GENETIC DIVERGENCE IN SUNFLOWER (*HELIANTHUS ANNUUS)* INBREDS

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Abstract

Increase in seed yield is the main objective of sunflower breeding programs. Genetic variability is the basic prerequisite for each breeding program. Determination and knowledge of genetic variability facilitate the selection of superior genotypes in different generations of selfing. In breeding programs, much effort is expended each year to find superior lines among a large number of tested lines. Genetic divergence has been analyzed in 125 sunflower inbred lines for the following traits: 1) number of florets per plant, 2) number of seeds per plant, 3) percentage of pollination per plant, 4) autofertility and 5) autocompatibility. The selected lines differed in the values of the analyzed traits. Relationships between and within groups of lines were analyzed by a discriminant analysis, which uses mean values of individual plants, and groups of plants and lines. Mahalanobis squared distance served as a measure of similarity. Based on the analyzed traits, it was concluded that 23 inbred lines do not belong to the groups they were allocated in on the basis of the pedigree method of selection. The accuracy of classification varied from 68 to 88%.

Introduction

Sunflower (*Helianthus annuus* L.) is economically the most important species of the family Asteraceae. Increase in sunflower seed yield is the main objective of sunflower breeding programs. Genetic variability is the basic prerequisite for each breeding program. Determination and knowledge of genetic variability facilitate the selection of superior genotypes in different generations of selfing. To be able to develop new sunflower hybrids with desired characteristics, breeders must have at their disposal genetically divergent parental lines. Several methods for measuring genetic distance have been proposed. Mahalanobis D2 distance is extensively used in plant breeding (Teklewold et al., 2000). To assess relationships among lines, Mahalanobis squared distance was used as a measure of similarity (Kovačić, 1994). The objectives of this study were, based on the number of florets and seeds and the traits derived from them, to establish the usefulness of the analyzed traits for classifying inbred lines and to identify the best genotypes.

Materials and Methods

Divergence was studied in 125 sunflower lines in different generations of selfing. The lines, which had been derived from a high-protein variety Kolos, differed in the mean values

of the analyzed traits. The lines were grown using the conventional production technology. The planting was performed at the optimum date, at the distances of 70 cm between the rows and 35 cm within the row. Samples for analysis consisted of 9 plants per each line in the S6 generation. Three types of pollination were used to pollinate individual flowers of the lines in the S6 generation: autogamy, geitonogamy and open pollination (Kovačic et al., 1996).

A total of 1125 plants were analyzed. Floret and seed counts and pollination percentage calculation were done in the laboratory, by hand counting the numbers of pollinated and unpollinated florets on the heads of individual plants selected for the subsequent generation of selfing. Pollination rates for the three types of pollination were determined on the basis of relationships among the following traits: 1) total number of florets, 2) number of pollinated florets or number of seeds, and 3) ratio of seed number to floret number-percentage of pollination. Autofertility (AF) and autocompatibility (AC) were determined according to George and Shein (1980). Discriminant analysis is a method of multivariate analysis aimed at partitioning the analyzed material into groups and allocating observations into the predefined groups. This is achieved by forming linear combinations of discriminant functions of independent variables which will assure the maximum possible discrimination among the predefined groups and maximum possible accuracy in classifying the observations. This is realized by meeting the Fisher discriminant criterion, i.e., by maximizing the relationship between the variance among the groups and the variances within the groups. The number of discriminant functions may be smaller by 1 than the number of groups or the number of independent variables, whichever of these two is smaller. To determine the relationships among the lines, Mahalanobis squared distance was used as a measure of similarity among the observations:

$$D^2 = (\bar{x}_r - \bar{x}_s)\bar{S}(\bar{x}_r - \bar{x}_s)$$

where x = centroids of lines *r* and *s*; $\bar{S}$ = covariance matrix (Kovačić, 1994).

Results and Discussion

Relationships among and within the groups of S6 lines were established by the discriminant analysis using the mean values of the analyzed traits from individual plants and groups of plants according to the different types of pollination and lines. Mahalanobis squared distance was used as a measure of similarity. Distance matrices for the analyzed lines are given in Table 1. Similarities or differences among the lines were estimated on the basis of the results of allocation of individual measurements of discriminant scores. On the basis of the analyzed traits it was concluded that 23 inbred lines do not belong to the groups they were allocated in on the basis of the pedigree method of selection. The accuracy of classification varied between 68% and 88% (Table 2).

