REZULTATE PRELIMINARE PRIVIND PERFORMANTA AGRONOMICA A PRUNULUI TRANSGENIC „HONEYSWEET" IN CONDITIILE GEO-CLIMATICE ALE ROMANIEI

PRELIMINARY RESULTS ON AGRONOMIC PERFORMANCE OF 'HONEYSWEET' TRANSGENIC PLUM UNDER GEO-CLIMATIC CONDITIONS OF ROMANIA

Zagrai Ioan, Zagrai Luminita-Antonela, Guzu Georgeta
Research Station for Fruit Growing Bistrita, Romania

Abstract

'HoneySweet' is a transgenic plum protected against *Plum pox virus* (PPV) infection based on RNA interference mechanism. While field trials in different European PPV endemic countries clearly demonstrated the highly effective and stable resistance to PPV in 'HoneySweet' plum, and no safety concerns were found by regulatory authorities in the U.S., information about its agronomic performance is very limited. The aim of the present work is to gain further information relating to the agronomical and phenotype performance of 'HoneySweet' transgenic plum under PPV endemic area and geo-climatic conditions of Romania. Thus, an experimental plot including 'HoneySweet' and two conventional plum ('Reine Claude d'Althan' and 'Stanley' cvs.) was on Spring 2013. Data collected on 2016 revealed a higher yield potential for HoneySweet transgenic plum compared to 'Reine Claude d'Althan' cv. and a similarity with 'Stanley' cv. Average fruit weight highlights the superiority of the 'HoneySweet' transgenic compared with the two conventional plum varieties. There is also a superiority of 'HoneySweet's' transgenic plum in terms of fruit attractiveness when compared with 'Reine Claude d'Althan' and 'Stanley' cvs.

Cuvinte cheie: 'HoneySweet', prun transgenic, rezistenta, Plum pox, performanta agronomica.
Key words: 'HoneySweet', transgenic plum, resistance, Plum pox, agronomic performance.

1. Introduction

The plum is the dominant fruit tree species in Romania but it is severely affected by *Plum Pox virus*, making fruit production very problematic. *Plum Pox virus* (PPV) which causes Sharka disease is very detrimental because it often produces severe symptoms on fruits such as malformations and flesh necrosis and also premature dropping.

Sharka disease was described for the first time around 1917 in Bulgaria (Atanasoff, 1932). Since then, the disease has progressively spread not only to the most part of European continent (Roy and Smith, 1994) but also in worldwide countries stone fruits producing countries worldwide except Australia, New Zeeland and South Africa (Barba et al., 2011). In Romania, the *Plum Pox virus* is spread across all plum growing areas, causing serious yield losses, especially to susceptible varieties (Minoiu, 1997). A PPV epidemiology assessment, performed in the main plum growing areas in Romania, developed the highly critical and uncontrollable situation generated by *Plum Pox virus* in plum orchards (Zagrai et al., 2010).

There is no cure neither treatment when trees are infected by PPV and the diseases cannot be successfully eliminated in endemic areas by strict methods such as quarantine measures, propagation of virus-free plant materials, chemical treatments against aphid vectors, rouging of infected trees. Therefore, it is almost unanimously accepted that obtaining PPV-resistant plum and their large scale growing remains the most efficient control strategy against PPV. Long time the disease impact was limited by development of tolerant cultivars through conventional breeding (Dosba et al., 1994), which unfortunately allowed the spread of pathogen (Kegler et al., 1998). In addition, since PPV is under strict quarantine measures, tolerances do not represent a method of Sharka management (Ravelonandro et al., 2011). Therefore, resistance to PPV remain the strongly desired trait. Unfortunately, the paucity of naturally high level resistance to PPV in plum has hampered the efforts to control Sharka disease. Also, progress in conventional breeding for resistance is slowed down by the long generation time and the polygenic nature of the resistance involved (Barba et al., 2011). As a complementary approach to conventional breeding, biotechnology may offer solution to control Sharka disease. The transgenic 'HoneySweet' with pathogen-derived resistance is a notorious example of the success of genetic engineering involved in effective control of PPV, particularly in endemic countries (Scorza el al., 2016), as is the case of Romania.

