General Considerations in Physiological Breeding

INTRODUCTION

Application of Physiology in Wheat Breeding

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How can disciplinary research in physiology complement wheat breeding? This introductory chapter is intended to provide broad guidelines to help breeding programs: 1) assess whether physiological criteria should be included in a breeding strategy; 2) evaluate specific physiological selection traits and determine their usefulness in breeding. The other chapters in this book provide more explicit information on how physiological approaches can be used in breeding work for a variety of environmental conditions.

Physiological criteria are commonly though not explicitly used in breeding programs. A good example is selection for reduced height, which improves lodging resistance, partitioning of total biomass to grain yield, and responsiveness to management. Another is differential sensitivity to photoperiod and vernalizing cold, which permit adaptation of varieties to a wide range of latitudes, as well as to winter- and spring-sown habitats. Despite a lack of detailed understanding of how photoperiod and vernalization sensitivity interact with each other and the environment, the relatively simple inheritance of photoperiod (*Ppd*) and vernalization (*Vrn*) sensitivity genes and their obvious phenotypic expression (i.e. earliness versus lateness) has permitted them to be modified in many breeding programs. The same is true for the height reduction (*Rht*) gene. In the future an increased understanding of the genetic basis of these traits may enable breeding programs to exploit them further.

Selection for reduced height and improved adaptation to environment has had a profound impact on modern plant breeding, and the improvement in yield potential of spring wheat since the Green Revolution has been shown to be associated with a number of other physiological factors (Reynolds et al., 1999). Nonetheless, most breeding programs do not put much emphasis on selecting physiological traits *per se* (Rajaram and van Ginkel, 1996). Exceptions would include: 1) the stay-green character, which has been selected for in relation to improved disease resistance and is associated with high chlorophyll content and photosynthetic rate in Veery wheats, for example Seri-82 (Fischer et al., 1998), and 2) more erect leaf angle, a common trait in many high yielding bread and durum wheat plant types that was introgressed into the CIMMYT germplasm pool in the early 1970s (Fischer, 1996).

A recent survey of plant breeders and physiologists addressed the question of how physiological approaches in plant breeding could have greater impact (Jackson et al., 1996). According to the survey, while the impacts of physiological research on breeding programs have been limited in the past, future impacts may arise through:

- Focusing physiological work on an appropriate range of germplasm (which will depend on the specific breeding objectives);
- Working with larger populations to enable extrapolation of findings to breeding methods;
- Identifying traits for use as indirect selection criteria, in addition to those already used in core breeding programs;
- Identifying traits for use as selection criteria in introgression programs;
- Conducting selection trials in more representative environments, and
- Developing tools that could be quickly and easily applied to large numbers of segregating lines.

In this and the following chapters, many of these suggestions are incorporated into a research framework for assessing the value of physiological selection traits in a breeding context.

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Assessing the Potential of Physiological Selection Criteria to Complement Breeding Strategies

The Art, Science, and Empiricism of Breeding

Breeding is frequently referred to as a blend of science and art, as well as an empirical process. The science refers to the routine application of established facts, such as the documented role of specific genes in conferring disease resistance or environmental adaptation. The art of breeding refers to the intuition gained by working with germplasm and the integration of that experience with established knowledge. In other words, intuition enables good breeding decisions to be made based on our incomplete knowledge of the biology and ecology of plants. Empiricism refers to the use of multiple crossing and selection strategies to achieve a single objective, sometimes loosely referred to as "the numbers game." Physiological understanding adds to the science, and as such complements the intuitive knowledge required to conduct good breeding. Use of physiological selection criteria can improve the probability of success by making empirical selection more efficient.

Theoretical Basis for Using Physiological Traits

Assuming significant genetic diversity for a trait is established, the question of how its use as a selection criterion would improve breeding efficiency can be addressed. Without experimentation, this cannot be predicted with any certainty, any more than a breeder can know in advance which of many crosses will produce the desired variety. An essential question is whether selection for a given physiological trait as part of an integrated breeding approach will achieve results more quickly and efficiently than selecting parents and/or progeny for performance alone.

Many traits may appear to be of potential benefit to yield. To assess which trait(s) should be prioritized, alternate hypotheses may be tested empirically, based on a conceptual model that incorporates current understanding of physiological and biochemical constraints to performance (Figure 1). For example, if the principal yield-limiting factor is water stress, physiological understanding would suggest that genotypes with deeper roots will have an advantage over others, assuming moisture is available deeper in the soil profile. However, selection for yield alone will not guarantee that good lines have the deepest roots, because drought tolerance may be conferred through genetic superiority of other mechanisms, such as osmotic adjustment, accumulation and remobilization of stem reserves, superior spike photosynthesis, heat tolerant metabolism, and good emergence and establishment under moisture stress (Figure 1).

Genetic advance in dry environments has been quite limited in most breeding programs. Slower progress in moisture stressed environments compared to irrigated environments is usually ascribed to the heterogeneity of selection nurseries under dry conditions, which renders performance-based selection unreliable. Selection for specific traits in more controlled environments is likely to be more effective. In addition, if more than one mechanism is involved in drought tolerance, deliberate selection with a view to combining synergistic traits is likely to achieve results sooner than adopting a strategy whereby parents and progeny are selected on the basis of performance alone (see chapter by Richards et al.).

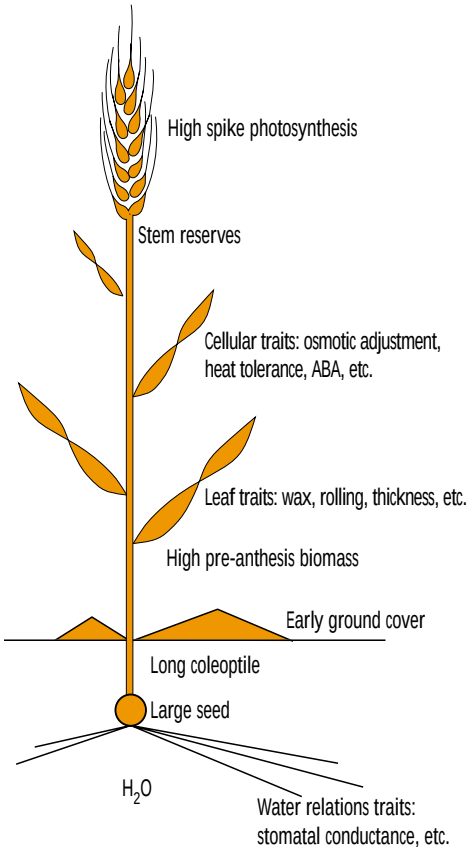

Figure 1. A conceptual model for drought tolerance in wheat. The theoretical ideotype has high expression of the following traits (not all of which would be useful in all drought environments): seed size & coleoptile length (improve early crop establishment), early ground cover & pre-anthesis biomass (reduce evaporation of soil moisture), stem reserves / remobilization & spike photosynthesis (help grainfilling during severe post-anthesis stress), stomatal conductance (indicative of roots which are able to extract soil water at depth), osmotic adjustment (maintains cell functions at low water potential), accumulation of abscisic acid (pre-adapts cells to stress), heat tolerance (heat stress may be caused by low leaf transpiration rates under drought), leaf anatomical traits e.g. waxiness, pubescence, rolling, thickness (reduce risk of photo-inhibition), high tiller survival and stay-green (easily observed integrative traits indicative of good drought tolerance).

Establishing the Genetic Bases of Physiological Traits

Once the value of a physiological trait has been established, it may be useful to determine its genetic basis, such as the number and location of genes involved in its expression. (Genetic studies, including the identification of molecular markers, can be conducted on the same kinds of populations developed to establish genetic gains associated with selection of phenotypic traits.) In theory, such an investment would enable fingerprinting for stress tolerance, or other desirable traits, on fixed lines in any breeding program worldwide. This information would allow strategic crossing programs to improve the likelihood of pyramiding drought tolerance traits, without necessarily having to measure phenotypic expression in the parents or progeny. If a trait is genetically complex and its expression a function of epistatic and other interactions among genes, then genetic markers would need to be identified in several different genetic backgrounds to gain comprehensive information about which *loci* may be involved.

Although identifying adaptive physiological mechanisms and their genetic markers may be time-consuming and costly, once the initial investment is made, the information is permanently available. The information can be used at different stages of the breeding process, depending on the resources available.

In a relatively low investment scenario, information on important physiological traits can be collected on potential parental lines. For example, it might be worth screening an entire crossing block or a subset of commonly used parents to produce a catalogue of useful physiological traits or their genetic markers. The information can be used strategically in designing crosses, thereby increasing the likelihood of transgressive segregation events that bring together desirable traits. In a scenario where more resources are available to screen for physiological traits, the same selection criteria could be applied to segregating generations, in yield trials, or any intermediate stage, depending on where genetic gains from selection are optimal.

The rest of this chapter will describe generalized procedures for evaluating physiological criteria within a breeding program, and how they might be applied.

Standard Procedures for Incorporating Physiological Criteria into a Breeding Strategy

The procedure for incorporating physiological criteria into a breeding program has two phases, each of which consists of a number of experimental steps (see boxes).

Phase 1. Identifying Traits Associated with Performance

Define the target wheat-growing environment

Before designing a research program, it is crucial to define the physical and agricultural characteristics of the target environment (Table 1), for a number of reasons. First, this information enables selection traits to be chosen which are most relevant to the environmental factors limiting performance. For example, when aiming to improve performance under drought, there is no point in selecting for deep rooting capacity if target environments are characterized by soil profiles that lack additional water at depth. Similarly, it would not be beneficial to select for early ground cover with a view to conserving soil moisture if farmers practice residue retention or soil mulching.

Second, an accurate description of the target environment is important to facilitate experimental design by permitting identification of appropriate research sites (based on temperature profile, latitude, soil type, etc.) as well as choice of experimental treatments (i.e. sowing dates, crop management, etc.). By matching the conditions of the experimental environment with those of the target environment as far as possible, results are more likely to be representative of the target environment as a whole. In many instances it is advisable to replicate trials across a number of locations within the target environment. It may not be possible to mimic the target environment precisely at any experimental site, and for strategic reasons these sites may be managed differently. Nonetheless, decisions of this type should be based on as complete a knowledge base as possible of the target environment (see chapter by Hobbs and Sayre).

Identify physiological traits/ selection criteria

When considering the incorporation of physiological criteria into a breeding strategy, previously published work on traits and methodologies can be

Steps for Incorporating Physiological Criteria into a Breeding Strategy

Phase 1: Identifying Traits Associated with Performance

- Define the target wheat-growing environment
- Identify physiological traits/selection criteria
- Choose genotypes appropriate for evaluating trait expression
- Design the experimental environment
- Develop protocols to optimize trait expression
- Measure trait expression and its association with performance

Table 1. Key parameters defining target and experimental environments.

Parameter	Units
Climate	
Maximum daily temperature	ºC (monthly mean)
Minimum daily temperature	ºC (monthly mean)
Sunhours or incident radiation	h/day or joules/m^2/d
Annual rainfall	mm/month
Relative humidity	(% max/min)
Crop environment	
Average yield	t/ha
Preceding crop(s)	e.g. summer crop species
Biotic stresses	Typical % yield loss
Soil	
Soil type	e.g. clay/loam/sand
Soil pH	pH
Physical properties	e.g. compaction zones
Organic matter	%
Rooting depth	approx in cm
Management	
Typical fertilizer rates	NPK etc. kg/ha
Typical irrigation schedule	Frequency/mm applied (if available)
Disease, pest, and weed control	Frequency

consulted. Much of this literature has been reviewed—for example, by Blum (1988) for abiotic stresses, by Evans (1993) for yield potential, and by Loss and Siddique (1994) for wheat under drought. In addition, many traits and references will be included in subsequent chapters related to yield potential, heat stress, drought stress, etc. To be of potential use in breeding, a trait must meet two broad criteria 1) evidence of significant genetic variability for the trait must exist, and 2) selection for the trait must be economically advantageous based on relative costs and benefits (see chapter by Brennan and Morris).

In choosing traits it may be helpful to think in terms of their level of integration at the whole plant level. Traits can be classified into two broad categories: simple traits associated with a particular morpho-physiological attribute, such as leaf waxiness, and integrative traits produced by the net effect of a number of simpler traits—for example, canopy temperature. Being a function of several simpler traits, integrative traits are potentially powerful selection criteria for evaluating breeding progeny; however, the heritability (measured as the ratio of genetic variance σ^2_g to phenotypic variance σ^2_p) of such traits is generally lower than that of simple traits. Clearly the heritability of a target trait will influence the ease with which it can be measured and employed in a breeding program.

Choose genotypes appropriate for evaluating trait expression

The initial choice of germplasm is critical since conclusions will hinge on it being representative of current breeding objectives. Crossing block materials and advanced breeding lines are good sources. Germplasm collections may provide useful sources of genetic diversity, especially if the accessions originate from environments where the yield constraints are similar to those in the target environment. Ideally germplasm should have a number of common characteristics that will facilitate experimental work and interpretation of the results (Table 2). Differences in height, maturity, adaptation, disease susceptibility, etc., are all factors potentially confounding to the trait under study, and variation may increase experimental error. It is unlikely that all material will meet all of the criteria; however, research findings will be greatly enhanced if most of them are met.

If significant genetic diversity for the trait cannot be found in agronomically acceptable backgrounds, it may be necessary to introgress the trait into commonly used parental lines before trait evaluation can begin. Since this requires considerable resources, there has to be good reason to believe that the trait is economically advantageous, based on, for example, studies in related species or preliminary studies on lines in which modest genetic diversity for the trait indicated a strong association with performance.

An important additional point to check before proceeding is the current status of germplasm coming out of ongoing selection programs with respect to the trait of interest. If that material already shows high expression for the trait of interest with little significant genetic

Table 2. Desirable characteristics for germplasm used in physiological breeding experiments.

- Generally adapted to the target environment
- Acceptable range of maturity
- Acceptable agronomic type
- Resistant to prevalent diseases and pests
- Broad genetic background
- Not contrasting in height class†
- Similar response to photoperiod and vernalization†

† Unless characteristics are those under investigation.

diversity, then the methodologies being used are effective in making genetic gains for the trait. Developing physiological selection methodologies is only worthwhile if they can achieve greater efficiency than existing approaches. Otherwise, the only role of physiological measurements may be to identify new and better genetic sources of the trait.

The number of lines studied must be sufficient to ensure a range of genetic diversity for the trait of interest, preferably in several diverse but locally adapted genetic backgrounds. It may be sensible to start with a large number of lines for preliminary observations. This could run into a hundred or so, depending on the complexity of the trait; the precise number will depend on the likelihood of finding genetic diversity for the trait of interest. Once diversity has been established from preliminary observations in a controlled test cycle, numbers can be reduced (e.g. 20-50) to include the best germplasm, encompassing the full range of genetic diversity, for more detailed observations in subsequent cycles.

Design the experimental environment

Trials should be managed optimally because factors such as disease pressure and irregular irrigation can introduce errors into the expression of physiological traits. Besides choosing an appropriate test site that is representative of the target environment, a number of management factors need to be considered (Table 3). Some factors, such as sowing dates that mimic temperature and photoperiod regimes, should be as representative as possible of the target environment to avoid expression of traits not relevant to the experimental objectives. Others, such as land preparation and pest control, should be managed optimally to reduce experimental error. Seed quality is another factor which should be managed optimally to ensure good stand establishment. In addition, seed should come from the same growing environment. Differing seed sources may constitute a serious confounding factor, for example when studying responses to micro-elements known to be stored to varying degrees in the seed, depending on local growing conditions (see chapter by Ascher-Ellis et al.). More details on management of trials is given in the chapter by Hobbs and Sayre.

Develop protocols to optimize trait expression

The efficiency of physiological trait selection will be related to how well a trait is expressed and measured. Therefore, for any trait, experimentation must take place to establish how and when measurements should be made to maximize genetic resolution for its expression. Three groups of factors may interact with the expression of a trait: 1) macro-environment, i.e. temperature, radiation, irrigation status, nutritional status, and soil type; 2) micro-environment, i.e. small daily fluctuations in temperature and radiation, etc., as well as small environmental differences among plots or between plants caused, for example, by soil heterogeneity, weeds, pests, etc.; and 3) physiological factors, i.e., age of plant or its organs, diurnal rhythms of plants, and small amounts of genetic diversity that may exist within so-called fixed lines.

Table 3. Management factors in trait evaluation work.

Factors that should be representative of target environment:

- Crop rotation
- Sowing date (to mimic target photoperiod and temperature regime)
- Planting method
- Seed rate
- Fertilizer regime
- Irrigation regime

Factors that should be managed optimally:

- Seed quality and source
- Land preparation
- Pest and disease control
- Weed control
- Appropriate statistical design

For example, leaf chlorophyll is relatively simple in its expression in that it is not affected by diurnal changes. However, its expression may vary under different nutritional regimes. Chlorophyll is also a function of leaf age, and some standardization in measurement will be necessary to take this into account. One might choose to measure chlorophyll in the flag-leaf at flowering or in the youngest fully expanded leaf at regular intervals after sowing. On the other hand, traits like leaf conductance or canopy temperature depression (CTD) are strongly affected by temperature and relative humidity (i.e. vapor pressure deficit) and have a diurnal function. Studies in CIMMYT (Amani et al., 1996) have shown that CTD is best expressed on warm, sunny, cloudless afternoons, in well-watered plots. The trait is also affected by phenology and, while pre-heading readings are usually higher, readings made during grainfilling are best associated with yield potential.

Avoiding confounding factors and use of experimental design. While it is impossible to completely avoid confounding factors in field experiments, much can be done to minimize their effect through planning, good crop management, and appropriate experimental design. For example, it has already been mentioned that germplasm should be chosen to avoid excessive contrasts in maturity date and height (Table 2). In addition, if test sites are carefully chosen and managed

optimally, plot-to-plot variability can be reduced. For traits affected by weather conditions, investigators may need to be flexible in the timing of data measurement, collecting readings only under relatively favorable conditions.

Fortunately, appropriate statistical procedures can help attenuate some of these problems. The use of replication in combination with appropriate blocking structures is very powerful and will help control the effects of heterogeneity inherent in all field experimental sites. Covariance analysis can help reduce the confounding effect of plant age and phenology, provided the trait and maturity class are not genetically linked. Multiple sampling will reduce errors associated with measurement (human and instrument error). These strategies, in combination with advanced data analysis procedures (i.e., spatial analysis), will reduce error associated with environmental variability.

Genotype by environment interaction. The factors that can affect the expression of a trait (i.e., macro-environment, micro-environment, and physiological factors) may show interaction with genotype, accounting for what is collectively called genotype by environment interaction (G×E). Some traits demonstrate little G×E; that is to say that genotypes ranked based on these traits will largely maintain this rank across different environments, regardless of the absolute expression of the trait. These traits are highly heritable, as environment has little influence on their expression. Therefore, selection for these traits will be effective across locations and years. In general, the greater the genetic complexity of a trait, the greater is the probability of obtaining significant G×E.

Measure trait expression and its association with performance

Once experimental protocols have been refined, data must be collected in at least two or three environments (these may be different representative sites and/or years), and assessed for 1) significant and consistent expression of the trait of interest, and 2) association of the trait with performance among genotypes. For the latter, correlation between the trait and performance should be tested using the mean values for both, averaged across replications within environments. (Correlations should not be interpreted from individual replication data, since these may be highly confounded by environmental differences among replications; ideally genetic correlations should be calculated.)

Any interpretation of data from unrelated fixed lines is speculative, since the association between traits and performance may be confounded by other genetic factors, such as differences in phenology and plant type. For this reason, assuming the above criteria are met, a second phase of experimentation is needed to demonstrate a definitive genetic linkage between the trait and performance in more closely related materials such as homozygous sister lines. Genetic gains resulting from selection and measured as improved performance can then be estimated.

Phase 2. Estimating Heritability of Traits and Response to Selection

Make experimental crosses with parents contrasting in trait

The initial objective of producing experimental germplasm is the generation of homozygous sister lines, i.e., recombinant inbred lines (RILs), which may be F_4 (or later) generation derived so as to be reasonably homozygous and homogenous.

Experimental germplasm can, in theory, be derived from any cross. In practice the parents should be sufficiently well adapted to the target environment to permit field experimentation, and show genetic diversity for the selection trait under investigation; preferably, they should also meet the additional criteria listed in Table 2. Lines on which initial studies were made are a good source, since they will have been well characterized in Phase 1. Two or three crosses would probably be a minimum since traits of interest may interact within different genetic backgrounds.

Develop randomly-derived homozygous sister lines

To demonstrate a genetic linkage between traits in homozygous lines, ideally there should have been no selection pressure applied during their development, thereby ensuring that all lines are randomly derived. One way to achieve this is through using the single seed descent (SSD) method. However, SSD is a resource-intensive way of producing germplasm. The cheapest alternative is to simply advance each generation in bulk without deliberate selection. However, the disadvantage of bulking is that natural selection and

Steps for Incorporating Physiological Criteria into a Breeding Strategy

Phase 2: Estimating Heritability of Traits and Response to Selection

- Make experimental crosses with parents contrasting in trait
- Develop randomly-derived homozygous sister lines
- Test genetic links between quantitative traits and performance
- Estimate heritability and genetic gains from selection
- Apply physiological traits to complement breeding

genotypic competitivity (e.g., tillering ability, height) will influence gene frequencies. A reasonable compromise is to grow bulks at low density starting with F_2 seed, such that individual plants can be recognized. To minimize the effects of interplant competition, the bulk is maintained by harvesting a single spike from each plant, rather than the whole plant.

An alternative to the above is to produce doubled haploid populations. This strategy allows the researcher to produce a large number of totally unselected and genetically fixed lines in a relatively short space of time; however, the technique is quite costly and resource dependent (Snape, 1989).

Relevance of "unselected" germplasm to breeding objectives. There are a number of practical problems associated with working on "unselected" materials. The most obvious relates to relevance to a breeding program, in which unsuitable materials are normally discarded as early as possible. Conclusions based on studies with unselected material may thus not be relevant to a practical breeding program.

A second problem relates to potentially confounding factors inherent in unselected material with respect to measuring physiological traits. For example, expression of many physiological traits may be seriously confounded by phenotypic variability in maturity date or height. Unless the choice of experimental germplasm has avoided all potentially confounding factors (Tables 2 and 4), segregating lines are likely to show considerable variation for such characteristics.

As a compromise between maintaining maximum genetic diversity on the one hand and, on the other, obtaining results that are relevant to breeding objectives and still experimentally valid, negative selection can be practiced on experimental germplasm to remove totally unsuitable material. While the precise nature of such characteristics will vary with the target environment, some common ones are listed (Table 4), bearing in mind that lines with undesirable characteristics should never be removed if they are in any way related or linked to the trait under study. Undesirable traits are the same ones that would normally be discarded during the breeding process.

Test genetic links between quantitative traits and performance

The relevant generation at which to look for a genetic link between a trait and performance will depend on how complex the trait is. Due to the nature of genetic segregation, the number of heterozygous *loci* in a genotype is approximately halved after each generation of self-fertilization. Thus, based on probability, any given genotype will be approximately 50% homozygous at all *loci* in the F_2, 75% in the F_3, 87.5% in the F_4, etc., such that the probability of being genetically stable increases with each subsequent generation. As a consequence, traits controlled by few genes are more likely to become fixed in earlier generations than more complex ones. In addition, the more genes involved in the expression of a trait, the greater the number of different genotypes possible.

Table 4. Undesirable agronomic characteristics that may confound results in experimental breeding.

- Extremes of height
- Unsuitable phenological development
- Strong lodging tendency
- Very poor tillering ability
- Shriveled kernels
- Very low yield potential
- Chlorotic and necrotic symptoms
- Susceptibility to diseases that are widespread and difficult to control

Since in practice it is difficult to establish the number of genes involved for any but the most simple of traits, we must assume that a trait is relatively complex or relatively simple based on segregation ratios for qualitative (or Mendelian) traits or, in the case of quantitatively inherited characters, the degree of G×E observed in the trait's expression. The heritability of a trait in early generations will also indicate its genetic complexity. For simply inherited characters such as the presence or absence of awns, the heritability of expression is 100%; however, the expression of quantitatively inherited characters such as grain yield will be greatly influence by G×E.

Based on the above considerations, one might harvest a number of (relatively) randomly derived lines anywhere from F_4 to F_8 by taking the seed of individual plants in the requisite generation and multiplying it for yield testing. The procedure is then the same as described in Phase 1, when looking for the association between a trait and performance in fixed lines. The genetic link will be validated when tested in an adequate number of environments.

Evaluating physiological traits independently of biotic stress. A major confounding factor in evaluating physiological traits is disease incidence. Generally speaking diseases should be controlled chemically, if not genetically, so as not to confound conclusions. This is reasonable considering the very different nature of the genetic mechanisms involved. For example, when comparing resistance to biotic versus abiotic stresses, it is clear that germplasm with improved abiotic stress tolerance will maintain its superior performance indefinitely under a defined physical environment. Resistance to

pests and diseases, on the other hand, may break down very rapidly. Hence, within the context of experimental germplasm at least, it makes no sense to restrict genetic variability for a physiological trait based on disease resistance criteria that may become obsolete in time.

One exception to the above would be particularly intractable disease problems that are of major economic importance in the target area, where maintenance breeding is costly. In that case, not only would it be necessary for germplasm to be derived from at least one resistant parent, but susceptible progeny would need to be eliminated systematically. Although it may be decided to control diseases in experimental work, disease expression should be encouraged in ongoing breeding work so that susceptible lines can be eliminated before they are evaluated for physiological criteria.

Estimate heritability and genetic gains from selection

If a trait is highly heritable, it is more efficient from a breeding point of view to select lines as early as possible in the breeding process. If shuttle breeding is being applied, the environment most suitable for expressing a specific trait may coincide with a particular generation or generations. In either case, it is necessary to establish the heritability of the trait at that generation, so that genetic gains can be evaluated using the formula

$$R = ih^2\sigma_p \qquad (1)$$

where i = intensity of selection or the proportion of the population included in the selected group and σ_p is the phenotypic standard deviation.

In this case, realized heritability (estimated as σ^2_g/σ^2_p) is perhaps the most relevant parameter to calculate. This is done by measuring trait expression in a population of lines or bulks from a cross and, using an arbitrary selection intensity, dividing the population into high and low groups. These are advanced one generation, and then the trait is measured again. The smaller the difference between the high and low groups in subsequent generations, the lower the heritability. This can be represented by the following equation:

$$h_r = (F_{n+1}\ \text{high} - F_{n+1}\ \text{low}) / (F_n\ \text{high} - F_n\ \text{low}) \qquad (2)$$

Where F_n high is the mean trait value of the plants selected for high expression in the n^{th} generation; F_n low is the mean trait value of plants selected for low expression in the n^{th} generation; F_{n+1} high is the mean value in F_{n+1} of the same high lines from F_n; and F_{n+1} low is the mean value in F_{n+1} of the same low lines in F_n (Falconer, 1990).

Apply physiological traits to complement breeding

When a trait shows a strong association with performance in unrelated fixed lines (Phase 1) as well as in homozygous sister lines (Phase 2), it is probably worth applying selection pressure for the trait in preliminary trials (PTs). However, the nature of the association should also be examined, i.e., the distribution of the values of the trait in relation to performance of lines. For example, when the distribution is linear at first but flattens off at higher yields (Figure 2), selection for the trait may only be effective in eliminating the poorest material.

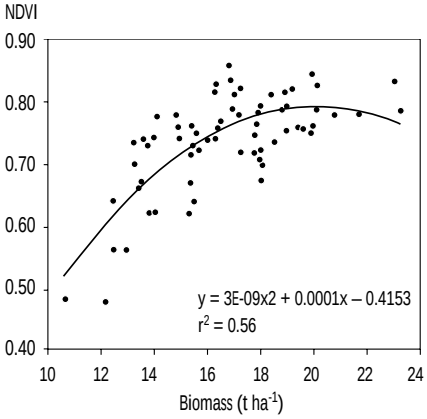

Figure 2. Relationship between spectral reflectance index (NDVI, normalized difference vegetation index) measured during grainfilling and biomass of irrigated spring wheat advanced lines, Obregon, northwestern Mexico, 1996-97.
Source: Reynolds et al. (1999).

When a trait shows high heritability and good association with performance in sister lines, it may lend itself to early generation selection (EGS), instead of or in addition to selection in PTs (Table 5). Selection pressure might be applied in F_3 plants or $F_{3:4}$ plots etc., depending on the sensitivity of trait expression to planting method. Even for a trait that is relatively weakly associated with performance, but highly heritable, early generation selection may be a useful tool for eliminating the poorest material (Table 5). Assuming that the data point convincingly to the use of a physiological trait in breeding, it must be selected for within the overall framework of the breeding program. Disease resistance, agronomic type, and industrial quality are among economically important traits that are essential if a new variety is to succeed. A theoretical scheme for incorporating physiological traits into a conventional breeding program is presented below (Table 6).

Table 5. Criteria for applying physiological traits in a breeding program.

Trait heritability	Association of trait with performance	
	Strong	Weak
Low	Selection in PYTs	No application
High	Early and/or late generation selection	Negative selection in early generations

Table 6. Theoretical scheme for incorporating some physiological selection criteria into a conventional breeding program showing different alternatives for when traits could be measured, depending on resources available.

	Breeding generation when selection should be conducted			
	All generations	F3	F4-F6	PYTs / Advanced lines
Simple traits				
Disease	visual			
Height	visual			
Maturity	visual			
Canopy type		visual	†	
Complex traits				
Yield			visual	yield plots
Industrial quality			grain	grain
Lodging		small plots	small plots	yield plots
Canopy temperature depression			small plots	yield plots
Stomatal conductance		plants	small plots	yield plots
Leaf chlorophyll		plants	small plots	†
Spectral reflectance			small plots	yield plots

† Selection in early/intermediate generations is probably sufficient, as GxE is low.

In summary, the advantages of EGS of physiological traits over later generation selection are 1) resources may be saved by eliminating physiologically inferior material from the program, and 2) the likelihood of discarding favorable genetic diversity is decreased. The potential disadvantages are 1) without close interdisciplinary collaboration, time may be wasted by measuring traits on agronomically unsuitable material, or even promoting it, and 2) in early generations large numbers of plants must be tested, and some currently available physiological tools are either too expensive or cannot be applied quickly enough.

Conclusions

This chapter attempts to provide basic guidelines for evaluating and applying physiological selection traits in breeding work. If adopted, physiological criteria represent a refinement of a breeding program and in no way replace traditional methods of selection. Nonetheless, breeding efforts aimed at meeting ongoing challenges, such as breaking yield barriers and improving performance under abiotic stresses, are more likely to achieve success if physiological understanding is used to complement traditional approaches.

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CHAPTER 1

Directions for Physiological Research in Breeding: Issues from a Breeding Perspective

P.A. Jackson[1]

Plant breeding has traditionally applied a trial-and-error approach in which large numbers of crosses are made from many sources of parental germplasm. Progenies are evaluated for characters of direct economic interest (e.g., grain yield and grain quality) in target environments. Good performing parental germplasm, crosses, and progenies are selected for further use or testing. In many programs "breakthroughs" in improvement are made simply by finding superior sources of parental germplasm among the numerous sources tested. This conceptually simple approach has been highly successful in many crop species and numerous breeding programs.

The approach has often succeeded in the absence of in-depth knowledge about the physiological basis for superior performance. In some crops such knowledge has been obtained by doing retrospective analyses of prior genetic gains. Breeders have not applied this knowledge to a significant extent as a guide to further improvements, but instead have taken any avenue of improvement that happens to arise from direct selection for yield and economic performance.

Given the success of such approaches to date, to what extent can plant breeding programs benefit from physiological research? A recent survey of plant breeders and physiologists (reported in Jackson et al., 1996) indicated a general view among both groups that in the future physiological research would have an increasing role in plant breeding programs. However, the same survey also indicated that many respondents felt outputs from physiological research to date had not developed into practical improvements to the extent they had expected or thought was desirable.

There has been considerable discussion in the literature about the potential role of physiological research in plant breeding. Much of this discussion has been from a physiological perspective—i.e., examining the potential merits of different plant traits for improving yield under different environmental conditions. The aim of this chapter is to take a breeding perspective of ways in which physiological understanding may be applied in traditional breeding approaches. The assumption is that plant breeding programs will continue to rely heavily on large scale evaluation of parental and progeny populations. Physiological understanding could enhance and refine this approach.

Applying Physiological Understanding in Breeding

Illustrated in Figure 1 are the ways in which physiological understanding may be applied in a breeding program; this provides the basis for the subsequent discussion. The figure also shows possible ways to enhance this process, all of them based on physiological research or understanding.

Understanding yield-limiting factors

Understanding the biological factors limiting the performance of genotypes across target environments is essential for improving breeding programs through physiological research. Examples of such factors are moisture stress during different phenological periods (Fischer, 1979; Woodruffe and Tonks, 1983), soil fertility constraints (Carver and Ownby, 1995), production of sink capacity (grain number) and subsequent partitioning of dry matter to grain (Gallagher et al., 1975), canopy light interception during reproductive growth (Lawn and Byth, 1974), and presence of a plant disease. The defining feature of a limiting factor in this context is that improving genetic response to that specific factor would result in higher yields. In all physiological research targeting crop improvement, knowledge of what these limiting factors are for a particular crop species x target environmental domain combination is

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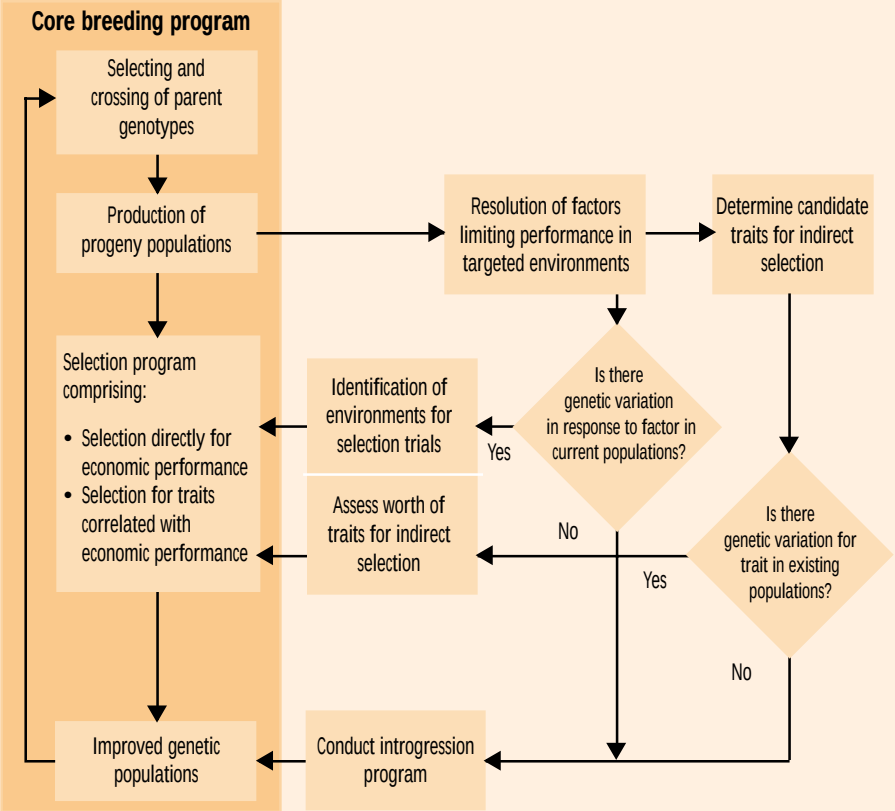

Figure 1. Key steps in a generalized breeding program (on the left) and the potential role of physiological research or understanding.

either taken from previous experience or research, or established at the outset as an objective of the research.

Knowledge of the important limits to better performance may lead to more effective approaches to crop improvement, which will be discussed later. An example of the application of such knowledge is the use of disease screening trials in breeding programs. In such cases, the breeder has previously recognized that the disease in question is an important limiting factor in at least some target environments. Genotypes will be deliberately evaluated in the presence of that disease at some stage of the selection process, and an appropriate weighting given to the results. Furthermore, if necessary, sources of parental germplasm that exhibit favorable responses to the disease may also be sought outside the breeding program. Thus breeding is more focused and effective than when such a limiting factor is not known.

While a breeder may have some knowledge of the major constraints to higher yields in his or her target environments, often it may be superficial or deficient. Physiological research can help to fill the gap. Different approaches have been used, including focused agronomic trials in which factors suspected of limiting performance are manipulated to verify and quantify their effect (Nix, 1980). At a slightly more sophisticated level, simple but very informative conceptual models of yield accumulation processes (e.g., Fischer, 1979) may be developed. Finally, crop growth models to quantitatively assess constraints have been advocated (e.g., Muchow and Carberry, 1993) and may have particular value where highly variable seasonal conditions exist. However, it should be noted that development of crop growth models is expensive and, in many cases, may not be necessary to gain an adequate level of knowledge.

Identification of the environmental or physiological constraints to higher yield does not automatically lead to more effective breeding and selection approaches. However, it is a starting point for developing the approaches discussed below.

Choosing environments for selection trials

Most resources in large plant breeding programs are devoted to conducting selection trials for progeny lines. The aim is to select superior lines that will be more precisely evaluated in subsequent trials, and eventually to release worthwhile lines. Selection trials are usually conducted in environments considered representative of target production areas.

Limiting factors are significant enough to be the object of selection trials if: 1) they are economically important, i.e., they have a widespread or large impact on yield in the target region, and 2) there is genetic variation for response to them, i.e., they allow discriminating among the genetic materials being evaluated. Enhanced knowledge of these factors will help the breeder choose sites for selection trials and decide how to manage them. The breeder evaluates genetic materials so as to be able to observe genotypic response to the limiting factors. Based on these observations, he can select genotypes with the desired combination of favorable responses.

If the occurrence or intensity of the limiting factors varies across the target region, this will lead to genotype x environment (G×E) interactions in multi-environment trials. Large G×E interactions may result in smaller realized gains from selection if the

factors causing the interactions are not adequately sampled at a particular stage of selection. If there is adequate sampling of such factors in selection trials, selection should produce good results.

A stratified random sampling of environments will usually be conducted across the target region if limiting factors are not known or poorly defined. The stratification may be based on, for example, geographic location, soil type, management regimes (e.g., irrigation versus rainfed), or other factors that might possibly affect the relative response of genotypes. This approach will be effective to some extent; however, sampling of some factors may be deficient with such a hit-and-miss approach, and the relative weighting to apply to results from different trials during selection will be unknown.

In this situation physiological research clearly has a role to play by helping to identify the significant constraints to higher yields and determine the level of genetic variation for response to those constraints in particular populations. Using the approaches outlined in the previous section, physiological research on one or a few genotypes may help identify economically important constraints (determined as previously described). However, comparing responses of an adequate sample of genotypes representative of those being evaluated is necessary to determine the extent to which the various factors elicit genetic variation. This may sometimes be done in conjunction with selection trials already established in the breeding program (e.g., Jackson et al., 1994). Improved knowledge of the factors involved in generating variation among materials being tested will nearly always facilitate more focused and efficient selection strategies.

