Challenges to Use of Genetic Selection for Reducing Cadmium Concentration in Crops.

Dr. Cynthia Grant
Agriculture and Agri-Food Canada Brandon Research Centre, Box 1000a, R.R.#3, Brandon, MB R7A 5Y3
Cynthia.Grant@agr.gc.ca

Abstract
Cadmium (Cd) is a trace element that is present in the soil naturally and from anthropogenic sources. Cadmium can accumulate in plants to levels that do not impair plant growth but may be detrimental to human health when the plant is consumed. Therefore, management practices have been evaluated to reduce the risk of Cd accumulation in crops. Natural variation occurs in the uptake and distribution of cadmium (Cd) among crop species and among cultivars within species. Considerable attention has been paid to selection of hyperaccumulating crops for phytoremediation to lower the concentration of Cd in soils by crop removal. Plant breeding can also play an important role by reducing the uptake and movement of Cd in to the edible portions of the plant or to increase the concentration of nutritionally beneficial trace elements that may reduce the absorption of Cd by the gut. Selection programs for a low Cd content of various crops, including durum wheat, sunflower, rice and soybean have been established and low-Cd durum wheat cultivars and sunflower hybrids have been developed. In durum wheat (*Triticum turgidum* L. var *durum*), low Cd concentration is controlled by a single dominant gene. The trait is highly heritable, and incorporation of the low-Cd allele can help to reduce the average grain Cd to levels below proposed international limits. The allele for low Cd concentration does not appear to affect major economic traits and should not cause problems when incorporated into durum cultivars. The cost of Cd selection in a breeding program is initially large both in terms of Cd determination and reduced progress towards development of other economic traits, but declines as more breeding lines in the program carry the low-Cd trait and are utilized in new crosses. Production of low-Cd crop cultivars can be used as a tool to reduce the risk of movement of Cd into the human diet, but an integrated approach including selection of suitable genetics in combination with other agronomic practices that can reduce Cd accumulation should be employed to limit Cd content in crops. To ensure long-term sustainability, it is also important to minimise Cd input into soils to avoid long-term Cd accumulation and deterioration of soil quality.
Keywords: Cadmium, genetics, selection

Introduction
Cadmium (Cd) is a trace element that is present in the soil naturally but is also added to soils from anthropogenic sources, such as application of sewage sludge and manure, irrigation water, fertilizers and soil amendments, and in atmospheric deposition [1] . Cadmium can accumulate in the human body over time from ingestion of food containing Cd, leading to a risk of chronic toxicity with excessive intake [2; 3] . The risk of Cd accumulation is particularly great from staple food crops that make up a large proportion of dietary intake. Therefore, it is desirable to limit the concentration of Cd in crops used for human consumption in order to reduce potential health risks.

Natural variation occurs in both the uptake and the distribution of Cd in crop species and in cultivars within species [4; 5; 6] . The accumulation and distribution of trace elements in plants is affected by factors including the size and morphology of the root system, production of root exudates that can mobilize elements, root association with microorganisms such as mycorrhizal fungi and root-colonizing bacteria, the amount and activity of transport systems across cell membranes, presence of intracellular binding sites, vacuolar sequestration, xylem loading and unloading, phloem transport and retention in the root [7; 8; 9; 10; 11; 12; 13] . These factors are under genetic control and therefore, plant breeding has been investigated as a means of reducing Cd concentration in the edible portions of crops [4; 6; 14; 15; 16] . Cultivar selection is an attractive method for changing the trace element profile of crops, as the benefit will persist in the seed.

Breeding and selection of low cadmium crops
If crops are not specifically selected for a low-Cd characteristic new cultivars may be randomly either lower or higher than traditional cultivars. For example, when modern sunflower hybrids were developed, it appears that the genotypes converted to inbreds had higher kernel Cd concentrations than random sunflower genotypes, resulting in an elevated Cd concentration in hybrid sunflower cultivars [17; 18] . Many of the Canadian durum wheat cultivars developed prior to the early 1990's also had relatively high concentrations of Cd in the seed [19] . However, genetic variability within a species provides an opportunity to actively select for lower Cd concentration [4; 6; 14; 15; 20; 21; 22] .

