Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(3): 1961-1966

Received: 29-03-2020
Accepted: 30-04-2020

SR Manjunatha
Department of Sericulture, Department of Crop Physiology and @Department of Agril. Entomology, UAS, GKVK, Bangalore, Karnataka, India

Manjunath Gowda
Department of Sericulture, Department of Crop Physiology and @Department of Agril. Entomology, UAS, GKVK, Bangalore, Karnataka, India

Nataraja Karaba
Department of Sericulture, Department of Crop Physiology and @Department of Agril. Entomology, UAS, GKVK, Bangalore, Karnataka, India

KC Narayanaswamy
Department of Sericulture, Department of Crop Physiology and @Department of Agril. Entomology, UAS, GKVK, Bangalore, Karnataka, India

KS Jagadish
Department of Sericulture, Department of Crop Physiology and @Department of Agril. Entomology, UAS, GKVK, Bangalore, Karnataka, India

Corresponding Author:
SR Manjunatha
Department of Sericulture, Department of Crop Physiology and @Department of Agril. Entomology, UAS, GKVK, Bangalore, Karnataka, India

Performance of silkworm hybrid PM x CSR2 on drought tolerant transgenic mulberry lines

SR Manjunatha, Manjunath Gowda, Nataraja Karaba, KC Narayanaswamy and KS Jagadish

Abstract
Crop plants adaptable for water stress conditions are gaining importance due to scarcity of water resource, so as mulberry in sericulture potential areas. In this context newly evolved drought tolerant mulberry lines namely, VR, ST, BT, MT and RD have been developed and silkworm bioassay was conducted on these lines utilizing V instar silkworms of hybrid PM X CSR2 with V1 as a check (wild type), at the Department of Crop Physiology, UAS, GKVK, Bangalore, during June 2016. Fifth instar silkworms were reared on these mulberry lines and their influence on silkworm growth and few economic traits were assessed. The results revealed that these lines had no deleterious effects on silkworm growth and cocoon yield. The food consumption and food digestion of the silkworms were normal on all the transgenic mulberry lines and no significant differences were observed though wild type (V1) registered better values. Similarly, Effective rearing rate (ERR) calculated based on the V instar initial number and V instar larval weight were on par in all the lines tested. However, wild type fared better than the transgenic line (100% ERR and 39.19 g/10 larvae). Among the cocoon characters, cocoon yield by number, single cocoon weight and pupal weight were on par in all the lines, while cocoon yield by weight, single shell weight, shell ratio and silk productivity were significantly different and the wild type recorded higher values of 1817.25 g / 1000 worms, 0.36g, 19.26% and 5.21 cg/day, respectively. These findings suggest that drought tolerant transgenic mulberry lines have no adverse effect on silkworms and line ST performed better than other transgenic lines evaluated, being next only to wild type (V1).

Keywords: transgenic mulberry, PM X CSR2, rearing parameters and cocoon parameters

Introduction
Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen, and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability and known as the “Queen of Textiles” the world over. On the other hand, it stands for livelihood opportunity for millions owing to high employment oriented, low capital intensive and remunerative nature of its production. The very nature of this industry with its rural based on-farm and off-farm activities and enormous employment generation potential has attracted the attention of the planners and policy makers to recognize the industry among one of the most appropriate avenues for socio-economic development of a largely agrarian economy like India.
Mulberry (*Morus indica* L.) is the sole food plant of the silkworm (*Bombyx mori* L.) which has been exploited commercially in the sericulture industry. A major determining factor for enhancing the production of silk is to produce quality mulberry foliage under extreme agro-climatic conditions by developing plants that are able to adapt to different climatic conditions. It has been estimated that various abiotic stress conditions, such as moisture, salinity and alkalinity can cause yield losses in mulberry in the range of 50 to 60 per cent (Rao, 2002) [1]. Development of mulberry varieties for tolerance to water stress under semi-arid conditions holds tremendous potential as nearly 48 percent of the Indian cultivars of mulberry fall under rain-fed, water stressed conditions. Yield losses under stressful conditions are always associated with reduction in leaf quality. There is an urgent need to evolve mulberry types having stress tolerance traits that can grow and produce sufficient foliage under environmental stresses.
There are transgenic approaches attempted in mulberry with varied degrees of success (Das *et al*., 2011; Checker *et al*., 2012; Sajeevan *et al*., 2017) [4, 3, 2]. Recently, mulberry transgenic plants expressing *Arabidopsis* gene, *AtSHN1* to improve post-harvest water loss and impart

stress tolerance has been developed (Sajeevan *et al*., 2017) [2]. The transgenic plants displayed dark green shiny appearance with increased leaf surface wax content (Sajeevan *et al*., 2017) [2]. In these transgenic plants increased wax content altered leaf surface properties. The transgenic plants showed significant improvement in leaf moisture retention capacity and there was slow degradation of proteins in detached leaves compared to non-transgenic plants. The study demonstrated the possibility of specific trait manipulation using transgenic approach in mulberry.

