Assessment of Walnut Grafting Success under Locally Fabricated Hot Callusing Technology with IoT based Environmental Control

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ABSTRACT

Walnut grafting under open conditions often achieves a relatively low success rate of 0-20%, primarily due to difficulties in maintaining optimal environmental conditions. Grafting is typically carried out in February and March when the plants are dormant, during which the temperatures fall below 15°C. However, successful walnut grafting requires an optimal temperature of 27°C and a relative humidity of 90%, conditions that are difficult to maintain in an open environment. Hot callusing technology presents a solution to achieve and sustain these optimal conditions. This study, conducted at the Agriculture Research and Development Centre (ARDC) Wengkhar and Drepong village, Mongar Dzongkhag, aimed to develop an effective and sustainable hot callusing technology. The system was developed using locally available materials integrated with IoT (internet of things) technology to automate and monitor environmental conditions. The efficacy of locally fabricated hot callusing systems on the success rate of walnut grafting was evaluated both on-station and on-farm. The study initiated on-station in 2023 with 180 seedlings achieved a graft success rate of 78%. The second study conducted on-farm in 2024 with 1,170 grafted seedlings, achieved a 76% success rate. For both the sites, the grafting was done in February month when the plants were still at dormancy stage. Unlike the past studies, this study made use of vertical space and IoT for automation and monitoring. Further, investment analysis demonstrated a positive Net Present Value (NPV) and a high Internal Rate of Return (IRR), confirming the economic viability of this technology for walnut nursery enterprise development. The findings indicate that the locally fabricated hot callusing system, coupled with IoT technology, provides a sustainable and profitable solution for walnut grafting in Bhutan, with the potential for broader applications in agricultural enterprise development.

Keywords: *Walnut grafting; Success rate; Hot callusing technology; Temperature; Humidity; Internet of Things (IoT)*

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1 Introduction

Walnut is one of the potential cash crops in Bhutan with good domestic and export market opportunities. In 2021, the country produced approximately 129.31 metric tonnes of nuts from 8911 bearing trees, contributing to 0.30% of the total fruit production (National Statistics Bureau, 2024). However, most of the walnut orchards in Bhutan consist of seedling-origin trees, which take a longer time to bear fruits and exhibit yield variability (RNR-RC Yusipang, 2003).

Recognizing the importance of walnuts as a high-value crop, the Million Fruit Tree Plantation Project (MFTP) has identified walnuts as one of the priority commodities for promotion. In 2024, as part of the MFTP Phase III, 33,817 walnut seedlings were distributed nationwide for plantation of which 91% of the total walnut seedlings were imported from the neighbouring countries, while only 9% were produced domestically (National Seed Centre, 2024b). This significant gap underscores the high demand for walnut seedlings within Bhutan. Further, grafted walnut seedlings fetch higher prices in the market ranging from Nu 175 to 205 per seedling (National Seed Centre, 2024a).

However, walnut trees are among the most challenging fruit crops to graft with a low graft success rate ranging from 0-20% when grafted under open conditions (Avanzato & Atefi, 1995; Gandev, 2015; Lagerstedt, 1983). A critical factor for successful grafting is the formation of callus tissue, which facilitates the union between grafted parts (scion and the rootstock plant). The rapid formation of callus tissue is essential and is influenced by several factors including temperature, light, pressure, moisture, and chemical growth regulators (Lagerstedt, 1983). Among these factors, temperature and humidity are the main factors affecting the callus formation in walnuts (Rongting & Pinghai, 1991). It requires an optimum temperature of 27°C and a Relative Humidity (RH) of 90-95% at the graft union for three weeks for successful callus formation (Avanzato & Tamponi, 1987; Karadeniz, 2005). Several studies have reported that walnuts do not form callus tissue when the temperature falls below 20°C (Hassan et al., 2019; Reil et al., 1998). Generally, grafting is performed in February and March when the buds are dormant. However, during this period, the temperature is below 20°C and RH is below 70%,

making it difficult to achieve the required conditions for successful grafting in open environments (National Center for Hydrology and Meteorology, 2023).

To address this, Hot Callusing Technology (HCT) offers a solution by selectively heating the graft union to promote callus formation while keeping the rest of the plant unheated (Avanzato & Atefi, 1995; Gandev, 2017; Lagerstedt, 1983). Hot callusing is the method of heating the graft union of the plants to accelerate the callus formation while leaving other plant parts unheated (Lagerstedt, 1983). This technology, first developed by Lagerstedt Harry B in 1981, has been widely studied and has demonstrated a graft success rate of 80-85% in various trials (Avanzato & Atefi, 1995; Erdogan, 2004; Gandev, 2017; Vanzyl, 2009). In 1997, the National Centre for Organic Agriculture (NCOA), the then Renewable Natural Resources Research Centre (RNR-RC) Yusipang, Thimphu carried out a trial on walnut grafting by developing the HCT (RNR-RC Yusipang, 2003). The study found it very promising for commercial production of grafted walnut seedlings in the country. However, the technology was not widely adopted due to challenges such as interrupted power supply, difficulty in monitoring and maintaining the required callusing temperature by analogue thermostats, and the nonavailability of other essential spare parts of the HCT in Bhutan (RNR-RC Yusipang, 2003). Therefore, this study aimed to redesign and develop an enhanced hot callusing system with the following objectives:

- i. Develop an efficient and sustainable hot callusing system with locally and readily available materials.
- ii. Integrate IoT technology for precision control and monitoring of callusing parameters (temperature, humidity) and other automation processes to reduce labour costs.
- iii. Evaluate the effectiveness of the prototype for potential enterprise development.