One way this information may be used in breeding programs is via the use of managed environments for selection trials. In Queensland, Australia, wheat (Cooper et al., 1995) and barley (Jackson et al., 1994) lines were found to exhibit large variation in grain number and grain yield under favorable growing conditions (influenced by water and nitrogen availability) at around anthesis. The responsiveness of different lines to good conditions at around anthesis accounted for a large proportion of G×E interactions exhibited by breeding populations in a sample of production environments. It was therefore suggested that wheat and barley lines should be evaluated in one or more high input (water and N) environments in the early stages of selection (Jackson et al., 1994). This would allow effective discrimination among lines for adaptation across the target environments.

Cooper et al. (1995) tested the value of using a small number of high input environments for selection trials in wheat. They showed that mean yields of wheat lines across three such environments had a high genetic correlation with mean yields across a larger number (16) of random production environments. They concluded that high input managed environments could be used at the preliminary yield evaluation stage to facilitate efficient and effective discrimination among unselected lines. This approach should provide more reliable selection data, at a lower cost, than using a larger number of random production environments for selection trials.

Using physiological traits as indirect selection criteria

Identification of yield limiting factors may suggest physiological traits that breeders could use as indirect selection criteria. Although this topic has received considerable attention in physiological research and literature, successful application in breeding programs appears rare. Possible reasons for this lack of success and ways to enhance application in breeding programs are discussed below.

Once identified, physiological traits affecting response to important limiting factors may be used in two ways: 1) as indirect selection criteria in progeny populations in core breeding programs, and 2) to define objectives for introgression activities (Figure 1).

Genetic response for yield using another trait as an indirect selection criterion may be predicted from the following formula (Falconer and Mackay, 1996):

$$CR = i\, h_x \cdot r_g \cdot \sigma_{gy}, \qquad (1)$$

where CR is the correlated response for yield, from selection based on character x; i is the standardized selection differential (related to the proportion of genetic population selected); h_x is the square root of the heritability for character x; r_g is the genetic correlation between character y and character x; and σ_{gy} is the genetic standard deviation for character y.

The genetic correlation is the correlation between genetic effects for yield and the trait used for selection, and may be estimated from analyses of covariance. Many physiological studies reported in the literature have described only phenotypic correlations (i.e., correlations based on means), which may be seriously biased (either upward or downward) by error effects or correlated environmental effects. Genetic correlations are more relevant than phenotypic correlations for examining the value of traits to be used as selection criteria.

Equation 1 suggests that using a trait as an indirect selection criterion will be effective only under rare circumstances. First, the heritability (i.e., the ratio of

variation due to genetic variance compared with error, G×E interaction) of the trait has to be high. Second, the genetic correlation between the trait and yield (or other character of primary interest) also has to be high. A further consideration is the cost of measuring the trait. When this cost is high (assuming fixed budgets), smaller numbers of genotypes may be screened, reducing the intensity of selection and the component *i* in equation 1. Despite the fundamental importance of these parameters in determining the value of traits for use as selection criteria, they are often ignored in discussions of the subject in the literature.

For a trait to be relevant to breeding programs, it is important that its heritability and genetic correlations be estimated in genetic populations representative of those being evaluated. This is because such parameters will differ among different genetic populations (Falconer and Mackay, 1996), and misleading or irrelevant information could be obtained if atypical populations are used. For example, the use of highly diverse genotypes would produce estimates of genetic variance, heritability, and, probably, genetic correlations that are greater than those of more homogeneous genetic populations in advanced breeding trials. Similarly, these genetic parameters in highly selected varieties or lines in advanced breeding trials would probably be very different from those in less selected early generation materials.

It is also important that an adequate sample of the representative genetic population(s) be used in estimating genetic parameters. Breeders usually consider a minimum of 30-40 genotypes to be an adequate sample for estimating variance components and other statistical data. Methods for determining standard errors of estimated genetic statistics have been developed (Falconer and Mackay, 1996) and are helpful in assessing whether sampling and experimental design are adequate for precise estimation.

In practice, there are few cases where indirect selection alone will be more effective than direct selection for yield, particularly if labor-efficient and low-cost methods have been developed for conducting large scale yield trials, as is usually the case. However, sometimes it is possible to identify traits having all the desired features—high heritability, high genetic correlation with characters of economic interest, low cost of measurement. Greater gains can be made by using such traits together with yield as a selection index than by using yield alone. The use of selection indices has been reviewed elsewhere (Baker, 1986).

Well documented examples of the successful application of indirect selection have been reported by Fischer et al. (1989) and Bolanos and Edmeades (1993a; 1993b). The latter selected maize populations using a selection index based on grain yield across environments where water was managed and other traits such as relative leaf elongation under stress, anthesis to silking interval, and leaf death score. Fischer et al. (1989) compared recurrent selection using a selection index to selection for grain yield *per se*. Gains in grain yield under severe moisture stress conditions were greater when using the selection index than based on yield *per se* (Table 1). These authors suggest that traits other than yield were more useful as selection criteria because they had greater heritability than yield under severe moisture stress. This may be because such traits were less influenced by competition effects in small plots and by soil variability, which is sometimes a problem in trials under severe moisture deficits.

By extension, the identification of individual traits for use as selection criteria is selection toward an ideotype in which the ideotype predicts what the characteristics of a genotype ideal for a target environmental domain should be. Perhaps the major limitation of such an approach is that it does not, by itself, account for the level of genetic variation in genetic populations, nor for genetic correlations among traits. In some cases, there may be little genetic variation for traits viewed as desirable and, therefore, selection based on such traits will be ineffective and wasteful.

Table 1. Performance of maize populations after recurrent selection using different criteria.

	Grain yield at soil moisture deficit level			Character†		
Population‡	Mild	Medium	Severe	ASI	RLE	LDS
Original	5240	2780	1520	6.5	75	4.2
Yield-mild	6170	2580	1330	6.3	69	4.0
Yield-severe	5420	3160	1680	4.7	77	3.8
Index	5950	3670	2300	3.7	81	3.1
SE	496	399	297	0.6	4	0.3

† ASI: anthesis to silking interval (days); ALE: relative leaf extension (%); LDS: leaf death score.

‡ Original: the population before any selection; yield-mild: the population selected based on grain yield under limited moisture stress; yield-severe: the population selected based on grain yield under severe moisture stress; index: the population selected based on a selection index.

Source: Adapted from Table 3 in Fischer et al. (1989).

Negative genetic correlations may exist between many physiological traits and other useful characters (e.g., Misken and Rasmusson, 1970); this may result in low or zero genetic correlations with economically important characters. For example, many yield components are negatively correlated (Adams, 1967), so that gains from selecting for one component will inevitably result in a decrease in other important components. Genetic linkage or pleiotropy may cause negative genetic correlations and will either reduce the rate of progress made in introgression (in the case of linkage) or reduce the value of the trait being introgressed (with pleiotropy). Rasmusson (1991) found pleiotropy and trait compensation were major factors limiting progress in an extensive barley breeding program applying an ideotype approach.

Thus selection for traits that could be useful as selection criteria simply on the basis of physiological understanding may result in small or no gains for characters of direct interest, such as yield. Further, this approach may not identify traits having a positive genetic correlation with yield and in which gains via breeding may be easiest to achieve. If yield itself (or other characters of economic interest) is used as the key selection criterion, physiological traits influencing yield, and for which genetic variation exists, will automatically be changed.

In summary, when considering the use of physiological traits as indirect selection criteria, expected results should be compared with predicted gains using yield itself. The search for, and assessment of, traits that may be useful as selection criteria should be based on estimation of their heritability, their measurement cost, and their genetic (not phenotypic) correlation with yield. Using traits in association with yield as a combined selection index should also be considered.

Using physiological understanding to define objectives of introgression programs

The aim of the main steps in a core breeding program (Figure 1) is normally the direct development of new cultivars, and the selection of parents and progeny is usually based on overall estimates of economic performance. However, in a more strategic phase of genetic improvement, the breeder may select parent germplasm on the basis of a specific trait such as disease resistance or tolerance to some abiotic stress. The aim here is to introgress—i.e., introduce—a specific trait into locally adapted breeding stocks (Simmonds, 1993). Successful introgression of germplasm from diverse outside sources has often resulted in quantum gains in improvement. Examples include N.E. Borlaug's use of dwarf wheat germplasm as parental material to reduce lodging, and the use of *Saccharum spontaneum* in sugarcane to improve ratooning and stress tolerance in noble varieties (Berding and Roach, 1987).

Donor germplasm may be identified from any source outside the locally adapted materials being selected. For example, it may include improved materials from other breeding programs which, though not locally adapted, may have desirable characters; materials from germplasm collections of the same species; or materials from related species. Physiological understanding may be useful or even necessary for choosing donor material to be used in introgression. Its role here would be to define specific traits or responses of particular value for which little genetic variation exists in core breeding populations (see chapter by Skovmand et al.).

It should be emphasized that introgression is sometimes a difficult, long-term process and often unsuccessful; it therefore requires careful consideration and planning. The donor germplasm of the trait being introduced will nearly always be inferior from an overall agronomic point of view. For this reason, several generations of backcrossing toward locally adapted material and selection between each generation of crossing are required to combine adequate expression of the trait in a suitable agronomic background.

In the future, genetic engineering approaches will increasingly be used to provide 'new' genes, efficiently incorporate them into adapted germplasm, and control their expression. The aim here is in many ways similar to that of introgression based on more conventional breeding approaches. However, the new approaches are potentially more powerful in that they make a wider range of genes accessible for improving plant traits. This research will require an important and complementary effort by plant physiologists and biochemists to define or suggest specific genetic manipulations needed to overcome constraints to better productivity or improved quality in target environments.

Conclusions

This chapter outlines three ways in which knowledge developed from physiological research may be used to assist plant breeding programs:

- to improve sampling of environments for selection trials;
- to identify traits that may be used as indirect selection criteria, most commonly in an index combined with direct measurements of economic performance;
- to assist in determining the objectives of introgression programs and, increasingly in the future, of genetic engineering approaches.

Physiological research needs to be more closely integrated with active breeding programs for its application to yield practical outcomes. If this occurs, physiological research will be conducted on relevant genetic populations and, more importantly, outputs and suggestions from physiological research can be rapidly evaluated, compared, and redeveloped in the context of existing breeding approaches. A plant breeding perspective of the research and potential opportunities for its application should be determined using simple quantitative genetics models (see equation 1). Both the physiologist and the breeder need to continually address hard questions about how physiological research will improve existing breeding approaches. This should help maintain a focus on producing practical breeding outcomes.

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CHAPTER 2

Searching Genetic Resources for Physiological Traits with Potential for Increasing Yield

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World demand for wheat is growing at approximately 2% per year (Rosegrant et al., 1995), while genetic gains in yield potential of irrigated wheat stand at less than 1% (Sayre et al., 1997). Thus global demand for wheat is growing at about twice the current rate of gain in genetic yield potential, with progress in rainfed environments being even lower. Meeting these demands by continuing to expand agricultural production into remaining natural ecosystems is environmentally unacceptable, and the economic costs of increasing yields through the intensification of agronomic infrastructure are high. Hence a cost-effective and environmentally sound means of meeting global demand for grain is through the genetic improvement of wheat.

Increases in wheat yield potential to date have resulted mostly from manipulation of a few major genes, such as those affecting height reduction (*Rht*), adaptation to photoperiod (*Ppd)* and growth habit (*Vrn).* Future gains in yield potential, especially under stressed conditions, will almost certainly require exploitation of the largely untapped sources of genetic diversity housed in collections of wheat landraces and wild relatives. Though these sources of genetic diversity have been exploited to improve disease resistance in wheat (e.g. Villareal et al., 1995), little use has been made of them for physiological improvement. Nonetheless, many traits reportedly have potential to enhance yield, and high expression of these can be found in germplasm collections. Seed multiplication nurseries can be used for characterizing and evaluating germplasm collections for non-disease and non-destructive traits (DeLacy et al., 2000). Since seed regeneration activities are carried out anyway, they can be an economic way of collecting data. Recent work (Hede et al., 1999; DeLacy et al., 2000) has indicated that agronomic traits (including those with low heritability) measured on small, seed-increase hillplots or miniplots can be used for such purposes.

Genetic Resources

The genetic resources available to plant physiologists and breeders are found in several Triticeae genepools recognized by Von Botmer et al. (1992) and described as concentric circles (Figure 1). The concept of the genepool was first proposed in 1971 by Harlan and deWet (Harlan, 1992), who suggested a circular way of demonstrating the relationships among genepools. The primary genepool consists of a given biological species including, in the case of a crop species, its cultivated, wild, and weedy forms. Gene transfer within species of the primary genepool is not

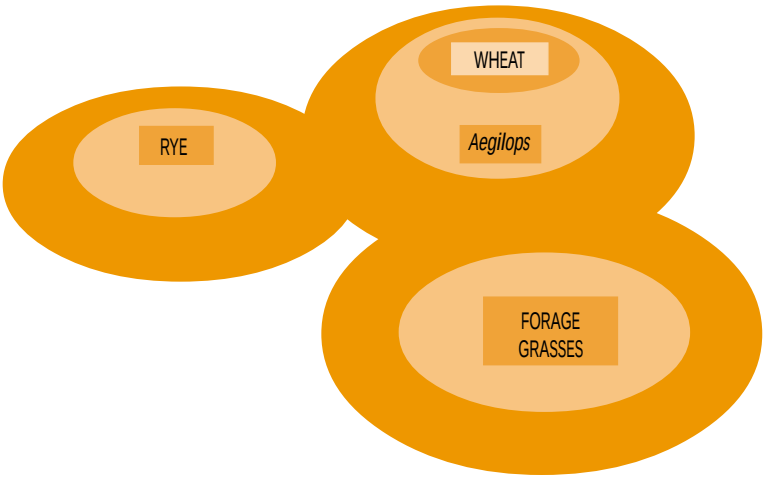

Figure 1. Schematic diagram of the concentric circles illustrating genepools of the Triticeae.
Source: Adapted from Botmer et al. (1992).

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difficult. Table 1 lists the diploid, tetraploid, and hexaploid species in cultivated wheat's primary genepool, listing their common names and indicating the genomes of each. The secondary genepool comprises the cenospecies, i.e., a group of related species with which gene transfer is possible but difficult. Most species in wheat's secondary genepool, along with their synonyms and genomic constitution, are given in Table 2. Wheat's tertiary genepool is composed of related genera of annual and perennial grasses from which gene transfer can only be achieved through the use of special techniques. The genera and species in the tertiary genepool, described by Dewey in 1984, are too numerous to be listed here.

Genetic resources of cultivated plant species were categorized by Frankel (1977) and the Food and Agriculture Organization of the United Nations (FAO) Commission on Plant Genetic Resources (FAO, 1983); however, this categorization is not followed by all centers involved in genetic resource conservation and utilization. The categories are:

- modern cultivars in current use
- obsolete cultivars, often the elite cultivars of the past, many of which are found in the pedigrees of modern cultivars
- landraces
- wild relatives of crop species
- genetic and cytogenetic stocks
- breeding lines

Recently the International Plant Genetic Resources Institute (IPGRI) and FAO jointly developed a list of multi-crop passport descriptors to provide coding schemes consistent across crops. These descriptors should be compatible with future IPGRI crop descriptors and with those used by the FAO World Information and Early Warning System (WIEWS) on Plant Genetic Resources (PGR) (Hazekamp et al., 1997). The descriptors are: 1) unknown; 2) wild; 3) weedy; 4) traditional cultivar/landrace; 5) breeding/research material; 6) advanced cultivar; and 7) other. Because they are more generic (so as to fit multi-crop classification), these descriptors are also less useful in single-crop classification systems.

The classification system used in CIMMYT's wheat collection is based on the categories outlined by Frankel and the FAO Commission on PGR (Skovmand et al., 1992). Recently, however, a list including 21 categories was defined in the GRIP project (Skovmand et al., 2000) to describe the biological status of materials in CIMMYT's collection and other genetic resources. When such specific categories are applied to collections, utilization efficiency is enhanced, making it easier for users to know exactly what they are working with.

Table 1. Summary of taxa in the primary and secondary genepools of cultivated wheat.

I. Sect. **Monococcon** Dumont; Ploidy level: diploid; genome type (female x male parent): AA **('A')**

1. ***Triticum monococcum*** L.
- a. ssp. *monococcum*; cultivated form; einkorn or small spelt wheat
- b. ssp. *aegilopoides* (Link) Thell.; wild form; synonym: *T. boeoticum*

2. ***Triticum urartu*** Tumanian ex Gandilyan; wild form

II. Sect. **Dicoccoidea** Flaksb.; Ploidy level: tetraploid; genome type (female [B] x male [A] parent): BBAA **('BA')**

3. ***Triticum turgidum*** L.; cultivated and wild forms
- a. ssp. *turgidu*; rivet, cone, or pollard wheat
- b. ssp. *carthlicum* (Nevski) A. Loeve & D. Loeve; Persian wheat, Persian black wheat
- c. ssp. *dicoccon* (Schrank) Thell.; emmer wheat
- d. ssp. *durum* (Desf.) Husn.; macaroni wheat, hard wheat, or durum wheat
- e. ssp. *paleocolchicum* (Menabde) A. Loeve & D. Loeve
- f. ssp. *polonicum* (L.) Thell.; Polish wheat
- g. ssp. *turanicum* (Jakubz.) A. Loeve & D. Loeve; Khorassan wheat
- h. ssp. *dicoccoides* (Koern. ex Asch. & Graebn.) Thell.; wild emmer wheat

4. ***Triticum timopheevii*** (Zhuk.) Zhuk.
- a. ssp. *timopheevii*; cultivated and wild forms
- b. ssp. *armeniacum* (Jakubz.) van Slageren; wild form

III. Sect. **Triticum;** Ploidy level: hexaploid; genome type (female [BA] x male [D] parent): BBAADD **('BAD')**

5. ***Triticum aestivum*** L.
- a. ssp. *aestivum*; bread wheat
- b. ssp. *compactum* (Host) Mackey; club, dwarf, cluster, or hedgehog wheat
- c. ssp. *macha* (Dekapr. & Menabde) Mackey
- d. ssp. *spelta* (l.) Thell.; large spelt, spelt or dinkel wheat
- e. ssp. *sphaerococcum* (Percival) Mackey; Indian dwarf wheat or shot wheat

6. ***Triticum zhukovskyi*** Menabde & Ericz.; Ploidy level: hexaploid; genome type (female [GA] x male [A] parent: GGAAAA **('GAA')**

Source: van Slageren (1994).

The concentric circles proposed by Harlan and deWet (Harlan 1992) to describe the different genepools have been very useful, and the concept has provided a rational basis for comparing taxonomies. However, it gives the impression that separations among the pools are clear-cut, with distinct divisions between one pool and another, though Harlan (1992) did state that the lines of demarcation may be fuzzy. Furthermore, the circles do not reflect the relative difficulty of utilizing the genepools, nor the cost of utilizing genetic resources within a genepool or within a species.

A schematic diagram of the effort needed to transfer traits from genetic resources to farmers' fields is given in Figure 2. Within the primary genepool, the utilization cost increases as the genetic distance increases. Within a species there are also levels of genetic resources (from current high yielding cultivars to landraces) that may determine the cost of using those resources.

As one moves away from the primary genepool, the effort required to utilize genetic resources in the secondary and tertiary genepools increases geometrically. It is difficult to release a commercially acceptable cultivar that does not have previously released cultivars in its pedigree (Rajaram, pers. comm.) because crosses with species in the secondary and tertiary genepools tend to disunite favorable gene complexes, which affects performance. Technology extends the genepools and reduces costs, as, for example, embryo rescue has done in the recent past and genetic engineering promises to do in the future. Also, species in the secondary genepool, such as *Aegilops tauschii*, can now be used as readily as species in the primary genepool through the production of hexaploid synthetic wheats using embryo rescue followed by chromosome doubling using colchicine (Mujeeb-Kazi, 1995).

Table 2. Species of *Aegilops*, their genomic formula and synonyms (when available) when *Aegilops* and *Amblyopyrum* are placed within *Triticum emend.*

	Species of *Aegilops*	Genome	Species of *Triticum*
1	*Aegilops bicornis* (Forssk.) Jaub. & Spach	S^b	*Triticum bicorne* Forssk.
2	*Aegilops biunciales* Vis.	U<u>M</u>	*Triticum macrochaetum* (Shuttlew. & A. Huet ex Duval-Jouve) K. Richt.
3	*Aegilops caudata* L.	C	*Triticum dichasians* Bowden
4	*Aegilops columnaris* Zhuk.	U<u>M</u>	*Triticum* – none
5	*Aegilops comosa* Sm. in Sibth. & Sm.	M	*Triticum comosum* (Sm. in Sibth. & Sm.) K. Richt.
6	*Aegilops crassa* Boiss.	D<u>M</u>	
		DD<u>M</u>	*Triticum crassum* (Boiss.) Aitch. & Hemsl.
7	*Aegilops cylindrica* Host	**DC**	*Triticum cylindricum* (Host) Ces., Pass. & Gibelli
8	*Aegilops geniculata* Roth (*Ae. ovata*)	<u>M</u>U	*Triticum* – none
9	*Aegilops juvenalis* (Thell.) Eig	DMU	*Triticum juvenale* Thell.
10	*Aegilops kotschyi* Boiss.	SU	*Triticum kotschyi* (Boiss.) Bowden
11	*Aegilops longissima* Schweinf. & Muschl.	S^l	*Triticum longissimum* (Schweinf. & Muschl.) Bowden
12	*Aegilops neglecta* Req. ex Bertol.	U<u>M</u>	
		U<u>M</u>N	*Triticum neclectum* (Req. ex Bertol.) Greuter *Triticum recta* (Zhuk.) Chennav.
13	*Aegilops peregrina* (Hack. in J. Fraser) Maire & Weiller	SU	*Triticum peregrinum* Hack. in J. Fraser
14	*Aegilops searsii* Feldman & Kislev ex Hammer	S^s	*Triticum* – none
15	*Aegilops sharonensis* Eig	S^l	*Triticum longissimum* (Schweinf. & Muschl.) Bowden spp. Sharonense (Eig) Chennav.
16	*Aegilops speltoides* Tausch	S	*Triticum speltoides* (Tausch) Gren. ex K.Richt.
17	*Aegilops tauschii* Coss.	D	*Triticum aegilops* P.Beauv. ex Roem. Ex Schult.
18	*Aegilops triuncialis* L.	UC	
		CU	*Triticum triunciale* (L.) Rasp. (var. *triunciale*) (*T. triunciale* spp. Persicum)
19	*Aegilops umbellulata* Zhuk.	U	*Triticum umbellulatum* (Zhuk.) Bowden
20	*Aegilops uniaristata* Vis.	N	*Triticum uniaristatum* (Vis.) K.Richt.
21	*Aegilops vavilovii* (Zhuk.) Chennav.	DMS	*Triticum syriacum* Bowden
22	*Aegilops ventricosa* Tausch	DN	*Triticum ventricosum* (Tausch) Ces. Pass. & Gibelli
	Species of *Amblyopyrum*		
1	*Amblyopyrum muticum* (Boiss.) Eig	**T**	*Triticum tripsacoides* (Jaub. & Spach) Bowden

Source: van Slageren (1994).

Global wheat genetic resources and their availability

About 640,000 accessions of *Triticum* spp., *Aegilops* spp., and X *Triticosecale* (a man-made crop, a cross between wheat and rye) can be found in collections around the world (Table 3). The degree of duplication in these collections is difficult to ascertain without some type of global wheat genetic resources database. Given this situation, the level of priority that should be placed on collecting more materials is uncertain, except where

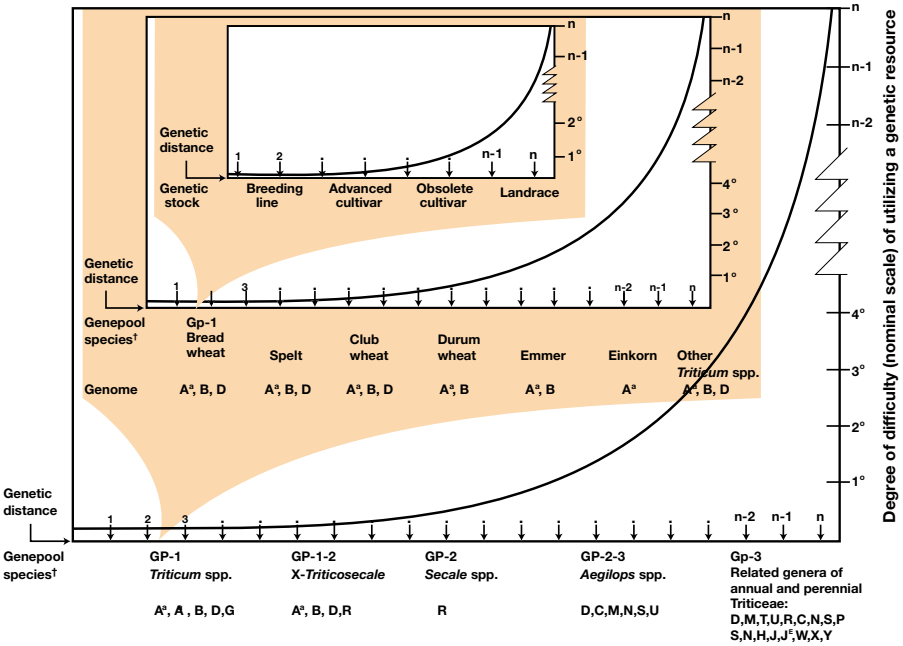

Figure 2. Schematic diagram of the effort required to transfer adaptive traits from genepools of wheat to farmers' fields.

† Not in strict phylogenetic order.

there is a real threat of genetic erosion to native species. Accessions in collections around the world may or may not be properly preserved, and some may not even be catalogued. It may thus be more cost effective to place such collections in secure storage than to collect more materials in the field. Most major wheat-producing countries have *ex-situ* collections, and genetic resources can be obtained from these collections by writing to the curator.

Table 3. Number of accessions available in collections around the world.

Type of wheat	Number of accessions
Hexaploid	266,589
Tetraploid	78,726
Diploid	11,314
Unspecified *Triticum*	252,530
Aegilops spp.	17,748
Triticale	23,659
Total	640,603

Source: Information collated from IBPGR (1990).

Conservation can be either *in situ* or *ex situ*, but most wheat genetic resources have been conserved *ex situ*. Only in the last few years has *in-situ* conservation been seriously considered; the World Bank recently supported such an undertaking in Turkey. The exception is the natural habitats in Eastern Galilee, Israel, where the Ammiad wild wheat study was undertaken in the 1980s. Shands (1991) and Hawkes (1991) summarized a symposium where the findings in this *in-situ* field laboratory were discussed. *Ex-situ* conservation of Triticeae genetic resources is easy and cost effective (Pardey et al., 1998), since they are adapted to long-term storage conditions.

The key to accessing wheat genetic resources is the development of a database, or interconnected database systems, with the capacity to manage and integrate all wheat information, including passport, characterization, and evaluation data. In the early 1990s, the CIMMYT Wheat Program established just such a strategy for integrating and managing all data pertaining to germplasm regardless of where they were generated (Skovmand et al., 1998). The goal was to facilitate the unambiguous identification of wheat genetic resources and remove barriers to handling and accessing information. The resulting database, the International Wheat Information System (IWIS), seamlessly joins the conservation, utilization, and exchange of genetic materials. The system is fast, user-friendly, and available on an annually updated CD-ROM (Skovmand et al., 2000a).

IWIS has two major components: the Wheat Pedigree Management System, which assigns and maintains unique wheat identifiers and genealogies, and the Wheat Data Management System, which manages performance information and data on known genes. Another information tool, the Genetic Resource Information Package (GRIP), was developed using IWIS for data warehousing. One of the functions of GRIP attempts to collate passport information across genebanks to identify duplications and unique genetic resources (Table 4) (Skovmand et al., 2000b).

The Genetic Resources Information Network (GRIN) and the System-Wide Information Network for Genetic Resources (SINGER) are other publicly available databases on wheat and Triticeae genetic resources. GRIN contains information about the USDA Small Grains Collection stored in Aberdeen, Idaho, and can be reached on the Internet (http://www.ars-grin.gov/) . SINGER (http://singer.cgiar.org/), developed and made available under the leadership of System Wide Genetic Resources

Program (SGRP), gives access to all FAO/CGIAR Center accessions, including wheat and other cereals.

In the 1980s there was an increasing trend towards greater application of intellectual property protection (IPP), which contrasted with the pervading attitude during the 1960s and 1970s, where IPP within the context of international plant improvement was seen as an obstacle to progress. Since then, the view that strong IPP can help maintain technological leadership has gained respectability, especially in the United States (Siebeck, 1994). Several international initiatives have resulted, such as the strengthening of the UPOV Convention in 1991, which narrowed the breeder's privilege to use protected cultivars as breeding parents. However, according to Siebeck (1994), the most significant initiative was instigated as part of the Multilateral Trade Negotiating Round in the General Agreement on Tariffs and Trade that ended in 1993. At the insistence of industrialized nations, strengthening of IPP was included as a key negotiating point. Efforts in UPOV and GATT to widen IPP on inventions and breeding technologies were paralleled by efforts to regulate international access to genetic resources.

The "International Undertaking on Plant Genetic Resources," established by FAO in 1983, was an attempt to stop genetic erosion and protect plant genetic resources. The Undertaking initially subscribed to the rule of free germplasm exchange and recognized plant genetic resources as the "heritage of mankind." However, disagreements later arose over the issue of genetic resources ownership. The idea of compensation was introduced in 1989 and modified in 1991, when FAO adopted the common heritage principle but subordinated it to "the sovereignty of states."

Unlike the FAO Undertaking, which was voluntary, the Convention on Biological Diversity (CBD) of 1992 is an internationally ratified treaty among nations. The CBD officially recognizes sovereign control by individual nations over biological diversity and resources within their territories. The convention excludes material collected before 29 December 1993, when the CBD took effect, but any germplasm collected after that date in a country that has signed the CBD comes under the provisions of the Convention. A result of the discussions on the ownership of genetic resources was the signing of an agreement between the CGIAR and FAO that places the germplasm collections held in trust by the CGIAR system under the auspices of FAO.

Consequently, plant genetic resources may not be freely available to everyone in the future but likely to be made available under some type of intellectual property rights (IPR) agreement. For example, the accessions in the CIMMYT collection that come under the FAO/CIMMYT in-trust agreement are shared under a Materials Transfer Agreement that states the accessions can be utilized but not protected by IPR. However, products derived from research and breeding with such materials can be protected, since they are deemed to be different and belong to the scientist or breeder who developed them.

Table 4. Biological status classification used in GRIP II.

GRIP code	Status
BL	Breeding Line
CV	Cultivar
LV	Landrace
X	No data
AL	Addition Line
BL	Apomixis Line
BL	Breeding Population
BL	Cross
BL	Genetic Population
GS	Genetic Stock
ML	Multi Line
MTL	Mutation Line
NIL	Near Isogenic Line
RCMS	CMS Restorer
RF	Fertility Restorer, non specific
SL	Substitution Line
TL	Translocation Line
CMS	Cytoplasmic Male Sterile
GMS	Genetic Male Sterile
RG	Genetic Restorer
MS	Male Sterile, non specific

Source: Skovmand et al. (2000b).

The Search for New Genetic Variation

A classic method of identifying new genetic variation is the recognition of potentially useful traits by experienced scientists and research staff in the course of routine maintenance activities, special studies, or as an offshoot of prebreeding and breeding exercises. This should not be underestimated, given that much of the useful novel variation deployed in cultivated crops today was recognized in this way.

Augmented use of seed multiplication nurseries

Seed multiplication nurseries can be used to characterize and evaluate germplasm collections for non-disease and non-destructive traits. Since routine seed regeneration activities have to be carried out anyway, they can be an inexpensive means of collecting data. Recent work has indicated that traditional agronomic traits (including those with low heritability) measured on small, seed-increase hillplots can be used for such purposes (Hede et al., 1999; DeLacy et al., 2000). Curators of

germplasm banks have traditionally avoided these traits, which are useful for plant improvement programs. Descriptions of germplasm based on "useful" attributes are immediately advantageous to practical plant improvement programs because they indicate where useful variation may be found in the collection.

A description based on useful attributes also allows more directed search strategies than those derived from traditional characterization attributes or random DNA markers with high heritabilities. Provided that random markers adequately cover the genome, they give information on the amount of variation at and between sites, thus indicating whether adequate collection has been done. However, until adequate linkages to known functions have been established, they, like traditional characterization attributes, provide little information on the type of variation present.

When low(er) heritability data are used, means and variances change in different seasons, years, and places. This has limited their use for germplasm description, but many, if not most, attributes useful for plant improvement programs are of this type. Much of the difficulty encountered in integrating such information from sets of data acquired at different times can be avoided by appropriate data analysis. After standardization by the range or standard deviation within sets, means and variances for each attribute are the same.

As an example, DeLacy et al. (2000) reported on an analysis of a seed multiplication nursery made up of 465 accessions of bread wheat landraces collected in 1992 from 24 sites in three states of Mexico. They were examined in unreplicated hillplots in a screenhouse for 15 morphological, agronomic, and grain quality attributes as part of the routine regeneration process conducted by the CIMMYT Wheat Genetic Resources Program. A pattern analysis (combined use of classification and ordination methods) of the data provided a good description of the accessions and collection sites (Figure 3). Since economically useful attributes were used, the analysis provided relevant information for both germplasm curators and potential users, who now have a description of the accessions from which to choose relevant breeding material.

The data were analyzed using range standardized squared Euclidean distance (rsSED) as the dissimilarity measure. These SEDs are calculated among attributes that are range standardized (Williams, 1976) and employed to ensure each attribute contributes equally to the analysis. Ordination was performed by singular value decomposition (Eckart and Young, 1936) of the Gower complement similarity measure to the rsSED (Gower, 1967; DeLacy et al., 1996). The relationships between accessions and attributes from the ordination were displayed on a biplot (Gabriel, 1971).

Both the accession and attribute plotting points can be interpreted as vectors on the biplot, but since the accessions were investigated in terms of the attributes, attributes were represented as vectors and accessions as points. As the data were centered (i.e., the attribute mean was subtracted from all values for that attribute so the grand mean becomes zero), the origin on the biplot represents average values for all attributes. The percentages on each axis represent the proportion of total variation, measured by the total sum of squares (TotSS), accounted for by each vector. In this case the aim is to represent the original 15 dimensional space defined by the 15 attributes in a low dimensional space. Since not all the variation is modeled, some distortion of the relationships among attributes and accessions will occur when depicted on the biplot.

The attribute vectors are drawn in a positive direction, i.e., in the direction of increasing value for that attribute. The length of each vector is proportional to how well each attribute was modeled, since each vector should be the same

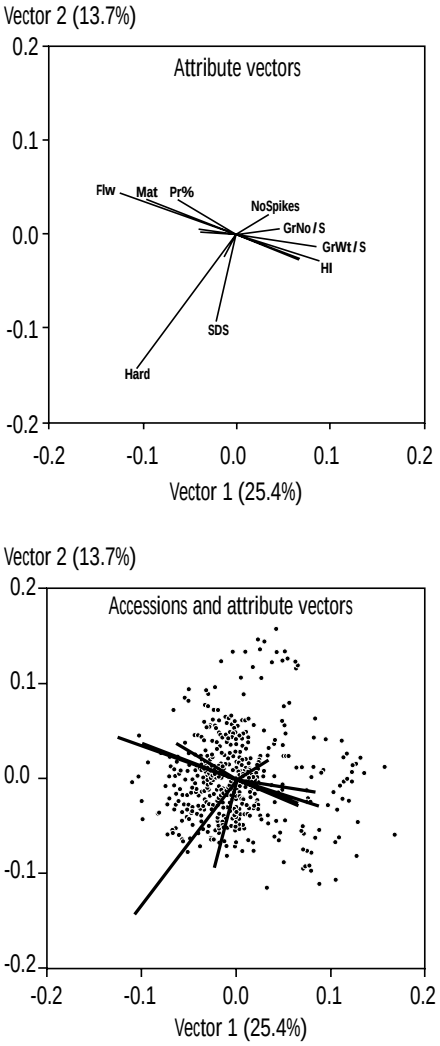

Figure 3. Attribute vectors and accession plotting points for the biplot for vectors 1 and 2 from the ordination based on 15 morphological, agronomic, and grain quality attributes of 465 individual spike accessions of wheat landraces collected from 24 sites covering four states in Mexico.

length if they were all equally well modeled. The angles of the vectors to each other in the biplot represent the phenotypic correlation between the attributes over all values of the accessions for each attribute. An angle of zero indicates a correlation of +1, an angle of 90º a correlation of 0, and an angle of 180º represents a correlation of -1. The length on the attribute vector to the point where a perpendicular dropped from the genotype plotting point to the vector is proportional to the modeled (predicted) value of that genotype for that attribute.

Grain hardness was well modeled, eight attributes (Flw, Mat, Pr%, SDS, GrWt/S, HI, GrSize, GrY; see Table 5) were modeled reasonably well, and six (SpikeS, GlumeS, FlagS, Ht, NoS, GrNo/S) were poorly modeled by vectors 1 and 2 (Figure 3). As the two dimensional representation of the 15 attribute space accounts for 39% of the original variation, some distortion will occur. Grain yield, its components (No/S, GrNo/S, GrWt/S, GrSize), and harvest index are positively correlated and highly negatively correlated with maturity (Flw, Mat), protein content of the grain, size of the flag leaf, and plant height as the two groups of attributes have vectors at close to 180^0 to each other. In contrast, the two quality attributes (Hard, SDS) and glume and spike size are positively correlated with each other (parallel vectors) but independent of the other two groupings of attributes (vectors at 90º).

Accessions plotted to the right in Figure 3 have high yield and high values for yield components, but are early maturing and have low protein percentage in the grain. Clearly those to the left are late, have higher protein percent, but are low yielding. Vector 2 separates those with hard grain and high SDS, at the bottom, from soft wheats with low SDS (Figure 3) at the top. Vector 3 separates those with high grain size, grain weight per spike, SDS, and large glume and flag leaf size (bottom) from those with low values for these attributes and which are hard and have a high number of spikes (top of graph). Hence, accessions occupying different positions in the biplots have different character combinations that can be read directly from the plots, always remembering that some distortion must occur, as not all variation is represented in the low dimensional space. Accessions in different positions on the biplot have different attribute combinations in the "description space."

Augmented use of disease or "special attribute" nurseries

Useful, low-heritability traits can be measured in trials that are routinely planted by the genetic resources program for other purposes, as well as in seed multiplication nurseries with the same value-added characteristic. Thus even nurseries planted for evaluating accessions for disease resistance can be utilized for measuring other traits, the exception being trials where disease is so severe that plants are heavily damaged or dead.

