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Utvärdering av transgena grundstammar beträffande deras effekter på frukt kvalitet i äpple

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Contents

SUMMARY

Plant biotechnology has played an important role in plant breeding for introducing desired characteristics into cultivars. This technique is particular important for improving apple cultivars as it can considerably reduce the breeding time. Through genetic engineering, it is possible to change only one gene which is responsible for one desirable character. Apple semi-dwarf rootstock M26 was previously transformed with the *rolB* gene and showed a greatly increased rooting capacity. A field trial was established in Alnarp to evaluate the effects of these *rolB* transgenic M26 and M9 rootstocks on growth and development of non-transgenic apple cultivars grafted onto them. It was especially important to evaluate if the fruit quality was affected by the transgenic rootstocks; because fruit quality is of great concern to the apple consumers, which in turn affect the market value of the variety.

In this report, the fruit quality was analyzed for the cultivars Elise, Elstar and Jonagold grafted on one *rolB* transgenic clone of rootstock M26 and two transgenic clones of M9 with the *rolB* gene, named *rolB*1 and *rolB*2, as well as non-transgenic M26 and M9 as controls. Quality parameters analyzed include fruit size, fruit weight, fruit colour, firmness, acidity (TA), total soluble solids (TSS) and ratio of TSS to TA, vitamin C and total phenols.

The results showed that Elise on M26 and M26 (*rolB*) had greater size than those on transgenic and non-transgenic M9. The amount of acidity of Jonagold on M26 (*rolB*) was significantly higher as compared to M26. M26 (*rolB*) had higher TSS in Elise than non-transgenic M26, M9 and transgenic M9 (*rolB*1) The fruit firmness was significantly higher in M9 and M9 (*rolB*2) both in case of Elise and Elstar than non-transgenic M26, M9 and transgenic M9 (*rolB*1). Ratio of TSS/acidity was higher in M9 (*rolB*1) in variety Elstar than non-transgenic M26, M9 and transgenic M26(*rolB*). There was no significant difference in total phenols either in the peel or the pulp, except in case of M26 (*rolB*), in peel of Elise, which was significantly lower than M26, M9, M9 (*rolB*1) and M9 (*rolB*2). Vitamin C content was significantly lower in M26 than in other transgenic M26 (*rolB*), M9 (*rolB*1) and M9 (*rolB*2) in Elise. The conclusion from this study is that the *rolB* does not seem to affect the quality of fruit negatively; therefore it can be used for improving the rooting ability of apple rootstocks and for increasing the production potential for the plants.

1. INTRODUCTION

1.1 Apple:

Apple (*Malus domestica*) is a pome fruit that belongs to family Rosaceae with 34 chromosomes. Apple is deciduous tree or shrub, rarely with spiny branches, buds ovoid, white to pink or carmine flowers. Since apple was under selection and improvement by humans for thousands of years, it is difficult to know their origin with certainty. The genus consists of about 15 primary species, including 2 from Europe, 4 from North America and the others from Asia. It is the most important fruit crop in the temperate zone, where it is widely spread and cultivated, suggesting its considerable economic role in this region.

During the recent years apple production was increased in every part of the world where conditions are optimum for its production. The total yield reached up to more than 64 million tons during 2009 (FAO, 2010). Apple fruit is very important and popular for its nutritious values as it contains important dietary substances; such as vitamins, saccharides, pectin, minerals and fiber, which make apple a very important source of human nutrition. Apple is also an important raw material for food processing industry for juice, jams, jellies and marmalades (Dvořák et al. 1976).

Most of the production of apple is confined centrally to a small number of varieties. Its great diversity for climatic adaptations has made apple the most widely distributed tree fruit of the temperate zone. Some varieties mature in 70 days, while others take 180 or more days, some are very hardy but others are very tender, some have long chilling requirements and others have less (Westwood, 1991).

Apple breeding is a long term work and labour intensive. The current breeding objectives include fruit quality, high yield, good agronomic performances and durable resistance against apple scab (*Venturia inaequalis*), fire blight (*Erwinia amylovora*) and powdery mildew (*Podosphaera leucotricha*). Traditionally, primitive *Malus* species has been used in apple breeding work as a source of disease, insect resistance and fruit quality. In the last 15 years, combining biotechnology and classical breeding attracted many scientists working in apple breeding. Plant biotechnology can reduce the breeding time by directly introducing desired characteristics into apple cultivars while keeping the main genetic framework unchanged. Through genetic

engineering, it is possible to change only one gene which is responsible for one desirable character. Fruit quality is the most important concern nowadays in apple breeding.

1.1.1 Rootstock:

Fruit trees are propagated commonly on a rootstock, with a scion cultivar on them through grafting. Rootstocks have great influence on fruit production because they can effectively control the tree growth and development (tree size and tolerance against adverse soil conditions) of scions grafted onto them (Drake *et al*., 1988; Fallahi *et al*., 1985; Ferree *et al*., 2001).

Dwarfing rootstocks are used in the whole world for commercial production of apple. As dwarfing rootstocks improve different characteristics including tree size reduction, early bearing, high density planting, easy management and high yield efficiencies. There are a number of dwarfing rootstocks available which are not very good in anchorage or they have not dwarf characteristics. So commercially important rootstocks need to be improved (Zhu and Welander, 1999).

Rootstock, as a part of a fruit tree, strongly influences fruit quality, vigour and productivity. It was reported that rootstocks affect fruit quality in different ways such as ripening and storage ability, mineral composition and firmness (Drake *et al.*, 1988; Marini *et al.*, 2002; Brown and Wolf, 1992). Jonagold grafted on semi-dwarf rootstock M26 showed good orchard performance on light soils and very vigorous response on fertile soils (Skrzynski and Poniedzialek 1999).

Although rootstock is one of the most important abiotic factors influencing the quality of fruit, it's still not very clear about mechanisms behind the rootstock effects (Castle 1995). In practice, different combinations of cultivar and rootstock may have different effects on fruit quality. It is thus difficult to decide which rootstock that is suitable for a given scion cultivar, which will in turn, affect future orchard management and profitability (Skrzynski and Gastol, 2006).

The reports published about the effects of rootstocks on scion cultivars in apple, in field conditions are different and inconsistent. This might be because of other factors affecting the fruit quality like light interception, seed count, inflorescence position, temperature, training system and thinning to evaluate the commercial importance of a rootstock, which were not involved in

the studies (Yahya *et al.*, 2004). Studies showed that more dwarfing rootstocks gave much better light penetration and photosynthetic productivity (Baugher *et al.*, 1994).

M9 rootstock is the most commonly used one in Western Europe and some other countries for commercial production. It is not very winter hardy as the root system can be damaged under very low temperatures (Adam *et al.*, 2002). It is difficult to root from cutting and best method for propagating is through stool layering, which is proved to be very efficient. Nursery men selected different types of M9 for better propagation and rooting ability. There is clearly a need to improve this rootstock for better rooting and this was achieved by genetic engineering (Zhu *et al.*, 2001).

Rootstock M26 was a result of the crossing between M9 and another Malling series M16. Commercial use of this rootstock was started in 1959. This rootstock was found to be more winter hardy and to have better rooting abilities as compared to M9, which makes it even more successful in northern Europe (Ferrea and Carlson, 1987). M26 rootstock has good rooting ability. Without the application of IBA, it has been found that the rooting ability and dwarfism did not meet the commercial level in Western Europe. So it is very important and of commercial interest to make them root better (Zhu *et al.*, 1999).

1.1.2 Fruit Quality:

Fruit quality is one of the most important parameter from consumer's point of view. Kader (1985) defined that quality is a combination of attributes and properties that gave the commodity value to humans for food. Fruit quality is actually a combination of appearance, taste, aroma, fruit firmness, nutritive values, and other features. Consumer's requirements in high quality are increasing and it is becoming more and more challenging for fruit quality improvement, especially regarding fruit appearance, taste, and aroma (Autio *et al.*, 1996). Different cultivars have different parameters regarding fruit quality; for Jonagold, fruit flesh firmness accounts for 50% in its quality measurement (Pladett *et al.*, 1992).

