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Selfing Mating Effect on Growth Traits and Silymarin Production of Some Selected Lines among Milk Thistle (*Silybum marianum* L.) Varieties

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Abstract: Ten selected lines for each purple and white head flower varieties of milk thistle, *Silybum marianum* were assessed for five growth traits and silymarin production among three generations: open parents, selfing progenies and selfing offspring. Highly significant variations were existed between lines, varieties and generations as well as their interactions in all tested traits. The line characters for each variety were subjected to analysis of variance only for open parents opposite to selfing offspring and seemed highly significant variablities. The selfing offspring generation produced higher mean value in all purple variety traits except no. of head flower. Contrarily, the parent generation produced higher values in all white variety traits except fruit yield (FY). Lines 34 and 9 for purple and white varieties respectively were the best lines in both open and selfing generations. Coefficient of variation, genotypic and phenotypic coefficients of variation as well as broad sense heritabilities and genetic advance for most of studied growth traits were improved in the selfing offspring generation to indicate that milk thistle traits were governed with additive gene effects. Fruit yield traits had the highest parent offspring regression and narrow sense heritability in both varieties. On the other hand, lines 34, 22 and 28 in the purple as well as 9, 2 and 13 in the white variety were the highest, medium and lowest fruit yield, respectively and subjected for fruit content of silymarin using HPLC. Concentration and total yield of six detected silymarin compounds showed wide variations between lines, varieties and generations ranged from 11.92 to 62.85 mg g^{-1} and between 329.8 to 2121.3 mg $plant^{-1}$, respectively. Selfing mating improved the silymarin contents in the purple lines, but reduced the content of white lines. Improving of silymarin content was returned to increasing of silybin components. Interested notice is that silymarin production has the same pattern of fruit yield trait, so selection must based on this trait to produce new improved yielding silymarin genotypes.

Key words: Selfing mating · milk thistle · varieties · silymarin components

INTRODUCTION

Milk thistle, *Silybum marianum* (L.) Gaertn is an important medicinal annual or biennial plant belonging to family Asteraceae. The plant fruits contain the 3-oxyflavone silymarin, an isomeric mixture of three flavonolignans i.e. silychristin, silydianin and silybin [1]. These compounds are of considerable pharmacological interest owing to their strong anti-hepatotoxic and hepatoprotective activity [2, 3]. Silymarin is actually used for therapy of liver diseases and the flavolignan silybin is the most effective compound [4].

Two different varieties have been described under *marianum* species: *marianum marianum* with purple flowers and *marianum albiflora* with white flowers [5]. Both varieties are distributed widely and cultivated in the Mediterranean area and share the same ecological biosphere [6, 7]. In Egypt, milk thistle grows widely as a winter crop on canal banks, while the plant cultivation in new reclaimed lands is more suitable for the plant growth traits and silymarin production [8].

There is lack of research effects on milk thistle breeding for producing characterized lines rich in silymarin. Therefore a breeding program involving cross or selfing mating might be a successful strategy in this respect. On the other hand, the variation studying (between and within varieties) is also important for genetic improvement of the plant growth and yield components [9].

The present investigation aims to study the mating system effect on milk thistle growth traits and silymarin production.

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MATERIALS AND METHODS

Fruits of two *Silybum marianum* varieties: *marianum* (with purple flowers) and *albiflora* (with white flower) were obtained from Medicinal & Aromatic Plant Breeding Group, National Research Centre (NRC), Egypt. Ten selected lines (according to high number of head-flowers per plant) were chosen for each variety. The experiments were carried out at the experimental station of NRC, Shalakan, Qalubia Governorate, Egypt. A randomized complete design with three replications was used for three generations: open parents, selfing 1 (selfing progenies) and selfing 2 (selfing offspring) at 2003/2004, 2004/2005 and 2005/2006 seasons, respectively. Each replicate had 5 rows for each variety with 4 m length and 60 cm in between. The fruits were sown at October in hills 50 cm distance. After complete seedling, the plants were thinned to leave one plant per each hill. Two random plants were selected for each row before opening the flowers. The selected plant flowers were covered with pergamen pages tightly for parent generation to produce selfing progenies (selfing 1) and for progeny generation to produce selfing offspring (selfing 2). Five growth traits: plant height (PH), number of main branches/plant (MB), number of total branches/plant (TB), number of total head flower/plant (HF) and fruit yield/plant (FY) were recorded.

