

## Morphogenesis of *Rosa hybrida* Linn var. Christian Dior *in vitro*

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**ABSTRACT** *In vitro* studies on morphogenesis of an ornamental plant, *Rosa hybrida* Linn. var. Christian Dior were carried out on explant sources such as leaf, stem and shoot. Various plant hormones such as BA (6-Benzylaminopurine), NAA ( $\alpha$ -Naphthaleneacetic acid) and TDZ [1-phenyl-3-(1,2,3,4-thiadiazol-5-yl) urea] were used to initiate cultures and to observe the morphogenetic response of the species. Different concentrations of sucrose and pH were tested in order to obtain plant regeneration. Multiple shoots were obtained from shoots and stems cultured on MS supplemented with 2.0 mg/L BA + 0.1 mg/L NAA and 4.0 mg/L BA.

**ABSTRAK** Kajian *in vitro* untuk melihat corak morfogenesis telah dijalankan ke atas tumbuhan hiasan, *Rosa hybrida* Linn. var. Christian Dior menggunakan eksplan seperti daun, batang dan pucuk. Hormon BA (6-Benzylaminopurine), NAA ( $\alpha$ -Naphthaleneacetic acid) dan TDZ [1-phenyl-3-(1,2,3,4-thiadiazol-5-yl) urea] digunakan untuk melihat morfogenesis tumbuhan ini. Beberapa kepekatan sukrosa dan pH yang berbeza telah digunakan untuk mendapatkan regenerasi lengkap. Tunas berganda telah diperolehi daripada eksplan tunas pucuk dan batang yang dikultur pada media MS yang ditambahkan dengan 2.0 mg/L BA + 0.1 mg/L NAA dan 4.0 mg/L BA.

(morphogenesis, *Rosa hybrida*, *in vitro*)

### INTRODUCTION

The Rosaceae consist of 100 genera with about 2,000 species. Most of the members of the family are ornamental plants or fruit trees [1]. There are about 120 species in the genus *Rosa* [2]. The most popular ornamental species is *Rosa hybrida* Linn. This species is believed to have originated from Asia [3]. *Rosa hybrida* has been used for making jam, jelly and also has some medicinal values [4]. Other uses include production of perfumes and cosmetics.

Some tissue culture work has been done on this species [5,6]. Dubois *et al.* [7] obtained dwarf plants of *R. hybrida* with desirable characteristics such as faster growth rate, early flowering and more adventitious shoots. Secondary metabolites such as carotenoids, steroids, terpenoids and tannins have also been detected in tissue culture of the species.

In this paper, we report the morphogenetic response

of *R. hybrida* var. Christian Dior, to different hormone treatments, different pH and sucrose.

### MATERIALS AND METHODS

Explant sources of *Rosa hybrida* were obtained from Sungai Buluh, Selangor. Due to difficulty in getting seeds, we used explant from leaves, petioles, shoots and young stems from 2 month-old intact mature plants. The intact explants were carefully sterilized to minimise contamination. First, the explants were washed in sterile distilled water (3X), and then soaked in 50% sodium hypochlorite containing a few drops of Tween-20 for about 8 minutes. They were then soaked in 20% sodium hypochlorite for another 12 minutes and subsequently rinsed in three changes of sterile distilled water. Prior to culturing, explants were dipped in 70% alcohol for a few seconds and finally washed in autoclaved distilled water. The explants were then cultured on MS [8] medium supplemented with various concentrations of BA [9], NAA and the level of sucrose was varied to achieve plant regeneration. The explants were also subjected to different pH to see whether it had any effect on morphogenesis of this species.

### RESULTS AND DISCUSSION

Table 1 shows the response of *R. hybrida* to different concentrations of BA (6-Benzylaminopurine). No shoot formation was observed when MS supplemented with the various concentrations of BA (0.5-8.0 mg/L) except for 4.0 mg/L BA. Callus formation was observed in NAA (0.5-10.0 mg/L) and MS combination (Table 2). A newly reported hormone for woody plants, TDZ (Table 3), and MS supplemented with different combination of NAA and BA failed to regenerate the tissue (Table 4).

Shoot formation was obtained from stem cultured on MS + 4.0 mg/L BA after 10 weeks (Fig. 1). Multiple shoots were obtained from shoot tip explants after culturing on MS + 2.0 mg/L BA and 0.1 mg/L NAA for 4 months (Fig. 2).

