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RESEARCH ARTICLE

THE RELATIONSHIP AND CHANGING RATE OF STRAWBERRY CROWN CARBOHYDRATE AS RESULT OF CHILLING HOURS AND COLD STORAGE

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ABSTRACT

The relationship between the strawberry crown content (Carbohydrates and Nitrogen) and the accumulated neutral chilling hours, moreover, the changing rate of content as affected by artificial cold storage was investigated in Sana'a University. Strawberry Seedling was digging at the three different periods during winter season. Crown carbohydrates and nitrogen was analysis at the time of digging and after cold storage. The result indicated that; increasing chilling hours led to increasing crown contents of all types of carbohydrate and no significant on nitrogen level. Changing rates occurs in seedling was calculated to seedling as effect of Cold storage, during the digging date (natural chilling) and between digging date and after storage. This study concluded that: Natural chilling more effective than that carried out using cold storage in the dark. The cold storage at 2 C° only suggested for short period, but not as an alternative the natural chilling. The data from this study could use for mange strawberry in nursery.

KEYWORDS

Natural chilling hours, cold storage, strawberry, Crown content

1. INTRODUCTION

Commercial production of strawberries depends on the annual renewal of planting either using fresh seedlings or frigo (using seedlings cold stock at -2 C° until planting and this is called waiting-beds). Cold seedling is widely used in many countries (Lieten et al., 1995).

Bare-root transplants, successful plant establishment is dependent on crown/root reserves for the formation of new feeder roots and leaves. Once autotrophic function of the plant is re-established, plant reserves become less important. Furthermore, the first inflorescence may be initiated in the nursery (Kirschbaum et al., 2010), constitutes a major energy-demanding sink. Indeed, the growth of the first fruits might depend on the total non-structural carbohydrates status of the transplant (Nishizawa et al., 1998).

The high content of starch in the crowns was deemed necessary for the success of long-term of strawberry bare-root seedling storage (frigo) or to compensate for the requirements of chilling, where strawberry plants collect starch in the root and crown as a result of low temperature and length of day during autumn and winter (Maas, 1986; Durner et al., 1984; Eshghi et al., 2007). In Yemen the temperature in winter not extremely low as comparing in Europe countries, where the maxim chilling differ from 120 to 400 hours calculated as less than 7 C°.

Forcing strawberry after a short period of dormancy influences not only the vegetative growth but also the floral capacity (Kronenberg and Wassenaar, 2004). However, there are important differences between the values of starchy content. For example, in the Elsanta variety, Lieten (1997) found an important positive correlation between the number of chilling hours and the root content of sucrose at the time of digging and the subsequent crop. The strawberry plant grows at its best performance when content high level of the carbohydrate (Al-madhagi et al., 2014; López et al., 2002). On the other hand, cooling affects the size of the crown and the number of crowns (Al-Madhagi et al., 2018), early and greater fruit *(López-Galarza et al., 2009; Cocco et al., 2016)*. Therefore, the properties of plants prepared for storage are of great importance.

In spite of the availability of high altitudes in our country, the risk of planting high altitudes may damage plants if they take off late or not using row cover, while storage is considered a good protection for seedlings from natural nurseries in high altitudes. In addition, storage maybe necessary to reduce the cost of the planting strawberry where the production occurs in Yemen at short day seasons at late autumn, winter and first month of spring. In contrast, low-altitude cultivation will be lower starch content (Ruan et al., 2009), which plant performance is bad after planting.

This study was carried out to: determine the possibility of this system in Yemen. In Yemen conditions winter temperatures are not low to compensate for the cold requirements for strawberry plants and plants transferred after March are clearly affected with temperature and photoperiod (Al-Madhagi et al., 2018), For this reason, maybe use either chilled or refrigerated plants for a long period.

The aim of this research is to determine the accumulation rate and the higher levels of starch, soluble and simple sugars content and the effect of cooling on these components as a guide to determine the most appropriate digging date of seedling from the nursery. In addition, is to determine the difference between natural and artificial chilling of strawberry.

