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Research Article

Transplanting Time of Strawberry Runners Influences Plant Growth, Post-harvest Quality and Antioxidant Properties of Different Cultivars Grown in Subtropical Highlands of Azad Jammu and Kashmir

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Abstract

Strawberry is a well-known aggregate fruit. It has an exceptional mouth feel taste with high nutritional characteristics. Transplanting time of strawberry runners mainly affects growth of plant, yield and quality of fruit. But transplantation time for different cultivars of strawberries is not yet standardized in Azad Kashmir. Thus, the study was designed to evaluate the field performance of three strawberry cultivars viz. Chandler, Seascape and Tribute on three different transplanting dates i.e., 15th March, 30th March and 15th April. Plant vegetative growth, fruit yield and antioxidant characteristics were determined. Transplantation time significantly influenced most of the parameters under study. Runners transplanted on 30th March showed the highest survival percentage (90.0%), number of leaves per plant (9.97) in cv. Chandler followed by cv. Seascape and cv. Tribute. Runner production per plant was high in the runners transplanted on 15th March in all the cultivars under observation. Total soluble solids, sugars, vitamin C, anthocyanin and antioxidant activity was significantly enhanced in the fruit of plants transplanted on 30th March. Overall, results suggested that transplantation of strawberry runners from 15th March to 30th March is ideal for gaining high yield and nutritious strawberry fruit in the area of Azad Kashmir.

Keywords: Anthocyanin content, Flavonoids, Chandler, Seascape, Tribute, strawberry.

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is getting economic importance now a day due to its nutritional value, taste and charming red color (Hossain et al., 2016). Strawberry grows in almost all over the world, but it was introduced in subcontinent during 1960's (Kumar and Ahad, 2012). It is a temperate fruit crop, but it can grow well in tropical and sub-tropical regions. Due to its broad growing spectrum the area under cultivation of crop is increased rapidly in sub-continent (Singh and Asrey, 2009).

These small berries aid as a major source of minerals, vitamin C and antioxidants (Giampieri et al., 2012; Mahmood et al., 2012). Because of extraordinary dietary contents

and economic status, different cultivars with innovative cultural techniques are getting importance in order to fulfill the market demands. The charming red color, cosmetic look and high nutritional quality of strawberry also increased consumer demand. This red color is mainly due to anthocyanin which mainly contains traces of cyanidin and pelargonidin 3-monoglucoside (Belakud et al., 2015). Because of their high nutritional contents and therapeutic health benefits, strawberries serve as a nutritious food (Basu et al., 2014). These therapeutic benefits of strawberries are usually due to the synergetic effects of bioactive factors present in strawberries. But this is also documented that these bioactive compounds vary with transplantation time and varieties. Thus, the time of transplantation is a crucial factor in strawberry growth, yield, nutritional quality and sweetness of fruit highly depends on day length and temperature (Paul et al., 2017). Degree of sweetness is characterized by acid sugar ratio which depends upon cultivar, ripeness, weather, altitude and time of transplantation (Zheng et al., 2009). Thus, the time of transplantation for strawberry is a significant factor for partitioning assimilates, which has great impact on yield and growth of strawberry (Rahman et al., 2014).

Rawalakot being a subtropical high land climatic zone and it is hypothesized that strawberries can be grown in this region. Thus, no studies have been reported for cultivation of different varieties of strawberries with different planting times in Rawalakot. Hence, this study has been aimed at selecting the suitable transplanting dates and fruit composition of three varieties of strawberry.

Materials and Methods

Experimental Material and Field Site Description

This study was conducted in the years 2019 and 2020 at experimental farm of Department of Horticulture, Faculty of Agriculture, University of Poonch, Azad Kashmir (Latitude 33-360N and Longitude 73-750E). A polyethylene sheet (PE 200 µm) was used to provide shade. In this study, different cultivars of strawberry viz. Chandler, Tribute and Seascape were selected and three transplanting dates i.e., 15th March, 30th March and 15th April. Disease free and healthy strawberry runners of cv. Chandler were collected from National Agriculture Research Centre (NARC), Islamabad while Seascape and Tribute were collected from Mountain Agriculture Research Center Juglot, Gilgit-Baltistan and shifted to 12-inch diameter plastic pots.

