IN-VITRO INDUCTION OF GRAPEVINE (*Vitis Vinifera* L.) SHOOTS USING 2-iP

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ABSTRACT

The research concerning induction of grapevine shoots grown in vitro have been conducted during period of May to July 2016 at The Laboratory of Plant Tissue Culture, Faculty of Agriculture, Udayana University. The objective of the research was to investigate the most appropriate explant position and the optimal concentration of 2-iP in stimulating of grapevine shoot in vitro. Nodal segment from grapevine plant grown in the green house was used as planting material. The experiment was laid out in the factorial design, with two factors. The first factor was the position of explant (lay-down and up-right position) and the second factor was concentration of 2-iP (i.e. 0, 3, 6, 9, 12 ppm). Each combination was replicated ten times. The results showed that the treatment of 6 ppm 2-iP with up-right explant position was the most appropriate condition in stimulating growth of grapevine shoot. With up-right position of explant, the percentage of shoot producing-explants at 10 weeks after planting was 100% at the treatment of 6 ppm 2-iP, compared to 4%, 12%, 40%,and 40% at the treatment of 0 , 3, 9 and 12 ppm 2-iP respectively. The average number of shoot per explant was 5.5 for 6 ppm-treatment, while it was less than 3 for other treatments.

Keywords: grapevine shoots, induction, 2-iP, explant position

INTRODUCTION

Vitis vinifera L. var. Alphonso Lavallee is the most common table grape grown in Bali. The gape is a black variety and known as ‘anggur Bali’ in Indonesia, and ‘Ribier’ in Australia. Astawa *et al.* (2015) reported tha ‘anggur Bali’ is less tasty due to its low sugar content compared to other black varieties of table grape found in the market in Bali. Dwiyani (2007) reported that the farmers harvest early their grape due to awareness of pathogen attacks. Early harvesting caused low sugar content of fruits and leads to less tasty of the fruits in the market. This is true for non-climacteric fruit like table grape, in which the sugar content do not improve after harvesting (Wills *et al.* 1981). Therefore, Improvement of genetic characteristic of grapevine is considerably important. The current research provided the regeneration system of grapevine through in vitro technique with the use of cytokine, i.e. 2-iP. It is well known that cytokines stimulate the in vitro proliferation and

development of shoots of grapevine species grown in vitro (Goussard, 1987). The regeneration system resulted from the current research can be used for Agrobacterium-mediated transformation with in vitro technique for grapevine. Beside that, the system may also useful in providing in vitro propagation method for table grape.

MATERIALS AND METHODS

Mother plants were obtained from a vineyard located at District of Kalianget, Buleleng Regency, North of Bali, Indonesia. In vitro experiment was carried out at The Plant Tissue Culture Laboratory, Faculty of Agriculture, Udayana University, Denpasar, Bali, Indonesia during the period of March to August 2016.

.Nodes of shoots were used as explants. Explants were sterilized using 2 gL^{-1} of benomyl-containing fungicide (rinse for 10 minutes in the outside of laminar), and 10% sodium hypo chloride for five minutes inside the laminar, then they were washed using sterilized distilled water for three times before planting them at the media. The full strength of New Phalaenopsis (NP) medium (Islam *et al*, 1998) was used as basic medium. The experiment was laid out in the factorial design, with two factors. First factor was the position of explant (lay-down and up-right position) and the second factor was concentration of 2-iP (i.e. 0, 3, 6, 9, 12 ppm). The observed Variables were the percentage of explant that produced shoot, the average number of shoot per explant and the average length of shoot. Data was analyzed using Analysis of Variance (Steel and Torrie, 1980). The treatment means was compared based on Least Significant Difference (LSD) at 1% level of probability.

RESULTS AND DISCUSSION

The effect of explant position and 2-iP concentration and their interaction can be seen at Table 1. Explant position had significantly effects only on the percentage of explants that produced shoots, however, concentration of 2-iP had significantly effects on almost variables observed, except on the length of shoots. The effect of interaction of Explant position and 2-iP concentration was not significant for all variables

Table 1.The Effect of Explant position (E) and 2-iP concentration (C) and their interaction(ExC) on the variables observed at 10 weeks after planting

Variables	E -Effects	C- Effects	E x C- Effects
The percentage of explant that produced shoots	*	*	ns
The number of shoots per-explants	ns	*	ns
The length of shoots	ns	ns	ns

Remarks: ns = non-significant; * = significantly different at 5 % level of probability according to Analysis of Variance

Table 2. The Effects of Treatments on the Percentage of explants that produced shoots

Explant Position Treatment	Concentration of 2-iP (ppm)					Notation of mean differences between Explant Position Treatment
	0	**3**	**6**	**9**	**12**	
Up-rigt	4.2% A (d)	12.0 % A (c)	100.0% A (a)	40.0% A (b)	40.1% A (b)	**39.26% a**
Lay-down	2.2% B (d)	10.3% A (c)	50.2% B (a)	25.0% B (b)	30.3% A (b)	**23,6% b**
Notation of mean differences between the treatments of 2-iP concentration	**3.2% d**	**11.15% c**	**75.1% a**	**32.5% b**	**35.2% b**	

Remarks: The same capital letter behind the value indicates no significant differences among values in the same column based on The Least Significant Difference (LSD) at 5% level of probability, while those of small letters within brackets indicates no significant differences among values for the same raw and vice versa. The same letter in the notation of mean differences indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 5% level of probability.

