The Americas Journal of Plant Science and Biotechnology ©2011 Global Science Books

Epigenetics Serves Genetics: Fusarium Head Blight (FHB) Resistance in Elite Wheat Germplasm

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ABSTRACT

Current gene introgression-based approaches have made only modest progress toward the goal of developing Canadian wheat cultivars with strong resistance to Fusarium head blight (FHB). Re-examining comparisons of gene induction in resistant and susceptible near-isogenic lines of 'Sumai 3', a well-established source of FHB resistance, suggested that resistance was conditioned by effective expression of gene alleles already present in both resistant and susceptible lines. Our alternative approach, based on modifying expression of existing gene alleles, rather than introgressing new ones, aims to preserve the valuable traits accumulated over time by breeders while changing the small number of traits needing improvement. Starting from a single seed of the doubled haploid Canadian wheat cultivar, 'McKenzie', successive generations of selfed lines inoculated with *Wheat streak mosaic virus* (WSMV) evolved resistance to WSMV infection that became genetically fixed with repeated rounds of selection. Some of these lines also evolved improved FHB resistance which has proved stable in three years of testing. Improved resistance to leaf spots, while not specifically selected for, was also recorded in one family of lines, suggesting the versatility of such approaches to crop improvement. In a preliminary agronomic trial conducted in the absence of deliberately applied disease pressure, the evolved line with the best apparent combination of new traits confirmed its promise by equalling or surpassing its 'McKenzie' progenitor in the parameters that define the cultivar class. To honour the Scottish philosopher who prepared the ground for evolutionary thinking, we provisionally name this line Hume.

Keywords: crop improvement, 'McKenzie', *Wheat streak mosaic virus* (WSMV)

INTRODUCTION

Fusarium head blight (FHB) is one of the most serious and economically damaging diseases affecting wheat in Canada. There are comparatively few sources of resistance and the best available have backgrounds that do not provide the agronomic and quality traits Canadian wheat requires (Gilbert and Tekauz 2000; Somers *et al.* 2003). The problem is particularly acute for the durum wheat class (Kumar *et al.* 2007). Recent reviews of the available literature point to a dearth of genetic resources that might be deployed. The disease is driven by the environment and the pathogen survives the harshest winters on stubble.

