Genetic Enhancement of Food Safety Attributes of Durum Wheat

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Consumer interest in food safety has increased dramatically in recent years. This has been spurred by issues such as outbreaks of bovine spongiform encephalopathy ("mad cow disease"), foot-and-mouth disease, avian influenza in farm animals, *E. coli* in spinach, and melamine adulteration in pet foods. Mycotoxins in food crops arising from fungal growth in the field or in storage have long been a concern. Concerns about pesticide residues have fostered steady growth in demand for "organic" food products. These issues have caused a major shift in priorities in publicly funded agriculture and food sciences research in the developed world. Research to address the establishment of appropriate regulations for the control of potential contaminants and the development of production strategies to mitigate them has become a high priority.

Genetic enhancement provides a cost-effective means for mitigating some food safety issues in crop plants. For example, genetic resistance to diseases can reduce mycotoxin and, consequently, fungicide residue levels on grain. The cultivar development strategy for durum wheat (*Triticum turgidum* L. var *durum*) in Canada emphasizes genetic resistance to insects and diseases to meet the strict requirements of registration of cultivars (4), so application of fungicides and insecticides is rare. In contrast, two to three fungicide applications and one to two insecticide applications are common for intensive wheat production in France (1), where genetic resistance has not been a breeding focus.

doi:10.1094/CFW-52-4-0179
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- Breeding for disease and insect resistance reduces mycotoxin, fungicide, and insecticide residue levels on grains.
- Breeding for fusarium resistance is a major focus of wheat improvement programs in parts of North America, Europe, and Asia.
- Although progress has been slow in wheat, especially durum, due to the genetic complexity of fusarium resistance, biotechnology tools are reaching the stage of practical use in breeding for resistance.
- Development of DNA markers permits pyramiding of genes to reduce the rate of resistance breakdown to insect infestation by pests such as the Hessian fly and the orange wheat blossom midge. The first midge-resistant common wheats were submitted for registration in Canada in 2007.
- Incorporation of a single dominant gene into durum cultivars has reduced cadmium concentrations for those cultivars to well below proposed international limits.

New biotechnology tools are accelerating progress in breeding for disease resistance utilizing natural genetic variation within wheat or its close relatives. DNA markers have proved to be very useful tools to accelerate selection for desirable genes. As genes for factors such as disease resistance are identified, there will be more opportunities for the use of genetic transformation. However, the use of transformation in development of wheat cultivars is restricted by the lack of consumer acceptance of genetically modified organisms in foods.

Genetic Resistance to Disease

Diseases which cause mycotoxin contamination of grain are of primary interest to the food industry and consumers. Fusarium head blight or "scab," caused by several species of *Fusarium*, is the primary disease of concern due to mycotoxins such as the trichothecene deoxynivalenol (DON). This is recognized as a significant food safety hazard, and regulations have recently been tightened to limit its presence in grain and food products. Fusarium damage also has negative effects on pasta quality, particularly color (7).

The risk of fusarium infection of durum crops depends on a number of factors. Infection is lower in semiarid production regions than in more humid or irrigated regions. Crop management factors such as crop rotation, wherein infection is greater following fusarium susceptible crops such as corn, and timing of fungicide application also influence the degree of infection.

Breeding for fusarium resistance is a major focus of wheat improvement programs in parts of North America, Europe, and Asia. Progress has been slow due to the genetic complexity of fusarium resistance, which is controlled by several major genes and an unknown number of minor genes. Durum wheat does not possess as great a level of resistance as common (bread) wheat (*T. aestivum* L.), so progress has been even slower. Attempts are being made to move resistance from common wheat and other wheat relatives into durum, but these efforts have been challenged both by the genetic complexity of resistance and the detrimental affects of other genetic factors transferred with the resistance genes on processing quality of the resultant durum lines. DNA markers are being utilized to identify some of the major genetic factors related to fusarium resistance (22) and to assemble these into improved wheat genotypes (21) (Fig. 1). Breeding for reduced fusarium symptoms, such as fusarium-damaged kernels, should reduce DON levels as shown by the data published by Dexter et al. (7).

Ergot (*Claviceps purpurea*) is another disease that poses a health risk due to alkaloids in the fungal sclerotia that contaminate grain. Although these can be readily removed by efficient grain cleaning prior to milling, ergot is still an important factor in commercial grading due to the additional cost of cleaning. The disease has been viewed as an endemic problem, with incidence varying with growing conditions; occurrence of alternate grass host species on field verges, which become sources of inoculum; and crop factors such as sterility of wheat florets, which provides an increased opportunity for ergot infection.

There is evidence of genetic variation for resistance in durum wheat, with one genotype appearing to have good resistance (17). The same study indicated that the durum genotypes evaluated produced fewer and smaller sclerotia than common wheat. Crosses with the apparently resistant durum cultivar indicate that the trait is heritable, and thus amenable to selection in a breeding program (J. M. Clarke and J. G. Menzies, unpublished).

