



Field Performance of Strawberry (*Fragaria x ananasa* Duch) Varieties 'Sweet Charlie' and 'Festival' Grown from Different Sub-Cultured Tissue Culture Meristem in La Trinidad, Benguet

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Abstract

The field performance of two strawberry (*Fragaria x ananasa* Duch) varieties 'Sweet Charlie' and 'Festival' were investigated using various sub-cultured tissue culture meristem grown in La Trinidad, Benguet condition. 'Sweet Charlie' plants from sub-culture (S) 2 to 8 were the earliest to produce its first harvestable fruits from transplanting. Longer days were needed by plants from S9 and S10 in terms of days from transplanting to flowering and from flowering to fruit set. Longest fruit were observed from plants in S10, while comparative equational diameter were observed from various sub-cultures of 'Sweet Charlie', except S9 which was observed to have smaller diameter. Sweetest fruits were gathered from plants in S2. In 'Festival' variety, plants from S3, S5 and S6 were the earliest to produce the first harvestable fruits from fruit set. Longer fruits were observed from S2-S5 and S8, while the sweetest fruits were taken in plants from S10, comparable to S3 to S9. In terms of yield, fruits from S3 had the highest marketable yield, comparable to that from S4, S5, and S8. Non-marketable yield was also highest in plants from S3, comparable to that from S5, S7, and S9.

KEYWORDS

Fragaria x ananasa Duch
sub-culture
in vitro propagation
fruit size
sugar content

Introduction

Strawberry (*Fragaria x ananassa* Duch.), a member of the family Rosaceae, is a major fruit crop around the world. Its fruit is botanically termed as achenecetum, an aggregate fruit of achenes and the edible part is the fleshy receptacle, thus also an accessory fruit. The fruits are rich in bioactive phytochemicals, especially phenolic compounds with high antioxidant capacity, and as a part of daily diet could be beneficial for human health (Hannum, 2004). Strawberry are day length dependent plants with cultivars being long day,

short day or day neutral. The high degree of genetic heterozygosity present in *Fragaria* spp. enabled the development of several strawberry cultivars adapted to widely varying environment conditions and resistant to several diseases and pests. Not only the genetic variability, but also a high adaptability and plasticity of the strawberry plant itself give this crop such a remarkable range of adaptation (Darrow, 1966). These result to cultivation of strawberry in different countries under temperate and sub temperate environment (Biswas et al., 2008).

Production of strawberries has been attempted to meet the demands for improved yields, fruit size and quality traits. However, the narrow genetic base of the cultivated strawberry, combined with the polyploidy nature of the crop constrain traditional breeding methods. *In vitro* approaches, which had been used in horticultural production in the past 30 years (Boxus, 1974), are an alternative efficient strategy to implement strawberry improvement (Palei et al., 2015). Although some problems are still remaining such as multiple shoots regeneration ability, *in vitro* propagation is the commonly used method for mass propagation of strawberry. However, *in vitro* propagation of strawberry vary from cultivars to cultivars and from location to location (Mir et al., 2010). This shows the continuous need to develop and improve *in vitro* approaches suitable to a particular local setting to meet the demand for strawberry production.

Strawberry is the “One Town One Product” (OTOP) of the municipality of La Trinidad, Benguet and is a treasured crop among the farmers (La Trinidad Municipal Profile). Located at about 1,300 meters above sea level (masl), some areas in Cordillera particularly in Benguet and Mountain Province offer the semi-temperate requirement of strawberry, having a temperature range of 10-25°C. Strawberry farmers experienced dramatic increase in income using planting materials from tissue-cultured meristem provided by Benguet State University (BSU). It has been a practice of local farmers to use strawberry runners produced from such materials. However, in 2010 and 2011, strawberry farmers noticed a decrease in production since the first use of runners from tissue cultured meristem in 2008. In 2010, they noticed the crops to flower profusely but eventually yielding marble sized fruits. In 2011, farmers claimed that the plants scarcely produced flowers and fruits. Farmers attributed these decrease in yields to the runners produced from the tissue cultured meristem, in turn, blaming the tissue-cultured meristem.

