

Scotland's Rural College

## Pre-breeding Strategies

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# Chapter 25

## Pre-breeding Strategies



Sivakumar Sukumaran, Greg Rebetzke, Ian Mackay, Alison R. Bentley, and Matthew P. Reynolds

**Abstract** In general terms, pre-breeding links needed traits to new varieties and encompasses activities from discovery research, exploration of gene banks, phenomics, genomics and breeding. How does pre-breeding given its importance differ from varietal-based breeding? Why is pre-breeding important? Pre-breeding identifies trait or trait combinations to help boost yield, protect it from biotic or abiotic stress, and enhance nutritional or quality characteristics of grain. Sources of new traits/alleles are typically found in germplasm banks, and include the following categories of ‘exotic’ material: obsolete varieties, landraces, products of interspecific hybridization within the Triticeae such as chromosome translocation lines, primary synthetic genotypes and their derivatives, and related species mainly from the primary or secondary gene pools (Genus: *Triticum* and *Aegilops*). Genetic and/or phenotyping tools are used to incorporate novel alleles/traits into elite varieties. While pre-breeding is mainly associated with use of exotics, unconventional crosses or selection methodologies aimed to accumulate novel combinations of alleles or traits into good genetic backgrounds may also be considered pre-breeding. In the current chapter, we focus on pre-breeding involving research-based screening of genetic resources, strategic crossing to combine complementary traits/alleles and progeny selection using phenomic and genomic selection, aiming to bring new functional diversity into use for development of elite cultivars.

**Keywords** Simple and complex traits · Genetic diversity · Physiological breeding · Proof of concept · Genetic gains

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## 25.1 Learning Objectives

- Understand the rationale, objectives, approaches and tools used in wheat pre-breeding.

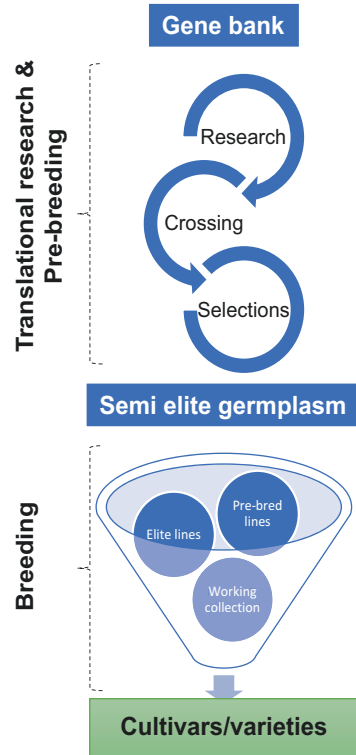
## 25.2 Introduction

Why is there a focus on pre-breeding? Plant breeders typically prefer to cross among elite lines [1] (see Chap. 7), except when specific and otherwise unavailable traits are needed such as disease resistance, this being the main route for introducing genetic diversity in conventional breeding. However, such repetitive use of elite breeding lines may limit the ability of new cultivars to adapt to emerging threats such as harsher climates and an ever-evolving spectrum of biotic threats. The use of well characterized primary synthetic hexaploids and landraces is a relatively straightforward way to widen genetic diversity and represents a key objective of pre-breeding. If tetraploid and hexaploid genomes lack genetic variation for biotic and abiotic stress tolerances, wild species can be used in interspecific hybridization (wide crossing) to add specific new diversity (see Chap. 18). Hence pre-breeding ensures continuity of supply for novel and diverse genetic variability in readily useful backgrounds that can enter breeding pipelines [2–4] and help broaden the wheat gene pool generally. Physiological pre-breeding can be practiced by crossing with novel sources of traits as well as among elite material in order to deterministically stack complementary physiological traits to raise yield potential and adaptation to abiotic stress [5]. In general, the activities that precedes the development of a variety and initial reshuffling of genes by a breeder is termed ‘pre-breeding’ (Fig. 25.1).

## 25.3 Definitions

- Gene bank & Genetic resources: More details can be found in Chap. 17.
- Traits: Any physiological, morphological, biochemical, or genetic character of a plant including resistance/susceptibility to biotic stresses that can be used to differentiate two genotypes is called a trait.
- Simple traits and complex traits: Simple traits are often categorical, determined by few genes and are simple to phenotype and genotype. Complex traits are usually quantitative and determined by many genes with small effects. The heritability estimates of complex traits are commonly lower than the simple traits due to the many possibilities for interaction with genetic background, growth stage and environment [6].
- *In silico*: An experiment performed by computer or by computer simulations.

**Fig. 25.1** A general sequence of events from gene bank to varieties



## 25.4 Aspects of Practical Pre-breeding

### 25.4.1 Access to Genetic Resources Through Gene Banks

The first step in pre-breeding is access to gene bank material (collection and conservation of germplasm; see Chap. 17) relevant to the breeding of the cultivars. Genetic resources broadly include modern cultivars in current use, obsolete cultivars, landraces, wild relatives, and genetic and cytogenetic stocks, breeding lines, and synthetic wheats etc. CIMMYT’s wheat gene bank contains over 150,000 samples of wheat, the single largest collection of germplasm for any crop consisting of wild relatives, landraces, synthetics, cultivars, semi-elite lines, and mapping populations. More details can be found in Chap. 17.