2. MATERIALS AND METHODS

2.1 Period of bare-root Seedling digging

The experiment was carried out at the Faculty of Agriculture, Sana'a University during the winter season at the research farm. Strawberry seedlings were digging at different dates to represent the stages of plant dormancy as describe in Table (1) to be the difference between the dates of digging 45 - 50 days. The temperature per hour was taken from the

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Meteorological Agency, and number of chilling hours during the experiment calculated as less than 7 C°. In all digging date some seedling randomly chosen to analysis the carbohydrates and the other seedling were stored at a refrigerator temperature of 2±1 C° until the spring season in February.

Table 1: Describe the experiment (chilling period and cold storage)

Neutral dormancy period	Neutral chilling hours	Cold storage period (day) at 2±1 C°
Before the natural dormancy	0	120
Half of the period of natural dormancy	30	60
End of the dormancy period	139	0

2.2 Crown Dry Matter %

Ten crown of bare-root strawberry seedling was taken at the time of digging and after cold storage period. Crown sample were dried in oven at temperature of 70 C° for period, until the dry weight stapled. And the percentage of dry matter was calculated as the following equation: Fresh Weight / Dry Weight × 100.

2.3 Carbohydrate measurement

The method of Eshghi et al. (2007) was modified and used for sugar analysis as described below. Ground, dried tissue (1 g) was placed in a centrifuge tube, four millilitres of 80% (v/v) ethanol was added to the Falcon tube and vortexed. The tube was placed in a water-bath at 65 °C for 20 min. After centrifugation for 5 min at 1109 x *g* at 4 °C the supernatant was pipetted into another centrifuge tube. The remaining plant material was then re-extracted with 4 ml 80% (v/v) ethanol, centrifuged and the supernatants compounded.

The starch was analysed from the same samples. After removal of all the soluble material in the samples, it subjected to the acidity method using 0.7 N HCL to convert into soluble sugar. Then the solution was hydrolysed in a boiling-water bath for 2.5 hours, chilled at room temperature, with an equivalent with of 0.5 N NaOH. Then, the samples were diluted and the soluble sugar. The carbohydrate level (simple, soluble carbohydrate sugars, starch) was estimated using the Anthron colorimetric method using a Spectrophotometer at a wavelength of 620 nm, according to López et al. (2002), and the results were calculated as glucose (g) in every 100 g sample dry. The percentage of the crown content of different type of carbohydrate (Total carbohydrates (TC), dry weight (DW), starch (S), soluble carbohydrates (SC) was analysis at every date of digging (after receiving quantity of natural chilling) and after received period of cold storage (Table1). Ten seedlings were randomly chosen from, and the fresh and dry weight of the crown part was recorded.

2.4 Nitrogen level

The level of nitrogen was estimated total nitrogen using Keldahl method

2.5 Changing rate %

Changing rate of carbohydrate of stages shown in Table 2 was calculated by using the following equation:

$$\frac{\text{Average before cold storage} - \text{Average after cold Storage}}{\text{Average After Cold Storage}}$$

Table 2: Changing rate of different carbohydrate in before and after cold storage and digging

Stages	Symbol
Δ before and after storage (first dig)	Δ C1
Δ between digging first and second before storage	Δ D1
Δ between digging first and third before storage	Δ D1-3
Δ between first dig after cold and last digging without chilling	Δ C1D3
Δ before and after storage (second digging)	Δ C2
Δ between digging second and third before storage	Δ D2
Δ between second dig after cold and last digging without chilling	Δ C2D3

2.6 Data Analysis

The data subjected to analysis of:

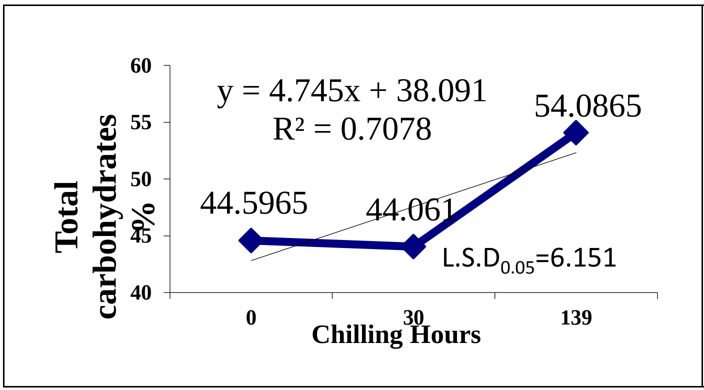
- Effect of chilling were analysis by variance and the averages compared with LSD less than 0.05 by using GenStat 12.1 software.
- Correlation between: i. Chilling hours (CH) and the crown content, and ii. Between the crowns content was calculated by using SPSS 21 software.
- T test Paired Samples between the different changing rates was analysis by using SPSS 21 software.

