

# Character Association in Improved Mulberry Genotypes Exhibiting Delayed Leaf Senescence

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Mulberry (*Morus spp.*) is a perennial tree cultivated for its foliage to rear the domesticated silkworm, *Bombyx mori* L. Mulberry has improved through conventional breeding in general aims to improve the quantity and quality of leaf yield, which have direct bearing on silk productivity. Leaf senescence is one of the major constraints, which restricts the quantity of quality leaf availability for silkworm rearing. High yielding mulberry varieties often show leaf fall in the range of 20 – 33% in tropical sericultural belts. Hence, in order to increase the leaf availability, it is essential to delay the senescence of leaves. Keeping this in view, the present study was undertaken on 9 mulberry genotypes, which were developed systematically for delayed senescence. The interrelationship among factors that contribute to growth, yield and low foliar senescence were investigated. Correlation between agronomic traits and leaf yield revealed the existence of strong positive associations among plant height, total shoot length (TSL), nodal distance (ND), leaf fall (LF), number of leaves/ plant (NLP), fresh and dry weight of 100 leaves (FWL & DWL), leaf area (LA), leaf area index (LAI), above ground biomass (AGB) with leaf yield. However, leaf harvest index (LHI) had a strong negative correlation with leaf fall % and leaf yield. Significant improvement in the important growth and yield attributing characters viz., FWL, DWL, LA, AGB, LHI and LAI contributed to a higher yield in CT44. Path co-efficient analysis revealed the direct positive effect of the characters viz., AGB (1.233), LHI (0.449), NLP (0.217), and LA (0.181), on leaf yield. From the studies it is concluded that low leaf fall coupled with high LHI can be considered for the selection of varieties with delayed leaf senescence in mulberry.

Abstract

**Keywords:** Delayed senescent mulberry, High yielding mulberry, LAI, LHI, Leaf productivity, Leaf quality.

## INTRODUCTION

Sericulture, an agro-based industry encompassing the activities of mulberry cultivation, silkworm rearing, reeling and weaving, provides employment to more than 7,00,000 people in West Bengal, India (Dutta and Nanavaty, 2005). The Gangetic plains have a tropical humid climate with alluvial soil having high water holding capacity. As the region is warm and humid, mulberry grows luxuriantly in all the seasons except in late autumn and winter when the temperature and soil moisture go down below tolerable level (<15°C). This low growth coupled with high leaf senescence, generate a high leaf scarcity for silkworm rearing during this period. In West Bengal, the current popular mulberry variety, S1635, shows high seasonal variation in leaf production a sharp decrease in November (Autumn) and February (Winter), when the bivoltine silkworm rearing is at its peak (Moorthy and Das, 2007). This sharp decline in the leaf yield is mainly due to the early leaf maturity induced leaf fall during the colder months (Vijayan *et al.*, 1999). The average leaf fall % in S1635 under normal conditions was reported to be 31% under 60 × 60 cm spaced plantations, and it goes upto 33% under close planting systems (60 × 10 cm) of irrigated conditions in West Bengal (Rahman *et al.*, 1999). In order to develop a season insensitive variety with uniform pattern of growth and leaf yield through out the year, attempts have been made to identify mulberry accessions with less response to seasonal variations, from the germplasm and used them in breeding. As a consequence, a number of hybrids have been developed and are being evaluated for their suitability for silkworm rearing and responses to seasonal variations. In order to select hybrids indirectly without the need of being subjected to various seasonal changes, it is essential to identify reliable selection parameters. This study was taken up with such an objective of working out the interrelationship among various parameters of growth, leaf yield and leaf senescence so that some of these parameters can be used for selecting the hybrids at an early stages of its growth.

## MATERIALS AND METHODS

### 1. Plant Materials and Experimentation

The field experiments were conducted at the mulberry farm of Central Sericultural Research and Training Institute, Berhampore, West Bengal by planting 8-month-old saplings of 9 selected mulberry genotypes *viz.*, CT6, CT9, CT11, CT15, CT44, CT94, CT159, CT185, and S210 along with a check (S-1635) raised from their hard wood stem cuttings. The new mulberry genotypes were evolved from the cross involving 3 female and 5 male parents selected from germplasm (Table 1). Experimental plantation was laid out in a randomized block design with 3 replications. The size of the individual sub-plots within the blocks was 17.64 m<sup>2</sup> and the number of plants was 49 in a square plot under 60 cm x 60 cm spacing. Inter-block as well as inter-genotype distance was 150 cm. The experimental plants in the field were maintained as per recommended package of practices for irrigated plains of West Bengal (Ray *et al.*, 1973) and irrigated at an interval of 15 days during dry seasons. The crop was protected against the attack of insect pests and diseases by spraying 0.1 % Rogor (30% EC) and Bavistin (Carbendazim 50% EC), respectively, as and when required. Leaf harvests were made after one year of plantation in accordance with the 5 silkworm commercial crop schedule, which are in vogue in Murshidabad district of West Bengal (Moorthy and Das, 2007).