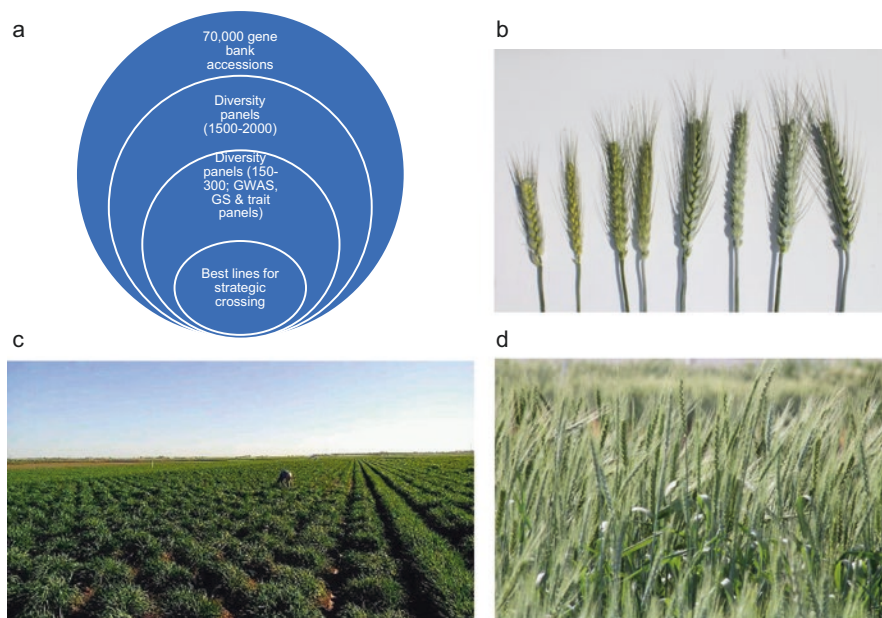
### 25.4.2 Screening Genetic Resources

In practice, it is not feasible to screen 150,000 collections from a gene bank at a single time in the field or greenhouse for all traits of interest due to logistical limitations. The best approach when working with large numbers of accessions is to first

phenotype for simple agronomic traits in the field while applying high throughput phenotyping via remote or proximal sensing to expand the range of traits that can be measured [7]. For example, traits such as plant height, phenology, lodging and other agronomic traits, together with grain yield, are the most important economically, and may be screened visually or at high throughput using proxies derived from spectral reflectance indices (SRI). SRIs are also used for a range of physiological parameters such as hydration status, photosynthetic pigments, in season biomass, canopy temperature, stay-green etc. For details of screening using SRIs please (see Chap. 27). Once this is done, smaller panels of lines (typically 150 to 300 entries) are made to phenotype and genotype in detail and determine marker trait associations/QTL. When selecting candidate parents for strategic crossing, major gene markers – such as for *Ppd*, *Vrn*, *Rht* and those for kernel weight – can provide key supplementary information to guide targeting and help avoid excessive segregation among progeny for height and maturity class. Genomic selection models have also been proposed in the context of parental selection [8] (Fig. 25.2).

### 25.4.3 Trait and Marker Discovery in Germplasm Panels

Germplasm panels need to be constructed in such a way that they have sufficient statistical power to be used to identify genetic markers associated with target traits as well as heritable phenotypes. Screening to characterize traits of interest may



**Fig. 25.2** Process of utilizing the gene bank accessions (a) define a number of entries from the gene bank as initial set and reduce it to a number where detailed phenotyping can be done (b) examples of trait diversity present in the genebank (eg. spike length and size) (c) snapshot of phenotyping initiation to booting by growing 2000 accessions in the field at Sonora, Mexico, and (d) primary synthetic hexaploid panel formed by crossing durum wheat with *Aegilopsis*

include evaluation in environments to assess yield potential and response to biotic and abiotic stresses. The traits (Fig. 25.3), which may be genetically simple or complex in nature, need to be studied using various phenotypic screening approaches e.g., visual selection, high throughput phenotyping, and novel methods for screening. A detailed review of methods based on physiological parameters can be found elsewhere [9]. For details and strategies to breed specifically for diseases, drought stress, and nutrition, please refer to Chaps. 9, 10 and 11, respectively.

Gene discovery is based on use of populations constructed with the purpose of mapping traits into genomic regions of the wheat chromosomes. Basically, two main methods are followed: (1) genome-wide association mapping; and (2) QTL (Quantitative trait loci) mapping [10]. Genome-wide association mapping exploits linkage disequilibrium to provide high resolution and fast mapping. If the population is large enough, heritability high, and the trait architecture simple, this method can pinpoint the gene of interest. QTL mapping or linkage mapping is complementary to GWAS, where lines contrasting in a character of interest are used to generate a mapping population. In its simplest form, this requires the pre-characterization of donor material in order to identify contrasting parental lines and then generation time to develop RILs from biparental or back-cross populations. This approach can complement GWAS (Genome wide association study), allowing independent validation of the effect of an identified marker [11, 12]. Once the markers are identified in a diversity panel or a RIL population those need to be validated for further use. Please refer to Chap. 28 to learn about the validation and MAS (Marker-assisted selection) approaches [13].