Table 1. Mahalanobis D^2 distances among averages of the analyzed S_6 lines and posterior probability for allocation to group.

S_4	S_5	S_6	Red. br.		L-358/9	Priors	L-466/4	Priors	L-863/4	Priors	L-865/4	Priors	L-932/2	Priors
358/9	3589	35893/3	1		32.20	0.98	40.00	0.02	53.60	0.00	80.30	0.00	66.00	0.00
		35893/9	2		11.10	0.93	16.40	0.07	26.60	0.00	50.60	0.00	37.30	0.00
		35893/14	3		10.10	0.53	10.40	0.47	21.30	0.00	40.80	0.00	28.40	0.00
		35893/29	4	-->	15.00	0.37	14.00	0.63	29.30	0.00	50.40	0.00	35.70	0.00
		35893/a	5		8.60	0.60	9.40	0.40	25.60	0.00	49.40	0.00	30.30	0.00
		35899/1	6		27.10	0.73	29.10	0.27	46.50	0.00	73.20	0.00	57.00	0.00
		35899/4	7		7.70	0.87	11.50	0.13	22.70	0.00	39.60	0.00	31.10	0.00
		35899/34	8		13.30	0.97	20.30	0.03	36.80	0.00	63.30	0.00	48.30	0.00
		35899/a	9		6.40	0.82	9.40	0.18	24.10	0.00	49.70	0.00	34.60	0.00
		35899/b	10		9.30	0.71	11.00	0.29	24.00	0.00	44.20	0.00	32.80	0.00
		358913/1	11		23.20	0.85	26.70	0.15	46.10	0.00	64.40	0.00	58.20	0.00
		358913/23	12		20.60	0.99	29.60	0.01	41.60	0.00	64.90	0.00	48.00	0.00
		358913/28	13		6.70	0.70	8.30	0.30	24.90	0.00	49.00	0.00	32.00	0.00
		358913/30	14		20.30	0.80	23.20	0.20	34.70	0.00	54.50	0.00	40.20	0.00
		358913/a	15		16.00	0.68	17.50	0.32	34.10	0.00	59.10	0.00	41.50	0.00
		358916-1/2	16		11.30	0.56	11.80	0.44	25.60	0.00	43.40	0.00	37.80	0.00
		358916-1/3	17		18.90	0.94	24.50	0.06	33.40	0.00	48.30	0.00	35.50	0.00
		358916-1/21	18	-->	32.50	0.21	29.90	0.79	49.40	0.00	71.60	0.00	57.70	0.00
		358916-2/2	19		24.80	0.69	26.50	0.31	37.80	0.00	55.40	0.00	50.20	0.00
		358916-2/4	20		45.00	0.58	45.60	0.42	56.00	0.00	73.60	0.00	62.40	0.00
		358917/2	21		24.20	0.82	27.20	0.18	36.30	0.00	62.00	0.00	40.00	0.00
		358917/4	22		17.30	0.99	26.80	0.01	30.60	0.00	49.10	0.00	36.60	0.00
		358917/6	23		22.10	0.48	22.30	0.45	26.00	0.07	49.50	0.00	34.80	0.00
