



E-ISSN: 2278-4136

P-ISSN: 2349-8234

[www.phytojournal.com](http://www.phytojournal.com)

JPP 2020; Sp 9(4): 86-90

Received: 18-05-2020

Accepted: 20-06-2020

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## Assessing the impact of nuclear polyhedrosis virus (Grasserie) on economic traits of bivoltine silkworm (*Bombyx mori* L.) breeds under temperate climatic conditions

Raghavendra SM, KA Sahaf, NA Ganaie, Somagaini Pavankumar, Prithiv Raj V and Nayeema Jan

**Abstract**

The present investigation was carried out to assess the impact of nuclear polyhedrosis virus (Grasserie) on economic traits of bivoltine silkworm (*Bombyx mori* L.) breeds under temperate climatic conditions. Ten mature larval weight was recorded (40.9g) in case of SANISH8 and it was minimum (35.6g) in APS4. Among the cocoon traits SH<sub>6</sub> recorded the maximum cocoon weight of 1.49g, NB<sub>4</sub>D<sub>2</sub> recorded highest shell weight of 0.30g and shell ratio of 20.12 per cent. However in susceptible breed cocoon traits found minimum cocoon weight of 1.38 g (CSR<sub>4</sub>), shell weight of 0.26 g (APS<sub>4</sub>) and shell ratio of 18.56 per cent (APS<sub>4</sub>). Breeds which were less susceptible to BmNPV viz., NB<sub>4</sub>D<sub>2</sub> and APS<sub>8</sub> yields more cocoons by weight. Among the improved breeds of silkworm the average filament length, filament size and raw silk were found significantly in BmNPV inoculated. However, the maximum average filament length of 816 m (DUN-6), filament size of 2.61 (DUN-22) and raw silk of 29.17% (DUN-6) was noticed. However, in susceptible breeds average filament length of 709 m (APS<sub>4</sub>), filament size of 2.28 (APS<sub>4</sub>) and raw silk of 24.58% (CSR<sub>2</sub>). Genotypes APS<sub>8</sub> and NB<sub>4</sub>D<sub>2</sub> has showing better economic characters as compared to other silkworm genotypes.

**Keywords:** BmNPV, *Bombyx mori* L., Grasserie and Nuclear Polyhedrosis virus

**Introduction**

Sericulture is one of the oldest agro-based industries in the world. It is commonly rural based on-farm and off-farm activity and has huge employment generation potential. Sericulture has been perceived as one of the most suitable avenue for socio-economic development especially in a developing nation like India particularly for farmers with little land possessions as well as to other weaker section of the society. Sericulture in India has ended up being an exceptionally profitable money crop which gives alluring returns consistently. India ranks second in respect of mulberry raw silk production being next only to china. Mulberry silk accounts for 70.1 per cent (21,273 MT), tasar silk 10.8 per cent (3,268 MT), eri silk 18.6 per cent (5,637 MT) and muga silk 0.56 per cent (170 MT) of the total raw silk production of 30,348 MT (Anonymous, 2017) [1]. Sericulture occupies a unique position in Indian economy and assumes more importance in alleviating the problems of the rural people. Since it is practiced from days of hore, it has now become the most significant rural industry due to its low investment, maximum employment generation and quick turn over. Jammu and Kashmir is the traditional sericulture state of India and is practiced in 20 districts. The cocoon and the raw silk production in J&K during 2016-2017 have been 973 MT and 145 MT respectively (Anonymous, 2017) [1]. Presently, about 30,000 rural families are directly or indirectly associated with the silk industry.

The successful cocoon crops are highly dependent on several factors viz., nutritional status, silkworm breed, rearing technology employed, environmental factors during rearing, the latter contributing considerably to the cocoon crop. The major problems faced by the silk industry are frequent disease outbreaks since most of the commercially reared silkworm species, including *Bombyx mori*, are highly susceptible to the diseases like pebrine, flacherie, grasserie and muscardine. In India approximately 40 percent crop losses are attributed to these diseases (Sheebarajakumari *et al.*, 2007; Subha Rao *et al.*, 1991) [9, 12]. Among the diseases, viral diseases are most commonly occurring diseases (Samson *et al.*, 1990) [7]. The virus formerly known as *Borrelina bombycis* is presently termed as *Bombyx mori* L. nuclear polyhedrosis virus (BmNPV). This disease is caused by an occluded baculovirus. The nuclear polyhedrosis of silkworm is commonly known as 'Grasserie' disease (French).