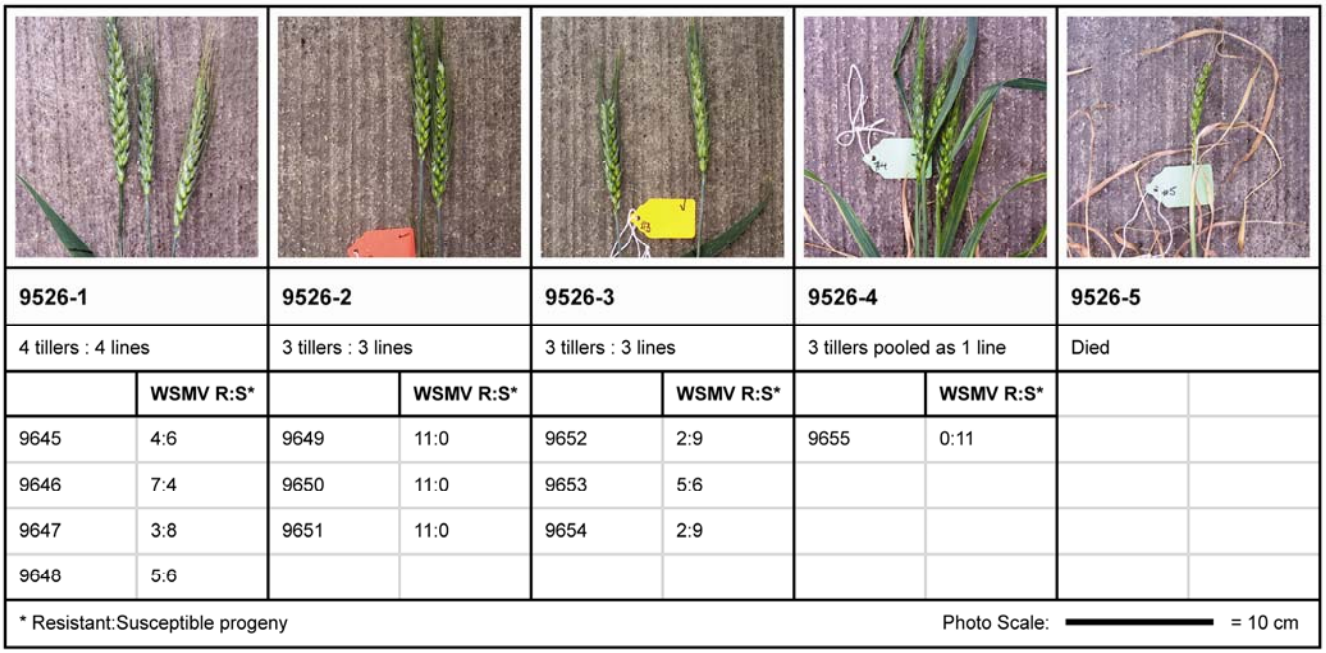
Most efforts at crop improvement undertaken today assume that the germplasm to be improved is deficient in a trait of interest because it *lacks the appropriate gene*, or more precisely, the *right gene allele*. Accordingly, after identifying a source of the appropriate gene with the right gene allele, the improvement effort then concentrates on introgressing the gene(s) into the desired germplasm. To the extent possible, breeders seek to preserve and enhance the already desirable traits of the target germplasm while avoiding any undesirable traits of the source. Since the advent of systematic plant breeding, the most common practice has been to cross and back-cross with recurrent selection, employing sources of resistance which are often unadapted (e.g. the Chinese FHB-resistant wheat cv. 'Sumai 3') or derived from alien germplasm (Rudd *et al.* 2001; Shen *et al.* 2004; Fedak and Han 2005). More recently, methods using cytogenetic and molecular tools employing such expedients as polymerase chain reaction (PCR), amplified fragment length polymorphisms (AFLPs), and quantitative trait loci (QTLs) (Yang *et al.* 2005; McCartney *et al.* 2007) have identified chromosomal locations of potential resistance genes and facilitated their introgression. A further refinement, pursued in the development of genetically modified organisms, is to target the exact genes and 'genetically transform' the desired germplasm directly (Shin *et al.* 2008). However, the goal of strong FHB resistance in a desirable background continues to elude our best efforts. Resistance is polygenic and all chromosomes in hexaploid wheat, except 7D, have been implicated for conditioning some level of FHB resistance (Buerstmayr *et al.* 2009).

When dealing with large populations many now think it is more efficient to track stretches of DNA linked to disease resistance QTLs rather than phenotypes. The virtue of this approach is that it does not need to confront the variability of phenotypic expression of FHB symptoms in the early and intermediate generations. Recent analyses, however, indicate that in practical terms, marker-assisted selection improves only slightly on the modest success of conventional introgression protocols because even major QTLs explain only a small part of the total variance (Miedaner *et al.* 2009). The traditional approaches, while producing bread wheat with improved resistance to FHB have failed to provide full protection against losses of grain yield and quality. A new approach is needed.

The origin of FHB-resistant 'Sumai 3' and recent microarray studies of gene expression point to an alternative perspective. 'Sumai 3' is the progeny of a cross between parents which are moderately susceptible to FHB. Microarray gene expression studies of 'Sumai 3' and two near-isogenic lines (NILs) (Xu *et al.* 2002), selected for susceptibility to FHB, revealed the presence of the same general plant defence genes induced in response to inoculation by *Fusarium graminearum* Schwabe in both the FHB-resistant 'Sumai 3'

Received: 5 December, 2009. Accepted: 11 May, 2011.

Original Research Paper



9526-1		9526-2		9526-3		9526-4		9526-5	
4 tillers : 4 lines		3 tillers : 3 lines		3 tillers : 3 lines		3 tillers pooled as 1 line		Died	
	WSMV R:S*		WSMV R:S*		WSMV R:S*		WSMV R:S*		
9645	4:6	9649	11:0	9652	2:9	9655	0:11		
9646	7:4	9650	11:0	9653	5:6				
9647	3:8	9651	11:0	9654	2:9				
9648	5:6								

* Resistant:Susceptible progeny Photo Scale: = 10 cm

Fig. 1 Progeny of a single symptom-free tiller of line 9526 following rub-inoculation with *Wheat streak mosaic virus* (WSMV) at mid-tillering growth stage.

and its susceptible NILs (Golkari *et al.* 2009). No genes could have been introgressed from a resistant parent, and resistance was not initiated by recognition phenomena; rather it was the fortuitous alignment of diverse plant defence pathways that conferred the unexpected resistance.