Balanced sugar to acid ratio could provide fresh refreshing taste in apple fruit. Most commercial cultivars, like "Jonagold", have already known minimal values for their acceptability in selling periods. Soluble solids should be between 13-14% and flesh firmness lower than 45N (Hoehn *et al.*, 2001).

Apples vary in chemical composition like many other fruits. This variation is found due to different factors like, variety, environmental conditions, location and agricultural practices. Every plant has its own chemical composition (Lee *et al.*, 2003; Sturum *et al.*, 2003). Phenolic compounds are naturally occurring secondary metabolites in plants which determine the outer and inner quality parameters of apple fruits, including appearance, flavor and health related properties. Phenolic compounds play an important role against the oxidative action of free radicals (Lattanzio, 2003; Treutter *et al.*, 2001). It was reported that apple peel have much more contribution in antioxidant activity than apple pulp and that is why it is more important to eat apple with peel (Eberhardt *et al.*, 2000). Polyphenolic compounds are very useful against development of cancer, coronary heart diseases and they lower the risks of brain and immune disinfection, stroke and other diseases. Hock *et al.* (1988) and Usenik *et al.* (2004) reported that phenolic compounds play an important role in antioxidant expression in plant defense mechanism. The phenolic compounds of a plant tissue can determine the level of susceptibility and tolerance to pests and fungal diseases.

It was reported that rootstocks can affect fruit size, fruit firmness, soluble solids and postharvest characters (Castle, 1995). Goffings and Herregods (1994) suggested that fruits grafted on rootstocks including P and M series should have fruit firmness between 4.7-5.1 Kg/cm^2 as an acceptable range. The best values for sugar and acid ratio were calculated at harvest for P60 and M26 rootstocks, which fully satisfy the consumers demand for fresh consumption owning a balanced sugar to acid ratio (Vangdal, 1985; Sekse, 1992).

Different physiological disorders were noticed in rootstocks M26 and M9 after post harvest phase including bitter pit and breakdown. It was found that different quality parameters including fruit firmness, titratable acidity, total soluble solids and sugar to acid ratio are rootstock dependant (Skyrzynski and Gastol, 2007).

1.1.3 Genetically Modified Crops:

Genetic engineering is very popular nowadays in the modern plant breeding and it is becoming more and more important. Through the use of gene technology, it is possible to change different traits which are not very easy with conventional plant breeding methods (Smolka *et al.*, 2010). Genetic improvement of apple rootstock by crossing and selection is a very slow process due to

apple's long juvenility and heterozygosity. By genetic engineering it's easy to change one desirable character without changing the whole genetic setup (Zhu and Welander, 1999). Transgenic crops are becoming an important part of agricultural landscape in some of the modern countries. Since 1996, genetically modified crops area was increased by 10% per year. This is one of the biggest growth rates for any technology to be adopted (Dunwell, 2004).

ISAAA reported that the production area for GMO was increased up to 140 million hectares in 40 countries in 2010. Most of the cultivated crops are annuals with increased pest and pesticide or herbicide resistance; on the other hand the commercial production of fruit tree is very limited (ISAAA, 2010). The only GM cultivated tree species are poplar, with insect resistance grown in China, and papaya with virus resistance grown in both China and USA (James, 2008).

Although a number of dwarfing rootstocks are available in commercial production, there is no ideal rootstock for apple. It is thus necessary to improve the currently available rootstocks using genetic engineering (Zhu *et al*., 2001, 2003). There are a number of reports about transgenic fruit trees with different improved agronomic traits. However, there are very few field trials on these trees, probably mainly due to a long life cycle of trees and the public concern about GMOs.

For GM fruit trees, Zhu and Walender (1999) found that *rolA* gene reduced the root length in Gravenstein apple grafted on transformed M26 rootstock, which in turn cause the reduction in tree size. In a study for evaluating the effect of transgenic rootstocks in apple under field conditions Smolka *et al.* (2010) suggested that transgenic rootstocks *rolB* modified rootstocks should be used in combination with vigorous scion cultivars to get good yields and growth characteristics. However, there is need to evaluate growth and development of GM fruit trees under field conditions to increase our knowledge about it (Smolka *et al*., 2010).

In a study for increasing the rooting ability of dwarfing pear (*Pyrus communis*) rootstock BP10030, Zhu *et al.* (2003) found that rooting ability was significantly increased by the introduction of *rolB* gene in the rootstock. Rooting percentage and rooting number was increased both *in vitro* and *ex vitro*. On the other hand, it also reduces the length of stem as compared to non-transgenic ones.

1.1.4 *rolB* Gene:

In a previous investigation the *rolB* gene was isolated from *Agrobacterium rhizogenes* which usually causes hairy root disease in dicotyledonous plants after infection in nature (White *et al.*, 1985; Spena *et al.*, 1987). It was reported that the *rolB* gene can improve rooting in different plant species (Capone *et al.*, 1989; Zhu *et al.*, 2001). The *Agrobacterium rhizogenes* contains four rooting loci (rol), named as *rolA*, *rolB*, *rolC* and *rolD* on its T-DNA and these genes are responsible for changes in different characteristics of plants upon infection. The *rolA* gene was found to be associated with shorter internodes, and the *rolB* and *rolC* genes are responsible for improving the rooting ability of the plant. So it is important to use the *rolB* and *rolC* genes to improve rooting ability of vigorous apple rootstocks and the *rolA* gene to give them more dwarfs characteristics (White *et al.*, 1985: Cardarelli *et al.*, 1987).

1.2 Objective:

The objective of this experiment was to study the fruit quality of non-transgenic apple scion cultivars on the transgenic rootstocks M26 and M9, transformed with *rolB* gene, in comparison with the non-transgenic controls.

2. MATERIALS AND METHODS:

2.1 *Materials:*

Three cultivars of non-transgenic apple, Elise, Elstar and Jonagold grafted onto the transgenic rootstocks of M26 (M2), non-transgenic M26 (M1), non-transgenic M9 (M3) and transgenic M9 (M4, M5) were used in this study. The trees were planted in the field trial at SLU, Alnarp in Southern Sweden in 2004. The fruits were harvested in September 2009, wrapped in paper bags and placed in storage room at 4 ℃ for five weeks. In December the fruits were cut into pieces and put in plastic bags and stored in -80℃ until 6 April 2010.

Combinations of selected varieties with transgenic and non-transgenic rootstocks

Variety/Treatments	**Non-Transgenic**	**Transgenic**	**Non-Transgenic**	**Transgenic**	**Transgenic**
Elise	M26	M26 (rolB)	M9	M9 (rolB1)	M9 (rolB2)
Elstar	M26	M26 (rolB)	M9	M9 (rolB1)	M9 (rolB2)
Jonagold	M26	M26 (rolB)			

2.2 *Methods:*

For the analysis of the weight, size, colour and firmness, twenty fruits were chosen from each combination. For the chemical analysis twenty apples were selected from each combination and divided into five samples of four apples each. Every apple was cut into two pieces and then every half was cut into three pieces. Aliquots of samples for the different analyses were made by randomizing the pieces. The samples for vitamin C and phenols were placed in -80 °C, while the samples for sugar and acidity were placed in -50 °C.

2.2.1 Weight and size

Twenty apples from each combination were weighed separately. The size of the apples was measured one by one at the largest diameter.

2.2.2 Color

The colour of the apples was measured by using Minolta Chroma Meter CR-200 (Konica Minolta Japan) according to user manual. The results were presented as Hue angle, which is determined as $H = \tan - 1\ (b/a)$ where *a* represents chromaticity on a green (-) to red (+) axis, and b represents chromaticity on a blue (-) to yellow (+) axis, whereas H ranges from 0 (red colour) to 90 (yellow colour).

2.2.3 Firmness

Firmness was measured by Penetrometer FT-327 (Italy), with a piston size of 11.1 mm. The tension was measured from two sides of the apple, one from the sunny side (mostly red) and one from the opposite side (mostly yellow). The peel was removed from both locations before penetrating the piston.

