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Screening of F1 progeny for selection of superior hybrids in mulberry (*Morus* spp.) - A simple approach
PART I: Screening of seedlings in relation to seed size

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Abstract: In mulberry (*Morus* spp.), the process of selection of promising hybrids from F1 population requires the screening of a large number of progenies and a long period. To develop a simple and faster approach for screening, studies were conducted using F1 seeds of two crosses. The details of screening studies conducted in relation to seed-size and seedling-size are reported separately in two parts. In this part, the F1 seeds were size-graded as small, medium and large seeds; their progenies were raised separately and screened in nursery. There was a considerable degree of variation in size of seeds and medium-size class seeds were in high percentage in both the crosses. The length, width and weight of seeds were also varied between the seed size classes significantly in both the crosses. The seed size classes differ with high significance in shoot length and Root collar diameter and also differ significantly in root length and weight of seedlings. The positive correlation between the seed size and growth of seedlings, seed size and germination, seed size and seedling survival in nursery indicated that size-grading of seeds and rejection of small seeds in the beginning of screening process itself, may help to increase the efficiency of screening by increasing the chances of getting superior hybrids from limited progenies. However, confirmation on the performance of large seedlings from small seed size class may help to draw conclusion. Hence, the studies are continued with size-grading of seedlings in the next part of screening study.

Key words: F1 hybrids screening; Mulberry breeding; Root collar diameter; seed-size; seedling growth.

Introduction

Mulberry (*Morus* spp.), an important commercial crop, is cultivated extensively for its foliage and fruits. Its foliage is the sole food for silkworm (*Bombyx mori* L.) which gives most valued silk. As it is cross pollinated, heterozygous and perennial crop, its genetic improvement through hybridization and selection becomes a difficult task. Due to high degree of heterogeneity in its F1 progeny, the screening and selection of promising hybrids is a lengthy and tedious process and requires the screening of a large number of progeny. Though, this process is called selection but in a strict sense the process may be called as method of rejection rather than selection (Sarkar, 2009). Simple techniques to identify potential grade of seeds and seedlings, reliable parameter to assess the potentiality of seedlings precisely, proper methods to distinguish the superiority among the progeny *etc.* will help in reducing the duration and burden of this process to a large extent. In mulberry, except the reports of enhancement of germination of ageing seeds (Basavaiah *et al.*, 1990), genotype specific variation in seed viability and seed vigour (Dandin *et al.*, 1991) a preliminary observation of variation in germination between heavy and light seeds of four F1 progenies (Prasad *et al.*, 1992) and lengthy procedure for screening of F1 progenies (Sarkar, 2009), studies related to variability in seed- and seedling-size and its application in screening F1 progeny are scanty.

Seed plays an important role in the transfer of genetic characters and improvement of qualitative and quantitative traits of production. One of the most important factors in maximizing crop yield is planting high quality seed. Seed-size is an important physical indicator of seed quality that affects vegetative growth and is frequently related to yield, market grade factors and harvest efficiency (Zareian *et al.,* 2013) and generally larger seeds have better performance than small seeds (Ambika *et al.,* 2014).

A major programme of developing superior triploids for leaf in mulberry through hybridization and selection was undertaken in the department. Under this programme, with an objective of developing a simple approach for screening and selection of F1 hybrids by identifying the potential grade of seeds and seedlings; reliable parameters to assess the performance of seedling and assessing the suitability of comparison tree method (Sidhu, 1997) studies were made. The details of techniques adopted, observations made and inferences drawn during screening at nursery and on seedlings transplanted (transplants) are reported separately as

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Part-I and Part-II, respectively. In this report (Part-I), the extent of variation in the size of F1 seeds and the impact of seed-size on germination, seedling survival and initial growth of progenies are presented.

Materials and Methods

This study was conducted at Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru, during 2012-15.

Seed procurement: To get sufficient quantity of good quality seeds, two crosses *viz.,* MUT-1 × Ber.C-776 (Cross A) and MUT-1 × V-1 (Cross B) were conducted. The F1 seeds of these crosses were extracted from the ripened fruits. Clean seeds kept in paper covers were stored for about a month in a desiccator at room temperature.

Seed grading: A bulk lot of 3600 seeds of each cross were divided into four groups of 900 seeds each for the purpose of treating them as four replications. The seeds of each replication were sieved, first through a sieve of 2.0 mm diameter pore size and considered the seeds which remained in the sieve as large seeds. Again, the seeds which passed through the sieve were sieved with another sieve of 1.4 mm pore size. Now the seeds remained in the sieve was considered as medium sized seeds and those which passed through the sieve were considered as small seeds. The percentages of small, medium and large seeds in the bulk lots were determined.

Seed weight and size: From each seed-size class, four batches of 100 seeds were taken and their weight was recorded. Then by drawing ten seeds from each batch of 100 seeds considered for recording weight, the length and width of forty seeds were recorded using Verniar Dial Caliper (Mitutoyo, Japan) of 0.01 mm dial graduation.