The pots were placed 15 cm apart from each other in order to give proper growing space. Farmyard manure and soil mixed in (1:1) ratio before transplantation. Each pot was filled with 4 kg of farmyard manure and soil mixture. Urea (0.5 g) in one liter of water and was given (150 ml each pot) after 1 month of transplanting and then fortnightly till fruiting. Other agronomic practices such as irrigation and weeding were also done in order to ensure better growth and quality of strawberry plants. Result reproducibility was confirmed by two trails. The 1st trial was done from March 2019 to August-September 2019 and the 2nd from March 2020 to August-September 2020 for all the parameters under study.

Data about vegetative and reproductive growth, postharvest quality attributes and health related compounds of strawberry fruit was recorded.

Vegetative Attributes of Strawberry Plants

Vegetative parameters such as survival percentage (%), number of leaves per plant, root length (cm) and chlorophyll content of leaves (g/ml) were determined after 90 days of transplantation. Chlorophyll was measured by the method given by Zahid et al. (2014).

Reproductive Attributes of Strawberry Plants

Reproductive parameters such as runners per plant, flowers per plant, fruits per plant were determined after 90 days of transplantation.

Physical Attributes of Strawberry Fruit

Average fruit weight (g) and fruit diameter (cm) were measured using weighing balance and vernier calliper, respectively.

Postharvest Quality attributes of strawberry fruit

Total Soluble Solids (%)

Total soluble solids were done at room temperature using a digital refractometer (Kyoto Company, Japan). One drop (each sample) of fruit juice was mounted on a dry prism of refractometer prism and data was noted in percent (%).

Titratable Acidity (%)

Fruit pulp (5 g) from randomly selected fruits was homogenized, followed by mixing in purified water (20 ml). The mixture was then filtered to take pure extract. Sodium hydroxide (0.1 N) was used for titration of extract (5 ml). A few phenolphthalein drops were used as an indicator until light pink color appeared. The following formula was used to calculate titratable acidity.

$$TA\% = \frac{NaOH\ (ml)\ \text{x normality x ascorbic acid (equi. wt.)}}{Sample\ (g)\ \text{x aliquot volume}}$$

Total Sugars (%)

Fruit juice (25ml) was mixed with concentrated HCl (2.5 ml) and was preserved for one night. Neutralization of solution was done using 1N NaOH solution and phenolphthalein was added as an indicator. This solution was titrated against Fehling's solution (10 ml) from the following formula;

$$\text{Total sugar}\ (\%) = \frac{\text{invert sugar (mg) x dilution factor}}{\text{Titrate value x sample volume}} \times 100$$

Health Related Compounds in Strawberry Fruit

Vitamin C (mg /100g fresh fruit weight)

Vitamin C was assessed by using 2,6-dichlorophenol indophenols (dye). The extracted sample (5 ml) was poured in a conical flask (100 ml) along with 5 ml of 4.0% meta phosphoric acid solution followed by titration with 2 dyes until light pink color.

Total Anthocyanin (mg of cyanidine-3-glucoside 100 g-1 fresh fruit weight)

Total anthocyanin was measured by using pH dilution method (Zheng et al., 2007). First dilution was made by using potassium chloride buffer (pH 1.0) while second dilution was made by using sodium acetate buffer (pH 4.5) against a blank and equilibrated for 15 min. Absorbance for potassium chloride dilution was measured at 515 nm and for sodium acetate buffer was measured at 700 nm, using UV-Vis spectrophotometer. Pigments of anthocyanin were calculated as mg of cyanidine-3-glucoside per 100 g fresh fruit weight.

Total Phenolic Contents (µg gallic acid 100g-1 fresh fruit weight)

Spectrophotometric method was used to measure total phenolic contents. Strawberry juice (0.1 ml) plus Folin-Ciocalteau (0.5 ml) and sodium carbonate (1.5 ml) were mixed. A volume (10 ml) was made by adding distilled water. Mixture was placed in a hot water bath at 40 °C for 2 hours. Absorbances were recorded at 765 nm by using UV-Vis spectrophotometer and results were articulated in µg gallic acid per fresh fruit weight.

