

*Chapter 19*

**SYSTEMIC HEURISTIC APPROACHES GUIDE THE  
INTERACTION OF ENHANCED GENETIC DIVERSITY  
AND COMPLEX STRESSES TO GENERATE BETTER  
WHEAT GERMPLASM FASTER AND AT LOWER COST**

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**ABSTRACT**

Since the time man has domesticated plants for food, we have sought to expand the range of benefits they can provide. Starting in the late 19<sup>th</sup> century, we began to apply the principles of modern science in order to achieve these aims faster, more consistently and on a wider scale. In modern times, the goals included higher, more sustainable yields in the face of changing environments. Recently we have started to place increasing emphasis on improving aesthetic and sensory experience and addressing health concerns. The complexity of the goals is clear.

In this review, we attempt to make the case that geneticists are ready to exploit new possibilities arising from a better appreciation of the complexity of inheritance and its interactions with the environment. In doing so, we should seek to preserve and consolidate the undeniable extensive gains made by what might be now regarded as classical or conventional plant breeding approaches. Standing on such a solid foundation, we will show that by recognizing and more fully respecting the complex, adaptive and newly-discovered, self-modifying nature of biological information, we have developed novel, complementary approaches to current plant breeding practice. With the benefit of



this new perspective, we may better see the way to reach our goals faster, with less costly screening, and for a wider range of environments.

## THE CLASSIC ERA OF GENETICS WELCOMES ITS SUCCESSOR

At the end of the 20<sup>th</sup> century, genetics was based on concepts that appeared self-sufficient. It was believed that genes controlled all life and that by studying genes we would gain a complete understanding of life itself and be able to control it. Although this view was not universally shared [1], it is a surprising discovery that the human genome contains far fewer genes than expected (22,000 rather than 100,000 based on data as of early 2009), which is prompting a more general re-thinking of earlier gene-centered views. Recent genomic studies have confirmed, and are continually extending, the concept that single genes can be involved in producing several different proteins. Moreover, the information encoded in a gene's nucleotide sequence is not all-determining [2]. Genomics, the study of genes and their expression, continues to raise new questions. The expressed information of genes: proteins, metabolites or regulating nucleic acid intermediates are now widely seen not merely as final products of a "DNA blueprint" but as players themselves on the stage of biological complexity. No dominant unifying theory that integrates these phenomena has yet emerged [2, 3]. The traditional Mendelian concepts of "gene" transmission have been maintained, because these laws explain much of the range of phenomena involved, but recent scientific findings call on us to revise our definitions and starting assumptions. The concept of "one gene, one product" can no longer be considered widely valid, and the role of DNA as the sole fundamental hereditary material of all living organisms has been called into question. This hitherto absolutely central tenet of genetics now needs to accommodate new insights derived from recent advances in molecular genetics, biochemistry and the observation of living organisms.

Plant genetics, both as an academic discipline and as a practical guide for breeders, was established on a gene-driven model. Progress was seen to consist in building new genotypes by adding new or better genetic units. This concept did not take into consideration the progress achievable from a narrow genetic base [4]. These authors advanced the idea that *de novo* variation arises frequently within germplasm through an array of mechanisms. Such a view may well be vindicated as the genome has been shown to be modified by stress [5, 6], a finding that is consistent with the earlier conclusions of McClintock [7, 8].

## WHAT CONTROLS HEREDITY?

Much confusion arises from different definitions and uses of the concept of a gene [9]. An early attempt to provide clarity restricted the definition of a gene to a message-encoding portion of DNA, but evidence has continued to be adduced that DNA base sequences cannot be the sole locus of heredity. The definition of a gene as a stretch of DNA encoding RNA that is translated into a protein is clearly too limited. Jacob and Monod (1961) described the first model of gene regulation via *cis*-acting regions that bind regulatory elements and other genes (trans-acting elements) producing regulatory RNAs that can lead to regulatory proteins [10].



This gave a first glimpse of a world of interactions at a time when applications to breeding were unclear. Currently known additional levels of control over heredity include a range of new regulatory roles dependent on non-coding DNA [11]. Many genes (in a broader sense) act by generating non-translated RNAs that play critical roles in controlling the cell's machinery through transcriptional and posttranscriptional regulation of gene expression [2, 12]. These non-translated RNAs vary in size and function, and some have a very small size (small interfering RNAs, or siRNAs and micro-RNAs, or miRNAs) [13, 14]. Proteins, as products of genes, also interact with each other, with RNA and DNA in highly complex ways which in turn can also be affected by the environment.

Many genes belong to groups characterized by coordinated expression in order to accomplish a given metabolic function. Others, seemingly less important at first glance, are capable of very subtle interactions among themselves and with the internal and external environment, which may regulate the activity of the organism. Most proteins may be involved in networks which in turn, through interactions, exponentially increase the complexity of life's basic mechanisms. The analytical simplicity of the link between gene and function is necessarily lost. Geneticists must therefore seek different analytical frameworks for coping with a system in which cause-effect relationships are replaced by complex interactions. Among the most critical issues is the challenge posed by epigenetic mechanisms and various levels of their interactions recently shown to be involved in *de novo* genetic variation in plants and animals [15].

In the past, the relationship between DNA and the remainder of the cell in its environment was likened to a computer program and the data it processes. As modern concepts evolve, we no longer know where the program is, because DNA could as well be viewed as the data and the rest of the mechanisms of life as the program. Indeed, it is likely that many elements act like both a program and data [16]. Could this ambiguity of roles be peculiar to, or even defining of, life [17]? Numerous authors have argued that the ultimate unit of selection must be the 'gene' itself. This starkly highlights the conundrum arising from different understandings of the 'gene' concept as employed by molecular biologists, evolutionists or plant breeders [9].

A highly reductionist view of the gene may fail to adequately recognize that selection must operate on living individuals in finite populations facing unpredictable events [18]. Fitness cannot be measured relative to a constant environment; adaptability to changing environments also matters. Organisms are complex temporary constructs of DNA, RNA, proteins and many other substances. In sexually reproducing plants, these components interact in hierarchical networks in ways that may vary between generations and occasionally yield unforeseen results. While the system for inheriting and expressing information involves canalization mechanisms that maintain phenotypic stability across environments [19, 20], it also displays adaptive abilities not readily explained by the information's most visible flows. An important part of this adaptability may arise from the accumulation of phenotypically invisible changes of DNA code over time. Information important for the evolution of organisms may accumulate in pseudogenes, formerly seen as defective or inactive "junk DNA". Many pseudogenes are now known to affect heredity [2].

Some geneticists define the gene as "any portion of chromosomal material that lasts for enough generations to serve as a unit of natural selection" [21]. RNA or proteins might also serve as vectors of genetic information instead of or in addition to DNA [22]. Lewontin (2000) has expanded on DNA-centered heredity by presenting views about genetics that are



more open-minded about probabilistic uncertainty and environmental interactions [23]. Individuals plants—and animals as well— carry a certain amount of sub-optimal or deleterious genetic factors; but this judgment of value must be restricted to a defined environment, without any claim to predict adaptive value elsewhere [23]. Thus, QTLs like pHva1 and C1H2, which are associated with lower yield in some dry environments, correlate with a yield advantage in other dry environments [24]. This should not be unexpected, since even within small geographical areas, dry climates present a remarkable diversity of secondary stresses [25].

One has to wonder what can happen over many generations to those parts of the DNA that are neutral to selection or essentially silenced because their information is never expressed. This represents the raw material from which evolutionary processes may fashion 'useful' components over time. An essential feature that distinguishes eukaryotes from prokaryotes is indeed a vastly higher proportion of non-coding DNA [11]. Specific genes differ in their natural mutability and respond to stress and chaotically varying environments with diverse mechanisms that exploit mutations and epigenetic events [26, 27].

What appears as phenotypic stability may actually mask numerous mutation events. In some species, phenotype stability appears so robust that it appears unrelated to the rate of molecular evolution [28]. In others, however, a simple structure like a heat shock protein [20] or a new micro-RNA may alter the functions of a whole network of interactions. Epigenetics introduces levels of complexity that no longer permit the illusion that we could possibly fully account for how the vectors of heredity act. The approach we will present is designed to be able to make continuing progress, even in the face of these necessary limitations to any detailed understanding of the underlying mechanisms.

## NETWORKS, NODES, CENTRAL MECHANISMS AND PLEIOTROPY

The model gaining increasing support postulates that several hundred different genes belonging to many pathways can regulate or modify to some extent the expression of a single gene in a given tissue [29]. Pseudogenes with an atypical structure are also involved [2]. A gene often has to work together with a group of other genes as part of a regulated metabolic process that operates in a specific environmental framework. This constitutes a synexpression group, and a multitude of these are present in each eukaryote. This communication system is necessarily complex and operates within sophisticated multi-nodal networks that give plants a remarkable flexibility in the way they use their genome [30]. Synexpression groups usually have recognizable goals or functions (e.g., management of metabolism, energy, signaling, defense, transcription, cell fate, protein fate, cell cycle, cell transport, adjustment to environment), and are organized in strongly hierarchical groups (cores, modules, attachments, etc.) that represent levels of functional organization of protein complexes [31]. Genes involved in multiple pathways are less frequently tied within a specific synexpression group [32]; however their connectivity makes them part of a higher level of networking.

Pleiotropy (one gene influencing more than one trait) thus exerts important effects [33] and cannot be a rarity; indeed it has acquired an increasingly important role as our understanding has evolved. The genes involved in signaling are even more pleiotropic than those regulating transcription [34]. As the evolution of plants might well occur largely



through changes in transcriptional regulators [35](Barrier *et al.* 2001), especially by modification of their cis-acting regions [34], the biological complexity of a species could therefore well be more influenced by the diversity of non-coding DNA than by coding DNA [2, 11]. From the new models of gene interactions, it follows that polygenic control and epistasis are much more common than was anticipated in the last century. Another concept that may need more attention is that of 'centrality', that is the existence of a central core of genes linked to those functions most critical to the organism's survival [36].

## INTRODUCTION TO A SYSTEMIC HEURISTIC APPROACH

With the application of formal laws of heredity — discovered by Mendel and refined by many others over the course of the 20<sup>th</sup> century— we have come to base most of our scientific approaches to crop improvement on the introgression of known determinants of desirable traits into a target germplasm. Among the many successes achieved by these focused approaches, there are, for example, the high-yielding, semi-dwarf cereals with genetic resistance to specific pests and races of pathogens that helped avert wide-scale famines in the latter half of the 20<sup>th</sup> century. However, the very specificity of such targeted approaches has often left them poorly suited to confront the challenges arising from such diverse threats as highly adaptable pathogens, pests, and chaotically varying environments [37].

How might we best go about exploiting the clear and demonstrated strengths of the well-established 'classical' or conventional approaches while addressing their limitations? We can start by acknowledging that while we may have some understanding of the core components of an organism's genetic information and its expression, we must confront our near-total incomprehension of the myriad complexities of how a plant translates its genetic information and adapts to its environment. In this review, we aim to show that astutely conceived, 'systemic' approaches can dramatically extend the range of usable genetic resources by taking advantage of the very complexities that pose such difficulties for classical, introgression-based approaches. We can then harness the expression of these newly-found resources in schemes that align with biological cycles to make continuing, incremental progress to set goals even when all the working parts of underlying mechanisms cannot be fully known, still less how they interact.

A *systemic, heuristic approach* — so named because it considers germplasm, genes and diverse biotic/abiotic environments as parts of a broad, interacting system — more fully respects the complex and adaptive, self-modifying nature of biological information than conventional counterparts that base progress on the introgression of known genes. The term '*heuristic*' is added to explicitly emphasize that the approach successively incorporates the relationships, regularities or 'rules of thumb' that emerge with repeated cycles of observation. Thus, as the germplasm evolves, the methods adapt in response to lessons learned at every step, working protocols are validated, and simple, inexpensive expedients are developed and used. In seeking to more closely emulate the approach that evolution itself has taken, we must therefore: a) generate the greatest diversity of useful expression in the germplasm of interest; b) select under the pressure of many interacting, biologically relevant stresses; and c) continually advance the best candidates to further cycles, without undue delay.



## SEEKING GREATER DIVERSITY: EXPLOITING WHAT IS EXPRESSED AND BRINGING FORTH WHAT IS YET TO BE EXPRESSED

The diversity we seek should not only encompass what can be distinguished at the gene level, but also the global scope of genes' interactions with the environment and with the actions or products of other genes. The new approach also reveals the greatest possible extent of hidden genetic diversity; indeed, many usable genes do remain undetected in the parental phenotype for reasons as diverse as epistatic inhibition, epigenetic effects, multi-gene interactions, or environments that inhibit expression. By choosing to use complex crosses that unleash maximal diversity and then applying severe stresses at contrasting test sites, the presence of such genes can be revealed. Genetic diversity that is not immediately or visibly expressed can be thus as useful as its more evident counterpart.

An example of this principle put into practice can be seen in the development of crop improvement regimes pioneered in Brazil starting in the late 1960s. By starting with the classical genetic combinatorial diversity in populations arising from  $F_1 / F_1$  crosses, the application of carefully controlled stress ensures that individual seedlings can be evaluated and selected for compatible resistance to biotic and abiotic factors [38]. Rapid progress in exploiting desirable genes that are additive and/or dominant can be achieved by immediately re-crossing  $F_1$  or  $F_2$  plants (having a pedigree comprising already 4 or more parents) in order to quickly generate new  $F_1$  or  $F_2$  lines containing an even higher number of parental gene sources. In further crossing steps, those lines can again become parents for pyramiding of additive/dominant genes carrying resistance to several stresses and diseases, leading to near-exponential increase in the number of parents that can be exploited (Figure 1).

