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Standardization of pit size and organic filling mixture for raising tissue cultured date palm in hot arid region of western Rajasthan

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ABSTRACT

The study aims to provide comprehensive guidelines for optimizing pit size and the composition of filling mixtures, thereby fostering the growth, root development and overall health of tissue cultured Barhee date palm cultivars. The experiment was undertaken in 2019- 2021 at the ICAR-Central Institute for Arid Horticulture research farm located in Bikaner, Rajasthan, India. Fully hardened tissue-cultured saplings of the Barhee cultivar of date palm were planted with a spacing of 5 x 5 m in the month of July. Different planting pit sizes (1.0 m x 1.0 m x 1.0 m, 0.75 m x 0.75 m x 0.75 m, and 0.50 m x 0.50 m x 0.50 m) and filling mixtures of field soil, clay and FYM (1.0: 1.0:1.0, 2.0:0.5:0.5, 0.5:2.0:0.5 and 0.5:0.5:2.0) were used as treatment combinations for evaluating the growth of plants. The results indicated that tissue-cultured plants of the Barhee cultivar raised in a pit size of 1 m^3 with a filling mixture of field soil, clay, and FYM in the ratio of 0.5:0.5:2.0 performed better.

Introduction

The date palm (*Phoenix dactylifera* L.) is a member of the Arecaceae (Palmae) family and stands as one of the earliest cultivated fruit plants in human history. According to archaeological findings, Iraq is recognized as the source of origin date palm (Johnson *et al.* 2013). In India, the cultivation of date palms is primarily concentrated in the western parts of Rajasthan, the Rann of Kutch and the semi-arid regions of Gujarat and Punjab area which also known for its harsh, hot and arid climatic conditions (Bhansali, 2009). Rajasthan occupies almost 60% of the total of these regions. However, this region is known for its unforgiving environment characterized by harsh climatic conditions, arid soil with high salinity, extremely low rainfall, extreme temperature fluctuations and poor soil fertility.

Tissue culture has revolutionized date palm cultivation by providing disease-free and genetically consistent plantlets, offering a promising solution to strengthen date palm production in this region. Among the various imported tissue-cultured date palms, the Barhee cultivar has emerged as a prominent choice. Nevertheless, the success of tissue-cultured Barhee date palms in Western

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Rajasthan hinges on several critical factors such as the size of the planting pit and the composition of the filling mixture which determine root and shoot development and hence productivity of crop.

The size of the planting pit directly influences root development through prolonged moisture retention and increased access to plant nutrients, while the composition of the filling mixture significantly impacts soil structure, aeration and moisture-holding capacity (Mauki and Kilonzo, 2022).

Pitting size and filling mixtures has been one of the most important aspects of tree planting programs that are crucial for tree survival (Jo and Park, 2017). Lack of information about appropriate pits size and filling mixture have been reported as major constraints in raising tissue cultured date palm particularly in regions featured by unreliable rainfall and low soil productivity (Ndegwa *et al.*, 2017). Different pitting size and filling mixture are important in improving root-soil contact, improves soil aeration and water infiltration (Allan, 1998). Therefore, this study aimed at size of planting pit and filling mixtures for the successful tree sapling establishment in arid regions of Rajasthan. Thus, this study aspires to offer practical recommendations for date palm growers in Western Rajasthan, enabling them to refine their practices and optimize the cultivation of Barhee date palms in this demanding environment.

Materials and Methods

The experiment was conducted during 2019-2021 at ICAR-Central Institute for Arid Horticulture, Bikaner (The experimental site was situated on 28°N latitude, 73°18'E longitude and at an altitude of 234.84 m above sea level) Rajasthan, India. The soil of the experimental site was sandy, desertic, poor in fertility and water-holding capacity. The soil nutrient content was examined at three different depths (30-90 cm depth) and samples were analysed and recorded. The average rainfall of this region was about 230 mm/annum. May-June was the hottest month (mean maximum temperature 42.9°C and mean minimum temperature 29.6°C) and December-January was the coldest month of the year (mean maximum temperature 23.7°C and mean minimum temperature 8.9°C). Occasional frost was also experienced during January and February.

This site was chosen due to its suitability for stimulating the arid conditions prevalent in the region. The detailed soil properties are given in Table 1. The treatment consisted of three pit sizes and four filling mixtures (Table 2). The experiments were laid out in a factorial randomized block design with three replications. Fully hardened tissue-cultured saplings of the Barhee cultivar of date palm, each possessing 2-3 leaves, were planted with the spacing of 5 x 5 m.