While plenty of experiments, including field trials in different European PPV endemic countries, clearly demonstrated the high level of 'HoneySweet' resistance to PPV and no safety concerns was found

by regulatory authorities in the U.S., limited information are available about its agronomic performance that is very important for practical application.

2. Material and methods

The aim of the research is to gain further information relating to the agronomical and phenotype performance of 'HoneySweet' transgenic plum and compatibility of this event under PPV endemic area and geo-climatic conditions of Romania.

The experimental field was established at Fruit Research & Development Station Bistrita under appropriate permission delivered by Romanian Ministry of Environment. Three years old trees of both transgenic and conventional plums were planted on the spring of 2013. The experimental plot design consists in 12 blocks of four trees (two trees 'HoneySweet' + two conventional - one tree 'Stanley' + one tree 'Reine Claude d'Althan') interspersed with *P. cerasifera* (four plants) and *P. spinosa* (four plants) for coexistence studies (data not show). Thus, a total of 56 trees, of which 24 conventional plum (12 trees of the 'Reine Claude d'Althan' cv. and 12 of the 'Stanley' cv.), 24 transgenic plum ('HoneySweet' cv.) and 8 wild relatives were planted at spaces of 4.5 m between the rows and 3.5 m between trees on row, 635 trees/hectare respectively were used.

Data on agronomical performance of 'HoneySweet' transgenic in comparison with the two conventional varieties grown in the same experimental plot were collected on 2016. The experimental plot maintenance was as for commercial orchards, excepting the number of the phytosanitary treatments, which were lower. That because the study is performed in the context of Plum pox virus-free plum crops and decreasing the environmental pollution caused by the abuse of pesticides used for aphids control. Thus, five phitosanitary treatments were performed, of which three of them only with insecticides.

The methodology of the experimentation was the common used for field trials. Thus, the yield was determined by weighing, on trees and repetitions, than reported on the surface unit. For the assessment of fruit quality, first determination was made on the weight of the fruit harvesting and the percentage of seeds in fruit weight. For each variant, one medium samples was taken with 20 fruits each from different parts of the tree crown. To evaluate the shape and symmetry of the fruit, biometric determinations were performed with the help of the electronic buckler by measuring the fruit length (mm), the fruit width (mm), and the fruit thickness (mm) to 20 fruits of each variant. Also, the uniformity, attractiveness, fruit colour, firmness, and pulp colour were compared between transgenic and the two conventional plums.

3. Results and discussion

The time of fruit harvesting of the three varieties in the experimental plot was different. Thus, under the climatic conditions of 2016, 'Reine Claude d'Althan' cv. was the first harvested on August 11th, followed by HoneySweet cv. on August 16th, and the last one harvested was Stanley cv. on August 29th.

The yields recorded on 2016 showed higher or lower oscillations between three plum varieties (Table 1). Thus, in the 12 blocks, 'HoneySweet' cv. recorded an yield ranged between 7.1 and 11.7 t/ha, 'Stanley' cv. between 6.4 and 12 t/ha, while the 'Reine Claude d'Althan' cv. recorded an yield ranging from 2.5 to 8.9 t/ha. The minimum and maximum values were recorded in block 9, respectively in block 5 in 'HoneySweet' transgenic plum, in block 10, respectively in block 5 in the 'Stanley' cv., and in block 10, respectively block 2 in the 'Reine Claude d'Althan' cv. It was noted that the 'HoneySweet' transgenic plum variety recorded higher yield than 'Reine Claude d'Althan' cv. in all 12 blocks of the experimental plot. The differences of the yield between two varieties ranged from 2.2 - 4.0 t/ha and between 19.9 - 49.4% in the favour of the 'HoneySweet' transgenic plum. When was compared with the 'Stanley' cv. the situation is different. Thus, in eight blocks (1, 3, 5, 6, 7, 8, 9 and 11) the 'HoneySweet' transgenic plum recorded slightly lower yield than the 'Stanley' cv., the difference ranging between 2.5 - 9.9%. The situation is changed in blocks 4, 10 and 12, where the yield of 'HoneySweet' transgenic plum exceeded that of the 'Stanley' variety with 2.5%, 12.3%, respectively 7.6%. There was only one situation where the 'HoneySweet' transgenic plum had the same yield as the 'Stanley' cv. (block 2).