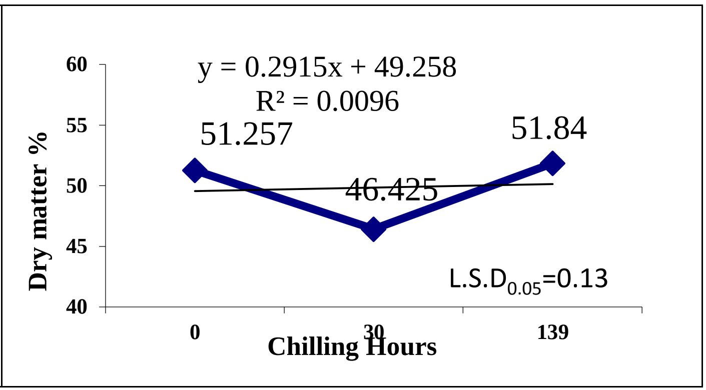
3. Results

3.1 The Relationship between the Carbohydrate Content of Strawberry Crowns and the Accumulated Chilling Hours

Chilling hours (CH) significantly affect (P=0.05) on strawberry crown contents of total carbohydrates (TC), dry weight (DW), starch (S), soluble carbohydrates (SC) and nitrogen (N) levels, the maximum rate of these characteristics was in seedling digging at the last date (139 chilling hours), with an increased about 24.12%, 15.2%, 63.67%, 69.3% and 19.3% compared to crown content in seedling digging at the first date (0 chilling hours), respectively Figure (1). There was no significant difference on effect of chilling hour and crowns content of simple sugars (SS) and dry matter (DM).

The liner regression between the chilling hours and the crowns content of dry matter and different type of carbohydrates is indicated in Table (3). Where the regression relationship between chilling hours and total carbohydrates (TC) was significantly (R^2 = 0.871), and the equation could be writing as TC = 43.377 + 0.75 ch. Meanwhile the regression between the chilling hours and starch (S) was significant (R^2 = 0.781) and the regression equation was as follows: S = 37.741 + 0.07 ch. liner regression between chilling hours and dry matter (DM) was significantly (R^2 = 0.170) and the regression equation were DM = 47.950 + 0.019 ch. And the regression between chilling hours and nitrogen was significantly (R^2 = 0.785) with regression equation was N = 0.468 + 0.03 ch. Soluble carbohydrates (SC) was significantly affected by chilling hour (R^2 = 0.773) and the regression equation SC= 0.205 + 0.001 ch.





