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Morpho-anatomical traits for preliminary screening of mulberry (*Morus* spp.) genotypes for moisture stress

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Abstract

Preliminary selection of mulberry (*Morus* spp.) genotypes for drought tolerance/resistance was done by following a joint scoring technique on multiple parameters like, stomatal frequency, stomatal size, leaf thickness and cuticle thickness. Using this technique a total of twenty one genotypes were selected from a population of forty for further evaluation. The selected genotypes would be evaluated under hot spot condition for getting a sustainable, high yielding, drought resistant mulberry. Ranking on the basis of scoring of each genotype separately for individual morpho- anatonical traits showed that three genotypes, No. 50, 4 and 5 were the top scorers with high leaf thickness values and less stomatal size. Similarly, genotypes with minimum stomatal size, more leaf and cuticle thickness were ranked high. Genotypes exhibiting significant superiority for any three of the four parameters over the check were considered for selection. The joint score technique was therefore used here as a screening tool for its ease and effectiveness to select mulberry genotypes on the basis of multiple characters.

Key words: Mulberry, morpho-anatomical traits, screening, joint-score technique, moisture stress

Introduction

Mulberry (*Morus* spp.) is cultivated predominantly as a rainfed crop in India [1]. The recommended high yielding mulberry varieties are however, not performing well under moisture stress condition. On the other hand, the leaf yield potential of mulberry varieties recommended for rainfed sericulture is still very low [2, 3]. Therefore, a suitable and quick screening technique for preliminary selection of a large gene pool on the basis of drought resistance/tolerance and subsequently evaluating the selected genotypes through multilocational trial is considered for identification of genotype for the regions suffering from chronic water deficit condition

It is fact that stomatal frequency, stomatal size, leaf thickness and cuticle thickness have high correlation with moisture retention capacity of leaves [4]. Stomata and cuticle retard water loss from leaves and water retention has been proposed as a test of drought resistance [5]. Besides, thick cuticled leaves with small stomata are able to retain more moisture and thus can overcome water deficit period by regulating water loss.

The present study was carried out with the objective to select genotypes tolerant/resistant to moisture stress condition based on morpho-anatomical traits and to make decision jointly by following joint score technique for further field trial experiment [6]. An attempt had earlier been made in mulberry to evaluate genotypes on the basis of survival and growth parameters in nursery following this technique [7]. In fact, preliminary selection of genotypes by joint score technique based on morpho-anatonical traits is still lacking in mulberry.

Materials and methods

Mulberry, the only food plant of silkworm (*Bombyx mori*) is a perennial crop and usually multiplied through cuttings. Cuttings survival and leaf yield potential are the most important parameters to be looked into for selection of mulberry genotypes towards mulberry crop improvement programme for commercial exploitation. Forty elite strains of mulberry, originated from different hybridization programme with high survival (~80%) and better yield (–1888 kg/plant^{-1}) were selected for conducting this study during 2003. Considering the morpho-anatomical parameters namely stomatal frequency, stomatal size, leaf thickness and cuticle thickness as the contributing traits to drought avoidance/tolerance, these genotypes were subjected to joint score analysis for preliminary screening [6].

For stomatal studies 5th, 6th and 7th leaves from 60 days old shoots of all the genotypes were collected at 9 AM in a beaker containing water. Subsequently a thin film of quickfix was applied on the lower surface in the central portion of the leaf blade. Stomatal impressions were peeled out and the frequency was determined. Twenty microscopic field observations were recorded for each genotype. The process was repeated three times in each case. Stomatal frequency (mm^{-2}) was determined by using Πr^2. The average length and breadth of guard cells was determined on 20 measurements for all the three leaves and repeated five times for final consideration. Standard procedures were followed for the measurements of leaf and cuticle thickness. Thickness of leaf and cuticle was measured in μm. In all the cases ten readings were taken five times and the mean was calculated.

