Monitoring of *Vitis vinifera* endophyte population

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Abstract
Due to increasing demands to agricultural production it is necessary to fine processes which will increase health of cultural plants and will have positive impact on landscape with potential to increase production. Our work is concerned on grapevine plants (*Vitis vinifera*) endophytic population. We monitor endophytic population in leaves and stems of four grapevine varieties Pinot Gris, Pinot Noir, Rhine Riesling and Müller Thurgau during year from two vineyards with different ways of wine production, biodynamic production and conventional with use of pesticides. Our concern is to monitor functional and structural changes in endophyte populations and its seasonal changes. Functional changes were determined by measurement of selected plant growth promoting properties of endophyte populations. For monitoring changes in population structure fatty acids methyl esters analysis was used.
Keywords: grapevine, endophyte

1. Introduction

Endophytic bacteria are bacteria living in inner parts of plants. They can have positive, negative or neutral impact on plant host, which also depends on actual conditions in which plant is cultivated. Presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase genes in endophyte population is not beneficial if plant is cultivated in optimal conditions. Same it is for nitrogen fixation and inorganic phosphates solubilization. If in soil is large amount of nitrogen or phosphorus bacteria that can provide plant with nitrogen by fixation of nitrogen from air or solubilize phosphates would not provide any benefit for plant. Therefore it is good to determine potential of endophytic population in case that conditions change to not optimal.

This could not be achieved only by determining community structure because not all strains of particular bacterial species have same set of genes. Bacteria of same species can also variate in metabolic capabilities. For this reason it is good to determine both structure of endophytic population and also metabolic potential of this population. This is reason why we monitor structure of endophytic population and also its properties which could have impact on grapevine plant. We choose to determine plant growth promoting properties, namely nitrogen fixation, inorganic phosphates solubilization, siderophores production and indole acetic acid (IAA) production. Changes in community structure were monitored by fatty acids methyl esters (FAMEs) analysis.

Till now biomass (leaves and stems) of grapevine was collected and analyzed in autumn 2018 and winter 2019 (only steams). For the future it is planned to collect and analyze biomass in spring, summer and autumn 2019.

2. Materials and Methods

Leaves and steams of grapevine plants were on vineyard collected into 10 mM $MgS0_4$ solution. Till surface sterilization samples of plant biomass were stored on ice. Surface sterilization of plant material was done by 6.25 % sodium hypochloride solution followed by 70 % ethanol solution and then washed 4 times in sterile distilled water. Surface sterilized plant material was air dried in laminar box and 10 g of material was homogenized in 50 ml of 0.2 M phosphate buffer (pH 7.1) after centrifugation (1000x*g*, 10 minutes) supernatant was used for inoculation of appropriate media. All media were cultivated at 24°C, 130 RPM in dark. During 7 days samples were collected and concentration of phosphorus (Joergensen et al. 1995), ammonia (Baethgen and Alley 1989), IAA (Loper and Schroth 1986) and siderophores production (Arora and Verma 2017) was determined. Second part of surface sterilized plant biomass was lyophilized, homogenized and was used for preparation of samples for FAME analysis on GC-FID (Fang et al. 2001). Concentrations of FAMEs were used for principal components analysis (PCA) in program Past 3.22.

3. Results

3.1. Nitrogen fixation

Nitrogen fixation was not recorded in any of endophytes extracted from grapevine samples collected in autumn 2018 or winter 2019.

3.2. Phosphates solubilization

Results in figure 1 indicate that there are no significant differences in phosphate solubilization activity.

