

# The future of genetically engineered plants to stabilize yield and improve feed



Gaganpreet Kaur Dhariwal and André Laroche

Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, 5401 1st Avenue S., Lethbridge, AB CANADA T1J 4B1

## Implications

- Recent biotechnological developments that tackle different crop improvement challenges, including both decreased yield and feed quality with ever increasing impact of different biotic and abiotic stresses, are readily becoming available to imprint lasting positive economic impacts on the cost of crop production and feeding animals.
- The challenge in improving digestibility of feed for the benefits of improving growth per unit of fed biomass, which is also shared by the bioethanol industry using lingo-cellulose as feedstock, can be achieved through diversifying feed source.
- In this article, where the available biotechnological options and possibilities for use of the genetically engineered plants to stabilize yield and improved feed quality have been reviewed, emphasis has been given on accelerating the use of genome editing tools.

**Key words:** animal feed, biotechnology, CRISPR/Cas9, disease resistance, genes, grains, digestibility

## Plant Adaptation to Its Environment

Plants being sessile organisms are extremely sensitive to both abiotic and biotic stresses and must adapt quickly to their environment as escaping is not an option. In Northern and Southern temperate countries, cold is a prevalent stress for perennial and winter annuals forage and grain crops used to feed animals. In warmer countries, events such as unseasonal heat and waves of extreme heat periods or the opposite, flooding, can be also extremely devastating and significantly decrease yield potential of crops. Around the globe, drought events occur in irregular patterns and can cause important yield losses. One of the many impacts of climate change is the increase in frequency of erratic meteorological events such as unseasonal or torrential rain storms or heat waves that can decrease or severely impact crop yield (Gaudet et al., 2012). On the other hand, diseases are also affecting crop yield potential. It takes several years to identify different disease resistance genes and incorporate them in highly performing plant cultivars. This process has been accelerated by the utilization of marker-assisted selection when a DNA marker or other marker types are available. So far, in crop species, a limited number of individual disease resistance genes conferring resistance to different isolates within pathogen species have been identified. Plants are resistant to at least 99.9% of potential pathogens they encounter. Depending on crop species and geographical regions, fungi,

virus, bacteria, or insect can represent a challenge to crop species. Taking examples of the situation of cereals in Western Canada and the North American Great Plains, fungal pathogens represent a major threat. For example the three wheat rusts caused by *Puccinia graminis* f. sp. *tritici*, *P. triticina*, and *P. striiformis* f. sp. *tritici*, causal agents of stem, leaf, and stripe rust, respectively, can be a real issue for wheat crops. Individual resistance genes, i.e., conferring resistance to different races of pathogens, have been introduced in wheat varieties for close to 100 yr or so. However, given the perpetual war between plants and pathogens, pathogens have found ways to evolve and mutate to be able to defeat specific resistance genes present in plants and can infect plants and decrease yield to levels up to 80 to 90% in the worst conditions depending on the aggressiveness of new isolates, susceptibility level of the wheat crop, environmental conditions, and developmental stage of the crop. In North America and around the world, numerous examples of this have occurred over and over. In case of the stem rust pathogen, the Ug99 isolate first observed in Uganda in 1999 and its subsequent derivatives have defeated numerous stem rust resistance genes. These isolates have moved to the Middle East and represent a threat to the world agriculture as accidental introduction on different continents remains a possibility. Very few stem rust resistance genes are effective against Ug99 and derivatives. These genes are being incorporated into the world wheat germplasm as fast as possible. The stripe rust isolate Warrior first identified in United Kingdom and in 2011 and is a newly aggressive isolate causing a lot of issues to the wheat growers as effective resistance of many varieties against this pathogen has been overcome (Hovmöller et al., 2015). It takes time to identify and incorporate effective resistance genes into germplasm.