Total Flavonoid Contents (mmol quercetin 100g-1 FW)

Fine pulverized fruit sample (0.1 g) was agitated in hot water (10 ml) followed by mixing with of aluminum chloride (0.1 ml) and 0.1M potassium acetate (0.1 M) were mixed. After 30 minutes activity was noted at absorption 430 nm UV-visible spectrophotometer (UV-400 Spectrophotometer Hamburg, Germany) and the result were expressed as m mol of quercetin per 100g fresh fruit weight.

Antioxidant Activity (mg FeSO4 100g-1 FW)

Ground fruit sample (2 g) plus water (10 ml) was centrifuged at 10,000 rpm for 10 minutes. Supernatant was used as extract. Extract (0.04 ml) plus FRAP reagent (3 ml) was mixed and incubated in a water bath for 4 minutes at 37o C. Absorbance was taken at 593 nm in spectrophotometer and results were stated as 1 mg FeSO4 per g fresh fruit weight.

Statistical Analysis

Randomized complete block design (RCBD) with three replicates was used for the above mentioned experiment and it was repeated twice, and the data was combined for analysis as the key results of single analysis revealed at par results across the trail. Data collected was subjected to analysis of variance (ANOVA) using statistical software (Statistic 8.1) and means were separated at $P < 0.05$using Tukey's test.

Results

Vegetative Parameters

During the study years it was observed that survival percentage was significantly ($P < 0.05$) different among transplanting dates and cultivars under study (Table 1). The highest survival percentage was recorded in cv. Chandler (90.0%) transplanted on 30th March followed by 15th March transplantation. The lowest survival percentage was recorded in cv. Tribute (36.66%) transplanted on 15th April. It was observed that vegetative growth of plants mainly depends on the time of transplantation. Cold temperature has a vibrant role in survival and growth of plants. The demand of cold for survival of strawberry in very important as plants need cold temperature during transplantation in the field (da Costa et al., 2017). Results of our experiment are also in covenant with Hidaka et al. (2016), who stated that early transplantation and cold environments help plants to withstand in the stress environment and which helps in increased survival percentage. This difference in survival percentage of different cultivars might be due to environmental and geographical differences (Hamidi et al., 2016). Similarly, other vegetative parameters such as leaves/ plant, root length (cm) showed a significant difference at $P < 0.05$ (Table 1). The number of leaves was higher (9.97) in cv. Chandler followed by cv. Tribute (8.66) transplanted on 15th March. Whereas the lowest number of leaves for all the three cultivars were recorded on 15th April. These

results showed that low temperature was favorable for development and growth of strawberry plants (Singh et al., 2007). Leaves per plant decreased with delayed transplantation which might be owed to less time for vegetative growth whereas, number of leaves were higher in early transplantation (Anna et al., 2003). Root length was significantly ($P < 0.05$) higher in early transplantation (15th March) for all three cultivars. Whereas the lowest root length (6.33 cm) was recorded in cv. Tribute on 15th April. A sharp difference in root length with transplantation time is due to an increase in temperature during April. Roots are more sensitive to high temperature than any other part of plant (Menzel and Smith, 2014). An early plantation helps in increased root length which provides vigor to plant and helps in increased uptake of nutrients (Qadeer et al., 2001).

Table 1. Outcomes of transplanting time on survival percentage (%), number of leaves per plant and root length (cm) of strawberry cv. Chandler, Seascape and Tribute.

Transplanting dates	Survival percentage (%)			Number of leaves/plants			Root length (cm)		
	Cultivars			Cultivars			Cultivars		
	Chandler	Seascape	Tribute	Chandler	Seascape	Tribute	Chandler	Seascape	Tribute
15th March	86.6 ab	70.0 bc	66.66 bc	7.34 bc	5.14 de	4.22 ef	15.66 a	13.00 b	9.00 cd
30th March	90.0 a	80.0 ab	56.66 cd	9.97 a	8.66 ab	6.18 cd	14.33 ab	9.66 c	7.16 de
15th April	66.66 bc	50.0 cd	36.66 d	6.44 cd	3.93 ef	2.73 f	14.00 ab	9.33 c	6.33 e

Different letters indicate significant ($P < 0.05$) difference among treatments (transplanting dates x cultivars).