The average yields for the three varieties achieved under the conditions on 2016 on the whole experimental plot revealed a similarity of 'Stanley' and 'HoneySweet' cvs. yields, with a slightly favourable trend for 'Stanley' cv. (Fig. 1). Thus, 'HoneySweet' transgenic plum recorded an average yield of 8.8 t/ha and 'Stanley' cv. of 9.1 t/ha. A much lower average yield at a level of 5.5 t/ha was noticed to 'Reine Claude d'Althan' cv.

In the climatic conditions of the year 2016, the average fruit weight of the 'HoneySweet' transgenic plum in the 12 blocks of the experimental plot ranged from 59.7 to 64.3 g, while the 'Reine Claude d'Althan' and 'Stanley' cvs. varieties produced fruits with an average weight of 41.2 - 49.2 g, and 29.1 – 36.1 g respectively. It was noted large amplitude of fruits weight (8.1 g) in 'Reine Claude d'Althan' cv., which has indicated a non-uniformity size also visually observed. A similar situation was found to 'Stanley'

cv. which recorded amplitude of fruits weight of 7.0 g. The fruit of the transgenic plum variety were the most uniform in terms of fruit weight the amplitude being 4.6 g.

The results of average fruit weight in the whole experimental plot revealed a superiority of the 'HoneySweet' transgenic plum compared with the two conventional cvs. for this trait. More precisely, the average of fruits weight on 'HoneySweet' was almost double compared with that of 'Stanley' cv. (62.1 g versus 31.4 g) and to a difference of 17.8 g compared with that recorded in 'Reine Claude d'Althan' cv. (Fig. 2). As expected, the results on the average weight of the seeds showed that 'HoneySweet' fruits have significantly bigger stone than 'Reine Claude d'Althan' cv. (2.15 g versus 1.65 g) and only slightly bigger than 'Stanley' cv. (2.15 g versus 1.95 g). However, the weight of the seeds singularly analyzed it is not a reliable indicator for appropriate appreciation of the quality of fruits for this trait. Therefore, the weight of the stones was correlated with the weight and size of the fruit, and stone / fruit ratio (%) was determined. Thus, the results showed that the 'HoneySweet' transgenic plum recorded a low stone / fruit ratio (3.5%), similar with that determined in 'Reine Claude d'Althan' cv. (3.7%), and almost twice less than determined on 'Stanley' cv. (Fig. 3)

The biometric measurements on fruits confirmed the biggest fruit size to the 'HoneySweet' transgenic plum compared with the two conventional varieties (Fig. 4). The average values of biometric measurements in the 'HoneySweet' has indicated a fruit length of 52.0 mm, a fruit width of 46.0 mm and a a fruit thickness of 45.0 mm. These average values but also individual values showed a regular shape, more precise oval at 'HoneySweet' transgenic plum. The average values of biometric measurements in the 'Reine Claude d'Althan' cv. has indicated a fruit length of 40.4 mm, a fruit width of 44.8 and a fruit thickness of 42.3 mm, which confirms the relatively spherical, slightly flattened shape of the fruits at this variety. The average values of biometric measurements in the 'Stanley' cv. has indicated a fruit length of 46.9 mm, a fruit width of 34.5 mm and a fruit thickness of 33.4 mm, which confirm the oval, but strangled shape of the fruit in this variety.

