Soil application of hydrolysed protein based biostimulants coupled with microelements in Friuli Venezia Giulia vineyards

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Abstract. Soil pills biostimulants were tested for two following seasons in two vineyards grown with Sauvignon blanc and Glera, respectively, in comparison with an equivalent chemical fertilisation. The plant water status was slightly improved in case of Sauvignon blanc that experienced in both seasons conditions of severe water stress, resulting in higher acidity and lower pH. The Glera vineyard did not express the same improvements, likely because being under irrigation, it did not experience any drought during both seasons. The soil pills showed also a positive effect on wine sensory properties, improving the tropical descriptor of Sauvignon blanc wines, and the flavour descriptors of Glera.

1 Introduction

The EBIC (European Biostimulants Industry Council) defines that biostimulants affect interactions inside the plant and between the plant and the beneficial microorganisms around it. When applied to plants or to rhizosphere, biostimulants can stimulate the natural processes improving nutrients absorption and efficiency, tolerance to abiotic stresses and crop quality.

Biostimulants derive from biological or inorganic matrices such as fermenting microbes from animal or plant matrices, microbial cultures, macro- and microalgae, protein hydrolysates, humic and fulvic acids, compost, manure and industrial waste [1]. The subgroup of hydrolised proteins includes all those compounds consisting of polypeptides, oligopeptides and amino acids [2] obtained from animal or vegetable processing waste following chemical and/or enzymatic hydrolysis [3-5].

Plants can absorb amino acids both through roots [6] and leaves [7]. The application of protein hydrolysates on leaves and roots increases root absorption and improves the water and the nutrients use efficiency [8-10]. The vine plants absorption of mineral elements occurs in different timings along the growing season; nitrogen is mostly needed before flowering for plant growth, while potassium, calcium, magnesium and boron are highly absorbed after berry-set until veraison. Moreover, usually the supply of nitrogen in a single application could increase plant vigour with several problems of canopy management during the summer season, and grape sanity at harvest because of the development of rots.

Given the high solubility of biostimulants based on hydrolysed protein and the consequent fast assimilation of amino acids by soil microorganisms [11], one of the challenges for the future could be to prolong the biostimulant effect by modulating the release of nutrients over time. There are two possibilities to match this objective, namely increase the density by pressing the granules, and ii. coating the granules with a layer of hydrophobic matrices.

SICIT GROUP SPA has developed and tested various techniques to obtain a coating allowing a smart controlled release of hydrolysed protein sand nutrients over time, and the aim of the experiments developed was to verify the effects of soil application of such formulates on plant physiology, grapevine yield and maturation, and wine chemical and sensory characteristics.

2 Materials and Methods

Three different soil biostimulant formulates with controlled release were studied (SR-BTB, short-time release; MR-BT, medium-time release; LR-BTB, long-time release biostimulant). The formulates are based on a nuclear structure characterised by a concentration of nitrogen ranging from 4.43 and 4.74%, enriched with magnesium (1.27-1.30%), boron (10.5-10.9%), iron (0.34-0.36%) and sulphur (19.8-24.2%). The three formulates were first studied at laboratory scale, in order to understand the release kinetics of nitrogen and also the other four co-formulated mineral elements.

Release patterns of different formulations have been carried out in water according the method EN 13266 [12]. Pills have been solubilized until all reached 90% of total N.

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2.1 Field experiments

With the aim to understand the effects of the formulates at field level, two experiments were carried out during the seasons 2021 and 2022. A vineyard of Sauvignon blanc (clone R3) was selected in San Floriano del Collio (Collio DOC area), and was characterised by a loam-silt-clay texture with negligible coarse. The plant density accounted for 5050 plants/ha (2.2 m x 0.9 m between rows and along the row, respectively), and the vines were trained using a Guyot pruning system. Differently, a vineyard of Glera (clone ISV-ESAV 14) was selected in Cordenons (Friuli Grave DOC area), where the texture is loam-sand with abundant coarse. In that vineyard the plant density was 4166 plants/ha (3.0 m x 0.8 m between rows and along the row, respectively), and the vines were trained using a double arched "Cappuccina" pruning system. This vineyard was watered constantly during both seasons with a drip irrigation system, to avoid conditions of water stress that are negative for sparkling wine production.