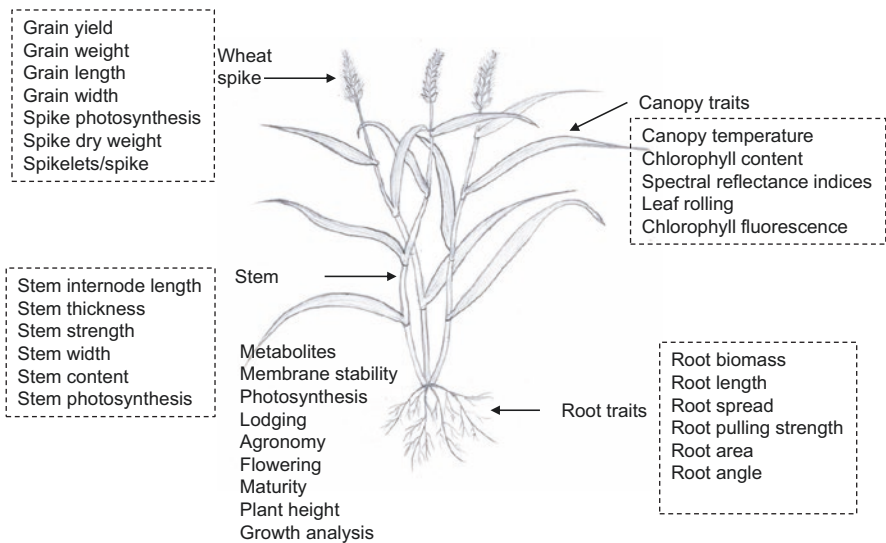


Fig. 25.3 A list of general traits in wheat used for pre-breeding

### 25.4.4 Trait Value and Prioritization. Which Traits and Why?

Phenotypic and genetic screening using markers in germplasm panels under relevant environments result in the identification of lines with high value traits/alleles, and can suggest trait combinations to boost yield and climate resilience. This step also identifies the heritability estimates of the trait, the genetic complexity of the trait, and if it is a simple or complex trait (Table 25.1). The traits fall within a continuum of simple to complex in nature, reflecting gene action from major effect to minor effect and therefore high heritability to low heritability. Even though heritability is assumed to be a genetic background and environment dependent parameter, some of the traits generally have high heritability, compared to others which are determined by multiple genes with minor effects. Large  $G \times E$ , also makes it important to match traits with environments for breeding. Hence there is ‘no one trait fits all environment’ rule in pre-breeding. The main criteria for use of a trait in crossing and selection is its association with yield or other key performance trait -like biomass, kernel size or root capacity- and how easy it is to screen during generation advancement directly or using proxies like SRIs.

**Table 25.1** Traits can be grouped based on number of genes involved, trait heritability, and the selection methods-phenotypic and genetic- that can be used for pre-breeding

Trait grouping	
<b>Simple traits</b>	<b>Complex traits</b>
Major genes	Multiple genes with small effects
Traits with high heritability	Traits with relatively low heritability
Categorical traits	Quantitative traits
Easily measurable	Time consuming
Low $G \times E$	High $G \times E$
Eg. Flowering time, plant height, grain weight	Eg. Photosynthesis, grain yield, radiation use efficiency, water use efficiency
<b>High heritability</b>	<b>Low heritability</b>
Plant height, flowering time, maturity; grain size and color, spike size	Grain yield, most physiological traits, metabolites, spectral reflectance indices that are influenced by environmental fluxes
<b>Methods used</b>	
Marker assisted selections/marker assisted backcrossing	Trait-based selections, genomic selection
Fine mapping and cloning of genes possible	Cloning of genes not possible
Single plants can be measured	Need to measure multiple plants in the field

#### 25.4.4.1 Trait Integration

Incorporation of yield boosting, yield protecting or nutritional/quality trait(s) into elite backgrounds is the key goal of pre-breeding and to deliver proof of concepts of their value in appropriate target environments through trialing. Here breeding methods differ for simple vs. complex traits and if there is availability of molecular markers.

#### 25.4.4.2 Pre-breeding for Simple Traits

Trait- and marker-based incorporation of simple traits is possible if based on the availability of robust linked markers. Simple traits or major gene-based traits are relatively easier to incorporate since their selection in subsequent generations can be done phenotypically or through marker assisted selection approaches. For example, reduced plant height, controlled by the famous green revolution alleles *Rht-B1b* and *Rht-D1b* is relatively easy to select visually. In addition, the identification of molecular markers with technologies such as *Kompetitive allele specific PCR* (KASP) and gene cloning is possible with relatively fewer generations and less time compared to complex traits. Another example of a trait that is easy to screen and incorporate is developmental traits. Although multiple genes related to flowering time have been identified in wheat, the different genes determining spring to winter growth habit remains a major screen in every pre-breeding activity. Other examples include genes for traits such as vernalization (*Vrn*), photoperiod (*Ppd*), plant height (*Rht*), *earliness per se* (*Eps*), thousand grain weight (*TaGW2*) and rust genes for leaf rust (*Lr*), stripe rust (*Sr*), and yellow rust (*Yr*) (For details on rusts refer to Chap. 8). Marker-assisted selection and marker-assisted backcrossing are normally used in selection for simple traits.