Many factors affect crop productivity, in which climate is a major one. In 2011 cropping season, the weather was not favorable for strawberry production. Based on the record of the Municipal Agriculture Office of La Trinidad, Benguet, there was a drop-in production from 21t ha- to 16t ha- due to extreme weather changes (Catajan, 2016). Climate change brought off season downpours and sudden heat waves which resulted to the flooding

of strawberry fields, rotting of fruits, destruction of flower buds and hardening of fruits. Nonetheless, the clamors of strawberry farmers in the locality prove the need to provide better planting materials of strawberry. Though strawberry researches had been prioritized in BSU, very few actually focused on *in-vitro* propagation method. Most studies conducted on strawberry in the locality focused on its disease, e.g. virus and nematodes (Villanueva, 1993, 1994, 2010, 2013; Villanueva et al., 2010; Oloan & Villanueva, 2007), fertility system (Padua, 2010; Cuyahon & Marquez, 2010), and varietal evaluation (Guitelen et al., 1982; Hermano, 1982). The last published work on *in vitro* propagation method of strawberry in the locality was conducted by Balcita et al. way back in 2001 where they determined the best media for propagation. This, along with the current immediate need for better planting materials, highlights the necessity of improving *in vitro* propagation method of strawberry in the locality.

One critical stage of *in vitro* method is sub-culturing, the proliferation of propagules to produce a voluminous planting materials. In this stage, more than 10 sub-culturing can be producing variation or abnormality (Lopez-Aranda et al., 1994). This study was conceptualized to determine the effect of continuous sub-culturing on the field and yield performance of strawberry cv. ‘Sweet Charlie’ and ‘Festival’ under La Trinidad, Benguet conditions. The authors hope that the result of the study would contribute in improving the *in vitro* propagation protocol of strawberry which, in turn, improves yield performances.

Materials and Methods

This study was conducted at the Horticulture Research and Institute (HORTI) Tissue Culture Laboratory and at the BSU Balili Experimental Area. Figure 1 presents the over-all process used in the study in producing tissue cultured strawberry.

Culture Initiation/ Establishment Stage

Explant collection and sterilization. Runner tips of strawberry variety ‘Sweet Charlie’ and strawberry ‘Festival’ variety were used as planting materials for establishment in the laboratory. The runner tips were trimmed and washed with soap and tap water for 15 minutes; sterilized with 50% sodium hypochlorite for 30 minutes and



