Pak. J. Bot., 50(3): 1225-1232, 2018.

DYNAMICS OF FRUIT WEIGHT, MESOCARP SIZE AND CELLULAR MORPHOLOGICAL DYNAMICS IN 12 GRAFTING SWEET CHERRY (*PRUNUS AVIUM* L.) CULTIVARS IN ONE YEAR

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Abstract

Rootstock and scion have significant effects on fruit production in sweet cherry, but their interplay has not been fully detected for the cellular mechanism at different fruiting stages. In this study, three sweet cherry scions of 'Lapins', '8-102' and 'Rainier' were planted with four rootstocks of 'Mahaleb', 'Dwarf', 'ZY-1', and 'Latin' resulting in 12 combined cultivars. Fruits were sampled at four growing stages. On 28 May, 2015 parameters of fruit weight, length, and diameter were greatest in trees with combined scion of '8-102' × rootstock of 'ZY-1', followed by the combination of '8-102' × 'Dwarf'. On 24 June, 2015 fruit cell number was found to have a positive correlation with fruit length and weight. In conclusion, scions of '8-102' and 'Rainier' is recommended for grafting on rootstock of 'Latin' for desired mature fruit quality through abundant mesocarp cell division.

Key words: *Prunus avium*; Fruit quality screen; Grafting; Genotype; Woody fruit tree.

Introduction

The sweet cherry (*Prunus avium* L.) is a highly-valued seasonal product in regions under temperate climates. Sweet cherries are well known to produce well-being antioxidants, e.g. anthocyanins, phenolic acids, and flavonols (Jakobek *et al.*, 2009). Size and weight are two determinants of price for sweet cherry fruits (Kappel *et al.*, 2012). The landrace variety had fruit weight of about 6 g but that of modern varieties can reach about ~14 g (Franceschi *et al.*, 2013). To obtain new cultivars is an available approach to harvest fruit weight at least over 2 g in wild varieties (Franceschi *et al.*, 2013). In many US markets, fresh cherries are upgraded as soon as their fruit size reached 24 mm in length (Whiting *et al.*, 2006). The 2009 sweet cherry crop in the Pacific Northwest of the USA was record-breaking because many orchards were overset with an abundance of under-sized fruit over 2g in weight and 20 mm in length (Zhang & Whiting 2011). In other regions, fruit weight and size are also two critical parameters determining fruit quality for sweet cherry cultivars.

Rootstock and scion have the interplay to affect fruit production in sweet cherry. Whiting *et al*., (2005) showed the effect of rootstock variation on the maturity time in scions. Sweet cherry rootstocks were predominantly cultivated from seedlings but there was growing interest in new clonal rootstocks with the potential of greater vigor control of the scion. Large size, stable firmness, and desired sweetness are all considered important fruit quality traits for the employment of scion during grafting (Kappel *et al.*, 2012). Grafting is a widely used technique in the production of horticultural cultivars (Koepke & Dhingra, 2013). The combination of various genotypes by scions and rootstocks in a single generated plant results in an interesting biological variation over the known genetic paradigms. Rootstock variation has been found to affect the fruit quality about morphology and weight (Daza *et al.*, 2008; Rato *et al.*, 2008). Studies have suggested that both rootstock and scion can affect final fruit size and quality in sweet cherry (Zhang & Whiting, 2011). Robinson *et al*., (2006) found the control of fruit yield of scions by rootstock. The interest for the increase of the sweet cherry production resulted in the increased interest for the breeding of existing, and the selection of new, more appropriate rootstocks (Jakobek *et al.*, 2009). Understanding the basic cellular mechanism for the different cultivar variation is essential to develop tools for enhancing fruit size either through breeding or through the manipulation of fruit growth using horticultural practices (Johnson & Malladi, 2011). Thus, much has been known to generate new sweet cherry cultivars with desired fruit quality but cellular mechanism has not been fully detected to interpret the interplay between rootstock and scion on sweet cherry fruit growth.

Sweet cherry fruit is composed of a thin protective exocarp, a fleshy mesocarp, and a stony endocarp (pit) containing the seed. Cellular morphology of fleshy mesocarp can further be defined by its cell number and cell size (Zhang *et al.*, 2010). Cell level processes have been commonly studied and it has well known about the correlation between cell number and fruit size in blueberry [*Vaccinium ashei* (Johnson & Malladi, 2011)], peach [*Prunus persica* (Scorza *et al.*, 1991)], olive [*Olea europaea* (Rapoport *et al.*, 2004)], strawberry [*Fragaria* × *ananassa* (Cheng &Breen 1992)], melon [*Cucumis melo* (Higashi *et al.*, 1999)], apple [*Malus* × *domestica* (Denne, 1960)], and tomato [*Solanum lycopersicum* (Bertin *et al.*, 2009)]. Otherwise, cell expansion was indicated as the driving factor for fruit growth at the later stages and some positive correlations were found between cell size and fruit growth for some given fruit tree species (Bohner & Bangerth 1988; Gillaspy *et al.*, 1993; Harada *et al.*, 2005; Looney 1993; Okello *et al.*, 2015; Wismer 1995). In contrast, other evidences demonstrated no correlation between them (Cheng & Breen, 1992; Olmstead *et al.*, 2007; Scorza *et al.*, 1991; Zhang *et al.*,