In terms of fruit attractiveness, the comparative assessment between 'HoneySweet' and the two conventional varieties ('Stanley' and 'Reine Claude d'Althan') revealed the superiority of transgenic variety. Thus, the bigger size of the fruit is clearly in the favour of 'HoneySweet' cv. Then, the shape of the 'HoneySweet' fruits is regular, oval. The background colour of the epidermis is blue and uniform, very pleasant. The pulp, yellowish-green, is firm and succulent. Seed is small relative to the weight of the fruit, which adds appreciation of the variety. The overall appearance of the fruits (Photo 1) in the 'HoneySweet' variety is very pleasant, with a positive visual impact on the consumers.

4. Conclusions

The preliminary results revealed a higher yield potential for 'HoneySweet' transgenic plum compared to 'Reine Claude d'Althan' cv. and a similarity with 'Stanley' cv., with a slightly favourable trend for the later.

Average fruit weight highlights the superiority of the 'HoneySweet' transgenic plum variety to the conventional 'Reine Claude d'Althan' and 'Stanley' cvs. for this feature. Moreover, the fruits of the transgenic plum variety were more uniform in size compared to the conventional varieties.

There is a superiority of 'HoneySweet's' transgenic plum in terms of attractiveness when compared with 'Reine Claude d'Althan' and 'Stanley' cvs. There is also a superiority of 'HoneySweet's' transgenic plum, in particular to the 'Stanley' cv. in terms of stone / fruit ratio.

References

1. Atanasoff D.,1932. Plum pox. A new virus disease. Yearbook University of Sofia, Faculty of Agriculture and Silviculture 11: 49- 69.
2. Barba M., Hadidi A., Candresse T., Cambra M., 2011. *Plum pox vir*us. In Virus-Like Diseases of Pome and Stone Fruits, 185-197.
3. Dosba F., Lansac M., Eyquaqrd J F., 1994. Resistance des *Prunus* a la sharka. EPPO Bulletin 24 : 691-696.
4. Kegler H., Fuchs E., Gruntzig M., Schwartz S., 1998. Some results of 50 years of research on the resistance to *Plum pox virus*. Acta Virologica, 42: 200-2015.
5. Minoiu, N., 1997. Bolile si daunatorii prunului. In: Prunul, pp 343-374. Cociu I., Botu I., Minoiu N., Modoran, I., Editura Conphys, Pitesti.
6. Ravelonandro M., Scorza R., Hammond RW., 2011. Biotechnological Approaches for Resistance to Viruses, Viroids and Phytoplasmas. In Virus-Like Diseases of Pome and Stone Fruits, 395-399.
7. Roy AS, Smith IM.,1994. *Plum pox* situation in Europe. Bulletin OEPP/EPPO 24:515-523.
8. Scorza R., Ravelonandro M., Callahan A., Zagrai I., Polak J., Malinowski T., Cambra M., Levy L., Damsteeght Z., Krska B., Cordts J., Gonsalves D., Dardick C., 2016. HoneeSweet (C5), the First

Genetically Engineered *Plum pox virus* – resistant Plum (*Prunus domestica* L.) Cultivar. HortScience 51 (5): 601- 603.
9. Zagrai I., Zagrai L., Kelemen B., Petricele I., Pamfil D, Popescu O, Preda S., Briciu A., 2010. Typing and distribution of *Plum pox virus* isolates in Romania. Julius-Kuhn-Archiv, 427: 342-346.ISBN978-3-930037-67-3.

Tables and figures

Table 1. **The yields recorded on HoneySweet transgenic plum and on two conventional plums (Reine Claude d'Althan and Stanley) from experimental plot at Bistrita, Romania -2016**

