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Research Article

# Electrical Stimulation as a Potential Technique for Enlarging Table Grape Berry Size by Enhancing Cell Division

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**Abstract:** Enlarging table grape berry size enhances commercial value. The texture and feel of large berries broken down in mouth are important considerations for consumer purchase in East Asia. We report herein that electrical stimulation enlarges table grape berry size by enhancing cell division. In two growing seasons, Shine Muscat and Pione, which are field-grown grapevines cultivated in Yamanashi Prefecture, Japan, were exposed to electrical stimulation using a solar panel from bud burst to harvest. Soluble solids content and total acid content in berries exposed to electrical stimulation were similar to those in untreated grapevines (control), whereas grapevines exposed to electrical stimulation had enlarged berry size irrespective of cultivar. Microscopic analysis of berry skins demonstrated that cell number per unit area of skin epidermis in grapevines exposed to electrical stimulation was higher than that of control, suggesting that electrical stimulation enlarged berry size by enhancing cell division but not cell elongation. This suggestion was supported by gene expression profiling of grape cultured cells

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exposed to electrical stimulation. Electrical stimulation upregulated the transcription of kinesin-like protein *KIN-5C* and nuclear pore complex protein *NUP88*, which regulate cell cycle and cytokinesis, respectively, but not expansins, which play a role in plant cell elongation. One of the merits of exposing grapevine to electrical stimulation is that the electrical stimulation may reduce labor hours and load compared with other techniques, such as irrigation and girdling for berry enlargement. These findings would help us develop a novel and innovative viticultural technique to enhance the commercial value of table grapes.

**Key words:** berry size; cell division; electrical stimulation; table grape; viticultural practices

## Introduction

Grape berry size is one of the determinants of table grape quality. The berry fresh weights of table grapes Kyoho (*Vitis vinifera* × *V. labrusca*), Pione (a tetraploid hybrid of *V. vinifera* × *V. labrusca*), and Shine Muscat (*V. vinifera* × *V. labrusca*), which are widely cultivated in Japan, are more than five times those of wine grapes (*V. vinifera*), such as Chardonnay and Merlot. For example, Kyoho berry measures approximately 3 cm in diameter and weighs 10 to 12 g. Because consumers prefer table grape cultivars having large berries as edible fruit, table grape berries have grown in size. The texture and feel of large berries broken down in mouth are the most important considerations when purchasing grapes in East Asia (Iwatani et al. 2011, Saito and Yamada 2003). Large berries are generally unpopular as wine grapes because an increase in berry size decreases total soluble solids in berries and anthocyanin contents in berry skins (Roby et al. 2004). Thus, unlike wine grapes, the cultivation of table grapes focuses on berry size at the expense of other berry characteristics.

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Berry enlargement is regulated by cell division and expansion (Carmona et al. 2008). Cell division by mitosis starts after anthesis and ceases approximately four to six weeks after anthesis. This is followed by cell expansion, which subsides near véraison. After véraison, berry enlargement is entirely due to cell expansion. The rate of cell division and expansion after anthesis appears to be one of the major determinants of the variation in berry fresh weight among grape cultivars (Houel et al. 2013). Viticultural techniques are known to increase berry size. Large berries of Thompson Seedless (*V. vinifera*) increased in size with increasing volume of water applied by irrigation (Zúñiga-Espinoza et al. 2015). Girdling also increased berry weight of Early Red Seedless (*V. vinifera*) by more than 1.5 times (Crupi et al. 2016). However, these techniques require precise area and/or timing of treatment depending on the cultivar and the field environment as well as the expertise of vine growers.

Interest in techniques to increase berry size of table grapes has intensified in the scientific community. The use of chemical compounds such as plant hormones is a simple way to increase grape berry size. Ethylene application at véraison increased Cabernet Sauvignon berry diameter through sap intake and cell elongation (Chervin et al. 2008). Upregulating the expression of genes related to water exchange (aquaporins) and cell wall modification (polygalacturonases, xyloglucan endotransglucosylases, pectin methyl esterases, cellulose synthases, and expansins) contributed to ethylene-induced berry expansion (Chervin et al. 2008). In particular, expansins, which are cell-wall-localized proteins that loosen cell wall, were involved in berry cell expansion in wine grapes (Schlosser et al. 2008, Suzuki et al. 2015) and a table grape (Ishimaru et al. 2007). Gibberellic acid (GA) application at the onset of the cell enlargement stage also increased berry weight of Early Red Seedless by 130% (Crupi et al. 2016).

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In a previous study conducted over two growing seasons, we proposed the hypothesis that electrical stimulation improved grape berry composition in field-grown wine grapes (Mikami et al. 2017). When two electrodes were screwed into the trunks of grapevines and connected to solar panels from two weeks before flowering to harvest, electrical stimulation enhanced anthocyanin and resveratrol accumulation in berries relative to those of control and electrode-treated grapevines. Although the acceptable level of electrical voltage and/or current to field-grown grapevines remains to be determined, no harmful effect of our electrical stimulation system on the reproductive and vegetative growth (flowering, véraison, and harvest timing), morphology in shoot, leaf, and bunch, and photosynthetic performance of field-grown grapevines were observed over a two-year growing season (Mikami et al. 2017). Resveratrol is one of the phytoalexins in grapevine (Jeandet et al. 1995). Recently, electrical stimulation acts as an abiotic elicitor of plant defense response to fungal diseases, resulting in decrease of the incidence of gray mold and/or ripe rot on bunches and downy mildew on leaves of field-grown grapevine (Mori et al. 2021). Thus, our electrical stimulation system can alter physiological status in grapevine through the transcriptional upregulation of genes related to phenylpropanoid biosynthesis, stilbenoid biosynthesis, and anthocyanin biosynthesis. In this study, to enlarge berry size of field-grown table grapes, electrical stimulation using improved solar panels was applied to two table grape cultivars, Shine Muscat and Pione, from bud burst to harvest in 2018 and 2019 growing seasons. We demonstrated that electrical stimulation enhanced cell division as an abiotic stress generator, thereby enlarging berry size of table grapes.