Linked markers to a limited number of resistance genes for different crops are available. These markers, generally DNA based, represent DNA fragments located in the vicinity of a resistance gene and are used to follow up and assess transfer of specific genes into a specific line. Although recipient lines could also be evaluated using a pathogen, it is usually much faster and efficient to assume the presence of a functional gene using a DNA marker. In case where plants will carry pyramided (2 or more) resistance genes against the same pathogen, molecular tools must be used to verify the presence of all genes since whether the line carries one or more genes, it will be resistant to the pathogen in conventional screening. Pyramiding resistance genes is part of the modern arsenal of plant breeders to protect functionality of independent resistance genes as pathogens can mutate/evolve and defeat a single gene. It is a lot more difficult and much less frequent for a pathogen to mutate to defeat simultaneously three genes at the same time. In a way, the effectiveness of pyramiding genes was discovered accidentally as some crop varieties from time to time have exhibited enhanced and long-lasting resistance as exemplified by a resistance to stripe rust. After genetic dissection of the resistance mechanism, it was found that presence of multiple genes assembled without know-



Stripe rust close up.

ing it explained the long-lasting resistance (Chen, 2005).

As the interaction between pathogens and plants has been identified as a perpetual ongoing war, plants and pathogens have evolved a successful strategy in an attempt to defeat the opponents (Jones and Dangl, 2006). Defeated resistance genes are sometimes of limited usefulness as their loss of effectiveness can be regional or continental only and they remain effective elsewhere around the world. However, the potential to become defeated elsewhere is higher. Opposite situations have been reported as well. The stripe rust resistant *Yr10* resistance gene was defeated in the

mid-1980s in the Pacific Northwest, USA. Varieties carrying this single gene were pulled out from commercial fields in this region. With this gene being absent in the environment, it appears that the genetic elements involved in defeating it were not maintained/conserved in many stripe rust isolates as 30 yr later, some old club wheat varieties carrying this single *Yr10* gene appear to be resistant to the majority of stripe rust isolates as they are grown commercially again (X. Chen, personal communication, 2015). This situation is probably not going to last very long as it is very likely that stripe rust isolates able to defeat *Yr10* will increase in abundance and will defeat *Yr10* again in that region.

Although *Yr10* was defeated in the mid-2000's in southern Alberta, this gene is still functional in central Saskatchewan. This suggests that regional climate conditions and environment also affect where different isolates of the same pathogen can grow and survive as usually isolates will migrate from southern Alberta to that region due to the dominant wind.

Global warming is asymmetric in a way that northern and southern hemispheres will experience higher temperature increases than tropical areas. This will have different impacts on plant productivity. From a disease point of view, this may enable pathogens to successfully overwinter at higher latitudes, causing higher disease incidence and impact on yield reduction. It may also enable different pathogens to move from lower latitudes to Canada and cause issues for which the current crop germplasm does not carry resistance at all against these new pathogens. An example of this is wheat leaf beetles that are becoming more prevalent and represent a threat to cereal crops in western Canada. On the other hand, global warming may have a positive impact of prolonging the growth season as the number of frost-free days could increase significantly from just over 100 d on the Canadian Prairies. Late-maturity cultivars generally help at increasing yield, so it would be advantageous toward the challenge of increasing crop productivity to feed the world by 2050.

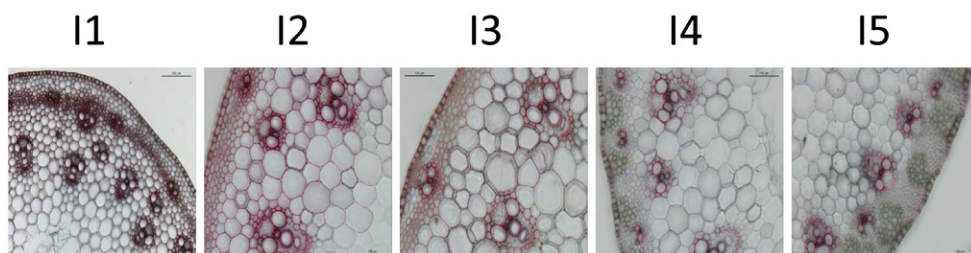
## Stability of Food and Feed Supplies

Any event causing a substantial yield decrease in either forage or grain yields will have an economic impact on the cost of feeding animals as the price per unit will increase. Farmers keep a close eye on feed materials and on factors affecting crop productivity. In North America, farmers and feedlot operators are used to adjusting cattle diet based on the price of available feeding stocks.

