THE USE OF ELICITORS IN VITICULTURE: A TOOL TO OBTAIN HIGHLY COLORED WINES WITH A REDUCE ALCOHOL CONTENT?

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Abstract:

Context and purpose of the study

Climate change is causing a gap between the technological and phenolic maturity of grapes, resulting in wines with high alcohol content and low polyphenol concentration. Another phenomenon associated with high temperatures and whose effect is more pronounced if the harvest is delayed is the decrease in the acidity of the grapes, mainly in malic acid, and an increase in pH caused by the accumulation of potassium derived from the increase in temperature. Therefore, climate change and the effects it causes on the vine leads to unbalanced wines, with high alcohol content and lack of color, with green tannins, astringency and excessively low acidity if not corrected.
The use of elicitors has been shown to lead to an increase in the concentration of polyphenols in grapes and given these observations, the objective of this work was to evaluate the efficacy of the foliar application of three different chemical elicitors (Lalvigne Mature, Harpin αβ and Bion) to increase and accelerate the synthesis of phenolic compounds in the treated grapes, allowing harvesting them with less sugar and, therefore, allowing obtaining i wines with less alcohol.

Material and methods

The study has been carried out for three consecutive years (2019, 2020 and 2021) in a vineyard of 'Monastrell' grapes, located in the D.O. Jumilla (Murcia, Spain). Three different commercial elicitors were applied by foliar spray, Lalvigne Mature (yeast derivative, Lallemand), Harpin αβ (a protein molecule that consists of four fragments from other harpin proteins found in bacterias that cause diseases in plants, Plant Health Care) and Bion (benzothiadiazole, Syngenta). In 2019 and 2020, two applications were made, at veraison and 15 days later. In 2021 only Harpin was applied and a third dose was added 15 days after the second foliar application.
Grapes from each treatment were vinified following a traditional red winemaking process and the physicochemical and chromatic parameters were analyzed at the time of bottling.

Results

The results show that treatment with elicitors can be an effective tool to alleviate the consequences of climate change in the enological sector, obtaining wines with 8-15% less alcohol, but with a polyphenol content similar or even greater than that of a wine made from control grapes without any treatment and similar to that obtain in wines from control grapes harvested with a higher ripening degree.
These results vary according to the elicitor used and the year of treatment and its climatological characteristics. The Harpin elicitor is the most consistent in its results year after year, being very effective when three treatments are applied to the grape separated from each other 15 days from each other (harvest of 2021). When this elicitor was applied, wines presented around 15% less alcohol than a wine made from the most ripen control grapes, and with similar chromatic parameters, even more polymeric anthocyanins were measured, which may help to the stability of the color in these wines.

Keywords: Climate change, Polyphenols, Grape ripening, Wine quality

1. Introduction

The quality of the wine is determined by numerous factors, among which the quality of the grape and its maturity state, although not only technological maturity (this related to the sugar content of the pulp) but also phenolic and aromatic maturity. The harvest date will be defined by a balance between the different maturation characteristics.

The general increase in temperatures that is occurring in recent years due to a climate change is having an impact on the wine industry, causing an acceleration of the grapes technological maturity and a delay in phenolic maturity (Keller, 2010), since temperatures above 30ºC may affect the synthesis of anthocyanins. Therefore, when technological maturity is reached (expressed as the level of sugars in the pulp), the skin and seeds may remain unripe from the phenolic point of view, and wines from these grapes will present low color. However, if harvest is delayed for reaching a correct phenolic maturity, grapes will accumulate high content of sugars and that will lead to a high alcohol content in wines.

Therefore, it seems clear that climate change and the effects it causes on the vine will lead to unbalanced wines, with high alcohol content (that can exert a number of negative effects that pose microbiological, technical, sensory and economic challenges) and lacking in color, with green tannins, high astringency and low acidity, if the later is not corrected during the first steps of vinification (Mira de Orduña, 2010).

In response to the important challenge of mitigating the effects of climate change on the vine, different techniques have been studied in recent years, including both agronomic practices and oenological techniques, looking for a better balance between the technological and phenolic maturity of the grape and for improving the composition and final quality of the wines (Novelo and de Palma, 2013, Dequin et al., 2017).