Table 2 shows the effects of treatment on the number of explant that produced shoots. The results showed that 6 ppm of 2-iP was the most appropriate concentration for inducing shoots from node of grapevine node grown in vitro, while the position of up-right was the best condition in stimulating shoots of grapevine node grown in vitro. Table 3 shows the effects of treatments on the number of shoot per-explant. It was clearly indicated that the number of shoots per-explants was affected by the concentration of 2-iP. Concentration of 6 ppm resulted in the greatest number of the number of shoot per-explant compared to other concentration.

There was no effects of treatments on the length of shoots (Table 4).

A cytokinin, 2-isopentenyladenine (2iP) is used for stimulating cell division as well as shoot induction (Chuenboonngarm *et al*. 2001). Mustafa and Taha (2012) reported that 2-iP resulted in the highest number of leaves of some fig (*Ficus carica*) cultivars grown in vitro compared to Benzyl adenine, Kinetin and Adenin sulphate with concentration of 2.5 mgL^{-1}-each. However, Poudel *et al* (2005) found that 2-iP resulted in highest survival rate in culture establishment of *Vitis vitifolia* var. Ryuukyuuganebu grown in vitro compared to Kinetin and Benzyl adenin (BA) with concentration 0.5µM.

Table 3. The Effects of Treatments on the Average Number of Shoots per-Explant

Explant Position	**Concentration of 2-iP (ppm)**					Notation of mean differences between Explant Position Treatment
	0	**3**	**6**	**9**	**12**	
Up-rigt	2.4 A (b)	3.0 A (a)	5.0 A (a)	3.3A (b)	3.0 A (b)	**3.34 a**
Lay-down	2.0 A (a)	2.2 A (a)	6.0 A (a)	2.3 A (a)	2.1 A (a)	**2.92 a**
Notation of mean differences between the treatments of 2-iP concentration	**2.2 b**	**2.6 b**	**5.5 a**	**2,8 b**	**2.55 b**	

Remarks: The same capital letter behind the value indicates no significant differences among values in the same column based on The Least Significant Difference (LSD) at 5% level of probability, while those of small letters within brackets indicates no significant differences among values for the same raw and vice versa. The same letter in the notation of mean differences indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 5% level of probability.

Table 4. The Effects of Treatments on the average length of shoot (cm)

Explant Position	Concentration of 2-iP (ppm)					Notation of mean differences between Explant Position Treatment
	0	**3**	**6**	**9**	**12**	
Up-rigt	2.00 A (a)	2.00A (a)	3.00A (a)	2.00A (a)	3.00A (a)	2.40 a
Lay-down	1.60A (a)	1.66A (a)	2.60A (a)	2.00A (a)	1.50A (a)	1.90 a
Notation of mean differences between the treatments of 2-iP concentration	1.80 a	1.83 a	2.80 a	2.00 a	2.25 a	

Remarks: The same capital letter behind the value indicates no significant differences among values in the same column based on The Least Significant Difference (LSD) at 5% level of probability, while those of small letters within brackets indicates no significant differences among values for the same raw and vice versa. The same letter in the notation of mean differences indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 5% level of probability.

establishment of *Vitis vitifolia* var. Ryuukyuuganebu grown in vitro compared to Kinetin and Benzyl adenin (BA) with concentration 0.5µM. This is in accordance with Husaini *et al*. (2011) who proposed that the plant cultivars is one of many factors that affect morphogenesis (organogenesis and embryogenesis) in vitro. In the current research, we did use 2-iP only and found that concentration of 6 ppm resulted in the highest number of the percentage of explants that produced shoots, the average number of shoots per-explant, and the average length of shoot, compared to other concentration i.e. 0,3,9, 12 ppm. With this research, we suggested that in vitro morphogenesis of grapevine was higher with higher concentration of 2-iP up to 6 ppm. However, it showed contradictive effects with higher than 6 ppm. The result was in line with Poudel *et al*. (2005) who proposed that in vitro propagation of grapevine.is much affected by the concentration of cytokine and auxin. Figure 1 shows the condition of culture at four weeks after planting.