		358917/9	24	-->	39.80	0.15	36.30	0.82	43.30	0.03	62.20	0.00	46.50	0.01
		358917/11	25		24.60	0.52	25.20	0.38	28.20	0.09	53.50	0.00	35.20	0.00
466/4	4664	46642/9	26		7.60	0.43	7.00	0.57	21.40	0.00	39.30	0.00	29.30	0.00
		46642/11	27		20.50	0.37	19.90	0.49	22.40	0.14	34.30	0.00	30.10	0.00
		46642/18	28		21.70	0.08	16.70	0.92	26.60	0.01	48.90	0.00	33.30	0.00
		46642/19	29		15.60	0.30	13.90	0.70	27.80	0.00	53.30	0.00	35.00	0.00
		46642/23	30		16.70	0.18	13.70	0.79	19.90	0.04	42.80	0.00	29.60	0.00
		46643/1	31		28.40	0.01	19.30	0.99	38.30	0.00	62.90	0.00	47.70	0.00
		46643/7	32		14.00	0.27	12.10	0.71	19.90	0.01	36.50	0.00	24.50	0.00
		46643/16	33		24.80	0.05	19.00	0.95	32.70	0.00	57.70	0.00	39.50	0.00
		46643/17	34		20.60	0.21	18.00	0.79	33.60	0.00	59.80	0.00	39.10	0.00
		46643/25	35	-->	9.90	0.72	11.70	0.28	20.30	0.00	36.30	0.00	25.50	0.00
		46644/4	36	-->	8.60	0.74	10.70	0.26	23.70	0.00	45.30	0.00	30.80	0.00
		46644/14	37		23.00	0.15	19.50	0.85	38.00	0.00	63.80	0.00	46.80	0.00
		46644/15	38	-->	11.40	0.86	15.00	0.14	33.10	0.00	57.10	0.00	43.10	0.00
		46644/19	39		20.30	0.27	18.40	0.71	25.70	0.02	47.70	0.00	33.90	0.00
		46644/21	40		11.20	0.49	11.10	0.51	19.70	0.01	40.10	0.00	34.40	0.00
		46645/8	41	-->	8.80	0.73	10.90	0.26	19.20	0.00	46.30	0.00	23.50	0.00
		46645/13	42		26.90	0.23	24.50	0.77	39.60	0.00	58.60	0.00	48.40	0.00
		46645/14	43		23.50	0.08	18.70	0.91	30.30	0.00	55.10	0.00	37.20	0.00
		46645/16	44		39.40	0.08	34.70	0.91	43.90	0.01	69.30	0.00	62.50	0.00
		46645/22	45	-->	15.00	0.49	15.30	0.43	18.80	0.07	36.00	0.00	22.50	0.01
		46646/16	46		21.80	0.10	17.50	0.88	25.00	0.02	41.40	0.00	37.00	0.00
		46646/19	47		8.50	0.17	5.30	0.82	13.70	0.01	32.00	0.00	21.00	0.00
		46646/21	48		83.80	0.08	78.90	0.92	108.20	0.00	124.60	0.00	117.30	0.00
		46646/25	49		15.90	0.16	12.50	0.84	25.10	0.00	41.80	0.00	35.00	0.00
		46646/26	50		49.10	0.00	38.10	0.76	40.40	0.24	52.40	0.00	57.50	0.00

