DOI : 10.5958/0974-0112.2016.00012.8

Indian J. Hort. 73(1), March 2016: 19-24

Morphological-biochemical-physiological traits assisted selection for *kusmi* lac production on *ber* (*Ziziphus mauritiana* Lam.) varieties

J. Ghosh*, V.D. Lohot, V. Singhal, S. Ghosal and K.K. Sharma

ICAR-Indian Institute of Natural Resins and Gums, Namkum, Ranchi 834010, Jharkhand

ABSTRACT

***Ber* (*Ziziphus mauritiana* Lam.) is a common lac host for *kusmi* and *rangeeni* strain of *Kerria lacca*. In the present study, morphological, biochemical and physiological traits of 23 *ber* cultivars / varieties were analyzed during 2011-12 and 2012-13 to identify traits for high lac production under rainfed growing areas. *Ber* varieties were significantly different for morphological, biochemical and physiological traits. Canopy, shape, branching pattern of tree (morphological traits); initial settlement density, mean settlement length and sex ratio (among lac attributing traits); total sugars, soluble protein and chlorophyll content index (among biochemical and physiological traits) had significant association with scrapedlac. Best sub-set regression using R^2 for scrapedlac revealed that only sex ratio has predicted 62% of scrapedlac yield. Four traits, *viz*., canopy and branching pattern of tree, sex ratio of lac insect and soluble protein in leaves of *ber* varieties were able to assess it up to 67% and these traits may be used as marker, while selecting host *ber* varieties for *kusmi* lac production. Based on these parameters, out of 23 *ber* varieties four (Kaithali, Jogia, Seb × Gola (F_1) and Banarasi Karaka) were identified as promising for lac cultivation in rainfed regions of Jharkhand.**

Key words: *Ber*, *Ziziphus mauritiana* Lam., *Kerria lacca*, *kusmi* lac.

INTRODUCTION

Jujube or Indian plum or *ber* (*Ziziphus mauritiana* Lam.) is an economically important tropical fruit tree grown all over the drier parts of the Indian sub-continent for its fresh fruits (Awasthi and More, 1). It is a fast-growing and hardy tree that can bear extremes of temperatures and thrives well under dry conditions (Pareek, 7). It plays an important role in supporting livelihood income, employment, folk medicine, timber and livestock fodder. Genetic diversity of *Ziziphus* spp. in India is high and about 20 species are found between 8.5-32.5° North and 69-84° East (Azam *et al*., 2). The genetic relationship among fruit cultivars of *Z. mauritiana* Lam. (*ber*) had been realized for utilization of some genotypes in fruit as well as lac production (Saha *et al*., 8).

Ber tree is commercially exploited for lac cultivation in India extensively along with other host trees *palas* (*Butea monosperma*) and *kusum* (*Schleichera oleosa*). It is a very good host for both *rangeeni* and *kusmi* biotypes of bivoltine, *Kerria lacca*, (Kerr). Lac is the scarlet resinous secretion of scale insect which yields three useful materials resin, dye and wax. Thousands of these tiny insects colonize the suitable branches of host trees and secrete the resin as a protective covering. Only wild *ber* varieties were exploited for commercial lac cultivation in India till date. Therefore, the present study was carried out to identify morphological-biochemical-physiological traits for winter *kusmi* lac production on *ber* plants.

*Corresponding author's E-mail: jghoshranchi@gmail.com

MATERIALS AND METHODS

The experiment was conducted at ICAR-IINRG, Ranchi, Jharkhand, India during 2011-12 and 2012-13. Twenty three cultivars / varieties of *ber* were procured from ICAR-Central Arid Zone Research Institute, Jodhpur and planted in July 1996. Each genotype had ten trees in a row and spaced at 4 m. Trees were arranged in diagonal system of planting. Integrated nutrient management on growth and yield in *ber* was adopted as per recommended schedule except foliar spray of thiourea. All trees were pruned in February 2011 to ensure proper shoot development at inoculation time. Adequate new branches emerged at inoculation time in July. *Kusmi* broodlac was inoculated for the first time in few branches of all the cultivars/varieties of *ber* used in this study to raise winter *kusmi* crop (July 2011-Feb 2012). Broodlac was harvested at maturity in February 2012 coinciding with pruning. These *ber* varieties were again inoculated in July 2012 to raise another winter crop (July 2012 - Feb 2013). Data were recorded for tree morphology, lac yield attributing traits and biochemical-physiological traits in leaves in three replications.

