

Electronic Journal of Plant Breeding



Research Article

Genotype x Environment interaction and stability analysis for leaf yield of mulberry (*Morus alba* L.) under alkali affected soils

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Abstract

The objective of this study was to determine the stability analysis and genotype x environment interaction of alkali tolerant mulberry genotypes at different alkali soils (untreated/unreclaimed, reclaimed with inorganic/organic amendment) on leaf yield to understand their adaptation. Five alkali tolerant mulberry genotypes along with two improved genotypes and one ruling local check were evaluated in an alkali hotspot. The large variation in mean leaf yield/microplot, regression coefficient (b_i) and deviation from regression (S_{di}^2) indicates the different responses of genotypes to soil reclaimed with amendments. Average two year leaf yield/microplot, maximum of 18.240 kg was produced by the AR-12 followed by the AR-14, AR-29, AR-08, V1, AR-10 and S-34. Genotypes AR-12 and AR-14 showed high leaf yield (AR-12: 18.240 kg, AR-14: 16.15 kg), the low deviation from regression (S_{di}^2) (AR-12: -0.04, AR-14: 0.03) and their regression coefficient values (b_i) were close to unity (AR-12: 1.41, AR-14: 1.34) and could be classified as stable genotypes.

Key words

Genotype x Environment Interaction, Stability analysis, Leaf Yield, *Morus alba*.

INTRODUCTION

Sericulture is the combination of four major components i.e. cultivation of host plants for quality leaf production, rearing silkworm for cocoon production, reeling of cocoon to take out silk yarn, and fabric production. India produces, all the four major commercial silk fibers (35468 MT), in which more than 70 per cent (25344 MT) of the silk comes from mulberry silkworm (Anonymous 2019). Mulberry silkworm is monophagous exclusively feed on *Morus* spp, and it accounts for more than 60 per cent of the cocoon production cost (Rangaswami *et al.*, 1976; Venkatanarasaiah, 1992), therefore, mulberry cultivation has a significant role to play for the sustainability of sericulture in any country. In India, mulberry grown in a total area of 2,35,001 ha (Anonymous 2019), for expansion of area under mulberry by horizontally i.e., exploring and expanding more and more new areas such as soils affected with alkalinity, salinity and acidity,

which are apparently not suited for growing agricultural crops is the quicker and easier option for the growth of sericulture.

Alkali soils can be defined as salt affected soils with exchangeable sodium percentage (ESP) of more than 15, pH more than 8.5 and electrical conductivity (EC) less than 4 mmhos/cm² (Richards, 1954). These alkali soils could be effectively utilized either by reclamation or by growing alkali tolerant genotypes. Reclamation of alkali soils involves reversing of the process, which caused deterioration of these soils i.e., replacing excess exchangeable sodium with calcium supplied either through outside source or mobilising precipitated calcium carbonate present in the soil. For this purpose, gypsum (direct sources), pyrites, sulphur, acid (indirect sources) and pressmud, green manure and farmyard manure

(organic matters) are used (Somani and Totawat, 1993; Haq *et al.*, 2001), which brings in desirable chemical and physical properties of soils for high productivity.

Varietal adaptability to environmental variations is essential for the stabilization of crop production both over area and years (Singh and Narayan, 1993). Hence, several potent genotypes have to evaluate at different environments and years before picking certain desirable genotypes. The comparative performance of different genotypes often varies from one environment to another i.e. genotype environment interaction exist (Rashid *et al.*, 2002), and the presence of G × E Interaction (GEI) in any genetical study leads to overestimation of genetical and statistical parameters (Sharma, 1998) which makes the breeder hard to decide which genotypes should be selected. GEI results from changes in the magnitude of the differences between genotypes in different environments, there are two types of GEI present, i) non cross-over GEI, in which the ranking of genotypes remains constant across environments and the interaction is significant because of changes in the magnitude of the response, ii) crossover GEI, in which significant changes in rank occurs from one environment to another where one genotype may be chosen for one environment and other genotype for other. Thus, breeders look for non-crossover type of interaction when picking genotype for wider adaptation (Won *et al.*, 1998). The study of GEI offers suitable information to identify stable genotypes over a range of environments (Reddy *et al.*, 1998). Testing genotypes at more location is considered important than testing for many years for stability studies (Joshi *et al.*, 2003).

