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Technique, Time, and Etiolation Applications Influencing the Grafting Success in Avocado (*Persea americana* Mill.)

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ABSTRACT

In a two-phase experiment during 2018 and 2021, we evaluated the propagation success of avocado against three types of grafting (cleft, veneer, and splice) and two budding techniques (T and patch) practiced in the 3rd week of February, April, June, August, October, and December, considering the atmosphere of the six seasons (spring, summer, rain, autumn, late autumn, and winter, respectively) in Bangladesh. Cleft grafting was suitable during spring, demonstrating statistical superiority by exhibiting earlier bud sprouting (25.58 days) and better sapling qualities (16.27 cm scion growth and 14.57 leaves sapling^{-1}). It produced a maximum number of transplantable propagules after ten months of grafting compared to all other combinations. However, commercial multiplications could not be satisfied with only a 48.00% success rate at the best combination. A post-grafting etiolation was therefore applied to the spring season's cleft grafts. Out of 72-hour, 120-hour, 168-hour, and 216-hour etiolation and control (non-etiolation), etiolation up to 120-hour significantly augmented the success rate compared to the control treatment. The 216-hour etiolation treatment failed to produce any successful grafts. The earliest bud sprouting (21.37 days) with maximum bud sprouts (86.00%), transplantable grafts (78.67%), and superior quality grafts was obtained from the 120-hour etiolation compared to the other treatments, while the control treatment produced only 49.33% transplantable grafts. Thus, the 120-hour etiolation made 59.46% more transplants than the non-etiolation treatment. T-budding in winter was the worst technique and time for avocado grafting. Veneer grafting and the autumn season were the second-best options. Therefore, post-grafting treatment appeared best for avocado vegetative propagation.

Introduction

Avocado (*Persea americana* Mill.), the only edible fruit species of the Lauraceae family, is a high-value produce well acknowledged for its nutritional and health benefits. This 'new world fruit' is high in fats, proteins, and minerals but is low in carbohydrates. It contains fat-soluble vitamins like A and B along with median levels of D and E, thereby creating protection against heart diseases and lessening blood pressure by reducing LDL cholesterol (Duarte et al., 2016; Araújo et al., 2018; Bhuyan et al., 2019). The fruit is valuable for its omega fatty acids (oleic and linoleic), phytosterols, tocopherols, and squalene (dos Santos et al., 2014). This tropical and subtropical fruit originated in Mexico and Central America in 291 B.C. (Galindo-Tovar et al., 2008). There, the plant spread to Mexico, Chile, the USA, South Africa, Spain, Palestine, Australia, New Zealand, and Peru due to its broader adaptability to soil and environmental conditions (Silva, 2014). In the Indian region, this fruit was introduced later in the 19th century, with its domestication mainly from Srilanka (Tripathi et

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al., 2014). Since then, avocado cultivation as a homestead fruit has become popular in South Asian countries, including Bangladesh. In Bangladesh, small-scale commercial production of this premium fruit crop has successfully supported the increase in demand. Its flowers and new flashes initiate in the spring (February-March). Fruits are harvested during September-October (Azad et al., 2020) in Bangladesh when there exists a shortfall of monsoon (lean season) to ascertain recommended nutrition for the consumers (BBS, 2022). However, establishing new avocado orchards has been the subject of practice in recent years. However, one of the most challenging problems entrepreneurs faced was the availability of quality saplings.

In commercial avocado orchards, seedlings and clonally propagated rootstocks are common. Seed propagation does not maintain true-to-type and usually takes longer (more than ten years) to bear flowers and fruits for commercial harvest (Bender and Whiley, 2002). Vegetative propagation, on the other hand, gives identical offspring to the mother plant along with adversity tolerance and sustainable productivity. Different methods of vegetative propagation, cutting, budding, and grafting are practiced worldwide in avocados (Wolstenholme, 2003; Chithiraichelvan et al., 2006; Tripathi and Karunakaran, 2019). Cutting propagation can be successful with the pretreatment of mother plants with gibberellic acid, etiolation, girdling, etc. (Escobedo and Escobedo, 2011; Richards and Rupp, 2012; Thakur et al., 2014). However, adventitious roots and the absence of a tap root system in the cuttings may fail to support the vigorous tree growth and heavy fruit bearing as well as from heavy winds and gusty storms.

Besides, rooting of cutting has sometimes shown limited success with utterly low rates of root induction, inconsistent rooting, longer time for root induction, and difficulty in management under field conditions (Shepherd and Bender, 2002; Ahmed et al., 2001). In recent years, micropropagation has become common in successful avocado multiplication (Thao et al., 2003; Hiti-Bandaralage et al., 2017; Abo El-Fadl et al., 2022) though *in-vitro* propagation difficulties are also frequent (Premkumar et al., 2003; Nhut et al., 2008; Estay et al., 2016). *In-vitro* multiplication technology might be cumbersome as it requires trained individuals, sophisticated facilities, and expensive materials, which make it a pricey technique. Acclimatization is also a hindrance (Barceló-Muñoz and Pliego-Alfaro, 2003; Kumar and Rao, 2012; Asayesh et al., 2017). Transplants that develop in tissue culture lack the leading tap root of the seedling, which is necessary for anchorage in orchard soil. Therefore, grafting and budding can benefit vegetative propagation of avocados with several advantages, including early flowering compared to seedlings and a tree size smaller than own-rooted trees because they begin to bear fruit earlier (Janick et al., 2010). However, successful rootstock-scion union is the prerequisite for propagation success that requires proper selection of rootstock and grafting time of the year based on suitable growing conditions (Mng'omba et al., 2010) and grafting expertise (Akinnifesi et al., 2008).

Temperature conditions during cellular activity, callus formation, and healing should be favorable for success in budding or grafting. Furthermore, the degree of graft success is also affected by the grafting techniques (Soleimani et al., 2010). Cleft, veneer, splice, side, and wedge grafting commonly make grafting techniques in fruit crop propagation. I-budding, T-budding, and patch budding are prevalent budding techniques in Bangladesh. Avocado is a plant in which vegetative propagation is to some extent laborious as root development in cutting and shoot union in grafting. Successive sapling growth is restricted inherently to produce propagules in numbers. Measures such as etiolation and PGR application assist in achieving success in vegetative propagation (Escobedo and Escobedo, 2011; Thakur et al., 2014).

Thus, the current research hypothesized that varied methods and combined management approaches might bring success in avocado vegetative propagation. Therefore, a two-step experiment was undertaken with several grafting and budding techniques at different times of the year to sort out and suggest the best method and time of vegetative propagation, incorporating etiolation treatment for avocado propagation in the sub-tropical climate of Bangladesh.

Materials and Methods

Experimental site

The experiment was conducted at the Fruit Research Farm of Pomology Division, Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI) (23.99 °N latitude and 90.41 °E longitude) from February 2018 to October 2021 in two separate phases. At about 34 meters above sea level, the site was characterized by moderate to heavy rainfall, high humidity, high temperature, and relatively long days from April to September. It had scanty rainfall, low-to-moderate humidity, low-to-moderate temperature, and a short day period from October to March (Fig. 1).

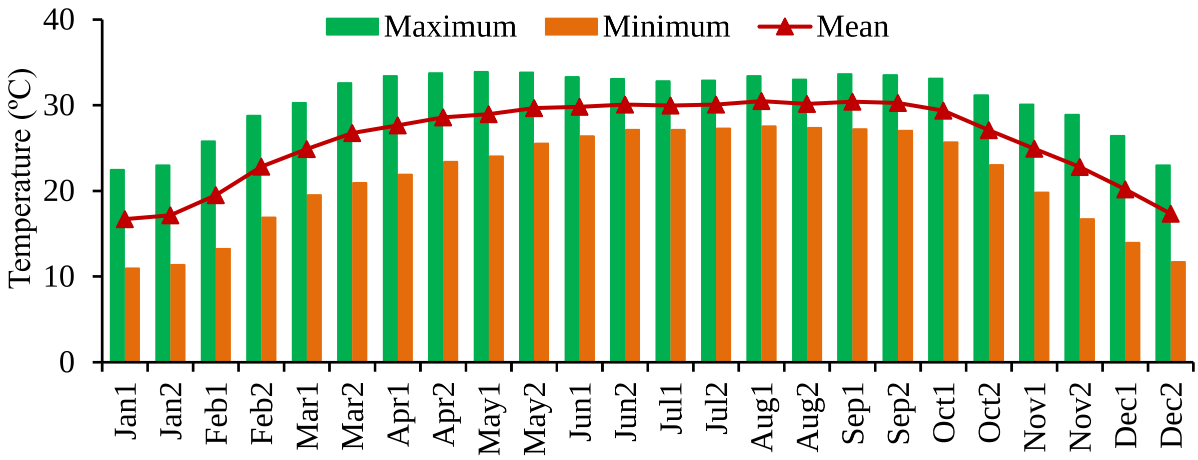

Fig. 1. Average temperature (maximum, minimum and mean) measurements at different months of the year during the experimental period (2018-2021). Here, in the horizontal axis, Jan, Feb, Mar, Apr, Jun, Jul, Aug, Sep, Oct, Nov and Dec represent the first three letters of the twelve months of the year starting from January to December, respectively. Whereas, the digits 1 and 2 adjoining the month representing letters denote the first and second half of the months, respectively.