#### 25.4.4.3 Pre-breeding for Complex Traits

(a) Phenomic approaches:

Strategic crossing, in which parents are selected to complement each other for ‘source’ and ‘sink’ related traits, is a successful pre-breeding strategy which has shown significant genetic gains in spring wheat for yield potential, heat and drought stressed target environments [14, 15]. In general, source refers to traits that are directly or indirectly associated with carbon assimilation (e.g. canopy architecture, radiation use efficiency, roots, above ground biomass, etc.). The sink is represented by grain number and potential size as well as the traits that enable yield formation such as spike architecture/fertility and traits showing negative trade-off with final spike dry weight such as specific internode growth [16]. Some traits and process may serve both source and sink roles such as spikes which also photosynthesize and sinks of labile carbohydrate -stored mainly in stems-, that are remobilized as sources of assimilate for grain filling



especially under stress. The phenotype based approach to crossing occurs necessarily in the absence of sufficient genetic understanding of complex traits and how their alleles may interact. However, crossing among lines with complementary traits -backed by previous research- can stack the odds of accumulating favorable alleles in progeny selected for yield and complementary secondary traits.

In this scheme, progenies from F<sub>2</sub> to F<sub>6</sub> undergo a modified bulk method of selection (Fig. 25.4) employing selection for integrative traits like canopy temperature and NDVI (Normalized Difference Vegetation Index) on whole families for example, since the genetic value of individual plants cannot be measured accurately for complex traits. The resulting progenies represent well characterized, semi-elite lines that generally encompass alleles from diverse or exotic backgrounds such as synthetics, landraces and other genetic resources in a useful genetic background. These semi-elite materials can be used as parents by breeders aiming to achieve specific adaptation to their environments as mentioned in Chap. 3 [17, 18].

(b) Genomics based pre-breeding

The genetics of complex traits is not straight-forward to study through GWAS and QTL mapping. As trait complexity increases, the potential for G × E also increases. A typical QTL identified for a trait may be 15–20 cM in size, which may contain 1000s of genes, which need to be narrowed down through fine mapping for efficient use. If fine mapped, they still may not explain a high proportion of the phenotypic variance, so such QTLs and marker-trait associations (MTAs) need to be further refined and validated before applied in pre-breeding.

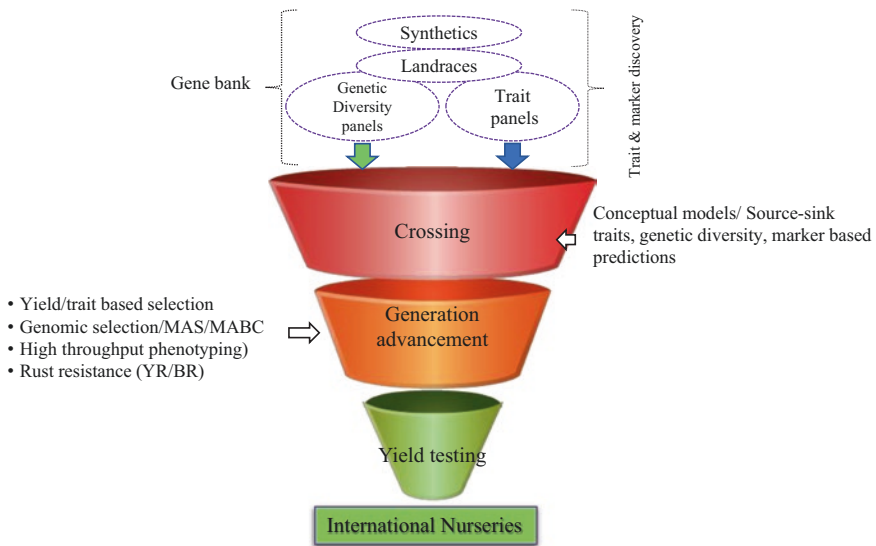


Fig. 25.4 Overview of the current IWYP and HeDWIC pre-breeding pipelines at CIMMYT

A more useful molecular breeding approach for complex traits like yield is genomic selection and prediction-based pre-breeding. Here genomic selection methods can be used for predicting parents, predicting the outcome from crosses, predicting the performance of progeny generations, and in selecting individual plants. However, these approaches are not routine in pre-breeding programs and are at the simulation or development stage. For example, rapid generation advance through speed-breeding combined with genomic selection may be advantageous for some traits [19].