## Materials and Methods

### Plant materials.

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Shine Muscat (*V. vinifera* × *V. labrusca*) and Pione (a tetraploid hybrid of *V. vinifera* × *V. labrusca*) were cultivated in commercial vineyards located in Fuefuki City, Yamanashi Prefecture, Japan. Three-year-old Shine Muscat was cultivated in the shelf style in a greenhouse. Ten-year-old Pione was cultivated in the shelf style in an open field. For the shelf style cultivation, two long canes (approximately 20 buds each) was severed as the overhead arbor and branched near the top of the trunk (1.5 m above the ground) (Figure 1).

Grape cultured cells prepared from meristems of *V. vinifera* cv. Koshu were maintained on modified Gamborg's B5 medium at 27 ºC in the dark (Fujita et al. 2018).

**Electrical stimulation of field-grown grapevines.**

Ten Shine Muscat and five Pione grapevines were used for electrical stimulation. Electrical stimulation was carried out at bud burst (February 20, 2018 and January 18, 2019 for Shine Muscat, and April 25, 2018 and April 16, 2019 for Pione). Two electrodes (steel screws, 40 mm length) were screwed into a grapevine trunk (5 cm above ground for the positive electrode and 130 cm above ground for the negative electrode, respectively) and connected to a solar panel (Figure 1). The solar panel had the following electrical characteristics: maximum voltage 11.6 V ± 5%, maximum current 100 mA ± 5%, and working temperature -35 ºC to 85 ºC. As described previously (Mikami et al. 2017), illuminance exceeding 8,000 lux induced full capacity of the solar panel, whereas voltage was low when illuminance was below 8,000 lux. No voltage was detected in grapevines at night. The electrodes and the solar panels were detached from the grapevines after harvest (end of May for Shine Muscat and end of August for Pione, respectively), suggesting that in our experimental design, the grapevines connected to the solar panels were stimulated in the daytime but not at night from bud burst to harvest. Untreated

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grapevines were used as control. The same treatment on the same grapevine was performed in 2018 and 2019 growing seasons.

**Berry characteristics.**

To compare berry characteristics of grapevines exposed to electrical stimulation with those of control, bunches at harvest were collected and berry characteristics were measured following a previously described method (Kobayashi et al. 2020). Briefly, forty bunches (four bunches from each grapevine) for Shine Muscat and ten bunches (two bunches from each grapevine) for Pione, respectively, were randomly collected from grapevines exposed to electrical stimulation or control on May 17, 2018 and May 23, 2019 for Shine Muscat and August 21, 2018 and August 23, 2019 for Pione, respectively. Each bunch was weighed using an electronic balance (EK2000i, A & D Co., Tokyo, Japan). Ten berries (three from the top of the bunch, four from the middle of the bunch, and three from the bottom of the bunch) for Shine Muscat and seven berries (two from the top of the bunch, three from the middle of the bunch, and two from the bottom of the bunch) for Pione were collected from each bunch, respectively. The transverse diameter of each berry was measured using a digital caliper (AD-5764A-100, A & D Co., Tokyo, Japan). The weights of the ten or seven berries were measured using an electronic balance.

After measurement of berry weight, juices were obtained from the ten or seven berries by hand-pressing. Soluble solids content (Brix) and total acid content (g/100 mL) in the juices were measured with a refractometer (PAL-BX/ACID2, Atago, Tokyo, Japan).

Thin slices of berry skins were prepared from the ten Shine Muscat berries using a razor. Skin thickness (thickness of epidermal and subepidermal cell layers) was measured under a light microscope (BX51,

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Olympus, Melville, NY). A small piece of skin (5 mm square) was also cut out from each of the ten Shine Muscat berries using a razor. The skin pieces were treated with an enzymatic solution (pH 5.6) containing 2.5 g/L pectolyase (Kyowa Chemical Products, Osaka, Japan), 91 g/L mannitol, and 5 g/L sodium dextran sulfate for 4 h at room temperature. Then, the skin pieces were washed with distilled water, mounted on a glass slide, and covered with a cover slip. Cell number in a 0.5 $mm^2$ area of the epidermis at four points of each piece of skin was counted under a light microscope.

Skins of the seven berries from each bunch of Pione were frozen immediately in liquid nitrogen. Extraction of anthocyanins from berry skins and measurement of total anthocyanin content were performed as described previously (Moriyama et al. 2020). Briefly, berry skins were homogenized in a mortar containing liquid nitrogen using a pestle. One g of the pulverized sample was macerated in 10 mL of HCl-methanol [36:1 (v/v)] at room temperature in the dark overnight. The absorbance ($OD_{520}$) of the solution was measured using a spectrometer (UV-1800, Shimadzu, Kyoto, Japan). Total anthocyanin content was calculated according to a previously published formula (Bakker et al., 1986) and converted into malvidin-3-glucoside equivalent as mg per gram of berry skin fresh weight.

