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Screening of F1 progeny for selection of superior hybrids in mulberry (*Morus* spp.) - A simple approach
Part II: Screening of transplants in relation to seedling size

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Abstract: In continuation of studies on the effect of seed size on growth, made under part-I, the effect of seed size and seedling size are studied jointly by size-grading of seedlings into three classes under each seed-size class, with 2700 F1 promising genotypes of two crosses. The combined population was screened in two steps, adopting comparison tree method. In step-1, transplanted seedlings (transplants) with closer spacing were screened based on shoot weight for a period of two crops and 487 genotypes were shortlisted based on higher superiority percent. In step-2, these 487 transplants were replanted with wider spacing and screened using three parameters for a period of four crops. In each crop, the genotypes which showed superiority percent value more than Minimum Selection Standard for the parameters studied, were shortlisted. Among 22 genotypes shortlisted finally, 18 have come from large seed-size class and the remaining four are also from long seedling-size class of medium seed-size class. This clearly established the importance of size-grading of seeds and seedlings in screening process. As the chance of getting any promising genotype from small seeds is remote, they could be rejected before sowing and as it is also true with small seedlings they could be rejected at nursery stage screening. The tracking of juvenile performance of shortlisted genotypes indicated that, shoot length and root collar diameter are more reliable parameters for assessing the growth of seedlings at nursery. Comparison tree method is found to be highly useful for shortlisting of transplants in mulberry.

Key words: Comparison tree method; F1 hybrids; Mulberry breeding; Screening; Seedling size.

Introduction

Mulberry (*Morus* spp.) an important tree crop, grown for its foliage and fruits is cross pollinated and highly heterozygous. Due to high degree of heterogeneity in its F1 population and long generation period, the screening and selection of promising hybrids becomes lengthy process and requires the screening of a large number of hybrid seedlings. Developing a technique to identify promising genotype at seedling stage or even at seed stage will reduce the burden of screening process to a large extent.

Seedling vigour is deeply related to productivity to the extent that high vigour seeds are expected to form better stands and produce higher yields (Filgueiras, 1981). It is established that, three aspects of seed quality *viz.,* seed germination, vigour and size may influence crop yield through both indirect and direct effects (Ellis, 1992). Seedling morphometric attributes like root collar diameter, seedling height and terminal bud condition have long been used to grade forest tree seedlings into different classes of implied survivability and growth after transplanting in the field (Venkatesh *et al.,* 2003). Synthesis of data from experimental studies on the advantages of large-seeded species establishing under particular hazards (such as shade, drought or herbivory) confirmed that seedlings of large-seeded species perform better than those of small seeded species in most situations (Moles and Westoby, 2004). In forest tree breeding, Comparison tree method, has been recorded as an easy and effective method of Plus tree selection (Sidhu, 1996) and has been employed in many plants (Solanki *et al.,* 1984; Kackar *et al.,* 1993; Sidhu 1997 and Rai and Parthasarathi, 1989). In mulberry, information on screening of F1 hybrids after transplanting in the screening plot is scanty. Keshavcharyulu *et al.,* (2006), in a programme of developing promising mulberry genotypes for sub-optimal irrigated conditions through advanced generation breeding approach have employed comparison method to short-list the genotypes. As stated in part I of study, under a broad objective of developing a simple approach for screening and selection of F1 hybrids in mulberry, this part of investigation has been undertaken to identify the potential grade of seedling-size.

Materials and Methods

The studies were undertaken at Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, during 2012-15.

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Seedling grading and labeling

In the previous part of screening studies, 450 healthy promising seedlings were isolated under small (S), medium (M) and large (L) seed-size classes (totaling 1350 seedlings/cross). For the continuation of screening process in this part of study, these 450 seedlings were further classified as short (s), medium (m) and long (l) seedlings and divided 150 seedlings to each class of seedling-size. To keep track of the performance of every seedling from nursery to final selection, all the 1350 seedlings of each cross were labeled with serial numbers with identity of cross (A and B), seed-size class (S, M and L) and seedling-size class (s, m and l). To examine the genotypes shortlisted at the end of this study, for their past performance at nursery stage, the data scored on four growth parameters for all the 1350 seedlings of each cross was documented with label. The screening plots of seedlings were maintained by applying manure @ 20 MT/ha/yr and NPK @ 150 – 50 – 50 kg/ha/yr with regular irrigation. Further, screening and selection was performed in relation to seedling size and combined population of both the crosses by employing Comparison tree method (Sidhu, 1997) in two steps.