Rootstock and scion selection

Rootstock plants for vegetative propagation were developed by sowing seeds of BARI Avocado-1 (the only registered avocado variety in Bangladesh) in polythene bags (30 cm × 20 cm) filled with potting medium (1:1:1 ratio) garden soil: sand: compost during September-October 2017. The rootstocks grew well to at least 12-15 months old at the start of the experiment. The main stem of the 40-45 cm tall stock plants became 12-15 mm in diameter at 20-25 cm (probable grafting height) from the ground. On the other hand, about 4-6 month-old shoot tips from the desired mother plant were selected as scions and had dark green leaves (12-15 mm in diameter), free from disease and insect infestation and devoid of new leaf buds rather than having active dormant terminal as well as lateral buds. The scions were also from the variety BARI Avocado-1, with superior fruit qualities (Azad et al., 2020). The selected scion shoots on mother plants were defoliated a week before operation and detached from the mother plant just before grafting/budding practices.

Grafting treatment and design

Initially, we used five different types of vegetative propagations, namely, cleft grafting (P1), veneer grafting (P2), splice grafting (P3), T-budding (P4), and patch budding (P5) at six different times in 2018 and 2019. The grafting times were according to the six seasons of Bangladesh, namely, D1: spring (mid-February to mid-April), D2: summer (mid-April to mid-June), D3: rain (mid-June to mid-August), D4: autumn (mid-August to mid-October), D5: late autumn (mid-October to mid-December) and D6: winter (mid-December to mid-February). Grafting/budding occurred on the first week of each of the six seasons, so the grafts received the distinct weather attributes of the season during graft union and initial establishment. The experiment was laid out in a randomized complete block design (RCBD), replicating each treatment thrice. Every time, twenty scions (shoot tips; 10-12 cm long) were grafted separately in cleft, splice, and veneer grafting, and another twenty buds (scion) occurred from each of the T-budding and patch budding techniques in each replication (Table 1).

Grafting procedure and aftercare

Grafting and budding were done with a sharp grafting/budding knife. Rootstocks and scions were matched at grafting to ensure proper alignment of vascular tissues (cambium) for improved graft-take (Hartmann et al., 2007). The graft union was wrapped carefully with polythene tape. Immediately after the grafting operation, the scion shoots or buds, including some main stems of the rootstocks were covered with 30 cm × 6 cm transparent polythene covers for clear visualization of different growth phases. The grafts were kept under partial shade for two weeks. The grafts were irrigated regularly as and when necessary. The off-shoots arising below the graft join from the rootstocks were removed carefully and timely. The polythene covers were unwrapped as and when new leaves emerged from the scion parts or finally on the 45th day after the grafting operation.

Table 1. Bark peeling and slipping condition of avocado at different grafting seasons of the year.

Grafting seasons	Bark slipping condition	Ease of bark peeling
Spring	Highly slipping	Bark peeled easily
Summer	Slipping slightly	Bark peeled moderately
Rain	Slipping slightly	Bark peeled moderately
Autumn	Moderately slipping	Bark peeled easily
Late Autumn	Highly slipping	Bark peeled easily
Winter	Poorly slipping	Bark not easily peeled

Here, Spring: mid-February to mid-April, Summer: mid-April to mid-June, Rain: mid-June to mid-August, Autumn: mid-August to mid-October, Late autumn: mid-October to mid-December and Winter: mid-December to mid-February.

Application of etiolation

Seasonal variations and grafting techniques for successful avocado multiplication were observed in 2018 and 2020. In the second step, further evaluations were made with the etiolation application to the grafts. However, etiolation was not applied to all graft combinations performed in the first phase. Rather, the most effective grafting method (cleft grafting) at the best time (spring season) was selected after the results of the prior experiment. Regulated etiolation treatment was given to the spring season's cleft grafts during 2020-2021. Here, four dark chambers of 1 m × 1 m in size were prepared by covering them with colored cloths from all sides. The grafts were placed in the artificially prepared chambers immediately after propagation for four different durations viz., E1: 72-hour (hour), E2: 120-hour, E3: 168-hour and E4: 216-hour along with control/no etiolation (E0). Grafting procedures were similar to earlier ones, and 50 grafts per replication were placed under each etiolation treatment.

Data collection

The grafts were allowed to grow separately in the nursery condition as per treatment and design. The grafted and budded saplings were monitored carefully to observe the bud break and the number of days from grafting and budding operation to the bud sprout. The sprouting percentage was recorded by dividing the number of sprouted buds by the total number of grafts or buds. Then, the successful graft was estimated at 120 days after grafting (DAP) by dividing the number of successful grafts or buds by the total number of grafts or buds under each replication. Ten months after grafting and budding, we measured scion length (growth), several new shoots, and leaves per propagule. An increase in scion length and total length of bud sprouts were regarded as scion growth for grafting and budding, respectively. The scion diameter above the graft joint and the diameter of new sprouts from the buds were also measured. In this case, we did not use a statistical comparison for the first phase due to the separate types of propagating methods, i.e., grafting and budding. Finally, we counted the transplantable saplings under each treatment and determined the percentage of transplantable saplings by the total number of propagated materials. Here, bud breaking at the scion tip was considered as sprouting. Scions developed a normal green color in successful grafts. Unsuccessful grafts appeared with black/dark discoloration starting from the apex towards the graft union. An average of at most ten random observations from each replication (or less where not available) was taken in each treatment value against the growth-related variables, such as scion length and diameter, number of new shoots, and number of leaves per propagule.

Statistical analysis and interpretation

The collected data were tabulated and standardized in percentages where statistical analysis was required. Both one-way and two-way analysis of variance (ANOVA) were performed for analyzing the second and first phase data, respectively, where treatment values were separated using Fisher's protected Least Significance Difference (LSD) test ($p \leq 0.05$). All data were presented as mean values ± standard errors (SE) of three replications. Statistics 10.0 software program was used for data analysis.

Results

The results of the present research experiment appear in two sections. As per the materials and methods, the first section describes the effects of different grafting methods applied at different seasons of the year on the grafting success of avocados. The second section presents the results after administering the etiolation treatment to the best combination achieved in the first phase.

Step 1. Efficacy of grafting method and time of practice on grafting success of avocado

Days taken to bud sprouting

Propagation methods and grafting seasons individually and in combination had statistically significant ($p \leq 0.05$) impacts on the days required for bud sprouting in avocados (Table 2 and Table 3). Among the methods, bud sprouting occurred fastest (30.87 days) in cleft grafting, while a significant delay in sprouting was noticed in splice grafting (35.36 days from propagation). On the other hand, spring season grafting required a statistically minimum period for bud-sprouting (27.61 days), which was statistically identical to autumn season grafting (28.87 days). The maximum duration for bud sprouting was recorded in winter season propagation (39.46 days). Furthermore, a combination of propagation method and propagation time showed that cleft grafting in the spring (P1D1) led to the fastest sprouting (25.58 days), which had statistical similarity with P1D4, P2D1, P3D1, P4D1, and P5D1 treatments. However, bud-sprouting was slowest (41.07 days) in veneer grafting practiced in the winter season (P2D6), having statistical similarity with that of P3D5, P3D6, P4D6, and P5D6 combinations.