2 Materials and Method

2.1 Study area

The first trial was initiated on-station at Wengkhar in 2023 to test the prototype. A total of 180 seedlings were grafted and placed in a hot callusing tube in a 4 x 4.5 m net house. After the successful testing of the prototype on-station, the second trial was initiated on-farm at Drepong village under Mongar Dzongkhag in 2024 in one of the private nurseries in a 5 x 10 m net

house. A total of 1170 grafted seedlings were placed in the hot callusing tube in a 5 x 10 m net house.

2.2 Seedlings Propagation

- *a. Propagation of rootstocks:* Seedlings of 1-2 years old attaining pencil size were used as rootstocks.
- b. Scion Collection: Prior to grafting, scion woods were collected in December from the healthy and productive matured softshell walnut trees. Scion woods with 2-3 buds were cut and waxed to prevent moisture loss during storage until they were used for grafting in February.
- *c. Grafting:* Grafting was done in February when the plants were in their dormant stage. To reduce contamination, all grafting knives were cleaned with ethanol before any cuts were made. Rootstocks were carefully selected so that the scion and rootstock diameters match at the graft union. Bench graft with side veneer grafting technique was used to graft the seedlings. All the grafted seedlings were then placed in the hot callus chamber.

2.3 Design and fabrication of hot-callusing apparatus

The hot-callusing apparatus that we designed was mainly based on the design described by (Lagerstedt, 1983) with some modifications using locally available materials. The fabricated hot-callusing apparatus provides heat only to the graft union area to accelerate callusing while the other parts of the plant (scion and root) remain unheated. The fabricated apparatus comprises of the following parts:

a. Semi-permanent structure: A 10 x 5 m size greenhouse tubular frame was used to construct a shade house with 70% shading to in-house all the hot-callusing related equipment. The main purpose of the structure was to protect delicate grafted walnut seedlings, IoT devices, and sensors from direct exposure to sunlight and rain. Generally, a total of 54 callusing tubes can be fitted in a 10 x 5 m structure. However, in our study, we installed only 39 callusing tubes in the structure.

b. Hot-callusing tube: A 75 mm diameter PVC pipe, commonly available at local hardware stores was used as the primary material for the hot-callusing tube (Figure 1). On one side of the PVC pipe, 30 callusing chambers were cut to enclose the graft union area. Inside each pipe, 6 mm thick plastic foam was lined as a heat insulator and an 8 mm copper tube was inserted to circulate hot water. This hot water circulation maintained the necessary temperature for hot-callusing. Some of the hot-callusing tubes were equipped with temperature sensors to monitor the callusing temperature. Each callusing tube accommodated 30 grafted plants by maintaining 10 cm between two plants. With 39 hot-callusing tubes in a structure, it accommodated about 1170 grafted seedlings. Following this design, the final production of the hot-callusing tubes was outsourced to a local private firm.



Figure 8. 75 mm diameter PVC pipe as hot-callusing tube and 110 mm PVC roof gutter as root holder pipe

c. Root Holder PVC: PVC roof gutter pipe of 110 mm diameter was used as a platform to hold the root system of the grafted walnut seedlings (Figure 1). The root holder pipe was filled with soil mixed with mosses and fitted with an automated drip irrigation system

d. Boiler Tank: A 200 L plastic barrel was used as the main water reservoir and heating unit for the hot-callusing system (Figure 2). A water level sensor was fitted inside a barrel to enable automatic refilling when the water level dropped. A barrel was also fitted with a 2500-watt electric water heater to heat the circulating water and a temperature sensor to monitor the water temperature. To minimize heat loss to the surrounding environment, the barrel was insulated by wrapping black plastic foam. The water temperature within the tank was consistently maintained between 50-55 °C.



Figure 9. 200 L plastic barrel insulated with black foam to retain water temperature

e. Water Circulation Pump: To circulate the hot water from the reservoir tank to the hotcallusing tubes, we used a regular household water pump with 1 HP capacity which was connected to a 240 V AC power supply through pump relay switch. The pump relay switch was then connected to the IoT based controller which will switch ON and OFF the pump based on the temperature setpoints detected by the temperature sensor fitted inside the hotcallusing tube.