2006). For sweet cherry cultivars, both division and expansion of mesocarp cells have been reported to relate to fruit growth (Olmstead *et al.*, 2007; Param & Zoffoli, 2016; Yamaguchi *et al.*, 2004). Therein, fruit cell number contributed more to fruit growth in sweet cherry than cell size as indicated by (Yamaguchi *et al.*, (2004) and Olmstead *et al.*, (2007). Current knowledge about cellular mechanism for fruit growth in sweet cherry is derived from studies in over-year terms, quite little is known about dynamics of cell number and cell size at different fruiting stages within a growing season.

In the present study, scions from three sweet cherry cultivars were grafted to four rootstocks; hence generated were 12 new cultivars. Fruit growth and cell number and cell length were measured at four fruiting stages and analyzed for the difference among cultivars and correlation between parameters. It was hypothesized that: (i) cell number and length changed among cultivars at some given fruiting stages and (ii) both cell number and cell length contributed to the final fruit weight and morphology.

Materials and Methods

Plant material: Scions were obtained from three sweet cherry cultivars of 'Lapins', '8-102' and 'Rainier'. 'Lapins' is a late-maturing sweet cherry cultivar common in Pacific Northwest (Einhorn *et al*., 2013). '8-102' is the cultivar 'Wanhongzhu' selected by Dalian Academy of Agricultrual Sciences. Both 'Lapins' and '8-102' are being planted in Beijing and Liaoning (Wang *et al.*, 2015). 'Rainier' was developed in 1952 at Washington State University, and is now widely developed in China (Wen *et al.*, 2014). Rootstocks were obtained from four sweet cherry cultivars of 'Mahaleb', 'Dwarf', 'ZY-1' and 'Latin'. 'Mahaleb', *P. mahaleb*, is a major rootstock in Central and Southern European countries as well as in Asia Minor, Central Asia, and China (Kappel *et al.*, 2012). 'Dwarf' was derived from the hybrid of *P. cerasus*. 'ZY-1' was introduced to China since 1988 from Italy by Chinese Academy of Agricultural Sciences with significant traits of dwarfing stocks as well. 'Latin' was selected from the hybridization of *P. avium* and *P. cerasus* by Beijing Academy of A&F Sciences.

Fruit sampling: Fruits were sampled at four days of 28 May, 3 June, 11 June, and 24 June, 2015. These four dates were chosen because they fell in the significant fruiting stages. On 28 May, 3 June, 11 June and 24 June, 2015 sweet cherry trees underwent the first fast-growing, the ordinary fruit size, the second fast-growing, and the fully mature periods, respectively.

At each date, four fruits were randomly sampled from four twigs in a scion-rootstock combined tree at four orientations of east, west, south, and north. These four fruits were bulked and averaged after measuring for the mean of the tree. Bulked averages of five trees were assigned as five replicates (n=5).

Paraffin-section production: Fresh mesocarp samples were dived in FAA stationary liquid (90 ml of ethyl alcohol at the concentration of 70%, 5 ml of methanol, and 5 ml of glacial acetic acid) whose volume was two- to three-fold greater than the sample's one. To facilitate the transparency of sliced pieces through dehydration, samples were pre-treated through the following series manipulations: 70% ethanol for 1-2 h, 80% ethanol for 1-2 h, 95% ethanol for 1-2 h, 100% ethanol for 2-3 h, 50% ethanol and 50% xylene for 2 h, 100% xylene for 1 h, and 100% xylene for 1h. Subsequently, samples were coated by 50% paraffin and 50% xylene at 37-42°C for 4 h, cooled at the room temperature, and reserved overnight. In the next day, samples were continuously coated by 70% paraffin and xylene at 60°C for 4h, 100% paraffin at 60°C for 4h, 100% paraffin at 60°C for 4h (overnight at room temperature), and 100% paraffin at 60°C for 4h. Paraffin-coated samples were moved to the self-made box and over-dried at 60°C to shape. Shaped samples coated with griffin were moved to KYD –TK then moved to the room temperature to cool the paraffin. Samples were sliced into pieces at the depth of 8-10 μm with the angle between blade and sample of 20°~30°. Mesocarps were sliced along the length twice to leave the slide with two sides across pit. Sliced sample sheets were firstly moved to water for cooling at room temperature and secondly dredged out by the object slide with one face painted by 1g of gelatin, 100 ml of deionized water, 15 ml of glycerinum. Dredged slides were moved to KYD –TK at 42°C and oven-dried at 30°C overnight.