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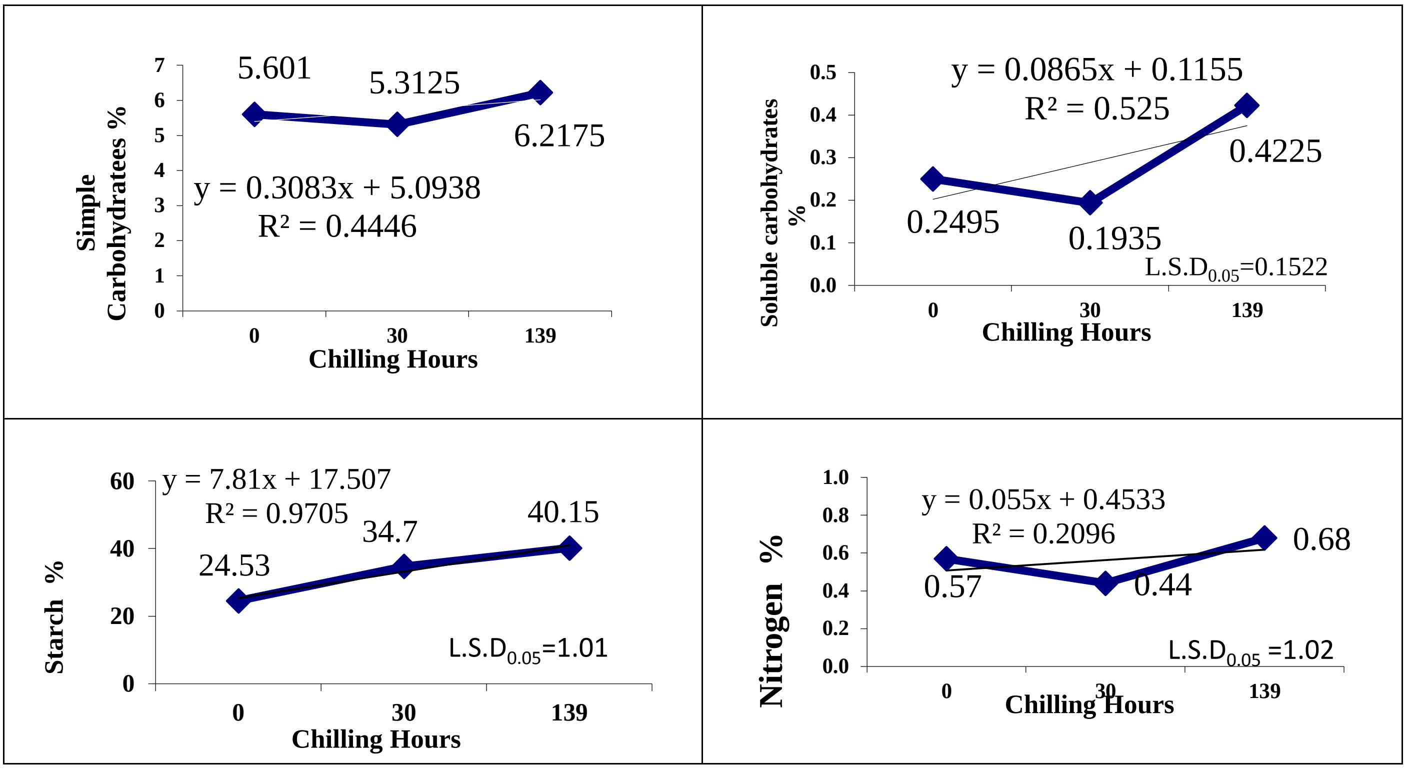


Figure 1: Effect of of Chilling Hour on the level of different carbohydrate

Table 3: Enter Regression Analysis on influence of Chilling Hour on the level of different carbohydrate.

Dependent Variable	modal	*B*	**SE *b***	Beta (B)	Sig.	**R**	***R²***	**Δ*R²***	Std. Error of the Estimate
SS	(Constant)	5.402	0.33	0.558	0.000	0.558	0.311	0.139	0.5952
	CH	0.005	0.00		0.250				
SC	(Constant)	0.205	0.03	0.879	0.003	0.879	0.773	0.716	0.0584
	CH	0.001	0.00		0.021				
S	(Constant)	37.741	1.52	0.884	0.000	0.884	0.781	0.726	2.7059
	CH	0.070	0.02		0.019				
TC	(Constant)	43.377	1.18	0.933	0.000	0.933	0.871	0.839	2.1009
	CH	0.075	0.01		0.007				
DW	(Constant)	32.767	7.56	-0.008	0.002	0.008	0.170	-1.000	9.5228
	CH	-0.001	0.09		0.587				
N	(Constant)	0.468	0.06	0.886	0.001	0.886	0.785	0.731	0.1072
	CH	0.003	0.00		0.019				
DM %	(Constant)	47.950	2.21	0.413	0.002	0.413	0.170	-0.245	3.2148
	CH	0.019	0.03		0.587				

ΔR^2 = adjusted r^2, Chilling hours (CH), Total carbohydrates (TC), dry weight (DW), Dry matter (DM), starch (S), Soluble carbohydrates (SC),Simple sugar (SS) and Nitrogen (N)

By analyzing the correlation as shown in Table (4) between the chilling hours and the crown content, it was found that the chilled hours were positively correlated with the dissolved sugars 0.896 (*P*= 0.05), starch 0.88 (p = 0.05), total carbohydrates 0.93 (*P*= 0.01) and nitrogen 0.88 (*P*= 0.05), meanwhile, there was no significant correlated with simple sugars and dry matter.