Block. no	Variant	Fruits amount (kg / pom)	Average fruits amount (kg / pom)	Average yield (t / ha)	Difference to HoneySweet	
					(t/ha)	%
1	Reine Claude d'Althan / (CA1.1.)	6,5	6,5	4,1	-4,0	-49,4
	Stanley / (CS 2.1)	13,5	13,5	8,5	+0,4	+4,9
	HoneySweet / (T 1.2)	12,5	12,75	8,1	-	
	Honey Sweet / (T 2.2.)	13,0				
2	Reine Claude d'Althan / (CA 1.4)	14,0	14,0	8,9	-2,2	-19,8
	Stanley / (CS 2.3.)	17,5	17,5	11,1	0,0	0,0
	HoneySweet / (T1.3.)	19,0	17,5	11,1	-	
	Honey Sweet /T 2.4)	16,0				
3	Reine Claude d'Althan / (CA 1.6)	11,0	11,0	7,0	-1,4	-19,5
	Stanley / (CS 1.7)	14,5	14,5	9,2	+0,8	+5,7
	Honey Sweet / (T 2.7)	14,5	13,75	8,7	-	
	Honey Sweet / (T 2.6)	13,0				
4	Reine Claude d'Althan / (CA 2.8)	7,0	7,0	4,4	-3,7	-45,7
	Stanley / (CS1.8)	12,5	12,5	7,9	-0,2	-2,5
	Honey Sweet / (T 1,9)	12,5	12,75	8,1	-	
	Honey Sweet / (T2.9)	13,0				
5	Reine Claude d'Althan / (CA 2.11)	9,5	9,5	6,0	-5,7	-48,7
	Stanley / (CS 2.12)	19,0	19,0	12,0	+0,3	+2,5
	Honey Sweet / (T1.11)	18,0	18,5	11,7	-	
	Honey Sweet / (T 1.12)	19,0				
6	Reine Claude d'Althan / (CA 1.13)	12,5	12,5	7,9	-2,4	-23,3
	Stanley / (CS 1.14)	17,5	17,5	11,1	+0,8	+7,7
	Honey Sweet / (T 2.14)	17,5	16,25	10,3	-	
	Honey Sweet / (T 2.13)	15,0				
7	Reine Claude d'Althan / (CA 3.1)	7,0	7,0	4,4	-3,1	-41,3
	Stanley / (CS 4.2.)	12,5	12,5	7,9	+0,4	+5,3
	Honey Sweet / (T 3.2)	12,0	11,75	7,5	-	
	Honey Sweet / (T 4.1)	11,5				
8	Reine Claude d'Althan / (CA 4.3)	9,0	9,0	5,7	-2,4	-29,6
	Stanley / (CS 3.4)	14,0	14,0	8,9	+0,8	+9,9
	Honey Sweet / (T 3.3)	12,5	12,75	8,1	-	
	Honey Sweet / (T 4.4)	13,0				
9	Reine Claude d'Althan / (CA 4.7)	5,0	5,0	3,2	-3,9	-54,9
	Stanley / (CS 3.6)	12,0	12,0	7,6	+0,5	+7,0
	Honey Sweet / (T 3.7)	11,5	11,25	7,1	-	
	Honey Sweet / (T 4.6)	11,0				
10	Reine Claude d'Althan / (CA 4.9)	4,0	4,0	2,5	-4,8	-65,7
	Stanley / (CS4.8)	10,0	10,0	6,4	-0,9	-12,3
	Honey Sweet / (T 3.8)	10,0	11,5	7,3	-	
	Honey Sweet / (T 3. 9)	13,0				
11	Reine Claude d'Althan / (CA 3.12)	9,0	9,0	5,7	-4,6	-46,2
	Stanley / (CS 4.12)	17,5	17,5	11,1	+0,8	+4,7
	Honey Sweet / (T 3. 11)	18,0	16,75	10,6	-	
	Honey Sweet / (T 4. 11)	15,5				
12	Reine Claude d'Althan / (CA 4.14)	8,5	8,5	5,4	-2,5	-31,6
	Stanley / (CS 3. 14)	11,5	11,5	7,3	-0,6	-7,6
	Honey Sweet / (T 3.13)	12,0	12,5	7,9	-	
	Honey Sweet / (T 4. 13)	13,0				