To a greatly extended combinatorial diversity, such an approach also adds diversity that arises from recombination, which reaches its peak in the  $F_1$  itself. Enhanced diversity is not the only benefit, as recombination is nature's way to break deleterious linkages. In self-pollinated crops, the range of useful recombinatory events falls off from  $F_2$  onwards, reflecting the increasingly homozygous state of each succeeding generation. Crossing must therefore be done in the earliest generations possible for a given trait. There is no valid reason to delay crossing in a stress and disease selection scheme; it can be done using selected  $F_1$ s or  $F_2$ s as parents [39]. Using  $F_1$ s as parents right away to transfer dominant and additive traits identified under stress is perfectly logical. Clear benefits arise from selecting for dominant and additive traits at the  $F_1$  or at the earliest possible generation [38].

To obtain the greatest range of genetic variation while seeking epistatic and recessive traits, it is essential to select at  $F_2$  or later (Caetano, unpublished). For disease and stress responses that are modulated by a number of recessive or epistatic factors, a plausible strategy is to incorporate these genes or characteristics into plants of short stature, and to select for desirable genotypes in  $F_2$  or  $F_3$ . This takes advantage of the fact that the shortest plants are generally more susceptible to many diseases for which resistance is additive and/or recessive. This is the case when selecting for resistance to 'Fusarium head blight' (FHB) [40-44] and tolerance to 'barley yellow dwarf virus' (BYDV) [45]. The underlying hypothesis is that the occurrence of a short  $F_2$  plant resisting both BYDV and FHB reflects integration of a system of effective and compatible resistance genes. Those events are very rare but can be identified using complex stress.



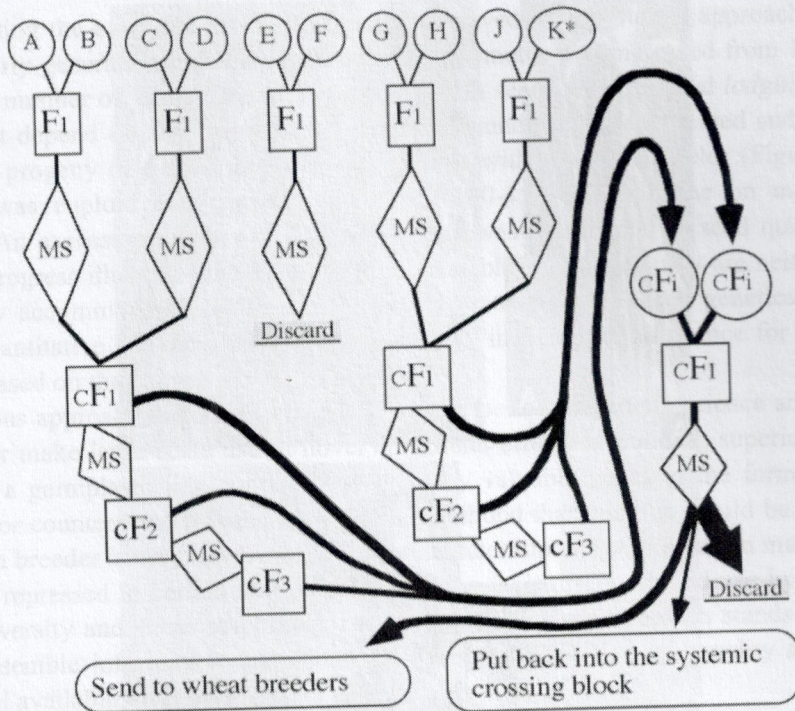


Figure 1. Example of one possible systemic scenario. Genetic diversity flows through a series of rapid crossing cycles, with constant selection pressure. Pure line genotypes are listed as A to K. Steps performed under multiple stress selection are labeled MS;  $cF_1$  represents  $F_1$  developed from heterozygous parents (often  $F_1/F_1$ ). Crosses are done on  $F_1$  and  $cF_1$  plants grown under complex stress, or on the next generations also under complex stress. Most of the material is thus discarded before a  $F_2$  can be grown. A high rate of elimination of genotypes makes room for a greater range of genetic diversity to be introduced at any crossing cycle. The parental genotype  $K^*$  represents a parent that carries unfavorable heredity together with desired traits (and/or hidden traits). It is often better to avoid a high dosage of such parents (landraces, winter/spring, interspecific and foreign germplasm) in order to obtain a higher proportion of usable progenies. The fate of the discarded cross E x F represents what complex stress must impose on 95% – 99% of  $F_1$ s in the first cycles of a systemic, heuristic approach.

Genotypes that express phenotypes of short stature and multiple disease resistances have been generated for some time by this procedure in Brazil [46] and more recently in Canada (Comeau and Langevin, unpublished). Such genotypes can be used recurrently during the introgression of new genes. Caetano has shown that many epistatic traits can be recovered by backcrossing two or three times. If a fourth backcrossing proved necessary, it might be opportune to seek other sources of genetic diversity that can express the desired characteristic.

Certain factors that necessarily arise in complex interactions add elements of unpredictability to inheritance, but one should not be deterred by this. Chromosome recombinations, new epistatic interactions, epigenesis and mutations might all contribute, in direct and indirect ways, to the generation of novel genetic types that cannot be foreseen in conventional genetic models. By applying iterative, heuristic selection protocols based on biotic stresses and morphology to the diversity generated in  $F_1/F_1$  populations, Caetano showed that, after even a fairly small number of successive  $F_1/F_1$ ,  $F_2/F_2$  or other early generation crosses, a genetic leap toward one of the targeted goals could be achieved. This will be illustrated by the example of increasing peduncle diameter.





Figure 2. Morphological and physiological variation in phenotypes generated by the systemic approach, which potentially affect resistance to stress and diseases. A. Wheat line with vertical leaves pointing downwards (Brazil), favorable to penetration of light and gases and promoting rapid drying of spikes; this also reduces accumulation of fungal spores near ligules. A'. Usual leaf angle, in ordinary germplasm, to illustrate that disease spores accumulate near the ligule; disease then kills leaf tissue at this point (rusts in this case), strangling translocation. B. Wheat line with a long peduncle, low nodes (arrows), short leaf and acceptable spike (Quebec), useful to improve penetration of light and gases, also helping resistance to lodging and drought; peduncle tissue maintains photosynthesis longer than leaf tissue. C. Wheat with very wide diameter of floral peduncle and stem, and 7 florets per spikelet; it is very resistant to lodging, but too many florets per spikelet increases FHB damage. Note the width of a lead pencil added for reference, at the bottom of the photo (Brazil). D. Line with a higher number of underground internodes (arrows), bearing roots on each node—a feature that aids mineral absorption and anchors the plant better, reducing lodging (Brazil). E. Ordinary bread wheat plot closely invaded by weeds (Brazil). F. Plot of competitive and allelopathic bread wheat developed by the systemic approach (Brazil), able to kill neighboring weeds in about 5 weeks.



Exploiting the extended range of variation achieved by a systemic approach employing frequent early-generation crosses, floral peduncle diameter was increased from 1-2 mm to 4 mm over a number of years. This trait correlated with seeds per spike and *lodging* resistance, and did not depend on any major gene. Peduncle diameter then augmented suddenly to 7.5 mm in the progeny of a cross between two parents with 4-mm peduncles (Figure 2C). The genotype was euploid and carried spikes with 140-150 florets borne on more than 20 spikelets. An excessive number of florets/spikelets was detrimental to seed quality, but the saltatory progress illustrates the kind of real, repeatable phenomena that are neither foreseen nor readily accommodated within a conventional introgression-based genetics framework. Indeed, quantitative genetics, as currently practiced, makes little allowance for such sudden progress based on the expression of minor genes.

Previous approaches from any recent or distant period of genetic science are unlikely to discover or make large-scale use of novel epigenetic effects in building superior genotypes. Indeed, if a germplasm line contains one or many valuable genes in the form of elements repressed or counteracted by other genes, the likelihood that this line would be selected as a parent by a breeder is very low. And yet, valuable determinants of expression may be masked, hidden or repressed in certain wheat lines, awaiting their discovery and use by explorers of genetic diversity and stress responses. The systemic, heuristic approach stands out as being able to assemble information that benefits from the hidden side of heredity and that fully exploits all available diversity.

## KNOWN DRIVERS OF GENETIC VARIATION BEYOND MENDEL

Beyond the variation that arises from meiosis and the normal workings of Mendelian genetics, one must also consider the variation that arises from DNA mutations and epigenesis. The effect, frequency, and nature of mutations are also affected by the actions of their attendant repair mechanisms such as 'mismatch repair proteins' (MMRs); the frequency of mutations and other events (epistasis, etc) is known to increase if MMRs are inactivated or repressed [47]. The spatial distribution of these mutations is not random, as the plant chromosome contains mutation 'hot spots', perhaps in part related to the length of near-identical sequences [48,49] (longer ones favoring recombination) and in part to epigenetic and positional effects that protect regions of the chromosome against changes. DNA sometimes undergoes deletions and rearrangements of a variable extent [50-52]. Interspecific crosses allow elimination and silencing of DNA but can also resurrect the expression of certain DNA sequences [53, 54]. The actions of transposons and retro-transposons, by inserting and removing themselves from critical structural or regulatory DNA sequences, constitute yet another set of several mechanisms capable of generating genetic diversity [55].

DNA can undergo methylation, which does not directly alter the base-sequence encoding of messages, but which may alter the expression of genetic information via epigenetic effects [15]. Epigenesis is essential for organizing patterns of gene expression that impinge on tissues, phenology and the environment. Besides DNA methylation and de-methylation, mechanisms such as gene silencing, genomic imprinting, transposition, paramutation, [56, 57], histone modifications and chromatin remodeling [26, 58, 59], alone or in combination, can affect how the information coded in DNA is actually expressed. Vast stretches of the



genome can be epigenetically silenced, while other areas remain largely active. The idea that it is worthwhile to explore how epigenetic effects add a useful dimension to plant breeding strategies gains support from the evidence that epigenetic heredity transmits better in plants than in animals [60]. The number of generations over which epigenetic effects endure — and perhaps ultimately act in a manner difficult to distinguish from 'classic' Mendelian genes [15] — can be highly variable.

Variation can therefore be found even without sexual crossing. The concept that epigenetic mechanisms modify, indeed enrich, the expression of messages encoded in DNA base sequences has profound implications for the progress that can be achieved from a narrow genetic base [4]. It calls into question the absolute necessity of the role of genetic diversity as conventionally conceived and, as a necessary corollary, further posits that *de novo* variation — through a variety of mechanisms — arises frequently within the germplasm selected by the breeder. The hypothesis that newly generated variation can make an important contribution is now beginning to be confirmed by practical examples, such as the exploitation of *de novo*-evolved resistance to 'wheat streak mosaic virus' (WSMV) [61-64] and FHB [62, 65]. The disarming simplicity and rapidity [62] with which such useful *de novo* variation can be generated should not perhaps be considered too shocking, as the plant genome has been shown capable of being modified by stress [5, 6], a finding consistent with the earlier conclusions of McClintock (1978, 1984) [7,8]. These successes can be seen as examples of having found ways to make the use of nature's reserves of genetic diversity. Emulating nature in this way may soon play an essential role in the future of applied genetics.

What are the engines that drive the accumulation of such reserves in nature's genetic diversity? Considerations from first principles suggest that the need to prevail in chaotic environments would favor the evolution of systems capable of faster evolution [66, 67], and observations of natural ecosystems support the view that stressful environments can indeed act as generators of new genetic diversity. Studies conducted in Israel showed that some species related to wheat and barley exhibited increased genetic diversity on sites subjected to higher stress [68]. This diversity encompassed both structural genes and regulators. In *Hordeum spontaneum*, part of this genetic diversity appeared to result from the action of retro-transposons. Although stress can speed the elimination of many phenotypes, it may, seemingly paradoxically, also increase genetic diversity in the long term, provided extinction is avoided. Stress can also reveal the existence of genetic diversity that was inactive or rarely expressed. Many 'genotype x environment' (G x E) interactions are altered by a simple change in a "heat shock" protein which normally buffers systems to yield normal phenotypes. A number of mutations could thus remain hidden until they are revealed through the action of stress or environmental change [19, 20].

Learning that crop germplasm experiences *de novo* variation as it interacts with the stresses it encounters in the environment points to — at first glance — unsettling consequences for modern seed production systems. Such variation would run directly counter to the stated goal of faithfully reproducing and increasing germplasm that is supposed to consist of genetically invariant, highly-inbred or even doubled haploid (DH) lines. To maintain the desired genetic invariance, there are rules that impose "roguing" (manual removal) of off-types. In Canada, for example, initial seed stocks of many crops are propagated at the Seed Increase Unit, Indian Head, SK. While roguing in a drought year is mostly business as usual, the next year's seed production poses special challenges because off-types are more frequent, and roguing must be done with the greatest care (D. Gehl, *personal communication*). The



suggestion that this can derive from genetic variability induced by drought stress is consistent with the observation of higher rates of *de novo* resistance to WSMV resistance found in populations grown from seed harvested in drought years (Haber, unpublished). Similarly, DH lines, grown in a manner that avoids outcrossing, should not produce off-types. However, these have been found to be frequent in the DH lines obtained from the cultivar Laval-19 even if grown in the greenhouse [69] and in the DH cultivar McKenzie when grown under pressure from WSMV infection [62].

