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Assessment of agronomic performance of PPV resistant 'HoneySweet' transgenic plum in ecological conditions of the Northern Romania

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ABSTRACT

'HoneySweet' is an RNAi based genetically engineered plum resistant to *Plum pox* virus (PPV). Field trials in different European PPV endemic countries clearly demonstrated that 'HoneySweet' plum shows a highly effective and durable resistance to PPV infection. No safety concerns were found by regulatory authorities in the U.S. Expanded studies to assess the agronomic performance of 'HoneySweet' plum were performed under PPV endemic and ecological conditions of Northern Romania. An experimental plot including transgenic plum, 'HoneySweet', and two conventional plum cultivars, 'Reine Claude d'Althan' and 'Stanley', was established in spring of 2013. Agronomic performance data collected during 2016-2018 revealed a higher yield potential for transgenic 'HoneySweet' compared to 'Reine Claude d'Althan' and a similarity with 'Stanley'. Average fruit weight highlighted the superior attributes of transgenic 'HoneySweet' compared with the two conventional plum cultivars. 'HoneySweet' transgenic plum fruits were larger, had lower stone/fruit ratio, and more optimal shape and colour relative to 'Reine Claude d'Althan' and 'Stanley', and a high level of resistance to PPV infection. These data and observations revealed that 'HoneySweet' transgenic plum has good productivity and market potential and is well adapted to PPV endemic and ecological conditions of the Northern Romania.

Keywords: genetically engineered plum, yield potential, fruit attractiveness

INTRODUCTION

The plum (*Prunus domestica* L.) is the dominant fruit tree species in Romania (FAOSTAT, 2019) but it is severely affected by the *Plum pox* virus (PPV), which causes Sharka disease. PPV is considered the most detrimental viral pathogen of stone fruits (including plum, apricot and peach) because it often produces severe symptoms on fruits such as malformations, flesh necrosis, gummosis, and premature fruit drop making plum production especially problematic. Sharka disease was described for the first time around 1917 in Bulgaria (Atanasoff, 1932). Since then, it has progressively spread around the Mediterranean basin, Middle East, and from there throughout the world to Western Europe, Asia, Africa, North and South America, becoming a global concern (Roy and Smith, 1994;

Barba *et al.,* 2011). To date, PPV surveys confirm that only New Zealand and Lebanon are PPV-free (EPPO, 2021).

PPV is naturally spread by aphids in a non-persistent manner (Labonne *et al.,* 1995). Sharka disease has agronomic and political consequences because it causes serious economic losses (Cambra *et al.,* 2006) resulting from severe yield declines. Due to its economic impact, PPV infection represents one of the main limiting factors for the profitability of the stone fruits crops in many European countries (Dunez and Sutic, 1988; Nemeth, 1994; Cambra *et al.,* 2006, Barba *et al.,* 2011), including Romania (Toma *et al.,* 1998; Zagrai *et al.,* 2010a), where PPV is endemic and can seriously compromise the yield of susceptible plum cultivars (Minoiu, 1997; Macovei and Diaconu, 2001; Isac and Zagrai, 2006). A PPV epidemiology assessment, performed in the main plum growing areas in Romania, demonstrated the highly critical and uncontrollable situation generated by *Plum pox* virus in plum orchards (Zagrai *et al.,* 2010b).

There is no curative treatment of virus infected trees in the field. Although strict methods such as quarantines, propagation of virus-free plants, chemical treatments against aphid vectors, removal of infected trees are recommended for Sharka containment, no consistently effective measures have been developed in endemic areas. For a number of years, the economic impact of Sharka was reduced by the development of tolerant cultivars through conventional breeding (Dosba *et al.,* 1994). While tolerant cultivars generally show few fruit symptoms, they allow the virus to proliferate and spread (Kegler *et al.,* 1998). Therefore, host plant resistance to the virus remains the most viable and efficient solution for control of PPV (Ravelonandro *et al.,* 2011; Scorza *et al.,* 2013). Since resistance to PPV is a key trait necessary for plum production, breeding programs were initiated to produce plum cultivars highly resistant to PPV. The scarcity of naturally high-level resistance to PPV in cultivated *Prunus domestica* L. has hampered breeding efforts to control Sharka disease. The most significant result in conventional breeding for PPV resistance in plum is limited to selection for a hypersensitive response (Hartmann and Petruschke, 2000; Neumüller *et al.,* 2010) although hypersensitivity-based resistance may be virus strain-dependent (Polák and Jarošová, 2012). Nevertheless, hypersensitivity represents a mechanism that requires continued research attention (Scorza *et al.,* 2013).