Initial settlement density was measured by counting number of crawlers settled in one square centimeter area at three levels of branches (lower, middle and upper). Mean settlement length was calculated by taking an average of settlement length of crawlers on ten branches per tree. Per cent initial insect mortality was taken at 30 days after lac inoculation by counting dead crawlers in settled length of one square centimetre. Female to male sex ratio was counted during sex differentiation stage. Harvested broodlac was weighed for each *ber* variety. The resinous cover was scraped off from the twigs, weighed as scrappedlac. Fresh leaf samples were taken from inoculated and uninoculated (control) trees of each *ber* variety for biochemical analysis. The sugars were determined by Nelson's arsenomolybdate method (Nelson, 5). Non-reducing sugar was calculated by subtracting reducing sugar from total sugars. Soluble protein was estimated by Lowrey's method (Lowrey *et al.*, 3). Chlorophyll content index (CCI) was measured by chlorophyll content meter (CCM 200, Opti-Sciences). Observations were recorded from mature leaves of three plants of each variety in inoculated and un-inoculated trees at 11 to 12 noon and average was considered for analysis. Data were analyzed for genetic variability in all traits under investigation, interaction of lac insect with *ber* varieties on biochemical and physiological traits, correlation coefficient of traits with lac yield and best subset regression as per standard biometrical approaches. CAZRI Gola is well established fruit variety and susceptible to lac insect and thus considered as check in this studies.

RESULTS AND DISCUSSION

Initial settlement density (ISD), mean settlement length (MSL), sex ratio (SR), % initial mortality (IM%), broodlac yield (LY) and scrapedlac yield (SY) were significantly different among *ber* varieties (Table 1). Initial settlement density ranged from 64.8 (Sanaur 5)

Table 1. Lac attributing characteristics on *ber* varieties for *kusmi* lac production.

Variety	ISD	MSL	SR	IM%	LY /plant	Output ratio	SY/ plant
Dandan	85.0	51.5	37.2	25.7	377.5	3.8	184.9
Aliganj	85.6	45.0	39.9	38.6	439.7	4.4	111.9
Seb × Katha F_1	113.8	54.3	34.7	18.5	265.8	2.7	84.5
Bagwadi	99.9	46.7	31.0	30.2	501.3	5.0	208.6
Illaichi	89.4	44.0	34.2	16.7	487.7	4.9	333.8*
Thornless	134.0	45.2	29.4	15.8	631.5*	6.3*	263.1
Maharwali	124.8	34.4	35.7	10.0	324.2	3.2	118.4
Kali	151.1	49.8	26.2	11.2	495.7	5.0	343.4*
CAZRI Gola	87.6	32.7	33.3	20.0	433.3	4.3	270.2
Reshmi	111.3	34.8	38.4	18.1	207.0	2.1	47.6
Katha	155.2	58.6	24.0	12.3	617.2*	6.2*	298.6
Seb × Gola F_1	120.9	55.9	34.8	21.3	670.3*	6.7*	326.5*
Seb × Tikadi BC_1	119.1	48.4	27.3	17.4	460.3	4.6	169.8
Chhuara	141.9	54.7	26.2	19.5	486.7	4.9	318.0
Umran	110.8	53.2	24.8	21.9	472.5	4.7	294.1
Jogia	167.6	58.4	22.0	30.8	690.8*	6.9*	419.3*
Banarsi Karaka	114.7	60.7	21.2	21.3	648.3*	6.5*	438.0*
ZG-3	86.1	48.7	24.3	15.6	536.3	6.4*	297.8
Seb	76.7	30.3	27.3	25.5	280.8	2.8	184.7
Sanaur 5	64.8	22.1	30.0	19.9	268.3	2.7	96.8
Kaithali	129.0	42.5	22.3	16.3	731.3*	7.3*	496.6*
Banarsi Pebandi	90.4	38.2	24.4	25.6	595.0	6.0	478.7*
Mundia	174.3	37.1	25.8	7.0	421.7	4.2	341.4*
Range	64.8-174.3	22.1-60.7	21.2-39.9	7.0-38.6	207.0-731.3	2.1-7.3	47.6-496.6
Mean	114.03	45.52	29.3	20.0	480.1	4.9	266.4
CD at 5%	41.19	19.99	4.52	13.6	176.39	1.76	51.45