Considerations from first principles suggest that the need to prevail in chaotic environments would favor systems capable of rapid evolution (Dennett 1991, 1995), and observations of natural ecosystems support the view that stressful environments can indeed act as generators of new genetic diversity (Nevo 2001; Comeau *et al.* 2010). Plants have evolved to thrive in a chaotic environment. Their lack of means to escape or limit their exposure to stress favoured the evolution of mechanisms that optimize rapid-response strategies based on existing genetic information (Boyko and Kovalchuk 2008). Such modifications, occurring without change to the original DNA base sequence, are by definition epigenetic and can become heritable. Proceeding from this perspective we pursued an approach that would emulate evolution rather than seek to improve phenotypes by introgressing specific genes. Accordingly, the attendant methods emphasized variation among progeny, selection, and rapid advancement to the next generation.

Wheat plants that are susceptible to infection with *Wheat streak mosaic virus* (WSMV) respond with leaf yellowing (streak mosaic), stunted growth and poor seed set. In a susceptible response the infection is systemic, and all tissues of the mother plant (leaves, stems and roots, but not seeds) become host to multiplying virus. The disease is readily induced in plants under controlled conditions and symptoms can be reliably scored and interpreted, making it an ideal and manageable biotic stress. The absence of a susceptible host response is also usually easy to determine unambiguously. Thus the careful observer should be prompted to examine the phenotype of the progeny of any plant or plant part that appears symptom-free.

An integral part of the proposed approach is that the underlying methods evolved, and continue to evolve, with successive rounds of experiments. One of the most useful results of the work has been the validation of concepts from which specific methods can be readily adapted and applied to specific problems: in the case at hand, disease resistance in wheat germplasm that meets agronomic requirements. In this context early and interim results (2006 and 2007) were published (Haber *et al.* 2008). We recapitulate earlier results here only to support the case that the newly-expressed traits have indeed been consolidated and continue to be inherited in a consistent manner.

MATERIALS AND METHODS

An iterative method involving rub-inoculation with WSMV at mid-tillering and examination of reactions of the tillers to infection over successive generations was used (Haber *et al.* 2008). Wheat seeds were planted in fibre pots (17 cm diameter) in a soil mixture containing 6 parts soil: 1 part peat moss:1 part sand and the plants fed with 20: 20: 20 (N, P, K) (Peters Professional, Scotts-Sierra Horticultural Products Co. Marysville, OH) water soluble fertilizer biweekly. Plants were grown with 16 h light and 8 h dark starting at a constant 15°C for two weeks. They were subsequently maintained at 18°C (light period) and 15°C (dark period) until mid-tillering, Zadoks growth stage (ZGS) 45 (Zadoks *et al.* 1974) when they were inoculated with WSMV. Plants were lightly dusted with carborundum powder and rub-inoculated with a cotton swab dipped in WSMV suspension at a concentration of 1 g WSMV-infected leaf tissue ground in 10 ml distilled water. Plants were then maintained at a constant 20°C and examined for disease symptoms between 8 to 12 days after inoculation. To prevent accidental crossing, spikes were covered with glassine bags as soon as they emerged. When spikes were ready for harvest they were handled individually; seed from each individual head was threshed, weighed, bagged, and sealed before proceeding to the next spike.

A single seed of the WSMV-susceptible doubled haploid (DH) wheat variety 'McKenzie' was the starting material. The resulting plant was grown to maturity from which 20 seeds were chosen as the founding population. These were grown under pressure from WSMV infection in 2006 and responses recorded. A single tiller of one plant (source of line 9526) remained free of symptoms and contained no infectious virus as determined by back-assay to susceptible test plants. Five seeds from line 9526 were grown as sub-lines, again subjected to WSMV pressure and responses of tillers carefully recorded (**Fig. 1**). Progeny of 9526-2, conspicuously free of foliar symptoms, yielded sub-lines 9649, 9650, and 9651 from the three tillers. While all progeny of each of these sub-lines were resistant to WSMV infection, line 9650 was chosen as the source of four next-generation sub-lines to be evaluated for reaction to FHB (**Table 1**). Titres of infectious virus were determined by back-assay (Seifers *et al.* 2006) and based on resistant responses to WSMV infection, seed from certain tillers were selected for advance to the next cycle.