Material and methods

All experiments were conducted in green house/ containment facility of the Department of Crop Physiology, and Department of Sericulture, University of Agricultural Sciences (UAS), GKVK campus, Bengaluru, Karnataka. The plant species used for the study were transgenic plants of *Morus indica* L. and cultivated mulberry genotype V1. All the transgenic materials (VR series- Rd29A::*HVA;* ST series-CaMV35S::*HVA1;* BT series-CaMV35S::*bch1;* MT series-CaMV35S::*Osmotin* and RD series- Rd29A::*Osmotin*) were developed at the University of Delhi, South Campus, New Delhi and transferred to the Department of Crop Physiology, UAS, Bengaluru as per the Department of Biotechnology, Government of India guidelines. These plants were multiplied and grown in the containment facility.

The transgenic mulberry lines were raised and maintained in pots, which were initially pruned at once and were regularly irrigated once in two days. Each plant was provided with 5.00 g N, 2.88 g P and 3.00 g K after 15 days of pruning. Leaves were harvested on 45^{th} day for silkworm rearing. Five selected mulberry transgenic lines developed at University of Delhi, South campus, New Delhi and a non-transgenic mulberry, V1 maintained at Department of Crop Physiology, University of Agricultural Sciences, Bengaluru, was used for the study and the details are as follows. T1– Silkworm rearing with Transgenic Mulberry line-1 (VR series-Rd29A::*HVA1*), T2–Silkworm rearing with Transgenic Mulberry line-2 (ST series-CaMV35S::*HVA1*), T3– Silkworm rearing with Transgenic Mulberry line-3 (BT series-CaMV35S::*bch1*), T4-Silkworm rearing with Transgenic Mulberry line-4 (MT series CaMV35S::*Osmotin)*, T5–Silkworm rearing with Transgenic Mulberry line-5 (RD series-Rd29A::*Osmotin*) and T6–Silkworm rearing with Mulberry non-transgenic variety V1/Control

The silkworm bioassay was done in two trials, the trial using fifth instar silkworms of PM×CSR2, while the second trial was from egg to egg. Before the commencement of rearing, the silkworm rearing room and equipments were cleaned and washed in water. The appliances were then sun dried in bright sunlight for a period of 8 -10 hours. Later, rearing room and appliances were disinfected with two per cent formalin solution @ 800 ml per 10 m^2 as suggested by Dandin *et al.* (2003) [5]. Ten larvae randomly selected from each replication of every treatment were weighed on a sensitive balance at the end of each instar, just before they settled down for moult. Grown up larval weight was recorded on fifth day of fifth instar. The duration of each instar was recorded in all the treatments and replications. The duration of each moult was recorded in each treatment, replication-wise.

Ten larvae randomly selected from each replication of every treatment were weighed on a sensitive balance at the end of each instar, just before they settled down for moult. Grown up larval weight was recorded on fifth day of fifth instar**.** The duration of fifth instar was recorded in all the treatments and replications. The time taken from the beginning of ripening to time taken till the 50 per cent of worms start spinning was recorded treatment-wise and replication-wise. Cocoon yield by number and weight was recorded for each treatment, replication-wise and estimated for 1000 worms. The ERR was calculated as the number of cocoons harvested out of number of worms reared in each replication.

$$ERR\ (\%) = (Nc / Nw) \times 100$$

Where,
Nc = Number of cocoons harvested and Nw = Number of worms reared

Ten cocoons per replication were randomly drawn from each replication on fifth day after spinning and individually weighed using an electronic balance and average taken. Simultaneously, the corresponding pupal and shell weights were also recorded. The cocoon shell ratio was calculated by the following formula,

$$\text{Cocoon shell ratio } (\%) = (Ws / Wc) \times 100$$

Where,
Ws = Cocoon shell weight (g) and Wc = Cocoon weight (g)

The productivity of the silkworm fed with six transgenic mulberry varieties were calculated replication wise by the formula,

$$\text{Silk productivity (cg/day)} = (Ws / Fd) \times 100$$

Where,
Ws = Weight of cocoon shell (cg) and Fd = Fifth instar duration (days).