In both vineyards a completely randomised experimental design was set, with 4 replicates of 15 vines for each treatment in comparison. Two treatments were compared: BT, soil pills, mixture containing30%, 40% and30%of SR-BT, MR-BT and LR-BT, respectively at the dosage of150 kg/ha of soil pills mixture (6.9, 11.5,16.0, 2.0, 0.5 kg/ha of N, Mg, S, Fe, B, respectively); CHE, chemical fertilisation adding the same units of N, Mg, S, Fe and B. The application of both BT and CHE treatments was made at pre-flowering time (18 May 21 and 18 May 22), by distributing manually the different granules in the single parcels in case of BT, and dissolving the chemical fertilisers in water and distributing the solution in case of CHE. Would be interesting to have a soil analysis because if nitrogen is abundant in the soil probably the effect of fertilization is negligible.

During the growing season, measurements of shoot growth and plant water status (stem water potential, Ψ_{STEM}) were carried out. To measure Ψ_{STEM}, fully expanded (mature) leaves were bagged and covered with aluminum foil one hour before the measurement and then excised with a razor blade. The leaves were then placed in a Scholander–type pressure chamber (©Soil Moisture Co., Santa Barbara, USA) with the petiole protruding from the chamber. The leaf was pressurized using a nitrogen tank, and Ψ_{STEM} was recorded in a few seconds when the initial xylem sap emerged from the cut end of the petiole.

From veraison on, 50-berry samples were collected from each replicate to analyse the trends of maturation till harvest. Total soluble solids (°Brix) and pH were measured using a manual refractometer (ATC-1, Atago, Tokyo, Japan) and a pH meter (HI2211, Hanna Instruments, Woonsocket, RI), respectively. Titratable acidity was determined by titration of the juice with0.1 M NaOH until pH 7.

2.2 Vinification, aroma and sensory analysis

At the time of harvest, the grapes were collected, and the yield parameters were determined on 10 vines/plot (number of clusters and average cluster weight).

Approximately 30 kg of grapes were picked and immediately transported to the experimental winery of the University of Udine. The grapes were pressed at 2-bar pressure using the A20 pneumatic press provided by Grifo Macchine Enologiche (Piadena, CR, Italy).15-10 L of grape juice, were placed in glass carboys, where the vinification was initiated by inoculation with Premium® Prosecco (Enologica Vason S.p.A., Verona, Italia), at a temperature set at 18 °C. At the end of fermentation, the wines were subjected to tartaric stabilization (about two weeks at 4 °C). As regard Glera, the base wines were processed to obtain sparkling wines using the Martinotti–Charmat method carried out in 7-L stainless-steel autoclaves, maintained at 18 °C. More details are reported in [13]. In January, at the end of vinification, both Sauvignon blanc and Glera wines were bottled.

The sensory evaluation of wines produced was undertaken for each year separately, approximately nine months after bottling. The wines were evaluated by a median panel of ten people. The panellists were recruited between researchers and students from the University of Udine, as well as local oenologists and producers, well acquainted with the studied cultivars and relative wines. The wines were purchased randomly to the panel.

2.3 Statistical analysis

t-test ($p<0.05$) was used to test the differences between treatments separately for each date in case of Ψ_{STEM}, and at harvest for yield and grape basic maturation parameters. Data of aroma and sensory analysis were processed through principal component analysis using R software [14].

3 Results and discussion

Release patterns for N of the three formulations are reported in Fig. 1.

