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Analysis of the 'Superior Seedless' Vines' Berries Shattering during their Shelf-Life after being Grafted onto Several Rootstocks

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ABSTRACT

Grapevines, are widely cultivated and have a significant global economic impact. Berry breaking is a critical practice for grape growers as it directly impacts fruit quality, ensuring optimal flavor development and desirable characteristics in the final product. An experiment was conducted over the 2021 and 2022 seasons at a commercial farm with sandy soil, focusing on 12-year-old vines. The experiment involved grafting 'Freedom,' 'Ruggeri 140Ru,' 'Paulson 1103P,' and 'Superior Seedless' rootstocks, and evaluating the results. Harvest dates were determined by testing bundle samples using SSC (16%) and storing them at room temperature for four days. The Paulson 1103P rootstock showed a lower rate of berry breaking compared to other rootstocks. The activity of xylanase (XLN; EC:3.2.1.8), polygalacturonase (PG; EC:3.2.1.15), and cellulase (CEL; EC: 3.2.1.4), was also comparatively reduced. Throughout the four days, the Paulson 1103P rootstock demonstrated superior physical and chemical quality traits. The levels of cell wall hydrolysis enzymes, including PG, XLN, and CEL, were lower in vines grafted onto the Paulson 1103P rootstocks. The influence of the Paulson 1103P rootstock on physical and chemical quality traits persisted throughout the first four days of shelf life. The results suggest that 'Superior Seedless' vines grafted onto the Paulson 1103P rootstock exhibited a more pronounced decrease in berry breaking rate until the end of the four-day trial storage period under ambient air conditions.

Keywords: Polygalacturonase, Xylanase, Cellulase

INTRODUCTION

Grapevines, which lose their leaves seasonally, are grown worldwide and are a significant contributor to the global economy. The history of grapes dates back 13,000 years to Egypt, where they were not only discovered but also featured on the walls of ancient Egyptian temples and tombs, including Queen Nefertari's tomb (Kassem et al., 2011). Research has been conducted to address challenges in sustainable grape cultivation, including soil diseases, phylloxera, and nematodes that commonly arise during production (Köse et al., 2014). Climate change impacts drought resistance have also been studied (El-Gendy, 2013; Cousins and Striegler, 2005; Jogaiah et al., 2013).

There has been a significant focus on using rootstocks in vineyard plantations to improve grape quality and yield. However, there is limited research on how rootstocks affect fruit market behavior. Some studies suggest that the impact of rootstocks on fruit cluster quality is mainly seen after harvest. Previous research has addressed post-harvest concerns, such as preventing berry shattering in "Superior Seedless" fruit clusters by treating them with 2 mM salicylic acid. To address yellowing during export after harvest, a strategy was implemented as described by Lo'ay and Dawood, (2017). In separate research, exogenous cyanocobalamin therapy has been used to enhance the berry color of 'Crimson Seedless' grapes, as explored by Lo'ay in (2017).

For over 130 years, grapevine rootstocks have, played a crucial role in grape farming by contributing to the development of desirable traits in grape clusters. Initially, rootstocks were used in reclaimed areas and underwent

evaluation (Wolpert et al., 1994). For example, the Freedom rootstock shows improved resistance to nematodes, and phylloxera (El-Gendy, 2013). The 1103 P rootstock, known for its robustness and resistance to phylloxera, thrives in clay and limestone soils, and also shows resistance to salt and resilience to prolonged dry periods. The 'Ruggeri' 140 Ru rootstock, characterized by high vigor and strong phylloxera protection, is also well-known for its resistance to different soil conditions and salinity (Wolpert et al., 1994).

In the study, we observed significant differences in berry shattering among clusters grafted onto different rootstocks at different stages of ripeness. The aim of the study is to investigate the impact of specific rootstocks used for "Superior Seedless" grapes on berry shattering. This research also aims to understand the ability of berry pedicels to break down cell walls during their shelf life.

