THE ASSESSMENT OF DISTANCE BETWEEN *SUGARY-1* INBRED LINES DEVELOPED AT THE AGRICULTURAL RESEARCH AND DEVELOPMENT STATION TURDA

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ABSTRACT

The sweet corn has not benefited from yield gains due to genetic improvement as field corn has. Possible reasons include the narrowness of the genetic base of sweet corn, the lack of defined heterotic groups and the greater effort devoted to improving field corn. Most of the publicly available inbreds were derived from three cultivars: Golden Bantam, Stowell's Evergreen, Country Gentleman. Ten new *sugary-1* inbred lines developed at the Agricultural Research and Development Station Turda were examined by pedigree information, morphological traits, additive genetic effects (ĝ), phenotypic heterosis (H%), electrophoresis of the seed storage proteins (zein). Based on cluster analysis of these data sets the ten *sugary-1* inbred lines can be divided into two major groups, very early inbreds that contain Golden Bantam or Northern Flint germplasm (TA 28, SW 87) and late inbreds with Southern Dent (TD 282, TD 103, TD 102). Several inbreds were not closely aligned with either group (TA 22, TD 101). The amount of genetic diversity present in these inbreds can be considered sufficient to obtain high yield and good taste sweet corn hybrids.

Key words: cluster analysis, genetic diversity, *sugary-1* inbred relationships

INTRODUCTION

In the last 50 years, yield gains due to the genetic improvement of sweet corn were modest as compared to those of field corn (Duvick, 1984). Several reasons can account for this situation, such as:

- the narrow genetic base of sweet corn; according to some authors (Galinat, 1971; Doebley et al., 1988) it is derived mainly from Northern Flint and its evolution tend to seriously narrow variability (Doebley et al., 1986);
- non-Northern Flint germplasm, represented by the „Evergreen" and „Country Gentleman" cultivars, has emerged from the introgression of the *sugary-1* gene in the dent germplasm (Galinat, 1971; Tracy, 1990);
- excessive use of „Golden Bantam" type in developing *su-1* inbred lines (as a source of yellow endosperm and for its good eating quality) (Huelsen, 1954; Galinat, 1971). Most of present sweet corn germplasm contains, in various proportions, „Golden Bantam" genes in its pedigree (Gerdes and Tracy, 1994).

However, RFLP analyses emphasize a significant diversity in sweet corn germplasm, in spite of the intense use of „Golden Bantam", „Stowell's Evergreen" and „Country Gentlemen" germplasms (Tracy, 1994; Gerdes and Tracy, 1994; Revilla and Tracy, 1995). On the other hand, in contrast with field corn, sweet corn lacks defined heterotic groups (Goodman, 1985).

Several methods were used in order to establish the relations of several germplasm sources and the genetic diversity of sweet corn: the pedigree method (Smith, 1988; Smith et al., 1990), morphological distinctions (Rhodes and Carmer, 1966; Smith, 1988; Goodman and Brown, 1988; Revilla and Tracy, 1995a; Haş et al., 2002), electrophoretic analysis of izoenzyms (Doebley et al., 1988; Revilla and Tracy, 1995b; Rotari et al., 1996; Haş et al., 2002), genetic traits such as heterosis (Smith, 1988), RFLP analysis (Gerdes and Tracy, 1994), molecular markers (Drinić et al., 2000). Morphological traits are capable of showing both identity and distinctness, but these data are affected by environmental interaction. Heterosis data provide estimates of genetic relatedness between lines.

According to Smith and Smith (1989a, 1989b), using morphological traits, chromatographic data and electrophoretic analysis of izoenzyms represents a starting point in establishing genetic diversity of inbred lines, whereas heterotic or RFLP analyses offer clearer data on the relations between lines. The purpose of this study has been to evaluate the genetic diversity and the relationship of ten *sugary-1* inbred lines newly developed at the Agricultural Research and Development Station Turda. To this end, specific methods were used:

- phenotypic methods based on biometric analysis and phenotypic distance values;
- genetic methods based on additive genetic distance values, as well as the analysis of the genetic parameters correlations and the degree of the phenotypic heterosis;

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- biochemical methods based on the electrophoresis of seed storage proteins (zein) and the visual evaluation of densitometer scans.

