Scientia Horticulturae 226 (2017) 285–292

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

# Electrical stimulation: An abiotic stress generator for enhancing anthocyanin and resveratrol accumulation in grape berry

Masachika Mikami[a], Daisuke Mori[b], Yoshiyuki Masumura[b], Yoshinao Aoki[a], Shunji Suzuki[a,*]

[a] Laboratory of Fruit Genetic Engineering, The Institute of Enology and Viticulture, University of Yamanashi, Yamanashi, Japan
[b] Environment Division, Nihonshinko Co., Ltd., Osaka, Japan

ARTICLE INFO

*Keywords:*
Abiotic stress
Anthocyanin
Electrical stimulation
Grapevine
Resveratrol

ABSTRACT

Plants are known to utilize electrical signals under several physiological conditions and electrical stimulation from the outside induces physiological changes in plants. In this study, to improve grape berry composition in field-grown grapevines, electrical stimulation of grapevine using solar panels was undertaken as an abiotic stress generator in 2015 and 2016 growing seasons. Electrical stimulation had a notable effect on grapevine growth and development as well as photosynthetic performance. Berry weight and tartaric acid and total phenolic contents in berries of grapevines exposed to electrical stimulation were similar among the grapevines tested. Brix in berries of grapevines exposed to electrical stimulation and electrode-treated grapevines was higher than that in control grapevines in both years. Electrical stimulation increased anthocyanin and resveratrol contents in berries of grapevines in both years relative to those of control grapevines and electrode-treated grapevines. The alteration of Brix and anthocyanin and resveratrol contents in berries was supported by the results of microarray analysis demonstrating the transcriptional upregulation of genes related to sucrose metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, stilbenoid biosynthesis, and anthocyanin biosynthesis in grape cells exposed to electrical stimulation. Taken together, the results suggested that electrical stimulation of grapevine enhanced anthocyanin and resveratrol biosynthesis by activating their biosynthetic pathways.

## 1. Introduction

One of the major goals of viticulture is to improve grape quality for winemaking. Grape quality is intricately influenced by sugars, acids, phenolics, and other chemical components, including aroma compounds (Lund and Bohlmann, 2006). Soluble solids and titratable acid contents in grape berries are used as ripeness criteria for wine grapes (Coombe et al., 1980). Anthocyanins, which are water-soluble flavonoids, are an important contributor to red wine color (Glories, 1978). Aroma compounds also play crucial roles in wine quality and taste. Grape berries contain various aroma compounds, such as terpenoids (Wirth et al., 2001) and thiols (Tominaga et al., 1998). As wines are "artistic" products of a complex mixture of chemical compounds in grape berries, the chemical compounds in grape berries are one of the targets for improving wine quality in viticulture.

A number of practical techniques were developed to improve grape quality for winemaking. Anthocyanin composition in grape berry skin was altered by leaf removal (Matus et al., 2009; Chorti et al., 2010) and cluster thinning (Guidoni et al., 2002). Leaf removal also changed amino acid composition through exposure of bunches to sunlight (Martínez-Lüscher et al., 2014). Training systems changed $C_6$ and $C_9$ volatiles in grape berry by altering fatty acid composition (Xu et al., 2015). Pruning and cluster thinning techniques to manipulate yield of grape bunches induced changes in wine sensory properties (Chapman et al., 2005). Girdling increased berry weight and enhanced anthocyanin accumulation in berry skins (Brara et al., 2008).

It has been reported that chemical compounds or microorganisms act as abiotic or biotic elicitors, respectively, to improve grape quality. Foliar treatments with methyl jasmonate and yeast extract increased grape and wine anthocyanin contents (Portu et al., 2016). Beneficial bacteria enhanced the accumulation of resveratrol, which is trans-3,4′,5-trihydroxystilbene, in grape berry (Aoki et al., 2017). As the application of abiotic and biotic elicitors to grapevine is easy and similar to fungicide application, this technique may be an alternative to improving grape and wine quality.