**Table 1.** The effect of different concentrations of hormone BA on explants of *Rosa hybrida* var. Christian Dior under 16 hours light and 8 hours dark at 23-26°C.

BA (mg/L)	Explants	Observations	Average weight of fresh callus (g)*
0.5	leaf	no response	-
	petiole	no response	-
	stem	no response	-
1.0	leaf	no response	-
	petiole	no response	-
	stem	no response	-
1.5	leaf	yellowish callus formed after 3 weeks	0.13
	petiole	yellowish callus formed after 3 weeks	0.03
	stem	yellowish callus formed after 2 weeks	0.08
2.0	leaf	yellowish callus formed after 2 weeks	0.17
	petiole	yellowish callus formed after 2 weeks	0.05
	stem	yellowish callus formed after 2 weeks	0.09
3.0	leaf	yellowish callus formed after 2 weeks	0.16
	petiole	yellowish callus formed after 2 weeks	0.03
	stem	yellowish callus formed after 2 weeks	0.09
4.0	leaf	yellowish callus formed after 3 weeks	0.11
	petiole	yellowish callus formed after 3 weeks	0.03
	stem	yellowish callus formed after 3 weeks	0.07
5.0	leaf	yellowish callus formed after 3 weeks	0.09
	petiole	yellowish callus formed after 3 weeks	0.03
	stem	yellowish callus formed after 3 weeks	0.06
6.0	leaf	yellowish greenish callus formed after 4 weeks	0.08
	petiole	yellowish greenish callus formed after 4 weeks	0.03
	stem	yellowish greenish callus formed after 4 weeks	0.04
7.0	leaf	yellowish greenish callus formed after 4 weeks	0.05
	petiole	yellowish greenish callus formed after 4 weeks	0.02
	stem	yellowish greenish callus formed after 4 weeks	0.04
8.0	leaf	green callus formed after 4 weeks	0.06
	petiole	green callus formed after 4 weeks	0.02
	stem	green callus formed after 4 weeks	0.04

\*average weight of fresh callus per explant after 8 weeks

Table 5 summarises the effects of different levels of sucrose on callus formation. For leaf explants, callus formation was higher when greater amount of sucrose was added to the medium. Addition of 50 mg/L sucrose gave the highest amount of callus (Table 5). Callus formation from petiole was not encouraging as previously discovered in other species, e.g. chilli [10]. Addition of sucrose had no effect on the formation of callus from petioles. Stem explants also did not form large amount of callus. However, increasing concentrations of sucrose to 40 and 50 g/L did increase callus formation (Table 5).

Although shoot formation was not observed during the study, observations on other hybrids of *R. hybrida*

**Table 2.** The effect of different concentrations of NAA on explants of *Rosa hybrida* var. Christian Dior under 16 hours light and 8 hours dark at 23-26°C.

NAA (mg/L)	Explants	Observations	Average weight of fresh callus (g)*
0.5	leaf	no response	-
	petiole	no response	-
	stem	no response	-
1.0	leaf	yellowish callus formed after 2 weeks	0.11
	petiole	yellowish callus formed after 2 weeks	0.05
	stem	yellowish callus formed after 2 weeks	0.08
1.5	leaf	yellowish callus formed after 2 weeks	0.12
	petiole	yellowish callus formed after 2 weeks	0.05
	stem	yellowish callus formed after 2 weeks	0.07
2.0	leaf	yellowish callus formed after 2 weeks	0.12
	petiole	yellowish callus formed after 2 weeks	0.05
	stem	yellowish callus formed after 2 weeks	0.07
4.0	leaf	yellowish callus formed after 2 weeks	0.14
	petiole	yellowish callus formed after 2 weeks	0.07
	stem	yellowish callus formed after 2 weeks	0.09
6.0	leaf	white yellowish callus formed after 2 weeks	0.15
	petiole	white yellowish callus formed after 2 weeks	0.08
	stem	white yellowish callus formed after 2 weeks	0.09
8.0	leaf	clear yellowish callus formed after 2 weeks	0.09
	petiole	clear yellowish callus formed after 2 weeks	0.05
	stem	clear yellowish callus formed after 2 weeks	0.07
10.0	leaf	whitish callus formed after 2 weeks	0.09
	petiole	whitish callus formed after 2 weeks	0.06
	stem	whitish callus formed after 2 weeks	0.08

\*average weight of fresh callus per explant after 8 weeks

**Table 3.** The effect of different concentrations of TDZ on explants of *Rosa hybrida* var. Christian Dior under 16 hours light and 8 hours dark at 23-26°C.