MATERIAL AND METHODS

Ten *sugary-1* inbred lines developed at the Agricultural Research and Development Station Turda and 30 F_1 hybrids obtained by crossing these lines in an incomplete diallel system with reciprocals were used in this study. They were planted on Corn Breeding Laboratory Turda, in two years. The experimental design was a randomized complete block with three replicates. Ten plants were selected at random from each plot at harvest (at 21 days after silking date) and they were measured (22 traits/plant). Environment (year), block and hybrids or parental lines were the factors involved in the combined analysis of variance. A factorial (m • n + n • m) matting design [Design 2 of Comstock and Robinson (1952) and adapted by Căbulea et al. (1994)] was employed for analyzing the 2-yr data. The backgrounds of all lines are given in table 1 (Haş, 2000).

Table 1. Background and genealogy information for the ten new *sugary-1* inbred lines developed at the Agricultural Research and Development Station Turda

No	Inbred line	Background	Genealogy
1.	SW 87	Supersweet- S.U.A.	2500-2-1-1
2.	TA 22 *su*	Syn. Q 206 Canada	3603-1-1-7
3.	TA 27 *su*	Reward – S.U.A.	5103-6-3-5
4.	TA 28 *su*	Golden Beauty – S.U.A.	7188-1-1-3
5.	TD 103 *su*	How Sweet It Is – S.U.A.	7208-1-1-1
6.	TA 25 *su*	Reward – S.U.A.	3610-2-4-1
7.	TA 26 *su*	Reward – S.U.A.	5093-1-1-1
8.	TD 101 *su*	Aux 5651 – S.U.A.	3607-2-1-3
9.	TD 282 *su*	Silver Queen – S.U.A.	3870-10-2-1
10.	TD 102 *su*	Aromatnaja – R. Moldova	7208-3-1-1

For the preliminary analyses, traits were assigned to one of three groups (Smith et al., 1991), according to the complexity of their genetic control (Table 2). Trait Group 1 contained characters that seem to be controlled by many independent loci. Trait Group 2 contained vegetative and reproductive characters that seem to be controlled by one to few independent loci of moderate genetic complexity. Trait Group 3 was comprised of characters that are under simple, generally major locus, genetic control. Assigning morphological traits into different groups allowed the option of weighting distances between lines on the basis of the complexity of genetic control for each trait. This provided the opportunity to more effecttively measure morphological distance in terms of the extent of genetic diversity between pairs of inbred lines (Table 3).

Weighted and unweighted morphological distances between lines were calculated by using all distance values, even when the distance for an individual trait between a pair of lines fell below the 5% level of significance.

Distance values were calculated as follows (Smith et al., 1991):

$$\sum_{i=1}^{n}\left[\frac{(T1(i)-T2(i))^2}{\mathrm{var}(T(i))}\right]^{1/2}$$

where T1 is the value of the i^{th} trait for line 1. T2 is the value of the i^{th} trait for line 2, and var (T(i)) is the variance of the difference for the trait.

The evaluations of yield heterosis was calculated according to Hallauer and Miranda (1981):

$$H\,\% = \frac{F1 - \frac{(P1 + P2)}{2}}{\frac{(P1 + P2)}{2}}$$

Biochemical measures of distance were calculated using isoenzymic data from zein endosperm seed storage proteins chromatography, and also from electrophoretic profile of zein proteins (Rotari et al., 1996).

Since pedigrees of all lines were known, it was possible to determine differences between lines derived from morphological or biochemical data.

Polygenic diversity was evaluated by calculating additive genetic effects (ĝ) within the factorial system:

$$\hat{g}_i \text{ or } \hat{g}_j = \frac{\sum x_i}{i} - \frac{\sum x_\bullet}{i \times j}, \text{ (Căbulea, 1975)}$$

x_i = sum of values where parent „i" is constant;

$\Sigma x_\bullet$ = sum of all values in the factorial system.

Correlation coefficients (r) of the additive genetic effects (ĝ) for the analyzed traits were also calculated in order to emphasize the degree of coancestry.