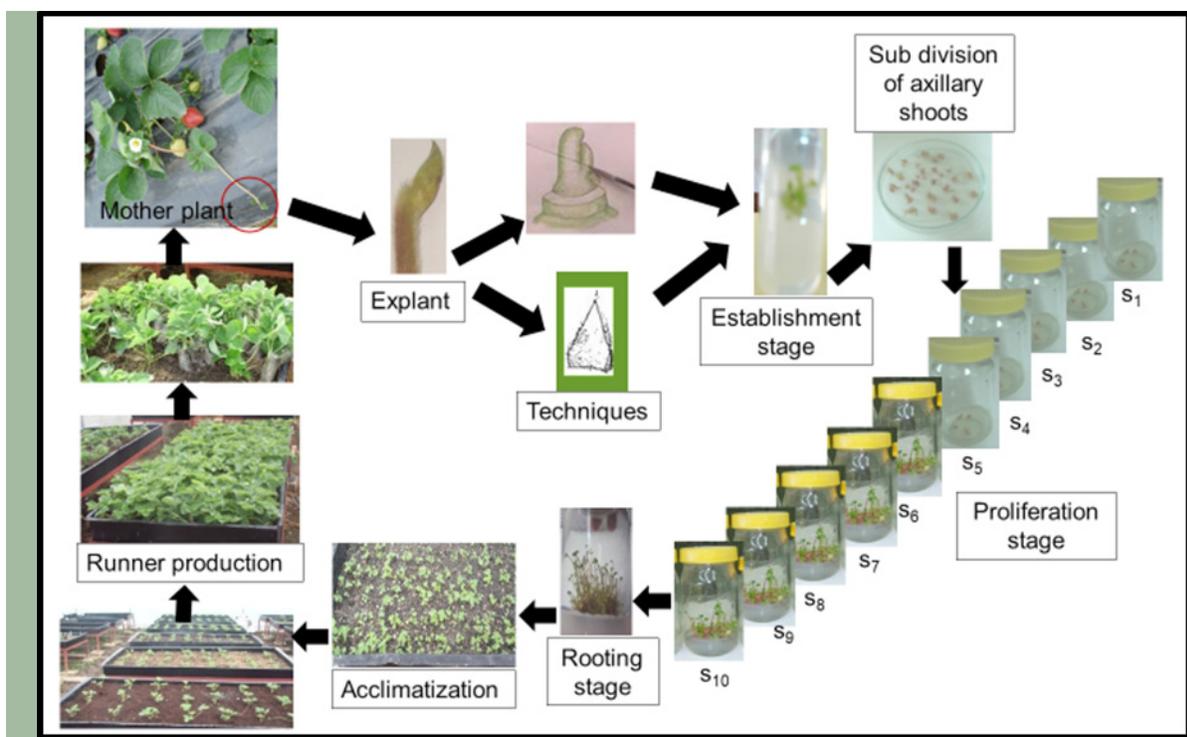


Figure 1. Process in producing tissue cultured strawberry

washed with sterilized distilled water three times (Dumaslan, 2007).

Initiation and inoculation of explant.

Sterilized runner tips were excised inside a laminar flow. Using a binocular microscope, the base of the runner tips were cut off and outer leaves were removed, leaving two to three leaf primordia. Inoculation was done in a Boxus (1974) medium with the addition of 1mg l^{-1} benzyl adenine (BA) and 1mg l^{-1} Gibberelic acid (GA_3) having 40g l^{-1} of sucrose and 6g l^{-1} of agar (Dumaslan, 2007).

Multiplication of adventitious shoots. The established explants were transferred to a fresh culture medium inoculated at proliferation stage. After 3-4 weeks, adventitious buds produced were subdivided into smaller pieces and then transferred into fresh multiplication medium (Dumaslan, 2007). Cultures were maintained in a Boxus (1974) medium, which contains mineral nutrients, 4% sugar, 0.6% agar and growth regulators such as 1ppm benzyl adenine (BA) and 1ppm Gibberelic acid (GA_3). The cultures were subdivided every 30 days up to the 10-subculture stage.

The shoots produced were separated in each sub-culture stage and were planted in rooting medium containing 1ppm Indole butyric acid (IBA)

and activated charcoal. The different sub-culture stages served as the treatment, as shown in Table 1. Plantlets were maintained in the culture room for 3-4 weeks to produce root. When plantlets have produced enough roots, these were transferred outside of the laboratory to adapt to the ambient environment. Different stages of cultures were inoculated and maintained in a growth chamber with a temperature of $10\text{-}24^\circ\text{C}$ with 16 hours light and 8 hours dark for rapid growth (Dumaslan, 2007).

Acclimatization. Plantlets were brought to the rain shelter for acclimatization for 4 weeks. Acclimatized plantlets were planted in the plots prepared and allowed to produce runners.

Field Experiments

Land preparation, mulching and transplanting. Four-meter area of 85m^2 was thoroughly prepared and sub divided into three blocks with 27 plots, each measuring $1\text{m} \times 2.5\text{m}$. The field experiment was laid out in RCBD with three replications. The plots were applied with processed chicken manure (PCM) at 1 can per plot and mulched with black polyethylene. Holes were made on the plastic mulch with a distance of $30\text{cm} \times 30\text{cm}$ between hills and rows following



the recommendation of Solimen et al. (2010). The runners produced by the different sub-culture from tissue cultured meristem of strawberry varieties 'Sweet Charlie' and 'Festival' were uprooted and transplanted in the prepared plots covered by the plastic mulch having the holes on the planting distance.

Maintenance and Cultural Management.

Plants were irrigated twice a week. Strawberry plants were sprayed with insecticide once a week to control pests during the vegetative stage. Complete fertilizer was applied at 20 bags ha^{-1} plus chicken manure at 100 sacks ha^{-1} that was soaked in water for seven days (Solimen et al., 2010). Mixed fertilizers were drenched equally to the plots every two weeks with a rate of 50 kg ha^{-1} urea and 50 kg ha^{-1} of complete fertilizer. Weeds and old leaves were removed after harvesting. All the cultural practices were provided equally to the different treatments to ensure the optimum growth of the strawberry plants.

Data Gathered and Statistical Analysis

Data gathered in the study include number of days from transplanting to flowering, days to fruit set, days to first harvest, days from fruit set to first harvest, weight of marketable and non-marketable fruits per plot, fruit sizes (equatorial and polar diameter), sugar content and computed yield per hectare (t ha^{-1}). Polar diameter was measured from base to tip of the fruit while equatorial diameter was measured at widest portion of the fruit (Figure 2). Data gathered were analyzed using ANOVA and LSD *as post hoc*.