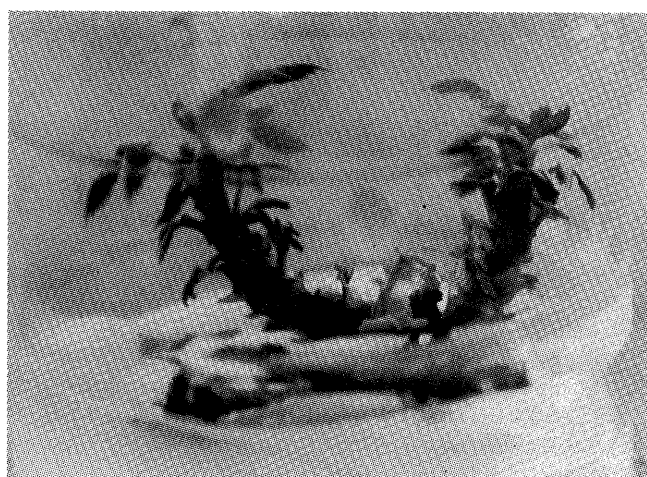
TDZ (mg/L)	Explants	Observations	Average weight of fresh callus (g)*
0.5	leaf	no response	-
	petiole	no response	-
	stem	no response	-
1.0	leaf	no response	-
	petiole	no response	-
	stem	no response	-
1.5	leaf	no response	-
	petiole	no response	-
	stem	no response	-
2.0	leaf	no response	-
	petiole	no response	-
	stem	no response	-

\*average weight of fresh callus per explant after 6 weeks

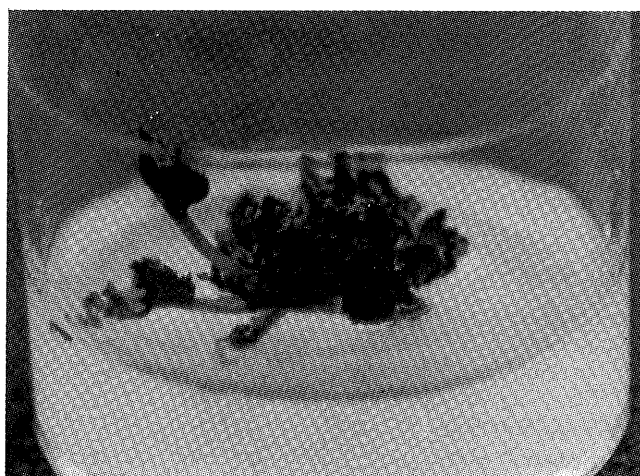
**Table 4.** The effect of different concentrations of BA and NAA on explants of *Rosa hybrida* var. Christian Dior under 16 hours light and 8 hours dark at 23-26°C.

Combination of hormone (mg/L)	Explants	Observations	Average weight weight of fresh callus (g)*
1.0 BA and 0.1 NAA	leaf	greenish callus formed after 2 weeks	0.28
	petiole	greenish callus formed after 2 weeks	0.09
	stem	greenish callus formed after 2 weeks	0.13
1.0 BA and 0.5 NAA	leaf	yellowish callus formed after 5 weeks	0.03
	petiole	yellowish callus formed after 3 weeks	0.01
	stem	yellowish callus formed after 2 weeks	0.01
1.0 BA and 1.0 NAA	leaf	yellowish greenish callus formed after 5 weeks	0.65
	petiole	yellowish greenish callus formed after 5 weeks	0.20
	stem	yellowish greenish callus formed after 4 weeks	0.25
2.0 BA and 0.1 NAA	leaf	yellowish callus formed after 4 weeks	0.04
	petiole	yellowish callus formed after 3 weeks	0.01
	stem	formation of shoots after 3 weeks and greenish callus after 3 weeks	0.02
2.0 BA and 0.5 NAA	leaf	yellowish callus formed after 4 weeks	0.38
	petiole	yellowish callus formed after 1 weeks	0.11
	stem	yellowish callus formed after 2 weeks	0.14
2.0 BA and 1.0 NAA	leaf	yellowish greenish callus formed after 1 week	0.35
	petiole	yellowish greenish callus formed after 1 week	0.18
	stem	yellowish greenish callus formed after 1 week	0.21
3.0 BAP and 0.1 NAA	leaf	brownish callus formed after 10 weeks	0.01
	petiole	brownish callus formed after 5 weeks	0.01
	stem	brownish callus formed after 4 weeks	0.01
3.0 BAP and 0.5 NAA	leaf	yellowish callus formed after 2 weeks	0.37
	petiole	yellowish callus formed after 2 weeks	0.15
	stem	yellowish callus formed after 2 weeks	0.20
3.0 BAP and 0.5 NAA	leaf	yellowish callus formed after 2 weeks	0.21
	petiole	yellowish callus formed after 2 weeks	0.09
	stem	yellowish callus formed after 2 weeks	0.13