Rules that require the removal of variants as they arise assume that such variants were often 'mistakes', most often attributed to improper cleaning of harvesting equipment or unintended cross pollination events. However, evidence exists that stressful conditions generate more variants. Brazil is widely known as a country in which wheat production encounters abundant stresses from soils, climate, pests and diseases [70]. In this setting, bread wheat exhibits rather extensive *de novo* cytogenetic variation [71-75]. Such consistent high frequencies of cytogenetic anomalies have not been similarly reported in countries that have milder climate and less disease. Among the factors that could conceivably account for this, some effects of temperature, climate, pesticides and soil acidity were demonstrated, but the largest proportion of anomalies was attributed to diseases, pests and viruses attacking the plants [75, 76]. Genotypes with Norin 10 germplasm in their pedigree were more sensitive to these effects and consequently displayed a high proportion of off-types [74]. While the majority of such individual variants will not be expressing desirable traits, the selection of occasional beneficial variants may lead to evolution, 'descent with modification' [77]. It is not necessary to have established exact cause-effect relationships in order to identify and exploit phenomena arising from selection under the long term effects of stress. The Brazilian workers have made steady progress in their chosen directions, achieving, for example, straw strength [78] and leaf area duration [79] parameters clearly beyond the parental range of variation as well as a very powerful root anchoring and a very high degree of allelopathy (weed repression) combined with competitive ability (Figure 2E, 2F).

A systemic approach – one that considers germplasm, genes and diverse biotic/abiotic environments as parts of a broad, interacting system – must integrate Mendelian phenomena, knowledge of genetic diversity and the heritability of interacting factors in a variable environment, as well as an expanding array of non-Mendelian phenomena. We will therefore need to review a number of concepts that illuminate the choice of complex methods used and the range of challenges that must be addressed. This will be more than a mere conceptual *tour de force* as, before concluding, we will illustrate feasible progress with some early practical applications. Among these is an example that exploits success in generating certain useful, heritable traits *de novo*. These early applications point the way to an all-encompassing approach combining genetic diversity (in the broad sense that we expand on below) with the targeted use of stress to generate and identify additional usable *de novo* variation.

## A NEW DEFINITION OF GENETIC RESOURCES

We need to think hard about what factors contribute to genetic diversity and how to make best use of them. Conserving genetic diversity has often been compared to the safe storage of the elemental building blocks that that can be used in a simple and defined way to construct



desirable plant types. However, we now increasingly understand that critical genes, by being extensively regulated through complex modules acting in hierarchies, can embody aspects of genetic diversity that go beyond the theory of individual gene action. How the genes of a complex organism interact is an essential part of its genetics, so to be usable, genetic diversity must consist of genes organized within regulatory systems that offer adaptive value. Moreover, stress and interspecific hybridization can alter profoundly the genetic composition of chromosomes and the epigenetic regulation of gene function. Therefore, in assessing the role of alien genetic diversity, one must recognize that the manner in which these genes interact with the overall genome can profoundly alter their behavior [53, 80]. In a different genetic background, many genes may lose part or all of their activity, or conversely, hitherto inactive genes may awaken from their dormancy. Finally, when seeking to expand the range of genetic diversity by crossing with related species, one must not overlook micro-introgression phenomena such as those observed in interspecific rice crosses [81].

The view that genetic diversity reflected simply the diversity of independent genes in an assembled collection was always an over-simplification, and now we can clearly see that it is unhelpful in guiding the identification and use of genetic resources. Co-adapted linkage blocks and complex gene combinations that increase the plasticity, adaptation and/or energy efficiency of a genotype represent entities that have more value than simple genes. This is like the difference between a box full of mismatched electronic parts and a partly assembled electronic system. Proven assemblies have more value.

Germplasm developers collaborate with breeders in the practical endeavor of improving crops by identifying and characterizing new useful genes and traits for future incorporation into breeders' lines. In following the commonly dispensed advice to focus attention on just one or a few stresses (e.g. resistance to a fungus, a virus or an abiotic stress like drought or waterlogging), germplasm developers may introgress potential gene sources into more adapted backgrounds and consider their task complete when they transfer this germplasm to breeders. They may also send unimproved resistance sources directly to the breeders who will need to do the hard work related to introgression into adapted germplasm. However, in all of those focused approaches, the competition of the expression of various traits for available photosynthetic energy is simply overlooked [82], so that improving one trait can easily cause the deterioration of several others [83-85]. Moreover, if the resistance to stress arises as a consequence of the action of mechanisms or biochemical pathways that conflict with agricultural goals of yield or quality, the germplasm developer and the breeder will be working at cross purposes [45]. A significant proportion of resistance genes investigated may thus never become compatible with their envisioned uses, even after massive research efforts and investments.

As a wheat breeder and keen student of the wider science and philosophy literature, M. Lévesque incisively criticized the notion that approaches which strictly followed the precepts of pure Cartesian logic constituted the best or only tools to advance understanding of life sciences. As ordinary soldiers in the legions who sought plant improvement in the 1970s and '80s under the banner of classical gene introgression, the authors of this chapter all came to share growing doubts about the all-conquering power of those ideas and methods of the past century. In reflecting on similar doubts, Simmonds (1995) posed the question: "*How frequent are superior genotypes in plant breeding populations?*" [86]. His severe verdict now calls on us to recognize that we tend to fail to identify the best genotypes.



If valuable genotypes are indeed not 'extremely rare' but our selection needs to be more efficient, we must then turn our attention to just how we should choose and apply those stresses that are most helpful to selection. This sheds new light on the role selection plays; experienced plant breeders see the analogy between plant breeding and natural selection [87]. To make progress as we seek to emulate Nature, we will put less emphasis on 'pushing' genes through pedigrees – and more on 'pulling' by selection out of a pool into which we have flowed more sources of variation. "*More Darwin, Less Mendel*", if a rousing slogan were needed.

## REFINING THE ART AND SCIENCE OF SELECTION

Plant breeders may belong to different schools of thought about whether it is best to select for improved agronomic traits under high or low stress, but after 40 years of effort, it remains impossible to combine improved yield in favorable environments with high resistance to drought [25]. In conventional breeding programs, the manner in which potential wheat cultivars are exposed to stress is often limited temporally and spatially. Cereal breeders may initially focus on identifying good quality germplasm that yields well [86, 88] or lines that resist a specific disease, yet their ability to identify the best genotypes in early generations is generally unsatisfactory. Simmonds (1995) argues that the main reason for the frequently low rates of success lies in inefficient selection rather than extreme rarity of valuable genotypes [86]. While breeders can eliminate the excessively tall, diseased or late-maturing lines [89] by visual inspection, they cannot identify successful candidates in early generations if they rely on screening for so few traits at a time. Moreover, screening simply to reveal the effects of one specific stress, whether biotic or abiotic, fails to account for any interactions among genotype, pathogen or environmental stresses. Such 'clarity' often comes at the price of losing information that might well prove to be vital for the success of a genotype in a particular region.

Researchers often limit the number of selection criteria they employ in order to reach their goal more quickly and to obtain results that can be explained with analytical simplicity [62]. This may be resistance to a fungus, a virus, or to an abiotic stress like drought, cold, or waterlogging. After identifying potential gene sources, the developer may start to introgress the desired trait into more adapted backgrounds but then neglect the fact that various traits and biochemical mechanisms compete for available photosynthetic energy [82] with benefits and penalties that vary between environments [24]. In improving one character, he may easily cause the deterioration of others [85]. Moreover, a particular form of resistance to a stress may be conditioned through mechanisms or biochemical pathways whose activity, by limiting yield or quality, may not be compatible with agricultural goals. A single gene can affect a large number of traits [33]. For example, resistance to biotic and abiotic stresses can be mediated by reorienting biochemical pathways in response to environmental signals mediated by a single kinase [90], so that the plant loses some biotic stress resistance while adjusting to resist abiotic factors.

Many traits that a plant expresses are correlated, and this may sometimes make a breeder's task easier; for example, drought-resistant plants are often resistant to cold, and the converse is also observed [91]. Unfortunately, most of the correlations between traits pose



challenges. Some genes for resistance to salinity, drought or disease have pleiotropic or linkage drag effects that geneticists have humorously nicknamed "resistance to yield" [92]. After substantial investments in research, resistance genes might be declared to be successfully introgressed, but the germplasm is never used. Research conducted in Quebec between 1980 and 1994 to improve resistance to BYDV in wheat illustrates the point. This long-term study generated wheat lines that were irreproachable from the standpoint of resistance and tolerance, but the vast majority were judged unsuitable for the breeders' purposes [45, 77]. The BYDV tolerance was polygenic and strongly associated with excessive height (Figure 3), lateness, low protein content and unacceptable grain shape. Very few useful derivatives were obtained even after two backcrosses to adapted cultivars. Furthermore, any improvement in agronomic traits using those lines as parents came at the cost of losing virus tolerance (Comeau, unpublished).

Whenever a breeding project that focuses on disease resistance or quality falls short of its agronomic targets, it is too easy to 'round up the usual suspects' and blame 'linkage drag'. It follows that one should keep trying to break the postulated linkages by, for example, using larger populations. This might work, if only – so goes the wishful thinking – those desirable alleles were sufficiently distant from 'the villains' that spoil agronomic traits. Sometimes, this simple idea does work. Too often, however, pleiotropy and epistasis lie behind these many correlations that are so difficult to overcome. In a nutshell, this is one of the real challenges of plant breeding. The plant's response to the complete set of biotic and abiotic stressors is rarely considered, let alone optimized [70, 93]. To breed germplasm more successfully, one should assemble more quickly the mechanisms the plant can use to adapt to its agroecosystems rather than simply rely on single genes. Tollenaar and Wu (1999) reported that maize yield improved in response to better adaptation to a series of stresses relevant to their target agricultural environment [94]. Troyer (2006) similarly concluded that maize genetic improvement came from the lineages gradually adapting better to their new environments rather than from increased heterosis [87].

Contrary to a widely held view, even the best growing conditions create new kinds of stresses. A dense canopy exacerbates a number of stresses in maize, a finding likely to apply to many species [94]. Such canopies increase competition for minerals, CO<sub>2</sub> and light and, by changing the microclimate within the canopy, promote diseases. Stems etiolate as the plants try to capture more light, causing the stems to become too slender and lodge. In the case of wheat, efforts focused on obtaining high yields result in reduced grain quality (specific weight, proteins) [95].

While they may not be expressed in their current phenotype, the determinants of desirable traits may exist as readily available hidden potential in certain parental lines. This can serve as a basis for breeding for horizontal resistance, which, conditioned by a large number of minor genes, is more stable across environments and years [37]. It follows that the utility of a gene source is much better judged by the performance of a cross's progeny than by parental phenotype. Moreover, it is best to assess this under complex stress and/or contrasting environments. Recent work has, in fact, started to reveal this hidden potential within elite germplasm [15, 62, 96]. To increase the likelihood of yielding desirable phenotypes, an ideal parent must provide a wide range of valuable traits, including well regulated responses to changing stresses [97].



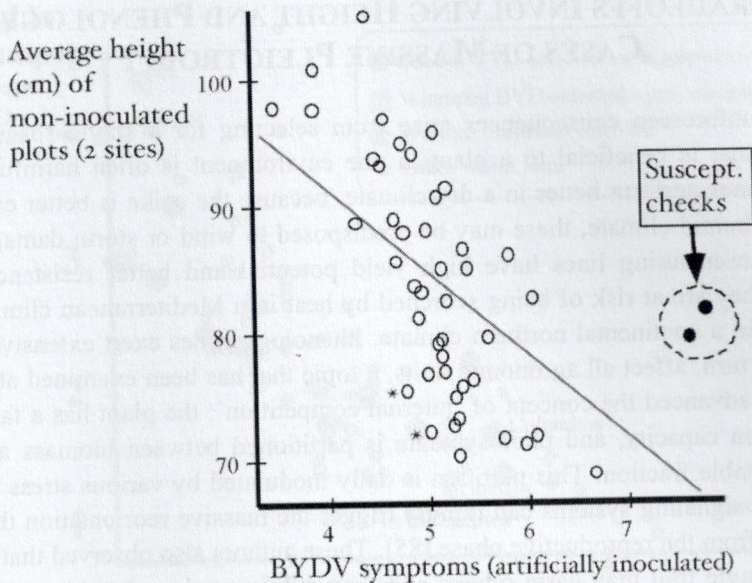


Figure 3. Relationship between BYDV symptoms in virus inoculated plots and the height of non-inoculated plots, for a series of elite BYDV-tolerant wheat genotypes tested in Quebec (1992). The most BYDV-tolerant genotypes were tall. Genotypes presenting a useful compromise are asterisked. The regression line includes two BYDV sensitive check cultivars. Data by Comeau and Collin, 1992.

Fungi, viruses, bacteria, insects, mites and nematodes interact with abiotic factors to cause serious crop damage, and in wheat, FHB or rusts on their own, may cause enormous economic losses.

As it befits a highly-evolved system, a plant can respond to stresses in a complex and sophisticated manner by flexibly coordinating the interacting and hierarchical activities of a fairly small number of underlying basic mechanisms. For example, in wheat, DNA microarray-based studies of gene expression have shown that each tissue responds in a different manner to *Fusarium* [98, 99], although the mechanisms of perception and response to different stresses share many common pathways [100].

