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Effect of seed rates on seed germination and seedling growth of Mulberry (*Morus* sp.)

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Mulberry (*Morus* sp.) is a fast growing, deciduous, woody perennial plant, usually pollinated by wind (anemophily). *Morus* sp. produces seed which is used to raise seedlings to be used for root grafting, whereas scion of an improved variety is grafted over the rootstock got through seedling raising. Seed rate is an important parameter that decides population of plants per unit area with optimum exposure to sunlight, proper space, aeration, and nutrition. In this study, we tried to find the optimum seed rate in mulberry for raising of quality seedlings under Kashmir climatic conditions. The investigation was carried out at College of Temperate Sericulture, Mirgund, SKUAST- Kashmir, wherein different seed rates were tested for seedling raising in mulberry. Amongst different seed rates tested, treatment T_3 wherein 28 seeds were sown per square foot was at par with treatments T_1 and T_2 having 20 and 24 seeds per sq. foot, respectively. The study indicated that 20-28 seeds per square foot could be successfully adopted for raising of quality seedlings.

Keywords*:* Anemophily, Rootstock, Sericulture

Sericulture is an agro-based industry and the final product of this industry is silk. Mulberry is recognized for its delicious fruit, which is consumed fresh, or in the form of juice. The fruit of mulberry is a multiple type and is regarded as third generation fruit. The mulberry (*Morus* sp.) leaf is the sole food source for the mulberry silkworm (*Bombyx mori* L.) and contributes 38.20% towards the success of a cocoon crop[1]. The silk industry depends on the mulberry plant which can be propagated through various methods *viz*., seeds, cuttings, layering, grafting, and tissue culture, etc. Mulberry can be easily propagated through cuttings, however has limitations which includes lack of variations resulting in reduced adaptability of daughter plants, restriction of raising only region-specific plant varieties, reduced vigour by subsequent generations and lack of tap root system in vegetatively propagated plants with least robustness in them[2]. Under temperate conditions of Kashmir, high yielding popular varieties of mulberry like Goshoerami, Ichinose and KNG, etc. suffer successful propagation through cuttings under open field conditions[3]. The poor rooting ability of these cuttings is due to due to low propagation rates and poor survival[4]. Such situations suggest propagation through seed for preparation of stock material.

Although a number of technologies for plant propagation are available in the field, stakeholders continue to adhere to the traditional system of propagation like grafting which encompasses the union between rootstock obtained from the seedlings and scion of desirable variety. For raising such stocks, sowing of mulberry seeds assumes importance as the root part of the seedlings raised after seed sowing is used as stock material. Further, seeds are also an important source to obtain hybrid plants in breeding studies. In Kashmir, the seeds are extracted from freshly collected ripe fruits in the month of June and are sown immediately to get seedlings. Although propagation of mulberry through grafting is resorted to heavily, germination percentage of seed sown and the number of seedlings raised continues to be far less than the expectation. Moreover, the seedlings raised are not healthy, and thus succumb to both biotic and abiotic stress leading to increased cost of production of mulberry plants to be supplied to field for plantation.

Germination of mulberry seed and the subsequent growth of seedlings are affected by various factors[5,6]. The ‘seed rate’ in the nursery bed plays an important role in the germination and overall growth and development of the mulberry seedlings. Seed rate is the quantity of seed sown per unit area with optimum exposure to sunlight, proper space, aeration, and nutrition. For obtaining higher yield as well as quality crop, the optimum seeding rate is one of the important production factors. Seed densities above the optimum may increase disease incidence and lodging, production costs, and reduce plant height and yield. Keeping the above facts in view, the present study was undertaken, to find the optimum seed rate in mulberry for raising quality seedlings of *Morus* sp. under Kashmir climatic conditions.