Regarding of the explant position, 'up-right' position resulted in a greater number of the percentage of explants that produced shoots, indicating that the position is more

appropriate than 'lay-down' position. We proposed that with 'up-right' position, the wound in the explant was directly expose in to the media, causing the cytokine (in this case, 2-iP) was easier absorbed by the explant and stimulated the shoot growth.

In conclusion, 6 ppm of 2-iP in MS medium with explant position of up-right is the most appropriate method in producing embryonic shoots of V. vinifera in vitro. This method can be used to provide embryonic shoots as a target for Agrobacterium-mediated transformation in table grapes.

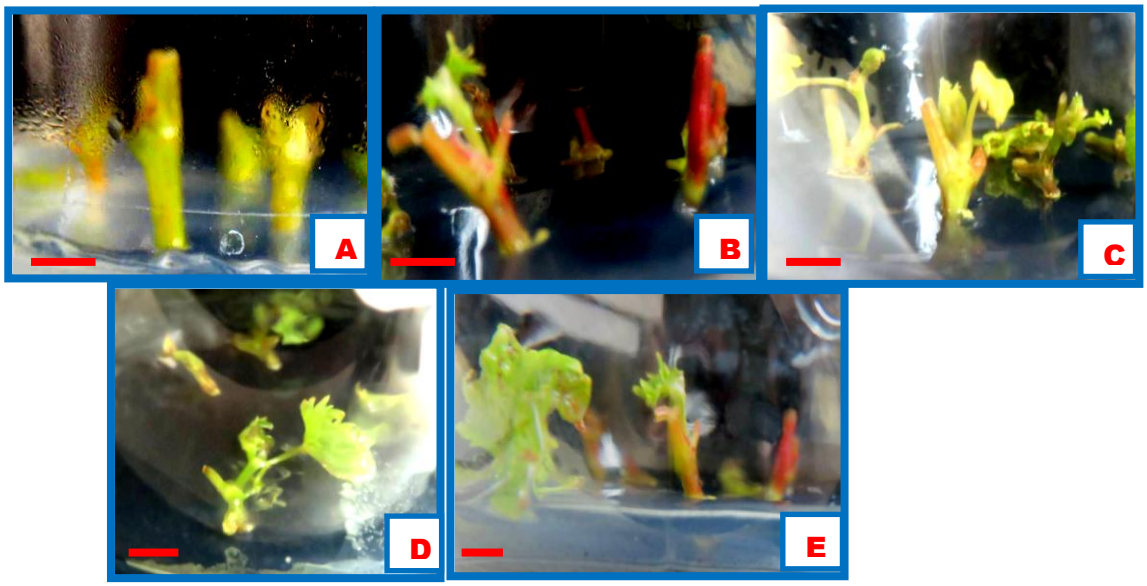

Figure 1. Cultures of grapevine nodal segments in various concentration of 2-iP at four weeks after planting. A=0 ppm; B=3 ppm; C=6 ppm; D=9 ppm and E=12 ppm; Bar=1cm

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REFERENCES

Astawa ING, R Dwiyani, NNA Mayadewi, IM Sukewijaya, NLM Pradnyawati. 2015. Perbaikan Kualitas Buah Anggur Bali (Vitis vinifera var. Alphonso lavallee) melalui Aplikasi GA3 Sebelum Bunga Mekar. Agrotrop 5 (1): 37-41

Chuenboonngarm, N., Charoonsote, S., and Bhamarapravati, S. 2001.

Effect of BA and 2iP on Shoot Proliferation and Somaclonal Variation of Gardenia jasminoides Ellis in vitro Culture. Science Asia 27: 137-141

Dwiyani, R. 2007. The Soil of Bali Island and Potentials for Farming. In Atmadilaga and Brahmantyo (Eds). Indonesian Geographical Expedition Bali 2007. Center for Land Resource Survey PSSAD- Bakosurtanal, Bogor, Indonesia. pp:29-33

Goussard PG. 1987. Anatomical responses to cytokinins of abscised grapevine shoot apices cultured in vitro. South African Journal for Enology and Viticulture 8 (2): 53-59.

Islam, MO., Ichihasi,S., Matsui, S. 1998. Control of growth and development of protokorm like body derived from callus by carbon sources in Phalaenopsis. Plant Biotechnol 15:183-187.

Mustafa, NS. and Toha, RA. 2012. Influence of plant growth regulators and subculturing on in vitro multiplication of some fig (*Ficus carica*) cultivars. J. of App. Sci. Res. 8(8): 4038-4044

Poudel, PR., Kataoka, I., and Mochioka, R. 2005. Effect of plant growth regulator on in vitro propagation of Vitis ficifolia var. Janebu and its interspecific hybrid grape. Asian J. of Plant Sci. 4(5): 466-471.

Wills, RHH., Lee, TH., Graham, D., Mc Glasson, WB. and Hall, EG. 1981. Post Harvest. An Introduction to the Physiology and Handling of Fruits and Vegetables. South China Printing Co., Hongkong. 163p.