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'CIMAP SIL-9': A Dwarf and High Silymarin Yielding Variety of Milk Thistle (*Silybum marianum)*

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ABSTRACT

Silybum marianum commonly known as milk thistle is a herb belonging to the family Asteraceae. The ripe seeds of the plant contain silymarin which is used as medicine for treating diseases like liver cirrhosis, jaundice, hepatitis and liver poisoning. Other major medicinal properties of Silybum are anticancer, antidepressant, antioxidant, cardio protective, demulcent, digestive tonic, hepatoprotective, hepatoregenerative, immunostimulatory and neuroprotective. A new variety of Silymarin named as "CIMAP SIL 9" has been developed by CSIR-CIMAP through directed breeding efforts. The developed variety has silymarin content of 8% and has an appropriate height of 80-90 cm, making its harvesting very easy. Whereas, the check (CIM Liv) is very tall which makes its handling very difficult. The variety therefore has a dual advantage of dwarfness combined with very high content of silymarin.

INTRODUCTION

Silybum marianum (L.) Gaertn also known as milk thistle belongs to the Family Asteraceae and is a well-known medicinal plant. It is native to the Mediterranean region of Europe and widely dispersed to many countries throughout the world. Different plant parts of silybum are being used for last 2000 years in the preparations of various traditional medicines. The most important bioactivity of this medicinal plant is hepato protection. Since 20th century, it has been used for the treatment of diseases like liver cirrhosis, jaundice, hepatitis and liver poisoning. Other major medicinal advantages offered by *Silybum* are anticancer, antidepressant, antioxidant, cardio protective, demulcent, digestive, hepatoregenerative, immunostimulatory and neuroprotection.

All medicinal applications of Milk thistle are due to active constituent silymarin which is present in higher concentration in seeds as compared to other

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plant parts. Silymarin is an isomeric mixture of flavonolignans such as silybin A and B, isosilybin A and B, isosilychristin, silydianin and silychristin and the flavonoid taxifolin. Among these Silybin, isosilybin, silychristin and silydianin are principle components of silymarin. All the biological activities of milk thistle are due to the presence of these phytochemicals. Apart from curing human diseases this plant also plays an important role as veterinary medicine and also used in several cosmetic formulations such as body creams and lotions.

Two main logics behind the breeding experiment in milk thistle at CSIR-CIMAP are dwarfness and silymarin content. Presently considering all the available resources in terms of their yield potential and growth (dwarfness) for making it profitable for the industries, the proposed strain is having an appropriate height of 80-90 cm, making its harvesting very easy. Whereas, the check (CIM Liv) is very tall which makes its handling difficult. If released, the industries involved in silymarin extraction may get their raw material in the country itself rather than importing it from abroad. Till now, the biggest discouragement for industries was a lower silymarin content in existing varieties, making the extraction non profitable to them. Once the industries are getting the silymarin content more than the imported one, they will start buying indigenous material thereby increase in the acreages of Silybum in India. This may lead to more opportunity of diversification in agriculture and ultimately more income enhancement of poor farmers. The proposed strain is having the dual advantage of dwarfness combined with very high content of silymarin. Besides, this is a crop best suited to marginal and dry land area.

ORIGIN OF VARIETY

This variety is outcome of extensive breeding work carried out during last 8 years. So far the best available variety of silybum is “CIM Liv”, which is growing tall and having the silymarin content of mere 2.8%. Whereas, industries want a variety containing silymarin content of around 6% making it profitable from extraction. Besides, it was also observed that a tall variety is making the harvesting / picking much more difficult. Therefore, the present efforts were started in year 2004 for developing a new variety having an appropriate height (about 3-4 feet) and silymarin content of not less than 6-7%. Figure 1 (a) and (b) shows the comparative growth pattern of CIM-LIV and CIMAP SIL 9 in the field.

MATHODOLOGY

The variability provides the platform on which plant breeders exercises the selection and isolate the superior genotypes for commercial cultivation. So far only one breeding population i.e. CIM Liv was available. Therefore, attempt was made to induce the variability using ^{3} Irradiation.

(a)

(b)

Figure 1: Comparative growth pattern of (a) CIM-LIV and (b) CIMAP SIL 9 in the field.