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Material and Methods

The experiment was conducted at the College of Temperate Sericulture, Mirgund (SKUAST-Kashmir) during 2015. Ripe fruits of mulberry were collected randomly from mulberry plants in the month of June. The fruit was stored in air tight room at ambient conditions for 3-4 days for easy kneading, after which the seeds were extracted from the fruit. After extraction the seeds were shade-dried. The seed viability was confirmed by tetrazolium chloride and flotation tests. The seeds were pre-soaked in water for 24 h and sown during the first week of July in polytubes having an exposed area of one square foot. The polytubes were filled with a medium comprising of garden soil and well decomposed Farm yard manure (FYM) by mixing 100 g of FYM per 2 kg of garden soil. The design used was 'completely randomized design' (CRD) with eight treatments (T_1-T_8 with 20, 24, 28, 32, 36, 40, 44 and 48 seeds/ sq. ft., respectively). To preserve moisture and protect the polybags from rains, locally available dried weeds were used as mulches[7,8]. Each treatment was replicated thrice. Observations were recorded after 11th day of germination on the following parameters.

Germination percentage rate

Germination started after eleven days of sowing the seeds. From 11th day observations were taken regularly and germinated seeds were counted daily to calculate germination percentage. It was calculated according to the formula as in Sagar *et al*.[9].

$$\text{Germination percentage} = \frac{\text{No.of seeds germinated}}{\text{No.of seeds sown}} \times 100$$

The germination rate was calculated by the formula as per Wani *et al*.[10].

R= $\sum n/\sum Dn$ ("R" is the germination rate; "n" is the number of seeds germinated in days and "D" is the days counted from the beginning of the test.)

Roots per seedling, root length & weight

To calculate roots per seedlings, the seedlings were uprooted, washed thoroughly to remove the adhering soil and the roots counted manually. The length of the longest root was measured using a normal scale in centimeter from the base to the tip of the seedling. To weigh the whole root, it was trimmed from the seedling at the origin and was dried between the folds of a blotting paper. The weight of the root portion of these seedlings was recorded in grams using a digital balance. After that, the average value of five seedlings was taken.

Shoot weight

The shoot left after cutting the root portion of the seedlings was weighed one by one for five seedlings using the same digital balance in each treatment to calculate the average shoot weight.

Root volume "mL"

The root mass after was dried in the blotting paper and was used for root volume estimation using a graduated glass cylinder by water displacement technique. Whole root mass was dipped completely in the water present in a cylinder and the rise in water level was used to calculate root volume.

Seedling thickness & height and Leaves per seedling

The thickness of the seedlings was measure by Vernier Calliper. Three readings of each seedling were taken at three different places *viz*., bottom, a middle and top portion and then average thickness per seedling was calculated. Similarly, the height of seedling was measured by a normal scale in centimeter from base to the tip of the seedlings.

The leaves of the seedlings were counted manually.

Root-shoot ratio

The root-shoot ratio was calculated as per the formula given by Wani[11]:

$$\text{Root-shoot ratio} = \frac{\text{Weight of the root}}{\text{Weight of shoot}}$$

Seedling vigour index (SVI)

The seedling vigour index was computed by the formula suggested Shatpathy *et al*.[12]:

SVI= Germination (%) × Seedling length "cm"

For all the parameters except germination percentage and germination rate, five seedlings per treatment per replication were taken to calculate the average value. The day of sowing was taken as the first day and the total number of seeds germinated on each day was counted and recorded.

Statistical analysis

The data collected was compiled and analysed statistically. The significance of 'F' & 't' was tested at a 5% level of significance. The software package used for analysis was "OPstat". Whenever the F test was found significant at 5% probability, critical difference values were used to compare the treatment means.

Results and Discussion

Germination rate and percentage

The germination rate was maximum (0.085) in T_1 which was statistically at par with T_2 and T_3 and

Table 1— Effect of seed rates on the germination of seed, shoot and root parameters of mulberry seedlings