### 2. Growth Parameters

The data on different growth and yield attributing parameters *viz.*, plant height, number of branches per plant, total shoot length, nodal distance, leaf fall (%), number of leaves per plant, fresh and dry weight of leaves (g), single leaf area (cm<sup>2</sup>), leaf yield, above-ground biomass, leaf harvest index (%), leaf area index (LAI) and foliage yield were recorded for 3 consecutive years.

### 3. Statistical Analysis

Data were analyzed for analysis of variance (Sharma, 2000). Critical difference (CD) at 5% level of significance was estimated to compare the different genotypes with check consulting Fisher and Yate's table. Character associations among different traits and leaf yield were calculated through correlation and path analysis by following the method described by Panse and Sukhatme (1967).

## RESULTS AND DISCUSSION

Genotypes CT44 and CT11 showed superiority over other genotypes including the check S1635 in annual leaf yield. These genotypes yielded 47940 kg ha<sup>-1</sup> year<sup>-1</sup> and 43990 kg ha<sup>-1</sup> year<sup>-1</sup>, which were higher than the leaf yield of the check variety S1635 by 17.1 % and 7.5 % respectively (Table 3). This higher yield was observed consistently in all the 5 different commercial crop seasons and the 3 years (Fig. 1 & 2). The data on different growth and other leaf yield attributing characters revealed variations among them (Table 3 and Fig. 3). The leaf fall %, which indicates the rate of senescence, was found to be significantly lower in CT44 than S1635 in all the 5 crop seasons (Fig. 4), consistently, with mean values of 9.80 and 20.13% for the respective genotype (Table 3).

It is a known fact that leaf senescence is a complex and highly organized process resulting in several changes in gene expression and metabolic processes. These metabolic changes are considered to be important to maintain the continuous growth and development of the plant. During the senescence, nutrients are mobilized from the senescing leaves to younger parts of the plant to support their growth (Hörtensteiner and Feller, 2002). In fact, senescence in plants is highly regulated and modulated expression of many different genes (Buchanan-Wollaston *et al.*, 2003). A microarray analysis of *Arabidopsis* has revealed that during senescence, changes in the expression of more than 800 genes take place (Buchanan-Wollaston *et al.*, 2005). Comparison of changes in gene expression patterns further revealed involvement of salicylic acid (SA), jasmonic acid (JA) and ethylene pathways. Therefore, it is obvious that a complex polygenic trait like leaf senescence cannot be controlled or improved by manipulating one or a few genes or traits. Hence, concerted efforts are required by integrating the genetic, physiological and biochemical aspects to improve the leaf retention by delaying the process of senescence. Information on character association provides an opportunity to manipulate those traits that contribute greatly towards delayed leaf senescence or retention of leaves to increase the leaf yield of the plant.

Correlation coefficients among leaf yield and other yield attributing characters revealed that leaf yield was significantly correlated positively with plant height, total shoot length, nodal distance, leaf fall%, total number of leaves per plant and above ground biomass (Table 4). Similar results were also reported by Sarkar *et al.*, (1992), Rahman *et al.*, (1995), Vijayan *et al.*, (1997b), Tikader and Rao (2001). The character leaf fall %, which shows the leaf senescence rate, also had a significant positive correlation with other yield attributes *viz.*, plant height, total shoot length and had a significant negative correlation with number of branches/plant and LHI. This may be due to the plants having more number of branches had slow growth rate and low leaf fall %, while the plants with comparatively lesser number of branches had a high growth rate (PH) and also had a high leaf fall %. The higher LHI in delayed senescent genotypes (low leaf fall %) may also be due to more allocation of dry matter in the foliage than in the stems. In the present investigation, number of branches per plant did not show significant correlation with the leaf yield. This differs with the findings of Sahu *et al.*, (1995), Vijayan *et al.*, (1997b) and Susheelamma *et al.*, (1998) who reported the positive association of number of branches per plant with leaf yield in mulberry. However, number of branches had a significant positive correlation with number of leaves per plant (NLP) and negative correlations with plant height and leaf fall %. Nodal distance showed positive correlations with fresh (FWL) and dry (DWL) weights of leaves, LA and AGB (Table 4).

