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Genetic Variability and Antiphytoviral Activity of Wild and Inbred Genotypes of *Silybum marianum* Varieties

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ABSTRACT

Wild and inbred genotypes of *Silybum marianum* were assessed for five quantitative characters, silymarin production and its potent antiviral activities. Purple, white and wild varieties as well as their genotypes showed significant genetic variations in all characters except for the number of main branches/plant. Correlation coefficients showed highly significant values in all varieties while significant regression values were computed for plant height and total branches each with head flowers, in addition to seed yield with total branches in all. Six silymarin components were detected showing high variation among varieties and genotypes. Purple genotype P_{34} and white W_9 were the best genotypes as they presented the maximum mean value for all studied characters and the highest silymarin content (30.20 and 62.85 mg/g, respectively). The seed methanolic extract of all genotypes had an inhibitory effect on *Tomato mosaic virus* (ToMV) infectivity. The extract reduced ToMV infection with different percentages: 80% for wild, 71.4, 69.6, 68 for white genotypes (W_9, W_2 and W_{13}), and 60, 58.4 and 46.4, for purple genotypes (P_{34}, P_{22} and P_{28}). All silymarin constituents had a highly significant negative correlation with the number of local lesions. However, both *in vivo* and *in vitro* tests showed the inhibitory effect of the methanolic extract on virus infectivity. However, ToMV post-inoculation rubbing with the methanolic extract reduced ToMV infectivity more effectively than ToMV pre-inoculation. However, the wild *S. marianum* extract more effectively reduced ToMV infectivity *in vivo* than *in vitro*.

Keywords: milk thistle, silymarin, ToMV, virus infectivity

INTRODUCTION

Silybum marianum (L.) Gaertn is one of the most important medicinal plants belonging to the family Asteraceae and is known as lady's thistle, milk thistle or *Shook El-Jamal*. The active chemical component of milk thistle seeds is 3-oxyflavone silymarin, an isomeric mixture of three flavonolignans, i.e. silychristin, silydianin and silybinin (Ibrahim *et al.* 2007). These compounds have considerable pharmacological interest owing to their strong anti-hepatotoxic and hepatoprotective activity (Sanchez-Sampedro *et al*. 2005). Silybinin works as antioxidant, scavenging free radicals and inhibiting lipid peroxidation, in addition to altering the membrane structure of liver cells, blocking the absorption of penetrating toxins into the cell and stimulating the production of new liver cells to replace damaged cells (Schumann *et al*. 2003).

Two different varieties (*marianum* with purple head flowers and *albiflora* with white head flowers) are classified under the species *marianum* (Hetz *et al.* 1995). Both varieties are widely distributed in the Mediterranean area and share the same ecological biosphere (Sadaqat *et al.* 1983). Both varieties have been successfully cultivated through a continuous breeding program from 2002 until now (Ottai and Abdel-Moniem 2006; Ibrahim *et al.* 2007; Ottai *et al.* 2009).

Separately, *Tomato mosaic virus* (ToMV) is a virus that seriously attacks and causes significant loss of tomato yields. Plant extracts are applied as a natural product and as a safe method to control virus infection to varying degrees. However, this is only true when they are mixed with the virus prior to inoculation or when applied within a short period of time before or after inoculation (El-Dougdoug 1997). The effect of plant extracts was attributed to chemical contents within them such as anti-phytoviral agents, e.g., tannic acid, phenolic compounds, flavonoids, polysaccharides, sterols, alkaloids and proteins (Menounos *et al.* 1986; Othman *et al.* 1991).

The aim of this study was to assess genetic variability of quantitative growth characters and antiphytoviral activity of the wild variety and inbred genotypes of purple and white *S. marianum* varietiess.

MATERIALS AND METHODS

Plant materials

Three double genotypes of *Silybum marianum* Cod (34, 22 and 28) and Cod (9, 2 and 13) of purple (*marianum*) and white (*albiflora*) varieties, respectively were selected from a continuous breeding program of the Genetics and Cytology Department, National Research Center (NRC), Egypt as plant material for this study. In addition, wild Egyptian *S. marianum marianum* plants were chosen from the desert at Ras Sadder, Sinai, Egypt. This plant was identified by the NRC herbarium.