Table 1. (continued)

S_4	S_5	S_6	Red. br.		L-358/9	Priors	L-466/4	Priors	L-863/4	Priors	L-865/4	Priors	L-932/2	Priors
863/4	8634	86343/D	51		34.20	0.00	35.60	0.00	23.90	0.58	38.90	0.00	24.60	0.41
		86343/8	52		50.30	0.00	51.90	0.00	35.50	0.94	52.00	0.00	41.00	0.06
		86343/20	53		15.70	0.21	14.50	0.39	14.50	0.39	33.20	0.00	21.60	0.01
		86343/25	54		32.00	0.05	27.50	0.46	27.50	0.46	39.70	0.00	32.40	0.04
		86343/27	55		27.10	0.01	21.00	0.14	17.50	0.83	30.40	0.00	25.60	0.01
		86346/10	56		55.20	0.00	52.30	0.02	44.20	0.88	48.50	0.10	60.10	0.00
		86346/10	57		31.00	0.00	29.80	0.00	16.00	0.75	30.50	0.00	18.20	0.25
		86346/11	58		139.70	0.00	136.40	0.00	114.90	1.00	134.40	0.00	130.50	0.00
		86346/14	59	-->	64.70	0.00	53.20	0.00	32.00	0.05	33.40	0.03	26.30	0.92
		86346/16	60		26.90	0.00	25.40	0.00	13.70	0.72	15.70	0.27	22.10	0.01
		86347/14	61	-->	72.20	0.00	64.50	0.00	44.00	0.25	41.90	0.73	50.00	0.01
		86347/16	62		75.50	0.00	70.70	0.00	43.20	0.90	47.70	0.10	54.50	0.00
		86347/19	63	-->	54.10	0.00	50.50	0.00	28.60	0.08	39.60	0.00	23.60	0.92
		86347/23	64	-->	34.60	0.06	29.50	0.82	33.40	0.12	51.70	0.00	47.30	0.00
		86347/27	65		46.80	0.00	40.50	0.00	21.50	0.90	25.90	0.10	33.80	0.00
		863410/1	66		37.90	0.00	33.80	0.00	21.90	0.89	40.40	0.00	26.10	0.11
		863410/4	67	-->	18.50	0.03	18.40	0.03	13.30	0.37	27.00	0.00	12.50	0.57
		863410/10	68		54.10	0.00	45.90	0.00	34.90	0.98	46.20	0.00	43.20	0.02
		863410/26	69		30.30	0.26	34.20	0.04	28.30	0.69	37.30	0.01	38.40	0.00
		863410/31	70	-->	52.90	0.00	50.80	0.00	25.60	0.03	18.80	0.97	33.80	0.00
		863412/6	71		42.50	0.00	35.00	0.07	29.90	0.91	46.60	0.00	38.60	0.01
		863412/7	72		33.60	0.00	28.70	0.00	12.90	0.51	21.40	0.01	13.00	0.48
		863412/15	73		22.90	0.02	17.90	0.29	16.20	0.68	33.30	0.00	27.60	0.00
		863412/17	74	-->	23.70	0.00	20.30	0.01	13.70	0.24	23.50	0.00	11.40	0.75
		863412/19	75	-->	34.00	0.04	37.10	0.01	33.10	0.07	52.10	0.00	28.00	0.88
865/4	8654	86543/7	76		152.20	0.00	147.60	0.00	126.50	0.00	109.00	1.00	132.50	0.00
		86543/11	77		89.00	0.00	82.80	0.00	58.50	0.23	56.00	0.77	72.00	0.00
		86543/19	78		61.20	0.00	56.20	0.00	32.10	0.38	31.20	0.60	38.40	0.02
		86543/20	79	-->	40.10	0.00	33.50	0.00	16.30	0.60	22.70	0.02	17.20	0.38
		86543/24	80		114.20	0.00	112.50	0.00	90.10	0.00	69.70	1.00	91.40	0.00
		86549/6	81		127.90	0.00	124.60	0.00	98.00	0.00	68.40	1.00	99.60	0.00
		86549/7	82		54.60	0.00	50.80	0.00	33.20	0.14	29.50	0.86	39.40	0.01
		86549/14	83		66.70	0.00	63.90	0.00	42.50	0.00	30.90	1.00	44.30	0.00
		86549/20	84		48.90	0.00	45.80	0.00	40.10	0.01	31.70	0.98	46.10	0.00
		86549/24	85		54.90	0.00	53.90	0.00	30.90	0.33	29.90	0.55	32.90	0.12
		865417/13	86		52.00	0.00	45.30	0.00	22.90	0.01	13.30	0.99	35.80	0.00
		865417/17	87	-->	27.20	0.00	23.30	0.02	16.10	0.66	23.00	0.02	17.70	0.30
		865417/20	88		110.60	0.00	110.50	0.00	86.90	0.00	57.70	1.00	90.00	0.00
		865417/21	89		120.10	0.00	116.00	0.00	90.50	0.00	76.10	1.00	100.50	0.00
		865417/30	90		100.90	0.00	96.70	0.00	65.70	0.01	56.10	0.92	61.20	0.07
		865418/2	91		102.40	0.00	97.00	0.00	68.60	0.00	46.90	1.00	73.80	0.00
		865418/8	92		64.60	0.00	57.40	0.00	46.00	0.00	32.90	1.00	55.50	0.00
		865418/15	93		57.90	0.00	53.50	0.00	38.30	0.35	37.10	0.65	50.60	0.00
		865418/20	94		86.90	0.00	80.10	0.00	49.80	0.00	29.80	1.00	61.40	0.00
		865418/26	95		106.90	0.00	102.50	0.00	89.30	0.00	71.90	1.00	102.20	0.00
		865424/12	96		56.50	0.00	55.70	0.00	34.10	0.03	27.10	0.97	41.00	0.00
		865424/15	97		69.80	0.00	68.50	0.00	42.20	0.05	36.30	0.89	41.80	0.06
		865424/21	98		52.70	0.00	49.40	0.00	32.30	0.03	25.20	0.97	43.20	0.00
		865424/22	99	-->	84.10	0.00	72.70	0.00	46.20	0.02	40.40	0.42	39.80	0.56
		865424/27	100		121.30	0.00	110.90	0.00	83.00	0.00	57.10	1.00	86.60	0.00

Table 1. (continued)

