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# OPTIMIZATION OF DIFFERENT TYPES OF HEALING CHAMBERS FOR GRAFTED BITTERGOURD (*Momordica charantia* L.) SEEDLINGS

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**Abstract.** Ampalaya or bittergourd is an important vegetable crop in the Philippines. The study was conducted to determine the survival rate of grafting ampalaya with patola using cleft method of grafting and optimize type of acclimatization in rearing grafted ampalaya seedlings. The experiment was laid out in Randomized Complete Block Design with four (4) treatments replicated three (3) times with ten (10) sample plants per replication. The following treatments were as follows: $T_0$- Control, $T_1$- Individual acclimatization (without humidifier), $T_2$-Individual acclimatization (with humidifier) and $T_3$–Group acclimatization. Results revealed humidifier can supply an improvised healing chamber with a dimension of (L- 2.3 m, W-1.28 m and H- 1.08 m) and was deemed necessary in rearing grafted seedlings to attain better survival. Group acclimatization has the highest percentage graftake and survival. Ten (10) days with humidifier plus six (6) days hardening with a total acclimatization period of 16 days from grafting until prior transplanting was sufficient in establishing vascular connection between scion and stock with respect to substantial mean rainfall amount of 61.6 mm experienced during the acclimatization period favoring the acquisition of optimum range of relative humidity in a healing chamber necessary for the survival of grafted ampalaya seedlings. The relative humidity experienced during the acclimatization period specifically on the first 10 days of grafted ampalaya seedlings inside the healing chamber was 99% and a temperature ranged of 25-30°C.

**Keywords:** Bitter gourd, grafting cucurbits, humidifier, relative humidity, temperature

## INTRODUCTION

Ampalaya (*Momordica charantia* L.) is a tropical fruit commonly called bitter gourd, balsam pear or bitter melon(Anbarasan & Tamilmani, 2013). It is an important vegetable crop in the Philippines.)Wang and Lin (2005) of AVRDC also described bitter gourd (ampalaya) as a herbaceous climbing annual with ridged stems that can reach up to5 m long. With these botanical characteristics, bitter gourd is likely to be succulent having more water content and thus vulnerable to transpiration and feasibly needs healing chambers when grafted. In vegetable production, grafting is already used for more than 50 years in many parts of the world. Grafting is not associated with the input of agrochemicals to the crops and is therefore considered to be an environment-friendly operation of substantial and sustainable relevance to integrated and organic crop management systems(Rivard and Louws, 2008). Apparently, grafting technology was also anticipated by Villocino (2011) to be useful in developing a low-input sustainable horticulture in the future.

Cucurbit grafting is rare in the United States as stated by Davis et al. (2008), but with continued loss of quality disease-free farmland along with the phase-out of methyl bromide in 2005, the U.S. cucurbit industry observed grafting as an attractive option. Davis, Perkins-Veazie, Sakata, et al.(2008) concluded that grafting cucurbits promotes plant vigor, increased yield in the presence of disease, tolerant to abiotic stresses and resistant to soilborne plant pathogens. In many Mediterranean countries, grafting is being used to control root-knot nematodes, bacterial wilt, and other soilborne pathogens, as an alternative to methyl bromide applications (Leonardi & Romano, 2004). However, in grafting, acclimatization process needs to be studied as to variations of temperature in location specific areas. Such that, healing chambers are improvised and designed in conformance to the number of grafted plants and the capacity of humidifier. The size and design of healing chambers varies depending on the production scale. Healing chambers can be small, such as plastic bags that wrap a few potted seedlings at a time for a home gardener, or they can be larger structures within a commercial greenhouse. Regardless of the chambers' size, they must provide suitable environmental conditions to ensure a fast and successful graft union. Johnson & Miles (2011) also cited that healing chambers are an economical option for creating a humid environment. Maintaining temperatures within the optimal range and high RH is of primary concern when healing grafted vegetable seedlings. Temperature within the healing chamber is directly impacted by temperature within the greenhouse. Although some large-scale commercial grafting operations often use environmentally controlled growth chambers to hold plants during the healing process, these chambers are not cost effective for most operations (Daley et al., 2014) .These problems led to the conduct of this study with the general objective of optimizing different types of acclimatization to ensure higher percentage of grafting survival. This study aimed to determine the survival rate of grafting ampalaya with patola using cleft method of grafting and determine the optimum type of acclimatization in rearing grafted ampalaya seedlings.