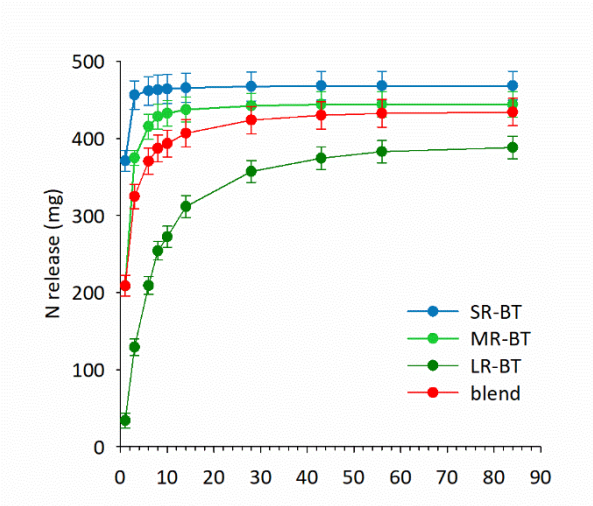

Fig. 1. Release pattern of nitrogen in water. SR-BT, MR-BT and LR-BT are short-, medium- and long-time release tablets, respectively, while the blend represent the release time of the mix of tablets used in the field experiments. Bars represent standard deviation (n=3).

SR-BT and MR-BT were remarkably similar, reaching 75% of N released in 1 and 2 days, respectively. The LR-BT, conversely, showed a much slower kinetic, reaching 75% of N release in 19 days. The release pattern of other macro-nutrients (K, S, and Mg) and micro-nutrients (Fe and B) was similar to that of N (results not shown). Moreover, the blend tested in the experiments revealed a release pattern related to the relative composition in SR-BT,MR-BT and LR-BT.

The season 2021 started with a rainy spring, while in the summer months the temperatures increased above the average values of the period (Fig. 2A). From the end of July, the values of Ψ_{STEM} revealed a condition of severe water stress on Sauvignon vines (Fig. 3A). Even if the difference was not significant, a better water status was observed in case of the BT treatment as compared to CHE. The rainy period at the beginning of the season allowed the soil biostimulants to solubilizethus releasing the mineral elements according to the coating formulation. As regards the vineyard of Glera, the application of water with irrigation maintained a good plant water status and no differences were observed during the three measurements carried out in 2021 (Fig. 3B).

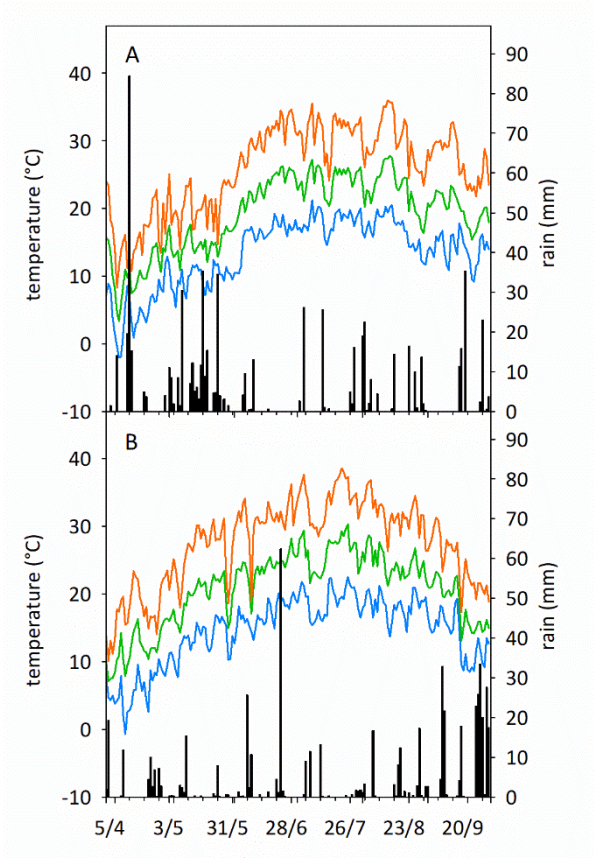

Fig. 2. Trends of meteorological conditions during the season 2021 (A) and 2022 (B) from at the weather station of Capriva del Friuli (ARPAFVG–OSMER, http://www.meteo.fvg.it/). Orange, green and blue lines report maximum, average and minimum temperature (axis on the left), while vertical histograms indicate daily rainfall (axis on the right).