S_4	S_5	S_6	Red. br.		L-358/9	Priors	L-466/4	Priors	L-863/4	Priors	L-865/4	Priors	L-932/2	Priors
932/2	9322	93221/14	101		65.40	0.00	61.00	0.00	47.80	0.00	56.70	0.00	37.10	1.00
		93221/17	102		63.10	0.00	56.60	0.00	33.00	0.40	41.60	0.01	32.30	0.59
		93221/21	103		38.10	0.00	32.00	0.00	20.40	0.05	29.40	0.00	14.60	0.95
		93221/23	104		62.10	0.00	56.20	0.00	34.90	0.00	45.50	0.00	17.20	1.00
		93221/24	105		28.70	0.00	30.20	0.00	13.70	0.14	22.70	0.00	10.10	0.86
		93223/4	106		41.60	0.00	39.00	0.00	30.20	0.11	36.40	0.00	26.00	0.88
		93223/15	107		82.30	0.00	80.90	0.00	64.50	0.00	83.80	0.00	50.60	1.00
		93223/16	108		42.90	0.00	39.80	0.00	21.60	0.21	33.70	0.00	19.00	0.79
		93223/22	109		41.30	0.00	37.70	0.00	20.00	0.09	27.30	0.00	15.40	0.90
		93223/34	110		55.30	0.00	58.10	0.00	34.30	0.08	38.60	0.01	29.50	0.91
		93227/4	111		61.60	0.00	57.40	0.00	41.50	0.03	43.30	0.01	34.80	0.95
		93227/13	112		44.70	0.00	38.90	0.00	19.50	0.01	27.10	0.00	10.70	0.99
		93227/16	113	-->	33.90	0.00	29.90	0.00	17.80	0.84	29.90	0.00	21.10	0.16
		93227/23	114		29.00	0.00	26.90	0.00	18.70	0.02	28.70	0.00	10.70	0.98
		93227/24	115		44.30	0.00	43.40	0.00	21.60	0.03	27.90	0.00	14.70	0.97
		932213/5	116		51.90	0.00	48.10	0.00	27.30	0.00	25.20	0.01	16.70	0.98
		932213/12	117		45.00	0.00	49.10	0.00	41.40	0.01	49.10	0.00	32.40	0.99
		932213/13	118		45.60	0.00	46.80	0.00	29.80	0.02	44.00	0.00	21.60	0.98
		932213/15	119	-->	17.80	0.17	15.20	0.63	19.90	0.06	40.90	0.00	18.10	0.14
		932213/16	120		44.20	0.00	41.10	0.00	26.50	0.28	42.90	0.00	24.60	0.72
		932216/6	121		39.70	0.00	32.90	0.00	20.10	0.07	29.50	0.00	14.80	0.93
		932216/7	122	-->	44.30	0.00	39.20	0.00	24.30	0.64	38.60	0.00	25.50	0.36
		932216/10	123	-->	13.00	0.22	11.00	0.63	14.80	0.09	34.80	0.00	16.00	0.05
		932216/16	124		31.80	0.00	32.40	0.00	17.80	0.04	32.90	0.00	11.50	0.96
		932216/19	125		41.20	0.00	37.70	0.00	22.10	0.02	33.60	0.00	14.30	0.98

-->incorrect classification

Table 2. Classification matrix and percentage of accuracy of allocation of S_6 lines to the source groups

Source group	L-358/9	L-466/4	L-863/4	L-865/4	L-932/2	Accuracy %
L-3589x	22	3	0	0	0	88
L-4664x	5	20	0	0	0	80
L-8634x	0	1	17	2	5	68
L-8654x	0	0	2	22	1	88
L-9322x	0	2	2	0	21	84
Total	27	26	21	24	27	82

Based on Mahalanobis squared distance and posterior probability, three lines (35893/29, 358916-1/21 and 358917/9) should be transferred from the group of S6 lines which originate from line L-358/9 to the group of lines which originate from line 466/4 (Table 1). Five inbred lines derived from line 466/4 (46643/25, 46644/4, 46644/15, 46645/8 and 46645/22) are most similar to the lines originating from L-358/9. Of the 25 lines in the third group, eight should be moved elsewhere. Lines 86346/14, 86347/19, 863410/4, 863412/17 and 863412/19 are most similar to the lines originating from L-932/2; lines 86347/14 and 863410/31 to the lines originating from L-865/4; and line 86347/23 to the lines originating from L-466/4. Two out of the three lines that draw origin from group L-865/4 (86543/20 and 865417/17) are most similar to the lines originating from L-863/4. Line 865424/22 is most similar to the lines from group L-932/2. Based on Mahalanobis squared distance and posterior probability, two lines

(932213/15 and 932216/10) should be transferred from the group of S_6 lines which originate from line L-932/2 to the group of lines which originate from line L-466/4 (Table 2). Lines 93227/16 and 932216/7 are most similar to the group of lines derived from line L-863/4.

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