallauer and Carena 2009). From heterozygous

genotypes identified as superior in the RS procedure, inbred progeny could be advanced to form parental lines for Product Development (PD). This integrated breeding approach was first formulated in the 1970s (Sprague and Eberhart 1977). It gained priority with the advent of genomic selection (GS, Gaynor et al. 2017; Rembe et al. 2019). A key feature of GS applications in RS and PD, i.e., commercial line development, is the chance to shorten cycle times (Fig. 2.3). Mass selection (fast and slow) in a population of heterozygous genotypes aims to improve GCA with the opposite pool by using genomic-estimated GCA effects (GEGCA) as a selection criterion. Adopting a simple genetic model, additive and GCA effects can be linearly converted into each other. By applying advanced glasshouse technology (Hickey et al. 2017) cycle length can be reduced from traditionally one year to six or even less months. As pointed out by Rembe et al. (2019), persistency of prediction accuracy over several cycles has been analyzed only based on computer simulations (Müller et al. 2017) and experimental proof-of-concept, in terms of a high realized long-term gain, has still to be provided.

An RS scheme based on genomic and phenotypic selection (PEGCA + GEGCA in Fig. 2.3) and well adapted to hybrid rye breeding (for details see Fig. 2.4) involves intercrossing of superior S1-genotypes selected in the previous selection cycle, dissecting the population into S1 candidate lines and assessing their GCA to the opposite heterotic pool. Phenotypically estimated GCA (PEGCA) effects of candidate lines are based on using CMS testers to produce testcross seed. Then testcrosses are grown in multi-environmental trials, which allow high accuracy assessment of grain dry matter yield (GDY) as the most important trait. At the same time, phenotypic data assessed from testcrosses, can be used regularly to update the genome-wide prediction model.

Specifically, genomic prediction of GCA effects can address different sources of genetic variabilities, such as the variance within crosses (V_{wc}) and within S_0-plants (V_{wSo}). This is less costly compared to the phenotypic approach or is
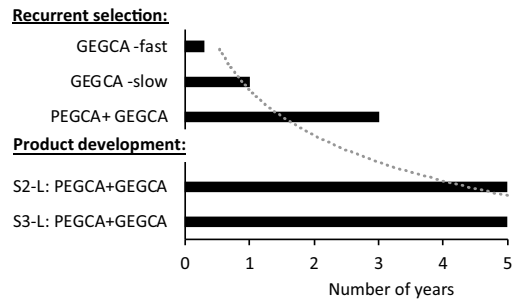
Fig. 2.3 Cycle length in Recurrent Selection and product development schemes; GE = *G*enomic *E*stimated, PE = *P*henotypic *E*stimated; GCA = *G*eneral *C*ombining *A*bility

even not feasible with the latter (V_{wSo}) in the given RS procedure. GS models are trained with historical data from the previous cycles. Well performing S1-SP and their derived S2-L identified in the RS scheme can directly be fueled into product development.

2.6.2 Product Development

The RS procedure presented above might be integrated into concrete breeding schemes for line development. In the scheme, purely based on phenotypic selection (PS, Fig. 2.5), we start with intercrossing S2-L, self the progeny in two subsequent generations down to receive S2-L seed. With S2-L, we can exploit both V_{bc} and V_{wc}, and also half of V_{wSo}, to select for line per se performance. The main selection pressure is exerted on traits such as resistance to lodging, plant height, heading date or disease resistances, inbreeding tolerance and grain quality characters, e.g., 1000-grain and test weight and falling number. Once the lines have overcome this first hurdle, candidate lines are outcrossed to CMS testers in year 4. In the same year, S2-SP are selfed to S3-L to speed up the further inbreeding process in case the respective S2-L is selected. In the following year 5, the testcross seed is used for phenotyping in multi-environmental trials. At least for so-called 'second cycle' lines, their phenotypically assessed GCA estimates (PEGCA) for GDY will be the main selection

Year	Generation	Technical procedure	Selection criterion	Exploitable additive variance (V_A)			Selection stage	Comments
				V_{bc}	V_{wc}	V_{wSo}		
1	1	< S1-SP × S1-SP' >	GEGCA $_{S1\text{-}SP\ within\ S1\text{-}L}$		0.50		3	Intercross GEGCA selected S1-SP and generate pair cross seed
	2	< S0-SP >	GEGCA $_{S0\text{-}SP}$	0.25			1	Self S0-SP, which have been GEGCA selected
2	3	[T-CMS × S1-L]						Produce testcross seed of selected S1-L by outcrossing them to CMS
3	4	T-CMS • S1-L	$I = b_g$ GEGCA + b_p PEGCA	0.75	0.25		2	Combine PEGCA (from testcross mean) and GEGCA (from parental S0-SP) into index I for final selection of S1-L to be intercrossed with their selected S1-SP