The progress in conventional breeding for resistance is slowed down by the long generation time and the often-polygenic nature of resistance (Barba *et al.,* 2011). As an alternate or complementary approach to conventional breeding, biotechnology may offer solutions to the control of Sharka disease. 'HoneySweet' - an RNAi based transgenic plum resistant to *Plum pox* virus - is the well-known example of the success of genetic engineering in controlling PPV, particularly in endemic countries (Scorza *et al.,* 2016), such as Romania (Zagrai *et al,* 2011; Zagrai and Zagrai, 2020). While exhaustive long-term studies, including field trials in different European PPV endemic countries, clearly demonstrated the high level of 'HoneySweet' resistance to PPV (Ravelonandro *et al.,* 1997; Malinovski *et al.,* 2006; Zagrai *et al.,* 2011; Scorza *et al.,* 2016; Polák *et al.,* 2017) and while no safety concerns were found by regulatory authorities in the U.S. (Scorza *et al.,* 2007), only limited information is available about its agronomic performance. Such information is very important in practical terms. The aim of the present work was to fill this gap and gain further information relating to the agronomic and phenotypic performance of 'HoneySweet' transgenic plum and the potential utility of this transgenic cultivar in a PPV endemic area such as Northern Romania.

MATERIALS AND METHODS

Plant material and experimental plot

The experimental field was established at the Fruit Research & Development Station Bistrița under appropriate permission provided by the Romanian Ministry of Environment (Authorisation no. 1/1032/GA/16.05.2012, European no. B/RO/11/01). Three year old trees of both transgenic ('HoneySweet') and two widely grown conventional plums ('Reine Claude d'Althan' and 'Stanley') grafted onto Myrobalan BN 4Kr seedling rootstocks were planted in the spring of 2013. The experimental plot design (Figure 1) consisted of 12 blocks of four trees each (two trees of transgenic plum 'HoneySweet' and two of conventional plum - one tree of 'Stanley' and the other one of 'Reine Claude d'Althan') interspersed with *Prunus cerasifera* (four plants) and *Prunus spinosa* (four plants) for coexistence studies (data not shown). Thus, a total of 56 trees, of which 24 conventional plums (12 trees of 'Reine Claude d'Althan' and 12 of 'Stanley'), 24 transgenic plum ('HoneySweet') and 8 wild relatives were planted at spaces of 4.5 m between the rows and 3.5 m between trees on row. As a result of the density used, a total of 635 trees per hectare were taken into account to determine the yield.