The obtained data were subjected to statistical analysis of variance, averages ($\bar{x}$), Standard Deviations (SD), ranges and coefficient of variations (CV%) using SPSS program [10]. Genotypic and phenotypic coefficients of variations (GCV and PCV, respectively) were estimated according to Burton [11]. Broad and narrow sense heritability (h_b^2 and h_n^2, respectively) were also estimated according to Robinson [12]. Genetic advance (GA) and parent offspring regression (B_{ij}) were calculated using Johanson [13] and Dewey & Lu [14], respectively.

Silymarin components were determined for three selected lines in each variety according to its FY (highest, medium and lowest). One gram of air dried fruits were defatted in a Soxhlet apparatus with 150 ml of petroleum ether (40-60°C) for 12 h. The residue was extracted with 50 ml of methanol at 65-70°C over 8 h. The methanolic solution was concentrated to a dry residue. The extract was dissolved in 10 ml of methanolic solution [1].

HPLC analysis was carried out using Shimadzu HPLC, LC-6A. A phenomenex C-18 (250×4.6 mm ID) column was used, eluting with MeOH-H_2O-AcOH 40:60:5, at a flow rate of 1 ml min^{-1} and the detection at 280 nm according to Alikaridis *et al.* [15]. A commercially available (Aldrich, 25492-4) mixture of flavonolignans was used as reference standard for the identification and assay.

RESULTS AND DISCUSSION

Analysis of variance: Ten selected lines of two *Silybum marianum* varieties (*marianum* and *albiflora*) among three generations (parents, selfing progenies and offspring) were assessed for plant height (PH), number of main and total branches/plant (MB and TB, respectively), number of head flowers/plant (HF) and fruit yield/plant (FY) to evaluate the genetic variability. Analysis of variance in Table 1 revealed that highly significant variations were existed between generations and lines in all traits and between varieties in PH, MB and FY. Also, significant differences were recorded for interaction variance of generation with varieties (G×V) in all traits and with lines (G×L) in the traits except MB. The obtained varietals variations are in agreement with the results of Hets *et al.* [7]. Meanwhile, the significant variation among lines showed considerable amount of genetic variability could be utilized for breeding program to improve the plant characters. In this respect, Ram *et al.* [9] found the same differences between 15 accessions of milk thistle.

The second generation (selfing progenies) was the segregated generation of parent selfing pollination, so it had not any consideration in the following discussion. The line characters of both milk thistle varieties (purple and white head flowers) were subjected for analysis of variance only for parent and offspring generations and illustrated in Table 2. Highly significant variations were performed between lines of purple variety in all traits. Same differences were cleared in the traits of white variety lines, except MB it had no significant variation. These differences confirmed that the corresponded lines have considerable genetic variations which can be used as a base of new strains. Other studies, Hetz *et al.* [7], Ram *et al.* [9], Omidi Tabrizi *et al.* [16] and Eslam [17] found that high genetic variations for different characters between genotypes of milk thistle or other crops like sunflower and safflower.

Growth traits among generations, varieties and lines: Data in Table 3 showed the mean value of the five studied quantitative characters for the ten selected lines of both milk thistle varieties (purple and white head flowers) among parent and selfing offspring generations. Selfing offspring produced higher mean values than the parents

Table 1: Analysis of variance (MS) of five growth traits for 10 selected lines in two *Silybum marianum* varieties among three generations: parents, progenies and offspring

		Characters				
Parameters	D.F.	PH	MB	TB	HF	FY
Replicates	2	96.45	7.81	190.46	192.88	5.98
Varieties (V)	1	843.77**	24.06**	220.42	112.07	333.76**
Generations (G)	2	2036.13**	37.92**	10530.15**	25177.95**	1075.97**
Lines (L)	9	967.33**	12.82**	443.93**	761.55**	91.86**
Interactions						
V×G	2	3154.84**	15.62*	1930.62**	2968.02**	31.16**
V×L	9	70.95	2.62	58.86	28.14	1.93
G×L	18	282.90**	3.58	185.37*	165.77*	10.45**
Residual	136	74.19	2.62	68.51	66.54	1.99

* Significant at p<0.05, ** Significant at p<0.01

Table 2: Analysis of variance (MS) of five growth traits for 10 selected lines in purple and white *Silybum marianum* varieties of parents and selfing offspring generations

