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### **177 Utility of BoCAL-1 and BoAP1-a Genotypes in Identifying Broccoli and Cauliflower Accessions**

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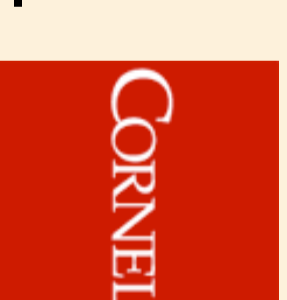
Broccoli and cauliflower are different botanical varieties of *Brassica oleracea*. Mutant alleles at the loci *BoCAL* and *BoAP1* can cause arrest at curding that is characteristic of cauliflower. These genes control early floral differentiation, necessary for the progression from a cauliflower-like inflorescence to the flower buds of broccoli. To what extent is the cauliflower-to-broccoli variation within the USDA-PGRU collection determined by mutant alleles of these genes? We surveyed the broccoli collection to examine the correlation between genotype and phenotype. Earlier work showed that *BoCAL* alone was not an effective predictor of cauliflower phenotype in this collection. The redundant function of *BoCAL* and *AP1* in determining inflorescence arrest raises the possibility that the combined genotype can explain the phenotypic variation. We found that not to be the case. Two accessions varied in phenotype and segregated at both loci, but the combined genotypes were not associated with the expected phenotypes. Two additional accessions varied in phenotype and segregated at one locus, but with no association between genotype and phenotype. One line varying widely in phenotype was fixed for both loci. One line that was a stable intermediate phenotype segregated for *BoCAL*. A commercial broccoli cultivar had the cauliflower allele at both loci. The genetic basis of the cauliflower phenotype in the USDA *B. oleracea* collection is due more to alleles of genes affecting the expression of *BoAP1* and *BoCAL* than to variation in these alleles of the genes themselves.

# Utility of *BoCAL-a* and *BoAP1-a* Genotypes in Identifying Broccoli and Cauliflower Accessions

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ABSTRACT

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## INTRODUCTION

Broccoli and cauliflower are different botanical varieties of the same species (*Brassica oleracea*), with different phenotypes. Commonly found mutant alleles at the loci *BoCAL-a* and *BoAP1-a* have been implicated in causing arrest at curding that is characteristic of the cauliflower phenotype (Smith and King, 2000). These genes cause early floral differentiation, necessary for the progression from a cauliflower-like inflorescence to the flower buds of broccoli. Accessions within the USDA PGRU broccoli collection exhibit phenotypic variation ranging from cauliflower to broccoli. We surveyed this broccoli collection to test the correlation between *BoCAL-a* and *BoAP1-a* genotype and curding phenotype.

## OBJECTIVES

1. Determine whether the genotype at CAULIFLOWER (*BoCAL-a*) and APETALA1 (*BoAP1-a*) are diagnostic for the cauliflower and broccoli forms of *Brassica oleracea*.
2. Determine whether variation among and variation within lines is affected by alleles at the *BoCAL-a* and *BoAP1-a* loci.

## MATERIALS AND METHODS

We surveyed the USDA collection of broccoli to test the correlation between the *BoCAL-a* and *BoAP1-a* genotypes and phenotype (Fig. 1) in 19 accessions and 8 F1 hybrids of *B. oleracea*. All the accessions were scored for phenotype in 2001, 10 variable or non-flowering accessions were rescored for phenotype in 2002. The genotype was determined by amplifying a region of the *BoCAL-a* gene containing the mutation sequencing the amplicon to determine whether the variable nucleotide was a G (wt) or a T (mutant), or by amplifying a variable length polymorphism that distinguishes alleles of *BoAP1-a*. All the lines were planted in the field and evaluated for the stage of inflorescence arrest at harvest maturity.