Table 1. Physico-chemical properties of experimental site

S.No.	Characteristics	Mean value	Reference
1.	Particle size analysis		Bouyoucos, 1962
	Sand (%)	89.50	
	Silt (%)	06.50	
	Clay (%)	04.00	
	Textural class	Loamy sand	
2.	Bulk density (Mg/m^{-3})	1.60	Black, 1965
3.	Particle density (Mg/m^{-3})	2.65	Black, 1965
4.	Water holding capacity (%)	5.60	Black, 1965
5.	Infiltration rate mm hr^{-1})	24	Johnson, 1963
6.	pH (1:2)	8.5	Jackson, 1979
7.	Electrical conductivity (dSm^{-1}) (1:2)	1.10	Jackson, 1979
8.	Organic carbon (g kg^{-1})	0.09	Walkley and Black ,1934
9.	Available nitrogen (kg ha^{-1})	130	Subbiah and Asija, 1956
10.	Available phosphorus (kg ha^{-1})	15	Olsen *et al.*, 1954
11.	Available potassium (kg ha^{-1})	164	Jackson, 1973
10.	DTPA extractable iron (mg kg^{-1})	29.60	Lindsay and Norvell, 1978
11.	DTPA extractable zinc (mg kg^{-1})	0.35	Lindsay and Norvell, 1978
12.	DTPA extractable copper (mg kg^{-1})	2.60	Lindsay and Norvell, 1978
13.	DTPA extractable manganese (mg kg^{-1})	13.54	Lindsay and Norvell, 1978

Table 2. Experiment details

Pit sizes (P)	1.00 m x 1.00 m x 1.00 m	P1
	0.75 m x 0.75 m x 0.75 m	P2
	0.50 m x 0.50 m x 0.50 m	P3
Filling mixtures (M)	1.0 : 1.0 : 1.0 (Field soil: Clay: FYM)	M1
	2.0 : 0.5 : 0.5 (Field soil: Clay: FYM)	M2
	0.5 : 2.0 : 0.5 (Field soil: Clay: FYM)	M3
	0.5 : 0.5 : 2.0 (Field soil: Clay: FYM)	M4

Data collection

Vegetative parameters

Parameters like plant height, number of leaves and plant spread were recorded after 12 and 24 months after planting. The plant growth parameters like height, plant spread was recorded with the help of steel tape and the number of leaves by manual counting method.

Leaf nutrient status

For date palm middle part of the physiologically mature leaves was taken for analysis of N, P, K, zinc and iron determination. One gram oven-dried grounded sample was taken in a digestion tube to which 1m mL of diacid mixture (nitric acid: perchloric acid = 5:2) zinc and iron analysis and 10 mL were added and triacid mixture (nitric acid: perchloric acid: sulphuric acid = 9: 4: 1) for P and K analysis were added. The contents were kept overnight for pre-digestion. The tubes were loaded into the digester and then heated the digestion chamber. The temperature was raised to 200 degrees centigrade for 2-3 hours till the solution turned colorless with cessation of emission of white dense fumes from the digesting samples. The tubes were kept for cooling down. The digested material was then filtered and volume made up to 100 mL with distilled water and stored for further estimation. The determination of total N and P content were carried out by using the modified micro Kjeldahl method and vanadomolybdate yellow color method, respectively. The zinc and iron were determined by using atomic absorption spectrophotometer. Petiole samples were prepared with a laboratory homogenizer using about 0.5 g of fresh material. 80% acetone was used as an extraction solvent. The total chlorophyll content (a + b) was analytically determined using a spectrophotometer, measuring absorbance at wavelengths of 662 nm and 644 nm. The chlorophyll content was then calculated using the formula provided by Witham *et al*. (1971).

Statistical Analysis

Data obtained on various characters were analyzed statistically according to the factorial randomized block design as suggested by Gomez and Gomez (1984). The critical difference (CD) was calculated to understand the significance or non-significance of difference between treatment means at 5% level of significance.

