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Impact of Adaptation on Wild Milk Thistle (*Silybum marianum*) Genotypes

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ABSTRACT

Silybum marianum (L.) Gaertn. (Asteraceae) grows in the Egyptian desert. It is an important medicinal plant due to the presence of silymarin, which used as an active ingredient in the treatment of various liver diseases. The plant is exposed to gathering processes, which may lead to extinction. Therefore, 10 wild genotypes were selected from the desert and variability of seven quantitative characters was studied *in situ*, where genetic differences were evaluated between genotypes. Moreover, the wild harvested seeds were cultivated for two successive adaptive seasons in 2006/2007 and 2007/2008 in an improved environment (agricultural old clay land) to study the impact of environmental changes (*ex situ*) on the genetic character expression of genotypes. Highly significant differences existed among genotypes for all characters in both adaptive seasons, confirming the different genetic backgrounds of these genotypes. These characters had improved in the improved environment. Total silymarin content varied among genotypes. There was little variation in protein bands in the adaptive process.

Keywords: conservation, correlation coefficient, genetic improvement, protein bands, quantitative, characters, silymarin content

INTRODUCTION

Silybum marianum (L.) Gaertn. (Asteraceae) grows in the Egyptian desert, especially in Sinai. It is an important medicinal plant for its natural product silymarin which is used as an active ingredient against various liver diseases (Omer 1996; Flora *et al.* 1998; Ottai and Abdel-Moniem 2006; Ibrahim *et al*. 2007). There is a lack of research on the domestication and improvement of this plant especially since it is exposed to gathering processes in the desert (Ram *et al.* 2005; Ottai and Abdel-Moniem 2006).

Plant adaptation in agricultural environments (such as on old clay land in this study) is an important means for conservation, domestication and genetic improvement (Sadaqat *et al.* 1983; Omer 1996). Improvement of plant characters can be achieved through a clear understanding of the nature and amount of variability present in genotypes (Ram *et al.* 2005). On the other hand, the synthesis of protein and secondary metabolites i.e. flavonoids differs when plants are cultivated on sandy soil vs. old clay soil (Bilgrami *et al*. 1980; Omer *et al*. 1984). Therefore, the aim of this study was to assess the effect of adaptation environment on the expression of growth characters, silymarin composition and electrophoresis profile of water-soluble protein in *S. marianum* genotypes.

MATERIALS AND METHODS

The plant material consisted of five replication plants for 10 genotypes of *Silybum marianum* selected from Sinai deserts. Seven quantitative characters (plant height, linear growth, number of main and total branches/plant, number of capsules/plant, seed yield/plant and seed index) were investigated in original desert lands *in situ* for the 2005/2006 wild season. At the end of the wild season, the seeds were harvested separately and cultivated in an *ex situ* agricultural environment (in old clay land at Qena Governorate) for two adaptive seasons, 2006/2007 and 2007/2008. A randomized complete design with three replicates was used. Each replicate had 5 lines 5 m in length and 60 cm in between. The seeds were directly sown in October in hills at a 50 cm distance. Thinning was done after 30 days of cultivation to leave one plant/hill. Data were recorded on the selected plants in each replicate for the seven above mentioned characters and analyzed by SPSS program (2001).

Silymarin content and its constituents were determined according to Hammouda *et al.* (1991). Correlation coefficient was estimated using SPSS (2001).

Seeds of wild and both adaptive seasons of genotype 6 were used for protein electrophoresis analysis. SDS-polyacrylamide gel electrophoresis was performed according to Lammli (1970). A sufficient amount of seeds was defatted with petroleum ether. The samples preparation and the extraction of water soluble protein were performed according to Stegemann *et al.* (1980). Gels were scanned and analyzed using a Gel Doc Bio-Rad System. The degree of electrophoresis similarity was calculated according to Socal and Sneath (1963).

RESULTS AND DISCUSSION

Ten genotypes of *S. marianum* were selected from the desert and assessed for seven growth characters to evaluate genetic variability among genotypes and impact of environmental changes from a desert to an agricultural environment on the plant characters. Significant variations were detected among genotypes revealed their considerable amount of genetic variability (**Table 1**). Significant variation was also detected between seasons and their interaction with genotypes. Several authors (Hetz *et al.* 1995; Ram *et al*. 2005; Ottai and Abdel-Moniem 2006; Ibrahim *et al*. 2007) found similar genotypic variation of *S. marianum*. **Table 2** indicates that all characters except for seed index showed further improvement in the first up to the second adaptive season at the improved agricultural environment for all genotypes. Sadaqat *et al.* (1983) reported that the plant adaptation in agricultural environment (in old clay land) is an important mean for conservation and improvement.