Table 2. Morphological traits measured in the present study

Trait	Units	Description
Trait Group 1		
Ear weight	g	Weight of ear without husk (mean of 30 ears/year/line)
Husk weight	g	Weight of husk/ear
Ear length	cm	Length of ear
Husk length	cm	Length of husk
Ear diameter	mm	Diameter of ear without husk
Number of kernel rows	no.	Number of kernel rows per ear
Cob diameter	mm	Diameter of cob
Kernel content:		
- dry matter(DM)	%	Kernel content in dry matter at harvest
- sucrose	% / %DM	Kernel content in sucrose at harvest
- total sugar	% / %DM	Kernel content in total sugar at harvest
- WSP*	% / %DM	Kernel content in phytoglycogen at harvest
- fats	% / %DM	Kernel content in fats at harvest
- proteins	% / %DM	Kernel content in proteins at harvest
- starch	% / %DM	Kernel content in starch at harvest
Eating quality	note	The taste of kernels at harvest
GDU** to silking	Σ^0 C	Heat units accumulated from sowing to 50% silk emergence
GDU** to harvest	Σ^0 C	Heat units accumulated from sowing to harvest (21 days after silking)
Plant height	cm	Distance from soil level to base of tassel
Ear height	cm	Distance from soil level to base of main ear
Tiller number	no.	Mean number of tillers per plant
Trait Group 2		
Ear topfill	note	1. uncovered; 5.top ear covered by kernel;
Kernel depth	cm	Length of kernel (mean of 5 per ear)
Pericarp thickness	μ	Thickness of pericarp
Leaf number	no.	Number of leaves per plant
Leaf area	cm^2	(length x width leaf of main ear)/0.75
Tassel branch number	no.	Number of branches per tassel
Ear shape	note	1. Conical shape; 5. cylindrical shape;
Trait Group 3		
Silk color	note	1. red; 5. white;
Kernel color	note	2. white; 5. yellow;
Cob color	note	1. red; 5. white;

Table 3. The 45 possible pairs of the 10 *su 1* inbred lines

No.	The pairs of inbred lines	No.	The pairs of inbred lines	No.	The pairs of inbred lines
1.	SW 87 x TA 22	16.	TA 22 x TD 282	31.	TD 103 x TA 25
2.	SW 87 x TA 27	17.	TA 22 x TD 102	32.	TD 103 x TA 26
3.	SW 87 x TA 28	18.	TA 27 x TA 28	33.	TD 103 x TD 101
4.	SW 87 x TD 103	19.	TA 27 x TD 103	34.	TD 103 x TD 282
5.	SW 87 x TA 25	20.	TA 27 x TA 25	35.	TD 103 x TD 102
6.	SW 87 x TA 26	21.	TA 27 x TA 26	36.	TA 25 x TA 26
7.	SW 87 x TD 101	22.	TA 27 x TD 101	37.	TA 25 x TD 101
8.	SW 87 x TD 282	23.	TA 27 x TD 282	38.	TA 25 x TD 282
9.	SW 87 x TD 102	24.	TA 27 x TD 102	39.	TA 25 x TD 102
10.	TA 22 x TA 27	25.	TA 28 x TD 103	40.	TA 26 x TD 101
11.	TA 22 x TA 28	26.	TA 28 x TA 25	41.	TA 26 x TD 282
12.	TA 22 x TD 103	27.	TA 28 x TA 26	42.	TA 26 x TD 102
13.	TA 22 x TA 25	28.	TA 28 x TD 101	43.	TD 101 x TD 282
14.	TA 22 x TA 26	29.	TA 28 x TD 282	44.	TD 101 x TD 102
15.	TA 22 x TD 101	30.	TA 28 x TD 102	45.	TD 282 x TD 102

RESULTS AND DISCUSSION

1) Morphological data

We tested the ability of morphological data to identify genetically related and unrelated inbred lines from a diverse germplasm.

Correlations of distance measures based on morphological data set and those derived from heterosis and inbreds origin, show that associations among lines on the basis of morphology were essentially at random to any association that might be expected on the basis of heterosis or of inbred line origin. Even when morphological data were additionally constrained by known origin, using canonical variate analysis (Smith and Smith, 1989) correlations

between those modified morphological distances with heterosis measures were very low (Figure 1).

