Standardization of softwood grafting in Avocado (*Persea americana* Mill) var. TKD1

ABSTRACT

In recent years, Hass avocados (*Persea americana* Mill) have become more widely cultivated in Colombia. One of the difficulties facing the nation is boosting orchard output by ensuring graft plants have the best agronomic, genetic, and phytosanitary criteria. In this study, the growth and development of avocado rootstocks derived from seeds of various weight ranges will be monitored for 90 days. Four weights of avocado seeds (S_1, S_2, S_3, S_4) (30–40 g, 40–50 g, 50–60 g, and 60-70 g) and four scion maturity of (D_1, D_2, D_3, D_4) (60 days old, 70 days old, 80 days old, 90 days old were used as treatments in a factorial completely randomized experimental design. The outcome showed that the best germination rate of 75% for S_4 and the highest survival percentage (94.98%) for S_4D_4. After 90th DAG the maximum number of new shoots per plant (5.75), number of leaves/plants (18.15), leaf area (20.83 cm^2), and carbohydrate content (6.57%) were reported. Based on the results of the experiment, it was determined that the optimum method for propagating avocados was softwood grafting using 60–70 g seed size (rootstock) and 90-day-old scion. These plants had better vigor and faster germination rates, all of which could reduce the length of time spent in the nursery while still ensuring the quality of the material, which is influenced by improved seedling growth.

Keywords: *seed weight, Germination, scion maturity, carbohydrate, Avocados*

Introduction

The Avocado (*Persea americana* Mill) originated from tropical America mainly from Mexico and Central America. Increasing the production of the orchards by ensuring graft plants meet the finest agronomic, genetic, and phytosanitary standards is one of the nation's difficulties. One of the healthiest fruits, it has higher protein content (up to 4%), higher fat content (up to 30%), and lower sugar content (1.0%). Avocado is typically eaten raw, as a filling for sandwiches, or in salads. Additionally, the pulp can be kept by freezing and utilized in milkshakes and ice cream. Although avocados may thrive in a variety of climates and soil types, they are sensitive to poor drainage and cannot resist waterlogging. Early in the 20th century, Sri Lankan farmers brought avocados to India (Ghosh, 2000).

In some regions of Tamil Nadu, Kerala, Karnataka, and the North Eastern Himalayan states, it is grown on a small basis. However, many other states in the nation have gardens where plants can be seen growing. Avocado farming is currently becoming more and more popular due to its nutritional benefits and rising health consciousness. Avocado is planted as one of the mixed crops in the coffee-based cropping system in Kodagu (Karnataka), The Palani, Shervaroy Hills (Tamil Nadu), and other locations.

The production of seeds in various regions without fulfilling the minimal quality requirements has led to a large amount of planting material that, when used in the field, starts to pose issues, mostly at the root level. The cultures with a wide range of responses to edaphoclimatic and sanitary circumstances are a natural consequence of significant genetic variety (Ben-Ya'acov and Michelson, 1995). As a result, the rootstock's seed must come from mature, fruitful trees that have produced at least two crops, are well-formed, and have excellent, healthy fruits Bernal et al. (2014).

In terms of the growth and development of avocado fruit, the rootstock gives the tree nutrients, water, and other molecules like hormones and proteins through the root system that are important for healthy fruit development. Lazare et al. (2020) state that while the scion produces the required photo assimilates through photosynthesis, an appropriate rootstock-scion connection is still required to ensure high yields and good fruit quality.

Yields and fruit quality are affected by abiotic elements including light intensity, water stress, temperature, and soil fertility, biotic ones like pests and diseases, and genetic factors like the rootstock (Mickelbart, 2012). An excellent root system that can withstand unfavorable conditions and ensures the early growth of the fruit can favor these elements, limiting the fruit's permanence over the tree (Lira et al., 2020). Therefore, seed weight and scion maturity for softwood grafting must be standardized as a result.

Materials and Methods

The experiment on "Standardization of softwood grafting in avocado (*Persea americana* Mill) var. TKD1" was carried out in a mist chamber from February to July 2023 at the Horticultural Research station Thadiyankudisai during the year 2022-2023. The latitude and longitude of the location are 10°S and 77°E with an elevation of 1098 m

above Mean Sea Level. The average rainfall per year is about 1400 mm. The experiment had 16 treatments and 3 replications, and it was set up in a factorial completely randomized design (FCRD). In this softwood grafting is followed and scion materials are collected from a 20-year-old avocado tree of variety TKD 1 at the Horticultural Research Station Thadiyankudisai, scion materials for softwood grafting were gathered.