Table 1 Evolution of Hume.

	2007				
2007 FHB Nursery	Line 9650 – 4 head rows				
Row number	136	137	138	139	'McKenzie'
FHB Index	5.0	32	4.5	8.0	17.5
DON ppm	0.9	1.4	2.6	2.7	1.1
Height cm	75	85	85	85	92
2007 Fall Greenhouse	Line 136 named Hume				
	20 plants grown. Stressors: WSMV and *Fusarium graminearum* applied.				
	|				
	19/20 immune to WSMV 17/20 resistant to FHB				
	|				
	Earliest tiller with resistance to both WSMV and FHB selected: 20 plants grown and 5 early heads selected				
	2008				
2008 FHB Nursery	Line 9970 - 5 head rows				
Row number	2738	2739	2740	2741	2742	'McKenzie'
FHB Index	0.25	0.25	1.0	0.25	1.75	22.5
Height cm	78	78	79	79	74	92
	Rows 1, 3 and 4 sent to New Zealand for off-season increase					
	2009				
2009 FHB Nursery	NZ11369	NZ11370	NZ11371	'McKenzie'	
FHB Index	6.2[a]	9.5	6.8	17.5	
Leaf spots	18.2 [a,b]	26.8	25.0	42.0	

[a] Mean of 4 replications
[b] Percent lesion coverage of Flag and Flag $^{-1}$ leaves (0.6 Flag + 0.4 Flag $^{-1}$)

Table 2 Timeline of evolution of Hume from 'McKenzie' wheat.

Line number	Date	Observations
9064	2006 Feb	One tiller of one plant of 'McKenzie' wheat is resistant to WSMV infection
9526	2006 Sept	Five plants from the single tiller vary in response to WSMV infection
9650	2007 Feb	WSMV resistance shown to be heritable, some plants of short stature (progenitor of Hume)
9650 row 136	2007 Aug	FHB resistance in field nursery demonstrated , short stature: Named Hume
9918	2007 Nov	FHB and WSMV resistance confirmed under controlled conditions, short stature
9970	2008 Apr	Uniform resistance to WSMV infection, short stature
9970 rows 2738-2742	2008 Aug	FHB resistance in field nursery, short stature
NZ11369,70,71	2008 Nov to 2009 Apr	Seed increase of three rows in New Zealand
NZ11369,70,71	2009 Aug	FHB and leaf spot disease resistance in field nursery
Pool of NZ lines	2009 Aug	Agronomic yield trial and preliminary quality evaluation

Four head rows of line 9650 (nursery rows136-139) exhibiting a resistant reaction to WSMV infection were evaluated for reaction to FHB in a field nursery in 2007 using standard procedures (Gilbert and Woods 2006). The sub-lines, as well as appropriate checks, were planted as 1 m rows and inoculated at 50% anthesis and again 2 to 3 days late with 40 mL of a macroconidial suspension of *F. graminearum* at a concentration of 5×10^4 macroconidia/mL and mist-irrigated for 30 min after each inoculation. Lines were scored for FHB incidence and severity 18 to 21 days after inoculation. Data are presented as an FHB index, the product of incidence (1-10 scale where 1 is resistant and 10 susceptible) and severity (1-10 scale). The continued expression of resistance to WSMV was confirmed under controlled conditions in experimental rounds that followed evaluation of FHB reaction in the field nurseries. At maturity, inoculated wheat spikes were harvested, ground in a Bunn coffee grinder (espresso setting, Bunn® Canada, Bunn Corp., Aurora, ON). Samples were assayed for the pathogen-produced mycotoxin, deoxynivalenol (DON) by ELISA (Sinha and Savard 1996).