**Microarray analysis of grape cultured cells exposed to electrical stimulation.**

Microarray analysis of grape cultured cells exposed to electrical stimulation was performed according to a previously described method (Mikami et al. 2017). Briefly, grape cultured cells were grown at 27 ºC for 2 weeks on modified Gamborg's B5 medium. Positive and negative electrodes (steel needle, 2 mm length, 0.5 mm diameter) were entirely pricked on a mass of grape cultured cells and connected to a solar panel, same as field-grown grapevines. The solar panel was set under two fluorescent lamps (approximately 4000 lux). Untreated grape cultured cells were used as control. After

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the electrical stimulation for 4 h at room temperature, the cell mass was homogenized in a mortar containing liquid nitrogen using a pestle. Total RNA isolation from the cell mass 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). Briefly, 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. The signal intensity of each spot, signal evaluation and normalization were analyzed 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 ($p < 0.01$).

**Real-time RT-PCR.**

First-strand cDNA was synthesized from total RNA of electrically stimulated or control grape cultured cells using a PrimeScript RT Reagent Kit with gDNA Eraser (Takara) according to the manufacturer's instructions. Real-time RT-PCR was performed with SYBR Premix Ex Taq II (Takara). PCR amplification was performed for 40 cycles at 95 ºC for 5 s and at 60 ºC for 1 min after an initial denaturation at 95 ºC for 30 s. The nucleotide sequences of the primers used for real-time RT-PCR were as follows: *V. vinifera* kinesin-like protein KIN-5C (5'-AATGGAGGCCCTTCTTGACG-3' and 5'-ACGAGTATGGAGCTGTCCCT-3', LOC100240753), *V. vinifera* transducin beta-like protein 3 (5'-TCAAAGGCCACAAAGGGGTT-3' and 5'-AGCACTGAGCAAGGTCCATC-3', LOC100252767), *V.*

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*vinifera* replication protein A 14 kDa subunit B (5'-TGGATACATCAAGCCCTGCA-3' and 5'-ATTCCCCATTTGCAAGCAGG-3', LOC100244193), *V. vinifera* nuclear pore complex protein NUP88 (5'-ATGCAATGGTGGGGAGGAAG-3' and 5'-CTGCAATCCAGACTGGCTGA-3', LOC100265724), and *V. vinifera* β-actin (5'-CAAGAGCTGGAAACTGCAAAGA-3' and 5'-AATGAGAGATGGCTGGAAGAGG-3', GenBank accession no. AF369524). β-Actin was used for data normalization. The dissociation curves were evaluated to verify the specificity of the amplification reaction. Using the standard curve method and Thermal Cycler Dice Real Time System Single Software ver. 3.00 (Takara), the expression level of each gene was determined as the number of amplification cycles needed to reach a fixed threshold. Data are expressed as relative values to β-actin and presented as means ± standard deviations.

**Statistical analysis.**

Data are presented as means ± standard deviations of biological replicates. Statistical analysis was performed by ANOVA variance analysis and then Student's t-test using Excel statistics software 2012. To rule out the possibility that berry characteristics might be dependent on meteorological conditions on each growing season, statistical analysis for berry characteristics in each growing season was independently carried out.

## Results

**Electrical stimulation enlarges berry size in field-grown grapevines.**

The electrical stimulation of field-grown grapevines, Shine Muscat and Pione, is shown in Figure 1. Full capacity of the solar panels (approximately 11.6 V) was induced in fine weather, whereas no voltage was detected in the grapevines at night.

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196 To evaluate whether electrical stimulation affects the growth and development of grape bunches
197 and berries, we observed the vegetative growth of field-grown grapevines exposed to electrical
198 stimulation over two growing seasons. Electrical stimulation had no notable effect on flowering,
199 véraison, and harvest timing of grapevines exposed to electrical stimulation compared with control
200 irrespective of cultivar or growing season (data not shown).

201 No phenological differences in bunches were observed among the grapevines tested, although
202 bunch weight of Shine Muscat exposed to electrical stimulation was larger than that of control in 2018
203 growing season (Figure 2A). Interestingly, berries of Shine Muscat exposed to electrical stimulation
204 weighed more than those of control in both 2018 (Figure 2A) and 2019 growing seasons (Figure 2B). In
205 2019 growing season, berry diameter of Shine Muscat exposed to electrical stimulation was larger than
206 that of control (Figure 2B). Skin thickness is one of the most important characteristics of Shine Muscat
207 berry because consumers eat Shine Muscat with intact skin. Whereas skin thickness of berries from
208 grapevines exposed to electrical stimulation was larger than that of control in 2018 growing season
209 (Figure 2A), skin thickness of berries was similar for the two grapevines tested in 2019 growing season
210 (Figure 2B). Although soluble solids content of Shine Muscat exposed to electrical stimulation was
211 lower than those in control in 2018 growing season (Figure 2A), soluble solids content of Shine Muscat
212 exposed to electrical stimulation were comparable to those in control in 2019 growing season (Figure
213 2B). Similarly, total acid content of Sine Muscat exposed to electrical stimulation were higher than those
214 in control in 2019 growing season (Figure 2B), but total acid content of Shine Muscat exposed to
215 electrical stimulation were comparable to those in control in 2018 growing season (Figure 2A).