The names indicate either the yellowish colour of diseased insect or its swollen fat like appearance. Infection with grasserie, the worms show the symptoms of shiny body, aimless crawling, inter-segmental swelling, white fluid coming out after skin rupture and dead worms hanging from the rearing stands and trays.

Grasserie disease usually affects fourth and fifth instars silkworms under natural conditions (Chitra *et al.*, 1975) [2]. If the infection is noticed during early instars, worms fail to spin cocoons and die, but worms can spin the cocoons and die inside producing melted cocoons, if infection occurs during later instars. *Bombyx mori* nuclear polyhedrosis virus (BmNPV) is prevalent during all the rearing seasons. The silkworms infected by BmNPV show symptoms at the advanced stage of development and by that time the larvae have almost completed the feeding period, consuming the entire quantum of leaves and dies within a day or two. In India the loss due to BmNPV has been accounted for to the extent of 20-50 per cent in commercial sericulture (Samson *et al.*, 1990) [7]. Rearing of bivoltine breeds and hybrids increase both quantity and quality of raw silk production however, these silkworm breeds are more susceptible to Grasserie as compared to multivoltine breeds (Sivaprakasam and Rabindra, 1995; Liushixian, 1984) [11, 5]. Obviously this causes a big constraint for the successful adoption of bivoltine sericulture in a tropical country like India. Need was felt to identify silkworm breeds tolerant to BmNPV before taking up any breeding programme, specifically for evolving BmNPV tolerant breeds. Therefore, it would be essential to assess the susceptibility status of the available silkworm breeds against BmNPV. Hence, keeping in view the economic importance of silkworm breeds and magnitude of impact caused by nuclear polyhedrosis virus, the present study was proposed.

### Materials and Methods

Investigations were made during spring, 2017 to assess the impact of nuclear polyhedrosis virus (Grasserie) on economic traits of bivoltine silkworm (*Bombyx mori* L.) breeds in College of Temperate Sericulture, SKUAST-Kashmir, Mirgund. The experiment comprised of ten treatments (10 silkworm genotypes) (Table-1) and design of the experiment was completely randomized design (CRD) with each treatment replicated thrice. The DFL's of breeds were surface sterilized and eggs are incubated at 25±1°C and 75±5% relative humidity. After hatching, the larvae of all breeds were reared up to third instar by following standard rearing practices and then separated into replications of 200 worms each for imposing the treatments. After preparation of BmNPV inoculum, the inoculation was carried out per orally by feeding silkworm with virus suspension on leaf.

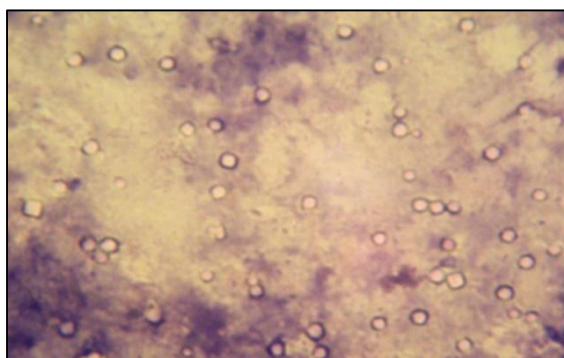


Fig 1.

### Polyhedral bodies causing viral disease (Grasserie) of Silkworm (*Bombyx mori*)

The larvae were allowed to feed on the treated leaves for 16 hrs and subsequently fed with untreated mulberry leaves. Survived larvae were reared till cocooning and mounted for spinning. The cocoons were harvested on 6<sup>th</sup> day of spinning and assessed on 7<sup>th</sup> day for economic parameters. All these data's were subjected to statistical analysis. The materials used and the techniques employed are presented below:

**Table 1:** Treatment details of economic trait assessment of different bivoltine silkworm breeds against nuclear polyhedrosis virus

Treatments	Silkworm genotype
T <sub>1</sub>	APS-4
T <sub>2</sub>	APS-8
T <sub>3</sub>	CS-6
T <sub>4</sub>	CSR-2
T <sub>5</sub>	CSR-4
T <sub>6</sub>	DUN-6
T <sub>7</sub>	DUN-22
T <sub>8</sub>	NB4D2
T <sub>9</sub>	SH-6
T <sub>10</sub>	SANISH-8

### Economic trait assessment parameters

Fifth instar larval duration (hours), total larval duration (hours), average larval weight, single cocoon weight (g), single shell weight (g), shell ratio (%), ERR by number & weight, filament length, filament size and raw silk (%).