## MATERIALS AND METHODS

### *Experimental design and field layout*

The experiment was laid out in Randomized Complete Block Design with four (4) treatments replicated three (3) times with ten (10) sample plants per replication. T0 (Control) was subjected outside the chamber but under the shed area. These are grafted seedlings kept under shed without plastic cover. While T1 were grafted seedlings individually wrapped with plastic cover placed under the shed. T2 are grafted seedlings in cup placed initially with individual polypropylene transparent plastics prior to acclimation in the improvised healing chamber with humidifier. And T3 were grafted seedlings in cup placed directly inside the improvised healing chamber with humidifier.

These were the following treatments: (Different types of acclimation), T0- Control, T1- Individual acclimatization (without humidifier), T2- Individual acclimatization (with humidifier) and T3 – Group acclimatization. Statistical analysis of results was performed using the STAR program of the International Rice Research Institute (Star, 2014).

### *Materials used*

*For the seedling production:* Seedling trays, sterilized potting medium (carbonized rice hull and garden soil), small and portable sprayer, starter solution, ampalaya and patola seeds. Ampalaya variety Galaxy (harvestable 48 days after seeding) seeds and different patola rootstocks were used.

*For the Grafting operation:* Colored ethyl alcohol thermometer (dry and wet bulb), digital thermometer probe with RH sensor, humidifier, improvised grafting chamber, PP plastic bag(polypropylene plastic bags (5 x 7 x 002) that fits on individual plastic cups, blades, rubber bands, alcohol, grafting clips and a grafting chamber size of 2.30 meters in length, 1.28 meters in width and 1.08 meters in height.

*Production of seedlings*

*Preparation of germinating medium.*

A sterilized potting medium of carbonized rice hull and garden soil at a ratio of 2:1 respectively was prepared. Cellular trays were prepared for the germination of ampalaya (scion) and plastic cups for all patola (rootstocks) with holes in the bottom for drainage.

*Sowing of seeds*.

Patola seeds as rootstocks were directly sown in individual plastic cups. All ampalaya seeds (scion) were sown in cellular trays on the same day the patola seeds (rootstock) were sown and were placed in the seedling nursery that serves as a temporary shed to avoid seedlings from being disturbed and to control watering and pests eradication.

*Care of germinated seedlings*.

Seedlings were watered every day until grafting operation. However, four (4) hours prior to grafting seedlings were suppressed from water. As soon as the rootstocks were 15 days old, grafting operation was then executed.

*Grafting operation.*

The cleft method of grafting was used in this experiment. Rootstocks were cut 15 minutes ahead before grafting starts. Rootstock tissue needs to be less turgid so that cracking can be avoided when perforated. Then true leaves, apical and lateral meristems were removed properly using a blade to avoid rootstock shoot growth. Thereafter, the stock was perforated vertically using the blade prior to the insertion of ampalaya scion. Grafting clips were used to keep the point of union in place.

The scion (ampalaya) was grown in seedling trays, while rootstocks (patola) were grown in plastic cups to facilitate grafting operation. The seed of the rootstock and scion were planted together in the same day. After fifteen (15) days, rootstock and scion seedlings were ready for grafting. Once grafting was done, the tray of grafted seedlings for Treatment 2(Individualized acclimatization) grafted seedlings planted on plastic cups was enclosed individually with a polypropylene plastic enough to cover the seedlings to have a humid microclimate and were tightened with rubber bands and placed in the improvised grafting chamber with humidifier. On the other hand, Treatment 3(Group acclimatization) was placed directly in the improvised grafting chamber equipped with humidifier. These grafted seedlings were placed inside its respective chamber for four (4) days suppressed from sunlight. Then, the opaque or black cover was removed with another four (4) days inside the chamber with the humidifier on. After eight (8) days inside the chamber, humidifier was then removed and plastic cover was opened for the next two (2) succeeding days thereby initially hardening the plants and was totally removed for the remaining six (6) succeeding days to fully prepare the grafted seedlings under direct sunlight in the open field condition. Sixteen (16) days after grafting, the seedlings are then ready for field planting.