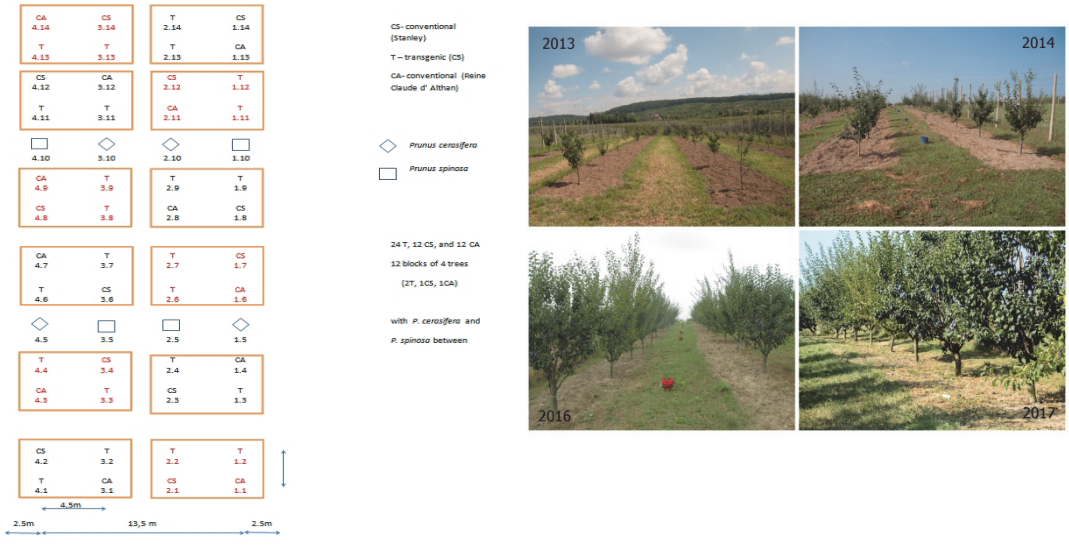

Figure 1. The design of experimental field plot

Plot maintenance

The experimental plot maintenance was consistent with regional commercial orchards, except that the number of phytosanitary treatments was lower. While one aspect of the study was aimed at evaluating the performance of PPV resistant 'HoneySweet' versus PPV susceptible plum cultivars it was also of interest to evaluate the potential of decreasing the environmental pollution caused by the insecticides used for aphid control (Zagrai *et al.*, 2020). Thus, only three (2016), or four (2017 and 2018) phytosanitary treatments with insecticides were applied versus the 7 to 8 treatments generally applied for plum production in the Northern Romania plum growing area.

Data collected

Agronomic performance of 'HoneySweet' was evaluated for three consecutive years, 2016, 2017 and 2018. Information on the agronomic performance was related to the yield potential and the quality of fruits such as fruit weight, fruit size, fruit attractiveness. The

nutritional value was also assessed by determining the content of dry matter, carbohydrates, proteins, lipids, minerals, vitamins and polyphenols (Bobiș *et al.,* 2017).
The experimental methodology was that commonly used for this kind of field trials (Cociu and Oprea, 1989). Yield was determined by total fruit weight, per tree, per repetition, and then reported on the surface unit as potential tons/ha. Then the yield recorded on the 'HoneySweet' was compared with those recorded on the two other well known cultivars, 'Reine Claude d'Althan' and 'Stanley', grown in the same plot. To assess fruit quality, sampling was made by collecting 20 fruits throughout the canopy of each tree. Then, the weight of the fruits was determined by using a laboratory electronic scale. To evaluate the shape and symmetry of the fruit, biometric measurements related to the length (mm), width (mm), and thickness (mm) of fruits were performed using an electronic calliper. Then, stone weight and the ratio of stone weight to fruit weight were determined. Also, the uniformity, colour, firmness, and pulp colour of the 'HoneySweet' fruits were compared with those of conventional plums, 'Reine Claude d'Althan' and 'Stanley' to assess fruit attractiveness. The moment of harvesting was at maturity of fruits when they reached the maximum size and taste features.

Statistical analysis

Statistical analyses were done using XLSTAT program (Addinsoft, New York, USA), ANOVA modelling data, Duncan multiple range test, 95% confidence interval, in order to evaluate differences in yield, weight of fruit and stone, and also percentage of stone weight in fruit.

RESULTS AND DISCUSSIONS

Harvest maturity and yield potential

The fruit ripening period in the Northern Romania was different depending on the plum cultivar. Thus, in the period of 2016-2018, the fruits of 'Reine Claude d'Althan' were harvested in the first part of August, followed by 'HoneySweet' transgenic plum 1 to 5 days later. The maturity of 'Stanley' fruits was recorded in the second half of September.
The fruit yields of cultivars studied under the climatic conditions of 2016-2018 are shown in Figure 2.

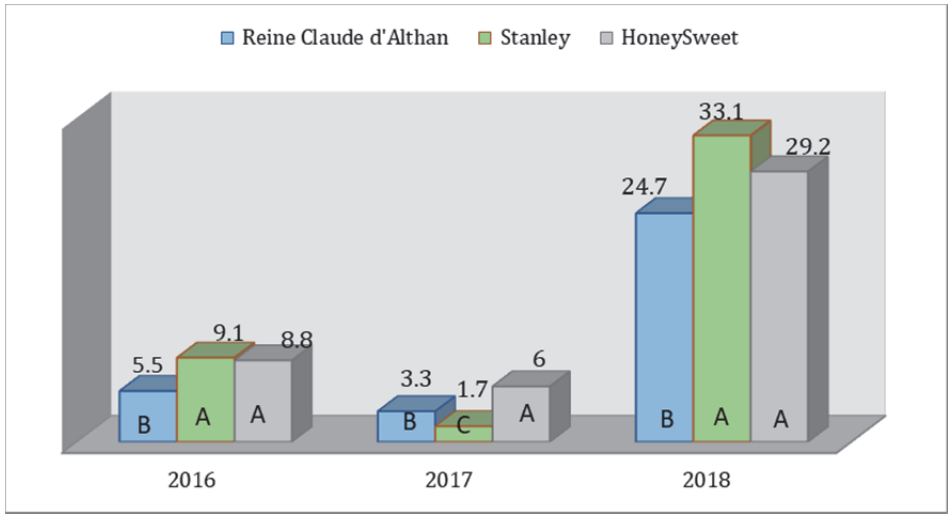

Figure 2. The fruit yields (t/ha) of plum cultivars in the experimental plot established at SCDP Bistrița