From previous studies, it became clear that abiotic stress applied to grapevine is responsible for the alteration of berry composition. In this study, we investigated the applicability of electrical stimulation to grapevine as an abiotic stress generator. Higher plants utilize electrical signals under several physiological conditions (Pickard, 1973).

*Abbreviation:* AP, action potential; PINII, proteinase inhibitor II; VP, variation potential

* Corresponding author at: Laboratory of Fruit Genetic Engineering, The Institute of Enology and Viticulture, University of Yamanashi, Kofu, Yamanashi 400-0005, Japan.
*E-mail address:* suzukis@yamanashi.ac.jp (S. Suzuki).

http://dx.doi.org/10.1016/j.scienta.2017.09.005
Received 25 July 2017; Received in revised form 31 August 2017; Accepted 4 September 2017
Available online 09 September 2017

Electrical excitability is frequently associated with response to environmental stimuli in higher plants (Fromm and Lautner, 2007). Electrical signals are classified into action potential (AP) and variation potential (VP). AP rapidly transmits electrical signals, whereas VP propagates electrical signals slowly. For example, AP in *Mimosa pudica* was transmitted within rachis at the speed of 20–30 mm $s^{-1}$ when the tip of leaf pinna was stimulated mechanically by touching (Fromm and Lautner, 2007). When *M. pudica* leaves were stimulated by cutting, VP was generated in the rachis, passing through the secondary pulvinus at the base of the pinna and moving into neighboring pinna at the speed of 5–6 mm $s^{-1}$ (Fromm and Lautner, 2007). Correspondingly, electrical stimulation from the outside induces physiological changes in plants. Treatment of tomato leaves with electrical current induced proteinase inhibitor II (PINII) gene transcription, leading to the alteration of stomatal aperture (Herde et al., 1995).

To date, no studies of the effect of grapevine electrical stimulation on berry composition have been carried out. Our objective is to evaluate the utility of electrical stimulation as an abiotic stress generator for improving grape quality. In the present study, we demonstrated that electrical stimulation of grapevine enhanced anthocyanin and resveratrol biosynthesis by activating their biosynthetic pathways.

## 2. Materials and methods

### 2.1. Plant materials

Field-grown grapevines and potted seedlings were used. *Vitis vinifera* cv. Merlot, grafted onto rootstock Kober 5BB, was cultivated in the experimental vineyard of The Institute of Enology and Viticulture, University of Yamanashi, Japan. The grapevines were approximately 30 years old and trained to the Guyot-style system. Self-rooted Merlot seedlings were cultivated in pots for approximately 2 months. Grapevine cultured cells prepared from meristems of *V. vinifera* cv. Koshu were maintained on modified Gamborg's B5 medium at 27 °C (Katoh et al., 2009).

### 2.2. Electrical stimulation of field-grown grapevines

Six grapevines were prepared for electrical stimulation from two weeks before flowering to harvest in 2015 and 2016 growing seasons (May 20 to September 9 in 2015 and May 9 to September 13 in 2016, respectively). Electrical stimulation was applied two weeks before flowering (Fig. 1A). Two electrodes (steel screws, 30 mm length) were screwed into the trunks of grapevines (at 20 and 60 cm above ground) and connected to two solar panels (upper, minus electrode; lower, plus electrode, Fig. 1B). The solar panels were set 2.5 m above ground (Fig. 1A). The solar panels had the following electrical characteristics: maximum voltage 5 V ± 5%, maximum current 80 mA ± 5%, and maximum power 0.4 W ± 5%. For the control experiment, grapevines with only electrodes (without solar panel) or without any treatment were also prepared. Each grapevine received the same treatment for electrical stimulation in both years.

### 2.3. Electrical stimulation of grapevine cultured cells

Grapevine cultured cells were grown at 27 °C for 2 weeks on modified Gamborg's B5 agar plates (Katoh et al., 2009). Electrical stimulation was applied by inserting two electrodes (0.5 mm diameter) into the cell mass and connecting them to a solar panel (Fig. 1C and D). Non-treated cultured cells were used as control. After 4 h electrical stimulation, the cell mass was frozen immediately in liquid nitrogen and stored at −80 °C for microarray analysis.