Legend:
S0-, S1-SP	S0 and S1 single plants, resp.
L, T-CMS	Line, cytolasmatic male sterile tester from the opposite breeding pool
GEGCA, PEGCA	Genomically or Phenotypically Estimated General Combining Ability effects, resp.
< > , [ ]	intercrossing by pair crosses, selfing by paper bags or testcross seed production in plastic walls, resp.
☐	phenotypic or genomic assessment of the selection criterion
V_{bc}, V_{wc}, V_{wSo}	Variance between and within crosses, within S0-plants, resp.
b_g, b_p	weights for GEGCA and PEGCA in an index

Fig. 2.4 Technical description of an RS scheme involving phenotypic (PEGCA) and genomic (GEGCA) selection of GCA (General Combining Ability) effects

criterion. Lines with a positive PEGCA will continue to a second selection stage. This second selection stage—not displayed in the schemes below—will usually be the last step before using candidates as parental lines for commercial hybrids. Synchronously, excellent S2-lines are intercrossed to build up an improved population for the next breeding cycle. The steps described so far in scheme PS follow a classical and well-known phenotypic selection procedure (Geiger and Miedaner 2009), thus being an obvious object of comparison for alternate breeding schemes.

Alternatively, combined genomic and phenotypic selection (COM S2-L, Fig. 2.5) could be designed and an additional selection stage implemented with selection on GEGCA for GDY after the line per se test with historical data from previous cycles. Thus, assuming a sufficiently high prediction accuracy, candidate lines with an inferior GEGCA are discarded. The breeding program will benefit insofar as the large effort in producing and phenotyping testcrosses can be reserved to the putatively better part of the candidate population. In year 5, PEGCA effects from phenotyping testcrosses and GEGCA effects from genomic prediction enter into a selection index combining both sources of information, which can be weighted, e.g., by a classical Smith-Hazel approach (Dekkers 2007) or by empirical weighting factors. The index should be more accurate compared to PEGCA alone for two main reasons: (1) The latter might be impeded by loss of testing environments caused by technical hazards or high genotype x environment interaction. (2) If model training comes from aggregating data across multiple breeding cycles, GEGCA can deliver highly valuable complementary information to PEGCA as it mitigates biases from genotype x year interaction. As described for PS, S2-L with a positive index value will continue to a second selection stage and will be intercrossed for setting up a new breeding population.

An alternative combined genomic and phenotypic selection (COM S3-L, Fig. 2.5) affords selfing of a number of S2-single plants to S3-L within all those S2-L tested for per se performance in year 3. After having identified the S2-L with the best GEGCA, the breeder selects the best S3-L via GEGCA within those S2-L. Thus, compared to the other two schemes, we can exploit a larger part of the segregation variance (V_{wSo}). Only those S_3-L excelling by a superior

Scheme	PS		COM S2-L		COM S3-L	
Year	Technical procedure	Selection criterion	Technical procedure	Selection criterion	Technical procedure	Selection criterion
1	$< $S2-L xS2-L' $>$		$< $S2-L xS2-L' $>$		$< $S3-L xS3-L' $>$	
	$< $So-SP $>$		$< $So-SP $>$		$< $So-SP $>$	
2	$< $S1-SP $>$		$< $S1-SP $>$		$< $S1-SP $>$	
3	$a1=No/N1$	L *per se* $_{S2-L}$	$a1=No/N1$	L *per se* $_{S2-L}$	$<$S2-SP: $a1=No/N1$	L *per se* $_{S2-L}$
			$a2= N2/N1$	GEGCA$_{S2-L}$	$a2= N2/N1$	GEGCA$_{S2-L}$
					$a3= N3/N2$	GEGCA $_{S2-SP: S2-L}$
4	$<$S2-SP$>$	[T x S2-L]	$<$S2-SP$>$	[T x S2-L]		[T x S3-L]
5	$a2= Nf/N1$ T-CMS x S2-L	PEGCA$_{S2-L}$	$a3= Nf/N2$ T-CMS x S2-L	I= b$_p$ PEGCA$_{S2-L}$ +b$_g$ GEGCA$_{S2-L}$	$a4= Nf/N3$ T-CMS x S3-L	I= b$_p$ PEGCA$_{S3-L}$ +b$_g$ GEGCA$_{S3-L}$
6	$< $S2-L xS2-L' $>$		$< $S2-L xS2-L' $>$		$< $S3-L xS3-L' $>$	T xS2-L

L *per se* $_{S2-L}$ = Line per se performance of S2-L

Fig. 2.5 Technical description of breeding schemes based on phenotypic selection (PS) and combined genomic and phenotypic selection in two generations (COM S2-L and COM S3-L) Nx = number of lines per stage x, Nf = final number of lines); other abbreviations see Fig. 2.4

GEGCA are outcrossed to testers. As previously described for COM S2-L, an index combining PEGCA and GEGCA from S3-L is used as a selection criterion. S3-L with a positive index value continues to a second selection stage and is intercrossed for setting up a new breeding population.