During the study years it was observed that chlorophyll a, b and total chlorophyll content of leaves was significantly ($P < 0.05$) different among transplanting dates and cultivars under study (Figure 1). Highest chlorophyll 'a' content of leaves (8.72 g/ml) were recorded in cv. Chandler transplanted on 30th March which was significantly ($P < 0.05$) different with the chlorophyll 'a' content (6.64 g/ml) of cv. Chandler transplanted on 15th April. Whereas lowest chlorophyll 'a' (4.54 g/ml) was recorded in cv. Tribute transplanted on 15th March which was non-significantly different with chlorophyll 'a' contents of cv. Tribute transplanted on 30th March and 15th April (Figure 1a).

Highest chlorophyll 'b' content of leaves (10.1 g/ml) was recorded in cv. Chandler transplanted on 15th March which was significantly ($P < 0.05$) different with the chlorophyll 'b' contents of leaves for same cultivar transplanted on 15th April (8.30 g/ml). Whereas, lowest chlorophyll 'b' (5.80 g/ml) was recorded in cv. Tribute transplanted on 30th March which was not significantly different with chlorophyll 'b' contents of cv. Tribute transplanted on 15th March and 15th April (Figure 1b). Highest total chlorophyll content of leaves (17.92 g/ml) was recorded in cv. Chandler transplanted on 30th March which significantly ($P < 0.05$) different from the total chlorophyll content (7.97 g/ml) of leaves of cv. Tribute transplanted on 15th April. In our results late transplantation showed a declining tendency of net photosynthetic rate for different cultivars. Whereas, an increased photosynthetic rate helps in decreased moisture loss and increased fruit yield (Yu et al., 2015). Chlorophyll 'a' is considered as the most important pigment of plants which helps in photon collection. While chlorophyll 'b' helps in transferring of energy to a different molecule of chlorophyl 'a' and also helps in photosynthesis by

collecting different light wavelengths (Malkin and Niyogi, 2000). Chlorophyll pigments are essential elements in absorption of light and transformation of different assimilates. In our results, the change in chlorophyll content of leaves is might be due to the increase in temperature as high temperature is responsible for degradation of chlorophyll due to photo oxidation which occurs due to increase in activity of chlorophyllase enzyme.