2.2.4 Total soluble solids

The total sugar was estimated by measuring the total soluble solids (TSS). The analysis of TSS (Total soluble solids) was carried out by Precision Digital Refractometer 80 RFM (England). The samples were prepared and stored in Eppendorf tubes at -20 °C. For the measurement of TSS, one drop of juice was placed on the lens of the Refractometer, which gave the approximate value of the total soluble solids in the samples.

2.2.5 Acidity

Juice for the measurement of acidity was prepared by mixing the pulp with the same amount of distilled water in a mini mixer. The homogenized mixture was centrifuged at 4 °C for 15 minutes at 10,000 rpm. The supernatant was removed and put in empty tubes. From the clear juice, 10 ml was collected, which was diluted with 20 ml of distilled water. The titrations were performed under the magnetic stirring until the pH value of 8.1, with 1M NaOH as titrant by using Metrohm 691 pH Meter (Metrohm, Switzerland). Titration was calculated and used for the calculation of acidity.

2.2.5.1 Calculation of acidity

Acidity was expressed in mg/100g of fresh weight. The formula used for the calculation of acid was C=a*t*c*100*67.05/j*p, where a is volume of the titrant, t is total weight of the homogenized mixture with water, j is the volume of the fruit juice used for the titration, p is the fresh weight of the sample and 67.05 is a constant for malic acid.

2.2.6 Sugar/acidity ratio

The relationship between sugar and acid was calculated as the ratio of TSS and acid content expressed as titratable acid (TA) as a percentage.

2.2.7 Vitamin C

The vitamin C content was analyzed in the form of L-ascorbic acid by using HPLC. 5 g of fresh weight was homogenized with 25 ml 1.5% metaphosphoric acid; with an Ultra-Turrax homogenizer (T8, IKA-Werke, Germany) was used. Then the homogenized mixture was centrifuged at 13000 rpm for 10 minutes at 4°C. 500 µl of supernatant was added to an Eppendorf tube and 600 µl of 7 mM of DDT was added to reduce the dehydroascorbic acid to ascorbic acid. The sample was centrifuged for 5 minutes at 10000 rpm after 30 minutes of reaction. Supernatant of 600 µl was transferred to a HPLC vial for analysis with the help of LaChrome Merck Hitachi (Burladingen, Germany) with software of D-7000 HSM HPLC, Germany and the column Phenomenex Synergi 4u polar RP. The mobile phase contained 20 mM KH_2PO_4 buffer and 4% methanol with a pH of 2.3 adjusted by H_3PO_4. Five micro liters were injected; detection was carried out at 280 nm with a flow rate of 1ml/min. The whole process was performed under green light conditions to minimize the degradation of vitamin C.

2.2.7.1 Standard curve for ascorbic acid

Three different concentrations of ascorbic acid were prepared (82.53µg/µl, 374.15 µg/µl and 740.264 µg/µl). The samples were run in HPLC with the injection volume of 5 µl, and the standard curve was drawn by plotting the integration area in Microsoft Excel.

2.2.7.2 Calculation of vitamin C

The amount of vitamin C content was measured in the form of ascorbic acid, which was calculated by the integration area from HPLC analysis compared with the standard curve values using the formula y=1329.7x, where y is the integration area, this formula was obtained from the calculation of standard curve. The amount of ascorbic acid was obtained using the formula, then ascorbic acid in ng/100g was calculated by the formula c=100*(x/dry matter content) and finally to get the ascorbic acid value in mg/100g fresh weight by dividing the above value by 1000000.

2.2.8 Phenols

2.2.8.1 Peel and pulp

Total phenols were analyzed separately in peel and pulp by using a modified Folin-Ciocalteu colorimetric method (Dewanto *et al*. 2002). Samples of peel and pulp were chopped separately and extracted in 1.5 ml 50% ethanol for 10 minutes. Then the samples were centrifuged at 13000 rpm for 15 minutes. In 1 ml cuvette, 63 µl of supernatant, together with 250 µl of water and 63 µl of Folin-Ciocalteu agent were added. After leaving the samples for 6 minutes for reaction, 625 µl of 7% Na_2CO_3 was added to raise the pH for phenols to be oxidized to phenolates (Dewanto *et al*., 2002). Samples were covered with parafilm and allowed to stand for 75 minutes. Then the samples were measured at 765 nm with Shimadzu Recording Spectrophotometer UV-240 Grapicord (Shimadzu, Japan).

2.2.8.2 Standard curve for Gallic acid

A standard solution was made by adding 1mg of gallic acid in 1ml of 50% ethanol. Different concentrations of 0%, 5%, 10%, 20%, 40% and 60% gallic acid in 50% ethanol were prepared. The standards were treated in the same way as the samples and measured on 765 nm.

2.2.8.3 Calculation of total phenols

The amount of total phenols was calculated by comparing the absorbance of the samples with the standard curve using the formula y=0.0247, where x is concentration of the gallic acid in percentage and this formula was obtained from the standard curve drawn from the values of the standards used. The amount of total phenols was calculated by formula c = x/100 * v / m, where

x is concentration of gallic acid in percentage and v is extraction volume in ethanol and m is fresh weight of sample in gram.

2.2.9 Statistical Analysis

The data was analyzed in SAS/STAT® Software 7.0 with Analysis of Variance (ANOVA) with a significance of p= 0.05 by Duncan's Multiple Range Test to determine the significance among the samples. Standard curves for vitamin C and phenols were drawn by regression lines in Microsoft Excel 2007.

3. RESULTS

3.1 Fresh fruit weight

3.1.1 *Elise:*

In this cultivar fruit weight in non-transgenic M26 and transgenic M26 (*rolB*) was not significantly different, whereas fruit weight was significantly higher for M26 and M26 (*rolB*) than M9, M9 (*rolB*1) and M9 (*rolB*2). Non-transgenic M9 and transgenic M9 (*rolB*1) were not significantly different from each other, but they showed significantly higher fruit weight as compared to M9 (*rolB*2). (Figure 1)

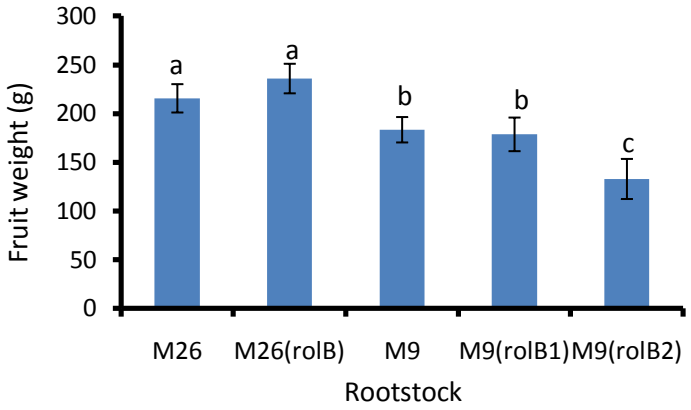

Figure 1: The fresh weight of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.1.2 *Elstar:*

In Elstar, non-transgenic M26 showed significantly higher fruit weight as compared to all other combinations. M26 (*rolB*) and M9 were not significantly different in fruit weight, but they had significantly higher weight than M9 (*rolB*1) and M9 (*rolB*2). M9 (*rolB*1) was significantly higher in fruit weight than M9 (*rolB*2). (Figure 2)

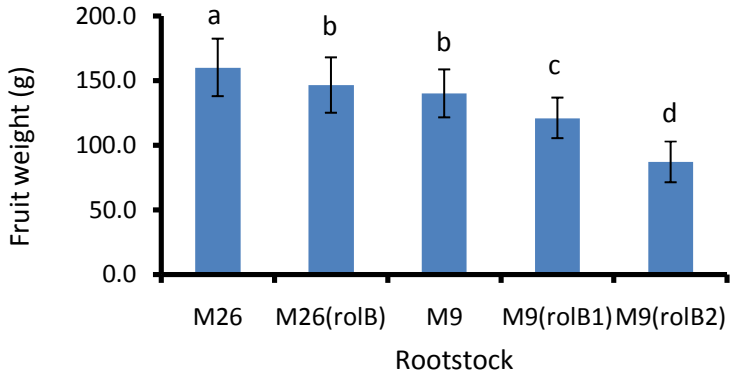

Figure 2: The fresh weight of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.1.3 *Jonagold:*

There was no difference in fruit weight between the non-transgenic M26 and transgenic M26 (*rolB*) rootstock in case of Jonagold. (Figure 3)

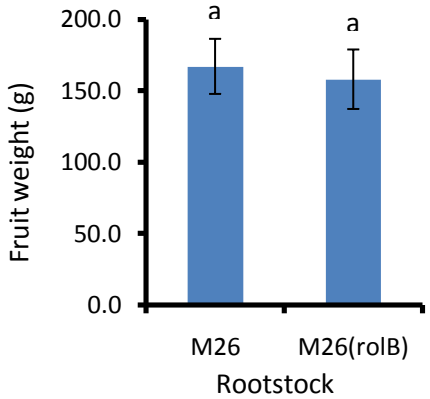

Figure 3: The average fruit weight of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letter are not significantly different at p= 0.05.