These results are in conformation with the findings of Vijayan *et al.*, (1997b), Sarkar *et al.*, (1992; 1987), Tikader and Rao (2001), Banerjee *et al.*, (2007) and Tikader and Kamble (2008; 2009). Vijayan *et al.*, (2010) also got similar results with saline stressed mulberry genotypes. The high leaf yield noticed in the variety CT-44 was due to the corresponding enhancement in leaf area, fresh and dry weights of 100 leaves, leaf harvest index and aboveground biomass (17% increase over the leaf yield of S1635) and ND was also within the optimum range (4.5–5.5 cm) for getting higher leaf yield in mulberry (Vijayan *et al.*, 1997b). Similarly, higher leaf retention due to delayed leaf senescence has also contributed to the better leaf yield potential of CT-44.

Since leaf yield is a complex character selection for it based on the one or two characters may not be sufficient to provide reliability in the process. Hence, in order to identify those characters that have major contributions to the leaf yield, the path coefficient analysis is used to elucidate the direct and indirect relationships. The results of the present study (Table 5) revealed that direct contributions of agronomic traits on leaf yield ranged from 1.233 in AGB to -0.292 in LAI. The second highest direct effect on leaf yield was shown by LHI (0.449) followed by NLP (0.217), LA (0.181), number of branches per plant (NB) (0.120) and plant height (PH) (0.107). The direct effect of LA, NB, PH was very low. However, LA and PH are likely to be contributing towards leaf yield indirectly through AGB. Besides, positive correlations and direct negative effects on leaf yield were also found in the characters *viz.*, LAI (-0.292), TSL (-0.146), ND (-0.010) and DWL (-0.021) but their indirect effect was via AGB. The delayed senescence had moderate to low TSL, which in turn had direct negative effect (-0.146) on leaf yield. The only character that had a negative correlation with leaf yield was LHI (-0.343) but it showed a direct positive effect on leaf yield and an indirect negative effect through AGB. Leaf fall had low positive direct effect (0.081) on leaf yield and showed indirect negative effect through LHI (-0.175) and TSL (-0.078). This supports the hypothesis that the plants having low or delayed leaf senescence (leaf fall) had a high LHI, which in turn contribute to the leaf yielding capacity in mulberry.

Since in mulberry leaf yield is the primary product, its improvement entails great significance. Leaf yield is a polygenic trait contributed by a number of important associated traits. Although, much improvement was made in leaf yield using conventional breeding methods (Vijayan *et al.*, 2011), developing a variety having stable leaf yield potential still eludes the scientists. Most of the high yielding varieties are prone to seasonal variations as during low temperature they yield very less as compared to their yield during summer and rainy seasons. Since the main purpose of mulberry cultivation is to feed the silkworm, availability of sufficient quantity of leaf during the season when the silkworms can be reared is important. The best seasons for silkworm rearing are winter and spring. However, during these seasons the mulberry leaf yield is the minimum. Hence, it is essential to develop a variety, which has the capability to sustain good growth during these seasons. The delayed senescence observed in CT-44 along with higher leaf yield contributed by plant height, number of branches and leaf weights make CT-44 the best mulberry variety available for bivoltine sericulture areas of West Bengal.

Thus, from the study, it can be concluded that using a systematic and integrated approach and integrated approach using knowledge of genetic, physiological and biochemical aspects, it is possible to develop mulberry varieties with stable leaf yield irrespective of the seasonal or climatic changes.

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## Tables

Table 1. Pedigree details of newly evolved delayed senescent mulberry genotypes.

Sl. No.	Genotype	Pedigree	Parent character	
			Female	Male
1	CT6 (C2043)	<i>M indica</i> HP x CHF-13	Large leaf area	Low senescence
2	CT9 (C2044)	-do-	-do-	-do-
3	CT11 (C2045)	-do-	-do-	-do-
4	CT15 (C2046)	-do-	-do-	-do-
5	CT44 (C2047)	<i>M indica</i> HP x CHF-12	-do-	-do-
6	CT94 (C2048)	Berhampore local x Zing	-do-	Cold tolerance
7	CT159 (C2049)	<i>M indica</i> HP x CHF-13	-do-	Low senescence
8	CT185 (C2050)	<i>M indica</i> HP x CHF-23	-do-	-do-
9	CT210 (C2051)	KPG-II x Maliha	Cold tolerance	Thick leaves

Genotype's name in parenthesis is as per the Serial of Elite germplasm register maintenance by our Institute.