Results and Discussion

Vegetative parameters

Results revealed that the plant vegetative growth was significantly affected by the different treatments. After 12 and 24 months of planting the highest plant height, number of leaves i.e., 118.00 cm and 169.15 cm, (7.38 and 11.40) were observed in plants under the pit size of 1m^3, respectively. Whereas, the highest plant height (120.45 and 160.20 cm), number of leaves (7.33 and 10.57) were recorded under filling mixture M4 (Table 3). The interaction effect between pit sizes and filling mixtures resulted in significant differences in plant growth. Specifically, the combination of pit size P1 and filling mixture M4 produced the highest plant height, with measurements of 130.85 cm and 185.60 cm, respectively. Additionally, this combination led to the highest number of leaves, with counts of 8 and 13.5. (Table 3 and 4). Furthermore, data presented in Table 5 reveals that plants growing under filling mixture M4 produced maximum plant spread (93.3 x 91.7 and 170.0 x 171.7) after 12 and 24 months after the planting, respectively. The maximum plant spread (95.0 x 96.0 and 178.8 x 181.2) was found in plants under the pit size P1 after 12 and 14 months after planting, respectively. The interaction effect between pit size and filling mixture was significant, with the maximum plant spread observed in the combination of pit size P1 and filling mixture M4. The findings of this study align with previous research conducted by Pahla*et al*. (2013) and Amanullah *et al*. (2013). Their research indicated that the performance of planted seedlings, specifically in terms of height, is positively affected by an increased application of manure. Vincent and Davies (2003) further elucidated/that larger planting pit sizes were associated with several advantages for seedlings. These advantages include a reduction in soil compaction within the rooting zone, enhanced water infiltration, and increased water availability during the critical early stages of establishment, all of which collectively influence tree growth positively. These results are consistent with earlier studies conducted by Ghafoor and Gopan (1988), Abdel-Hameed and Ragab (2004), Mohamed and Gobara (2004), Mansour *et al.* (2004) and Diab (2006) documented favorable effects on vegetative growth and leaf nutrient composition when different date palm cultivars received annual applications of organic fertilizers.

Among the different filling mixtures, M4 provided more FYM to the plant. FYM provides a slow-release source of nutrients, including nitrogen, phosphorus and potassium, which are crucial for plant growth (Tang *et al.*, 2007 and Yang *et al.*, 2004). Moreover, FYM enhances the physical properties of soil. It improves soil structure, increases water-holding capacity and promotes better aeration. This improved soil environment is conducive to root development, which is a fundamental component of vegetative growth. A healthy and well-developed root system can efficiently absorb water and nutrients from the soil, providing the necessary support for above-ground vegetative growth.

Leaf nutrient content

The maximum values of the leaf nitrogen (1.69 and 1.70%) and phosphorous content (0.28 and 0.28%) of the leaf were obtained on the palm that growing under P1 and filling mixture M4, respectively. The interaction effect between two factors was found to be significant and the highest leaf nitrogen and phosphorous content was found maximum under P1M4 *i.e.* 1.80 and 0.30% respectively (Table 6). Moreover, the highest potassium content was exhibited by plants grown under the pit size P1 (2.08%) and filling mixture M1 and M2 (2.02%) compared to the other treatments. The pit size and the composition of filling

Table 3. Effect of pit sizes and filling mixtures on the plant height (cm) of tissue cultured date palm cv. Barhee

Pit size	After 12 of planting					After 24 months of planting				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
P1	125.25	105.40	110.50	130.85	118.00	175.50	155.00	160.50	185.60	169.15
P2	110.00	105.00	115.00	120.50	112.63	150.50	135.40	140.50	155.00	145.35
P3	100.80	90.00	95.00	110.00	98.95	135.50	125.30	132.20	140.00	133.25
	112.01	100.13	106.83	120.45		153.83	138.57	144.40	160.20	

	After 12 of planting		After 24 months of planting
SEm±	Pit size	1.235	Pit size	2.114
	Filling mixture	2.546	Filling mixture	3.213
	Pit size x Filling mixture	1.980	Pit size x Filling mixture	2.876
LSD (0.05)	Pit size	2.964	Pit size	5.074
	Filling mixture	6.110	Filling mixture	7.895
	Pit size x Filling mixture	4.951	Pit size x Filling mixture	6.902

Table 4. Effect of pit sizes and filling mixtures on the number of leaves of tissue cultured date palm cv. Barhee