Measures on fruit cells: Cell morphologies were measured by BH bio-microscope with the visual field at 0.1 mm. Each mesocarp sample was measured for ten views distributed on the whole area of slide. Because most cells were round with length and diameter (d) close to each other (Fig. 1), volume of the cell (V) was calculated by the formulation:

$$V = 4/3 \times \pi \times d^3 \qquad (1)$$

The volume of fruit was also calculated by the formulation (1); hence number of cells can be calculated as the production of fruit volume divided by cell volume.

Statistical analysis: Data were analyzed as a factorial design with the scion and rootstock as two factors. Repeated analysis of variance (ANOVA) was performed at four sampling dates based on the General Linear Model (GLM) procedure to test the significance of effects using SAS (SAS Institute Inc., NC, USA). When any effect about scion or rootstock variations was detected to be significant, means were compared or ranked according to LSD test α=0.05 level. Both Pearson (linear) and Spearman (non-linear) correlations were made between any two of parameters by the CORR procedure in SAS.

Results

All ANOVA results have been shown in Table 1, where the interaction of scion and rootstock was significant as effects on fruit weight, length, and diameter on 28 May, 2015. Both cell length and cell number responded to the interaction on all four days.

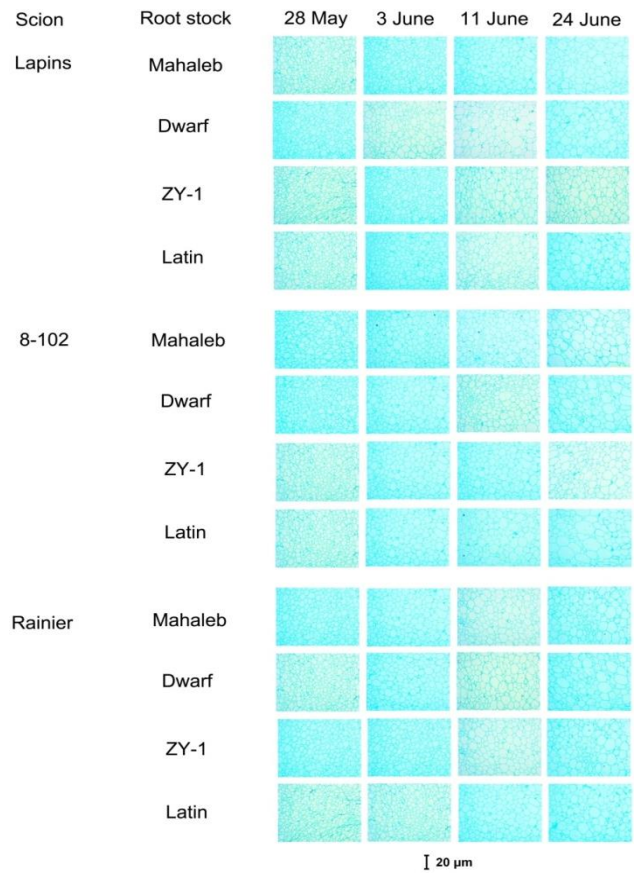

Fig. 1. Cell features in mesocarps of sweet cherry scions of 'Lapins', '8-102', and 'Rainier' planted on rootstocks of 'Mahaleb', 'Dwarf', 'ZY-1', and 'Latin' on 28 May, 3 June, 11 June, and 24 June, 2015.

Fruit weight and growth: On 28 May, 2015 all parameters of fruit weight, length, and diameter were greatest in trees with combined scion of '8-102' × rootstock of 'ZY-1', followed by the combination of '8-102' × 'Dwarf' (Table 2). The scion of 'Lapins' had moderately greater fruit length and diameter. The combination of scion '8-102' × rootstock 'Mahaleb' had the least fruit weight, length, and diameter.

The scion '8-102' had greater fruit weight than the scion 'Lapins' from 3 June to 24 June, 2015 (Fig. 2A). On 3 June, 2015 the scion '8-102' had greater fruit length and diameter than the scion 'Rainier', but on 24 June, 2015 the differences disappeared (Fig. 2B, C). Generally, the rootstock 'Latin' had greater fruit weight and fruit length than 'Dwarf' and 'Mahaleb', but difference of fruit length between 'Latin' and 'Dwarf' disappeared at 24 June, 2015 (Fig. 3A, B). No difference was detected for fruit diameter among rootstocks at 3 June and 24 June, 2015, but 'Latin' and 'Mahaleb' had the greatest and least fruit diameters at 11 June, respectively (Fig. 3C). At final sampling of mature fruits, fruit weight ranged between 6.3~7.5 g, fruit length and diameter ranged between 2.0~2.5 cm.