MATERIALS AND METHODS

1. Fruit supplies and a plan for the experiment

The study was conducted on 12-year-old vines during the 2021–2022 seasons in a commercial orchard near the Monufia Governorate in Egypt. The "Superior Seedless" cultivar was grafted onto different rootstocks, including Freedom, Ruggeri - 140Ru, and Paulsen - 1103P. Additionally, Superior Seedless was grafted onto the claim root (control) under sandy soil conditions with drip irrigation, reaching a height of 2 to 3 meters, and following frame management guidelines. Sampling was done in batches once the soluble solid content reached 16%. Upon reaching the Pomology Department Lab, the 240 fruit clusters were

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divided into two groups. The first group, consisting of 120 clusters, was used for non-destructive assessments, including rachis color hue angle, water loss rate, and berry breaking percentage. These clusters were then divided into four sets, each containing 30 clusters associated with a specific rootstock, and distributed among three repetitions. Destructive analysis was conducted as outlined previously. Two bunch samples from each repetition were selected daily to evaluate chemical characteristics. The batches were then stored at room temperature for four days.

2. Examining the physical qualitative qualities

Berry firmness, which indicates berry texture, was measured using an Effegi- penetrometer with a 2 mm diameter plunger. The force required to separate the berries was measured in Newtons (N). The shattering rate and color hue angle profile were also documented. Additionally, the degree of water loss was quantitatively measured (Artés-Hernández et al., 2006).

a. Evaluation of chemical quality attributes

To determine total soluble solids (SSC%) using a Carl Zeiss hand refractometer, a subset of berries was selected from multiple bunch tests and divided into three replicates. The acidity, measured as tartaric acid (TA%), was adjusted using 0.1N NaOH. The SSC/TA ratio was then calculated, considering the berries' developmental stage (AOAC, 1995).

b. The activity of cell wall degradation enzymes

In the homogenization process, one gram of berry pedicels was combined with 20 mM Tris-HCl buffer at pH 7. The resulting mixture was centrifuged at 16,000 rpm for 20 minutes at 4 °C, and the clarified supernatant was stored at -20 °C for subsequent analysis of polygalacturonase (PG), xylanase (XLN), and cellulase (CEL) enzymes. Enzyme activity was evaluated by measuring the reduction in galacturonic acid, xylose, and carboxymethyl cellulose as substrates (Miller, 1959).

c. Statistical analysis

Duncan's Multiple Range Test was employed to determine the significance of each evaluated treatment at a probability level of $P < 0.05$ (CoStat software version 6.303).

RESULTS AND DISCUSSION

Non-distractive measurements

In Figure 1, statistical significance at a P value of 0.05 is observed for rootstocks. The 'Paulson' rootstock shows better water loss mitigation in fruit clusters throughout the shelf-life period compared to other rootstocks. The proportion of water loss gradually increases until the fourth day of the shelf-life experiment, with the second day showing the lowest water loss percentage. Compared to 'Freedom,' 'Ruggeri,' and 'Superior Seedless,' the 'Paulson' rootstock consistently exhibits substantially lower water loss percentages (6%) on three occasions. Despite water loss continuing to increase until the end of the experiment, 'Paulson' maintains the lowest water loss compared to other rootstocks, registering at approximately 14% on the fourth day. No indications of berry shattering (BS) or rachis browning index (RBI) were observed at harvest until the second day of shelf life. However, by the second day, both BS and RBI parameters became apparent, with the "Paulson" rootstock showing the lowest BS percentage, gradually

increasing to 2.73% by the end of the shelf-life period.. Conversely, the RBI increased from 1.13 at harvest to 2.13 by the fourth day, indicating mild browning.