Rusts are another important disease complex of durum, including leaf rust (*Puccinia triticina*), stem rust (*P. graminis*), and stripe rust (*P. striiformis*), all of which can substantially reduce grain yields and cause economic losses but do not pose a mycotoxin risk. These diseases are controlled by repeated fungicide application or by genetic resistance. Maintenance of genetic resistance is an ongoing challenge due to mutations in the pathogens that overcome the resistance of the host plant. In the past, breeders successively added new resistance genes as the previous one was overcome. Biotechnology tools that have led to the discovery of resistance genes, or DNA markers closely linked to the genes, are reaching the stage of practical use in breeding for resistance. This facilitates "pyramiding" of multiple resistance genes into new cultivars, which should slow the ability of the pathogen fungi to overcome the resistance.

Genetic Resistance to Insects

There are several examples of genetic resistance to insects in durum wheat. Deployment of genetic resistant cultivars reduces the need of insecticides for crop protection, lowering potential residues on harvested grain and preventing the destruction of beneficial insects and other wildlife.

The Hessian fly (*Mayetiola destructor* Say) is a destructive pest of wheat in many production areas in North America and North Africa, causing yield loss through stem breakage and reduced translocation of assimilates into developing kernels. Breeding for resistance is widespread in both durum and common wheat in affected production regions. Historically, the insect has overcome wheat resistance genes fairly rapidly, necessitating constant deployment of new resistance sources. Development of DNA markers will permit pyramiding of genes to reduce the rate of resistance breakdown (9,14).

The orange wheat blossom midge, *Sitodiplosis mosellana* (Gehin) (Diptera: Cecidomyiidae), has become a pest of durum wheat in Canada. The midge larvae begin feeding on the developing wheat grain soon after anthesis and cause yield loss and reduced market quality in the case of nearly mature kernels damaged by feeding. Low tolerances for severely midge-damaged kernels in the No. 1 (0.1%) and No. 2 (0.25%) Canada Western Amber Durum grades (2) minimizes the reduction of gluten strength associated with midge damage. A natural antibiosis mechanism to combat the wheat midge was identified in common wheat (8), controlled by a single gene (15). A survey of diverse durum germplasm, however, found no antibiosis (12), so the antibiosis mechanism has been transferred from common wheat to durum through the use of a DNA marker (23). The first midge-resistant common wheats were submitted for registration in Canada in 2007.

The Sunn pest (*Eurygaster* spp.) is widespread in the Mediterranean region, and like the midge, it both reduces yield potential and dough strength through the secretion of proteolytic enzymes into kernels. There is recent evidence of variable resistance to the pest in common wheat (11,20), and there consequently exists the potential for selection in cultivar improvement by focusing on specific glutenin fractions affected by the protease (20).

The wheat stem sawfly, *Cephus cinctus* (Norton), is also a major insect pest in the Canadian durum production area. Related species occur in North Africa. Sawfly larvae cause yield loss by feeding on the inside of the stem wall, reducing the amount of nutrients available to the kernel, and by girdling the stems at ground level just before harvest, which causes them to fall over. This insect cannot be effectively controlled through the use of insecticides, but solid stems provide a physical barrier and resistance to sawfly larvae damage (10). No solid-stem durum cultivars are currently grown in Canada, but there is renewed interest in breeding solid stem cultivars due to increased incidences of sawfly infestation.

Reduced Heavy Metal Concentration

Excessive levels of essential micronutrients or nonessential trace elements, such as heavy metals, can be toxic to humans and animals (16). Levels of the nonessential heavy metal cadmium in food products have been a concern for many years, particularly in staple foods such as the cereal grains, which represent a large portion of the diet (24). Discussions under the FAO Codex Alimentarius seek to establish international standards, and the

Fig 1. Genetic variation for Fusarium resistance in experimental durum lines: intermediate resistance (left) and highly susceptible (right).

European Union recently instituted a limit of 0.2 ppm for cadmium in grains. Soils of the North American wheat production area contain elevated natural amounts of cadmium deriving from the parent material from which the soils formed. Human activities such as industrial contamination or application of cadmium-containing fertilizer are not a factor in the cadmium levels found in durum wheat from North America (19).

Research identified natural genetic variation for cadmium concentration and showed that low cadmium concentration in grains is controlled by a single dominant gene (3). Incorporation of this highly heritable trait into cultivars reduces the average grain cadmium to levels well below proposed international limits, averaging 50% lower than conventional durum cultivars. The allele for low concentration appears to be specific for cadmium and has generally no effect or inconsistent effect on concentrations of other ions, and it does not appear to detrimentally affect any major economic traits (5). A study in North Dakota demonstrated that there can also be up to a threefold variation in grain cadmium concentration among durum wheat lines grown in the same trial (13), apparently caused by genetic factors different from the single allele identified in Canadian breeding programs. A DNA marker (18) is now used to select for low grain cadmium concentration in Canadian durum breeding programs. The low cadmium cultivar Strongfield (6) entered commercial production in Canada in 2006.

Summary

Genetic enhancement of durum wheat plays an important role in the mitigation of food safety concerns. It can help address issues such as regulatory compliance for mycotoxin contamination and heavy metal concentrations, such as cadmium. Genetic enhancement also plays a significant role in the reduction of agro-chemical residues on grain. The food safety implications of genetic resistance lie in the reduced requirement for fungicides and insecticides to control diseases and pests. Biotechnology tools such as DNA markers are beginning to play a significant part in these genetic enhancement activities.

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