Fig 2: Larvae of different genotypes of silkworm (*Bombyx mori* L.) breeds

## Result and Discussion

### Larval characters

Among the inoculated lot NB<sub>4</sub>D<sub>2</sub> revealed least fifth instar duration of 173 hrs which was significantly different from rest of breeds (Table-2). Longest fifth instar duration was recorded in CSR<sub>4</sub> (221 hrs.). The maximum per cent increase over control of 16.58 was observed in CS<sub>6</sub>, whereas minimum per cent increase over control of 3.46 was observed in NB<sub>4</sub>D<sub>2</sub>. Vijay-kumari *et al.* (2001) [13] have also reported prolongation in the larval period in fifth instar. NB<sub>4</sub>D<sub>2</sub> revealed least total larval duration of 653 hrs which was significantly different from rest of breeds. Longest total larval duration was recorded in CSR<sub>4</sub> (701 hrs.). The maximum per cent increase over control of 4.75 was observed in CS<sub>6</sub>, whereas minimum per cent increase over control of 0.91 was observed in NB<sub>4</sub>D<sub>2</sub>. The reason for prolongation of larval duration in the inoculated batches can be due to loss of feeding ability because of decreased digestive enzymes activity (Gururaj *et al.*, 1999) [3] and variation in hormone titers (Mikhailov *et al.*, 1992) [6]. The average larval weight of different breeds under study ranged from 35.6 g to 40.9 g. Among the inoculated silkworm breeds, SANISH<sub>8</sub> recorded the maximum larval weight of 40.9 g, followed by CSR<sub>4</sub> (40.7 g) and NB<sub>4</sub>D<sub>2</sub> (40.5 g); whereas minimum larval weight of 35.6g was recorded in

APS<sub>4</sub>. The maximum per cent decrease over control of 9.41 was observed in APS<sub>4</sub>, whereas minimum per cent decrease over control of 0.48 was observed in CSR<sub>4</sub>. Horic and Watanabe (1980) [14] has also reported that under BmNPV infection larval weight gets reduced due to the accumulation of body substances, depletion of fuel reserves such as haemolymph, trehalose, fat bodies and glycogen.

### Single cocoon weight (g)

The single cocoon weight of different breeds under investigation ranged from 1.38 g to 1.50 g (Table-3). Among the inoculated silkworm breeds, SH<sub>6</sub> recorded the maximum single cocoon weight of 1.50 g, followed by SANISH<sub>8</sub> (1.49 g), DUN-22 (1.47 g), NB<sub>4</sub>D<sub>2</sub> (1.47 g), CSR<sub>2</sub> (1.45 g), CS<sub>6</sub> (1.43 g), DUN-6 (1.43 g) APS<sub>4</sub> (1.42 g) and APS<sub>8</sub> (1.42 g), while least single cocoon weight of 1.38 g recorded in CSR<sub>4</sub>. This reduction in cocoon weight in BmNPV infected silkworms has been attributed to diminish in total proteins at the later phase of infection (Watanbe and Kobayashi, 1969). The control batches maintained for each breed showed higher single cocoon weight (g) in contrast to the inoculated breeds. The maximum per cent decline over control of 7.38 was observed in CSR<sub>4</sub>, while least per cent decrease over control of 0.66 was noticed in SANISH<sub>8</sub>.

**Table 2:** Effect of BmNPV infection on the larval characters of different breeds of silkworm, *Bombyx mori* L.

Genotypes	Fifth instar larval duration (hours)			Total larval duration (hours)			Average larval weight (g)		
	Inoculated	Control	Percent increase over control	Inoculated	Control	Percent increase over control	Inoculated	Control	Percent decrease over control
APS <sub>4</sub>	204	191	6.37	684	671	1.90	35.6	39.3	9.41
APS <sub>8</sub>	178	161	9.55	658	641	2.58	35.8	38.0	5.78
CS <sub>6</sub>	193	161	16.58	673	641	4.75	38.8	40.5	4.19
CSR <sub>2</sub>	202	194	3.96	682	674	1.17	38.7	40.5	4.44
CSR <sub>4</sub>	221	197	10.8	701	677	3.42	40.7	40.9	0.48
DUN-6	190	181	4.73	670	661	1.34	39.3	41.0	4.14
DUN-22	202	191	5.44	682	671	1.61	39.1	40.6	3.69
NB <sub>4</sub> D <sub>2</sub>	173	167	3.46	653	647	0.91	40.5	41.2	1.69
SH <sub>6</sub>	196	171	12.75	676	651	3.69	38.5	40.6	5.17
SANISH <sub>8</sub>	192	169	11.9	672	649	3.42	40.9	45.8	10.06
Mean	195	178		675	657		38.9	40.8	
SEm±	4.12	1.0		3.08	1.0		0.80	0.6	
CD at 5%	12.52	2.9		9.17	2.9		2.38	1.8	