In the fall of 2007, 20 plants of Line 136, named Hume and numbered 9918 in this generation, were grown under WSMV and *F. graminearum* pressure. Selected progeny (seed of the five earliest maturing tillers now designated as sources of the lines 9969-9973) were evaluated under WSMV pressure alone (**Table 2**). Five head rows of line 9970, selected for early maturity and uniform plant morphology, were advanced as lines 2738 to 2742 for evaluation in the 2008 FHB nursery. The three earliest maturing lines, 2738, 2740, and 2741 were increased as separate lines in an off-season nursery and entered for a further year of evaluation in the 2009 FHB nursery. Seed of the three lines was also pooled for an independently conducted agronomic trial in 2009 carried out in the absence of artificial inoculation of any disease agent. Yield, height, lodging, test weight and 1000 kernel weight were assessed in 1 X 3.66 m plots and replicated three times.

Preliminary quality assessments were made on the grain harvested from the agronomic trials to provide measures of protein, flour yield, and mixing development time to provide the measures of quality assessment normally used by breeders to determine whether gemplasm is suitable for further advancement.

RESULTS

The five plants of Line 9526 varied in response to WSMV infection (**Fig. 1**). The plants numbered 9526-1, -2, and -3 were resistant to WSMV, 9526-4 was as susceptible as its 'McKenzie' progenitor, and 9526-5 was much more susceptible, dying three weeks after inoculation. Each tiller of plant 9526-1,-2, and -3 became the source of individual sub-lines while the three tillers of plant 9526-4, which experienced partial sterility, were pooled into a single line. Thus spike #1 of line 9526-1 became line 9645, spike #2 became line 9646 etc. The three lines derived from 9526-2, (9649 to 9651) were uniformly resistant to WSMV infection as seen in **Fig. 1**, whereas lines descended from 9526-1 and -3 yielded both resistant and susceptible individuals. Plants in the single line descended from 9526-4 were uniformly susceptible.

Line 9650, used as a donor parent of WSMV resistance in crosses with the susceptible 'McKenzie', behaved like a Mendelian source of a single, dominant gene (**Table 3**). The four head rows from line 9650, placed as rows 136 to139 in the 2007 FHB nursery, varied in their reaction to FHB. Three were more resistant than 'McKenzie' while one was clearly more susceptible (**Table 1**). The most resistant line,

Table 3 Inheritance of *de novo* resistance to wheat streak mosaic virus infection in a cross between seed from a tiller of Line 9650 (derived by selfing from 'McKenzie') and 'McKenzie'.

	Generation	Resistant : Susceptible
Line 9650 Plant 6 (n=10)	F_1	10 : 0
Seed of 1 early plant (n=107)	F_2	74 : 33[a]

[a] $P:H_0 = 0.199$

Table 4 *De novo* resistance to *Wheat streak mosaic virus* (WSMV) infection in progeny of Hume recorded 28 days after inoculation.

Temperature	WSMV
	Immune: Symptomatic
18°C	18 : 0
21°C	13 : 5

Table 5 Agronomic and quality traits of Hume and 'McKenzie'.

Properties	Hume	'McKenzie'
Yield (kg/ha)	6992	6036
Height (cm)	94	96
Lodging (1-9 scale)	4.0	4.3
Test weight (kg/hL)	78.7	79.4
1000-kernel weight (g)	36.9	36.4
Protein (%)	13.5	15.2
Flour yield (%)	72.6	73.1
Mixing development time (min)	2.3	1.4

row 136, had an FHB Index of 5.0 compared to 17.5 for the original 'McKenzie'. Row136 accumulated less DON and was considerably shorter than its progenitor. It was named Hume. In autumn 2007, 20 plants of Hume were grown under WSMV and *F. graminearum* pressure and selected progeny, designated lines 9969 to 9973, advanced under pressure from inoculation with WSMV alone. All lines uniformly expressed resistance. Selected for its earlier maturity and uniform plant morphology, Line 9970 was the source of 5 head row lines entered as rows 2738 to 2742 in the 2008 FHB nursery (**Table 1**). The promising level of FHB resistance was confirmed. The three earliest maturing of these were increased as separate lines in an off-season nursery (Lines NZ11369 to11371) and re-entered with four replicates in the 2009 FHB nursery. Again, improved FHB reaction compared to that of 'McKenzie' was documented (**Table 1**).