For comparing the efficiency of different breeding schemes, we estimate the annual gain from selection as the evaluation criterion. If model calculations are used as analytical tools restrictions, for e.g., the budget, effective population size should be incorporated to enable a fair and realistic comparison of alternative approaches and to allow for optimum calculations. In the following, GDY is taken as the only trait under selection, which is realistic, particularly for second cycle breeding populations that have already reached a good performance level for the other traits of interest. The respective variance components for GCA effects, their interactions with locations, years, years × locations, and single plot error are assumed to adopt a ratio of 1.0: 0.25: 0.25: 1.0: 2.0, regarded as typical for Central European conditions (taken from calculations of Laidig et al. 2017).

For all breeding schemes described above a fixed budget is assumed. Without presenting further details here, all activities necessary to process the breeding schemes above are assigned for glasshouse, nursery, genotyping, testcross seed production and phenotyping in yield plots, valued in money terms and summed up until the predefined budget maximum is reached. For line test, a selection rate of $\alpha = 0.1$ is predefined according to practical experience. This allows easy calculation of start-up costs per selected line, including costs for intercrossing, selfing and lines discarded in the per se test.

Further, technical risk prevention and high accuracy strategy (Wilde 1996) is assumed. When assessing PEGCA, all breeding schemes use a fixed number of testers ($T = 2$), locations ($P = 4$) and replications ($R = 2$). For most variance component ratios, these assumptions lead to lower gains from selection but also to lower variance in gains compared to other scenarios with lower testing but higher selection intensity. Assessing the GCA effect of individual candidate lines accurately produces some further positive side effects: (1) the false-positive rate of inferior candidates included into the second and final selection stage, intercrossing and other cost-driving activities can be reduced and (2) updating the genomic prediction model will benefit if an accurate phenotypic test is used for recalibration.

For all breeding schemes $N_f = 60$ has been fixed as the number of candidate lines entering into a final phenotypic evaluation. With this test, the putative best lines are identified as hybrid parents and hybrid performance can be predicted based on parental GCA effects.

For the calculation of genetic gains (Fig. 2.6), well-known approximations (Utz 1984) to obtain the selection intensity and the gain in multi-stage selection are applied. For selection indices combining information from phenotyping testcrosses (PEGCA) and from genomic prediction (GEGCA), weights were calculated by use of the Smith-Hazel index approach.

No detailed discussion on how to maximize genetic gain can be presented here and only selected topics are highlighted in the following. Instead of an optimum allocation of breeding resources, only an allocation fitting to the above-mentioned risk prevention strategy is considered. Under this restriction the effect of the GS selection rate (Fig. 2.6) on total genetic gain is investigated for a range of prediction accuracies $(0.1 \leq r_{GEGCA, GCA} < 0.5)$ which seems to be realistic according to empirical results. As can be derived from the graphs, $r_{GEGCA. GCA}$ should exceed a minimum threshold value of around 0.3

to make COM S2-L and COM S3-L becoming more attractive than PS. Further, a moderate GS selection rate of around 0.4–0.5 is advisable to reduce the risk of achieving a genetic gain lower than that of PS. Comparing COM S2-L and COM S3-L, the latter benefits from a larger proportion of segregation variance and thus can achieve the highest genetic gains.

Assuming a GS selection rate of 0.4, the allocation of breeding resources for the three breeding schemes is displayed in Fig. 2.7. Compared to PS, budget demands for glasshouse, nursery and genotyping increase substantially from about 30% to 54% (COM S2-L) and 66% (COM S3-L), respectively. Correspondingly, expenses for testcross seed production and yield plots decline from 62% (PS) to 38% and 26% for the combined schemes, respectively. Numbers of entries to be processed at subsequent selection stages, as shown in Table 2.6, reflect the budget demands for each selection stage. These figures highlight the necessary re-organization of a breeding program when integrating GS as a tool.

Summarizing the results of the model calculation, implementation of GS into the breeding process is highly attractive in terms of a higher
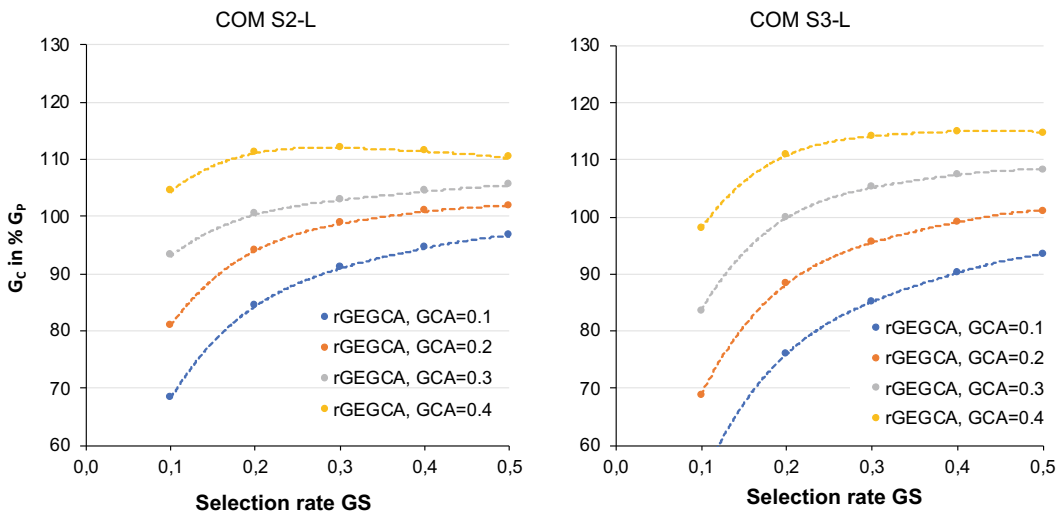
Fig. 2.6 Total gain relative to gain from phenotypic selection (G_C in % G_P) from the combined genomic and phenotypic selection schemes COM S2-L and COM S3-L for varying prediction accuracies of GCA ($r_{GEGCA, GCA}$) and varying selection rates from genomic selection (GS)