Results and Discussion

Days to Flowering and Fruiting in Sub-culture Treatments

Figure 3 presents the flowering and fruiting stages of strawberry under local condition. The number of days it took for the plant to reach these stages were recorded in the study and compared between treatments.

The number of days from transplanting to flowering for the variety 'Festival' did not differ significantly in the different sub-culture treatments. Under variety 'Sweet Charlie', sub-cultures 2 to 8 (S2 – S8) were significantly earlier to flower after transplanting as compared to S9 and S10 (Table1). In terms of fruit setting, S2, S3, and S4 under strawberry variety "Sweet Charlie" was significantly earlier at 4.33 days and latest in S10 (Table1). Fruit setting did not differ significantly in sub-cultures of variety 'Festival'.

However, significant differences were observed in sub-cultures of both 'Sweet Charlie' and 'Festival' in terms of number of days from fruit setting to first harvest. Fruits of S6 under 'Sweet Charlie' matured significantly earlier at 17.33 days followed by S8 and S10 at 18.33 and 19 days, respectively. For variety 'Festival', fruits of S10 matured significantly earlier at 12.67 days from fruit set while S3 was the last to be harvested after 19.87 days.

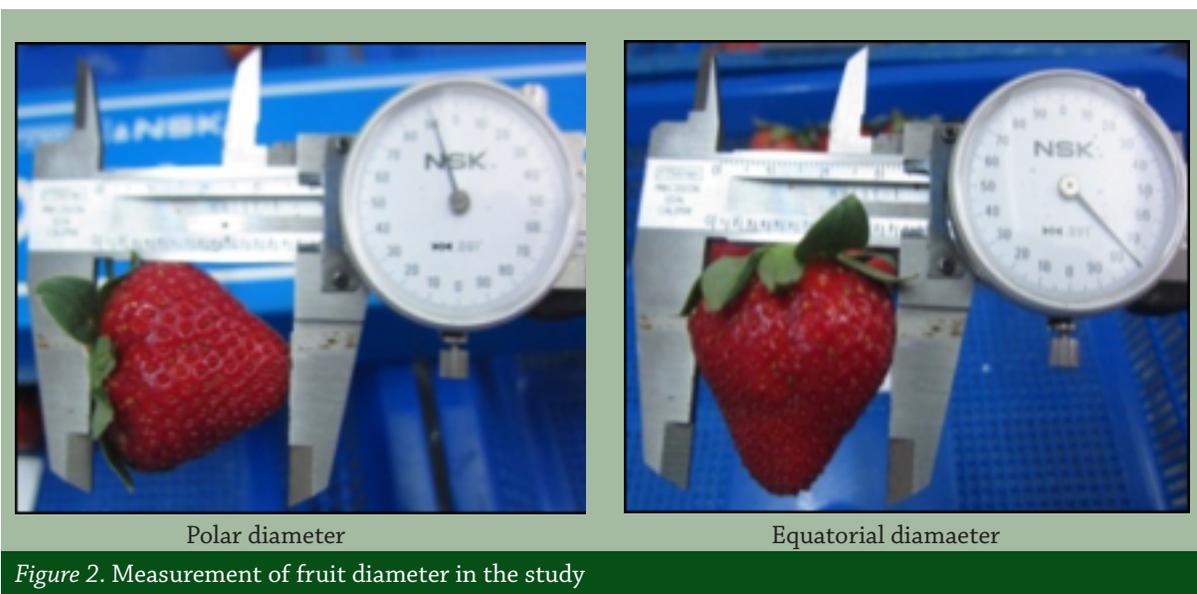


Figure 2. Measurement of fruit diameter in the study



Nonetheless, the sub-culture stages under varieties ‘Sweet Charlie’ and ‘Festival’ did not differ significantly in number of days from transplanting to first harvest. Variety “Sweet Charlie” at S4 was

the earliest to first harvest at 84 days compared to S9 and S10 that was delayed at 101-102, though not significantly different. Meanwhile, sub-cultures of variety ‘Festival’ had relatively similar days to