Sprouting percentage

The sprouting percentage was also significantly ($p \leq 0.05$) influenced by the main and combined effects of propagation time and method (Table 2 and Table 3). Cleft-grafted propagules produced statistically maximum bud sprouts (62.08%) compared to the others. The minimum number of bud sprouts was observed in T-budding (41.53%) and had statistical consistency with the patch budding method (43.33%). Among the six propagation seasons, statistical superiority in sprouting percentage was noted from spring propagated propagules (63.00%), followed by autumn propagation (57.17%). The lowest bud sprouting was recorded in winter season's propagules (41.33%), followed by summer and rainy season propagation. Again, in combination, cleft grafting in the first quarter of spring exhibited (P1D1) maximum bud sprouting percentage (73.33%), which was statistically at par with P1D4 and P2D1 treatments. Minimal bud-sprouting (32.50%) was observed in T-budding in winter (P4D6), which was statistically similar to that of P4D2 and P5D6 combinations.

Successful graft-take

Following the sprouting percentage, the maximum successful graft-take was recorded in cleft grafting (47.36%) and minimum graft-take in T-budding (28.75%). Similarly, spring season propagation had the highest successful graft-take (49.17%), and the winter season practice offered the lowest percentage of successful graft-take (29.00%) (Table 2). The combination also followed the same trend as earlier, where the statistically maximum rate of successful graft-take (61.67%) was recorded in spring season cleft grafting (P1D1), and the minimum amount (21.67%) in T-budding during the winter season (P4D6) (Table 3).

Scion growth

The increase in scion length (cm) of avocado saplings differed statistically due to the main and combined effect propagation method and time of practice (Table 2 and Table 3). Scion growth was estimated as maximum in cleft grafting (12.57 cm) and minimum in splice grafting (10.74 cm), which was statistically similar to patch budding (11.17 cm). Again, an increase in scion length was measured maximum in spring propagated saplings (14.55 cm) and minimum in winter propagated propagules (9.43 cm). Autumn season-propagated plants had the second-best scion growth in length (12.93 cm). Once again, the combination showed that an increase in scion length was recorded as the highest (16.27 cm) in cleft grafting performed in the spring (P1D1) compared to all the other treatment combinations having statistical disparity. Inversely, minimum scion growth was noticed in length (9.07 cm) in splice grafting of the winter season (P3D6), which was statistically at par with P1D6, P2D6, P3D3, P3D5, P4D5, P4D6 and P5D6 combinations.

Table 2. Main effect of grafting methods and time of grafting on propagation success and sapling growth of avocado.

Treatment	Days taken to sprouting	Sprouting percentage (%) [X]	Successful graft-take (%) [X]	Scion growth (cm)	No. of new shoots	No. of leaves	Transplantable sapling (%) [X]
Grafting/budding							
Cleft grafting	30.87 ± 1.28[c]	62.08 ± 0.72[a]	47.36 ± 0.61[a]	12.57 ± 0.47[a]	2.28 ± 0.08	11.26 ± 0.13[a]	35.83 ± 2.52[a]
Veneer grafting	32.96 ± 1.32[b]	56.39 ± 0.61[b]	41.81 ± 0.56[b]	11.64 ± 0.41[b]	2.26 ± 0.02	10.31 ± 0.36[b]	29.03 ± 2.51[b]
Splice grafting	35.36 ± 1.42[a]	54.44 ± 1.08[b]	40.28 ± 1.84[b]	10.74 ± 0.07[c]	2.18 ± 0.04	9.92 ± 0.15[bc]	27.50 ± 2.16[b]
T-budding	33.34 ± 1.25[b]	41.53 ± 1.37[c]	28.75 ± 1.88[c]	11.56 ± 0.37[b]	2.37 ± 0.02	9.48 ± 0.09[cd]	19.44 ± 2.22[c]
Patch budding	33.98 ± 1.14[b]	43.33 ± 1.10[c]	30.56 ± 0.97[c]	11.17 ± 0.47[bc]	2.36 ± 0.08	9.25 ± 0.34[d]	21.39 ± 2.03[c]
$LSD_{0.05}$	1.28	2.71	3.20	0.54	0.16	0.61	2.79
LS	**	**	**	**	ns	**	**
Grafting time							
Spring	27.61 ± 1.07[c]	63.00 ± 0.58[a]	49.17 ± 2.13[a]	14.55 ± 0.44[a]	2.44 ± 0.04[a]	12.38 ± 0.60[a]	38.00 ± 1.93[a]
Summer	33.84 ± 1.44[b]	47.83 ± 1.17[d]	33.83 ± 1.17[c]	11.01 ± 0.50[c]	2.18 ± 0.05[bc]	9.05 ± 0.11[c]	23.17 ± 1.95[c]
Rainy	34.88 ± 1.23[b]	48.50 ± 0.76[d]	34.50 ± 0.76[c]	10.73 ± 0.34[c]	2.27 ± 0.07[ab]	9.22 ± 0.13[c]	23.00 ± 2.71[c]
Autumn	28.87 ± 1.38[c]	57.17 ± 1.42[b]	43.83 ± 1.36[b]	12.93 ± 0.57[b]	2.42 ± 0.09[a]	11.81 ± 0.18[a]	33.33 ± 1.99[b]
Late Autumn	35.16 ± 1.23[b]	51.50 ± 2.47[c]	36.17 ± 3.33[c]	10.56 ± 0.19[c]	2.39 ± 0.05[a]	9.96 ± 0.12[b]	25.33 ± 3.01[c]
Winter	39.46 ± 1.33[a]	41.33 ± 1.74[e]	29.00 ± [d]	9.43 ± 0.25d	2.03 ± 0.08[c]	7.84 ± 0.15[d]	17.00 ± 2.14[d]
$LSD_{0.05}$	1.40	2.97	3.51	0.59	0.18	0.67	3.06
LS	**	**	**	**	**	**	**
CV (%)	5.76	7.89	12.71	6.96	10.71	9.10	15.70

[X]: Value of treatment mean with respect to twenty observations per mean.

Values are means ± standard errors of three independent replications. Different letters within the column indicate statistically significant differences among the treatments according to Fisher's protected LSD (least significance difference) test (p≤0.05). Here, Spring: mid-February to mid-April, Summer: mid-April to mid-June, Rain: mid-June to mid-August, Autumn: mid-August to mid-October, Late autumn: mid-October to mid-December, and Winter: mid-December to mid-February. LS: Level of significance, **: significant at 1% level of probability, ns: not-significant.

Table 3. Interaction effect of grafting method and time of grafting operation on grafting success of avocado.