ISD = initial settlement density, MSL = mean settlement length (cm), SR = sex ratio, IM% = initial mortality percent, LY = broodlac yield (g), Output ratio = broodlac output ratio, SY = scrapedlac yield (g), * = significantly higher than check, CAZRI Gola to 174.3 (Mundia). MSL varied from 22.1 cm (Sanaur 5) to 60.7 cm (Banarasi Karaka) with mean of 45.5 cm. The lowest value of SR was recorded in Banarsi Karaka (21.0), whereas, its highest value (40.0) in Aliganj. Aliganj had highest IM% (38.6) followed by Jogia and Bagwadi. Six cultivars/varieties, *i.e.*, Thornless, Katha, Seb × Gola F_1, Jogia, Banarsi Karaka and Kaithali were having significantly higher brodlac yield than CAZRI Gola. Eight cultivars/ varieties (Illaichi, Kali, Seb × Gola F_1, Jogia, Banarsi Karaka, Kaithali, Banarsi Pebandi and Mundia) had significantly higher scrapedlac yield than CAZRI Gola. An increase of 50 to 69% in broodlac yield and 17 to 84% in scrapedlac were recorded as best varieties (Kaithali, Jogia, Seb × Gola (F_1) and Banarasi Karka) over check (CAZARI Gola).

Sucrose ingested by the phloem sap feeding insects is utilized both as respiratory substrate and also for synthesis of rehalose, mannitol etc. Trehalose has been reported as the main sugar in other phloem sap sucking insects such as aphids, followed by glucose and small amount of sucrose and fructose (Moriwaki *et al*., 4). *Ber* varieties were significantly different with respect to reducing sugars (RS), non reducing sugar (NRS), total sugars (TS) and soluble protein (SP) and chlorophyll content index (CCI). RS in the leaf was found decreased in all *ber* varieties upon inoculation with lac insect. RS varied from

Table 2. Biochemical and physiological traits in *ber* varieties and their interaction.

Variety	RS-I	RS-C	NRS-I	NRS-C	TS-I	TS-C	SP-I	SP-C	CCI-I	CCI-C
Dandan	9.6	10.1	38.1	52.6	47.7	62.7	70.4	83.1	14.1	15.9
Aliganj	10.6	10.8	34.4	40.9	45.0	51.7	81.6*	91.00	16.4	19.57
Seb × Katha F_1	9.8	10.1	17.6	27.2	27.3	37.4	62.4	63.7	12.8	15.7
Bagwadi	15.4*	16.1	55.7	55.2	71.1*	71.3	58.3	62.0	17.9	20.7
Illaichi	15.6*	16.4	49.2	52.1	64.8	68.5	47.5	54.0	21.3*	25.6
Thornless	11.5	12.2	27.4	34.6	38.8	46.8	93.0*	110.9*	16.2	20.5
Maharwali	10.0	13.0	42.2	46.6	55.3	59.6	109.0*	122.5*	18.6	21.9
Kali	9.6	14.5	19.8	33.8	29.4	48.3	77.8*	92.1	12.5	28.3
CAZRI Gola (check)	9.2	12.5	47.1	74.0	56.3	86.5	61.9	76.6	14.8	39.2
Reshmi	6.5	6.6	32.5	32.4	39.0	40.0	103.6*	115.2*	17.5	21.4
Katha	9.5	14.3	54.8	57.6	64.3	71.9	85.3*	101.0*	18. 7	24.5
Seb × Gola F_1	7.4	11.9	45.6	46.6	53.1	58.5	107.8*	131.5*	12.5	13. 7
Seb × Tikadi BC_1	6.3	6.8	44.9	55.6	51.2	62.4	127.1*	145. 9*	17.7	18.6
Chhuara	6.1	9.0	37.1	38.1	43.3	47.2	124.3*	149.9*	13.4	35.4
Umran	11.5	12.2	68.8*	69.9	80.3*	82.1	142.4*	163.2*	18.0	37.7
Jogia	6.8	8.1	34.3	44.3	41.4	52.4	103.5*	119.7*	11.7	12.7
Banarsi Karka	10.9	16.7	46.1	57.2	57.0	73.9	73.9	83.3	15.6	21.4
ZG-3	10.1	11.9	34.7	35.7	45.8	48.7	144.2*	151.1*	14.6	19.7
Seb	11.3	12.3	34.8	35.4	45.1	49.7	128.1*	128.8*	14.0	16. 7
Sanaur 5	13.9	14.8	37.9	41.3	51.8	56.0	141.9*	142.3*	8.2	12.8
Kaithali	15.2*	15.6	31.1	32.8	46.3	48.4	136.4*	137.4*	12.1	12.7
Banarsi Pebandi	11.4	11.7	38.8	43.9	50.3	55.6	180.2*	180.7*	12.8	14.5
Mundia	13.7	14.3	32.9	33.6	46.6	47.9	161.1*	164.2*	11.00	14.8
Range	6.1-15.6	6.6-16.7	17.6-68.8	27.2-69.9	27.3-80.3	37.4-86.5	47.5-180.2	54.0-180.7	8.2-21.3	12.7 -39.2
Mean	10.6	12.3	39.3	45.3	50.0	57.7	105.3	116.1	14.9	21.0
CD at 5%	4.9	5.4	12.0	14.9	12.5	15.4	14.6	16.5	4.4	6.5
Cultivar (A)	3.34		8.99		8.64		11.89		3.83	
Inoculation (B)	N/A		2.65		2.55		3.51		1.13	
A × B	4.72		N/A		12.21		16.81		5.42	