Selection step-1:

In this step, the seedlings were transplanted under closer spacing of 40 cm × 30 cm in Randomized Complete Design and screened for only shoot weight. To begin with, the transplants were pruned at uniform height of 20 cm. One or two branches/seedling was allowed to grow further. After five months, the data on shoot weight of all the genotypes was collected (crop I). The data was analyzed to understand the variation in shoot weight of seedlings of various seedling size classes and seed size classes. Then, by following the method of Minimum Selection Standard (MSS) developed by Pitcher and Dorn (1967) selection was performed. The genotypes which met the MSS in terms of shoot weight (220 g) were noted. Again, after three months, the data on shoot weight of hybrids noted under crop I was recorded (crop II). The mean value of shoot weight (271.34 g) of all the selected genotypes (397+ 320) of crop I (initial population) is used to calculate the superiority percentages in crop II, using the following the formula:

$$\text{Superiority \%} = \frac{\text{Value of each individual plant for a parameter}}{\text{Mean value of initial population (Comparison crop)}} \times 100 - 100$$

At the end of step-1, genotypes showing more superiority percentage than MSS (7.27%) for shoot weight were shortlisted and their assessment is continued in step 2.

Selection step 2:

In step 2, only the 487 genotypes selected in previous step were transplanted again into a new plot under wider spacing of 60 cm x 60 cm and screened. The genotypes belonging to various seed- and seedling-size classes were screened in four crops period. After four months of transplanting, the data on three quantitative parameters *viz.,* length of longest shoot, number of branches/plant and leaf yield/plant were recorded and plants were pruned. As a Comparison crop, the mean values of three parameters of this were used for the calculation of superiority percentages of genotypes in three succeeding crops (crop IV, V and VI) and as such no selection was made in this crop. Again, after three months, data on three parameters mentioned above were recorded in all the genotypes and superiority percentages were calculated. The genotypes which showed higher superiority percentage than MSS for all the three parameters were shortlisted (crop IV). By repeating this selection procedure of comparison tree method, the genotypes were selected and shortlisted during crop V and crop VI.

Reliability and acceptable range of juvenile parameters

At the end of screening process, an attempt has been made to study the degree of reliability with acceptable range in four growth parameters *viz.,* shoot length, root collar diameter, root length and shoot weight which were employed to assess the superiority of seedlings at nursery by retrieving the data collected in part I of study. The data collected from all the 1350 seedlings in respect of these growth parameters was computed into three ranges as low, medium and high. Later, the number of transplants shortlisted during crop I to crop VI was scored under respective range of these parameters. The parameter which included more number of hybrids was considered as reliable one and their range which include more number of hybrid was considered as most acceptable range to screen the hybrids at seedling stage in nursery.

Statistical analysis: One-way ANOVA was carried out for shoot weight of crop I of selection to identify the differences between different seedling sizes under each seed size and cross. Factorial analysis was also carried out to find out the significance in the difference between three factors (cross, seed size and seedling size) and their interaction, using Mini-Tab software for shoot weight. Treatments (size of seeds and size of seedlings) means were compared using Tukey's test at 5% and 1% probability levels.

Results and Discussion

Seedling size classes and transplantation

In the previous part of screening, the rejection of inferior genotypes from nursery bed has been achieved partially using four growth parameters under each seed-size class. In this part, to identify a potential grade of seedling-size, seedlings under each seed-size class were size-graded into short, medium and long seedlings. While studying the effect of seed and seedling size on survival and growth of lobolly pine, Sluder (1979) observed that the effect of seed size and seedling size may be more precisely defined by using three size classes for each factor and they should be studied jointly. Generally, the seedlings at the first selection

stage are planted in rather close spacing to accommodate more number of plants in a unit area continuously for many years (Sarkar, 2009). The screening of a total of 2700 seedlings has been performed first under closer spacing, using less space and manpower. After two crops, the shortlisted 487 genotypes were replanted again in a new plot under wider spacing, with a purpose of avoiding uneven competition between the weak and vigorous seedlings and also promoting the expression of full potentiality of genotypes under proper spacing in new plot. Care has been taken to minimize the transplantation shock to the seedlings.