Molecular approaches for identifying useful genetic diversity

Genetic diversity from wheat's wild relatives has already been exploited through wide-crossing to improve disease resistance (e.g., Villareal et al., 1995). Useful characteristics also exist in primitive or landrace varieties, of which there are over 66,000 in the CIMMYT genebank. It would be extremely time-consuming to evaluate all these landraces, wide crosses, and wild relatives for all useful yield traits, such as those described above, in field trials (Figure 2). Potential exists for identifying the loci encoding quantitatively-inherited yield traits using QTL analysis in mapping of delayed backcross generations (Tanksley and Nelson, 1996). When molecular markers linked to traits of interest are identified, they could be used to screen uncharacterized germplasm collections for the same marker and linked alleles. These lines could then be evaluated in controlled experiments to observe how well the molecular marker is linked to phenotypic expression of useful traits. Where there are reasonable associations,

Table 5. Fifteen morphological, agronomic, and grain quality attributes measured on 465 individual spike accessions of wheat landraces collected from four states in Mexico.

Name of attribute	Abbreviation	Description
Flag leaf size	FlagS	Flag leaf length (cm)
Spike size	SpikeS	Spike length (cm)
Glume size	GlumeS	Glume length (cm)
Days to maturity	Mat	Days from sowing
Days to anthesis	Flw	Days from sowing
Height of plant	Ht	Height to tip of glume (cm)
Number of spikelets	No/S	Number of spikelets per spike
Grain number per spike	GrNo/S	Number of grains per spike
Grain weight per spike	GrWt/S	Grams
Grain size	GrSize	1000 kernel weight (g)
Grain weight per plot	GrY	Grams
Harvest index	HI	Grain weight as a proportion of total biomass
Grain hardness	Hard	Percent hardness (NIR analysis, calibrated with particle size index using 0.5 mm sieve in grinder)
Grain protein percentage	Pr%	Percent protein (NIR analysis, calibrated against Kjeldahl N x 5.7)
SDS sedimentation	SDS	Sodium dodecyl sulfate (SDS) sedimentation volume (ml/lg flour)

markers could be used to screen untapped genetic stocks, enabling new sources of genes with potentially useful alleles to be exploited in breeding.

How to use the identified traits

Genetic resources with desirable traits usually need to be tested and improved to be of use in wheat improvement (Figure 2). Most often these resources have many undesirable characteristics, such as extreme disease susceptibility, low yield, and highly specific environmental adaptation, in addition to the needed trait.

These resources therefore need to undergo prebreeding before they can be used in improvement work. Figure 4 demonstrates two prebreeding schemes, each with a different purpose: the open-parent, cyclical crossing program and a backcrossing program aimed at producing isogenic lines. These two program have different purposes and different end results; moreover, the first is progressive, while the second is unprogressive in terms of yield potential.

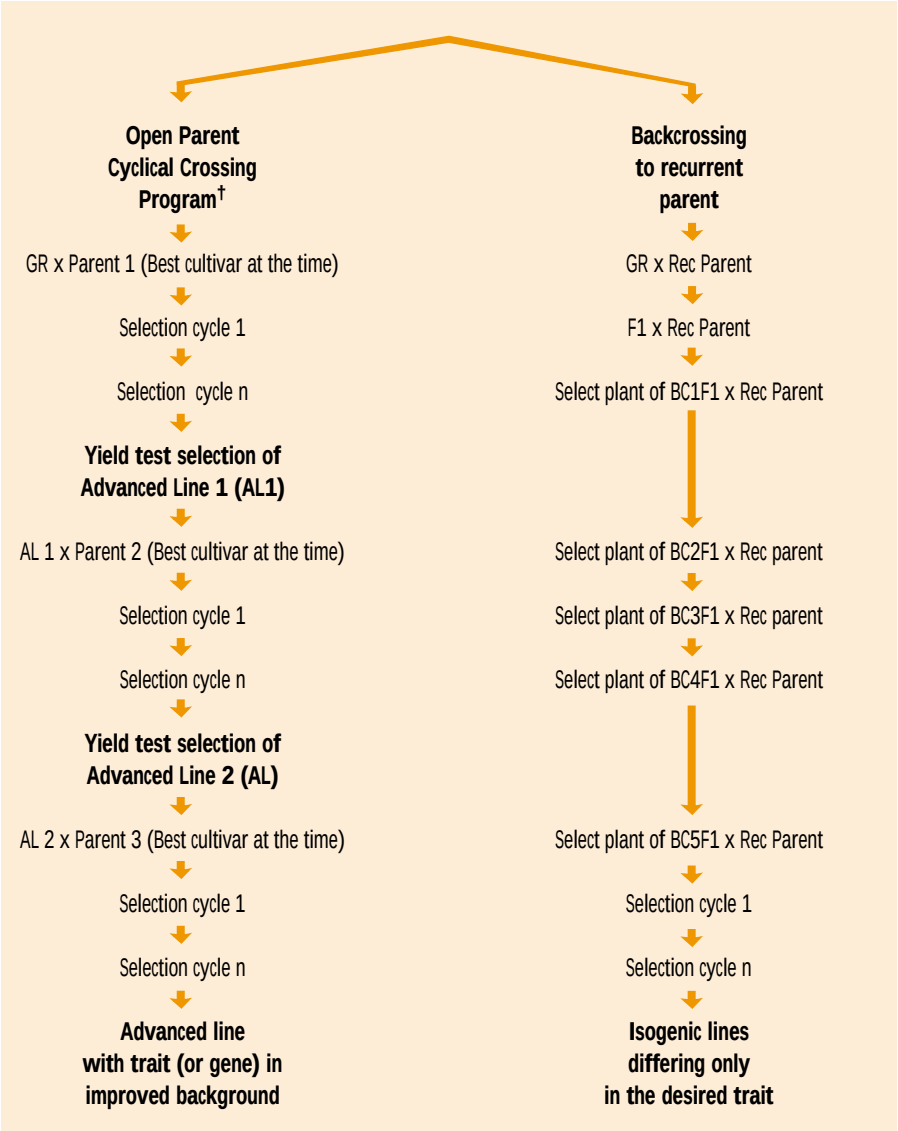

Figure 4. Utilization of genetic resources: prebreeding schemes.
† Source: Rasmusson (2001).

The open-parent, cyclical crossing program described by Rasmusson (2001) is utilized when the introgressing a trait known to be of value. Rasmusson was striving to introgress characters from two-row barley into six-row barley and found that the initial cross yielded germplasm with no putative candidates for cultivar release, with the best lines yielding about 20% less than the improved parent. The second cycle of the program, where the improved parent was the best current cultivar, produced progenies that yielded about 98% of the best parent's yield. The third cycle, again using the best current cultivar as a parent, yielded 112-119% of the checks. Using this scheme, germplasm with the desired trait is produced that could be competitive in a cultivar-release program.

A backcrossing program to generate isogenic lines is applied when the identified trait has as yet no proven value. The recurrent parent is crossed repeatedly to the genetic resource with the desired trait. In each backcross generation, selection is done for the tails of the populations, i.e., lines with the trait and lines without the trait. Lines that differ genetically only for the trait in question are the end result of this program. Additional trials can be conducted to assess the value of the trait, but bearing in mind that the germplasm produced will not outperform the recurrent parent.

Future utilization of genetic resources

As evidenced by the above, genetic resources have played a significant role in wheat improvement and will continue to do so, by providing breeders with the genetic variation they require to effect

future improvements. Variation will be needed 1) to further increase wheat's yield potential; 2) to provide new sources of disease and pest resistance and maintain the yield levels achieved so far; 3) to develop germplasm adapted to more marginal environments; and 4) to improve quality. To date the main contribution of genetic resources has been as new sources of disease and pest resistance, thanks to which achieved yield levels have been maintained.

There are few examples of genetic resources contributing to the three other objectives. One example is the dwarfing genes, especially *Rht1* and *Rht2*, that became available through the Japanese wheat Norin 10, which in turn inherited them from Shiro Daruma, a Japanese landrace (Kihara, 1983). Persistent efforts were required to transfer these dwarfing genes into a genotype of value (Borlaug, 1988; Krull and Borlaug, 1970), which illustrates the difficulty of using genes from unadapted materials. It also shows that desirable characteristics other than apparent ones may result from such germplasm, as evidenced by the fact that while incorporating strong straw to avoid lodging, Krull and Borlaug (1970) obtained better fertility and tillering capacity. It is now obvious that dwarfing genes *Rht1* and *Rht2* have a direct effect on yield over and above the benefits derived from diminished lodging (Gale and Youssefian, 1986).

A survey conducted by Cox (1991) revealed that most introductions to the United States were used to improve disease and pest resistance (Table 6). The only yield-related traits listed in the table are reduced height, stiff straw, large seed, and yield per se. No instances of improving yield in marginal environments are listed and only two where quality was improved: higher protein and gluten strength.

In another report (Fischer, 1996), traits involved in improving yield that were introduced from genetic resources are described. Erect leaf habit was introduced into CIMMYT germplasm from *Triticum sphaerococcum*, and a number of lines were developed through prebreeding. This germplasm was used in both the bread and durum wheat programs and let to the release of one bread wheat (Bacanora 88) and two durum wheat cultivars (Altar 84 and Aconchi 89).

Physiological traits are often identified as having contributed to improving yield potential, but usually in retrospective, after the germplasm has been developed. We need to be more proactive and identify potentially useful traits and then introduce the trait into the improvement program.

Traits to raise yield potential of irrigated wheat

To boost yield in irrigated situations, it is widely believed that genetic improvement must come about through simultaneously increasing both photosynthetic assimilation capacity and partitioning of assimilates to promote high grain number and growth rate (Richards, 1996). However, another way to increase grain number could be to increase the intrinsic fertility of the spike.

Multi-ovary florets is a trait being studied in CIMMYT's wheat germplasm bank. Spikes with this trait may have up to six kernels per flower (Chen et al., 1998), but individual kernel weights tend to be low. The trait is currently being introgressed into high yielding lines with good agronomic traits. Data for the F1 shows that the trait is expressed better in some backgrounds than others. However, average kernel weight of the F1s was in all cases higher than that of the multi-ovary donor and, in many cases, of both parents. Total grain weight per spike was generally higher than that of the parents (Table 7).

High leaf chlorophyll content has been identified in landrace collections: the best genotypes showed substantially greater leaf chlorophyll concentration than the check Seri-M82. While the trait does not guarantee higher leaf photosynthetic rate in all backgrounds, it

Table 6. Contributions to germplasm improvement of introduced genetic resources.

Yield potential	Cases	Resistance	Cases	Marginal environments	Cases	Quality	Cases
Reduced height	15	Strawbreaker	2		None	High protein	2
Yield	6	Powdery mildew	9			Gluten strength	1
Large seed	1	Stripe rust	4				
Stiff straw	1	Leaf rust	12				
		Stem rust	12				
		Septoria leaf blotch	3				
		Bunt	3				
		Soilborne mosaic virus	1				
		Cereal leaf beetle	1				
		Hessian fly	3				
		Snow mold	1				
		Greenbug	1				
		Wheat curl mite	1				

Source: Adapted from Cox (1991).

Table 7. Expression of the multi-ovary trait and yield components in F1 lines, wheat screenhouse, Mexico, 1999.

Yield component: Line / cross	Kernel no. / spike	Kernels / floret	Kernel wt (mg)	Grain wt / spike (g)
Multi-ovary line	124.0	2.17	37.5	4.65
Pastor	**69.3**	**1.00**	**51.5**	**3.57**
Multi-ovary line / Pastor	125.9	1.81	42.1	5.30
Pastor / Multi-ovary line	108.5	1.65	45.0	4.88
Baviacora M 92	**72.7**	**1.00**	**57.5**	**4.18**
Multi-ovary line / Baviacora M 92	84.6	1.04	62.8	5.31
Baviacora M 92 / Multi-ovary line	73.5	1.03	60.0	4.41
Esmeralda M 86	**95.3**	**1.00**	**53.0**	**5.05**
Multi-ovary line / Esmeralda M 86	91.8	1.13	59.1	5.43
Esmeralda M 86 / Yanglin	96.3	1.20	53.8	5.18

has been shown to be associated with increased leaf photosynthetic rate and higher yield in improved durum wheat cultivars grown under irrigated conditions (Pfeiffer, pers. comm.). These two findings suggest that combining higher chlorophyll content with greater spike fertility (for example, due to multi-ovary florets), which creates higher demand for photosynthesis, may help increase yield potential under irrigated conditions.

Traits to raise yield under stress conditions

Wheat yields are reduced by 50-90% of their irrigated potential by drought on at least 60 million ha in the developing world. At CIMMYT attempts are underway to improve drought tolerance by introgressing stress adaptive traits into empirically selected drought tolerant germplasm. Our current conceptual model of a drought resistant cultivar encompasses high expression of the following traits: seed size, coleoptile length, early ground cover, pre-anthesis biomass, stem reserves/remobilization, spike photosynthesis, stomatal conductance, osmotic adjustment, accumulation of abscisic acid, heat tolerance, leaf anatomical traits (such as glaucousness, pubescence, rolling, thickness), high tiller survival, and stay-green (Reynolds et al., 1999). CIMMYT's germplasm collection is being screened, as resources allow it, for high expression of many of these traits.

High stomatal conductance permits leaf cooling through evapotranspiration; this, along with higher leaf chlorophyll content and stay-green, is associated with heat tolerance (Reynolds et al., 1994). Recent studies identified high expression of these traits in bank accessions, and both traits showed high levels of heritability under heat stress (Villhelmsen et al., 2001). As a result, these accessions are currently being crossed into good heat tolerant backgrounds.

Pubescence and glaucousness protect plant organs from excess radiation under stressful conditions (see Loss and Siddique, 1994). Searches are under way for these and a number of other leaf traits, such as leaf rolling, leaf thickness, and upright posture, which may well play similar roles under stress.

Osmotic adjustment (Blum et al., 1999) and stored stem fructans (Blum, 1998) have been implicated in stress tolerance. Searches are underway for high expression of these traits among germplasm bank accessions, although laboratory protocols are required for their identification. High spike photosynthesis is another trait that could contribute to yield under stress but which is very time consuming to measure. For traits that are difficult to measure (and/or that show marked genotype by environment interaction), it is logical to develop genetic markers, which can be used to confirm their presence more unequivocally than by measuring phenotypic expression.

Conclusions

The last 30 years have witnessed an unprecedented level of international wheat germplasm exchange and the development of a greater degree of genetic relatedness among successful cultivars all over the world. The concept of broad adaptation has thus been well vindicated. However, greater genetic relatedness is seen by some as increasing genetic vulnerability to pathogens, although such vulnerability depends more on similarities in resistance genes, which may actually be more diverse now than before. Various new factors (including the growing strength of national breeding programs in the developing world and the advent of breeders' rights) should result in increased diversity among cultivars and may lead to the exploitation of hitherto overlooked specific adaptation in wheat.

This would be especially important if climate change accelerates. Just as increasing nitrogen supply and improving weed control have been almost universal factors driving wheat cultivation in the last 50 years, higher atmospheric concentrations of CO2 and global warming with resulting warmer temperatures could significantly influence breeding objectives in the next 50 years.

To boost yield in irrigated situations, spike fertility must be improved simultaneously with photosynthetic capacity. CIMMYT's wheat germplasm bank has identified a source of multi-ovary florets that have up to six kernels per flower. Other lines from landrace collections have very high chlorophyll concentrations, which may increase photosynthetic capacity. High chlorophyll concentration and high stomatal conductance (which permits leaf cooling) are associated with heat tolerance. Recent studies identified high expression of these traits in bank accessions, and both traits were heritable under heat stress. Searches are underway for drought tolerance traits related to remobilization of stem fructans, awn photosynthesis, osmotic adjustment, and pubescence.

Seed multiplication nurseries can be used for characterizing and evaluating germplasm collections for physiological traits. Characterization data can be analyzed using pattern analysis, which provides a good description of the accessions. The advantage of using these augmented seed nurseries is that cohorts of high(er) yielding lines are identified that can be used directly or examined for "new" traits. Genetic diversity from wheat's wild relatives has been exploited through wide-crossing to improve disease resistance. Further potential exists for identifying quantitative traits using QTL analysis in delayed backcross generations. Once markers linked to traits of interest are identified, germplasm collections could be rapidly screened for unique alleles at these markers.

Genetic resources are fundamental to the world's food security and central to efforts to alleviate poverty. They contribute to the development of sustainable production systems and supplement the natural resource base. Conserved germplasm is especially rich in wild crop relatives, traditional farmer cultivars, and old varieties, which together represent an immense reserve of genetic diversity. Materials conserved both *ex* and *in situ* are a safeguard against genetic erosion and a source of resistance to biotic and abiotic stresses, improved quality, and yield traits for future crop improvement. As D.C. Rasmusson recently stated (pers. comm., 2000), "a little genetic diversity goes a long way."

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CHAPTER 3

Genetic Basis of Physiological Traits

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During the past two decades, molecular tools have aided tremendously in the identification, mapping, and isolation of genes in a wide range of crop species. The vast knowledge generated through the application of molecular markers has enabled scientists to analyze the plant genome and have better insight as to how genes and pathways controlling important biochemical and physiological parameters are regulated. Three areas of biotechnology have had significant impact: the application of molecular markers, tissue culture, and incorporation of genes via plant transformation.

Molecular markers have enabled the identification of genes or genomic regions associated with the expression of qualitative and quantitative traits and made manipulating genomic regions feasible through marker assisted selection. Molecular marker applications have also helped us understand the physiological parameters controlling plant responses to biotic and abiotic stress or, more generally, those involved in plant development. This chapter discusses different types of molecular markers, the basic principles and practical considerations involved in their application in plant improvement, and some contributions they have made to wheat molecular genetics.

The Genome

Although the expression of genes can be modified by environmental factors, the nuclear genome of plant cells carries a genetic blueprint in the form of deoxyribonucleic acid (DNA) that contains information for cell maintenance and replication. The nuclear genome contains the largest amount of DNA and the highest number of genes encoded, but plant cells also contain DNA in their chloroplasts and mitochondria. Nuclear genomes of crop species are estimated to contain thousands of genes, some unique and others in multiple copies. However, the amount of DNA in the nuclear genome represented by transcribed genes is only a fraction of total DNA found in the genome.

Nuclear DNA is packaged and organized into chromosomes along with histones and non-histone proteins. The interactions between DNA and proteins play an important role in gene expression. While DNA encodes genetic information in the form of messenger RNA (mRNA), proteins are involved in the packaging of DNA and in regulating its availability for transcription. Transcribed gene products are transported across the nuclear envelope to be translated into proteins using the cellular apparatus.

Genes are distributed along the chromosomes, and the number of chromosomes a plant cell contains varies among crop species. There is considerable diversity in genome composition and organization of different organisms (Table 1). With the aid of molecular techniques, it has been possible to study and understand the organization of the nuclear genome of several plant species. Plant genome analysis encompasses genome mapping, gene tagging, quantitative trait (QTL) analysis, and synteny mapping.

Table 1. DNA content per haploid genome in different organisms.

Organism	2n	Picograms †	Mega base pairs 10^6bp / 1C	Length (cm)
E. coli	(1)	0.0047	4.2	0.14
Chloroplast (maize)	(c)	0.0002	0.160	0.006
Mitochondrion (maize)	(m)	0.0007	0.570	0.02
Arabidopsis thaliana	10	0.15	150	4.4
Oryza sativa	24	0.45	430	13.1
Triticum aestivum	42	5.96	5,700	173
Zea mays	20	2.6	2,500	75
Homo sapiens	46	3.2	3,900	102

† 1 picogram = 1 pg = 0.965 x 10^9 bp = 29 cm.

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The DNA Molecule

In higher organisms, a DNA molecule consists of a sequence of nuclear acids linked by chemical bonds. Each nucleotide contains a heterocyclic ring composed of carbon and nitrogen atoms (the nitrogenous base), a five-carbon sugar in ring form (a pentose), and a phosphate group. There are two kinds of nitrogenous bases: purines and pyrimidines. Each nucleic acid is composed of only four types of bases: two kinds of purines, known as adenine (A) and guanine (G), and two kinds of pyrimidines, cytosine (C) and thymine (T).

The nitrogenous base is linked to the pentose sugar by glycosidic bonds. When the phosphate group is added to the pentose sugar, the base-sugar-phosphate complex is called a nucleotide. Nucleotides are linked together into a chain by a backbone consisting of an alternating series of sugar and phosphate residues with the bases attached to the sugar molecules. In higher organisms, DNA consists of two strands of nucleic acids that are wrapped around each other in antiparallel form in a double helix. The sides of the two strands are composed of sugar and phosphate molecules, and the bases are inside the double helices. The two strands are held together by hydrogen bonding between the purine of one strand with a pyrimidine of the opposite strand. Base A always pairs with a T via two hydrogen bonds, whereas a G always pairs with a C via three hydrogen bonds. The composition of bases along one strand of the DNA chain is exactly complementary to its partner strand, which allows both strands to carry the same genetic information. This is essential for the self replicating capability of DNA.

The particular order of the bases arranged along the sugar-phosphate backbone is called the DNA sequence. This sequence provides precise genetic instructions for creating a particular organism with its own unique traits. The size of a genome is usually stated as the total number of base pairs in the haploid genome (Table 1).

Genes and Chromosomes

The gene is the basic physical and functional unit of heredity. Each gene is a nucleic acid sequence that carries information encoded to represent a particular polypeptide. Polypeptides provide the structural components of cells and tissues as well as enzymes for essential biochemical functions. The plant genome is estimated to comprise 20,000 to 100,000 genes.

Genes vary widely in length, often extending over thousands of bases, but only about 10% of the genome is known to include protein-coding sequences (exons) of genes. Interspersed within genes are intron sequences, which have no coding function. The rest of the genome is thought to consist of other noncoding regions (such as control sequences and intergenic regions), whose functions are still obscure. The configuration and methyation level of a DNA molecule play a role in gene expression, since expressed regions are generally characterized by a high level of methylation. Some genes have few copies; others may be present in multiple copies per haploid genome. Such repeated sequences may be present in tandem copies at a chromosomal locus or in different chromosomes dispersed throughout the genome.

The vast amount of DNA present in each plant cell is tightly packaged, with the help of histone and non-histone proteins in the nucleus, into microscopic structures known as chromosomes. Genes are scattered along the chromosomes, which vary in number from species to species. During gametic formation the somatic chromosome number is divided in half by cell division (meiosis), which ensures that the zygote (after the male and female gametes unite) will contain the same number of somatic chromosomes as the parents.

The chromosomal form of the nuclear genome varies significantly during cell division. During interphase, the chromatin in the chromosomes remains diffuse and therefore less visible under the microscope, it becomes more condensed and highly visible for cytogenetic manipulation during meiosis and mitosis. Cytological studies of individual chromosomes during metaphase through chromosome banding techniques have helped characterize and identify the individual chromosomes of the wheat karyotype. Moreover, classical cytological studies and current molecular cytogenetic techniques have aided in identifying chromosomal abnormalities and subtle interchanges.

The Wheat Genome

The numerous species of the genus *Triticum* can be classified into three ploidy groups: diploids (2n=2X=14), tetraploids (2n=4X=28), and hexaploids (2n=6X=42). Of the *Triticum* species, cultivated *T. aestivum*, known as bread wheat, is the principal commercial type, whereas *T. turgidum* (durum wheat) is principally used for making pasta. Cultivated bread wheat is an allohexaploid (2n=6X=42), composed of three distinct genomes, A, B and D. Current evidence suggests that it originated from natural hybrids of three diploid wild progenitors native to the Middle East. *Triticum urartu* Tum. is recognized as the donor of the A genome. Although *Aegilops speltoides* was considered the donor of the B genome, current evidence suggest that the real donor is either extinct or an undiscovered species belonging to the *Sitopsis* section of *Aegilops* (Pathak, 1940; Kimber and Athwal, 1972; Miller et al., 1982). *Triticum tauschii*, also

known as *Aegilops tauschii*, is widely recognized to be the donor of the D genome (Kimber and Feldman, 1987).

Among crops, wheat possesses one of the largest (about 16 billion bp per haploid genome) and most complex (hexaploid) genomes, with a high percentage of repetitive sequences (90%), which makes it quite challenging to study and manipulate at the molecular level. However, polyploids have a greater ability to tolerate loss or higher dosages of chromosomes, referred to as aneuploidy. Because of its hexaploid nature and economic importance as a food source, bread wheat is the most cytogenetically studied of the crop species. The complete range of aneuploid lines (nullisomics, monosomics, trisomics, and tetrasomics; Sears, 1953, 1954) and a great diversity of chromosome deletion stocks (Endo and Gill, 1996) have been made available in wheat. These cytogenetic stocks have been utilized in numerous studies aimed at locating genes on chromosomes and chromosome arms, as well as establishing relationships among the chromosomes of hexaploid wheat based on their origin and function.

DNA Markers

Markers are "characters" whose inheritance pattern can be followed at the morphological (e.g., flower color), biochemical (e.g., proteins and/or isozymes), or molecular (DNA markers) levels. These characters are called *markers* because they provide, although indirectly, information about the genetics of other traits of interest in a given organism. The main disadvantage of morphological markers is that they are easily influenced by the environment. In contrast, molecular markers are based on variations in genomic DNA sequences; since they are neutral, they have no phenotypic effect on the plant. The main advantages of molecular markers are that they can be numerous, are not affected by the environment, and can be scored at virtually any stage of plant development.

DNA markers can be based on restriction fragment length polymorphisms (RFLPs) or on the polymerase chain reaction (PCR) technique.

Restriction Fragment Length Polymorphisms

The RFLP technique was the first to be widely used in plant genome analysis. RFLP linkage maps of a number of species including wheat and maize have already been made. In this technique, a DNA sample taken from a particular plant is treated with restriction enzymes. Restriction enzymes recognize unique sequences in the double-stranded DNA and cleave both strands to produce numerous DNA fragments of varying length. These DNA fragments are separated, based on their size, on an agarose matrix in gel electrophoresis, denatured to make the DNA single-stranded, and then blotted onto nylon or nitrocellulose membranes using the Southern transfer technique. The DNA in the membrane is then hybridized with a probe isolated from the same, or a related, plant species, and whose chromosomal location is known. The probe, labelled with radioactivity or chemilluminiscent substances, hybridizes to complementary sequences in the fragmented DNA sample. Because of molecular differences in the plants being studied, the tagged or hybridized fragments will differ in length, which allows the samples to be uniquely characterized as molecular polymorphisms. Since the chromosomal location or "map position" of the probes is known, researchers can trace the length polymorphisms to chromosomal regions. These molecular polymorphisms or molecular markers can then be treated as any other Mendelian difference between contrasting samples.

Although the RFLP technique is time-consuming and somewhat cumbersome compared to more recent marker technologies, it is still extensively used in a wide range of crop species.

Markers Based on Polymerase Chain Reaction

Described in the early 1980s, PCR-based assays have revolutionized molecular marker assay systems. PCR-based techniques are robust, amenable to automation, and widely applied in large-scale marker development or implementation procedures.

PCR-based assays are based on an *in vitro* procedure for the enzymatic synthesis of DNA, in which two oligonucleotide primers hybridize to opposite strands flanking the region of interest in the target DNA (Figure 1). The procedure enables small amounts of specific DNA fragments (which may be mixed with large amounts of contaminating DNA) to be amplified exponentially. In a typical PCR-based assay, the "building blocks" required to synthesize a new strand of DNA are mixed with the template containing the target DNA together with primers in a tube along with thermostable DNA polymerase. They pass through cycles of differential temperatures involving template denaturation, primer annealing, and extending the annealed primers by DNA polymerase. The end result is an exponential accumulation of the target sequence, which can then be resolved on separation matrices such as agarose or acrylamide and viewed as discrete bands after staining.

Several types of PCR-based markers are being used in plant genome analysis:

- Random amplified polymorphic DNA (RAPDs)
- Sequence-tagged sites (STSs)
- Simple sequence repeat (SSR) or microsatellite
- Amplified fragment length polymorphism (AFLP)

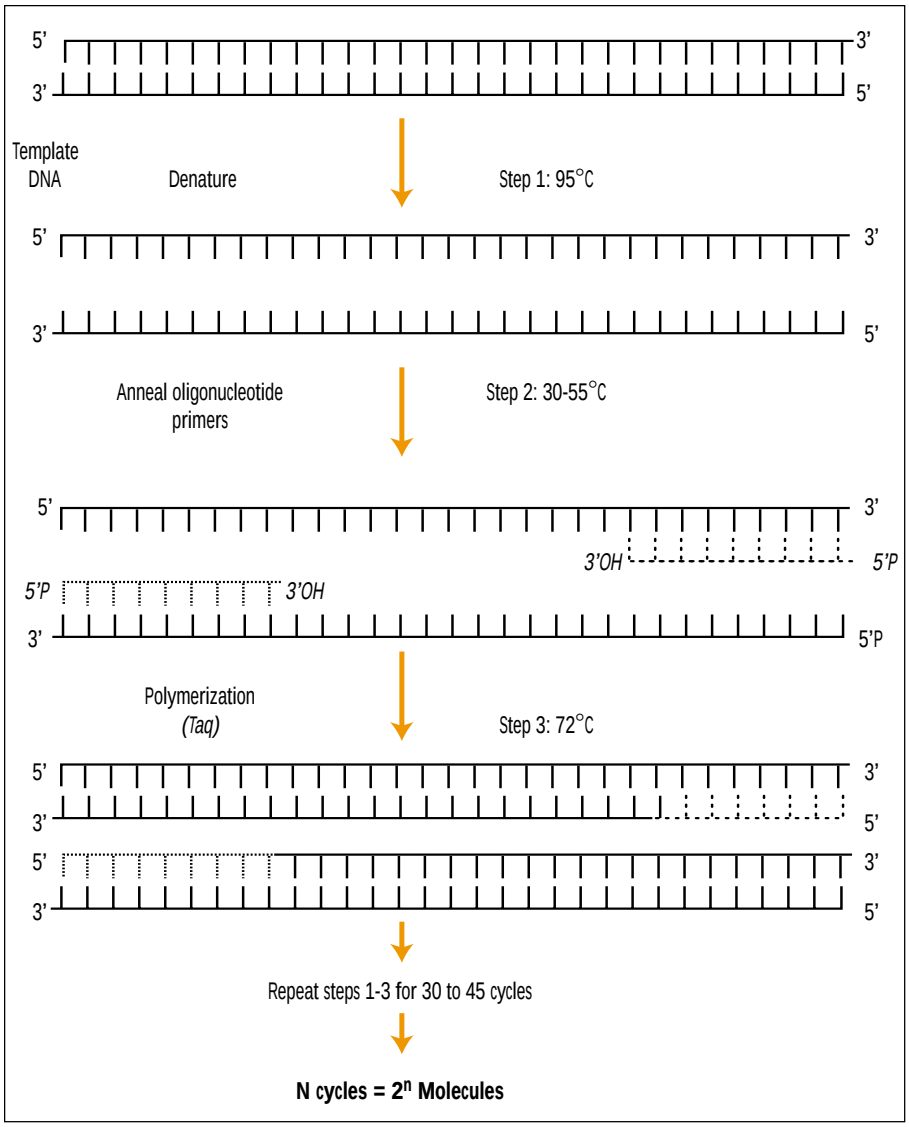

Figure 1. Polymerase chain reaction: DNA amplification.

Random amplified polymorphic DNA

As its name implies, the random amplified polymorphic DNA (RAPD) technique (Williams et al., 1990; Welsh and McClelland, 1990) is used to randomly amplify certain sequences. The primers used contain randomly synthesized oligonucleotides and are usually short (about 10 bp). RAPD polymorphisms are the result of either a nucleotide base change that alters the primer binding site or an insertion or deletion within the amplified regions. The major advantage of RAPDs is that they are suitable for all species because randomly synthesized primers (which are widely available) are not species specific. Disadvantages include the dominant nature of RAPD markers (only presence or absence of a bond, which means that the heterozygous cannot be identified), their randomness, and the resulting lack of repeatability due to non-specificity of the amplification products, specially in species such as wheat, where the genome is very large.

Sequence tagged sites

Sequence tagged sites (STSs) are mapped loci for which all or part of the corresponding DNA sequences have been determined (Olson et al., 1989; Talbert et al., 1994). This information can be used to synthesize PCR primers that amplify all or part of the original sequence. Since the primers are designed to amplify one specific locus and are longer than those used in RAPD analysis, STS assays are more robust and therefore more reproducible and reliable than RAPD analysis. Differences in the length of amplified sequences from different individuals can serve as genetic markers of the locus.

If no polymorphism is detected upon PCR amplification, the amplified fragments can be cut with restriction enzymes to observe length differences among samples, which can then be used as markers. This technique is sometimes referred to as cleaved amplified polymorphic sequences (CAPS).

The STS technique holds great promise for marker-assisted selection schemes, since it can be applied on a large scale and specific loci can be followed through successive plant generations in conventional breeding programs.

Simple sequence repeats

Simple sequence repeats (SSRs), also known as microsatellites, are composed of tandem repeats of two to five nucleotide DNA core sequences such as (AT)n, (GT)n, (ATT)n, or (GACA)n spread throughout eukaryotic genomes (Tautz and Renz, 1984). The DNA sequences flanking microsatellites are generally conserved within individuals of a given species, allowing the design of PCR primers that amplify the intervening SSRs in all genotypes (Weber and May, 1989; Litt and Luty, 1989). Variation in the number of tandem repeats results in different PCR product lengths (Figure 2). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeating units. The main

advantages of SSRs are the co-dominant nature of the observed polymorphisms (which means that homozygous A and B, as well as heterozygous AB, can be identified), the robustness of the assay, and the large number of polymorphisms observed. Their main disadvantage is the significantly high cost involved in sequencing genomic libraries in the development of SSRs.

Amplified fragment length polymorphisms

Amplified fragment length polymorphisms (AFLPs) combine the specificity of RFLP analysis with the robustness of the PCR assay and are designed to amplify a subset of restriction digested DNA (Vos et al., 1995). Usually two restriction enzymes, a rare cutter and a frequent cutter, are used in combination to digest genomic DNA (Figure 3). The DNA fragments thus generated are ligated with double-stranded adaptor sequences. The adapter-ligated fragments are subjected to two rounds of PCR amplifications. In the first round, primers complementary to the adapter sequences, plus an additional nucleotide at the 3' end, are used. A second PCR reaction is performed on the modified fragments with primers having the same sequence used in pre-amplification, plus one to three additional nucleotides, to amplify a subset of the pre-amplified DNA products. The numerous amplified sequences are sorted using high resolution electrophoresis. Differences in the length of the amplified segments are related to differences in the DNA composition of two given individuals. The primary advantage of AFLPs is the large number of fragments that can be compared per analysis.

Utility of DNA Markers

RFLP markers have been used to construct linkage maps for crop species, such as maize, tomato, and rice. Many RFLP markers with tight linkage to genes controlling economically important traits in various crop species have been identified. Once the sequence of an RFLP marker of interest is known, a PCR-based marker (STS) can be developed for large-scale screening (Ribaut et al., 1997). RFLPs are reliable markers, and the same probe can usually be hybridized on different crop genomes, making RFLP markers useful for comparative mapping studies. However, RFLP analysis requires large quantities of quality DNA, and detection of RFLPs by Southern blot hybridization may be laborious and time consuming, which may make this assay undesirable for plant breeding projects with high throughput requirements.

The invention of PCR-based assays has provided the basis for a large number of innovative methods for recognizing DNA polymorphisms among individuals, as described above. For mapping and large-scale screening, SSRs are the most desirable PCR-based markers. Once large numbers of SSRs are available that provide good coverage for a crop genome, large-scale SSR assays can be reliably performed at an early plant development stage, because: 1) a small amount of tissue is required; 2) DNA preparation is faster due to the small amount of template DNA required; and 3) large sample sizes are handled more efficiently. Moreover, SSRs are reliable, co-dominant, abundant, and uniformly dispersed within plant genomes. In July 2000, a

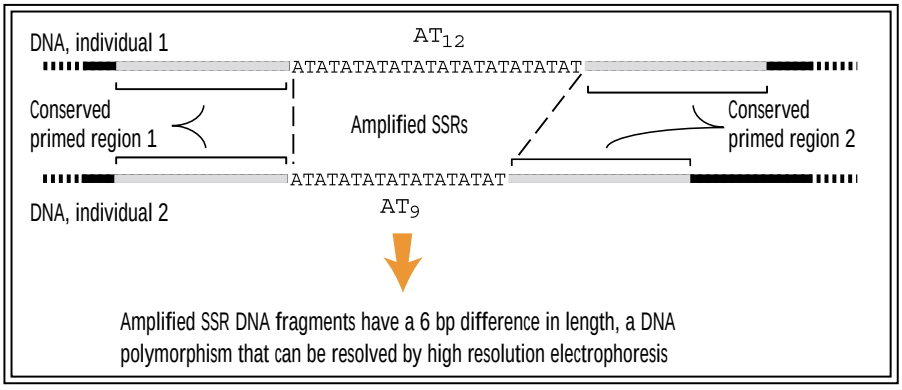

Figure 2. Example of a microsatellite: a dinucleotide repeat showing a polymorphism between two different individuals.

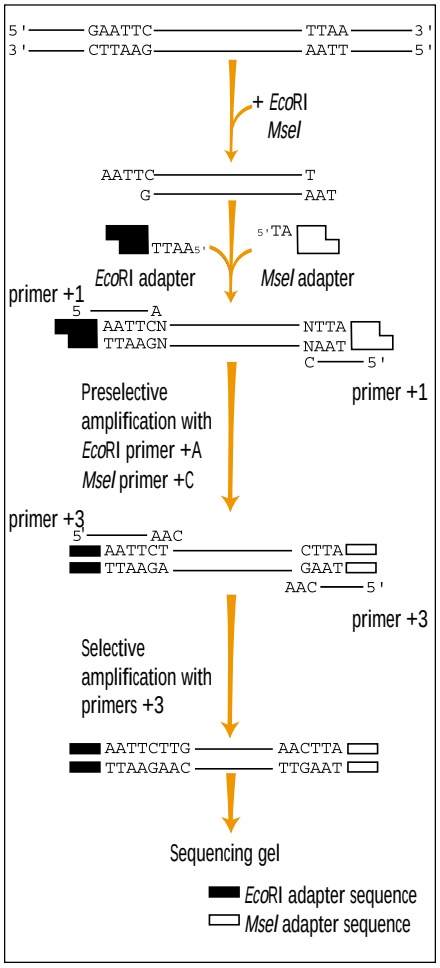

Figure 3. AFLP method.

collection of 500 SSRs became available in wheat, but 1000 to 1500 SSRs would be needed to develop a complete linkage map for QTL identification.

Geneticists today have powerful tools to conduct genomic analysis and trait dissection in crop species. The most suitable marker will depend mainly on the purpose of the investigation and the type of markers available for a particular crop (see a comparison of marker systems in Table 2).

Genomics

The newest area of investigation aimed at understanding the plant genome encompasses genome-wide approaches. "Functional genomics" can be defined as the development and application of genome-wide experimental approaches to assess gene function (Heiter and Boguski, 1997). The ultimate goal of genomics would be to characterize every gene present in a given genome. Approaches being utilized to achieve this goal are large-scale sequencing of expressed sequence tags (ESTs), large-scale functional analysis of plant genes (where thousands of DNA or RNA sequences can be analyzed on microscopic slides), and application of insertional mutagenesis or reverse genetics. These technologies are high throughput and require automation.

