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CIM-Sanjeevani: A high artemisinin yielding population of Artemisia (*Artemisia annua*)

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ABSTRACT

Artemisia annua L. (Asteraceae), commonly known as quinghao, is a traditional medicinal herb, native to China and widely grown in Asia and Europe. The biologically active compound artemisinin isolated from the herb of the A. annua is used as an anti-malarial drug. CSIR-CIMAP has now developed genetically improved variety of Artemisia annua with artemisinin content of 1.2%. The new variety CIM-Sanjeevani has been developed using classical breeding method of poly crossing between two existing varieties i.e. Jeevan Raksha and CIM-Arogya followed by population enrichment with desirable genes. Two way selections were practiced, which involves selection for maternal parent and simultaneous rejection of poor pollinators from the population. The plants of variety CIM-Sanjeevani has a yield potential of producing average 50-55 kg of artemisinin from an average dry herb yield of 43 – 45 q/ha in a single harvest. The plants of the variety CIM-Sanjeevani have intermediate morphology between variety Jeevan Raksha and CIM-Arogya. This variety is about 10 days late in flowering as compared to two earlier varieties and therefore also suited for three harvests. This variety will benefit both farmers and industries involved in Artemisia cultivation / business. Industry may have the benefit of reduced cost of production by a margin of 20%.

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INTRODUCTION

Artemisia annua, also known as sweet wormwood, sweet annie, sweet sagewort and annual wormwood (Chinese: quinghao), is an annual, aromatic herb, usually single-stemmed with alternate branches. It is native to temperate Asia but naturalized throughout the world. It belongs to the family Asteraceae and has fern-like leaves, bright yellow flowers and a camphor-like smell. It is a determinate short-day plant.

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The chemical artemisinin, which is synthesized naturally in the leaves of *Artemisia*, is a potent anti-malarial agent, and can kill the most deadly malarial parasite, *Plasmodium falciparum*. It is selectively toxic to the asexual erythrocyte stage of the parasites. The World Health Organization [7] recommends artemisinin-based combination therapy (ACT) for the treatment of cerebral malaria. Artemisinin and its semi-synthetic derivatives such as arteether, artemether, artesunate and dihydroartemisinin have been recommended to treat resistant and cerebral malaria. Demand for ACTs was increased from around 100 million treatments in 2008 to 250 million by 2015. However, the low yield of *Artemisia* creates problems for production and there are concerns that artemisinin supply will not reliably keep pace with increasing demand for ACTs [1]. Current research also shows that artemisinin drugs are effective against cancer, *Leishmania* [8, 5], *Trypanosoma* [4], and some viruses [2, 3]. In addition, *A. annua* has a high content of flavonoid compounds which are responsible for its high antioxidant activity.

So far the best available variety of *Artemisia* is "CIM Arogya" developed by CSIR-CIMAP containing artemisinin content of around 1%. After the availability of semi synthetic artemisinin (SSA) in the market and continuously falling international prices of artemisinin, it has been felt that the production cost of the natural artemisinin must be brought down in order to make it sustainable / competitive in the international market. The options available for reducing the prices of artemisinin are

1. Reduce its processing cost and thereby its overall production cost.
2. To extract or recover higher quantities of artemisinin from the same amount of raw *Artemisia* herb at same processing cost and thereby reducing its overall production cost.

Plant breeder can explore the second option by developing an artemisinin rich *Artemisia* population. Therefore, the present efforts were started in year 2004 for developing a new variety having artemisinin content of not less than 1.2% (an increase of at least 20% over existing variety CIM Arogya).

MATERIALS AND METHODS

CIM - Sanjeevani is the outcome of extensive breeding work carried out during last 12 years. It has been developed from poly cross progenies between two existing varieties i.e. Jeevan Raksha and CIM Arogya. It involves one cycle of original poly crossing between two varieties, three generation of synthesis, six generation of gene enrichment and two generation of evaluation. Two way selections were practiced, which involves the selection for maternal parent and rejection of poor pollinators from the population simultaneously.