The temperature sensitivity of the WSMV resistance in an early Hume line, increased one generation without WSMV pressure, was evaluated in a back-assay. Its *de novo* resistance conferred immunity and prevented completely the establishment of infectious virus in plants maintained at 18°C. This also applied to a majority of plants maintained at 21°C; five out of 18 developed mild symptoms and some titre of infectious virus 28 days after inoculation (**Table 4**).

The agronomic trial showed that Hume's superior disease resistance was not achieved at the cost of lower yield in the absence of intense disease pressure. Hume out-yielded 'McKenzie' by 15.8%. Other important agronomic and quality parameters were similar to 'McKenzie' with the exception of protein content and mixing development time (**Table 5**).

DISCUSSION

Identifying newly expressed traits in an iterative selection regime

In generating Hume from 'McKenzie' no genes were introgressed. The precautions taken in all early rounds of the protocol (up to 2007 FHB field trial) were designed to prevent the accidental introduction of genes by crossing or seed admixture. The protocol also ensured that if the apparent alteration of inherited traits merely reflected experimental error, or the apparent resistance were real, but not highly heritable, this would become clear in subsequent rounds of testing. Thus when traits appeared that had not been seen in the preceding generation these alterations would necessarily have arisen either from genetic mutations or from changes in the expression of genes already present. Genetic mutations, changes in coding DNA base sequences that are induced and heritable, do not likely account for the alterations we observed (Akimoto *et al.* 2007). First, the frequency with which such changes were observed, round after round, is far higher than the accepted convention of 10^{-6} per allele per generation in the absence of powerful mutagenic action (Akimoto *et al.* 2007). Second, the appearance of altered traits in individual tiller(s) of wheat plants subjected to the protocol cannot be as easily accommodated by genetic mutation as by the concept that in different parts of the same plant there might be altered expressions of inherited traits.

With respect to the first appearance of resistance to WSMV infection on a single tiller of an otherwise symptomatic plant, two explanations might plausibly account for the phenomenon: a) failure of the virus to enter the cells of the growing point as the plant extended a new tiller; or b) the new tiller, unlike the other tillers, expressed effective resistance to the infection. The newly appearing resistance was not limited to the generation in which it might have been induced, but was also expressed in at least some individuals of the next generation (**Fig. 1**). With repeated selection, in turn, at least some sub-lines came to express the new trait uniformly. To be useful, such *de novo* traits must persist from generation to generation even when the original triggering stress of their evolution is no longer being applied. No generation was subjected to WSMV stress between November 2007 and September 2009, and tests of small numbers of plants from harvested seed confirmed that virus resistance continued to be uniformly expressed in later generations.

Sub-lines that expressed the new trait of WSMV resistance uniformly were also able to serve as Mendelian gene donors in crosses. Thus, a trait which first appeared as an epigenetic variation appears capable of being fixed genetically and therefore becomes available to conventional gene introgression protocols.

The process that induced the apparent change in expression of WSMV resistance was also inducing changes in the expression of other traits. The most visible of these was the reduced height, compared to 'McKenzie', seen in every generation of lines descended from line 9526-2, the ancestor of Hume. When 'McKenzie' sub-lines that had been altered by the protocol were tested in an FHB nursery, clear variations in FHB resistance were also observed, thus indicating that the driver of initially epigenetic changes in WSMV resistance had also induced variation in the trait of FHB resistance. We made a pragmatic decision to emphasize the descendants of 9526-2 as they combined a greater number of newly-expressed desirable traits in early rounds of the protocol. For example, while some lines descended from 9526-3 expressed both WSMV and FHB resistance, they were much taller than 'McKenzie' and thus less suitable candidates for advancement (Haber *et al.* 2008).

Given that the expression of several traits appear altered in Hume compared with 'McKenzie', the concern logically arises that the expression of desirable traits in 'McKenzie' might also be altered. The early results from trials conducted in the absence of disease pressure (**Table 5**) indicate that Hume remains recognizably a member of the Canada western spring wheat class.