### Single shell weight (g)

The single shell weight of different breeds under study ranged from 0.26 g to 0.30 g. Among the inoculated silkworm breeds, NB<sub>4</sub>D<sub>2</sub> recorded the maximum single shell weight of 0.30 g, followed by DUN-6 (0.29 g), SH<sub>6</sub> (0.29 g), SANISH<sub>8</sub> (0.29 g), APS<sub>8</sub> (0.28 g), CS<sub>6</sub> (0.28 g), CSR<sub>2</sub> (0.28 g), CSR<sub>4</sub> (0.28 g) and DUN-22 (0.28 g), whereas minimum single shell weight of 0.26 g in (APS<sub>4</sub>). The control batches maintained for each breed showed higher single shell weight (g) in comparison to the inoculated breeds. The maximum per cent decrease over control of 9.67 was observed in CSR<sub>4</sub>, whereas minimum per cent decrease over control of 3.22 was observed in NB<sub>4</sub>D<sub>2</sub> (Table 3).

### Shell ratio (%)

The shell ratio of different silkworm breeds ranged from 18.56 to 20.12 per cent. Among the inoculated silkworm breeds NB<sub>4</sub>D<sub>2</sub> recorded the maximum shell ratio of 20.12 per cent, followed by SANISH<sub>8</sub> (19.88%), CSR<sub>4</sub> (19.83%), SH<sub>6</sub> (19.78%), APS<sub>8</sub> (19.65%), CS<sub>6</sub> (19.44%), CSR<sub>2</sub> (19.24%), DUN-22 (19.22%) and DUN-6 (18.80%), whereas minimum shell ratio of 18.56 per cent in APS<sub>4</sub>. The control batches maintained for each breed showed higher shell ratio in

comparison to the inoculated breeds. The maximum per cent decrease over control of 6.83 was observed in DUN-6, whereas minimum per cent decrease over control of 0.31 was observed in CSR<sub>2</sub> (Table-3). The present findings are in corroboration with the earlier findings on the effect on shell ratio (Satish and Govindan, 1988) [8].

### Cocoon yield by number per 10,000 larvae

The cocoon yield by number per 10,000 larvae of different breeds ranged from 3733 to 7267 (Table-4). Among the inoculated silkworm breeds, APS<sub>8</sub> recorded the maximum cocoon yield by number per 10,000 larvae of (7267), followed by NB<sub>4</sub>D<sub>2</sub> (6767), SH<sub>6</sub> (6167), CS<sub>6</sub> (5867), SANISH<sub>8</sub> (5433), DUN-6 (5333), DUN-22 (4633), APS<sub>4</sub> (4500) and CSR<sub>2</sub> (4067), while least cocoon yield by number per 10,000 larvae of 3733 in CSR<sub>4</sub>. The control batches maintained for each breed showed higher cocoon yield by number per 10,000 larvae in contrast to the inoculated breeds. The maximum per cent decrease over control of 53.72 was observed in CSR<sub>4</sub>, whereas minimum per cent decrease over control of 22.13 was observed in APS<sub>8</sub>. Sidhu and Singh, (1968) [10] reported that multivoltine breeds yield more number of cocoons than bivoltine breeds at moderate levels of BmNPV infections.

**Table 3:** Effect of BmNPV infection on the single cocoon weight, single shell weight and shell ratio of different breeds of silkworm, *Bombyx mori* L.