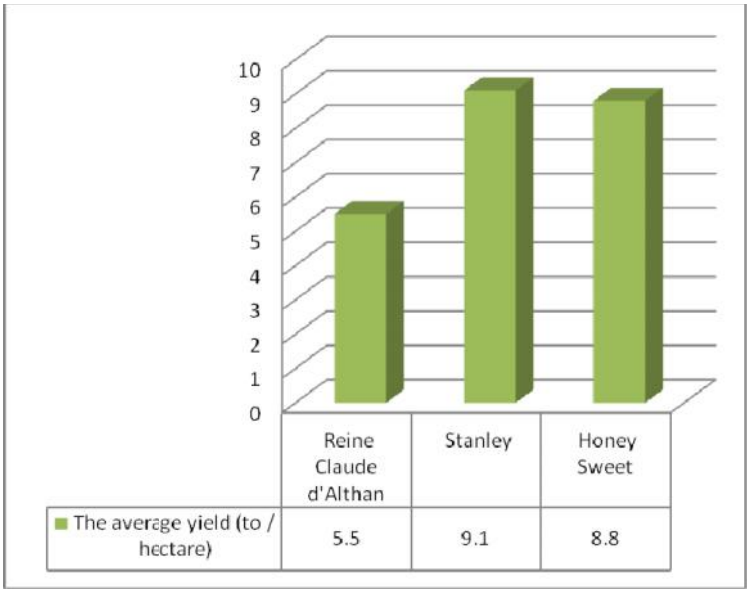

Fig. 1. The average of yields on three varieties studied in the experimental plot at Bistrita on 2016

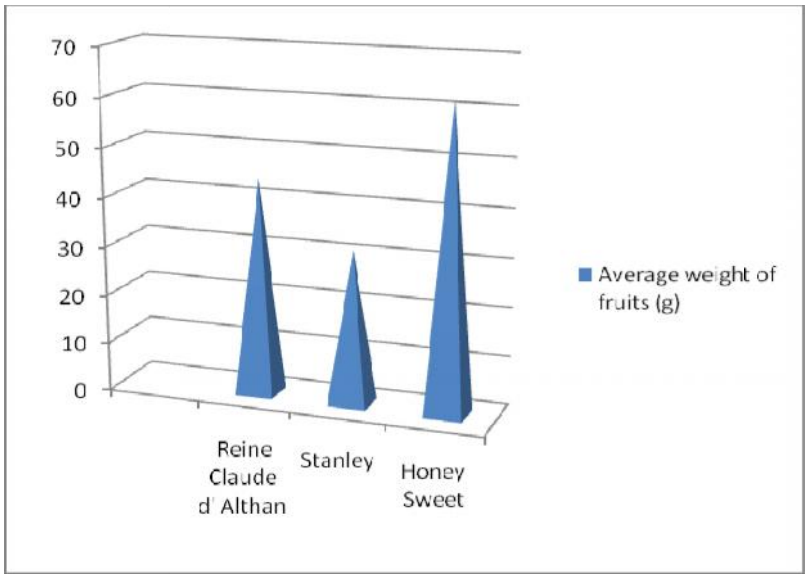

Fig. 2. The average of weight of fruits on three varieties studied in the experimental plot at Bistrita on 2016