Factor 1: Size of Seed (Rootstock)

S_1 – 30-40g

S_2 – 40-50g

S_3 – 50-60g

S_4 – 60-70g

Factor 2: Age of Scion (Softwood)

D_1 – 60 days old

D_2 – 70 days old

D_3 – 80 days old

D_4 – 90 days old

List 1. List of Treatment combinations used for the study

S*D	D_1	D_2	D_3	D_4
S_1	S_1 D_1	S_1 D_2	S_1 D_3	S_1 D_4
S_2	S_2 D_1	S_2 D_2	S_2 D_3	S_2 D_4
S_3	S_3 D_1	S_3 D_2	S_3 D_3	S_3 D_4
S_4	S_4 D_1	S_4 D_2	S_4 D_3	S_4 D_4

The biometrical observations were made on five randomly chosen plants from each replication to evaluate the morphological traits, for rootstock such as the number of days taken for sprouting, plant height, leaf area, shoot length, and observations after grafting with scion number of days taken to bud sprouting, number of new shoots/plants, survival percentage of grafting, leaf area (cm^2), carbohydrate content. The data for the number of days taken for sprouting for rootstock was recorded after the emergence of 1st sprouting and expressed in numbers. The plant height of rootstock plants was measured on the 30th, 45th, and 60th day after sprouting and expressed in cm. The leaf area was calculated at 60 days after sprouting.

The after-grafting data regarding the number of days taken to sprouting was calculated by observation of plants on alternate days from the days of planting and their mean value was used to calculate the days taken for the first sprout. The number of shoots per plant and number of new leaves per plant were recorded of fully developed plants were recorded 30,60,90 days after propagation The data on the percentage of survival were recorded after 90 days of planting the grafting/budding/cutting, carbohydrate content, and leaf area calculated after 90 days of propagation. The data were then statistically evaluated using the Panse and Sukhtame (1954) approach.

Result and Discussion

1. Germination percentage (%)

(Figure 1) Avocado seed germination rates varied according to seed size, with S_4 (60–70 g) showing the highest rate and S_3 (50–60 g), S_2 (40–50 g), and S_1 (30–40 g) showing the lowest. Seed size and germination percentage were shown to be directly correlated, with 85% for S_4 (60-70 g), 75% for S_3 (50-60 g), 69% for S_2 (40-50 g), and 60% for S_1 (30-40 g). The size and quantity of seed reserves, which should be related to a stronger nutritional reserve in bigger seeds, are related to the success of seed germination and the establishment of seedlings. During the formation of the avocado fruit, significant starch reserves build up in the seed, generating a pool of carbohydrates reserves that are gradually broken down to sucrose, which is then used for the initial growth of the embryo and seedling Tefay et al. (2012). This outcome is related to the avocado studies by Mejia et al. (2022), Ndoro (2018), and Adjei et al. (2011).

2.Number of days taken for sprouting (days)

According to a study of the data in (table 1), different seed sizes and scion maturities, as well as the minimum (19.25) number of days required for sprouting in S_4, were documented. The minimal number of days required for avocados to sprout (20.75) was seen in D_4, indicating that the scion's maturity had a major impact on this factor. The interplay between avocado seed weight and scion age also affected the length of time it took for avocados to sprout, with S_4 (60-70 g) and D_4 (90 days old) requiring the least amount of time 17 days to sprout. In softwood grafting the size of the seed and scion maturity plays a major role in seedling growth, whereas larger seed size and 90 days old scion should be used for grafting. During avocado fruit development, substantial starch reserves build up in the seed, generating a pool of carbohydrate reserves that are gradually broken down to sucrose, which is needed for initial embryo and seedling growth. The results conformed with those of Cordula et al. (2014), Tefay et al. (2012), and Ndoro (2018) in avocado.

3.Number of shoots per plant

(Table 2) The results showed that the highest (5.37) and minimum (2.55) shoots per plant were recorded in S_4 and S_1, depending on the seed size and scion maturity. The maximum (4.46) and minimum (3.78) shoots were seen during D_4 and D_1, indicating that the scion maturity had a substantial impact on the time taken for avocado seeds to sprout. The interaction between avocado seed size and scion maturity also had an impact on the number of shoots, with the greatest (5.75) and minimum (1.95) values seen in S_4 D_4 and S_1 D_1, respectively. The average number of stems on the seedlings with the largest seeds (above 70 g) was three, which is the most stems ever seen Paixo et al. (2016). This is probably because larger seeds contain more nutrients, which encourages the development of multi-stem rods. The number of shoots produced is more influenced by the greater seed size and the scion's age in days. Similar results were obtained in avocados by Souza et al. (2020) and Paixo et al. (2016).