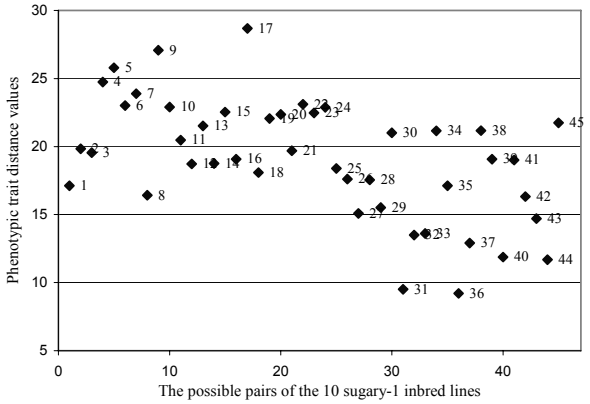

Figure 1 - Phenotypic trait distance values among the possible pairs of the 10 *sugary-1* inbred lines (Group I)

The morphological data showed that the inbred line pairs had range distances from:

- 9.2 to 11.9 (Group I) (Figure 1) inbred lines more similar for morphological traits:

Inbred line pairs	Morphological distances	Phenotypic heterosis (%)
TA 25 ↔ TA 26	9.2	35
TD 103 ↔ TA 25	9.5	65
TD 101 ↔ TD 102	11.7	22
TA 26 ↔ TD 101	11.9	47

- 21.2 to 28.7 (Group I) (Figure 2) inbred lines less similar for morphological traits:

Inbred line pairs	Genetic distances	Phenotypic heterosis (%)
TA 22 ↔ TD 102	28.7	36
SW 87 ↔ TD 102	27.0	15
SW 87 ↔ TA 25	25.8	44
TA 27 ↔ TD 102	23.0	52

Although morphological data have traditionally provided the sole set of descriptors for the purposes of cultivar identification and registration, they cannot reliably be used to estimate distances between maize cultivars that are reflective of their origin or genetic associations.

2) Genetic data

The genetic data (for ear weight) showed that the related pairs had a range of interline additive genetic effect distances from:

- 8.9 to 13.4 (Group I) (Figure 2) for more similar genetically inbred lines:

Inbred line pairs	Genetic distances	Phenotypic heterosis (%)
SW 87 ↔ TA 26	8.9	27
SW 87 ↔ TA 25	11.5	35
SW 87 ↔ TA 22	12.4	53

- 25.7 to 32.9 (Group I) (Figure 2) for less similar genetically inbred lines:

Inbred line pairs	Genetic distances	Phenotypic heterosis (%)
TA 27 ↔ TD 101	30.8	43
TA 27 ↔ TD 102	30.3	52
TA 22 ↔ TA 27	30.3	47

The inbred lines: TA 25, TA 26 and TA 27 have a common origin, but only TA 25 and TA 26 are more similar for their morphological and genetic distances:

Inbred line pairs	Morphological distances	Genetic distances	Phenotypic heterosis (%)
TA 27 ↔ TA 25	22.5	29.0	45
TA 27 ↔ TA 26	19.7	20.9	30
TA 25 ↔ TA 26	9.2	12.8	35

The morphological distances between inbred lines were highly correlated with additive genetic effects (Group I); r = 0.59*** (Figure 3).

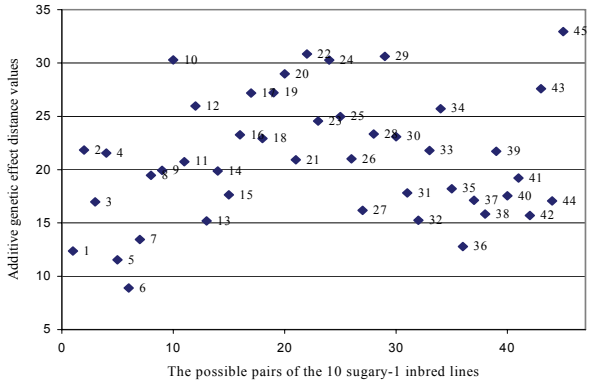

Figure 2. Additive genetic effect distance values among the possible pairs of the 10 *sugary-1* inbred lines (Group I)