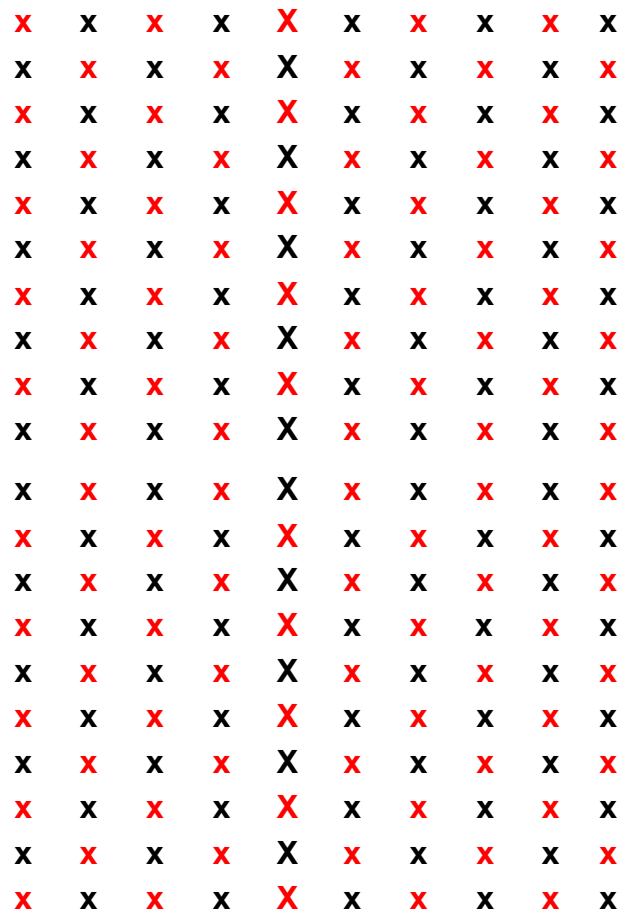

Figure 1: Layout plan of two poly cross nurseries of *Artemisia* planted side by side in the year 2004. Black cross represent the variety Jeevan Raksha and the red cross represent the variety CIM Arogya (or *vice versa*).

(A) Synthesis Cycle:

First Year: In the year 2004 two specially designed poly cross nurseries involving Jeevan Raksha and CIM-Arogya were planted side by side. Both crossing nurseries have 10 rows of 10 plants each. All the plants were analyzed for artemisinin content. Maternal selection was exercised for plants with artemisinin content of >0.8%.

Second Year (2005): Plant progenies from the selected plants of poly cross nursery along with selected high type progenies of CIM-Aroyga were again grown in the crossing block. All the plants were analyzed for artemisinin content. Maternal selection was exercised for plants with artemisinin content of >0.95%. Poor pollinators were removed.

Third Year (2006): Plant progenies from the selected plants of second year were grown along with selected high type progenies of CIM-Aroyga in the crossing block. All the plants were analyzed for artemisinin content. Maternal selection was exercised for plants with artemisinin content of >1.1%. Poor pollinators were removed.

Fourth Year (2007): Plant progenies from the selected plants of third year were grown along with selected high type progenies of CIM-Aroyga in the crossing block. All the plants were analyzed for artemisinin content. Maternal selection was exercised for plants with artemisinin content of >1.2%. Poor pollinators were removed.

(B) Enrichment Cycle:

Fifth to Tenth Year (2008 to 2013): Plant progenies from the selected plants of previous year were grown in the crossing block. All the plants were analyzed for artemisinin content. Maternal selection was exercised for plants with artemisinin content more than 1.2%. Poor pollinators were removed. The process was repeated consecutively for six generations. This population was evaluated for two years along with Jeevan Raksha and CIM-Arogya as check in a Randomized Block Design with three replications. The enrichment process was continued during evaluation also. Data from both evaluations are presented in the Table 1 and Table 2 respectively. Besides, the graph showing the generation wise selection response is presented (Figure 2).

Evaluation of the material: The details of the evaluation are provided as below.

First stage evaluation: CIM-Sanjeevani populations along with two checks (Jeevan Raksha and CIM-Arogya) were evaluated in single plot of 9^2 m (Gross 3.5x3.5m) replicated thrice. Data were recorded on plant height (cm), average number of primary branches / plant, fresh herb yield / plot (Kg)

Table 1: Results of ANOVA and comparative performance of CIM- Sanjeevani population along with two checks during the cropping season of 2014.