4. Number of leaves per plant

(Table 3) The effect of seed size and scion maturity on the number of leaves per plant showed that the highest value (17.59) and lowest value (12.40) of leaves were recorded in S_4 and S_1. The effect of scion maturity was also significant with respect to the time it took for avocados to sprout, with the highest (15.62) and lowest (14.43) leaves being observed during D_4 and D_1. The interaction effect of seed size and scion maturity in avocados also had an impact on the number of leaves, with the highest (18.15) and lowest (11.98) values found in S_4D_4 and S_1D_1. The rootstock and scion have an impact on how quickly grafts grow by increasing the production of photosynthates and dry matter, improving soil nutrient and water intake, and increasing the amount of synthetic food material. The scion's ideal maturity aids in the production of additional leaves. These findings concur with those in avocados from Mejia et al. (2021) and Olorunmaiye et al. (2011). Nimbalkar (2011) on karonda attained similar outcomes.

5.Survival percentage (%)

(Table 4) According to the study's findings, S_4 and S_1 had the highest and lowest survival rates, respectively (93.98% and 60.83%). Regarding the avocado's taken-to-survival percentage, the effect of scion maturity was also significant; the highest (84.53%) and lowest (74.27%) percentages were seen on the D_4 and D_1, respectively. The interaction between seed size and scion maturity in avocados also had an impact on survival percentage, with S_4D_4 and S_1D_1 showing the highest and lowest survival percentages (94.98 and 52.34%, respectively). The sufficient amount of carbohydrates and other food components in the scion and rootstock, as well as the mobilization of the stored food components for new development, maybe the cause of the survival percentage. This in turn leads to high meristamatic activity in the scion. Tanuja and Thippesha (2017) in the sapota study, Maheswari and Nivetha (2015) in the jackfruit study, and Angadi et al. (2011) in the wood apple study all provided support for this conclusion.

6. Leaf area (cm^2)

Analysis of the results (Table 5) showed that S_4 and S_1 had the highest and lowest leaf areas among seeds of various sizes and scion maturities, respectively, measuring 19.99 cm2 and 16.03 cm2. Regarding avocado leaf area, the maximum (18.81) and minimum (17.29) values were noted in D_4 and D_1 as the effect of scion maturity. The interaction impact of avocado seed size and scion maturity, which was found to have maximum (19.20) and minimum (15.16) values in S_4D_4 and S_1D_1, also affected the time it took for avocado buds to sprout. The early and vigorous union formation, better nutrient uptake, sufficient scion development, and suitable rootstock may have contributed to the increased number of leaves per plant and leaf area. The same outcomes were observed in studies on sapota by Tanuja and Thippesha (2017), mango by Alam et al. (2006), and guava by Rajatiya et al. (2022).

7. Carbohydrate content (%)

(Table 6) Carbohydrate levels were found to vary with seed size and scion age, with S_4 and S_3 having the highest (6.13%) and lowest (5.72%) levels, respectively. About the carbohydrate content of avocados, the effect of scion maturity was likewise significant; the highest (6.08%) and lowest (5.60%) values were seen on days D_4 and D_1. The interplay between avocado seed size and scion maturity also had an impact on the fruit's carbohydrate content, with maximum (6.57%) and minimum (5.28%) levels found in S_4D_4 and S_1D_1. The buildup of higher carbohydrate

contents is highly dependent on the scion maturity and seed size of the rootstock. Increased carbohydrate content, which promotes high graft take and increases the success rate, is a result of the proper seed size and maturation level. This finding agreed with Karna et al. (2017) for mango.

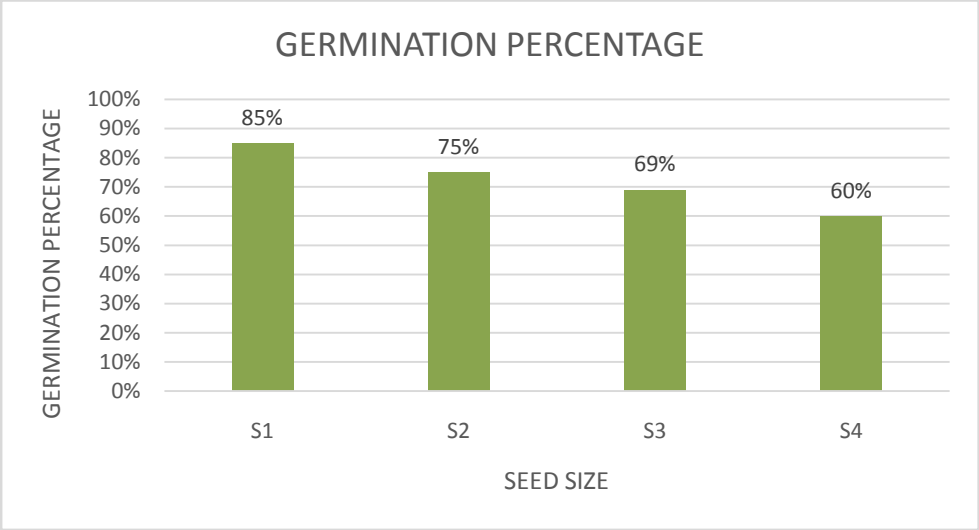

Figure: 1 Effect of seed size on germination percentage

Table 1: Influence of the size of the seed, scion maturity, and their interaction effect on the number of days taken to bud sprouting of Avocado