The duration of wound healing was initially patterned from the study of Villocino (2011) on his histology of graft union of grafted watermelon. From day one (1) of grafting until the grafted seedling was sixteen (16) days old, surrounding temperature or ambient temperature together with the chambers relative humidity (RH)  and inside temperature were monitored in a daily basis to record changes or fluctuations as to assess the survivability of the grafted seedlings.

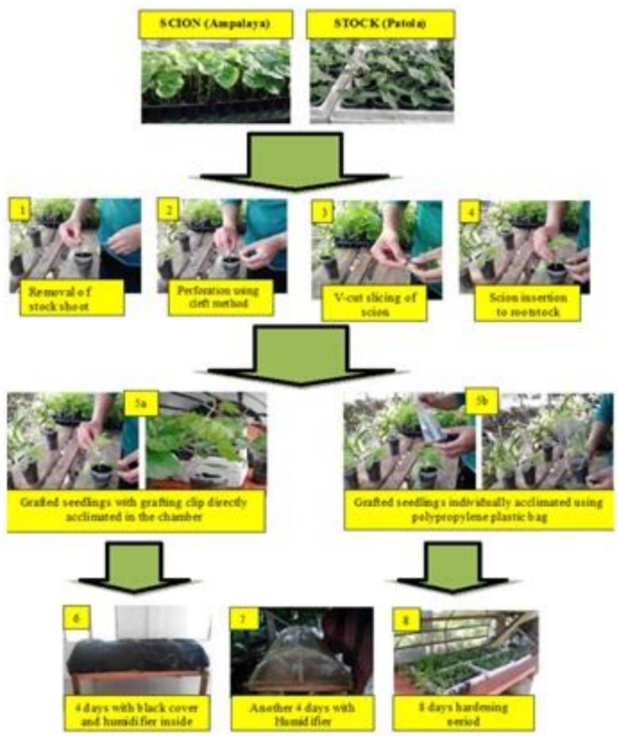


Figure 1. Process in grafting ampalaya with patola.

*Data gathered*

*1.Percent (%) graftake.* Successfully grafted ampalaya sample plants10 days after grafting were recorded and computed by dividing the successful grafted plants over the total number of ampalaya plants grafted, multiplied by 100.
*2.Percent survival.* This was recorded by counting the number of successfully grafted plants prior to transplanting. This was computed using the formula:

% Survival = Number of successfully grafted plants/Number of plants grafted x 100.

*3.Percent (%) Relative humidity (RH).* A digital thermometer probe with RH sensor was used to record readings from the time grafted seedlings were placed inside the grafting chamber until transplanting. While dry bulb and wet bulb readings were gathered using the mercurial thermometer for the RH of the experimental area.
*4.Temperature ($^{\circ}$C) of chamber.* Digital thermometer with relative humidity (RH) reading was recorded from different types of chamber (different treatments) from the start of grafting until hardening.
*5.Ambient temperature ($^{\circ}$C).* Data were gathered using the thermometer by recording the surrounding temperature per day basis from grafting until the day grafted seedlings were transplanted.

**RESULTS AND DISCUSSION**

*General Observation*

The Agroclimatic data obtained from the Philippines Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) divulged a mean rainfall amount of 61.6 mm from the day ampalaya seedlings were grafted until the 10$^{th}$ day they were acclimatized. Articulating that this acclimatization week experienced a substantial amount of rain favoring the acquisition of optimum range of relative humidity in a healing chamber, necessary for the survival of grafted ampalaya seedlings.