Cultivation method

Seeds of all selected genotypes were sown in two seasons at the Experimental Station Farm of the NRC in Shalakan, Qualiobia governorate, Egypt in a completely randomized design with three replications. Each replicate had 5 rows 4 m in length and an inter-row distance of 60 cm. Each row had 5 hills after seedlings formed (after 30 days with 4 leaves), the plants were thinned to one plant per hill. Five quantitative characters were recorded for the second season: 1) Plant height (PH) in cm; 2) Number of main branches (MB); 3) Total number of branches (TB); 4) Number of head flowers (HF); 5) Seed yield per plant (SY) in g. Quantitative

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characters from the first season were not used since it is the segregated generation in which plant characters are not stable The obtained data were statistically analyzed, coefficient of variation (CV%), where CV% = (variance/ average) 100 and coefficient of correlation and regression were computed using SPSS software (2001).

Methanolic extraction

The powder of 10 g of dried seeds (= harvested seeds after complete maturity from the second season) of each genotype were extracted with 50 ml absolute methanol in a dark glass bottle under laboratory conditions for 24 h, then filtered by filter paper Whatman 4 (150 mm). The marc was re-extracted another two times in the same manner and under the same conditions. The combined extract was evaporated under reduced pressure till dryness, made up to 10 ml with the same solvent and kept in a refrigerator (AOAC 1970).

Assay of antiviral activity

To study the influence of the antiphytoviral methanolic extract on ToMV infectivity and variability, different methods were tested:

1. In vitro

One ml of methanolic extract for each milk thistle genotype was mixed with 1.0 ml of the infected crude sap of tomato plants and with 1.0 ml water as a control. The mixtures were incubated at room temperature for 0, 1 and 24 hr. The inhibitory effect on ToMV infectivity and virus yield were estimated by local lesions (LLs) assay (100-µl were mechanical inoculated with a glass spatula on the leaf upper surface) of *Chenopodium amaranticolor* at the same physiological age. These LLs were marked (observed by eye and marked with a pen) on the upper leaf surface and counted. Measuring single LLs, characters, similarity and characteristic morphology (center, surrounding halo, chlorosis, necrosis, size, fineness) were described.

2. In vivo

This experiment was planned to study the effect of methanolic extract of wild milk thistle on the multiplication of ToMV by rubbing for 0, 1, 4, 24 and 48 hr pre- and post-inoculation with ToMV. The upper surface of tomato seedling leaves was rubbed with a glass spatula at the 4-leaf stage with 100 µl of 10^{-1} alcoholic extract and then inoculated with 50 µl of ToMV suspension. In the control test distilled water was rubbed at pre- and post-inoculation with ToMV for the same periods of time.

3. Seed soaking

Apparently healthy tomato seeds (100 seeds) were soaked for 12 and 24 hr in a methanolic extract of each wild and three genotypes of purple and white milk thistle genotypes, and in distilled water as the control. Batches of 25 treated seeds were cultivated in sterilized soil (five seeds per pot and five pots per treatment). Tomato seedlings were mechanically inoculated at the 4-leaf stage with 100 µl of ToMV suspension. The inhibitory effect was assayed by assessing the number of LLs on *C. amaranticolor*, calculated according to the formula:

$$I = (1–C)/(Co \times 100)$$

where I is the percentage of infection, C is the virus concentration in the presence of the tested material and Co is the virus concentration in the control (El-Dougdoug 1997). Statistical analysis was carried out with student's Q test at 0.1% (Sendeccor and Cochran 1967). $T = (Q_1 – Q_2)\ n_1 \times n_2$ at P 0.1% = 3.39 using this test. Biovariability of ToMV isolate was detected by a single LL (variation of LL morphology).