Innovative tools such as DNA chips and microarray have been developed to serve the new approaches. DNA chip technology provides efficient access to genetic information using miniaturized, high-density arrays of oligonucleotide probes. A set of oligonucleotides is defined, artificially synthesized, and immobilized on silica wafers or chips to construct a high-density array; each probe has a predefined position in the array. Labeled (fluorescence) nucleic acids from the analyzed plant sample are hybridized on the array, and hybridization intensities are detected by a scanner that reports quantitative assessment of RNA levels in the sample for each gene represented in the array (Lemieux et al., 1998). Microarrays are similar to the DNA chip, except that they use cDNAs (Ex. EST clone inserts). These innovative approaches are expected to provide insight into how the plant genome functions and to identify more genes involved in regulating different pathways in response to stress conditions.

Application of Molecular Markers in Plant Breeding

Conventional plant breeding is based on the selection of superior individuals among segregating progenies of sexual matings. Selection for plant improvement has largely been carried out on the whole-plant or phenotype, which is the result of genotypic and environmental effects. Although conventional plant breeding has made tremendous progress in many crop species, it is often hampered by difficulties in selecting for agronomically important traits, especially when they are influenced by the environment. Moreover, testing procedures may be difficult, unreliable, or expensive, due to the nature of the target traits or the target environment (e.g., abiotic stresses). For those reasons, selection through molecular markers might be an efficient complementary breeding tool, especially when selection is done under unfavorable conditions. If individual genes influencing target traits can be identified and associated with molecular markers, the efficiency of incorporating them into new varieties could be greatly enhanced.

Fingerprinting

A fingerprint specifically and unambiguously identifies a living organism. Identification can be achieved based on polymorphisms determined through molecular markers. In crop species, fingerprinting is a valuable tool for establishing varietal purity, which is important for varietal protection,

Table 2. Characteristics and usefulness of different types of molecular markers in wheat molecular genetics.

	RFLPs	RAPDs	SSRs	STSs	AFLPs
Fingerprinting	++	-/+	+++	+	+++
Genetic diversity	++	+	++	+	++
Qualitative gene tagging	++	++	++	++	++
QTL mapping	++	-/+	++	++	++
MAS	+	-	+++	++	+/++
Comparative mapping	+++	-	-	+	-
Types of probe/primers	gDNA, cDNA	Random 10-mer oligonucleotides	Specific 16-30 -mer oligonucleotides	Specific 20-25 -mer oligonucleotides	Specific adapters and selective primers
Level of polymorphism	Medium	Medium	High	Medium	High
Inheritance	Codominant	Dominant	Codominant	Codominant	Dominant/co-dominant
Technical difficulty	Medium	Low	Low	Medium	Medium/High
Reliability	High	Low	High	High	Medium/High

Source: Modified from Rafalski and Tingey (1993).

currently a concern for the commercial seed industry and public breeding enterprises. Fingerprinting can also be used to estimate the genetic diversity of a set of cultivars or landraces and to establish phylogenetic relationships for evolutionary studies. Other applications of molecular fingerprinting include:

- genomic characterization and identification for propriety purposes;
- identification of superior alleles in genebank accessions (e.g., landraces);
- identification of duplications within genebank accessions to ensure the best use of available resources;
- correlation of genetic diversity with heterotic patterns.

Fingerprinting studies have been reported for specific wheat germplasm using sets of DNA markers including RFLPs, microsatellites, and AFLPs (Barrett and Kidwell, 1998; Bohn et al., 1999; Fahima et al., 1998). The genetic distance among accessions can be evaluated based on fingerprinting. This information allows better characterization of genetic relationships among accessions (e.g., establishment of gene pools) and can be used to identify parental lines with good allelic complementarity.

Genetic Mapping of Target Traits

Genetic dissection of a target trait can be defined as identifying and characterizing the genomic segments or genes involved in its phenotypic expression. Before genetic manipulation using molecular markers, genes or quantitative trait loci (QTLs) must be identified and characterized via a two-step process: 1) construction of a suitable segregating population by crossing two parental lines contrasting for the target trait(s), and 2) identification of markers closely linked to the gene(s) of interest for further allelic manipulation (for a summary of the process, see Figure 4). This provides useful information, such as:

- the number of genes or QTLs significantly involved in the expression of the target trait;
- the effect (additivity, dominance) of the identified genomic regions and their impact on phenotypic expression of the trait;
- the stability of gene expression across environments (QxE); and
- the presence of pleiotropic effects at some target genomic regions.

Unfortunately, the evaluation of epistatic effects remains difficult, due mainly to the reduced number of genotypes used for this kind of genetic dissection.

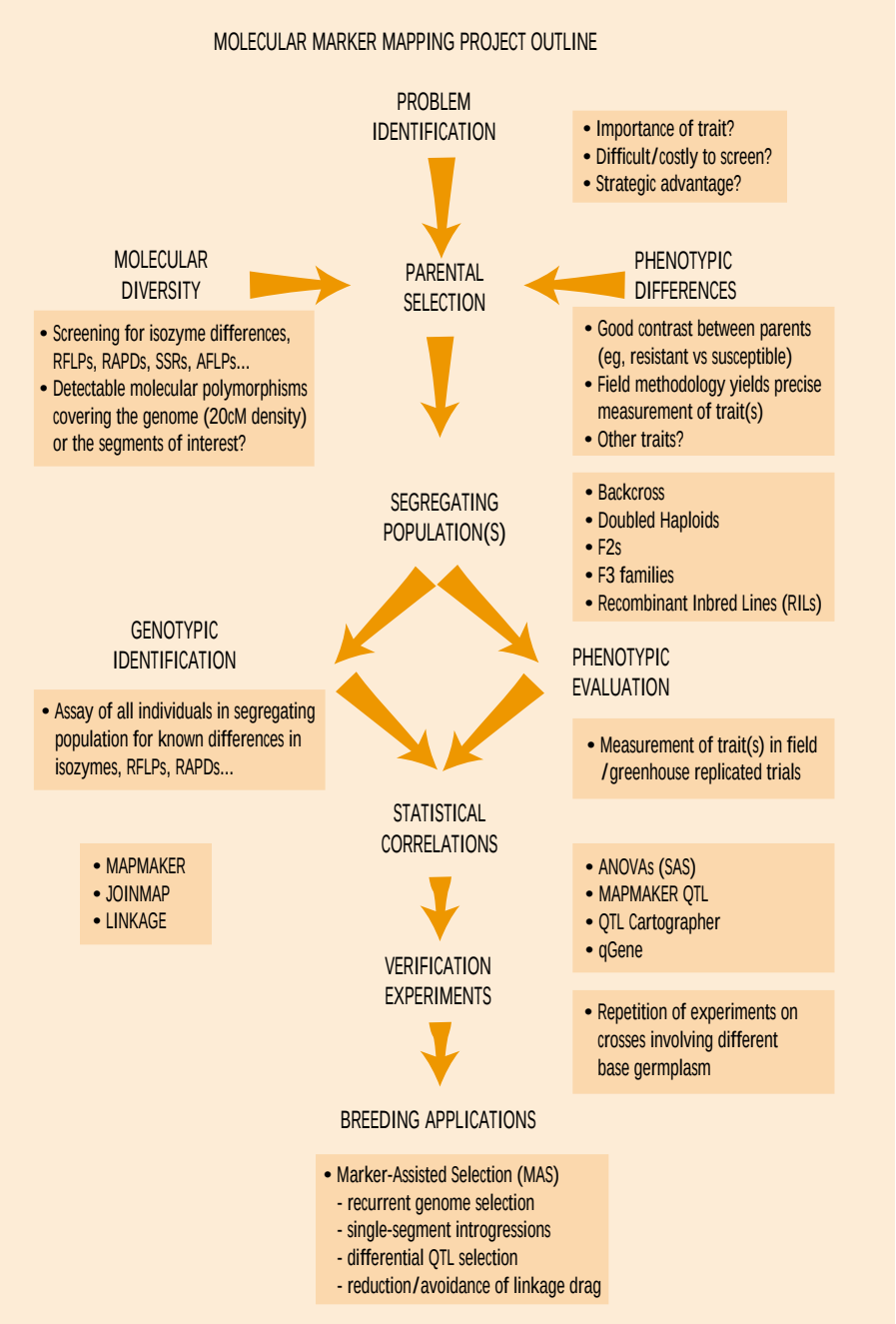

Figure 4. Description of how molecular markers can be used in genetic linkage mapping.

Germplasm for molecular analysis

Segregating populations. The most commonly used materials for genetically dissecting and mapping traits are segregating populations descended from two varieties showing divergence for the target trait(s). Effective marker identification in populations segregating for target traits depends upon laboratory data on the allelic composition of molecular markers at genomic locations distributed evenly within the genome and field evaluation of the trait(s). Based on both types of data, statistical procedures are used to find associations between markers and traits. When target traits are governed or influenced by several genetic factors, a genetic linkage map of the complete genome must be developed and a QTL analysis conducted to associate traits with markers over the complete genome. If the target trait is influenced by one or a few genes, lines can be classified for the trait and bulk segregant analysis used to associate trait alleles to molecular markers.

To develop a complete linkage map, genetically stable populations are advanced through several recombination cycles using self-pollination. In wheat, recombinant inbred lines (RILs) are best suited for such analyses, but a doubled haploid population can be developed to obtain stable, completely homozygous lines for marker analysis and field evaluations. In both cases the size of the segregating population has to be carefully considered, since populations that are too small will not allow precise gene characterization, specially when mapping quantitative traits, and large populations will consume resources unnecessarily. To genetically dissect a polygenic trait, a RIL population of about 200 to 300 families is considered suitable, but the number can be reduced if the trait is controlled by major genes.

Genetic stocks. The allohexaploid nature of bread wheat has significant disadvantages, but also some advantages in genetic analysis. For example, the presence of more than one set of genes allows wheat to tolerate the loss of complete chromosomes or chromosome arms (aneuploidy). Wheat's ability to tolerate aneuploidy has resulted in numerous genetic studies aimed at locating genes to chromosomes. Once genes are located on chromosomes, it is possible to produce detailed linkage maps for individual chromosomes and associate genes with markers. The chromosome constitution of commonly occurring wheat aneuploids is shown in Figure 5.

Aneuploids that lack a complete chromosome or a chromosome arm have in recent years become extremely important in locating biochemical or molecular markers to chromosomes. Initial screening of a new marker against a set of wheat lines, each lacking a different chromosome, will determine the marker's chromosomal location since in the absence of the chromosome carrying the gene, the marker will not be expressed. Although nullisomic (2n = 6x = 40 = 20") plants would be ideal for this analysis, they are difficult to maintain due to their low fertility; consequently, compensating nullisomic tetrasomic lines are utilized in which the absence of one chromosome is compensated for by two extra doses of a related homoeologous chromosome (Sears, 1953).

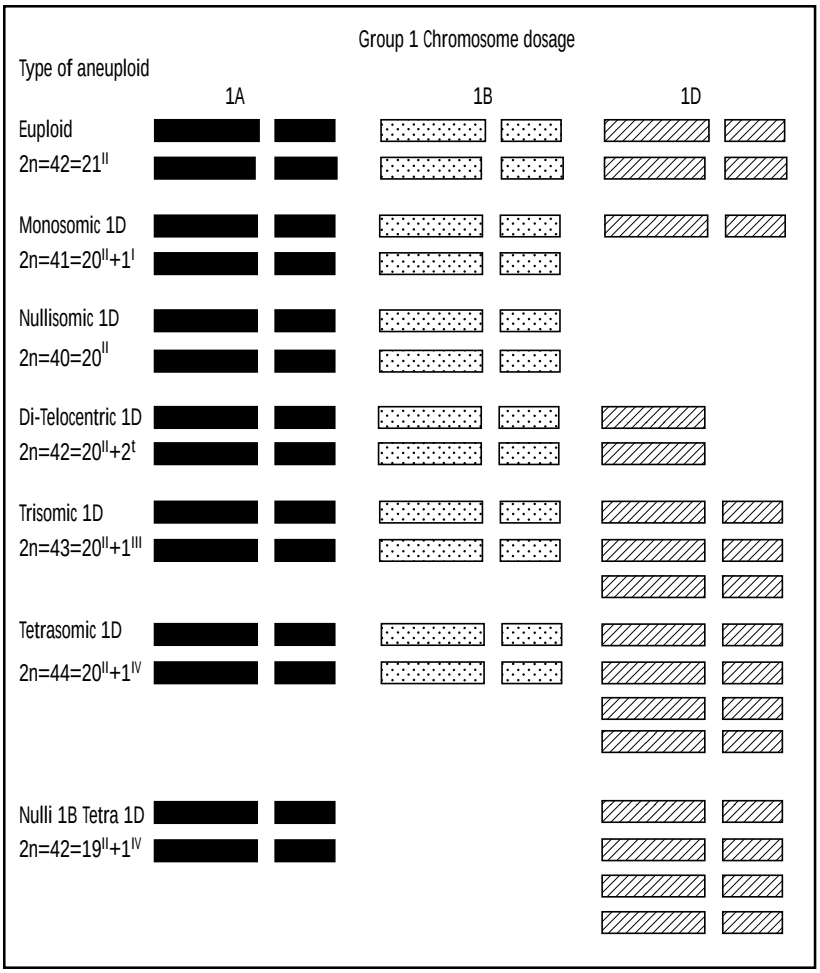

Figure 5. Genetic stocks in wheat.†

† Chromosome constitution assumes complete sets of groups 2-7 chromosomes.

Genetically stable stocks should be used where available to locate complex traits to individual chromosomes. The most suitable stocks are single-chromosome intervarietal substitution lines developed to introduce individual chromosomes from a donor variety into the genetic background of a recipient variety.

To develop intervarietal substitution lines, a series of plants of the recipient variety is needed in which the dosage of individual chromosomes has been reduced from 2 to 1. Known as monosomics, plants missing a single chromosome (2n = 6x = 41 = 20~i x 1~) are the most commonly occurring aneuploids. About 70 monosomic series are now available in different varieties worldwide (Worland, 1988). For simply inherited characters, monosomic series can be used to locate genes using test-cross procedures such as monosomic analysis (Sears, 1953), reciprocal monosomic analysis (McKewan and Kaltsikes, 1970), or backcross reciprocal monosomic analysis (Snape and Law, 1980). For more complex characters, monosomics are used as a base for developing intervarietal substitution lines by backcrossing individual chromosomes from a donor variety into the background of a recipient monosomic (Figure 6a) (Law and Worland, 1973). Once developed, intervarietal substitution lines are stable and true-breeding, and ideal for genetic analysis. By screening a complete series of 21 chromosome substitution lines any gene or trait can be readily located to individual chromosomes (Law and Worland, 1996).

Once the chromosomal location of a gene or trait has been determined using intervarietal substitution lines, these can be used to develop extremely precise genetic stocks known as single-chromosome recombinant lines (Law, 1966; Law and Worland, 1973). These lines are developed by initially producing an F1 between the critical substitution line and its recipient variety. In this F1, recombination is restricted to the single critical chromosome in an otherwise genetically homozygous background. Products of recombination of the critical chromosome are then fixed by crossing the recombining F1 plant onto a plant of the recipient variety monosomic for the critical chromosome. Monosomic progeny are extracted from the backcross and selfed to permit selection of disomic plants carrying a homozygous recombined chromosome (Figure 6b).

An alternative method of fixing recombination products is to pollinate the F1 between the recipient parent and the substitution line with maize pollen to produce haploid progeny that can be doubled with colchicine. Normally about 100 single-chromosome recombinant lines would be produced for the critical chromosome. The lines can then be classified for the trait under investigation in replicated field or growth room experiments. The trait allele can be readily associated with molecular markers by screening the recombinant lines with markers known to be polymorphic between the two parents and located on the critical chromosome.

Linkage map

Construction of a linkage map. During linkage map development, polymorphic molecular markers are used to genotype a segregating population. By statistically evaluating segregating marker alleles and linkages among different marker alleles from previous studies, markers can be placed in "linkage groups." When marker locations in the genome are known (e.g., RFLPs or SSRs), linkage groups can be assigned to chromosomes. When the genome of a crop species has adequate coverage with markers, the number of linkage groups observed should match the number of haploid chromosomes in the genome (i.e., the maize linkage map should have 10 linkage groups, that of wheat should have 21, etc.). Although constructing a linkage map is necessary for identifying genes controlling quantitative traits, a full linkage map is not always required to identify genes and associate them with markers when the target trait is regulated by major genes.

Principles of linkage map construction. To construct a linkage map, first potential parental lines are screened using molecular markers (one or a combination of molecular markers mentioned earlier) to identify DNA polymorphisms between the two. Once a suitable number of markers has been identified, they are used to determine the allelic composition for all genotypes in the segregating population. The segregation of a marker in a given population depends on the type of population. Segregation ratios are based on Mendel's first law of independent assortment. In an F2 population, a dominant marker should segregate 3:1, whereas a codominant marker that would allow the identification of heterozygotes should segregate 1:2:1. If a recombinant inbred line (RIL) or a doubled haploid (DH) population is used, segregation ratios should be 1:1 irrespective of whether the marker is dominant or co-dominant. A Chi-square test can be performed to determine the nature of the segregation of a marker.

After a number of markers has been genotyped across the population, the linkage among them is determined taking into account Mendel's second law of independent assortment (Figure 7). Table 3 presents the expected segregation for two unlinked loci in different populations. If the two loci are linked, significant deviations from the expected segregation ratios can be observed and confirmed statistically by performing Chi-square tests. If the linkage is confirmed, the frequency of recombination between the two loci can be calculated to establish the genetic distance between the two

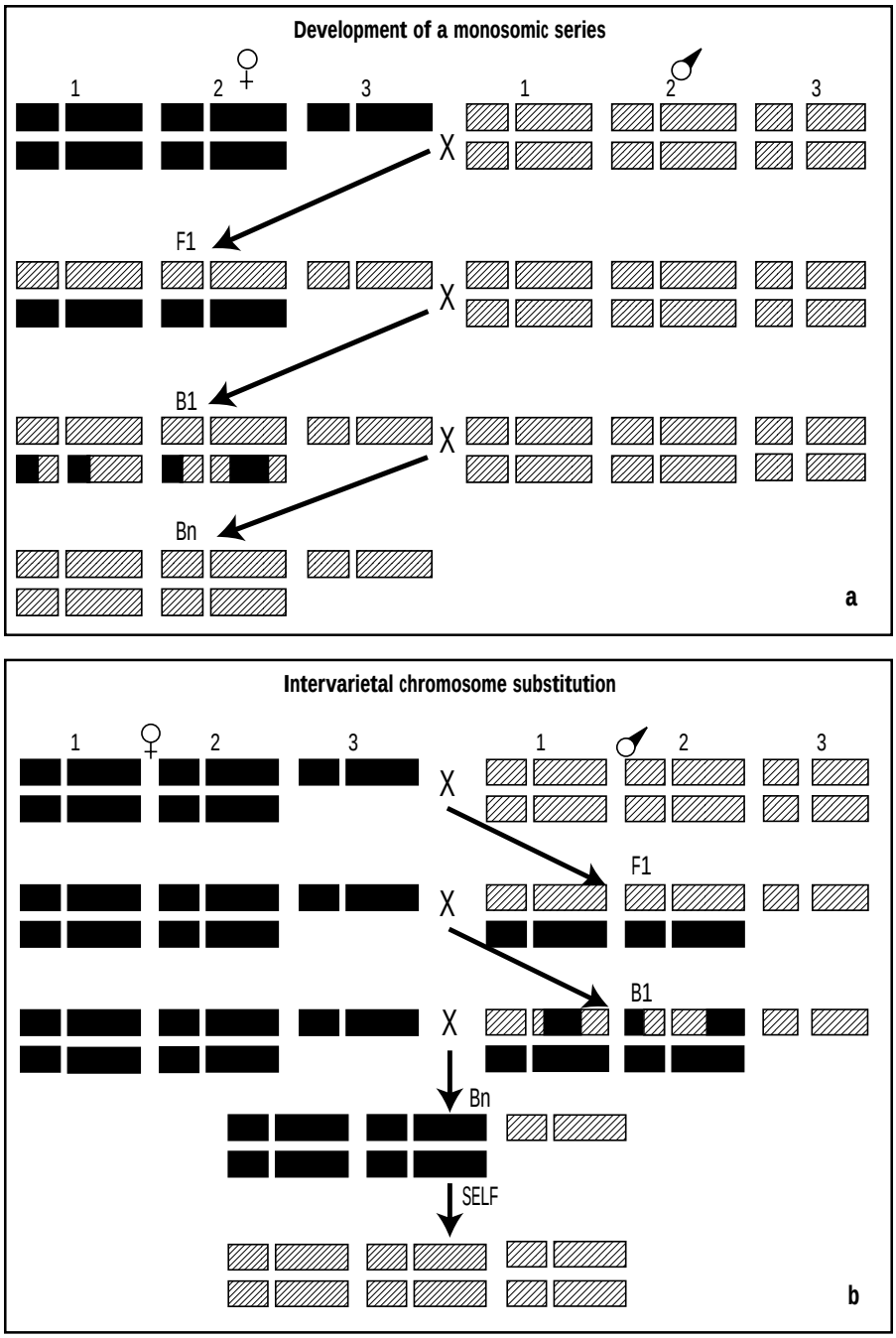

Figure 6. (a) Simplified scheme showing wheat with only 3 of its 21 pairs of homologous chromosomes. By repeatedly backcrossing the donor variety onto the recipient monosomic and selecting monosomic progeny after each backcross, a line monosomic for chromosome 3 is developed in the donor variety. (b) Chromosome 3 of a donor variety is introduced by backcrossing into the recipient variety. Initially the donor variety is crossed onto a chromosome 3 monosomic line in the recipient variety. Monosomic progeny are selected after each cross and backcrossed repeatedly onto the recipient monosomic to reconstitute its genetic background.

markers. When a large number of markers has been screened across a population, it is not feasible to use conventional statistical parameters such as Chi-square tests or computing recombination frequencies to establish linkage among markers. Furthermore, the presence of one recombination event between two adjacent loci would decrease the probability of another recombination event in adjacent loci. Computer programs that take into account all statistical parameters are available for use in linkage map construction.

Gene/QTL identification

Once the genetic map has been constructed, the next step is to find out if marker segregation within the population is associated with segregation of the target trait(s). Effective mapping studies aimed identifying molecular markers associated with target traits depend on two types of data: laboratory data on marker segregation and field data on the segregation of the trait(s). For example, in a population of RILs segregating for disease resistance, if a marker segregates in such a way that when a particular allele of the marker is present in a line, and that line shows disease resistance, a strong association between the marker allele and the trait can be inferred. In other words, the molecular marker has tagged the gene involved in the expression of resistance.

Phenotypic evaluation. Regardless of the type of data being evaluated, the quality of the phenotypic data is crucial for the success of gene/QTL analysis, since laboratory data on marker segregation within a population has to be correlated with field data to identify the QTLs. Therefore, phenotypic evaluation, whether in the field, greenhouse, growth-chamber, or laboratory, must be carefully planned and conducted with adequate replications to reduce the error. To evaluate a segregating population in the

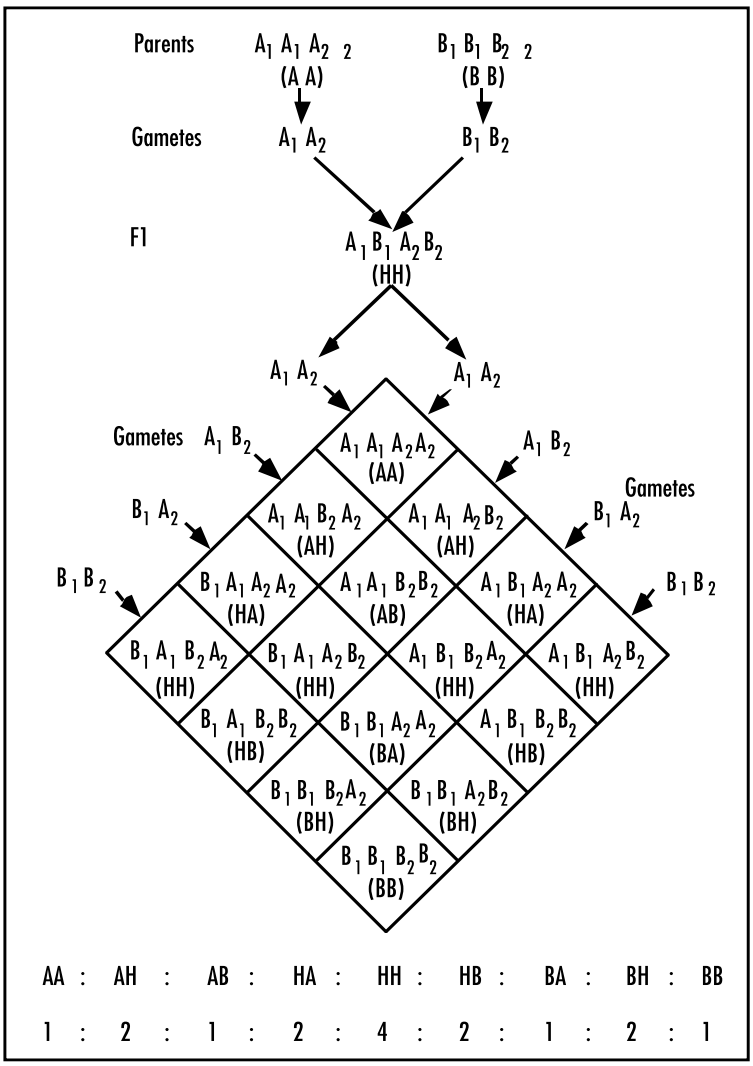

Figure 7. Expected genotypic classes for two codominant independent loci in an F_2 population.

Table 3. Expected allelic segregation of two unlinked loci in various populations.

Population	Dominant markers	Codominant markers
BC_1F_1	1:1:1:1	1:1:1:1
F_2	9:3:3:1	1:2:1:2:4:2:1:2:1
RIL	1:1:1:1	1:1:1:1
DH	1:1:1:1	1:1:1:1

field, we strongly recommend the use of field designs that include several replications, an alpha (0,1) lattice being the most commonly used to produce phenotypic data for QTL analysis. The accuracy of the protocols used in data collection is also very important.

Except for monogenic traits, phenotypic evaluation should not be conducted on a single-plant basis, but always on several plants of a family representing a given genotype, whether it be an F3 family representing an F2 genotype or several plants of a RIL family which, by definition, have the same genotype. As an example, to identify genes involved in the expression of osmotic potential in a segregating population for drought tolerance, the following must be determined:

- at what vegetative stage should leaf tissue be harvested;
- how to harvest genotypes at the same vegetative stage when they might segregate for precocity;
- what time of day to harvest, since temperature changes will induce changes in plant-water status;
- what kind of plant tissue is most suitable for the analysis;
- how to harvest many samples in a short period of time;
- how to avoid changes in water content of the tissue sample between harvest and cell sap extraction;
- how to extract cell sap from different tissue samples in a reproducible fashion;
- how often the osmometer should be calibrated to obtain reproducible measurements;
- how many replicates are needed to ensure, for example, increased accuracy.

Depending on the type of physiological test (e.g., hormone quantification), the number of samples that can be reasonably analyzed might be limited. In this case, the size of the segregating population has to be carefully considered. In a small population (e.g., fewer than 100 F2 plants or than 60 RILs), only genomic regions expressing a large percentage of phenotypic variance might be reliably identified. The complexity of taking

accurate measurements of most physiological traits makes identifying markers linked to genes involved in physiological responses more attractive.

Bulked segregant analysis (BSA). When a trait is regulated by a major gene, bulked segregant analysis (BSA) might be useful for identifying the location of the target genomic region (Michelmore et al., 1991). Bulked segregant analysis has been used to identify DNA sequences linked to a target region in several crop species (Michelmore et al., 1991; Eastwood et al., 1994). Any segregating population originating from a single cross can be used for BSA. Bulked segregants can be made for any locus or genomic region once the segregating population has been constructed.

Phenotypic distribution within a segregating population should indicate whether the trait is regulated by one or a few major genes (e.g., 1:3 distribution) or several minor genes (normal distribution). When the target trait is regulated by major gene(s), the two tails of the distribution can be safely identified through careful phenotypic selection; this material would be suitable for BSA (Figure 8).

The BSA method involves screening two pooled DNA samples from individuals with contrasting traits from a segregating population originating from a single cross. Each pool, or bulk, contains individuals selected to have identical putative genotypes for a particular genomic region (target locus or region) but also random genotypes at loci unlinked to the selected region. Therefore, the two bulked DNA samples differ genetically only in the selected region and present random allelic segregation for all other loci. For example, if markers are to be identified for disease resistance, equal amounts of DNA from the 5-10 most resistant individuals are bulked and taken as a "resistant" pool. Similarly, DNA from the 5-10 most susceptible individuals from the same population is bulked and treated as the "susceptible" pool. The two pools contrasting for the trait are then analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools would most likely be genetically linked to the loci determining the trait used to construct the pools.

Once polymorphic markers for the two pools have been identified, the linkage between a marker and the target locus is confirmed and quantified using the segregating population from which the bulks were generated. It is often necessary to find the marker's genomic location, which also establishes the genomic location of factors controlling the trait of interest. If RFLPs or SSRs are used in the BSA, their genomic location is often known. However, if markers such as RAPDs or AFLPs are used, their genomic locations have to be established using several approaches. If these markers segregate in another population for which a linkage map has already been developed, map locations can be established using this secondary population. Using single-chromosome intervarietal substitution lines is another alternative.

The last and most tedious alternative is to develop a complete linkage map for the cross from which the bulks were

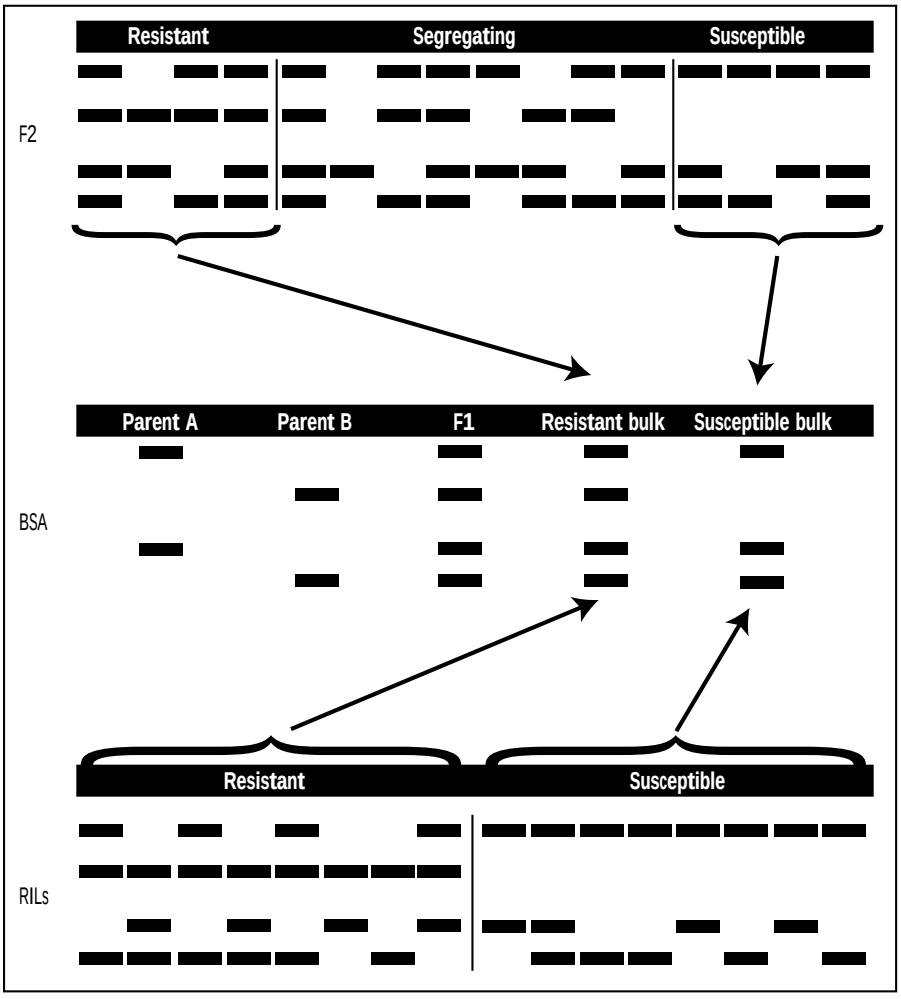

Figure 8. Bulk segregant analysis.

generated. If the linkage between the marker of interest and the target gene is confirmed using the population, the marker may be used for marker-assisted selection. The success of the approach will depend on 1) the genetic divergence between the parents in the target region, 2) the accuracy of phenotypic observations, and 3) the number of major genes involved in the expression of the target trait.

Identification of QTLs. For polygenic traits, phenotypic distribution within a segregating population is usually normal, which implies that several genes are involved in the expression of the target trait, each of them expressing a portion of total phenotypic variance. Bulked segregant analysis is not normally appropriate when target trait(s) are governed by several genes; in this case, constructing a complete linkage map is preferable. If the linkage map is constructed using DNA extracted from F2 plants, field evaluation can be conducted on F3 families derived by self-pollinating each individual F2 plant. Once the linkage map is constructed and phenotypic evaluation conducted, phenotypic correlations are commonly used to associate markers with traits and to genetically dissect complex traits into Mendelian factors. Computer programs are used to assess the correlation between phenotypic values of different genotypes within the segregating population and the allelic composition at each loci used to produce the linkage map. If this correlation is statistically significant at a given locus, the genomic region is assumed to be involved in the expression of the phenotypic trait (Figure 9). The statistical packages used in this procedure can be as simple as an F test or as complex as composite interval mapping.

It is not our purpose in this chapter to describe in detail the different mathematical approaches to QTL identification. However, commonly used approaches can be divided into three categories: simple correlation test, simple interval mapping (SIM, Lander and Botstein, 1989), and composite interval mapping (CIM, Zeng, 1994), and will be described briefly.

The simplest test to identify if there is any statistically significant association between the markers and a phenotypic data is a t –test (2 variables: homozygous parent 1 and homozygous parent 2) or an analysis of variance (F test, 3 variables: homozygous parent 1, homozygous parent 2 and heterozygous). The statistical test is conducted on each molecular marker independently to identify markers associated with the trait.

In SIM, which employs computer programs such as mapmaker/QTL software (Lander and Botstein, 1989) using mixture models and maximum likelihood techniques, a test value can be attributed to each cM on the linkage map. Therefore, a QTL peak (i.e., the point where the highest level of statistical significance is obtained) can be identified at any point on the map, not at a specific marker position, as in the previous approach. The SIM procedure differs from the previous approach in that it considers more than one marker at a time. Although SIM is a more "integrated" method than a simple correlations test, its major limitation is that it does not identify QTLs when they are linked together. For two linked QTL in coupling phase (favorable genetic contribution from the same parental line at two QTLs), SIM will identify only one QTL covering a large chromosome segment and overestimate its impact on trait expression. When two linked QTLs are in repulsion (favorable genetic contribution from different parental lines at two QTLs), SIM may not identify any of the QTLs.

Composite interval mapping, the third approach, takes into account the limitations of SIM (mentioned above). It considers markers as cofactors and has three phases:

- Unlinked markers close to QTL peaks (one marker per QTL) are identified using SIM analysis.
- The analysis is conducted again, but using the identified markers as cofactors to reduce residual variation throughout the genome, thereby eliminating false positive QTLs and identifying "new" minor QTLs.

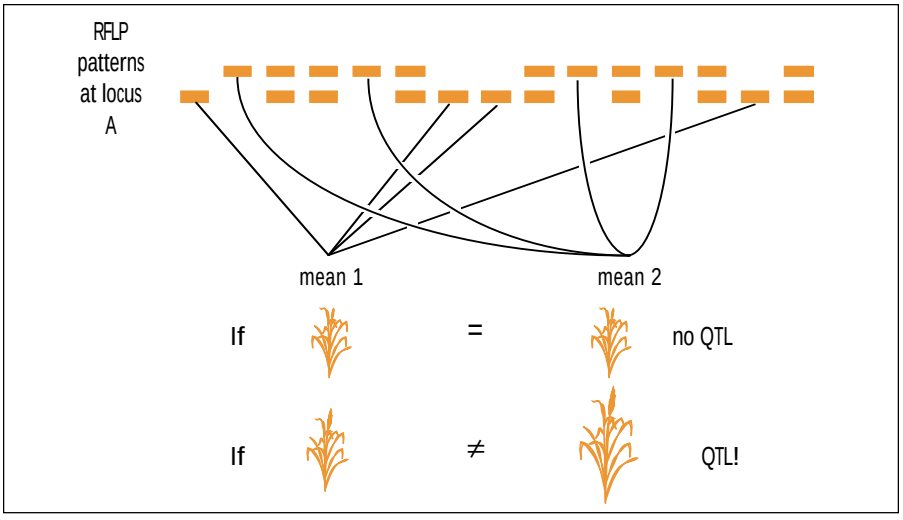

Figure 9. Illustration of a t-test for QTL detection at one RFLP marker.

- At all intervals throughout the genome, markers flanking a tested interval are used as cofactors to block the effects of possible QTLs linked to the interval of interest. The chosen distance between the tested interval and a cofactor is defined as a "window" for testing for the presence of a QTL.

When conducted on a detailed linkage map, CIM allows more precise identification of a QTL in the genome and better identification of coupled QTLs. In addition, it allows analysis of individual field data sets, as well as analysis of combined phenotypic data from different environments (locations, years, or treatments), and therefore also evaluates the QTL by environment interaction (Q x E). However, accurate evaluation of Q x E interactions, which requires top quality molecular and phenotypic data, remains a major constraint for validating marker-assisted selection (MAS) experiments. Moreover, even with new approaches like CIM, there remain clear limitations in evaluating epistatic effects between different regions of a genome.

Characterizing a QTL involves finding its precise location on a linkage map, determining the percentage of phenotypic variance expressed by each QTL independently or by several QTLs together, and quantifying the genetic effect (dominance and additivity) per QTL. Usually, a QTL is defined as major when it explains more then 30% of phenotypic variation. Although major QTLs may be involved in the expression of disease resistance, grain quality, or tolerance to abiotic stresses such as aluminum tolerance, they do not usually regulate the expression of very complex traits such as yield in water limited environments.

Progress in wheat molecular genetics

Use of molecular markers for mapping and gene identification. Progress in gene identification and marker development has been slow in wheat due to its hexaploid nature and the large size of its genome. However, in the recent past, a significant number of genes involved in various functions have been mapped to specific wheat chromosomal regions. Characterizing genes that control flowering in wheat has benefited from chromosome manipulations involving aneuploidy as well as molecular markers.