2.4 IoT based control and monitoring system

The successful formation of graft union in walnut seedlings placed inside the hot-callusing tube requires precision maintenance of a temperature (26-27°C) at the graft union (Lagerstedt, 1983). To accomplish this we used microcontrollers, sensors, and cloud IoT service for remote monitoring and control systems. The basic working principle of the system is as follows (Figure 3).



Figure 10. Basic architecture of Hot callusing IoT based climate control and monitoring system

- *a.* Microcontroller: We used a NodeMCU Microcontroller which is a low-cost, open-source development board based on the ESP8266 Wi-Fi chip (NodeMCU Documentation, 2023). It was designed for IoT applications and is highly popular for creating connected devices and automation systems. The NodeMCU is then uploaded with the latest version of Tasmota firmware. Tasmota is an open-source firmware designed to provide firmware for ESP8266 and ESP32 based Microcontroller (Tasmota, 2023). It is popular due to its ability to transform various devices into locally controlled, MQTT integration and support for a wide range of sensors and actuators. The microcontroller was then connected to the sensors, actuators, Wi-Fi and configured as main controller for automation
- b. Sensors: Sensors are fundamental components in any automation systems, providing the data needed for machines and devices to make autonomous decisions. Sensors measure various physical parameters such as temperature, humidity, light, pressure, motion, and gas concentrations, ensuring that automated processes respond effectively to changing conditions (Thompson, 2015). In this study, we used three types of sensors for measuring temperature, humidity and water level in the system as follows:
 - *i)* DS18B20 temperature Sensor: It was used to measure the temperature of the hotcallusing tube and the temperature of water in the boiler tank. The DS18B20 is a digital temperature sensor widely used in various electronics and automation projects due to

its accuracy, simplicity, and versatility. It communicates over a 1-Wire bus, which allows multiple sensors to be connected to a single data pin. The DS18B20 can measure temperatures ranging from -55°C to +125°C with an accuracy of ± 0.5 °C in the range of -10°C to +85°C (Maxim Integrated, 2020). Temperature data from these sensors regulates the temperature of hot-callusing tubes and water temperature of the boiler tank.

- *ii) HTU21D temperature and humidity sensor*: It was used to measure temperature and humidity inside the net-house. The HTU21D is a digital humidity and temperature sensor known for its high accuracy, compact size, and low power consumption. It measures relative humidity from 0% to 100% with an accuracy of $\pm 2\%$ and temperature from -40°C to +125°C with an accuracy of ± 0.3 °C (TE Connectivity, 2017).
- *iii) Magnetic water level sensor:* It is a device used to detect the water level in a tank or reservoir. It operates based on the principle of magnetic float switches, where a buoyant float rises and falls with the water level. As the float moves, it activates a reed switch inside the sensor, which can either open or close an electrical circuit, signaling the water level status to the microcontroller.
- a. Actuators: Actuators are devices responsible for converting electrical, hydraulic, or pneumatic energy into mechanical motion. They enable automated systems to perform physical tasks, such as opening valves, moving robotic arms, or adjusting the position of machinery etc. In this system we used two actuators that is, I HP water pump to circulate hot water in the hot-callusing tubes and 2500 Watts electric heater to heat water in the boiler tank
- b. 3G Wi-Fi router: To connect our microcontroller to the internet we used 3G Wi-Fi router which provides internet access using a 3G mobile network. It connects to the internet by receiving a 3G signal from a SIM card provided by a B-mobile operator and then broadcasts a Wi-Fi signal.
- c. IoT Cloud Service (eWeLink App): We used eWeLink Cloud Service as a main IoT platform. It allows users to control and monitor IoT devices remotely through smartphones or tablets. The app communicates with connected devices via cloud servers, enabling users to turn devices on or off, set schedules, or check device status from anywhere with an internet connection (eWeLink, 2023). The app is designed for ease of use, with drag-and-drop functions for automating tasks, creating schedules, and setting up scenes. It also provides space to store and retrieve sensor data for one hour interval for the total duration of 6 months.

- d. Setup and configuration of eWeLink App:
 - App download and registration: The first step in setting up the eWeLink app was to download it from the Google Play Store (for Android devices) or the Apple App Store (for iOS devices). After installing, we opened the app and created an account using email address or phone number. Then received a verification code and completed the registration process.
 - Adding Devices: Second step was to add our microcontroller devices by powering on and tapping the "+" button on the App home screen. The app typically connects via Wi-Fi and requires the input of our 3G Wi-Fi network credentials (SSID and password).
 - Device configuration and automation scenes: Once our devices were connected, we configured various settings, such as renaming the device and creating schedules for automated operation. We have created three automation scenes for controlling the hot-callusing system as follows:
 - Automation Scene 1: If the temperature inside the hot-callusing tube drops below 26 °C the water Pump will start and circulate the hot water in the hot-callusing pipe networks. If the temperature inside the hot-callusing tube rises above 27 °C the pump stops to circulate the hot water
 - Automation Scene 2: If the temperature of water in the boiler tank drops below 50
 ^o C the device will switch ON the water heater. If the water temperature rises above
 55 ^o C it will switch OFF the water heater.
 - Automation Scene 3: If the water level sensor detects a LOW signal the device will activate the electric valve and allow the water to flow in the boiler tank. If the level sensor detects HIGH signal the device will deactivate the electric valve.