Stripe rust is one of the most severe diseases of wheat around the world. Prior to 2000, stripe rust was considered a regional cool-temper-

ature disease in different regions of the world. Since 2000, some stripe rust isolates have become adapted to warmer temperature, and in North America, all the wheat grown on the Great Plains has become vulnerable to this pathogen previously causing yield reduction in California and the Pacific Northwest of USA and southern Alberta in Canada. In Europe, the Warrior isolate of stripe rust has overcome effective resistance genes in many wheat varieties. Although more than 80 different stripe rust resistance genes have been identified in different germplasms, a dozen or so have been used heavily, and only a few resistance genes are providing resistance. Numerous genes have been defeated.

Fusarium head blight caused by the fungus *Fusarium graminearum* and many other species of *Fusarium* decreased yields due to grain shriveling. However, the major issue with this family of pathogens is the accumulation of different toxins in infected grains of wheat, barley, and maize to name a few crops. The toxins accumulate in every grain, and consumption may induce abortion in animals and humans. Maximum levels of these mycotoxins are highly regulated in all countries in barley, wheat, and corn for human food (babies and adults) and animal feed. Above given toxin concentrations in grain, material must be discarded and is of no commercial value. Research to improve fusarium resistance is ongoing in many countries around the world but limited resistance improvement has been obtained in most cases so far. Resistance is multigenic and additive as many quantitative trait loci (QTL) have been identified, but these QTL are difficult to assemble to improve disease resistance. Despite this reality, incremental progress has been obtained during the last 25 yr. Improved resistance to *Fusarium* in selected lines has been obtained using selection under high disease pressure in *Fusarium* head blight (FHB) disease nurseries. In western Canada, Emerson winter wheat and, more recently, AAC Brandon and AAC Tenacious spring wheat have been identified exhibiting high resistance levels to FHB. However, the molecular mechanism involved in these resistant lines is still unknown, which slows down the accelerated development of FHB-resistant lines using molecular tools to ensure the presence of the necessary genes in additional lines to improve FHB resistance. An important complementary aspect is a decrease in mycotoxin accumulation as specific lines clearly accumulated less mycotoxins under disease pressure. These mycotoxins are highly resistant to degradation. Identification of an enzyme metabolizing these mycotoxins into non-potent molecules and their expression in developing seeds would greatly decrease the risks associated with mycotoxins in human food and animal feed. Drastic reduction in FHB mycotoxins would improve food and feed security tremendously. As feed grains represent a spin-off of human food, any transgenic approach that would reduce/eliminate the impact of FHB mycotoxins would also have to be socially acceptable for human food consumption around the world as, at least in North America, wheat and barley grains used as animal feed do not receive the grade for human food. The addition of an enzyme to feed to destroy mycotoxins could be an alternative option as it would be relatively easy to engineer microbes to overexpress large quantities of enzymes in a very economical way. However, neither enzyme nor complexes of enzymes are known to efficiently metabolize FHB mycotoxins. Such additives to wheat and barley grains could work but would increase the possibility of food cross contamination as mycotoxin-contaminated grains would circulate in the grain handling, storage, and transportation systems and would open ways to mishaps toward human-grade grains.