RS = reducing sugar, NRS = non-reducing sugar, TS = total sugars, SP = soluble protein, CCI = chlorophyll content index, C = control, I = inoculated, * = significantly higher than check, CAZRI Gola

6.1 (Chhuara) to 15.6 (Illaichi) mg/g fresh weight in inoculated condition. Varieties Bagwadi, Illaichi and Kaithali were having significantly higher RS than check CAZRI Gola (Table 2); however none of them had significantly higher RS than check in control condition. There was no significant difference in RS due to inoculation condition, but interaction between variety and inoculation condition was significant. Umran had significantly higher NRS-I and TS-I in inoculated condition than check. None of varieties surpassed CAZRI Gola for NRS and TS in control condition. Inoculation condition affected significantly on NRS and TS but interaction between cultivars and inoculation condition was observed significant only in case of TS. Obeed *et al*. (6) observed significant varietal differences for total soluble solids (TSS) and total, reducing, and non-reducing sugars in five *ber* varieties grown in Saudi Arabia.

The lac infestation activates the protein synthesis pathway in the plant to produce more protein for defense as well for development of both, lac insect and plant. This is due to the fact that amount of essential amino acids made available by phloem sap is insufficient to meet the insect's requirement. This shortfall is partly compensated for by the endosymbiotic proteobacterium which biosynthesizes lacking essential amino acids from sucrose and aspartate present in the phloem sap. Soluble protein (SP) ranged from 47.5 and 54.0 mg/g fresh weight (Illaichi) to 180.2 and 180.7 mg/g fresh weight (Banarasi Pebandi) in inoculation and control conditions, respectively. In our study, Thornless, Mahrawali, Reshmi, Katha, Seb × Gola, Seb × Tikadi (BC_1), Chhuara, Umran, Jogia, ZG 3, Seb, Sanour 5, Kaithali, Banarasi Pewandi, and Mundia recorded significantly more protein than check CAZRI Gola, thus finding their suitability for lac cultivation. Protein plays an important role as macromolecules in biological system, made up of different types of amino acids forming the building block, working as signal molecule in various pathways. It has been suggested that aphids can break protein, including senescence like changes, and take advantage of the increased translocation. Illaichi was the only variety having significantly higher CCI than check in inoculated condition; however CAZRI Gola had highest CCI in control condition (Table 2). These parameters were also significantly different in lac inoculated vs un-inoculated (control) conditions irrespective of variety, except for reducing sugar. The interaction between variety and inoculation *vs* control were significant for SP, CCI, RS and TS also and not significant for NRS in these *ber* varieties. As a whole sugar, protein and CCI decreased in inoculated conditions as compared to control due to the stress imposed by lac insect on plants.