There were three (3) preliminary trials conducted as to initially optimize the suitable chamber in rearing grafted seedlings. Two (2) trials for the chamber with a dimension of 5 meters in length, 3 meters and 60 centimeters in width and 2 meters and 50 centimeters in height ( L -5m, W- 3m and 60 cm, H- 2m and 50 cm) and one (1) trial before the final conduct of the study for the improvised chamber with a dimension of 2.30 meters in length, 1.28meters in width and 1.08meters in height( L- 2.3 m, W-1.28 m and H- 1.08 m). Each trial took 1.5 months to complete the entire observation from sowing, grafting, and hardening until the grafted seedlings were ready for field transplanting including its growth in the open field. Results evidently showed that a specific humidifier should be able to accommodate a number of grafted ampalaya seedlings such as to attain the appropriate humidity necessary for its survival. Miles et al. (2013) cited that RH is influenced by healing chamber dimensions and subsequent internal volume of air. The first two trials only obtained 10-35% survival of grafted seedlings which is not economically feasible for farmers in the vegetable business. However, in this study a one (1) liter capacity humidifier was able to provide the optimum range of relative humidity which is 85%-100% inside the healing chamber with the dimension of 2.30 meters in length, 1.28 meters in width and 1.08 meters in height.

Another constraint observed was the low percentage germination of BPI patola seeds used as one of the rootstocks. These seeds that were claimed to be Bacterial wilt resistant variety from the Bureau of Plant Industry (BPI) were found to have hard seed coat, such that soaking the seeds in water for 1-2 days was proven to break its dormancy.

In grafting cucurbits such as ampalaya and patola which are herbaceously succulent, temperature and humidity in acclimatizing grafted plants in the healing chamber was observed to be of great importance and that in selecting scion for grafting, a minimum of four (4) true leaves was preferable for the grafted plants to cope up with the growth of those non grafted. This study also revealed that ampalaya seeds fifteen (15) days after sowing were grafted, acclimated and hardened until 31 days prior to field transplanting. The grafted ampalaya seedlings were only a week late compared to those non grafted seedlings when planted in the field.

*Temperature and Relative Humidity*

Successful grafting of vegetables requires high relative humidity (RH) and optimal temperatures for a week following grafting to reduce transpiration of the scion until rootstock and scion vascular tissue are healed together and water transport is restored as stated by Johnson & Miles (2011).

On the third trial, grafted seedlings took too long about 15 days in the chamber. As a result most of the seedlings died and got rot. This was similar to the findings of (Miles et al., 2013) wherein he publicized that if the humidity in the healing chamber is too high or plants are left in the chamber for too long, plants may begin to develop physiological disorders. Adventitious roots may develop on the scion at the graft union as shown in Figure 2. And because the healing chamber is warm and moist, it provides an ideal environment for plant pathogens and mold. Subsequently, if the humidity in the healing chamber is too low or plants are taken out of the chamber too soon or too abruptly, scion leaves will begin to wilt and plants are likely to die.


Figure 2. A-Adventitious roots of scion growing at the graft union, B- wilted scion, C- Rotted scion and stock.

Lee Jung Myung (1994) recommended that the temperature inside the healing chamber should range between 77°F to 86°F or 25 °C to 30 °C. This claim was further explained by (Davis et al., 2008) that cell division is stimulated under this temperature range, which is fundamental for the healing process. They also noted that temperature inside the healing chamber may be a few degrees higher than outside. This was in concomitant to the result of this study as shown in Figure 3 below. However, there was a slight decrease in temperature inside the chamber on the 6$^{th}$ day. This was due to brown-out or unintended electrical disruption from the main electricity supply in town that hinder the humidifier from functioning well.