The objective of this study was to evaluate leaf yield magnitude and stability, find quality differences between the genotypes and years, find influence of soil alkali condition and genotype and identify most stable genotypes. This has been the first and only attempt of screening mulberry genotypes in natural alkali and after reclamation with inorganic and organic amendments. Selections based on the understanding of the genotypes with higher leaf yield under alkali soils, can be exploited by breeders to achieve maximum improvement while breeding for alkali tolerance.

MATERIALS AND METHODS

The study conducted at a field unit of Central Sericulture Research and Training Institute, Central Silk Board, Mysuru. Experimental field with black cotton soil, having properties like pH - 9.3 to 9.5, EC - 0.32 to 0.84 mmhos/cm, Exchangeable Sodium Percentage (ESP) - 42, Sodium Adsorption Ratio (SAR) - 30. For experimental purpose, natural alkali soil area used as unreclaimed alkali soil (condition-1), alkali soil reclaimed with inorganic amendment (condition-2) i.e. gypsum (70-80 % purity, > 2mm particle size) at 8 MT/ha and sulphur at 1 MT/ha and organic amendment (condition-3) i.e. pressmud contains relatively high soluble calcium (from sugar factory

employing sulphitation process), at 50 MT/ha. For mixing of inorganic/organic amendments, shallow ploughing with country plough was carried out followed by planking before the onset of monsoon. This was followed by an ample irrigation to achieve a stand of 5-7 cm water on the soil surface for at least 15 days. In between, puddling was practiced to mix the amendments thoroughly in the soil for effective reclamation. After 15 days, excess water was drained out of the experimental plot through separate channels, plots were irrigated and the water drained out so as to remove the excess salts and this process was repeated for an effective reclamation. After reclamation, the surface of the soil was allowed to dry completely. Then the land was prepared with proper leveling with little or no slope along the width to facilitate movement of water along the length in a uniform sheet with desired depth of application. Soil analysis of all the treatments for its chemical properties like Soil pH, Electrical Conductivity (EC), Exchangeable Sodium Percentage (ESP), Sodium Adsorption Ratio (SAR), were analyzed following the method suggested by Jackson (1973).

Parameters	Un-reclaimed alkali soil	After reclamation with	
		inorganic amendments*	organic amendments#
pH	9.50	7.90	8.30
Electrical Conductivity (mmhos/cm)	0.58	0.63	0.40
Exchangeable Sodium Percentage (%)	42.00	12.00	18.60
Sodium Adsorption Ratio (%)	30.00	8.00	14.00

* Gypsum @ 8 MT/ha. + Sulphur @ 1MT/ha.

Pressmud @ 50 MT/ha.

Five mulberry genotypes relatively tolerant under alkali soil i.e. AR-12, AR-14, AR-10, AR-08 and AR-29 and two improved checks i.e. V1, S34 and one ruling Local check were used in the experiment (**Fig. 1**). Sixty four plants were maintained per genotype and replication in the net plot. Each net plot/replication was surrounded by a row of border plants. Three experiments were maintained separately and each of the experiment was conducted following Randomized Block Design with three replications. The plantation was established in the field during the monsoon season by planting six month old saplings with 90 cm x 90 cm spacing. All regular intercultural operations were attended as per the recommended package of practices.

After an initial period of establishment of one year, the plants were pruned at a stump height of 30 cm from the ground level. After pruning and digging, farmyard manure was applied at 20 MT/ha/year in two split doses and thoroughly mixed with the soil by ploughing. The fertilizer



Fig. 1. Mulberry genotypes tolerant to alkali soils (AR-12, AR-14, AR-10, AR-08, AR-29), improved genotypes (V1 and S34) and check (Local)

schedule followed was 300:120:120 kg of NPK/ha/year in five split doses of 60:60:60 kg NPK/ha after I and III crop and 60 kg nitrogen/ha after II, IV and V crops. Leaves from all the plants from the net plot were harvested by leaf picking method and weight was recorded using a digital weighing balance. The genotypes were evaluated for leaf yield in three different soil reclamation conditions (unreclaimed, reclaimed with inorganic and organic amendments), which were considered as different environments, leaf yield/ha/year was computed pooling all the five harvests during each year.