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Berries of Pione exposed to electrical stimulation also weighed more than those of control in 2018 growing season (Figure 3A). Although the berry weight of Pione exposed to electrical stimulation was comparable to that of control in 2019 growing season (Figure 3B), anthocyanin content, soluble solids content, and total acid content of Pione exposed to electrical stimulation were higher than those in control.

Taken together, the results suggest that grapevines exposed to electrical stimulation could produce large berries without adversely affecting berry characteristics.

**Electrical stimulation increases cell number in berry skin epidermis.**

To determine how electrical stimulation enlarges berry size, cells in berry skin epidermis were observed under a light microscope. Cells in skin epidermis of Shine Muscat exposed to electrical stimulation were smaller than those of control (Figure 4A). Precisely, cell number per unit area of skin epidermis of Shine Muscat exposed to electrical stimulation was higher than that of control (Figure 4B). This result suggests that electrical stimulation enhanced cell division but not cell elongation.

**Electrical stimulation induced transcription of genes related to cell division.**

Microarray analysis was performed to determine the molecular mechanisms associated with berry enlargement in grapevines exposed to electrical stimulation. Microarray data was deposited in DDBJ/ENA/GenBank under accession numbers PRJDB12347 (BioProject), SAMD00406430 and SAMD00407628 (BioSample for grape cultured cells without or with electrical stimulation, respectively), and E-GEAD-455 (GEA accession number).

Numerous genes were differentially expressed (fold change $> 2$, $p < 0.01$) in grapevine cultured cells exposed to electrical stimulation. The expression of 1071 genes was upregulated in the cells,

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whereas electrical stimulation downregulated the expression of 1659 genes in the cells (data not shown). Our previous study demonstrated that the transcriptional upregulation of genes related to sucrose metabolism, phenylpropanoid biosynthesis, flavonoid biosynthesis, stilbenoid biosynthesis, and anthocyanin biosynthesis in the cells (Mikami et al. 2017). In the present study, we focused on genes encoding proteins involved in cell division and elongation.

In relation to cell cycle, DNA replication, and cytokinesis, the transcription of kinesin-like protein KIN-5C (*KIN5C*), transducin beta-like protein 3 (*TBL3*), replication protein A 14 kDa subunit B (*RPA14*), and nuclear pore complex protein NUP88 (*NUP88*) was upregulated in grape cultured cells exposed to electrical stimulation compared with control cells (Table 1). To confirm the reproducibility of the microarray analysis, real-time RT-PCR was performed. Upregulation of *KIN5C* and *NUP88* gene expression was detected in grape cultured cells exposed to electrical stimulation compared with control cells (Figure 5).

Expansin is a molecule that plays a role in cell wall extension in plants (Shcherban et al. 1995), and grape expansins were found to promote cell expansion (Suzuki et al. 2015). Microarray analysis demonstrated that electrical stimulation did not significantly affect the transcription of expansin genes (Table 1).

## Discussion

Recently, we demonstrated that electrical stimulation on grapevine trunk upregulated the expression of genes related to plant defense response in leaves, resulting in decrease of the incidence of fungal diseases on berries and leaves (Mori et al. 2021). However, we still could not identify the signals that are transmitted from trunks exposed to electrical stimulation to berries and leaves. One possibility is

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to produce signal molecules in trunks exposed to electrical stimulation. In *Arabidopsis* plants, electrical stimulation is systemically responsible for plant defense response through the salicylic acid-dependent defense pathway (Mori et al. 2021). Another possibility is to simply transmit electrical signals in grapevines exposed to electrical stimulation. Electrical signals in plants are classified by their transmission speed. The action potential (AP) rapidly transmits electrical signals in plants (for example, 20–30 mm/s in *Mimosa pudica*), whereas the variation potential (VP) propagates electrical signals slowly in plants (for example, 5–6 mm/s in *M. pudica*, Fromm and Lautner 2007). AP was transmitted from root to shoot through the phloem in maize exposed to drought stress (Fromm and Fei 1998). Electrical signal transmission from root to leaf through the phloem was also observed in avocado exposed to soil drying and rewatering (Gil et al. 2008). In leaves of fava bean exposed to thermal stimulation, electrical signals were generated and transmitted to distant, non-stimulated leaves, thereby enhancing ethylene emission from the non-stimulated leaves (Dziubinska et al. 2003). The detection of signal molecule and/or electrical signal transmission in grapevine exposed to electrical stimulation is necessary to identify signals transmitted from trunk to berry and leaves.

Both cell division and cell expansion during berry development are determinants of berry size (Houel et al. 2013). Expansins are involved in berry cell expansion, which is related to berry size enlargement (Ishimaru et al. 2007, Schlosser et al. 2008). An exhaustive genome-wide analysis of expansin also confirmed a strong correlation between gene expression of expansin and berry development (Dal Santo et al. 2013). However, electrical stimulation induced the expression of cell cycle- and cytokinesis-related genes but not expansin genes in grape cultured cells, suggesting that electrical stimulation may activate cell division but not cell elongation. This suggestion is supported by

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the microscopic observation that electrical stimulation enlarged berry size by enhancing cell division but not cell elongation. In the present study, skin thickness of berries was measured. However, so far, we could not demonstrate any results regarding to cell wall thickness of berries exposed to electrical stimulation. Further investigations employing transmission electron microscopic analysis of the cell wall as well as biochemical analysis of the cell wall composition including pectins would reveal detailed information about the effect of electrical stimulation on cell division.