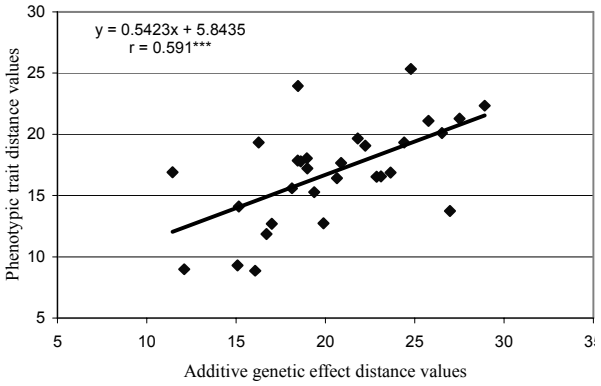

Figure 3. Scatter plot of inbred pairs for phenotypic distance values vs. additive genetic effects (Group I)

Genetic diversity was also analyzed based on the action of additive genes and the genetic effects (ĝ) specific to the 10 inbred lines (for ear weight at harvest).

The differences at the genetic level are emphasized graphically (Figure 4) and through the correlation coefficients (rĝ), where we can observe that the 10 inbred lines are classified according to the degree of relationship between them. It clearly shows that the highest level of additive likeness is observed between: TD 103 – TA 25 (rĝ = 0.93), TA 25 – TD 102 (rĝ = 0.87), TD 101 – TD 102 (rĝ = 0.79), TD 101 – TD 282 (rĝ = 0.79),TD 102 – TD103 (rĝ = 0.74),TA 26 – TA 27 (rĝ = 0.79), TA 27 – TA 28 (rĝ = 0.75).

Figure 4 also represents the differences, on the additive level between the 10 *su*-1 inbred lines. Significant differences are shown by the genetic correlation coefficients between the following inbred lines:

- SW 87 as compared to TD 103 (rĝ = -0.85), TA 25 (rĝ = -0.64);
- TA 27 as compared to TD 102(rĝ = -0.96), TD 101(rĝ = -0.92), TA 25(rĝ = -0.89) TD 103 (rĝ = -0.74), TA 22 (rĝ = -0.60);
- TA 28 as compared to TD 101 (rĝ = -0.84), TA 25 (rĝ = -0.84), TD103 (rĝ= -0.80) TD 282 (rĝ = -0.72), TD 102 (rĝ = -0.64).

The differences between the compared inbred lines reflect differences in homozygous loci level with cumulative effects.

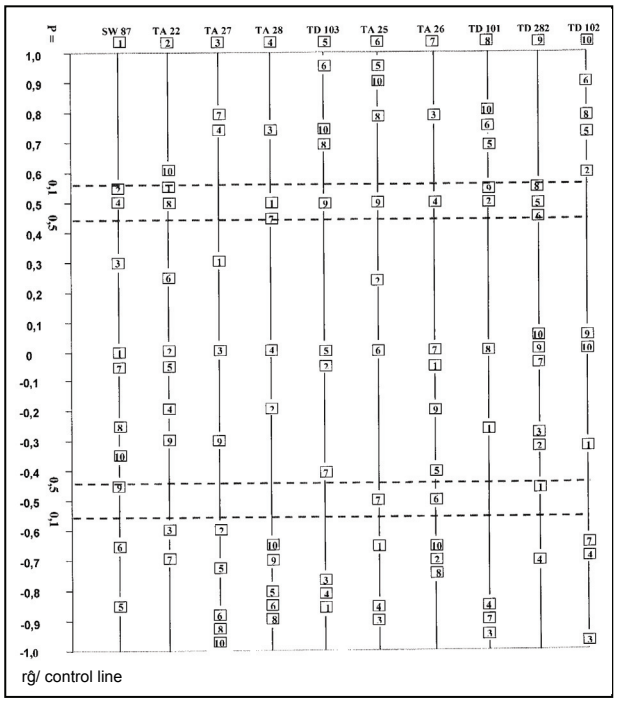

Figure 4. Genetic diversity based on genotypic correlation levels (rĝ) between ten *sugary-1* inbred lines

As far as the action of additive genes is concerned, the evaluation of data on the relations between the 10 *sugary-1* inbred lines (both through phenotypic differences and through correlation coefficients) shows no total correspondence between the two methods.