Treatments / sources	Plant height(cm)	Primary branches /plant	Fresh herb/plot (Kg)	Dry herb/plot (Kg)	Fresh herb/ha (q)	Dry herb/ha (q)
CIM-Arogya	196.83	76.87	21.5	3.43	238.89	38.15
CIM-Sanjeevani	216.13	82	25.33	3.8	281.48	42.02
Jeevan Raksha	233.53	105	18	2.7	200	30
Treatment M.S.S (2)	1011.08**	673.42**	40.36**	0.94**	4983.16**	116.16**
Error M. S. S. (4)	8.36	16.73	2.28	0.03	281.16	4.24
SEM ±	1.67	2.36	0.87	0.11	9.68	1.19
SD	2.36	3.34	1.23	0.15	13.69	1.68
CD (5%)	6.56	9.29	3.42	0.42	38.06	4.68
CD (1%)	10.86	15.36	5.67	0.70	62.98	7.74
CV	1.34	4.65	6.98	5.60	6.98	5.60

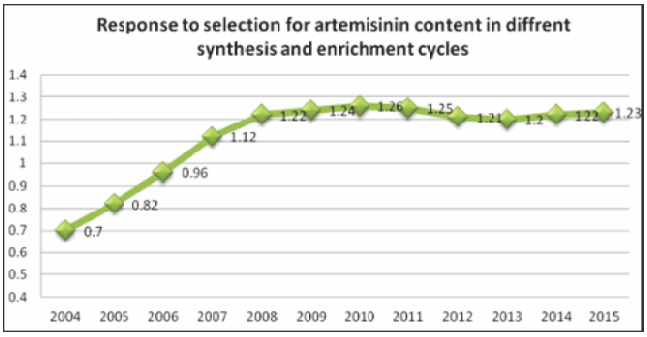

Figure 2: Graph showing response to selection for artemisinin content in different synthesis and enrichment cycles.

and dry herb yield / plot (Kg). Fresh herb yield / ha (q) and dry herb yield / ha (q) were estimated based on respective plot yield (Table 1).

Experimental details for initial evaluation

Nursery sowing:	First week of January
Transplanting:	First week of March
Harvesting time:	First fortnight of June
Plot size:	Single bed of 3.5x3.5m (gross), 9^2 m net
Replications:	Three
Row to row distance:	50cm
Plant to Plant distance:	30cm

Second stage evaluation: CIM-Sanjeevani population along with two checks (Jeevan Raksha and CIM Arogya) were again planted in 4.5m X 4.5m plots with three replications in a Randomized Block Design (RBD). Normal agronomic practices were followed. The evaluation of the material was done for plant height (cm), average number of primary branches / plant, fresh herb yield / plot (Kg) and dry herb yield / plot (Kg). Artemisinin content was analysed replication wise from the samples taken just before harvest. Fresh herb yield / ha (q), dry herb yield / ha (q) and artemisinin yield / ha (Kg) were estimated based on respective plot yield (Table 2).

Experimental details for second stage evaluation

Nursery sowing:	First week of January
Transplanting:	First week of March
Harvesting time:	First fortnight of June
Plot size:	Single bed of 4.5x4.5m (gross), 16^2 m net
Replications:	Three
Row to row distance:	50cm
Plant to Plant distance:	30cm

The analysis of variance (ANOVA) was performed by using Statistical Software 4.0 version, available in the Division of Genetics and Plant Breeding of the CSIR–CIMAP as prescribed by Singh and Chaudhary [6].

Table 2: Results of ANOVA and comparative performance of CIM- Sanjeevani population along with two checks during the cropping season of 2015.