Grafting/ budding	Time	Days taken to sprouting (d)	Sprouting percentage (%) X	Successful graft-take (%) X	Scion growth (cm)	No. of new shoots	No. of leaves	Transplantable graft (%) X
P1	D1	25.58^{n}	73.33^{a}	61.67^{a}	16.27^{a}	2.53^{a-f}	14.57^{a}	48.33^{a}
	D2	31.90^{h-k}	56.67^{e-g}	41.67^{d-f}	11.70^{f-h}	2.17^{d-j}	10.27^{e-h}	29.17^{d-f}
	D3	31.05^{i-l}	56.67^{e-g}	41.67^{d-f}	12.03^{fg}	2.20^{c-i}	9.67^{f-i}	29.17^{d-f}
	D4	25.95^{n}	70.83^{ab}	59.17^{ab}	14.33^{bc}	2.40^{a-h}	13.40^{ab}	48.33^{a}
	D5	33.75^{f-j}	65.00^{b-d}	43.33^{d-f}	11.00^{g-j}	2.40^{a-h}	10.93^{d-f}	35.83^{b-d}
	D6	36.98^{b-e}	50.00^{h-k}	36.67^{f-i}	10.07^{i-l}	2.00^{h-j}	8.70^{i-l}	24.17^{f-j}
P2	D1	26.85^{mn}	67.50^{a-c}	53.33^{bc}	14.70^{b}	2.70^{a}	10.23^{e-h}	41.67^{ab}
	D2	32.72^{g-j}	54.17^{g-i}	39.17^{d-g}	11.33^{g-i}	2.17^{d-j}	8.53^{i-m}	28.33^{e-g}
	D3	35.85^{c-g}	53.33^{g-i}	38.33^{e-h}	10.40^{h-k}	2.13^{e-j}	9.03^{g-l}	24.17^{f-j}
	D4	29.18^{k-m}	60.83^{d-f}	45.83^{c-e}	12.83^{d-f}	2.43^{a-g}	10.93^{d-f}	35.83^{b-d}
	D5	32.08^{h-k}	56.67^{e-g}	41.67^{d-f}	11.53^{f-h}	2.27^{b-h}	9.13^{g-k}	26.67^{e-h}
	D6	41.07^{a}	45.83^{j-l}	32.50^{g-k}	9.03^{l}	1.83^{ij}	7.63^{lm}	17.50^{j-m}
P3	D1	28.30^{l-n}	65.00^{b-d}	53.33^{bc}	13.37^{c-e}	2.60^{a-c}	12.40^{b-d}	39.17^{bc}
	D2	35.72^{c-g}	50.83^{g-j}	35.83^{f-j}	10.47^{h-k}	2.03^{g-i}	9.30^{g-j}	25.83^{e-i}
	D3	37.85^{b-d}	50.83^{g-j}	35.83^{f-j}	9.87^{j-l}	2.03^{g-j}	9.30^{g-j}	23.33^{f-j}
	D4	30.72^{j-l}	61.67^{c-e}	46.67^{cd}	12.07^{e-g}	2.47^{a-f}	11.17^{c-e}	32.50^{c-e}
	D5	38.55^{a-c}	54.17^{g-i}	39.17^{d-g}	9.60^{kl}	2.17^{d-j}	9.70^{e-i}	25.83^{e-i}
	D6	41.05^{a}	44.17^{kl}	30.83^{h-l}	9.07^{l}	1.77^{j}	7.67^{k-m}	18.33^{j-l}
P4	D1	29.17^{k-m}	54.17^{g-i}	38.33^{e-h}	14.57^{bc}	2.17^{d-j}	10.90^{ef}	31.67^{de}
	D2	33.92^{e-i}	37.50^{m-o}	25.00^{k-m}	10.97^{g-j}	2.07^{f-j}	8.87^{h-l}	15.00^{k-m}
	D3	32.83^{g-j}	40.00^{l-n}	27.50^{k-m}	11.30^{g-i}	2.43^{a-g}	9.30^{g-j}	18.33^{j-l}
	D4	29.32^{k-m}	44.17^{kl}	31.67^{g-k}	12.67^{d-f}	2.67^{ab}	11.03^{c-f}	21.67^{g-k}
	D5	36.13^{c-f}	40.83^{lm}	28.33^{j-m}	10.20^{i-l}	2.53^{a-e}	9.60^{f-i}	19.17^{i-l}
	D6	38.68^{a-c}	32.50^{o}	21.67^{m}	9.63^{kl}	2.33^{a-h}	7.17^{m}	10.83^{m}
P5	D1	28.13^{l-n}	55.00^{f-h}	39.17^{d-g}	13.83^{b-d}	2.20^{c-i}	13.80^{ab}	29.17^{d-f}
	D2	34.97^{d-h}	40.00^{l-n}	27.50^{k-m}	10.57^{h-k}	2.47^{a-f}	8.27^{i-m}	17.50^{j-m}
	D3	36.82^{b-f}	41.67^{lm}	29.17^{i-m}	10.03^{i-l}	2.57^{a-d}	8.80^{h-l}	20.00^{h-l}
	D4	29.20^{k-m}	48.33^{i-k}	35.83^{f-j}	12.77^{d-f}	2.13^{e-j}	12.50^{bc}	28.33^{e-g}
	D5	35.27^{d-g}	40.83^{lm}	28.33^{j-m}	10.47^{h-k}	2.57^{a-d}	10.43^{e-g}	19.17^{lm}
	D6	39.50^{ab}	34.17^{no}	23.33^{lm}	9.37^{kl}	2.23^{c-i}	8.03^{j-m}	14.17^{lm}
$LSD_{0.05}$		3.14	6.65	7.84	1.31	0.4	1.49	6.84
CV (%)		5.76	7.89	12.71	6.96	10.71	9.1	15.7
LS		**	**	**	**	**	**	**

X: Value of treatment mean with respect to twenty observations per mean.
Different letters within the column indicate statistically significant differences among the treatments according to a Fisher's protected LSD (least significance difference) test ($p \leq 0.05$).
Here, P1: cleft grafting, P2: veneer grafting, P3: splice grafting, P4: T-budding and P5: patch budding; D1: Spring (mid-February to mid-April), D2: Summer (mid-April to mid-June), D3: Rain (mid-June to mid-August), D4: Autumn (mid-August to mid-October), D5: Late autumn (mid-October to mid-December), and D6: Winter (mid-December to mid-February). **: significant at 1% level of probability.

Number of shoots and leaves

Statistically significant variations ($p \leq 0.05$) occurred in the number of new shoots and leaves per propagule, against the main effect of propagation time, method, and their combination, except for several new shoots under the propagation method (Table 2 and Table 3). The number of new shoots per propagule ranged from 2.18 to 2.37 in the propagating methods. However, the maximum number of leaves was counted in cleft grafting (11.26 leaves propagule^{-1}) and a minimum number of leaves was noted in veneer-grafted saplings (9.25 leaves propagule^{-1}). On the other hand, a statistically higher and similar number of new shoots per propagules was found where propagation occurred in four of the six seasons, namely, spring, rainy, autumn, and late autumn. Similarly, the number of leaves per propagule was counted maximum in spring propagation (12.83 leaves sapling^{-1}). The minimum number of leaves occurred in winter propagation (7.84 leaves propagule^{-1}). Once again, the highest number of leaves (14.57 leaves propagule^{-1}) was noted in saplings developed from spring-propagated cleft grafting (P1D1) having statistical unity with P1D4 and P5D1 combinations. Inversely, a significant minimum number of leaves (7.17 leaves propagule^{-1}) was recorded in T-budding in winter (P4D6), which was at par with P2D2, P2D6, P3D6, P5D2, and P5D6 combinations.

Transplantable grafts

The propagation method and time of propagation caused significant variations ($p \leq 0.05$) in the number and percentage of transplantable avocado grafts (Table 2). Transplantable grafts were recorded significantly maximum in cleft grafting (35.83%) followed by veneer and splice grafting (29.03% and 27.50%, respectively). The minimum number of transplantable grafts of avocado was observed in T-budding (19.44%), which was statistically at par with patch budding (21.39%). Again, propagation operations performed during spring produced the highest percentage of transplantable grafts (38.00%), followed by autumn season propagation (33.33%). The lowest number of transplantable grafts was obtained from winter season propagation (17.00%). The combination of propagation method and time of propagation also revealed statistical disparity ($p \leq 0.05$) in the percentage of transplantable grafts (Table 3). Cleft grafting in the spring season (P1D1) produced a maximum number of transplantable grafts (48.33%), which had statistical similarity with P1D4 (48.33%) and P2D1 (41.67%) combinations. On the contrary, a minimum number of transplantable grafts (10.83%) resulted from T-budding in winter months (P4D6), which was statistically at par with P2D6, P4D2, P5D2, P5D5, and P5D6 treatments.

Step 2. Evaluating etiolation efficiency on avocado propagation success

Based on the average of two successive propagation results of this avocado research in the first phase of the experiment, a second step involved new technological intervention where etiolation with an uninterrupted dark condition was applied to the propagules, thereby producing the best propagation method in the best time, i.e., cleft grafting in spring to improve the propagation success. Only 48.33% of transplantable grafts in the best combination were in the first phase). Here, cleft grafted propagules were kept in the dark environment (absence of sunlight) for four uninterrupted durations from 72-hour to 216-hour along with control (normal nursery condition of alternate day and night).

Days to sprouting

Etiolation treatment exhibited statistically significant variation ($p \leq 0.05$) in the number of days required from grafting to bud sprouting (Table 4). Since the etiolation number of days to bud sprouting was reduced by 2-3 days, where non-etiolated (control) propagules initiated the sprouting in a maximum of 25.80 days, it had statistical parity with the 72-hour etiolation treatment (23.90 days). The 120-hour etiolated cleft grafts began sprouting within 21.37 days as a minimum duration, showing statistical similarity to all other etiolation treatments.

Percentage of bud sprouts and successful graft-take

The number of bud sprouts and successful graft-take were also significantly ($p \leq 0.05$) influenced by the different etiolation treatments on spring season cleft grafted propagules (Table 4). The percentage of bud sprouts in 216-hour etiolation was recorded as the fewest, compared to all other treatment groups, with only 8.67% against 72.67% to 86.00% in other observations. Maximum bud sprouting was noted in 120-hour etiolation (86.00%), being statistically identical to that of the 72-hour etiolation (80.00%). Again, no successful graft-take was found in the longest etiolation (216-hour) treatment. Among the others, 120-hour etiolation gave maximum graft-take (82.67%), followed by 72-hour etiolation (75.33%), and minimum successful graft-take was recorded in the control treatment (59.33%), having statistical similarity with 168-hour treatment (64.00%).

Table 4. Effect of etiolation on grafting success and avocado sapling quality.