A sample of five cocoons per replication was randomly drawn and stifled in a hot air oven at 70ºC for three hours. The cocoons were kept in open for three hours to remove the moisture content and were dried under shade. The cocoons were cooked individually in boiling water for three to four minutes to soften the sericin layer. These cooked cocoons were reeled on an 'epprouvette'. Length of the silk filament was determined by the number of revolutions recorded and converted into meters by the formula,

$$\text{Length of silk filament (m)} = R \times 1.125$$

Where,
R = Number of revolutions; 1.125= Circumference of epprouvette.

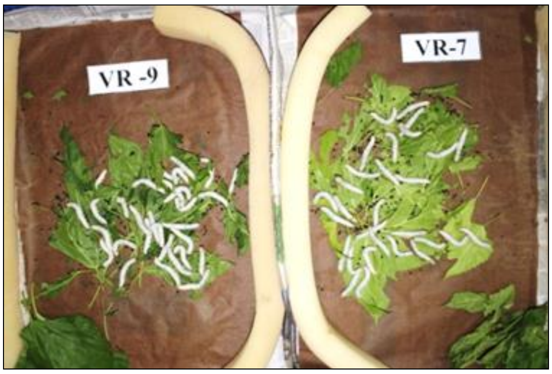
The single cocoon filament reeled using epprouvette was dried in an oven at 70ºC and weighed using electronic balance. The denier was worked-out by using the formula,

$$\text{Denier (D)} = (Fw / Fl) \times 9000$$

Where,
Fw = Single cocoon filament weight (g) and Fl = Single cocoon filament length (m)

Plate 1: Transgenic mulberry lines used for silkworm bioassay

Plate 2: Silkworm rearing setup for bioassay on transgenic mulberry lines



a) VR series

b) ST series

c) BT series

d) MT series

e) RD series **f) V1 Variety**

Plate 3: Silkworms reared on different transgenic mulberry lines

Results and Discussion

In the first trail the response of mulberry silkworm PM×CSR2 was determined by feeding transgenic mulberry leaves during the fifth instar only. These worms were fed with V1 mulberry leaves raised under irrigated conditions following recommended practices (Plate 3).

Significant differences were observed for fifth instar larval weight of silkworm hybrid PM×CSR2 reared on mulberry leaves of transgenic mulberry lines and V1 variety (Table1). Highest larval weight was observed in V1 variety (39.19 g/10 worms) followed by ST series (37.97 g/10 worms) and BT series (36.02 g/10 worms). Lowest larval weight was observed in MT series (33.79 g/10 worms) leaves fed silkworms.

Response of fifth instar silkworms fed on transgenic mulberry

Significant differences were observed for fifth instar larval weight of silkworm hybrid PM×CSR2 reared on mulberry leaves of transgenic mulberry lines and V1 variety (Table1). Highest larval weight was observed in V1 variety (39.19 g/10 worms) followed by ST series (37.97 g/10 worms) and BT series (36.02 g/10 worms). Lowest larval weight was observed in MT series (33.79 g/10 worms) leaves fed silkworms. Fifth instar larval duration showed non-significant differences among the silkworms reared on different transgenic mulberry lines and V1 variety (Table1). However, longer larval duration was observed in silkworms fed with leaves from RD series (7.48 days) and shorter larval duration was observed when fed with V1 mulberry leaves (7.28 days) followed by VR series (7.29 days). It has been found that improved mulberry varieties DD (Puttaswamy *et al*., 2001) [6], S1635 (Saratchandra *et al*., 2002) [8] and V1 (Rayar, 2011; Bhede *et al*., 2013; Durande *et al*., 2013; Sutar *et al*., 2014; Rathod *et al*., 2015) [7, 11, 9, 10] recorded higher fifth instar larval weight.

Non-significant differences was observed for time taken for 50 per cent spinning among the worms fed with mulberry leaves from different transgenic lines and V1 variety (Table 1). However, silkworms fed with VR series mulberry leaves took longer time for 50 per cent spinning (25.16 h) while worms fed on V1 mulberry leaves took shorter time to reach 50 per cent spinning (24.87 h). Effective rate of rearing based on fifth instar initial larval number was statistically on par among the silkworm batches fed with leaves of transgenic mulberry lines and V1 variety (Table1). However 100 per cent ERR was observed in silkworms fed with V1, ST series and RD series mulberry leaves. Lowest ERR was recorded in BT series (92.50%).