Table 4: Correlation between the Chilling Hours (CH) and the Crown Content

	SC	SS	S	TC	DM	N
CH	0.896*	0.558	0.884*	0.933**	0.413	0.884*

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed). TC = Total carbohydrates, DM= dry Matter, S= starch, SC soluble carbohydrates , and SS = simple sugar

Table 5: Correlation between the Studied Characters

Characters	SC	SS	S	TC	DM	N
SC	1	0.774	0.842*	0.925**	0.691	0.980**
SS			0.443	0.570	0.275	0.649
S				0.983**	0.685	0.878*
TC					0.668	0.940**
DM						0.803

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed). TC = Total carbohydrates, DM= dry Matter, S= starch, SC soluble carbohydrates, and SS = simple sugar

Analyzing the correlation between the studied characters is shown in Table (5). It was observed that, there was a positive correlation between crowns content of total carbohydrates and nitrogen level by 0.940 (*p*= 0.01), Nitrogen level and soluble carbohydrate 0.98 (*p*= 0.01), and starch level was positively correlated with soluble carbohydrate content by 0.84 (*p*= 0.01).

3.2 Changing Rate of Strawberry Crowns Content (Carbohydrates and Nitrogen) as Effected by Cold Storage

It is clear that cold storage has an effect on the total carbohydrate content of the crowns. Cooling reduced the percentage of total carbohydrates in the crowns, where the changing rate was about -0.25 and -0.26 in the seedling dogged at first and second dates (Δ C1, Δ C2), respectively. Compared to the total carbohydrates content at last digging date (Seedling received 139 naturally chilling hour) and between the total carbohydrates content on seedling from digging at the first and second date after cold storage (ΔC1D3, ΔC2D3), it is clear that, the rate of total carbohydrates on seedling of the last digging was significantly, positively higher about 61% and 65% than the total carbohydrate content on seedling of digging at the first and second date after cooling storage (Fig.2d).

As shown in Figure 2c, cold storage significantly reduced the crown content of starch by 34.3% and 33.1% on seedling of the first and second digging dates, respectively. It is also noted that the changing rate of starch(S) was significantly higher in non-refrigerated seedling at the third digging date (139 naturally chilling hour) by 75.3% and 73% compared to the crowns content of starch after cooling storage of the seedling of the first and second digging date, respectively.

Cold storage also increased the nitrogen content of crowns, where the rate of increase of 35% and 33% on seeding at the first and second digging dates, respectively, and compared between the content of crowns nitrogen between fresh seedling (139 naturally chilling hour) and nitrogen content of chilled storage crowns of seedling of first and second digging was increased by 23.1% and 57.5% respectively.

The changing rate of soluble carbohydrates (SC) was only significant on Δ C1 (between before and after storage of first dig), But it is clear that, the changing rates of (ΔC1D3, ΔC2D3), was negatively with no significant (Fig 2a).

The changing rate of DM% was significantly positive on the Δ C1 (1.25) and negative in second digging (-0.12) (fig 2e). The average rate of change DM% between Seedling received 139 naturally chilling hour and between the total carbohydrates content on seedling from digging at the first and second date after cold storage (Δ C1D3, Δ C2D3), it is clear that, the rate was significantly negatives with about 55% and 21% lower than the DM% on seedling of digging at the first and second date after cooling storage (Fig.2-e).

