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The Effect of Application of *Diplazium Esculentum* Leaf Extracts and GA3 on the Berry Quality of Grape (*Vitis Vinifera* L.) Cultivar of 'Prabu Bestari'

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Abstract. This study investigated the effect of extract of Diplazium esculentum leaves and GA3 on the berry quality of Vitis vinifera c.v. Prabu Bestari. The objective of this research was to find out the effect of the application of D. esculentum leaf Extract and GA3 on the berry quality of grape c.v. Prabu Bestari. This study was conducted in the Vineyard at the Experimental Station of the Faculty of Agriculture, Udayana University during April to October 2021. A total of five different treatments were applied on inflorescences of grape (by dipping them), i.e. T0= control, only distilled water; T1 = 200 cc leaf extract was diluted with distilled water in to 1 L (200 cc L^{-1}); T2=400 cc L^{-1}; T3 = 600 cc L^{-1}; T4= 500 ppm GA3 (as control positive). There were five treatments arranged in a randomized block design with 6 repetition. The results showed that all treatments were not able to induce seedless, as seeds still presented inside berries, however, the number of seeds in the treatment of 500 ppm GA3 reduced significantly compared to other treatments. Weight per seed decreased significantly at 400 cc L^{-1} D. esculentum extract, 600 cc L^{-1} D esculentum extract and 500 ppm GA3.

Keywords: Berry quality; Prabu Bestari; seedless

INTRODUCTION

Gibberellin or gibberellic acid or GA3 is a very important phytohormone and is mass-produced for commercial use in agriculture, tissue culture, plantations, etc. and has economic and industrial value. Gibberellin affects plant growth and development processes, for example, seed germination, shoot elongation, flowering, fruit development, breaking seed dormancy, enzyme synthesis, reversal of genetic dwarfism, parthenocarpy in fruits, wilting inhibition, and so on (Urbanova & Leubner-Metzger, 2016). The negative effect of GA3 application on table grapes was reported by García-Rojas *et al*. (2018) in inducing lignification of the pedicel and causing berry drop in postharvest, and also the residual effects and toxicity of synthetic GA3 on mammals and humans were reported by Sun *et al*. (2020). However, the use of GA3 in the agricultural sector is still very important.

GA3 has been reported to have the effect of improving grape quality by reducing russet (brown spots appearing on the surfaces of some fruits), elongating bunches (Xu et al., 2019), having an effect on berry thinning and berry size (Van der Vyver, 2016), and being able to induce seedlessness in seeded cultivars (Cheng et al., 2013). According to Dimovska *et al.* (2014), 'seedlessness' is one of the important attributes in determining the quality of grape berries

GA3 can be produced from plants, fungi, bacteria and algae (Camara et al., 2018). Gibberellic acid (GA3) on the market today in Indonesia are obtained from abroad and according to Gupta & Chakrabarty (2013), are produced from the fungus of *Gibberella fujikuroi*. This GA is produced by the industry (factory) abroad and sold in Indonesia at a very high price. In developing countries where the basis of life relies on agriculture, the use of growth regulators and fertilizers to maximize production is an important

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point that needs attention. Thus, growth regulators must be produced simpler and more economical from easily available materials to reduce production costs in the agricultural sector. Obtaining GA3 from sources other than *G. fujikuroi* has also been carried out by Bilkay *et al.* (2010) from the fungus *Aspergillus niger*.

Speculation of the growth regulators presence in plant material extracts has been reported by Leovici *et al*. (2014), who applied young coconut water, cow urine, and extract of mung bean sprouts on sugarcane (*Saccharum officinarum* L.). Astawa *et al.* (2016) reported that the application of extracts of corn and young bamboo shoots (in Indonesia, called 'rebung') on grapes gave effects that were similar to the effects of GA3 from previous studies (Astawa et al., 2015). Further research by Astawa & Khalimi (2023) found that the extract of leaves of *Diplazium esculentum* contained GA3 in the amount of 260µg GA3/cc.

D. esculentum is one of the important species of wild ferns, which is frequently consumed by people in Indonesia and also several other countries in Asia. The analysis of phytochemical and pharmacological activities showed that *D. esculentum* contains antioxidant, antimicrobial, antidiabetic, immunomodulatory, CNS stimulant, and antianaphylactic activities (Semwal et al., 2021). It was also reported that *D. esculentum* can treat several human diseases (Essien et al., 2019; Kadir et al., 2014; Tag et al., 2012; Zannah et al., 2017), however,in the current study we used *D. esculentum* as a source of plant growth regulators and applied into the two grape cultivars for improving their berry quality. The use of *D. esculentum* as a source of plant growth regulator in the agriculture sector has not yet been reported. This study aimed to determine the effect of the application of *D. esculentum* leaf extract and GA3 on the berry quality of seeded grape c.v. Prabu Bestari.