Table 2. Leaf yield performance of 9 selected mulberry genotypes in 5 different crop seasons (Figures average of 3 years each year comprising 5 commercial crop seasons).

Genotype	Leaf yield (t ha <sup>-1</sup> season <sup>-1</sup> )					Total (t ha <sup>-1</sup> year <sup>-1</sup> )	Yield gain over check (%)
	Sept.	Nov.	Feb.	Apr.	July		
CT6 (C2043)	8.8	7.1	6.3	8.0	9.4	39.8	--
CT9 (C2044)	7.0	7.4	7.0	8.0	8.4	38.0	--
CT11 (C2045)	8.6	7.7	8.1	8.6	10.7	43.9	7.5
CT15 (C2046)	7.8	7.0	7.6	8.5	9.3	40.4	--
CT44 (C2047)	9.6	9.1	7.9	9.5	11.5	47.9	17.1
CT94 (C2048)	6.2	5.5	4.7	7.1	8.4	32.0	--
CT159 (C2049)	7.1	6.2	6.3	7.4	8.9	36.1	--
CT185 (C2050)	8.2	7.1	5.0	8.6	9.4	38.5	--
CT210 (C2051)	7.4	8.0	6.2	8.2	8.8	38.8	--
S1635 (Check)	7.7	7.7	7.0	8.2	10.1	40.9	--
Mean	7.8	7.3	6.6	8.2	9.5		
CD at 5%					Genotype	1.8**	
					Season	0.2**	
					Season x Genotype	0.8**	
CV%						11.0	

\*\* Significant at 1 % level (p<0.01) Genotype's name in parenthesis is as per the Serial of Elite germplasm register maintenance by our Institute.

Table 3. Performance of 9 selected delayed senescent mulberry genotypes on different growth and yield attributing characters.

Genotype	NB (plant-1)	PH (cm)	TSL (cm)	ND (cm)	Leaf fall (%)	NLP	FWL (g)	DWL (g)	LA (cm <sup>2</sup> )	LAI	AGB (t ha <sup>-1</sup> Year <sup>-1</sup> )	LHI (%)
CT6 (C2043)	7.3	96.5	565.9	4.6	15.7	100.0	369.0	79.1	207.6	5.7	65.4	61.6
CT9 (C2044)	8.3	88.8	609.2	4.5	14.1	111.0	350.6	75.2	202.6	6.1	63.0	61.1
CT11 (C2045)	7.7	98.4	611.4	4.7	16.7	101.7	431.9	86.3	235.6	6.6	70.9	61.8
CT15 (C2046)	7.6	100.8	598.5	4.5	14.3	109.8	367.0	77.0	204.4	6.2	67.9	59.8
CT44 (C2047)	8.2	92.5	593.9	5.1	9.8	97.3	509.2	103.7	289.6	7.7	75.6	64.3
CT94 (C2048)	7.1	97.0	557.5	4.3	12.9	107.3	293.2	63.5	178.0	5.2	57.4	57.2
CT159(C2049)	8.1	100.2	636.7	4.4	14.5	118.5	304.5	62.8	180.4	5.8	63.7	57.8
CT185(C2050)	8.2	94.1	607.9	4.5	17.8	105.0	367.1	76.3	214.3	6.2	64.3	61.2
CT210(C2051)	7.4	102.9	653.0	4.4	16.5	118.2	374.7	75.4	213.8	6.9	65.5	59.9
S1635(Check)	8.2	95.4	651.6	4.6	20.1	109.0	410.3	82.4	210.3	6.3	68.1	60.3
CD at 5%	0.3**	4.1**	31.9**	0.1**	1.1**	5.7**	19.2**	4.8**	12.1**	0.4**	3.2**	1.2**
CV%	10.4	10.3	12.6	7.2	17.1	12.9	12.2	15.0	13.7	17.9	11.7	5.0

PH- Plant height; NB- No. of branches; ND- Nodal distance; SWL- Fresh weight of 100 leaves; DWL - Dry weight of 100 leaves; SLA- Single leaf area; AGB- Aboveground biomass; LHI – Leaf harvest index; TLA- Total leaf area; LAI – Leaf area index; LF-Leaf fall %; \* and \*\* - significant at 5 and 1% level, respectively.