Our research group focused the attention on the use of chemical elicitors, agrochemicals which were primarily designed to improve resistance to plant pathogens, by triggering plant defense mechanisms, one of which is the increase the levels of phenolic compounds. As chemical elicitors, various compounds and preparations have been used looking for an increase in phenolic compounds. Liu et al. (2005) found how the use of benzothiadiazole (active ingredient of the commercial product BION) in fruits such as mangoes or peaches induced the activation of the enzyme phenylalanine amino lyase, causing an increase in the content of phenolic compounds. The use of benzothiadiazole in grapes has also been shown to increase the biosynthesis of resveratrol precursors and anthocyanins (Ruiz-García et al.,2012) and the biosynthesis of volatile compounds (Vitalini et al., 2014).

Some proteins, such as harpin proteins, activates the plant's growth and defense genes resulting in improved plant growth, increased yield and quality. The use of Harpin protein has shown to increase the concentration of polyphenols by activating the enzyme phenylalanine amino lyase (Qiao et al., 2010). It has also been used in other fruits such as passion fruit (Boro et al., 2011) and pear (Saour et al., 2010) as a pre-harvest treatment to control diseases caused by pathogens, and postharvest to prevent their deterioration.

Also, yeast extracts contain several compounds that may act as elicitors. In this respect, yeast cell walls are made up of mannoproteins, glucans and chitin, and most of these compounds are regarded as triggers of various modes of plant defense. Portu et al. (2016) showed that foliar treatments carried out with yeast extract increased grape and wine anthocyanin and stilbene content when compared to a control.

Given these previous results, the objective of this work was to evaluate the efficiency of the application of three different chemical elicitors to Monastrell grapes, to determine if they increased the phenolic compounds and chromatic characteristics in wines and if, by using them, it was possible to accelerate phenolic maturity and by harvesting grapes with lower sugar content, to obtain quality wines with lower alcoholic content.

2. Material and methods

2.1 Experimental design

The study has been carried out for three consecutive years (2019, 2020 and 2021) in vineyards of the Monastrell grape variety of the DO Jumilla. The compounds used have included:

- LalVigneTM Mature (Lallemand Bio S.L., St Simon, France), a compound consisting of 100% specific fractions of derivatives of Saccharomyces cerevisiae. It acts exogenously stimulating the plant causing an increase and acceleration of the biosynthesis of secondary metabolites (phenolic and volatile compounds), positive for the quality of the wine, contributing to an improvement of phenolic maturity.

- Harpin αβ (Plant Health Care S.A., Madrid, Spain), a protein of bacterial origin, discovered as a by-product of Erwinia amylovora, which is a combination of 4 different proteins HrpN (Erwinia amylovora), HrpW (Pseudomonas syringae), HrpZ (Pseudomonas syringae), and PopA (Ralstonia solanacearum).
- BION® 50WG (Syngenta S.A., Santiago, Chile), chemical inducer composed of the active substance acibenzolar-S-methyl, of the benzothiadiazole family, which works as an analogue of salicylic acid, that is, activates the defense process mediated by salicylic acid, this process is called Systemic Acquired Resistance (SAR).

The first and second year of the study (2019 and 2020) the three elicitors were applied in the vineyards, each in a part of the vineyard, leaving untreated rows that served as Control. The three elicitors were applied at a dose of 1 kg / hectare, and two applications were made: at the beginning of veraison, and 10-15 days after the first application. The third year (2021) only the Harpin αβ elicitor was applied and an extra application was added, 15 days after the second application. Each year, treated and control grapes were harvest when grapes reached 23 Brix. Also, a second harvest of control grapes were done when grapes reached 25 Brix.

2.2 Vinifications

Each type of grape, separately, underwent a normal winemaking process and the physicochemical and chromatic parameters at the time of bottling were analyzed in triplicate.

2.3 Analytical determinations

The wines were characterized by measuring the alcoholic strength, pH and total acidity, according to official methods of the OIV (2004). The chromatic analyses, determined by spectrophotometry, were as follows: color intensity, total polyphenol index (Ribéreau-Gayon et al., 2006), total and polymeric anthocyanins (Boulton, 2001) and total tannins (Smith, 2005).

3. Results and discussion

The chromatic characteristics of all the studied wines have been evaluated at bottling. The characteristics of the wines made with the elicitor-treated grapes were compared with the two wines made with non-treated grapes (control wines) harvested both on the same date as elicitor-treated grapes (23º Brix aprox, C23) and when grapes reached 25ºBrix (C25).