INITIAL EVALUATION

Four seed lots consisting of 50 seeds each of "CIMAP 1891" were subjected to 40Kr ^{3} Irradiation. All experiments were conducted at CSIR-CIMAP research Farm Lucknow under normal fertility regime and intercultural practices. The sowing was done in November 2010 with plot size of 3.5x3.5m (gross), plant to plant and row to row distance was kept 50cm. The progeny plots of these lots were raised separately and plot bulk were harvested in April to May 2011. These bulks were also analyzed for silymarin content. Seed lot 4 was further grown and single plants were selfed and harvested separately. For next four generations half-sib progeny selection was exercised (single plant open pollinated progeny populations). Out of final 12 populations harvested in bulk (Table 1); four top silymarin yielding population along with CIM Liv as check were evaluated in a Randomized Block Design with three replications.

Data from both evaluations are presented in the Table 1 and Table 2 respectively. Besides, the generation wise selection and detailed pedigree of the strain is also presented (Figure 2). A total of 69, 50, 13, 26, 41 and 13 plants were analyzed in II, III, IV, V, VI and VI generation(s) respectively.

Total 12 advanced breeding populations were evaluated in single plot of 9^2 m (Gross 3.5x3.5m). Data were recorded on plant height (cm), average. no. of capsules/ plant, average seed yield / plant (g), seed yield / plot (g), average seed yield / capsule (g), 1000 seed weight (g) and silymarin content (%) in seeds. Silymarin yield / plot (g) was calculated and on the basis of this, four best strains 1112-S9bulk (63.91g), 1112-S8bulk (43.79g), 1112-S11bulk (37.97g) and 1112-S6bulk (37.40g) were identified for yield trial (Table 1).

Table 1: Performance of the *Silybum marianum* advanced breeding populations in the initial evaluation trial at CIMAP, Lucknow

S. no.	Line No.	Plant height (cm)	Average no. of capsules/ plant	Average Seed yield / plant (g)	Seed yield / plot (g)	Average seed yield / capsule (g)	1000 seed weight (g)	Silymarin (%)	Estimated silymarin yield / plot (g)
1	1112-S6	79	24.17	41.67	500	1.72	21.4	7.48	37.40
2	1112-S7	113	17.73	19.69	294	1.11	18.4	4.70	13.82
3	1112-S8	74	14.71	33.94	577	2.31	22.5	7.59	43.79
4	1112-S9 (CIMAP SIL 9)	80	25.06	48.13	770	1.92	22.0	8.30	63.91
5	1112-S10	80	12.85	25.46	331	1.98	20.8	7.29	24.13
6	1112-S11	78	22.57	40.79	571	1.81	21.2	6.65	37.97
7	1112-S1	98	6.63	13.63	109	2.06	23.3	9.99	10.89
8	1112-S2	100	8.71	18.43	129	2.12	25.9	6.75	8.71
9	1112-S3	96	8.38	21.50	172	2.57	25.4	9.57	16.46
10	1112-S4	105	5.73	11.18	123	1.95	26.0	9.06	11.14
11	1112-S5	98	7.88	14.88	119	1.89	25.7	7.97	9.48
12	1112-S12	96	11.13	20.50	164	1.84	17.6	6.16	10.10
	SD	12.62	7.02	12.29	227.87	0.35	2.86	1.49	17.82
	Mean	91.42	13.80	25.82	321.58	1.94	22.52	7.63	23.98
	CV	13.80	50.90	47.60	70.86	18.05	12.70	19.60	74.32
	SE	3.65	2.03	3.55	65.86	0.1	0.83	0.43	5.15
	CD (1%)	6.57	3.65	6.39	118.55	0.18	1.49	0.77	9.27
	CD (5%)	8.03	4.47	7.81	144.89	0.22	1.83	0.95	11.33

SD – Standard Deviation, CV- Coefficient of variation, SE- Standard Error, CD (1%) and CD (5%)- Critical Difference at 1% and 5% level of significance respectively.

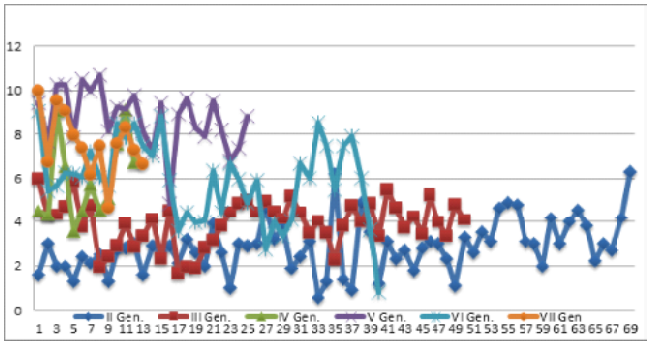

Figure 2: Graph showing generation wise gradual increase in the silymarin content in the breeding progenies.