METHODS

The study was conducted at a vineyard located at The Field Experimental Station of The Faculty of Agriculture, Udayana University in Denpasar Bali during May to middle of October 2022. Attributes for grape quality include sweetness, appearance, color, texture, flavor and aroma (Cristina Agulheiro-Santos et al., 2021; Romero et al., 2020), seedlessness (Karaagac et al., 2012), and berry size (Cristina Agulheiro-Santos et al., 2021). This study's observed attributes were sweetness, berry size, and seedlessness.

The extract of *D. esculentum* leaves was made by blending 1000 g of leaves with 500 cc of distilled water. After blending, the mixture was centrifuged and the solution was separated from the dregs. The extract (solution) was used and the dregs were discarded. The extract solution was further diluted again with distilled water in a certain volume of distilled water according to the treatment below.

The experimental design was prepared using a Randomized Block Design, with 5 treatments as the following: T0= control, only distilled water; T1 = 200 cc leaf extract was diluted with distilled water in to 1L (200 cc L^{-1}); T2=400 cc L^{-1}; T3 = 600 cc L^{-1}; T4= 500 ppm GA3. Thus, there were 5 treatments that were repeated 6 times.

The application was conducted by immersion the inflorescence (aged one week after forming, before anthesis) in the treatment solutions (Figure 1). The immersion was done twice. The second immersion was done one week after the first immersion.

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Figure 1. Method of application of extract of *D. esculentum* leaves and GA3 solution. The inflorescences used in the study (left) and imersion of inflorescences in the treatment solution (right)

The variables observed were berry sugar content, the weight of ten berries, number of seeds per berry, seed weight per berry, weight per seed, and berry diameter. The sugar content (°Brix) was calculated using a hand-held refractometer, weights were measured using a digital scale, while the diameter of the berry was measured transversely in the center of the berry using a caliper.

RESULTS AND DISCUSSION

Weight of 10 berries was not affected by the treatment, while variables of oBrix, number of seeds per berry, berry diameter and weight per seeds were significantly affected by the treatment (Table 1).

Table 1. Effects of treatments on variables observed

Treatments	Variables				
	°Brix	Weight of 10 berries (g)	Number of seeds per berry	Weight per seed (g)	Berry diameter (cm)
Control (T0)	9.41b	43.92 a	2.87 a	0.79 a	1.67 a
200ccL^{-1} extract (T1)	10.17b	44.31 a	2.79 a	0.73 a	1.62 a
400ccL^{-1} extract (T2)	10.13b	47.59 a	2.78 a	0.65 b	1.60 a
600ccL^{-1} extract (T3)	12.51a	47.42 a	2.73 a	0.58 c	1.58 b
500 ppm GA3 (T4)	13.03a	49.48 a	2.54 b	0.48 d	1.55 b
LSD 5%	0.88	5.94	0.23	0.12	0.08

Remarks: The same letter behind values of mean of treatment indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 5% level of probability and vice versa for different letter.

The percentage of Brix (%Brix) is the sugar content of an aqueous solution. One percent Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. Sugar content is one of the measures used to judge the various stages of maturity (Wilson, 2021). In the current study, grape maturity was stimulated by the treatment of *D. esculentum* extracts and GA3, as illustrated in Fig. 2. The picture was taken 30 days after treatment. It is clear that the treatment affected the maturity of berries. The treatment of T3 and T4 increased significantly % Brix (Table 1), which indicated that maturity was enhanced. An increase in the sugar content of grapes due to synthetic GA3

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treatment has been reported by Poudel *et al.* (2022) and Xie *et al.* (2022). Meanwhile, the effect on accelerating ripening with the administration of extract of plant material such as lemon grass and roselle extracts which are believed to be the source of GA3 was reported by El-Salhy *et al*. (2019). However, the application of *D. esculentum* leaf extract in increasing ripening of berry grapes as the result of the current study has not been reported elsewhere. Therefore, the increase in berry sugar content due to immersion of inflorescences before anthesis into *D.esculentum* extract solutions became the aspect of novelty of this study.

Berry size is represented by berry diameter and weight of 10 grape berries. The treatment of 600ccL^{-1} *D. esculentum* extract and 500 ppm GA3 decreased the diameter of berries as presented in Table 1. The shape of the berry lengthened as seen in Fig.2. Although the length of the berry was not measured, it can be seen visually that berries were elongated. The result is in contrast with the study of Pahi *et al.* (2020), which revealed that application of GA3 after berry set (when the berry was 8-10 mm in diameter) increased berry diameter. This indicated that the application time of GA3 is an important factor which influences the effect obtained.