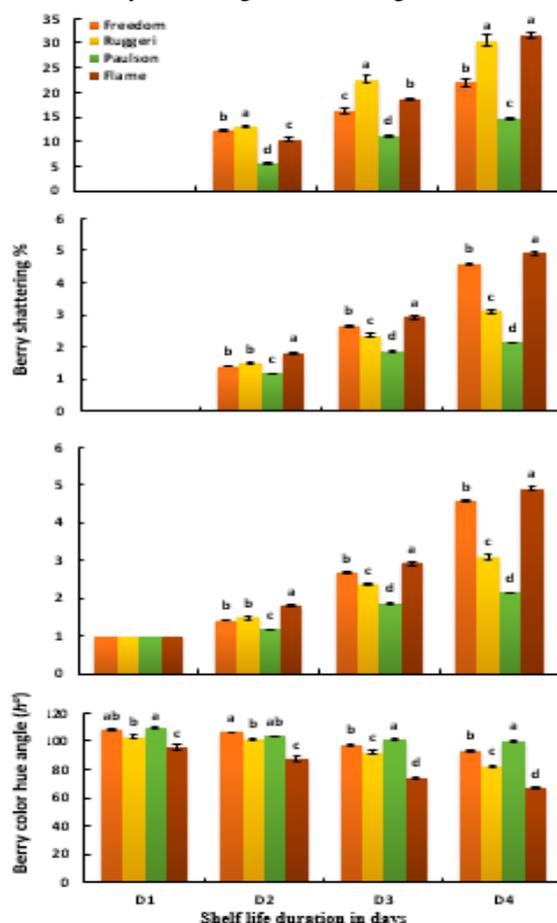


Figure 1. Illustrates the impact of rootstocks on some traits over a four-day shelf life period.

These findings demonstrate the impact of different rootstocks on the physical quality of grapes. The 'Paulson' rootstock seems to reduce water loss in stored fruit clusters by developing a strong natural epidermal structure with wax layers on the berry surface, which acts as a barrier against dehydration during storage. Fruit clusters from vineyards grafted on the 'Paulson' rootstock exhibited the lowest incidence of rachis browning index (RBI), possibly due to a higher content of ascorbic acid, which acts as an antioxidant, and reduces the production of reactive oxygen species (ROS) during storage. The 'Paulson' rootstock minimizes browning symptoms, by reducing the activation of polyphenol oxidase (PPO) enzymes, which catalyze the oxidation of phenolic compounds. Additionally, it is believed that factor such as sensitivity to mechanical force during handling, a potential climacteric behavior of the pedicel and brush of berries under stress from water loss, contribute to berry loss from bunches. The 'Paulson' rootstock may provide resistance to fruit clusters during storage, by potentially developing an abscission layer near the distal tip of the berry's pedicel (Crisosto et al., 2001).

Berry pedicels and cell wall degrading enzyme activities (CWDEs) and cell membrane permeability

Table 1 shows the changes in cell wall disintegration by XYL, CEL, PG, and PT enzymes, as well as the percentage of cell membrane permeability over the shelf life (days).

A significant interaction between storage duration and rootstocks for the enzyme activities related to cell wall breakdown is evident, with a p-value ≤ 0.001 .

The data obtained indicates a gradual increase in the activity of key factors that influence cell wall degradation from harvest to the end of the experiment. Overall, the rootstocks lead to an increase in enzyme activity for all enzymes, regardless of the specific rootstock. Fruit clusters grafted on the "Superior Seedless" (control treatment) rootstock showed a faster increase in enzyme activities over the four-day experimental period compared to those on other rootstocks. The 'Paulson' rootstock, on the other hand, exhibited a reduction in enzyme activities during storage, with lower values for XYL (8.89), CEL (2.33), PG (32.44), and PT (0.59) compared to other rootstocks.

Variations in enzyme activities related to cell wall disintegration, such as XYL, CEL, PG, and PT, changed

throughout the shelf life (days), due to a combination of factors. These factors include inherent differences in enzymatic activity among rootstocks, variations in fruit physiology and metabolism over time, and the interplay between storage conditions and rootstock characteristics. The interaction between storage duration and rootstocks emphasizes the complex relationship between these factors and their impact on enzymatic processes involved in cell wall degradation. The gradual increase in enzyme activities from harvest to the end of the experiment suggests ongoing biochemical processes associated with fruit ripening and senescence, which may be influenced by rootstock effects. The 'Paulson' rootstock, showed a reduction in enzyme activities during storage, highlighting the variability in enzymatic responses among different rootstock varieties, and the importance of rootstock selection in influencing fruit quality attributes and post-harvest behavior (Landi et al., 2014).