Altering the expression of existing genes as an alternative to gene introgression

The broad assumption underlying efforts to improve FHB resistance in wheat cultivars has served most breeding programs well. Its historical success has allowed gene introgression to be seen as the key enabler of progress and become so deeply embedded in current practice that few pause to consider alternatives. Every official wheat breeding prog-

ram today uses traditional crossing and backcrossing techniques to introgress and to complement the slate of desirable traits present in the recurrent parent of a crossing block. The implicit assumption is that what are currently described as *resistance genes* can be transferred, and that once transferred, they will express the associated trait.

Most programs in Canada now also use some form of marker-assisted selection (MAS) to identify and track selected and desired traits across generations. In early generations MAS can substitute for the onerous task of directly evaluating phenotypes in FHB field nurseries. It gives a reliable, objective, reproducible and automated assessment of the presence or absence of markers or the genes themselves, the purported proxies of resistance. However, the value of MAS becomes more difficult to establish, the larger the number of determinants for the trait. When attempting to introgress FHB resistance in wheat, for which at least three major determinants have been identified (Buerstmayr *et al.* 2009), the benefits of MAS both in terms of ease and effectiveness are open to question (Miedaner *et al.* 2009). A review of some 52 studies on QTL mapping and MAS for FHB resistance in wheat published since 1999 demonstrates the vast effort and amount of time invested in such strategies (Buerstmayr *et al.* 2009). As noted earlier, the largest QTLs explain only a small part of the variation associated with FHB resistance (Miedaner *et al.* 2009) and success has been modest at best. The most recent guide to variety selection for Manitoba lists just three with moderate resistance to the disease (Seed Manitoba 2011 2010), but their resistance does not derive from a known source such as 'Sumai 3'.

Beyond these technical considerations, a more profound concern arises from recognizing that most of the efforts to introgress the FHB resistance of 'Sumai 3' into cultivars overlook the moderate susceptibility of 'Sumai 3''s own parents (Liu and Wang 1991). Whence the resistance? References to transgressive segregation neither explain the phenomenon nor guide the researcher in choosing other pedigrees where crosses of susceptible parents might lead to more resistant descendants. When susceptible near-isogenic lines (NILs) of 'Sumai 3' (Xu *et al.* 2002) were compared with their resistant parent, it was striking that not only were the genetic differences negligible, but all the lines contained PR genes that responded to induction by *F. graminearum* (Golkari *et al.* 2009). Did the susceptible NILs already contain the genes needed to resist infection but fail to express their information in a coordinated, effective manner?

If we choose to proceed from this assumption, we must confront not only our extensive ignorance of the details of the phenomena involved but also our incomprehension of the mechanisms that might mediate their expression (Comeau *et al.* 2010). Fortunately, progress towards more effective resistance in the face of such ignorance is possible with an evolutionary approach that identifies and advances the individuals which best express desired combinations of traits in repeated rounds of selection. The likelihood of success generally increases with more extensive ranges of variation from which winning individuals might be selected.

It has recently been shown that subjecting the progeny of complex (3- and 4-way) crosses to regimes of repeated cycles of multiple, interacting (biotic and abiotic) stresses can be an effective approach for evolving desirable traits not adequately expressed in the parental lines (Comeau *et al.* 2010). In evolving populations that more fully express sets of desirable traits, this approach necessarily requires that very large plant populations be assessed, particularly in the early rounds. Moreover, the final selected products are, by the very choice of their complex parentage, much more difficult to characterize than the descendants of simple crossing and backcrossing regimes. One is faced with the seeming paradox of wanting a pool of variants that are altered with respect to the expression of small numbers of traits that need to be improved, while wishing to avoid undesirable variation in the much larger number of traits one seeks to conserve.

What if one chose to alter directly the expression of genetic information already present rather than introduce new genetic information from another source? Epigenetic change, the modification of gene expression without change in the underlying coding base sequences, may be one way to accomplish this. A recent study of the effects of a potent DNA demethylating agent on rice seedlings demonstrated that changing the genome's methylation status revealed in cultivated rice a specific resistance to bacterial blight that had previously only been obtainable by gene introgression from wild relatives (Akimoto *et al.* 2007).