Cell length and number: Fruit cell length was greatest in the combination of scion 'Lapins' × rootstock 'Latin' from 28 May to 24 June, 2015, but many differences were not significant on 3 June, 2015 (Fig. 4A-D). On 28 May, 2015 the combinations of scion '8-102' × rootstock 'Latin' and 'Rainier' × 'Mahaleb' had the least fruit length, but from 3 June on the least fruit length changed to occur in combinations of scion 'Lapins' × rootstock 'Mahaleb' and '8-102' × 'Mahaleb'. On 28 May, 2015 cell number was greatest in rootstocks 'Dwarf' and 'ZY-1' combined with scion '8-102' and in 'Latin' × 'Rainier' (Fig. 4E-H). From 3 June, 2015 on, cell number in the scion 'Lapins' started to increase. On 24 June, 2015 cell number in rootstock 'Mahaleb' was lower than most other combined scion and rootstocks.

Correlation analysis: Both fruit length and fruit diameter had a significant Spearman correlation with fruit weight from 28 May to 24 June, 2015 (Table 3). Cell number had a significant Pearson correlation with fruit weight on 28 May and 24 June and with fruit length on 3 June, 2015. In addition, on 3 June 2015 cell length had a significant Spearman correlation with fruit weight (n=12, R=0.5990, P=0.0396) and fruit diameter (n=12, R=0.6410, P=0.0247). Therefore, cell number was further regressed with fruit weight and fruit diameter and two non-linear regressions were found (Fig. 5 A, B). On 24 June, 2015 fruit cell number was also found to have a significant Spearman correlation with fruit length (n=12, R=0.6046, P=0.0373) and another non-linear regression was found between them (Fig. 5C).

Discussion

Whiting and his research group reported the crop load of 'Bing' sweet cherry on rootstocks 'Gisela 5' and 'Gisela 6' (*P. cerasus* × *P. canescens*) and found that fruit weight differed among rootstocks (Whiting *et al.*, 2005; Whiting & Ophardt, 2005). These studies emphasized the year-long fruiting performance in scion affected by rootstocks, but were insufficient to supply evidence for the comparison among combinations of multiple scions and rootstocks. Our study complemented the results from former studies at specific fruiting stage and revealed that the scion × rootstock interplay could affect fruits' growth before the pit-hardening. In our study, the fruit length of most of our sweet cherry scions and rootstocks reached the valuable grade of 24 mm. Both heredity and environmental factors affect the setting, growth, and maturity of fruits (Zhang & Whiting, 2011; Okello *et al.*, 2015). However, no difference of fruit length or fruit diameter was detected among scions on 11 June, 2015 when difference was found among rootstocks. These results suggested that at the second fast-growing stage when fruit size was rapidly growing and mainly contributed to by rootstock but not scion. This was probably the result of nutrient and water input into fruits and practices of fertilization and water supply should be considered during this time. Rootstock had no effect on fruit diameter at the first and second fast-growing stages, but rootstock had significant effect at the pit-hardening stage. These results suggested that fruit diameter growth may be related to hardening and affected by nutrient and water supply from rootstock uptake. Specific mechanism needs more work to confirm in future.

Table 1. *P* values from ANOVA analysis effects of sweet cherry scion (C), rootstock (T) and their interaction (S × T) on fruit weight, length and diameter and cell length and cell number in fruits on 28 May, 3 June, 11 June and 24 June, 2015.

Fruit parameters	*df*	28 May	3 June	11 June	24 June
Weight					
C	2	0.5390	**<0.0001**	**0.0266**	**0.0003**
T	3	**0.0002**	**<0.0001**	**0.0008**	**<0.0001**
C × T	6	**0.0001**	0.6553	0.7364	0.9856
Length					
C	2	0.7976	**0.0006**	0.2642	**0.0055**
T	3	**0.0012**	**0.0004**	**0.0011**	**0.0417**
C × T	6	**0.0126**	0.4405	0.6442	0.7781
Diameter					
C	2	0.3762	**0.0044**	0.4340	**0.0024**
T	3	**0.0314**	0.1710	**0.0025**	0.0900
C × T	6	**0.0081**	0.3455	0.2070	0.6462
Cell length					
C	2	0.1969	**0.0176**	0.3459	**0.0066**
T	3	**0.0062**	**<0.0001**	**0.0021**	**<0.0001**
C × T	6	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**
Cell number					
S	2	**0.0016**	**<0.0001**	0.5228	0.4338
T	3	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
S × T	6	**<0.0001**	**0.0022**	**0.0019**	**0.0048**

Table 2. Fruit weight, length and diameter across sweet cherry scions and rootstocks on 28 May, 2015.