			Purple head flower variety					White head flower variety				
Generations	Sources of variance	D.F	PH	MB	TB	HF	FY	PH	MB	TB	HF	FY
Parents	Between lines	9	220.60**	0.58**	20.35**	41.85**	4.35**	417.10**	5.35**	220.00**	508.85**	7.89**
	Within lines	2	0.20	0.00	0.10	0.10	0.00	0.90	0.00	0.40	0.40	0.06
	Error	18	0.90	0.03	0.85	0.85	0.15	1.90	0.05	1.90	3.65	0.12
Selfing offspring	Between lines	9	428.14**	20.36**	579.49**	331.38**	67.28**	579.25**	1.47	138.23**	319.72**	44.87**
	Within lines	2	6.05	0.05	0.45	0.05	0.06	1.25	1.25	11.25	22.05	0.31
	Error	18	4.49	1.61	26.12	24.72	3.42	14.58	1.25	27.92	23.05	2.02

* Significant at p<0.05, ** Significant at p<0.01

in all characters, except HF for purple variety, while parents of the white variety had the higher mean values in all traits except FY. In varietal comparison among the parents, higher values i.e. 166.2, 83.3 and 121 were recorded for PH, TB and HF of the white variety opposite 131.5, 67.4 and 91.3, respectively for purple. While among selfing offspring, purple produced the higher values for all traits than white. The selected lines had varied expressions in all traits among either varieties or generations. Line 34 was the highest line among purple variety in PH, TB and FY in the parents as well as PH, MB, TB and FY in the offspring, while line 9 was the highest line among white variety in PH, MB and FY in the parents addition to FY in the offspring. Lines 28 in purple and 13 in white produced the lower values of PH, MB and FY in both generations. Meanwhile, lines 22 in purple and 2 in white were in mediate and approximated to the general averages of all traits in both generations (Table 3). These results revealed that variations between the parents and selfing offspring and were agreement with those findings of Wyatt [18], Bookman *et al*. [19], Kephart [20] and Khan and morse [21] on several related crops. Also, the differences between lines were reported by Eslam [17] and Patial *et al*. [22] in safflower.

Genetic parameters: Data in Table 4 presented some evaluated genetic parameters for the traits of the two milk thistle varieties in both generations. Coefficient of variation (CV%) as well as genotypic and phenotypic coefficients of variation (GCV and PCV, respectively) increased in selfing offspring for all traits. Highest variation coefficients were cleared in PH of purple variety (7.55, 7.98 and 8.01, respectively) and in MB of white variety (16.84, 14.45 and 14.65 in the some respect) at the parent generation. Meanwhile, MB of purple and HF of white presented the maximum variations (37.81, 31.4 and 33.99 opposite to 22.19, 14.24 and 17.48, respectively) in the offspring generation. However, significant broad sense hertabilities (h_b^2) were presented for all traits in both varieties and/or generations except TB of white variety at offspring. Increasing of genetic advances (GA) was noticed in the offspring, except purple PH as well as

Table 3: Average and standard deviation values of five growth traits for 10 selected lines in each purple and white *Silybum marianum* varieties of parent and selfing offspring generations