2.5 Vertical stacking design of hot-callusing tubes

In this study we developed a vertical stacking design to accommodate more grafted walnut seedlings by utilizing the vertical space and optimizing horizontal space (Figure 4). The structure was divided into 3 rows with 1.4 m distance between each row. In each row, a total of 18 callusing pipes were installed vertically by maintaining a distance of 0.6 m. With this design, a total of 54 callusing tubes can be fitted in a 5x10 m structure. However, in this study, a total of 39 callusing tubes were installed. Each callusing tube accommodated 30 grafted plants with a total of 1170 grafted seedlings in 39 callusing tubes.



Figure 11. Vertical stacking design of hot callusing tubes

2.6 Irrigation layout

Drip irrigation was installed in the root zone along the gutter using Jain Drip and the irrigation was scheduled twice for 30 minutes in a day (Figure 1).

2.7 Sensitivity analysis

A sensitivity analysis was conducted to evaluate the profitability of the technology under different market conditions, considering five distinct scenarios. As the model is particularly sensitive to changes in the price of walnut seedlings, scenarios involving a 10% increase and a 10% decrease in annual revenue were considered. Additionally, since variations in the discount rate also influence the model's outcomes, changes of $\pm 2\%$ in the discount rate were incorporated into the analysis. The following scenarios reflect these adjustments;

- a. Normal: Based on current market conditions.
- b. Optimistic: Assuming a 10% increase in annual revenue and a 5% decrease in annual variable costs.
- c. Pessimistic: Assuming a 10% decrease in annual revenue and a 5% increase in annual variable costs.
- d. Higher Discount Rate: Assuming a 2% increase in the discount rate.
- e. Lower Discount Rate: Assuming a 2% decrease in the discount rate.

3 Results and Discussion

3.1 IoT-based monitoring and automation

An IoT-based control and monitoring system was set up and tested one day prior to the start of the walnut grafting process. Then the system has been kept operating continuously for over 30 days, with sensor data including callusing temperature, boiler water temperature, outside temperature, and humidity logged to the eWeLink cloud server at hourly intervals. The recorded data was later downloaded from the eWeLink server for further analysis and visualization. The IoT system effectively controlled and maintained the temperatures of both the hot-callusing chamber and the boiler tank within the specified range. However, there was a sudden temperature drop in both the hot-callusing chamber and boiler tank is water heater, which was later replaced with a new unit. Additionally, it indicated that walnut grafting in open air would be challenging during the experiment period due to the relatively low outside temperature. A more efficient way to maintain the temperature of the hot-callusing chamber was observed by increasing the temperature of the circulating water from the boiler. However, when the water temperature exceeded 60°C, the LDP pipe network became difficult to maintain, as the pipes began to break down and leak due to the high heat.



Figure 12. Record of daily callusing temperature, outside temperature, boiler water temperature and outside relative humidity for a month from an eWeLink server

3.2 Callus formation and Graft success

The grafted seedlings were immediately placed in a hot-callusing chamber, where the temperature at the graft union was maintained between 26-27°C for 30 days. The heat from the chamber accelerated the natural wound response in the plant. As a result, both the rootstock and scion began producing callus tissue at the cut edges. Callus is a mass of undifferentiated cells that forms as the plant's healing mechanism. The callus tissue bridges the gap between the rootstock and scion, promoting cell differentiation and vascular tissue connection over time. This connection is vital for water and nutrient transport between the two parts (Hartmann & Kester, 1959). Callus formation was visually inspected on a weekly basis. It was observed that the grafted seedlings began forming callus within 21 days of being placed in the hot-callusing chamber (Figure 6).



Figure 13. Callus formation at the graft union of walnut seedling

The on-station trial showed 78% graft success where 140 seedlings out of 180 formed a successful graft union. Similarly, the on-farm trial at Drepong also showed 76% graft success with 889 seedlings out of 1170 forming successful graft unions. The success rate of 76% on-farm and 78% on-station is relatively higher compared to the graft success rate in the open condition which has a maximum success rate of 30% at Wengkhar condition. The higher success rate in the HCT would be a result of localized heating of graft union while leaving other plant parts unheated due to which, buds remain dormant during grafting time and start growing at the end of uniting process (Lagerstedt, 1983; Soleimani et al., 2010).

The previous study also recorded graft success between 75-80% in the hot callusing system at Yusipang (RNR-RC Yusipang, 2003). They also indicated that the higher success rate was attributed to the absence of moisture and nutrient translocation to the scion until the callus bridge between the scion and rootstock had formed, due to the focused heating of the graft union area (Lagerstedt, 1983). In this study, an optimal temperature of 25-27°C was maintained at the walnut graft union for four weeks. Research has shown that walnut seedlings when exposed to this temperature range form callus tissue at the graft union within 21-28 days of grafting, which is essential for the union between the scion and rootstock (Gandev, 2015; Lagerstedt, 1983; RNR-RC Yusipang, 2003).