Silymarin yield/plant and its constituents are illustrated in **Table 3**. Further improvement was also achieved in sily-

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Research Note

Table 1 Analysis of variance for seven quantitative characters of 10 *Silybum marianum* genotypes during wild and two adaptive seasons as well as combined analysis.

Season	SOVA	Df	Seed yield/plant	Capsules/ plant	Total branches/ plant	Main branches/ plant	Linear growth	Plant height	Seed index
Wild	genotypes	9	15.8**	48.8**	38.0**	7.4**	594.1**	719.0**	1.7**
season	Replicates	4	0.0	0.8	0.5	0.4	0.8	0.3	0.4
	Error	36	0.5	2.4	2.7	1.0	2.6	5.6	0.2
First	genotypes	9	22.2**	80.0**	54.4**	24.9**	560.1**	707.6**	1.4
adaptive	Replicates	4	19.5	2.7	2.3	0.5	2.2	0.2	2.4
season	Error	36	2.4	1.4	1.9	3.1	3.6	3.0	4.9
Second	genotypes	9	54.6**	2495.8**	424.8**	19.5**	254.8**	849.8**	1.9*
adaptive	Replicates	4	0.6	0.8	7.8	0.4	2.0	75.6**	0.4
season	Error	36	3.0	5.1	5.5	2.3	3.9	14.2	0.7
	genotypes	9	68.8**	1285.1**	311.9**	37.8**	762.0**	1704.9**	3.7
Combined	seasons	2	6139.3**	136506.8**	57074.5**	290.9**	88923.7**	165132.2**	2920.7**
analysis	geno. x seas.	18	11.9**	669.8**	102.7**	7.0**	323.5**	285.8**	0.6
	Error	120	2.1	2.9	3.3	2.1	3.3	8.0	1.9

Table 2 Mean values of seven growth characters for 10 *Silybum marianum* genotypes among wild and two adaptive seasons.

Genotypes	Season	Plant height (cm)	Linear growth (cm)	Main branches /plant	Total branches /plant	Capsules /plant	Seed yield /plant (g)	Seed index
1	Wild	74.0	55.0	5.0	14.0	16.0	6.1	3.8
	1st adaptive	169.0	147.0	8.0	58.0	60.0	23.6	17.2
	2nd adaptive	188.0	132.0	9.0	77.0	107.0	27.3	16.4
2	Wild	77.0	58.0	6.0	15.0	8.0	7.6	3.4
	1st adaptive	173.0	133.0	11.0	62.0	65.0	26.6	16.7
	2nd adaptive	190.0	132.0	10.0	80.0	124.0	27.8	16.3
3	Wild	91.0	69.0	7.0	17.0	20.0	8.5	3.1
	1st adaptive	151.0	129.0	6.0	55.0	57.0	22.2	17.8
	2nd adaptive	196.6	138.0	12.0	88.0	139.0	30.8	16.0
4	Wild	71.2	49.8	4.0	14.4	15.0	5.5	4.3
	1st adaptive	170.0	150.0	6.8	58.0	59.0	22.5	17.5
	2nd adaptive	180.6	128.0	9.0	75.0	104.0	26.6	17.1
5	Wild	66.0	43.0	4.2	10.6	14.2	4.8	4.4
	1st adaptive	153.0	117.0	7.0	56.0	58.0	22.3	17.3
	2nd adaptive	168.0	123.0	8.0	74.0	92.0	25.5	16.8
6	Wild	103.2	76.2	7.4	20.2	23.4	10.2	2.8
	1st adaptive	182.4	126.0	12.0	64.0	68.0	27.7	16.4
	2nd adaptive	208.0	146.0	13.8	98.2	152.0	33.1	15.1
7	Wild	93.6	71.6	7.0	18.8	22.0	9.4	2.9
	1st adaptive	171.0	135.0	11.0	60.6	63.0	24.0	17.0
	2nd adaptive	205.8	141.4	13.2	94.4	144.0	31.1	15.6
8	Wild	68.8	47.2	4.8	13.2	14.8	5.8	4.0
	1st adaptive	155.0	120.0	7.0	58.0	59.0	22.4	17.6
	2nd adaptive	175.0	125.0	8.8	69.0	90.2	22.0	16.8
9	Wild	83.6	63.0	6.2	15.8	19.0	8.0	3.1
	1st adaptive	180.0	126.0	8.8	62.0	65.0	25.9	16.8
	2nd adaptive	198.2	134.8	11.4	83.2	139.8	31.4	15.8
10	Wild	82.0	60.8	6.2	15.8	18.0	7.4	3.4
	1st adaptive	152.0	130.0	6.0	54.0	56.0	21.8	18.1
	2nd adaptive	197.0	134.4	10.8	81.8	128.2	28.0	16.2

marin yield/plant and its constituents from the wild plants in the desert environment up to the first up to the second adaptive seasons in the agriculture environment with different constituent values for different genotypes. This is attributed to the deletion of stress conditions of the desert by cultivation in old land rich in water, organic matter, micronutrients with suitable direct heat and humidity. Therefore, old land helps the plants to increase the synthesis of secondary metabolites (Bilgrami *et al.* 1980; Hammouda *et al.* 1991; El-sayed *et al.* 1993; Omer *et al.* 1994).