In the second season (2022), the rain was much lower (Fig. 2B), and the lack of water created higher resilience in the grapevines; however, at the end of August the values of Ψ_{STEM} showed again a condition of severe water stress similar to the previous season in case of Sauvignon blanc (Fig. 3C). The limited rain occurred during spring possibly have not allowed the solubilization of soil biostimulants, thus the effects on water status were less evident than in the season 2021.Once again, looking at the plant water status of the Glera grapevines, the values of Ψ_{STEM} were less negative not highlighting conditions of water stress (Fig. 3D).Our results demonstrated that to express the whole effect of BT, it is crucial to have good moisture conditions after their soil incorporation, eventually with irrigation if the season is dry in that period, since it allows the solubilisation of the mineral elements.

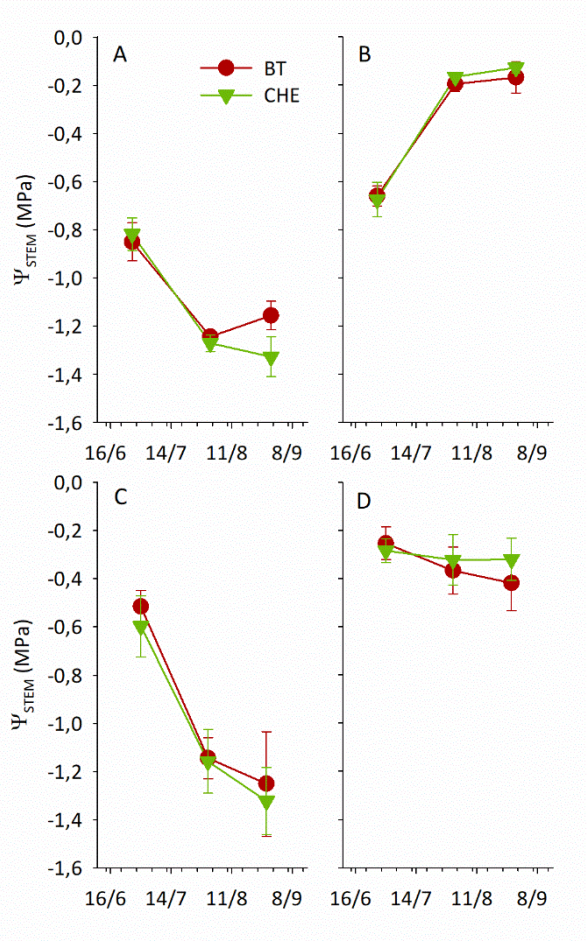

Fig. 3. Trends of Ψ_{STEM} of Sauvignon blanc (A,C) and Glera (B,D) in the seasons 2021 (A, B) and 2022 (C,B). Bars represent standard deviation (n=4).At each date, data were processed with *t*-test ($p<0.05$), and significant differences were not ascertained.(The lack of differences is probably due to the irrigation)

At harvest time yield parameters have been collected (Tab. 1). In particular, the yield was really low for Sauvignon blanc in 2021, due to a poor berry-set that resulted in a very low cluster weight average. As opposite in the following season, the yield parameters

were consistent with the average parameters of the variety in the area. As regards Glera, in both seasons the yield was much higher, both because of the variety characteristics, the pruning system adopted and the use of irrigation. Although the differences between CHE and BT were not significant, slightly higher number of clusters and yield were ascertained in both years in case of Glera, and in 2021 for Sauvignon blanc.

As regards basic maturation parameters (Table 1), the highest values of soluble solids were measured on Sauvignon blanc, particularly in the season 2021 when the yield was really low, and slightly lower values of soluble solids have been analysed on grapes in case of BT. Most probably, the better water status of the BT vines allowed for a reduced occurrence of berry shrinkage, thus soluble solids were not diluted. A lower sugar accumulation translates into a reduced alcohol level in wines, a condition that nowadays also matches the requests of the wine consumers. Moving on Glera, slightly higher values of soluble solids were measured in case of BT treatment. As far as this variety is concerned, the winegrowers are aware that an increase in the accumulation of sugars is welcome in order to ensure to reach at least 9.0 degrees of alcohol as requested by the production rules. To date, the increase of the soluble solids was 3.8 and 5.6%, in 2021 and 2022, respectively, values that are in line with what requested by the above mentioned rules.