Using intervarietal chromosome substitution lines and single-chromosome recombinant line populations, genes controlling vernalization response *Vrn1* and *Vrn3* have been located on the long arms of chromosomes 5A and 5D, respectively (Law et al., 1976), and *VrnB1* on chromosome 5B (Zhuang, 1989). Similar procedures have been utilized to identify genes controlling photoperiod response (*Ppd* genes) (Worland and Law, 1986). Plant height, important for determining adaptation and yield in wheat, is genetically complex; so far about 21 genes have been identified to be associated with this trait (McIntosh et al., 1995). A microsatellite marker has recently been developed that is linked to *Rht8* (Korzun et al., 1998).

Efforts have been made to genetically dissect complex physiological traits associated with drought tolerance such as accumulation of abscisic acid in rice and to investigate possible relationship between rice and wheat homeologous loci controlling abscisic acid accumulation (Quarrie et al., 1997). Using single-chromosome recombinant line populations and mapping, Quarrie et al. (1994) located a genetic factor controlling drought-induced abscisic acid production on the long arm of chromosome 5A in wheat. Molecular genetic tools have also been used to study complex traits such as carbohydrate metabolism and the association between abscisic acid concentration and stomatal conductance (Prioul et al., 1997). Comparative RFLP mapping in cultivated and wild wheat (*Triticum dicocoides*) has led to the identification of molecular markers associated with resistance to the herbicide chlorotoluron which is a selective phenylurea herbicide (Krugman et al., 1997). A list of wheat genes that control various physiological and agronomic parameters that have been identified with the use of molecular markers is presented in Table 4.

The existence of numerous sets of wheat near-isogenic lines (NILs) differing in the presence/absence of a resistance allele for various biotic stress factors (diseases and pests) has facilitated the mapping of genes for which such lines exist. Large numbers of genes conferring disease or pest resistance have been identified and associated with molecular markers (reviewed in Hoisington et al., 1999). When the chromosomal location of a particular gene is known from previous genetic studies but no NILs are available, markers mapped to that chromosome (Anderson et al., 1992) can still be used to score parental lines for polymorphisms, construct a single-chromosome map, and determine which markers are close to the gene of interest. This strategy was followed by Dubcovsky et al. (1996) to tag the *Kna1* locus in wheat, which is responsible for higher K+/Na+ accumulation in leaves, a trait correlated with higher salt tolerance.

In wheat, bulked segregant analysis, initially used mostly with RAPDs, can now be used with any type of marker

including AFLPs (Goodwin et al., 1998; Hartl et al., 1998), which have the advantage that a high number of DNA fragments can be amplified with one primer combination. Also, with AFLPs the problem of highly repetitive DNA is overcome by using methylation sensitive endonucleases such as *Pst*I and *SseI*.

Many genes that have been tagged with molecular markers in wheat have been introgressed from alien species (Hoisington et al., 1999). In the case of translocations from wheat's wild relatives known to carry genes for agronomically important traits, markers can be successfully established due to the high level of polymorphisms between the wheat and introgressed genome and the low level of recombination between the translocated segment and the corresponding wheat chromosomes.

Mapping QTLs in wheat. Utilizing a base map and linkage data from a range of other segregating wheat, rye, and barley populations, a consensus map with more than 1000 data points has been developed (Gale et al., 1995). This detailed linkage map has confirmed that the order of genetic loci across the A, B, and D genomes has been conserved (Gale et al., 1995).

A RIL mapping population developed utilizing 'Opata 85' and a synthetic hexaploid from CIMMYT has been used extensively in mapping and genome relationship studies (Van Deynze et al., 1995; Nelson et al., 1995a, b, c). The genetic map of this population, developed by the International Triticeae Mapping Initiative (ITMI), contains over 1000 RFLP loci. Two other published maps are available in wheat (Liu and Tsunewaki, 1991; Cadalen et al., 1997). Linkage maps in wheat have confirmed evolutionary chromosomal translocation rearrangements involving chromosomes 2B, 4A, 5A, 6B, and 7B, which were based on cytological evidence, and have established synteny among closely related grass species such as rice, maize, oats, and wheat (Ahn et al., 1993; Devos et al. 1994; Van Deynze et al., 1995; Borner et al., 1998).

The low number of quantitative traits dissected into their QTLs in wheat is a reflection of the focus on simply inherited traits and the difficulty of building comprehensive linkage maps. Given that the ITMI map is one of the densest and the population from which it was developed is segregating for a number of traits, it has been used to map important traits and several major genes. Known genes include vernalization (*Vrn1* and *Vrn3*), red-coleoptile (*Rc1*), kernel hardness (*Ha*), and powdery mildew (*Pm1* and *Pm2*) genes (Nelson et al., 1995a), as well as genes conferring and suppressing leaf rust resistance (Nelson et al., 1997). Quantitative trait loci have been identified for kernel hardness (Sourdille et al., 1996), Karnal bunt (Nelson et al., 1998), and tan spot (Faris et al., 1997).

Research on developing molecular markers for traits associated with drought tolerance in wheat started recently at CIMMYT. A RIL population is being utilized to identify genomic regions associated with a range of physiological parameters controlling drought tolerance.

Table 4. Genes identified and mapped with molecular markers for physiological and agronomic traits in wheat.

Traits	Genes	Species	Markers	Chromosomes	References
Physiological and agronomic					
Preharvest sprouting	QTL	*Triticum aestivum*	RFLP		Anderson et al., 1993
Vernalization	*Vrn1*		RFLP	5AS	Galiba et al., 1995; Korzun et al., 1997 Kato et al., 1998
	Vrn3		RFLP	5DS	Nelson et al., 1995a
Photoperiod response	*Ppd1*	*T. aestivum*	RFLP	2DS	Worland et al., 1997
	Ppd2	*T. aestivum*	RFLP	2BS	Worland et al., 1997
Dwarfing	*Rht8*		SSR	2DS	Korzun et al.,1998
	Rht12		SSR	5AL	Korzun et al., 1997
Cadmium uptake			RAPD		Penner et al., 1995
Aluminum tolerance	*Alt2*		RFLP RFLP	4D 4DL	Luo and Dvorak, 1996; Riede and Anderson, 1996
Drought induced ABA			RFLP	5A	Quarrie et al., 1994
Na+/K+ discrimination	*Kna1*	*T. aestivum*	RFLP RFLP	4D 4DL	Allen et al., 1995; Dubcovsky et al., 1996
Quality					
Kernel hardness	*Ha Hn* and QTL		RFLP RFLP	5D 5DS, 2A, 2D, 5B, 6D	Nelson et al., 1995a; Sourdille et al., 1996
Grain protein	QTL	*T. turgidum*	RFLP	4BS, 5AL, 6AS, 6BS, 7BS	Blanco et al., 1996
LMW glutenins		*T. turgidum*		1B	D'ovidio and Porceddu, 1996
HMW glutenins	*Glu -D1 -1*	*T. aestivum*	ASA	1DL	D'ovidio and Anderson, 1994
Flour color			RFLP/AFLP	7A	Parker et al., 1998

Marker-Assisted Selection

Based on information provided by mapping and genetic dissection of a given trait, the efficiency of using marker-assisted selection (MAS) can be evaluated and a suitable experiment designed. Molecular markers can be used for 1) tracing a favorable allele (including recessive) across generations, and 2) identifying the most suitable individual among segregating progeny, based on allelic composition across part or the entire genome. For tracing favorable alleles, it is critical to have molecular markers very close to the gene of interest.

Markers can sometimes be identified within genes that have been sequenced. For example, in maize, Opaque-2 mutant gene, which confers high lysine and tryptophan in the kernel, has been sequenced (Schmidt et al., 1990). Microsatellite sequences have been identified within the gene, and primers to amplify the microsatellites have been designed. This is optimal for tracing favorable alleles, since the marker is located within the gene sequence itself and co-segregates with the target gene.

However, in most cases, especially for polygenic traits, target genes have not been characterized at the molecular level. Therefore, the selected genomic regions involved in a marker-assisted selection experiment are chromosome segments or QTLs, identified as described previously. Two polymorphic DNA markers flanking the QTL region are required to follow the presence of elite alleles within the selected QTLs. When a QTL represents a large chromosome segment (more than 20cM), there should be a marker within the QTL to eliminate genotypes presenting a double recombination between the two flanking markers.

Depending on the nature of the genomic region (cloned gene, major or minor QTLs) involved in the expression of a target trait and the number of selected QTLs or genomic regions that need to be manipulated, several MAS schemes can be considered. Optimal strategies, in terms of the breeding approaches and the type of the molecular marker to be used efficiently in a MAS experiment, should also consider, apart from the technical constraints, the objectives of the experiments and the resources available. A favorable allele can be introgressed using the marker in target germplasm through backcrossing (BC), or can be selected in a segregating population whatever the level of recombination present (e.g., F2 or advanced populations). In a“marginal” MAS approach, quite important in open-pollinated crops, the most suitable parental lines for developing new materials through recurrent selection are identified.

Marker-assisted selection strategies

MAS for parental selection. Molecular markers can be used to genotype a set of germplasm, and the data used to estimate the genetic distance among evaluated materials. The degree of heterosis between lines can be predicted based on genetic distance. Moreover, germplasm can be characterized at specific loci known to be involved in the expression of a target trait, such as leaf rust in wheat, provided the germplasm has been well characterized phenotypically and molecular polymorphisms identified in a set of materials that differ in the expression of leaf rust resistance. This allows the identification of lines possessing the most suitable allelic composition for leaf rust at different loci.

Fingerprinting of potential parental lines can be very informative for breeders planning to make new segregating crosses. Although the information provided by molecular markers may not identify the best cross, it does help reduce the number of needed crosses. If by combining phenotypic and genotypic data the number of crosses can be reduced by half, this will greatly increase breeding efficiency.

The backcross-MAS approach. Marker-assisted selection using backcrossing (BC-MAS) has been used extensively—for example, to introgress target alleles when dealing with cloned genes or major QTLs. In a BC scheme, an elite allele at target loci is transferred from a donor to a recipient line. The use of DNA markers, which permit genotyping the progeny at each cycle, increases the speed of the selection process (Tanksley et al., 1989). An excellent example of the introgression of an elite allele at a target gene is the introgression of a transgene into elite maize inbreds (Ragot et al., 1994).

The following parameters must be considered in planning and executing a BC-MAS experiment: the number of target genomic regions involved in the selection, the size of the population screened at each cycle, the number of genotypes selected at each cycle, and the level of line conversion desired. The expected level of conversion is related to the number and distribution of DNA markers at non-selected loci, and to the recombination frequency between the target loci and the two flanking markers. All these parameters interactively have an impact on the number of cycles required to do BC-MAS. With the recent development of reliable PCR-based markers and single-nucleotide polymorphisms (SNP; Gilles et al., 1999), the capacity for screening large populations has been substantially improved (Ribaut et al., 1997).

In view of the non-linear relationship between reduction of the donor genome contribution at non-selected loci for different population sizes, in a BC-MAS experiment the number of target genes to be introgressed must first be defined. This facilitates calculating the

population size to be screened at each cycle, considering a target effective population size (defined as the number of individuals with favorable alleles at the target genes from which selection with markers can be carried out on the rest of the genome at non-target loci) of 50-100 genotypes. The effective population size and number of genotypes heterozygous at the target loci are essential for determining the selection model. Once the effective population size is defined, the desired recombination frequency between flanking markers and the target gene should be determined, as well as the number of genotypes selected at each cycle, based on the objectives and constraints of each project. Following this strategy, the number of BCs required to achieve the introgression can be easily predicted based on simulations (Frish et al., 1999).

In case of limited resources, or for concomitant introgression into a large number of recipient lines, as is often necessary in a breeding program, including an MAS step at an advanced BC cycle should be considered. Independently of the BC-MAS scheme considered, it is critical to make some effort to identify the most convenient set of markers.

New MAS strategies

The limitation of MAS for polygenic trait improvement. Despite the success of BC-MAS in certain applications, it has limited utility when several QTLs must be manipulated concomitently. Quantitative traits are difficult to manipulate due to their genetic complexity, principally the number of genes involved in their expression and epistatic interactions between genes (see Ribaut and Hoisington, 1998, for a review). Since several genes are involved in the expression of polygenic traits, they generally have smaller individual effects on plant phenotype. This implies that several regions, or QTLs, must be manipulated at the same time to have a significant impact, and that the phenotypic effect of individual regions is not easily identified. Field trials repeated over multiple years are required to accurately characterize QTL effects and evaluate their stability across environments. Epistatic interactions can induce a skewed evaluation of QTL effects *per se*, and if all the genomic regions involved in the interactions are not incorporated in the selection scheme, they can bias the MAS. In conclusion, although QTL identification has improved significantly, currently used MAS strategies have their limitations (especially related to cost effectiveness) and new ones should be considered.

Single large-scale MAS. In this proposed new approach, the selection of suitable parents and development of new lines are overseen by plant breeders, and DNA markers are used at an early stage of recombination to fix alleles at selected genomic regions (Ribaut and Betran, 1999). The MAS step, conducted only once, is based on the use of reliable PCR-based markers on large segregating populations derived from crosses between elite lines. Parental lines must be outstanding for the target trait and/or environment, and should have good allelic complementarity.

The new strategy offers two major advantages: 1) favorable alleles selected for improving a specific trait are derived from two or more sources of elite parental materials in a complementary scheme, disregarding the "recipient/donor" line concept, and 2) specific genomic segments are fixed only at regions previously identified for each donor line in the selection scheme, with no selection pressure applied outside the targeted genomic regions. This ensures good allelic variability in the rest of the genome for future line development under various conditions. This approach is also relevant for pyramiding favorable alleles at cloned genes or major QTLs in new germplasm.

Pedigree MAS. This approach is especially relevant for crops such as wheat, where pedigrees of elite germplasm are known. Fingerprinting elite wheat materials must be conducted in a set of lines actively used in the breeding program, and in elite materials to be released in subsequent years. Fingerprinting data may be combined with phenotypic data collected during different selection cycles to identify alleles favorable for traits of interest. For example, if an elite line contains alleles for yield performance in a target environment, their frequency should be higher than the expected random frequency in offspring derived from this elite parental line. This shift in allelic frequency reflects phenotypic selection by breeders and may be identified by comparing fingerprinting data of both parents and their offspring. Once the favorable alleles are identified, DNA markers closely linked to the target genomic regions can be used to accelerate fixation of favorable alleles in the next selection step: a new set of elite materials (offspring 1) to derive the next set of elite lines (offspring 2). Such MAS will probably be most efficient when conducted on F2 or F3 segregating populations.

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CHAPTER 4

Managing Experimental Breeding Trials

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The goal of most breeding trials is to assess the performance of a number of genetically diverse breeding materials in such a way that superior lines can be selected that perform better than local checks under specific farming conditions. This must be done without bias under conditions that mimic the conditions where the material will be recommended. If the breeder is going to select material for a specific biotic or abiotic stress, he must conduct the trial in an area that has this stress or condition. This will ensure a higher rate of success than planting only under favorable conditions that do not represent the target environment.

It is also important to manage breeding trials as carefully as possible to minimize experimental error to be able to evaluate differences between materials statistically. Confounding factors need to be kept to a minimum so that the breeder has confidence that his selections will stand up to testing under the client situation. This chapter will look at some of the factors that need to be considered when planning breeding trials.

Choosing the Experimental Site

Selecting the experimental site is critical for success. If a breeder wishes to select materials for salinity tolerance, he must select a site that represents the salinity situation in the areas where the variety will be released. His chances of success are increased by carefully selecting the site for the nursery. This selection will take into consideration the soil, climate, water regime, and biotic and abiotic stresses that will be encountered in the target environment. Factors that need to be considered include:

- Soil factors such as texture, pH, conductivity (salinity and alkalinity), and nutrient status. Soil texture will affect soil physical properties and the permeability and drainage of the soil. Soil pH and conductivity can have a large effect on plant growth, and crops and different cultivars will perform differently under different values for these parameters. Soil nutrient status will affect the yield potential of the germplasm. It is also important to consider soil nutrient status when breeders are specifically looking for tolerance to various nutrient factors such as phosphorus efficient lines or lines tolerant to micronutrient deficiencies.
- Climate is an important consideration especially when looking for abiotic or biotic stress tolerance. Should the breeder select a high or low rainfall area, cool or hot temperatures at the beginning or end of the growing season? Is frost an important consideration or heat during grainfilling?
- Is the crop being recommended for an irrigated or rainfed situation? Is waterlogging or poor drainage a characteristic of the recommendation domain? If selecting for rainfed areas, how do you handle the problem of soil moisture at planting? Do you pre-irrigate the plot so germination is good and then leave the rest of the season to natural rainfall? Is irrigation water available and is it of the quality you need or similar to that of the target environment?
- If the breeder is selecting material for specific biotic stresses, he should select an area where this stress occurs. Incidence of certain diseases and insect pests comes to mind.
- Abiotic stresses such as heat, salinity, and waterlogging also need to be addressed when selecting a site. Are abiotic stresses (for example, salinity or waterlogging) consistent across the selected field? If not, can they be statistically handled by experimental design and layout?

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Another question is whether the experimental area should be on station or in farmers' fields. Most breeding trials have traditionally been conducted on station, mainly because it gives the breeder control over experimental conditions, e.g., land preparation, fertilization, irrigation, crop protection, and security of the trial. However, there is often the problem how representative the station is of the client farmer situation. Unfortunately, many stations are not chosen for their similarity with farmers' fields but rather for convenience or availability. Experiment stations usually represent one or at most a few of the soils or environments faced by farmers.

There is therefore a tradeoff between experimental control and how representative on-station conditions are of the target environment. Probably the best solution is to conduct breeding trials under both situations. The first phase selects promising materials under the control of the station; later the materials are assessed under actual farmer situations. The latter should preferably include farmer participation and experimentation. The extra benefit of farmer participation is the feedback and assessment he can give the breeder. The new emphasis on farmer participatory breeding by some donor and development agencies utilizes this important feedback mechanism.

Influence of Crop Rotations

In many developing countries, especially in subtropical environments, wheat is often grown in double or triple cropping patterns. For example, in Asia, wheat is grown sequentially with rice, cotton, soybeans, and maize in the same calendar year. In Mexico, where the CIMMYT wheat varieties originate, wheat usually follows cotton, maize, or soybeans. It is therefore important that some breeding trials be grown on land with similar cropping patterns, for several reasons:

- The previous crop can strongly influence the harvest date and therefore the planting date for the wheat crop. For example, in Asia, long-maturing "basmati" rice is preferred in some locations because of its high quality and market price, its straw value and the fact that it needs less fertilizer. The next wheat crop will inevitably be planted late. Since there is genetic variability for performance of wheat varieties under late planting, breeding trials should at some point in the selection process be planted late after rice.
- The previous crop can have an effect on soil physical properties. When rice is grown in South Asia, the soil is puddled (wet cultivation) to lower permeability and water use. This profoundly affects the soil structure for the next upland crop, such as wheat. The poor structure following rice affects rooting and soil permeability. Waterlogging is common in wheat following rice, with plants turning yellow due to oxygen stress. Thus a breeder should evaluate his germplasm under these conditions to improve the chances of selecting adaptable varieties for rice-wheat farmers.
- Soil nutrient status is also affected. Some crops like rice are very exhaustive of nitrogen. For others, like potato, soil nutrient status is often better than normal due to high fertilizer application rate, which leaves residual fertility.
- Soil water status can also be affected. Deep-rooted crops such as sugarcane and cotton can deplete the soil profile of water. If the breeder grows his nursery after fallow, he will get different results than if he plants after the previous crop, unless he compensates for the low water status by irrigation.
- The previous crop will have a specific spectrum of pests and diseases. If weeds from the previous crop carry over to the wheat crop, measures must be taken to control the problem. Residues from the previous crop can also influence disease and insect incidence. In many countries combine-harvesting is becoming popular. This system leaves loose residue on the soil surface. In some cases, this residue can harbor diseases. In others, there are benefits to this mulch (especially rainfed areas) in the form of cooler temperatures and better water infiltration and conservation. Thus it is important to screen materials under these conditions.

Preparing the Nursery

Unless germplasm is being selected for reduced or zero-tillage situations, land preparation is a major first step in nursery management. The objective is to prepare the soil to favor wheat germination. Several factors need to be considered:

- Selection of the appropriate plow or harrow for the specific soil to ensure a good tilth, favorable for wheat germination. This may involve a series of steps from deep plowing, to harrowing, to compaction of the surface soil to ensure good seed-soil contact.
- Fields should be leveled as much as possible to reduce variability due to soil moisture, especially if applying irrigation. If germplasm is being selected under rainfed conditions and the fields are sloping, appropriate experimental blocking designs will be needed to reduce experimental variability and error.
- If the nursery is sown in farmers' fields, the plots should be prepared in the same way that farmers do, using the most representative areas (away from field edges, buildings, trees, etc.).

- Care should be taken to control any carryover weeds from the previous crop, to avoid creating a problem in the wheat crop.
- It may be necessary to pre-irrigate the field to ensure adequate soil moisture for germination of the wheat seed.

If farmers in the target area are beginning to use or are already using zero-till planting, strong consideration must be given to managing breeding nurseries in a similar fashion.

Planting Methods

If available, it is better to use commercially available seed drills specifically designed for planting breeding trials (Hege, Wintersteiger, Almaco, etc.). These systems can plant small plots with many different varieties without having to clean the seed drill after each plot. They also ensure consistent planting depth, even distribution of seed in rows, and optimize the chances of good germination, all if which reduce experimental error. If machinery is not available, hand planting is possible, but germination will be less regular and errors may be more common.

The trials should have a sufficient number of rows to eliminate border effects, i.e., the middle three or four rows of wheat to be harvested should be surrounded by at least one row of wheat to prevent bias in the experiment (Figure 1). Border rows will yield more than inner rows due to less competition for water, light, and nutrients. Where there is a lot of variability in plant height in the germplasm, even more space is needed between varieties to prevent experimental bias. This can be achieved by growing more border rows or by leaving a space between the plots.

Statistical Methods and Considerations

Error associated with yield estimates in field trials is increased by spatial variation at the experimental site. Factors causing variation may include uneven application of water and fertilizer, natural differences in soil fertility or water-holding capacity, spatial variation in soil physical properties, etc. Such variation can result in poor precision when estimating treatment effects. It is therefore important to reduce, as much as possible, the residual variation not accounted for by the variety effects. Residual variation can be reduced through the use of an appropriate experimental design (*a priori* approach) and of spatial methods of analysis (*a posteriori* approach), also called nearest neighbor analysis.

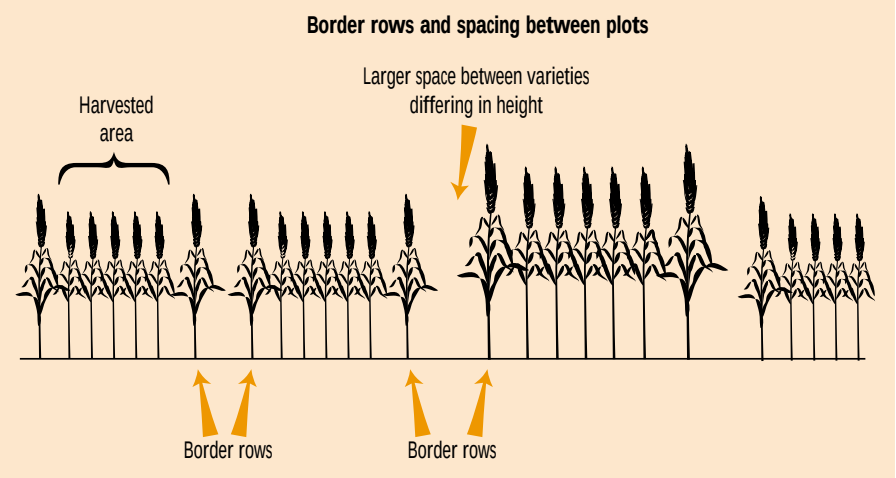


Figure 1. Layout of trials to avoid experimental bias and border effects.

Lattice designs

Most variety trials use complete or incomplete block designs and are analyzed by the traditional analysis of variance. Block designs attempt an *a priori* reduction of the experimental error considering spatial heterogeneity among blocks. If it is probable that environmental heterogeneity exists within an experimental complete block (i.e., replicate), for example in trials with large numbers of treatments, lattice designs can be used to adjust the means. The design of the experiment involves the restricted randomization of entries within sub-blocks so that means can be adjusted according to the variation among sub-blocks (Yates, 1938).

Incomplete block designs (such as alpha lattices) can regularly achieve better yield estimates and consume fewer resources than randomized complete blocks. Such efficient designs must be applied hand in hand with excellent field plot technique to produce accurate estimates of yield within locations. Designs and programs for analyzing lattice designs are included in the statistical package MSTATC, for example. Because they improve precision, lattice designs are highly recommended for field studies. The increased precision resulting from lattice adjusted data may permit trials to be grown with fewer replicates than would otherwise be necessary to establish significant differences. With today's analytical capabilities, it is rarely useful to consider more than two replicates for yield trials, and we believe unreplicated trials (in which only checks are replicated) may prove increasingly useful.

Spatial designs

Given that lattices do not consider the presence of spatial variability within sub-blocks, researchers have to find homogeneous sub-blocks in the field, without knowing their most appropriate shape, dimension, and orientation. The *a posteriori* approach for reducing residual variation can be applied when field variety trials are laid out in a rectangular array of rows and columns with replicates allocated contiguously. Spatial analysis can be performed to improve precision of estimates of variety effects and variety contrasts.

One approach is to adjust a plot for spatial variability by using information from its immediate neighbors. A useful measure for examining soil heterogeneity patterns is spatial autocorrelation of neighboring plots within rows or within columns (i.e., the correlation between residuals separated by various distances). If there is no spatial pattern, all correlations will be low. If there is pattern in the residuals, neighboring residuals will be more similar and so have higher correlation. Gleeson and Cullis (1987) proposed to sequentially fit a class of autoregressive, integrated, moving averages to the plot errors in one direction (rows or columns). This was in the context of randomized complete block experiments. They found that differentiating along the block and then fitting a moving average correlation structure to the residuals in that direction resulted in big gains in trial efficiency. Cullis and Gleeson (1991) extended the previous model to two directions (rows and columns) assuming that, in the field, rows and columns are regularly spaced.

Fertilizer Use

To express the full potential of a variety, sufficient nutrients must be applied to the experimental area. A soil test is useful for determining how much fertilizer to apply. Sufficient nitrogen, phosphorus, and potassium should be applied (as inorganic or organic sources, depending upon availability) to ensure these elements are not limiting. Care should also be taken to apply sufficient quantities of other nutrients, including micronutrients if they are known to be limiting. If varieties are planted when nutrients are limiting, they will be unable to express their true genetic potential, making it difficult to separate out the better lines since they will all yield poorly.

To prevent variation in the field, fertilizer should be applied as uniformly as possible, preferably by using a fertilizer spreader. When a spreader is not available, it is better to divide the fertilizer into smaller parcels or replications and apply each dose carefully to smaller areas. This is easily done for the basal application of fertilizer, but for the topdress application, the dose should be split by replication or plot, and the fertilizer carefully broadcast. If wheat is planted in a bed and furrow system, topdress fertilizer is best applied by machinery.

In farmers' fields, the fertilizer level recommended for assessment should be used and, in some cases, 50% above the recommendation, so the scientist can evaluate the potential of the variety in farmers' circumstances. The exception would be where the breeder is screening material for tolerance to specific nutrients. For example, boron is a known factor in wheat sterility. If a breeder wants to screen wheat lines for tolerance to low boron, he should have two sub-plots, one with applied boron and one without.

Irrigation

In rainfed trials, the experiment should be blocked to ensure uniform moisture distribution within replications. Where irrigation is applied, uniform application is necessary to avoid experimental error. Providing basins for each replication ensures that each plot will receive the same amount of water. In bed and furrow irrigation, replicating across the irrigation run ensures uniform water application within a replication. The advantage of bed and furrow configurations comes from more uniform water distribution, savings in water, and savings in land that would otherwise be needed to construct the basins and water distribution system.

Crop Protection Strategies

Unless the breeding trial is for evaluating germplasm against biotic stresses, crop protection measures should be used to ensure the varieties express their full yield potential. That may mean applying herbicides, insecticides, or fungicides, depending on the stresses present. Care should be taken when choosing the herbicide, since some lines may be susceptible. Labels should be carefully read and, when in doubt, other herbicides should be used or weeds controlled manually. Agronomists in the program should be screening germplasm against various herbicides so that this information is available to breeders.

Since breeders often wish to see the response of the lines to disease and insects, it is rarely necessary to spray for these factors.

Lodging

In the early years of the green revolution, lodging resistance was what enabled plants to break the yield barrier. The old, tall, traditional varieties lodged when fertility improved, and this restricted their yield potential. When new, shorter varieties with stiffer straw were introduced, fertilizer levels could be increased without lodging, which resulted in higher yields. However, even the new varieties lodged at higher fertilizer levels with late irrigation. Lodging is therefore still a major factor limiting yield potential and should be receiving more attention.

Several management methods can be used to reduce lodging and allow the full yield potential of the germplasm to be expressed. The following are some suggested options:

- A major reason for lodging is that the soil around the crown of the stem is wet and will not support the weight of the plant, especially when it is windy. This happens when wheat is planted on the flat and irrigation is given after flowering or during grainfilling. There are few options when wheat is planted on the flat except to apply irrigation in the late afternoon when winds tend to subside or to forego this irrigation. This may cause moisture stress and lead to lower yield, especially due to lower thousand-grain weight.
- Planting wheat on beds, especially with lower seed rates and two rows per 70-cm bed, can result in less lodging. In this system, later irrigation does not saturate the soil around the base of the plant as much as on the flat and so the plant is better supported by the soil. Plants are also exposed to more sunlight, much like border rows in solid stands. This makes the plants stronger and more able to resist lodging. In fact, planting wheat on beds will enable the plants to better express their yield potential than when planted on the flat because of better lodging resistance when more nitrogen is applied or grainfilling irrigation is given.
- Fertilizer timing can also be used to reduce lodging. If all the nitrogen is applied basally, the plants will be luxurious and competition for light will be high. This results in weaker plants more prone to lodging. If nitrogen can be reduced at planting and delayed until just around the first node stage (DC31, Zadoks' scale), it will be utilized more for grain than for excess foliage, and lodging will be less. Experiments have also shown that delaying nitrogen application until this later stage does not sacrifice yield potential but instead, because of less lodging, can give higher yields. Grain protein content will also be higher.
- Seed rate can be manipulated to help reduce lodging. If the plant stand is too thick, independent plants will be competing for light with adjoining plants and be weaker in the stem and more prone to lodging. By reducing the seed rate, lodging can be reduced.

If lodging is a factor in an experiment, it is important to take a good measure of it. Several factors are needed:

- Estimate of the area lodged in the sampled plot. The angle of the stem in relation to the vertical is also important. This datum can be used as a covariate in the analysis.
- The date when lodging occurred is important, though it is better to note the growth stage at which it happened. The timing and cause of lodging can usually be determined by reference to the occurrence of a storm or an irrigation event. Data should be collected as soon as lodging occurs since the angle and extent of lodging may change with time.

If breeders wish to measure the importance of lodging, they may consider growing one set of materials with support (e.g., using netting) and comparing with an unsupported check. Growth hormone sprays that reduce internode length may also reduce lodging and help measure lodging losses.

Harvesting and Sampling

Harvesting the crop and taking reliable samples are critical for breeding trials. If this is not done properly, all efforts to grow a good crop may be wasted. Taking samples as accurately as possible is essential for reducing experimental error. This will allow smaller differences between varietal means to be separated statistically.

In breeding trials, a sample area—rather than the whole plot—is generally used for estimating yield. As mentioned earlier, to reduce bias in the experiment, the border rows are removed and only the inner rows used for yield estimation. It is best to remove the outer rows and half a meter from either end of the plot. The remaining area can be cut and used for measuring yield and yield components. Plants should be cut close to the ground so that accurate harvest index and straw yield can also be determined.

Calculating Yield and Yield Components

Yield components can be calculated or measured individually. Three main harvesting methods are suggested for use when calculating yield components (Table 1). After harvest, yield components can be calculated according to formulas presented in Table 2.

Harvesting steps are similar across the three methodologies (Table 1); the procedure is described below under Method 1.

Method 1: Calculating yield components by harvesting total biomass

- Cut all aboveground biomass in a pre-determined area (A). Avoid border effects by sampling away from edges of plot.
- Sub-sample a set number (e.g., 50 or 100) of spike-bearing culms (i.e., spike, leaves, and stem) randomly from the plot sample and measure fresh weight (sub-sample fresh weight, or SSfw).
- Measure fresh weight of remaining plot sample (plot fresh weight, or Pfw).
- Dry the sub-sample of culms at 70ºC (facilitate by putting culms in a closed bag to avoid loss of grain, etc.) and weigh (sub-sample dry weight, or SSdw).
- Thresh plot sample for fresh grain weight (plot grain weight, or P-GW).
- Count 200 representative kernels and weigh fresh and after drying (200-grain fresh and dry weights, or 200fw/dw).

Method 2: Calculating yield components by harvesting a random sub-sample of culms

Yield components can be determined directly by taking random spike-bearing culms from the crop at physiological maturity.

- Take about 50 culms from rows (or area) to be harvested by grabbing a number of random sub-samples and cutting them off at ground level. All harvested rows should be represented in the sample.
- All culms in the sub-sample are dried at 70ºC.
- After measuring dry weight of the sub-sample (SS-dw), thresh to measure grain dry weight (sub-sample grain weight, or SS-GW).

This method has the advantage that hand harvesting is quick (<5 minutes/plot), and samples can be readily stored for processing when time is available. Note that with this method, measurement of harvest index is statistically independent of measurement of grain yield, whereas that of biomass is not independent of yield.

Table 1. Samples to measure when using three alternative harvesting methods for estimating yield, biomass, and yield components from experimental yield plots.

	Samples to be measured†						
Method	Plot biomass fw (g)	S-sample culms fw (g)	S-sample culms dw (g)	S-sample grain dw (g)	Plot grain fw (g)	200 kernels fw (g)	200 kernels dw (g)
1. Harvest total biomass	X	X	X		X	X	X
2. Harvest sub-sample of culms	X	X	X	X	X	X	
3. Reduced threshing	X	X	X	X		X	X

† S-sample= sub-sample; fw=fresh weight; dw=dry weight.

Table 2. Formulas for calculating yield components using three different harvesting methods.†

	Harvesting method		
Yield component	Method 1	Method 2	Method 3
Harvest index (HI)	P-GW*(200dw/200fw)/P-fw*(SSdw/SSfw)	SS-GW/SSdw	SS-GW/SSdw
Yield (g m^{-2})	[(P-GW*200dw/200fw)+(SSdw*HI)]/A	[(P-GW*200dw/200fw)+SS-GW]/A	Biomass*HI
Biomass (g m^{-2})	[(Pfw+SSfw)*(SSdw/SSfw)]/A	Yield/HI	[(Pfw+SSfw)*(SSdw/SSfw)]/A
1000-GW (TGW) (g)	200dw*5	200dw*5	200dw*5
Grains m^{-2}	Yield/TGW*1000	Yield/TGW*1000	Yield/TGW*1000
Culm dw (g)	SSdw/#culms sampled	SSdw/#culms sampled	SSdw/#culms sampled
Spikes m^{-2}	Biomass/culm-dw	Biomass/culm-dw	Biomass/culm-dw
Grains/spike	Grains m^{-2}/spike m^{-2}	Grains m^{-2}/spike m^{-2}	Grains m^{-2}/spike m^{-2}

† A=plot area harvested (m^{-2}); SS=sub-sample; fw=fresh weight; dw=dry weight; P=plot; GW=grain weight.
Note: Formulas assume that grain dried at 70ºC is at 0% moisture. Grain yield at x% moisture = Yield * [100/(100 – x)] (g/m^2).

Method 3: Calculating yield components with reduced grain-threshing requirement

Measuring grain yield and yield components with this method requires threshing only a sub-sample. The procedure is useful when larger-scale threshing machinery is lacking or when working with species that are difficult to thresh, such as *Triticum dicoccum* or synthetic hexaploid wheats. Measure the samples indicated for Method 3 in Table 1, and follow the relevant sampling procedures described under Methods 1 and 2.

Sampling Biomass in Lodged Crops

It is difficult to cut a given area in a lodged crop, especially if it has been sown by broadcasting. The process is facilitated by folding back spikes and stems to establish a starting reference line before inserting a quadrat. Great care must then be taken to collect only those plants whose crowns fall within the randomly located quadrat. If one side of a quadrat is separated from the other three sides, it is easier to insert the quadrat into the crop. In this case, the free fourth side is used to ensure the frame is properly square by placing it between the two free ends of the 3-sided frame.

Measuring Individual Yield Components in the Field

Plant population

A count of plant population should be made after the maximum number of plants has emerged and before tillering occurs (usually 10-14 days after adequate moisture becomes available for germination).

If plants are sown in rows, then 0.5 m length from each sampling row or from at least six such rows should be counted per plot. If broadcast, then samples of at least 0.5-1.0 m should be taken from each plot. The number of samples required will depend on the degree of variation within the plots, but at least two per plot should be recorded.

The mean plant density may disguise important variability in plant distribution (i.e., gaps that will cause yield reduction). This should be noted and measured by estimating the percent of the plot with missing plants.

Plant population can be used to assess the germination, vigor, and emergence of sown seed, and/or the extent of compensation under conditions advantageous to tillering. It is also needed if early growth per unit area is going to be monitored by successive measurements of growth per plant. Plant population typically varies between 50 and 300 plants/m^2. The number of plants/m^2 has a broad optimum and will vary with variety, climate, and management. However, under good rainfed conditions, 100 plants/m^2 could be considered the minimum for maximum yield, unless the crop is growing on residual moisture, in which case optimum density may be lower.

Spikes/m^2

The number of productive spikes can be measured nondestructively by counting in a given area or length of row, or calculated from sampling as demonstrated above.[3] Spikes per m^2 can be measured most easily before physiological maturity, which also reduces yield loss due to shattering caused by movement in the plots. In broadcast planting, direct measurement can be difficult, especially if crops lodge.

Spikes/m^2 can be used to assess the final number of productive spikes/ m^2 and can be combined with plant population count to assess the extent of tillering. Tillering typically ranges from 1 to 10 per plant. Spikes/m^2 is determined by events occurring from sowing to flowering and is dependent on variety, management, and environment.

Spikelets/spike

Sample a minimum of 10 spikes per plot at random (aim for a total of 30-40 spikes/treatment); select the culms from the base and count the number of spikelets. Take the average based on sample size. Most commonly, count the fully developed or grain-bearing spikelets (or at least those large enough to have at least one grain). Potential spikelet number is obtained by counting all the nodes on the rachis; it can exceed the developed spikelet number because of aborted spikelets at the base or tip of the spikes. Alternatively, under excellent environmental conditions, all potential spikelets may develop into grain-bearing spikelets.

The potential number of spikelets per spike is determined by the time of terminal spikelet formation (in wheat and triticale; barley does not form a terminal spikelet) around first node appearance. Subsequently, primordial spikelets at the base (and, later, at the tip) of the spike may abort because of stress. Normally, 10-25 spikelets form on each spike.

Grains/spikelet

Sample as for spikelets per spike. Count the spikelets, thresh, count grains, and calculate; or less accurately simply calculate from calculated grains per spike and measured spikelet number. When sampling large numbers of samples or plots, time may be saved by randomly counting one side of the spike and multiplying by two.