Scion and Stock-type	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)
Scion: Lapins			
Mahaleb	3.9 ± 0.54d	2.02 ± 0.1bc	1.95 ± 0.11bc
Dwarf	4.17 ± 0.4cd	2.07 ± 0.08bc	1.97 ± 0.09bc
ZY-1	4.21 ± 0.42bcd	2.14 ± 0.05ab	2.02 ± 0.06ab
Latin	4.3 ± 0.42bcd	2.05 ± 0.14bc	1.95 ± 0.11bc
Scion: 8-102			
Mahaleb	3.2 ± 0.33e	1.84 ± 0.07d	1.81 ± 0.08d
Dwarf	4.79 ± 0.39ab	2.14 ± 0.11ab	2.03 ± 0.1ab
ZY-1	5.09 ± 0.45a	2.21 ± 0.07a	2.12 ± 0.09a
Latin	3.8 ± 0.46d	2 ± 0.14c	1.94 ± 0.12bc
Scion: Rainier			
Mahaleb	4.15 ± 0.29cd	2.07 ± 0.11abc	1.95 ± 0.09bc
Dwarf	4.34 ± 0.41bcd	2.08 ± 0.1abc	1.96 ± 0.07bc
ZY-1	4.19 ± 0.42cd	2.06 ± 0.11bc	1.88 ± 0.08cd
Latin	4.55 ± 0.5abc	2.03 ± 0.07bc	1.95 ± 0.1bc

Our sweet cherry fruit weight at the mature stage (6-7 g) was comparable with the landrace variety of about 6 g but lighter than modern varieties (~14 g) (Franceschi *et al.,* 2013). The fruit weight of 'Bing' sweet cherry on rootstocks 'Gisela 5' and 'Gisela 6' ranged between 5g and 7g (Whiting *et al.,* 2005; Whiting & Ophardt, 2005). Robinson *et al.,* (2006) reported the crop load of 'Hedelfinger' planted on 'Gisela 5', 'Gisela 6', and 'MxM.2' and 'Lapins' and 'Sweetheart' planted on 'Gisela 5' and 'Gisela 6', wherein fruit weight of 'Lapins' reached 8.7 g. However, fruit weight of rootstocks 'Gisela 5' and 'Gisela 6' ranged between 5g and 7g in Whiting *et al.,* (2005) and Whiting & Ophardt (2005). In our study, the fruit weight of 'Lapin' was about 6 g at the maturity stage, which was comparable with Whiting's results but lower than Robinson *et al.,* (2006). Hence, results across studies proved that the combination between scion and rootstock can result in changes of fruit growth even with the same cultivar material.

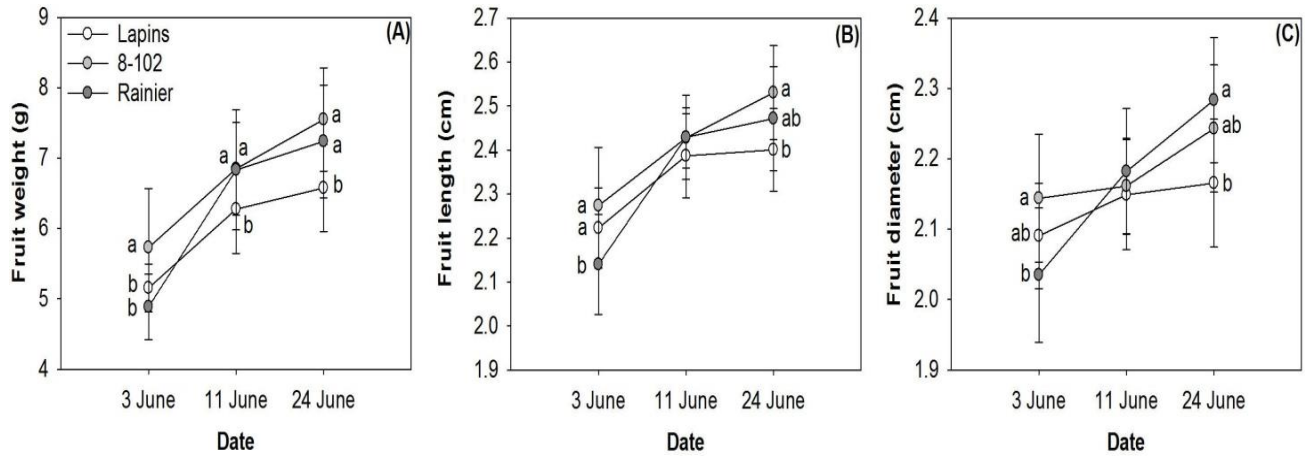

Fig. 2. Differences of fruit weight (A), fruit length (B), and fruit diameter (C) among sweet cherry scions on 3 June, 11 June, and 24 June, 2015. Different letters at a given day indicate significant difference at 0.05 level.