Table 1. Yield and basic maturation parameters as affected by BT application in Sauvignon blanc and Glera. CHE, chemical fertilisation; BT, soil tablet. Data analysed with *t*-test ($p<0.05$; n.s., not significant differences).

variety	year	treatment	cluster number	yield (kg/vine)	cluster weight (g)	solublesolids (°Brix)	titratableacidity (g/L)	pH
Sauvignon blanc	2021	CHE	13.86	0.60	43.01	24.03	5.38	3.27
		BT	13.99	0.59	42.62	23.63	5.80	3.19
		Sign. t	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	2022	CHE	16.80	2.93	177.04	19.55	6.06	3.34
		BT	18.69	2.82	151.68	19.23	6.02	3.38
		Sign. t	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Glera	2021	CHE	17.47	7.57	438.39	16.33	7.75	3.26
		BT	19.64	8.13	419.98	16.78	7.42	3.27
		Sign. t	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	2022	CHE	15.90	6.00	382.19	15.28	6.36	3.34
		BT	16.50	6.35	403.42	16.13	6.13	3.33
		Sign. t	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Titratable acidity is another important grape maturation parameter that accounts for wine quality. The application of BT allowed to maintain a 8.8% higher concentration of organic acids in case of Sauvignon blanc, while in the following season the same parameter was similar between the two treatments. As regards such result, the rain at the beginning of the season 2021 facilitated the solubilisation of the BT pills, positively affecting the maintenance of a higher level of acidity. As opposite, the grapes of Glera presented a slight reduction of the titratable acidity in both seasons when the vines were treated with BT. This result is not negative since the levels of such parameter were higher than in case of Sauvignon blanc, and the reduction has to be considered in any case optimal for the quality of the wines.

At last, the values of pH were slightly lower in the season 2021 for the variety Sauvignon blanc treated with BT. Together with higher titratable acidity and lower soluble solids, this is another important result since a lower value of this parameter accounts for a better freshness and a better stability of the wines during aging.

In the literature, there are only few articles reporting the effects of hydrolised proteins on grapevine performance, while there are many experiences carried out in horticultural crops. In particular, in the reference [15] the authors reported that the average cluster weight was improved with high rates of hydrolysed protein applications, and low dosages were instead responsible for an increase of soluble solids in grape berries. In a study carried out on Merlot in Northern Italy, a season-dependent effect on yield was revealed [16]; indeed, in one season, the hydrolysed proteins accounted for a significant increase of yield accompanied by a reduction of soluble solids, but in the following season both parameters, although showing similar trends, reported a negligible effect of the applied hydrolysed protein.

When the wines were served to the panel of experts, the evaluation have ascertained, in the case of Sauvignon blanc in 2021, slightly higher values of the descriptors body and tropical for BT, while, as opposite,

box tree, citrus, persistence and olfactive intensity were more perceived in CHE wines (Fig. 4). In the following season (Fig. 5), because of the higher temperatures and low rainfall during the summer, the expression of the different wine descriptors was generally lower than in 2021. Moreover, the wines of the BT treatment reported a slightly higher olfactive intensity. The results obtained for this variety are in line with what already discussed above. In particular it was clear the effect of BT in the first season 2021, when the rains at the beginning of the season facilitated the solubilisation of the minerals from the pills allowing the maintenance of a better plant water status and so also the biosynthesis of aroma compounds.

As regards Glera, the panel of experts could not discriminate between the wines in neither of the seasons examined in terms of olfactory and flavor descriptors. In any case, in both seasons the panelists showed more foam height and less bubble sizes in case of the BT treatment, and this aspect positively affected the perception of the flavour. The outcome related with the foam need to be further examined in future experiments in which hydrolised proteins will be used.

4 Conclusions

The results obtained with soil application of hydrolised protein as coated soil pills showed an improvement of the plant water status in case of Sauvignon blanc that experienced in both seasons conditions of severe water stress, resulting in better grape quality parameters (i.e. higher acidity and lower pH). The Glera vineyard did not express the same improvements, likely because being under irrigation, it did not experience any drought during both seasons.