Table 1 A. The impact of studied rootstocks as well as shelf life on the activity of cell wall-degrading enzymes (across two growth seasons of 2021 and 2022).

RT	ST			
	D1	D2	D3	D4
	XA			
F1	7.05 ± 0.049 ^{jk}	7.99 ± 0.255 ^{hi}	10.41 ± 0.248 ^{fg}	15.75 ± 1.178 ^d
R	7.32 ± 0.027 ^{ijk}	11.23 ± 0.111 ^{fg}	15.44 ± 0.576 ^e	22.27 ± 0.817 ^b
P	4.17 ± 0.061 ^l	5.27 ± 0.043 ^k	6.69 ± 0.125 ^{hij}	8.07 ± 0.021 ^{gh}
F2	6.01 ± 0.022 ^{ijk}	12.07 ± 0.014 ^f	18.76 ± 0.908 ^c	25.32 ± 0.460 ^a
	CA			
F1	1.71 ± 0.042 ^{ijk}	2.33 ± 0.033 ^h	5.62 ± 0.172 ^f	10.41 ± 0.220 ^c
R	2.00 ± 0.080 ^{hij}	2.79 ± 0.048 ^g	7.11 ± 0.412 ^e	14.66 ± 0.427 ^b
P	1.25 ± 0.016 ^k	1.55 ± 0.018 ^{jk}	2.00 ± 0.012 ^{hij}	2.11 ± 0.022 ^{hi}
F2	1.82 ± 0.054 ^{hijk}	3.26 ± 0.023 ^g	9.07 ± 0.079 ^d	15.16 ± 0.267 ^a
	PA			
F1	27.25 ± 0.573 ^f	33.95 ± 0.311 ^d	37.57 ± 0.261 ^c	38.33 ± 0.419 ^c
R	30.35 ± 0.351 ^e	34.25 ± 0.515 ^c	40.55 ± 0.419 ^b	40.11 ± 0.512 ^{ab}
P	21.81 ± 0.471 ^h	24.27 ± 0.525 ^g	26.24 ± 1.064 ^f	31.28 ± 0.509 ^e
F2	34.30 ± 0.488 ^d	39.17 ± 0.171 ^c	39.47 ± 0.476 ^{ab}	41.58 ± 0.371 ^a
	PEA			
F1	0.51 ± 0.012 ^h	0.59 ± 0.002 ^e	0.71 ± 0.003 ^{de}	0.72 ± 0.015 ^d
R	0.70 ± 0.015 ^d	0.76 ± 0.010 ^c	0.81 ± 0.005 ^b	0.79.5 ± 0.008 ^b
P	0.36 ± 0.001 ^j	0.37 ± 0.003 ⁱ	0.52 ± 0.012 ^h	0.62 ± 0.027 ^g
F2	0.65 ± 0.019 ^f	0.71 ± 0.005 ^{de}	0.74 ± 0.005 ^c	0.89 ± 0.005 ^a
	CMP			
F1	2.14 ± 1.422 ^k	11.37 ± 0.513 ^{gh}	15.13 ± 0.584 ^e	24.32 ± 1.041 ^{bc}
R	4.93 ± 0.025 ^{ij}	12.37 ± 0.600 ^g	20.45 ± 0.589 ^d	26.91 ± 0.544 ^a
P	4.07 ± 0.187 ^j	7.11 ± 0.183 ⁱ	7.34 ± 0.393 ⁱ	8.12 ± 0.425 ^h
F2	5.54 ± 0.025 ^{ij}	13.51 ± 0.597 ^f	21.62 ± 0.346 ^{cd}	24.91 ± 0.538 ^b

RT: Rootstocks types, ST: Shelf-life time (days), F1: Freedom, R: Ruggeri 140, P: Paulson 1103, F2: Flame seedless, XA: Xylanase activity, CA: Cellulase activity, PA: Polygalacturonase activity, PEA: Pectinase activity, CMP: Cell membrane permeability