Treatments / sources	Plant height (cm)	Primary branches/ plant	Fresh herb/ plot (Kg)	Dry herb/plot (Kg)	Fresh herb/ha (q)	Dry herb/ha (q)	Artemisinin Content (%)	Estimated artemisinin yield Kg/ha
CIM-Arogya	196.07	80.93	38.83	6.36	242.71	39.79	0.85	33.98
CIM-Sanjeevani	212.47	86.07	42.83	6.9	267.71	43.13	1.23	53.14
Jeevan Raksha	233.53	104.4	34.33	5.57	214.58	34.79	0.68	23.55
Treatment M.S.S (2)	1058.23**	456.58**	54.25**	1.35**	2119.31**	52.82**	0.24**	675.70**
Error M. S. S. (4)	4.65	3.93	1.33	0.01	52.08	0.56	0.004	11.62
SEM ±	1.24	1.14	0.67	0.07	4.17	0.43	0.04	1.97
SD	1.76	1.61	0.94	0.09	5.89	0.61	0.05	2.78
CD (5%)	4.89	4.50	2.62	0.27	16.38	1.70	0.15	7.74
CD (1%)	8.09	7.44	4.34	0.45	27.10	2.82	0.24	12.80
CV	1.01	2.19	2.98	1.91	2.98	1.91	7.10	9.23

Table 3: Distinguish characteristics of population CIM-Sanjeevani.

Characters	Value
Plant height (cm) Primary branches	205 - 225 cm 75- 90
Growth habit (Vegetative phase)	Determinate, hybrid morphology between Jeevan Raksha & CIM Arogya
Flowering period	8 – 10 days later than checks (after 30th Sep.)
Artemisinin content (%)	1.2%
Response to diseases / insect	Free from infection

RESULTS AND DISCUSSION

The variability provides the platform on which plant breeders exercises the selection and isolate the superior genotypes for commercial cultivation. The present breeding approach was based on the principal of recombining the superior genes from both the varieties and thereafter accumulation (enrichment) of favorable genes in the resultant progenies.

Analysis of variance is a statistical procedure which separates the total variation into different components. The results of analysis of variance for different characters in the year 2014 (Table 1) and 2015 (Table 2) are presented. The mean sum of squares due to genotypes showed significant difference for all characters under study at 1% level and 5% level of significance. Thus it represents that ample amount of genetic variability is present among the three genotypes and that the genotypes are genetically distinct.

In the first year of evaluation (2014) for the variety CIM- Sanjeevani, the character plant height had a mid-value (216.13) between CIM-Arogya (196.83) and Jeevan Raksha (233.53). For the character primary branches/plant also, CIM-Sanjeevani acquired a mid-value (82) between CIM-Arogya (76.87) and Jeevan Raksha (105). Whereas for the character dry herb/ha, the variety had a higher value (42.02) when compared to CIM-Arogya (38.15) and Jeevan Raksha (30). Similarly, in the second year of evaluation (2015), plant height and primary branches/plant had a mid-value (212.47,86.07) between CIM-Arogya (196.07,80.13)