In 2016, three years after the establishment of the plantation, all three cultivars yielded, between 5.5 to 9.1 t/ha, with the yield on 'Stanley' and 'HoneySweet', significantly higher than that recorded on 'Reine Claude d'Althan'. As the orchard was very young, the yields were expected to increase in 2017 in all cultivars. This did not happen because a damaging frost that occurred during blossom time which affected the yield, but differently depending for each cultivar. 'Stanley' was heavily affected by frost which translated to a strongly reduced fruit yield, 'HoneySweet' was also affected, but the differences between the two cultivars were statistically different. Also, the difference of yield in 2017 between 'HoneySweet' and 'Reine Claude d'Althan' was statistically different. In 2018, a higher yield was recorded on 'Stanley' than on 'HoneySweet', but the yield difference was not statisticaly different. That could be explained by a high level of flower production of 'Stanley' in 2018 as a result of an extremely low fruit yield in the previous year, caused by the frost. 'Reine Claude d'Althan' fruit production was significantly lower than the other two cultivars. Overall, in 2018 there was a major increase of yields for all three cultivars in the conditions of a favorable climate year.
The average yield of the period 2016-2018 revealed that 'HoneySweet' transgenic plum produced a 'Stanley'-like yield, while 'Reine Claude d'Althan' had a significant lower yield (Table 1). However, it should be noted that while 'HoneySweet' transgenic plum showed a yield similar to 'Stanley', the latter was heavily affected by frost during the 2017 bloom which led to a much reduced fruit yield. This may suggest that 'HoneySweet' could have a better behavior than 'Stanley' to frost during flowering. The differences between 'Reine Claude d'Althan' and the other two cultivars, 'HoneySweet' and 'Stanley', were significantly different. Since 'Stanley' is already well known as a very productive cultivar, 'HoneySweet' transgenic plum could also be considered as a plum ranging to the high yield group of plum cultivars.

Table 1. The average yields of transgenic 'HoneySweet' and conventional plum cultivars (2016-2018) at SCDP Bistrița

Cultivars	Average yields of 2016-2018 (t/ha)
'HoneySweet'	14.708[a]
'Stanley'	14.617[a]
'Reine Claude d'Altha'n	11.150[b]

Qualitative potential

Fruit weight
The average weight of 'HoneySweet' fruits during the three years of study, showed a range from 62.3 to 68.3 g, while 'Reine Claude d'Althan' and 'Stanley' produced fruits with an average weight of 42.4 - 46.9 g, and of 37.4 - 40.1 g, respectively. The results revealed a superiority of the 'HoneySweet' transgenic plum when compared with the two conventional plums for this trait as the average of fruit weights (Figure 3) of 'HoneySweet' was almost double that of 'Stanley' (64.9 g versus 38.9 g) and with a difference of 20.4 g when compared with that recorded on 'Reine Claude d'Althan', all these differences being statistically significant.

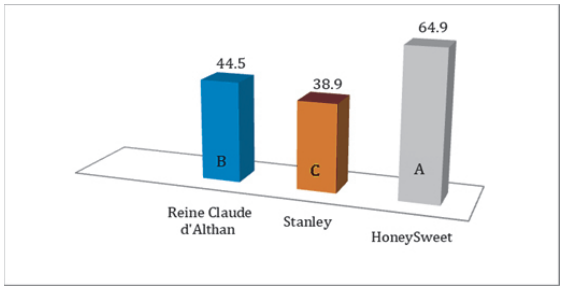

Figure 3. The average of fruit weight (g) - 2016-2018 - of plum cultivars

Stone weight and its percentage in the weight of the fruits

Figure 4 showed that 'HoneySweet' fruits have significantly larger stones than 'Reine Claude d'Althan' (2.4 g versus 1.7 g) and 'Stanley' (2.4 g versus 2.1 g). However, stone weight analysed alone is not a reliable measure of fruit quality as it is relative to overall fruit size. Therefore, the weight of the stone was compared to the weight of the fruit to produce a stone/fruit ratio (%) (Figure 5). 'HoneySweet' showed a relatively lower stone/fruit ratio (3.7%), similar to that of 'Reine Claude d'Althan' (3.8%), and significantly less than 'Stanley'. Thus, this fruit quality characteristic of 'HoneySweet' was superior to 'Stanley'