**Figure 1.** Cross-section of stem internodes 1 to 5 from triticale stained with phloroglucinol to detect lignin.

## Improving Digestibility of Forage and Grains

The challenge in improving digestibility of forage and grains for the benefits of improving growth per unit of fed biomass is also shared by the bioethanol industry using lingo-cellulose as feedstock. Enzymes can be added to decrease complex carbohydrates associated with the cell wall. Currently, it is possible to add engineered enzymes in microbial systems to a low cost that can improve the economics of the system as more sugars are available to be metabolized. Although carbohydrates constitute 70 to 80% of the cell wall, a molecule such as lignin interferes with their availability to be simplified by enzymes. For both animal feed and production of bioethanol, a common approach has been to decrease the lignin content of plant tissues. The efforts started more than 20 yr ago toward decreasing the total amount of lignin per unit mass and to decrease some specific lignin molecules. Lignin makes it difficult for enzymes to access the cellulose. Lignin deposition is seen around the plant fibers (Fig. 1). Lignin is also more abundant in mature tissues (Fig. 1, internode 1) than in immature tissues (Fig. 1, internode 5). Using this approach, it was feared that plants or energy trees would lose their ability to stand up, particularly under high wind conditions or in years of exceptional grain yield, and would become more susceptible to different pests. These fears did not materialize as plants did not become universally susceptible to all pathogens nor went systematically down because of a lack of standing ability under strong wind conditions. During different investigations, it was found that inhibiting one of the three pathways of the lignin biosynthesis was facilitating digestibility without affecting plant response to neither pathogens nor wind. This is also very useful for increasing bioethanol yields in energy crops.

## Plant Protection using Host-Induced Gene Silencing

Recent discoveries regarding small RNAs and mechanisms of gene silencing are providing new opportunities to explore plant gene functionality (Baulcombe, 2015) and have been used to study fungal pathogen–host interactions and potential strategies for novel disease control (Yin et al., 2015). Small RNAs (18–23 nucleotides) are part of a sophisticated plant defense system against viruses and for regulation of gene expression that can be taken advantage of to develop an active defense against other pathogens outside virus. Studies have shown that the expression of silencing constructs for fungal genes in plants can specifically silence their targets in invading pathogenic fungi via production of small RNAs. Host-induced gene silencing (HIGS) has been used to control fungal pathogens, including FHB, powdery mildew, (Baulcombe, 2015) and wheat rusts (Yin et al., 2015). The identification of common genes present in all three rust species could provide the basis for protecting wheat against

all three wheat rusts. This is an example on how to protect plants efficiently. Similarly, this virus-based system could be used to provide protection to other pathogens causing disease such as powdery mildew and FHB. This would not require genetic manipulation of the wheat plant but would necessitate the release of a genetically modified very mild virus in the environment. We are not aware of any attempt yet to commercialize this approach to improve plant resistance, nor are we aware of what the

regulators response to such an approach would be. These genetic modifications on a mild virus would stay clear from any wheat modification and would be likely advantageous for growers in marketing their crops.

## Transgenesis and Cisgenesis

Transgenesis and cisgenesis are rapid and very efficient techniques that enable introduction of genes in a specific line to improve one or more specific traits. Transgenesis traits have been commercialized in different crops starting more than 20 yr ago, and transgenic cotton, soybean, corn, and canola (rapeseed) have been marketed since on international markets. Country-specific regulations exist that may limit usage and marketing of these products. The initial traits of interest included herbicide resistance and protection against insects using potent bacterial genes. More recently, intervention to modify basic plant attributes such as fatty acid profiles in oilseed crops or modification of cell wall composition to improve transformation yield for the production of ligno-cellulosic bioethanol in energy crops have been done. It is anticipated that in the near future, more traits will be introduced to improve nutritive attributes of different crops. However, no transgenic rice, barley, and wheat lines have reached commercial markets yet. This is, in part, due to the hesitation of consumers to accept transgenics in their staple food and difficulties to distinguish conventional grain from with transgenic events. Fears of lucrative market losses due to uneasiness from consumers have stopped farmers from adopting and growing these transgenic crops. Efforts have been made in the past to utilize the transgenic resources, particularly to combat malnutrition in developing world; one of these efforts includes the development of Golden Rice to supplement essential vitamins for children. This project is still blocked for a variety of reasons after more than 10 yr of its initiation (Potrykus, 2010). It is difficult to predict if this potentially wonderful project will ever mature. To decrease fears about gene movement between organisms, the concept of cisgenesis was introduced as it would limit gene movement within a given biological species. So far, this has been used to assess and demonstrate functionality of candidate genes on an experimental basis, and these events have not been commercialized. An example was the demonstration of the functionality of the candidate *Yr10* stripe rust resistance gene (Liu et al., 2014). Such an approach would accelerate introduction of genes (e.g., disease resistance) from non-adapted lines into elite germplasm.