Determination and fractionation of silymarin

1.0 ml of prepared methanolic extraction for each genotype was analyzed using a Shimadzu HPLC, LC-6A. An Aphenomenex C-18 (250 × 4.6 mm i.d.) column was used, elution with MeOH-H_2O-AcOH (40: 60: 5), at a flow rate of 1 ml/min and detected at 280 nm, according to Alikaridis *et al.* (2000). A commercially available (Aldrich, 25492-4) mixture of flavonolignans was used as the reference standard for the identification and assay.

RESULTS

The genetic variation among three genotypes for each of the purple and white varieties as well as the Egyptian wild variety of *S. marianum* was assessed using quantitative characters, silymarin content and its potent antiviral activities.

Quantitative characters

The white variety showed highest PH (165.0 ± 10.8), MB (9.9 ± 1.2), TB (84.1 ± 4.9) and HF (122.7 ± 10.4), while the purple variety showed the highest SY (30.6 ± 1.3) (**Table 1**). The wild variety had the lowest value for all traits. CV% was highest for all characters of the wild variety. There was significant genetic variability between varieties for all studied traits. Moreover, there were significant differences in all characters between all purple and white genotypes, except for MB of the purple genotypes. P_{34} and W_9 showed the highest value for all studied characters. Conversely, P_{28} and W_{13} had the lowest values for all traits (**Table 1**).

Correlation and regression

Data in **Table 2** shows the estimated correlation and regression coefficients for each pair of characters and for each variety. All correlation coefficients were highly significant for all varieties. A completely positive correlation (1.00) was achieved between TB and HF and between PH and SY for the purple variety and between MB and HF for the white variety. All varieties (purple and white) showed significant regressions between HF and PH and between HF and TB and between SY and TB while the wild variety was characterized by a significant positive regression between MB and SY, the purple variety was characterized by significant positive regressions between HF and SY and, in the white variety, between PH and TB and between MB and SY (**Table 2**).

Silymarin components

The methanolic extract of milk thistle seeds contains silymarin, in addition to other chemical compounds. Therefore, silymarin content and its constituents were determined in the seed methanolic extract of the studied genetic resources using HPLC (**Table 3**). Three compounds (silychristin, silydianin, silybinin) and four silybinin constituents (silybin A, silybin B, isosilybin A, isosilybin B) were detected in different ratios in the seed extract of all tested genotypes. All silymarin components showed high variation among varieties and genotypes in descending order (white > purple > wild), except for silychristin and isosilybin B. The highest silychristin (7.67 ± 0.86) and isosilybin B (2.04 ± 0.23) contents were detected in wild and purple varieties, respectively. Purple genotype P_{34} and white W_9 produced the highest silymarin content (co-incidentally the quantitative characters in **Table 1** were also highest for these two genotypes). Except for silychristin content in the white variety, the silymarin content of all varieties and genotypes had a high CV%, > 10% and as high as 94.7% (**Table 3**).

Antiviral activity

The methanolic extract of the studied milk thistle resources was tested for its ability to inhibit ToMV on *C. amaranticolor*. The LLs caused by ToMV were used to evaluate the concentration and variability of ToMV infection and inhibition related to the different milk thistle varieties and their genotypes (**Table 4, Fig. 1**). Virus concentration was calcu-

Table 1 Mean values and coefficients of variation (CV%) of five quantitative characters for wild and three inbred genotypes of purple and white milk thistle varieties.

