# VARIETY DETERMINATION BY DIFFERENTIAL ISOZYME RESPONSES USING ELECTROPHORETIC STARCH GELS

Steven Godby and Veronica Gruppo

## ABSTRACT

Hybrid varieties of the sugarbeet, Beta vulgaris L., are thought to have a large population variability. Pure parental genetic lines are not attained by self-pollination as is done in some grains. The purpose of this research was to determine whether genetic variability is as great as believed or stable enough within a variety to use polymorphism at isozyme alleles to identify specific varieties. Thirteen varieties were studied in order to identify a wide range of characters. Thirty-six individual seeds/lot from three lots of each variety were used to monitor seed lot variability. Extracted isozymes from seedlings or imbibed seeds were collected on filter paper wicks, inserted into starch gels and electrophoresed. The resulting migration patterns were then recorded for nine alleles. The intravarietal responses by each of the thirteen tested varieties showed that seed lot differences appear to be minimal enough that varieties probably can be compared on an intervarietal basis. Comparisons can be made between varieties by using combinations of observed isozyme pattern distributions at specific alleles. There were two pairs of varieties that responded with very similar isozyme patterns for the majority of the alleles. There is a slight possibility that two of these varieties are related and cannot be identified with this procedure.

## INTRODUCTION

International competition within the sugarbeet industry has greatly increased the number of varieties available for the sugarbeet producer, yet to date there is not an acceptable method to identify a variety other than morphologic and agronomic descriptions. In at least one crop, corn, polymorphic isozymes have been used extensively as markers to identify cultivars by their unique responses. Sugarbeets, in contrast, have been presumed to have an intravariety variability that would prevent a reliable system using isozymes from being developed. The argument that isozymes should or should not be used to patent or register varieties has been discussed extensively with the discussion being

centered around acceptable ranges for the variablility of the isozyme responses, environmental effects on the isozymes, and the criteria for an acceptable isozyme variety marker (see Bailey, 1984). This research is concerned with all of these factors, but only to the point where varieties can be identified on an individual basis for a limited number of varieties.

Most of the written literature has shown that it is possible to use polymorphic enzymes as markers to identify varieties, but very little has been written about sugarbeet varieties. Levites and Garifullina (1987) observed and recorded unique responses within four varieties for six enzymes at nine loci. Using their data, they felt that there were significant intervarietal differences at some of the loci. Van Geyt and Smed (1984) described eight enzymes and their concomitant enzyme responses. Their research included several methods for determining isozyme responses as well as descriptions of the resulting banding patterns.

This paper describes the responses of nine polymorphic enzymes in thirteen varieties with the primary purpose of establishing a tool for varietal identification of the varieties that are tested and marketed in the Western Sugar Company's production area.

## MATERIALS AND METHODS

Three lots of seed were collected for 13 varieties of monogerm sugarbeet seed. The seed lots were sampled from seed sold in 1988 and 1989 in order to investigate possible differences in seed lots from year to year. Approximately 36 seeds were taken from each lot for a total of 108 individual seeds for each variety. Enzyme extraction was accomplished in two ways, depending on the enzyme that was being observed. For the enzymes alcohol dehydrogenase (ADH1), glutamate dehydrogenase (GDH2) and malic enzyme (ME1), the seeds were soaked at room temperature overnight to facilitate embryo removal. The imbibed embryo was removed, crushed in a buffered extract solution as described by Van Geyt and Smed (1984). The enzymes were then soaked up into filter paper soaked from the extraction solution.

For aconitase (AC0), isocitric dehydrogenase (IDH1) malate dehydrogenase (MDH1 and MDH2), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM), the seeds were soaked for four hours then put in wet paper towels for five days at 25 degrees centigrade. After five days, the germinated seedlings were crushed in a .005 M diethyldithiocarbamate solution for enzyme extraction. This solution was then wicked up into filter paper wicks.

Wicks saturated with enzyme extract were inserted into starch gels and eletrophoresed to isolate each individual seed's isozyme components.

The varieties that were sampled were from seed marketed in the Western Sugar Company production area by five sugarbeet seed companies. The varieties and companies are: ACH 184, ACH 164, and ACH 191 from the American Crystal Sugar Company; KW 3265, KW 3295, and Beta 4689 from Betaseed, Inc; HMI 55, HMI R2, HMI 5891, HMI 6176 and HMI 1605 from Hilleshog Mono-hy, Inc.; HH50 from Holly Sugar Company; Monohikari from Seedex, Inc.