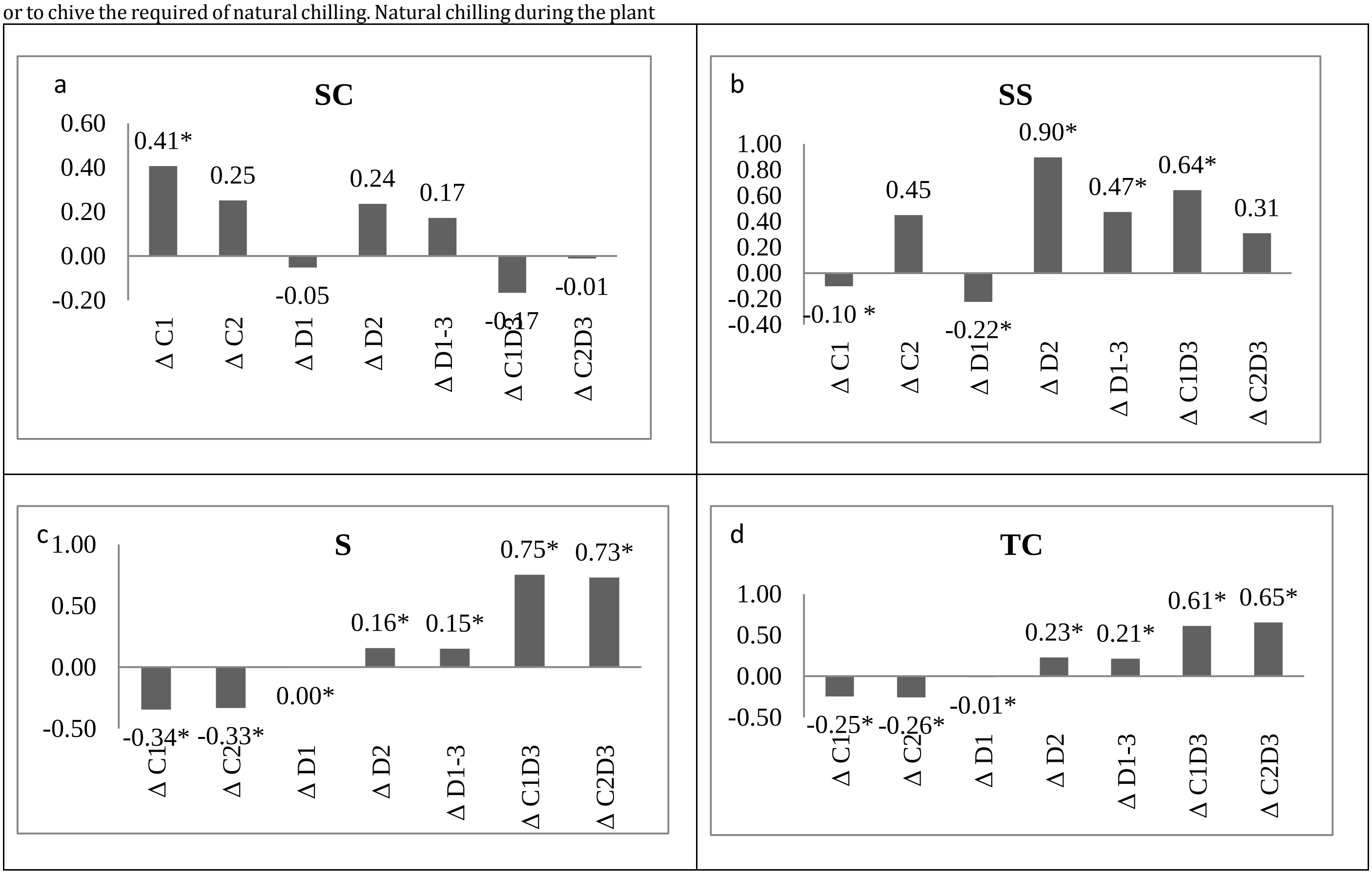
The changing rate of simple sugars SS is shown in Fig.2b, it was significantly negative -0.10 and none significantly positive 0.45 in the seedling dogged at first and second dates (Δ C1, Δ C2), respectively. Compared to the SS content at last digging date (Seedling received 139 naturally chilling hour) and between the SS content on seedling from digging at the first and second date after cold storage (ΔC1D3, ΔC2D3), it is clear that, the rate of SS on seedling of the last digging was significantly positive 64% and non significantly positive 31% than the total carbohydrate content on seedling of digging at the first and second date after cooling storage (Fig.2b).