Treatment (no. of seeds per sq. ft.)	Total no. of SG	No. of days for GN	GR	G%	Height of seedling "cm"	LPS	Mean thickness of seedling "cm"	Dry shoot wt. "g"	SVI	LRL "cm"	RPS	Root volume "mL"	Dry root wt. "g"	Root-Shoot ratio
$T_{1=20}$	17.00	11.67	0.085^{a}	85.00^{a}	15.57^{a}	10.06^{a}	0.33^{a}	0.83^{a}	1323.5^{a}	15.20^{a}	10.60^{a}	0.91^{a}	0.64^{a}	0.77^{a}
$T_{2=24}$	20.33	12.00	0.083^{a}	84.60^{a}	16.06^{a}	10.17^{a}	0.32^{a}	0.84^{a}	1358.7^{a}	15.47^{a}	10.30^{a}	0.87^{a}	0.65^{a}	0.77^{a}
$T_{3=28}$	23.33	12.00	0.083^{a}	83.30^{a}	16.00^{a}	10.03^{a}	0.30^{a}	0.82^{a}	1332.8^{a}	15.20^{a}	10.53^{a}	0.86^{a}	0.62^{a}	0.76^{a}
$T_{4=32}$	21.00	14.00	0.071^{b}	65.60^{b}	12.36^{b}	8.73^{b}	0.31^{a}	0.77^{b}	810.7^{c}	13.90^{b}	8.17^{b}	0.75^{b}	0.49^{b}	0.64^{b}
$T_{5=36}$	23.33	15.00	0.066^{b}	64.73^{b}	10.33^{c}	7.25^{c}	0.28^{b}	0.69^{c}	668.8^{d}	12.40^{c}	6.33^{c}	0.72^{b}	0.42^{c}	0.61^{b}
$T_{6=40}$	21.67	15.00	0.066^{b}	54.70^{c}	10.26^{c}	7.20^{c}	0.24^{c}	0.63^{c}	561.2^{e}	12.10^{c}	5.60^{d}	0.72^{b}	0.37^{d}	0.57^{b}
$T_{7=44}$	21.33	16.00	0.063^{c}	48.47^{d}	9.54^{c}	6.53^{d}	0.24^{c}	0.61^{d}	462.2^{f}	10.47^{d}	4.13^{e}	0.62^{c}	0.27^{e}	0.44^{c}
$T_{8=48}$	19.33	16.00	0.063^{c}	40.20^{e}	8.16^{d}	5.33^{e}	0.23^{c}	0.54^{e}	328.9^{g}	9.03^{e}	3.57^{f}	0.52^{d}	0.22^{f}	0.41^{c}
CD ($P \leq 0.05$)	-	-	0.007	4.50	0.84	0.45	0.03	0.06	81.9	0.81	0.50	0.05	0.03	0.07
SEm±	-	-	0.002	1.49	0.28	0.15	0.01	0.02	27.3	0.27	0.17	0.02	0.01	0.02
CV%	-	-	6.735	3.92	3.97	3.13	3.73	5.01	5.6	3.53	3.89	3.81	4.03	6.04

[SG, seeds germinated; GN, germination; GR, Germination rate; G%, germination percentage; SVI, Seedling vigour index; LRL, Longest root length; LPS, Leaves per seedlings; RPS, Roots per seedling]

significantly different from T_4, T_5, T_6, T_7, and T_8 (Table 1). The minimum germination rate (0.063 each) was recorded in T_7 and T_8 where 44 and 48 seeds were sown per sq. ft., respectively. Germination percentage was maximum (85%) in T_1. This was statistically at par with T_2 and T_3, where the germination percentage was 84.60 and 83.30%, respectively as shown in Fig. 1. The values were significantly different from T_4, T_5, T_6, T_7, and T_8. The least germination percentage (40.20%) was observed in T_8 where 48 seeds were sown per sq. ft. The results showed that the germination percentage and rate gradually decreased with increasing seed rates. The decrease in germination rate and percentage may probably be due to an increase in carbon dioxide concentration which in turn decreases oxygen level. Another possible reason could be that at high seed density much mortality occurs because high seed rate is more susceptible to attack by soil pathogens and hence becomes a constraint for seed germination[13,14]. At high density, much mortality occurs than at lower densities and consequently, a greater number of seeds germinate earlier in lower densities than at higher densities.