At an additive level, as well as on a heterotic intensity one, the highest likeness is observed between the two groups of lines:

- the SW 87 group, consisting of the following inbred lines: TA 28, TA 27, TA 22, TA 26, which appear to belong to the Northern Flint race;
- the TD 102 group, consisting of the following inbred lines: TA 25, TD 101, TD 103, which appear to belong to the Southern Dent;

The *su* TA 22 and TD 101 inbred lines seem to have an average reaction.

The inbred lines that showed the most striking resemblance on the additive level were: TA 22 - SW 87 and TD 102 - TD 103.

For the lines tested in this study, phenotypic heterosis can give a reliable estimate of relatedness among lines that would be expected on the basis of accurate knowledge of origin.

The diversity measured by yield heterotic intensity was found to be quite different from morphological differences.

The crosses TD 102 x SW 87 (H = 17%), TD 102 x TD 101(H = 19%), TD 102 x TA 28 (H = 35%), SW 87 x TA 27 (H = 35%) had the lowest yield heterosis level. Low-intensity heterosis level calls attention upon a possible genetic relation of the TD 102 inbred line with SW 87 and TD 101, as well as SW 87 with TA 27; all these are also confirmed by genealogy and the phenotypic differences (Figure 1).

Heterosis data were used as an indicator of genetic relationships between lines that closely mirror those to be expected on the bases of known origin.

For the ten *su* parental inbreds, which were studied here, we made a classification of the first three inbred lines by their *per se* value and by favorable value of additive effects (GCA) (Table 4). By the most favorable traits, which we considered in both ranks, the leaders were the *su* parental inbreds: TA 28, TA 22, SW 87.

The higher positive values, statistically significant of the correlation coefficients among *per se* values of parental inbred lines and their

additive effects are an argument in the favor of practicing the phenotypic selection of some traits such as: number of tillers per plant, ear length, kernel row number per ear.

Table 4. Classification of parental inbreds by their *per se* value and favorable additive effects in Design 2 experiment

Trait / Unit		Significance of additive variance	Correlation coefficients between *per se* value and additive effects	Classification of the best three inbreds by their *per se* value			Classification of the best three inbreds by their favorable additive effects		
Plant traits									
Plant height	cm	**	0.65*	1.**TA22**	2.TD103	3.TA25	1.TD103	2.**TA22**	3.TD101
No. branches/tassel	no.	**	0.86**	1.**TA22**	2.TD102	3.**TA28**	1.**TA28**	2.TA27	3.**TA22**
No. ears/plant	no.	**	0.50	1.TA27	2.**TA22**	3.SW87, **TA28**	1.TA27	2.**TA28**	3.SW87
No. tillers/plant	no.	**	0.84**	1.TA22	2.**TA28**	3.TD282	1.**TA22**	2.**TA28**	3.TD101
GDUs (sowing - silking)		**	0.60	1.SW87	2.**TA22**	3.TD28	1.**TA22**	2.**TA28**	3.TA27
Ear and kernel traits									
Ear weight	g	**	0.67*	1.TD102	2.TA26	3.**TA28**	1.TD103	2.**TA28**	3.TA25
Husk weight	g	**	0.39	1.SW87	2.TA27	3.**TA22**	1.**TA28**	2.SW87	3.**TA22**
Ear length	cm	**	0.91**	1.TD102	2.TD101	3.TD103	1.TD102	2.TD101	3.TA26
Row number	no.	**	0.89**	1.TA27	2.TA26	3.TD282	1.TA27	2.TA26	3.TD282
Kernel depth	cm	**	0.26	1.TD103	2.TA26	3.TA27	1.SW87	2.**TA28**	3.TD282
Kernel chemical composition									
Dry matter	%	NS	0.63*	1.**TA22**	2.TD103	3.TA26	1.TD282	2.**TA22**	3.TA25
Sucrose	% / % d.m	NS	0.36	1.**TA22**	2.SW87	3.TA27	1.**TA22**	2.TD101	3.TA25
Total sugar	% / % d.m	NS	0.52	1.TD282	2.**TA28**	3.TD103	1.TD282	2.TA25	3.**TA22**
Phytoglycogen	% / % d.m	NS	0.73*	1.TA25	2.**TA28**	3.TA27	1.TA25	2.TD103	3.**TA28**
Starch	% / % d.m	NS	0.07	1.**TA28**	2.TA27	3.TD282	1.TD282	2.TA25	3.TD101
Protein	% / % d.m	*	0.29	1.TA27	2.SW87	3.**TA22**	1.TD282	2.TA27	3.TA25
Fats	% / % d.m	NS	-0.34	1.SW87	2.TD282	3.**TA28**	1.TD102	2.**TA28**	3.**TA22**