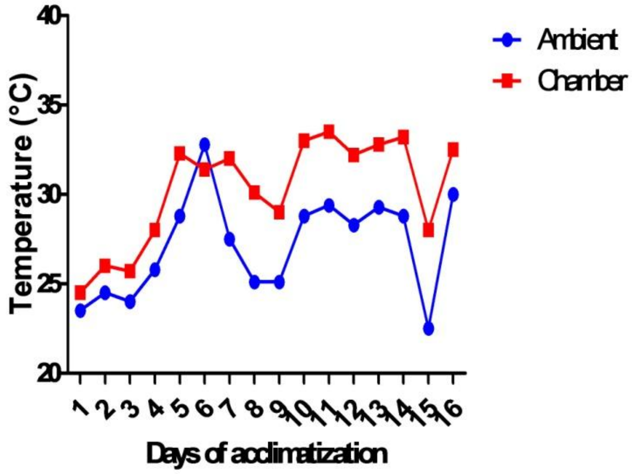


Figure 3. Daily mean temperature of grafted ampalaya from the day grafted until prior transplanting

The relative humidity (RH) of the surrounding during the first 10 days of acclimatization where within 85– 99%, and the RH inside the improvised healing chamber was averaging 99% as shown in Figure 4. The dates of the acclimatization period were from January 25 to February 2, 2016. This range of relative humidity was attained fortunately because during this week it was raining (Table 2), wherein even treatments without any acclimatization though were placed under shed obtained higher survival rates of grafted seedlings (Table 1).This claim associated the studies of Ozores-Hampton & Frasca (2013) stating that it is critical for the grafting success that the RH inside the healing chamber is 95% throughout the entire healing process, with special attention to the first hours after the grafted plants are placed inside. This also correlates with the studies of Lee Jung Myung (1994) wherein they reported the optimum RH to be 95% or higher for healing chambers and that Relative humidity must be maintained between 85% and 100%. High RH decreases the scion's transpiration rate, which prevents it from drying out (Johnson & Miles, 2011) and that humidifiers may be used in climates where the air RH is not very high to ensure the humidity inside the chamber will not decrease because of evaporation to the outside environment.

Healing of newly grafted plants in proper environmental conditions is the concern of this study such that during the healing process, the scion and the rootstock must establish vascular connection, which is considered the most critical process in the production of vegetable grafted transplants (Kubota, McClure, Kokalis-Burelle, Bausher, & Rosskopf, 2008). Therefore, reducing scion transpiration is crucial for the grafting survival (Miles et al., 2013). In order to reduce scion water loss to the surrounding environment, the air relative humidity (RH) should be high, ranging from 85% to 100%.

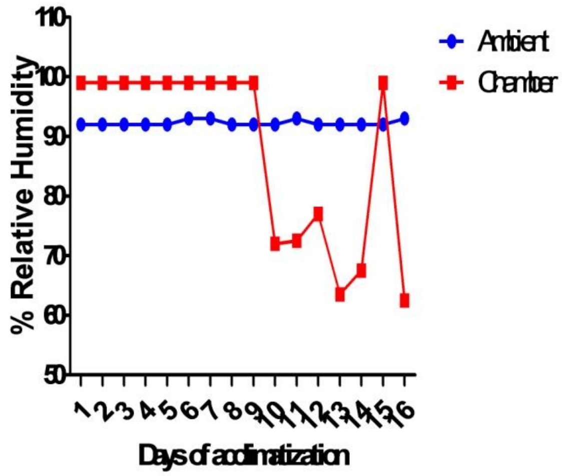


Figure 4. Percent relative humidity of grafted ampalaya from the day grafted until prior transplanting.

*Graftake and survival of grafted ampalaya seedlings*

Results in Table 1 revealed that T3 (Group acclimatization) have the highest percentage graftake and percentage survival. Though results on % graftake and % survival were not statistically significant, Table 1 evidently shows that T1 (Individual acclimatization without humidifier) was comparable with T2 (Individual acclimatization with humidifier) in terms of % graftake. However, T2 was higher in terms of % survival with 84.3% than with T1 (74.4 %) with respect to the amount of rainfall, temperature and relative humidity during the acclimatization period (Table 2). Regardless of the grafter being inexperienced, cleft method of grafting was found efficient in grafting ampalaya with patola and that humidifier with one (1) liter capacity can cater an improvised healing chamber with a dimension of L- 2.3 m, W-1.28 m and H- 1.08 m and was deemed necessary in rearing grafted seedlings to attain better survival results.