Analysis of variance (ANOVA) for genotype and leaf yield was performed by the method used for two-way analysis. Pooled analysis of data was performed in such a way that the total sum of squares is partitioned into various parts, which depict the interaction effects of genotypes with the environments. After testing the significance of the interaction the stability parameters were performed through the regression coefficient and deviation from regression as per the methods used by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). The regression coefficient as calculated in this case is considered to be the stability parameter, which is the regression of the performance of each genotype under different reclamation treatment on the environmental means overall the genotypes and calculated as

$$\text{Regression Co-efficient (b}_j\text{)} = \frac{\sum_j Y_{ij} l_j}{\sum_j l_j^2}$$

Where, Y_{ij} = mean of i^{th} genotypes on j^{th} reclamation environment

l_j = the environmental index which can be calculated as:

$$l_j = \frac{\sum_i Y_{ij}}{t} - \frac{\sum_i \sum_j Y_{ij}}{ts}$$

Where, t = number of mulberry genotypes to be tested and s = number of reclamation treatment (environment)

RESULTS AND DISCUSSION

Analysis of variance for genotype, year and their interactions under alkali soil, soil reclaimed with inorganic and organic amendment conditions (different environments) was performed and are presented.

Means of leaf yield of mulberry genotypes averaged across two years and three different soils environments (unreclaimed, reclaimed with inorganic, and reclaimed with organic) are given in **Table 1**. Mean leaf yield for the three soil environments ranged from 8.41 to 25.32 (MT). During the first year of the experiment, the average leaf yield on soils reclaimed with organic amendments (15.88

MT/ha./yr.) was significantly higher than that on soils reclaimed with inorganic amendments (13.78 MT) and unreclaimed alkali soil (12.06 MT). Interaction among different mulberry genotypes indicated that AR-12 (18.35 MT) is significantly superior over all other test genotypes, AR-10 recorded the lowest leaf yield (12.69 MT). All the test genotypes showed the significantly higher leaf yield over improved check, S34 and Local check. While studying the interaction between different treatments and genotypes, AR-12 (20.70 MT/ha./yr.) and AR-14 (19.75 MT) on soil reclaimed with organic amendments showed the significantly higher leaf yield than other genotypes under all the three treatments, whereas Local (8.41 MT) recorded the minimum leaf yield/ha./yr.

During the second year of the experiment, superiority of the treatments were found in soil reclaimed with organic amendments (18.51 MT/ha./yr.) followed by soil reclaimed with inorganic amendments (16.65 MT) and unreclaimed alkali soil (13.49 MT). Among the test genotypes, AR-12 (21.85 MT) followed by AR-14 (18.92 MT) and AR-29 (17.68 MT) exhibited significantly superior performance over improved check, S34 (14.68 MT) and Local check, (11.65 MT). However, leaf yield has increased in all the test genotypes when the soil was reclaimed with organic or inorganic amendments. Genotype \times reclamation interactions confirmed the superiority of AR-12 under soil reclaimed with organic amendments (25.32 MT), which exhibited a significantly higher leaf yield over other test genotypes under different treatments followed by AR-14 (21.89 MT) and AR-29 (19.86 MT) on soil reclaimed with organic amendments. Leaf yield was minimum in Local (9.83 MT) in unreclaimed alkali soil.

Average two years leaf yield of the experiment, soil reclaimed with organic amendments (17.19 MT/ha./yr.) was superior over soil reclaimed with inorganic amendments (15.22 MT) and unreclaimed alkali soil (12.78 MT) among the treatments. Interaction among different mulberry genotypes for leaf yield, it is indicated that AR-12 (20.10 MT) is significantly superior over all other test genotypes and showed statistically significant compared to improved check (S34) and normal check (Local). AR-14 (17.76 MT) and AR-29 (16.69 MT) have also significantly higher leaf yield while the performance of all genotypes were not so satisfactory and found minimum in Local (10.52 MT). Further, it was found that AR-12 (23.01 MT) followed by AR-14 (20.82 MT) under soil reclaimed with organic amendments and AR-12 (20.43 MT) followed by AR-14 (17.73 MT) under soil reclaimed with inorganic amendments were significantly superior over all other genotypes, whereas Local (9.12 MT) without reclamation recorded the minimum leaf yield/ha./yr., during the period of experiment.