Development of commercially acceptable low-Cd crop cultivar requires a long-term program. Breeders must: 1) find genetic variation in the Cd concentration in existing germplasm; 2) learn the inheritance of the low-Cd genetic character; 3) develop a breeding strategy to combine low-Cd traits with high yields, disease resistance and other quality traits in modern cultivars; and 4) develop inexpensive methods to combine the low-Cd characteristic with other desired traits. Reliance on identifying low-Cd phenotypes by chemical analysis of the grain is more costly than many other conventional breeding activities due to the high cost of analysis, so selection for low Cd may place an economic burden on the breeding program.

A large number of studies have been conducted over the years to determine genotypic differences in Cd concentrations a range of crops, but only recently have market forces and health concerns provided a stimulus for breeders to actively select for low-Cd cultivars. Programs are now in place to develop low Cd grain crops, including durum wheat [4; 19; 20; 23; 24; 25; 26] , rice [15; 21; 22; 27; 28; 29] , soybean [14; 15; 22] and sunflower [17; 18; 26] .

In Canada, a breeding program was established in 1991 to reduce the Cd content of Canadian durum wheat. Initial surveys in the early 1990s showed that the cultivar Hercules and its derivative Arcola had a lower grain Cd concentration than other Canadian cultivars, with the concentration in Arcola being about half that in the predominant Canadian durum cultivar Kyle. Considerable genetic variation was also identified in grain Cd concentration of cultivars and lines introduced from sources such as CIMMYT and ICARDA international nurseries. Grain Cd concentration was found to be controlled by a single gene, with low Cd dominant in the crosses studied [4] . The low-Cd trait was highly heritable so selection on a single plant basis was feasible. Cadmium accumulation in the grain of the low-Cd cultivars was limited because of restricted root to shoot translocation [8; 30] , associated with lower Cd concentration in the xylem sap and reduced xylem sap exudation [7] . Cadmium concentration of leaf tissue was highly correlated with grain Cd concentration (r=0.87 to 0.89) and therefore could predict the plant phenotype, which was useful in backcrossing the low-Cd trait into high Cd cultivars [4]

Near-isogenic high/low grain Cd concentration lines were developed from five durum wheat crosses, with each pair being genetically uniform except for the Cd concentration trait [4] . Under field conditions, the average grain Cd concentration was approximately 2.5 times greater for the high than for the low isolines (Fig. 1) [25] . Average yield, grain protein concentration, test weight, kernel weight, days to maturity and lodging score were similar between the high and low Cd isolines. Under field conditions, concentration of other important trace elements such as Zn was generally similar in the high and low Cd lines. But, a solution culture experiment that assessed accumulation o Cd and Zn under varying levels of Zn nutrition indicted that when Zn supply was limited during early growth, the low-Cd isoline might be associated with a reduction in Zn accumulation in the grain [31] The low-Cd characteristic has been incorporated into newly registered durum wheat cultivars, with the first commercially-successful low Cd cultivar, Strongfield being released in 2004 [24] . All new durum cultivars released in Canada will carry the low Cd trait.

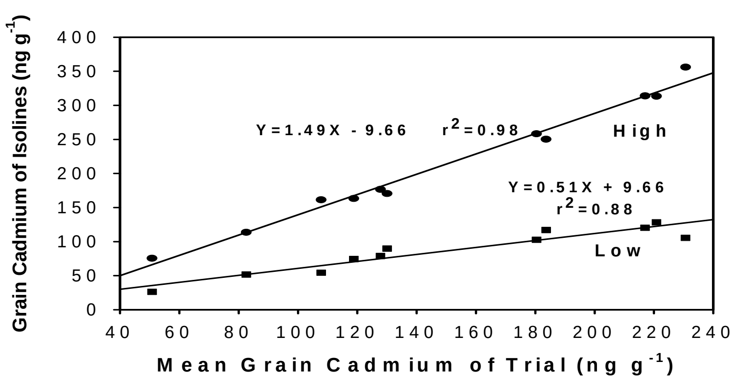

Figure 1. Grain cadmium concentration of high and low cadmium near-isogenic lines as a function of the average grain cadmium concentration of durum wheat grown at five locations in Saskatchewan and North Dakota in 1994 to 1996 [25] .