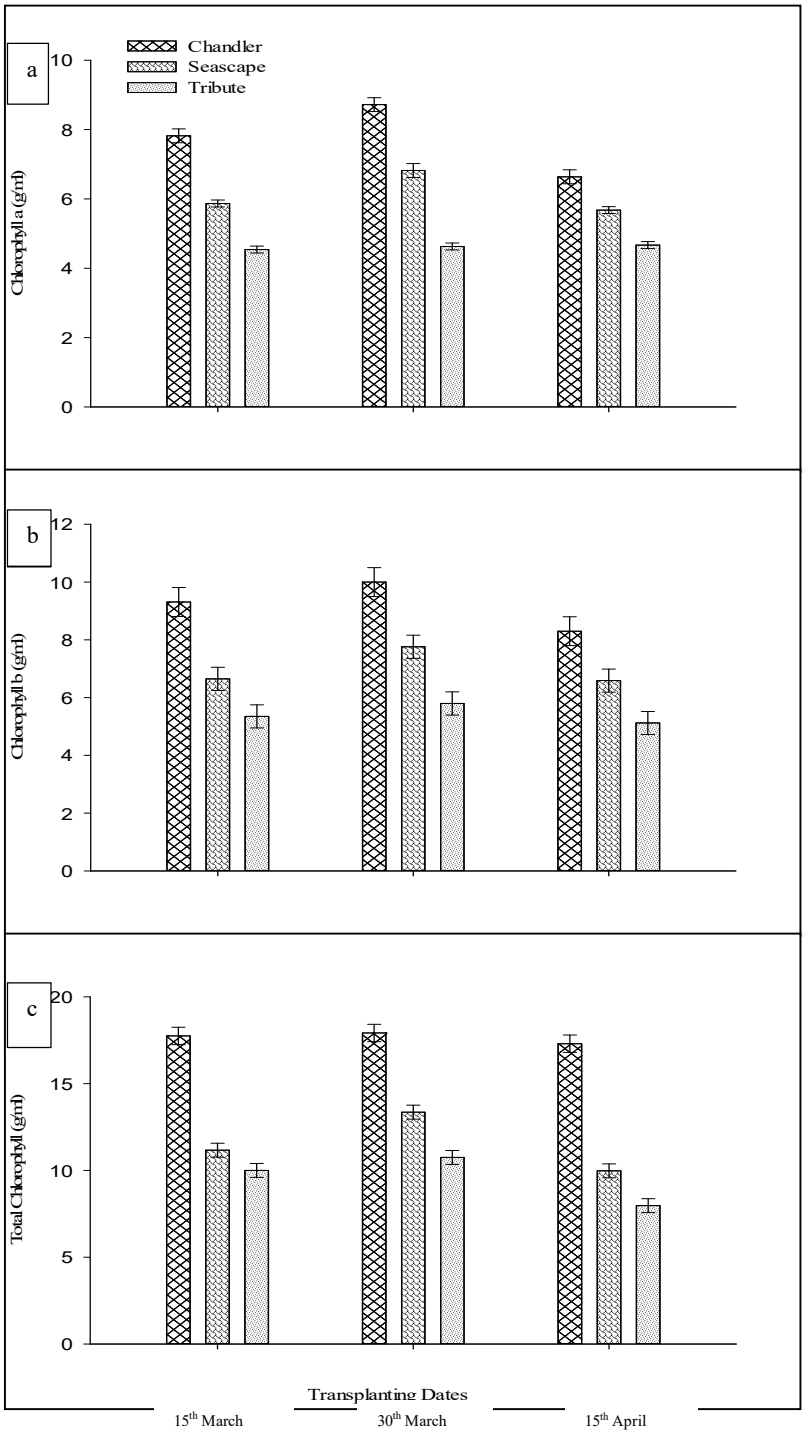

Figure 1. Effect of different transplanting dates on (a) chlorophyll 'a' (g/ml), (b) chlorophyll 'b' (g/ml) and (c) total chlorophyll (g/ml) of strawberry cv. Chandler, Seascape and Tribute. Vertical bars specify standard error of means for three replicates.

Reproductive Parameters

During the study years it was observed that results regarding reproductive parameters were significantly ($P < 0.05$) different among transplanting dates and cultivars under study (Table 2). The highest number of runners per plant (15.36) was recorded in cv. Chandler followed by cv. Seascape (12.20) and cv. Tribute (9.93) transplanted on 15th March. Whereas, plants transplanted on 15th April showed the lowest number of runners in all three cultivars (Table 2). Number of runner production is mainly genotype dependent. In our results, runners production is high in early transplantation which might be due to the fact that plants enjoy long time for vegetative growth results in high number of runners (Chowhan et al., 2016). Our results are supported by de Costa et al. (2017) that transplantation time is an important factor for the production of quality runners.

A highly significant ($P < 0.05$) difference was recorded in number of flowers of different cultivars. The highest number of flowers per plant (10.80) was found in cv. Chandler which was trailed by cv. Seascape (8.16) and cv. Tribute (4.60), transplanted on 15th March (Table 2). Whereas the minimum number of flowers (2.78) was observed in cv. Tribute which was non-significantly ($P > 0.05$) different from cv. Seascape (2.79) transplanted on 15th April (Table 2). Similarly, a highly significant ($P < 0.05$) difference was observed among number of fruits of different cultivars. The highest number of fruits per plant (7.63) was noted in cv. Chandler followed by cv. Seascape (4.36) and cv. Tribute (3.00) transplanted on 15th March (Table 2). Whereas the lowest number of fruits (1.81) were observed in cv. Tribute which was non-significantly ($P > 0.05$) different from cv. Seascape (2.06) and cv. Chandler (3.28) transplanted on 15th April (Table 2).