Table 4. Correlation among various growth and yield attributing characters that influence leaf yield in selected delayed senescent mulberry genotypes

Characters	PH	TSL	ND	LF	NLP	FLW	DLW	LA	LAI	AGB	LHI	LY
NB	-0.464**	0.251**	-0.043	-0.336**	-0.336**	0.415**	0.031	-0.111	0.305**	-0.020	0.123	0.018
PH		0.689**	0.072	0.695**	0.695**	0.371**	-0.013	0.136	0.397**	0.736**	-0.658**	0.610**
TSL			0.050	0.532**	0.532**	0.779**	-0.039	-0.003	0.677**	0.806**	-0.598**	0.699**
ND				0.049	0.049	-0.326**	0.287**	0.513**	0.066	0.262**	0.026	0.333**
LF						0.085	-0.038	-0.012	0.055	0.553**	-0.389**	0.544**
NLP							-0.137	-0.234**	0.705**	0.485**	-0.439**	0.354**
FLW							0.762**	0.801**	0.444**	0.364**	0.028	0.455**
DLW								0.681**	0.368**	0.194*	0.122	0.283**
LA									0.503**	0.344**	0.057	0.438**
LAI										0.662**	-0.341**	0.604**
AGB											-0.652**	0.922**
LHI												-0.343**

NB - No. of branches plant-1; PH - Plant height; TSL - Total shoot length; ND - Nodal distance; LF - Leaf fall (%); NLP - Total no. of leaves plant-1; FLW - Fresh weight of 100 leaves; DLW - Dry weight of 100 leaves; LA - Leaf area; LAI - Leaf Area Index; AGB - Aboveground biomass; LHI – Leaf Harvest Index; LY – Leaf Yield; \* and \*\* - significant at 5 and 1% level, respectively.

Table 5. Direct and indirect path co-efficient of different agronomic traits that influence leaf yield in the selected delayed senescent mulberry genotypes.

Characters	NB	PH	TSL	ND	LF	NLP	FLW	DLW	LA	LAI	AGB	LHI	r with LY
NB	<u>0.120</u>	-0.050	-0.037	0.000	-0.027	0.090	0.001	-0.001	-0.020	-0.089	-0.025	0.055	0.018
PH	-0.056	<u>0.107</u>	-0.101	-0.001	0.056	0.080	0.003	0.000	0.025	-0.116	0.907	-0.296	0.610**
TSL	0.030	0.074	<u>-0.146</u>	0.000	0.043	0.169	0.002	0.001	-0.001	-0.198	0.994	-0.269	0.699**
ND	-0.005	0.008	-0.007	<u>-0.010</u>	0.004	-0.071	0.012	-0.006	0.093	-0.019	0.323	0.012	0.333**
LF	-0.040	0.075	-0.078	0.000	<u>0.081</u>	0.018	-0.001	0.001	-0.002	-0.016	0.682	-0.175	0.544**
NLP	0.050	0.040	-0.114	0.003	0.007	<u>0.217</u>	-0.004	0.003	-0.042	-0.206	0.598	-0.197	0.354**
FLW	0.004	0.009	-0.010	-0.004	-0.003	-0.031	<u>0.030</u>	-0.016	0.145	-0.130	0.449	0.013	0.455**
DLW	0.004	-0.001	0.006	-0.003	-0.003	-0.030	0.023	<u>-0.021</u>	0.123	-0.108	0.239	0.055	0.283**
LA	-0.013	0.015	0.000	-0.005	-0.001	-0.051	0.024	-0.015	<u>0.181</u>	-0.147	0.424	0.026	0.438**
LAI	0.037	0.043	-0.099	-0.001	0.004	0.153	0.013	-0.008	0.091	<u>-0.292</u>	0.816	-0.153	0.604**
AGB	-0.002	0.079	-0.118	-0.003	0.045	0.105	0.011	-0.004	0.062	-0.193	<u>1.233</u>	-0.293	0.922**
LHI	0.015	-0.071	0.087	0.000	-0.032	-0.095	0.001	-0.003	0.010	0.100	-0.804	<u>0.449</u>	-0.343**

NB - No. of branches plant-1; PH - Plant height; TSL - Total shoot length; ND - Nodal distance; LF - Leaf fall (%); NLP - Total no. of leaves plant-1; FLW - Fresh weight of 100 leaves; DLW - Dry weight of 100 leaves; LA - Leaf area; LAI - Leaf Area Index; AGB - Aboveground biomass; LHI - Leaf Harvest Index; LY - Leaf Yield; \* and \*\* - significant at 5 and 1% level, respectively; Residual effect = 0.024; r = correlation value.