Varieties	Lines	PH	MB	TB	HF	SY
Wild	mean	81.0 ± 3.8 A	5.8 ± 0.4 A	15.5 ± 0.9 A	17.0 ± 1.4 A	7.3 ± 0.6 A
	CV%	14.8	21.0	17.8	26.1	24.1
Purple	P_{34}	147.0 ± 2.0 (c)	10.0 ± 0.2 (ns)	71.0 ± 2.5 (b)	96.3 ± 4.0 (b)	32.8 ± 0.6 (b)
	P_{22}	133.3 ± 1.5 (b)	10.0 ± 0.1 (ns)	68.3 ± 4.0 (b)	92.3 ± 4.5 (b)	30.7 ± 1.7 (ab)
	P_{28}	117.0 ± 3.0 (a)	9.0 ± 0.3 (ns)	63.3 ± 1.3 (a)	85.0 ± 1.5 (a)	28.4 ± 0.6 (a)
	mean	132.4 ± 8.7 B	9.7 ± 0.3 B	67.5 ± 2.3 B	91.2 ± 3.3 B	30.6 ± 1.3 C
	CV%	11.3	6.0	5.8	6.3	7.2
White	W_9	185.0 ± 0.0 (c)	12.0 ± 0.0 (b)	93.7 ± 2.5 (c)	141.7 ± 6.3 (c)	26.9 ± 0.7 (c)
	W_2	162.0 ± 3.5 (b)	9.7 ± 0.2 (ab)	81.3 ± 2.7 (b)	120.7 ± 4.6 (b)	23.9 ± 1.5 (b)
	W_{13}	148.0 ± 0.0 (a)	8.0 ± 0.0 (a)	77.3 ± 1.8 (a)	105.7 ± 1.8 (a)	21.4 ± 0.2 (a)
	mean	165.0 ± 10.8 C	9.9 ± 1.2 B	84.1 ± 4.9 C	122.7 ± 10.4 C	24.1 ± 1.6 B
	CV%	11.3	20.3	10.2	14.7	11.4

PH = plant height (cm); MB = No. main branches/plant; TB = No. of total branches/plant; HF = No. head flowers/plant; SY = seed yield (g/plant); CV% = coefficient of variance; ns = non significant.
(a, b and c) = means within genotypes are significantly different at $P < 0.05$; (ab) = means within genotypes are non-significantly different at $P < 0.05$; A, B and C = means within varieties are significantly different at $P < 0.05$.

Table 2 Correlation coefficients (lower diameter) and regression coefficients (upper diameter) of five quantitative characters for wild, purple and white milk thistle varieties.

Traits	Varieties	PH	MB	TB	HF	SY
PH	Wild		0.054	0.201	0.489**	0.109
	Purple		0.034	0.257	0.379*	0.147
	White		0.107	0.453**	0.967**	0.147
MB	Wild	0.997**		0.270	0.082	0.475**
	Purple	0.888**		0.159	0.094	0.230
	White	0.999**		0.230	0.111	0.728**
TB	Wild	0.999**	0.999**		0.305*	0.546**
	Purple	0.993**	0.935**		0.682**	0.558**
	White	0.989**	0.980**		0.464**	0.313*
HF	Wild	0.871**	0.845**	0.852**		0.145
	Purple	0.993**	0.935**	1.000**		0.380**
	White	0.999**	1.000**	0.982**		0.152
SY	Wild	0.970**	0.979**	0.979**	0.726**	
	Purple	1.000**	0.875**	0.990**	0.990**	
	White	0.996**	0.999**	0.972**	0.999**	

PH = plant height (cm); MB = No. main branches/plant; TB = No. of total branches/plant; HF = No. head flowers/plant; SY = seed yield (g/plant).
* and ** = significant at $P < 0.05$ and 0.01, respectively.
Different genotypes (both white and purple) were clustered because correlation and regression were computed as varieties (wild, purple and white) not as genotypes

Table 3 Mean values and coefficients of variation (CV%) of silymarin composition (mg/g seeds) of wild and three selected genotypes for each of purple and white milk thistle varieties.