The data were analyzed for variance test. The population means were arranged in descending order. Subtracting the critical difference value from the top most one, the remainder was noted. The genotypes of the same value within the range of topmost one and remainder were grouped together and numbered as one. The critical difference was then subtracted from the second one and likewise the genotypes of similar value of second one and remainder were grouped together and numbered as two. The process was repeated till all the values of treatments were covered. The groups were numbered as 1, 2, 3 .... so on. The score for each genotype was calculated by adding the group values in which genotypes falls and divided by the value obtained by multiplying the number of groups and last group. In this calculation, the topmost treatment with the highest value will get the least score. The treatment with the least value will get the highest score. The scores obtained by a genotype for various parameters were added to get the total score. Based on total score, the genotypes were ranked. The genotype with the least total score gets the first rank and the genotype with the highest total score gets the lowest rank. In this way, genotypes were shortened and those performing better than check may be carried forward in the next phase of the experiment for evaluation study along with the recommended genotype.

Results and discussion

Significant statistical difference among the genotypes for all the parameters indicated the presence of variability. Stomatal frequency was found maximum in genotype 17 (425 No. mm^{-2}) followed by 11, 21 and 2 in that order. Minimum stomatal size was recorded in genotype 5 (115 (μm^2) followed by 37. For this parameter 35 genotypes showed significant difference. Genotype 19 recorded maximum leaf thickness (242 μm) followed by 34, 40, 4 and 39 respectively. Leaf thickness of check genotype was recorded as 124 μm and 35 genotypes were significantly superior over the check for this parameter. Genotype 4 and 22, 20, 29, 5, 1, 8, 18 and 40 exhibited statistical significance for cuticle thickness over the check (Table 1).

Table 1. Stomatal frequency, stomatal size, leaf thickness and cuticle thickness of mulberry genotypes

Genotype	Stomatal frequency	Stomatal size (μm^2)	Leaf thickness (μm)	Cuticle thickness (μm)
1	195.60	170.00	171	7.50
2	401.80	161.00	161	5.00
3	261.20	500.00	159	5.00
4	264.20	190.00	207	9.00
5	198.00	115.00	175	7.51
6	169.20	145.00	155	5.00
7	380.20	145.00	192	6.50
8	279.40	160.00	183	7.50
9	311.20	200.00	153	8.50
10	327.40	200.00	156	7.00
11	421.40	155.00	174	6.00
12	175.60	250.00	171	6.50
13	313.40	162.40	182	6.50
14	200.20	190.00	150	5.50
15	295.00	190.00	153	5.00
16	210.20	215.00	176	5.00
17	425.00	180.00	138	5.50
18	268.40	180.00	190	7.50
19	307.20	155.00	242	5.00
20	395.00	154.80	175	8.50
21	408.00	154.80	197	6.50
22	401.20	170.00	180	9.00
23	214.00	202.80	195	6.00
24	199.00	210.00	164	5.50
25	194.00	200.00	199	6.00
26	263.80	165.00	134	5.00
27	267.80	200.00	171	6.10
28	214.20	177.60	138	6.50
29	251.00	210.00	157	8.00
30	264.60	400.00	197	6.00
31	198.00	250.00	152	5.00
32	190.80	200.00	158	5.00
33	166.20	220.00	165	5.50
34	189.80	120.00	232	5.00
35	207.00	255.00	132	6.00
36	146.80	172.80	149	5.00
37	203.80	190.00	185	5.00
38	346.80	220.00	168	5.50
39	169.20	185.00	202	5.50
40	186.00	170.00	208	7.50
Check	223.20	315.00	124	5.50
C.D. at 5%	38.12	83.24	27.46	1.61

Giving equal importance to each of the traits individually the joint scoring technique was used to select genotypes performing better over the check. Stomatal frequency, stomatal size, leaf thickness and cuticle thickness were considered for scoring the genotypes. Genotypes exhibiting significant superiority over the check for at least three of total four parameters were considered for selection. Based on total minimum joint score 21 genotypes were selected. Genotype 4, 8, 9, 18, 20 and 22 exhibited significant difference for all the parameters and were secured higher positions in ranking. It has been observed that every genotype which recorded statistical significance for cuticle thickness was selected and among them genotype 40,