Pit size	After 12 of planting					After 24 months of planting				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
P1	7.5	7.0	7.0	8.0	7.38	12.6	10.0	9.5	13.5	11.40
P2	6.6	6.2	6.8	7.0	6.65	9.8	7.8	7.5	10.2	8.28
P3	6.5	6.0	6.0	7.0	6.38	8.2	7.8	7.5	8.0	7.88
	6.87	6.40	6.60	7.33		10.20	8.53	8.17	10.57	

	After 12 of planting		After 24 months of planting	
SEm±	Pit size	0.465	Pit size	0.362
	Filling mixture	0.785	Filling mixture	0.386
	Pit size x Filling mixture	0.643	Pit size x Filling mixture	0.354
LSD (0.05)	Pit size	NS	Pit size	0.969
	Filling mixture	NS	Filling mixture	0.947
	Pit size x Filling mixture	NS	Pit size x Filling mixture	0.885

Table 5. Effect of pit sizes and filling mixtures on the plant spread (cm) of tissue cultured date palm cv. Barhee

Filling mixture	Pit size							
	After 12 month				After 24 months			
	P1	P2	P3	Mean	P1	P2	P3	Mean
M1	95 x 100	90 x 90	80 x 82	88.3x 90.7	190 x 185	155 x 150	140 x 145	160.7 x 160.0
M2	90 x 94	85 x 85	80 x 78	85.0 x 85.7	160 x 165	135 x 140	135 x 130	143.3 x 145.0
M3	95 x 90	85 x 82	75 x 75	85.0 x 83.3	165 x 170	135 x 125	135 x 130	145.0 x 141.7
M4	100 x 100	95 x 90	85 x 85	93.3 x 91.7	200 x 205	165 x 160	145 x 150	170.0 x 171.7
Mean	95.0 x 96.0	88.8 x 86.0	85 x 85		178.8 x 181.2	147.5 x 143.8	138.8x 138.8	

	After 12 month		After 24 months
SEm± Pit size	2.210 x 1.976	SEm± Pit size	3.243 x 2.975
Filling mixture	1.768 x 1.675	Filling mixture	2.753 x 2.435
Pit size x Filling mixture	1.892 x 1.647	Pit size x Filling mixture	2.895 x 2.657
LSD (0.05) Pit size	5.145 x 4.472	LSD (0.05) Pit size	7.132 x 6.342
Filling mixture	4.243 x 4.103	Filling mixture	6.242 x 6.132
Pit size x Filling mixture	4.540 x 4.117	Pit size x Filling mixture	6.580 x 6.139

Table 6. Effect of pit sizes and filling mixtures on the nitrogen and phosphorus in leaves of tissue cultured date palm cv. Banshee

Pit size	Nitrogen (%)					Phosphorus (%)				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
P1	1.75	1.60	1.60	1.80	1.69	0.29	0.27	0.27	0.30	0.28
P2	1.68	1.60	1.61	1.68	1.64	0.27	0.26	0.25	0.28	0.27
P3	1.60	1.58	1.60	1.62	1.60	0.26	0.26	0.26	0.27	0.26
	1.68	1.60	1.60	1.70		0.27	0.26	0.26	0.28	

	Nitrogen		Phosphorus
SEm± Pit size	0.021	SEm± Pit size	0.036
Filling mixture	0.031	Filling mixture	0.045
Pit size x Filling mixture	0.026	Pit size x Filling mixture	0.040
LSD (0.05) Pit size	0.051	LSD (0.05) Pit size	0.090
Filling mixture	0.078	Filling mixture	0.110
Pit size x Filling mixture	0.064	Pit size x Filling mixture	0.885

Table 7. Effect of pit sizes and filling mixtures on the potassium, zinc, and status in leaves of tissue cultured date palm cv. Barhee

Pit size	Potassium (%)					Zinc (ppm)				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
P1	2.10	2.05	2.05	2.10	2.08	78.25	75.40	75.00	80.00	77.16
P2	1.98	1.98	1.95	1.98	1.97	76.65	75.00	75.35	76.50	75.87
P3	1.98	1.98	1.97	1.98	1.98	75.00	75.00	75.25	76.00	75.31
	2.02	2.00	1.99	2.02		76.63	75.13	75.20	77.50	

	Potassium		Zinc
SEm± Pit size	0.026	SEm± Pit size	0.362
Filling mixture	0.037	Filling mixture	0.386
Pit size x Filling mixture	0.031	Pit size x Filling mixture	0.354
LSD (0.05) Pit size	0.071	LSD (0.05) Pit size	0.969
Filling mixture	0.088	Filling mixture	0.947
Pit size x Filling mixture	0.074	Pit size x Filling mixture	0.885

mixtures showed significant interaction and the highest potassium content was found under P1M4 (2.10%) and P1M1 (2.10%). However, the zinc content was maximum (77.16 and 77.50 ppm) in plants grown under the pit size P1 and filling mixture M4, respectively. The interaction between these factors was found to be significant and the highest zinc content was observed in response to P1M4 (80.00 ppm) (Table 7).