expected genetic gain. This finding is in line with the results of Marulanda et al. (2016) which are based on somewhat different assumptions. By model calculations, pitfalls and zones of risks can be identified. For example, taking care of high and stable prediction accuracy and an adapted selection intensity for all GS steps involved appears to be of high relevance.

2.7 Preconditions for Inscription of a Variety

Developing a new rye cultivar is cost- and time-intensive and particularly challenging because several traits have to be combined and most show complex inheritance. Before the registration of a variety to their respective National lists, official authorities test varieties for (1) Value for Cultivation and Use (VCU) and (2) Distinctness, Uniformity and Stability (DUS). Further, a suitable variety denomination is needed to start the registration process.

Taking Germany as an example for other EU member states: "the legal basis of national listing is the Seed Act (SaatG). It serves to protect consumers and ensure that agricultural and horticultural industries are provided with high-quality seed and plant material derived from healthy, high-quality and productive varieties" (Bundessortenamt 2019).

The German Federal Plant Variety Office ("Bundessortenamt") considers a variety to have VCU "if its qualities taken as a whole offer a clear improvement for cultivation, for use of the harvest or use of products derived from the harvest compared to comparable listed varieties" (Bundessortenamt 2019).

In Germany, VCU testing for rye requires a three-year test period with 10 to 20 locations per year. VCU testing in rye is usually carried out with two cultivation variants, i.e., an extensive variant without any use of fungicides and growth regulators and a more intensive variant mirroring the actual common practice of farmers with full use of growth regulators and fungicides whenever needed.

DUS tests are embedded into a system providing protection with an intellectual property right for new plant varieties at the National or European Community levels. Plant breeders' rights serve the plant breeding industry and breeding advancements. Anyone who breeds or discovers a new rye variety can apply for national plant breeders' rights at the Federal Plant Variety Office under the Plant Breeders' Rights Act (SortG). Plant breeders' rights can be obtained if a plant variety is new, distinct, uniform, stable and designated with a suitable denomination.

The definition of DUS criteria in the variety definition process is traditionally based on the

Fig. 2.7 Allocation of breeding resources to phenotypic (PS) and combined (COM) genomic and phenotypic selection in two generations (COM S2-L and COM S3-L); TC = testcross
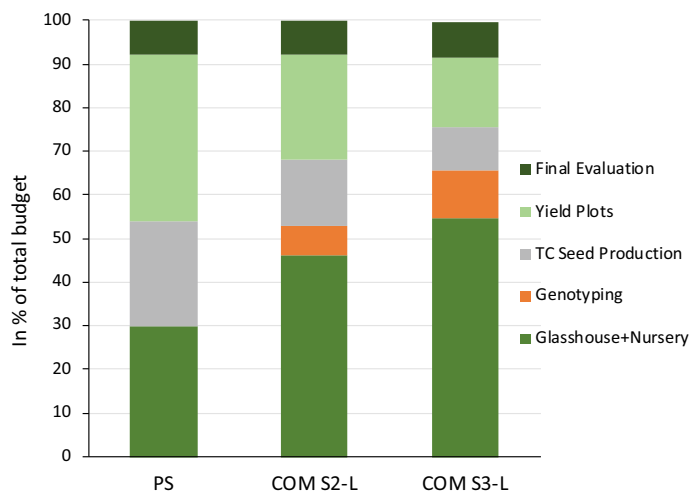
Table 2.6 Number of entries (N_0-N_f) processed in the breeding schemes at subsequent selection stages; N_f = final number

Number of entries	Breeding scheme		
	PS	COM S2-L	COM S3-L
$N0$	7090	10,960	7520
$N1$	**709**	1096	752
$N2$	–	**439**	902[a]
$N3$	–	–	**301**
N_f	**60**	**60**	**60**