Chemical and physical characteristics

Table 2 presents the berry firmness (BF) and separation force (BSF) of different 'Superior Seedless' rootstocks over time (days) in storage. A significant correlation between rootstocks and storage duration is observed at a P value of 0.001. Initially, both BF and BSF are higher at harvest time. Vines grafted onto the 'Paulson' rootstock, exhibit greater firmness (6.97 N) and higher separation pressure (6.44 N) compared to other rootstocks. Throughout the storage period, both BF and BSF gradually decrease across all treatments (rootstocks). 'Superior Seedless' demonstrates a substantial and increased reduction in both BF and BSF. When considering characteristics that appeal to customers, the firmness of berries (BF) and skin firmness (BSF) are crucial physical attributes. It is important to note that the elevated BF and BSF result from the berries' brush and pedicel maintaining a more stable insoluble gelatin content over the storage period. (Bassetto et al., 2005). The immediate impact on cell

wall degradation, induced by enzyme softening, may be better understood when considering the rate of reductions in BF and BSF.

During the four-day storage duration, there is a noticeable increasing enzyme activity responsible for breaking down cell walls (shown in Fig. 2). This increase in enzyme activity is consistent across all studied rootstocks ("Freedom," "Ruggeri," "Paul," and "Superior Seedless"), indicating a similar shelf life for each. However, the extent of enzyme activity increase varies among the different rootstocks. Notably, "Superior Seedless" fruit clusters show higher enzyme activation, which is associated with increased berry cracking during the shelf-life period. On the other hand, the 'Paulson' rootstock exhibits lower levels of activation and, consequently, less berry shattering.

By evaluating grape deterioration and its relationship with cell wall enzyme activity during storage, we can better understand the berry shattering process. It is

clear that the activities of XYL, PG, CEL, and PT are directly linked to the enzyme's activity. The Paulson 1103' rootstock consistently shows lower increases in cell

wall-degrading enzyme activity compared to other rootstocks, while other rootstocks exhibit a more rapid surge in enzyme activity.

Table 2. The impact of studied rootstocks as well as shelf life time on physical and chemical attributes (across two growth seasons of 2021 and 2022).

RT	ST			
	D1	D2	D3	D4
	BF			
F1	6.32 ± 0.020 ^b	6.62 ± 0.029 ^c	5.00 ± 0.033 ^e	4.50 ± 0.074 ^f
R	6.22 ± 0.056 ^c	5.11 ± 0.027 ^d	4.25 ± 0.005 ^f	4.00 ± 0.031 ^g
P	6.81 ± 0.066 ^a	7.62 ± 0.120 ^a	6.25 ± 0.162 ^b	5.97 ± 0.054 ^b
F2	4.61 ± 0.111 ^e	42.96 ± 0.110 ^e	3.17 ± 0.117 ^h	3.09 ± 0.019 ^h
	BSF			
F1	3.65 ± 0.030 ^d	3.56 ± 0.029 ^l	3.26 ± 0.026 ^j	3.11 ± 0.011 ^k
R	4.04 ± 0.031 ^c	4.11 ± 0.011 ^g	4.07 ± 0.047 ^k	2.85 ± 0.026 ^l
P	5.67 ± 0.029 ^a	7.23 ± 0.017 ^b	6.11 ± 0.018 ^c	6.07 ± 0.021 ^c
F2	4.21 ± 0.015 ^b	3.97 ± 0.021 ^h	2.89 ± 0.025 ^l	2.54 ± 0.061 ^m
	TSS			
F1	15.65 ± 0.333 ⁱ	18.66 ± 0.133 ^f	20.47 ± 0.324 ^e	21.69 ± 0.192 ^{bc}
R	18.43 ± 0.323 ^{fg}	20.56 ± 0.314 ^{de}	22.54 ± 0.233 ^b	22.32 ± 0.389 ^a
P	15.03 ± 0.477 ^g	17.36 ± 0.243 ^h	18.47 ± 0.156 ^{gh}	19.43 ± 0.133 ^{fg}
F2	18.67 ± 0.336 ^f	20.31 ± 0.433 ^e	21.11 ± 0.476 ^{cd}	22.09 ± 0.473 ^{ab}
	TA			
F1	0.311 ± 0.011 ^{ef}	0.294 ± 0.006 ^{fg}	0.307 ± 0.006 ^{gh}	0.261 ± 0.005 ^{ij}
R	0.321 ± 0.015 ^e	0.327 ± 0.003 ^{efg}	0.281 ± 0.019 ^{hi}	0.292 ± 0.005 ^{fgh}
P	0.431 ± 0.005 ^a	0.411 ± 0.005 ^b	0.391 ± 0.005 ^c	0.362 ± 0.005 ^d
F2	0.291 ± 0.005 ^{fgh}	0.291 ± 0.005 ^{hi}	0.251 ± 0.005 ^j	0.217 ± 0.007 ^k
	SSC/TA-ratio			
F1	51.87 ± 2.359 ^g	63.47 ± 2.166 ^f	68.92 ± 2.312 ^{ef}	82.10 ± 2.750 ^{bc}
R	55.68 ± 2.079 ^g	51.70 ± 1.644 ^f	76.65 ± 1.410 ^{cd}	76.78 ± 0.541 ^d
P	37.19 ± 0.198 ^j	42.24 ± 0.417 ^{ij}	47.24 ± 0.368 ^{hi}	53.95 ± 1.715 ^{gh}
F2	64.16 ± 2.197 ^f	69.72 ± 2.659 ^{de}	84.10 ± 2.419 ^b	101.80 ± 3.011 ^a