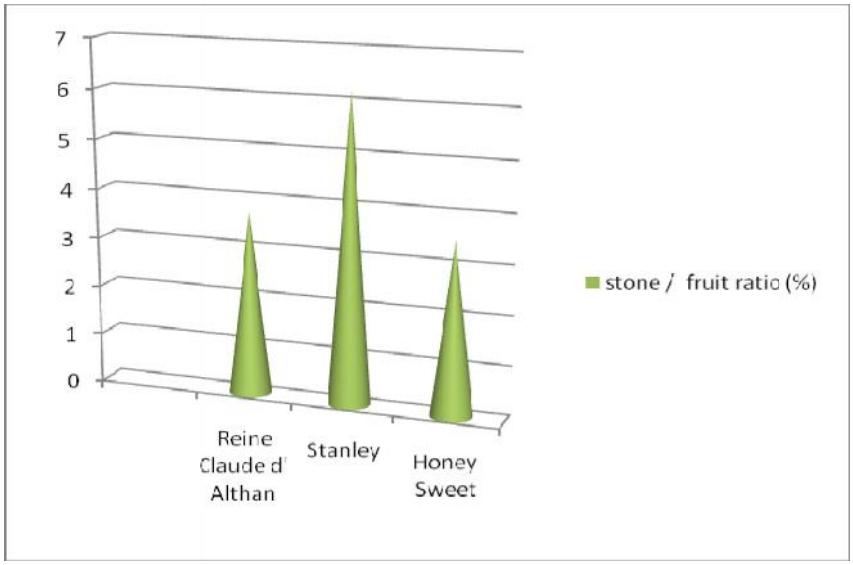

Fig. 3. The average of stone/ fruit ratio on three varieties studied in the experimental plot at Bistrita on 2016

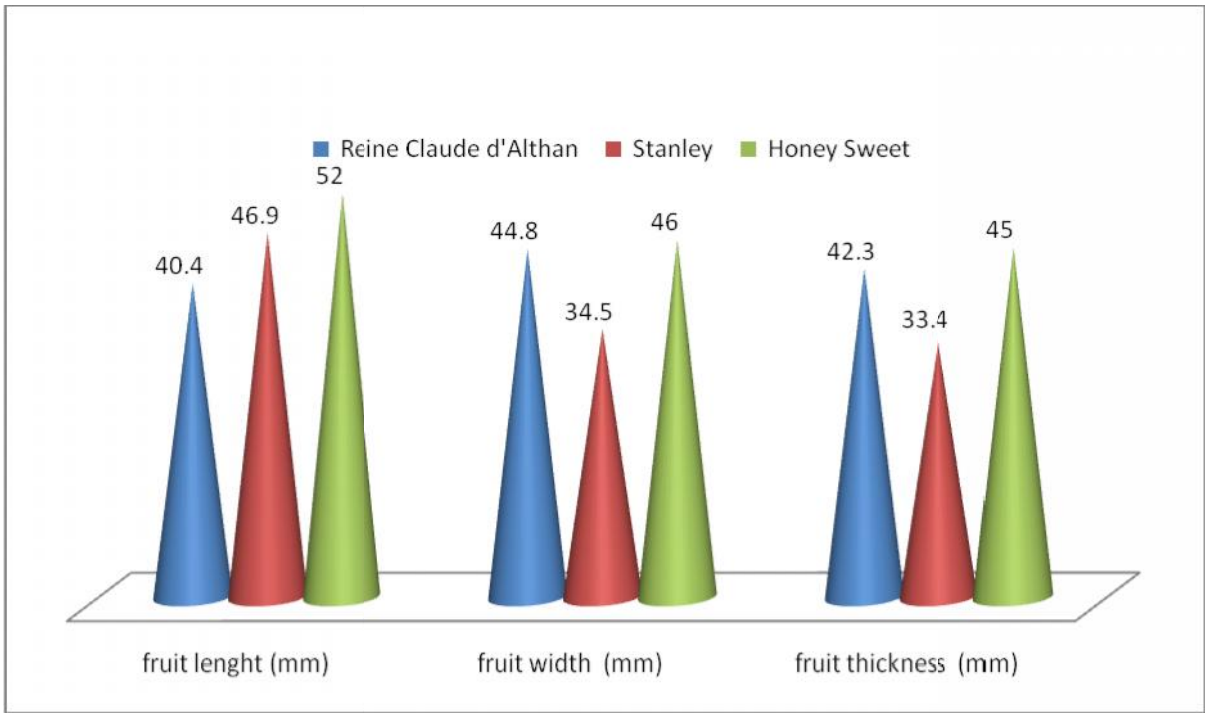

Fig. 4. The average values of biometric measurements of fruits on three varieties studied in the experimental plot at Bistrita on 2016

Photo 1. The overall appearance of the 'Honeysweet' fruits from experimental plot at Bistrita