[a]Assuming 3 S3-L/selected S2-L: N1 $\times$ 0.4 $\times$ 3 = 902
Bold = Number of entries phenotyped by testcrosses

analysis of phenotypic data in standardized field trials. In agreement with a general guideline, a rye-specific procedure has been defined in document TG/58/6 of the UPOV (https://www.upov.int/tgp/de/). This guideline applies to open-pollinated varieties, but also to hybrids and their parental components. Based on characteristics such as ploidy level (2n *versus* 4n) and seasonal type (winter *versus* spring), varieties are subdivided into major groups. Then, each of the groups' 20 characteristics is described in consecutive phenological states known to be optimal for expression. These characteristics reflect the variety-specific morphology and physiology of plant organs such as grain, stem, leaf, or ear. For each trait, the method of assessment is defined either by actual measurements (M), by visual assessments based on a single observation of a group of plants or parts of plants (VG), or visual assessments from observations of a number of individual plants or plant parts (VS). The statistical analysis of the data assessed in the field trials is detailed in a Technical Working Party paper (TWC/18/10) resulting in the so-called combined-over years distinctness (COYD) and uniformity criteria (COYU), (https://www.upov.int/tgp/de/).

An actual research project investigates the use of DNA markers for DUS testing in rye (Schmid, pers. commun.). Application of DNA marker technology might accelerate the DUS procedure and thus could lead to advantages for plant breeding companies, Plant Variety Protection granting authorities and the agricultural sector. The potential use of markers in DUS testing is based on the premise that the difference between varieties, the uniformity within varieties and the stability of varieties can be determined with appropriate measures of marker diversity and, therefore, complements currently used phenotypic traits used in DUS testing.

Only if both VCU and DUS tests are successfully passed, breeding companies are allowed to sell certified seed. When buying certified seeds, farmers pay royalties to breeders, which allows them to invest in further research activities and to develop new and superior varieties. Thus, the whole value chain including farmers, food and feed producers and finally consumers benefit from better varieties.

2.8 Breeding Goals

In practical breeding programs, the assessment of candidates always involves consideration of multiple traits under selection. A breeding goal could then be formalized by an index incorporating all traits of relevance. For several decades there is a well-developed theory available to construct such indices (Sölkner et al. 2008). For example, in the optimal Smith-Hazel selection index the weight given to a specific trait can be derived from estimating its respective economic value, heritability and genetic correlations between traits (Falconer and Mackay 1996).

Because often the latter parameters are difficult to estimate with sufficient accuracy, plant breeders or variety offices use multi-trait indices, which are constructed in a more simple and intuitive way and nevertheless aim to reflect the overall breeding goal. The trait performance of a

candidate line is then expressed as the difference to well-known standard entries or to the trial mean. These differences are then weighted and summed up as partial indices such as resistance or quality indices or as overall varietal indices. A candidate hybrid will be discarded by the breeder or the inscription will be refused by the variety office if a predefined threshold value is not achieved.

Taking Germany as an example for the Central European target environment, a generic overview on traits relevant for rye breeding (Table 2.7) is provided by the so-called German "descriptive variety list" (BSL 2019). The traits can be grouped into those associated with phenology, biotic and abiotic stress, yield, or quality. The high number of traits comes from the highly versatile use of rye for human food, animal feed stuff and different industrial uses (bioethanol, biogas). A more detailed description of the traits and their heritabilities can be found in Miedaner and Laidig (2019). Compared to other cereals, such as wheat and barley, rye has been regarded as feed stuff of lower value in the past. Based on new nutritional findings, rye may be experiencing a renaissance as a high value feed stuff for fattening pigs. Although crude fiber content is low, rye excels by high contents of fructans and arabinoxylans. These are metabolized in the colon to butyrate, which has positive effects on mucosal membrane health and animal welfare. Further, boar taint and infection by salmonella can be prevented. Phosphorous content in rye is low, but the grains own high phytase activity, which helps the pig to better utilize phosphate and reduce phosphate excretion (Kamphues et al. 2019).

Obviously, global climate change will also have an impact on re-defining actual and future breeding targets. Taking Central Europe and the years following 2000 as an example, there was massive drought stress in rye-growing areas in 2003, 2007, 2011, 2018 and 2019. Because drought stress occurs episodically and thus is unpredictable, farmers might adopt risk prevention strategies and replace more drought prone cereals such as wheat with the more stress tolerant rye (Schittenhelm et al. 2014). Clearly, this strategy only provides a benefit if rye hybrids show superior performance under drought but also under normal or even optimal conditions. To ensure broad adaptability of germplasm, managed drought stress environments should be included in test environments (Haffke et al. 2015).

Over the last two decades, the traditional market areas for hybrid rye in Central Europe could be expanded to other regions of the world including Russia and Canada (Fig. 2.8). With the diversification of the target areas, the breeder has to consider new breeding objectives such as frost tolerance or snow mold resistance (Miedaner and Wilde 2019). Further, genotype × environment interactions and specifically cross-over interactions have to be considered as a challenge which makes it difficult or even impossible to predict the performance of candidate genotypes across changing environments.