[3] If measured directly, the procedure and number of sub-samples are as for plant population.

Grains per spikelet is the result of both the number of competent florets/spikelet and kernels/competent floret (or grain set). Values for competent florets per spikelet typically vary from 1.5 to 5.0 and for kernels per competent floret from 0.6 to 0.99.

Grain set

Grain set refers to the percentage of competent or entire florets (florets with fully formed, plump green/yellow anthers at flowering) that actually produce grain (the opposite of percent sterility) and should reflect conditions around anthesis (e.g., pollen fertility, early grain survival), in contrast to grains per spikelet, which can be influenced by earlier conditions as well. However, at maturity, it is difficult to know which florets were competent. It is suggested that the basal two florets of the 6-10 central spikelets are always competent, and therefore a grain set index can be obtained by observing the percentage of such florets with grains. Sample as specified for spikelets or count 10 such spikelets in five random spikes per plot; the total of missed florets is the % sterility (= 100 – **%** grain set).

Alternatively, one can use matched spikes (i.e., spikes showing equal size and development). One spike is sampled at anthesis and the other at maturity, counting (destructively) competent florets at anthesis in one spike (i.e., florets showing normal anther development; non-competent florets will show whitened, flattened anthers with no fertile pollen, whose stamens never elongate) and grains at maturity in the other spike. At least 20 matched spikes per treatment are needed for reasonable accuracy; selection of matched spikes and counting at anthesis are time consuming.

Grain set is an indicator of stress events occurring around anthesis (e.g., drought, temperature extremes, boron deficiency, or genetic sterility, which can interact with the environment). It is a more precise and, hence, more useful measure than grains per spikelet or grains per spike.

Thousand-grain weight at maturity and during grainfilling

To measure thousand grain weight (TGW), count out two random samples of 100 entire grains (i.e., those possessing an embryo). Dry the grains at 70ºC (48 hours should be sufficient) and weigh. This will usually give sufficient accuracy. If weights differ by more than 10%, a third sample of 100 should be taken or recheck the counts.

To study grain growth during grainfilling, maximum accuracy is achieved by selecting groups of a sufficient number of spikes, matched for anthesis date and size, so that one can be randomly sampled from each group on each sampling date. Four to eight such groups (four to eight spikes each date) per treatment should be sufficient for accurately calculating grain growth rate by linear or curvilinear regression. The study can be based on all grains on the spike or in a given position on the spike (e.g., basal florets of central spikelets).

A reduction in TGW may be due to climatic or biological (e.g., pathogen) stress during grainfilling. Kernel weight (calculated as TGW/1000) is usually 20-50 mg. However, lower grain weight may not indicate stress during grainfilling, due to the plasticity of yield components. For example, if the plant population is high (leading to a high number of kernels/m^2), TGW may decrease without seriously affecting yield. TGW tends to be characteristic of a variety, and there are large differences between varieties even under good conditions. Within a variety, kernel weight usually shows a negative linear relationship to mean grainfilling temperature. Potassium deficiency can also result in low thousand grain weight.

Grain or kernel number (per m^2)

Kernel number per m^2 (KNO) is usually calculated by dividing grain yield (GY; in g/m^2) by kernel weight (KW; in mg):[4]

```
KNO GY * (1000/KW)
```

Kernel number can also be independently measured by directly determining spike number (SNO/m^2), and kernels per spike (KPS) from at least 20 randomly sampled spikes per plot (aim for 60-100 spikes):

```
KNO=SNO * KPS
```

Kernel number per m^2 acts as a summary of all events up to and a little beyond anthesis. For example, the combined effects of management and climate on plants/m^2, spikes/plant, spikelets/spike, and grains per spikelet are all combined in this single term. Competent floret number (the precursor of kernel number) is also well correlated with spike (inflorescence only) dry weight at anthesis, the relationship being on the order of 100 florets/1.0 g spike (10 mg/floret), although the range across varieties for grain number is 70-140 kernels/g spike dry weight at anthesis.

Under many conditions, yield is a function of KNO, which is particularly dependent on crop growth rate during rapid spike growth (emergence of the second-to-last leaf—or about 1 month before anthesis for spring wheat—until just after anthesis).

[4] Using this calculation, KNO is statistically linked to GY and may give rise to spurious correlations between GY and GNO if GY is not determined accurately.

Yield Estimation and Measurement

Visual estimates

Yield can be estimated by visual assessment (this requires experience and a knowledge of the variety and area) or based on yield components from mid-grainfilling onward.

Yield estimates from yield components

To calculate yield from yield components, first estimate the number of spikes/m^2 from *in situ* counts (outlined above). Next, randomly sample and then count the number of grains/spike. Then, assume a TGW based on the variety and conditions expected during the rest of the grainfilling period (typical TGW under reasonable grainfilling conditions and temperatures: 30-40). Calculate yield with the following equations:

Yield (g/m^2) = spikes/m^2 * grains/spike *(TGW/1000)
Yield (kg/ha) = yield (g/m^2) * 10

Variability of the field or treatment and desired accuracy will determine the number of spike counts made. For greatest accuracy many small samplings are best and feasible when dealing with non-destructive sampling. For example, for a 1-ha drill-planted field, take spike number counts in 20 random but well dispersed 2-row x 50-cm quadrats and combine these counts with kernel number counts in 50 random spikes; a reasonable estimate of kernels/m^2 should result (but be careful to select sampling sites and spikes at random). Counting kernels per spike (one side x 2) while walking between quadrat sites saves time and should take less than 30 minutes. Be sure row spacing is accurately known and/or measure spacing to confirm; replicate the measurements for accurate assessment. For example:

- Average row spacing: 15 cm
- Average spike count (2 row by 50 cm): 40 spikes
- Spike sample area: 2 rows * 15-cm row spacing * 50 cm = 0.15 m^2
- 40 spikes/0.15 m^2 = 266.7 spikes/m^2
- Average kernel count/spike = 21.5
- Kernels/m^2 = 266.7 * 21.5 = S734
- Assume TGW = 40 (based on experience)
- Yield = 5734 * 40/1000 = 229.4 g/m^2 = 2294 kg/ha

Yield estimates from samples

Yield can be estimated as outlined for yield components. Alternatively, borders can be discarded and yield estimated from the remaining plot that is harvested. In some instances, it is not possible (especially in on-farm trials) to harvest the entire plot or to thresh the entire plot sample. In these cases, follow the options outlined above or sample 5 x 1 m^2/field or 2 samples of 1 m^2/plot if there are 3 replicates.

Yield moisture content

The grain trade and farmers usually express yield at a given moisture content (e.g., 10% in Australia, 12 or 14% in Europe on a fresh weight basis). Therefore, conversion factors are required to adjust moisture. Moisture content is calculated as the weight of moisture relative to fresh weight:

[Moisture/(moisture + dry weight)]

The following formulas outline various moisture calculations. Yield and grain moisture calculations:

Field weight of harvested grain = FW (kg)
Harvested area = A (m^2)
Fresh weight of sub-sample = WS
Oven-dry weight of sub-sample = DS

Grain moisture conversions:

Grain moisture content (M%)
M% = [(WS – DS) * 100] /WS
Yield (unknown moisture, GYm)
GYm (t/ha) = (FW * 10)/A
Yield (0% moisture, GY(0%))
GY(0%) = [GYm * (100 - M)]/100
Yield (X% moisture, GY(X%))
GY(X%) = [Y(0%) * 100]/(100 – X)

Throughout the previous discussion, all weights of plant parts (including grain) refer to constant weight after drying at 70°C. However, the American Association of Cereal Chemistry (AACC) defines 0% moisture as that achieved by drying ground grain at 103°C. Therefore, other conversion factors are required in addition to the above to obtain a true 0% moisture reading. To convert the weight of grain dried for 20 hours at 70°C to moisture content as defined by AACC, divide the weight by 1.025 (because grain dried at 70°C has approximately 2.5% moisture). The factor drops to 1.012 as drying time at 70°C increases to 48 hours. This means that, to express grain at 10% moisture, the oven-dry weight (70°C for 24 hours) needs to be multiplied by 1.084 (i.e., 1.00/1.025).

Assessing Crop Residue

Direct measurement

Collect and bulk straw found within five random samples (or two per replicate) of at least 1.0 m^2. Oven dry straw (70°C) and weigh. Convert g/m^2 to t/ha by dividing by 100.

Due to the generally large spatial variation in ground cover, a visual estimate (by an experienced researcher) is often sufficiently accurate using the method described below.

Estimating percent residue cover using the line-transect method[5]

The line-transect method is one of the easiest methods to use for determining the percent residue cover on the soil surface. Accurate measurement is necessary to determine if enough cover is present to comply with the conservation plan.

The following is a step-by-step procedure for using the line-transect method to measure the percentage of residue cover.

Step 1. Use a 100- or 50-m measuring tape for measuring residue cover. Other measuring lengths or even knotted ropes can be used if the appropriate multiplication factor is used to calculate the percentage.

Step 2. Select an area that is representative of the whole field/plot. Avoid end rows or small areas that have been adversely affected by flooding, drought, weed or insect infestations, or other factors that may have substantially reduced yields.

The most accurate reading of residue cover is obtained by taking an average from at least three different representative locations in the field.

Step 3. Anchor one end of tape and stretch it diagonally across the rows so that it crosses several passes by the farming implements. Readings are more accurate when the tape is stretched diagonally across the rows than when taken in a window of residue left by the combine or where the amount of residue is smaller.

Step 4. Measure residue cover by counting the number of "m" marks that are directly over a piece of residue, as shown below. For an accurate reading,

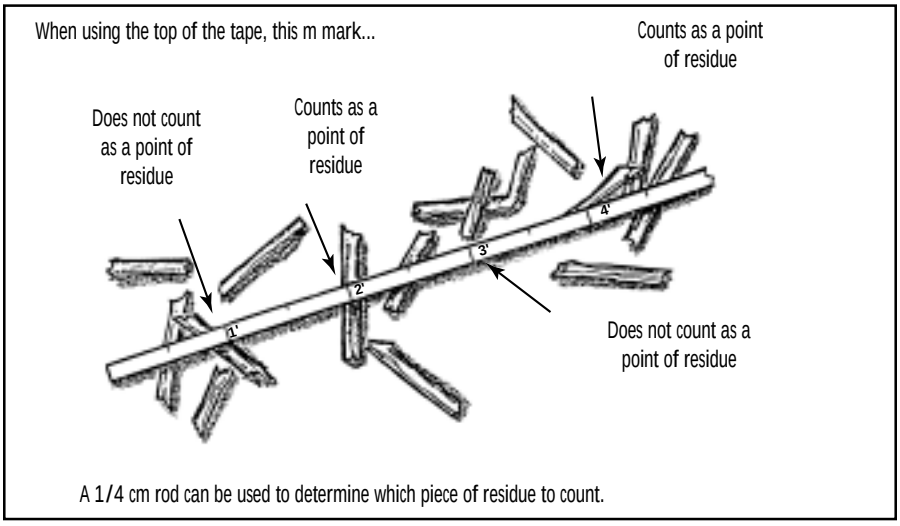
- do not move the tape while counting;
- look at the same side of the tape at each m mark;
- look straight down at the tape and the m mark, and
- count only those m marks that have residue directly under them.

To effectively reduce erosion, a piece of residue must be large enough to dissipate the energy of a raindrop during an intense storm. To be counted, the residue must be larger in diameter than this dot●

A convenient way to determine if a piece of residue is large enough to count is to use a 1/4 cm diameter brazing rod or wooden dowel. Touch the edge of the rod to the m mark. If the residue extends completely beyond all edges of the rod end, count it. If the rod covers the residue completely or if part of the rod end extends beyond the edge of the residue at any point, don't count it because a raindrop falling on this point would strike some unprotected soil.

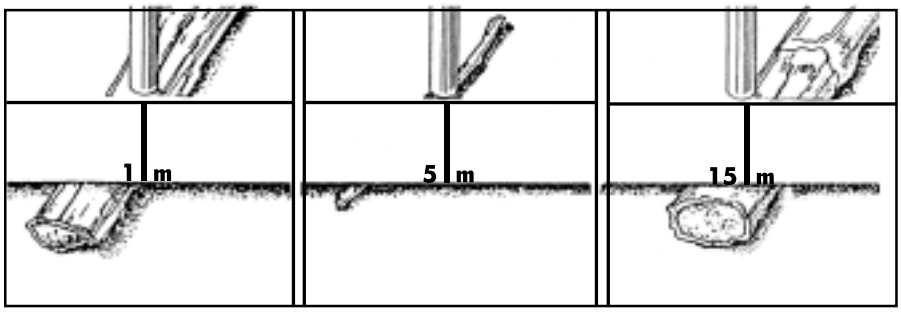
On a 100-m tape, the number of m marks that are directly over residue will be a direct measurement of the percentage of residue cover for the field. On a 50-m tape the number of m marks must be multiplied by 2. If other measurement lengths are used, the appropriate multiplication factor must be used, especially for plot-level measurement.

Residue amount can be used to assess soil cover and thus potential evaporation reduction but, more importantly, erosion control. Assuming uniform distribution, approximately 4 t of wheat straw lying horizontally are required to give 100% ground cover. Straw is also a potential source of disease infection for subsequent crops and may immobilize N during the decomposition process.



A 1/4 cm rod can be used to determine which piece of residue to count.

[5] This step-by-step description of the line-transect method was taken from a fact sheet by David P. Shelton, and Elbert C. Dickey, Extension Agricultural Engineers, University of Nebraska; Robert Kanable, Conservation Agronomist, Soil Conservation Service; Stewart W. Melvin, Extension Agricultural Engineer, Iowa State University; and Charles A. Burr, Extension Agricultural Specialist, University of Missouri. The fact sheet was produced through the cooperative efforts of representatives of Cooperative Extension, Midwest Plant Service, NACD's Conservation Technology Information Center, U.S. Environmental Protection Agency, and the USDA Soil Conservation Service.



Counts
raindrop strikes residue

Does not count
raindrop strikes both soil and residue

Does not count
raindrop strikes both soil and residue

Only those **"m"** marks having a piece of residue directly under them should be counted.

Ancillary data

It is always useful, especially for interpretating the results, to collect other site data. The following are some minimum data sets for consideration.

Climate data. The performance of any crop is very dependent on climate. Usually, there is a weather station close by that can provide this valuable supporting information. The following data would be useful:

- maximum, minimum, and average daily temperature
- precipitation
- radiation data or sunshine hours
- relative humidity (max-min, if possible)

Soil data. Soil data such as the following are useful for interpreting results:

- soil texture
- soil pH
- conductivity (a measure of salinity)
- infiltration (a physical measure of porosity)
- soil moisture holding capacity (permanent wilting point and field capacity)
- soil carbon level (a measure of organic matter)
- available P and K levels
- micronutrients status
- penetrometer measures to assess if plow pan exists
- water table depth
- irrigation water applied, numbers, and, if possible, amounts

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CHAPTER 5

Recent Tools for the Screening of Physiological Traits Determining Yield

J.L. Araus,[1] *J. Casadesus,*[2] *and J. Bort*[1]

This review presents practical guidelines for breeders of wheat (and other small grain cereals) interested in adopting a physiological approach to crop improvement. Some of the most promising tools for fast, reliable characterization of yield-determining traits will be discussed from an ecophysiological perspective. We will focus on the practical aspects and limitations of using relevant screening tools or selection criteria. The potential contributions of physiological research to plant breeding, as well as its inherent limitations, have been extensively reviewed from a breeding perspective (for example, Jackson et al., 1996). A theoretical framework for identifying yield determinants that are obvious candidates for evaluation has also been established (see below), although it has not been used in practical breeding.

The use of physiological traits as screening tools in breeding is still largely experimental—for different reasons. Sometimes the traits are very indirectly related to yield (Araus, 1996; Richards, 1996) or there is little ecophysiological understanding of the crop, especially when breeding for yield under stress. Nevertheless, breeding for crop escape has been very successful, and phenological changes have been the most important indirect factor in increasing wheat yields under Mediterranean conditions (Slafer et al., 1993; Loss and Siddique, 1994). However, in breeding for crop resistance, the evaluated traits and screening tools are often related to tolerance, not avoidance (see definitions in Larcher, 1995).

An indirect (i.e., physiology-based) breeding strategy could fail to produce yield gains and might even lead to a decrease in yield. Improved plant tolerance, though it protects the crop, can limit yield potential. The target environment where selection is carried out must be defined *a priori*, and the possibility of a negative breeding effort should not be ignored. In fact, plants that show the **most tolerant response during screening may also be the most sensitive**, in terms of yield loss, for example, because they are unable to delay the effect of stress at the cellular level.

The most promising methods allow for quick screening of "integrative" physiological traits (Araus, 1996), so called because they integrate physiological processes either in time (i.e., during the plant cycle) or at the organization level (e.g., whole plant, canopy). Other quick screening methods for evaluating, for example, the photosynthetic performance of plants under stress conditions have been proposed—among them, chlorophyll fluorescence measurements on single leaves. However, under field conditions fluorescence values may only reflect differences in phenology across genotypes. Nevertheless, remote sensing detection of fluorescence spectra at the canopy level could become a promising approach for breeding purposes (Lichtenthaler, 1996).

Identifying Physiological Traits for Use As Selection Criteria

One approach to search for traits that could be used in breeding programs is to identify the physiological processes determining productivity. A crop's yield potential (or harvestable part, Y) over a given period of time can be divided into three major processes (Hay and Walker, 1989). First, the interception of incident solar radiation by the canopy; second, the conversion of intercepted radiant energy to potential chemical energy (i.e., plant dry matter); and third, the partitioning of dry matter between the harvested parts and the rest of the plant. Whereas the first component depends on the canopy's total photosynthetic area, the second relies on the crop's overall photosynthetic efficiency (i.e., total dry matter produced per unit of intercepted radiant energy); the third is harvest index. Total biomass, which is the result

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of the first two components, can be physiologically defined as the result of canopy photosynthesis over time.

Other approaches may be followed, depending on agroecological conditions. For example, under water-limiting (e.g., Mediterranean) conditions, the most widely used framework, proposed by Passioura (1977), allows the study of indices that maximize yield per rainfall unit. Thus economic yield depends on the total water transpired by the crop, water use efficiency, and harvest index. Although these three components are not truly independent, Passioura's is a useful framework for searching for critical traits to improve yield under drought.

Yield can be divided into several integrative components or traits. Yield itself is the most integrative trait, because it is influenced by all factors **(**known and unknown**)** that determine productivity. However, there are many known limitations in a purely empirical breeding approach based only on yield. Therefore, any breeding strategy based on a physiological (i.e., analytical) approach should use screening tools or criteria to evaluate the integrative physiological parameters that determine harvestable yield with a single measurement. Although harvest index has been the most successful trait when modified to improve yield, the other two components of the above equations, which are responsible for total crop biomass, remain **(**basically**)** unchanged. In the following pages we will focus on tools used to evaluate physiological traits determining total biomass. We will discuss two different kinds of tools for screening integrative physiological traits: carbon isotope discrimination (Δ) and indices based on canopy spectral-reflectance.

The Δ not only evaluates genotypic differences in water use efficiency, but can also be affected by the total amount of water transpired by the crop (the first component of Passioura's equation) or by photosynthetic activity (the second component of the yield potential equation). Keeping in mind developing country breeders, we have included under the generic title "surrogates" other screening criteria, such as ash accumulation in different plant parts or criteria related to leaf structure. Though these features are not Δ surrogates in a strict sense, they are always related to yield and are both quicker and cheaper than Δ determinations; furthermore, no large facilities or highly qualified technical support are necessary to use them.

Canopy spectral reflectance is one of the most promising remote sensing techniques (see also Araus, 1996). Although at present the equipment is very expensive, in a few years its cost should drop dramatically. Another remote sensing technique, canopy temperature, provides integrated information on the crop's stomatal conductance at the canopy level (see chapter by Reynolds) (Reynolds et al., 1994) and has the advantage of being low cost. However, its usefulness is limited in severely stressed environments, and genotypic differences in phenology and canopy architecture can further limit its validity.

Carbon Isotope Discrimination

For C_3 plants, discrimination (Δ) of the heavier (^{13}C) stable carbon isotope over the lighter, more abundant (^{12}C) form (99%) during photosynthetic CO_2 fixation is an integrated measure of internal plant physiological properties and external environmental conditions that influence photosynthetic gas exchange (Farquhar et al., 1989). In C_3 cereals such as wheat, Δ is positively related to CO_2 levels in intercellular air spaces (Diagram 1) (Farquhar et al., 1982; Farquhar and Richards, 1984; Ehdaie et al., 1991) and, given a constant leaf-to-air vapor pressure difference, also negatively related to water use efficiency (WUE, measured either as net photosynthesis/transpiration, also called transpiration efficiency, or as plant biomass produced/water transpired) (Farquhar and Richards, 1984; Hubick and Farquhar, 1989). Plants with high WUE would be less able to discriminate against ^{13}C, and thus would accumulate more of the heavy carbon isotope in their tissues than less efficient water users.

When measured in plant dry matter, D provides an (integrated) indication of WUE throughout plant growth (Farquhar et al., 1982, 1989). D has been proposed as a possible screening tool for identifying variations in WUE in wheat (Farquhar and Richards, 1984; Ehdaie et al., 1991; Condon and Richards, 1993) and barley (Hubick and Farquhar, 1989). In fact, the permanent relationships between WUE and D during treatment and the high broad-sense heritability of D in wheat, together with other considerations, indicate that D may be useful for modifying the WUE and yield of water-limited wheat crops (Condon et al., 1987; Condon and Richards, 1992, 1993).

The relationship between Δ and water-use efficiency

Following on the model of Farquhar et al. (1982), Δ in C_3 plants may be defined in its simplest form as:

$$\Delta = a + (b - a)(p_i/p_a),$$

where a is the carbon-13 discrimination caused by diffusion in air (4.4 ‰), b is that caused by carboxylation by the

RuBP carboxylase enzyme (27 ‰), and (pi/p_a) is the intercellular to atmospheric CO_2 partial pressure ratio. Conversely, p_i/p_a may be defined as a function of Δ:

$$p_i/p_a = (\Delta - 4.4)/2.6.$$

If we assume that the temperature of the leaf (or other photosynthetic organ) is close to air temperature, and if daytime relative humidity is also known, WUE (the net assimilation to transpiration ratio) may be defined as a function of p_i/p_a:

$$WUE = p_a(1 - p_i/p_a)/V(1 - RH)1.6,$$

where V is the saturated partial water vapor pressure at a given temperature, RH is relative humidity, and 1.6 is the psychometric constant (Farquhar et al., 1982). Thus, WUE can be estimated from Δ values using the following equation:

$$WUE = pa[1 - (\Delta - 4.4)/22.6]/V(1 - RH)1.6.$$

An agronomic estimation of WUE (considered as the ratio of dry matter accumulated to total water transpired) can be also be derived from Δ using different equations (Hubick et al., 1986; Hubick and Farquhar, 1989; Craufurd et al., 1991; see also Araus et al., 1993).

Methodological considerations

Carbon isotope analysis is performed by mass spectrometry. Although the equipment needed for such testing is expensive and often beyond the capability of many laboratories and research stations, there are commercial firms that do the analyses reliably and at a reasonable price. Before sending the material to be analyzed, it must be ground very finely.

$^{13}C/^{12}C$ ratio values are expressed as carbon isotope composition ($\delta^{13}C$) values, where

$$\delta^{13}C\ (‰) = [(R\ sample/R\ standard)-1] \times 1000,$$

R being the $^{13}C/^{12}C$ ratio. The standard for comparison is a secondary standard calibrated against PeeDee belemnite (PDB) carbonate. Test precision is usually lower than 0.10 ‰. The value of the discrimination (Δ) against ^{13}C is calculated from δ_a and δ_p, where a refers to air and p to plant (Farquhar et al., 1989):

$$\Delta = (\delta_a - \delta_p \Delta / (1 + \delta_p)$$

On the PDB scale, free atmospheric CO_2 has a current deviation, δ_a, of approximately -8.0 ‰ (Farquhar et al., 1989).

Implications for plant breeding

What type of sample to take and when?

Considerable genotypic variations for Δ have been found in bread wheat (Condon and Richards, 1992), barley (Romagosa and Araus, 1991; Acevedo, 1993), and durum wheat (Araus et al., 1993a), but environmental factors may cause even larger changes in the value of Δ, which could compromise the effective use of Δ in breeding programs. After studying wheat Condon and Richards (1992) concluded that assessing genotypic variation in Δ would be most effective under well-watered conditions. In this regard, Richards and Condon (1993) pointed out that under adequate conditions, Δ is highly heritable and exhibits substantial genetic variation and few genotype x environment interactions.

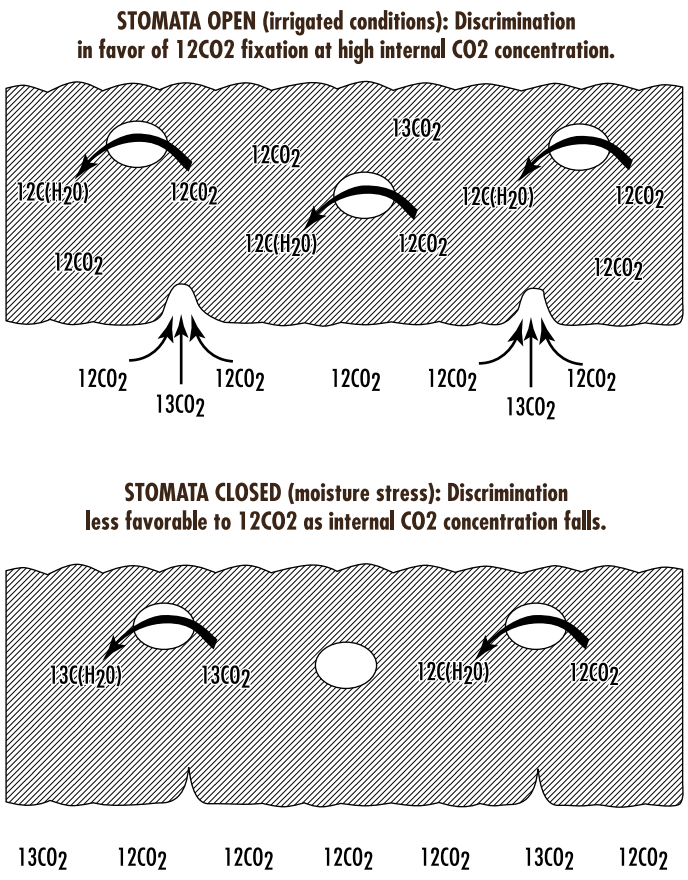

Diagram 1. Carbon isotope discrimination under irrigated and dry conditions.

As an alternative in rainfed environments, Condon and Richards (1992) proposed sampling for Δ at early crop stages, when terminal stress is lacking. However, the information available on rainfed environments often does not support these expectations. Though correlation between Δ and yield is usually weak or non-existent when dry material from seedlings is analyzed (Bort et al., 1998), it increases when upper plant parts are used in Δ analyses (Figure 1). The best genetic correlations between Δ and yield, together with the high broad sense heritability of Δ, have also been reported for the upper parts of durum wheat (Araus et al., 1998b).

The correlation between yield and Δ increases with plant age, perhaps due to the effects of progressive stress (particularly after anthesis) on yield. In fact, Δ usually decreases from the oldest to the youngest plant parts, even under well-watered conditions (Hubick and Farquhar, 1989; Acevedo, 1993; see also Figure 1). Such a decrease may be attributed to stomatal closure in response to declining soil water and/or increasing vapor-pressure deficit during the last period of crop growth (Condon and Richards, 1992; Condon et al., 1992; Araus et al., 1993b). Thus, mature kernels could be the most adequate plant part to sample. Under Mediterranean conditions, for example, the Δ of kernels rather than the Δ of lower plant parts may provide more information on which genotype is less affected by stress during grain filling.

Higher or lower carbon isotope discrimination? In water-limited environments, genotypes with low Δ should have greater biomass and hence potential for higher yields, assuming that all genotypes use the same amount of water for transpiration (Richards, 1996). In fact, selecting for high WUE (Passioura, 1977) or low Δ (Craufurd et al., 1991) has been proposed as an important alternative when defining plant breeding strategies under limited water conditions. However, Δ values often correlate positively with grain yield and/or total biomass in wheat (Condon et al., 1987; Kirda et al., 1992; Araus et al., 1993c, 1997b; Morgan et al., 1993; Sayre et al., 1995) and barley (Romagosa and Araus, 1991; Richards, 1996) under well-irrigated or rainfed conditions (Figure 1).

From an agronomic point of view, a positive relationship between Δ and yield may exist if plants are not using all available soil water. Assuming the same phenology, a genotype with high Δ will

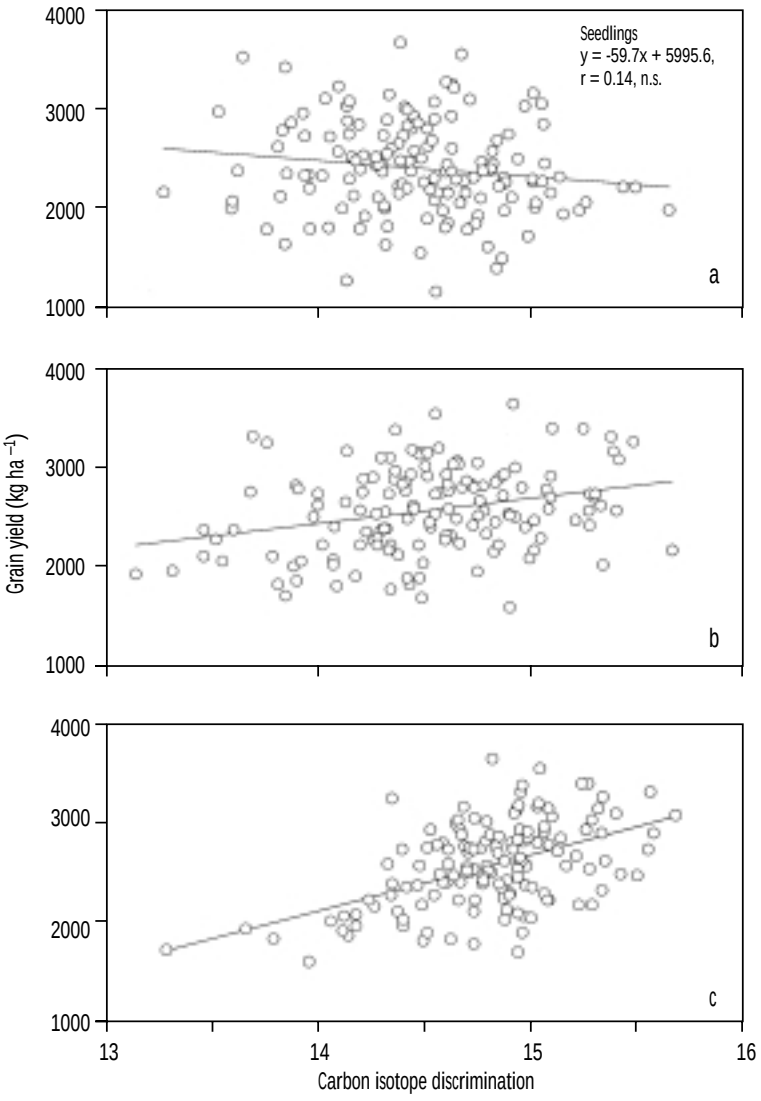

Figure 1. Relationships between grain yield and carbon isotope discrimination (Δ) measured in dry matter of seedlings (a), in the penultimate leaf sampled around heading (b) and in mature kernels (c) for a set of 144 durum wheat genotypes grown under rainfed conditions in the Tel Hadya, northwestern Syria. For more details, see Araus et al. (1997b).

be able to sustain a high level of transpiration. Therefore, Δ can be considered an indicator of WUE, but also depends on the water transpired by the crop, which is in fact the first parameter of Passioura's identity. The positive association between Δ and yield also suggests that variations in stomatal conductance rather than in intrinsic photosynthetic capacity are predominant in determining Δ (Romagosa and Araus, 1991; Condon et al., 1992). Higher Δ is related to a higher level of CO_2 in the cellular air spaces due to greater stomatal conductance (Farquhar and Richards, 1984), which leads to higher photosynthetic rates and, hence, higher yield even in the absence of stress. In this situation, WUE decreases (and Δ increases) because stomatal limitation reduces transpiration more than photosynthesis, even when yield may be positively affected by low stomatal limitation on photosynthetic rate.

Relatively high transpiration levels may have implications for water-limited environments. For example, when water supply can be provided to the crop under drought stress, (e.g., by deep soil moisture extraction), the high-yielding (i.e., high-transpiring) genotype will have the most advantage (Blum, 1993, 1996). In fact, relatively low canopy temperatures resulting from high stomatal conductance and transpiration are typical of the more drought-resistant genotypes (Garrity and O'Toole, 1995; see also Blum, 1996). In addition, when grown at above-optimal temperatures, such as the maximum daily temperatures typical during grain filling, the positive correlation observed between stomatal conductance and yield may also be related to heat avoidance (Reynolds et al., 1994).

Alternatively, mechanisms that prevent water loss, such as inherently lower stomatal conductance, may limit yield potential because of the intercellular levels of CO_2, thus decreasing photosynthesis. These genotypes will consistently show low Δ values (Morgan et al., 1993). In fact, stomata that close only in response to severe water stress may be more useful in terms of yield than stomata that permanently show low conductance values (Jones, 1987). Moreover, selection for low Δ (i.e., high WUE) may favor low-producing genotypes under drought conditions (i.e., drought-susceptible genotypes). Therefore, low Δ may not be a good selection criterion for improving yields in dry environments. Plant production under drought conditions depends not only on WUE but largely on the genotype's capacity to sustain transpiration (Blum, 1993).

Blum (1996) pointed out that when soil moisture is very limited, the high-yielding genotype may be at a disadvantage because of its high stomatal conductance. In fact, it has been reported for wheat and barley that this (crossover) happens if yield is reduced to below 2-3 t ha^{-1} (Ceccarelli and Grando, 1991; Blum, 1993). Other reports on durum wheat and barley do not support the existence of such a crossover in environments with a mean grain yield of 1.5 t ha^{-1} or lower (Romagosa and Araus, 1991; Araus et al., 1998b). In these environments it may still be worth selecting for yield potential, particularly if deep extractable soil moisture is available to provide a yield above that of the crossover of genotype yields (Richards, 1996). Summarizing, the above results support the hypothesis that under Mediterranean conditions high-yielding genotypes, which sustain greater stomatal conductance and transpiration losses during grain filling, can provide higher yields in a wide range of environments with different levels of drought stress.

Optimal yield conditions. Carbon isotope discrimination has also been proposed as a useful trait to select for yield potential (Araus et al., 1993c; Sayre et al., 1995; Araus, 1996). The positive correlation between Δ and yield would exist in the same context as above. A positive relationship between Δ and growth has also been reported for seedlings grown under adequate water conditions (Febrero et al., 1992; Lopez-Castañeda et al., 1995), even when under field conditions cold stress (usual at this early stage) may obscure such a relationship (Bort et al., 1998). Nevertheless, increased early growth and leaf area development may be inherently linked to decreased WUE (Turner, 1993) and thus to higher Δ. Blum (1996) pointed out that the data accumulated on carbon isotope discrimination and yield appears to support a consistent positive relationship between crop yield and photosynthetic capacity for various genetic materials of wheat and other crops (Hall et al., 1994). If selection for high photosynthetic capacity or higher crop productivity brings about an increase in stomatal conductance, then a concomitant increase in Δ (or a decrease in crop WUE) is expected (Blum, 1996).

Role of phenology in genotypical differences in Δ. In the absence of stress, Δ in wheat is independent of phenological differences (Sayre et al., 1995). However, under non-optimal conditions the role of phenology in the relationship between Δ and yield must be considered. Thus, as pointed out before, in Mediterranean environments phenology is the most important factor that accounts for increased wheat yields, as it affects assimilate partitioning, the pattern of water use, and other traits (see references in Slafer et al., 1993; Loss and Siddique, 1994). In addition, some of the genotypical differences in Δ, as well as their positive association with

yield, can be due to phenology. Thus early flowering lines are more likely to have high Δ than later-flowering lines due to the lower transpirative demand, which maintains higher stomatal conductances in the former (Ehdaie et al., 1991; Acevedo, 1993). Summarizing, under Mediterranean conditions early-flowering in wheat and other cereals is related to higher yields, which is in accordance with higher Δ in the earlier genotypes. Alternatively, there is genotypical variability in Δ that cannot be explained by phenology (Condon and Richards, 1993; Richards and Condon, 1993; Araus et al., 1998b) and is just due to differences in accumulated transpiration.

Δ Surrogates

Given the cost and technical skills involved in carbon isotope analysis, different "surrogates" of Δ have been investigated, such as mineral accumulation in vegetative plant parts (Figures 2 and 3) and leaf structure. Instead of being Δ substitutes or surrogates, these selection criteria probably allow the evaluation of traits, other than WUE, that determine yield. Regarding the first criterion, if passive transport driven by transpiration is (at least partially) the mechanism of mineral accumulation in vegetative parts, then mineral content will also be an indicator of the first parameter of Passioura's identity, i.e. total water transpired. The second trait corresponds to structural criteria that indicate the amount of photosynthetic tissue per unit leaf area and is therefore related to photosynthetic capacity. In addition, the mechanisms underlying the physiological association between Δ and either mineral accumulation (Walker and Lance, 1991; Masle et al., 1992) or the amount of photosynthetic tissue (Araus et al., 1997a, b) are not fully understood. However, the empirical relationships of these alternative criteria with Δ and yield may justify their use on a routine basis. Such tools might be used during the early phases of a breeding program, which usually involve large populations. If the facilities or the funds are available, later selections could be based on more precise and accurate, yet expensive, Δ analysis (Mayland et al., 1993). These two alternative criteria are discussed in the following paragraphs. Interestingly, these surrogates can be used in C_4 crops (such as corn or sorghum), where Δ is not as useful for evaluating WUE and yield itself as in C_3 plants (Farquhar et al., 1989; Henderson et al., 1998).

Mineral content in vegetative parts

Potassium, silicon, total mineral, or ash content accumulated in vegetative tissues have been proposed as surrogates of Δ in cereals, forage crops, and soybean (Walker and Lance, 1991; Masle et al., 1992; Mayland et al., 1993; Mian et al., 1996). Masle et al. (1992) reported for all the herbaceous C_3 species they assayed a positive linear relationship between total mineral content of vegetative tissues and the inverse of either WUE or Δ. Therefore, the amount of minerals accumulated by plants in the glasshouse or in the field could be a potentially useful indicator of Δ and WUE (Walker and Lance, 1991; Masle et al., 1992; Mayland et al., 1993).

In theory, total mineral and ash content seem to be better surrogates than the content of any single mineral, such as silicon or potassium (Masle et al., 1992; Mayland et al., 1993). Therefore, estimating plant mineral content, especially ash content, which requires only a muffle furnace, might be an attractive alternative to Δ for preliminary screening of large, genetically diverse populations (Masle et al., 1992; Araus et al., 1998b).