Cocoon yield by number also showed non-significant differences among the silkworm batches fed with mulberry leaves of different transgenic lines and V1 variety. The trend was as observed in ERR (Table1). Significant difference was observed for cocoon yield by weight when silkworms were fed with mulberry leaves of different transgenic lines and V1 variety (Table 1). Significantly highest cocoon yield was observed in V1 variety (1817.25 g/1000 worms), followed by ST series (1732.00 g/1000 worms) and RD series (1588.00 g/1000 worms). The least cocoon yield was recorded in MT series (1454.13 g/1000 worms).

The cocoon weight of silkworm hybrid PM×CSR2 was significantly maximum when they were fed on V1 variety mulberry leaves (1.89 g), followed by transgenic lines ST series (1.73 g) and least cocoon weight was observed in MT series (1.45 g) (Table 2 and Fig. 1). The pupal weight showed non- significant difference among the silkworm batch reared on different transgenic mulberry lines and V1 variety (Table 2). However highest pupal weight was observed in ST series (1.52 g) followed by V1 variety (1.51 g). The least pupal weight was observed in VR series (1.32 g).

Weight of the single shell showed significant variation among the silkworms fed with different transgenic mulberry lines and V1 variety. When the silkworms (PM×CSR2) were reared on V1 mulberry leaves resulted in maximum shell weight (0.36 g) and was followed by ST series (0.30 g) and BT series (0.29 g) transgenic line. Lowest shell weight was recorded in MT and VR series (0.27 g). Significant difference in shell ratio was evident among the five transgenic and V1 mulberry varieties (Table 2). It was statistically maximum when silkworms were fed with BT series transgenic mulberry leaves (19.37%) followed by V1 (19.26%). The least shell ratio was recorded in ST series (17.28%).

The silk productivity of silkworm breed, PM×CSR2 was significantly maximum when they were nourished with V1 mulberry leaves (5.21cg/day) followed by transgenic lines ST (4.27 cg/day), and BT (4.16 cg/day) series of transgenic mulberry lines. The least silk productivity was observed in VR series (3.81cg/day) (Table 2). Significant difference was observed for filament length among the silkworm batches reared on different transgenic mulberry lines and V1 variety (Table 2). Significantly longest filament length was observed in V1 variety (750.80 m). RD series (773.10 m) was the next best followed by ST series (643.90 m).

The transgenic mulberry lines and V1 variety resulted in significant differences for filament weight when fed to silkworms (Table 2). Significantly highest filament weight was recorded in RD series (0.28 g) followed by V1 variety (0.26 g) and ST series (0.25 g). Least filament weight was recorded in VR series (0.17 g). Filament denier was significantly different among the silkworms fed with transgenic mulberry lines and V1 variety leaves (Table 2). Significantly highest denier was recorded in ST series (3.45) followed by RD series and MT series (3.23). Least filament denier was observed in VR series (2.52).

It has been found that improved mulberry varieties DD (Puttaswamy *et al.*, 2001) [6], S1635 (Saratchandra *et al.*, 2002) [8] and V1 (Rayar, 2011; Bhede *et al.*, 2013; Durande *et al.*, 2013; Sutar *et al.*, 2014; Rathod *et al.*, 2015) [7, 11, 9, 10] recorded higher fifth instar larval weight. In the present study similar trend was observed wherein V1 variety was respectively). However, among the transgenic mulberry lines ST series and RD series were better for cocoon weight and shell weight, (1.73 and 1.59 g and 0.30 and 0.28 g, respectively), MT series for shell ratio (18.93%) and ST series for silk productivity (4.27 cg/day).

Pupal weight was however non-significant among the silkworms fed with transgenic mulberry lines and V1 variety though slightly higher in ST series followed by V1 variety. Rayar, (2011) [7] recorded better cocoon weight shell weight, shell ratio and pupal weight when bivoltine silkworms were reared on V1 variety. V1 variety supporting better cocoon weight and shell weight in PM×CSR2 breed has also been documented (Durande *et al.*, 2013; Sutar, 2014; Rathod *et al.*, 2015) [11, 9, 10]. In the present study transgenic mulberry resulting in better cocoon traits is encouraging though not supported by any previous study.