## Figures

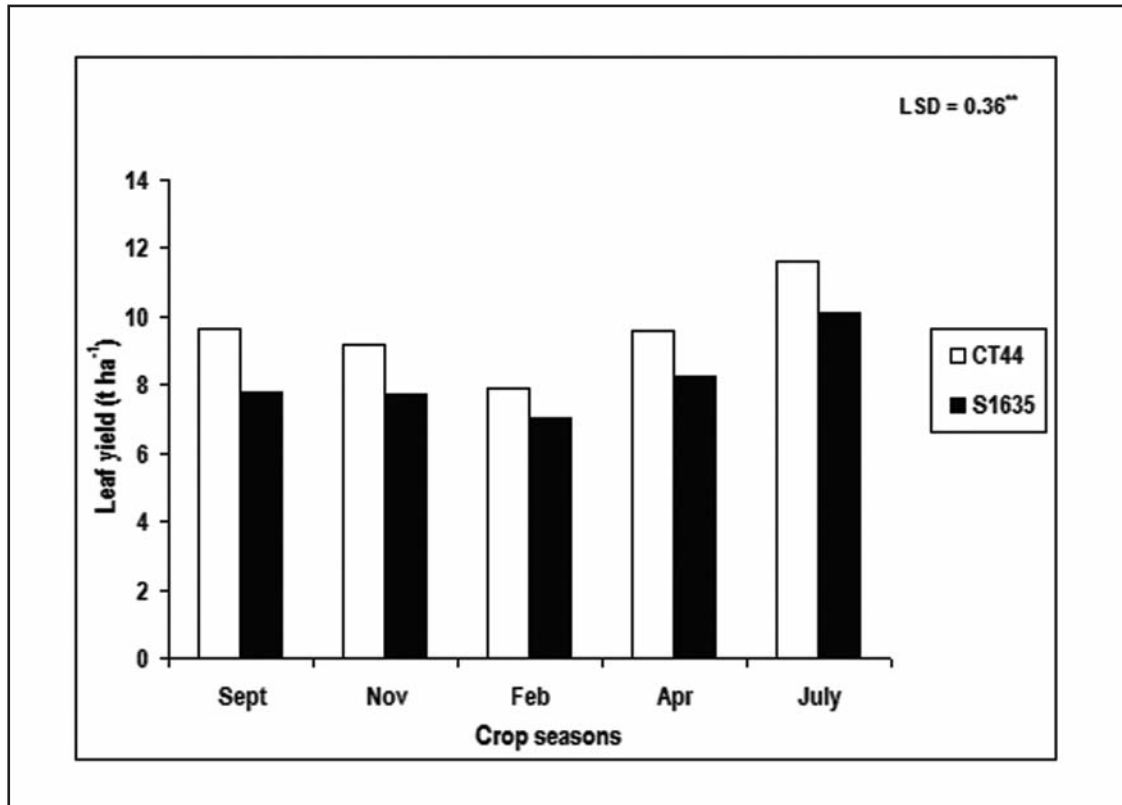


Fig. 1. Leaf yield performance of CT44 in comparison with the Check S-1635 in 5 different commercial silkworm crop seasons.