RT: Rootstocks types, ST: Shelf-life time (days), F1: Freedom, R: Ruggeri 140, P: Paulson 1103, F2: Flame seedless, BF: Berry firmness, BSF: Berry separation force, TSS: Total soluble solid, TA: Total acidity

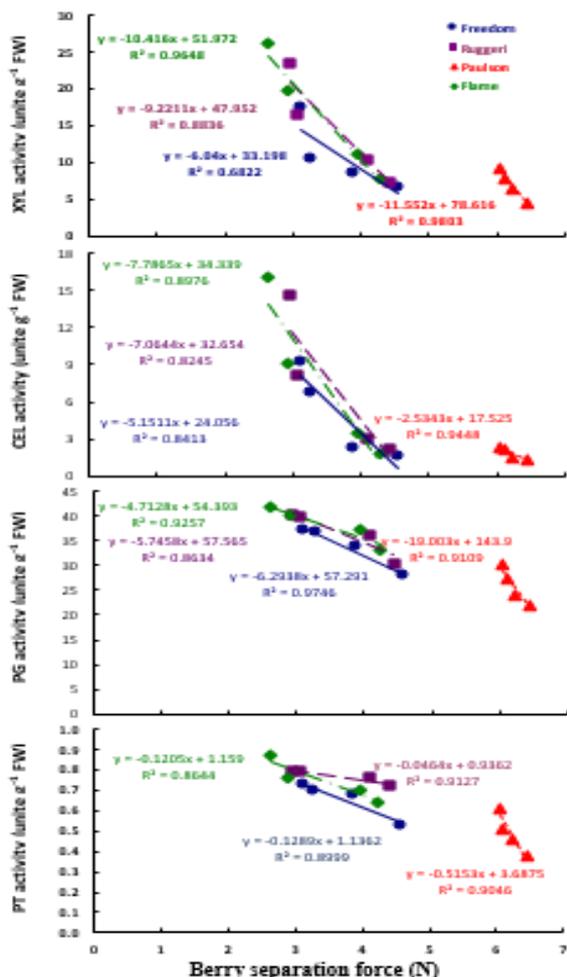


Figure 2. displays notable linear correlations

The study looked at four 'Superior Seedless' rootstocks and their effect on berry shattering over four days. The "Paulson" rootstock was found to have a significant impact on improving both the physical and chemical quality of the fruit in terms of shattering during storage. The decrease in berry shattering is linked to a reduction in the activity of enzymes that break down cell walls during storage. This could be due to higher levels of metabolic carbohydrates during berry development, leading to increase production of ascorbic acid (AA), which provides protection during storage or marketing. It is recommended to graft Superior Seedless plants onto Paulson rootstock to improve fruit quality and enhance grape marketing. This based on the idea that increased metabolic carbohydrates during berry development can lead to more stable fruit clusters, reducing the risk of damage during handling and marketing. Therefore, using 'Paulson' rootstock for grafting Superior Seedless vines can help minimize grape berry breakage and improve overall marketability.

CONCLUSION

In conclusion, the study highlights the significant impact of rootstock selection, particularly Paulson 1103P, on grapevine berry integrity and quality. The experiment shows that this rootstock reduces berry breakage and maintains lower levels of cellular wall hydrolysis enzymes, resulting in superior physical and chemical quality over a four-day storage period. Grafting 'Superior Seedless' vines onto Paulson 1103P rootstock could offer substantial benefits in fruit cluster behavior and shelf-life 20preservation. Further exploration and adoption of Paulson 1103P rootstock in grapevine cultivation practices is recommended to enhance overall fruit quality and economic outcomes.