The environmental effect along with genotype \times environment interaction (GEI) makes it difficult to verify and give general recommendations for a particular variety. However, several attempts have been made

Table 1. Leaf yield (MT/ha.) of different mulberry genotypes under different treatments

Genotype	First year				Second year				Average of two years			
	Unreclaimed alkali soil	Soil reclaimed with inorganic amendments*	Soil reclaimed with organic amendments#	Average	Unreclaimed alkali soil	Soil reclaimed with inorganic amendments*	Soil reclaimed with organic amendments#	Average	Unreclaimed alkali soil	Soil reclaimed with inorganic amendments*	Soil reclaimed with organic amendments#	Average
AR-12	15.96	18.40	20.70	18.35	17.77	22.47	25.32	21.85	16.87	20.43	23.01	20.10
AR-14	13.66	16.35	19.75	16.59	15.79	19.10	21.89	18.92	14.72	17.73	20.82	17.76
AR-10	11.15	12.06	14.85	12.69	11.54	14.20	15.37	13.71	11.35	13.13	15.11	13.20
AR-08	12.59	14.21	15.88	14.23	12.71	15.27	16.80	14.93	12.65	14.74	16.34	14.58
AR-29	13.71	15.46	17.88	15.69	14.76	18.44	19.86	17.68	14.24	16.95	18.87	16.69
V1	11.04	12.82	14.58	12.81	13.13	16.65	19.14	16.31	12.09	14.73	16.86	14.56
S34	9.97	11.46	13.11	11.51	12.38	15.15	16.51	14.68	11.18	13.30	14.81	13.10
Local	8.41	9.49	10.25	9.38	9.83	11.94	13.18	11.65	9.12	10.71	11.72	10.52
Average	12.06	13.78	15.88	13.91	13.49	16.65	18.51	16.22	12.78	15.22	17.19	15.06
C.D. at 5% for:												
Treatment (Reclamation)			0.34				0.32				0.28	
Genotype			0.55				0.53				0.45	
Treatment x Genotype			0.95				0.91				0.78	

* Gypsum @ 8 MT/ha. + Sulphur @ 1MT/ha.

Pressmud @ 50 MT/ha.

to specify, estimate and correct GEI (Koumber *et al.*, 2011). The ideal genotype should be high yielding under different environmental conditions, but as genetic effects are not independent of environmental effects, most genotypes do not perform satisfactorily in all environments (Carvalho *et al.*, 1983). When interaction between genotype and environment occurs, the relative ranking of genotypes for yield often differs when genotypes are compared across a series of environments and/or years. This poses a serious problem for selecting genotypes significantly superior in grain yield (Stafford, 1982). GEI are of major importance, because they provide information about the effect of different environments on cultivar performance and have a key role for assessment of performance stability of the breeding materials (Moldovan *et al.*, 2000). Stable genotypes have the same reactions across the environments. The GEI are major components of variation, i.e., the relative performances of the genotypes vary from one environment to another (Sarkar *et al.*, 1986). Differences in yield of different variety/ genotypes in response to different environmental conditions were observed in wheat, pigeon pea and many crops (Ulker *et al.*, 2006; Ramesh *et al.*, 2017). Improvement in cocoon yield and economic characters of cocoons confirms the better quality of mulberry genotypes under reclaimed conditions (Li and Sano, 1984; Tayade and Jawale, 1984; Chaluvachari and Bongale, 1995).

An analysis of variance for stability revealed highly significant differences ($P < 0.01$) for leaf yield among genotypes and environment + (G x E). This also revealed, not only the amount of variability existed among environments but also the presence of genetic variability among the genotypes. The sum of squares due to treatment and genotype x treatment are partitioned into treatment (linear), genotype x treatment (linear) and pooled deviation (non-linear) from the regression model. The highly significance ($P < 0.01$) of these components showed that both predictable and unpredictable components shared GEI.