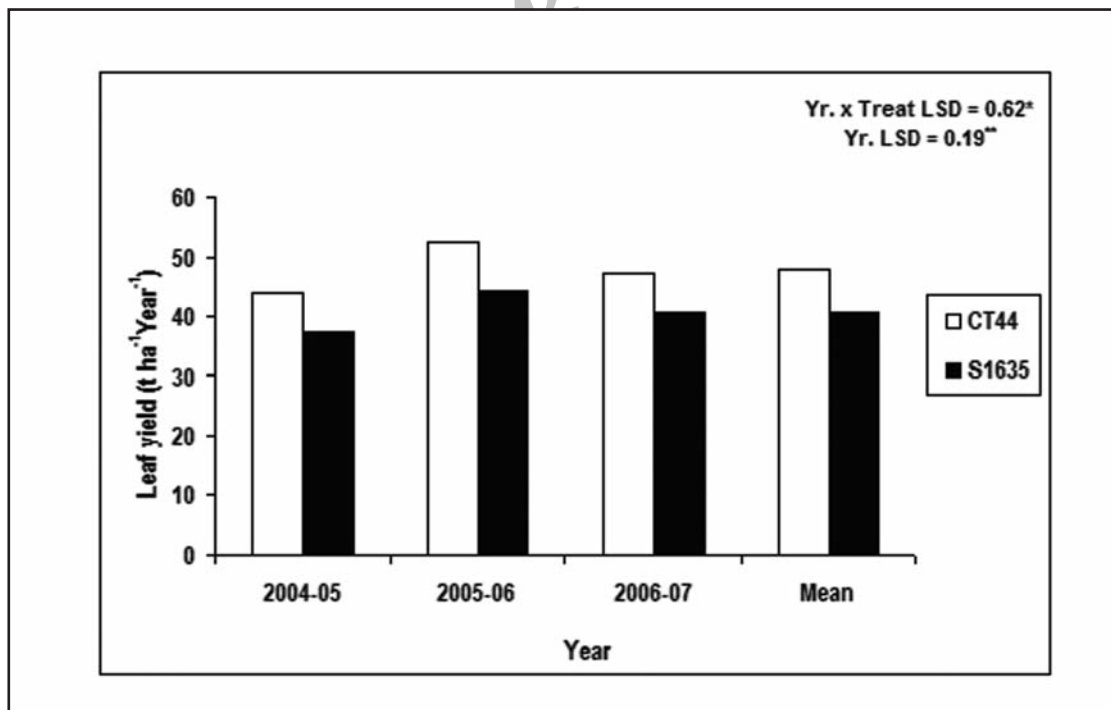


Fig. 2. Leaf yield performance of CT44 in comparison with the Check S-1635 in 3 different years of study.