Varieties	Lines	Silychristin	Silydianin	Silybinin					Silymarin content
				Silybin A	Silybin B	Isosilybin A	Isosilybin B	Total	
Wild	Mean	7.67 ± 0.86	1.39 ± 0.12	0.18 ± 0.06	0.17 ± 0.05	0.06 ± 0.02	0.09 ± 0.11	0.50 ± 0.03	9.55 ± 0.63
	CV%	19.30	15.50	57.70	45.80	58.90	94.70	47.3	11.42
Purple	P_{34}	6.30	9.25	3.21	5.00	3.98	2.46	14.65	30.20
	P_{22}	3.87	5.23	1.19	2.16	2.26	1.68	7.29	16.39
	P_{28}	2.64	3.86	0.50	1.36	2.56	1.98	6.40	12.90
	Mean	4.27 ± 1.08	6.11 ± 1.62	1.63 ± 0.81	2.84 ± 1.10	2.93 ± 0.33	2.04 ± 0.23	9.44 ± 2.61	19.83 ± 5.28
	CV%	43.60	45.8	86.20	67.40	31.30	19.30	47.90	46.10
White	W_9	7.17	16.77	13.03	19.50	4.85	1.53	38.91	62.85
	W_2	6.64	15.37	10.84	16.30	4.01	1.28	32.43	54.44
	W_{13}	5.93	4.23	1.46	2.13	0.50	0.13	4.22	14.38
	Mean	6.58 ± 0.36	12.12 ± 3.97	8.44 ± 3.55	12.64 ± 5.34	3.12 ± 1.33	0.98 ± 0.43	25.19 ± 10.65	43.89 ± 14.95
	CV%	9.50	56.70	72.79	73.10	74.00	76.19	73.20	59.00

CV% = coefficient of variance.

Table 4 Single local lesion (LL) variability of *Tomato mosaic virus* (ToMV) mixed with methanolic extract of wild and three inbred genotypes of purple and white milk thistle varieties and inoculated on *Chenopodium amaranticolor.*

Varieties/lines	No. of LLs	LL diameter (mm)	% of TMV inhibition	% of TMV infectivity	Similarity	Single LL morphology
Wild	25.0	1.0	80.0	20.0	Homologous	Regular, chlorotic, without halo
P_{34}	40.0	3.0	60.0	40.0	Homologous	Regular, chlorotic, without halo and center
P_{22}	50.0	1.0	58.4	41.6	Homologous	Irregular, chlorotic, with yellow halo
P_{28}	52.0	2.5	46.4	53.6	Homologous	Irregular, chlorotic, without halo and center
W_9	36.2	1.8	71.4	28.6	Heterologous	Irregular, necrotic, chlorotic, without halo and center
W_2	37.0	2.0	69.6	30.4	Homologous	Chlorotic ring with necrotic center
W_{13}	38.0	1.2	68.0	32.0	Homologous	Irregular, chlorotic, without halo
ToMV parent isolate (control)	105.0	3.0	00.0	100.0	Homologous	Regular, necrotic

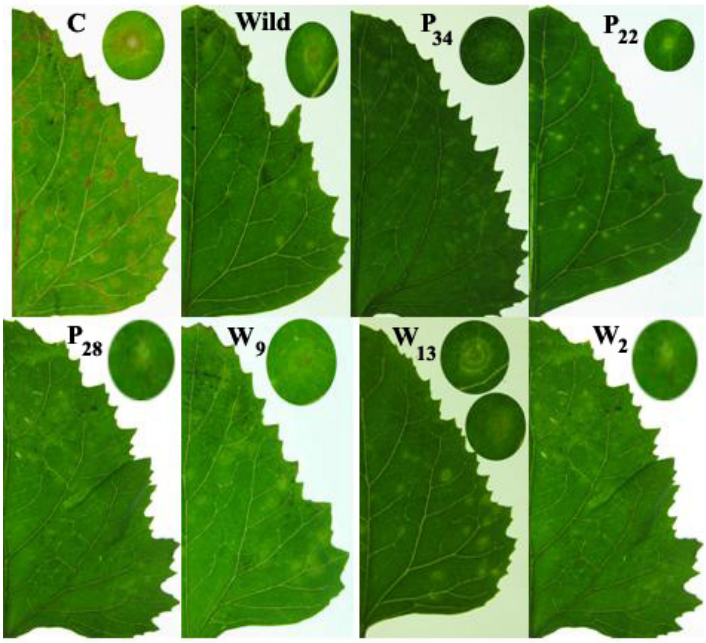

Fig. 1 Variability in local lesions to infection by ToMV following the application of methanolic extracts of wild and three inbred genotypes of purple and white milk thistle varieties. C = control, P_{34} = purple genotype No. 34, P_{22} = purple genotype No. 22, P_{28} = purple genotype No. 28, W_9 = white genotype No. 9, W_{13} = white genotype No. 13, W_2 = white genotype No. 2.