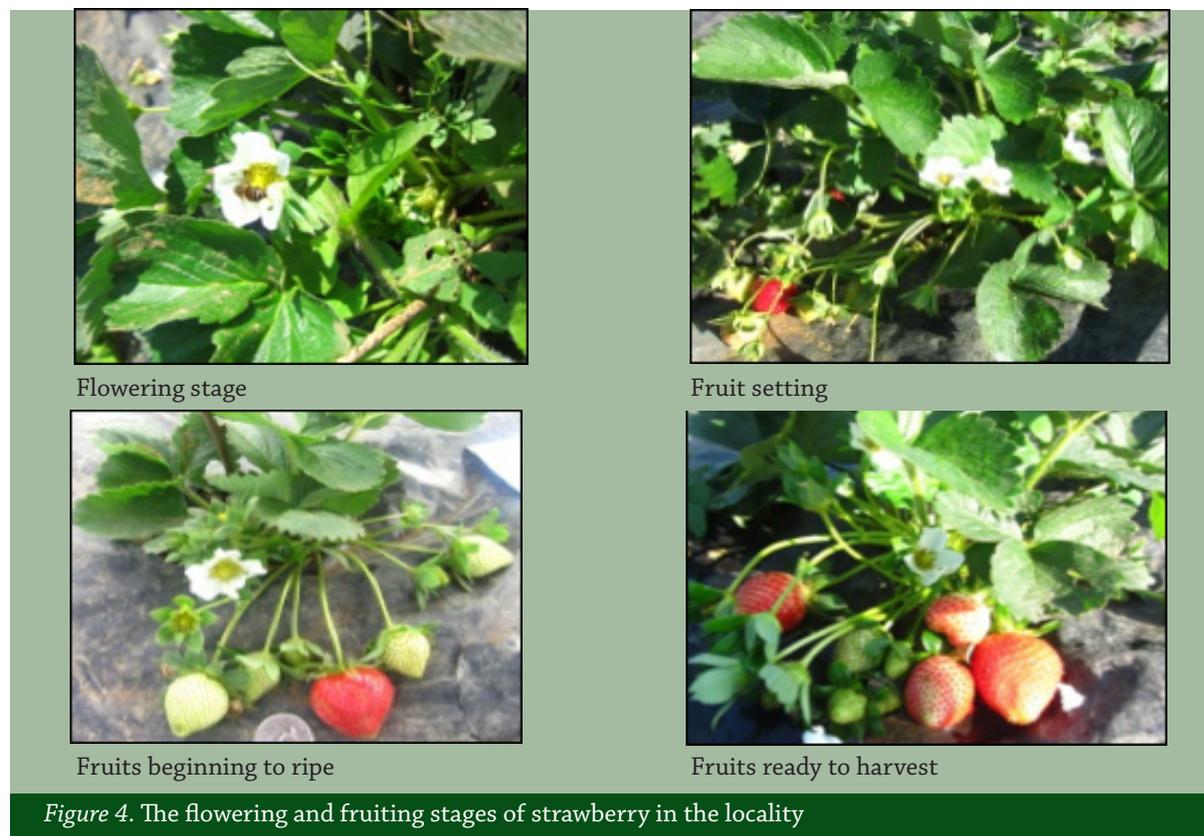


Table 1

Average number of days for each growth stage of strawberry cultivars

Sub-culture	Days from transplanting to flowering		Days from flowering to fruit setting		Days from fruit set to first harvest		Days from transplanting to first harvest	
	Sweet Charlie	Festival	Sweet Charlie	Festival	Sweet Charlie	Festival	Sweet Charlie	Festival
S2	67.00 ^a	69.33 ^a	4.33 ^a	6.33 ^a	19.33 ^{bc}	13.13 ^{cd}	90.66 ^a	88.79 ^a
S3	64.67 ^a	64.00 ^a	4.33 ^a	5.00 ^a	18.00 ^{ab}	19.87 ^a	87.00 ^a	88.87 ^a
S4	64.33 ^a	65.33 ^a	4.33 ^a	5.33 ^a	19.33 ^{bc}	14.60 ^{bcd}	83.66 ^a	85.26 ^a
S5	65.00 ^a	64.67 ^a	5.33 ^{abcd}	5.00 ^a	18.00 ^{ab}	17.87 ^{ab}	88.33 ^a	87.54 ^a
S6	65.33 ^a	65.00 ^a	4.66 ^{ab}	5.67 ^a	17.33 ^a	16.73 ^{abc}	87.32 ^a	87.40 ^a
S7	66.67 ^a	65.67 ^a	5.00 ^{abc}	5.00 ^a	18.00 ^{ab}	13.47 ^{cd}	89.67 ^a	84.14 ^a
S8	67.00 ^a	65.33 ^a	5.66 ^{bcd}	5.67 ^a	18.33 ^{ab}	15.07 ^{bcd}	90.99 ^a	86.07 ^a
S9	74.00 ^b	65.00 ^a	6.33 ^d	5.33 ^a	21.00 ^c	13.13 ^{cd}	101.33 ^a	83.46 ^a
S10	76.99 ^b	65.54 ^a	5.99 ^{cd}	5.00 ^a	19.00 ^{ab}	12.67 ^d	101.98 ^a	83.21 ^a

Means with the same letter in a column are not significantly different at 5% LSD



first harvest at 83-89 days with S10 being the earliest while S2 and S3 were the last to be harvested.