To explore potential molecular mechanism related to berry enlargement by electrical stimulation, we used grape cultured cells for gene expression analysis. Considering the difference in volume between field-grown grapevine and grape cultured cells, phenomena observed in grape cultured cells by electrical stimulation might not be analogous to what might be occurring in grapevines exposed to electrical stimulation. However, at least grape cells is certain to alter transcriptional profile in response to electrical stimulation. Gene expression of kinesin-like protein KIN-5C was markedly upregulated by electrical stimulation. Kinesin-like proteins are a large family of plus- or minus-end-directed microtubule motors related to plant mitosis, meiosis, and development (Liu et al. 1996). Phosphorylation of kinesin-like protein 1 by nucleus- and phragmoplast-localized protein kinase 1 was involved in the transition from metaphase to cytokinesis in tobacco cells (Nishihama et al. 2002, Sasabe et al. 2011). In contrast, kinesin-like protein SGL regulated grain length and plant height by affecting the expression of genes in GA biosynthesis controlling cell elongation in rice (Wu et al. 2014). Rice kinetin-like protein BC12/GDD1, which has transcription regulation activity, bound to a promoter of GA biosynthesis gene and then mediated cell elongation by regulating GA biosynthesis in rice (Li et al. 2011). The present study suggests that KIN-5C may regulate cell cycle, but not cell elongation, induced by electrical

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300 stimulation. Future studies of the overexpression or gene disruption of *KIN-5C* in grape and model
301 plants are expected to reveal the function of KIN-5C in the growth and development of grape berry. In
302 addition, to explore further the role of GA biosynthesis/catabolism on grape berry enlargement by
303 electrical stimulation, comprehensive analysis of GA signaling pathway in grapevines, including grape
304 berries and skins, exposed electrical stimulation are required.

305 Gene expression of nuclear pore complex protein NUP88 was also upregulated by electrical
306 stimulation. Nuclear pore complexes, which are aqueous channels connecting the nucleus and the
307 cytoplasm for RNA and protein movement, consist of multiple copies of approximately 30 different
308 proteins including nuclear pore complex protein (Raices and D'Angelo 2012). In *Arabidopsis* plant,
309 NUP88 is required for mitosis in gametogenesis and correctly promotes mitosis during gametogenesis
310 (Park et al. 2014). NUP88 also contributes to plant immunity by promoting the nuclear retention of plant
311 immune regulators in *Arabidopsis* rosette leaves (Cheng et al. 2009, Wiermer et al. 2010). As the null
312 mutant of NUP88 resulted in embryo lethality in *Arabidopsis* (Cheng et al. 2009), whether NUP88
313 contributes to plant growth and development by enhancing cell division is not yet clear. NUP88
314 overexpression in model plants will help us understand the physiological function of NUP88 in plant
315 growth and development.

316 How does electrical stimulation upregulate *KIN5C* and *NUP88* gene expression? Electrical signals
317 generated by mechanical damage induced the gene expression of proteinase inhibitor II in tomato (Herde
318 et al. 1995). In our previous study of a wine grape cultivar, we demonstrated the transcriptional
319 upregulation of genes related to sucrose metabolism, phenylpropanoid biosynthesis, flavonoid
320 biosynthesis, stilbenoid biosynthesis, and anthocyanin biosynthesis in grape cultured cells exposed to

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electrical stimulation (Mikami et al. 2017). Therefore, electrical stimulation might be able to alter widely transcriptional profiles in grape cells. In the microarray analysis conducted in the present study, we detected transcription factors upregulated by electrical stimulation (data not shown). To answer the above question, the precise functions of the transcription factors should be determined.

## Conclusion

We found that electrical stimulation applied directly to grapevine trunk may act as an abiotic stress generator, resulting in the alteration of berry characteristics. Our findings indicate that electrical stimulation may be used as a viticultural technique for enlarging table grape berry size, thereby increasing commercial value. One of the merits of exposing grapevine to electrical stimulation is that electrical stimulation may reduce labor hours and load compared with other techniques, such as irrigation and girdling. To explore further the applicability of electrical stimulation to table grape berry enlargement, critical field tests are necessary to identify the optimum conditions of our electrical stimulation system, including electrical voltage, timing of electrical stimulation, and cultivar adaptation to electrical stimulation, to ensure that electrical stimulation works effectively in table grapes.

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**Table 1** Transcriptional alteration in cell division and expansion in electrically stimulated grape cultured cells

| Gene symbol[a] | Gene description | Fold change[b] (electrical stimulation/control) |
|---|---|---|
| cell cycle | | |
| LOC100240753 | kinesin-like protein KIN-5C | 2.16 |
| LOC100252767 | transducin beta-like protein 3 | 2.13 |
| DNA replication | | |
| LOC100244193 | replication protein A 14 kDa subunit B | 2.23 |
| cytokinesis | | |
| LOC100265724 | nuclear pore complex protein NUP88 | 2.41 |
| cell expansion | | |
| LOC100245911 | expansin-A6 | 0.41 |
| LOC100260158 | expansin-A1-like | 0.30 |
| LOC100244103 | expansin-A10 | 0.25 |
| LOC100244917 | expansin-like | 0.10 |

[a]From The National Center for Biotechnology Information.

[b]Relative change in gene expression in grape cells exposed to electrical stimulation versus control cells.