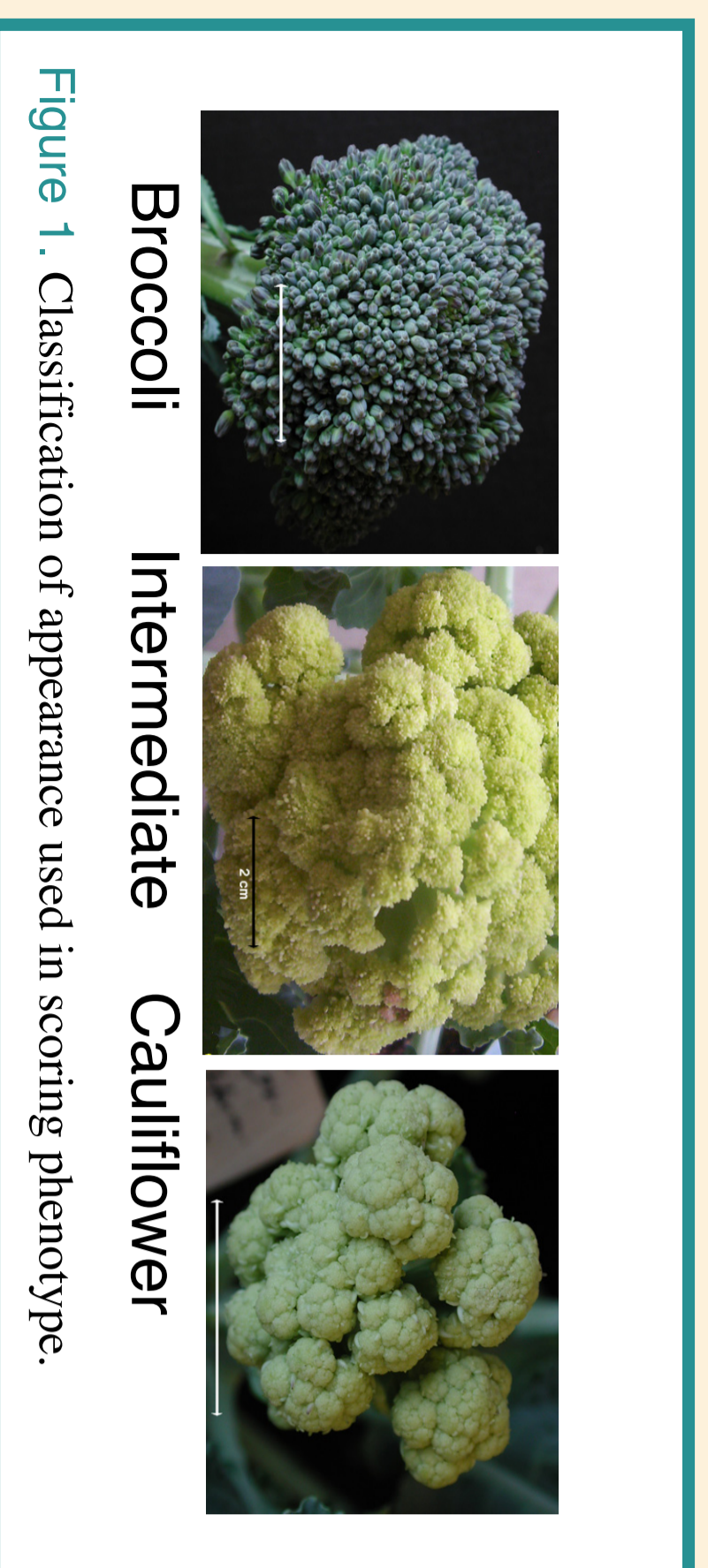


Figure 1. Classification of appearance used in scoring phenotype.

## LITERATURE CITED

Smith, L.B. and G.J. King. 2000. The distribution of *BoCAL-a* alleles in Brassica oleracea is consistent with a genetic model for curd development and domestication in cauliflower. *Molecular Breeding* 6:603-613

## Acknowledgements

Thanks to Graham King and Lee Smith at Warwick HRI for sharing their genotyping method for *AP1-a*. Susan Sheffer provided excellent technical support, and Gemaro Fazio provided access to his Bio-Rad GeneAnalyzer to genotype *BoAP1*.

## RESULTS

**VARIATION AMONG LINES:** The ability to predict the phenotype of an accession from a simple DNA assay would be useful for classifying the broccoli collection. The predictive power was assessed by comparing the accessions previously designated as broccoli in the collection.

Four different genetic models for control of the phenotype were considered, and the correlation between the prediction and the actual phenotype calculated (Table 1.) The additive allele model was strongest ( $P = 0.03\%$  in 2001,  $P = 1.6\%$  in 2002), but only the *BoCAL*-alone model was rejected ( $P = 30.9\%$  in 2002).