Table 1 shows the results of the physicochemical and chromatic parameters for each of the wines produced in 2019 and 2020. In 2019, it can be observed that some small differences in the alcoholic percentage were observed among the wines made with treated grapes, with Lalvigne being the elicitor with which the lowest alcoholic percentage was obtained with no significant differences with the control wine C23. The pH and total acidity are not affected with the use of elicitors, although it should be noted that, due to the low acidity presented by the grapes at the moment of crushing, it was adjusted to a value of 5.5 mg / L. As for the chromatic parameters, the use of the different elicitors increased the wine total polyphenol content and the concentration of tannins polymeric anthocyanins in their wines, if we compare them with the control wine of the same harvest date. The color intensity of the wines from treated grapes was similar to that of control grapes except for the wine made with Harpin-treated grapes, with which a wine with higher color intensity was obtained.

Comparing the characteristics of the wines made with the treated- grapes and the control wine from the second harvest (C25), we could observe, as expected, that C25 presented between 8 and 13% more alcohol, but its color intensity was similar to that of the wine made with the less ripen grapes treated with Harpin and its content in total polyphenols and anthocyanins was similar to all the wines from treated grapes.

Several authors have found that the use of elicitors increased the wine color. For example, the use of Lalvigne showed a notable impact on the phenolic maturity of grapes, causing an increase in the concentration of anthocyanins and tannins (Portu et al., 2016). Cruppi et al. (2020) also found that Harpin treated grapes increased their color and bioactive compounds.

When the experience was repeated in 2020, it could be observed that those wines made with the less ripen grapes had similar alcoholic percentage, except the wine made with grapes treated with Lalvigne, which has resulted in a wine with a slightly higher alcoholic content.

As regards the chromatic characteristics, an increase in color intensity was observed when grapes were treated with Lalvigne and Harpin whereas total phenols and anthocyanins were higher in the wines made from grapes treated with Harpin and Bion, and, contrary to 2019 findings, all the wines from treated grapes presented lower concentration of tannins than C23 wine. Although treating the grapes with BION increased total phenol content in wine, color intensity did not increase and these findings differ from the results found by Ruíz-García et al. (2012), who found that the application of benzothiadiazole (the active ingredient of BION) increases the amount of total anthocyanins, as well as an increase in color intensity in wines made with grapes treated with this elicitor with respect to a control wine made with untreated grapes.

Table 1: Physical-chemical and chromatic parameters of the different wines in two years of study (2019 and 2020)

		Alcohol (%)	pH	Total acidity	CI	TP	TA	PA	TT
2019	C23	12.66±0.00 a	3.61±0.01 b	6.43±0.30 b	10.54±0.12 a	44.84±1.32 a	446.38±5.29 b	21.59±0.44 a	1148.77±44.05 a
	Lalvigne	12.46±0.04 a	3.63±0.02 b	6.25±0.24 b	10.88±0.80 a	55.61±1.93 c	427.21±40.21 a	29.78±1.35 c	1713.72±56.83 c
	Harpin	13.18±0.08 c	3.57±0.03 b	6.32±0.20 b	11.70±0.10 b	51.62±2.23 b	432.72±10.73 b	30.13±2.95 c	1488.26±79.65 b
	Bion	12.85±0.04 b	3.50±0.01 a	6.45±0.26 b	10.34±0.58 a	52.03±1.57 b	409.11±8.39 a	25.93±1.31 b	1425.70±127.98 b
	C25	14.37±0.30 d	3.79±0.03 c	5.83±0.15 a	11.14±0.01 b	52.12±0.11 b	448.09±7.63 b	26.50±0.12 b	1233.00±113.46 a
2020	C23	11.77±0.08 a	3.91±0.03 b	4.79±0.21 a	8.25±0.14 a	32.52±0.71 b	143.50±7.56 b	20.22±1.34 c	1001.40±74.89 b
	Lalvigne	12.08±0.04 b	3.80±0.01 a	5.49±0.18 c	9.24±0.16 b	28.65±0.01 a	129.85±0.64 a	13.60±0.80 a	862.75±6.99 a
	Harpin	11.80±0.08 a	3.86±0.01 a	5.22±0.00 b	10.40±0.48 b	34.90±1.61 b	197.33±6.28 c	19.62±0.20 c	851.85±41.14 a
	Bion	11.49±0.14 a	3.86±0.06 a	5.56±0.21 c	8.47±0.07 a	36.69±0.58 c	145.95±4.91 b	15.02±0.80 b	904.95±18.77 a
	C25	13.58±0.05 c	3.80±0.07 a	6.34±0.04 d	9.23±0.18 b	42.44±0.25 d	316.93±19.63 d	21.22±1.68 c	990.53±116.24 b