4. Discussion

Annual production is an important planting system of strawberry, and the cold storage consider the point of save seedling during the worst condition or to chive the required of natural chilling. Natural chilling during the plant dormancy is the final stage during the cycle growth of plant in the year. Chilling is need for the initiation of flowers in strawberry (Ito.H and Saito.T, 1962; Darrow, 1966; Kinet et al., 1993; Lieten, 1997; Al-Madhagi et al., 2018). In addition, temperature has influence on the level and type of carbohydrates and endogenous hormone (Hicklenton and Reekie, 1998; Lee and Ahn, 2001; López et al., 2002; Ahmed and Ragab, 2003; Eshghi and Tafazoli, 2007; Ruan et al., 2009; Al-madhagi et al., 2014; Bauerfeind et al., 2015; Al-Madhagi et al., 2018). Strawberry crowns are an important source of carbohydrates and they might play a role during plant development specifically related to fruit sweetness(Macías-Rodríguez et al., 2002).Starch accumulation in roots is more likely determined by temperature rather than by photoperiod (Kirschbaum, 1998; Mière et al., 1996).

Quantity of chilling hour is depends to strawberry cultivars, where California and Florida cultivars are considered low-chill cultivars compared to European and Japanese cultivars (Bigey, 2002). Moreover, the behaviour of the plant after chilling is a result of the total accumulative of chilling hour. Besides, if the strawberry plant did not receive the satisfactory amount of chilling, the growth will be sluggish and would not form high fruits and longer durations of chilling lead to the shorter flower differentiation (Avigdori-Avidov et al., 1977; Smeets, 1982; Yanagi and Oda, 1993; Lieten, 1997; Al-Madhagi et al., 2018; Hamano et al., 2020).Chilling requirement associated with the accumulation of specific amounts of total non-structural carbohydrates, (TNC). For example, Camarosa' and Pajaro' needed 400 CU and 700 Ch in order to accumulate ~40 mg. g-1 FW of starch respectively (López et al., 2002).

In the current study the plant received the full amount of natural chilling hours (139 chilling hours calculated as less 7C°) significantly increased the crown contents of total carbohydrates (TC), dry weight (DW), starch (S), soluble carbohydrates (SC) and nitrogen (N) levels, that due to accumulative of photosynthesis carbohydrate in the crown of strawberry as result of stopped growth and flowering. However, A typical physiological process in dormant plant during the winter dormancy (decreasing temperatures and photoperiods) is the plant partitioning of nutrients spatially carbohydrates to the storage organs, as an adaptive strategy for surviving cold winters, and store for resuming growth in next spring (Ino et al., 2003; Bauerfeind et al., 2015; Fonteyne et al., 2018). That may could probably explain the reason of increasing total carbohydrates (TC), starch (S), soluble carbohydrates during the natural chilling.