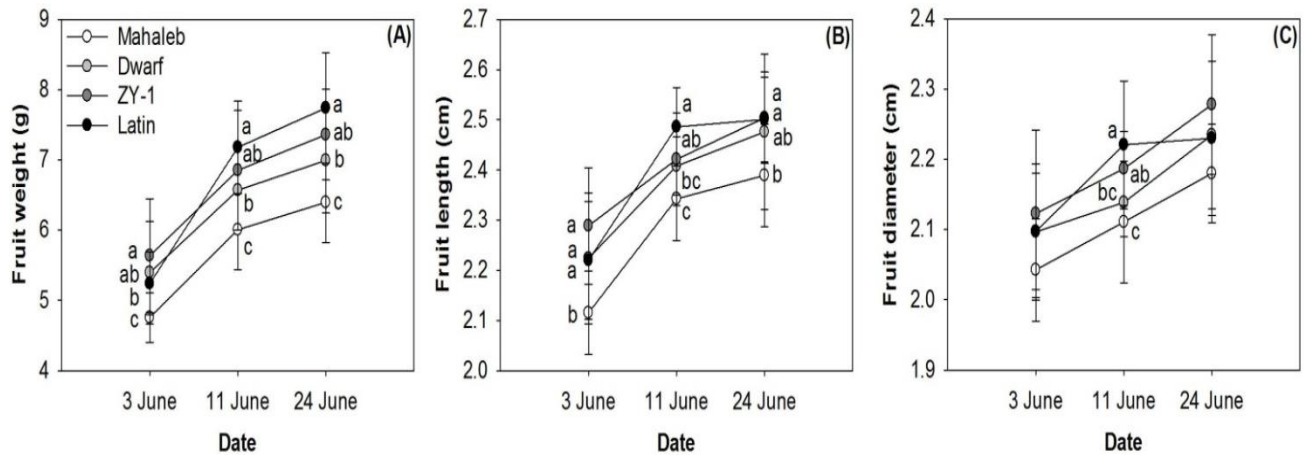

Fig. 3. Differences of fruit weight (A), fruit length (B), and fruit diameter (C) among sweet cherry rootstocks on 3 June, 11 June, and 24 June, 2015. Different letters at a given day indicate significant difference at 0.05 level.