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**Figure 1** Experimental design of electrical stimulation applied to field-grown grapevines. (A) Shine Muscat grapevines were cultivated in the shelf style in a greenhouse. (B) Pione grapevines were cultivated in the shelf style in an open field. Solar panels (SPs) were positioned approximately 1.5 m above ground. Electrodes were screwed into the grapevine trunks and connected to two solar panels (−, negative electrode. +, positive electrode). Bunches of Shine Muscat and Pione are shown in figures, respectively. Bar = 5 cm.

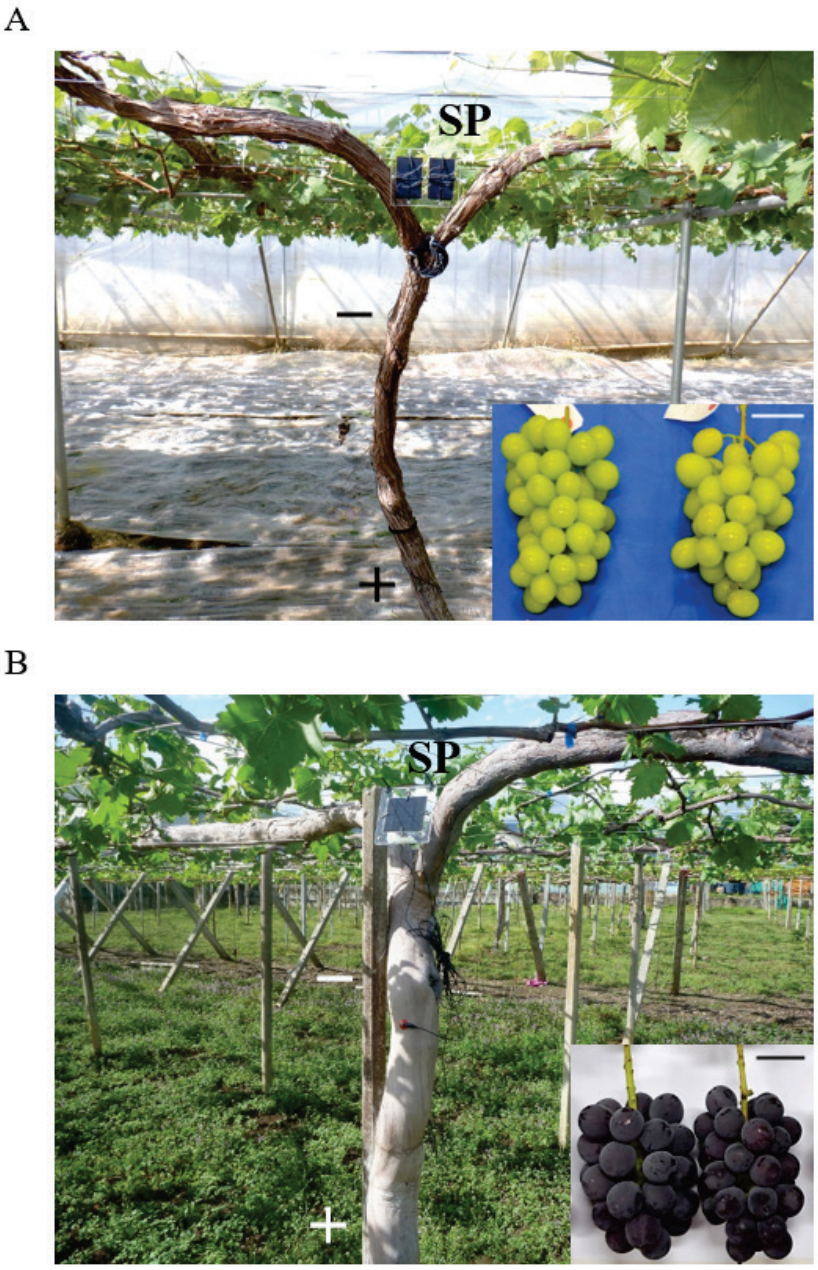

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**Figure 2** Berry characteristics of Shine Muscat exposed to electrical stimulation. (A) 2018 growing season. (B) 2019 growing season. Crosses (×) show means of twenty field-grown grapevines. * $p < 0.05$ vs. control. ** $p < 0.01$ vs. control. Control, untreated grapevines. Electrical, grapevines exposed to electrical stimulation.