Among all the factors that stress plants, those related to soil and water dominate. It is thus important to use those factors in selecting. Soils that are dry, acid or otherwise poor may cumulatively stress plants even more than the biotic stress exerted by pests and disease [93]. Symptoms induced by virus infections as well as those attributed to nematodes, root rot agents and a number of micronutrient deficiencies can be deceiving at times [45, 101]. Considering numerous possible interactions, identifying one cause of agricultural loss should never lead to the belief that all stresses have been sufficiently studied. Systemic genetics accords a prominent role to root health and physiological efficiency of roots, since absorbed minerals affect all aspects of the plant's life [102-104], including morphology, phenology, and resistance to stresses and plant enemies.



## TRADEOFFS INVOLVING HEIGHT AND PHENOLOGY: CASES OF MASSIVE PLEIOTROPY?

Multiple unforeseen consequences arise from selecting for a single specific stress. A characteristic that is beneficial to a plant in one environment is often harmful in another. Taller wheat lines perform better in a dry climate, because the spike is better extruded from the boot. In a humid climate, these may be predisposed to wind or storm damage and lodge too easily. Late-maturing lines have high yield potential and better resistance to certain diseases, but they are at risk of being scorched by heat in a Mediterranean climate, or killed by early frost in a continental northern climate. Phenology genes exert extensive pleiotropic effects that, in turn, affect all agronomic traits, a topic that has been examined at length [85]. These authors advanced the concept of 'internal competition': the plant has a fairly constant daily production capacity, and photosynthate is partitioned between biomass accumulation and the harvestable fraction. This partition is daily modulated by various stress factors [82], while complex signaling systems can rapidly trigger the massive reorientation that separates the vegetative from the reproductive phase [85]. These authors also observed that focusing on improving a single trait may harm others, and they put forward a systemic approach which was later generalized to encompass more than the phenological dimension. Yan and Kang (2003) subsequently proposed a more global model: a) the cultivar is not simply a collection of independent traits, and b) to improve crops by breeding, one must understand plant genetics, components of the biological system, and the relationships between these components and the target environment [97].

Certain resistance traits are easier to improve in environments where tall [77, 105] or late genotypes are more acceptable. Tall genotypes are adapted to the driest environments. The basis of the "Green Revolution" was the discovery that shorter genotypes permit better lodging resistance and yield potential. Breeders deployed Norin 10 *Rht* dwarfing genes to confer high resistance to lodging and thus enhanced yield when conditions were ideal. This improved tillering to permit more efficient use of nitrogen. However, when conditions are less than ideal, short-statured cereals are more susceptible to viruses [45, 101], FHB [41, 42, 106, 107], drought, root rots, and herbicides. A link between type I Fusarium resistance (to initial infection) and the modulation of the gibberellin hormonal regulation pathways by the dwarfing allele *Rht-D1b* was recently reported [44, 108]. Pleiotropy or very tight linkages may thus account for the increased sensitivity to FHB in short wheat genotypes that carry *Rht-D1b* [109]. Undesirable consequences that arose from the interaction of the 'shortened' stature with the rest of the genetic background were aggravated by the short span during which major rust-resistance genes were effective [37]. Other dwarfing genes were also used (like *Rht-B1b*, and *Rht8*); these might have fewer drawbacks, but their interactions with diseases have not been fully quantified.

Wheat germplasm selected for tolerance to BYDV generally yields well (Figure 4) and also copes well with root rots, waterlogging and degraded soils [45, 102], but it tends to be too tall and late maturing (Figure 3). To overcome these latter deficiencies, one must take into account a diverse array of parameters that include height, the harvest index under pressure from virus infection, as well as the stability of such practical traits as yield and specific weight under viral stress. A number of semi-dwarf wheat lines germinate unevenly because of their short coleoptiles and tend to achieve a lower specific weight [110].



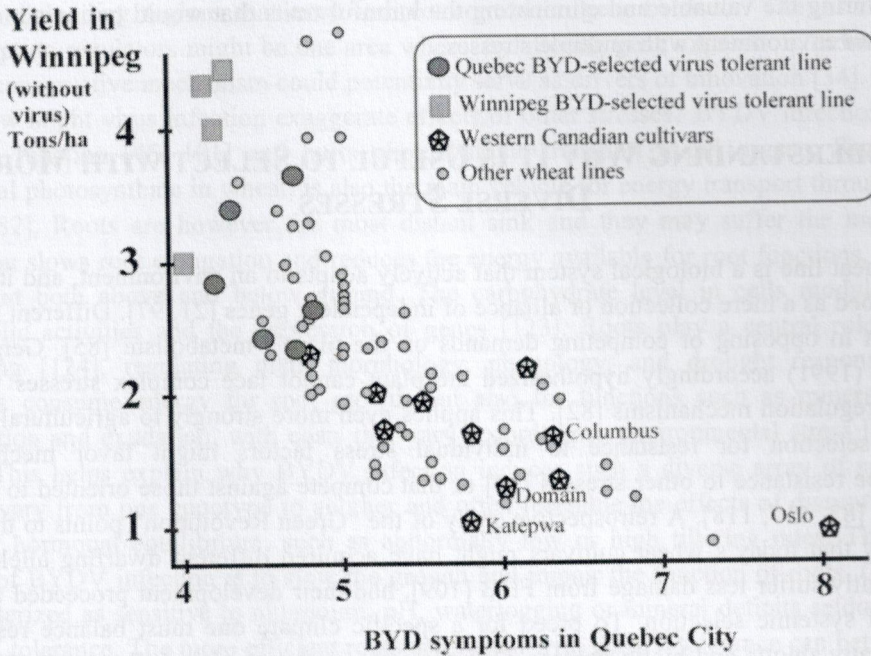


Figure 4. Yield of wheat lines in Winnipeg vs the BYDV symptoms in Quebec City artificial inoculation trials. Raw data correlation is  $-0.718$ ; or after  $\log(1+x)$  conversion of data sets, correlation is  $-0.791$ . No virus had been used in Winnipeg and no virus epidemic detected, but the spring conditions had been cool and wet. Lines that did best for both yield and virus tolerance were mostly the lines previously selected on the single basis of BYDV tolerance, either in Quebec or in Winnipeg. Registered cultivars (pentagon symbols) yielded rather poorly in this Winnipeg yield trial. 1994 data, Haber and Comeau.

The drought response of short wheat lines that are insensitive to gibberellins is not uniform. Such cultivars may exhibit good adaptation to the drier regions of Canada [111], but in Europe they are often intolerant of heat [112]. A plausible case can be made that dwarfing genes repress root elongation [113] especially after mid-season, and/or that they reduce uptake of water and nutrients, but these hypotheses await confirmation by more extensive data [114, 115].

By employing strategies such as 'shuttle breeding' between contrasting, 'stressful' locations, CIMMYT (Centro de Mejoramiento de Maiz y Trigo) programs gradually overcame their handicap and created medium height cultivars that perform remarkably well under difficult conditions [116]. In parallel to this, the link between sensitivity to stress and dwarfing genes has been the focus of intensive research in Quebec since 1985 [45]. For genotypes of normal height, systemic heuristic approaches have produced wheat lines with tolerance/resistance to the combined stresses of BYDV and FHB. The same approach is currently being applied to develop semi-dwarf wheat lines that resist both diseases. Faster progress has been obtained with BYDV than with FHB; currently, few lines with adequate resistance to FHB are shorter than 75-80 cm. It is evident that problems will be encountered when projects focus on just one or a few traits. The adverse consequences of neglecting correlations and ignoring the effects of the environment on gene expression can remain underestimated for decades. Systemic approaches avoid such problems, having the merit of



both capturing the valuable and eliminating the harmful traits that would be invisible unless tested in an environment with multiple stresses.

## UNDERSTANDING WHY IT IS USEFUL TO SELECT WITH MORE DIVERSE STRESSES

A wheat line is a biological system that actively adapts to an environment, and it cannot be described as a mere collection or alliance of independent genes [21, 97]. Different stresses can result in opposing or competing demands on the plant's metabolism [85]. Geiger and Servaites (1991) accordingly hypothesized the plant cannot face complex stresses without efficient regulation mechanisms [82]. This applies even more strongly to agricultural plants, because selection for resistance to individual stress factors might favor mechanisms that reduce resistance to other stresses [90] or that compete against those oriented to growth and yield [83, 117, 118]. A retrospective study of the "Green Revolution" points to the clear possibility that today's wheat cultivars might have acquired different dwarfing alleles, and consequently suffer less damage from FHB [109], had their development proceeded under a regime of systemic selection. To breed for a specific climate one must balance responses against many abiotic and biotic stresses. Dry climates present special challenges [117] as the sum of secondary stresses may exert greater effects than water shortage by itself [25]. Humid climates also contain a wide array of abiotic and biotic stresses [70, 93, 119]. All responses to stress have a cost; for example, adjusting to osmotic stress and/or salinity often involves synthesis of osmotica like glycinebetaine. The biochemical fate of assimilated carbon in betaine is terminal [120]; the energy expenditure cannot be recycled into useful biomass once the stress is relieved by rain or irrigation. This drought resistance mechanism thus reduces to some extent the energy available to all other biochemical pathways. Therefore, plants adapt most advantageously when they allocate photosynthate efficiently in complex and variable situations, and it follows that selecting under pressure from multiple stresses works best. The genetics of regulation is inherently complex, as it must optimize numerous signal/response pathways.

Are certain specific stresses in a systemic, heuristic approach more useful than others? As emphasized above, an effective selection system must include natural stresses that approximate those the plant will encounter as it grows. Although wheat grown in some parts of western Canada, for example, encounters BYDV and WSMV only sporadically, there are two reasons infection with viruses — and the host response to infection — are nonetheless central elements in systemic heuristic approaches we have developed for improving wheat. First, virus infection usefully exaggerates the effects of other stresses, making it easier to identify and select the most promising individuals. Second, virus infection can induce epigenetic variation, some of which may in turn become fixed genetically. Upon infection, the host response induces signals that move through the plant tissues, causing genome instability in the cells as it spreads [5, 6]. This may also affect germ cells. While we are currently only beginning to learn in detail how they work, such mechanisms may well have an adaptive value [7, 8, 51, 121].

Different stresses may have different effects, either through direct mutation or recombination-based innovation [122]. Recombination can occur within coding regions but



also in non-coding regions that exert indirect control on gene behavior. The *cis* regions of transcription regulators might be one area where, by altering gene expression, induced errors in this conservative mechanism could potentially serve as drivers of innovation [34].

How might virus infection exaggerate effects of other stresses? BYDV infection impairs phloem function [45, 101] and hurts photosynthate transport to all organs. Sucrose, the principal photosynthate in wheat, is also the main vehicle for energy transport throughout the plant [82]. Roots are however the most distant sink and they may suffer the most; virus infection slows root elongation and reduces the energy available for root functions, affecting the plant both above and below ground. The carbohydrate level in cells modulates both metabolic activities and the expression of genes [123]. Roots play a central role in plant signaling [124], regulating plant morphology, phenology, and drought responses. Root systems consume energy for root growth but also for functions such as mineral uptake, respiration and exudation, with costs that vary according to environmental stress [119, 125, 126]. This helps explain why BYDV infection induces such a diverse array of symptoms. These vary from one genotype to another and often resemble the effects of disruption of the normal hormonal equilibrium, such as abnormally low or high tillering rates. The earliest effect of BYDV infection is to slow the growth and impair the function of roots. Genotypes characterized as sensitive to aluminum, pH, waterlogging or mineral deficits seldom display BYDV tolerance. The more efficient roots associated with BYDV tolerance can better supply aerial and underground organs with minerals needed for resistance to and tolerance of stress [103]. Chlorophyll-bearing tissue that receives all required minerals at optimal levels will, in turn, be able to produce more photosynthate for root growth and function. Genetic improvement aims to optimize overall energy efficiency of root and shoot interactions so as to meet agricultural goals. The response to BYDV infection helps to identify energy-efficient plants, resulting in lines that produce more biomass and yield better even in absence of virus (Figure 4). One of Canada's highest yielding wheat cultivars in 2004-2009 was Hoffman, the one that also exhibited the best BYDV tolerance.

It is essential to analyze the agricultural ecosystem (soil, climate, micro-organisms) and a large germplasm base when considering the use of specific stressors and stress-inducing selection sites. Buddenhagen (1983) underscores the discriminating value of less favorable ecosystems that can accelerate genetic progress, highlighting as an example the remarkable advances attained in wheat genetics at EMBRAPA in Brazil [70]. The first users of the systemic method used early selection (beginning with  $F_1$ s) and continued selection in later generations in the presence of well-controlled but multiple stresses: flooded soils, poor soils, overly hot soils, and foliar diseases combined with inoculations with viral and fungal pathogens. Beginning with the  $F_1$ , the  $F_1 / F_1$ , or the  $F_2$  and continuing over three or four generations, the germplasm was tested in various soils or sites and/or exposed to stresses, different from one generation to the next. The approach promoted plasticity and broadened the area of adaptation. Although the rejection rate was high and sometimes apparently excessive, rare genotypes were identified that combined most of the desired traits [46].

We have been exploring, in turn, the contributions that: a) heritable variation and b) selection under pressure from multiple stresses make in the development of systemic heuristic approaches to crop improvement. These contributions cannot, however, be entirely considered distinctly from one another. Stresses can bring about heritable changes in plant populations, and the expression of heritable variation can, in turn, alter the nature of stresses plants encounter in the environment.