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تحليل تفتت حبات العنب "سوبريور سيدليس" خلال فترة تخزينها بعد تطعيمه على العديد من الأصول

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² قسم الفاكهة، كلية الزراعة، جامعة المنصورة، مصر

الملخص

تعتبر أشجار العنب، التي يتم زراعتها على نطاق واسع في جميع أنحاء العالم، بأنها تمتلك تأثيراً اقتصادياً عالمياً كبيراً. يعد تكسير ثمار العنب ممارسة مهمة لمزارعي العنب لأنه يؤثر بشكل مباشر على جودة الفاكهة، مما يضمن تطوير النكهة المثالية والخصائص المرغوبة في المنتج النهائي. في تجربة أجريت خلال مواسم 2021 و 2022 في مزرعة تجارية تحتوي على تربة رملية، كانت الأشجار التي تبلغ من العمر 12 عامًا نقطة التركيز. شمل التطعيم استخدام أصول 'Freedom' و'Ruggeri 140Ru' و'Paulson 1103P' و'Superior Seedless' مع التقييم اللاحق للنتائج لتحديد تاريخ الحصاد، تم اختيار عينات الحزم باستخدام SSC (16%)، تلاها تخزين لمدة أربعة أيام عند درجة حرارة الغرفة (25 ± 1 درجة مئوية ونسبة رطوبة هواء 43 ± 1). على عكس الأصناف الأخرى من الأصول، أظهرت أصل "Paulson 1103P" معدلاً أقل لتكسير الثمار. بالإضافة إلى ذلك، تم تقليل نشاط الإنزيمات الهيدروليزية لجدران الخلية، بما في ذلك Polygalacturonase (PG؛ EC:3.2.1.15)، و xylanase (XLN؛ EC:3.2.1.8)، و cellulase (CEL؛ EC:3.2.1.4)، بشكل مقارن. وعلاوة على ذلك، أظهرت أصل "Paulson 1103P" سمات جودة فيزيائية وكيميائية متفوقة على مدار الأربعة أيام. يُلاحظ أن نشاط الإنزيمات الهيدروليزية لجدران الخلية، مثل PG، و XLN، و CEL، بقي على مستوى أقل مقارنة بالأصناف الأخرى من الأصول المزروعة. وعلاوة على ذلك، استمر تأثير أصل "Paulson 1103P" على سمات الجودة الفيزيائية والكيميائية خلال الأربعة أيام الأولى من فترة الحفظ على الرف. تُظهر النتائج أن سلوك العنب "Superior Seedless" المطعمه على أصول "Paulson 1103P" أظهرت تقليلاً أكبر في معدل تكسير الثمار حتى نهاية فترة التخزين التجريبية (أربعة أيام) تحت ظروف الهواء الطبيعية.