Table 8. Effect of pit sizes and filling mixtures on the iron and total chlorophyll content in leaves of tissue cultured date palm cv. Barhee

Pit size	Total chlorophyll (mg g^{-1} FW)					Iron (ppm)				
	M1	M2	M3	M4	Mean	M1	M2	M3	M4	Mean
P1	2.30	2.05	2.05	2.50	2.23	172.00	166.00	167.00	179.00	170.25
P2	2.15	2.00	1.95	2.25	2.09	166.50	165.00	165.00	167.25	165.94
P3	1.98	1.98	1.95	2.02	1.98	155.00	155.25	155.70	158.00	155.99
	2.14	2.01	1.98	2.26		164.50	162.08	162.56	168.08	

	Total chlorophyll		Iron	
SEm±	Pit size	0.046	Pit size	0.682
	Filling mixture	0.067	Filling mixture	0.836
	Pit size x Filling mixture	0.063	Pit size x Filling mixture	0.644
LSD (0.05)	Pit size	0.115	Pit size	1.637
	Filling mixture	0.164	Filling mixture	2.090
	Pit size x Filling mixture	0.154	Pit size x Filling mixture	1.610

Data in Table 8 reveal that the leaf chlorophyll content was significantly affected by both the factors and maximum chlorophyll content (2.23 and 2.26 mg g^{-1} FW) was exhibited by the plants grown under the pit size P1 and filling mix M4, respectively. The interaction effect of two factors was found to be significant and maximum chlorophyll content (2.50 mg g^{-1} FW) was found under P1M4. The highest leaf iron content (170.25 and 168.08 ppm) was recorded with pit size P1 and filling mixture M4, respectively, the interaction of pit size and the filling mixture was significant for leaf iron content and the highest leaf iron content was recorded in response to P1M4 (179.00 ppm) followed by P1M1 (172.00 ppm). The results are in agreement with the findings of Mauki and Kilonzo (2022) who proposed larger pit size especially in dryland regions for the survival of various plant species.

A larger pit size offers a more root growth has and enhance opportunities to access a broader spectrum of nutrients in the soil. The extensive root network, nurtured by a larger pit, increases the capacity of plant to absorb nutrients, potentially leading to higher leaf nutrient content. This is particularly advantageous when aiming for a rich nutrient profile in plant leaves. Furthermore, larger pits can store more moisture, ensuring that the plant has consistent access to water. Adequate moisture availability is pivotal for efficient nutrient uptake through the roots. FYM is a reservoir of vital nutrients, such as nitrogen, phosphorus, potassium and micronutrients. When integrated into the soil, it significantly enriches the nutrient pool available to plants (Kaurch *et al.,* 2005). As plants draw these essential elements through their roots, the enhanced nutrient availability can result in elevated nutrient content within the leaves. The influence of FYM extends to improved nutrient retention and enhanced soil structure. By promoting favorable conditions for nutrient storage and release through enhanced soil structure and microbial activity, FYM facilitates efficient nutrient uptake by plant roots (Acharya *et al.,* 1988). This, in turn, results in greater nutrient content in plant leaves. The influence of FYM also extends to the enhancement of photosynthesis. Improved nutrient availability, soil structure and moisture retention collectively bolster the photosynthetic capacity of plants (Bhriguvanshi *et al*., 1988). Consequently it leads to increased nutrient assimilation and storage in the leaves, resulting in higher leaf nutrient content.

Conclusion

The results we obtained suggest that the Barhee cultivar tissue-cultured plants can perform better when cultivated in pits measuring 1 m^3 and filled with a mixture comprising 0.5 parts of field soil, 0.5 parts of clay and 2.0 parts of FYM. This study supports the idea that optimizing pit size and the composition of the filling mixture can have a beneficial impact on the establishment of date palm trees.

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