From these data, it is readily apparent that subculture stages did not affect significantly the days to flowering or fruiting in strawberry varieties 'Sweet Charlie' and 'Festival'. There were no observable trend in the flowering or fruiting of the subcultures.

Fruit Size and Sugar Content

Table 2 presents the fruit diameter and sugar content in the sub-cultures of 'Sweet Charlie' and 'Festival' varieties. Polar diameter of fruits in sub-cultures of 'Sweet Charlie' ranged at 2.41-3.01cm while equatorial diameter ranged at 2.63-3.40cm. Larger fruits were recorded in sub-cultures of 'Festival' with polar diameter ranging at 2.71-3.40cm and equatorial diameter at 3.41-3.89cm. 'Sweet Charlie' fruits from S10 had the highest polar diameter while S9 had the lowest equatorial diameter. On the other hand, polar diameter of 'Festival' fruits in S2 recorded the highest at 3.4 cm, which is comparable to that of S3, S4, S5, and S8. However, no significant differences were observed on the equatorial diameter of 'Festival' fruits in different sub-cultures.

The sugar content of the strawberry ranges

from 8.96-11.83 in 'Sweet Charlie' while 7.72-9.25 in 'Festival'. 'Sweet Charlie' fruits from S2 attained the highest sugar content at 11.83 °Brix. On the other hand, 'Festival' fruits from S10 was the sweetest at 9.25 °Brix, in which that from S3 to S9 were comparable. This confirms the popular observation that 'Sweet Charlie' is sweeter than 'Festival'. The sugar content recorded in the study were higher than those documented by Guitelen et al. (1982) which recorded only 6.70-7.73 °Brix for strawberries varieties planted in the same locality.

Yield Performance of 'Sweet Charlie' & 'Festival' Treatment

Figure 4 contrasts the appearance of marketable from non-marketable fruits of strawberry. The marketable yield of 'Sweet Charlie' variety was highest from S3 at 1,090.80g per 2.5 m² plot, but comparable to that from all sub-cultures except in S9 with low yield of 212.6g per 2.5 m² plot (Table 3). In 'Festival' variety, highest marketable yield was recorded from S3 at 1941 g/2.5m², comparable to that from S5, S8, and S4 at 1,833g, 1,543g and 1,457g per 2.5m² respectively.

In terms of non-marketable yield, 'Sweet Charlie' variety had 124- 183g/2.5m² plot while 'Festival' had 11-324g/2.5m² plot. The lowest non-marketable 'Sweet Charlie' fruits was obtained

Table 2

Average fruit size and sugar content of fruits harvested from plant grown for the different sub-culture of tissue cultured meristem

Treatments	FRUIT SIZE (cm)				SUGAR CONTENT	
	Sweet Charlie		Festival		Sweet Charlie	Festival
	Polar diameter	Equatorial diameter	Polar diameter	Equatorial diameter		
Sub culture 2	3.13 ^b	3.09 ^{ab}	3.40 ^a	3.78 ^a	11.83 ^a	7.92 ^{bc}
Sub culture 3	3.43 ^b	3.25 ^a	3.00 ^{ab}	3.82 ^a	8.96 ^b	8.95 ^{ab}
Sub culture 4	3.20 ^b	3.13 ^{ab}	2.84 ^{ab}	3.89 ^a	9.53 ^b	8.86 ^{abc}
Sub culture 5	3.16 ^b	3.19 ^{ab}	2.88 ^{ab}	3.63 ^a	9.70 ^b	8.71 ^{abc}
Sub culture 6	2.86 ^b	3.69 ^{ab}	2.58 ^b	3.43 ^a	9.00 ^b	7.72 ^{abc}
Sub culture 7	3.09 ^b	3.19 ^{ab}	2.82 ^b	3.61 ^a	9.40 ^b	8.54 ^{abc}
Sub culture 8	2.86 ^b	2.57 ^b	2.87 ^{ab}	3.52 ^a	9.48 ^b	8.58 ^{abc}
Sub culture 9	3.12 ^b	1.70 ^c	2.79 ^b	3.70 ^a	10.30 ^b	8.58 ^{abc}
Sub culture 10	5.15 ^a	2.65 ^{ab}	2.71 ^b	3.41 ^a	10.05 ^b	9.25 ^a
CV (%)	9.3%	9.4%	11%	8.6%	5.3%	8.0%