## MAKING MUTATIONS AND EPIGENESIS WORK FOR OUR PURPOSES

From the 1950s to about 1980, there were high expectations that numerous desirable traits could be obtained by applying often harsh chemical or physical mutagens and selecting for desirable new traits among the small proportion of survivors. Radiation mutagenesis, however, is a blunt instrument that causes excess genetic damage all too easily [127]. It shares with other artificial mutagens the tendency to act simultaneously on too many loci or parts of the chromosome, making it quite difficult to recover useful mutants. By contrast, stressors that resemble in type and intensity those the plant encounters in the environment induce generally more benign mutations, and desirable heritable changes among progeny are easier to select [62].

More recently, in pursuing the objective of chemically altering *epigenetic* (rather than genetic) information, Akimoto *et al.* (2007) also reported heritable phenotypic changes in rice plants treated with the demethylating agent 5-azadeoxycytidine (5-azadC) [15]. Although few (35 out of 1000) seedlings survived the treatment by 5-azadC, one, Line-2 was shown to have acquired resistance to the pathogenic bacterium *Xanthomonas oryzae* pv *oryzae*, the cause of rice bacterial blight. The resistance showed stable inheritance for at least seven generations.

Similar, desirable heritable changes in plant gene expression can be achieved in a safer, faster and less expensive manner using plant viruses. A crop improvement protocol incorporating some of the elements of the systemic heuristic approach presented in this review used WSMV as an inducing virus [62]. In part, this choice reflected the aim to evolve new sources of resistance to this economically important virus *de novo* from elite germplasm rather than be forced to introgress a known resistance gene (*wsm1*) carried on substituted or translocated alien chromatin from *Thinopyrum intermedium* [128]. More critically, any epigenetic effects of WSMV-based protocols would not arise from direct effects of virus infection on the host genome, as the viral single-stranded RNA replicates in the cytoplasm and thus does not interact with host DNA. The dramatic extent of *de novo* genetic variation that WSMV infection induced in progeny (conventionally seen as genetically invariant) descended from a single seed of the DH cultivar McKenzie can be seen in the rapid evolution and selection of novel desirable traits such as shorter stature and resistance to both WSMV and *Fusarium graminearum*, the principal causal agent of FHB in North America [62].

The rate at which novel – and subsequently heritable – phenotypes are evolved *de novo* in responsive wheat lines indicates that the ‘engine’ of variation that acts in WSMV-treated plants is far more powerful than the ‘normal’ order of  $10^{-6}$  mutation per allele per cycle that directly modifies DNA base sequences. There is some very preliminary evidence that the apparently epigenetic effects induced by WSMV infections are associated with modifications of histone proteins (Haber and Standing, unpublished). The relative roles of epigenetic events and mutations of DNA base sequences in these intriguing phenomena are yet to be quantified.

Another example of a central role played by virus infection in a systemic approach to crop improvement can be seen in the application of BYDV infection in repeated seasons of trials at carefully chosen sites that exposed plants to complexes of stressors simultaneously [77, 96]. To capture the useful ability to perform well in poorly drained, mineral-deficient soils, plants were grown under exactly these conditions, and the effect of these stresses and of the added inoculations with *F. graminearum* was exaggerated by infection with BYDV. Such an approach can be quite lethal, but the surviving plants are easily observed and selected.



Burning a haystack is indeed an effective way to expose a needle. In subsequent rounds, the survivors' descendants were exposed in repeated cycles to the stresses of infection with BYDV and *F. graminearum*, culminating in the selection of adapted germplasm with stable high yields and resistance to both diseases.

## RESISTANCE AND TOLERANCE AS INDEPENDENT PHENOMENA

A central conceit of crop improvement strategies that focus on the introgression of 'pathogen resistance genes' is that demonstrating the gene's efficacy in suppressing the pathogen's proliferation (i.e. 'resistance') is the same as protecting the host from the effects of the pathogen. Resistance often does indeed provide such protection but the phenomena are not synonymous. Resistance and tolerance are two independent phenomena, and thinking they are closely related can lead crop improvement efforts dangerously astray. Resistance relates to the plant's ability to fight off an attacker, regardless of the damage it sustains in the process. This mechanism may involve an appreciable expenditure of energy or use energy-inefficient pathways, which may sometimes result in reduced yield [83, 84, 129]. The term tolerance, on the other hand, merely refers to the reduction of losses. In our long-term studies on resistance to BYDV and to some closely related causal agents called cereal yellow dwarf virus (CYDV), we observed that, although by definition, resistant genotypes exhibit a lower level of infection, some may suffer considerable damage [100]. Other genotypes, by contrast, sustaining high viral titers (and serving as major reservoirs of virus) are classed among those that cumulatively sustain the least damage [45, 101]. Koch's postulate states that pathogenicity is proved by isolating a pathogen from its host and then reproducing disease symptoms by inoculating the agent into a disease-free host. While our counter-intuitive finding seems to violate this postulate, it should be remembered that some resistance genes against pathogens and insects may repress enemies of the plant, while having a degree of deleterious effect on the plant itself. To illustrate with a real example, plant host hypersensitive responses that are improperly regulated fail to remain confined to the immediate vicinity of the cells infected by the pathogen, thus damaging the host. This must be labeled resistance without tolerance. By contrast, some stay-green plants can show a high level of fungal colonization (over 50% of leaf area covered by mildew), yet their tissues contain the aggression and compensate for it well enough that the grain continues to be nourished (Langevin, unpublished). In this case, there is tolerance but only a little resistance.

Consistent with the understanding of the plant as a system that functions within the environment, these concepts should be logically extended to taking abiotic stresses into account. Plant species or genotypes can follow different strategies in managing the resources available to them and in the process gradually modify their environment. A plant can either grow slowly and conserve soil water or, alternatively, produce biomass rapidly, keep the soil cooler, and create a natural cover of leaves that shades the ground like a mulch. The soil is a few degrees cooler on a hot day under a cover of shade [130]. Since cooler soil water or microclimate can have major effects on water use efficiency [131], even a few degrees of cooling could have a significant effect. Could this natural mulch be described as a mechanism of resistance to heat, since it represents energy expenditure designed to combat the ulterior heat stress of the plant? A plant could also excrete enough exudates to increase manganese



availability in a Mn-deficient soil, with benefits for plant growth. Indeed, the plant could be understood as expressing 'resistance to poor soil', an *extended phenotype* [132] arising from the coordinated expression of genes narrowly understood to control specific components of its physiology. In short, the environment affects the plant, but the plant can also modify its environment [23]. We suggest that whenever the plant avoids abiotic stress through phenology or morphology, the plant could be viewed as tolerant (or escaping). Inasmuch as the plant spends energy to modify its abiotic environment towards milder conditions, the best terminology might be resistance to abiotic stress.

## AN EVOLUTIONARY APPROACH TO GERMPLASM DEVELOPMENT

Having set out first how we might pursue a greatly expanded range of usable genetic variation, and then how we might select cumulatively and creatively, how do we now assemble out of these considerations practical protocols that deserve membership in the family of systemic heuristic approaches?

Selecting the right stresses and the right protocols for applying them are choices every bit as critical as selecting the right parents. An essential aspect for any continuing progress is the ability to identify and advance the best adapted phenotypes. The systemic approach accomplishes this by identifying those happy combinations of valuable information and well-regulated effective expression that yield those individuals that will survive and thrive under the imposed regimes of complex stresses. This approach delivers the additional, subtle but powerful benefit that it drives out dysfunctional combinations of genes, often before seed set. Plants, by surviving and clearly showing the most vigorous phenotypes, do the difficult work of identifying the best individuals for us to advance to the next cycle. Here we are *emulating* evolution, with the difference that we are doing some of the selecting. We will know if we have made substantial contributions beyond the capabilities of approaches that focus on gene introgression, if we succeed in consistently meeting the efficiency criteria suggested by Wallace and Yan (1998): capturing numerous traits at the earliest possible stage [85]. Although we should not expect to arrive at any one protocol that will be effective in all settings, methods can be developed to eliminate genotypes that are not able to respond effectively to the complex stresses that will arise in the natural and agricultural environment.

True to the philosophy of *emulating evolution*, we should not seek to arrive at perfect or optimal expression of desired traits in a single leap. For practical reasons as well, it is wise to apply stresses less severely in the first year to avoid destroying every individual in the germplasm. This can be done through lower doses of inoculum and, in the case of stress from virus infection, inoculating at later plant growth stages. The total load of biotic and abiotic stresses can then be increased gradually over succeeding generations. The severity of FHB is more difficult to control because of interactions with the climate. It is logical to plan on increasing gradually the severity and number of diseases and abiotic stresses, provided experts can advise on an annual (or seasonal) basis how intensely to apply each factor. Our experience in Brazil and Canada emphatically supports our confidence in the excellent returns from making selections based on the use of complex stresses applied sequentially or simultaneously. The high rejection rate is a small price to pay for the enhanced ability to identify rare genotypes that combine most of the desired traits.



Selecting under the pressure of multiple, often interacting stresses thus differs from the 'screening' employed in conventional plant breeding in terms of the precision and severity of the stressors used, as well as in the larger number of criteria employed [77, 100]. The progeny of each cross is subjected to a regime of complex stresses which may include mineral deficiencies, viruses, drought, excess water, rusts, *Fusarium* and other biotic and abiotic stresses. The subsequent selection emphasizes morphology, phenology, and physiology that can facilitate stress tolerance and agronomic adaptation [83]. Over a 4-year period, we also selected with increasing rigor for visual grain quality, so that it would be easier to transfer any identified resistance into projected future cultivars. Although numerous stresses were applied and evaluations incorporated a large number of criteria, two biotic stressors in particular — BYDV and FHB — contributed much of the effect and weighed heavily in selection. Tolerance of BYDV infection correlates with physiological efficiency, generalized stress tolerance, yield and mineral uptake efficiency [45, 102, 133], thus helping to identify plants with better root traits, ability to produce biomass and yield stability, even if BYDV resistance or tolerance *per se* are not specifically needed [96]. The combination with FHB stress extends additional power to selection, as BYDV increases both FHB damage and vomitoxin levels [107], making it easier to identify in a population those individuals that perform better thanks to effective expression of putative FHB resistance genes.

A plant's phenology, morphology, agronomic traits and the expression of resistance to stress are related [83, 134]. The relationships are complex, but keen observations required by a systemic approach and tools of multivariate analysis [97] do reveal certain rules of thumb that emerge among the many possible combinatorial solutions for optimizing competing demands. After numerous cycles of variation and selection, some of those rules coalesce as broader solutions that can aid the pursuit of many different goals. As the systemic approach must select for these traits indirectly, care must be taken that phenology and morphology are not pushed in directions incompatible with agronomic value. For example, in seeking to determine the mechanism of resistance to FHB in a certain barley improvement program, Zhu *et al.* (1997) found that plant morphology explained most of the resistance [105]. In this case, height favors resistance, but excess height conflicts with high yield. We attempt to follow the ideotype defined by Caetano (unpublished) which seeks to express traits compatible with the penetration of gases and light into the canopy while avoiding excess load caused by large flag leaves and excess height (Figure 2A, 2A', 2B). A properly aerated canopy allows better light and CO<sub>2</sub> distribution, increasing the efficiency of the photosynthesis that supports defense mechanisms; it also creates a microclimate that is less favorable to diseases [83]. Lodging exerts negative effects in multiple ways. One should, therefore, select both for good physical properties of the straw and improved anchoring of the crowns (Figure 2D) in order to improve the load-carrying capacity and overall health of the crop.

It is advantageous for polygenic traits to be intertwined and woven together from the start. For example, solid-stem genotypes are insect-resistant but currently need major efforts to bring *Fusarium* resistance to more acceptable levels, a task made more difficult by the decades-long separate development of these two polygenic traits.

A similar problem is encountered when seeking to introgress resistance genes into germplasm with good bread-making quality. The genetics of quality are complex, as the genes determining this set of traits are found on every chromosome and are thus linked to any trait in the plant. Thus, if one breeds for *Fusarium* resistance while ignoring quality aspects [135], few, if any, of the generated lines will be suitable for making bread, as seen when



using Asian parents as sources of FHB resistance (Voldeng, unpublished). Selection protocols should, therefore, employ methods that can identify quality traits in early generations from just a few seeds [136] so that pursuing resistance does not come at the cost of losing quality.

## EXPLOITING AND FIXING SPECIFIC AND GENERAL COMBINING ABILITY

Hybrid vigor often results from both specific combining ability (SCA) and general combining ability (GCA) [87]. While 'marker-assisted selection' (MAS) can cope with a small number (rarely more than 3) of GCA alleles, it is not so well suited to deal with SCA, a phenomenon largely driven by epistasis but also dependent on dominance, epigenesis, and perhaps overdominance [87]. An overdominance effect cannot be captured for use in homozygous crops, as it results from a heterozygous locus, but proven overdominance happens to be rare [88, 137]. Systemic approaches, by contrast, deal well with complexity, as by their very design, they can capture and accumulate the desirable manifestations of heterosis, transgressive segregation or epistasis. Following evolutionary principles in the broadest sense, they enable further progress without needing to commit to one or other 'explanation' drawn from a repertoire of Mendel-based or strictly combinatorial genetic principles.