		Purple milk thistle									
		PH		MB		TB		HF		FY	
Generation	Lines	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Parent	34	147.0	2.0	10.0	0.2	71.0	2.5	96.3	4.0	32.8	0.6
	1	147.0	1.0	10.7	0.4	70.3	2.1	97.0	3.5	32..2	1.2
	23	134.0	4.0	10.0	0.5	70.7	2.0	94.7	2.5	31.2	0.7
	7	134.7	4.0	10.0	0.0	68.0	2.7	93.0	3.5	30.9	0.2
	26	133.0	3.0	10.3	0.5	69.3	1.1	92.7	3.5	30.8	0.4
	22	133.3	1.5	10.0	0.1	68.3	4.0	92.3	4.5	30.7	1.7
	17	126.0	4.0	9.0	0.1	65.7	3.2	88.3	6.5	29.9	0.4
	3	126.0	3.0	9.3	0.5	64.3	2.6	86.7	5.0	29.2	0.2
	18	117.0	4.0	9.7	0.2	63.0	2.4	86.7	2.0	28.9	1.0
	28	117.0	3.0	9.0	0.3	63.3	1.3	85.0	1.5	28.4	0.6
	G.$\bar{x}$	131.5		9.8		67.4		91.3		30.5	
Selfing offspring	34	192.0	3.5	19.0	0.4	100.0	1.7	98.7	2.7	50.2	2.0
	1	147.7	2.5	10.7	0.4	46.7	2.1	103.0	0.0	49.8	1.6
	23	183.7	2.5	7.7	0.5	78.3	3.2	72.7	2.5	48.0	0.7
	7	184.7	1.5	9.3	0.2	72.3	3.3	81.7	3.2	46.2	0.6
	26	184.3	2.5	10.0	0.1	84.3	2.7	76.3	4.5	44.8	0.8
	22	176.7	4.5	10.3	0.2	95.0	1.3	88.0	3.5	43.7	1.5
	17	169.7	2.5	6.0	0.3	45.0	1.1	86.7	3.0	41.6	1.3
	3	172.0	3.0	11.7	0.3	77.3	1.7	53.0	3.5	39.1	0.4
	18	186.7	3.5	10.0	0.0	99.3	2.5	83.3	2.5	38.2	1.1
	28	146.0	2.0	6.0	0.4	57.3	2.5	59.7	4.0	27.5	0.3
	G.$\bar{x}$	179.4		10.1		75.2		80.3		42.9	
		White milk thistle									
		PH		MB		TB		HF		FY	
Generation	Lines	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Parent	9	185.0	0.0	12.0	0.0	93.7	2.5	141.7	6.3	26.9	0.7
	8	183.3	3.5	11.3	0.7	96.0	0.0	144.0	0.0	26.5	0.9
	4	178.7	3.0	9.0	0.3	87.7	4.0	131.3	2.2	25.3	1.2
	5	177.7	2.7	9.7	0.3	85.3	3.5	129.7	2.9	25.0	0.0
	2	162.0	3.5	9.7	0.2	81.3	2.7	120.7	4.6	23.9	1.5
	15	160.0	0.0	9.7	0.6	80.3	4.5	118.7	3.5	23.2	1.3
	10	159.3	2.5	8.7	1.0	78.0	0.0	109.3	3.5	23.1	0.6
	24	157.3	2.1	8.3	0.2	76.0	0.0	106.3	4.1	22.7	0.3
	6	150.7	1.5	8.3	0.4	77.7	1.5	103.0	1.5	21.8	0.7
	13	148.0	0.0	8.0	0.0	77.3	1.8	105.7	1.8	21.4	0.2
	G.$\bar{x}$	166.2		9.5		83.3		121.0		24.0	
Selfing offspring	9	178.7	3.6	7.0	0.8	58.3	2.2	78.7	3.3	51.8	1.7
	8	156.3	2.7	6.3	0.3	52.7	1.6	58.3	2.7	45.7	1.4
	4	183.0	0.0	6.7	0.7	42.7	1.6	53.7	2.6	43.1	1.0
	5	151.7	2.0	6.0	0.0	61.0	0.0	81.0	0.0	42.9	1.1
	2	157.7	2.5	7.7	0.4	59.3	1.2	66.7	2.2	41.3	0.8
	15	158.3	1.5	8.3	1.0	53.3	1.3	69.7	3.4	40.4	0.8
	10	170.7	2.5	6.7	0.6	56.7	1.0	57.7	1.4	39.9	0.5
	24	172.0	3.5	9.0	0.0	59.3	2.1	70.3	2.1	38.4	1.4
	6	175.3	1.5	6.3	1.1	23.0	0.0	72.3	3.1	34.1	0.4
	13	120.0	0.0	4.0	0.0	51.3	2.5	26.0	0.5	33.1	0.5
	G.$\bar{x}$	162.4		6.8		51.8		63.4		41.1	

PH= Plant height. MB= No. of main branches. TB= No. of total branches. HF= No. of head flowers/plant. FY= Fruit yield/plant. G.$\bar{x}$ means general average

Table 4: Genetic parameters of five growth traits for purple and white *Silybum marianum* varieties of parent and selfing offspring generations