3.2 Fruit Diameter:

3.2.1 *Elise*

Fruit diameter was not significantly different in M26 and M26 (*rolB*), however they were found to be significantly different from the all other combinations. M9 and M9 (*rolB*1) were also not significantly different from each other but they were significantly larger in diameter as compared to M9 (*rolB*2). M9 (*rolB*2) was significantly different from other combinations having a smallest diameter. (Figure 4)

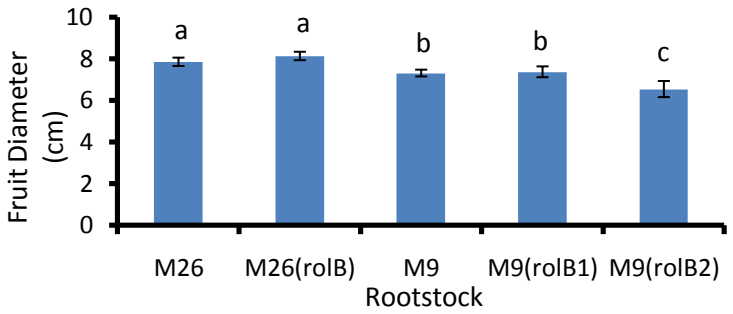

Figure 4: The diameter of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.2.2 Elstar

In Elstar, M26 (*rolB*) have shown significant difference from M9 (*rolB*2), but it was not significantly different from M26, M9 and M9 (*rolB*1). Same in the case for M9 (*rolB*2)(Figure 5).

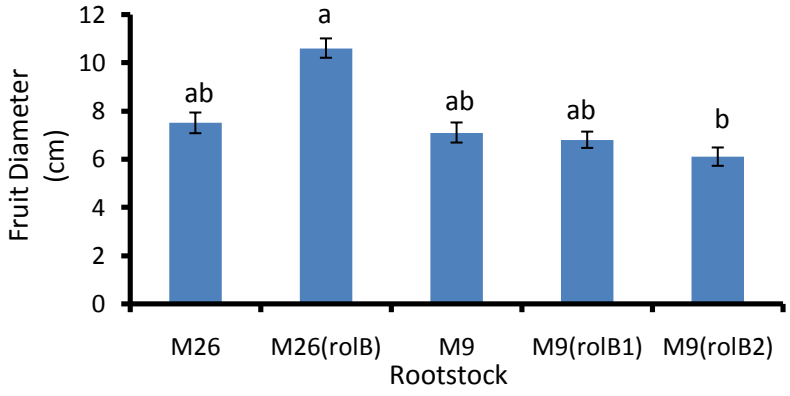

Figure 5: The diameter of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.2.3 *Jonagold*

There was no significant difference in fruit diameter between M26 and M26 (*rolB*) in case of Jonagold. (Figure 6)

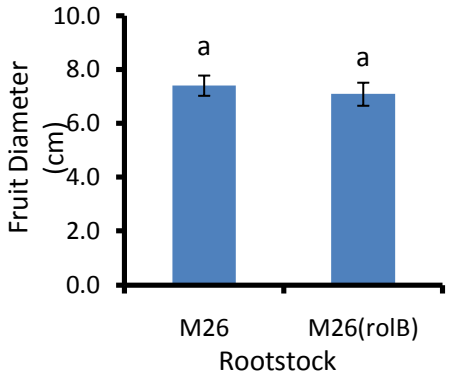

Figure 6: The diameter of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letter are not significantly different at p= 0.05.

3.3 Fruit Colour

3.3.1 *Elise*

In Elise, M9 (*rolB*1) is significantly different from M26 and M26 (*rolB*), but was not significantly different from M9 and M9 (*rolB*2), whereas M26 and M26 (*rolB*) were not significantly different from each other. (Figure 7)

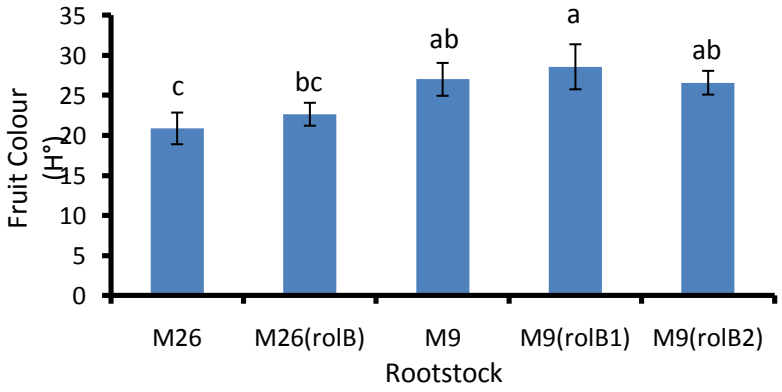

Figure 7: The colour of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.3.2 *Elstar*

In Elstar, M26 was significantly different from M26 (*rolB*), M9 (*rolB*1) and M9 (*rolB*2). There was no significant difference among M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) and same was the case between M26 and M9 (Figure 8)

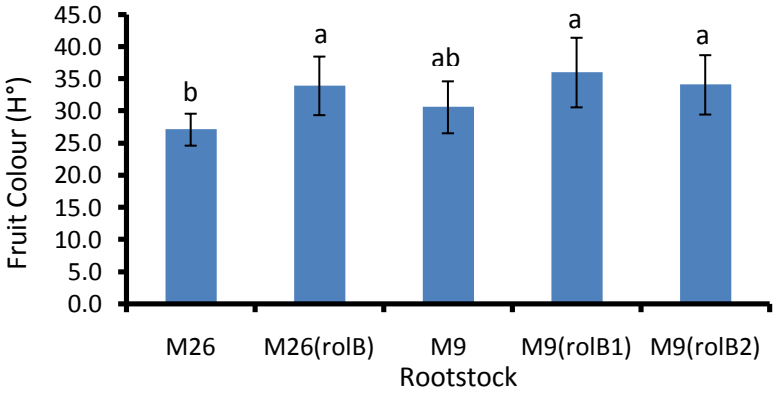

Figure 8: The colour of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.3.3 *Jonagold*

There was no significant difference in fruit colour between M26 and M26 (*rolB*). (Figure 9)

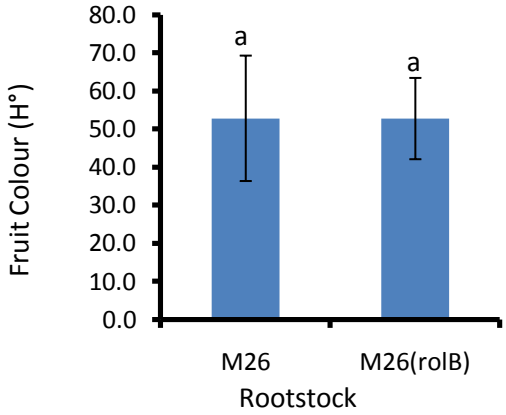

Figure 9: The colour of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letter are not significantly different at p= 0.05.