A recent example of *de novo* GCA is the development and commercial release of the first wheat cultivar with genetic resistance to WSMV infection [63]. While strong, well-characterized genetic resistance has been available in chromosome substitution [138] and translocation [139] lines for more than 25 years, wheat breeders attempting to use these sources never succeeded in developing resistant lines with acceptable agronomic and quality traits [140]. Genetic resistance to WSMV infection was then discovered in a single accession of CO960293 (an elite winter wheat line with a pedigree of WSMV-susceptible parents) grown in a disease nursery in 1999; follow-up studies confirmed that other accessions of the line and its pedigree parents were all susceptible [63]. It is thus plausible that this trait originated *de novo* from a mutation induced by WSMV stress. The resistance was characterized as dominant but temperature-sensitive. Being sourced from an elite genetic background (lacking alien chromatin derived from wild relatives of wheat), it had excellent overall agronomic GCA leading rapidly to the development and commercial release of the cultivar RonL [64].

When considering how best to exploit the matrix of interactions between stress and genetic diversity, one must understand that stress interacts with heterosis [141], epistasis, and SCA (which itself may contain a strong component of epistasis). Traits that are expressed based on epistasis can be stronger and better regulated than those expressed by either dominant or additive and recessive genes. Several studies, in both plants and animals [142], have also shown that stress enhances heterosis [143, 144], and that the hybrids with the greatest heterosis also have a significant SCA component. The effects of interacting multiple stresses that are essential to systemic heuristic approaches generally quickly reveal whether one has indeed used suitable parents to arrive at the best possible expression of a trait in given environments. When excellent hybrid vigor under stress is observed in  $F_1$ , it is good to know that, as a rule, most of this hybrid vigor can be fixed through selfing and recurrent selection. It



is thus the superior complementarity between alleles arising from SCA and GCA, more than the heterozygous state itself that confers hybrid vigor [87]. The performance of the genotype may be yet further enhanced by epigenetic factors.

The actual example of the rapid development of FHB-resistant germplasm adapted for use in eastern Canada illustrates clearly how a proper consideration of SCA aids the successful pursuit of a systemic heuristic approach. After producing and studying more than 60000  $F_1$  genotypes (2003-2008 crosses), we concluded that three-parent or four-parent combinations containing wheat lines QG22.24, SS Blomidon, plus *Fhb1* gene carriers like Alsen led to excellent SCA for FHB resistance, while the combination of QG22.24 and SS Blomidon in the pedigree sufficed to generate SCA for better biomass and root traits. Such observations guided the creation of the  $F_1/F_1$  cross AB143 (pedigree: QG22.24/Alsen//SS Blomidon/Alsen), in which vigorous plants were found nearly free from damage from all inoculated diseases.

Caetano has repeatedly observed that substantial SCA can be fixed through recurrent and direct selection, establishing a better base for subsequent progress. Canadian observations confirm this: our most valuable multi-resistant genotypes often showed transgressive behavior including SCA in  $F_1$ , and it was possible to fix rapidly the desirable behavior. Whenever the presence of SCA under stress is confirmed, selection pressure with controlled stresses should be maintained in subsequent crossing/selection steps, so as to avoid losing the gains that have been made. While one might choose MAS approaches to select the genes involved, any subsequent capture of desirable epistasis and SCA would be entirely accidental, as no current gene-tracking method is efficient at discovering new cases of outstanding expression of these phenomena. In sum, good SCA enables progress that may dramatically exceed the incremental advances expected in most introgression protocols. It is more than a little ironic that in the many efforts to introgress the FHB resistance of Sumai 3, so little has been invested in understanding how its effective expression arose from its modest inheritance. None of its parents is highly resistant [145], and the best progeny of crosses of Sumai 3 to other bread wheat lines seldom actually reach the resistance level of Sumai 3'. It is hard to escape the conclusion that the FHB resistance of Sumai 3 arose from a SCA event. The germplasm with the best FHB resistance generated in Canada by the systemic method also captured multiple SCA events, but with the added benefit that the high resistance was fixed in a much better agronomic background.

There are other examples where a systemic approach has yielded dramatically transgressive segregants, but in which the relative roles of GCA-SCA components have yet to be determined. By selecting parental  $F_1$  populations in an environment exposed to multiple stresses (BYD, FHB, phosphorus deficiency and stresses associated with very late sowing), a genotype was generated that could thrive in poor soils and produce large (21cm long) spikes [133]. While such a genotype was easy to identify under exposure to complex stresses, it would have been almost impossible to find using conventional approaches. Once more, the metaphor of burning a haystack for the creative destruction wrought by complex stresses is an apt one.

The relative rarity of beneficial SCA does not diminish its value as an objective. If extensive genetic diversity needs to be examined, it should be done. For this purpose, crossing  $F_1$  lines yields more diverse progenies – and better results – than crossing pure lines. In 2005 a comparative study of conventional  $F_1$  progeny (obtained using two homozygous parents) and complex  $F_1$  progeny ( $F_1/F_1$ ) with 4 parents under stress conditions showed that



complex crosses differed markedly from the conventional group and produced a higher percentage of desirable plants (Figure 5).

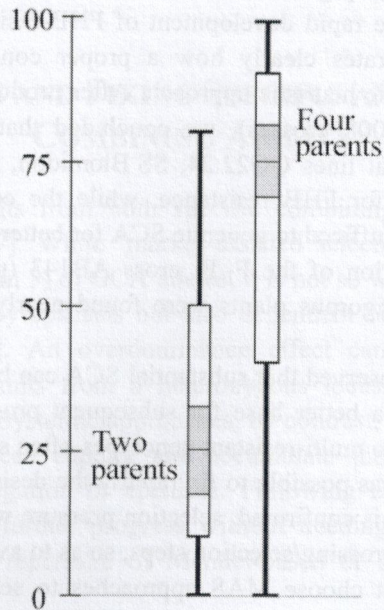


Figure 5. Percentage of relatively healthy looking grains (neither shriveled nor diseased) obtained under complex stress conditions, after harvesting F1 plants obtained either from fixed parents (i.e., two pure-line parents) or from intercrossing F1s (i.e. F1/ F1, or cF1) chosen in previous trials for their potential value, a strategy which thus involves four pure-line parents. Three stressors had a major effect: plants had been inoculated with BYDV and *Fusarium graminearum* in a year of prolonged drought (2005), but less severe stresses like rusts, powdery mildew, leaf spots and root rots were also reducing grainfill. Quebec data, 2006.

It should be emphasized that exceptional plants can only be seen when stress is applied; in the absence of stress the average looks as good as the very best. The enhanced genetic diversity of complex F<sub>1</sub> progeny should therefore be combined with the application of complex stresses. With optimal interaction of multiple stresses, the efficiency of introgression of usable genetic diversity can easily be increased many fold. Resistance to multiple stresses contributes to yield and biomass and stabilizes yield and quality in difficult years. The studies currently in progress have made it possible to identify in just four years those plants (and their derived lines) that carry multiple resistance factors — to FHB, BYD, rusts, powdery mildew and *Pythium* root rot. The systemic approach thus readily yields ‘wholes that are greater than the sums of their parts’ as it generates transgressive characteristics superior to those of the original parents.

## BREEDING USING NEW TOOLS TO SPEED AMELIORATION

In presenting a counterpoint to the position that crops are best improved by introgressing known, specific genes, one (or a few) at a time, we have explored how protocols that seek to emulate the processes of evolution can maximize usable variation and efficient selection.



Effective combinations of the most biologically relevant and readily applied of these protocols constitute a '*systemic heuristic approach*' that can be – and in Brazil already is being – used profitably to improve crops quickly and consistently in a wide range of environments. This latter point that a good cultivar should have broad adaptation [87] is becoming more challenging to address as budget limitations reduce the number of sites at which germplasm is tested. Our experience in Eastern Canada over the last 30 years, for example, has shown that precision in evaluating yield has declined with the number of test sites: in 1974 with 35 sites the margin of error was plus or minus 2%, in 2008 with 9 sites plus or minus 6%. This might be (partly) offset by more intelligent use of sites [146], but it is hard to escape a greater degree of variability of germplasm across years and sites as, below a certain threshold, nothing can really compensate for a lack of test sites [147]. By testing and selecting under complex stresses, systemic approaches may promote broader adaptation and address – at least partly – the deficiencies arising from reduced numbers of test sites. This is expected because  $G \times E$  attributed to years and testing locations is largely due to climate stress, soil stresses, and plant enemies.

Let us now examine what might follow from efforts to apply this broad, highly adaptable approach more widely, both as it affects the objective goals of crop improvement and the manner in which we collaborate and learn from each other. It is now widely recognized that collaboration on a wide scale is essential to deal with such widespread challenges as, for example, the inability of adapted quality wheat germplasm to resist FHB effectively. The contemporary form that the classical gene introgression-based approach takes in responding to this serious challenge presents an instructive example to highlight, by contrast, what systemic approaches can offer.

Developing cultivars that combine yield, quality, resistance to other important diseases and FHB resistance presents a challenge considerably beyond, for example, deriving a cultivar with resistance to a newly prevalent race of leaf rust by introgressing a known resistance source and backcrossing repeatedly with selection. Genetic resistance to FHB is conditioned by at least 3 distinct major genes, with substantial effects exerted by numerous additional genes whose effectiveness of expression is, in turn, heavily influenced by genetic background [148, 149]. Known sources of strong FHB resistance among hexaploid wheats exhibit poor GCA and SCA with most target elite germplasm [150], and sources of strong resistance have yet to be identified among elite durum wheats [151].

The picture is further complicated by the differential inheritance of at least two major types of FHB resistance: type I reduces infection, and type II slows down the fungal invasion after it has begun [152]. Other types of resistance have also been documented [152], but their inheritance and expression relative to types I and II need to be clarified. In the last 15 years, more than 50 studies examining 'quantitative trait loci' (QTLs) and closely-linked DNA markers of FHB resistance have found some 22 markers on 16 out of 21 chromosomes [153]. Although these efforts have aided the successful development of moderately resistant lines or cultivars that are adequate for many wheat growing areas, they have yet not generated commercial-class wheat with Sumai 3's level of resistance. For regions that experience more severe or frequent epidemics, the highest resistance is a critical goal, one that QTL-MAS approaches have so far failed to deliver. Some markers may not be linked sufficiently tightly to target genes, and others may co-segregate with traits such as shattering, height or lateness [109, 150], complicating their use.



Contemporary versions of focused gene introgression-based approaches have sought to avoid the combinatorial and selection challenges that this task presents by tracking DNA markers closely linked to quantitative trait loci of the FHB resistance trait (FHB-QTLs) through succeeding generations, rather than evaluating the phenotypes of necessarily very large populations in disease nurseries [154]. In such protocols of MAS, actual plant phenotypes are evaluated principally to 'confirm' the resistance predicted to be expressed in lines possessing DNA markers for the major FHB-QTLs and perhaps to refine models and linkage maps. Such an approach inescapably demands substantial commitments from dedicated laboratories and associated personnel before a single new-generation plant's response to disease and environment can be observed. By contrast, the largest demand placed on practitioners of systemic heuristic approaches is the need to continually observe and record the progress of actual plants as they grow, develop and mature in stressful environments under (often multiple) disease pressure, and then decide which are the (roughly) best 1 or 2% to advance to the next cycle.

The near-essential aspect of the need to collaborate on a wide geographic scale also takes on very different forms in the two approaches. Establishing mapping populations from the progeny of appropriately chosen parents, identifying their FHB-QTLs, developing and characterizing markers, and then tracking those markers through succeeding generations in a desired genetic background are large tasks [155] that are sensibly undertaken as collaborations among well-equipped (often national or international) centres. This clearly limits the extent of who can choose to collaborate in generating the germplasm to those who can meet this high threshold.

The systemic method's very high rejection rate makes it more affordable. In 2006, we managed over 10000  $F_1$  as individual plants under complex stress in the field. Indeed, if 10000  $F_1 / F_1$  seedlings had been managed in the conventional manner, 10000  $F_2$  populations would have been created, occupying in the next generation an area of about 3 m x 9 km. The cost of performing selections with viruses and *Fusarium* sp. would become prohibitive. With the systemic approach, we selected annually about 10000  $F_1 / F_1$  in a 500-m<sup>2</sup> to 800-m<sup>2</sup> area, and all but a few were rejected, as the vast majority succumbed to the multiple stresses. The extent of genetic diversity that can be accommodated within a similar research budget is thus 50 to 100 times greater. There are fewer  $F_2$  populations because stresses destroyed most of the  $F_1$ s, and the interacting effects of multiple stresses have also diminished the sizes of the  $F_2$  populations that do remain. To identify and exploit recessive and epistatic traits, such as occur, for example, in short genotypes or certain complex resistance backgrounds, it is still essential to exploit  $F_2$ s or  $F_3$ s. The systemic process facilitates progress here as well, as it does not require numerous large  $F_2$  and  $F_3$  populations. Should researchers choose to pursue an exceptional combination to a maximum extent, they can still opt to examine more and larger  $F_2$  and  $F_3$  populations if they consider the expense is warranted.