A similar program was undertaken in the United States to reduce the Cd concentration in sunflowers to comply with a guide value (*Richtwert*) of 0.6 mg Cd kg^{-1} dry weight (DW) established by Germany for Cd in confectionary sunflowers. Much of the sunflower crop in the Untied States is grown in North Dakota and Minnesota on poorly drained, fine textured soils, containing high background concentrations of chloride [17; 18; 32] . Under these conditions, sunflower seed had values reaching 1.33 mg kg^{-1} DW. In 1994, replicated field studies were conducted at four locations to evaluate two hundred sunflower genotypes, including plant introductions from various countries, USDA-ARS germplasm lines, and interspecific germplasm lines [17; 18; 26] . Several very low-Cd accessions were identified and a maintainer and a restorer line that were intermediate in Cd concentration were found among the USDA-ARS confection germplasm lines. The breeding program initiated in North Dakota has resulted in the release of lines with Cd concentrations substantially below the average value for Cd in sunflower and hybrids with a 50% reduction in kernel Cd concentration [33]

Table 1. Uptake of Cd by brown rice (μg plant^{-1}), concentration of Cd in brown rice, straw, and chaff (mg (kg DW)$^{-1}$), grain yield (g plant^{-1}), and partitioning ratio (% of total Cd uptake found in brown rice). Comparison of 38 cultivars [27]