The correlation coefficient was calculated among each pair of genotype growth characters as well as silymarin content and silymarin yield/plant in both wild environment and the second adaptive season in the agricultural environment, as illustrated in **Table 4**. Except for seed index, most correlations were significant and positive in both environments. Non significant associations were estimated between silymarin content with linear growth, number of main branches/ plant, seed yield/plant and seed index as well as between silymarin yield/plant with silymarin content only in the wild season. Seed index had a significant negative correlation with all characters in both environments. Ram *et al.* (2005) found the same significant correlations between numbers of capsules/plant with number of branches/plant and between seed yield/plant with silymarin content. They concluded that these results are phenotypic relatively and not necessarily of genetic origin and these relations are influenced by environmental factors limiting yield.

Genotype 6 was the best, achieving the highest value for all growth characters among both environments and had the highest silymarin yield and content with maximum silychristin, silydianin, silybins A and B in the second adaptive season at the agriculture environment. Therefore, its seeds were inspected for analysis of electrophoresis profiles of water soluble protein for wild, first and second adaptive seasons to insight the adaptation effect on its protein (**Fig. 1, Table 5**). Maximum of 17 bands ranging from 117 to 7 KDa were detected. Four of them with molecular weights of 79, 73, 33 and 13 KDa were commonly detected in the seeds of all seasons. Wild season seeds were distinguished by two bands (17 and 9 KDa) while the seeds grown in the improved environment at the first adaptive season distin-

Table 3 Silymarin yield/plant (g) and silymarin constituents (mg/g) of 10 *Silybum marianum* genotypes among wild and two adaptive seasons.

Genotypes	Season	Silychristin	Silydianin	Silybin A	Silybin B	Isosilybin A	Isosilybin B	Silymarin content	Silymarin yield/plant
1	Wild	7.14	1.39	0.18	0.20	0.06	0.02	8.99	0.06
	1st adaptive	4.23	3.77	2.05	1.14	5.70	1.69	18.58	0.44
	2nd adaptive	3.92	4.94	1.26	2.14	8.50	1.33	22.09	0.60
2	Wild	7.48	1.31	0.18	0.16	0.04	0.02	9.19	0.07
	1st adaptive	4.66	3.68	2.16	1.70	1.88	1.72	15.80	0.42
	2nd adaptive	3.87	5.23	1.19	2.16	2.26	1.68	16.39	0.46
3	Wild	8.56	1.26	0.09	0.09	0.02	0.01	10.03	0.09
	1st adaptive	4.88	10.20	6.33	5.17	3.14	1.43	31.15	0.69
	2nd adaptive	6.44	12.65	5.69	8.04	4.00	2.37	39.19	1.21
4	Wild	6.14	1.66	0.28	0.23	0.07	0.60	8.98	0.05
	1st adaptive	5.77	2.93	2.17	1.77	6.99	1.18	20.81	0.47
	2nd adaptive	4.14	4.66	1.31	2.14	14.88	0.96	28.09	0.75
5	Wild	5.65	1.76	0.36	0.30	0.08	0.03	8.18	0.04
	1st adaptive	2.17	4.11	1.50	2.00	1.17	0.89	11.84	0.26
	2nd adaptive	5.93	4.23	1.46	2.13	0.580	0.13	14.38	0.37
6	Wild	10.16	1.10	0.02	0.02	0.01	0.01	11.32	0.12
	1st adaptive	6.24	10.16	5.56	7.43	1.87	0.86	32.12	0.89
	2nd adaptive	7.17	16.77	13.03	19.50	4.85	1.53	62.85	2.08
7	Wild	9.77	1.16	0.11	0.20	0.12	0.09	11.45	0.11
	1st adaptive	10.22	7.77	5.43	6.16	2.34	1.04	32.96	0.79
	2nd adaptive	6.51	13.79	7.04	10.83	4.00	2.12	44.29	1.38
8	Wild	6.52	1.57	0.296	0.21	0.07	0.03	8.66	0.01
	1st adaptive	4.17	4.12	1.20	2.11	1.34	1.15	14.09	0.32
	2nd adaptive	2.64	3.86	0.50	1.36	2.56	1.98	12.90	0.28
9	Wild	8.03	1.29	0.13	0.12	0.03	0.02	9.62	0.08
	1st adaptive	5.18	9.83	7.87	9.45	1.86	1.15	35.34	0.92
	2nd adaptive	6.64	15.37	10.84	16.30	4.01	1.28	54.44	1.71
10	Wild	7.24	1.36	0.16	0.19	0.05	0.03	9.03	0.07
	1st adaptive	7.31	3.67	3.63	4.18	2.07	0.92	21.78	0.48
	2nd adaptive	6.30	9.25	3.21	5.00	3.98	2.46	30.20	0.85