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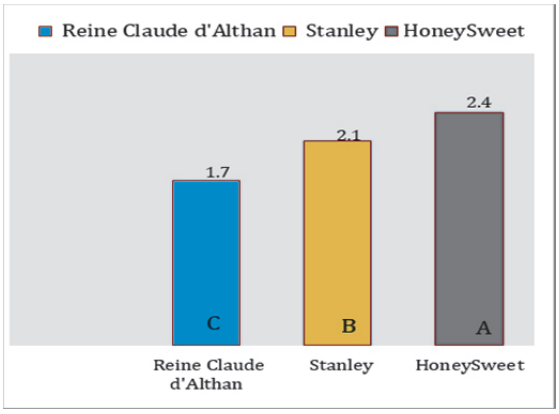

Figure 4. The average weight of stone (g) of plum cultivars (2016-2018)

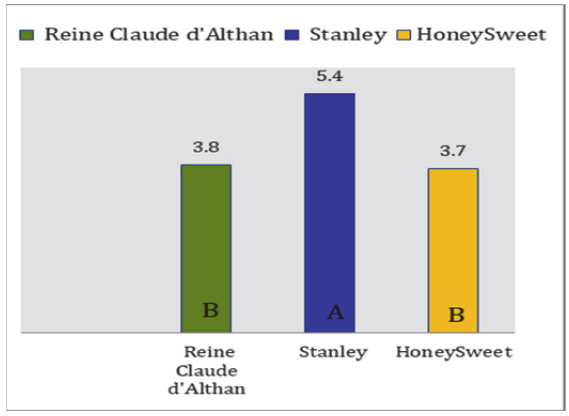

Figure 5. Percentage of stone weight in fruit of three plum cultivars

Biometric and phenotypic properties of the fruits

'HoneySweet' transgenic plum fruits were the largest of the three cultivars studied (Figure 6) with an average of fruit length (H)/fruit width (D)/fruit thickness (d) of 52.8/46.2/44.8 mm. It was also noted that individual values showed a more consistent regular, precise oval shape of 'HoneySweet' fruit – a desirable characteristic for growers and consumers. 'Reine Claude d'Althan' fruits had a ratio of 39.8/43.9/41.5 mm, which confirmed the relatively spherical, slightly flattened fruit shape. 'Stanley' fruits had a ratio of 49.4/37.0/35.7 mm, which produced an oval elongated fruit with neck-constriction.

Attractiveness of the fruits

In terms of fruit attractiveness, the comparative assessment between transgenic 'HoneySweet' and the two conventional cultivars ('Reine Claude d'Althan' and 'Stanley') revealed the superiority of 'HoneySweet' (Figure 7).

Obviously, the larger size of the fruit is clearly a favorable characteristic of along with the consistent oval shape. The background color of the epidermis is blue and uniform, very pleasant. The pulp, yellowish-green, is firm, succulent, most part non-cling stone. Although the 'HoneySweet' stone is larger in comparison to the other two cultivars in the test, it is small relative to the weight of the fruit, which is a positive fruit quality trait.

The appearance of the 'HoneySweet' fruits (Figure 8) is very attractive, with a positive visual impact on the consumers.

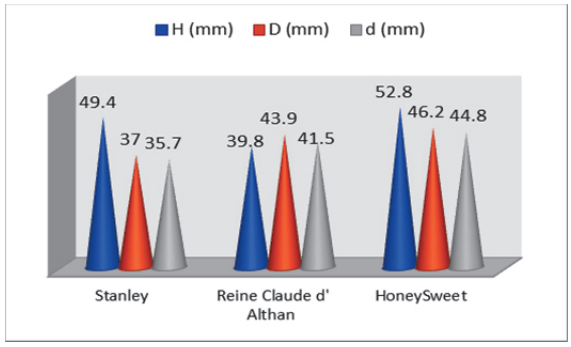

Figure 6. Biometric measurements – shape of the fruits (length, width and thickness)

Figure 7. Comparative fruit size of 'Stanley' (a), 'Reine Claude d'Althan' (b) and 'HoneySweet' (c)

Figure 8. Fruits of 'HoneySweet' transgenic plum

CONCLUSION

The high yield potential and fruit quality correlated with high level of resistance to natural PPV infection revealed that 'HoneySweet' transgenic plum is a valuable plum well adapted to PPV endemic and ecological conditions of Northern Romania.

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