Size of seed	Scion maturity				Mean
	D_1	D_2	D_3	D_4	
S_1	35.25	32.72	26.24	24.68	29.72
S_2	28.61	26.82	24.55	22.74	25.68
S_3	27.93	25.72	22.85	20.13	24.15
S_4	22.69	20.74	19.58	17.62	20.15
Mean	28.62	26.50	23.30	21.29	
	Seed size		Scion maturity		Interaction (S X D)
SE(d)	0.208		0.208		0.417
CD(p=0.05)	0.425		0.425		0.850

Table 2: Influence of the size of the seed, scion maturity, and their interaction effect on the number of shoots per plant of Avocado

Size of seed	Scion maturity				Mean
	D_1	D_2	D_3	D_4	
S_1	1.95	2.20	2.90	3.10	2.55
S_2	3.64	3.75	3.95	4.10	3.86
S_3	4.50	4.79	4.89	4.90	4.77
S_4	4.99	5.20	5.55	5.75	5.37
Mean	3.78	3.98	4.32	4.46	

	Seed size	Scion maturity	Interaction (S X D)
SE(d)	0.050	0.050	0.100
CD(p=0.05)	0.137	0.137	0.274

Table 3: Influence of the size of the seed, scion maturity, and their interaction effect on the number of leaves per plant of Avocado

Size of seed	Scion maturity				Mean
	D_1	D_2	D_3	D_4	
S_1	11.98	12.14	12.56	12.95	12.40
S_2	13.45	13.98	14.32	14.89	14.16
S_3	15.32	15.85	16.21	16.52	15.97
S_4	16.98	17.34	17.89	18.15	17.59
Mean	14.43	14.82	15.24	15.62	
	Seed size		**Scion maturity**		**Interaction (S X D)**
SE(d)	0.169		0.169		0.338
CD(p=0.05)	0.344		0.344		0.689

Table 4: Influence of the size of the seed, scion maturity, and their interaction effect on survival percentage of Avocado

Size of seed	Scion maturity				Mean
	D_1	D_2	D_3	D_4	
S_1	52.34	60.73	65.07	65.2	60.83
S_2	65.43	67.53	84.04	85.96	75.74
S_3	87.08	88.86	89.65	91.99	89.39
S_4	92.24	93.99	94.72	94.98	93.98
Mean	74.27	77.77	83.37	84.53	
	Seed size		**Scion maturity**		**Interaction (S X D)**
SE(d)	0.910		0.910		1.821
CD(p=0.05)	1.855		1.855		3.710

Table 5: Influence of the size of the seed, scion maturity, and their interaction effect on the leaf area of Avocado

Size of seed	Scion maturity				Mean
	D_1	D_2	D_3	D_4	
S_1	15.16	15.68	16.22	17.06	16.03
S_2	16.91	17.44	17.47	18.15	17.49
S_3	17.92	18.72	18.92	19.20	18.69
S_4	19.18	19.88	20.10	20.83	19.99

Mean	17.29	17.93	18.17	18.81	
	Seed size		**Scion maturity**		**Interaction (S X D)**
SE(d)	0.188		0.188		0.376
CD(p=0.05)	0.383		0.383		0.767

Table 6: Influence of the size of the seed, scion maturity, and their interaction effect on the carbohydrate content of Avocado

Size of seed	**Scion maturity**				**Mean**
	D_1	**D_2**	**D_3**	**D_4**	
S_1	5.19	5.28	5.55	5.72	5.43
S_2	5.72	5.77	5.82	6.05	5.84
S_3	6.09	6.10	6.12	6.13	6.11
S_4	6.26	6.29	6.30	6.57	6.35
Mean	5.81	5.86	5.94	6.11	
	Seed size		**Scion maturity**		**Interaction (S X D)**
SE(d)	0.055		0.055		0.110
CD(p=0.05)	0.112		0.112		0.225

Conclusion

According to the experiment's findings, for softwood grafting the seeds that are 60–70 g in weight and scions that are 90 days old are optimal for propagating avocados, followed by seeds that are 50–60 g in weight and scions that are 80 days old or older. Farmers have trouble propagating avocados because there aren't any standard rootstock and scion available. Therefore, standardizing rootstock and scion maturities for softwood grafting will enable the large-scale manufacture of high-quality planting material to farmers' advantage. It will also boost growth and yield, which will increase the farmers' revenue.

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