## Genome Editing

Different biological systems have been developed and investigated to modify the genome of living organisms. The most frequently used system is the CRISPR/Cas9 (Li et al., 2016). This technique has been successfully applied to different biological systems and is characterized by the exact



modification of a specific targeted sequence. Although the technology implies specific structural changes in targeted sequences in the genome of an organism, it is not considered as a technology involving genetically modified traits based on gene introduction from an unrelated species. Consequently, the CRISPR/Cas9 gene-editing technique has revolutionized biology, including plant biology. This new tool also opens numerous new avenues on how to improve plant responses to the environment. Some aspects include improving plant responses to different pathogens. Taking advantage of this technique, defeated resistance genes could be slightly modified based on sequence knowledge of effective genes to release new effective genes. This would be a lot simpler and faster than going after novel genes, characterizing their response to different pathogen isolates and determining how they interact with other resistance genes against similar and dissimilar pathogens. A vast majority of resistance genes have been introduced in wheat from relative wild species such as *Thinopyrum* species and necessitate at least a dozen generations before the genes can be placed in a good agronomic variety. Currently, a genome survey of wild wheat relatives is an efficient approach to identify new genes for a large variety of traits including resistance genes to many different pathogens. Using genome-editing technology would be a very efficient way to recycle defeated genes into second and third life as well as introducing novel sequences from newly characterized genes without introducing the gene itself in the plant.

Although much remains to be understood about the abilities of plants to survive cold and freezing temperatures, important transcription factors directing the plant response have been identified and characterized. In response to cold, drought, heat, and other abiotic stresses as well as numerous biotic stresses, an important consensus has emerged regarding the time of response to the stress and the magnitude of the response. By editing specific genes and transcription factors, which are master switch regulators, it is possible to improve plant response to specific stresses without disturbing the many other positive attributes expressed in crop varieties. Although CRISPR/Cas9 and related techniques have been used in many different biological systems from bacteria to humans, the status of this and related gene-editing techniques has not been considered by regulators around the world very frequently yet. One occurrence that we are aware of is the release of a new canola (rapeseed) variety in Canada in which a gene has been edited using the described approach. In this ruling, the plant was neither considered as a transgenic nor a genetically modified organism as it did not include introduction of DNA material from an exogenous organism. As derivatives of GMO crops and grains are used to feed animals around the world, whatever the decision of international regulators, this process should be used very soon if not already, for improving feed quality to animals. However, in crops targeted to human nutrition and for which surplus is shunted to feed, it may take longer to become used routinely for crop improvement.

Genome-editing techniques will have a lasting impact on crops to rapidly modify and improve specific traits. We are predicting that the first monocot to be commercialized with genome-edited gene(s) will be corn as there is a good differentiation between varieties targeted to human nutrition and animal feed. This should be eventually followed by barley and wheat.

## Literature Cited

- Baulcombe, D.C. 2015. VIGS, HIGS and FIGS: Small RNA silencing in the interactions of viruses or filamentous organisms with their plant hosts. *Curr. Opin. Plant Biol.* 26:141–146. doi:10.1016/j.pbi.2015.06.007
- Chen, X.M. 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *Tritici*) on wheat. *Can. J. Plant Pathol.* 27:314–337. doi:10.1080/07060660509507230

## About the Authors



**Dr. André Laroche** is a Research Scientist with Agriculture and Agri-Food Canada, in Lethbridge, Alberta. He has more than 35 years of research experience in the areas of plant molecular biology and molecular phytopathology. His research interests widely range from cereal functional genomics of biotic (snow mould resistance, smut and bunt, stripe and leaf rust) and abiotic stress tolerance (also winter hardiness), to biorefinery, methodology development, DNA marker assisted selection, and lipid metabolism, etc.