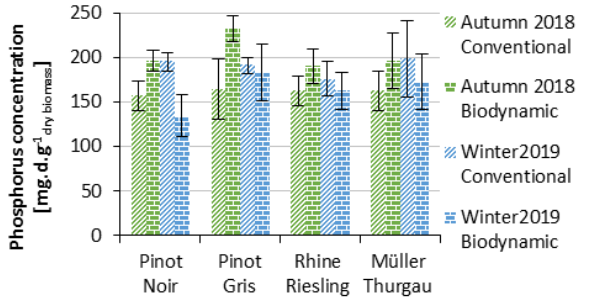

Figure 1. Ability of phosphate solubilization of endophytes in steams of tested grapevine varieties

3.3. Indole acetic acid production

In production of IAA (figure 2) were recorded differences between vineyards. Endophytes from three of four measured grapevine varieties produced more IAA if plants were treated conventionally, with use of pesticides.

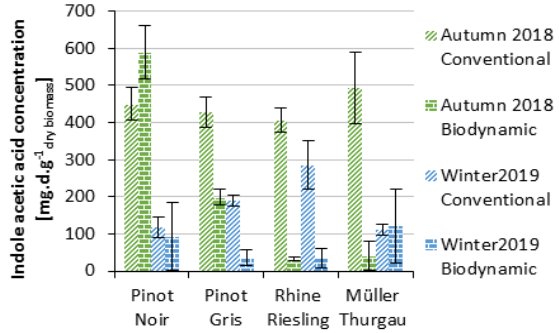

Figure 2. Indole acetic acid production of endophyte populations in steams of tested grapevine varieties

3.4. Siderophores production

Endophytes from plants collected in winter produced more siderophores than endophytes from plants collected in autumn (figure 3).

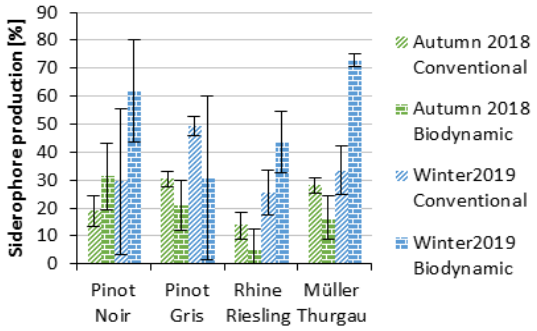

Figure 3. Siderophores production of endophyte populations in steams of tested grapevine varieties after 6 days of cultivation.

3.5. Fatty acids methyl esters analysis

From PCA (figure 4) can be concluded that endophyte community structure is impacted by season and part of plant more than grapevine variety and way of wine growing method in the vineyard.

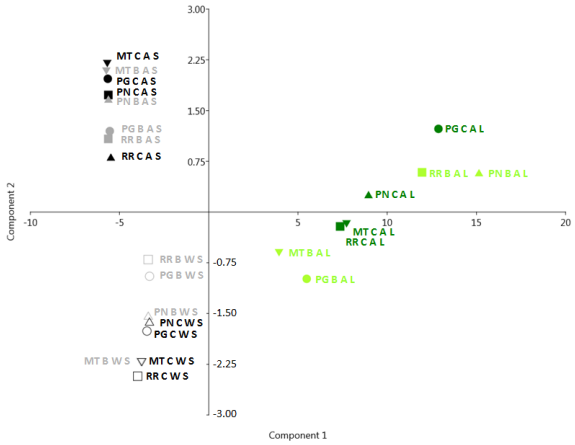

Figure 4. PCA of FAMEs. MT – Müller Thurgau, PG - Pinot Gris, PN - Pinot Noir, RR - Rhine Riesling, W – winter 2019, A – autumn 2018, C - conventional, B - biodynamic

4. Conclusions

In endophytes mixed population from grapevine steams were determined two activities connected with extracting nutrients from soil - phosphates solubilization and nitrogen fixation, one connected with biocontrol – siderophores production and one which influence plant growth and development – IAA. Changes in structure of endophytes community were monitored by FAMEs analysis. Till now data from only two seasons are available, more deep insight in endophyte grapevine population would be possible after whole year will be analyzed.

Acknowledgement
Authors would like to thank GA ČR 18-26463S for financial support.

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