Inevitably, there is an impact on the correlated genetic gain for the individual traits when an index mirroring a complex breeding goal is taken as a selection criterion. The higher the number of traits and the higher their economic weights and the more they are correlated in an undesired direction, the lower will be the gain for a given trait. Thus, the breeder faces a dilemma arising from this multi-trait situation. Briefly, some ways to escape from this dilemma can be sketched out.

1. Rye breeders can apply cost-efficient multistage selection procedures as outlined in Sect. 2.6 (this chapter). Because the genetic correlation of line per se performance of a candidate to its GCA effect is high for many traits, they can be easily selected at the beginning of line development.
2. High-throughput technologies such as GS or HYP are available, allowing for testing of a large number of candidate lines.
3. The breeder could establish satellite programs besides the main program with regard to increasing the diversity of target environments. QTL for traits that are mandatory for specific environments ("must-have QTL") can then be introgressed.
4. Finally, a breeding program could be restricted to just one segment × environment-

Table 2.7 Traits for different usages of rye for human food, animal feed stuff, and industrial purposes

| Type of trait	Grain			Whole-plant mass	
	Baking	Feed	Ethanol		
Early		Late			
Phenology-associated traits:					
Ear emergence	+	+	+	++	+
Ripening	+	+	+	0	+
Plant height	**0**	**0**	**0**	+	++
Susceptibility to stress-associated traits					
Winter killing	−−	−−	−−	−−	−−
Lodging	−−	−−	−−	0	−−
Culm buckling	**0**	**0**	**0**	**0**	**0**
Powdery Mildew	−−	−−	−−	−−	−−
Rhynchosporium	−	−	−	−	−
Leaf rust	−−	−−	−−	0	0
Ergot	−−	−−	−−	0	0
Yield-associated traits					
Ear density	+	+	+	+	+
No. of grains per ear	+	+	+	0	0
Thousand-grain weight	++	++	++	0	0
Grain yield (extensive)	++	++	++	0	0
Grain yield (intensive)	++	++	++	0	0
Dry matter yield	**0**	**0**	**0**	++	++
Quality-associated traits					
Falling number	++	+	+	0	0
Protein content	+	++	−−	0	0
Amylogram viscosity	++	−−	−−	0	0

++, +, 0, −, −− = Very high, high, neutral, negative, and very negative importance for the trait

combination or a few of them rather than diluting the capacity on all of them.

2.9   Breeding Progress

In Germany, hybrid and population varieties have been tested in the same VCU trials since the beginning of the 1980s and recently the respective data have been analyzed for the period 1985–2016 (Miedaner and Laidig 2019). Further, from the annual special harvest survey (Besondere Ernteermittlung, BEE 2017) estimates for the average national-wide on-farm performance are available. VCU trials offer an ideal tool to dissect the genetic and non-genetic part of the overall progress made over time. Separately for each type of variety, populations and hybrids, the genetic trend can be formalized as a regression coefficient of genetic effects of new candidate lines on years of testing (Laidig et al. 2017). Analogously, the non-genetic trend can be estimated for the innovation coming from agronomy. For grain yield and related characters (Table 2.8), a high increase at the trait level from 1985 to 2016 can be observed. Whereas at the beginning hybrids out-yielded populations by
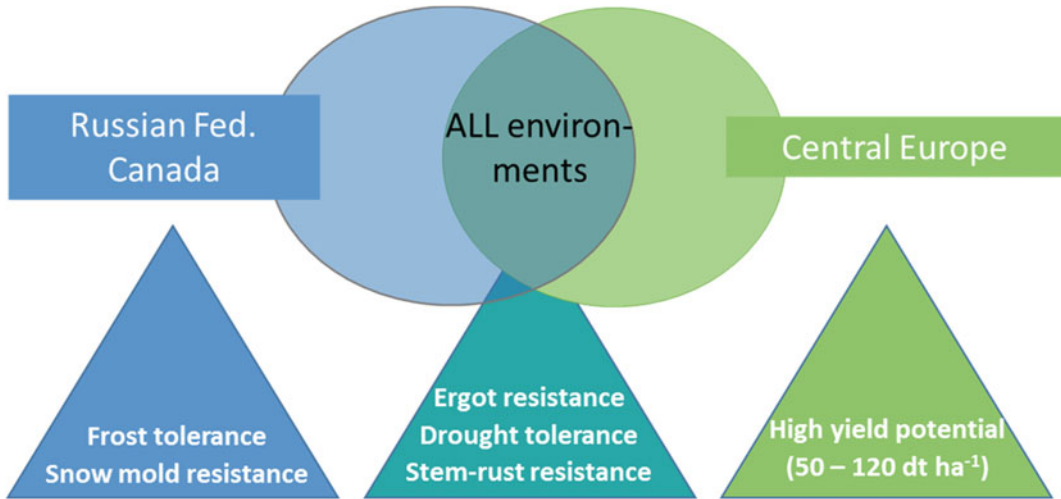
Fig. 2.8 Specific breeding goals for hybrid rye cultivars in different regions of the world