A robust connection was determined among the production of leaves, flowers and fruit across the whole growing season. Early transplantation of plants helps in development of leaves, roots and crown whereas, late transplantation helps in development flowers and fruits (Menzel and Smith, 2014). Floral induction of strawberry in tropical and sub-tropical regions is reduced with the increase in temperature as this temperature increase negatively influences working ability the photo assimilates within the plant crown (da Costa et al., 2017). In our result, the increase in temperature with late transplantation results in reduced flowering and fruiting period which causes low or no fruit production (Rahman et al., 2016). It is clear that vegetative development of strawberry may antagonize with reproductive stage of strawberry plants (Pérez-de-Camacaro et al., 2002).

Table 2. Outcomes of transplanting time on number of runners per plant, number of flowers per plant and number of fruits per plant of strawberry cv. Chandler, Seascape and Tribute.

	Number of runners/plants			Number of flowers/plants			Number of fruits/plants		
Transplanting dates	Cultivars			Cultivars			Cultivars		
	Chandler	Seascape	Tribute	Chandler	Seascape	Tribute	Chandler	Seascape	Tribute
15th March	15.36 a	12.20 b	9.93 c	7.22 bc	5.23 d	3.44 ef	5.72 b	3.66 cd	2.46 de
30th March	10.43 bc	8.70 cd	6.80 d	10.8 a	8.16 b	4.60 de	7.63 a	4.36 bc	3.00 cde
15th April	7.66 cd	6.83 d	4.56 e	5.63 cd	2.79 f	2.78 f	3.28 cde	2.06 e	1.81 e

Different letters indicate significant ($P < 0.05$) difference among treatments (transplanting dates x cultivars).

Physical Attributes of Strawberry Fruit

During the study years it was observed that results regarding physical parameters of fruit were significantly ($P < 0.05$) different among transplanting dates and cultivars under

study (Table 3). Maximum fruit weight (7.81 g) was recorded in cv. Chandler transplanted on 15th March. Whereas the less fruit weight (1.49 g) was observed in cv. Tribute transplanted on 15th April (Table 3). Similarly, Maximum fruit diameter (2.28 cm) was recorded in cv. Chandler transplanted on 15th March. Whereas, the lowest fruit diameter (0.78 cm) was recorded in cv. Tribute transplanted on 15th April (Table 3). Physical parameters of strawberry fruits are highly influenced by transplanting time and cultivars (Rahman, 2014). Fruit weight and fruit diameter are the important factors that influence the yield of plants. In our results smaller fruits are obtained in the plants transplanted on 15th April which revealed that end of growing season results in small sized berries (Menzel and Smith, 2014). Late transplantation results in small sized fruit with low yield which might be due to rise in temperature (Chaitanya et al., 2017). In our results minimum fruit diameter was recorded in late transplantation which showed a negative correlation with high temperature (Hossain et al., 2014).

Table 3. Outcomes of transplanting time on fruit weight (g), fruit diameter (cm) of strawberry cv. Chandler, Seascape and Tribute.

Transplanting dates	Fruit weight (g)			Fruit diameter (cm)		
	Cultivars			Cultivars		
	Chandler	Seascape	Tribute	Chandler	Seascape	Tribute
15th March	6.56 b	4.32 d	2.14 fg	1.69 b	1.27 cd	1.18 cd
30th March	7.81 a	5.63 bc	2.85 ef	2.28 a	1.62 b	1.45 bc
15th April	5.07 cd	4.01 de	1.49 g	1.39 bc	1.08 de	0.78 e

Different letters indicate significant ($P < 0.05$) difference among treatments (transplanting dates x cultivars).

Post-harvest Quality Attributes of Strawberry Fruit

During the study years it was observed that results regarding post-harvest quality parameters of fruit were significantly ($P < 0.05$) different among transplanting dates and cultivars under study (Table 4). Highest total soluble solids (8.03%), titratable acidity (0.59%0 and total sugars (17.92%) were observed in cv. Chandler transplanted on 30th March which was non- significantly ($P > 0.05$) different with the plants transplanted on 15th April. Whereas, minimum total soluble solids (4.31 %), titratable acidity (016%) and total sugars (7.90) in cv. Tribute transplanted on 15th April (Table 4). Rahman et al. (2014) reported that titratable acidity and total soluble solids reduced with late transplantation. Early transplanted plants exposed more to the suitable growing conditions and they also have more time for acid sugar accumulation which results in increase of total soluble solids (Anwar et al., 2016).