The current scheme in CIMMYT uses four different approaches as shown in Fig. 25.5. One approach constitutes a fungicide (disease-free) pipeline where all generations are grown with fungicide to avoid the loss of high value alleles linked with rust susceptible backgrounds; this is most typical when neither parent is a modern, disease resistant line. Another stream is for simple traits, where MAS and MABC (Marker-assisted backcrossing) is used to incorporate genes/alleles. The third approach is based on speed-breeding where a rapid bulk-based approach is used to advance generations. In the fourth approach, lines in each generation are screened for rust (yellow rust and brown rust based on the shuttle breeding process of Dr. Normal Borlaug) to incorporate rust resistance into high value, semi-elite lines intended for breeders in countries where rust is an issue. The final products are distributed to public and private breeding programs globally through CIMMYT’s International Wheat Improvement Network (IWIN) for yield testing. The better performing lines are used to cross, to reselect individual plants and incorporate locally important traits or disease resistance genes into their elite cultivars.

The collaborators in different countries share data back to IWYP and HeDWIC translational research and pre-breeding hubs, which is further used to select parents for breeding or pre-breeding or as semi-elite trait sources (<http://orderseed.cimmyt.org/>).

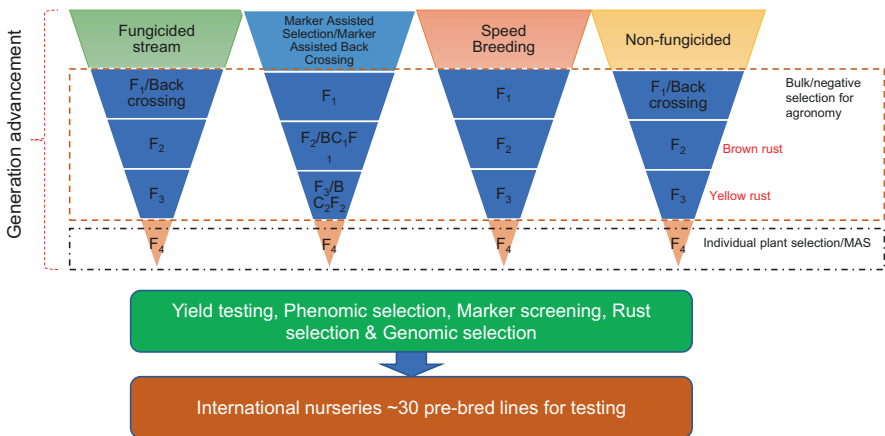


Fig. 25.5 Methods of pre-breeding practiced at CIMMYT

## 25.5 Proof of Concept *-in-silico* Approaches: Simulations

The cost and time required to run a pre-breeding program means that optimization and testing approaches may be best studied first by computer simulations and quantitative genetics theory. However, the process for major genes is well established. Backcrossing with selection on phenotype is effective if heterozygotes can be distinguished from the recurrent parent homozygous class. Selection on genome-wide markers can speed the process by reducing linkage drag and increasing the rate of recovery of the elite background: termed “background selection”. For recessive major genes this is not possible without a slow process of progeny tested after each cross to the recurrent parent. Markers tagging the QTL are therefore required for “foreground selection”. In the absence of a perfect marker for the trait, pairs of markers flanking the targeted QTL are desirable. Software to optimize the backcrossing the introgression of a major gene, including foreground and background selection has long been available (e.g. Popmin [20]) and more general-purpose software for genetic simulation (e.g. GeneDrop, AlphaSimR [21, 22]) can also be easily adapted to test alternative backcrossing strategies through gene-dropping approaches.

For quantitative traits, and if there are no tagged QTL to be introgressed, the situation is more complex and computer simulation is desirable to validate suitable strategies. If the desired phenotype or trait level is missing in the elite pool, then a cross between an elite and an exotic line can be followed by selection, either on phenotype, or through genomic selection, in the segregating generations. However, the trait to be introgressed will be in negative LD with the favorable traits already carried by the elite parent. Selection in an  $F_2$  population or among  $F_2$  derived lines may increase the frequency of favorable alleles carried by the resultant pre-breeding population, but the loss of the adapted background from the elite parent may be considerable. Equivalently, on making one or more backcrosses to the elite parent prior to selection, there is a strong risk that most favorable alleles in the exotic donor will be lost.

A further complication arises in instances where pre-breeding is intended to introduce novel variation for existing polygenic traits. In a cross, it is inevitable that most favorable alleles will be carried by the elite parent, but there may be novel variation in the exotic source, potentially at low frequency in an ancestral population but lost during domestication. In this case, there is a strong likelihood that favorable alleles carried by the exotic parent will be lost again, during selection. The chance of loss will be worse if selection takes place in generations derived from the backcross to the elite parent. This will occur whether selection is directly on phenotype or using genomic selection. The only way to unequivocally know that new variation has been introduced is to observe significant transgressive segregation over the elite parent and this is unlikely if the trait difference between the elite and exotic parents is large, as is usually the case. To overcome this “performance gap” other strategies have been tested in simulation. Gorjanc et al. [23] suggested establishing bespoke pre-breeding populations composed exclusively of exotic founders