Methodological considerations regarding ash content. Samples must be properly oven-dried and ground. Approximately 1 to 1.5 g of dry matter is placed in a pre-weighed porcelain crucible (empty crucible), the crucible with the sample is weighed (filled crucible), and the sample is burnt in a furnace at $450^{B}C$ for 12 h. Then the crucible with mineral residue (burnt crucible) is weighed again. Ash content is expressed (on a concentration basis) as a percentage of sample dry weight as follows:

$$\text{Ash content (\%)} = \frac{\text{(burnt crucible weight - empty crucible weight)}}{\text{(filled crucible weight - empty crucible weight)}} \times 100$$

Implications for plant breeding: Choice of environment and type of sample. The positive correlation between ash content and Δ may indicate that plants able to maintain higher stomatal conductance and transpiration will accumulate more ash in a transpirative organ, provided entry and accumulation of minerals in the plant take place (at least partially) through the transpirative stream.

Which are the best growing environments for using this surrogate? Mineral accumulation seems to be better related to Δ and even to yield under well-watered conditions (Masle et al., 1992; Mayland et al., 1993), although these traits can be useful under drought conditions (Figure 2a; Araus et al., 1998b). Measurements taken on plants grown in the greenhouse can give contradictory results (Walker and Lance, 1991). Another question is the kind of sample to use. Later developed leaves (flag or penultimate leaves) are best. Ash accumulated in the flag leaf may be positively related to the Δ of kernels (Araus et al., 1998*b*). Leaves must be mature to let minerals accumulate, but not senescent because minerals can remobilize to other plant parts. Thus, ash content measured at maturity in straw did not correlate with either Δ of mature grains or yield. (Voltas et al., 1998).

Mineral content in mature kernels as a criterion to complement Δ

A negative relationship between ash content (on a dry mass basis) in mature kernels and yield has been reported for barley and durum wheat, under both optimum and non-optimum (i.e., rainfed) conditions (Febrero et al., 1994; Araus et al., 1998b; Voltas et al., 1998). This may be explained by the fact that ash content on a kernel mass basis may be an indirect indicator of total reproductive sink per culm attained at maturity (Araus et al., 1998b). In fact, total kernel mass per spike is the product of two yield components developed last during the crop cycle: number of kernels per spike and kernel mass. Thus, kernel ash has been proposed as a criterion complementary to kernel Δ in assessing genotype differences in cereal yield under Mediterranean conditions (Febrero et al., 1994; Voltas et al., 1998). In theory (Diagram 2), the pattern of ash accumulation in kernels is different from that in vegetative tissues because, unlike mineral accumulation in vegetative tissues, grain filling does not take place via the xylem (driven by transpiration) (Slafer et al., 1993). Such differences in mineral accumulation could explain the complementarity of ash content and Δ in kernels as integrative traits predicting grain yield (Febrero et al., 1994; Voltas et al., 1998).

Summarizing, kernel ash combined with either kernel Δ or leaf ash can be partially complementary (i.e. independent) parameters when assessing differences in grain yield (Febrero et al., 1994; Araus et al., 1998b; Voltas et al., 1998). Selecting for low ash content in kernels, combined with either high Δ in kernels or, alternatively, high ash content in the flag leaf, deserves further attention in wheat breeding (Figure 3). This approach could be particularly interesting if it were coupled with a new analytical technique

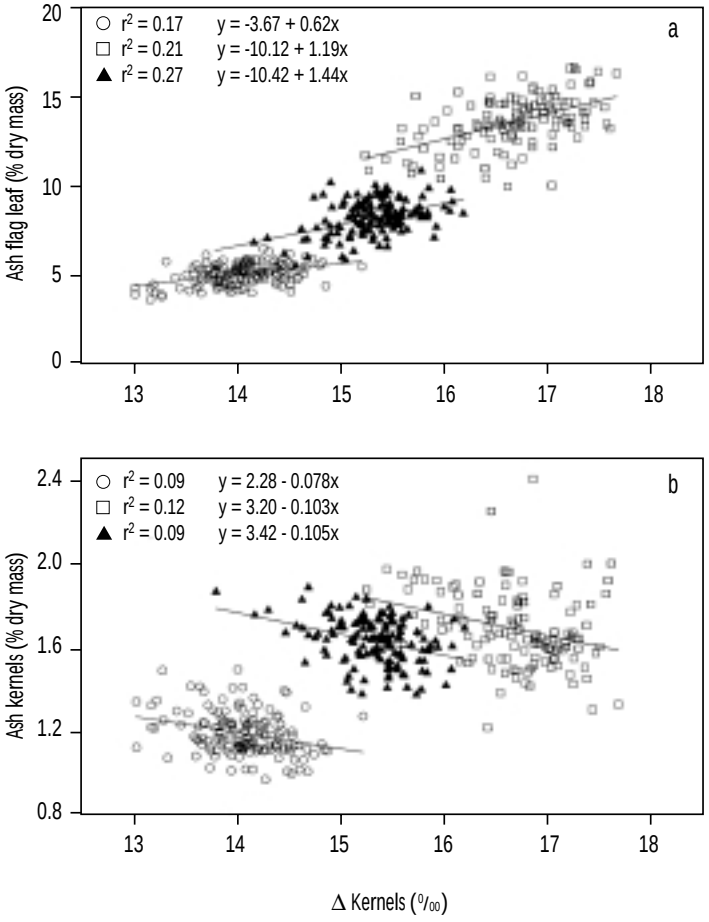

Figure 2. Relationship between carbon isotope discrimination (Δ) in mature kernels and ash content based on dry mass (a) of the flag leaf blades around three weeks after anthesis, and (b) in the same mature kernels. Plants were cultivated in three trials differing in water status: Breda, Tel Hadya rainfed, and Tel Hadya with supplementary irrigation.

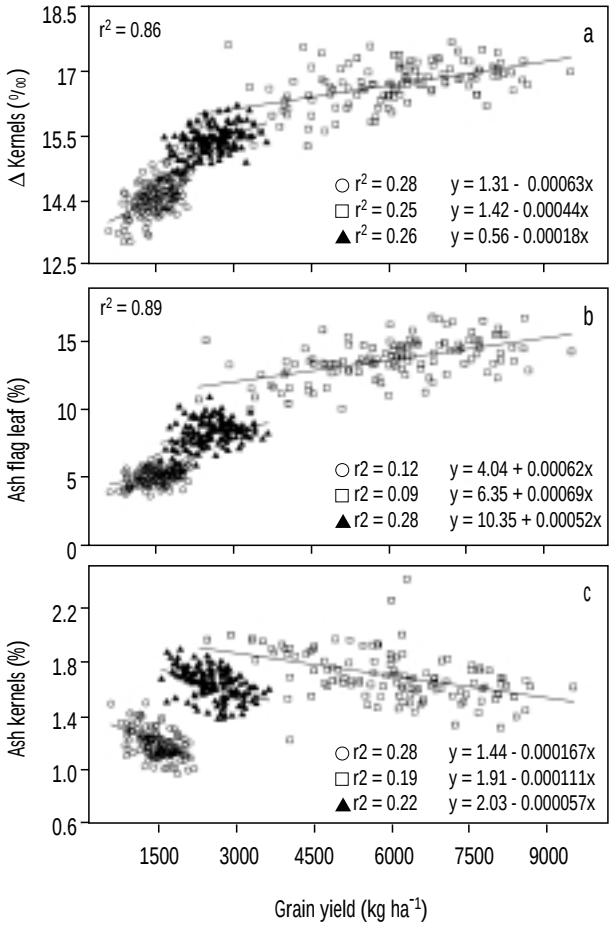

Figure 3. Relationship between grain yield and (a) carbon isotope discrimination (Δ) in mature kernels, (b) ash content (based on dry of the flag leaf around three weeks after anthesis, and (c) ash content of the same mature kernels.

such as near infrared reflectance spectroscopy (NIRS), which would allow a fast, reliable estimation of ash content and Δ in intact kernels (see Araus, 1996).

Leaf structural criteria

Changes in Δ can derive from changes in the balance between leaf stomatal conductance and photosynthetic capacity. In wheat, genotypic variations in Δ seem to derive from differences in both stomatal conductance and photosynthetic capacity, each contributing about the same (Condon et al., 1990; Condon and Richards, 1993; Morgan et al., 1993). If the intrinsic photosynthetic capacity of leaves is increased, Δ could decrease and WUE could be improved, without compromising yield potential (see above). Therefore, a negative relationship between Δ and yield could be expected even in the absence of stress.

Genotypic differences in photosynthetic capacity may depend on the amount of photosynthetic tissue per unit leaf area. Thus, single structural parameters such as dry mass per unit leaf area (LDM, the reciprocal of specific leaf area, also termed specific leaf dry weight, or SLDW) or total nitrogen or chlorophyll content per unit leaf area may be good indicators of the strength of photosynthetic tissue (see references in Araus et al., 1989; Nageswara Rao and Wright, 1994). For example, total chlorophyll content per leaf area may be evaluated in a fast, single and non-destructive way using a portable chlorophyll meter like the SPAD-502 (Soil-Plant Analysis Development Section, Minolta Camera Co., LTd., Japan). Usually the leaf parameter that correlates negatively best with Δ is LDM, followed by SPAD, which indicates that genotypes with thicker and/or more compact leaves have lower Δ. The results suggest that LDM and SPAD measurements can be used as single, rapid indicators of Δ in barley (Araus et al., 1997a) and durum wheat (Araus et al., 1997b) under optimal growing conditions (see also Lopez-Castañeda et al., 1995).

However, some of these correlations may exist under drought conditions and could be useful for breeding, but may be spurious in nature. In fact, growing conditions have a strong direct effect not only on Δ, but also on leaf structure, which in turn could lead to spurious relationships (Araus et al., 1997b). The correlations between Δ and leaf structure, rather than being sustained by a physiological relationship between the amount of photosynthetic tissue and Δ, may sometimes be indirect associations caused by a parallel effect of water status and phenology on leaf structure, grain Δ, and yield (Araus et al., 1997a, b). Summarizing, LDM should be used only in the absence of drought to determine segregating population differences in leaf Δ based on internal photosynthetic capacity. It is worth selecting for higher kernel Δ and grain yield based on higher LDM in rainfed trials, although there probably is no direct physiological basis behind such relationships (Araus et al., 1997b).

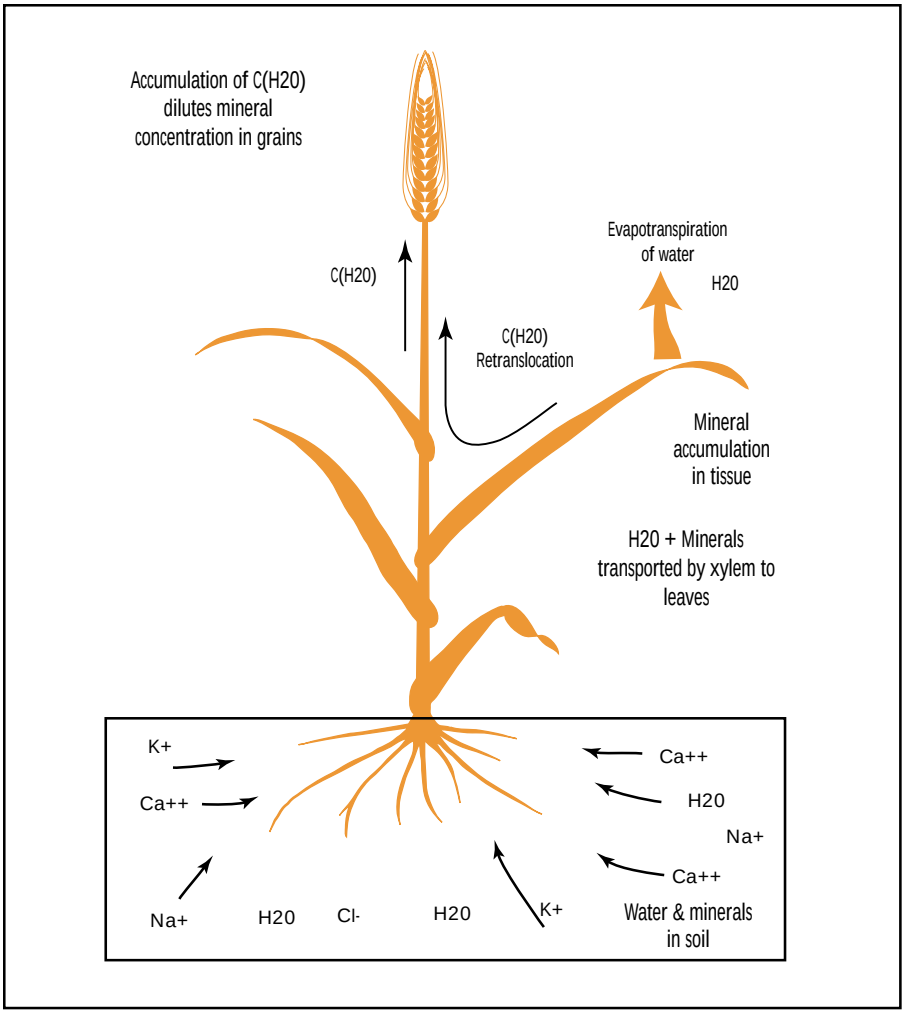

Diagram 2. Accumulation of ash in plants.

Spectral Reflectance Methods

The pattern of light reflection on leaves at different wavelengths through the photosynthetically active radiation (PAR, 400-700 nm) and near infrared radiation (NIR, 700-1200 nm) regions of the electromagnetic spectrum is very different from that of soil and other materials (Diagram 3). Leaf pigments absorb light strongly in the PAR region but not in the NIR, thus reducing the reflection of PAR but not of NIR. Such a pattern of pigment absorption determines the characteristic reflectance signature of leaves (Figure 4). Similarly, the light spectrum reflected by a canopy (either natural or agricultural) differs from that reflected by the bare soil and varies in a way that can be related to the overall area of leaves and other photosynthetic organs in the canopy, as well as to their pigment composition and other physiological factors (Figure 5). Therefore, the measurement of spectra reflected by vegetation canopies provides information that can be used to estimate a large scope of parameters. Some of them are related to the green biomass of the canopy, its photosynthetic size (i.e., total area of leaves and other photosynthetic organs), the amount of PAR absorbed by the canopy, and its photosynthetic potential. Other parameters are more related to the canopy's physiological status at the time of measurement and can be used to assess the extent of some nutrient deficiencies and environmental stresses. The physiological parameters that can be estimated by spectral reflectance techniques include chlorophyll and carotenoid concentrations, photosynthetic radiation use efficiency (PRUE), and water content.

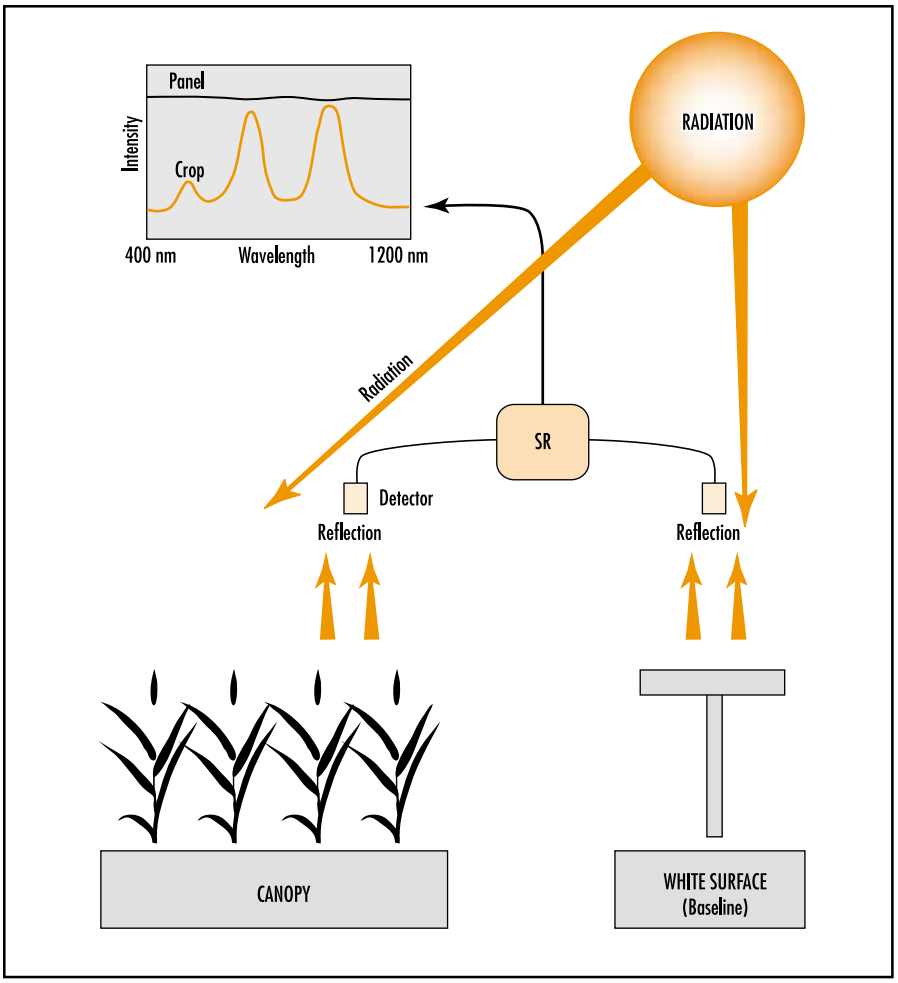

Diagram 3. Spectral reflectance from crop surfaces.

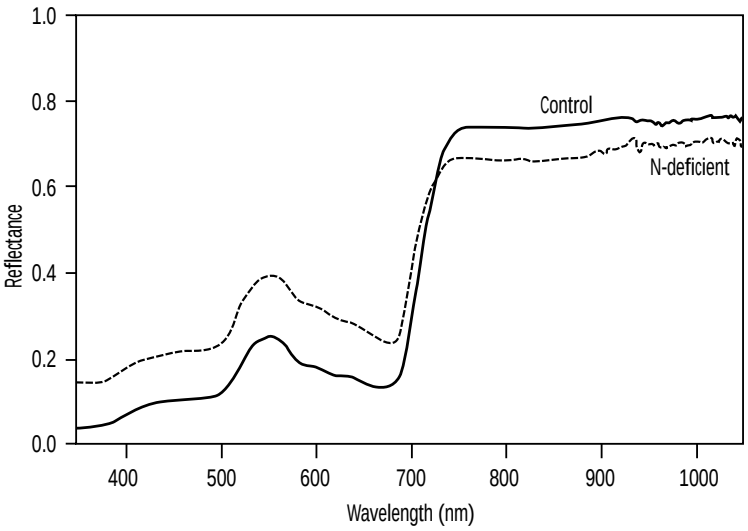

Figure 4. Reflectance signature of two wheat leaves differing in nitrogen status. Note the higher reflectance in the photosynthetically active radiation region of the nitrogen deficient leaf due to lower chlorophyll content in the leaf area.

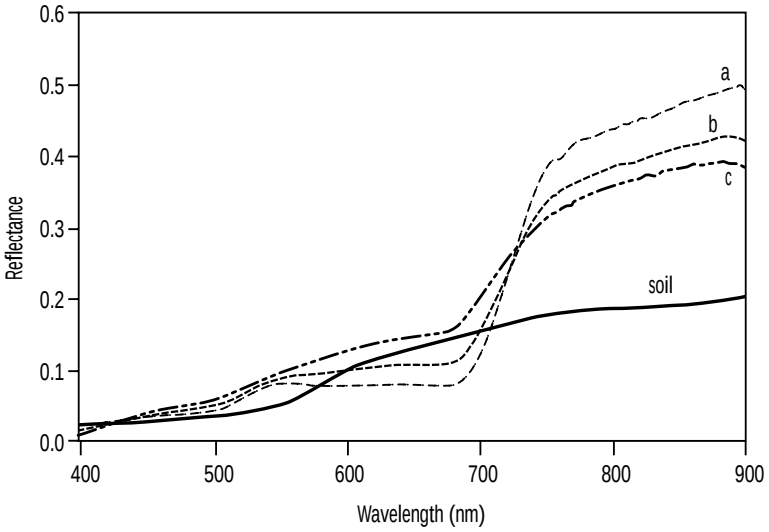

Figure 5. Changes in the pattern of canopy reflectance in a durum wheat plot. Measurements were taken every three days (a, b, c), during the last week of grainfilling, coinciding with fast crop senescence. Note the decrease, during senescence, in the amplitude of the change in reflectance in the red-NIR (around 700 nm) edge. Note also the increase within the PAR region of the reflectance in the red compared to the blue band due to a relatively faster decrease during senescence in chlorophyll compared to carotenoids. Soil reflectance is included for comparison.

Spectral reflectance indices

Spectral reflectance indices are formulations based on simple operations between reflectances at given wavelengths, such as ratios, differences, etc., which are widely used to quantitatively relate changes in reflectance spectra to changes in physiological variables. These indices have the advantage of summing up in a few numbers the large amount of information contained in a reflectance spectrum with narrow waveband resolution.

Originally used in remote sensing by aircraft and satellites, reflectances measured at the ground level are very useful for assessing agrophysiological traits. These traits can be evaluated simultaneously in each sample, at a rate of up to one thousand samples per day, which is much more tedious and time consuming with other methods. This makes spectroradiometric indices ideal for screening for yield potential or for resistance to different stresses.

Sample applications

Perhaps the most widespread application of reflectance indices is for assessing parameters related to canopy greenness. These parameters are related to the canopy's photosynthetic size and include green biomass, leaf area index (LAI) (total one-side leaf area of the crop relative to soil area), green leaf area index (GLAI) (similar to LAI, but includes only functional green leaves), and green area index (GAI) (similar to GLAI, but includes other photosynthetic organs such as green stems). The amount of green area in a canopy determines PAR absorption by photosynthetic organs, which in turn determines the canopy's potential production. The fraction of the incident PAR that is absorbed by the canopy (fPAR) can be estimated from LAI-related parameters or directly from reflectance measurements. Cumulative PAR absorption, which is one of the parameters determining total biomass and thus final yield (see the beginning of this chapter), can be assessed by measuring reflectance periodically during the growth cycle.

Some physiological parameters can also be quantified by spectral indices. Leaf pigments can be detected and quantified based on reflectance spectra and can be used as indicators of several physiological processes. Thus, the canopy's nutritional state can be evaluated through pigment concentration, as chlorophyll (Chl) concentration in leaves is (usually) closely correlated to its nitrogen content. Indices that are good indicators of Chl are (usually) also good indicators of N-content. In addition, plants with low N usually have a high carotenoid (Car) to Chl ratio, which can also be assessed by reflectance indices (Figure 5).

Pigment remote sensing can also be used for assessing the crop's phenological stage (Figure 5) and the occurrence of several stress factors (Blackburn, 1998; Peñuelas, 1998). For example, the Car to Chl ratio can be associated with senescing processes that result from the plant's natural ontogeny pattern or are triggered by different stresses. Also, phenological stages can be associated with different Car/Chl values. Several indices related to changes in pigment composition have been developed and can be used for the remote detection of nutrient deficiencies, environmental stresses, pest attacks, etc. In such contexts, by periodically assessing leaf area, leaf area duration (LAD) can also be used as an indicator of resistance to certain environmental stresses.

The photosynthetic capacity of a canopy can be estimated by using vegetation indices that correlate to the photosynthetic size of the canopy or indices related to the amount of chlorophyll. However, actual photosynthesis may not match

photosynthetic capacity due to the variability of photosynthetic use efficiency of the absorbed radiation, especially when plants are exposed to unfavorable conditions. The photochemical reflectance index (PRI) was developed to detect pigment changes in the xantophyll cycle associated with changes in PRUE (Filella et al., 1996). PRI has been shown to track the changes in PRUE induced by factors such as nutritional status and midday reduction, across different species and functional types.

Another potential application of reflectance indices is remote detection of relative water content (RWC) of plants. Different levels of water stress can be detected indirectly through their effects on vegetation indices related to leaf area, pigment concentration, or photochemical efficiency. In addition, specific indices have been developed for the direct assessment of RWC.

Measurement techniques

Instruments. The instruments required for measuring reflectance spectra are: 1) a field spectroradiometer that analyzes the spectrum of sampled radiation, 2) foreoptics that capture the radiation reflected by a given target, and 3) reference panels, supports, and levels for repeated sampling of incident radiation and radiation reflected by the canopy.

Modern narrow-band spectro-radiometers measure the irradiance at different wavelengths with a bandwidth of about 2 nm through the PAR and NIR regions of the spectrum. Most spectral indices use specific wavebands in the 400-900 nm range; only a few use longer wavelengths, such as the water index, which uses 970 nm (Peñuelas et al., 1993). The use of spectroradiometers with narrow band resolution allows the calculation of several parameters obtained from the first and second derivative of the reflectance spectra against wavelength, which can be used to complement the reflectance indices.

Radiation reflected by the canopy in the PAR and NIR regions is sampled by a foreoptic that limits the field of view to a given solid angle, usually between 10 and 25º. Sampled radiation is conveyed to the spectrum analyser through a fiber optic cable. Light reflected by the canopy is measured with the foreoptic held 1-2 m above the canopy on a fixed or hand-held support, such as a boom **(**Picture 1**)**, and with the help of the required levels or protractors to ensure that all measurements are taken at the same angle between the foreoptics and the sampled surface.

In order to cross-reference the intensity of reflected radiation at each wavelength to the intensity of incident radiation at the same wavelength, all sampled spectra must be converted to reflectance units, i.e., the ratio between the absolute spectrum reflected by the canopy and the absolute spectrum incident on the canopy. Regular measurements of the spectra incident on the canopy are then made. Incident spectra are measured by aiming the foreoptic at a white reference panel in the same orientation to the sun and to the foreoptic as the canopy. Reference panels are commercially available under the name Spectralon (Labsphere, PO Box 70, North Sutton, NH 03260) or they can be made of barium sulphate (Jackson et al., 1992).

Factors affecting the estimation of canopy parameters by spectroradiometrical methods. In addition to canopy variables estimated using spectroradiometrical methods, other factors related to the canopy or external to it will affect the measured

Picture 1. How to place the foreoptic while measuring radiation reflected by a wheat canopy.

reflectance spectra. Variation in canopy structure (such as changes in leaf erectness or appearance of reproductive organs), as well as in the angles between sun, sensor, and target surface, will affect the amount of shadow and/or soil background appearing in the field of view; this can cause non-desired variation in the measured spectra.

There are no standard methods to cope with the variability introduced by interference; most researchers using spectroradiometry adapt the details of their experimental protocols to the particular traits and objectives of their experiments. It is important to fix the measuring conditions used to obtain the spectra. Viewing angle and viewing height, row orientation, and time of day have to be determined when designing an experiment. Disturbance by factors beyond the researcher's control has to be considered when interpreting the results. Not all indices are equally affected by these factors, and indices also differ in their sensitivity to the parameter being measured. Some indices may be more appropriate than others, depending on the aims of the study, canopy characteristics, and measurement conditions.

To minimize the variability induced by sun position, it is preferable to take all measurements at about noon. Nevertheless, the angle of the sun is most important in canopies with low LAI (Kollenkark et al., 1982; Ranson et al., 1985). As for the viewing angle, nadir (sensor looking vertically downward) is perhaps the most commonly used set-up. This is because it has a lower interaction with sun position and row orientation, and delays the time at which spectra become saturated by LAI. On the other hand, nadir viewing is more affected by the soil background. When an off-nadir viewing angle is used, variability due to changes in solar elevation or sensor elevation is minimized if the angle between the sensor azimuth and the sun azimuth is 0-90º (Wardley, 1984).

In a row canopy with low soil cover, the amount of shadow within the canopy varies during the day, depending on the angle between sun azimuth and row orientation. Such angular changes can produce variation in the measured reflectance as great as 100% in red and lower in NIR wavelengths (Kollenkark et al., 1982). Peak variability occurs when the sun is shining down the rows (when sun azimuth equals row orientation), lightening the soil surface and thus giving a higher reflectance reading. For that reason, if reflectance is measured at about noon, rows oriented east to west are more appropriate than rows oriented south to north, especially if soil cover is poor.

Ratio indices are usually less sensitive to changes in viewing geometry and tend to cancel the effects for angular changes (Wardley, 1984). However, they can also be altered because at some wavelengths (such as in red) reflectance is (usually) more intensely altered than at other wavelengths (such as in NIR). Light incident on shaded leaves is poor in the wavelengths that have been absorbed by upper leaves, and their reflected spectra is even poorer. For that reason, the higher the number of shaded leaves that appears in the field of view, the larger the differences in the canopy's reflectance spectra between regions where radiation is absorbed by photosynthetic pigments and regions where it is not. If due to external factors such as viewing angle, sun angle, or wind, the number of shaded leaves in the field of view increases, this will lead to an increase in indices related to green biomass.

The relationship between indices and estimated canopy parameters has been reported to be disturbed by phenological changes that affect crop structure, such as those associated with anthesis in maize (Andrieu and Baret, 1993) or head emergence in wheat (Shibayama et al., 1986). Leaf erectness can also affect canopy reflectance. Model calculations and test results show that radiation reflected perpendicularly from plant canopies is considerably greater from planophile canopies than from erectophile canopies (Jackson and Pinter, 1986). The vertical elements of an erectophile canopy trap reflected radiation within the canopy, while in a more planophile canopy, more radiation is reflected vertically. These structural effects can alter indices used for estimating the same canopy parameter in a different way. For example, Jackson and Pinter (1986) observed that although indices SR and PVI (see later in this chapter) are both used for estimating GLAI, SR was higher in erectophile canopies of wheat, while PVI was higher in planophile canopies. Optical differences in the surface of plant organs, such as different glaucousness (Febrero et al., 1998), can also have some effect on the canopy's reflectance spectra.

Clouds increase the proportion of indirect radiation (i.e., diffuse) to total radiation incident on the canopy; this improves the penetration of light into the canopy. As a result, a greater proportion of incoming radiation is absorbed by photosynthetic pigments; this increases the vegetation indices and leads to an overestimation of green biomass. Wind during the measurements can momentarily alter canopy structure and disturb the relationship between the reflectance spectra and the canopy parameters to be estimated from the spectra (Lord et al., 1985).

Nearby objects, including instruments and the people operating them, can alter the measured spectra by reflecting radiation on the target surface. For that reason, they should be kept as far as possible from the field of view; the instruments should be painted a dark color, and people should wear dark clothes (Kimes et al., 1983).

Taking measurements. Systematic measurements of incident radiation must be made before and during the measurement of reflected radiation to account for possible variation in the incident spectra caused by atmospheric conditions or sun position.

Reference panels should be Lambertian surfaces, that is, they reflect the incident light equally in all directions and for all wavelengths. However, they are not perfect and the intensity of the reflection changes in an important way when panel orientation changes. For that reason, care must be taken to make all incident measurements keeping the panel at the same angle with the foreoptics and with the sun. Changes in the distance from the panel to the foreoptics are less important. This distance is set to ensure that the entire field of view is covered by the panel. Then the reflectance of the canopy samples can be measured making sure that the field of view of the instrument is covered with weed-free canopy and a uniform background, and with plant material homogeneous in structure (Bellairs et al., 1996).

Use of Canopy Reflectance Indices

Assessing the photosynthetic size of canopies using vegetation indices

Vegetation indices (VI) estimate parameters related to the photosynthetic size of a canopy based on the reflectances in the red and near infrared regions. Green biomass, LAI, GAI, GLAI, fPAR, etc., can be estimated through their positive correlation (either linear or logarithmic) with vegetation indices (Wiegand and Richardson, 1990a, b; Baret and Guyot, 1991; Price and Bausch, 1995). Measuring vegetation indices periodically during the crop growing cycle allows the estimation of LAD (which can be used as an indicator of environmental stress tolerance) and the total PAR absorbed by the canopy, which is one of the most important factors for predicting yield (Wiegand and Richardson, 1990).

Vegetation indices take advantage of the great differences in reflectance at red and NIR caused by vegetation. The most widely used VI are the simple ratio (SR) and the normalized difference vegetation index (NDVI), which are defined as:

$$SR = R_{NIR} / R_{Red}, \text{ with a range of 0 to } \infty,$$

where R_{NIR} is the reflectance at NIR and R_{Red} is the reflectance at red.

$$NDVI = (R_{NIR} - R_{Red}) / (R_{NIR} + R_{Red}), \text{ with a range of -1 to 1.}$$

SR and NDVI were originally used with the wide wavebands of former radiometers (for example, 550-670 nm for red and 710-980 nm for near infrared in AVHRR radiometers in satellites of NOAA series). With the high spectral resolution of today's radiometers, wavebands can be much narrower. Hall et al. (1990) used a waveband centered at 770 nm for NIR and another at 660 nm for red, while Peñuelas et al. (1997b) used 900 nm and 680 nm for NIR and red, respectively.

Some authors have reported improvements in NDVI performance after changing the wavebands used in the index. Carter (1998) describes an improved correlation with leaf photosynthetic capacity when using a modified NDVI where R701 (+/-2nm) and R520 (+/-2nm) were used for NIR and red, respectively.

Variations of these indices have been proposed to compensate for the effect of soil background. Thus the soil adjusted vegetation index (SAVI) was defined by Huete (1988) as:

$$SAVI = [(RNIR - RRed) / RNIR + RRed + L)] (1 + L),$$

where the parameter L was adjusted to minimize noise caused by soil for a large range of soil covers. For most crop conditions L=0.5, while for very low soil covers L=1 would be more appropriate, and L=0.25 would be appropriate for very high covers (Huete, 1988).

Other indices include parameters obtained from the soil's reflectance spectrum. One of them is the transformed soil adjusted vegetation index (TSAVI) which was defined by Baret and Guyot (1991) as:

$$TSAVI = a(R_{NIR} - aR_{Red} - b) / [R_{Red} + a(R_{NIR} - b) + 0.08(1+a^2)],$$

where a is the slope and b is the intercept of the linear equation

$$R_{NIRsoil} = a*R_{Red\ soil} + b.$$

An important drawback in estimating LAI by VI is the saturation of the VI with LAI. Saturation of NDVI starts at about LAI=1, and beyond LAI=2 it becomes insensitive to further increases in LAI (Gamon et al., 1995). Perpendicular vegetation index (PVI) partly overcomes the saturation problem inherent to NDVI (Richardson and Wiegand, 1977):

$$PVI = \{(R_{Red.soio} - R_{Red.vegetation})^2 + (R_{NIR.vegetation} - R_{NIR.soil})^2\}^{1/2}$$

Although PVI is more sensitive than NDVI to changes in the viewing

geometry, PVI does not become as clearly saturated as NDVI with changes in GLAI (Shibayama et al., 1986).

Examples of assessing LAI-related parameters by VI can be found in the literature (Baret and Guyot, 1991; Field et al., 1994; Price and Bausch, 1995). Ground level measurement of VI has been used successfully as a tool for assessing early biomass and vigor of different wheat genotypes (Elliott and Regan, 1993; Bellairs et al., 1996). Under experimental conditions of a wheat breeding program, Bellairs et al. (1996) reported young wheat canopies where LAI was less than 1.5, a coefficient determination (r^2) of 0.90-0.95 between biomass and NDVI. As for assessing the intensity of different plant stresses, Peñuelas et al. (1997b) showed that NDVI was a useful tool for measuring agronomic responses of barley to salinity.

A practical use of vegetation indices is for making yield predictions. Yield can be predicted from successive VI measurements taken during the growing season, based on the following assumptions (Wiegand et al., 1991): 1) plant stands integrate the growing conditions experienced and express net assimilation achieved through the canopy, 2) stresses severe enough to affect economic yield will be detectable through their effects on crop development and the persistence of photosynthetically active tissue in the canopy, 3) high economic yields cannot be achieved unless plant canopies fully utilize available solar radiation as the plants enter the reproductive stage, and 4) vegetation indices calculated from remote observations in appropriate wavelengths effectively measure the photosynthetic size of the canopy. Wiegand and Richardson (1990b) reported an r^2 of 0.5 for predicting wheat grain yield from PVI measured on four dates during vegetative growth. Similarly, Rudorff and Batista (1990) reported an r^2 of 0.66 between wheat yield and integrated VI from booting to completely senesced plants. If most uncertainty in yield prediction by VI is site-dependent, then calibrations of yield vs. VI across good and poor growing conditions within production areas can describe the results of past and future growing seasons acceptably (Wiegand et al., 1991).

Remote sensing of pigments

Estimating chlorophyll concentration. Several indices have been developed for estimating Chl concentration using canopy reflectance methods. The simplest indices are just reflectance at 675 and 550 nm. Reflectance at 675 nm (R675) is very sensitive to changes in Chl content. However, the relationship becomes saturated at relatively low Chl values (around 10 $\mu g\ cm^{-2}$) and is a good indicator of chlorophyll content only at very low concentrations. Absorption by Chl at 550 nm is lower than at 675 nm; therefore, the reflectance at this wavelength (R550) is less sensitive to changes in Chl content but is not saturated at such low concentrations, thus covering a range of higher Chl values (Thomas and Gausman, 1977; Jacquemoud and Baret, 1990; Lichtenthaler et al., 1996).

Both R675 and R550 are non-normalized indices that can be affected by external factors (Curran, 1983). Other indices use more than one wavelength. Analyzing wavelengths that were more sensitive to changes in Chla, Chlb, and Cars in soybean leaves grown at different N levels, Chapelle et al. (1992) developed the ratio analysis of reflectance spectra (RARS) indices, RARSa, RARSb and RARSc, which optimized the estimation of Chla, Chlb, and Cars, respectively, in soybean leaves. RARSa = R_{675} / R_{700} showed a determination coefficient of 0.93, with Chla ranging from 0.4 to 27 $\mu g\ cm^{-2}$; RARSb = R_{675} / (R_{650} * R_{700}) showed an r^2 of 0.82, with Chlb ranging from 1 to 7 $\mu g\ cm^{-2}$; and RARSc = R_{760} / R_{500} showed an r^2 of 0.94, with Cars ranging from 1.5 to 6$\mu g\ cm^{-2}$ (Chapelle et al., 1992). Blackburn (1998) reported that using R680 and R800, instead of R675 and R700, in RARSa significantly improved the prediction of Chla in a range of leaves from different species with different degrees of senescence. Other reflectance indices that can be used for estimating pigment concentration are summarized in Table 1.

Leaf chlorophyll content can also be assessed through its relationship with parameters derived from the position of the red edge. The red edge position (REP) is the wavelength in the 680-780 nm range where the change in reflectance when increasing the wavelength from red to NIR reaches its maximum. The REP shifts to slightly longer wavelengths as Chla values increase (Curran et al., 1990; Filella et al., 1995). By obtaining the first and second derivatives of the spectra in this area, several parameters that are good indicators of Chl content can be calculated. Among these parameters are the wavelength of the red edge (λ_{re}), the maximum amplitude in the first derivative of the reflectance spectra (dR_{re}), and the sum of amplitudes between 680 and 780 nm in the first derivative of the reflectance spectra ($\Sigma dR_{680-780}$). These REP-related parameters are suitable indicators of chlorophyll content in a wider and higher range of concentration than R675 and R550, with the additional advantage that they are less affected by external factors such as the geometry, incident intensity, and soil background (Filella and Peñuelas, 1995).