His current scientific interests involve the characterization of the stripe rust pathogen isolates and their interactions with wheat. He is also affiliated as Adjunct Professor with the Department of Biological Sciences, University of Lethbridge. **Correspondence:** Andre.Laroche@AGR.GC.CA



**Dr. Gaganpreet Kaur Dhariwal** is a NSERC Postdoctoral Scientist with Agriculture and Agri-Food Canada (AAFC) at Lethbridge Research and Development Centre in Lethbridge, Alberta. Dr. Dhariwal completed her doctorate in Botany with minors in Plant Breeding, Genetics, and Biotechnology from Punjab Agricultural University (PAU), India. At PAU, her research interest involved both basic and applied research; for basic research, she studied the physiological bases of hard seediness (due to lignin deposition) in one of the legume species (*Vigna radiata*) and identified an alternate dwarfing gene system in wheat using Virus Induced Gene Silencing (VIGS), introgressed Opaque 2 gene, and stripe rust resistance genes in quality protein maize and wheat, respectively, for her interest in applied research. At AAFC, her research focuses on understanding the role of different stress responsive genes (using VIGS and expression analysis), signalling network pathway components and microRNAs in disease resistance and drought tolerance in cereal crops as well as transfer of some of the important genes in winter wheat using biotechnological tools.

for her interest in applied research. At AAFC, her research focuses on understanding the role of different stress responsive genes (using VIGS and expression analysis), signalling network pathway components and microRNAs in disease resistance and drought tolerance in cereal crops as well as transfer of some of the important genes in winter wheat using biotechnological tools.

- Gaudet, D.A., A.M. Tronsmo, and A. Laroche. 2012. Climate change and plant diseases. In: K.B. Storey and K.K. Tanino, editors, *Temperature adaptation in a changing climate: nature at risk*. CABI International. p. 144–159.
- Hovmöller, M.S., S. Walter, R.A. Bayles, A. Hubbard, K. Flath, N. Sommerfeldt, M. Leconte, P. Czembor, J. Rodriguez-Algaba, T. Thach, J.G. Hansen, P. Lassen, A.F. Justesen, S. Ali, and C. de Vallavieille-Pope. 2015. Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. *Plant Pathol.* 65:402–411. doi:10.1111/ppa.12433
- Jones, J.D.G., and J.L. Dangl. 2006. The plant immune system. *Nature* 444:323–329. doi:10.1038/nature05286
- Li, J., X. Meng, Y. Zong, K. Chen, H. Zhang, J. Liu, J. Li, and C. Gao. 2016. Gene replacements and insertions in rice by intron targeting using CRISPR–Cas9. *Nature Plants* 2:16139. doi:10.1038/nplants.2016.139
- Liu, W., M. Frick, R. Huel, C.L. Nykiforuk, X. Wang, D.A. Gaudet, F. Eudes, R.L. Conner, A. Kuzyk, Q. Chen, Z. Kang, and A. Laroche. 2014. The stripe rust resistance gene Yr10 encodes an evolutionary-conserved and unique CC-NBS-LRR sequence in wheat. *Mol. Plant* 7:1740–1755. doi:10.1093/mp/ssu112
- Potrykus, I. 2010. Regulation must be revolutionized. *Nature* 466:561. doi:10.1038/466561a
- Yin, C., S.I. Downey, N.L. Klages-Mundt, S. Ramachandran, X. Chen, L.J. Szabo, M. Pumphrey, and S.H. Hulbert. 2015. Identification of promising host-induced silencing targets among genes preferentially transcribed in haustoria of *Puccinia*. *BMC Genomics* 16:579. doi:10.1186/s12864-015-1791-y