Initial performance of seedling size classes:

The analyzed data on mean values of shoot weight of all the hybrids under various seed and seedling size classes of crop I are presented in Table-1. It is evident from the data that the hybrid seedlings of cross A were found to have higher shoot weight than the hybrids of cross B and the interaction between cross and seedling size was highly significant. It was also clear that, the shoot weight increased with increase in seed size as well as seedling size in both the crosses and the difference in shoot weight between crosses, seed size classes as well as seedling size classes was also highly significant. Many studies in various plants have also established that seedlings from large seeds had a higher biomass (Sluder, 1979; Khurana and Singh, 2000; Rezapour *et al.*, 2013). The highly significant difference found between seedling-size classes and shoot weight in both the crosses clearly indicated that selection of hybrids based on seedling size has high relevance.

Table 1: Mean values of shoot weight of F1 progenies belong to three seedling size and seed size classes in crop-I.

Seed size class	Seedling size class	Shoot weight (g) Cross A	Cross B
Small	Short	131.91 ^{a}	128.68^{a}
	Medium	139.08 ^{a}	143.97^{ab}
	Long	174.44 ^{b}	157.03^{b}
Medium	Short	177.64 ^{b}	171.70^{bc}
	Medium	195.33 ^{c}	183.32^{c}
	Long	219.73 ^{d}	189.89^{dc}
Large	Short	200.81 ^{b}	202.04^{d}
	Medium	220.08 ^{d}	207.07^{de}
	Long	245.36 ^{e}	218.07^{e}
Significance		**	**

ANOVA and F values

Source	df	F-values
Cross	1	27.35**
Seed size	2	339.16**
Seedling size	2	69.04**
Cross x seed size	2	2.14^{ns}
Cross x seedling size	2	9.13**
Seed size x seedling size	4	0.52^{ns}
Cross x seed size x seedling size	4	0.64^{ns}
Error	2682	

ns, * and ** denotes non-significant, significant at 5% and significant at 1% probability levels, respectively.

Short-listing of genotypes under six crops

The selection and short-listing of seedlings has been performed by employing Comparison tree method. Kesavacharyulu, *et al.,* (2006), while screening 4026 F1 hybrid seedlings of 22 crosses of mulberry have also used the above said growth parameters and comparison tree method. This method has been employed in the selection of plus tree in *Acacia torilis, Prosopis cinenaria* (Solanki *et al.,* 1984 and Kackar *et al.,* 1993), *Dalbergia sissoo* (Sidhu, 1997) and *A. catechu* (Rai and Parathasarathi, 1989). In crop I of step 1, among 2700 initial population (based on MSS in shoot weight-220.0 g) a number of 717 genotypes were shortlisted. In crop II a number of 487 genotypes were shortlisted based on their superiority over MSS in shoot weight (7.27 %). During screening under step 2, the values in respect of three parameters of crop III were considered for comparison and 228, 76 and 22 genotypes were shortlisted in crop IV, V and VI respectively. The data on variability, MSS and mean superiority percentages in respect of four growth parameters collected on combined progenies of two crosses under various crops is presented in Table-2. The data clearly indicated that there is an appreciable improvement in all the parameters studied in the genotypes short-listed. In the comparison crop, the mean values in respect of length of longest shoot (cm), number of branches and leaf yield/plant (g) were 132.49 cm, 3.9 and 328.53 g, respectively. In sixth crop of selection the mean values for above said three parameters were 172.7 cm, 7.7 and 497.55 g with of 30.2, 99.23 and 51.44% improvement (S %), respectively over the comparison crop.

Table 2: Variability, MSS and mean superiority percentages in respect of four growth parameters in shortlisted genotypes.

Screening step		Selection step-1 (Closer spacing)		Selection step-2 (wider spacing)			
Crop		I (Initial population)	II	III (Comparison crop)	IV	V	VI
No. of genotypes shortlisted (Cross A + Cross B)		717	487	487	228	76	22
Shoot weight (g)	Mean	271.34	336.78	---	---	---	---
	S.D.	38.84	31.72	---	---	---	---
	CV	0.14	0.09	---	---	---	---
	MSS (g)	220.0	290.0	--	--	--	--
	MSS of S%	---	07.27	--	--	--	---
	Mean S%	---	24.11	---	---	---	---
Length of longest shoot (cm)	Mean	---	---	132.49	177.26	179.71	172.27
	S.D.	---	--	18.08	16.40	12.37	5.41
	CV	---	--	0.13	0.09	0.06	0.03
	MSS of S%			--	9.51	20.00	22.27
	Mean S%	--	--	--	33.96	35.64	30.02
Number of branches	Mean	---	--	3.9	5.5	6.8	7.7
	S.D.	---	--	1.25	1.12	0.96	0.60
	CV	---	--	0.32	0.20	0.14	0.07
	MSS of S%			--	28.20	53.84	79.49
	Mean S%	---	--	--	41.02	74.35	99.23
Leaf yield/plant (g)	Mean	---	--	328.53	480.83	505.44	497.55
	S.D.	---	--	105.2	90.25	71.05	32.43
	CV	---	--	0.32	0.18	0.14	0.06
	MSS of S%			--	15.66	21.75	34.23
	Mean S%	---	--	--	46.53	53.84	51.44