In addition to the wide variety of indices related to absolute Chl concentration, the normalized phaeophytinization index (NPQI) can be used to detect chlorophyll degradation.

$$NPQI = (R_{415} - R_{435}) / (R_{415} + R_{435})$$

(Peñuelas et al., 1995c)

NPQI was introduced as an indicator of pest attacks on apple trees (Peñuelas et al., 1995c). In some cases it also seems to indicate different phenological states in wheat (Casadesús and Araus, unpublished data).

One practical approach for estimating Chl concentration using reflectance indices is to test the performance of more than one index and choose the one most appropriate for the experiment. Another approach is to pool the information contained in a number of indices. In this sense, Filella et al. (1995) were able to assign different reflectance spectra to different N-status classes using a discriminant analysis based on R430, R550, R680, λ_{re}, dR_{re}, and NDPI (defined later in this chapter). Non-destructive portable chlorophyll meters based on absorbance measurements through the leaf provide fast and easy determinations of chlorophyll content and are commercially available at a relatively low price. For example, the SPAD-502 mentioned above calculates the ratio of absorbances at 650 nm λ (chlorophyll absorbance peak) and at 940 nm (non-chlorophyll absorbance) (Monje and Bugbee, 1992). Estimates of chlorophyll using canopy spectral reflectance methods are in general closely related to the amount of chlorophyll per soil area calculated from the reading of portable chlorophyll meters multiplied by the LAI (Filella et al., 1995). Chlorophyll assessment using canopy reflectance methods has the advantage that it directly integrates the chlorophyll content of all the leaves in the canopy. It also offers additional information such as canopy size and content of pigments other than chlorophyll.

Carotenoid to chlorophyll ratios. Estimating the Car: Chl ratio by reflectance indices can be useful for assessing the extent of some plant stresses, given that increases in Cars concentration relative to Chl are often observed when plants are subjected to stress (Young and Britton, 1990).

Both Chl and Car absorb in the blue, but only Chl absorbs in the red. Indices that are combinations of the reflectance in these two regions are correlated to the Car : Chl ratio. The simplest indices are pigment simple ratio (PSR) and normalized difference pigment index (NDPI), which are formulated in an analog way to SR and NDVI and defined to estimate the ratio of total pigments to Chla (Peñuelas et al., 1993):

$$PSR = R_{430} / R_{680}\ ,\ NDPI = (R_{680} - R_{430}) / (R_{680} + R_{430})$$

Both PSR and NDPI are affected by disrupting effects introduced by leaf surface and structure. A new index was developed to avoid such problems: the structural independent pigment index (SIPI), which was defined by Peñuelas et al. (1995a) as:

$$SIPI = (R_{800} - R_{435}) / (R_{415} + R_{435})$$

SIPI uses wavelengths showing the best semi-empirical estimation of the Car : Chla ratio, and its formulation minimizes the disrupting effects of leaf surface and mesophyll structure (Peñuelas et al., 1995a). R800 is used as a reference where neither Cars nor Chl absorb and are only affected by the structure. The best fit between the Cars : Chla ratio and SIPI for a variety of plants with Chla ranging from 0.06 to 54 μg cm^{-2} and Cars from 1 to 16 μg cm^{-2} was exponential, with an r^2 of 0.98 and the form, Cars : Chla = $4.44 - 6.77\exp^{-0.48\ SIPI}$ (Peñuelas et al., 1995a).

Table 1. Reflectance indices for estimating pigment concentration.

Pigment	Definition	Reference
Chl	R_{675}	Jacquemoud and Baret, 1990
	R_{550}	Jacquemoud and Baret, 1990
	$R_{750/550}$	Lichtenthaler et al., 1996 Gitelson and Merzlyak, 1997
	$R_{750/700}$	Lichtenthaler et al., 1996 Gitelson and Merzlyak, 1997
	$NDVI_{green} = (R_{NIR} - R_{540-570}) / R_{NIR} + R_{540-570})$	Gitelson and Merzlyak, 1997
	λ_{re}, dR_{re} and $\Sigma dR_{680-780}$	Filella et al., 1995
Chla	$RARSa = R_{675} / R_{700}$	Chapelle et al., 1992
	$RARSa^* = R_{680} / R_{800}$	Blackburn, 1998
	$PSSRa = R_{800} / R_{675}$	Blackburn, 1998
Chlb	$RARSb = R_{675} / (R_{650} * R_{700})$	Chapelle et al., 1992
	$PSSRb = R_{800} / R_{650}$	Blackburn, 1998
Cars	$RARSc = R_{760} / R_{500}$	Chapelle et al., 1992
Cars/Chla	$SIPI = (R_{800} - R_{435}) / (R_{415} + R_{435})$	Peñuelas et al., 1995

Indices related to the Cars : Chl ratio change during the crop growing cycle. They are low during vegetative growth and start to increase before the beginning of senescence (Filella et al., 1995). They can be used in assessing the nutritional state of a crop (Filella et al., 1995), shown by high values of the indices when N is low, and for detecting pest attacks (Peñuelas et al., 1995c).

Assessing radiation use efficiency by PRI

Canopy photosynthesis can be roughly estimated based on the estimation of the canopy's photosynthetic size or Chl concentration. However, these parameters are associated with potential canopy photosynthesis, which does not always correspond to actual photosynthesis, especially for plants growing in stressful environments. While VI are correlated with PAR absorption by the canopy (a slowly varying trait, in a range of days to weeks), the photochemical reflectance index (PRI) is correlated with photosynthetic radiation use efficiency (PRUE) of absorbed PAR, a rapidly varying process, in a range of hours.

Part of the PAR absorbed by Chl cannot be used for photosynthesis and is lost mainly through heat dissipation, which is linked to the xanthophyll-de-epoxidation cycle (Demmig-Adams and Adams, 1996). PRI reflects changes in reflectance of around 531 nm, which have been associated with pigment changes in the xanthophylls cycle (Gamon et al., 1992).

PRI was originally defined as physiological reflectance index (Gamon et al., 1992) but later the definition was slightly modified (its sign was changed) and the name of the index was revised as photochemical reflectance index (Peñuelas et al., 1995b). Here PRI refers to the second definition.

$$PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$$

(Peñuelas et al., 1995b)

PRI is correlated with the de-epoxidation stage of the xanthophylls cycle, with zeaxanthin, and with radiation-use efficiency (Filella et al., 1996). Higher PRI values indicate greater efficiency.

PRI has been shown to track changes in photosynthetic radiation use efficiency induced by different factors such as nutritional state and midday reduction, across different species and functional types (Gamon et al., 1997). However, it does not properly track changes in PRUE if there are structural changes in the canopy associated with stress, such as leaf wilting (Gamon et al., 1992). Also, this index is valid only for fully illuminated canopies and does not perform properly across wide ranges of illumination from shade to sun (Gamon et al., 1997).

Directly assessing plant water status

Some bands of radiation absorption by water exist in the 1300-2500 nm region, but due to its high absorptance in this region, reflectance becomes saturated (i.e., it does not respond to further increases in RWC) even in a canopy with low water content. In the 950-970 nm region, there is some weak absorption of radiation by water that is not saturated for a moderately dry canopy. The reflectance at 970 has been used in the definition of the water index (WI).

$WI = R_{900} / R_{970}$ (Peñuelas et al., 1993, 1997)

In WI, reflectance at 970 nm is taken as a wavelength sensitive to water content, while reflectance at 900 nm is taken as a reference which is similarly affected by canopy and leaf structures but with null absorption by water.

WI has been used to track changes in RWC, leaf water potential, stomatal conductance, and foliage minus air temperature differences when plant water stress is well developed (RWC<0.85) (Peñuelas et al., 1993). Peñuelas et al. (1997a) reported a correlation coefficient of around 0.55 between WI and RWC for a range of species measured at different times of the year in their natural Mediterranean environment. However, WI appears to be quite insensitive until the drying process is well advanced. For that reason, WI can be useful for assessing wild fire risk but has less utility in irrigation scheduling. As for stress detection, Peñuelas et al. (1997b) showed that WI was a good indicator of water status in response to salinity.

NDVI is also affected by the drying process and by structural and color changes in the plants. The ratio of WI and NDVI has a better correlation with RWC increases, especially in species that undergo important changes in NDVI throughout the year (Peñuelas et al., 1997a).

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CHAPTER 6

Economic Issues in Assessing the Role of Physiology in Wheat Breeding Programs

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Wheat breeding can be thought of as an economic activity, in the sense that it involves processes of physical transformation that are characterized by streams of costs and benefits. Decisions taken about the organization and operation of a wheat breeding program (including technical decisions such as the choice of parental materials, crossing techniques, selection methods, and evaluation procedures) are likely to have economic implications. To the extent that changes in the organization or operation of a wheat breeding program affect the streams of costs and benefits, the economic outcomes that can be expected from the program will increase or decrease.

Plant breeders are viewed by many, especially by those in the food production and processing industries, as a resource that can be used to enhance the overall performance of the agricultural sector (Brennan, 1997). Precisely because they have this capacity, plant breeders often face demands on their services that far exceed what they can realistically expect to deliver. In a world of limited resources, plant breeders therefore need some basis for deciding which among the many demands being placed on them should have priority. Although economic factors are often taken into account (explicitly or implicitly) when research priorities are established, the informal and ad hoc manner in which this is done frequently leads to decisions that are far from optimal in an economic sense. Basic economic analysis, grounded in the careful assessment of benefits and costs, can provide the foundation for making those decisions in a more informed and defensible manner.

Assessing Potential Changes to a Wheat Breeding Program

Under what circumstances might it be advisable to incorporate physiology into a wheat breeding program? In assessing the organization and management of an existing breeding program and deciding whether or not changes may be needed to meet a particular objective, it will often be useful to review the following preliminary questions before undertaking formal economic analysis.

Is the problem best addressed through breeding?

Before any changes are made to a breeding program, it is important to determine whether the results being sought could be obtained more quickly and/or cheaply by some other means. For example, if the research objective is to increase protein content in wheat, experience suggests that it will often be better to concentrate on breeding for higher yield, while leaving the challenge of raising protein content to agronomic management. This is because even though cultivars differ in their protein content, prospects for increasing protein content through breeding are limited. Research has shown that protein content is mainly influenced by environment (and by genotype *x* environment interaction), so the genotype effect is generally very small (Bingham, 1979). Furthermore, given the known negative relationship between yield and protein content (O'Brien and Ronalds, 1984), any increases in protein obtained through selecting higher-protein cultivars are likely to result in lower yields.

What level of breeding input is appropriate?

Once it has been decided that the research objective is best addressed through breeding, it is necessary to determine what level of breeding input is appropriate. An appropriate level of breeding input is one that can be justified in terms of the size of the expected benefits. Generally these will be related to the size of the target region: As the size of the target region increases, so will the level of breeding effort that is justified. Brennan (1992)

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and Maredia (1993) have shown that for small target regions, it often will be appropriate only to select from among breeding lines that have been imported from elsewhere. But once the target region increases beyond a certain size, it will be appropriate to establish a full-fledged local crossing program. The precise threshold values needed to justify expansion of an existing breeding program will depend, among other things, on the characteristics of the target environment (area, average yield levels, use of improved varieties, etc.) and the yield gains that can be expected by increasing the breeding input.

What breeding strategy is likely to be most efficient?

Once it has been determined that the problem is best handled through breeding and that the size of the target region justifies a full-fledged crossing program, a breeding strategy must be decided. Wheat breeding can be pursued in many different ways. The initial choice of source materials is of course critical; if the source materials selected for improvement contain a high proportion of favorable alleles for the problem being addressed, the chances of success are greatly enhanced. After choosing the source materials, the breeder must determine how much effort will be put into crossing, as compared to selection and evaluation of the resulting lines. In addition to a wide range of so-called conventional breeding methods, modern breeders also have the option of incorporating biotechnology techniques, such as the use of genetic markers, tissue culture, etc. In deciding what types of selection methods will be optimal, it is important that the decision be driven by what is best for the program and its outcomes, not simply by the availability of new tools or techniques (Brennan, 1997). Innovative technologies that appear to be tremendously appealing in the short run often turn out to be far less attractive over the longer term. For example, Brennan and O'Brien (1991) found that the incorporation of early-generation, small-scale quality testing into an Australian commercial wheat breeding program, while initially attractive, led to a lower economic return for the breeding program (Box A).

On what basis will varieties be released for use by farmers?

The procedures used to evaluate new varieties prior to their release also merit consideration, because evaluation procedures can have important economic implications for breeding programs.

Box A: Incorporating Quality Selection into a Wheat Breeding Program

A recurring question facing any wheat breeding program concerns when to begin selecting for quality characteristics—for example, protein content or gluten level. Opinions differ as to what the appropriate time is for starting quality testing. Some breeders feel that selecting for quality should be left until late in the improvement process, after significant progress has been achieved in raising yield potential. Others feel that selecting for quality in addition to yield should commence early in the breeding process, so that low-quality materials are screened out early on.

Brennan and O'Brien (1991) used an economic framework to evaluate the efficiency of two alternative approaches to the problem. Their study focused on two Australian wheat breeding programs, one which performed early generation quality testing, and another which did not (see table). Both programs used the same amount of labor, and the number of crosses and lines sown in the F_2 generation was identical. The two programs differed mainly in the stage at which quality testing was initiated, which caused different sets of lines to move through each program.

Small-scale tests for quality carried out early in the breeding process (F_2 stage) were found to be less expensive than tests carried out in later generations (F_6 stage). This led to the prediction that early generation testing would prove more cost effective. But when the economic returns of the program doing early generation quality testing were compared to those of the program in which quality testing was introduced at a later stage, they were found to be lower. The costs associated with the program that included early generation testing were slightly higher, but the benefits were markedly lower over the longer term. The program without early generation quality testing was able to concentrate exclusively on yield potential, enabling it to achieve much more rapid rates of yield gains. Admittedly, it also had a lower rate of quality increase, since less selection pressure was placed on quality, but when economic values were assigned to yield levels and quality factors, the additional yield gains more than compensated for the lower levels of quality. The study thus showed that in the absence of a substantial premium for quality, wheat producers and consumers will be disadvantaged if breeding programs opt to select for quality in the early generations at the expense of yield improvement.

Economic returns to early versus late selection for grain quality in wheat.

	Program A†	Program B‡
Expected increase over current varieties (%):		
- yield	4.6	2.3
- quality	0.2	1.1
Total costs‡ (US$ 000)	353	369
Total benefits‡ (US$ 000)	3,710	2,557
Benefit-cost ratio	10.5	6.9

† Quality-testing introduced in F_6 for Program A, and F_2 for Program B.
‡ Discounted to year of crossing at 5% per annum.
Source: Derived from Brennan and O'Brien (1991).

Before a new variety is released, breeders must decide how widely it should be tested and over what period of time, how well it need perform relative to other cultivars that are already in use, and what level of genetic diversity is desirable within and among released varieties.[3] Subjecting experimental varieties to rigorous testing prior to their release increases the likelihood that they will be commercially successful, but extensive testing can also significantly raise overall development costs.

If after reviewing these preliminary questions it still seems worthwhile to proceed, it may be appropriate to undertake more rigorous economic analyses. Although space limitations prevent us from presenting detailed step-by-step instructions, the next two sections provide a broad overview of key economic concepts needed for formally evaluating the desirability of incorporating physiology into a wheat breeding program. They provide a brief description of the basic procedures that would have to be followed.

Key Economic Concepts Relating to Investment Analysis

Basis for economic assessment

The decision of whether or not to incorporate physiology into a wheat breeding program can be approached like any other investment decision. In this respect, the key issue concerns the economic returns that will be generated as a result of the proposed organizational change. The basic economic question that needs to be addressed is really quite simple: What level of investment is justified by the expected benefits, taking into account alternative investment opportunities?

Before any formal economic analysis is undertaken, two important concepts need to be understood: opportunity cost and time value of money.

Opportunity cost. An opportunity cost is the benefit foregone by using a scarce resource for one purpose instead of its next best alternative use (Gittinger 1982). Opportunity costs play an important role in investment analysis, because most investments involve choices between mutually exclusive alternatives. Since the resources available to a breeding program are usually limited, whenever additional emphasis is put on one breeding objective, less emphasis must necessarily be put on other objectives. To return to the example cited earlier, if the decision is taken to target higher protein content, this will probably slow the expected rate of progress in breeding for higher yield. Thus, the opportunity cost of breeding for enhanced protein content will be the progress that would have been achieved (but had to be given up) in breeding for higher yield.

Of course, the tradeoffs may not always be so evident. In plant breeding, targeting one objective does not necessarily mean that progress toward other objectives will be suppressed, at least not directly. The rationale for adding a physiology component in fact may be to achieve the same outcome with greater efficiency. Thus, data from physiological measurements may be used to complement yield trial data; if the additional information improves the breeder's ability to predict cultivar performance in target environments, the need for extensive yield trials may be reduced or even eliminated (Reynolds et al., 1997). But even in cases such as this, the concept of opportunity cost remains valid, because the resources invested in taking the additional physiological measurements presumably could have been used in some other way to generate other types of benefits.

Time value of money. Economic analysis must take into account one important facet of value that stems from human behavior, namely, the time value of money. The time value of money refers to the fact that people place a higher worth on values realized earlier as compared to values realized later (Gittinger, 1982). Asked to choose between receiving $100 today and receiving $100 one year from today, most people would choose today. In economic analysis, the time value of money is taken into account through discounting, whereby costs and benefits expected to occur in the future are assigned lower values.

Discounting is important in any type of investment analysis, which by definition deals with streams of costs and benefits through time. It is particularly important in the analysis of agricultural research investments, given 1) the unequal distribution through time of expected costs and benefits, and 2) the uncertainty about future outcomes of agricultural research. In plant breeding, there are usually long lags—often 10 years or more—between the initial crossing and selection activities (which imply costs) and the eventual adoption of improved varieties by farmers (which generates benefits). Under those circumstances, the expected benefits may be discounted by decision makers, to the extent that the investment may no longer seem attractive (Box B).

3 The important issue of how many varieties should be released may also have to be decided by breeders, although usually this matter is left to some sort of government-appointed varietal certification and release committee.

Box B: Stream of Costs and Benefits Associated with a Breeding Program

To calculate the economic returns to a plant breeding program, it is necessary to estimate the costs and benefits associated with it. Figure 1 illustrates the stream of costs and benefits typically associated with a plant breeding program. During an initial period, net benefits remain negative because research costs are being incurred (for example, in the crossing, selection and evaluation of experimental materials) without any benefits being realized (Morris et al., 1992). Eventually the research produces an improved variety, which after undergoing a certification process is approved for release. Following a lag necessary for the production and distribution of seed, the variety is taken up by farmers, with the rate of adoption typically following an S-shaped (or logistic) curve. Providing the variety leads to improved yields in farmers' fields, the original research investment (made years earlier in most cases) now begins to generate benefits in the form of increased production. The stream of net benefits consequently turns positive, increasing as the area planted to the new variety expands, reaching a maximum at peak adoption, and then declining as the variety is gradually replaced by another, newer variety.

While the relative sizes of costs and benefits are obviously important in evaluating a research investment, their distribution through time is also important. Benefits realized far in the future are considered less valuable than benefits realized in the short term. To accommodate the *time value of money*, research costs and benefits are discounted. Figure 2 illustrates how discounting depresses the value of net benefits realized near the end of the period of analysis relative to those realized near the beginning. Because of the long time lags involved in research such as plant breeding, discounting is an important concept used in analyzing returns to investments in research.

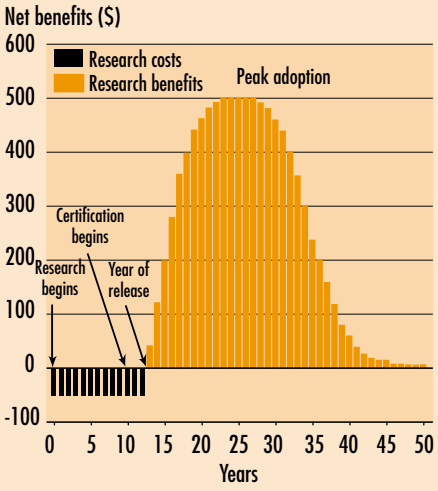

Figure 1. Undiscounted stream of costs and benefits associated with a plant breeding program.
Source: Figure 12 in Morris et al. (1992).

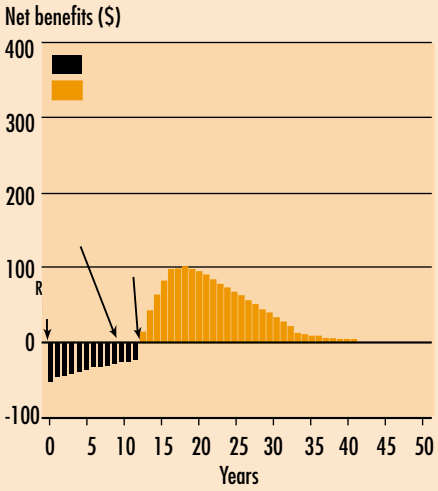

Figure 2. Effects of discounting the stream of costs and benefits associated with a plant breeding program.
Source: Figure 13 in Morris et al. (1992).

Discounting can dramatically alter the attractiveness of any investment opportunity. For example, spending $1 now to bring about a $2 return after 10 years at first glance might seem a sound investment. But with a 12% discount rate (often used to approximate the opportunity cost of capital in developing countries), $2 received 10 years in the future is worth only $\$2/(1.12^{10}) = \$0.64$ at today's prices. Seen in this light, the investment does not appear to be attractive.

Significantly, the effects of discounting are very sensitive to the distribution through time of the expected costs and benefits. If the same $1 investment is expected to generate the same $2 return after only five years rather than ten, the present value of the benefits (i.e., the value at today's prices) is $\$2/(1.12^{5}) = \$1.13$. Depending on whether or not more attractive investment opportunities are available, this return might seem quite attractive.

Approaches to economic analysis

How can economic analysis help a research administrator assess the desirability of establishing a physiology component to the breeding program? Economists would characterize the incorporation of physiology into an existing breeding program as a *marginal change*, since it does not involve reorganization of the entire breeding program, but only an incremental change, or a change "at the margin."

Two alternative approaches can be used to analyze marginal changes: 1) comparing only changes in costs and benefits expected to result from the addition of the physiology component (partial budget analysis), or 2) comparing the costs and benefits of the entire breeding program *with* the physiology component to the costs and benefits of

the entire breeding program *without* the physiology component (whole budget analysis). Which of these two approaches is preferable will depend on the quantity and quality of data that can be collected, the time available for the analysis, and perhaps the level of economic expertise on hand. The results obtained through the two approaches will generally be similar, although they may differ somewhat depending on the level at which the analysis is undertaken.

Whether the approach being used is based on partial budget analysis or whole budget analysis, the key to assessing the economic desirability of the proposed change is to correctly identify the costs and benefits that can be expected to vary—known as the *marginal costs* and *marginal benefits*.

Certain types of cost changes can readily be identified and estimated in advance. For example, if incorporating physiology involves the hiring of a physiologist, then it can safely be predicted that one additional cost will be the physiologist's salary and associated costs. Similarly, if the incorporation of physiology requires the construction of a new laboratory facility with specialized equipment and materials, that cost, too, can easily be foreseen and quantified.

But other types of cost changes will be much more difficult to predict. Decision making in plant breeding programs usually proceeds in a sequential manner, with decisions taken at early stages of selection often leading to unpredictable outcomes that in turn affect decisions taken in subsequent stages. For this reason, determining future cost streams is not always easy. Because of the "snowballing" effect, the cumulative changes in the cost structure of a breeding program over the long run may appear quite different from what could realistically have been anticipated at the time a management decision was originally taken. In studying two wheat breeding programs in Australia, Brennan and O'Brien (1991) found that even though the introduction of early generation quality testing reduced overall breeding costs in the first generation of plants, over the longer term total costs were higher because the sequential cost effect increased costs in subsequent generations (see Box A). The outcomes of research investments being inherently more unpredictable than the outcomes of many other types of investments, careful thought needs to be put into assessing the extent to which future costs are likely to be affected by any marginal changes.

Just as marginal costs are often difficult to predict, so, too, are marginal benefits. With investments that affect the organization and management of a plant breeding program, in some cases it may be relatively easy to identify and estimate expected marginal benefits. That will occur especially when the marginal benefits relate to changes in the value of final outputs of the program (e.g., the acquisition of a new source of germplasm that contributes directly to the development of higher yielding varieties). In other cases, it is extremely difficult to identify and quantify expected marginal benefits. That is particularly so when they will result from changes in current research procedures that are likely to affect the way future research is carried out (e.g., modifying early generation evaluation procedures in ways that are likely to have consequences for the numbers and quality of materials available in later generations).

When the research investment being considered is the addition of a physiology component to the breeding program, identification and quantification of expected marginal costs and benefits may be difficult because so many aspects of the existing program are likely to be affected. Under these circumstances, partial budget analysis based on expected changes in marginal costs and marginal benefits will often be inadequate, and it may be desirable to carry out a more complete economic analysis based on the program's total costs and returns. Brennan (1989a) describes a method for developing detailed estimates of costs and returns for an entire plant breeding program; these detailed estimates can be used to evaluate the economic attractiveness of significant (i.e., non-marginal) changes to the organization of the program. The total-budget approach described by Brennan is based on a comparison of expected costs and returns *without* a physiological component and expected costs and returns *with* the physiological component.

Evaluating the Desirability of Using Physiology in Breeding Programs

Estimating costs and benefits of the current program

Whenever a detailed economic analysis is to be carried out to determine the desirability of incorporating physiology into an existing breeding program, as a starting point it will often be useful to develop broad estimates of the total costs and returns of the current program.

A broad estimate of the current program's costs can be made in a "top-down" fashion based on aggregate budget information about the program's total operating costs, total annual capital costs, total annual salary costs, and total overhead costs. Alternatively, the current program's costs can be broadly estimated in a "bottom-up" fashion based on disaggregated cost data relating to each individual activity of the program (see Brennan and Khan, 1991). Either way, it is important to

include all relevant costs likely to be affected by the proposed change, including costs associated with crossing, evaluation, selection, disease screening, quality evaluation, and regional trials, as well as costs relating to variety release and registration activities.

The extent to which overhead and administrative costs (such as the salaries and support costs of head office personnel, finance officers, and human resources staff; library costs; information technology costs) need to be taken into account depends on whether or not these costs are likely to be affected by the proposed changes. If administrative overheads are likely to remain unaffected, it may be convenient to overlook them.

The benefits generated by a wheat breeding program can be measured at different levels. For researchers working in a breeding program, the benefits include not only improved varieties *per se*, but also scientific benefits such as novel research techniques, specialized laboratory equipment, and original knowledge. For the organization that sponsors the breeding program, especially if it is a profit-oriented private company, the benefits will often be measured in terms of the additional income earned through the sale of improved varieties (whether directly through commercial seed sales or indirectly through royalties or licenses). For society as a whole, the most important benefits that flow from a wheat breeding program tend to be the productivity gains achieved by farmers when they grow improved varieties produced by the program (measured as income increases or as cost reductions).[4] For simplicity, the emphasis in this chapter is on the latter type of benefit (farm-level productivity gains), although our analysis also applies to other types of benefits.

The benefits (or returns) generated by the current program can be broadly estimated based on the outputs from the program. In most cases, these will consist of improved varieties. In order to estimate the economic benefits associated with the adoption of improved varieties, usually it is necessary to answer the following questions:

- To what target regions are the varieties adapted?
- What advantage do the varieties confer (e.g., higher yield, improved quality)?
- What will be the average price of each incremental ton of grain produced, or the average price increase attributable to improved quality?
- What will be the rate and extent of adoption of the varieties following their release?

Once these questions have been answered (to the extent that they can be), it should be possible to estimate the benefits likely to be generated by the proposed change, if only in broad terms. For example, in his study of a public wheat breeding program in New South Wales, Australia, Brennan (1989a,b) made the following estimates:

- the program serves a target region that includes about 1.0 million ha planted to wheat each year, with average yields of 1.7 t/ha;
- each new variety produced by the program generates an average yield increase of 2.25% and an average improvement in the quality index of 1.09%;
- each 1% yield increase is worth $1.11/t, while each 1% improvement in quality is worth $0.81/t;
- on average, adoption of each variety peaks at about 16% of the target area in the seventh year following release; and
- each new variety has a productive life of 20 years.

Based on these broad estimates, Brennan was able to calculate the economic benefits generated by the breeding program, which total approximately $920,000 per year at peak adoption.

Estimating marginal costs and benefits of projected changes

The next task is to estimate the changes to both costs and benefits that would flow from incorporating physiology into the program. To a certain extent, these will necessarily be speculative, and they will in any case depend on the role envisioned for physiology within the larger breeding program.

Estimating future cost changes is frequently complicated by short-run versus long-run issues. In some cases, the physiology component can be expected to lead directly to cost savings, for example when the introduction of early generation screening methods is likely to reduce the number of lines that will have to be evaluated in later generations. Similarly, it has been shown that the introduction of certain tissue culture techniques can dramatically reduce the costs of multiplying experimental materials (Brennan, 1989b) (Box C). In other cases, however, the physiology component can be expected to lead to cost increases, at least in the short run, for example when the physiological tests undertaken can be expected to add measurably to the expense and/or time involved in screening.

[4] Depending on the degree to which farmers sell their products, and depending on the nature of the markets in which they sell their products, these productivity gains may be transmitted in part or in whole to consumers.

Particularly in these cases, it is important to determine whether the additional costs incurred in the short run are likely to lead to even greater cost savings over the longer term. Although additional expenditure in the short run is often justified on the grounds that the long-run payoffs will be large, this is not always the case. In their comparative analysis of two Australian wheat breeding programs, Brennan and O'Brien (1991) found that the slowdown in yield gains that resulted from incorporating early generation quality testing was not sufficiently compensated by the resulting gains in grain quality resulting from that higher selection pressure.

Estimating future benefits is complicated by the difficulty of precisely anticipating research outputs. All research is to some extent speculative, so the outcomes of any research investment can never be known with certainty. Nevertheless, it is usually possible to make educated guesses about the future values of key parameters that will determine the size (and distribution through time) of economic benefits. As stated earlier, the impact of incorporating physiology into an existing wheat breeding program potentially will be reflected in: 1) higher yielding varieties, 2) higher quality varieties, 3) better adapted (and therefore more widely grown) varieties, and/or 4) earlier release of new varieties. To the extent that it is possible to relate the incorporation of physiology to expected changes in the values of these key parameters, it will be possible to arrive at a rough estimate of expected benefits.

In estimating the benefits from a change to the program, it is important to remember that resources are limited, so any gains made in selecting for one objective must come at the expense of gains in selecting for another objective or objectives. These trade-offs must not be overlooked when benefits are being estimated.

Box C: Using Doubled Haploid Tissue Culture in a Wheat Breeding Program

Of the many forms of tissue culture available for use by wheat breeders, one of the most valuable is doubled haploid culture, which involves *in vitro* development of fixed lines from parental material. The technique is attractive because development of each generation of progeny can be initiated before the parents have achieved physiological maturity, thus accelerating the breeding process. Furthermore, it is carried out in the laboratory, rather than in the field, thus reducing the need for costly grow-out trials. By using doubled haploid tissue culturing techniques, wheat breeders can reduce the number of years needed to produce lines for advanced testing, while at the same time saving considerably on field production costs.

Brennan (1989b) examined the potential returns to a conventional wheat breeding program of adopting doubled haploid tissue culture techniques (see table). The anticipated impacts of adopting the techniques were modeled by assuming that: 1) the production of generations F_1 to F_5 would be compressed into just two years, as opposed to the usual five years, and 2) field production costs would be slightly reduced. In addition, it was implicitly assumed that lines produced using doubled haploid culture would be identical to the lines emanating from a conventional breeding program.

Brennan estimated the costs and benefits of the two alternative scenarios (conventional breeding without tissue culture and conventional breeding with tissue culture). His analysis showed that the use of tissue culture to accelerate the production of advanced breeding lines could be expected to generate handsome economic returns. Following the adoption of tissue culture, the net present value increased by more than $600,000 per line, and the benefit-cost ratio rose from 6.9 to 9.0. Thus, by slightly reducing production costs and significantly accelerating the production of advanced generation materials, doubled haploid tissue culture was shown to significantly increase the expected profitability of the breeding program.

Economic returns to the use of tissue culture in wheat breeding.

	Conventional breeding program†	Breeding program with tissue culture‡
Discounted costs ($A 000)‡	550	489
Discounted benefits ($A 000)‡	3,816	4,418
Net present value ($A 000)	3,266	3,929
Benefit-cost ratio	6.9	9.0

† All values in 1986 Australian dollars.
‡ Discounted at 5% per annum.
Source: Brennan (1989b).

Analyzing anticipated future flows of costs and benefits

Once the size of marginal costs and marginal benefits have been estimated, it is necessary to project their distribution through time. The simplest way to do this is by making year-by-year projections of marginal costs and marginal benefits. Given the relatively long period that can elapse between the time a research investment is initiated and the time tangible benefits are first realized in farmers' fields (known as the "research lag"), it is usually desirable to project marginal costs over a period of at least 10 years. The exact duration of the expected research lag will depend on the type of research that is being contemplated. In wheat breeding, some activities can be expected to have a relatively short research lag—for example, 3-4 years to introduce a grain

color characteristic that is controlled by a single gene. Other activities can be expected to have a much longer lag of 10 years or more—for example, incorporating drought tolerance, which is controlled by complex interactions among several different genes.

Once the size and distribution through time of research costs have been estimated, equivalent estimates must be made about expected flows of future benefits. In the case of a wheat breeding program, these will generally depend on the diffusion pattern of the new varieties produced by the program. Varietal diffusion patterns can vary widely, depending on the characteristics of the new variety or varieties, the degree to which farmers recognize and value the new characteristics, the effectiveness of the seed production and distribution system, and other factors. Some improved varieties are adopted very rapidly by a large proportion of farmers, resulting in a short, steep diffusion curve that reaches a ceiling level approaching 100% of the target region. Other improved varieties are adopted much more slowly and only by a relatively small proportion of farmers, resulting in a long, flat diffusion curve that tops out at a ceiling level well below 100% of the target area. Based on the assumptions made about the technology diffusion pattern (known as the "adoption lag"), the size and distribution through time of marginal benefits can be estimated.

Given the two types of lag involved in wheat breeding (research lag and adoption lag), it is advisable to consider an extended period when evaluating the desirability of incorporating physiology into an existing breeding program. As a general rule of thumb, marginal costs and benefits should be projected out over a 30-year period.

Next, the projected flows of costs and benefits must be discounted. Discounting is necessary to take into account the time value of money, i.e., the fact that costs and benefits realized in the future are valued less than costs and benefits realized in the present. To take into account the time value of money, discount factors are applied to future costs and benefits to convert them to their present value.

Discounting is carried out using the following formula:

$$D_n = U_n / ((1+r)^{n-1}),$$

where:

- D = discounted value of cost (benefit) in year n
- U = undiscounted value of cost (benefit) in year n
- r = discount rate
- *n* = year (where n = 1 is the present year, n = 2 is next year, etc.).

Alternatively, discount factors may be applied to future costs and benefits. Standard discount factors are readily available in most handbooks on project analysis and are readily generated by most financial spreadsheet programs.

Calculating measures of project worth

After projected costs and benefits have been discounted, they can be summed to obtain total discounted costs (TDC) and total discounted benefits (TDB). The TDC and TDB can be used to calculate two simple measures for use in assessing the attractiveness of any potential investment:

- net present value (NPV = TDB - TDC), and
- benefit-cost ratio (B/C ratio = TDB / TDC).

If the objective of the economic analysis is simply to determine whether or not the incorporation of physiology will be profitable, then it may be appropriate to proceed with the investment if it can be established that it will add to the overall economic returns generated by the breeding program. This will be the case if the NPV is positive (NPV > 0).

The main advantage of using NPV as a decision criterion is that it is easy to compute. One big disadvantage of the NPV measure, however, is that it fails to take into account the size of the proposed investment; without further investigation, there is no way to tell whether a given NPV was generated by a large investment or by a small one. This limits the usefulness of the NPV as a tool for deciding between alternative investment opportunities, because simply choosing the alternative with the highest NPV may not always be desirable. When asked to choose between investing $100 in one project expected to generate a NPV of $200 and investing $200 in another project expected to generate a NPV of $210, most people would prefer the first project–even though the NPV is lower.

If the objective of the economic analysis is to select among two or more alternative investment opportunities, then it will be preferable to use the B/C ratio as a decision criterion. Since the B/C ratio expresses (discounted) benefits per unit cost, it provides a measure of project worth that is not affected by the size of the project. Choosing the project with the highest B/C ratio, regardless of the size of the absolute size of the project, will ensure the highest possible returns to the investment.

Factoring in non-economic considerations

Measures of project worth such as the NPV and the B/C ratio provide useful information that can help in deciding whether or not to proceed with a proposed investment, but they should not be the sole basis for the decision. Most potential investments are characterized by costs and benefits whose value cannot easily be assessed, meaning they cannot be incorporated into the economic

"bottom line." For this reason, before taking a decision based on conventional measures of project worth such as the NPV and the B/C ratio, it is important to assess the extent to which non-economic considerations should be allowed to influence the final decision. Only after these non-economic considerations have been carefully considered can a balanced judgment be made concerning how to proceed.

Conclusions

With funds for agricultural research becoming increasingly scarce in most countries, research administrators face mounting pressure to ensure that available resources are used efficiently. Although there can be no question that a properly organized and managed physiology component has the potential to add value to wheat breeding activities, this does not necessarily mean that every wheat breeding program should include one.

This chapter has reviewed some basic concepts from investment analysis that can be used to assess the desirability of investing in a physiology component of a breeding program. We have described a series of steps that may be useful in helping to formalize decisions that all too often are still left to the "gut feeling" of scientists and research administrators:

- establish that the problem is appropriately addressed through breeding;
- estimate in rough terms the costs and benefits of the current breeding program;
- identify activities that are likely to change with the incorporation of physiology;
- estimate the economic consequences in terms of changes in costs and benefits;
- calculate economic measures of project worth (NPV and B/C ratio); and
- factor in any non-economic considerations.

Economic analysis is not infallible, so following these steps will not necessarily ensure that the "correct" decision will be taken. And as we have pointed out, the outcomes of research are by nature uncertain, so some of the parameters used in the economic analysis will necessarily be tenuous. But one big advantage of invoking an economic framework of analysis is that it forces decision makers to think somewhat more systematically about the many factors that are likely to influence the outcome of investment decisions; this in turn increases the likelihood of achieving a favorable outcome.

If decisions about the role of physiology in wheat breeding are taken based partly on economic considerations, then changes made to the organization and management of existing breeding programs are likely to lead to genuine improvements in efficiency. Improvements in efficiency in turn enhance the flow of new varieties emanating from the breeding programs, leading to increases in farm-level productivity that will eventually benefit both producers and consumers.

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