No model has a strong enough correlation to be useful for classifying germplasm. Even though the correlations are highly significant, the predictive value is low. A Pearson  $r$  of 0.26 means that the genetic model explains about 7% of the variation in phenotype among these accessions.

Discriminant analysis using all four models at once shows a posterior probability of a correct determination as 50%. (SAS DISCRIM procedure) Many broccoli are predicted to be cauliflower or intermediate. For the purpose of classifying germplasm, we require a value over 95%, preferably 99%.

	2001 (n=211)		2002 (n=101)	
	Pearson r	P	Pearson r	P
<i>BoCAL</i> alone	0.17	1.6%	0.10	30.9%
<i>BoAP1</i> alone	0.21	0.02%	0.20	4.3%
Additive alleles <sup>1</sup>	0.25	0.03%	0.24	1.6%
Dominant by locus <sup>2</sup>	0.26	<0.01%	0.21	3.9%

<sup>1</sup> Assumes loci have redundant function and dominant alleles are additive across loci  
<sup>2</sup> Assumes loci are additive but alleles are dominant within locus as predicted by Smith and King (2000)

Table 2. The specific predictions of each model in Table 1, and the number of plants fitting each outcome. Correct predictions (green) are in the diagonal from the upper left. The genotype codes are A = *BoAP1-a*, a = *Boap1-a*, C = *BoCAL-a*, c = *bocal-a*.

Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	24	2	2	-
Intermediate	68	5	1	1
Broccoli	180	42	17	-

Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	15	2	2	9
Intermediate	32	12	28	28
Broccoli	80	25	134	-

Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	14	2	10	-
Intermediate	31	14	27	1
Broccoli	53	34	127	19

Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	aacc	Aacc	AaCc	AACC
Genotype > Phenotype	14	11	41	1
Intermediate	31	41	3	3
Broccoli	53	164	32	-

**GENETIC VARIATION WITHIN LINES:** Within one mapping population, the genotype at *BoCAL-a* and *AP1-a* consistently predicts broccoli, cauliflower and intermediate phenotypes (Smith and King, 2000). We examined whether that was true in two accessions that varied both in genotype for these genes and phenotype (Table 3.) Neither accession exhibited such an association. In addition two accessions that varied in phenotype segregated at one of the two loci, but there was no association (Table 3 a and b). Segregation at *BoCAL-a* may not affect the phenotype (HRI 5295). Conversely, phenotypic variation was great in one line (Green Harmony) that was fixed for both loci.

The observed phenotypic variation that is not explained by these genes is likely to have three sources. First, there may be other segregating genes that directly influence inflorescence arrest. Second, environmental conditions, principally temperature, affect developmental arrest. Third, other segregating genes may influence the degree of environmental sensitivity.

Accession	Genotype	Phenotype
China (G 31824)	Segregated for <i>AP1/ap1</i> , fixed <i>cal</i> (Tab. 3a)	
Ramoso (P1 441510)	Segregated for <i>CAL/cal</i> , fixed <i>ap1</i> (Tab. 3b)	
India (P1 115881)	Segregated for <i>AP1</i> and <i>CAL</i>	
Precoce (G 30928)	Segregated for <i>AP1</i> and <i>CAL</i>	
HRI 5295	Segregated at <i>CAL</i> , fixed <i>AP1</i> , uniformly intermediate	
Green Harmony	Fixed genotype, despite varying phenotype. This accession is illustrated in Figure 1.	

Genotype > Phenotype	aacc	Aacc	AACC
Genotype > Phenotype	aacc	Aacc	AACC
Genotype > Phenotype	1	-	-
Intermediate	9	4	2
Broccoli	3	4	-

Genotype > Phenotype	Aacc	aacc	aACC
Genotype > Phenotype	Aacc	aacc	aACC
Genotype > Phenotype	2	1	-
Intermediate	1	1	1
Broccoli	5	9	14

## CONCLUSION

While the mutant *bocal-a* and *boap1-a* alleles are known to be able to cause a cauliflower phenotype, other genes appear to have substantial control over the same process in other genetic backgrounds. Although there is a highly significant effect of these genes on the phenotype, it is so small that these genes alone do not have utility in classifying germplasm.