3.4 Fruit Firmness

3.4.1 *Elise*

In case of fruit firmness, M9 and M9 (*rolB*2) showed no significant difference between each other, but they were significantly more firm as compared to all other combinations. M26, M26 (*rolB*) and M9 (*rolB*1) showed no significant difference between each other. (Figure 10)

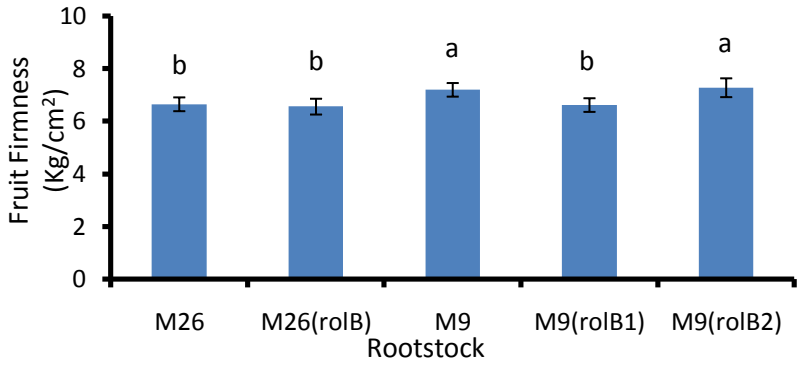

Figure 10: The firmness of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.4.2 *Elstar*

M26 (*rolB*), M9 and M9 (*rolB*2) showed no significant difference between each other in case of fruit firmness, but they were significantly different from other combinations. M26 and M9 (*rolB*1) were not significantly different from each other. (Figure 11)

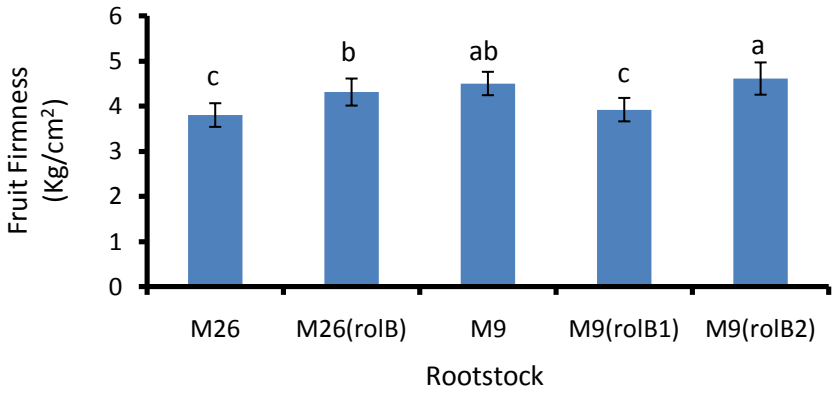

Figure 11: The firmness of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.4.3 *Jonagold*

There was no significant difference in fruit firmness between M26 and M26 (*rolB*). (Figure 12)

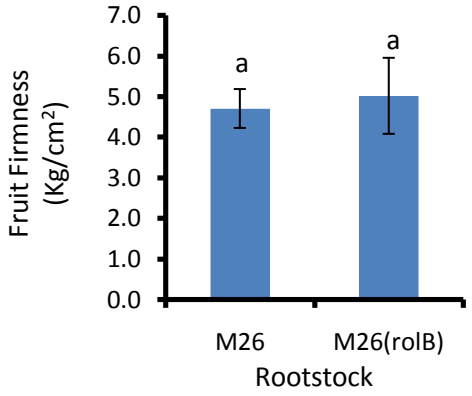

Figure 12: The firmness of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letters are not significantly different at p= 0.05.

3.5 Acidity

3.5.1 *Elise*

In case of acidity, M26 was significantly different from M9 and M9 (*rolB*1), but it was not significantly different from M26 (*rolB*) and M9 (*rolB*2). (Figure 13)

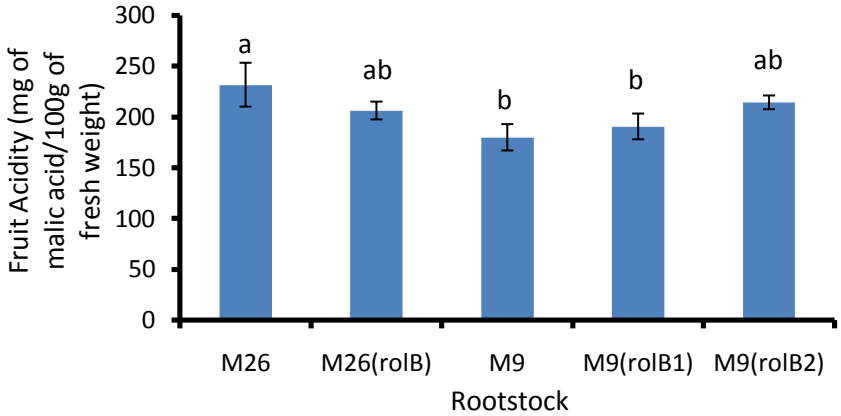

Figure 13: The acidity of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.5.2 *Elstar*

There was no significant difference in acidity between all transgenic and non-transgenic combinations. (Figure 14)

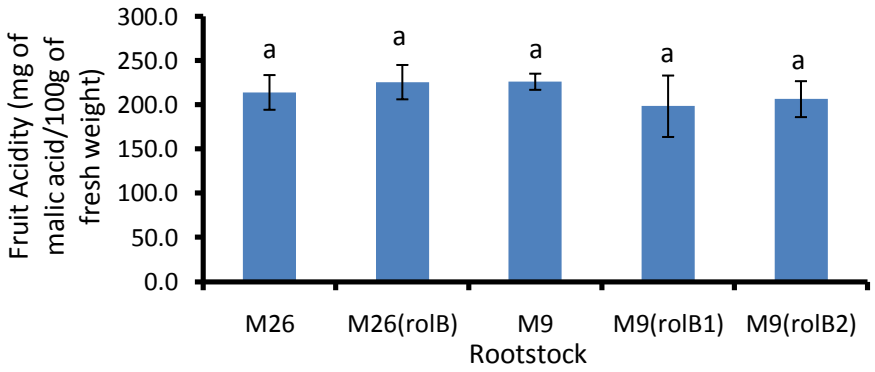

Figure 14: The acidity of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.5.3 *Jonagold*

In Jonagold M26 (*rolB*) was significantly higher than M26. (Figure 15)

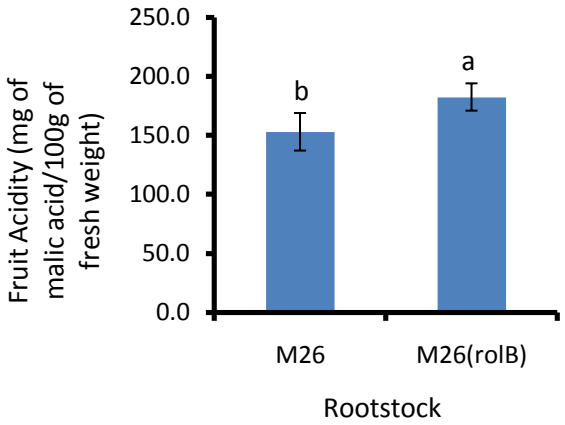

Figure 15: The acidity of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letter are not significantly different at p= 0.05.