Table 1: Effect of transgenic mulberry on rearing and yield performance of silkworm, PM×CSR2 reared from V instar

Transgenic / non-transgenic lines@	Fifth instar larval wt. (g/10 worms)	Larval duration (days)	Time taken for 50% spinning (h)	ERR (%)	Cocoon yield by number (No./1000 worms)	Cocoon yield by wt. (g /1000 worms)
VR SERIES	34.56^{abc}	7.29	25.16	96.67	966.66	1528.50^{abcd}
ST SERIES	37.97^{de}	7.38	25.11	100.00	1000.00	1732.00^{d}
BT SERIES	36.02^{abcd}	7.46	25.13	92.50	925.00	1512.37^{abc}
MT SERIES	33.79^{a}	7.43	25.09	95.83	958.33	1454.13^{ab}
RD SERIES	34.30^{ab}	7.48	25.12	100.00	1000.00	1588.00^{abcd}
V1	39.19^{e}	7.28	24.87	100.00	1000.00	1817.25^{a}
F TEST	*	NS	NS	NS	NS	*
Sem±	1.06	0.34	1.17	2.65	6.8	71.5
CD at 5%	3.15	-	-	-	-	212.44

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par. @ VR-Rd29A::*HVA1*, ST-CaMV35S::*HVA1*, BT-CaMV35S::*bch1*, *MT*-CaMV35S::*Osmotin*, RD-Rd29A::*Osmotin*, V1- *Victory1*

Table 2: Effect of transgenic mulberry on cocoon and filament parameters of silkworm breed PM×CSR2 reared from V instar

Transgenic / non-transgenic lines@	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)	Silk productivity (cg/day)	Filament length (m)	Filament wt. (g)	Filament denier
VR SERIES	1.53^{abc}	1.32	0.27^{a}	17.49^{ab}	3.81^{a}	596.45^{a}	0.17^{a}	2.52^{a}
ST SERIES	1.73^{de}	1.52	0.30^{abcd}	17.28^{a}	4.27^{abcde}	643.90^{abcd}	0.25^{bcd}	3.45^{c}
BT SERIES	1.51^{ab}	1.40	0.29^{abc}	19.37^{d}	4.16^{abcd}	614.25^{ab}	0.21^{ab}	3.02^{b}
MT SERIES	1.45^{a}	1.35	0.27^{a}	18.93^{abcd}	3.91^{ab}	622.35^{abc}	0.22^{bc}	3.23^{bc}
RD SERIES	1.59^{bcd}	1.41	0.28^{ab}	17.61^{abc}	3.99^{abc}	773.10^{e}	0.28^{d}	3.23^{bc}
V1	1.89^{f}	1.51	0.36^{e}	19.26^{cd}	5.21^{f}	750.80^{e}	0.26^{cd}	3.14^{bc}
F TEST	*	NS	*	*	*	*	*	*
Sem±	0.08	0.07	0.01	0.56	0.18	24.49	0.01	0.11
CD at 5%	0.23	-	0.04	1.65	0.53	72.76	0.04	0.32

Note: *: Significant at 5%, NS: Non- Significant, Figure with same super script are statistically on par. @ VR-Rd29A::*HVA1*, ST-CaMV35S::*HVA1*, BT-CaMV35S::*bch1*, *MT*-CaMV35S::*Osmotin*, RD-Rd29A::*Osmotin*, V1- *Victory1*

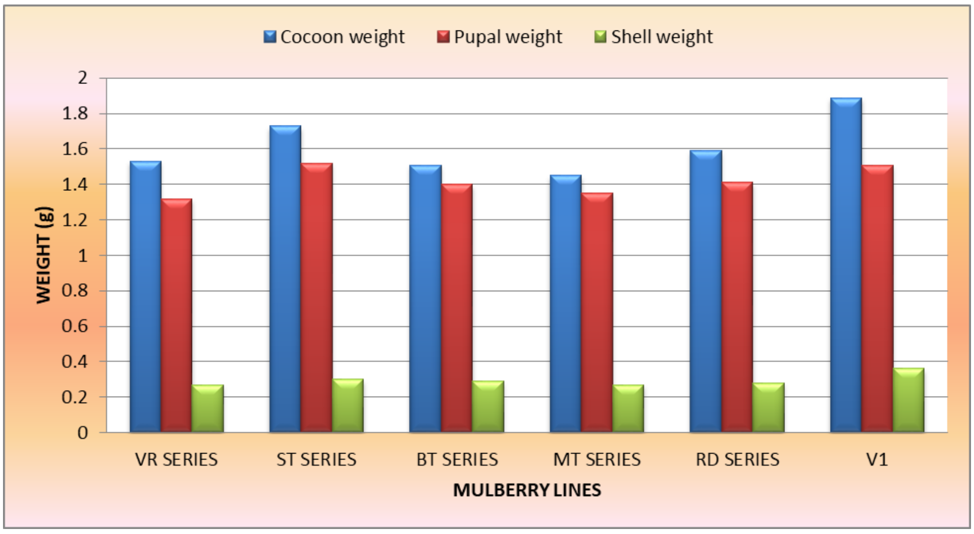


Fig 1: Effect of transgenic mulberry on cocoon parameters of silkworm breed PM×CSR2 reared from V instar