The circumstances in which new sources of WSMV resistance were discovered in hitherto susceptible elite winter wheat lines suggested strongly that these might also be examples of desirable traits arising from altered expression rather than introgression of known genetic sources of the trait (Seifers *et al.* 2006, 2007). In the earliest rounds the newly-evolved trait was not uniformly inherited, but with repeated selection, lines were identified that stably and uniformly expressed the new trait. This pattern is similar to the early evolution of WSMV resistance we describe in the study here, where we have proceeded prospectively and deliberately. Once the newly evolved trait was inherited for two or more generations, it continued to be uniformly inherited even when there were several intervening generations without exposure to pressure from WSMV infection (**Table 2**). Thus, as in the *de novo* evolution of bacterial blight resistance in cultivated rice (Akimoto *et al.* 2007) it is possible to generate desirable changes in the expression of important traits by altering the expression of the genetic information already present. However, in deriving resistant Hume from susceptible 'McKenzie', the iterative protocol was thrifty, efficient and rapid. It required just a few generations with only modest numbers of individuals in each generation to produce lines that stably and uniformly expressed the desirable trait of WSMV resistance.

If the trait of WSMV resistance could be evolved *de novo*, might we not also examine the treated populations for *de novo*, and ultimately heritable, expressions of other desirable traits? Our epigenetics-driven protocol, simple, safe, and readily applied, makes it much easier to pursue several, deliberately-chosen traits simultaneously in adapted wheat backgrounds. On the face of it, this approach should be more likely to preserve the valuable traits accumulated over time by breeders while changing the small number of traits needing improvement. Larger-scale tests in 2009 do show that the *de novo* evolution of resistance to WSMV and FHB in 'McKenzie' has not been purchased with a reduction in agronomic or grain quality characteristics. Hume's yield surpassed 'McKenzie's' by 15.8%. Other agronomic traits were not markedly altered (**Table 5**). Hume's higher yield is consistent with extensive late grain filling with starch resulting in a lower proportion of grain protein. In addition to consistent expression of resistance to WSMV infection and markedly improved reaction to FHB, Hume's response to leaf spot pathogens (first observed in the 2007 FHB Nursery) was confirmed in the sub-lines that were advanced to the 2009 Nursery (**Table 1**).

Potential for virus-mediated epigenesis as a versatile tool for crop improvement

The initial, path-finding, identification and exploitation of epigenetically-altered traits that can be fixed genetically has since been observed in wheat lines other than 'McKenzie'. As a doubled-haploid line it was chosen, among other reasons, for analytical simplicity, since its descendants would not show any effects of gene re-assortment. Protocols similar to the ones we have outlined here have successfully generated *de novo* resistance to WSMV and other disease agents in a range of winter and summer habit bread wheat lines, as well as durum wheat lines (Haber *et al.* 2006; Seifers *et al.* 2007).

The plasticity of the expression of heritable traits seen in higher plants repeatedly exposed to systemic stresses has

become an active research topic in recent years (Henderson and Jacobsen 2007). Many phenomena which have eluded explanations that depend solely on the simple expression of specific gene alleles are now being fruitfully explored as territory at the frontiers between genetics and epigenetics (Morris *et al.* 2004; Li *et al.* 2006).

In explaining why we chose the stress of WSMV infection we initially emphasized the pragmatic considerations of ease of use, clarity of host response and the goal of identifying new sources of genetic resistance to the virus. A more profound consideration may be that the plant host responds to systemic infection with a positive-sense, single-stranded RNA virus (like WSMV) by producing short, interfering (or silencing) *siRNA*, which a growing body of research findings is implicating in critical roles in the alteration of gene expression (Li 2008). While we can make no claim here that we have exploited any specific action of WSMV infection – or the host's response to infection – in enhancing the rate of epigenetic change, it is striking how effectively and rapidly our protocol achieves this effect.

We have shown here that substantial and useful changes in a wheat cultivar's expression of traits can be obtained without bringing in genetic information from any other source. It remains to be resolved how the credit and rewards for such improvements should be apportioned.

ACKNOWLEDGEMENTS

Funding from Western Grains Research Foundation and the technical expertise of Brian Gillis, Ron Kaethler, Kirsten Slusarenko, Courtney Wolfe, Melodie Budzinsky, and Jeff Ackerman are gratefully acknowledged.

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