3.3 Vertical space utilization

Most hot-callusing setups described in the literature did not incorporate vertical stacking designs (Gandev et al., 2018; Lagerstedt, 1983; RNR-RC Yusipang, 2003). In contrast, this study innovatively utilized the vertical space within the greenhouse, greatly improving its overall efficiency. Without vertical stacking, a 5 x 10 m greenhouse would have been limited to accommodating only 500 seedlings. However, by taking advantage of the available vertical height, the same greenhouse was able to house 1,170 grafted seedlings, which is more than doubling its original capacity. This design not only expanded the capacity for seedlings but also enhanced operational efficiency, resulting in more streamlined management and care processes.

The results of this study highlight the potential of vertical farming technology as a practical solution, especially in urban and peri-urban settings where land availability is constrained. The use of vertical space optimizes land use, enabling higher crop density in limited areas, which is crucial for addressing the growing demand for agricultural productivity in space-limited environments. Furthermore, the vertical stacking design promotes better airflow and light distribution, further supporting seedling health and growth.

3.4 Investment analysis

To evaluate the profitability of the technology, an investment analysis was conducted, considering the costs incurred for setting up HCT and the production of walnut seedlings over ten years. The initial investments for experiment setup and material costs amounted to Nu 244646, with a 5% increase in variable cost annually over the following ten years. Using a

discount rate of 12% which is the standard benchmark for economic analyses in developing countries, the analysis revealed that the entrepreneur could achieve the break-even point by the second year, with an Internal Rate of Return (IRR) of 61% exceeding the discount rate and a positive Net Present Value (NPV) of Nu 534358.677 (Table 1). Similar findings were also reported by RNR-RC Yusipang (2003) where they reported a positive NPV and a higher IRR for walnut grafting using HCT. The benefit calculations were based on a graft success rate of 77%, representing the average success rate achieved across both on-station and on-farm conditions. To account for potential uncertainties and market fluctuations, a sensitivity analysis was conducted across five different scenarios: normal, optimistic, pessimistic, higher discount rate, and lower discount rate. The results showed there was no major changes in the investment model though there are slight changes in the NPV and IRR value, indicating that the technology remains stable and profitable under varying conditions (Table 2). The detailed costing and the sensitivity analysis for different scenarios are given in Annexure 1 and 2.

Year	Cost (C)	Benefit (B)	Net Benefit (NB)	DF (1+r) ^{-t}	Net DF (NBxDF)	ANDF
0	244646	0	-244646	1.0000	-244646	-244646.0
1	22359	184684.5	162325.5	0.8929	144933.482	-99712.5
2	23609	184684.5	161075.5	0.7972	128408.402	28695.9
3	28440	184684.5	156244.5	0.7118	111211.749	139907.6
4	43109	184684.5	141575.5	0.6355	89973.7897	229881.4
5	92759	184684.5	91925.5	0.5674	52160.9974	282042.4
6	111640	184684.5	73044.5	0.5066	37006.6169	319049.0
7	25165	184684.5	159519.5	0.4523	72158.5207	391207.6
8	43109	184684.5	141575.5	0.4039	57179.9699	448387.5
9	27240	184684.5	157444.5	0.3606	56776.0651	505163.6
10	94009	184684.5	90675.5	0.3220	29195.0842	534358.7
			61%	NPV	534358.677	

Table 1. Investment analysis of hot callusing technology for walnut seedling propagation

Т	ab	le 2	2.8	Sensi	tivity	analy	vsis	of	the	hot	cal	lusing	tecl	hnol	ogv	und	er o	liffer	ent	scen	ario	os
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SN	Scenario	Variable Change	NPV (Nu)	IRR %)	Break-even
1	Normal	Without any changes	534358.677	61	2nd Year

2	Optimistic	Annualrevenueincreaseby10%,Annualvariablecostdecreaseby5%	664167.036	74	2nd Year
3	Pessimistic	Annual revenue decrease by 10%, Annual variable cost increase by 5%	404550.319	49	3rd Year
4	Discount rate	Increase by 2%	478248.229	61	2nd Year
5	Discount rate	Decrease by 2%	598075.79	61	2nd Year

4 Conclusion and Recommendations

The study demonstrated that the Hot Callusing Technology (HCT) achieved a graft success rate of 76-78% in walnuts, significantly improving grafting outcomes compared to conventional methods. Another innovation in this technology is the efficient use of vertical space, which maximizes greenhouse capacity and production efficiency. Additionally, the integration of IoT systems for monitoring and controlling environmental conditions makes the technology not only adaptable but also scalable, providing opportunity for youth entrepreneurs to adopt it as a viable business enterprise.