Etiolation duration	Days taken to sprouting	Bud sprouts (%) Y	Successful graft-take (%) Y	Scion growth (cm)	Scion diameter (cm)	No. of shoots sapling^{-1}	No. of leaves sapling^{-1}	No. of transplantable graft Y	Transplantable graft (%) Y	Transplantable grafts over control
Control	25.80 ± 1.10^{a}	72.67 ± 2.91^{c}	59.33 ± 2.90^{c}	16.47 ± 0.64^{b}	1.70 ± 0.06	2.27 ± 0.09^{b}	14.53 ± 0.75^{c}	24.67 ± 1.76^{d}	49.33 ± 3.53^{d}	
72-hour	23.90 ± 0.91^{ab}	80.00 ± 1.15^{ab}	75.33 ± 1.76^{b}	20.80 ± 0.61^{a}	2.00 ± 0.12	2.57 ± 0.12^{a}	16.93 ± 0.91^{bc}	34.67 ± 0.88^{b}	69.33 ± 1.76^{b}	40.54%
120-hour	21.37 ± 0.71^{b}	86.00 ± 2.31^{a}	82.67 ± 2.40^{a}	19.73 ± 0.72^{a}	1.93 ± 0.09	2.60 ± 0.06^{a}	21.37 ± 0.50^{a}	39.33 ± 0.88^{a}	78.67 ± 1.76^{a}	59.46%
168-hour	21.80 ± 0.35^{b}	76.67 ± 2.40^{bc}	64.00 ± 2.30^{c}	18.27 ± 0.77^{ab}	1.87 ± 0.07	2.23 ± 0.03^{b}	18.97 ± 1.23^{ab}	29.33 ± 1.45^{c}	58.67 ± 2.91^{c}	18.92%
216-hour	22.50 ± 0.76^{b}	8.67 ± 0.67^{d}	nd	nd	nd	nd	nd	nd	nd	nd
$LSD_{0.05}$	2.82	6.35	6.79	2.56	0.30	0.26	3.41	4.12	8.24	
CV (%)	6.48	5.21	4.83	6.82	8.1	5.30	9.52	6.40	6.40	
LS	*	*	**	*	ns	*	*	**	**	

Y: Value of treatment mean with respect to 50 (fifty) observations per mean.
Values are means ± standard errors of three independent replications. Different letters within the column indicate statistically significant differences among the treatments according to Fisher's protected LSD (least significance difference) test (p≤0.05). nd: not determined, LS: Level of significance, * and **: significant at 5% and 1% level of probability, respectively, ns: non-significant.

Sapling growth attributes

Various growth attributes like scion growth in length, number of new shoots, and leaves per sapling of the cleft grafts varied significantly ($p \leq 0.05$) due to etiolation application (Table 4). As there existed no successful graft-take in 216 hours, measurements of sapling growth attributes, scion growth in length, scion diameter, number of new shoots, and leaves per sapling were not made for that treatment for comparison. The maximum scion growth in length was recorded in 72-hour etiolated grafts (20.80 cm), being statistically similar to that of 120-hour and 168-hour etiolation treatments. An increase in scion length was measured minimum in the control treatment (16.47 cm). The scion diameter of the treated grafts did not vary statistically. It ranged from 1.70 cm to 2.00 cm. The number of new shoots and leaves was counted statistically as the highest in 120-hour etiolation (2.60 shoots transplants^{-1} and 21.37 leaves transplants^{-1}). A statistically similar number of shoots and leaves with the best treatment was noticed in 72-hour etiolation (2.57 shoots sapling^{-1}) and 168-hour etiolation (18.97 leaves sapling^{-1}), respectively. Again, statistically minimum number of shoots was observed in 168-hour etiolation (2.23 shoots sapling^{-1}), and the lowest number of leaves was counted in the control treatment (14.53 leaves sapling^{-1}).

Transplantable saplings

No transplantable grafts were obtained from the 216-hour etiolation treatment. Statistical variations ($p \leq 0.05$) occurred among the other treatments for transplantable graft percentage (Table 4). Maximum transplantable grafts were obtained from 120-hour etiolation treatment (78.67%), followed by 72-hour etiolation (69.33%). Non-etiolation resulted in a minimal number of transplantable grafts (49.33%), followed by 168-hour etiolation (58.67%). Moreover, the 120-hour etiolation treatment produced 59.46% more transplantable propagules than the control treatment. However, an increase by 40.54% and 18.92% occurred in total transplantable grafts in 72-hour and 168-hour etiolation treatments, respectively (Table 4).

Discussion

In horticultural crops, particularly fruit crops, seed propagation is avoided due to the large variability in offspring. It is impossible to obtain genetically identical plants to establish commercial orchards. Vegetative propagation is thus a way to have true-to-type plants as mother plants. Not all vegetative propagation techniques are suited to all crops and climatic conditions. Furthermore, successful propagation within each agro-climatic zone cannot be done year-round. Propagation success highly depends on numerous internal and external causes, including the genetic relationship between the stock and the scion and weather attributes during and after the grafting operation, such as temperature, relative humidity, moisture, and plant water status. Other factors include plant growth and developmental stage of scion and rootstock, operation method and timing, cultural management practices before and after grafting, contamination with extraneous microbes and hazards, growth-regulating agents, chemicals, and plant nutrition (Karadeniz, 2005; Hartmann et al., 2007; Lewis and Alexander, 2008; Ahmed et al., 2012; Beshir et al., 2019; Tripathi and Karunakaran, 2019). Therefore, in a specified region, propagation success depends on the types of grafting and the months of the year when practicing grafting.

In the present research on avocado, among five types of vegetative propagation, three grafting and two budding methods led to results during the first quarter of the six different seasons in Bangladesh. Cleft grafting in the spring proved the best technique and time when the maximum number of buds sprouted in the earliest period. The maximum number of transplantable grafts of superior growth occurred ten months after grafting. Veneer grafting was close to the best treatment, whereas T-budding made the worst results. Autumn grafting closely followed the best season compared to other months. The success of a grafting operation depends on the strength of the union formed. And the graft union formation depends on several events, e.g., molecular and physiological/biochemical responses and anatomical components in scions and rootstocks (Wang, 2011; Rasool et al., 2020). Any dislocation of vascular elements indicates weak or distorted joining that may have occurred, ultimately leading to graft failure. The appreciable success in cleft grafting in the present investigation may be due to the greater alignment of parenchymatous tissues of the rootstocks and scions. A lengthier diagonal cut surface at both sides of the scion, corresponding to the vertical flip of the rootstock, encourages precise intermingling and interlocking of vascular bundles to enable a quick union of the grafts, as noted previously by Tripathi and Karunakaran (2019). Another reason for the increased success rate in cleft grafting may be the identical diameter of the scion and rootstock, which promotes uniform callusing of cambium cells on both sides of the graft (Ayala-Arreola et al., 2010; Mng'omba and du Toit, 2013; Rasool et al., 2020).

Further to many other reasons, the grafting technique is the most essential factor in determining grafting success (Soleimani et al., 2010), which might be another reason for maximum success in cleft grafting in the experiment. The lower success in splice grafting, T-budding, and patch budding may be due to lesser contact with the growing cells. Whitsell et al. (1989) and Tripathi and Karunakaran (2019) stated that fewer cambium connecting areas led to a reduced success rate in budding. Maximum success up to 46% and 32.5% in avocado multiplication through cleft grafting was also reported earlier by Chithiraichelvan et al. (2006) and Tripathi and Karunakaran (2019), respectively. Again, the earliest bud sprouting in cleft grafting in the present study might be due to early and better contact of cambial layers of the stock and scion, resulting in early callus formation as compared to veneer grafting, splice grafting, T-budding, and patch budding. However, the firmness of holding the stock and scion without any tissue damage in the cleft graft might be another reason behind the earliest bud break (Roy et al., 1999). In addition, quick and perfect contacts between the scion and rootstock in grafting may facilitate easy translocation of soil nutrients from the rootstock to the growing tip, ultimately increasing the scion length, number of shoots, and number of leaves in the grafted saplings (Ghosh and Bera, 2015; Tripathi and Karunakaran, 2019). The cleft grafting technique also proved the best in several other fruit crops, including mango (Islam et al., 2004; Beshir et al., 2019), sapota (Singh and Bons, 2016), aonla (Jalal et al., 2019), walnut (Ahmed et al., 2012), and tamarind (Mayavel et al., 2022).