Conclusion

Rearing fifth instar PM×CSR2 silkworms on transgenic mulberry resulted in significantly higher fifth instar larval weight in V1 variety (39.19 g/10 worms) followed by ST series (37.97 g/10 worms) and BT series (36.02 g/10 worms). Fifth instar larval duration and time taken for 50 percent spinning were non-significant among transgenic lines. The cocoon weight of silkworm hybrid PM×CSR2 was maximum when they were fed on V1 variety mulberry leaves (1.89 g). The highest pupal weight was observed in ST series (1.52 g) followed by V1 variety (1.51 g). When the silkworm (PM×CSR2) were reared on V1 mulberry leaves resulted in maximum shell weight (0.36 g) and was followed by ST series (0.30 g) and BT series (0.29 g) transgenic lines. Shell weight was statistically maximum when silkworms were fed with BT series transgenic mulberry leaves (19.37%) followed by V1 (19.26%). The silk productivity of silkworm breed, PM×CSR2 was significantly maximum when it was nourished with V1 mulberry leaves (5.21 cg/day) followed by ST series (4.27 cg/day). The longest filament length was observed in V1 variety (750.80 m) followed by RD series (773.10 m).

References

1. Rao AA. Conservation status of mulberry genetic resources in India. Expert Consultation on Promotion of Global Exchange of Sericultural Germplasm Resources, Satellite session of *19th ISC Congress.* Bangkok, Thailand, 2002, 21-25.
2. Sajeevan RS, Nataraja KN, Shivashankara KS, Pallavi N, Gurumurthy DS, Shivanna MB. Expression of *Arabidopsis* SHN1 in Indian mulberry (*Morus indica* L.) increases leaf surface wax content and reduces post-harvest water loss. Front. Plant Sci. 2017. https://doi.org/10.3389/fpls.2017.00418
3. Checker VG, Chhibbar AK, Khurana P. Stress-inducible expression of barley *Hva1* gene in transgenic mulberry displays enhanced tolerance against drought, salinity and cold stress. Transgenic Res. 2012; 21:939-957.
4. Das M, Chauhan H, Chhibbar AK, Haq QMR, Khurana P. High-efficiency transformation and selective tolerance against biotic and abiotic stress in mulberry, *Morus indica* cv. K2 by constitutive and inducible expression of tobacco osmotin. Transgenic Res. 2011; 20(2):231-246.
5. Dandin SB, Jayaswal J, Giridhar K. Handbook of Sericulture Technologies, Central Silk Board, Bangalore, 2003, 259.
6. Puttaswamy S, Katti SR, Venketesh H, Raghu Raman R. Performance of NB_4D_2 - A bivoltine silkworm race reared on four mulberry varieties. Proc. Natl. Sem. Mulb. Seri. Res. India. 2001; 38(2):123-128.
7. Rayar SG. Performance of new bivoltine breeds of silkworm, *Bombyx mori* L. on improved mulberry varieties during rainy season. J Ent. Res. 2011; 35(1):69-74.
8. Saratchandra B, Rajanna L, Philomena KL, Paramesha C, Ramesh SP, Jayappa T. An evaluation of elite mulberry varieties for yield and quality through bioassay. Ann. Rep., CSR&TI, Mysore, 2002, 13.
9. Sutar AV, Khemnar SS, Gaikwad BB, Mudgalkar AB. Evaluation of different mulberry varieties against mulberry silkworm, *Bombyx mori* L. for rearing performance and economic traits. J Ent. Res. 2014; 38(4):251-254.
10. Rathod BS, Nalwandikar PK, Shetgar SS, Sawant CG, Sonkamble MM. Effect of feeding proportionately mixed leaves on double hybrid silkworm. The Bioscan. 2015; 10(1):01-04.
11. Durande GB, Gade RS, Jagtap RN, Tayade DS. Performance of PM×CSR_2 race of silkworm *Bombyx mori* L. on different varieties of mulberry. Bioinfolet. 2013; 10(3b):1023-1025.