lated as the mean of LLs. The wild variety had the lowest mean number (25 LLs), followed by white genotypes (36.2, 37 and 38 LLs for W_9, W_2 and W_{13}, respectively) and followed by purple genotypes (P_{34}, P_{22} and P_{28} with 40, 50 and 52 LLs, respectively) compared with the control, ToMV isolate which resulted in 105 LLs. However, ToMV infection was inhibited to different levels, measured as percentage inhibition (80, 71.4, 69.6, 68, 60, 58.4 and 46.4% for wild variety, white genotypes (W_9, W_2 and W_{13}) and purple genotypes (P_{34}, P_{22} and P_{28}), respectively). In addition, the inducer reduced the percentage ToMV infection to 20, 28.6, 30.4, 32, 40, 41.6 and 53.6%, respectively.

The variability of ToMV isolates was described by LL diversity. Frequency (i.e., phenotypic property of LLs) was used in a parent strain of ToMV in *C. amaranticolor* (as control) resulting in conspicuous LLs (homologous necrotic LL edge, brown, without a halo and 3 mm in diameter). The effect of the methanolic extract of the tested resources on ToMV was investigated by measuring single LL characters, similarity and characteristic morphology and mutagenic effect (**Table 4, Fig. 1**).

Correlation of silymarin composition and ToMV infection

Correlation and determination coefficients were calculated for silymarin composition (from the methanolic extract of the studied resources in **Table 5** with the produced mean number of LL for each purple and white variety). All silymarin constituents in both varieties had negative and highly significant correlations with the amount of LLs produced. Moreover, > 80% of determination coefficients were estimated for all silymarin constituents corresponding with the mean number of LLs.

Effect of methanolic extract on ToMV multiplication

The methanolic extract of wild milk thistle seeds, which contain silymarin, the effective substance of the plant, was tested as a biocontrol agent of ToMV *in vitro* and *in vivo* and also in trials involving tomato seeds.

1. In vivo: Post-ToMV inoculation

Treatment with the methanolic extract affected ToMV infectivity, especially after 1 hr, after virus inoculation on tomato plants. This period gave the lowest number of LLs (12.24), which increased as infection period increased to 24 hr (22.42 LLs) and then 48 hr (35.51 LLs) (**Table 6**). The extract did not reduced ToMV infectivity in a constant manner from 1-48 hr while exerting its inhibitory effect, although 1-4 hr could be considered the multiplication period in which ToMV penetrated and translocated throughout the tomato plant.

2. In vitro: Pre-ToMV inoculation

There was a highly significant reduction in the number of LLs when tomato plants *in vitro* were sprayed with the methanolic extract before ToMV inoculation, i.e., the methanolic extract had an inhibitory effect on virus infectivity; this effect tended to increase over time (**Table 6**). Generally, ToMV post-inoculation rubbing with the methanolic extract more effectively reduced ToMV infectivity than ToMV pre-inoculation. Therefore, the wild milk thistle methanolic extract was more effective in reducing ToMV infectivity in tomato plants *in vivo* than *in vitro*.

3. Effect of methanolic extract on seed germination and ToMV infection

Tomato seeds were soaked in the methanolic extract of wild, purple and white varieties for 12 and 24 hr in an attempt to decrease ToMV infection (**Table 7**). Non significant variability was observed in seed germination between both tested periods for all genotypes. This seed pre-treatment reduced the number of LLs and ToMV infection more than the treatment with tap water, i.e. the control (**Table 7**).

DISCUSSION

Variability in milk thistle plant characters

Five quantitative characters of seven selected genotypes of milk thistle, *Silybum marianum* var. *marianum* (purple flowers) and var. *albiflora* (white flowers) as well as an Egyptian wild variety were assessed for genetic variability. There was significant genetic variability among varieties and their genotypes; this variability could be utilized for a breeding program to improve the characters of this plant. Ram *et al*. (2005) also found significant variation in the

Table 5 Correlation and determination coefficients of local lesion number with each silymarin component of purple and white milk thistle varieties.