3) Biochemical data

The evaluation of diversity of the 10 *su-* inbred lines at molecular level took into account the genetic polymorphism of the seed storage proteins (zein) specific to each of the studied lines.

Comparison of the electrophoretic spectrum was based on the relative mobility of the electrophoretic bands (Figure 5) and on the visual evaluation of densitometer scans of electrophoretic spectrum of zein (Figure 6).

Thus:

- the SW 87 inbred line has some common elements in the proteic spectrum with TA 28 and TA 25, but it also has some differences;
- the SW 87 does not differ in electrophoretic spectrum from TD 101 and TD 282;
- the SW 87 line has slight quantitative differences as compared to TD 102.

The similarities between the 10 *sugary-1* inbred lines established, by the methods previously presented, are confirmed by the evaluation of the elements of the electrophoretic spectrum of zein.

Biochemical data were able to uniquely describe all the ten lines studied herein.

Our results were similar with those obtained by Smith and Smith (1989a, 1989b) in their study about field corn lines.

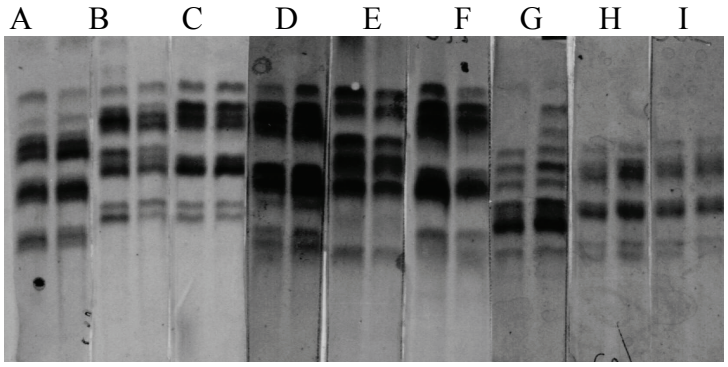

Figure 5 – Zein electrophoretic spectrum of nine *sugary-1* inbred lines: A (SW 87), B (TA 22), C (TA 27), D (TA 28), E (TD 103), F (TA 25), G (TA 26), H (TD 101), I (TD 282