The investment analysis further indicated the economic feasibility of HCT with a higher Internal Rate of Return (IRR), a positive Net Present Value (NPV), and a breakeven point by the second year of operation. Moreover, the sensitivity analysis revealed that the breakeven point remained consistent across five different scenarios, indicating that the technology is resilient, profitable, and adaptable to various economic conditions.

Given these findings, HCT is highly recommended for adoption by the National Seed Centre (NSC), private nursery operators, and young entrepreneurs for commercial production of grafted walnut seedlings. The combination of high success rates, efficient use of space, integration with IoT systems, and strong financial returns makes it a promising technology for scaling up walnut production on commercial scale.

5 Acknowledgment

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6 Authors' contribution statement

Pema Yangdon contributed to the implementation of research, data collection, result interpretation, and drafting of the manuscript. Dr. Tshering Penjor played a key role in the study's conception and design, overseeing the research process and contributing to manuscript preparation. Thinley Gyeltshen was responsible for data analysis and result interpretation. Lungki, Mandira Acharya, Tshewang Dorji, Tshering Pem, and Dema Yangzom supported the research through their involvement in data collection and implementation. Domang provided valuable input in study conception and design, while Sangay Dendup played a critical role in reviewing and editing the manuscript draft.

7 References

- Avanzato, D., & Atefi, J. (1995). Walnut grafting by heating the graft-point directly in the field. In *III International Walnut Congress 442* (pp. 291-294).
- Avanzato, D., & Tamponi, G. (1987). The effect of heating of walnut graft unions on grafting success. In International Symposium on Vegetative Propagation of Woody Species 227 (pp. 79-83).
- Erdogan, V. E. L. I. (2004). Use of hot callusing cable in walnut propagation. In *V International Walnut Symposium 705* (pp. 313-317).
- eWeLink. (2023). eWeLink app: Overview and features. eWeLink. https://ewelink.cc/
- Gandev, S. I. (2015). Application of hot callus and epicotyl grafting methods in walnut propagation. In *III Balkan Symposium on Fruit Growing 1139* (pp. 475-478).
- Gandev, S. (2017). Walnut propagation using a hot water installation and growing the obtained plants in containers. *Bulgarian Journal of Agricultural Science*, *23*(1), 83-85.
- Gandev, S., Nikolova, V., Dimanov, D., Ivanov, P., & Dimitrov, A. (2018). Propagation of a local walnut cultivar'Izvor 10'by in vitro techniques and hot callus method. In *III International Symposium on Horticultural Crop Wild Relatives 1259* (pp. 115-120).
- Hartmann, H. T., & Kester, D. E. (1959). Plant propagation: Principles and practices.
- Hassan, G., Bhat, Z., Sofi, J., Wani, A., Sofi, K., Wani, A., Khan, O., Dar, A., John, I., & Hajam, M. (2019). Propagation of Persian walnut (Juglans regia L.) under controlled climatic conditions. Journal of Pharmacognosy and Phytochemistry, 8(2), 1675–1677.
- Karadeniz, T. (2005). Relationships between graft success and climatic values in walnut (Juglans regia L.). Journal of Central European Agriculture, 6(4), 631–634.

Lagerstedt, H. B. (1983). Method and apparatus for hot callusing graft unions. In U.S. Patent.

- Maxim Integrated. (2020). Maxim Integrated's essential analog temperature sensor ICS deliver precision measurement to enable robust protection for goods and equipment [Marketing]. Maxim Integrated News. https://www.industryemea.com/news/30649-maxim-integrated%E2%80%99s-essential-analog-temperature-sensor-ics-deliver-precision-measurement-to-enable-robust-protection-for-goods-and-equipment
- National Center for Hydrology and Meteorology. (2023). Weather data of Mongar Dzongkhag from 2019-2023. https://www.nchm.gov.bt/
- National Seed Centre. (2024a). Circular for revised and updated selling prices of seeds and seedlings and other farm inputs. National Seed Centre; NSC/ISP-01/2024-2025/32/29 dated 25/07/2024.
- National Seed Centre. (2024b). Sales Report of fruit seedlings supplied for third phase Million Fruit Trees Project. National Seed Centre.
- National Statistics Bureau. (2024). Integrated Agriculture and Livestock Census of Bhutan 2023 (p. 212). www.nsb.gov.bt
- NodeMCU Documentation. (2023). NodeMCU Documentation. https://nodemcu.readthedocs.io/en/release/
- Reil, W. O., Forde, H. I., Mckenna, J. R., & Leslie, C. A. (1998). Propagation. In: D.E.Ramos (ed.). Walnut production manual.
- RNR-RC Yusipang. (2003, October). The hot callusing system- A walnut propagation techniques. Renewable Natural Resources Research Centre Western Region- Yusipang.
- Rongting, X., & Pinghai, D. (1991). A study on the uniting process of walnut grafting and the factors affecting. 160–171.
- 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.
- Tasmota. (2023). Tasmota Documentation—Tasmota. https://tasmota.github.io/docs/
- TE Connectivity. (2017). HTU21D digital humidity and temperature sensor. https://www.te.com/en/industries/sensor-solutions.html
- Thompson, J. (2015). The role of sensors in automation: How smart sensor is changing industries. 12(3), 42–52.
- Vanzyl, L. C. (2009). Grafting of walnut (Juglans regia L.) with hot callusing techniques under South African condition. University of the Free State.