The result of the combined analysis of stability for leaf

yield is given in **Table 2**. The Genotype x treatment (linear) interaction was highly significant (tested against pooled deviation) which demonstrated that genotypes respond differently to variation in environmental conditions and indicating existence of differences among the regression coefficients. The pooled deviations equal to pooled error, showing that the differences in stability were due to deviation from linear regression only. Further, the variation in stability of different genotypes performances was mainly due to genotypes by environment interaction. The stability was defined as adaptation of varieties to unpredictable and transient environmental conditions and the technique has been used to select stable genotypes unaffected by environmental changes (Allard and Bradshaw, 1964). Afzal *et al.* (2001) detected pooled analysis of variance overall environments, indicating that the genotype, environment and GEI mean squares were highly significant for yield. Therefore, an understanding of GEI provides valid insights into the selection of new stable genotypes in the diversified environmental conditions prevailing in a region. The mean squares due to G x E (linear) were non-significant, depicting lack of genetic differences among genotypes for linear response to varying environments, while the mean squares due to pooled deviations were significant, reflecting considerable differences among genotypes for non - linear response (Rasul *et al.*, 2006). Genotypes, environments and GEI variances were significant at $P < 0.01$ (Akcura *et al.*, 2009). Sood *et al.*, 2016 ; Anwar *et al.* (2007) analyzed stability of variance for grain yield and reported highly significant variances due to environments and environment (linear), while non-significant variance was obtained for genotype.

Calculated stability parameters for leaf yield are presented in **Table 3**. During the first year of the experiment, AR-12 and AR-14 had 'b_i' values much higher than one (1.25 and 1.54, respectively), indicating that these genotypes respond best under the reclamation treatments. Genotype, AR-10 with 'b_i' value around 1.0 (0.99) could be considered as a stable genotype and it did not respond significantly even when the soil was reclaimed. The high 'b_i' values for AR-

Table 2. ANOVA for stability of leaf yield of mulberry genotypes

Source of variation	df	Mean Sum of Squares		
		First year	Second year	Average of two years
Genotypes	7	21.022**	25.628**	22.621**
Treatment+(Genotype x Treatment*)	16	3.227	5.704	4.280
Treatment (Linear)	1	47.168**	84.915**	63.975**
Genotype x Treatment * (Linear)	7	0.573**	0.857**	0.615**
Pooled Deviation	8	0.057	0.044	0.024
Pooled Error	42	0.237	0.267	0.179

* Treatments are considered as environments

** Significant at 1%

Table 3. Estimates of stability and adaptability parameters of leaf yield (kg) for mulberry genotypes across different soil environments (unreclaimed, reclaimed with inorganic/ organic amendments)

Genotype	First year			Second year			Average of two years		
	Mean yield/ microplot (kg)	Regression Co-efficient (b_i)	Deviation from Regression (S_{di}^2)	Mean yield/ microplot (kg)	Regression Co-efficient (b_i)	Deviation from Regression (S_{di}^2)	Mean yield/ microplot (kg)	Regression Co-efficient (b_i)	Deviation from Regression (S_{di}^2)
AR-12	16.650	1.25	-0.02	19.84	1.51	-0.08	18.240	1.41	-0.04
AR-14	15.130	1.54	-0.06	17.16	1.19	0.05	16.150	1.34	0.03
AR-10	11.510	0.99	-0.22	12.43	0.77	-0.06	11.970	0.85	-0.01
AR-08	12.900	0.87	-0.07	13.54	0.81	-0.09	13.220	0.84	-0.06
AR-29	14.230	1.11	-0.07	16.04	1.03	0.03	15.140	1.06	-0.05
V1	11.620	0.93	-0.06	14.79	1.19	-0.05	13.210	1.09	-0.06
S-34	10.440	0.83	-0.07	13.32	0.83	-0.07	11.880	0.83	-0.05
Local	8.510	0.48	-0.04	10.57	0.67	-0.09	9.540	0.59	-0.05
Mean	12.625	1.000		14.712	1.000		13.669	1.000	
Standard Error	0.170	0.099		0.148	0.064		0.110	0.055	

12 and AR-14 indicated that these two genotypes could be utilized as one of components for efficient integration. During the second year of the experiment, AR-12 and AR-14 had ' b_i ' values much higher than one (1.51 and 1.19, respectively), indicating that these genotypes respond best under the reclamation treatments. Genotypes, AR-29 and V1 with ' b_i ' value around 1.0 (1.03 and 1.19, respectively) could be considered as stable genotypes though they did not respond significantly even when the soil was reclaimed. The high ' b_i ' values for AR-12 and AR-14 indicated that these two genotypes could be utilized as one of components for efficient integration.