Canopy, shape, branching pattern of tree (morphological traits); initial settlement density, mean settlement length and sex ratio (among lac attributing traits); total sugars, soluble protein and chlorophyll content index (among biochemical and physiological traits) had significant association with scrapedlac (Table 3). These traits may be considered while selecting *ber* varieties for raw lac. Multiple regression coefficients of all 24 *ber* varieties were estimated to find out the maximum contribution of various traits towards raw lac. Estimates provided eight best subset regressions in *ber* varieties with scrapedlac as dependent variable and eight traits as independent variables ignoring broodlac yield (Table 4). Scrapedlac yield has been predicted up to 62% by sex ratio only (model 1). Considering low Cp value, four traits, *viz*., canopy of tree, branching pattern, sex ratio and soluble protein have assessed scrapedlac up to 67%. However, it could be estimated up to 73% by all traits under investigation (model 8). Sushil *et al*. (9) developed a multivariate equation derived from 4 independent variables, *viz*., resin per female, dry cell

Table 3. Correlations coefficient among quantitative traits in *ber* varieties for lac production.

Trait	Morphological-biochemical-physiological trait	Broodlac yield	Scrapedlac yield
Morphological	Canopy of tree	**	**
	Shape of tree	*	*
	Branching pattern	**	*
Lac attributing	Initial settlement density	**	**
	Mean settlement length of insect	**	**
	Female to male sex ratio	*	*
Biochemical	Total sugars	NS	**
	Soluble protein	*	**
	Chlorophyll content index	NS	**

* and ** = significant at 1 and 5% probability levels, respectively.

Table 4. Best subsets regression using R square for scrapedlac yield.

Model#/ Variable	Cp	Rsqr	Adj Rsqr	Tree shape	Branch	Canopy	ISD	SR	TS	SP	CCI
1	0.811	0.621	0.603					*			
2	0.444	0.666	0.633		*	*		*		*	
3	0.927	0.695	0.647		*	*	*	*		*	
4	2.177	0.710	0.645		*	*	*	*		*	
5	3.550	0.722	0.640			*	*	*		*	*
6	5.328	0.726	0.623	*	*	*	*	*		*	*
7	7.148	0.729	0.603	*	*	*	*	*	*	*	
8	9.000	0.732	0.579	*	*	*	*	*	*	*	*

Shape of tree = Compact, Semi spreading, Spreading; Branching pattern = Short, medium, Long; Canopy of tree = Closed, semi open, open; ISD = initial settlement density, SR = sex ratio, TS = total sugars, SP = soluble protein, CCI = chlorophyll content index.

weight, density at crop maturity and life period and stated that this was the most efficient and convenient model for estimating lac productivity with 97.76% accuracy.

This is for general information that we cannot get lac yield as well as fruit yield simultaneously from *ber* varieties. It was evident from previous work at our institute that fruit yield reduced drastically up to 70% after lac inoculation in local wild *ber*. Moreover, in case of failure of lac crop, we get fruit value from these cultivars. The average fruit yield of *ber* varieties at the age group of 10 to 20 years is 50-72 kg fruit/ year in rainfed situation, whereas average broodlac output ratio is 6.2 to 7.3 from 2 kg broodlac inoculated per tree in recommended *ber* cultivars. Comparing fruit and lac yield, we get Rs 1,500 to 2,160 per tree per year by selling fruit and Rs 1,980 to 2,420 by selling broodlac per tree per year. With plant density of 600 trees/ ha (4 m × 4 m). Thus, we can get Rs 1,86,000 to 3,18,000 more income from lac cultivation in one hectare plantation.

In the light of above, 23 *ber* varieties known for its fruit value were screened for lac production through morphological, biochemical and physiological markers. Four traits of morphological (canopy and branching pattern), lac attributing (sex ratio) and biochemical (soluble protein) characters may be used as marker for winter *kusmi* lac production. Four *ber* varieties known for fruit purpose, *viz.,* Kaithali, Jogia, Seb × Gola (F_1) and Banarasi Karaka were identified as potential lac hosts (Fig. 1). These *ber* cultivars/varieties may be promoted at farmers' field to enhance the economy through lac production and in case of failure of lac crop, the fruit production thus, extending lac production in *ber* growing belts.