Selections versus seedling size:

The number of hybrids shortlisted during screening under six crops under various seed-size and seedling-size classes and two crosses indicated that more number of hybrids are shortlisted in cross A compared to cross B (Table-3). The data also indicated that there is a strong correlation between number of hybrids shortlisted and size of seeds as well of seedling size. All the three seedling size classes, including the long one under small and medium seed size classes, the number of shortlisted genotypes gradually decreased from crop I to crop V and reduced to zero in crop VI. Among the 22 genotypes shortlisted finally (from both the crosses) 18 were identified from large seed-size class and the remaining four also from long seedling-size class of medium seed-size class. Not even a single genotype was got selected from small seed-size class as well as short and medium seedling-size class of medium seed size class. With this, it could be inferred that the chance of getting any promising genotype from small seeds is remote and they could be rejected conveniently. Further, in large seed-size class also, more number of genotypes short listed from long seedlings. Hence, it is concluded that, size-grading of hybrid seeds and rejection of small seeds before sowing are important criteria to be considered while screening of F1 hybrid seedling population in mulberry. Also, size-grading of seedlings before transplanting into screening plot and rejection of short seedlings helps in reducing the burden of screening process. Various studies on forest trees have also established the significance of size-grading of seeds and seedlings in the improvement of growth and survival (Dorman, 1976; Sluder, 1979).

Table 3: Number of F1 hybrids shortlisted in respect of seed and seedling size classes under various crops.

Seed size class	Seedling size class	Selection step-1					Selection step-2					
		Crop I		Crop II		Crop III	Crop IV		Crop V		Crop VI	
	Cross	A	B	A	B		A	B	A	B	A	B
Small	Short	16	8	5	5	No selection (Comparison crop)	1	--	--	--	--	--
	Medium	25	17	10	10		1	--	--	--	--	--
	Long	30	19	20	20		5	3	2	1	--	--
Medium	Short	36	25	24	19		16	14	3	2	--	--
	Medium	40	22	32	22		18	17	4	3	--	--
	Long	70	60	45	28		15	14	7	6	3	1
Large	Short	50	46	35	19		14	13	6	5	2	1
	Medium	55	55	40	47		22	20	7	7	2	2
	Long	75	68	50	50		30	25	13	10	5	6
Total		**397**	**320**	**261**	**226**	**--**	**122**	**106**	**42**	**34**	**12**	**10**

Reliability and acceptable range of juvenile parameters:

The frequency of shortlisted F1 hybrids in respect of three ranges of data on four growth parameters scored as per their performance during screening at nursery stage is presented in Table-4. The data showed that, though maximum frequency of hybrids was found under high range of all the parameters, very high percentage of hybrids occurred under high range of shoot length and root collar diameter. Further, all the 22 hybrids finally shortlisted were found to occur under high range of shoot length and root collar diameter. On

the contrary, under root length and shoot weight, the shortlisted hybrids were found distributed under all the three ranges. This correlation clearly indicated that, the shoot length and root collar diameter provide the best estimate of seedling performance after transplanting. Accordingly, they are found to be more reliable parameters and their high ranges are the acceptable ranges which could be used for screening F1 seedlings at nursery stage in mulberry. In many studies on forest tree nursery also, seedling height and stem diameter are reported to provide best estimate of seedling performance after out planting (Mexel and Landis, 1990; Haase, 2008).

Table 4: The frequency of F1 hybrids shortlisted under PART-II in respect of four seedling growth parameters studied in PART-I of screening and selection.