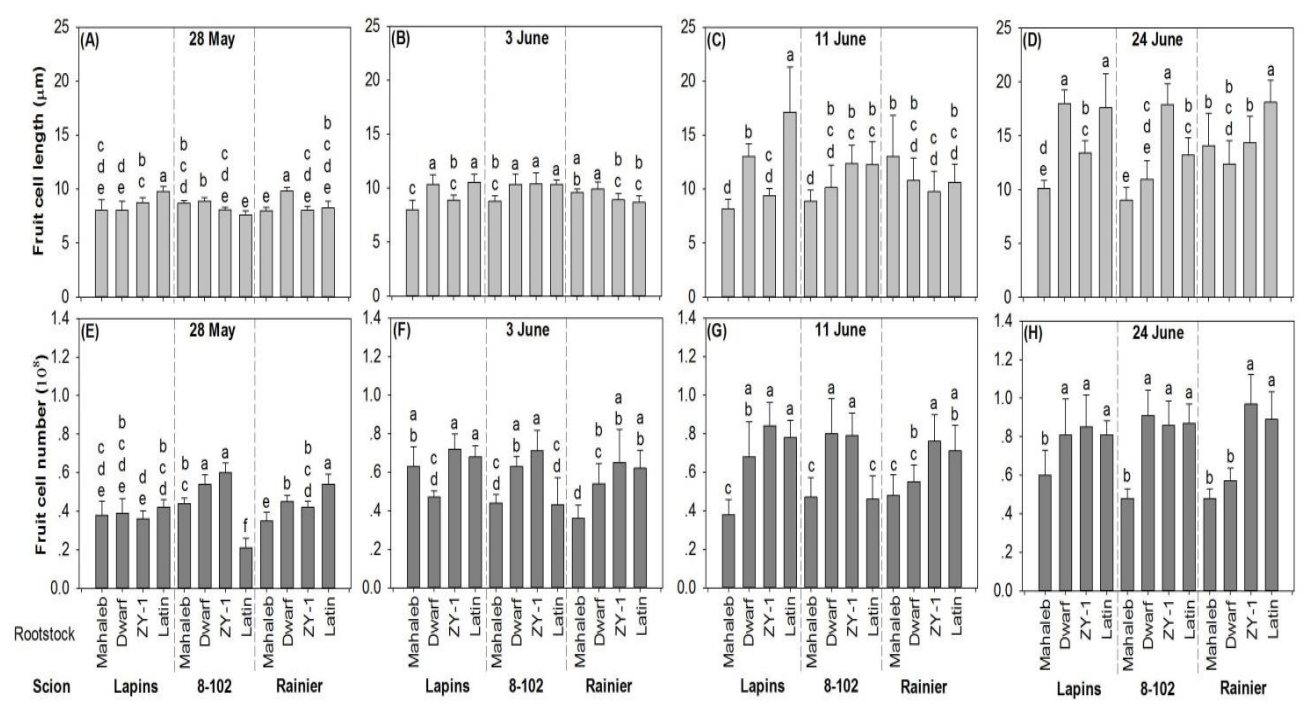

Fig. 4. Differences of cell length (up) and cell number (bottom) in mescarp of fruits from sweet cherry scions × rootstock combinations on 28 May, 3 June, 11 June, and 24 June, 2015. Different letters at a given day indicate significant difference at 0.05 level.

Table 3. Pearson correlations between parameters from fruit weight, fruit length, fruit diameter and cell length and cell number in fruits in sweet cherry cultivars on 28 May, 3 June, 11 June and 24 June, 2015.

	28-May				
	Fruit weight	**Fruit length**	**Fruit diameter**	**Cell length**	**Cell number**
Fruit weight		**R=0.8930**	**R=0.8523**	R=0.1102	**R=0.6527**
		P<0.0001	**P=0.0004**	P=0.7332	**P=0.0214**
Fruit length	**R=0.9573**		**R=0.9088**	R=0.0375	R=0.3708
	P<0.0001		**P<0.0001**	P=0.9079	P=0.2354
Fruit diameter	**R=0.9347**	**R=0.9142**		R=-0.01849	R=0.4035
	P<0.0001	**P<0.0001**		P=0.9545	P=0.1934
Cell length	R=0.4691	R=0.3798	R=0.5400		R=0.2755
	P=0.1239	P=0.2233	P=0.0699		P=0.3862
Cell number	R=0.5587	**R=0.7265**	R=0.4757	R=-0.1114	
	P=0.0590	**P=0.0075**	P=0.1180	P=0.7305	
			3-Jun		
	11-Jun				
	Fruit weight	**Fruit length**	**Fruit diameter**	**Cell length**	**Cell number**
Fruit weight		**R=0.9451**	**R=0.7390**	R=0.2496	R=0.4926
		P<0.0001	**P=0.0060**	P=0.4341	P=0.1037
Fruit length	**R=0.8756**		**R=0.9088**	R=0.2600	R=0.4436
	P=0.0002		**P<0.0001**	P=0.4145	P=0.1486
Fruit diameter	**R=0.6631**	**R=0.7622**		R=-0.2161	R=0.5672
	P=0.0187	**P=0.0040**		P=0.5000	P=0.0545
Cell length	R=0.1676	R=-0.0252	R=-0.1457		R=0.2445
	P=0.6026	P=0.9379	P=0.6513		P=0.4438
Cell number	**R=0.5951**	R=0.5333	R=0.4089	R=0.1336	
	P=0.0394	P=0.0742	P=0.1869	P=0.6790	
			24-Jun		

