UC Davis program for low-Cadmium durum wheat. Jorge Dubcovsky

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Background information

The presence of the low Cd allele *Cdu1*, decreases by more than half [(1)] the normally high grain Cd concentration shown by durum wheat cultivars when grown in Cd-rich soils as those present in the region where Desert Durum® cultivars are grown. The most probable mechanism by which Cd is reduced in the grain in this genetic system is the restricted Cd translocation from the roots to the shoots [(2)]. Based on the limited Cd translocation to the shoot, it is possible to screen low-Cd genotypes at early stages because plants carrying the low Cd allele show lower Cd concentration in the leaves as well as in the grain [(3)].

Proposed objectives and summary of results

After the first year of the grant all five specific objectives were completed.

1.- *Screen the highest yielding Desert Durum lines from the UCD breeding program and those submitted by the private programs to the Regional Wheat Trials with the molecular markers for the Cdu1 gene.*

Objective 1 was completed: seven lines were identified as putative carriers of the *Cdu1* gene based on the linked molecular marker OPC-20: 'Havasu' and YU803-52 (WestBread, LLC); D00627 and Ca501 (Arizona Plant Breeding); 'Rio Colorado' (CIMMYT); UC1308 and UC1374 (UCD).

2.- Evaluate the best Desert Durum lines from the public breeding program for Cd content in field experiments in Imperial Valley.

Objective 2 was completed: All the lines with the missing band in OPC-20 except for Ca501 were confirmed to be low Cd in field experiment 09250 in Imperial Valley. Seven lines were confirmed to be low Cd and also have the linked molecular marker OPC-20 (missing band): Havasu, YU803-52, D00627, Rio Colorado, UC1308, UC1374 (UCD).

3.- Establish a Desert Durum crossing block in the public breeding program including only low Cd varieties.

Objective 3 was completed: 15 F_1 hybrids were produced and F_2 seed has been obtained and transferred to the breeding program. The F_2 seed will be planted in the field at UCD in November 2009 to advance the F_3 generation. A few hybrid combinations were not obtained since the flowering times did not matched.

4.- Initiate the introgression of the Cdu1 low-Cd allele from Strongfield into the top varieties and breeding lines from the public breeding program.

Objective 4 was completed: Backcross one (BC_1) and BC_1F_2 were produced for the following lines: UC1113, Desert King, D99-425 BC2, and Kronos. For Wetmore and Do4AZ-335 we are one generation behind and we ar just producing the BC_1 crosses.

5.- Improve the molecular marker for the Cdu1 gene.

Objective 5 was completed: We developed a new molecular marker linked to OPC-20 but which shows a band linked in coupling with the low cadmium gene *Cdu1* instead of in repulsion.

1. Methodology for early screening

A greenhouse experiment was conducted in February 2008 to validate the methodology of an early screening for low Cd. Three plants of each of the following genotypes: Kofa (high Cd), Strongfield (low Cd), and UC1308 (low Cd), were grown within each pot using soil from the Desert Research and Station Center in El Centro, CA. Nine replications (pots) were used for each cultivar/breeding line. Approximately two months after planting, leaves were collected from all plants in each pot and dried in the oven. At the time of collection most plants had the flag leaf completely exposed. The material was then ground and sent for Cd determination at UC Davis. The results (Table 1) showed a significant difference ($P<0.0001$) in leaf Cd concentration between the high Cd cv. Kofa and the low Cd genotypes, Strongfield and UC1308.

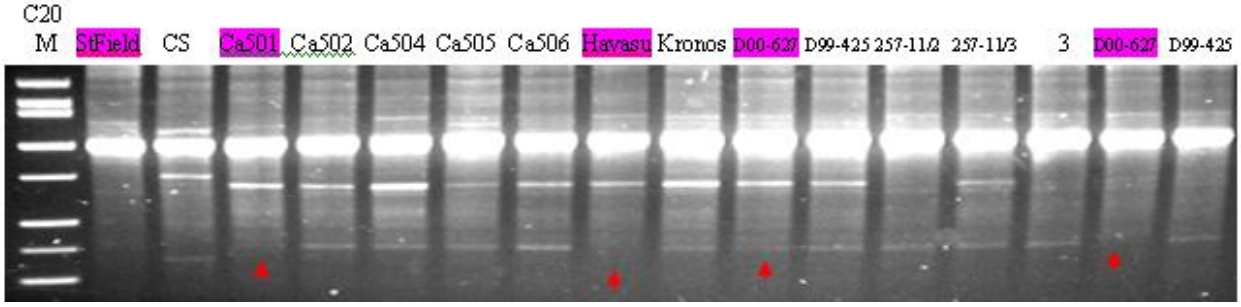
Table 1. Leaf Cd concentrations of three durum lines grown in the greenhouse.