ACKNOWLEDGEMENTS

Thanks are due to Director, ICAR-IINRG, Ranchi for the facilities and help received from the technical staff Mr S. Tripathi and Mr M.L. Rabidas.

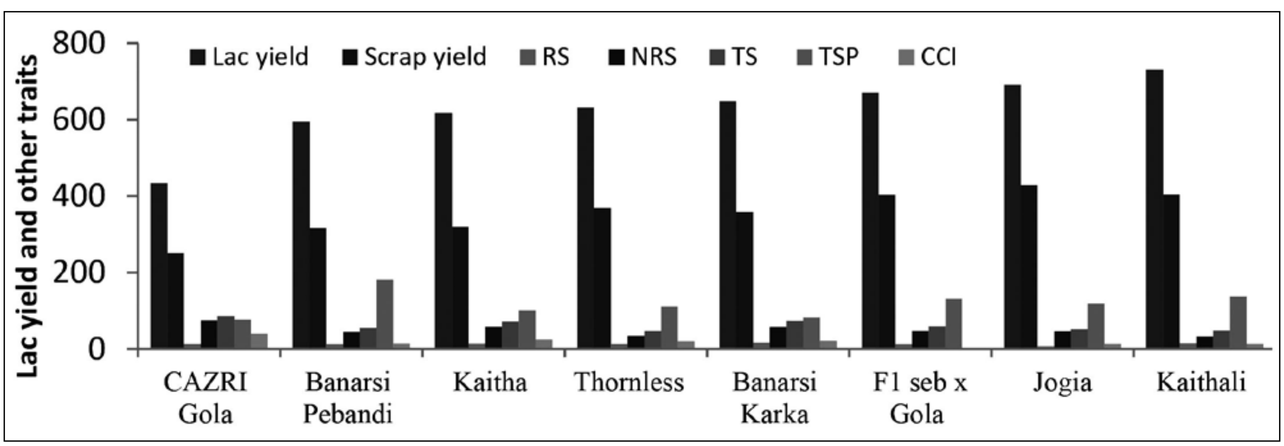

Fig. 1. Promising *ber* cultivars/varieties for *kusmi* lac production.

REFERENCES

1. Awasthi, O.P. and More, T.A. 2009. Genetic diversity and status of *Ziziphus* in India. *Acta Hort*. **840**: 33-40.

2. Azam Ali, S., Bonkoungeon, E., Bowe, C., DeKock, C., Godera, A. and Williams, J.T. 2005. *Ber and other Jujubes, in Fruits for the Future* (*2nd Revised Edn.*), William, J.T., Smith, R.W., Haq, N. and Dunsiger, Z. (Eds.), International Centre for Underutilized Crop, University of Southampton, Southampton, UK, 289 p.

3. Lowrey, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1952. Protein measurement with the folin phenol reagent. *J. Biol. Chem*. **193**: 265-75.

4. Norichika, Moriwaki, Kazuhiro, Matsushita, Masami Nishina and Yoshiaki, Kono. 2003. High concentrations of trehalose in aphid hemolymph. *Appl. Entom. Zool.* **38**: 241-48.

5. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153**: 375-80.

6. Obeed, R.S., Harhash, M.M. and Abdel-Mawgood, A.L. 2008. Fruit properties and genetic diversity of five *ber* (*Ziziphus mauritiana* Lamk.) cultivars. *Pakistan J. Biol. Sci.* **11**: 888-93.

7. Pareek, O.P. 2001. *Ber*, International Centre for Underutilized Crop, Southampton, UK, 229 p.

8. Saha, D., Srivastava, S.C. and Ramani, R. 2013. Genetic relationships among fruit cultivars and host plants of Indian lac insect in ber (*Ziziphus mauritiana* Lam.) revealed by RAPD and ISSR markers. *Indian J. Biotech.* **12**: 170-77.

9. Sushil, S.N., Mishra, Y.D., Chakrabarty, P.K. and Kumar, S. 2000. Estimating stick lac productivity of lac on *ber*, *Zizyphus mauritiana* Lamk., through regression model. *J. Entom. Res.* **24**: 343-48.

Received : March, 2015; Revised : February, 2016; Accepted : February, 2016