Cultivar	Cd uptake	Cd concentration			Grain yield	Partitioning ratio
		Brown rice	Straw	Chaff		
	(μg plant^{-1})	(mg (kg DW)$^{-1}$)			(g plant^{-1})	(%)
Nanjing 40 (A[a])	0.97[b] n	0.06 m [c]	2.63 j	0.01 n	19.55 hi	7.94 b
Xiushui 11 (A)	1.96 mn	0.08 lm	2.38 k	0.13 m	29.61 d	5.64 c
Jiahua (A)	1.93 mn	0.09 lm	3.56 h	0.08 m	25.98 ef	8.05 b
Suhujing (A)	2.96 m	0.14 kl	2.28 k	0.09 m	25.56 ef	7.09 bc
Yang 9850 (B)	3.61 lm	0.32 j	4.39 f	0.43 h	14.58 j	6.81 bc
Nanjing 16 (B)	3.96 lm	0.42 h	4.31 f	0.31 i	15.18 j	10.62 a
Chunjiang 026 (A)	4.12 l	0.21 k	2.91 ij	0.16 l	23.68 fg	5.31 c
Chunjiang 25 (A)	4.27 l	0.17 kl	3.36 h	0.22 jk	30.32 cd	5.36 c
Qingliuai (B)	4.29 l	0.32 j	4.41 f	0.22 jk	17.31 ij	5.31 c
Chunjiang 016 (A)	4.71 l	0.21 k	2.92 ij	0.33 i	27.06 de	7.32 bc
Chunjiang 012 (A)	6.20 k	0.29 j	3.88 g	0.19 kl	25.81 ef	11.74 a
Jia 948 (B)	6.67 k	0.52 g	5.21 e	0.42 h	16.56 j	11.47 a
Jinqiu (A)	6.91 k	0.23 k	3.04 hi	0.26 j	36.24 ab	6.10 c
Zhonghua (A)	8.49 j	0.30 j	3.32 h	0.36 hi	34.14 bc	8.14 b
Zhe 2 (B)	9.31 ij	0.55 fg	4.32 f	0.35 hi	21.85 gh	8.42 b
Zhejiang 1500 (A)	9.37 ij	0.34 ij	3.50 h	0.50 gh	33.27 c	10.81 a
Fenyouzhao 11 (B)	9.57 ij	0.57 fg	5.54 de	0.58 f	21.67 gh	10.07 a
Zhou 903 (B)	10.37 hi	0.55 fg	5.22 e	0.43 h	24.31 fg	9.13 ab
Huangjin (A)	10.59 hi	0.31 j	2.75 j	0.17 l	41.22 a	10.71 a
Hang 931 (B)	10.65 hi	0.73 e	5.42 e	0.54 fg	18.84 i	9.64 a
Wuyujing 3 (A)	11.08 h	0.37 i	3.59 h	0.19 kl	36.15 ab	9.03 ab
Zhe 733 (B)	11.48 h	0.63 f	5.63 d	0.53 fg	23.52 fg	8.81 b
K41 (B)	11.81 gh	0.56 fg	5.39 de	0.47 gh	27.22 de	9.01 ab
K12 (A)	13.59 g	0.44 h	3.31 h	0.31 i	37.29 ab	7.84 b
Wuyujing 7 (A)	14.57 fg	0.50 g	4.17 f	0.47 gh	35.18 b	8.54 b
Wufuxian (B)	16.46 f	0.78 de	5.27 e	0.82 c	27.24 de	8.02 b
Zhongyouzhao 1 (B)	17.05 f	0.85 cd	6.55 bc	0.77 d	25.90 ef	7.02 bc
Zhenxian 866 (B)	17.71 f	0.76 de	5.66 d	0.79 cd	30.09 d	7.09 ab
Zhenongda 104 (A)	20.58 e	0.87 c	6.73 b	0.65 e	38.99 a	8.51 b
Zhefu 802 (B)	20.63 e	0.81 d	6.36 c	0.59 f	32.88 c	10.74 a
Zhongsi 3 (B)	20.99 de	0.99 a	7.12 a	0.91 a	27.37 de	8.63 b
Yuanfenzaoxian (B)	22.22 d	0.85 cd	6.35 c	0.87 b	33.75 bc	8.86 b
Zhe 1 (B)	24.91 c	0.97 a	6.71 b	0.79 cd	33.15 c	8.61 b
Zhaoxiangxian (B)	26.66 b	0.95 a	6.95 ab	0.77 d	36.23 ab	9.14 ab
Xiushui 110 (A)	27.29 ab	0.83 cd	6.56 bc	0.91 a	39.68 a	10.40 a
Jiayu 293 (B)	27.92 a	0.97 a	7.00 a	0.67 e	37.17 ab	7.34 bc
Zhongdao 98186 (B)	28.11 a	0.87 c	6.62 bc	0.79 cd	30.54 cd	9.09 ab
Nanjing 41 (A)	28.58 a	0.91 b	5.87 d	0.79 cd	37.90 ab	8.47 b
Average ± SD	12.69 ± 8.55	0.53 ± 0.03	4.79 ± 1.49	0.47 ± 0.26	28.76 ± 7.35	8.44 ± 1.68
Range	0.96–28.58	0.06–0.99	2.28–7.12	0.01–0.91	14.58–41.22	5.31–11.47

[a] A and B represent Japonica and Indica rice types, respectively.
[b] All data are means of a 3-year experiment.
[c] Values followed by the same letters within each column are not different by Duncan's Multiple Range Test at 5%.

Rice is a major staple crop, comprising about 20% of the caloric supply world-wide and over 30% in Asia i (http://beta.irri.org/solutions/images/stories/wrs/wrs_nov08_2008_table16_calorie.xls). Negative health effects from Cd consumption have been primarily associated with consumption of rice [34] . Rice can accumulate high concentrations of Cd, but also contains very low concentrations of Zn, Fe and Ca [34] . In a subsistence diet relying primarily on rice, deficiencies of Fe, Zn and Ca can lead to increased Cd absorption by humans, increasing the risk of adverse health effects. Therefore, reducing the Cd concentration of rice is of particular importance. Considerable variability in the Cd concentration has been found in rice (Table 1) including Japonica, Indica and hybrid rice [15; 21; 22; 27; 28; 35; 36; 37; 38; 39; 40; 41; 42] and breeding programs have been initiated to select for low-Cd cultivars. The relative ranking of rice cultivars appears to be similar under paddy and upland conditions [21] thus the low Cd trait should persist when the cultivars are grown in different environments. Quantitative trait loci for Cd concentration in rice have been identified and mapped [37; 43] which should facilitate the breeding of new cultivars of low-Cd rice. Additionally, high Cd lines of Japonica-Indica hybrid rice are being investigated for their potential in phytoremediation of contaminated sites [44]