3.6 Total sugar

3.6.1 *Elise*

In Elise, M26 (*rolB*) was significantly different than M26, M9 and M9 (*rolB*1). M26 and M9 (rolB2) were not significantly different from each other. Whereas M26, M9 and M9 (*rolB*1) were not significantly different from each other. (Figure 16)

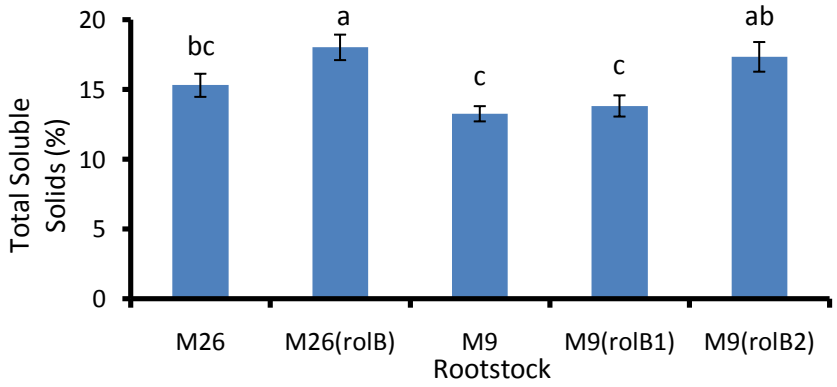

Figure 16: The total sugar of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.6.2 *Elstar*

There was no significant difference between all the combinations in Elstar in case of TSS. (Figure 17)

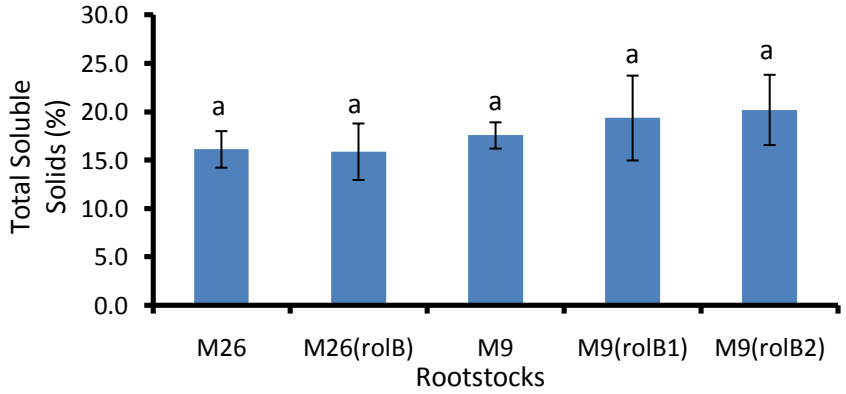

Figure 17: The acidity of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.6.3 *Jonagold*

There was no significant difference between M26 and M26 (*rolB*) in case of TSS. (Figure 18)

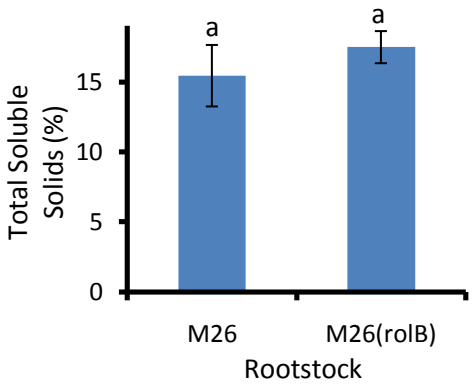

Figure 18: The acidity of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letter are not significantly different at p= 0.05.

3.7 Sugars/Acidity Ratio

3.7.1 *Elise*:

In Elise, M26 (*rolB*) was significantly different from M26. But there was no significant difference between M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2). (Figure 19)

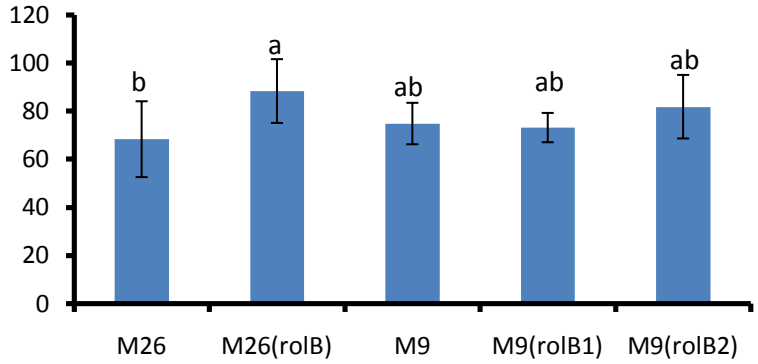

Figure 19: The sugar/acidity ratio of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.7.2 *Elstar*:

There was significant difference between both M26 and M9 combinations. M9 (*rolB*1) was significantly different than M26, M26 (rolB) and M9 but it was not significantly different from M9 (rolB2). M26, M26 (*rolB*) and M9 were not significantly different. (Figure 20)

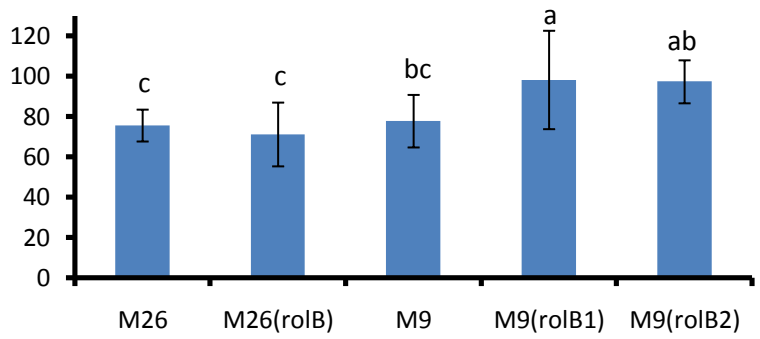

Figure 20: The sugar/acidity ratio of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.8 Vitamin C

3.8.1 *Elise:*

In case of vitamin C non-transgenic M9 was significantly different from M26. On the other hand M9 was significantly different from M26 (*rolB*) and M9 (*rolB*2). (Figure 21)

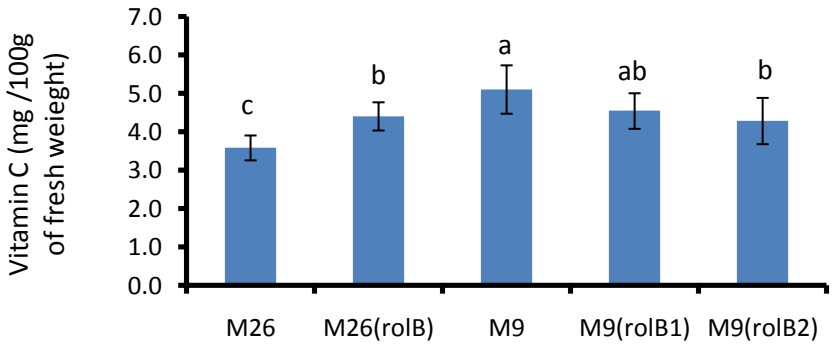

Figure 21: The vitamin C value of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.8.2 *Elstar*

The M26 (*rolB*), M9 and M9 (*rolB*1) was significantly higher in vitamin C content than M26. There was no significant difference between M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2). (Figure 22)

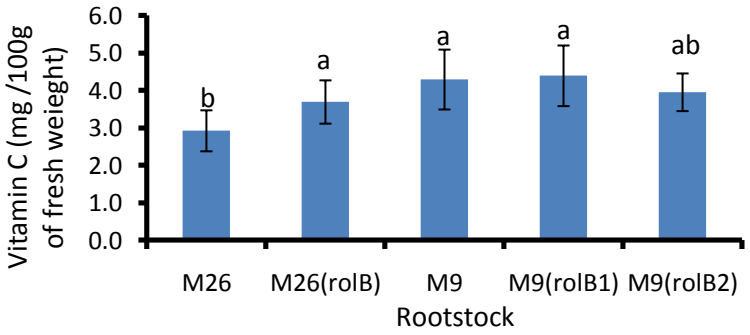

Figure 22: The vitamin C value of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.9 Total phenols in Pulp

3.9.1 *Elise*

There was no significant difference in amount of total phenols in all the combinations of Elise. (Figure 23)

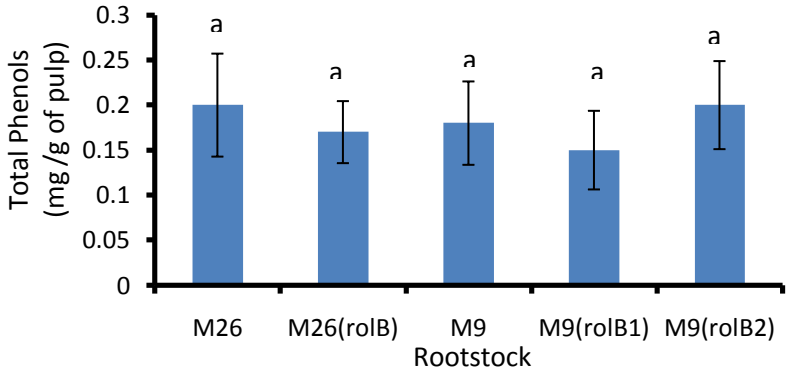

Figure 23: The total phenols in the pulp of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.9.2 *Elstar*