Table 2. Ranking of the mulberry genotypes

Genotype	Score value				Total score	Overall rank
	Stomatal frequency	Stomatal size	Leaf thickness	Cuticle thickness		
1	0.2333	0.3125	0.5789	0.4167	0.3854	5*
2	0.9667	0.2500	0.7368	1.0000	0.7384	38*
3	0.5667	1.0000	0.7632	1.0000	0.8325	41
4	0.5667	0.4375	0.1579	0.1667	0.3322	3*
5	0.2333	0.1250	0.5263	0.4167	0.3253	2*
6	0.1333	0.2500	0.8158	1.0000	0.5498	18
7	0.9000	0.2500	0.3158	0.7500	0.5539	19*
8	0.6000	0.2500	0.3947	0.4167	0.4154	6*
9	0.7333	0.5000	0.8421	0.2500	0.5814	22*
10	0.7667	0.5000	0.7895	0.5833	0.6599	32*
11	1.0000	0.2500	0.5526	0.8333	0.6590	31*
12	0.1667	0.6875	0.5789	0.7500	0.5458	17
13	0.7333	0.2500	0.4211	0.7500	0.5386	16*
14	0.2333	0.4375	0.8947	0.9167	0.6206	28
15	0.6667	0.4375	0.8421	1.0000	0.7366	37*
16	0.3000	0.5625	0.5263	1.0000	0.5972	24
17	1.0000	0.4375	0.9211	0.9167	0.8188	40
18	0.5667	0.4375	0.3421	0.4167	0.4407	8*
19	0.7000	0.2500	0.0526	1.0000	0.5007	13*
20	0.9667	0.2500	0.5263	0.2500	0.4982	12*
21	0.9667	0.2500	0.2632	0.7500	0.5575	20*
22	0.9667	0.3125	0.4737	0.1667	0.4799	10*
23	0.3667	0.5000	0.2895	0.8333	0.4974	11
24	0.2333	0.5625	0.6842	0.9167	0.5992	25
25	0.2333	0.5000	0.2368	0.8333	0.4509	9
26	0.5667	0.2500	0.9474	1.0000	0.6910	33*
27	0.5667	0.5000	0.5789	0.8333	0.6197	27*
28	0.3667	0.4375	0.9211	0.7500	0.6188	26
29	0.4667	0.5625	0.7895	0.3333	0.5380	15*
30	0.5667	0.9375	0.2632	0.8333	0.6502	30
31	0.2333	0.6875	0.8684	1.0000	0.6973	34
32	0.2333	0.5000	0.7632	1.0000	0.6241	29
33	0.1000	0.5625	0.6579	0.9167	0.5593	21
34	0.2333	0.1875	0.0789	1.0000	0.3749	4
35	0.2667	0.7500	0.9737	0.8333	0.7059	35
36	0.0667	0.3750	0.8947	1.0000	0.5841	23
37	0.2333	0.4375	0.3684	1.0000	0.5098	14
38	0.8333	0.5625	0.6053	0.9167	0.7294	36*
39	0.1333	0.4375	0.2105	0.9167	0.4245	7
40	0.2333	0.3125	0.1316	0.4167	0.2735	1*
Check	0.4000	0.8125	1.0000	0.9167	0.7823	39

*Genotypes selected

5 and 4 occupied the 1st, 2nd and 3rd rank respectively. Moreover, genotype 40 and 4 also recorded maximum leaf thickness and genotype 5 showed minimum stomatal size. Similar observation on morpho-anatomocal parameters associated with drought resistance had been reported in mulberry [4]. On the other hand, genotype 17, with maximum stomatal frequency; leaf thickness and cuticle thickness at par with check genotype and could not be selected. This may be because of the role of stomatal size in moisture retention and not stomatal frequency as indicated by several workers [8, 9 and 10].

Stomatal frequency, stomatal size, leaf thickness and cuticle thickness have significant relationship with drought resistance and higher water use efficiency [11]. Thus selection of genotypes was done by following joint score technique giving due weightage for all the parameters. Though there are several statistical methods available to rank the genotype on the basis of individual character, the technique used here is the most appropriate one to make decision jointly and select genotypes based on more than one character at a time without giving preference to any individual trait. It is found effective because of easy and quick identification of genotypes prior to conduct the next step of a breeding programme. This screening technique ensures drought tolerance among the selected genotypes under moisture stress condition. However this has no relation with high yield, as it depends on the function of many other yield attributing traits.

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