Choosing to follow a systemic heuristic approach allows people and institutions of vastly different endowment to collaborate meaningfully toward common goals. Generating, evaluating and advancing the germplasm are processes that can be well integrated with each other at any location. The emphasis on tracking markers, by contrast, entails clear separations between these tasks. After typically two or more years to generate the germplasm, the evaluation of phenotypes of actual plants is then usually carried out by members of other institutions acting in a subservient capacity. These contrasts also necessarily affect the quality and extent of the information that is gathered and how it is shared. While the quantitative data



files generated by one or many scientists may adequately identify a few individuals that have the highest resistance to Fusarium, they are essentially lists of numbers and as such cannot distinguish site effects or identify escapes. Moreover, such data fail to fully describe the important traits of a line, so that one often cannot identify which resistant lines are free from the common problems related to linkage drag or pleiotropy. Much of the germplasm that is identified as resistant to FHB should really be discarded because it also exhibits undesirable traits (e.g., shattering, lack of waterlogging resistance, lodging, lateness, susceptibility to rusts, etc.).

The nature of the material generated and the information to be adduced from it thus tend to condition 'centre-periphery' relationships between the generators and evaluators of germplasm in MAS-based approaches. Much valuable field information is either never recorded because it does not fit comfortably into the scheme or simply gets lost. Collaborators using a systemic heuristic approach may be, and often are, far flung but they can choose to work more like teams weaving different parts of a large tapestry. Each collaborator would consider the plant as a whole, looking for a large number of traits embodied in rare individual genotypes worth keeping.

It is not only a high threshold of commitments of facilities and personnel that limit the extent of who can choose to join MAS-based collaborations. The considerable lead times and need for germplasm in collaborative comparisons to be at equivalent states of gene or QTL introgression impose fairly rigid requirements for the co-ordination of joint efforts. The flexibility of systemic heuristic approaches, by contrast, allows new collaborators to join already established projects fairly readily at any time and make meaningful contributions within a year.

In seeking to provide relief from the need to evaluate phenotypes of very large numbers of individuals in each generation, current MAS-based approaches do not fundamentally address the challenge of complexity posed by polygenically-determined traits and hidden genetic diversity. In practice, few gene-introgression projects now aim to incorporate more than 3 QTLs, and 5 or 6 are considered to be at the outer limit of practicability [156]. MAS can help introgress specific genes or QTLs in cases where observing phenotypes cannot distinguish the expression of single or multiple determinants (e.g., pyramiding of rust resistance genes). However, it offers no compelling advantages when seeking to select traits determined by genes that are numerous and that interact with each other and the environment, as is the case for agronomic yield or FHB resistance.

The combinatorial and selection challenges that present-day MAS-based approaches seek to avoid or minimize are confronted in a very different way by systemic heuristic approaches. Combinatorial complexity, far from being a 'problem', is seen as being a resource out of which evolution (*'descent with modification'* guided by selection) can fashion ingenious responses to iteratively imposed demands to survive — and thrive — under complex stresses. Some of these 'ingenious' responses have already been described — in empirical terms — among the outputs of practical crop-improvement programs that employed elements of what we describe here as a systemic heuristic approach. A growing literature is now also, happily, beginning to demonstrate links between environmental effects, parasitism and epigenetic change, and how such change can become fixed genetically, thus providing theoretical footing for further exploitation of these phenomena.

Several disciplines are necessarily involved when one seeks to pursue systemic objectives. The complexity of objectives and the polygenic base of inheritance should not



deter plant geneticists from seeking to thus harness evolutionary processes, as even modest budgets permit success; an example is Kraft's development of multiple disease-resistant pea germplasm (*Pisum sativum*) [157]. Another example is Caetano's development of wheat germplasm combining very strong straw [78] with leaf area duration and "stay-green" habit [79] and resistance to all common biotic and abiotic stresses. A systemic process making frequent use of  $F_1 / F_1$  crosses and rigorously controlled complex stress sequences can select not only for valuable genes but more importantly, for those interactive networks of genes that respond effectively to complex stresses that vary among sites and between years. Moreover, such a process promotes physiological plasticity needed to allocate photosynthetic products daily to the parts of the plant that can guarantee stable yields and quality.

Approaches to crop improvement which iteratively select under the pressure of multiple interacting stresses are better able to identify and conserve the most versatile determinants of disease resistance: those that can act in a broad range of environments and in concert with valued agronomic traits to resist the effects of other stresses. Moreover, by performing several crossing cycles to build up additive resistance factors and break deleterious linkages, the genetic diversity of the germplasm can be explored and hidden value identified. In our own recent practice (from 2003 to 2008), we generated more than 60000 individual  $F_1$  seeds, exploring hundreds of gene sources from North and South America, Asia, Africa, Europe, Australia, and the Middle East. This extensive range of genetic diversity meshed perfectly with the severity of our selection. In one cross arising from a systemic heuristic approach to ameliorate such diverse germplasm, AB143, we demonstrated – as confirmed by recent marker data – that a major FHB resistance allele was derived from a cultivar (SS Blomidon) well known to be susceptible to FHB. Within the susceptible cultivar, the beneficial effect of the resistance allele was perhaps counteracted by one or many other genes. As its potential had not been identified, SS Blomidon was never used (with or without MAS) in crosses aimed at improving FHB resistance. The salutary import of these observations is that susceptible cultivars can serve as potential gene donors of FHB resistance.

The hidden allele was only discovered because we use a very large number of parents to generate  $F_1$  populations and then apply multiple interacting biotic stresses to wreak serious damage on  $F_1$  plants. SS Blomidon had initially been chosen solely as a source of resistance to mildew and leaf spots. Some crosses likely allowed the hidden FHB resistance allele to dissociate itself from the other(s) that had ruined its efficiency. Although it could not have been foreseen from the choice of parents and the combinatorial action of known alleles, the end result was a critical advance in FHB resistance, one of the most difficult traits that we select for [77, 96]. Progenies reached a much higher resistance than that of the best parent (Figure 6), and markers explained the high SCA for FHB. The susceptible SS Blomidon was an especially useful donor of FHB resistance because it did not confer alleles that cause agronomic defects through linkage drag. Now, after 4 years of systemic selection under multiple stresses, we might consider integrating components of MAS into our systemic approach to better understand what contributed to our progress and to conserve the many genes that are needed for maximum resistance to many diseases. Perhaps the more valuable role for MAS will prove to be its use in conserving progress already made rather than as a means to generate those rare happy gene combinations that constitute major leaps of progress when they are expressed. In a current example of such a hybrid methodology, the systemic approach generated promising germplasm from which MAS then eliminated 1B/1R translocations that harm dough quality (A. Laroche, unpublished).



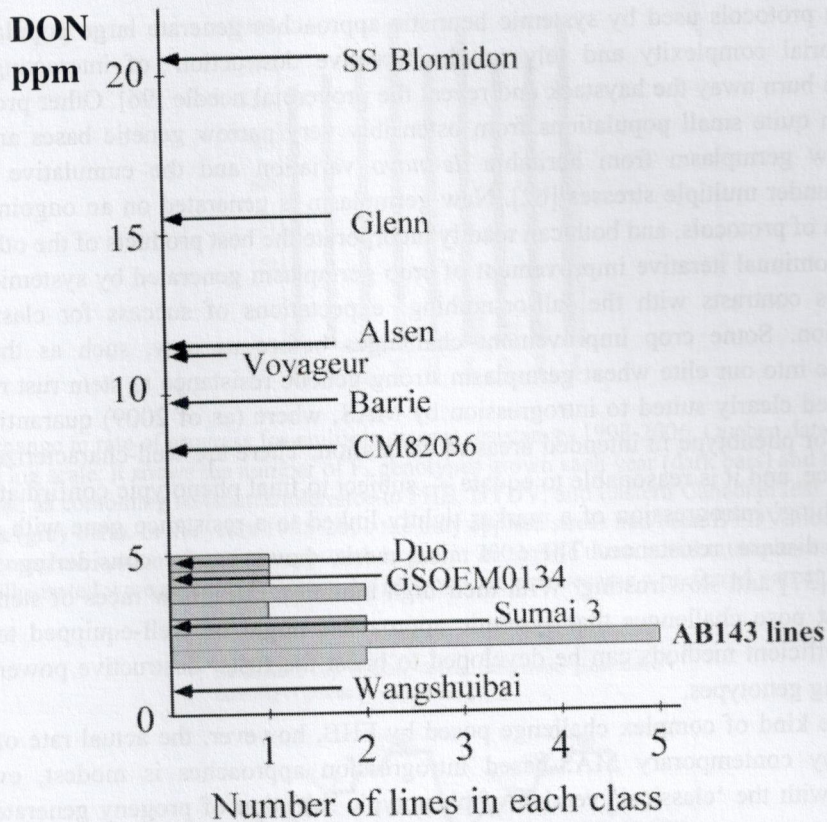


Figure 6. Comparison of various wheat lines for the vomitoxin (DON) content in a FHB inoculated nursery in 2006. The AB143 progenies (shaded bars) came from the cross QG22.24/Alsen//SS Blomidon/Alsen, the only  $F_1/F_1$  cross that performed well under multiple stress (FHB, BYDV, rusts, mildew and leaf spots) in 2003. Wangshuibai and Sumai 3 are known worldwide as among the most resistant to Fusarium Head Blight, and also tend to have the lowest toxin level. GS0EM-0134 is a Lethbridge line with strong type II resistance derived from CM82036. Duo is MR to FHB and has mostly type I resistance. Cultivars AC Barrie, AC Voyageur, and Alsen all behave as MS in Eastern trials. Glenn is also MS, but when infected is more likely to maintain visual grain quality SS Blomidon is FHB susceptible. According to 2008 data, QG22.24 has DON levels quite similar to SS Blomidon (data not shown), even though its visual symptoms indicate good type I resistance. DON data from M. Savard, ECORC.

### A NURSERY OF DISCOVERIES, NEW POSSIBILITIES AND NEW HYPOTHESES

Much of the expression of hereditary information is conditioned by the polygenic, epistatic or epigenetic effects of gene actions, and we should not pretend it is not so. Breeding for horizontal and polygenic resistance, often promoted as the way to longer lasting resistance [37], is best done using a systemic heuristic approach, with the added benefit that many diseases can be tackled simultaneously. Complex genetics should not frighten us off. Tools such as GGE biplots [97] and rich sets of observations generated over time help practitioners arrive at new “rules of thumb” and working strategies for dealing with a given complex problem.



Some protocols used by systemic heuristic approaches generate large populations with combinatorial complexity and rely on the 'creative destruction' of interacting complex stresses to burn away the haystack and reveal the proverbial needle [96]. Other protocols can work with quite small populations from ostensibly very narrow genetic bases and develop useful new germplasm from heritable *de novo* variation and the cumulative effects of selection under multiple stresses [62]. New germplasm is generated on an ongoing basis by both kinds of protocols, and both can readily incorporate the best products of the other.

The continual iterative improvement of crop germplasm generated by systemic heuristic approaches contrasts with the 'all-or-nothing' expectations of success for classical gene introgression. Some crop improvement challenges before us now, such as the need to incorporate into our elite wheat germplasm strong genetic resistance to stem rust race *Ug99*, seem indeed clearly suited to introgression by MAS, where (as of 2009) quarantine forbids selection for phenotype in intended areas of adaptation. There are well-characterized sources of resistance, and it is reasonable to equate — subject to final phenotypic confirmation — the 'all-or-nothing' introgression of a marker tightly linked to a resistance gene with success in achieving disease resistance. There is also merit, however, in considering horizontal resistance [37] and slow rusting. With their high mutation rates, new races of stem rust and yellow rust pose challenges that systemic approaches might be well-equipped to confront provided efficient methods can be developed to boost the rusts' destructive power as a tool for selecting genotypes.

For the kind of complex challenge posed by FHB, however, the actual rate of progress obtained by contemporary MAS-based introgression approaches is modest, even when compared with the 'classical' version of phenotypic selection of progeny generated from a cross between a recurring elite susceptible parent and an unadapted source of genetic resistance such as Sumai 3 (ref. [158]). Will the continued application of a systemic heuristic approach to this particular problem maintain such a clearly higher rate of progress? To date at least our progress has been rapid, as 3 years after selecting the AB143 F<sub>1</sub>, we had generated agronomic germplasm that combined multiple disease resistance with FHB resistance at the level of Sumai 3 (Figure 6). Germplasm which expresses such superior overall resistance in a good agronomic background will yield good candidate lines to serve as platforms for developing new standards of overall excellence in wheat breeding. Based on the first survivors under complex selection pressure, it became easier to generate a growing number of multi-resistant F<sub>1</sub> lines in the 2006 cycle (Figure 7) and thereafter. For at least part of the active germplasm, the systemic approach also makes it possible to introgress new genetic diversity at a rate unmatched by any existing protocol (Figure 8).

From the promising new base that was developed in Canada, we could now seek to incorporate greater genetic diversity of rust resistance and/or commercial gluten quality. In a few years their relative merits should be fairly clear, as our proposals are relatively simple to implement and compare against the performance of the established players.

There is a curious coda to any efforts to compare what progress might be made by these different approaches that start from different assumptions. If those who now seek to use MAS-QTLs to introgress into adapted wheat the genes that condition FHB resistance in Sumai 3 recalled some history, they might realize these genes only became associated, expressed and visible after a series of fortuitous crosses and selections under continued FHB pressure [145].