AR-12 and AR-14 had ' b_i ' values much higher than one (1.41 and 1.34 respectively), indicating that these genotypes respond best under the reclamation treatments. Genotype, AR-29 and V1 with ' b_i ' value around 1.0 (1.06 and 1.09 respectively) could be considered as stable genotypes and they did not respond significantly even when the soil was reclaimed. The high ' b_i ' values for AR-12 and AR-14 indicated that these two genotypes could be utilized as one of components for efficient integration. A genotype with a high regression coefficient (b_i) and regression line (S_{di}^2) reacts readily to changes in the environment and possesses considerable variability, whereas cultivars with a $b_i < 1.0$ and S_{di}^2 near to 0.00 react weakly to changes in growing conditions and are considered to be stable in yield (Shindin and Lokteva, 2000). Finlay and Wilkinson (1963) regarded those genotypes with a b_i near 1.0 and high mean leaf yield as being well adapted to all environments. Variability among environments is an important factor and mostly

determines the usefulness of b_i values (Ulker *et al.*, 2006). Baker (1988) considered deviation from regression (S_{di}^2) to be the most appropriate criterion for measuring phenotypic stability in an agronomic sense, because this parameter measures the predictability of genotypic reaction to environment; with high and desirable per se performance of a variety across environments is also a positive point to rate the variety as a better and highly stable genotype. Ozgen (1994), Ulker *et al.* (2006), Abdul *et al.* (2007), Akcura *et al.* (2009), Feiziasl, *et al.* (2010) and Hristov *et al.* (2011) and Anarase *et al.*, (2015) considered that a desirable genotype with stability and above average yield should have a regression line with a positive intercept and slope equal to 1.0 and the lower deviation from regression.

In the present study, differences among the treatments, environments as well as GEI were found to be significant. Genotypes, AR-12 and AR-14 found to respond best under the reclamation treatments, throughout the study period. It was observed that genotype, AR-10 during the first year and AR-29 and V1 during the second year were found to be stable though they did not respond significantly even when the soil was reclaimed. The high ' b_i ' values for AR-12 and AR-14 indicated that these two genotypes could be utilized as one of components for efficient integration. The variations in regression coefficient (b_i) values suggested that the eight genotypes responded differently to the different soil environments.

Ghosh *et al.* (2009), evaluated eight newly developed

mulberry hybrids and found, variance for deviation from regression (S_{di}^2) of hybrids S-1908 and C-2039 did not differ significantly from zero. The b_i value of hybrids S-1908 and C-2039 is also not significantly different from unity hence these may be considered to be stable ones. The hybrid C-2038 having b_i not significantly different from unity (1.023) and moderate but significant S_{di}^2 (0.35) emerged as a high yielder with high heterotic value for leaf yield. Similarly, ten mulberry varieties at seven centres evaluated for GEI and found that, variance for deviation from regression (S_{di}^2) of varieties C2017, RFS175 and Thalaghatapura did not differ significantly from zero (Ghosh *et al.*, 2013). The b_i values of only RFS175 out of these three are not significantly different from unity and may be considered to be a stable variety with moderate leaf yield. Sathyanarayana and Sangannavar (2020) evaluated eight mulberry genotypes for stability and GEI for bioassay parameters and found, AR-12, AR-14 and AR-10 showed ' b_i ' values much higher than one, indicating that these genotypes respond best under the reclamation treatments. Regression coefficient of each genotype was highly significant and positively correlated with mean.

The study provided some guidelines to the mulberry breeders who are engaged in developing superior genotypes tolerant to alkali soil by producing high and quality of mulberry leaf. Mulberry, being a vegetative propagated plant, once a tolerant variety is evolved, it can be perpetuated easily through cuttings provided it possesses a good rooting capacity. A proper planning of multiplication and the adoption of reclamation package as suggested is expected to be effective enough for augmentation of all the characters which influence the leaf yield and rearing performance of silkworm.

ACKNOWLEDGMENT

We wish to thank Dr. T. Mogili and Dr. A. Sarkar for their valuable technical support and guidance on this study besides Central Silk Board, Bengaluru for the financial support.

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