Items	Varieties	Silychristin	Silydianin	Silybin A	Silybin B	Isosilybin A	Isosilybin B	Total silybinin	Silymarin content
Correlation	Purple	-0.984**	-0.996**	-0.995**	-0.998**	-0.948**	-0.853**	-0.998**	-0.999**
	White	-0.999**	-0.912**	-0.941**	-0.940**	-0.943**	-0.935**	-0.940**	-0.936**
Determination	Purple	96.75	99.13	99.05	99.68	89.94	72.83	99.67	99.87
	White	99.84	83.25	88.59	88.26	88.84	87.50	88.43	87.56

* and ** = significant at $P < 0.05$ and 0.01, respectively.

Table 6 Antiphytoviral effect of methanolic extract for wild milk thistle on *Tomato mosaic virus* (ToMV) infectivity pre and post inoculation (*in vivo* and *in vitro*).

Treatments	Pre-TMV inoculation			Post- TMV inoculation		
	No. of local lesions	Infectivity %	Inhibitory effect	No. of local lesions	Infectivity %	Inhibitory effect
Sprayed with buffer	95	100	-	95	100	-
Sprayed with extract						
Time 0	7.50	92.10	7.90	15.15	84.05	15.95
1 hr	9.25	90.26	9.74	12.24	87.11	12.89
4 hrs	15.00	84.12	15.88	20.33	78.60	21.40
24 hrs	20.75	78.15	27.85	22.42	76.40	23.60
48 hrs	25.10	73.57	26.45	35.51	62.62	37.38

Table 7 Effect of soaking tomato seeds in methanolic extract of wild milk thistle on *Tomato mosaic virus* (ToMV) infection.

Seed treatments	% seed germination		ToMV infection					
			12 hrs soaking			24 hrs soaking		
	12 hrs	24 hrs	No. of local lesions	% of ToMV Infection	Inhibitory effect	No. of local lesions	% of ToMV Infection	Inhibitory effect
Tap water	95	98	95	100	-	95	100	-
Methanolic extract								
Wild	98	99	25.10	73.57	26.43	21.21	77.67	22.33
P_{34}	98	99	41.23	56.60	43.60	25.13	73.54	26.46
P_{22}	99	97	32.75	65.52	34.48	31.51	66.83	33.17
P_{28}	99	99	25.62	73.03	26.96	30.17	68.24	31.76
W_9	96	98	15.41	83.77	16.23	12.75	86.57	13.43
W_2	97	98	20.34	78.58	21.42	14.35	84.89	15.11
W_{13}	95	99	41.26	56.56	43.44	27.75	70.78	29.22

phenotypic and genotypic coefficient, broad sense heritability and genetic advance among 15 accessions of the purple milk thistle, where seed yield/plant and number of capsules/plant had the highest genotypic variation. In addition Ottai and Abdel-Moniem (2006) reported significant differences in mean values of top 10%, broad sense heritability, genotypic and phenotypic coefficients, selection intensity, genetic advance, correlation and determination coefficients in seven plant characters of purple and white milk thistle varieties among two seasons. Moreover, Ibrahim *et al*. (2007) found high variation in five characters and silymarin composition of 10 selected lines for purple and white milk thistle varieties for open and selfing generations. On the other hand, the wild plants show decreasing organic matter, especially in drought and unsuitable weather conditions, particularly sensitive to temperature and humidity (**Table 1**). All these as well as the interaction with the genetic environment play an important role in the variability of plant quantitative characters. Therefore, it is evident that the wild variety had the lowest value for all characters when compared with inbred genotypes which were grown in an agricultural environment (old clay land), as reported by Ottai *et al*. (2009) in similar genotypes. Moreover, random pollination in the wild plants leads to heterogeneity between them, where CV% was > 14% (**Table 1**) in the all characters related to this variety.