\*average weight of fresh callus per explant after 12 weeks being cultured



**Figure 1.** Formation of shoots in MS supplemented with 4.0 mg/L BA



**Figure 2.** Multiple shoots from shoot tip explant, cultured on MS supplemented with 2.0 mg/L BA and 0.1 mg/L NAA.

**Table 5.** The effect of different concentration of sucrose on explants of *Rosa hybrida* var. Christian Dior in media MS supplemented with 1.0 mg/L BA and 1.0 mg/L NAA, under 16 hours light and 8 hours dark at 23-26°C.

pH	Explants	Observations	Average weight of fresh callus (g)*
10	leaf	whitish callus formed after 2 weeks	0.08
	petiole	whitish callus formed after 2 weeks	0.06
	stem	whitish callus formed after 2 weeks	0.03
20	leaf	whitish callus formed after 2 weeks	0.07
	petiole	whitish callus formed after 2 weeks	0.05
	stem	whitish callus formed after 2 weeks	0.06
30	leaf	yellowish callus formed after 1 week	0.20
	petiole	yellowish callus formed after 1 week	0.06
	stem	yellowish callus formed after 1 week	0.08
40	leaf	yellowish callus formed after 1 week	0.29
	petiole	yellowish callus formed after 1 week	0.03
	stem	yellowish callus formed after 1 week	0.10
50	leaf	yellowish callus formed after 1 week	0.30
	petiole	yellowish callus formed after 1 week	0.08
	stem	yellowish callus formed after 1 week	0.15

\*average weight of fresh callus per explant after 6 weeks being cultured

**Table 6.** The effect of different pH on explants of *Rosa hybrida* var. Christian Dior in MS medium supplemented with 1.0 mg/L BA and 1.0 mg/L NAA, under 16 hours light and 8 hours dark at 23-26°C.

pH	Explants	Observations	Average weight of fresh callus (g)*
4.8	leaf	whitish callus formed after 1 week	0.03
	petiole	whitish callus formed after 1 week	0.05
	stem	whitish callus formed after 1 week	0.05
5.8	leaf	greenish callus formed after 1 week	0.26
	petiole	yellowish callus formed after 1 week	0.06
	stem	yellowish callus formed after 2 week	0.07
6.8	leaf	yellowish callus formed after 1 week	0.15
	petiole	yellowish callus formed after 1 week	0.05
	stem	yellowish callus formed after 1 week	0.09
7.8	leaf	yellowish callus formed after 1 week	0.10
	petiole	yellowish callus formed after 1 week	0.03
	stem	yellowish callus formed after 1 week	0.08
8.8	leaf	yellowish callus formed after 2 weeks	0.02
	petiole	yellowish callus formed after 2 weeks	0.02
	stem	yellowish callus formed after 2 weeks	0.06

\*average weight of fresh callus per explant after 6 weeks being cultured

[11] showed that increased sucrose concentrations might influence the formation of shoot.

The best callus formation was observed on this medium at pH 5.8 (Table 6) for all the explants tested. This finding supports the suggestion by Pierik [12] that pH 5.0-6.0 is the most suitable pH range for callus formation in most species. Murashige and Skoog [8] reported that all salts could be stabilised at pH between 5.7-5.8. In the present study direct regeneration and root formation were not observed from any of the explants tested. However, shoot formation was observed on MS supplemented with 2.0 mg/L BA and 0.1 mg/L NAA from stem and shoot explants.

**Acknowledgments** The authors would like to thank the Government of Malaysia for the research grant R & D no: 01/07/04/038/01.

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