Genotypes	Single cocoon weight (g)			Single shell weight (g)			Shell ratio (%)		
	Inoculated	Control	Per cent decrease over control	Inoculated	Control	Per cent decrease over control	Inoculated	Control	Per cent decrease over control
APS <sub>4</sub>	1.42	1.44	1.38	0.26	0.28	7.14	18.56 (4.42)	19.67 (4.54)	5.64
APS <sub>8</sub>	1.42	1.48	4.05	0.28	0.30	6.66	19.65 (4.54)	20.63 (4.65)	4.75
CS <sub>6</sub>	1.43	1.47	2.72	0.28	0.30	6.66	19.44 (4.52)	20.23 (4.68)	3.90
CSR <sub>2</sub>	1.45	1.49	2.68	0.28	0.29	3.44	19.24 (4.50)	19.30 (4.53)	0.31
CSR <sub>4</sub>	1.38	1.49	7.38	0.28	0.31	9.67	19.83 (4.54)	21.07 (4.69)	5.88
DUN-6	1.43	1.47	2.72	0.29	0.30	3.33	18.80 (4.44)	20.18 (4.68)	6.83
DUN-22	1.47	1.48	0.67	0.28	0.29	3.44	19.22 (4.49)	19.86 (4.56)	3.22
NB <sub>4</sub> D <sub>2</sub>	1.47	1.48	0.67	0.30	0.31	3.22	20.12 (4.66)	20.99 (4.68)	4.14
SH <sub>6</sub>	1.50	1.53	1.96	0.29	0.32	9.37	19.78 (4.55)	20.70 (4.65)	4.44
SANISH <sub>8</sub>	1.49	1.50	0.66	0.29	0.30	3.33	19.88 (4.44)	20.00 (4.58)	0.60
Mean	1.45	1.48		0.28	0.30		19.39	20.26	
SEm±	0.019	0.014		0.007	0.005		0.024	0.033	
CD at 5%	0.056	0.042		0.021	0.016		0.070	0.099	

**Cocoon yield by weight per 10,000 larvae (kg)**

The cocoon yield by weight per 10,000 larvae of different breeds under investigation ranged from 5.37 kg to 10.53 kg. Among the inoculated silkworm breeds, APS<sub>8</sub> recorded the maximum cocoon yield by weight per 10,000 larvae 10.53 kg, followed by SH<sub>6</sub> (9.70 kg), NB<sub>4</sub>D<sub>2</sub> (9.61 kg), DUN-6 (9.13 kg), CS<sub>6</sub> (8.53 kg), SANISH<sub>8</sub> (7.88 kg), DUN-22 (6.27 kg), APS<sub>4</sub> (6.14 kg) and CSR<sub>2</sub> (5.51 kg), whereas minimum

cocoon yield by weight per 10,000 larvae of 5.37 kg in CSR<sub>4</sub>. The control batches maintained for each breed showed higher cocoon yield by weight per 10,000 larvae, in comparison to the inoculated breeds. The maximum per cent decrease over control of 55.61 was observed in CSR<sub>4</sub>, whereas minimum per cent decrease over control of 23.91 was observed in DUN-6 (Table-4).

**Table 4:** Effect of BmNPV infection on the cocoon yield by number and by weight of different breeds of silkworm, *Bombyx mori* L.

Genotypes	Cocoon yield/10,000 larvae by number			Cocoon yield/10,000 larvae by weight (kg)		
	Inoculated	Control	Per cent decrease over control	Inoculated	Control	Per cent decrease over control
APS <sub>4</sub>	4500	8400	46.42	6.14	12.1	49.25
APS <sub>8</sub>	7267	9333	22.13	10.53	14.5	27.37
CS <sub>6</sub>	5867	8233	28.73	8.53	11.8	27.71
CSR <sub>2</sub>	4067	8267	50.80	5.51	11.9	53.69
CSR <sub>4</sub>	3733	8067	53.72	5.37	12.1	55.61
DUN-6	5333	8433	36.76	9.13	12.0	23.91
DUN-22	4633	8433	45.06	6.27	12.2	48.60
NB <sub>4</sub> D <sub>2</sub>	6767	9467	28.52	9.61	13.9	30.86
SH <sub>6</sub>	6167	8467	27.16	9.70	13.2	26.51
SANISH <sub>8</sub>	5433	8233	34.00	7.88	12.4	36.45
Mean	5377	8533		7.87	12.6	
SEm±	175.11	154.9		0.45	0.31	
CD at 5%	520.23	460.2		1.36	0.92	

Figures in parenthesis are arc-sine transformed values

**Average filament length (m)**

The average filament length of different breeds ranged from 709 m to 816 m. Among the inoculated silkworm breeds, DUN-6 recorded the maximum average filament length of 816 m, followed by SANISH<sub>8</sub> (783 m), DUN-22 (772 m), CSR<sub>2</sub> (768 m), SH<sub>6</sub> (759 m), NB<sub>4</sub>D<sub>2</sub> (755 m), APS<sub>8</sub> (747 m), CS<sub>6</sub> (733 m) and CSR<sub>4</sub> (724 m), while least average filament length of 709 m was observed in APS<sub>4</sub>. The control batches maintained for each breed showed higher average filament length in comparison to the inoculated breeds. The maximum per cent decrease over control of 9.79 was observed in APS<sub>4</sub>, whereas minimum per cent decrease over control of 0.48 was observed in DUN-6 (Table-5).