Shoot parameters of the mulberry seedlings

The seedling height was highest (16.06 cm in T_2, which was statistically at par with T_1, and T_3 registering respectively an average height of 15.57 and 16.0 cm. The values were significantly different from the values recorded in T_4, T_5, T_6, T_7, and T_8. The seedling height was the least (8.16 cm) in T_8. The number of leaves per seedling was highest (10.17) in T_2, which was statistically at par with 10.06 and 10.03 leaves recorded respectively in T_1 and T_3. The values were again significantly higher than the values recorded in other treatments T_4, T_5, T_6, T_7, and T_8. The least number (5.33) of leaves per seedling was recorded in T_8 where 48 seeds were sown per square foot. The thickness of seedling was maximum (0.33 cm) in T_1 which was statistically at par with T_2 and T_3, where the seedling thickness was 0.32 and 0.30 cm, respectively. The values were statistically significant over the values recorded in the rest of the treatments T_4, T_5, T_6, T_7, and T_8. T_8 where 48 seeds were sown per square foot registered the least value of thickness (0.23 cm). The highest value (0.84 g) for shoot weight was registered in T_2, which was statistically at par again with T_1 and T_3 and statistically significant over the values recorded in T_4, T_5, T_6, T_7, and T_8. The least value (0.54 g) for shoot weight also was observed in T_8 where 48 seeds were sown per sq. foot. Seedling vigour index was maximum (1358.7) in T_2. It was however at par with the values recorded in T_1 and T_3 where the seedling vigour index was 1323.5 and 1332.8, respectively. The least value for seedling vigour index (328.9) was observed in T_8.

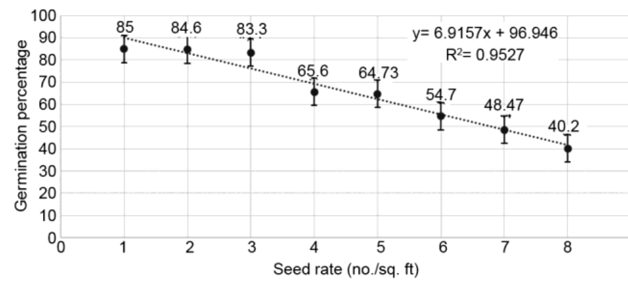

Fig. 1— Relation between germination percentage and seed rate

Seedling height, thickness, dry shoot weight, number of leaves per seedling and seedling vigour index also were comparatively better at lower seeding rates (T_1 to T_3) and usually showed a decline with an increase in seed rate. The above ground parameters such as seedling height, dry shoot weight, and leaves per seedling are comparatively better at lower seed rates than higher seed rates as demonstrated earlier[15-18]. The decline in the above ground growth parameters of mulberry seedlings beyond 28 seeds per square foot (T_3) could be due to increased competition between the seedlings for food, light, and space and the competition starts as early as germination[19]. The appropriate density of plants per unit area leads to better use of nutrients, moisture, and light resulting in better growth of plants. Low interplant competition among the seedlings at lower seed rates could be the reason for producing seedlings having a greater number of leaves per seedling, besides higher root, and shoot length. High density decreased the height of the seedlings which could be attributed to restrictions on plant food sources[20]. At lower density, more dry matter accumulation due to better solar radiation penetration thus increased dry shoot weight, the thickness of seedling, number of leaves per seedling. Increased plant density has been shown to have decreased dry shoot weight, number of leaves and thickness of seedling in sunflower[18]. Seedling vigour index too was better at lower seeding rate i.e., 20-28 seeds per square foot. Better source-sink relationship at lower densities of the plants could result in better accumulation and assimilation of photosynthates into the sink that resulted in better SVI at wider spacing or lower seed rate. Seedlings at low density have better vigour index. Similar findings have been reported in mungbean[21].