Parameter	Range	Cross A					Cross B				
		Step-1		Step-2			Step-1		Step-2		
		I	II	IV	V	VI	I	II	IV	V	VI
No. of hybrids shortlisted	--	397	261	122	42	12	320	226	106	34	10
Shoot length (cm)	50 - 57	27	11	05	0	-	41	18	8	01	0
	58 - 65	120	60	22	12	0	89	42	15	04	0
	66 - 73	250	190	95	30	12	190	166	83	29	10
Root collar diameter (mm)	5.4 – 5.8	22	10	0	-	-	13	0	-	-	-
	5.9 – 6.3	75	31	10	0	-	54	34	05	0	-
	6.4 – 6.8	300	225	112	42	12	253	192	101	34	10
Root length (cm)	31 - 38	112	60	21	06	02	90	35	21	05	2
	39 - 46	125	91	41	16	02	96	73	32	12	2
	47 - 54	160	110	60	20	08	135	118	53	17	5
Shoot weight (g)	51 - 58	124	59	24	10	01	85	71	35	9	3
	59 - 66	101	72	33	10	6	102	72	23	7	3
	67 - 74	172	130	65	22	5	133	83	48	18	4

Superiority of final selections:

The 22 selected hybrids are listed with their label and data on their performance in respect of three growth parameters and superiority percentage (Table-5). The data showed that the hybrids belong to medium seed-size and seedling-size classes were inferior to the hybrids belong to large seed-size class. Further, among the 22 shortlisted hybrids, only two (B-L-l-137 and A-L-l-55) showed higher superiority percent for all the three parameters studied and the remaining hybrids were superior in respect of one or two parameters. Finally, to select the genotypes for PYT, along with the data on parameters assessed, smoothness of leaf, thickness of leaf, size of leaf, intensity of green colour in leaf, branching nature, internodal distance *etc.* were also considered. At the end, eight hybrids from cross A and five hybrids from cross B which are marked (√) in the last column of Table-5 were judged as superior ones for leaf yield and quality related parameters. These hybrids are being evaluated in primary yield trial, under the major programme of developing superior mulberry triploids.

Table 5: Identity and Superiority percentages in relation to three growth parameters of 22 hybrids shortlisted and 13 genotypes identified for PYT.

Parameter				Longest shoot		Branches/plant		Leaf yield/plant		13 Genotypes identified for PYT
Cross	Seed size	Seedling size	Label of shortlisted genotypes	Length (cm)	S %	No.	S %	Yield (g)	S %	
A	Medium	Long	A-M-l-25	164	23.78	7	79.49	441	34.23	--
			A-M-l-36	177	33.59	7	79.49	499	51.89	√
			A-M-l-120	168	26.80	7	79.49	462	40.63	--
	Large	Short	A-L-s-42	162	22.27	7	79.49	475	44.58	--
			A-L-s-53	173	30.58	8	105.13	515	56.76	√
		Medium	A-L-m-63	175	32.09	8	105.13	510	55.24	√
			A-L-m-74	176	32.84	8	105.13	505	53.72	√
		Long	A-L-l-4	179	35.10	8	105.13	518	57.67	√
			A-L-l-12	176	32.84	8	105.13	514	56.45	√
			A-L-l-55	174	31.33	9	130.77	531	61.63	√
			A-L-l-121	175	32.09	8	105.13	540	64.37	√
			A-L-l-146	165	24.54	7	79.49	473	43.97	--
B	Medium	Long	B-M-l-111	165	24.54	8	105.13	462	40.63	--
	Large	Short	B-L-s-115	177	33.59	7	79.49	493	50.06	√
		Medium	B-L-m-81	168	26.80	8	105.13	473	43.97	--
			B-L-m-96	177	33.59	8	105.13	527	60.41	√
		Long	B-L-l-29	167	26.05	7	79.49	442	34.54	--
			B-L-l-76	172	29.82	8	105.13	538	63.76	√
			B-L-l-81	177	33.59	8	105.13	545	65.89	√
			B-L-l-89	166	25.29	8	105.13	478	45.50	--
			B-L-l-128	180	35.86	8	105.13	462	40.63	--
			B-L-l-137	177	33.59	9	130.77	543	65.28	√
		Mean		172.27	30.03	7.77	99.30	497.55	51.45	--
		S.D.		5.42	--	0.60		32.43	--	--

Conclusion

In mulberry size-grading of F1 seeds into small, medium and large size classes and rejection of small seeds before sowing, size-grading of seedling into short, medium and long size classes and rejection of short seedlings before transplanting are important criteria to be followed to enhance the efficiency of screening and selection of superior hybrid. Adoption of comparison tree method for short-listing the promising genotypes is useful in mulberry. The two juvenile growth parameters- shoot length and root collar diameter are found to be more reliable parameters to assess the superiority in nursery. The present study is resulted in the identification of 13 promising hybrids of mulberry (triploids) apart from developing a simple approach for screening and selection of superior hybrids in mulberry.

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