Genetic variability in soybean [5; 14; 15; 22; 35; 45] and flax [26; 46; 47; 48] has been reported. Cadmium concentration in young tissue of soybean was correlated well to the final Cd concentration of the mature seed, which would facilitate breeding [15] Based on the importance of soybean as a staple food crop, development of low-Cd soybean cultivars should be a priority (Arao et al., 2003; Ishikawa et al., 2005a; Morrison, 2005; Arao and Ishikawa, 2006). Flax is often consumed as a health food, so there is interest in reducing the Cd content to enhance its healthy image. However, only small amounts of flaxseed are normally consumed in the human diet. Although there is interest in developing low-Cd flaxseed to encourage its consumption as a health-promoting food, a breeding program for reduced Cd would be of less importance than in the staple crops such as durum wheat, rice and soybean.

Challenges to use of genetic selection for reducing Cd concentration in crops

Use of plant breeding to develop well-adapted, low-Cd cultivars has great promise, but there are there are still challenges in utilizing this approach to reduce Cd concentration in crops. Development and testing of a new cultivar is time consuming, because the low-Cd characteristic has to be combined with many other essential traits such as high yield, agronomic suitability, quality, and disease resistance. For example, it was 10 years from the initiation of the Canadian breeding program for low-Cd durum wheat to the release in 2001 of the first low-Cd cultivar, named AC Napoleon. AC Napolean was not widely accepted by Canadian farmers and the first commercially successful low-Cd durum wheat cultivar was AC Strongfield, registered in 2004 [24; 49] Although low-Cd rice lines have been developed and included in crosses in Japan for several years, it may still be 4 or 5 years before a commercial cultivar acceptable to Japanese consumers is available (Arao – personal communication). The process of selecting for low Cd may be even more difficult and time-consuming in out-crossed crops than in crops that are self-pollinated.

The initial phase of conversion to low Cd cultivars is extremely cost, both in terms of Cd analyses as well as because less progress will be made in breeding for other economic traits due to the inclusion of low Cd as an essential selection characteristic. Chemical analysis for Cd is expensive, but is necessary to confirm that lines are actually low in Cd. Detection of low-Cd status early in growth allows selection at an earlier stage and will reduce the time and cost of the breeding program [15; 23; 35; 50] . The cost of selecting cultivars in a breeding program declines as more breeding lines carry the low-Cd trait. For example, the Canadian durum wheat programs now require much less Cd testing than when the program was initiated because a large pool of low Cd germplasm is available for use in new crosses. In addition, the development of a RAPD marker in durum wheat improved the efficiency of the screening process at a much lower cost than using chemical analysis [19] . Markers have also been found associated with a major gene affecting Cd accumulation in oat [51] .

Plant breeders already have a long list of characteristics that are required in each new cultivar that is produced. Yield, quality and disease resistance are basic factors that must be included, but traits such as herbicide tolerance, lodging resistance, drought resistance, insect resistance, and days to maturity are also considered. There is additional pressure to select for characteristics such as nutrient use efficiency and nutritional quality. The value of investing time and resources in selection for the low-Cd characteristic must be assessed relative to investment in selection for other characteristics when determining breeding priorities. In addition, Cd concentration in crops may be affected by other selection activities. If a crop is selected for aluminum (Al) tolerance so that it performs better on acid soils ($pH<5.5$), the low pH may encourage excess Cd accumulation, making it necessary to include genes to limit Cd uptake. Programs to encourage high accumulation of essential trace elements such as Zn in grain may inadvertently encourage Cd uptake as well, because of the biochemical similarity of these elements.