Root parameters of the mulberry seedlings

The length of the longest root was maximum (15.47 cm) in T_2, which was statistically at par with the values recorded for T_1 and T_3 (15.20 each) and significantly different from the values recorded in T_4, T_5, T_6, T_7, and T_8 (Table 1). The lowest value of root length (9.03 cm) was found in T_8 where 48 seeds were sown per sq. ft. The number of roots per seedling was maximum (10.60) in T_1, which was statistically at par weight 10.53 and 10.30 roots per seedling recorded respectively in T_3 and T_2. The values were significantly higher than the values recorded in T_4, T_5, T_6, T_7, and T_8. The lowest number of roots per seedling (3.57) was found in T_8 where 48 seeds were sown per sq. ft. Root volume was maximum (0.91 mL) in T_1, which was statistically at par with T_2 and T_3, recording respectively the root volume of 0.87 and 0.86 mL. The values were statistically significant over the values recorded in rest of the treatments amongst which the least value (0.52 mL) was recorded in T_8 where 48 seeds were sown per sq. ft. Root weight was maximum (0.65 g) in T_1 (20 seeds per sq. ft.), which was statistically at par with the root weight of 0.64 and 0.62 g, respectively in T_2 (24 seeds per sq. ft.) and T_3 (28 seeds per sq. ft.) and significantly different from the values recorded in other treatments. The lowest value of root weight (0.22 g) was recorded in T_8 where 48 seeds were sown per square foot. T_1 and T_2, registered the maximum root-shoot ratio (0.77 each), which was statistically at par with T_3, registering the root-shoot ratio of 0.76 and significantly different from T_4, T_5, T_6, T_7, and T_8. The lowest value of the root-shoot ratio (0.41) was found in T_8 where 48 seeds were sown per square foot. The rooting parameters of mulberry seedlings *viz*., longest root length, number of roots per seedling, root volume, dry shoot weight, and root-shoot ratio were higher in the seed rates from T_1 to T_3 involving 20-28 seeds per square foot and thereafter showed a decreasing trend. The possible reasons could be the availability of more space which favours quick differentiation and development of root tissues, offers little obstruction for spreading of roots in the medium, hence increased length of longest root, number of roots per seedling, dry root weight and root volume. Root length, number of per seedling and root volume increases under wider spacing. Similar results were reported in black gram[22] and in soyabean[23]. Overall, the growth and development of mulberry seedlings was better at the seed rate 20-28 under the temperate conditions of Kashmir.

Conclusion

Observations in the present study suggest sowing mulberry seed at the rate of 20-28 seeds per square foot seemed to be the best rate of seed to be sown in the given area for optimal germination percentage, germination rate, seedling height, number of leaves per seedling, mean thickness of seedling, dry shoot weight, seedling vigour index, length of longest root, number of roots, dry root weight, root-shoot ratio compared to other treatments. At this rate, the seed sown along with the growing seedlings possibly had proper space and distance for optimum sunlight penetration and proper space for the root system to develop and subsequent uptake of water and soil nutrients which resulted in proper growth and development of the mulberry seedlings. This rate shall ensure effective use of seeds and land available for producing more seedlings per unit area for graft making which is the conventional method of raising mulberry plants in the valley.

Conflict of interest

The authors declare no conflict of interest.

References

1 Wani MY, Mir MR, Mehraj S, Rather RA, Ganie NA, Baqual MF, Sahaf KA & Hussain A, Effect of different types of mulches on the germination and seedling growth of mulberry (*Morus* sp.). *Int J Chem Stud*, 6 (2018) 1364.

2 Leaky RRB, Plant Cloning: Macro-Propagation. In: *Encyclopedia of Agriculture and Food Systems*, Vol. 4, (Eds. ; Elsevier, Amsterdam), 2014, 349.

3 Prakash K, Nivedha D, Pushpadarini RM, Ramazeame K, Vasanth L, Dhivya S, Dinesh G, Raghavi V, Logesh R, Nirmala P, Thivagaran R, Priyadharsini B, Bhuvaneswari R, Devaraj S, A & Ishwarya M, Root and shoot growth of semi-hard wood cuttings of Mulberry (*Morus indica* L.) influenced by water imbibitions using wet cloth wrapping technique. *Int J Scient Progr Res*, 7 (2017) 2250.

4 Casales FG, Van der Watt E & Coetzer GM, Propagation of Pecan (*Carya illinoensis*): A review. *Afr J Biotechnol*, 17 (2018) 156.

5 Ugena L, Hylova A, Podlesakova K, Humplik JF, Dolezal K, Diego ND & Spichal L, Characterization of biostimulant mode of action using novel multi-trait high-throughput screening of Arabidopsis germination and rosette growth. *Front Plant Sci*, 9 (2018) 1327.