about 10% in 1985, their superiority increased to about 20% in 2016. The gap observed between types of varieties is confirmed by corresponding annual genetic trends differing by a factor of about 3 for hybrids [0.773 dt (ha year)$^{-1}$] and populations [0.237 dt (ha year)$^{-1}$]. Assuming a simple genetic model and *ceteris paribus* conditions, annual genetic progress should be similar for both types of variety (Sprague and Eberhart 1977). Breeding budget will be one of the factors probably contributing most to the actual observed large difference between them. In the case of hybrids, royalties paid by farmers each year in a foreseeable amount are re-invested into large research and development budgets benefitting from economy of scale effects and availability of modern technology. In contrast, in case of population cultivars, lower royalties and wide use of farm-saved seed substantially restrict financial returns to breeding companies. Compared to the genetic trends, the non-genetic annual trends are often lower reflecting the fact that effects from innovation in agronomy can be exploited only to a limited extent in VCU trials.

A comparison of grain yield achieved in the year 2016 on VCU (100.7 and 84.1 dt ha^{-1} for hybrids and populations, resp.) and on-farm level (55.7 dt ha^{-1}) reveals a considerable performance gap (Table 2.8). There is little impact

from such performance gap under the assumption that the ranking of cultivars in the VCU and the on-farm environment is identical. However, as pointed out by Falconer and Mackay (1996, p. 322), a character measured in two different environments should not be regarded as one character, but as two. Potentially, genes required for high yield level in VCU trials represent a set of genes different from those required for the more stress-prone on-farm environment and thus the genetic correlation between the two traits might be lower than 1. Lowering the yield level in VCU trials might appear to be an obvious solution. However, trials on low-yielding or stress-prone environments often are impeded by higher error variances. Thus, there might be a trade-off between increasing the genetic correlation between the selection (VCU trial) and the target (on-farm) environment on one side and the accuracy of yield assessment on the other side.

A large difference is also found for the overall trends between VCU trials [0.866 and 0.551 dt (ha $\times$ year)$^{-1}$ for hybrids and populations, respectively] and on-farm [0.383 dt (ha $\times$ year)$^{-1}$]. Probably, a major part of this difference is due to the fact that prices for rye substantially decreased due to agro-political decisions made in the period of review which lead to shifting rye cultivation from better to less fertile soils. When

Table 2.8 Trait levels and regression coefficients of yield-related traits. Percent trends (%) given rel. to 1985 (Miedaner and Laidig 2019)

Trait	Unit	Source	Type	Trait level		Linear annual trends						r_g to
				1985	2016	Genetic		Non-genetic		Overall/on-farm		Grain
						abs[a]	%	abs	%	abs	%	Yield[b]
Grain yield	dt ha^{-1}	VCU trial	HYB	73.9	100.7	**0.773**	1.05	0.112	0.15	**0.866**	1.17	
			POP	67.0	84.1	**0.237**	0.35	**0.242**	0.36	**0.551**	0.82	
			HYB%	110.3	119.7							
		on-Farm		43.8	55.7					**0.383**	0.87	
Ear density	ears m^{-2}	VCU trial	HYB	475.9	593.1	**2.494**	0.52	1.510	0.32	**3.783**	0.80	0.55
			POP	487.9	539.8	**0.871**	0.18	0.793	0.16	1.675	0.34	
			HYB%	97.5	109.9							
Single-ear yield	g ear^{-1}	VCU trial	HYB	1.64	1.79	**0.007**	0.40	−0.002	−0.12	0.005	0.31	
			POP	1.46	1.64	**0.002**	0.17	0.002	0.15	**0.006**	0.41	—
			HYB%	112.3	109.1							
Number of kernels per ear	kernels ear^{-1}	VCU trial	HYB	51.7	49.0	0.046	0.10	0.002	−0.26	0.038	−0.17	−0.28
			POP	45.7	43.4	0.037	0.22	−0.067	−0.5	0.033	−0.17	
			HYB%	113.1	112.9							
Thousand-grain mass	g	VCU trial	HYB	34.1	37.2	**0.130**	0.30	−0.041	−0.03	0.090	0.29	−0.22
			POP	34.2	38.3	0.018	−0.09	**0.111**	0.43	**0.109**	0.38	
			HYB%	99.7	97.1							

[a]Regression coefficients, **bold letters**: significant at 5% level or higher; HYB = hybrid cultivar, POP = population cultivar, HYB%: hybrid relative to population performance
[b]Genetic correlations according to Laidig et al. (2017) (1989–2014)

farmers had to decide between genetic (population *vs.* hybrids) or agronomic (extensive *vs.* intensive use of fungicides, fertilizers and growth regulators) intensification, they often chose the first alternative as can be seen from a market share of hybrids that is now at more than 80% in Germany (Miedaner and Laidig 2019).