Means with the same letter in a column are not significantly different at 5% LSD





Figure 4. Marketable vs non-marketable fruits harvested in the study in the locality

from S9 treatment, comparable to that of S2, S5, and S6. In 'Festival', the lowest non-marketable yield was recorded from S2 at 111.2g/2.5m² plot. This result is comparable to that from S4 to S10.

Combining the marketable plus non-marketable yield and interpolating that into hectare yield, the total yield of the treatments were estimated. The estimated total yield of different sub-culture treatments under 'Sweet Charlie' variety ranged from 0.85-4.36t/ha while 'Festival' at 3.79-7.26t/ha. S3 in both varieties had the highest estimated yield. The data also showed higher yield

of 'Festival' than 'Sweet Charlie' variety. It can be gleaned from Table 3 that the yield of 'Sweet Charlie' variety tends to decrease after S7. However, there were no observable trend in yield of 'Festival' that could imply potential effects of sub-culturing on its productivity. This result agrees with the findings of Lopez et al. (1994) wherein the proliferation rate of strawberry at S3 to S8 did not significantly affect strawberry productivity. It was only at more than 10 subcultures that the production was significantly lowered.

Conclusions

The study was conducted to investigate the effect of sub-culturing during the *in vitro* propagation on the field performance of strawberry varieties 'Sweet Charlie' and 'Festival' under La Trinidad condition. In 'Sweet Charlie', plants from S2 to S8 were comparably faster to produce the first harvestable fruits from transplanting than that from S9 and S10. Longer fruits were observed from S10 while the rest of the sub-culture had similar values. Equatorial diameter was highest in fruits from S3 although comparable to that from all the sub-cultures, except from S8 and S9. Sugar content was the highest in plants from S2. In terms of marketable yield, were not significantly different in all sub-cultures, except in S9 which was significantly lower.

Table 3

Average marketable, non-marketable and total yields of 'Sweet Charlie' and 'Festival' varieties grown from the different subcultures of tissue cultured meristem

Treatments	Marketable yield (grams/2.5m ² plot)		Non-marketable yield (grams/2.5m ² plot)		Total yield (t/ha)	
	Sweet Charlie	Festival	Sweet Charlie	Festival	Sweet Charlie	Festival
Sub culture 2	753.10 ^a	949 ^d	154.0 ^{ab}	111.2 ^b	3.00	3.79
Sub culture 3	1,090.80 ^a	1,941 ^a	175.1 ^a	324.3 ^a	4.36	7.76
Sub culture 4	932.20 ^a	1,457 ^{bc}	158.2 ^a	216.1 ^{ab}	3.73	5.82
Sub culture 5	784.90 ^a	1,833 ^{ab}	122.7 ^{ab}	183.0 ^{ab}	3.14	7.33
Sub culture 6	788.30 ^a	1,387 ^{bcd}	138.9 ^{ab}	129.9 ^b	3.15	5.55
Sub culture 7	964.20 ^a	1,244 ^{cd}	182.8 ^a	162.8 ^{ab}	3.85	4.97
Sub culture 8	777.60 ^a	1,543 ^{abc}	165.9 ^a	157.1 ^b	3.11	6.17
Sub culture 9	212.60 ^b	1,257 ^{cd}	123.5 ^b	171.5 ^{ab}	0.85	5.03
Sub culture 10	727.80 ^a	1,244 ^{cd}	165.1 ^a	131.4 ^b	2.91	4.97

Means with the same letter in a column are not significantly different at 5% LSD



In 'Festival', no significant differences were observed on the number of days from planting to first fruit harvest and equatorial diameter. Nevertheless, plants from S2 produced the longest fruits, comparable to that from S3, S4, S5, and S8. Plants from S10 was observed to be the sweetest, comparable to that from S3 to S9. In terms of yield, plants from S3 had the highest marketable yield, but comparable to that from S4, S5, and S8.

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