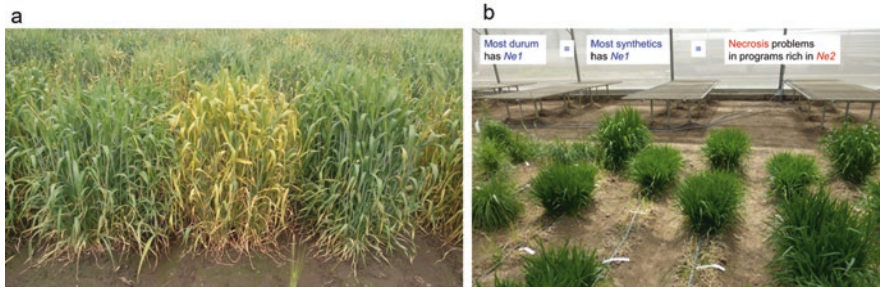
and then improving these rapidly through genomic selection, prior to introgression to elite lines through backcrossing with selection in the usual manner. Simulations showed this was more effective than the standard approach of direct backcrossing of elite to exotic. However, there is a chance that this approach does not capture novel variation but merely reintroduces alleles at a low frequency in the exotic pool which are already at a high frequency or fixed in the elite pool. It is unknown if this is a problem in practice. Simulating a process of crop domestication, breeding, then pre-breeding to recover lost variation could indicate its likelihood but has not been reported as far as we are aware. Such simulations should be possible with, for example AlphaSimR [22] which incorporates the coalescent based simulator into the package to generate founder haplotypes and already includes a models for wheat and maize domestication.

To circumvent the problem of the performance gap Yang et al. [24] tested the very simple approach of partitioning the genomic prediction equation into a component for markers for which the favorable allele was carried by the elite parent and one for which the favorable allele was carried by the exotic parent. Since most genomic prediction methods provide regression coefficients for every marker in the model, this amounts to partitioning regression coefficients into those with a positive sign and those with a negative sign, provided alleles carried by the two parents are coded consistently across markers (e.g. alleles from the elite parent are coded as 1 and those from the exotic as 0). In simulations they found, as expected, that genomic (or phenotypic) selection ignoring this partition would result in selection of a predominantly elite background and novel variation from the exotic would be lost. Partitioning the genomic prediction equation into two parts allowed a controlled approach to the introgression process, without excessive loss of novel exotic variation. They also tested this approach in barley and maize NAMs and found it effective.

Similar approaches have been developed by Allier [25] and tested in simulation: whereby the proportion of genome from the donor source is treated as a second trait. Simultaneous selection on two traits, the target trait for introgression and the proportion of donor genome, can then be used to ensure that the donor genome is not entirely lost, though there is no guarantee that the donor genome that is maintained in the selected lines is favorable. In practice, selection would be on an index of the two traits.

## 25.6 Pre-breeding Challenges

The primary challenges in practical pre-breeding are the identification of subsets of donor material which are likely to harbor novel and useful genetic variation for breeding and the scale of activities required to advance and assess material carrying diversity from pre-selected 'exotic' material. This creates complexity in delivering final products for uptake that meet core breeding objectives and can be smoothly integrated into established pipelines. Linkage drag is a major challenge when working with wild relatives and occurs between a high value allele of a primary trait



**Fig. 25.6** Two main opportunities and challenges while synthetics are used for pre-breeding (a) new lines resistant to yellow rust and (b) necrosis of the new synthetics  $\times$  elite crosses

associated with a high value allele of a secondary trait, for example a yield potential trait may increase lodging susceptibility. Yellow rust is one of the most devastating wheat diseases in the world and some pre-breeding wheat lines are susceptible to it. Cross incompatibility is another issue; when a new primary synthetic is crossed with an elite line it may not germinate or die after a few days (Fig. 25.6). Primary synthetic wheat especially performs differently, its spikelets are difficult to thresh and in some cases, shattering is an issue. Another issue associated with pre-breeding is the time required to develop the elite lines from the semi-elite material. It may require another full breeding cycle to come up with elite lines. The most critical step and challenge in pre-breeding is to know the genes identified in the genetic material are really novel and are not already present in the elite cultivars.

## 25.7 Technologies that Can Assist or Speed-Up Pre-breeding

Most of the new technologies mentioned in this volume (see Chaps. 27, 28, 29, 30, 31 and 32) will help to accelerate or increase precision of pre-breeding. Some are mentioned below.

### (a) Trait screening methods

Some of the traits that are important to increase the yield potential of wheat are too complex to screen using normal visual selection [26]. This may need complicated equipment and a long processing time e.g. above ground biomass, root traits, harvest index. Development of genetic markers, prediction models, and genomic selection approaches can assist in pre-breeding of these traits. Please see Chap. 32 to learn about selection indices and their use in pre-breeding and breeding.

### (b) Genetic markers

Genetic markers are highly useful for marker assisted selection and marker assisted back crossing for simple traits where the markers explain a large amount of variation. Some the traits that are routinely used and genes discov-

ered are flowering time and plant height related genes. However genetic studies on RUE (radiation-use efficiency) and BM (biomass) also help to understand the genetic structure of the trait, its heritability estimate and complexity [16, 27, 28].