Driving forces for yield progress can be identified when analyzing yield components. Significant annual genetic trends can be found particularly for ear density, but also for single-ear yield. Whereas at the beginning of the reviewed period, ear density in hybrids was slightly lower (97.5%) compared to population varieties, it was substantially higher at the end (109.1%). This finding is fully in line with trends summarized in a pivotal paper on corn breeding (Duvick 2005). According to the author "newer hybrids yield more than older hybrids because of continuing improvement in ability of the hybrids to withstand the stress of higher plant density, which in turn is owed to their greater tolerance to locally important abiotic and biotic stresses." Obviously, actual rye hybrids are able to keep a higher number of tillers alive during pre-flowering reduction phases and they can maintain a sufficient sink in the later grain filling phase. The number of kernels per ear was already high in hybrid cultivars compared to population cultivars at the beginning (51.7 vs. 45.7) and did not improve further.

2.10 Conclusions and Future Developments

Rye breeding, as with breeding programs for many species, managed in the last decade to integrate molecular markers into commercial breeding schemes and to step forward to genomics-based breeding (Miedaner et al. 2019). For monogenic traits, like pollen-fertility

restoration or rust resistances, KASP markers can be easily applied in large populations for the selection of the favorite plants at the seedling stage. The advent of medium-density SNP chips opened the way for assigning unknown material to heterotic groups and for developing genomic selection procedures that are now a must-have in each breeding program (see Sect. 2.6). These molecular techniques will make it easier to develop specific rye materials for new target areas and new traits.

For cross-pollinating rye, hybrid breeding allowed the systematic development of cultivars with specific performance traits, like biomass yield and disease resistance. This will foster the possible expansion of rye production to new target areas, like Russia, Canada, or the USA. While in Russia, bread and ethanol production is the main goal, in North America, stakeholders must be convinced that rye is an excellent foodstuff for livestock, because the use for bread making in these areas is limited. Another main advantage of rye, especially in areas with poor soils and regular drought stress, is the high stress-resilience of the crop. This will become even more important with the restriction of nitrogen use in industrial countries. In Denmark, for example, the rye acreage grew by 3.5 times since 2009 when the "Green Growth Agreement" released nitrogen application restrictions (https://www.statbank.dk/AFG07). Global climate change is predicted to result in less predictable weather and more episodic extreme weather events which will also contribute to the necessity for abiotic stress tolerance as a major breeding target in the future. Rye provides an excellent starting base. For this purpose, phenotyping in controlled environments is of key relevance. Techniques such as the use of robotics or automatic and remote sensing of plant stands will allow more reproducible results than classical field experiments in terms of abiotic stress. Key genomic regions identified by these techniques can be directly incorporated into elite germplasm. Further, the search for more effective alleles in the same genomic region will be facilitated and gene bank accessions (Varshney et al. 2018) can be effectively exploited. In any

case, breeders should be aware that results from advanced phenotyping are indirect assessments (Fischer et al. 2014, Chap. 9). In consequence, their correlation to field performance under stress has to be verified.

Although the relative contribution of phenotyping will be reduced by the implementation of genomic selection (see Fig. 2.7), it will become even more important to produce high-quality phenotypic data. Breeders will need these data for manifold purposes including updating calibrations, identifying top performing commercial candidates, assessing their stability for yield and disease resistance across multiple locations. Organizing high-quality phenotypic data will be a challenge because advancement in this field is much slower compared to the ability to genotype large arrays of germplasm in the laboratory.

For the sake of commercial plant breeding as well as for the well-being of food production for a growing population, a steady increase of grain yield will be pivotal. This requires continuing investment into breeding programs and breeding research, but also a targeted broadening of gene pools (see Sect. 2.5 of this chapter). Because the hybrid breeder cannot benefit from breeding programs of competitors in the same effective way as the breeder of self-pollinating crops, it will get more and more important to widen the genetic variation by effective reciprocal recurrent selection programs supported by genomics and by the exploitation of new genetic materials. The main driver for effective genomic selection will be the prediction accuracy that is routinely achieved in commercial programs; this parameter will greatly benefit from the use of multiple cycles of training populations (Auinger et al. 2016). However, the use of new genetic materials will decrease the degree of relatedness between training and breeding populations. Therefore, the recalibration of genomic selection models by phenotyping training populations adjusted to the actual breeding progress will be a continuing task for the breeder.

Breeding rye as an internationally underutilized crop faces a particular challenge when it comes to training students with skills in quantitative genetics, plant molecular biology

and classical breeding. To efficiently apply the new genomic tools, they should be given the opportunity to study in a multi-disciplinary and interactive learning environment.

The economic effects of plant breeding research investments are found to be highly profitable from a societal point of view (Witzke et al. 2004) and at the same time beneficial for reducing CO_2 emissions (Lotze-Campen et al. 2015).

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