On the other hand, the highest success in spring season grafting followed by autumn grafting may be attributed to moderate temperature coupled with moderate-to-high humidity to provide a congenial environment at the early growth stage for better graft union (Fig. 1). Besides, during the first quarter of spring there existed healthy and matured scion sticks just before the resumption of the active growth phase of the mother plants at the onset of monsoon with adequate supply of moisture and nutrients. Faster cambial activity and higher accumulation of carbohydrates in scion shoots and the sprouting of dormant bud observed during the spring contributed to the increased number of sprouts and subsequent grafting success (Whitsell et al., 1989; Hartmann et al., 2007). Again, after the monsoon splashes off new shoots during the summer and rainy months, matured scions with dormant buds become available in the autumn, ensuring better success in avocado grafting. Although grafting can be done any time during the dormant season, the chances for successful healing of the graft union are best if the work is done in early spring, just when the buds of the scion are beginning to swell, but before active growth has started (Hartmann et al., 2007; Prasanth et al., 2007; Beshir et al., 2019). The ideal temperature for cellular activity and callusing for grafting, in general, varies between 25-35 ºC, and callus formation and wound healing hardly occurs below 20 ºC (Knight and Campbell, 1999; Erdogan, 2006; Islam et al., 2014). Therefore, the greater effectiveness of spring grafting may be attributed to a combination of favorable temperature, humidity, and active scion and stock tissue states at this time of year, which allows for the greatest regeneration of parenchyma cells in the cambium region (Ahmed et al., 2012). The failure of a union in grafting during winter months in Bangladesh is due to low temperature and low humidity in the atmosphere below the critical requirements for wound healing and graft joining (Islam et al., 2014; Nahar et al., 2015). The propagules of spring grafting further got a favorable environment in the next six to eight months for proper growth with an adequate supply of nutrients and moisture, which consequently helped for excellent scion growth in length and more shoots and leaves. Moderate grafting success in the summer and rainy months might be due to the congenial temperature and humidity conditions (Whitsell et al., 1989; Tripathi and Karunakaran, 2019; Kaur and Kaur, 2019).

Moreover, in the second phase of the experiment, the success rate of spring-propagated cleft grafts remarkably enhanced by 50% and above due to etiolation application up to a certain limit. The intricate process of grafting involves several phases, such as callus bridge development, vascular cambium alignment, the induction of a wound recovery response, the production of the secondary xylem and phloem, and so on (Hartmann et al., 2007; Melnyk et al., 2015; Rasool et al., 2020). Vascular connection reformation between the scion and stock at wound recovery is crucial as the graft wound disrupts the plant’s vascular dynamism (Asahina and Satoh, 2015). Thus, joining the vascular systems is required to facilitate water uptake and nutrient transport to achieve graft junctions. Again, callus formation of parenchymatous cambium cells as the prior step of graft joining requires specific environmental conditions, particularly in a well-regulated dark period (Monteuuis and Bon, 2000; Rahemi et al., 2016). The etiolation influence is highly evident in callus induction in in-vitro micro-propagation (Barceló-Muñoz and Pliego-Alfaro, 2003; Thao et al., 2003;

Khan et al., 2006; Hiti-Bandaralage et al., 2017; Lee and Seo, 2018; Restrepo Osorio et al., 2018; Sarikhani and Sarikhani-Khorami, 2021; Develi and Miler, 2023) as well as force rooting in cuttings where callus development is of utmost criteria for root induction as governed by darkening (Richards and Rupp, 2012; Thakur et al., 2014; Duman et al., 2020). With the regulated etiolation, the stems experience reduced lignification in the secondary xylem, increased auxin and starch concentration, and lower periderm thickness (Maynard and Bassuk, 1996; Nanda and Melnyk, 2018; Rasool et al., 2020), which might favor the callus development and vascular alignment for successful graft union. Several phytohormones help stimulate the intricate physiological relationship between the rootstocks and scions for grafting, as noticed in *Arabidopsis thaliana* (Kümpers and Bishopp, 2015). Mattsson et al. (2003) and Nanda and Melnyk (2018) showed that auxins from the vascular strands of the stock and scion under far-red light stimulate the differentiation of cambium cells, resulting in the formation of vascular connection and re-joining between both junctions. Thus, they prompt the emergence of tissues using cell expansion for further vegetative growth in the later stages. Again, reports stated that controlled etiolation accumulates sugars (starch/CHO) in the wounds and that sugars provide the essential energy required for cellular differentiation during wound healing and early supplement of food for new growth, i.e., scions (Maynard and Bassuk, 1996; Nanda and Melnyk, 2018). Pribil et al. (2014) and Armarego-Marriott et al. (2020) showed that etioplasts develop in tissue in the absence of light (etiolation), which regenerates into chloroplasts when subjected to light rays. But prolonged etiolation might completely bar the conversion of etioplast to chloroplast and subsequent vascular regeneration for stock-scion joining, as in the present experiment with 216-hour etiolation, which had no success on transplantable graft production. Finally, as rooting finds vigor by etiolation, proliferated roots in the stock plants might further take part in adequate nutrient uptake for superior propagule growth in the months after perfect graft joining (Fig. 2). Therefore, grafting technique, time, and pre-and post-grafting treatment are determinant for vegetative propagation in avocado, a difficult-to-propagate species.

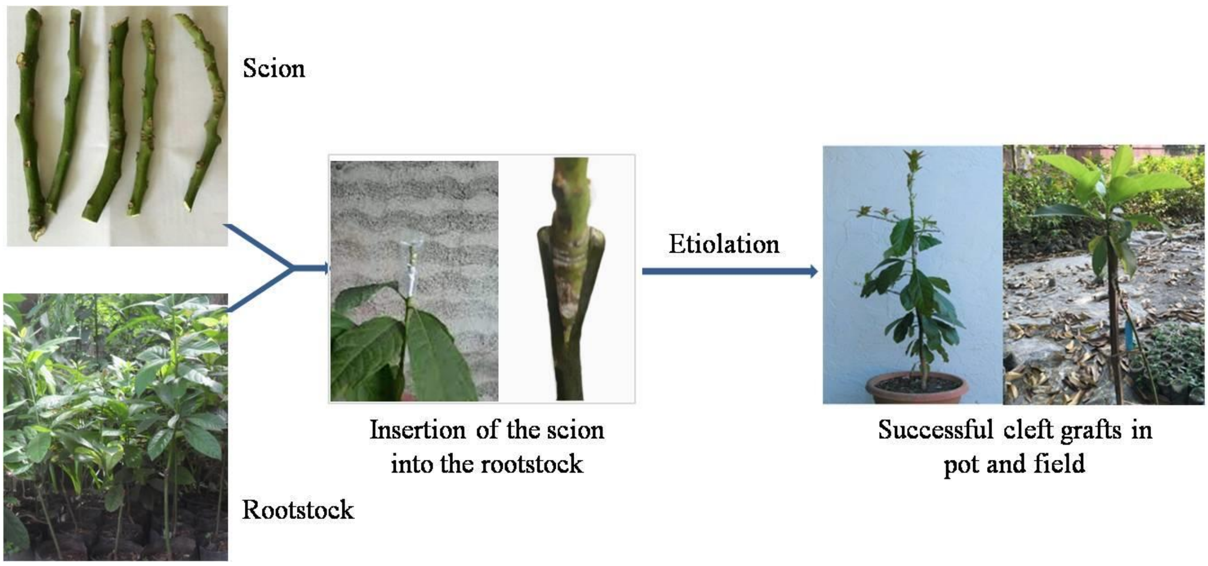

Fig. 2. Photographs on different steps of avocado cleft grafting in the present experiment.

Conclusion

In the present experiment, the highest grafting success was achieved in cleft grafting, followed by veneer grafting. The lowest success rate occurred in T-budding. On the other hand, the maximum number of saplings was obtained from grafting in the first quarter of the spring season, and then to a lesser extent in the autumn season. The minimum number of saplings was obtained from the winter season's grafting. There was an increase in the spring season's cleft grafts by applying the etiolation treatment for 120 hours. The percentage of transplantable saplings increased by 59% compared to the control, but grafting failure occurred at 216 hours of etiolation. Thus, appropriate grafting (cleft) at appropriate times of the year (spring season) with post-grafting etiolation (regulated) have a direct and positive influence on the successful vegetative propagation of avocado in sub-tropical environmental conditions in Bangladesh.

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Conflict of Interest
The authors indicate no conflict of interest for this work.

Data availability statement
The authors confirm that the data supporting the findings of this study are available within the article.