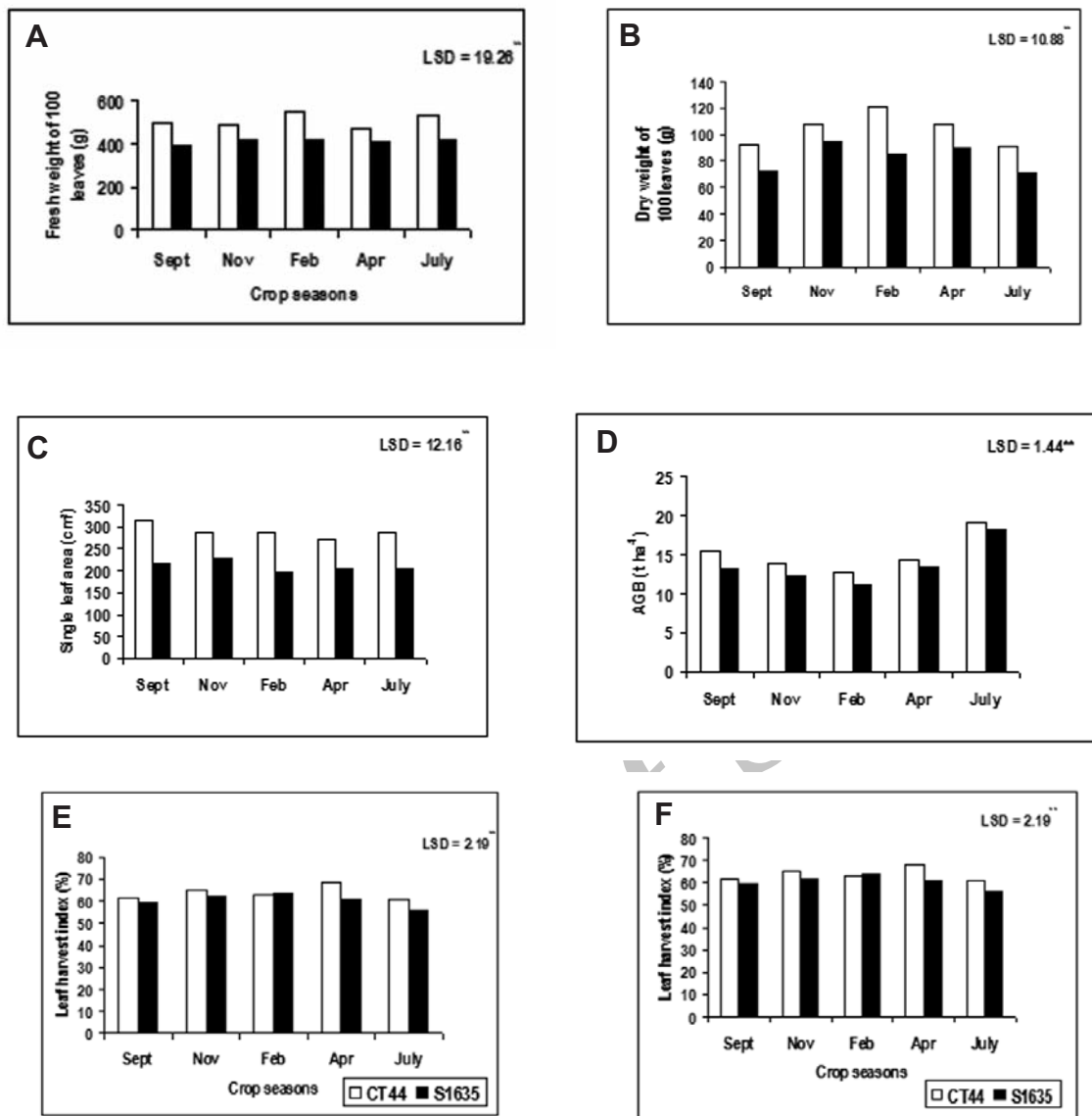


Fig. 3. Comparative performance of CT44 with the Check S-1635 for Fresh weight of 100 leaves (A), Dry weight of 100 leaves (B), Single leaf area (C), Aboveground biomass (D), Leaf harvest index (E) and Leaf area index (F) in 5 different crop seasons.

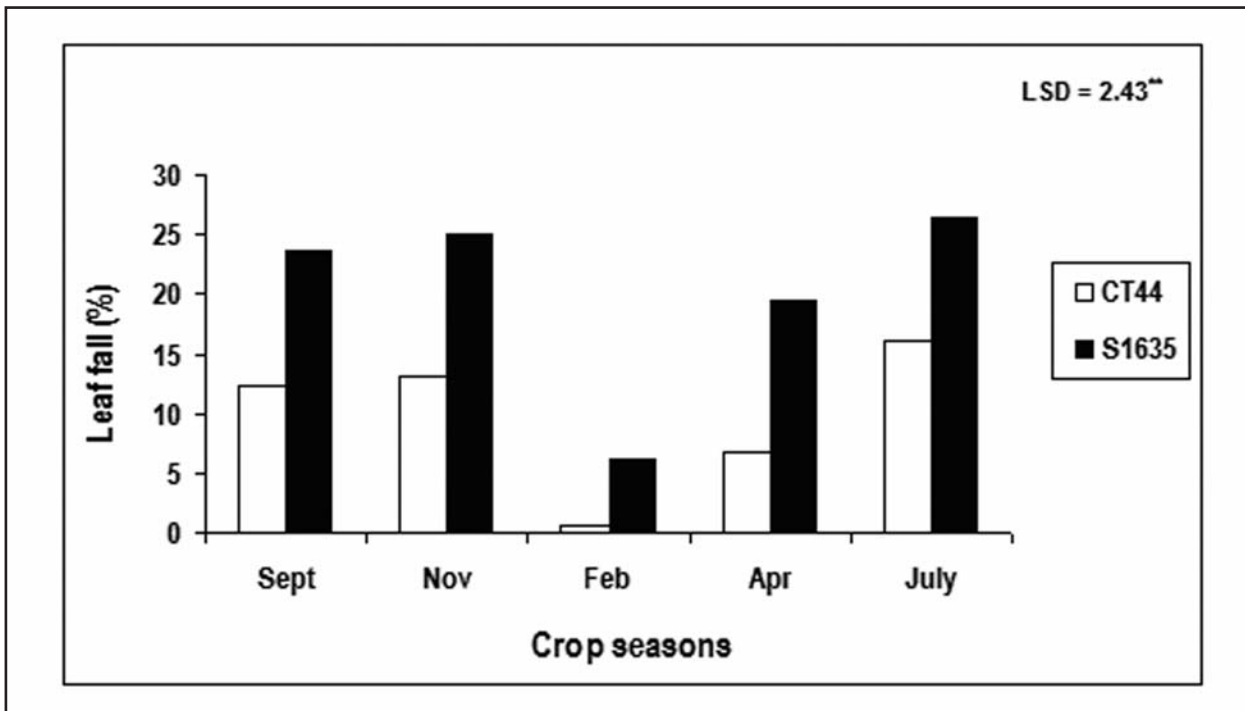


Fig. 4. Performance of CT44 for leaf fall (senescence rate) (%) in comparison to check S-1635 in 5 different crop seasons.

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