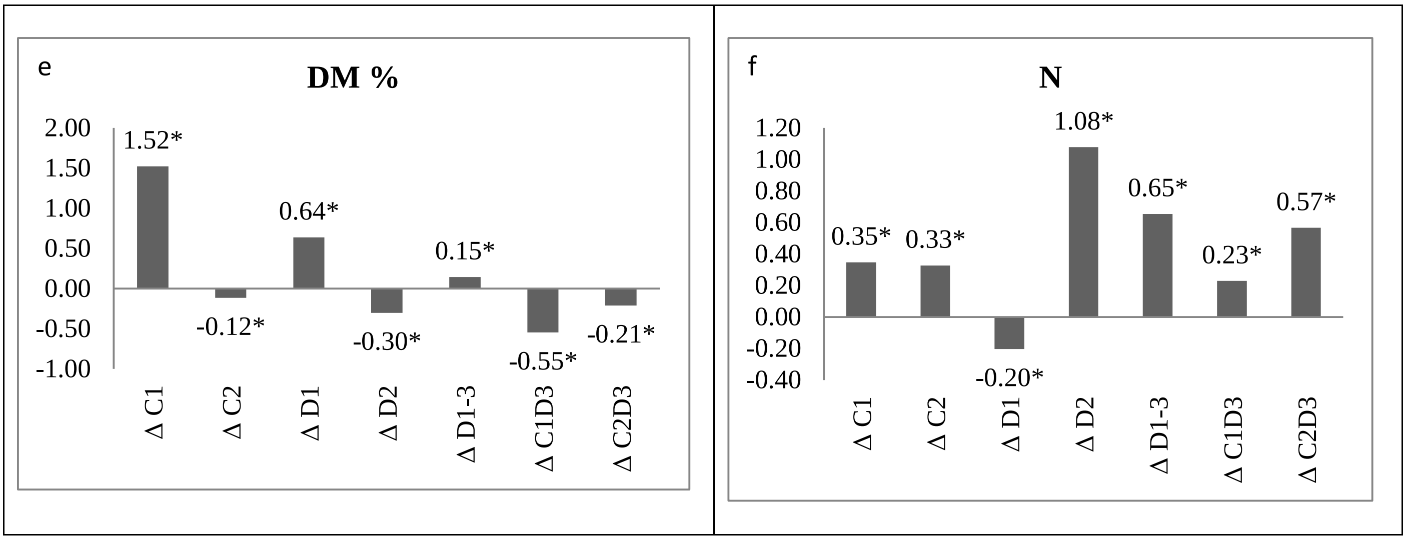


Figure 2: Changing rate % of carbohydrate Δ before and after storage (first dig)Δ C1 , Δ between digging first and second before storage Δ D1 , Δ between digging first and third before storage Δ D1-3 ,Δ between first dig after cold and last digging without chilling Δ C1D3 , Δ before and after storage (second digging)Δ C2 ,Δ between digging second and third before storage Δ D2 , Δ between second dig after cold and last digging without chilling Δ C2D3. *Significant at *0.05* of t test Paired Samples Test

However, from the current data the level of different type of carbohydrate are increasing with low or non hydrolysis down to the undemanding type of carbohydrate, where, no significant difference was observed on the content of simple sugars (SS), dry matter (DM) and nitrogen during the plant received natural chilling.

On the other way, cold storage increased the percentage of soluble carbohydrates (SC) and nitrogen level as well as reduced starch and total carbohydrates in the seedling. It may explain the decline of starch content during cold storage is due to the assimilation process and converting starch into the soluble sugar that is used as an energy supply for the survival live(Rosa et al., 2009). The total of non-structural carbohydrate reserves drop during cold storage due to plant respiration where the respiration of the dormant strawberry depending on root temperature. Nevertheless, the role of non-structural carbohydrate reserves is crucial for long term storage since plant respiration consumes sugars during the storage period (Kirschbaum et al., 2010). More natural chill is ordinarily important for the build-up of reserve carbohydrates necessary for successful long-term seedling storage (Gallace et al., 2019).

Anyway, the changing rate as effected by cold storage was greatest when compared between seedling received neutral chilling (last dig with 139 ch) and seedling received 0 ch, and this getting data corroborate that the cold storage cannot be as a replacement for the neural chilling. Status of seedling as comparing between the levels of carbohydrate in seedling received natural chilling and cold storage conformed that the field chilling more effective than that carried out using cold storage in the dark. And this point is taking the same direction with that previous found by (Tehranifar et al., 1998).

Strawberry does not true dormancy, where the short-day cultivars adapted to cool winters will grow in the tropical regions, and the full production will be achieved by required chilling (Arney, 1955; Al-madhagi et al., 2014). In fact, The starch present in the plant at the nursery stage contributes to the subsequent growth and development of strawberry (Al-madhagi et al., 2014). And the growth and development of strawberry after chilling is responsive to the endogenous hormone and the level of carbohydrate (Waithaka et al., 1980). Al-Madhagi et al. (2018) Found that the addition chilling hour above natural chilling is only suggested for 15 and 30 days, and the seedling store for more than 30 d will produced fewer flowers and more runners. Finally, if Atkinson et al. (2013) suggested that change crop management practices and growing systems to tolerate low chill is in the current climate change in Europe, what than we can suggest for Yemen condition only more studies and importing new strawberry cultivars.

5. Conclusions

Natural chilling hour is more effective than using cold storage in the dark. Cooling storage at 2°C is not advice to long seedling storage. It could be suggest that cold storage (2°C) for short period maybe using for addition chilling but not as an alternative the natural chilling. More studied and trail are needed by using newest cultivars. In this study we used artificial chilling, which for the direction of future research it suggest to submit to application the seedling to the artificial cold storage after the seedling harvest all natural chilling hour

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