CI, color intensity; **TP,** total polyphenol; **TA,** total anthocyanins (mg/L); **PA,** polymeric anthocyanins (mg/L); **TT:** total tannins (mg/L)

Keeping in mind that the objective of this study was to obtain wines with a lower alcoholic content without affecting their chromatic parameters, the treatment of the less ripen grapes with elicitors presented an alcohol content that was 12% lower than the control wine of the second harvest (C25), but they presented similar or higher color intensity, especially the wine made with grapes treated with Harpin, which, in addition, achieves higher values of total phenol content.

Table 2 shows the results of the physicochemical and chromatic parameters for each of the wines produced in 2021.

Table 2: Physical-chemical and chromatic parameters of the different wines in 2021

	Alcohol (%)	pH	Total acidity	CI	TP	TA	PA	TT
C23	13.48±0.07 a	3.66±0.01 b	5.05±0.03 a	13.46±0.18 b	49.83±0.72 a	482.23±2.11 a	22.72±0.33 a	1463.62±0.85 b
Harpin 2T	13.28±0.28 a	3.65±0.01 b	5.17±0.04 a	12.27±0.14 a	47.98±0.18 a	475.51±13.3 a	28.34±0.19 b	1201.58±15.75 a
Harpin 3T	13.17±0.33 a	3.57±0.02 a	5.26±0.06 b	14.23±0.14 c	52.31±0.39 b	534.01±16.76 b	26.31±0.99 b	1462.63±28.24 b
C25	15.39±0.02 b	3.79±0.02 c	5.22±0.13 b	14.92±0.04 c	54.32±1.16 b	502.48±4.27 b	28.91±0.71 b	1433.35±21.79 b

CI, color intensity; **TP,** total polyphenol; **TA,** total anthocyanins (mg/L); **PA,** polymeric anthocyanins (mg/L); **TT:** total tannins (mg/L)

Based on the results obtained from previous years, this year it was decided to apply only the elicitor Harpin αβ, and to add an extra application of the elicitor to grapes, thus in 2021 we compared four different wines, two control wines (C23 and C25) and with the less mature Harpin treated grapes two different wines, with only two Harpin applications or three Harpin applications. The normal application of Harpin (two applications of the elicitor) only increase the polymeric anthocyanin content of the wines and no increase in total phenol content or tannins were observed with respect to the control wine of the same harvest date (C23). However, when the elicitor was applied three times, a wine with a similar concentration of tannins and a greater amount of polyphenols and total and polymeric anthocyanins was obtained, which resulted in a greater color intensity. In addition, if we compare this wine with the wine made with grapes from the second harvest (C25), we obtain similar values in all chromatic parameters, but the wine from Harpin with three treatments having around 14.5% less alcohol.

4. Conclusions

The present work aimed to determine, in three consecutive years (2019, 2020 and 2021) if three commercial chemical elicitors, Lalvigne mature, Harpin αβ and Bion, are able to accelerate the phenolic maturity of grapes to make it possible to harvest grapes with lower sugar content and, thus, to obtain high colored wines with less alcohol content. The results showed that treatment with elicitors could be an effective tool to alleviate the consequences of climate change in the oenological sector, obtaining wines with between 11 and 15% less alcohol, but with a polyphenol content similar or greater than a control wine without treatment, although these results vary according to the elicitor used and the climatological characteristics of the year of treatment. The elicitor Harpin presented always positive results, being most effective when three treatments were applied to the grape separated each other 15 days. With this elicitor, wines presented around 15% less alcohol than a control wine from grapes harvested at an advance maturation stage with similar chromatic parameters. Moreover, the wines from Harpin treated grapes presented more polymeric anthocyanins, which may improve the wine color stability during wine aging.

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