Table 1. Percent (%) graftake of grafted ampalaya seedlings 10 days after grafting and its percent (%) survival prior to transplanting after 16 days from grafting.

| Treatment | % graftake | %survival |
|---|---|---|
| T0- Control | 70 | 96.6 |
| T1- Individual acclimatization (without humidifier) | *80* | 74.4 |
| T2- Individual acclimatization (with humidifier) | 80 | 84.3 |
| T3- Group acclimatization | 86.7 | 87.3 |
| CV (%) | 47 | 17.62 |

*Means with the same letter are not significantly different at 5%* LSD.

This was further explained by Kubota et al., (2008) declaring that during the healing process, the scion and the rootstock must establish vascular connection, which is considered the most critical process in the production of vegetable grafted transplants. The complete vascular connection establishment takes approximately five to eight days, during which the scion is unable to uptake water through the rootstock (Rivard & Louws, 2008) suggesting the use of healing chambers with humidifier.

(Okatan, 2017; Johnson & Miles, 2011) demonstrated that it takes an average of 5–8 days after grafting for the rootstock and scion (top of grafted plant) to establish vascular connection and 14 days for the graft union to fully heal. During the first week after grafting, the scion is unable to receive water from the rootstock. It is therefore important to maintain proper environmental conditions to prevent water loss from the scion and promote rapid formation of the graft union.

(Davis, Perkins-Veazie, Sakata, et al., 2008) stated that in general, grafting compatibility is related to taxonomic affinity, but there are significant exceptions. Graft incompatibility as reviewed by (Davis, Perkins-Veazie, Hassell, et al., 2008) is differentiated from graft failure, which often results from environmental factors or lack of skill of the grafter. That is why only one method was used in this study.

Table 2. Average weekly rainfall per month within the acclimatization period of grafted bittergourd seedlings.

| **WEEKS** | **AVERAGE RAINFALL (mm)** | | |
|---|---|---|---|
| | January 2016 | February 2016 | March 2016 |
| 1 | 1 | 1.31 | 0.0 |
| 2 | 2.9 | 11.48 | 2.42 |
| 3 | 2.0 | 2.32 | 0.73 |
| 4 | 61.6 | 5.97 | 1.51 |
| 5 | 9.6 | - | - |
| Average | 15.42 | 5.27 | 1.16 |

The cleft method of grafting was selected because this was more efficient than other methods recommended in grafting cucurbits and that percent (%) graf take was assessed as well as its survival. However, when grafting conditions have been successfully ensured, graft incompatibility could be attributed to other factors such as failure of rootstock and scion to affect a strong union, failure of the grafted plant to grow, or premature death of either rootstock or scion after grafting.

Therefore, reducing scion transpiration is crucial for the grafting survival (Johnson & Miles, 2011) and in order to reduce scion water loss to the surrounding environment, the air relative humidity (RH) should be high, ranging from 85% to 100%.

## CONCLUSION

This study concluded that humidifier with one (1) liter capacity of water can supply an improvised healing chamber with a dimension of ( L- 2.3 m, W-1.28 m and H- 1.08 m) and was deemed necessary in rearing grafted seedlings to attain better survival results. T3 (Group acclimatization) have the highest percentage graftake and percentage survival. Ten (10) days with humidifier plus six (6) days hardening with a total acclimatization period of 16 days from grafting until prior transplanting was sufficient in establishing vascular connection between scion and stock with respect to substantial mean rainfall amount of 61.6 mm experienced during the acclimatization period favoring the acquisition of optimum range of relative humidity in a healing chamber necessary for the survival of grafted ampalaya seedlings. The relative humidity experienced during the acclimatization period specifically on the first 10 days of grafted ampalaya seedlings inside the healing chamber was 99% and a temperature ranging from 25 to 30 °C.

It is recommended to cut and use scion with a minimum of four (4) true leaves to cope up with the growth of ungrafted seedlings. Test and compare the performance of healing chambers with water below against the use of humidifier. Test different chamber dimensions with different sizes of humidifier and conduct and evaluate acclimatization periods of grafted seedlings with the use of humidifier during summer or sunny season.

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