Line	N	Leaf Cd conc. mg kg^{-1}
Kofa	9	2.53 ± 0.10
Strongfield	9	0.89 ± 0.10
UC1308	7	0.84 ± 0.13

Conclusion: This methodology can be applied to screen for low Cd genotypes in the greenhouse. This methodology does not require the complete development of the plant and the production of grain, accelerating the determination of the phenotype.

2. Screening of California and Arizona varieties

The standard donor of the low-Cd trait is the Canadian cv. Strongfield (Agriculture and Agri-Food Canada [4]). We used the RAPD marker OPC-20 [5] to assess the presence/absence of the low-Cd trait in a number of genotypes. The absence of a 350 bp fragment is associated with the *Cdu1* allele for low cadmium.



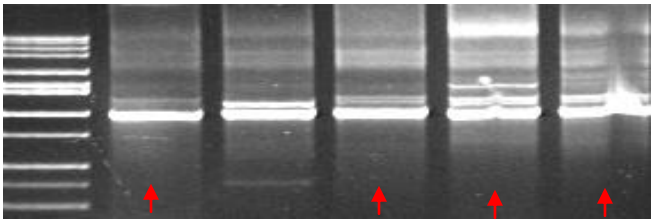


Fig. 1. Screening of CA and AR durum lines with marker OPC-20. Red arrows indicate the absence of the 350-bp band associated with the low-Cd allele

We detected the absence of the 350-bp fragment in the following lines

WestBread, LLC: 'Havasu' and YU803-52
Arizona Plant Breeding: D00627 and Ca501
CIMMYT: Rio Colorado
UC Davis: UC1308 and UC1374

All these lines are predicted to have the low-Cd allele, but since there is the possibility of recombination between the OPC20 marker and the Cdu1 gene it was necessary to confirm these results in field experiments.

3. Field evaluation of Cd levels

To confirm the grain Cd concentration of the lines showing the low Cd allele included in the crossing block, a row experiment was planted in December 2008 at the Desert Research and Station Center in El Centro, CA. A total of 15 lines were included in this experiment (Table 3) that was designed as an RCBD with 4 replications. This experiment was harvested in May 2009 and tests for Cd were completed in August 2009.The results are presented in Table 2 below.

Table 2. Cadmium levels in mg kg^{-1} from experiment 09250 (Imperial Valley, Randomized Complete Block Design with 4 replications)

Pedigree	Rep1	Rep2	Rep3	Rep4	**AVG.**	Class
Ca501	0.46	0.32	0.37	0.33	0.37	High
D00-627	0.27	0.21	0.23	0.23	**0.24**	**Low**
Desert King	0.36	0.29	0.32	0.34	0.33	High
Desert King-*Gpc*	0.39	0.36	0.38	0.38	0.38	High
Kronos	0.52	0.31	0.36	0.38	0.39	High
Kronos-*Gpc*	0.32	0.37	0.37	0.39	0.36	High
Rio Colorado	0.22	0.19	0.20	0.22	**0.21**	**Low**
Strongfield	0.17	0.19	0.21	0.18	**0.19**	**Low**
UC1113	0.28	0.33	0.27	0.27	0.29	High
UC1113-*Gpc*	0.27	0.34	0.38	0.29	0.32	High
UC1308	0.19	0.18	0.18	0.20	**0.19**	**Low**
UC1308-*Gpc*	0.22	0.20	0.21	0.21	**0.21**	**Low**
UC1374	0.22	0.21	0.25	0.27	**0.24**	**Low**
YU803-52	0.20	0.20	0.23	0.22	**0.21**	**Low**

Four measurements of Cd level in the soil of this experiment showed an average of 0.4 ppm

This field experiment corroborated that almost all the lines that have the missing band for the OPC20 marker had low levels of Cd in the grain (<0.20 mg kg^{-1}).

The only exception was line Ca501, which likely has a recombination event between the marker and the low cadmium gene *Cdu1*. Based on this last result this last line was eliminated from the crossing blocks between low Cd lines.