Si No	Particular	Unit	Quantit y (No)	Rate (Nu)	Amount (Nu)	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10
Fixed	Cost														
1	Greenhouse	No	1	4350 0	43500										
2	Latching Solenoid	No	1	4500	4500					4500					4500
3	Smart Irrigation Controller	No	1	7500	7500					7500					7500
4	Water Pump	No	1	9500							7500				
5	Power Relay Switch	No	1	4500	4500				4500				4500		
6	Temperature and Humidity Thermostat	No	2	6500	13000					13000					13000
7	Router	No	1	4250	4250						4250				
8	Hot Callusing Pipe	No	40	1680	67200						67200				
9	PVC Gutter Pipe	No	40	1030	41200					41200					41200
10	HDPE Pipe	Meter	100	28.06	2806							2806			
11	CPVC Tank Nipple	No	2	72	144			144			144			144	
12	CPVC Ball Valve	No	1	187	187			187			187			187	
13	Greenshed Net	Roll	1	3200	3200			3200			3200			3200	
14	Grafting Knife	No	6	1000	6000				6000				6000		
15	Secateur	No	6	1500	9000				9000				9000		
16	250litres barrel	No	1	2500	2500										
17	Water Heating Rod	No	1	1350	1350			1350			1350			1350	
Total .	A				210837			4881	19500	66200	83831	2806	19500	4881	66200
Varial	ble Cost		T	1			I	I	Γ			Γ		Γ	Γ
1	Scionwood	No	1170	4	4680	4680	4680	4680	4680	4680	4680	4680	4680	4680	4680
2	Grafting Tape	No	5	250	1250		1250		1250		1250		1250		1250

Annexure 1 Net Present Value Model for Hot Callusing Technology

3	Rootstock	No	1170	9	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530	10530
4	WiFi Bill	Monthly	1	99	99	99	99	99	99	99	99	99	99	99	99
5	Electricity Bill	Monthly	1	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
Total	В			•	18059	16809	18059	16809	18059	16809	18059	16809	18059	16809	18059
Labor	Cost														
1	Ground Preparation	No	6	600	3600										
2	Greenhouse Installation	No	4	600	2400										
3	Callus Pipe Installation	No	5	600	3000					3000	3000				3000
4	Automation Setup	No	2	600	1200			1200		1200	1200				1200
5	Grafting	No	5	600	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
6	Monitoring	Hours/wee k	2	75	150	150	150	150	150	150	150	150	150	150	150
7	Setting Up Grafted Plants in Callusing Pipe	No	2	600	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
8	Setting up rooting media for the grafted plants	No	2	600	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Total	C				15750	5550	5550	6750	5550	9750	9750	5550	5550	5550	9750
Grand	l Total (A+B+C)				244646	22359	23609	28440	43109	92759	111640	25165	43109	27240	94009
With 5	% increase in cost				256878.3	23476.95	24789.45	29862	45264.45	97396.95	117222	26423.25	45264.45	28602	98709.45
With 5	i% decreases in cost				232413.7	21241.1	22428.6	27018.0	40953.6	88121.1	106058.0	23906.8	40953.6	25878.0	89308.6
Reven	ue														
	Total Grafted plants	1170		77											
	Market price	205		901	184684.5	184684.5	184684.5	184684.5	184684.5	184684.5	184684.5	184684.5	184684.5	184684.5	184684.5
	With 10% increase in revenue				203152.9	203152.9	203152.9	203152.9	203152.9	203152.9	203152.9	203152.9	203152.9	203152.9	203152.9
	With 10% decrease in revenue				166216.0 5										

Annexure 2 Sensitivity Analysis of hot callusing technology under different scenarios

Scenaro	1:	Without	changing	anything

Discount	rate	0.12

Year	Cost (C)	Benefit (B)	Net Benefit (NB)	DF (1+r)-t	Net DF (NBxDF)	ANDF	
0	244646	0	-244646	1.0000	-244646	-244646.0	_
1	22359	184684.5	162325.5	0.8929	144933.482	-99712.5	
2	23609	184684.5	161075.5	0.7972	128408.402	28695.9	Break-even
3	28440	184684.5	156244.5	0.7118	111211.749	139907.6	
4	43109	184684.5	141575.5	0.6355	89973.7897	229881.4	
5	92759	184684.5	91925.5	0.5674	52160.9974	282042.4	
6	111640	184684.5	73044.5	0.5066	37006.6169	319049.0	
7	25165	184684.5	159519.5	0.4523	72158.5207	391207.6	
8	43109	184684.5	141575.5	0.4039	57179.9699	448387.5	
9	27240	184684.5	157444.5	0.3606	56776.0651	505163.6	
10	94009	184684.5	90675.5	0.3220	29195.0842	534358.7	
			61%	NPV	534358.677		