There was no significant difference between all the combinations in case of total phenols in the pulp. (Figure 24)

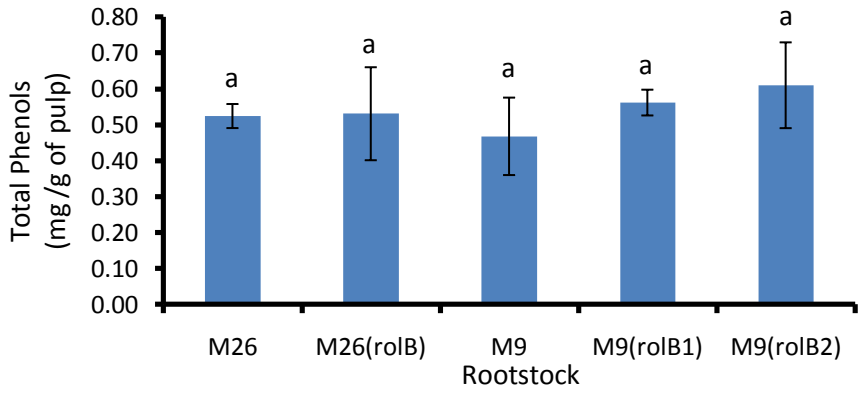

Figure 24: The total phenols in the pulp of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.9.3 *Jonagold*

There was no significant difference between both the combinations in case of total phenols in pulp. (Figure 25)

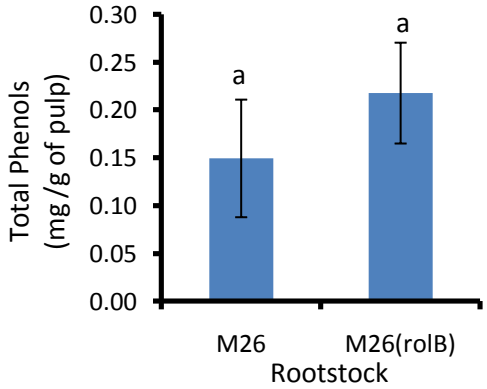

Figure 25: The total phenols in the pulp of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letter are not significantly different at p= 0.05.

3.9.2 Total Phenols in Peel

3.9.2.1 *Elise*

M26, M9, M9 (*rolB*1) and M9 (*rolB*2) were significantly higher than M26 (*rolB*). On the other hand M26, M9, M9 (rolB1) and M9 (rolB2) were not different from each other. (Figure 26)

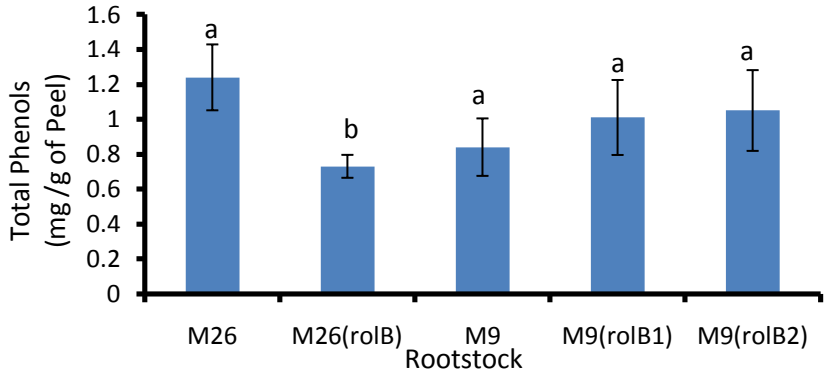

Figure 26: The total phenols in the peel of "Elise" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.9.2.1 *Elstar*

M9 and M9 (*rolB*2) were significantly higher than M9 (rolB1), but they were not significantly different from each other. M26, M26 (*rolB*) and M9 (*rolB*1) were not significantly different from each other. (Figure 27)

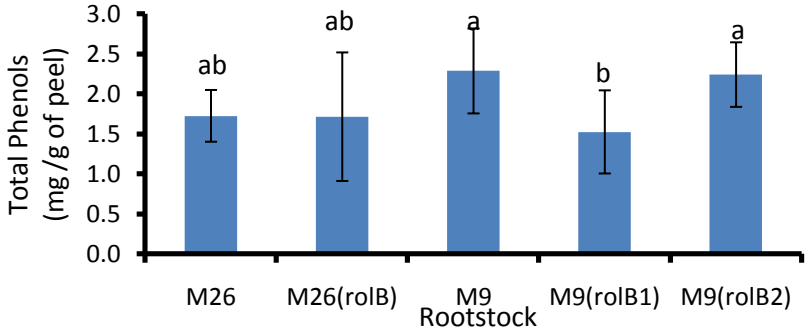

Figure 27: The total phenols in the peel of "Elstar" fruits grafted on M26, M26 (*rolB*), M9, M9 (*rolB*1) and M9 (*rolB*2) respectively. Columns with the same letter are not significantly different at p= 0.05.

3.9.2.3 *Jonagold*

There was no significant difference between both combinations for total phenols in peel. (Figure 28)

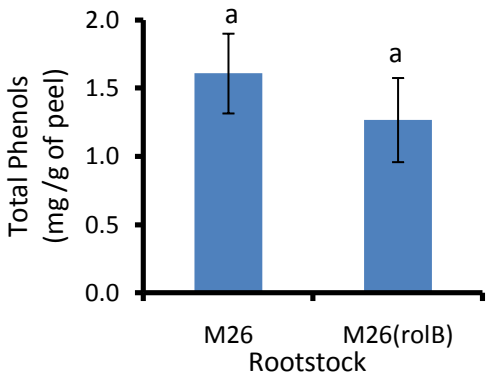

Figure 28: The total phenols in the peel of "Jonagold" fruits grafted on M26 and M26 (*rolB*). Columns with the same letter are not significantly different at p= 0.05.

4. DISCUSSION

4.1 Weight and size:

For the cultivar Elise, the results showed no significant difference in fruit weight in transgenic and non-transgenic rootstocks with exception of M9 (*rolB2*) which had lower values. Fruit size followed the same pattern as fruit weight. M26 and M26 (*rolB*) showed much higher weight of 235.75 g and larger size (8.13 cm) than M9 and M9 transgenic clones, *rolB*1 and *rolB*2 (Fig. 1 and 4). In a previous study, Banach and Gastol (2006) stated that M9 rootstock showed heavier fruit than M26 in case of cv. Jonica, which is in contrast with this study. In our case, it is possible that M26 (*rolB*) had more vigorous growth than M9 which can positively affect fruit development. However, in this study, we also found that the cultivar Elstar on non-transgenic M26 showed heavier fruit weight (Fig.2) than transgenic M26 (*rolB*), whereas on non-transgenic M9, Elstar showed heavier fruit weight than M9 (*rolB*1) and M9 (*rolB*2). This result was in accordance with Banach and Gastol's (2006) report. In another study, Jonica apple grafted on M26 and M9 showed heavier fruit (Skrzynski and Gastol, 2006). Palmer *et al.* (1997) found that heavy crop load does affect the fruit size usually resulting in smaller fruits. It was also reported that M26 showed better fruit weight and large fruit size than B.9 and Mark (Autio., 1991; Barden, Marini, 2001). In our study, fruit weight of Jonagold had not shown any significant difference between non-transgenic M26 and transgenic M26 (*rolB*), however, a previous study showed that transgenic M26 (*rolB*) does affect the fruit weight and size (Catrin, 2009). These results suggest that cultivar and rootstock combination, fruit load and other environmental conditions affect fresh fruit weight. It is likely that such a change is not related to the presence of the *rolB* gene.