(c) Gene editing

This may work well for simple traits where the causative variants are known. It can be used to create new variation to test or to create a desired variant [29]. For complex traits this may still not work since edits to the causative genes may have only minor effects. Please see Chap. 29 for more details.

(d) Speed breeding

Rapid generation advancement can contribute to two different areas of pre-breeding (1) to develop RILs or BC populations to study the genetics of the traits (2) to advance the pre-breeding populations through bulking [30]. Genomic selection and prediction models together with rapid generation advance may be helpful once the training and testing populations are defined and well substituted when needed [31]. Refer to Chap. 30 for more details.

(e) Reference genomes

Reference genome helps to identify and cross check the novel alleles [32]. It also helps in comparison of different marker systems based on physical positions. In addition, it also assists with the prediction of candidate genes for further studies, cloning and studying haplotypes [33]. (See Chap. 28)

(f) Gene cloning

Even though gene cloning is not necessary for pre-breeding, having a cloned gene helps to fix them in elite cultivars and to identify the novel genes [34].

## 25.8 Linking Pre-breeding with Agronomy to Exploit $G \times M$ Synergies

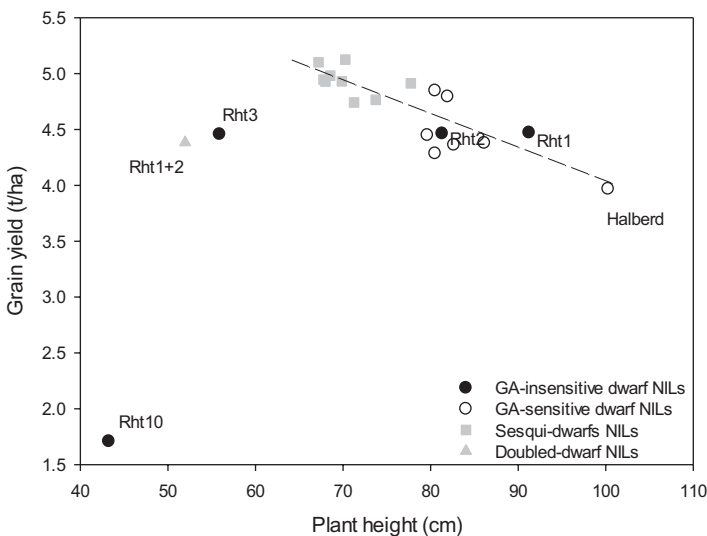
Together with improved crop agronomy and management, pre-breeding has potential to deliver traits and understanding in exploiting opportunities in genotype  $\times$  management interaction. Breeders carefully consider the target environment and farming system when selecting as adaptation and commercial success relies on varieties that perform reliably and at reduced cost to increase grower profitability. Among the most common considerations in modified management are changes in sowing date, reduced tillage including stubble retention, reduced herbicide-use through increased crop competitiveness, disease and insect resistance, and increased nutrient-use efficiency [27]. Opportunities exist in identifying traits that will support wider improvements in farm adaptation.

The gene pools typical of successful commercial breeding programs are fine-tuned (or ‘co-adapted’) for specific packages of alleles likely to deliver new varieties with improved performance across a wide range of disease, development, quality, and other key adaptation parameters. Key to the delivery of new traits/alleles is a

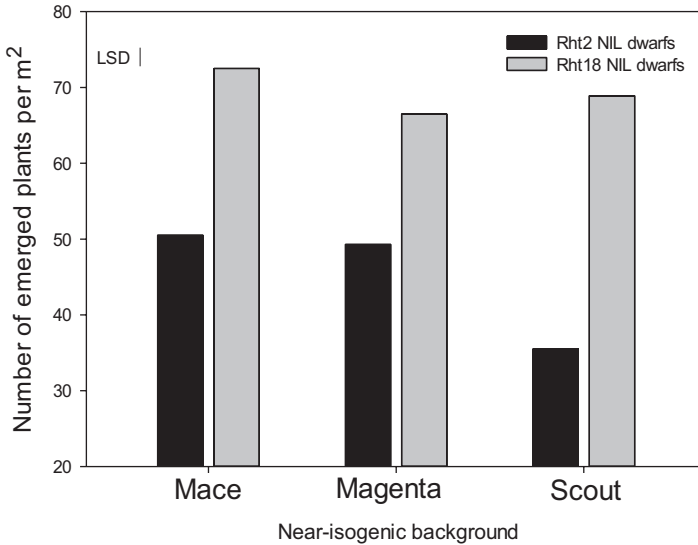
greater understanding and access to a wider gene pool as described for pre-breeding in this chapter. For example, the green revolution has delivered improved grain yields through deployment of height-reducing *Rht-B1b* (syn. *Rht1*) and *Rht-D1b* (syn. *Rht2*) gibberellic acid (GA) insensitive dwarfing genes. However, while these genes reduce plant height, they also reduce seedling growth and particularly coleoptile length. Other GA-sensitive dwarfing genes have been identified that also reduce plant height to increase grain yield (Fig. 25.7). One dwarfing gene, *Rht18*, increases coleoptile length an average of 50% to increase field plant establishment 50 to 90% with deep sowing (Fig. 25.8). Genetic increases in coleoptile length will improve crop establishment with deep sowing to reach deep soil moisture, stubble retention and warmer soil temperatures.

