

cite this poster as:

Sheffer, S.M., J.A. Labate, T.N. Björkman, L.D. Robertson, and A.M. Baldo. 2004. *BoGSL-ELONG* as a candidate diagnostic marker in phenotypically diverse *Brassica* populations. Eastern Great Lakes Molecular Evolution Meeting VIII. Ithaca, NY.

BoGSL-ELONG as a Candidate Diagnostic Marker in Phenotypically Diverse *Brassica* Populations

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Rationale

~ Domestication has led to extreme morphological divergence among botanical varieties within *Brassica oleracea* (the cole crops – broccoli, cauliflower, collard etc.). The glucosinolate gene *BoGSL-ELONG* was previously isolated from *Brassica oleracea*. A 30-bp deletion mutant allele was reported to be associated with cauliflower, and the wild-type allele with collard and broccoli (Li and Quiros, 2002).

~ One of the objectives of the Plant Genetic Resources Unit, a germplasm repository, is to better characterize and utilize the seeds collections. The purpose of this study is to test whether the cauliflower phenotype, but not the broccoli phenotype, is associated with the *BoGSL-ELONG* mutant allele in a broader range of phenotypically segregating populations.

~ This would provide a diagnostic marker to distinguish between broccoli and cauliflower seeds and seedlings without the usual time and money it takes to grow the plant to maturity.

Materials and Methods

~ Plants were sampled from 21 diverse *B. oleracea* populations, DNA was isolated using 50 to 100 mg plant leaf tissue, CTAB extraction protocol (Colosi and Schaal, 1993) and Spex Certiprep™ genogrinder.

~ *B. oleracea* primers IPMS9 and IPMS2 (Li and Quiros 2002) were used to amplify the *BoGSL-ELONG* gene under the following PCR conditions: 94°C for 3 minutes, 35cycles of 94°C for 1 minute, 52°C for 1 minute, and 72°C for 1 minutes, followed by a 10 minute extension at 72°C.

~ PCR products were scored on 2% MetaPHOR agarose, 0.5x TBE gels based on band molecular weight.

~ Phenotypic data describing plants scored as broccoli or cauliflower were collected for 2001 and 2002 field seasons on a total of 345 plants sampled from 21 populations.

Table 1. Twenty-one phenotypically diverse *Brassica* populations genotyped for *BoGSL-ELONG*.

Population ID	Name	Sample size
PI 46222	Violetto	11
PI 462206	Cavolo Ramoso calabrese	10
PI 443022	Norwegian broccoli	9
PI 441510	Ramoso	37
PI 430580	Yeh Erh Fu	8
PI 249556	Bhug-Gana	22
PI 231210	Romano	5
PI 115881	Cauliflower	49
HRI 5295	Cavolfiore Violetto di Sicilia	38
G 32213	De Cicco	10
G 32210	High Sierra F1	10
G 31824	Broccoli China	38
G 30928	Cavolo Broccolo Precoce	39
G 30778	Packman F1	3
G 30774	Shogun F1	2
G 30769	Green Harmony F1	9
G 30416	Zeus F1	16
G 30415	Premium Crop F1	3
G 30414	Pinnacle F1	2
G 30413	Green Comet F1	2
G 30009	Broc 3	22
Total		345 plants

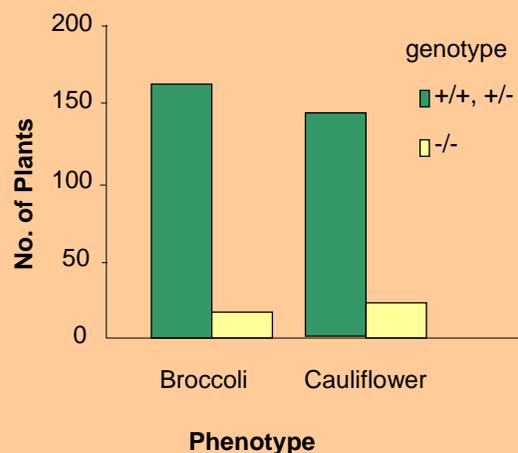
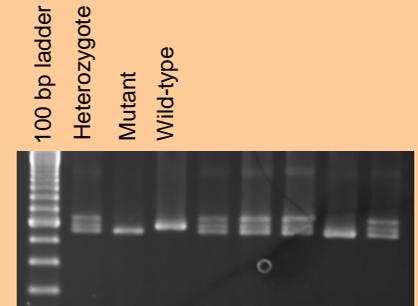


Fig.1 Frequencies of genotypes within phenotypic classes. +/+ = wild-type; +/- = heterozygote and -/- = mutant allele.

Fig.2 *BoGSL-ELONG* genotypes on 2% metaPHOR agarose gel



Results and Conclusions

~ Based on previous results (Li and Quiros, 2002) we predicted that the following genotypes would be associated with the broccoli and cauliflower phenotypes, respectively: +/+ and +/- with broccoli and -/- with cauliflower.

~ Preliminary data do not show *BoGSL-ELONG* to function as a diagnostic marker distinguishing between cauliflower and broccoli phenotypes. Frequencies of genotypes are not associated with their predicted phenotypes.

~ Two additional PCR products were observed among the 21 populations sampled (data not shown). We will sequence all of PCR products observed in this study to better distinguish between the various alleles.

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.

Li, G. and C.F. Quiros. 2002. Genetic analysis, expression and molecular characterization of *BoGSL-ELONG*, a major gene Involved in the aliphatic glucosinolate pathway of *Brassica* species. *Genetics* 162: 1937-1943.