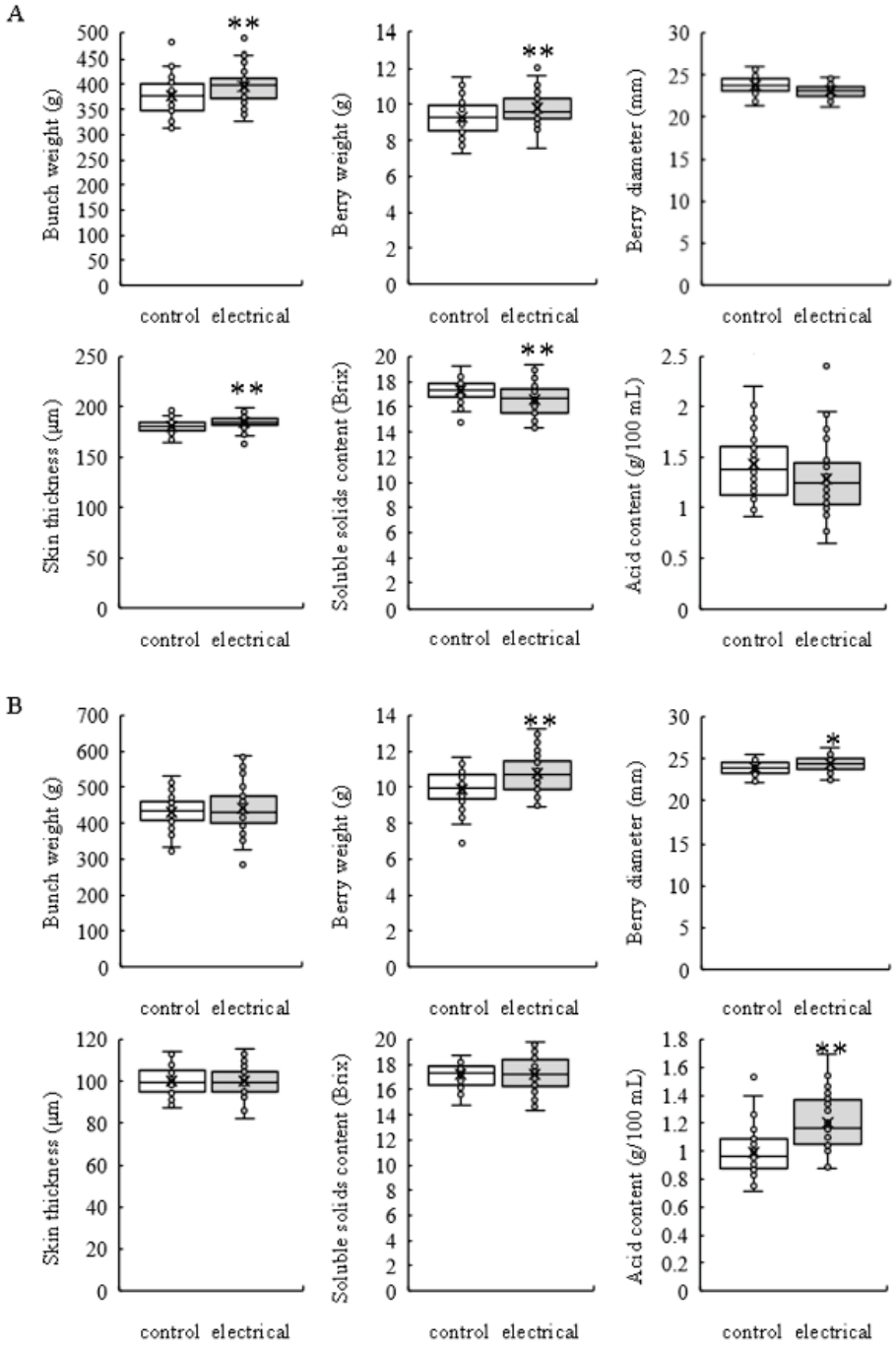

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**Figure 3** Berry characteristics of Pione exposed to electrical stimulation. (A) 2018 growing season. (B) 2019 growing season. Crosses (×) show means of five field-grown grapevines. * $p < 0.05$ vs. control. Control, untreated grapevines. Electrical, grapevines exposed to electrical stimulation.