		Purple head flower variety					White head flower variety				
Generation	Genetic items	PH	MB	TB	HF	FY	PH	MB	TB	HF	FY
Parent	$\bar{x}$	131.40	9.60	67.10	90.90	30.50	165.90	9.20	83.00	120.60	24.00
	Range	117-147	9-10	63.71	85-97	28.4-32.8	148-185	8-12	76-96	103-144	21.4-26.5
	CV%	7.55	5.38	4.58	4.79	4.63	8.23	16.84	8.50	12.52	7.88
	GCV%	7.98	4.48	4.65	4.98	4.75	8.68	14.45	12.58	13.18	8.22
	PCV%	8.01	4.77	4.85	5.08	4.92	8.72	14.65	12.69	13.27	8.34
	h_b^2	0.99	0.88	0.92	0.96	0.93	0.99	0.97	0.98	1.00	0.97
	GA%	11.76	3.67	5.35	6.44	7.02	11.41	29.60	15.34	14.55	11.83
Selfing offspring	$\bar{x}$	175.80	9.80	74.90	79.90	42.90	162.00	6.50	51.40	63.00	41.10
	Range	146-192	6-18	45-10	53-10	27.5-50.2	120-18	4-9	23.61	26-81	33.1-51.0
	CV%	9.49	37.81	26.68	19.63	15.87	11.25	20.83	22.19	25.12	13.22
	GCV%	8.30	31.40	22.16	15.49	18.65	10.39	4.94	14.24	19.01	11.31
	PCV%	8.39	33.99	23.19	16.69	19.14	10.65	17.28	17.48	20.44	11.82
	h_b^2	0.98	0.85	0.91	0.86	0.95	0.95	0.08	0.66	0.87	0.91
	GA%	9.36	80.93	30.38	24.83	16.35	12.46	2.72	11.57	22.90	24.22
	B_{ij}	0.14	0.40	0.15	0.70	0.92	0.36	0.14	0.17	0.284	0.60
	h_n^2	0.02	0.16	0.02	0.49	0.49	0.13	0.02	0.03	0.081	0.92

Table 5: Silymarin composition of three selected lines (highest, medium and lowest FY) in each purple and white *Silybum marianum* varieties of parent and selfing offspring generations

	Parent generation						Selfing offspring generation					
	Purple variety			White variety			Purple variety			White variety		
Silymarin composition	34	22	28	9	2	13	34	22	28	9	2	13
	Silymarin concentration (mg $seed^{-1}$)											
Silychristin	6.30	3.87	2.64	7.17	6.64	5.93	3.98	5.13	2.70	6.38	4.37	4.10
Silydinin	9.25	5.23	3.86	16.77	15.37	4.23	11.35	11.21	5.42	4.00	3.14	3.47
Silybin A	3.21	1.19	0.50	13.03	10.84	1.46	7.46	6.58	0.61	1.93	1.60	1.47
Silybin B	5.00	2.16	1.36	19.50	16.30	2.13	11.71	10.29	1.19	2.78	2.69	2.40
Isosilybin A	3.98	2.26	2.56	4.85	4.01	0.50	5.05	4.20	1.16	0.60	0.44	0.40
Isosilybin B	2.46	1.68	1.98	1.53	1.28	0.13	2.70	2.18	0.91	0.13	0.09	0.08
Total silymarin	30.20	16.39	12.90	62.85	54.44	14.38	42.25	39.59	11.99	15.82	12.28	11.92
	Silymarin accumulation (mg $plant^{-1}$)											
Silychristin	206.60	118.80	75.00	192.90	158.70	126.90	200.00	224.40	74.30	330.50	180.50	135.70
Silydinin	303.40	160.60	109.60	451.10	367.30	90.50	570.00	489.90	149.10	207.20	129.70	114.90
Silybin A	105.30	36.50	14.20	350.50	259.10	31.20	474.50	287.50	16.80	100.00	66.10	48.70
Silybin B	164.00	66.30	38.60	524.60	389.60	45.60	587.80	449.70	32.70	144.00	109.00	79.40
Isosilybin A	130.50	69.30	72.70	130.50	95.80	10.70	253.50	183.50	31.90	31.10	18.20	13.20
Isosilybin B	80.70	51.60	56.20	41.20	30.60	28.00	135.50	95.30	25.00	6.70	3.70	2.60
Total silymarin	990.50	503.20	366.30	1690.80	1301.10	307.70	2121.30	1730.10	329.80	819.50	507.20	394.50

white MB and TB were decreased. On the other hand, FY had the highest parent offspring regression (B_{ij}) and narrow sense heritability (h_n^2) in both varieties opposite to purple PH and white MB had the lowest B_{ij} and h_n^2 (0.137 and 0.136 as well as 0.019 and 0.018, respectively). The results showed that milk thistle varieties presented different responses for the selfing mating in all studied traits to confirm the different physiology, heredity and