Significant correlation coefficients among each pair of studied characters revealed that each trait has equal importance in milk thistle breeding. However, in all varieties only one trait (total branches) regressed significantly with head flowers and seed yield to show its particular importance above all others. Tabrizi (2002), Sarang *et al*. (2004), Ram *et al*. (2005) and Ottai and Abdel-Moniem (2006) also found similar correlations in safflower and milk thistle plants, respectively while Ram *et al*. (2005) concluded that correlation and regression results like these are relatively phenotypic and not necessarily of genetic origin and are influenced by environmental factors limiting yield.

Variability in silymarin content of milk thistle extract

The wild variety, growing in the desert, had a lower silymarin content than the inbred varieties, as determined by HPLC. El-Sayed *et al*. (1993) and Omer *et al*. (1994) attributed similar results to the stress conditions in the edaphic and climatic conditions of desert, where the field environment which has rich water, organic matter, micronutrients with suitable heat and humidity, which would help the plants increase their synthesis of secondary metabolites.

Virus inhibition of milk thistle extract

Seeds of milk thistle contained the most powerful methanolic extracts as virus inhibitors and should be subjected to further studies. Methanolic extracts include virus inhibitors such as 3-oxyflavone silymarin, an isomeric mixture of three flavonolignans, i.e. silychristin, silydianin and silybin (Cacho *et al.* 1999; Ibrahim *et al.* 2007). Seed extracts showed the highest affect on ToMV infectivity in the infected crude sap. This inhibitory effect was attributed to the chemical content of seed extracts as antiphytovirals interfere with the host defense mechanism, associated with the precipitation of virus (Meyer *et al*. 1995). Varna (1978) attributed the inhibition effect on different viruses to flavonoid compounds (including silymarin) which change the host susceptibility by blocking the infection on sites of the leaf surface. Our results illustrate that silymarin components from the methanolic extract of milk thistle seeds have various and interesting photobiologic actions, one of them being the inhibition of infectivity of RNA viruses, in this case ToMV.

Mechanisms of resistance to ToMV

It can exert a photosensitivity effect by either one of two mechanisms. In Type I, direct photoreaction with the substrate. In type II, energy transfer to oxygen which reacts with the substrate even through the main biologic and therapeutic effects are generally ascribed to type I mechanism and in particular to photoreactions with DNA and RNA (Sambrook *et al.* 1984). Silymarin interacts at the molecular level with nucleic acid (Varna 1978). This interaction occurs in two successive steps, formation of a preliminary molecular complex in the ground state and subsequent covalent photo addition of the silymarin to the macromolecule.

In all other antivirals tested, there are theories to explain the mode of action of virus inhibitors. The fist suggests that the inhibitors form a non-infected complex with the virus particle by means of aggregations of virus particle with inhibitors (Francki 1964). The second theory denotes that, the inhibitors effects the host cells which became physiologically altered and are thus no longer as susceptible to the virus (Simons *et al*. 1963). In the third theory, the inhibitory process of antiphytovirals is believed to operate through suppression of gene function II (interference with the translation of the genetic message or inhibition of protein synthesis). The fourth theory is suggested to involve one or more the events which take place on the ribosome. In the fifth theory, a specific type of inhibitory action on protein synthesis loading to the formation of correctly sequenced polypeptides on the ribosome. The sixth theory is based on the foundation that antiphytovirals inefficiently inhibits the t_{RNA}-ribosome interaction. A clue to its negative inhibitory effect on ToMV is provided by the fact that the antiphytoviral is only confined to the 70S ribosome, whereas has no detectable action on 80S particles, mainly found in tomato leaf ribosome, beside small quantities of 30S, 60S and 112S components (Vonkammen 1963). In the seventh theory, the antiphytovirals, which are known as broad spectrum and are effective against some large viruses, do inhibit protein biosynthesis on both 70S and 80S ribosomes although the 70S particles are somewhere sensitive to antiphytovirals. Furthermore, milk thistle is much more effective against protein synthesis (El-Dougdoug 1997) and nucleic acids (Vordanova *et al*. 1996; El-Dougdoug 1997).

PERSPECTIVES

These results can be useful in a milk thistle breeding programme in which plant selection of milk thistle must be based on high silymarin content.

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