As regards the yield and quality parameters, there was little effect of the pills application, and this result could be possibly due to the low amount of hydrolised protein used. Moreover, in our experiment we compare the effect of soil pills with a chemical fertilization in which the same amount of mineral elements was applied, thus a real effect of the treatment as compared with an unfertilized control was missing.

In the future experiments the hydrolysed proteins should be tested with different application rates in order to understand the minimum dosage responsible for a significant effect on plant water status, yield and grape quality parameters.

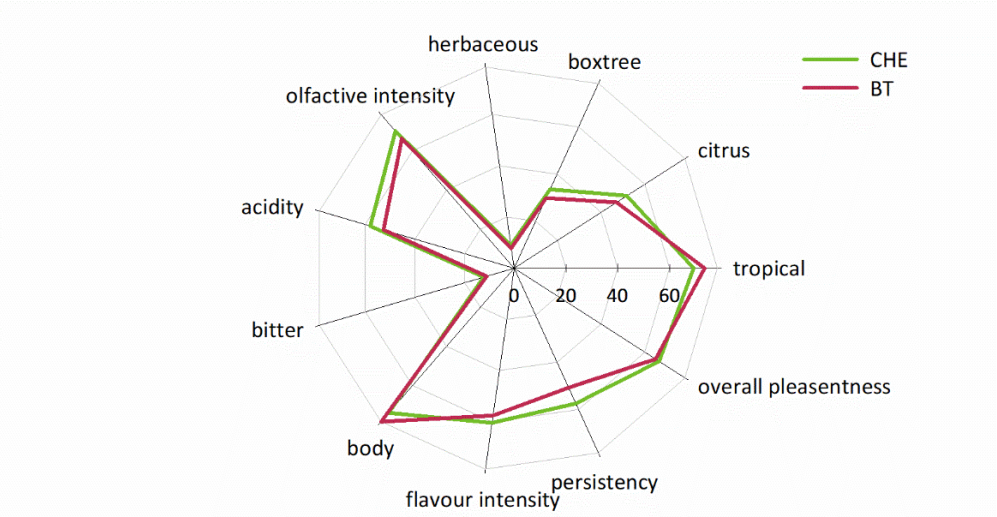

Figure 4. Radar chart representing the sensory evaluation of Sauvignon blanc wines of the season 2021. Differences between treatments were ascertained with *t*-test.

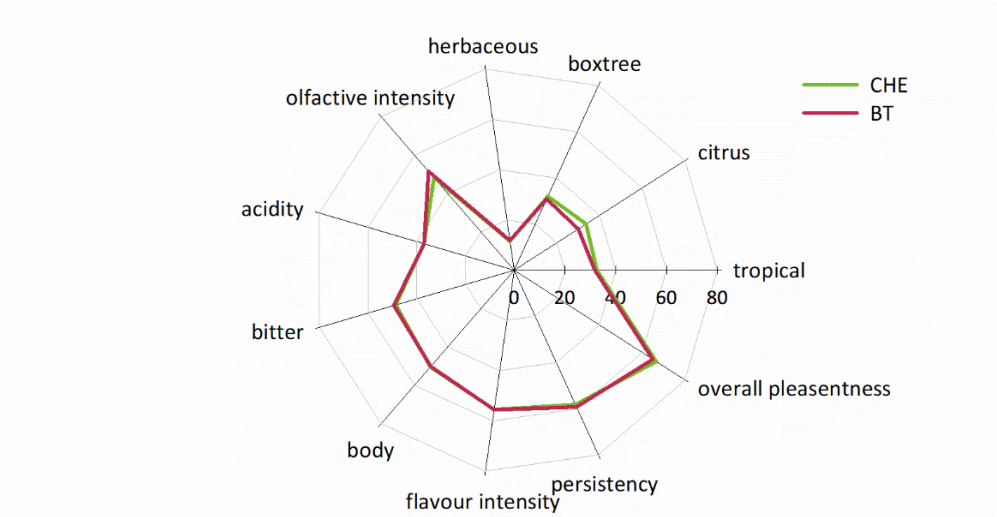

Figure 5. Radar chart representing the sensory evaluation of Sauvignon blanc wines of the season 2022. Differences between treatments were ascertained with *t*-test.

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