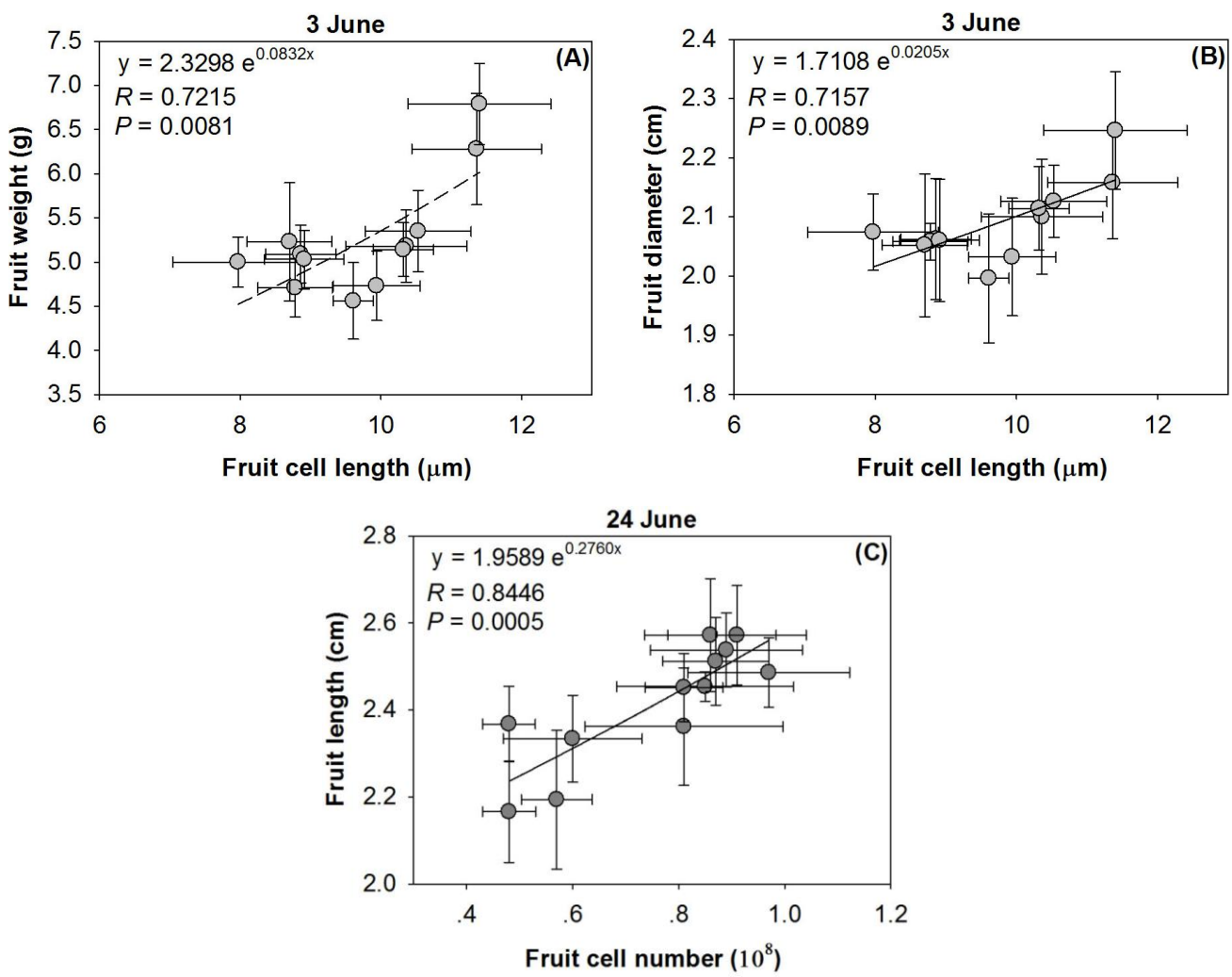

Fig. 5. Non-linear correlations between mesocarp cell length and fruit weight (A) and fruit diameter (B) on 3 June, 2015 and between mesocarp cell number and fruit length (C) on 24 June 2015 in sweet cherry cultivars.

Over-year studies have shown different cell size and cell number in mesocarp of mature fruits in various sweet cherry cultivars (Olmstead *et al.,* 2007; Param & Zoffoli, 2016; Yamaguchi *et al.,* 2004). On 24 June, 2015 cell number was positively correlated with fruit length and weight where as cell length had no relationship with fruit growth during this time. These results highly coincided with Olmstead *et al*., (2007) and partly agreed with Yamaguchi *et al.,* (2004) who also found the positive relationship between cell length and fruit weight. Our correlation results explained the reason of lowest fruit weight and length at the final sampling in the rootstock 'Mahaleb' (Fig. 3A, B), which also had lowest number of mesocarp cells no matter with what scions (Fig. 4H). This correlation occurred throughout the whole fruiting process except at the second fast-growing stage when the correlation between fruit size and fruit weight should have established during the pit-hardening period. The ceases of cell division and expansion at this stage can result by at least three explanations. The first may be that the intercellular space grew without cellular changes; the second may be that cells were filling with carbohydrates and nutrients without expansion; the third may be that fruit growth was mainly derived from pit growth instead of mesocarp growth during this time. Specific reason can only be confirmed when new studies were conducted to measure on cell performance in pit, mesocarp and epiderm meanwhile.

Mesocarp cell length in our results showed positive relationship with fruit diameter and weight at the pit-hardening stage. During this time, cell number was also positively correlated with fruit length and both fruit length and diameter were positively correlated with fruit weight (Table 3). These results together suggested that at pit-hardening stage fruit length grew with cell division and fruit diameter grew with cell expansion and fruit weight was mainly growing with cell size through diameter increment. These results concurred with Yamaguchi *et al*., (2004) although they measured on mature fruits.

Acknowledgments

This study was financially supported by The 12th Five-year Science and Technology Support Project (2013BAD02B03), National Natural Foundation of China (31272123 and 31401827). Workers and staff in the orchard of Institute of Forestry and Pomology, Beijing Academy of Agriculture & Forestry Sciences are greatly acknowledged for their effort to raise sweet cherry trees for this study. Editors and anonymous reviewers are kindly thanked for their work to promote the quality of our article.

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(Received for publication 4 May 2017)