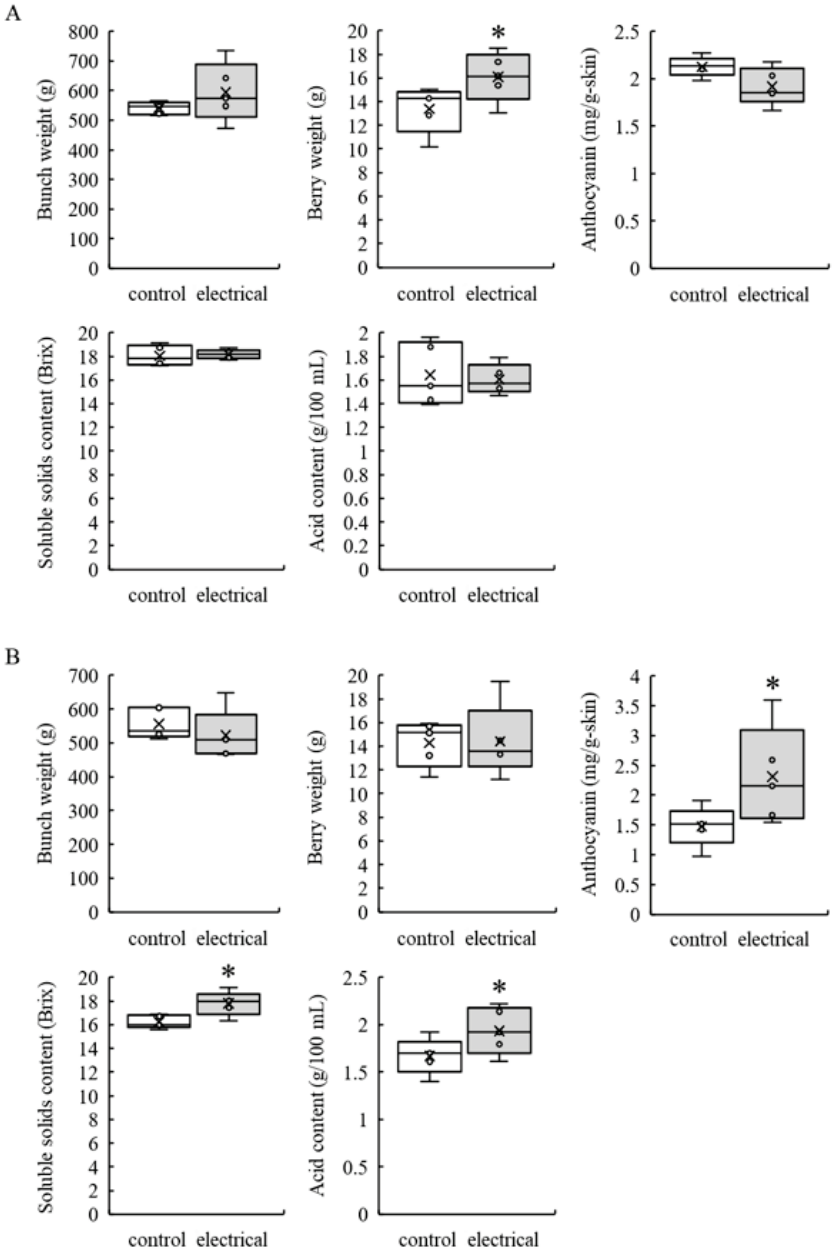

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**Figure 4** Cell number in berry skin epidermis of Shine Muscat exposed to electrical stimulation. (A) Phenotype of cells in berry skin epidermis of control (upper) and grapevines exposed to electrical stimulation (lower). Bar = 100 μm. (B) Number of cells in 0.5 mm2 of skin berry epidermis. Crosses (×) show means of twenty areas (four points per berry in five berries). * $p < 0.05$ vs. control. Control, untreated grapevines. Electrical, grapevines exposed to electrical stimulation.

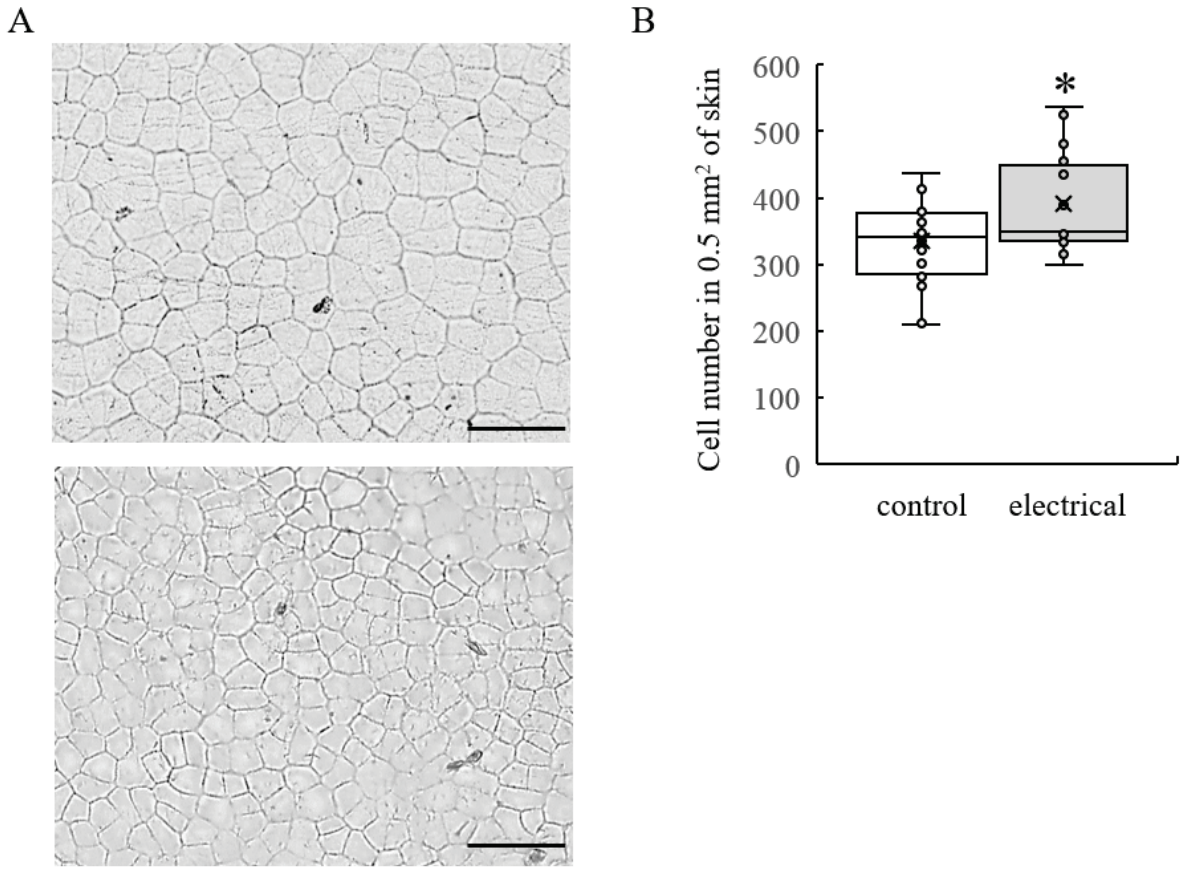

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**Figure 5** Transcription of genes related to cell division in grape cultured cells exposed to electrical stimulation. Grape cultured cells exposed to electrical stimulation for 4 h were used for real-time RT-PCR. Data were calculated as gene expression relative to actin expression. Crosses (×) show means of four independent experiments. KIN5C, kinesin-like protein KIN-5C. TBL3, transducin beta-like protein 3. RPA14, replication protein A 14 kDa subunit B. NUP88, nuclear pore complex protein NUP88. * $p < 0.05$ vs. control. Control, untreated grape cultured cells. Electrical, grape cultured cells exposed to electrical stimulation.