The zymogram for each isozyme was stained and scored using scoring systems described by Levites and Garifullina (1987) for ADH1, MDH1, MDH2 and ME1; Van Geyt & Smed (1984) for GDH2, IDH1, PGI, and PGM; and Seed Testing of America's scoring system for ACO.

Table 1 is the range of the lot responses for each variety and isozyme. The data compiled in Table 2 reflects the results for each variety in terms of the average percentages of different protein pattern responses observed for each allele.

## RESULTS AND DISCUSSION

There was a wide range of responses both between and within varieties (see Table 1 and Table 2). Generally, the isozyme response distributions appeared to be consistent among the three production lots which were tested for each variety. Isozyme response distributions did not seem to be affected by differences in production year or location.

The isozyme distribution patterns for MDH1, PGI, PGM, ME1, GDH2,and IDH offered the greatest potential for differentiating varieties. There were several varieties that showed distinctive response distributions on a single allele, but a minimum of two isozyme alleles were usually needed for a confident determination of variety (see Table 3).

Identifying varieties within single companies is a primary concern and appears to be possible for eleven of the thirteen varieties. Each seed company applies a different colored dye to their seed, thus enabling the division of the varieties into small groups for comparison. The grouping by company has proved itself important because two varieties, Monohikari and HMI 1605, are very similar at most of the alleles. Table 2 shows the observed isozyme response patterns for both varieties. There appears to be a difference at the PGI allele, but because there are no other signigicant differences at any other alleles, it is very difficult to make a confident varietal determination due to the lot to lot variability (Table 1). This allele may prove to be a significant marker once several more lots of both varieties are sampled, but until then these two varieties can be identified using their isozyme response patterns in conjunction with their dye color, unless one of the companies begins marketing both varieties.

TABLE 1. Intravarietal allele variability expressed as the range between the high and the low isozyme response for the three lots tested per variety.

| ALLELE | ACH 164 | ACH 191 | HH 50 | HMI 55 | HMI 5891 | HMI 6176 | KW 3265 | KW 3295 | MONO-HIKARI | BETA 4689 | ACH 184 | HMI 1605 | HMI R2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PGI-1 | 3 | 3 | 16 | 11 | 14 | 14 | 3 | 11 | 3 | 24 | 17 | 28 | 13 |
| PGI-2 | 17 | 17 | 5 | 3 | 8 | 6 | 3 | 0 | 3 | 6 | 3 | 0 | 11 |
| PGI-1,2 | 19 | 18 | 12 | 8 | 8 | 8 | 8 | 10 | 6 | 21 | 9 | 28 | 10 |
| MDH1-N | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 6 | 3 | 6 |
| MDH1-M,N | 11 | 8 | 3 | 11 | 14 | 8 | 25 | 25 | 12 | 24 | 18 | 0 | 23 |
| MDH1-N,P | 11 | 25 | 0 | 3 | 17 | 25 | 11 | 6 | 17 | 3 | 0 | 33 | 11 |
| MDH1-M,N | 17 | 17 | 3 | 8 | 19 | 22 | 19 | 19 | 18 | 24 | 24 | 33 | 18 |
| MDH2-S | 8 | 0 | 8 | 0 | 0 | 3 | 6 | 3 | 0 | 3 | 6 | 3 | 0 |
| MDH2-F | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| MDH2-F,S | 8 | 0 | 11 | 0 | 0 | 3 | 6 | 3 | 0 | 0 | 6 | 3 | 0 |
| ACO-1 | 24 | 16 | 16 | 17 | 0 | 28 | 17 | 8 | 0 | 6 | 0 | 3 | 17 |
| ACO-1+ | 24 | 16 | 16 | 17 | 0 | 28 | 17 | 8 | 0 | 6 | 0 | 3 | 18 |
| PGM-1 | 9 | 17 | 9 | 14 | 14 | 20 | 22 | 3 | 40 | 22 | 32 | 6 | 11 |
| PGM-2 | 3 | 3 | 0 | 0 | 3 | 9 | 7 | 8 | 3 | 3 | 6 | 0 | 0 |
| PGM-1,2 | 12 | 17 | 9 | 14 | 17 | 29 | 25 | 11 | 43 | 22 | 31 | 6 | 11 |
| IDH1-1 | 14 | 0 | 6 | 35 | 11 | 17 | 17 | 12 | 17 | 24 | 6 | 20 | 8 |
| IDH1-2 | 6 | 0 | 0 | 8 | 6 | 4 | 14 | 14 | 6 | 0 | 0 | 3 | 6 |
| IDH1-1,2 | 19 | 3 | 6 | 38 | 8 | 17 | 8 | 18 | 21 | 24 | 6 | 20 | 8 |
| ADH1-F | 24 | 0 | 14 | 0 | 0 | 4 | 9 | 8 | 0 | 0 | 0 | 9 | 0 |
| ADH1-F,S | 23 | 0 | 13 | 0 | 0 | 4 | 9 | 8 | 0 | 0 | 0 | 9 | 3 |
| GDH2-B | 6 | 4 | 5 | 5 | 19 | 27 | 20 | 24 | 27 | 9 | 3 | 36 | 8 |
| GDH2-B,C | 4 | 9 | 4 | 8 | 15 | 27 | 7 | 18 | 27 | 9 | 6 | 28 | 6 |
| GSH2-C | 17 | 6 | 0 | 0 | 6 | 0 | 39 | 5 | 0 | 0 | 0 | 8 | 3 |
| ME1-S | 5 | 10 | 8 | 26 | 13 | 5 | 6 | 0 | 0 | 14 | 10 | 6 | 21 |
| ME1-F | 7 | 3 | 5 | 3 | 0 | 18 | 11 | 17 | 0 | 39 | 10 | 3 | 0 |
| ME1-F,S | 2 | 7 | 7 | 24 | 13 | 16 | 6 | 17 | 0 | 26 | 17 | 9 | 21 |