The Cd concentration of both low- and high-Cd cultivars will be influenced by both soil and management practices [52] In field studies across a range of environments and soil types, low-Cd lines of durum wheat were consistently lower than the high Cd lines at each site-year, but at sites where Cd availability in the soil was high, both

both low- and high-Cd cultivars produced Cd concentrations near the proposed 0.2 mg kg^{-1} limit [25] When flax was grown on a site in Manitoba, Canada that had high levels of phytoavailable Cd, both Vimy and McGregor flax cultivars contained excessive Cd concentrations, although McGregor was genetically lower [53] . Application of fertilizers, addition of Cd in fertilizers, biosolids or other soil amendments, the use of high-chloride irrigation water, soil salinity and soil acidification can all increase Cd phytoavailability in soil and Cd accumulation in low- or high-Cd crops [52; 54; 55; 56; 57; 58; 59; 60; 61; 62; 63; 64; 65] . Correction of Zn deficiencies, flooding of rice paddies combined with application of organic matter and possibly liming or addition of organic residues can reduce Cd uptake by crops [59; 64; 65; 66; 67; 68; 69; 70; 71] . Combining management practices that limit Cd accumulation with use of low Cd cultivars would be more effective at reducing Cd movement into the food chain than growing low-Cd cultivars alone.

The risk of toxicity from Cd in food is influenced not only by Cd concentration but also by concentrations of other trace elements such as Zn and Fe [34] Concentration of Fe and Zn is very low in cereal crops grown in many parts of the world. In response to this problem, breeding programs have been initiated to increase the concentration of essential trace elements and enhance the nutritional value of staple crops such as rice, wheat and maize [72; 73; 74; 75; 76; 77; 78] . Breeding programmes to increase concentrations of essential trace elements would have the combined benefit of enhancing the nutritional value of staple crops while potentially reducing the bioavailability of Cd, particularly if low-Cd was included as a selection criterion.

Although management practices and use of appropriate cultivars can decrease Cd in crops there is still a risk of long-term accumulation of phytoavailable Cd in agricultural soils that could increase the Cd concentration in both low- and high-Cd cultivars. Therefore, practices need to be implemented to reduce Cd input into soils in order to slow the rate of accumulation. Long-term accumulation of Cd in soils from fertilizers can be limited by reducing the amount of Cd added to the soil, through the use of use low-Cd fertilizers and by minimizing the rate of fertilizer used [52] . Production of low-Cd P fertilizer requires either that the source phosphate rock is low in Cd or that the Cd is removed during processing. Low-Cd phosphate rock is limited in supply and current methods of Cd removal would significantly increase the cost of the fertilizer, posing a major constraint to P management in low-value agricultural crops. It may be desirable to target the use of low-Cd P fertilizers to crops that tend to receive high P application rates and to soils where the Cd phytoavailability is high. Both Cd accumulation in soils from Cd in fertilizers and the indirect effects of fertilizers, including N and KCl, on enhancing Cd phytoavailability will increase with the rate of fertilizer application [52; 64; 79] . Therefore, it is desirable to minimize the rate of fertilizer applied to cropping systems. Excess applications of nutrients should be avoided, both to limit Cd input and to reduce the impacts on Cd phytoavailability. Reduction in fertilizer application rate may reduce crop yield unless the rate reduction is accompanied by an increase in nutrient use efficiency. Adoption of improved management practices, such as effective soil testing, fertilizer banding, and use of more efficient fertilizer sources suited to the soil, crop, and environment can allow reductions in P use while maintaining crop productivity.

Summary

Plant breeding can play an important role in reducing Cd in the human diet by decreasing Cd accumulation in the edible portions of the plant or by increasing the concentration of beneficial trace elements that may reduce the absorption of Cd by the gut. Selection programs for low Cd content of various crops, including durum wheat, sunflower, rice and soybean have been established and low-Cd durum wheat cultivars and sunflower hybrids have been developed. The cost of Cd selection in a breeding program is initially large both in terms of Cd determination and reduced progress towards development of other economic traits, but declines as more breeding lines in the program carry the low-Cd trait and are utilized in new crosses. Production of low-Cd crop cultivars can be used as a tool to reduce the risk of movement of Cd into the human diet, but an integrated approach including selection of suitable genetics in combination with other agronomic practices that can reduce Cd accumulation should be employed to limit Cd content in crops. To ensure long-term sustainability, it is also important to minimise Cd input into soils to avoid long-term Cd accumulation and deterioration of soil quality.

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