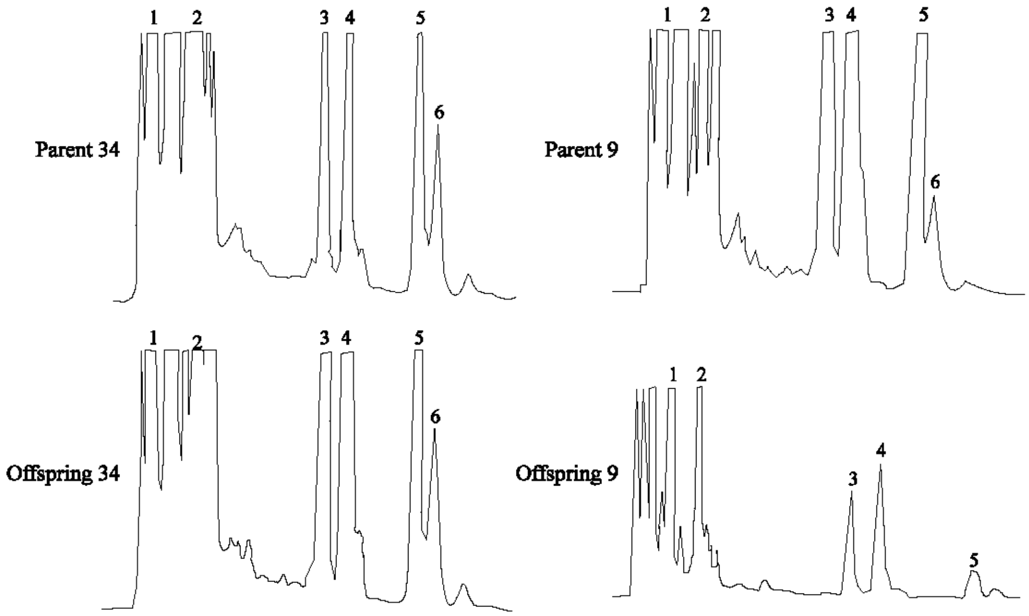

Fig. 1: HPLC chromatogram of silymarin compounds for parent and offspring generations of line 34 (the highest FY of purple variety) and line 9 (the highest FY of white variety) of milk thistle, *S. marianum*. 1. Silychristin, 2. Silydinin, 3. Silybin A, 4. Silybin B, 5. Isosilybin A, 6. Isosilybin B

behavior of each variety comparing with another one [7]. High values of CV%, GCV, PCV, h_b^2 and GA in the offspring indicating that milk thistle traits are governed with additive gene effects as mentioned by Reddy *et al.* [23]. The future breeding program of this plant will be based on FY trait which had significant B_{ij} and h_n^2as mentioned before [24].

Silymarin content: Silymarin in the fruits of the highest, medium and lowest lines for the trait of FY (line 34, 22 and 28 in the purple variety as well as 9, 2 and 13 in the white variety, respectively) was determined using HPLC and shown in Table 5 and Fig. 1. Six silymarin compounds: silychristin, silydinin, silybin A, silybin B, isosilybin A and isosilybin B were detected in the extract of all tested lines (Fig. 1). All silymarin components - both in terms concentration (mg g^{-1} fruit) and accumulation (mg $plant^{-1}$) showed a high variability among lines, varieties and generations. All white lines produced higher components in the parent generation compared with the selfing offspring. Parents of white lines 9 and 2 had four times of total silymarin concentration and twice amount of silymarin per plant as compared with corresponding offspring ones. Increasing of silymarin contents returned mainly to increasing of silybin components, the most therapeutically effect. While the purple lines 34 and 22 had the higher levels of all silymarin components in offspring than parents, except silychristin in line 34. In the offspring of 34 and 22 lines, total silymarin reached to 42.25 and 39.59 mg g^{-1} as well as 2121 and 1730 mg $plant^{-1}$ corresponded with 20.2 and 16.39 mg g^{-1} as well as 990.6 and 503.2 mg $plant^{-1}$ for their parents, respectively. The purple line 28 had the higher silymarin B, isosilybins A and B as well as total silymarin in the parents, but higher silydinin in the offspring. Total silymarin and its components in most cases were varied between the lines and took the same pattern of lines FY distribution at both generations. The lowest fruit yield lines 28 and 13 did not appear any significant changes in total silymarin contents among generations.

Generally, all silymarin components varied depending on lines, varieties and generations. These results are in agreement with the findings of Hetz *et al.* [7] and Ram *et al.* [9]. Although, the selfing mating improved the content of silymarin in the purple lines, the contents of parents were the best value in the white lines confirming that each variety has different physiology and heredity behavior systems as mentioned by Hetz *et al.* [7]. Also, silymarin production take the same pattern of FY trait, therefore, selection must be based on FY to produce genotypes have high silymarin content [24, 25]. These results are important for genetic improvement program for growth traits and silymarin production of the milk thistle plant.

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