TABLE 2. Average percent isozyme responses observed in composite samples of thirteen sugarbeet varieties.

| ALLELE | ACH 164 | ACH 191 | HH 50 | HMI 55 | HMI 5891 | HMI 6176 | KW 3265 | KW 3295 | MONO-HIKARI | BETA 4689 | ACH 184 | HMI 1605 | HMI R2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PGI-1 | 1 | 9 | 35 | 5 | 50 | 71 | 16 | 8 | 15 | 45 | 19 | 46 | 11 |
| PGI-2 | 48 | 16 | 20 | 9 | 7 | 2 | 13 | 25 | 1 | 3 | 1 | 0 | 8 |
| PGI-1,2 | 51 | 75 | 45 | 86 | 44 | 27 | 71 | 66 | 84 | 52 | 80 | 54 | 81 |
| MDH1-N | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 |
| MDH1-M,N | 21 | 4 | 99 | 27 | 11 | 40 | 51 | 40 | 4 | 51 | 33 | 0 | 26 |
| MDH1-N,P | 14 | 37 | 0 | 5 | 43 | 23 | 7 | 4 | 72 | 2 | 0 | 65 | 5 |
| MDH1-M,N | 65 | 59 | 1 | 69 | 45 | 37 | 42 | 57 | 25 | 47 | 65 | 33 | 68 |
| MDH2-S | 95 | 100 | 94 | 100 | 100 | 99 | 91 | 94 | 100 | 99 | 89 | 99 | 100 |
| MDH2-F | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| MDH2-F,S | 5 | 0 | 6 | 0 | 0 | 1 | 8 | 7 | 0 | 0 | 10 | 1 | 0 |
| ACO-1 | 72 | 73 | 84 | 87 | 89 | 56 | 86 | 88 | 100 | 88 | 100 | 99 | 87 |
| ACO-1+ | 28 | 27 | 16 | 13 | 11 | 44 | 14 | 12 | 0 | 12 | 0 | 1 | 13 |
| PGM-1 | 3 | 5 | 10 | 6 | 7 | 17 | 14 | 4 | 28 | 14 | 14 | 24 | 95 |
| PGM-2 | 1 | 2 | 0 | 0 | 1 | 7 | 16 | 14 | 1 | 2 | 9 | 0 | 0 |
| PGM-1,2 | 96 | 94 | 90 | 94 | 93 | 76 | 70 | 82 | 71 | 84 | 78 | 76 | 5 |
| IDH1-1 | 61 | 98 | 93 | 51 | 16 | 34 | 38 | 28 | 29 | 53 | 83 | 33 | 39 |
| IDH1-2 | 5 | 0 | 0 | 4 | 36 | 26 | 18 | 17 | 3 | 0 | 0 | 5 | 13 |
| IDH1-1,2 | 35 | 2 | 7 | 45 | 48 | 40 | 44 | 55 | 69 | 47 | 17 | 63 | 48 |
| ADH1-F | 69 | 100 | 90 | 100 | 100 | 99 | 97 | 97 | 100 | 100 | 100 | 97 | 99 |
| ADH1-F,S | 31 | 0 | 10 | 0 | 0 | 1 | 3 | 3 | 0 | 0 | 0 | 3 | 1 |
| GDH2-B | 37 | 65 | 87 | 89 | 57 | 64 | 17 | 18 | 44 | 93 | 89 | 60 | 90 |
| GDH2-B,C | 53 | 34 | 13 | 11 | 40 | 36 | 65 | 72 | 56 | 7 | 11 | 38 | 9 |
| GDH2-C | 11 | 1 | 0 | 0 | 3 | 0 | 18 | 10 | 0 | 0 | 0 | 3 | 1 |
| ME1-S | 10 | 30 | 46 | 78 | 18 | 5 | 2 | 0 | 0 | 10 | 6 | 2 | 55 |
| ME1-F | 10 | 5 | 26 | 1 | 0 | 69 | 94 | 77 | 100 | 19 | 4 | 93 | 0 |
| ME1-F,S | 80 | 65 | 28 | 22 | 82 | 26 | 4 | 23 | 0 | 71 | 90 | 5 | 45 |