4.2 Colour:

In case of Elise, M9 (rolB1) was significantly different than M26 and M26 (*rolB*) (Fig.7). The poorest colouring was found in rootstock M9 (*rolB*1). Smolka *et al.* (2010) was found that M26 showed the reddest fruits compared to all other transgenic clones whereas M26 (*rolB*2) had the poorest coloured fruits. It was revealed that vigorous rootstocks gave much better red colour as compared to week and non-vigorous rootstocks (Kvikliene and Kviklys, 2006). Pätzold and Fisher (1991), Baab, (1998) was found that M9 favored better fruit colouring than M26 and P22, which is contradictory with our studies.

Elstar follows the same pattern as Elise; M26 was more reddish in colour than any other transgenic combinations (Fig. 8). Our results was confirmed by Smolka *et al.* (2010), who found that both non-transgenic rootstocks showed better red colour than transgenic ones. The results for Jonagold revealed that there was no significant difference in fruit colour between the transgenic and non-transgenic rootstocks. The colour of the apple may depend on the location of the apple on the crown where the fruit is situated. Changes in colour may also depend on the temperature and environmental conditions (Inglesias *et al.,* 2002; Magdic & Dobricevic, 2007).

4.3 Firmness:

The firmness was changed in all three varieties grafted onto the transgenic rootstocks compared to the control rootstocks. In Elise, M9 and M9 (*rolB*2) showed much firmer fruit (Fig 10), whereas M26, M26 (*rolB*) and M9 (*rolB*1) showed a lowest value in fruit firmness, which confirms the results of Drake *et al*. (1991) and Autio, (1994). In contrast, Johnson (1992) revealed that rootstock and flesh firmness have negative correlation, fruit size and flesh firmness, the smallest the size of fruit is, the highest the flesh firmness at harvest. In a study Tomala and Sloinska (2006) found that fruit mass is covariate for the firmness in Elise fruit at harvest which confirm the statement of Johnson that rootstock does affect the firmness resulting in firmer fruit.

In our case, Elstar M26 (*rolB*) M9 and M9 (*rolB*2) showed much firmer fruit than M26 and M9 (*rolB*1) (Fig. 11). M9 was the second best in flesh firmness reported by Skyrzynski and Gastol (2006) that showed the optimum value of firmness (4.5 kg/cm) for the consumer acceptance. Apple softens during different phases; once the softening was started it is difficult to stop it rather than to slow down the process by controlled atmospheric conditions for long term storage. Different harvest and postharvest factors influence the process of softening like the cellular mechanisms which regulate the onset of softening (Johnston *et al.,* 2002).

The fruit firmness in Jonagold onM26 and M26 (*rolB*) haven't shown any significant difference (Fig. 12). Skrzynski and Gastol (2006) reported that M26 showed much lower values of flesh firmness in 'Jonica' both before and after storage. In another study, Catrin (2009) had not found any significant difference among transgenic and non-transgenic rootstocks in case of Jonagold.

4.4 Titratable acidity:

In Elise, neither transgenic M26 nor M26 (*rolB*) and M9 (rolB2) showed any significant difference in titratable acidity (Fig. 13). The M9 and transgenic M9 (*rolB*1) were non-significantly different. However, Smolka *et al*. (2010) also didn't find any significant differences between transgenic and non-transgenic rootstocks in most of the cases. In Elstar there was no significant effect of rootstocks on titratable acidity (Fig. 14). In Jonagold, rate of acidity was higher in case of M26 (*rolB*), which was in contrast with the results of Catrin, (2009). Skrzyński, (2007b) reported that rootstock does not affect the value of titratable acidity. In another study Barritt *et al.* (1997) also reported that. It is possible that different conditions other than the rootstock may affect the value of acidity, including storage period, growing season and soil conditions. So the amount of acid can differ in both transgenic and non-transgenic as well as in M26 and M9, but the value does depend on the variety (Catrin, 2009).

4.5 Total sugars:

For the cultivar Elise, the analysis for TSS, M26 (*rolB*) and M9 (*rolB*2) showed the highest values than non-transgenic controls and M9 (*rolB*1) (Fig.16). TSS was increased for the transgenic rootstocks compared to the controls. This was in contrast with results of Smolka *et al*. (2010), where no significant difference was found for TSS. Tomala *et al.* (2006) found that rootstocks affect the concentration of TSS and they had got higher values of TSS both in M9 and M26, but there was variation among the values during successive years. TSS can also be affected by long storage period, which could be the case in this study.

In 'Elstar' there was no significant variation among the transgenic and non-transgenic combinations (Fig.17). This is not in line with the results of Smolka *et al*. (2010), probably for a similar reason as stated for Elise. There was no significant difference between transgenic M26 (*rolB*) and non-transgenic M26. Autio *et al*. (1996) found that rootstocks of P series tend to produce more TSS than M26 and M9 and they also claimed that the soluble solid contents were negatively correlated to stem cross sectional area.

For Jonagold, there was no significant difference between transgenic and non-transgenic rootstocks (Fig. 18). Smolka *et al*. (2010) and Catrin (2009) had also reported a similar result.

4.6 Ratio of TSS/TA

Transgenic M26 (*rolB*) showed higher ratio than non-transgenic M26, while no difference was found in M9 in Elise (Fig. 19). In Elstar M9 (*rolB*1) higher than M9 (Fig.20).

The ratio of TSS/TA is an important factor for determining the quality of apple. In this study almost all the combinations showed the higher values of ratio between TSS and TA in Elise than the previous studies, which can be possibly be due to longer period of storage, so that more organic acids were converted to sugars (Vangdal, 1985). In another study Catrin (2009) found that this ratio was higher in case of M9, M9 (*rolB*1) and M9 (*rolB*2) for Jonagold, we have got almost the same results in Elstar (Fig.20).

Vangdal (1985) revealed in a study that the higher value for sugar to acid ratio was obtained in rootstocks including M and P series. He also suggested that the rootstock from M series satisfies the expectations of the consumers because of a balanced ratio of sugar to acid. M26 was proved to be the best with optimum value of this ratio.

4.7 Vitamin C

The content of ascorbic acid did not differ much in this study and the values were very low as compare to previous studies. In case of Elise, the M9 and M9 (*rolB*1) were not significantly different from each other, whereas M26 and M26 (*rolB*) were significantly different from each other, which is consistent with the results of Smolka *et al*. 2010. In case of Elstar, M9 and M9 (*rolB*1) were significantly higher than M26. The lower ascorbic acid values could be the results of longer storage period which can reduce the vitamin C content by disturbing the enzymatic activities in the cell (Davey *et al.*, 2000).

4.8 Total Phenols

There was no significant difference in the results of phenols calculated in apple pulp for all three varieties. Smolka *et al.* (2010) found no significant difference in his results between all the combinations. In Elstar there was no significant difference between transgenic and non-transgenic combinations except the M26 (*rolB*) which was significantly lower in case of phenol content in peel, which was confirmed by Smolka *et al.* (2010). Catrin (2009) had not found any significant difference in case of Jonagold which is the same as our results. Scalzo *et al.* (2005)

revealed that apple varieties do have different levels of the total phenol contents as influenced by rootstocks. There are surely also others factors responsible for the difference in the content of phenols.

There was no significant difference in case of peel in all combinations in most of the cases but the content of phenols was higher in peel than in pulp. This confirms the results by Smolka *et al.* (2010) and Catrin (2009).

5. CONCLUSION

This study gave us an overview that the transgenic rootstocks do not seem to affect negatively the fruit quality of non-transgenic scion cultivars. The parameters studied were not altered for the cultivars grown on the transgenic rootstocks in most cases. In case of the differences found, it is unlikely that such differences are caused by the rootstocks as there was no consistent tendency. It is possible that the factors like tree age and canopy contributed to such changes as the plants from whom the fruit samples taken were four and half year old. Other factors including fruit size, number of fruits, the length of fruit storage, microclimate would have affected the quality parameters. Beside all above factors it has been studied that the transgenic rootstocks have lower growth parameters as compared to non-transgenic rootstocks regardless of the scion used. The fruit quality factors might have been affected due to the slower growth rate of the transgenic rootstock and scion combinations. However, we did not found any consistency of changes between transgenic and non-transgenic rootstocks; it does not seem that the *rolB* gene has a certain effect on the fruit quality.

6. REFERENCES

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