Scenaro 2: Revenue increases by 10% and cost decreases by 5%

_							
-	ANDF	Net DF (NBxDF)	DF (1+r)-t	Net Benefit (NB)	Benefit (B)	Cost (C)	Year
-	-232413.7	-232413.7	1.0000	-232413.7	0	232413.7	0
	-69992.4	162421.339	0.8929	181911.9	203152.95	21241.05	1
Break-even	74080.0	144072.385	0.7972	180724.4	203152.95	22428.55	2
	199449.4	125369.378	0.7118	176134.95	203152.95	27018	3
	302530.1	103080.651	0.6355	162199.4	203152.95	40953.55	4
	367802.2	65272.1893	0.5674	115031.9	203152.95	88121.05	5

Discount rate 0.12

			74%	NPV	664167.036	
10	89308.55	203152.95	113844.4	0.3220	36654.8499	664167.0
9	25878	203152.95	177274.95	0.3606	63927.1241	627512.2
8	40953.55	203152.95	162199.4	0.4039	65509.6172	563585.1
7	23906.75	203152.95	179246.2	0.4523	81081.8779	498075.4
6	106058	203152.95	97094.95	0.5066	49191.3234	416993.6

Scenaro 3: Revenue decreases by 10% and cost increases by 5%

Net DF Year ANDF Cost (C) Benefit (B) Net Benefit (NB) DF (1+r)-t (NBxDF) -256878.3 -256878.3 -256878.3 0 0 1.0000 256878.3 0.8929 1 23476.95 166216.05 142739.1 127445.625 -129432.7 166216.05 141426.6 0.7972 112744.42 -16688.3 2 24789.45 166216.05 3 136354.05 0.7118 97054.1195 80365.9 29862 Break-even 4 166216.05 120951.6 0.6355 76866.9284 157232.8 45264.45 68819.1 5 166216.05 0.5674 39049.8055 196282.6 97396.95 166216.05 48994.05 0.5066 24821.9105 221104.5 6 117222 7 166216.05 139792.8 0.4523 63235.1634 284339.7 26423.25 8 45264.45 166216.05 120951.6 0.4039 48850.3226 333190.0 9 166216.05 137614.05 0.3606 49625.006 382815.0 28602 166216.05 67506.6 0.3220 404550.3 10 21735.3185 98709.45 49% NPV 404550.319

Scenaro 4: Discount rate increases by 2%

Year	Cost (C)	Benefit (B)	Net Benefit (NB)	DF (1+r)-t	Net DF (NBxDF)	ANDF	-
0	244646	0	-244646	1.0000	-244646	-244646.0	-
1	22359	184684.5	162325.5	0.8772	142390.789	-102255.2	
2	23609	184684.5	161075.5	0.7695	123942.367	21687.2	Break-ev

Discount rate 0.14

Discount rate

0.12

107

			61%	NPV	478248.229	
10	94009	184684.5	90675.5	0.2697	24459.1548	478248.2
9	27240	184684.5	157444.5	0.3075	48415.4343	453789.1
8	43109	184684.5	141575.5	0.3506	49630.5735	405373.6
7	25165	184684.5	159519.5	0.3996	63749.9459	355743.1
6	111640	184684.5	73044.5	0.4556	33278.0916	291993.1
5	92759	184684.5	91925.5	0.5194	47743.2242	258715.0
4	43109	184684.5	141575.5	0.5921	83824.0613	210971.8
3	28440	184684.5	156244.5	0.6750	105460.587	127147.7

Scenaro 5: Discount rate decreases by 2%

Year	Cost (C)	Benefit (B)	Net Benefit (NB)	DF (1+r)-t	Net DF (NBxDF)	ANDF	_
0	244646	0	-244646	1.0000	-244646	-244646.0	-
1	22359	184684.5	162325.5	0.9091	147568.636	-97077.4	
2	23609	184684.5	161075.5	0.8264	133120.248	36042.9	Break-even
3	28440	184684.5	156244.5	0.7513	117388.805	153431.7	
4	43109	184684.5	141575.5	0.6830	96697.9715	250129.7	
5	92759	184684.5	91925.5	0.6209	57078.5031	307208.2	
6	111640	184684.5	73044.5	0.5645	41231.716	348439.9	
7	25165	184684.5	159519.5	0.5132	81858.7264	430298.6	
8	43109	184684.5	141575.5	0.4665	66046.0156	496344.6	
9	27240	184684.5	157444.5	0.4241	66771.8375	563116.5	
10	94009	184684.5	90675.5	0.3855	34959.3305	598075.8	
			61%	NPV	598075.79		

Discount rate 0.1