Ethical statement
The field operations in this experiment were carried out following guidelines and recommendations of "Biosafety Guidelines of Bangladesh" published by the Ministry of Environment and Forest, regularly monitored and supervised under the guidelines of BARI. The authors considered all ethical issues in using plant materials.

References

Abo El-Fadl RE, Ahmed ME, Abd Alhady MRA. 2022. *In vitro* propagation of avocado (*Persea americana* Mill.). Egyptian Journal of Desert Research 72(1), 73-87. https://doi.org/10.21608/ejdr.2022.133868.1102

Ahmed MF, Kantharajah AS, Holford P. 2001. Factors affecting adventitious shoot and root formation from the avocado cultivar 'Hass' (*Persea americana*, Mill.) in vitro. Gartenbauwissenschaft 66(6), 293-297.

Ahmed N, Singh SR, Srivastava KK, Shagoo PA, Hayat S. 2012. Effect of different environments, grafting methods and times on sprouting, graft success and plant growth of walnut (*Juglans regia*). Indian Journal of Agricultural Sciences 82(12), 14-18.

Akinnifesi FK, Leakey R, Ajayi OC, Gudeta S, Tchoundjeu Z, Matakala P, Kwesiga FR. 2008. Trees in the tropics: Domestication, utilization and commercialization. Wallingford, UK: CABI Publishing.

Araújo RG, Rodriguez-Jasso RM, Ruiz HA, Pintado MME, Aguilar CN. 2018. Avocado by-products: Nutritional and functional properties. Trends in Food Science & Technology 80, 51-60. https://doi.org/10.1016/j.tifs.2018.07.027

Armarego-Marriott T, Sandoval-Ibañez O, Kowalewska Ł. 2020. Beyond the darkness: recent lessons from etiolation and de-etiolation studies. Journal of Experimental Botany 71(4), 1215-1225. https://doi.org/10.1093/jxb/erz496

Asahina M, Satoh S. 2015. Molecular and physiological mechanisms regulating tissue reunion in incised plant tissues. Journal of Plant Research 128, 381-388. https://doi.org/10.1007/s10265-015-0705-z

Asayesh ZM, Vahdati K, Aliniaeifard S, Askari N. 2017. Enhancement of ex vitro acclimation of walnut plantlets through modification of stomatal characteristics *in vitro*. Scientia Horticulturae 220, 114-121. https://dx.doi.org/10.1016/j.scienta.2017.03.045

Ayala-Arreola J, Barrientos-Priego AF, Colinas-Leon MT, Sahagun-Castellanos J, Reyes-Aleman JC. 2010. Scion-interstock relationships and anatomical and physiological leaf characteristics of four avocado genotypes. Revista Chapingo. Serie Horticultura 16(2), 147-154.

Azad AK, Miaruddin M, Wohab MA, Sheikh MHR, Nag BL, Rahman MHH. 2020. Handbook on agro-Technology. Bangladesh Agricultural Research Institute. Gazipur 1701, Bangladesh. [in Bengali].

Barceló-Muñoz A, Pliego-Alfaro F. 2003. Micropropagation of avocado (*Persea americana* Mill.). Micropropagation of woody trees and fruits 519-542. https://doi.org/10.1007/978-94-010-0125-0_17

BBS. 2022. Statistical Yearbook of Agriculture-2021. Bangladesh Bureau of Statistics (BBS), Statistics and Informatics Division (SID), Ministry of Planning, Government of the People's Republic of Bangladesh.

Bender GS, Whiley AW. 2002. Propagation. The avocado: botany, production and uses, 189-211.

Beshir W, Alemayehu M, Dessalegn Y. 2019. Effect of grafting time and technique on the success rate of grafted mango (*Mangifera indica* L.) in Kalu District of Amhara Region, North Eastern Ethiopia. Cogent Food & Agriculture 5(1), 1577023. https://doi.org/10.1080/23311932.2019.1577023

Bhuyan DJ, Alsherbiny MA, Perera S, Low M, Basu A, Devi OA, Barooah MS, Li CG, Papoutsis K. 2019. The odyssey of bioactive compounds in avocado (*Persea americana*) and their health benefits. Antioxidants 8(10), 426. https://doi.org/10.3390/antiox8100426

Chithiraichelvan R, Kumar S, Karunakaran G, Sakthivel T. 2006. Standardization of propagation techniques in avocado (*Persia americana* Mill). National Symposium on underutilized horticultural crops. June 8-9 at IIHOUR, Bangalore, Abs pp. 68.

Develi BE, Miler N. 2023. Impact of light quality on *in vitro* potato microtubers formation. International

Journal of Horticultural Science and Technology 10 (special issue, light in horticulture), 89-100. https://doi.org/10.22059/ijhst.2023.353476.613

dos Santos MAZ, Alicieo TV, Pereira CM, Ramis-Ramos G, Mendonça CR. 2014. Profile of bioactive compounds in avocado pulp oil: Influence of the drying processes and extraction methods. Journal of the American Oil Chemists' Society 91, 19-27. https://doi.org/10.1007/s11746-013-2289-x

Duarte PF, Chaves MA, Borges CD, Mendonça CR. 2016. Avocado: characteristics, health benefits and uses. Ciência Rural 46, 747-54. https://doi.org/10.1590/0103-8478cr20141516

Duman Z, Hadas-Brandwein G, Eliyahu A, Belausov E, Abu-Abied M, Yeselson Y, Faigenboim A, Lichter A, Irihimovitch V, Sadot E. 2020. Short de-etiolation increases the rooting of VC801 avocado rootstock. Plants 9(11), 1481. https://doi.org/10.3390/plants9111481

Erdogan V. 2006. Effect of hot callusing cable on graft success in walnut (*Juglans regia*) propagation: nursery results. Indian Journal of Agricultural Sciences 76(9), 544-546.

Escobedo V, Escobedo JA. 2011. Adventitious root formation without rooting medium in etiolated shoots of 'duke' avocado (*Persea americana*) growing on nurse plants. Acta Horticulturae 923, 227-232. https://doi.org/10.17660/ActaHortic.2011.923.34

Estay C, Cautín R, Neaman A, Castro M. 2016. Clonal propagation of the avocado: effects of the rooting step on graft union formation and development. Agricultural Science and Research: Latin American Journal of Agricultural Sciences 43(2), 233-241. https://doi.org/10.4067/S0718-16202016000200006

Galindo-Tovar M, Ogata-Aguilar N, Arzate-Fernández AM. 2008. Some aspects of avocado (*Persea americana* Mill.) diversity and domestication in Mesoamerica. Genetic Resources and Crop Evolution 55, 441-50. https://doi.org/10.1007/s10722-007-9250-5

Ghosh SN, Bera B. 2015. Studies on standardization of propagation methods of some minor fruit crops in India. International Journal of Minor Fruits, Medicinal and Aromatic Plants 1(1), 31-36.

Hartmann HT, Kester DE, Davies FT, Geneve RL. 2007. Plant propagation principles and practices. 7th ed. Prentice Hall Pvt. Ltd., New Delhi, India. 880p

Hiti-Bandaralage JC, Hayward A, Mitter N. 2017. Micropropagation of avocado (*Persea americana* Mill.). American Journal of Plant Sciences 8(11), 2898. https://doi.org/10.4236/ajps.2017.811197

Islam MM, Rahim MA, Choudhury SH. 2014. Effect of scion wood maturity and time of grafting on the success, survivability and growth in cleft grafting of pummelo. Bangladesh Agricultural University, Bangladesh.

Islam MN, Rahim MA, Farooque AM. 2004. Standardization of time and grafting techniques in mango under Bangladesh condition. Asian Journal of Plant Sciences 3(3), 378-386.

Jalal A, Tripathi S, Kumar A. 2019. Standardization of propagation techniques in different cultivars of aonla (*Emblica officinalis* Gaertn). International Journal of Pure & Applied Bioscience 7, 71-77.

Janick J, Scofied S, Goldschmit EE. 2010. Principles of grafting and budding. In: Vegetative propagation. pp 415-463.

Karadeniz T. 2005. Relationships between graft success and climatic values in walnut (*Juglans regia* L.). Journal of Central European Agriculture 6(4), 631-634.

Kaur A, Kaur A. 2019. Effect of propagation time and methods on the success rate of grafted mango cv. amrapali. Agricultural Research Journal 56(4), 644-650. https://doi.org/10.5958/2395-146X.2019.00100.5

Khan JA, Jaskani MJ, Abbas H, Khan MM. 2006. Effect of light and dark culture conditions on callus induction and growth in citrus (*Citrus reticulata* Blanco). International Journal of Biology and Biotechnology 3, 669-672.