6 Ramu VS, Paramanantham A, Ramegowda V, Mohan-Raju B, Udayakumar M & Senthil Kumar M, Transcriptome analysis of sunflower genotypes with contrasting oxidative stress tolerance reveals individual and combined biotic and abiotic stress tolerance mechanisms. *PLoSONE*, 11 (2016) 1.

7 Wani MY, Mir MR, Baqual MF, Zia-ul-Haque S, Lone BA, Maqbool SA & Dar SA, Influence of different manures on the germination and seedling growth of mulberry (*Morus* sp.). *J Pharmacogn Phytochem*, 6 (2017) 04.

8 Wani MY, Mir MR, Mehraj S, Rather RA, Ganie NA, Baqual MF, Sahaf KA & Hussain A, Effect of different types of mulches on the germination and seedling growth of mulberry (*Morus SP.*). *Int J Chem Stud*, 6 (2018) 1367.

9 Sagar A, Tajkia JE, Haque ME, Fakir MSA & Hossain, AKMZ, Screening of sorghum genotypes for salt-tolerance based on seed germination and seedling stage. *Fundamental Appl Agric*, 4 (2019) 735.

10 Wani MA, Khan FU, Nazki IT, Din A, Iqbal S & Qadri ZA, Influence of various treatments on pre and post germination properties of *Callistephus chinensis* (L.) NEES cv. Powderpuff. *Bangladesh J Bot*, 48 (2019) 449.

11 Wani MA, Studies on flowering and seed production of China aster (*Callistephus chinensis* L.) cv. Powder puff. Ph. D. thesis submitted to SKUAST-K (2018) 163.

12 Shatpathy P, Kar M, Dwibedi SK & Dash A, Seed priming with salicylic acid improves germination and seedling growth of rice (*Oryza sativa* L.) under PEG-6000 induced water stress. *Int J Curr Microbiol Appl Sci*, 7 (2018): 907.

13 Phil S, Allen PS, Finch-Boekweg H & Meyer SE, A proposed mechanism for high pathogen-caused mortality in the seed bank of an invasive annual grass. *Fungal Ecol*, 35 (2018) 108.

14 Kumar P, Phor SK, Mor VS, Kumar S & Mittal S, to study the effect of seed rate and row spacing on quality parameters of fenugreek (*Trigonella foenum-graecum* L.). *Int J Chem Stud*, 6 (2018) 454.

15 Ram H, Singh JP, Bohra JS, Singh RK & Sutaliya JM, Effect of seedlings age and plant spacing on growth, yield, nutrient uptake and economics of rice (*Oryza sativa*) genotypes under system of rice intensification. *Indian J Agron*, 59 (2014) 256.

16 Vijayalaxmi G, Sreenivas G, Rani PL & Madhavi A, Effect of plant densities and age of seedlings on growth and yield parameters of kharif rice. *Int J Sci Environ Technol*, 5(2016) 1153.

17 Garcia MG, Bazo GL, Braccini AL, Lima LH, Suzukawa AK & Hossa KR. Effects of different sowing dates and row spacing on physiological quality of canola seed. *Aust J Crop Sci*, 10 (2016) 1417.

18 Fani E. The effect of seed density on yield and yield components of irrigated wheat in the North East of Khouzestan. *Agric Sci Dev*, 3 (2014) 292.

19 Keddy PA & Cahill J. Competition in plant communities. *Plant Ecol*, 4 (2018) 123.

20 Khosravi M. The effect of seed density yield components of irrigated wheat. *Agric Sci Dev*, 3 (2014) 992.

21 Shantharaja CS, Mahajan SS, Rajora MP & Bhatt RK. Effect of sowing dates, plant density, seed treatment and fertilizers on performance and quality seed production in Mungbean. *Vegetos*, 29 (2016) 2.

22 Amruta N, Devaraju PJ, Mangalagowri SP, Kiran HP & Teli K. Effect of integrated nutrient management and spacing on seed quality parameters of black gram. *J Appl Nat Sci*, 8 (2016) 340.

23 Mahesh K, Balaraju P, Ramakrishna B & Ram-Rao SS Effect of brassinosteroids on germination and seedling growth of radish (*Raphanus sativus* L.). *Am J Plant Sci*, 4 (2013) 2305.