Table 4. Outcomes of transplanting time on total soluble solids (%), titratable acidity (%) and total sugar (%) of strawberry cv. Chandler, Seascape and Tribute.

Transplanting dates	Total soluble solid (%)			Titratable acidity (%)			Total sugar (%)		
	Cultivars			Cultivars			Cultivars		
	Chandler	Seascape	Tribute	Chandler	Seascape	Tribute	Chandler	Seascape	Tribute
15th March	8.00 a	6.70 ab	5.10 bc	0.54 a	0.49 a	0.31 bc	17.75 a	11.17 bc	10.0 cd
30th March	8.03 a	6.76 ab	5.30 bc	0.59 a	0.55 a	0.33 b	17.92 a	13.36 b	10.7 c

15th April	6.51 ab	5.06 bc	4.31 c	0.35 b	0.21 cd	0.16 d	17.30 a	9.98 cd	7.90 d

Different letters indicate significant ($P < 0.05$) difference among treatments (transplanting dates x cultivars).

Health Promoting Factors of Strawberry Fruit

During the study years it was observed that results regarding health promoting factors (vitamin C, total anthocyanin, total flavonoids, total phenols, total antioxidants) of fruit were significantly ($P < 0.05$) different among transplanting dates and cultivars under study (Figure 2). Fruits obtained from cv. Chandler transplanted on 30th March have maximum vitamin C (0.20 mg/100 g fresh fruit weight; Figure 2a), total anthocyanin (81.2 mg/100g fresh fruit weight; Fig 2b), total flavonoids (2.49 mg/100g fresh fruit weight; Fig 2c), total phenol (0.71 µg gallic acid/100 g fresh fruit weight; Fig 2d) and total antioxidant activity (2.56 mg FeSO4/ g fresh fruit weight; Figure 2e). The lowest amount of these compounds was noted in the fruits of cv. Tribute transplanted on 15th April (Figure 2).