4. Improvement of the marker for Low Cadmium

The current molecular marker OPC-20 is a dominant marker (in repulsion) that can only differentiate plants carrying two copies of the low-Cd allele from the rest. This marker cannot differentiate plants carrying one (heterozygous) or two (homozygous) copies of the high-Cd allele, which is a problem for Marker Assisted Backcrossing strategies.

Using an STS marker developed by Dr. Curtis Pozniak from Canada (CADF1/CAD394R) we discovered a larger fragment (~1kb, yellow arrow) that was almost always present when the OPC-20 350-bp band was absent.

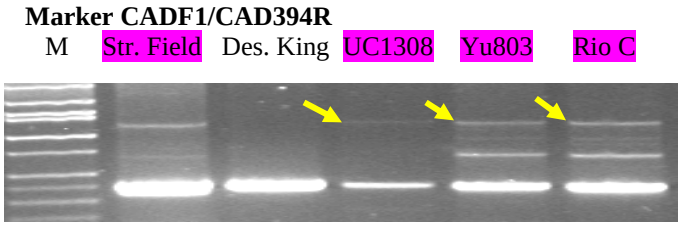


Fig. 2. Dominant marker associated with the presence of the low-Cd allele

Conclusion: Since the CADF1/CAD394R band is present in the lines with the low-Cd allele (OPC-20 was absent), it can be used to differentiate the heterozygous carriers of the low-Cd allele from the dominant lines for the high-Cd allele. This is a very useful tool to accelerate a marker assisted backcrossing program.

5. Mapping of the ~1kb band STS marker

To determine the relative position of the ~1kb STS marker relative to OPC20, we analyzed the Cd levels of five lines that showed recombination between the last two markers (in 180 tested).

Table 3. Recombinants between OPC20 – Cdu1 - ~1kb band STS marker.

Genotype	OPC20	Cd	1-kb band
High Cd lines	OPC20+	High Cd	1kb NO
Low Cd lines	OPC20abs	Low Cd	1kb yes
Recombinant lines			
Ca510	OPC20abs	High Cd	1kb NO
UC1406 / UC1308	OPC20abs	Low Cd	1kb NO
WWW D6523 / UC1308	OPC20abs	High Cd	1kb NO
Platinum / UC1308	OPC20abs	Low Cd	1kb NO
UC1375 / UC1308	OPC20abs	Low Cd	1kb NO

Conclusion: the fact that the 1 kb marker ~1kb STS marker disappears in the two recombinants between OPC20 and *Cdu1* and that it is also missing in 3 lines that are both low Cd and have the missing OPC20 band, indicates that the ~1kb STS marker maps on the other side of the *Cdu1* gene relative to OPC20.

6. Low Cadmium crossing block

On November 2008, a crossing block was planted at three different planting dates. The design and lines included are presented in Table 4. The objective of this crossing block is to generate segregating populations in which the low-Cd trait is fixed.

Table 4. Design of the crossing block for low-Cd.

Line	UC1374	Strongfield	Havasu	D00627
UC1308-Gpc		X	X	X
UC1374		X	X	X
Strongfield			X	X
Havasu				F
Rio Colorado	X	X	X	X
YU803	X	X	X	F

X = Successful cross; **F** = Failed cross

The F_1 seed produced from the different crosses were planted in Tulelake, CA in May 2009 and harvested September 9 2009. The F_2 seeds from these segregating populations will form the basis of a durum breeding program fixed for low Cadmium.

In the crosses between low and high-Cd durum lines, we will continue to use the OPC20 and the STS molecular markers to increase the frequency of the low-Cd allele.

7. Marker assisted backcrossing program

One problem for the previous approach is that almost all the current best varieties and breeding lines are high-Cd and are not included in the crossing block. This can delay the identification of low-Cd varieties with a yield potential and quality similar to the current high-Cd varieties.

Therefore, in parallel with the breeding program described above we are advancing a marker assisted selection backcrossing effort to accelerate the introgression of the low-Cd allele in the top yielding varieties and breeding lines.

For the *Cdu1* allele we have completed the crosses and first generation of backcrosses (BC_1 and BC_1F_2) in the following genetic backgrounds: Dessert King, UC1113, APB- D99-425, and Kronos. For Wetmore and Do4AZ-335 we are one generation behind and we are just producing the BC_1 crosses

References

[1] Clarke, J.M., D. Leisle, and G.L. Kopytko. 1997. Inheritance of cadmium concentration in five durum wheat crosses. Crop Sci. 37: 1722–1726.

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