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# Genotypes at the *BoCAL* Locus in Broccoli, Cauliflower, and Purple Cauliflower Accessions

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## Rationale

- Broccoli and cauliflower are different botanical varieties of the same species (*Brassica oleracea*), with very different phenotypic traits. The genetic basis for these differences is not well understood.

- There are several genes influencing differences in flower morphology between broccoli and cauliflower. A mutant allele at the locus *BoCAL* has been implicated in conferring the cauliflower phenotype (curding) (Kempin et al., 1995; Smith and King, 2000).

- We tested the correlation between the *BoCAL* mutant allele and cauliflower phenotype in a wide variety of broccoli, cauliflower, and purple cauliflower accessions.

- Understanding the genetic basis of curding can lead to insights about the evolution of morphological diversification within species (Purugganan et al., 2000). Such knowledge will be useful to breeders and curators working with *B. oleracea*.



Fig 1a. De Cicco, broccoli

*B. oleracea* v. *italica*



Fig 1b. Yeh Erh Fu, cauliflower

*B. oleracea* v. *botrytis*



Fig 1c. Violet Queen, purple cauliflower

*B. oleracea* v. *italica*

## Materials and Methods

Table 1. Twenty six *B. oleracea* accessions genotyped at the *BoCAL* locus.

Name	I.D.	Sample size
Norwegian Broccoli	PI 443022	9
Ramoso	PI441510	5
Yeh Erh Fu	PI430580	8
Russian Broccoli	PI 285596	8
Ramono	PI 231210	6
Broccoli China 2	G 31825	8
Broccoli China	G 31824	25
Broc.3	G 30009	17
Wellsbourne	04 5295	21
Unnamed	04 7519	3
Broccoli Natale Lopa	PI 462210	1
Broccollette Neri e Cespuglio	PI 462209	1
Cavolo Ramoso Calabrese	PI 462206	9
De Cicco	G 32213	10
Cauliflower	PI 115881	3
Zeus F1	G 30416	1
Pinnacle F1	G 30414	1
Premium Crop F1	G 30415	1
Shogun F1	G 30774	1
Packman F1	G 30778	1
Green Comet F1	G 30413	1
Green Comet-b F1	G30413	1
Green Harmony F1	G30769	1
Violet Queen	G30439	10
Cavolo broccolo precoce	G30928	5
Violetto	PI462222	6
Total		163

-DNA extraction from ~50 to 100 mg plant leaf tissue was performed using a miniprep DNA extraction protocol (Colosi and Schaal, 1993).

- The *BoCAL* gene was isolated from the genome by PCR.

- An *SpeI* restriction enzyme digest was performed to distinguish between the wild-type and mutant *BoCAL* alleles.

- Genotypes were visualized on 2% agarose, 0.5x TBE gels and scored as either homozygous wild-type (+/+), heterozygous (+/-), or homozygous mutant (-/-) (Fig. 2).

- Phenotypes were observed for all plants in the field.

## Results

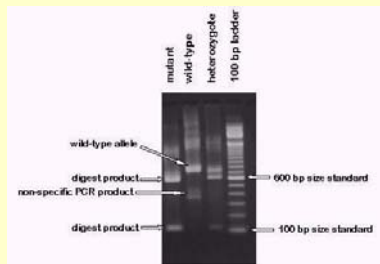


Fig 2. Agarose gel electrophoresis of PCR amplified fragments representing mutant and wild-type *BoCAL* alleles after restriction digestion with *SpeI*, as compared to 100-base pair size standard.

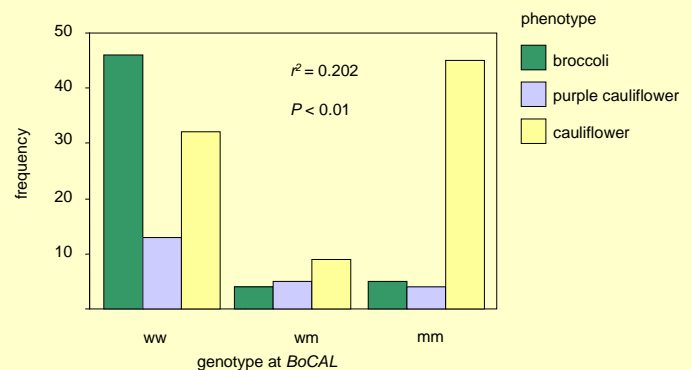


Fig. 3. Frequency of *BoCAL* genotypes in broccoli, cauliflower and purple cauliflower plants (w = wild-type allele, m = mutant allele).

## Conclusions

- Results indicated that genotype at the *BoCAL* locus was significantly correlated with phenotype (Fig. 3).

- All three genotypic classes contained all three phenotypes, and the mutant allele was neither necessary nor sufficient to generate the cauliflower phenotype.

- There was no evidence that the gene action of the wild-type allele was dominant.

- Additional loci and additional mutations at *BoCAL* could explain our findings. It is also possible that this *BoCAL* mutant allele is found at a relatively high frequency in cauliflower but is not a causative agent of curding phenotype.

- Selection for morphological change in *B. oleracea* continues to be important (Fig. 4). Understanding the molecular basis of curding could be valuable for marker-assisted selection.



Fig 4. Zeus, small-beaded broccoli

*B. oleracea* v. *italica*

## References

- Colosi, J.C. and B.A. Schaal 1993. Tissue grinding with ball bearing and vortex mixer for DNA extraction. *Nuc. Acids Res.* 21:1051-1052.
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