TABLE 3. Possible isozyme test sequence for determining varieties.

| VARIETY | ALLELE1 | ALLELE2 | ALLELE3 |
|---|---|---|---|
| ACH 164 | PGI1 | ADH | MDH1 |
| ACH 191 | MDH1 | PGI | GDH |
| ACH 184 | GDH | IDH | MDH1 |
| HMI 55 | ME1 | PGM | MDH1 |
| HMI 5891 | IDH | MDH1 | ME1 |
| HMI 6176 | PGI | ME1 | MDH1 |
| HMI 1605 | PGI | MDH1 | ME1 |
| HMI R2 | PGM | ME1 | PGI |
| MONOHIKARI | PGI | MDH1 | ME1 |
| HH 50 | MDH1 | IDH | PGI |
| BETA 4689 | MDH1 | PGI | GDH |
| KW 3265 | PGM | PGI | ME1 |
| KW 3295 | PGM | PGI | ME1 |

Two other varieties KW 3265 and KW 3295, present a more difficult problem, because their isozyme response patterns also are very similar and they both are marketed by Betaseed, Inc.

The PGI and ME1 alleles reveal some possibilities for two secondary responses, but because the observed differences are so slight and lot to lot variation looks sigificant, more lots must be sampled.

The MDH2 isozyme distribution patterns revealed very little potential for varietal determination for the varieties that were tested. However, this allele may be useful for testing genetic purity, since all of the sugarbeet varieties appear to respond very similarly at this locus.

The isozyme distribution patterns for ACO and ADH1 are too simlar within all the varieties to be useful for varietal identification. The possible exceptions to this are the distinctive responses of the varieties HMI 6176 for ACO and ACH 164 for ADH1. It is not clear whether these responses could be useful, because of the extreme variability observed between seed lots (see Table 1).

## CONCLUSIONS

The accumulated data implies that each of the thirteen varieties that have been tested have a relatively distinct "genotype" of isozyme distribution patterns when all of the pertinent alleles are considered at once. Nine of the varieties probably can be identified by progressively testing specific alleles and comparing the resulting isozyme distribution patterns to the database accumulated by testing several lots of each variety.

For the varieties that could not be distinguished, more seed lots must be tested. The number of seeds and seed lots that must be tested will be determined by the amount of population variability observed within these individual varieties. In addition, future studies of sugarbeets would be advanced by background information regarding the origin and development of individual lines.

## ACKNOWLEDGEMENTS

We would like to express our gratitude to the Western Sugar Company - Grower Joint Research Committee for their financial support.

## LITERATURE CITED

Bailey, D.C. "Isozymic Variation and Plant Breeders Rights." In Tanksley, S.D. and T.J. Orton, ed. Isozymes in Plant Genetics and Breeding, Part A. Amsterdam: Elsevier Science Publishers B.V.; 1983: 425-440.

Levites, E.V. and F.S. Garifullina (1987). "Use of Isozymes as Genetic Markers for Identification of Sugar-Beet Varieties." Proceedings of I.S.T.A. International Symposium, Leningrad, U.S.S.R. 104-109, 1987.

Stuber, C.W. "Techniques and Scoring Procedures for Starch Gel Electrophoresis of Enzymes from Maize (Zea maize L.)." Technical Bulletin 286, North Carolina State University, Raliegh, North Carolina, March, 1988.

Van Geyt, J.P.C.F. and E. Smed (1984). "Polymorphism of Some Marker Enzymes of the Sugarbeet (Beta vulgaris L.) Investigated by Polyacrylamide Gel Electrophoresis and Starch Gel Electrophoresis." Z. Pflanzenzuchtg. 92, 295-308, 1984.