S**eed germination and survival**: Seed germination was studied in the laboratory through paper towel technique and also in field conditions by sowing seeds in sand beds inside a poly-house. In paper towel technique, a total of 200 seeds (four replications of 50 seeds each) were sown for each seed-size class and the percent germination was determined. For further screening and selection of hybrid seedlings, 700 seeds of each size class of both the crosses were sown with four replications of 175 seeds each in separate sterile sand beds (micro-beds). The micro-beds were arranged with a Complete Randomized Design. The seed beds were maintained with regular watering and with plant protection measures. By recording the number of seeds germinated in each micro-bed at 20 days after sowing (DAS) the percent germination was determined. The survival percent of seedlings was determined by counting the available seedlings at 60 DAS in each micro-bed.

Seedling growth:
At 60 DAS, length of seedlings was recorded by randomly selecting 100 seedlings from each micro-bed. At 150 DAS all the seedlings were uprooted and their root system was washed. By selecting 100 seedlings randomly from each micro-bed, the data on three parameters *viz.,* shoot length, root length and weight of seedlings were collected. In addition, root collar diameter (RCD) of the seedlings was measured using Verniar Dial Caliper (Haase, 2008).

Statistical analysis: The data collected on various parameters were subjected to statistical analysis using Mini-Tab software. One-way and two-way Analysis of Variance (ANOVA) was performed to determine the differences among the variables.

Results and Discussion

Seed size variation:
The data on quantity in bulk lot, weight, length and width, percent seed germination on paper and in sand beds and also seedling survival of three seed size classes of Cross A and Cross B are presented in Table-1.

In both the crosses there was a highly significant variation in seed size and the medium sized seeds occur in highest percentages. Occurrence of high percentage of medium size seeds is also reported in many crop plants (Bicer, 2009). Weight of 100 seeds also varied significantly between the crosses. Such variations in seed weight between the cultivars (Sadeghi, 2011), genotypes (Ashirvar, 2012) and species (Li *et al.,* 2015) have been recorded in other plants. The seeds of both the crosses also varied significantly in length and width between three size classes as reported in *Afzelia quenzensis* (Mtambalika *et al*., 2014). It is reported that, seed-size is a plastic trait of the plants (Souza and Fagundes, 2014) and the individual seed-size (mass) in a species varies from nearly constant (Fenner, 1995) to as high as 16-fold (Hawk and Maun, 1989) and several factors may be responsible for this type of variation (Shaukat *et al.,* 1999).

Variation in seed germination and survival: The percent germination was almost same in three seed-size classes of both the crosses under paper towel method while it differed significantly under sand bed method. But in a study on heavy and light hybrid seeds of four crosses of mulberry, Prasad *et al*., (1992) have not recorded any difference in percent seed germination in nursery bed. Such genotypic variation in seed germination has been reported in mulberry (Dandin *et al.,* 1991) and also in many others plants (Malavasi and Malavasi, 1996 and Mtmbalika *et al*., 2014). As it is reported in *Pongamia pinnata* (Manonmani *et al*., 1996), *Vateria indica* (Gunaga *et al*., 2007), *Mammia suriga* (Gunaga *et al.,* 2011), *Alangium lamarckii* (Ashirwar, 2012) and also in some multipurpose trees (Negi and Todaria, 1997), in the present study also the percent germination was minimum in small seeds (89.16 %) and maximum in large seeds (95.44 %). Such variations in

seed germination with seed-size and increase in germination percentage with increase in seed size have been reported (Ashirwar, 2012; Gunaga *et al.,* 2011; Mtmbalika *et al.*, 2014). On the contrary, better germination and seedling vigour in small to medium sized seeds than those of bigger ones has been reported in some other plants (Dar *et al.,* 2002; Peksen *et al.*, 2004; Farhoudi and Motamedi, 2010).

The survival of seedlings at 60 DAS was also found different among three seed-size classes and also found to increase with the increase in seed-size which is in agreement with the observation of Upadhya and Cabello (2000) in Irish potato (*Solanum tuberosum*). Murali (1997) has opined that seed germination and survival is controlled by many intrinsic and extrinsic factors and is species specific. Different size of seeds having different level of starch and other food storage may be one factor which influences the expression of germination and growth of the plants (Wood *et al.,* 1977). In *Arabidopsis thaliana*, Krannitz *et al.,* (1991) have recorded the effects of inter specific genetic differences in seed-size on seedling survival in the absence of external nutrient supply.

Table 1: Quantity in bulk lot, weight, length, width and percent germination of three size classes of F1 seeds and survival of their seedlings in Cross A and Cross B.