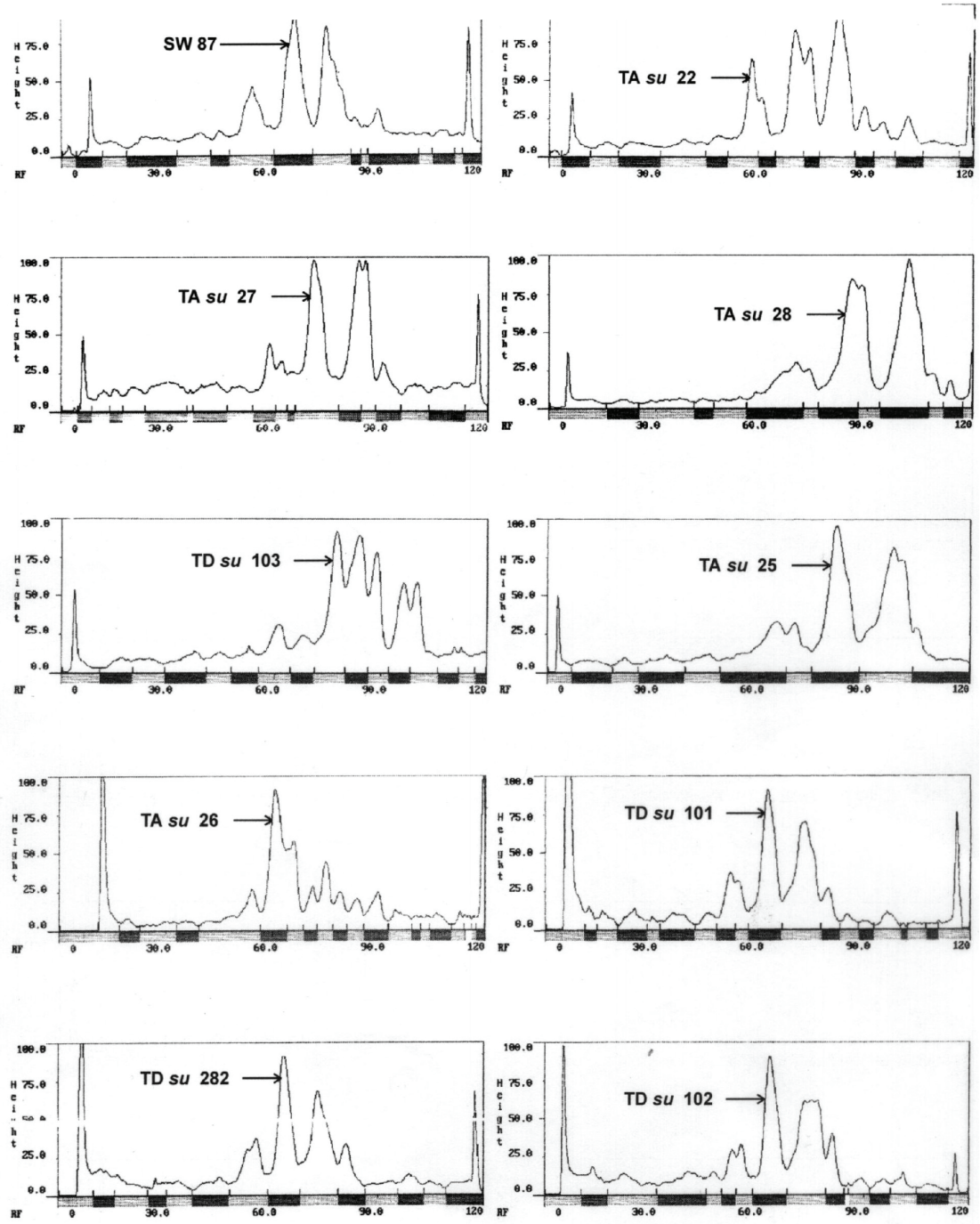

Figure 6 - Densitometer scans of zein electrophoretic spectrum of ten *sugary-1* inbred lines

CONCLUSIONS

Research for the evaluation of genetic diversity in *sugary-1* inbred lines can have a positive influence on the efficiency of sweet corn breeding and on the strategy of developing performance hybrids.

The analysis of these data showed that:

- the 10 *sugary-1* inbred lines, newly developed at the A.R.D.S. Turda are phenotypically and genetically differentiated and can be classified into three germplasm groups;

- morphological, genetic and biochemical data allow the identification of the following groups of the studied *su* lines: early inbreds, belonging to Northern Flint race (SW 87, TA 22, TA 28); late inbreds, apparently resulting from Southern Dent germoplasm (TD 103, TD 101, TD 102, TD 282); TA 22 and TD 101 *su* lines seem to have an average behavior;

- there was a relative correspondence between the results obtained in establishing the relations of the 10 *su* lines, through the following methods: relation coefficients (rĝ), the intensity of phenotypic heterosis (H%), visual evaluation of densitograms and the description of the electrophoretic spectrum of zein;

- morphological and biochemical data might be able to provide initial screens of identity and distinctiveness, but heterosis data appear to provide the most reliable estimates of overall genotypic relatedness;

- correlations between morphological distances with heterosis measures were very low; thus, although morphological data have traditionally provided the sole set of descriptors for the purposes of cultivar identification and registration, they cannot reliably be used to estimate distances between sweet corn cultivars that are reflective of their pedigree or genetic associations.

- the studied *sugary-1* inbred lines present the possibility of developing performance hybrid formulae, for commercially valuable hybrids with superior productivity, earliness and quality of the ear and of the kernels (particularly eating quality).

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