Knight RJ, Campbell CW. 1999. Ecological adaptation and the evolution of modern avocado cultivars. Revista Chapingo Serie Horticultura 5, 49-54.

Kumar K, Rao IU. 2012. Morphophysiological problems in acclimatization of micropropagated plants *in-ex vitro* conditions-A Reviews. Journal of Ornamental and Horticultural Plants 2(4), 271-283.

Kümpers BM, Bishopp A. 2015. Plant grafting: making the right connections. Current Biology 25(10), R411-R413. https://doi.org/10.1016/j.cub.2015.03.055

Lee K, Seo PJ. 2018. Dynamic epigenetic changes during plant regeneration. Trends in Plant Science 23(3), 235-247. https://doi.org/10.1016/j.tplants.2017.11.009

Lewis WJ, Alexander DM. 2008. Grafting & Budding: A Practical Guide for Fruit and Nut Plants and Ornamentals. Landlinks Press, Australia.

Mattsson J, Ckurshumova W, Berleth T. 2003. Auxin signaling in Arabidopsis leaf vascular development. Plant Physiology 131(3), 1327-1339. https://doi.org/10.1104/pp.013623

Mayavel A, Chitra P, Bhagatsingh CDA, Nagarajan B. 2022. Optimization of grafting season on cleft grafting for deploying commercial propagation of tamarind (*Tamarindus indica*) in Tamil Nadu. International Journal of Environment, Agriculture and Biotechnology 7(1), 198-202. https://dx.doi.org/10.22161/ijeab.71.23

Maynard BK, Bassuk NL. 1996. Effects of stock plant etiolation, shading, banding, and shoot development on histology and cutting propagation of *Carpinus betulus* L. fastigiata. Journal of the American Society for Horticultural Science 121(5), 853-860. https://doi.org/10.21273/JASHS.121.5.853

Melnyk CW, Schuster C, Leyser O, Meyerowitz EM. 2015. A developmental framework for graft formation and vascular reconnection in Arabidopsis thaliana. Current Biology 25(10), 1306-1318. https://doi.org/10.1016/j.cub.2015.03.032

Mng'omba SA, du Toit ES. 2013. Effect of diagonal cut surface length on graft success and growth of *Mangifera indica, Persia americana,* and *Prunus persica*. HortScience 48(4), 481-484. https://doi.org/10.21273/HORTSCI.48.4.481

Mng'omba SA, Akinnifesi FK, Sileshi G, Ajayi OC. 2010. Rootstock growth and development for increased graft success of mango (*Mangifera indica*) in the nursery. African Journal of Biotechnology 9(9), 1317-1324. https://doi.org/10.5897/AJB10.668

Monteuuis O, Bon MC. 2000. Influence of auxins and darkness on in vitro rooting of micropropagated shoots from mature and juvenile *Acacia mangium*. Plant Cell, Tissue and Organ Culture 63(3), 173-177. https://doi.org/10.1023/A:1010611126950

Nahar A, Choudhury SH, Rahim MA, Susmita Ray IS. 2015. Effect of scion defoliation and stock leaf retention on the grafting success and survivability of lime. Europian Academic Research 3(9), 9721-9728.

Nanda AK, Melnyk CW. 2018. The role of plant hormones during grafting. Journal of Plant Research 131(1), 49-58. https://doi.org/10.1007/s10265-017-0994-5

Nhut DT, Thi NN, Khiet BLT, Luan VQ. 2008. Peptone stimulates in vitro shoot and root regeneration of avocado (*Persea americana* Mill.). Scientia Horticulturae 115(2), 124-128. https://doi.org/10.1016/j.scienta.2007.08.011

Prasanth JM, Reddy PN, Patil SR, Pampanagouda B. 2007. Effect of cultivars and time of softwood grafting on graft success and survival in mango. Agricultural Science Digest 27(1), 18-21.

Premkumar A, Barcelo-Munoz A, Quesada MA, Mercado JA, Pliego-Alfaro F. 2003. Influence of sucrose concentration on in-vitro rooting, growth, endogenous sugars and ex vitro survival of juvenile avocado. The Journal of Horticultural Science and Biotechnology 78(1), 46-50. https://doi.org/10.1080/14620316.2003.11511585

Pribil M, Labs M, Leister D. 2014. Structure and dynamics of thylakoids in land plants. Journal of Experimental Botany 65(8), 1955-1972. https://doi.org/10.1093/jxb/eru090

Rahemi AR, Taghavi T, Dale A. 2016. A simple method to improve hazelnut grafting. Journal of Nuts 7(2), 83-87. https://doi.org/10.22034/jon.2016.527093

Rasool A, Mansoor S, Bhat KM, Hassan GI, Baba TR, Alyemeni MN, Alsahli AA, El-Serehy HA, Parey BA, Ahmad P. 2020. Mechanisms underlying graft union formation and rootstock scion interaction in horticultural plants. Frontiers in Plant Science 11, 590847. https://dx.doi.org/10.3389/fpls.2020.590847

Restrepo Osorio C, Gómez Velásquez FA, Gil Correal A, Torres Bonilla JM, Urrea Trujillo AI. 2018. *In vitro* propagation of avocado (*Persea americana* Mill.) cv. Hass through morphogenesis. Acta Agronómica 67(1), 160-167. https://doi.org/10.15446/acag.v67n1.61474

Richards MR, Rupp LA. 2012. Etiolation improves rooting of bigtooth maple cuttings. HortTechnology 22(3), 305-310. https://doi.org/10.21273/HORTTECH.22.3.305

Roy S, Sinha AK, Singh USP. 1999. Detached methods of propagation in mango (*Mangifera indica* L.). Journal of Allied Biology 9(1), 14-16.

Sarikhani H, Sarikhani-Khorami H. 2021. Effect of light quality on micropropagation and some morphological properties of cadaman avimag (*Prunus persica × P. davidiana*) rootstock. International Journal of Horticultural Science and Technology 8(1), 51-65. https://doi.org/10.22059/ijhst.2020.298841.351

Shepherd J, Bender G. 2002. A history of the avocado industry in California. California Avocado Society Yearbook 85, 29-50.

Silva TA, Ledesma N. 2014. Avocado History, Biodiversity and Production. In: Nandwani, D. (eds.) Sustainable Horticultural Systems. Sustainable Development and Biodiversity 2, 157-205. https://doi.org/10.1007/978-3-319-06904-3_8

Singh R, Bons HK. 2016. Standardization of propagation techniques in sapota [*Manilkara achras* (Mill.) Fosberg] under north Indian conditions. Research on Crops 17(3), 534-537.

Soleimani A, Rabiei V, Hassani D. 2010. Effect of different techniques on walnut (*J. regia* L.) grafting. Journal of Food, Agriculture & Environment 8(29), 544-546.

Thakur M, Sharma DD, Singh K. 2014. Studies on the effect of girdling, etiolation and auxins on rooting of olive (*Olea europaea* L.) cuttings. International Journal of Farm Sciences 4(2), 39-46.

Thao NTP, Ozaki Y, Okubo H. 2003. Callus induction and plantlet regeneration in ornamental *Alocasia micholitziana*. Plant Cell, Tissue and Organ Culture 73, 285-289. https://doi.org/10.1023/A:1023025717271

Tripathi PC, Karunakaran G. 2019. Standardization of time and method of propagation in avocado. Journal of Applied Horticulture 21(1), 67-69.

Tripathi PC, Karunakaran G, Sakthivel T, Sankar V, Senthilkumar R, Muralidhara SR, Venkataravanappa V, Madhu GS, Begane N. 2014. Avocado cultivation in India. Bulletin, Central Horticultural Experiment Station Indian Institute of Horticultural Research Chettalli, Kodagu, Karnataka, 14.

Wang Y. 2011. Plant grafting and its application in biological research. Chinese Science Bulletin 56, 3511-3517. https://doi.org/10.1007/s11434-011-4816-1

Whitsell RH, Martin GE, Bergh BO, Lypps AV, Brokav WH. 1989. Propagating avocados: principles and techniques of nursery and field grafting. University of California, Division of Agriculture and Natural Resources. Publication no. 21461.

Wolstenholme B. 2003. Avocado rootstocks: What do we know; are we doing enough research. South African Avocado Growers' Association Yearbook. 26, 106-112.