### 2.4. Chlorophyll content in leaves

To evaluate the photosynthetic performance of field-grown grapevines exposed to electrical stimulation, leaves were collected twice at véraison (July 24, 2015 and July 28, 2016, respectively) and harvest (September 9, 2015 and September 13, 2016, respectively). The fifth leaves from the bottom of shoot having 12–16 leaves were used. Three leaves were collected from one grapevine. Three leaf segments (approximately 1 $cm^2$) were cut out from one leaf and were incubated together in 1 ml of dimethylformamide at 4 °C for 24 h. Chlorophylls were measured with a spectrophotometer at 663.8 nm and 646.8 nm. Chlorophyll a + b contents were calculated according to a previously published report (Porra et al., 1989).

### 2.5. Berry characteristics

To compare berry characteristics of field-grown grapevines exposed to electrical stimulation with those of control grapevines, bunches having ripening berries were collected at harvest judged from both Brix and acidity of grape berries (Eichhorn-Lorenz Stage 38 on September 9, 2015 and September 13, 2016, respectively). Five bunches sampled from a grapevine were used for measurement of berry characteristics. Fifty berries were collected from each bunch. Berry weight was determined from the 50 berries and calculated as weight per berry. Juices were prepared by hand-pressing each bunch. Brix of juice was assessed with a refractometer (Atago, Tokyo, Japan). Tartaric acid was measured by titrating the juice with NaOH using an automatic titrator (Auto Titrator COM-1600, Hiranuma Sangyo, Ibaraki, Japan). After centrifuging the juice at 16,000 × g, the supernatant was filtered through 0.2 μm membrane filter (Pall, East Hills, NY) and the filtrate was subjected to further analysis. Measurement of total phenolics in the filtrate was performed according to a previously described method (Singleton and Rossi, 1965). The assay for resveratrol content was performed using HPLC as described previously (Aoki et al., 2017). Technical grade *trans*-resveratrol (Sigma, St Louis, MO) was used as the standard. To calculate resveratrol content in the juice, calibration curves were made by measuring various known concentrations of the standard solution.

Skins of 50 berries from each bunch were peeled off and used as sample. Anthocyanins in the peeled skins were extracted and measured as described previously (Yokotsuka et al., 1999). Anthocyanin content was converted into malvidin-3-glucoside equivalent as mg per gram of fresh skin weight.

### 2.6. Microarray analysis

Grapevine cultured cells were collected 4 h after electrical stimulation. The cells were homogenized in a mortar containing liquid nitrogen using a pestle. Total RNA isolation from the pulverized cells was performed with a Fruit-mate for RNA Purification (Takara, Otsu, Japan), followed by a NucleoSpin RNA Plant (Takara) according to the manufacturer's instructions.

Total RNA was subjected to microarray analysis using GeneChip *Vitis vinifera* (Grape) Genome Array (Affymetrix, Santa Clara, CA). Biotin-labeled cRNA synthesis using GeneChip 3′IVT PLUS Reagent Kit (Affymetrix), hybridization using GeneChip Hybridization, Wash and Stain Kit (Affymetrix), and signal detection using GeneChip Scanner 3000 7G (Affymetrix) were performed according to the manufacturer's instructions. Analysis of the signal intensity of each spot and signal evaluation and normalization were performed using Affymetrix GeneChip Command Console Software 4.0 (Affymetrix) and Affymetrix Expression Console Software 1.4 (Affymetrix). Genes upregulated by electrical stimulation were defined as follows: Background < 100, Fold change electrical stimulation/control > 2 (P-value < 0.01).

### 2.7. Statistical analysis

Data are presented as means ± standard deviations of ten biological replicates from two arbitrary grapevines. Statistical analysis was performed by Tukey's multiple comparison test using Excel statistics