Importantly, pre-breeding through improved physiological understanding of crop growth has permitted the identification and deployment of new dwarfing genes now being used in commercial breeding programs worldwide. Another example of physiological understanding is in the breeding of the polygenic early vigor trait important in drought tolerance and weed competitiveness.

Early vigor, defined as more rapid leaf area development following seedling emergence, is associated with wider leaves and greater biomass early in the season. As much as 60% of rainfall is evaporated from the soil representing a substantial loss in water needed for growth. Barley has greater early vigor to reduce soil evaporation loss and increase crop water-use efficiency. Barley is also more competitive with weeds owing to its shading of weeds early in the season. Wheat is very



**Fig. 25.7** Relationship of plant height and grain yield for gibberellic acid (GA) -insensitive and -sensitive single and doubled dwarfing gene near-isolines (NILs), and original tall parent Halberd at the Yanco Managed Environment Facility in 2018 (Line of best fit is  $Y = 7.061 - 0.031 \cdot X$ ,  $r^2 = 0.74$ ,  $P < 0.01$ ). (Reprinted with permission from [35])



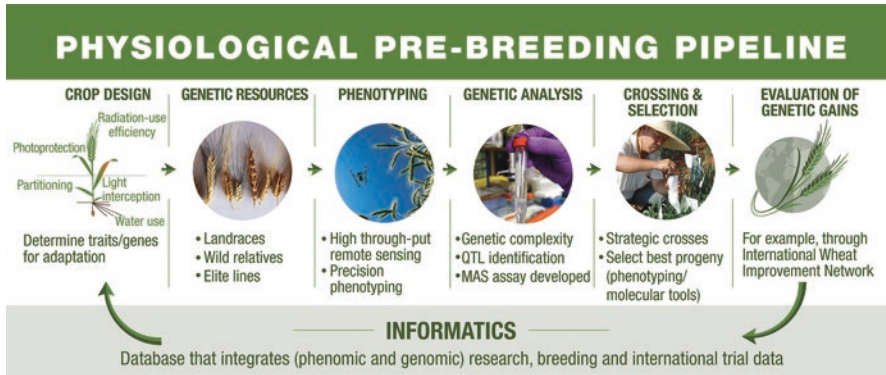
**Fig. 25.8** Average numbers of emerged seedlings (per m<sup>2</sup>) for backcross three-derived *Rht2* and *Rht18* near-isogenic lines in Mace, Magenta and Scout genetic backgrounds when sown at 12 cm sowing depth at Merredin, Western Australia in 2018 [27]

conservative in its early growth, yet a large global screen of international wheats identified a set of genetically-unrelated landraces used in intermating in the development of a structured high vigor, recurrent selection population. Selection over six cycles produced progeny with 40–50% greater seedling leaf area than original parents and vigor equivalent to barley. Resulting high vigor progeny have been used as parents in the development of populations for selection of improved weed competitiveness, higher water-use efficiency and improved nutrient uptake [36].

## 25.9 Key Concepts

For a non-crop scientist, the distinction between pre-breeding and breeding may not be obvious, but while pre-breeding involves many of the same steps as breeding (in order to deliver adequate proof of concept) it focuses more on the identification of specific trait sources and achieving new trait combinations, as well as their selection where feasible in early progeny generations, to deliver well characterized germplasm for use as novel parents in breeding. The germplasm contains new sources of traits or alleles -and therefore increased genetic diversity- to underpin the requirement of future cultivars while broadening the wheat gene pool.





**Fig. 25.9** A general scheme of physiological pre-breeding pipeline. (Modified with permission from [5])

## 25.10 Conclusions

A newly emerging approach in pre-breeding is based on conceptual models for grain yield under yield potential (Chap. 21), heat stress (Chap. 22), and drought stress (Chap. 23). This approach divides grain yield into sub-component traits i.e., plant biomass (source) and harvest index (sink). These traits are further dissected into several sub-component traits [14]. This approach (Fig. 25.9) has gained more acceptance recently due to the challenge of identifying reliable markers for complex traits and with rapid advances in field based phenomics and genetic gains have been achieved using strategic crossing in this way.

In conclusion, pre-breeding is an essential activity in plant breeding to bring new traits and genetic diversity into elite germplasm. Many breeding programs perform this activity and a clear distinction between the breeding and pre-breeding may not exist. The key step in pre-breeding is to successfully incorporate novel genetic variation into the elite cultivar without linkage drag or disturbing the equilibrium of the genes in the elite genotype. Pre-breeding, especially if it involves discovery and translational research and possibly wide crossing with wild relatives, as well as strategic crossing and progeny selection, can be a long-term process but is necessary to exploit the full biological potential of crops.

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