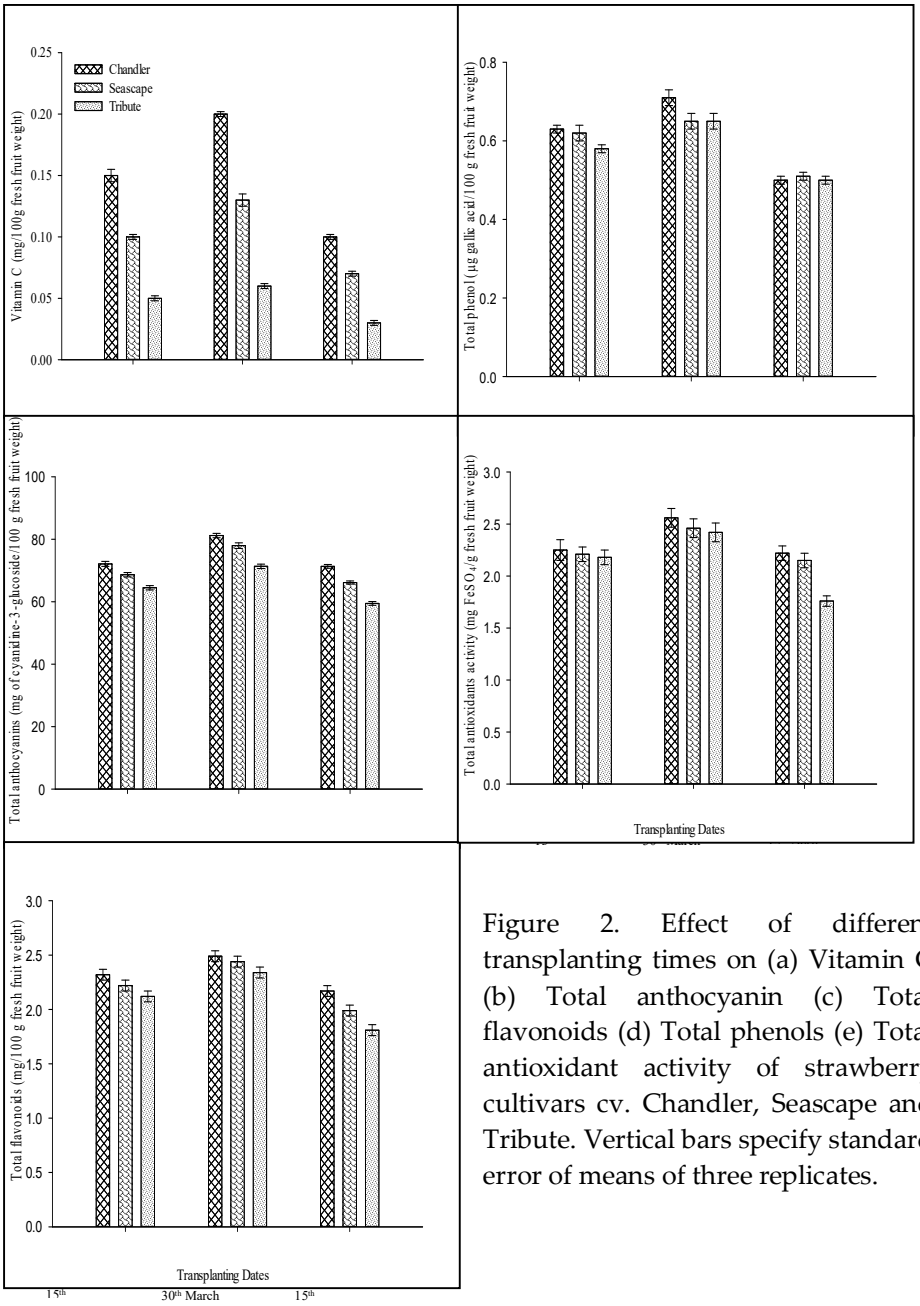

Figure 2. Effect of different transplanting times on (a) Vitamin C (b) Total anthocyanin (c) Total flavonoids (d) Total phenols (e) Total antioxidant activity of strawberry cultivars cv. Chandler, Seascape and Tribute. Vertical bars specify standard error of means of three replicates.

Different concentrations of vitamin C, tocophenols carotenoids, and flavonoids possess a significant effect on the human body (Vinha et al., 2014). The difference in amount of these antioxidant compounds in strawberry grown in different transplanting dates is due to the growing seasons which helps in increased number of vitamins and antioxidants (Anwar et al., 2016). Vitamin C production is tissue specific and also considered as genetic factor (Sun et al., 2012). Synthesis of vitamin C in plants trails L-galactose pathway and considered as anabolic and which is linked to photosynthesis in plants (Wheeler et al., 1998). Higher chlorophyll content in plants grown on 30th March lead to augmented vitamin C in fruits might be owed to the better photosynthesis. Improved number of leaves and high chlorophyll in our outcomes are liable to the high photosynthesis, thus aiding in buildup of vitamin C in fruits. Thus it may assume that high vitamin C content in fruit of early transplanted plants could be linked with prolonged fruit duration (Anwar et al., 2016).

Anthocyanins, flavonoids and phenols are also very compounds in relation to human health which are found in strawberry. All these are liable for diverse antioxidant activities. Polyphenols are widely present in strawberries (Williner et al., 2003). Few scientists proposed that strawberries consist of more than 50% polyphenols (Atkinson et al., 2006). In our results fruits harvested from the plants transplanted on 30th March have more total anthocyanin contents, total flavonoids and total phenols are due to the enhanced chlorophyll content and prolonged fruit duration (Aghofack-Nguemezi and Schwab, 2014).

The altered quantity of anthocyanin and phenolic compounds affects an augmented antioxidant activity, sanctions the theory of farming (transplanting time) is important for building high amount antioxidants in fruits (Panico et al., 2008). Martínez et al. (2013) stated that internal quality and health related compounds are correlated with chlorophyll content.

Conclusions

Results of this experiment revealed that transplantation date 15th March have more promising results in terms of runner production while transplantation date 30th March showed more promising results in terms of all other parameters such as yield and other biochemical and antioxidant characteristics. However, further studies at different farmer fields should be carried out to have an overall conclusion on the subject of effect of transplantation date on vegetative, reproductive and antioxidant characteristics of different strawberry cultivars.

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