**Filament size (d)**

The filament size of different breeds under evaluation ranged from 2.28 to 2.61 (Table-5). Among the inoculated silkworm breeds DUN-22 recorded the maximum denier of 2.61, followed by CSR<sub>2</sub> (2.56), CS<sub>6</sub> (2.54), DUN-6 (2.52), SH<sub>6</sub> (2.48), SANISH<sub>8</sub> (2.48), NB<sub>4</sub>D<sub>2</sub> (2.34), APS<sub>8</sub> (2.33) and CSR<sub>4</sub>

(2.32), whereas minimum denier of 2.28 in APS<sub>4</sub>. The control batches maintained for each breed showed higher denier in comparison to the inoculated breeds. The maximum per cent decrease over control of 8.62 was observed in APS<sub>8</sub>, whereas minimum per cent decrease over control of 2.24 was observed in DUN-22.

**Raw silk (%)**

Raw silk percentage of different breeds under study ranged from 24.58 to 29.17 per cent. Among the inoculated silkworm breeds DUN-6 recorded the maximum raw silk of 29.17 per cent, followed by CS<sub>6</sub> (28.82%), DUN-22 (28.53%), NB<sub>4</sub>D<sub>2</sub> (28.39%), SH<sub>6</sub> (26.50%), SANISH<sub>8</sub> (26.39%), APS<sub>4</sub> (26.37%) CSR<sub>4</sub> (26.35%) and APS<sub>8</sub> (25.89%), whereas minimum raw silk percentage of 24.58 per cent in CSR<sub>2</sub>. The control batches maintained for each breed showed significant raw silk percentage in comparison to the inoculated breeds. The maximum per cent decrease over control of 17.18 was observed in CSR<sub>2</sub>, whereas minimum per cent decrease over control of 1.24 was observed in DUN-22 (Table-5).

**Table 5:** Effect of BmNPV infection on the average filament length, filament size and raw silk of different breeds of silkworm, *Bombyx mori* L.

Genotypes	Average filament length (m)			Filament size (d)			Raw silk (%)		
	Inoculated	Control	Per cent decrease over control	Inoculated	Control	Per cent decrease over control	Inoculated	Control	Per cent decrease over control
APS <sub>4</sub>	709	786	9.79	2.28	2.43	6.17	26.37 (5.23)	29.61 (5.53)	10.94
APS <sub>8</sub>	747	775	3.61	2.33	2.55	8.62	25.89 (5.18)	27.58 (5.34)	6.12
CS <sub>6</sub>	733	747	1.87	2.54	2.70	5.92	28.82 (5.46)	29.39 (5.51)	1.93
CSR <sub>2</sub>	768	773	0.64	2.56	2.62	2.29	24.58 (5.05)	29.68 (5.35)	17.18
CSR <sub>4</sub>	724	735	1.49	2.32	2.44	4.91	26.35 (5.22)	29.17 (5.49)	9.66
DUN-6	816	820	0.48	2.52	2.58	2.32	29.17 (5.49)	30.04 (5.57)	2.89
DUN-22	772	784	1.53	2.61	2.67	2.24	28.53 (5.43)	28.89 (5.46)	1.24
NB <sub>4</sub> D <sub>2</sub>	755	768	1.69	2.34	2.52	7.14	28.39 (5.42)	30.08 (5.57)	5.61
SH <sub>6</sub>	759	764	0.65	2.48	2.68	7.46	26.50 (5.24)	27.56 (5.34)	3.84
SANISH <sub>8</sub>	783	805	2.73	2.48	2.67	7.11	26.39 (5.23)	29.25 (5.49)	9.77
Mean	757	782		2.45	2.59		27.10	28.93	
SEm $\pm$	1.101	1.265		0.032	0.057		0.038	0.019	
CD at 5%	3.269	3.758		0.094	0.171		0.114	0.116	

Figures in parenthesis are square root transformed values

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