Experimental details for initial evaluation

Planting time : November 2010

Harvesting time : April-May 2011

Plot size : Single bed of 3.5x3.5m (gross)

P to P and R to R distance : 50cm

SECOND STAGE EVALUATION:

Four half sib progeny population along with check (CIM-Liv) were planted in 3.5m X 3.5m plots with three replications in a Randomized Block Design (RBD) during the month of November 2011. The row to row and plant to plant distance was kept 50 cm. Normal agronomic practices were followed. The evaluation of the material was done for plant height (cm) and seed yield / plot (g). Per hectare yield data and silymarin yield was estimated (Table 2). In terms of seed yield and silymarin yield, the strain 1112-S9 performed best. It yielded almost 365 % higher silymarin yield as compared to check. The PCR profile of S9 and the check CIM-Liv with UBC53 is also presented in figure 3.

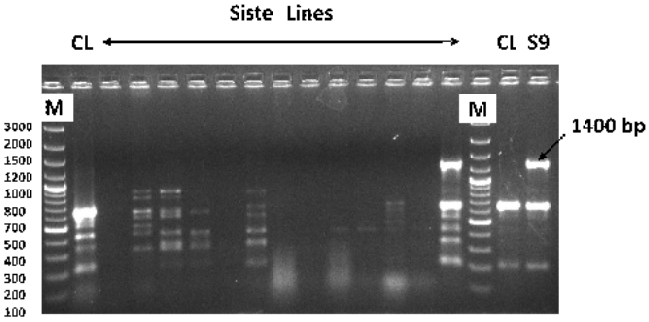

Figure 3: PCR profile of proposed CIMAP SIL 9 and CIM-LIV with UBC53.

Pedigree of the strain

0405-40Kr³Irad/0506-Lot4 bulk/0607-S17, SP Self/0708-S30, SPOP/0809-S8, SPOP/ 0910-S2, SPOP/1011-S10, SPOP/1112-S9 bulk.

Table 2: Yield performance of the *Silybum marianum* selected advanced breeding populations in the yield evaluation trial at CIMAP, Lucknow

S. No	Line No.	Average Plant height (cm)	Average Seed yield / plot (9 m) in g	Average Estimated Seed yield kg / ha	Silymarin %	Average Estimated silymarin yield kg / ha
1	1112-S6	79.33	623.33	692.6	7.48	51.81
2	1112-S8	75.33	703.33	781.5	7.59	59.31
3	1112-S9	80.00	910.00	1011.0	8.3	83.92
4	1112-S11	78.67	680.00	755.6	6.65	50.24
5	Check (CIM Liv)	191.00	733.33	814.8	2.8	22.81
	SD	46.77	104.22	115.8	1.49	20.37
	Mean	100.9	730.00	811.1	7.63	53.62
	CV	46.37	14.28	14.28	19.60	37.98
	SE	1.23	26.93	29.92	0.43	5.26
	CD (1%)	2.29	50.09	55.65	0.77	9.78
	CD (5%)	2.84	62.21	69.12	0.95	12.15

SD – Standard Deviation, CV- Coefficient of variation, SE- Standard Error, CD (1%) and CD (5%)- Critical Difference at 1% and 5% level of significance respectively.

Table 3: Comparison of the variety CIMAP SIL-9 with CIM-LIV

Attributes	CIMAP SIL-9	CIM Liv
Average Plant height (cm)	80	191
Average Seed yield / plot (9 m) in g	910	733
Average Estimated Seed yield kg / ha	1011	815
Silymarin %	8.3	2.8

Statement of distinction:

Till now, the biggest discouragement for industries was a lower content of silymarin in existing varieties, making the extraction non profitable to them.

Plants of variety CIMAP SIL 9 are having silymarin content of more than 8%. And an appropriate height of 80-90 cm, making its harvesting very easy. Whereas, the check (CIM Liv) is very tall which makes its handling very difficult. Table 3 represents the comparative characterstics of CIMAP SIL 9 and CIM-Liv and Table 4 represents the distinguished characterstics of CIMAP SIL 9.