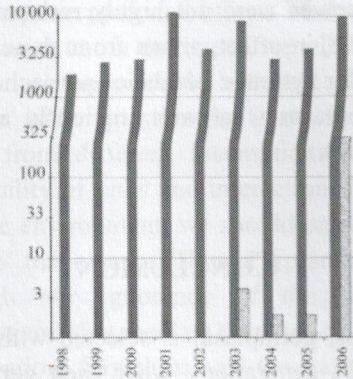


Figure 7. Change in rate of progress for multiple disease resistance: 1998-2006, Quebec data. The Y axis is on a log scale; it shows the number of F<sub>1</sub> genotypes grown each year (dark bars) and the number of F<sub>1</sub> selected as combining resistance/tolerance to FHB, BYDV, and Eastern Canadian leaf and stem rust isolates (gray bars). In the years 1998-2001 the only applied stress had been BYDV inoculation. The more complete systemic heuristic approach began in 2003. The three initial multiple-resistant 2003 selections illustrated were all from the cross AB143; one of them became a preferred parent, from 2003 to now.

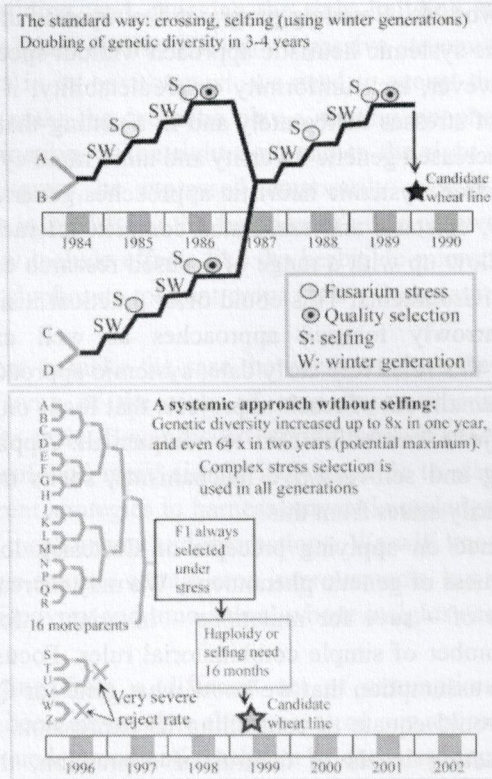


Figure 8. Time span needed for the conventional (above) and systemic methods (below) to introgress diversity from many parents and lead to a rather homozygous line. On the time axis, periods of indoors growth are in white, and field culture periods in grey. This is just an example of possible strategy. The conventional method has one or very few stress selection factors; the systemic method applies severe complex stresses (many diseases plus abiotic factors) at each generation. A high discard rate (>98%) makes room for new crosses at each cycle.



As the parental lines themselves were not highly resistant, the very determinants they now seek to introgress must have, in effect, arisen from an accidental version of a systemic heuristic approach. Our outline for systemic heuristic approaches aims to achieve deliberately and consistently for elite germplasm what was previously accomplished accidentally and fortuitously for experimental lines.

## CONCLUSION

In many breeding programs, germplasm is selected without intensive stresses in early generations. During the  $F_2$  to  $F_4$  generations, when their germplasm is not yet sufficiently homozygous, breeders reject either randomly or based on height and phenology. Ineffective performance in relation to stress factors is thus allowed to persist for years. And yet, when climate and/or soil conditions become unfavorable, intensive and complex biotic and abiotic stresses are naturally at work. The requirements for registering a cultivar can be complex, but — whatever Mendelian laws may state — this cannot excuse such over-simplified approaches to developing germplasm or crop breeding. The goal is to have a plant whose yield and quality remain acceptable when it faces difficult circumstances and does not pay a penalty when conditions are favorable. Breeding programs that operate in stressful environments get closer to the spirit of the systemic heuristic approach without specifically trying to do so. As natural stress may, however, lack uniformity or predictability, it is warranted to apply and manage complex sets of stresses deliberately and in a setting that captures the scientifically supported benefits of increased genetic diversity and more rapid cycles of recurrent selection.

When put into practice, systemic heuristic approaches generate abundant empirical data that bear on pleiotropy, epistasis and associated complex interactions. Understanding these will require that we follow up with a range of focused research endeavors linked to a larger number of traits and environments. This could offer practical insights about risks that result from conventional narrowly focused approaches as well as extend our theoretical understanding. Even at this relatively early date, systemic approaches have already achieved certain successes that remain out of reach to projects that focus on simple gene introgression; simplified focused projects cannot be said to conquer all. Applied genetics [85] involves hierarchical, interacting and self-reflexive mechanisms, so its methods should respect the complexity that necessarily arises from this.

A rigorous insistence on applying precepts of Cartesian logic has impoverished our appreciation of the richness of genetic phenomena. We may increasingly grasp that genes are not simply repositories of — save for mutations — invariant information whose expression conforms to a small number of simple combinatorial rules. Focused or targeted approaches have been based on the assumption that we know what gene (or QTL) confers a trait, so that tracking its presence would equate to controlling its expression. In beginning to reveal the myriad complex interacting levels of control of expression, the emerging discipline of epigenetics already warns insistently that focused approaches offer tool sets for applied genetics that are far too limited. Breeders have thus not yet benefited from the full range of inheritance mechanisms accumulated by evolution. McClintock (1984) emphasized the importance of understanding that plants (and indeed all organisms) in responding to the other

players in their environment thus saw a larger picture.

In admitting that primarily the greater types of methods that are inadequate to deal with enemies, the rhizosphere examine the iterative make progress, even incomprehension of the increasingly rich sets that advance to better seeking to do so, we perhaps mitigating the about [16, 102].

Interactions, with crazy aunt in the attic that developing the genes were bricks in command and control responding to other notes in the score will expressed in proper genes may often fail produce unintended  $G \times E$  in today's field can accompany the truly inescapable.

We have attempted approach might consistently and on we also believe their assumptions and use ignorance of the data to succeed not just to cultivate 'nurseries many other crop species.

Pursuing a path conventional practice need to reflect on the most telling arguments brought new advanced germplasm thus create approaches. They cannot avoid and the



players in their environment capture profound effects of stresses on their genomes [8]. She thus saw a larger picture that, much to our detriment, we still largely ignore.

In admitting that complexity is unavoidable, we must recognize it is not merely or even primarily the greater 'difficulty' of the question that is at issue, but that we must use different types of methods that start from different assumptions. Where inductive reasoning is inadequate to deal with the totality of links and interactions between genes, cells, plants, its enemies, the rhizosphere or the environment, we should emulate evolutionary processes and examine the iterative 'solutions' that emerge. Thus at a practical level, we will still be able to make progress, even though our ignorance of the full range of phenomena or incomprehension of the mechanisms that mediate them is –at first – humbling. Over time, the increasingly rich sets of observations furnish us with the material to build and test hypotheses that advance to better theoretical understanding. An added benefit is that, without necessarily seeking to do so, we will be continually combining different strands of knowledge and perhaps mitigating the dangers of its fragmentation - which philosophers have warned us about [16, 102].

Interactions, with their unavoidable attendant complexity, have often been treated like the crazy aunt in the attic: embarrassing – and usually best avoided. It is not a linguistic accident that developing the first GMOs was heralded as the beginning of 'engineering' of plants: genes were bricks in a building, and choosing the right building materials would provide command and control. The plant, however, is not a passive structure. Since it is constantly responding to other players in its environment, we need to accept that just putting the right notes in the score will not make the orchestra play a rousing symphony. The genes must be expressed in proper coordination, at the right time and to the right extent. Indeed, inserted genes may often fail to express, be expressed temporarily only to be rapidly silenced or produce unintended effects in modifying the function of other pathways [159]. For example, G x E in today's fields now include effects like the herbicide-micronutrient interactions that can accompany the use of glyphosate tolerant crops [160]. Complexity in living organisms is truly inescapable.

We have attempted here to make the case that what we describe as a systemic heuristic approach might achieve some of the aims of crop improvement cheaper, faster, more consistently and on a wider scale. Beyond such straightforward practical benefits, however, we also believe there is profound merit simply in being able to start from different sets of assumptions and use different strategies to harness current knowledge while coping with vast ignorance of the details of mechanisms and interactions. We will know that we are beginning to succeed not just when we achieve certain crop improvements, but when we are being seen to cultivate 'nurseries of discovery and innovation' whose insights can enrich the resources of many other crop species.

Pursuing a path starting from a different set of assumptions than those based in conventional practice has been essential to the success of our approach. This highlights the need to reflect on how we understand and teach life sciences at large. In the end, results are the most telling argument. Over the past 35 years in Brazil, every systemic breeding cycle has brought new advances in multiple resistance traits. Breeders are now beginning to use the germplasm thus created. Recent progress in Canada now also supports the case for systemic approaches. They promise us a way to respect with disciplined imagination the complexity we cannot avoid and thus give new impetus to plant genetics for decades to come.



## LEXICON

**Backcross:** repeated use in crosses of the same parent (often agronomic), generally to insert a desired trait (or gene) into an acceptable genetic background.

**Cis-acting** means "acting from the same molecule" (*i.e.*, intramolecular). It may be considered the opposite of trans-acting which generally means "acting from a different molecule" (*i.e.*, intermolecular). In the context of transcription regulation, cis-acting elements are usually considered to be DNA sequences that, via the binding or action of transcription factors or other trans-acting elements, directly regulate the expression of genes on the same chromosome.

**Epistasis** is the interaction between the genes at two or more loci, so that the phenotype differs from what would be expected if the loci were expressed independently. The gene whose phenotype is expressed is said to be epistatic, while the phenotype altered or suppressed is said to be hypostatic. A more restrictive view of Epistasis: An interaction between nonallelic genes, especially an interaction in which one gene suppresses the expression of another.

**General combining ability or GCA:** the part of the heredity that can be explained as based on additive gene effects. This leads hybrid behavior towards mid-parent values for the effects of each locus. Lines with good GCA thus tend to be cultivars or advanced breeding lines.

**Heterosis** is a phenomenon observed in hybrids which display greater vigor, size, resistance, or improvements in valued traits, compared to the parents. Shull (inventor of the term) felt that overdominance explained hybrid vigor; this hypothesis lost credibility (Simmonds page 90). Despite numerous studies, this phenomenon observed a century ago is not fully explained, and the possibility is that a lot of it relates to epistasis, dominance, and gene interaction effects. The beneficial role of the heterozygous state of a given locus has been proven in only very rare cases.

**Heterozygous:** status of chromosomes of a given pair which have different DNA sequences or alleles at a given chromosomal position. Cross-pollinated species are quite heterozygous, and this status offers benefits from higher genetic diversity (through SCA, epistasis, heterosis), but is also compatible with the maintenance of suboptimal alleles in low frequencies.

**Heuristic:** an adjective for methods that help in problem solving, in turn leading to learning and discovery. These methods in most cases employ experimentation and trial-and-error techniques. In more precise terms, heuristics stand for strategies using readily accessible, though loosely applicable, information to control problem solving in human beings and machines.

**Homozygous:** status of chromosomes of a given pair which have (at a given locus or at many loci) identical alleles or DNA sequence at a given chromosomal position. Strict autogamous (self-pollinated) species tend to be very homozygous. This status is not compatible with carrying large loads of suboptimal alleles.

**Horizontal resistance** is resistance that is not specific to a race or strain of a pathogen or plant enemy. In most cases this involves genetic complexity of mechanisms. This term is used by opposition to vertical resistance, which is generally due to a single gene, and more easily circumvented by simple gene-for-gene evolution of the plant enemies and pathogens.



**Lodging** is the loss of plant verticality, owing to various forces of nature: wind, rain, diseases of roots and crowns, poor root anchoring, superficial root system, physical properties of the plant tissues (cellulose/lignin ratio), and spike weight. Oversize flag leaves also increase load and wind drag. Height is very important due to the Archimedes lever principle. Lodging is very damaging as it impacts on yield, diseases, seed quality, seed fungal toxins, and cost of harvesting.

**Overdominance:** condition in which at a given locus, the heterozygous effect is overexpressed if compared to the homozygous effect of either one of the alleles involved. It is hard to demonstrate due to the need to prove it relates directly to a given locus, and not to a closely linked factor. It was formerly thought that overdominance explained heterosis, but Simmonds (1979) doubts that this phenomenon exists in plants, except in rare and unusual circumstances.

**Phenology:** control of the timing of events (tillering, flowering, ripening, etc) in an organism's development.

**Pleiotropy** describes the genetic effect of a single gene on multiple phenotypic traits. Pleiotropy exists because there is not a one-to-one relationship between the parts of an organism that a gene influences and our pragmatic definition of "traits". As our knowledge of the organization of biochemical networks, morphogenesis and signal-response mechanisms increases, the high frequency and complexity of interactions becomes clear, and pleiotropy gets to be viewed as a rule rather than an exception.

**Recurrent selection (or breeding):** genetic improvement for one or many goals, over many cycles of intermating and selection of germplasm derived from a limited number of parents. It is a common approach when dealing with complex traits.

**Specific combining ability or SCA:** the part of the heredity that does not fit allelic midparent values and thus cannot be explained as based on additive gene effects. This includes effects of dominance, epistasis, perhaps overdominance, and effects related to other interactions between genes and with diverse environments. Proven overdominance is very rare.

**Trans-acting element:** usually a DNA sequence that contains a gene. This gene codes for a protein (or microRNA or other diffusible molecule) that will be used in the regulation of another target gene. The trans-acting gene may be (or not be) on the same chromosome as the target gene, but the activity is via the intermediary protein or RNA that it encodes. (Cis-acting elements, on the other hand, do not code for protein or RNA). Both the trans-acting gene and the protein/RNA encoded are said to "act in trans" on the target gene.

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