Parameter	Seed size classes in Cross A				Seed size classes in Cross B			
	Small	Medium	Large		Small	Medium	Large	
Quantity in bulk lot (%)	29.00	40.95	30.05	**	32.72	38.67	28.61	**
Weight of 100 seeds (g)	0.27	0.29	0.34	*	0.23	0.27	0.30	*
Length of seed (mm)	1.36	1.73	2.09	**	1.25	1.59	1.99	**
Width of seed (mm)	1.10	1.22	1.27	*	1.05	1.11	1.20	*
Germination on paper (%)	98.96	99.30	99.60	ns	98.70	98.90	99.15	ns
Germination in sand bed (%)	92.41	93.23	94.30	*	92.62	94.40	95.44	*
Seedling survival (%)	86.01	87.29	88.33	*	79.16	88.82	81.66	**

ANOVA and F-values

Source	df	Seeds in bulk lot	Length of seed	Width of seed	Weight of 100 seeds	Germination on paper	Germination in sand bed	Survival
Cross	1	0.02^{ns}	5.52^{*}	6.42^{**}	5.37^{*}	1.23^{ns}	2.61^{ns}	6.00^{*}
Seed size	2	128.97^{**}	425.80^{**}	7.46^{**}	8.68^{**}	0.88^{ns}	24.64^{**}	161.70^{**}
Cross x Seed size	2	12.26^{**}	0.75^{ns}	0.14^{ns}	0.22^{ns}	0.03^{ns}	7.41^{**}	9.79^{**}

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

Performance of seedlings in seed size classes

Data on the performance of seedlings measured in terms of four growth parameters at 60 DAS and 150 DAS are recorded in Table-2. It is evident that the seedlings of three seed-size classes of both the crosses differ significantly in their shoot length both at 60 DAS and 150 DAS. While studying the performance of four hybrid populations of mulberry, Prasad *et al.,* (1992) have observed that variability in seedling height was found to increase steadily up to 60 days and which later leveled up. They have also inferred that seed-size had no influence on seedling vigour at the later period. High degree of variations in seedling height (shoot length) in relation to seed-size has also been reported in other plants (Venkatesh *et al.,* 2003; Rezapour *et al.,* 2013; Mtmbalika *et al.,* 2014). The present study, revealed highly significant differences between the seedlings of all three seed-size classes of both the crosses in root collar diameter also which is in agreement with the observations of Gunaga *et al.,* (2011) and Mtmbalika *et al.,* (2014). Many studies on forest trees have clearly established that seedling height and root collar diameter provide the best estimate of seedling performance after out-planting (Mexal and Landis, 1990; Haase, 2008). In respect of root length and weight of seedlings, the seedlings of the three seed size classes in both the crosses have showed only significant differences. Based on these results it is inferred that shoot length and root collar diameter are comparatively more reliable parameters than root length and weight of seedlings to assess the performance of seedlings.

The present study established a positive correlation between seed-size and the performance of seedlings in the nursery. It is also indicated that large seeds are distinctly superior in terms of germination of seeds and survival and growth of seedlings especially over the seedlings of small seeds. Hence, it is inferred that size-grading of seeds and rejection of small seeds at nursery stage of screening may become essential steps to improve the efficiency of screening of F1 progeny in mulberry hybridization programme. In this part of screening, by sowing a total of 4200 seeds, 2700 seedlings are selected as promising ones to subject them for further testing in screening plot.

Identification of promising genotypes for screening under part-II

For the continuation of screening study under part-II, 450 healthy seedlings under each seed-size class were required. To identify these seedlings, 500 good seedlings from each seed-size class were studied with four parameters. The seedlings showing superiority in three out of four parameters studied were identified as superior genotypes, and selected for further studies.

Table 2: Shoot length, root collar diameter, root length and weight of seedlings belong to three size classes of F1 seeds in Cross A and Cross B.

Growth parameter	Seed size classes in Cross A				Seed size classes in Cross B			
	Small	Medium	Large		Small	Medium	Large	
60 Days After Sowing (DAS)								
Shoot length (cm)	8.30	11.65	13.15	**	7.30	10.55	12.40	**
150 Days After Sowing (DAS)								
Shoot length (cm)	33.20	47.70	54.71	**	30.20	48.36	51.30	**
Root collar diameter (mm)	3.50	4.80	5.44	**	3.18	4.82	5.08	**
Root length (cm)	32.31	33.02	34.29	*	31.45	32.06	33.26	*
Weight (g)	27.01	27.71	28.62	*	26.30	26.95	28.06	*

ANOVA and F-values

Source	df	60 DAS	150 DAS			
		Shoot length	Shoot length	Root collar diameter	Root length	Weight
Cross	1	21.37**	22.35**	30.00**	11.12**	5.55**
Seed size	2	101.44**	964.67**	855.82**	441.49**	170.91**
Cross x Seed size	2	1.00ns	9.71**	8.88**	26.81**	2.82**

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

Conclusion

The inference drawn with regard to the necessity of size-grading of seeds and rejection of small size seeds before sowing has to be decided only on the basis of performance of seedlings belong to small seed-size class in the screening plot. Also, the inference drawn with regard to the reliability of four seedling growth parameters has to be confirmed, only after studying the past performance of genotypes shortlisted under part-II screening study.

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