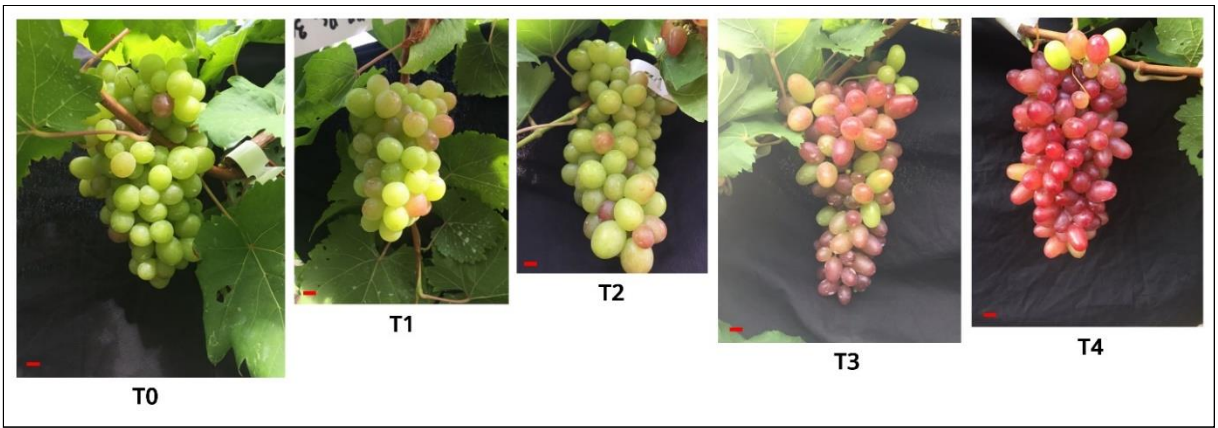

Figure 2. The effect of application of *D.esculentum* leaf extract and GA3 on grape maturity

Regarding seedlessness, the application of *D.esculentum* extracts and 500 ppm GA3 were not able to induce seedlessness, as indicated by the presence of seeds in the fruit. However, the size of the seeds in the T2, T3 and T4 treatment was smaller than in the other treatments which indicated by weight per seed. The number of seeds per berry was only affected by the treatment of 500 ppm GA3, but no effects with the treatment of D. esculentum extracts. Unlike the current study, synthetic GA3 with concentrations of 100 ppm that applied on *Vitis vinifera* L. cultivar Cabernet Franc (CF) and cultivar Cabernet Sauvignon (CS) before anthesis did not give significant results on the number of seeds (Gao et al., 2020).

In the current study, both D.esculentum extracts and 500 ppm GA3 applied before anthesis did not affect seedless, as seeds were still present inside berries. However, when GA3 applied 15 days after full bloom (anthesis), Cheng *et al.* (2013) found that complete abortion of seeds occurred on two seeded grape cultivars, 'Kyoho' and 'Red Globe'. It can be concluded that time of GA3 application greatly affected seedless on grape.

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The low GA3 content of the plant material was suspected to be the result of the non-significant effect of application of *D. esculentum* extract. However, concentration of 600ccL^{-1} extract had given an effect on the quality of grapes. Perhaps the resulting extract does not need to be dissolved for maximum effect. Machado *et al.* (2000) obtained 100 mg of GA3 from 1 kg of coffee husk by solid-state fermentation (SSF) process. When cassava pulp was added to the substrate of coffee husks, 492.5 mg of GA3 kg^{-1} substrate was obtained also using the SSF process (with the help of the fungi *Gibberella fujikuroi* and *Fusarium moniliforme*) (Machado et al., 2002). So, here it can be seen that type of plant material and method of extraction affect the amount of GA3 produced. In the future, the use of non-fermented *D. esculentum* leaf extract as used in this study needs to be improved. The use of microorganisms for fermentation and the addition of other materials as a fermentation substrate (such as cassava) can be used for further research. Further research related to the extraction method, application time and concentration of GA3 derived from plant material was still required.

CONCLUSION

All treatments (*D. esculentum* extract treatments and 500 ppm GA3) were not able to induce seedless, as seeds still presented inside berries, however, the number of seeds in the treatment of 500 ppm GA3 reduced significantly compared to other treatments. Seed size (indicated by weight per seed) decreased significantly at 400ccL^{-1} *D esculentum* extract, 600ccL^{-1} *D. esculentum* extract and 500 ppm GA3. The treatment of 600ccL^{-1} *D. esculentum* extract and 500 ppm GA3 enhanced berry maturity.

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