Table 4 Correlation coefficients of seven growth characters as well as silymarin content and silymarin yield/plant in *Silybum marianum* genotypes among the wild and the second adaptive season.

Characters	Season	Linear growth	Main branches /plant	Total branches /plant	Capsules /plant	Seed yield /plant	Seed index	Silymarin content	Silymarin Yield /plant
Plant height	Wild	0.970**	0.713**	0.823**	0.880**	0.891**	-0.704**	-0.379**	0.917**
	2nd adaptive	0.879**	0.759**	0.846**	0.922**	0.757**	-0.558**	0.803**	0.807**
Linear growth	Wild		0.757**	0.853**	0.905**	0.908**	-0.736**	-0.262	0.932**
	2nd adaptive		0.705**	0.902**	0.913**	0.778**	-0.531**	0.828**	0.840**
Main branches	Wild			0.648**	0.762**	0.687**	-0.511**	-0.168	0.681**
	2nd adaptive			0.800**	0.783**	0.673**	-0.507**	0.715**	0.724**
Total branches	Wild				0.866**	0.850**	-0.562**	-0.285*	0.780**
	2nd adaptive				0.902**	0.797**	-0.572**	0.827**	0.844**
Capsules /plant	Wild					0.843**	-0.664**	-0.296*	0.857**
	2nd adaptive					0.863**	-0.541**	0.868**	0.877**
Seed yield	Wild						-0.576**	-0.276	0.851**
	2nd adaptive						-0.469**	0.809**	0.818**
Seed index	Wild							0.121	-0.687**
	2nd adaptive							-0.524**	-0.538**
Silymarin content	Wild								-0.271
	2nd adaptive								0.988**

guished with eight bands (117, 110, 97, 62, 46, 23, 21 and 19 KDa) as opposed to only one band with 7 KDa characterized for seeds of the second adaptive season.

Protein bands hardly varied in the first adaptive season which faced the hard environmental exchanges from wild to old land. But, when the plants acclimated with the new adaptive environment in the second adaptive season the protein bands returned back to be similar with the original wild parent plants except for three bands of 17, 9 and 7 KDa. Therefore, a maximum similarity index value 66.67% was calculated among the pair of genotype 6 in the wild vs. in the second adaptive season (**Table 6**).

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Table 5 Water-soluble protein densitometry profiles of *Silybum marianum* genotype 6 for the wild as well as first and second adaptive seasons.

No. of bands	MW (KDa)	Wild season	First adaptive season	Second adaptive season
1	117	-	+	-
2	110	-	+	-
3	97	-	+	-
4	79	+	+	+
5	73	+	+	+
6	62	-	+	-
7	60	+	-	+
8	46	-	+	-
9	37	+	-	+
10	33	+	+	+
11	23	-	+	-
12	21	-	+	-
13	19	-	+	-
14	17	+	-	-
15	13	+	+	+
16	9	+	-	-
17	7	-	-	+
Total bands	17	8	12	7

Table 6 Overall electrophoresis similarity index values of *Silybum marianum* genotype 6 among the wild as well as first and second adaptive seasons.

Seasons	Similarity index
Wild x first adaptive	25.00
Wild x second adaptive	66.67
First adaptive x second adaptive	26.67

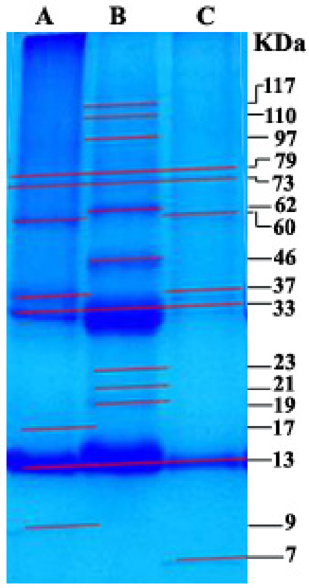

Fig. 1 Electrophenogram illustrating the variation in banding pattern of soluble protein of the seeds of wild and both adaptive seasons of genotype No. 6. (**A**) Wild genotype, (**B**) first adaptive season; (**C**) second adaptive season.

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