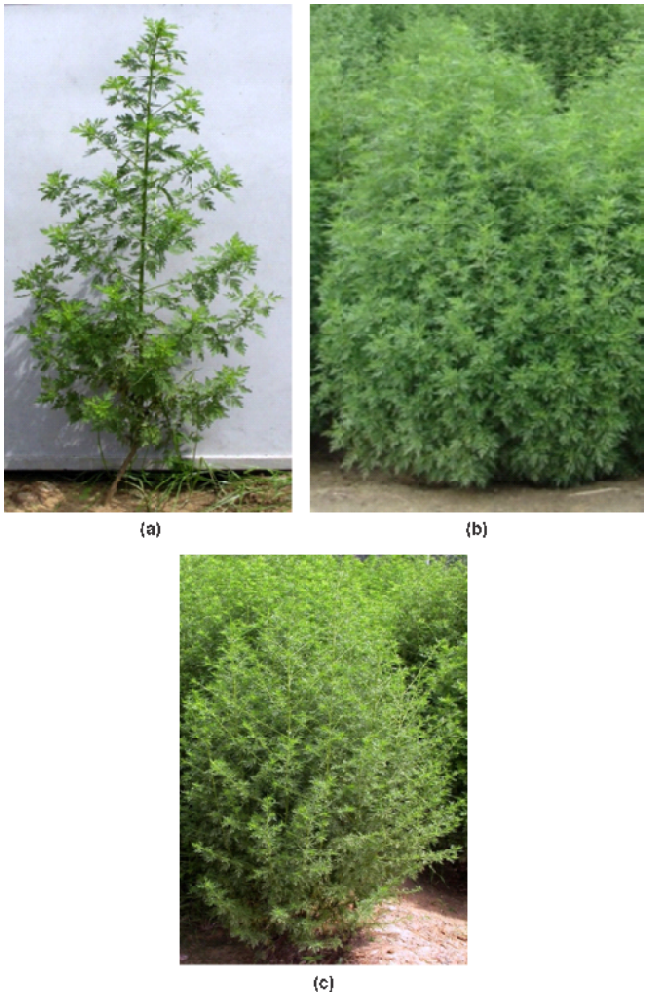

Figure 3: Close up view of individual plant morphology of released varieties of *Artemisia annua* (a) Jeevan Raksha, (b) CIM Arogya, (c) CIM Sanjeevani.

and Jeevan Raksha (233.53,104.4) and a higher value (43.13) for dry herb/ha. The population of this variety had distinctly higher average artemisinin content (1.2%) as compared to CIM-Arogya (0.85%) and Jeevan Raksha (0.68%). When estimated in terms of artemisinin yield/ha, CIM-Sanjeevani had the potential of yielding 55.14 Kg/ha of artemisinin as compared to the two checks, CIM-Arogya (33.93Kg/ha) and Jeevan Raksha (23.55 Kg/ha).

The recorded values for growth and yield parameters are provided in the Table 3. All the characters were recorded at the time of harvest. The plant height ranged between 205-225 cm and primary branches between 75-90. The variety showed an intermediate morphology between CIM-

Arogya and Jeevan Raksha with determinate type of growth habit. The population is about 10 days late in flowering as compared to the two checks and therefore also suited for three harvests. The plants of this variety were found to be free from leaf deformation disease and attack of mango mealy bug.

It was necessary to have a variety which may be able to give higher amount of artemisinin at the same cost of production and thereby resulting in the reduced cost of production in terms of per Kg of artemisinin. The proposed population of CIM-Sanjeevani has 1.2% of artemisinin and has the potential to make artemisinin business more lucrative / sustainable to the pharma industries involved in the artemisinin production. Besides, this variety will benefit both farmers and industries involved in Artemisia cultivation. Farmers can have extra income Rs. 10000 to 15000 from one hectare crop (giving 10-12% higher yield of dry leaves as compared to CIM-Arogya). Industry may have the benefit of reduced cost of production. The cost of production will be reduced by a margin of 20%.

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REFERENCES

1. Anonymous. 2015. The CNAP Artemisia Research Project, CNAP, Department of Biology (Area 7), The University of York, Heslington, York, UK.
2. Khan MMAA, Jain DC, Bhakuni RS, Zaim M, Thakur RS. 1991. Occurrence of some antiviral sterols in *Artemisia annua*. *Pl Sci* **75**:161–165.
3. Li S, Chen C, Zhang H, Guo H, Wang H, Wang L, Zhang X, Hua S, Yu J, Xiao P, Li R, Tan X. 2005. Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antiviral Res* **67**:18–23.
4. Mishina YV, Krishna S, Haynes RK, Meade JC. 2007. Artemisinins Inhibit *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense* In Vitro Growth. *Antimicrob Agents Chemother* **51**:1852–1854.
5. Sen R, Bandyopadhyay S, Dutta A, Mandal G, Ganguly S, Saha P, Chatterjee M. 2007. Artemisinin triggers induction of cell-cycle arrest and apoptosis in Leishmania donovani promastigotes. *J Med Microbiol* **56**:1213–1218.
6. Singh RK, Chaudhary BD. 1979. Biometrical methods in quantitative genetic analysis. New Delhi, Kalyani publication, P 120.
7. WHO Position Statement, June 2012. Effectiveness of Non-Pharmaceutical Forms of Artemisia annua L. against malaria.
8. Yang DM, Liew FY. 1993. Effects of qinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis. *Parasitol* **106**:7–11.