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香川大学農学部学術報告第48巻 第1号 33～38，1996

MICROPROPAGATION OF YELLOW-FLOWERED CYCLAMEN THROUGH ADVENTITIOUS ORGANOGENESIS IN MEDIUM CONTAINING 2, 4-DICHLOROPHENOXYACETIC ACID

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Plant regeneration from the tuber of aseptic seedling of yellow-flowered cyclamen 'Kage Yellow' in medium containing 2, 4-dichlorophenoxyacetic acid (2, 4-D) was examined. Many shoots were formed in subculture after 8 weeks of primary culture.

The optimal medium for shoot formation was 1/3 strength Murashige and Skooge (1/3 MS) medium supplemented with 0.5 μM 2, 4-D in primary culture and 1/3 MS medium without plant growth regulators in subculture. Shoot formation was enhanced when cultured in the dark. Much higher number of plantlets were regenerated through organogenesis using the medium with 2, 4-D than through organogenesis on the medium with BA and through embryogenesis. Plant regeneration through organogenesis using medium with 2, 4-D was considered as a useful method for the micropropagation of yellow-flowered cyclamen.

Key words : 2, 4-dichlorophenoxyacetic acid, micropropagation, organogenesis, yellow-flowered cyclamen.

Introduction

The breeding of new yellow-flowered cyclamen cultivars has been tried since a yellow-flowered individual was found in an inbred population of the white-flowered diploid cultivar 'Pure White' (1). The yellow petal color is regarded as an invaluable characteristic in cyclamen. However, it takes a long process to obtain superior yellow-flowered cyclamen cultivars because the yellow-flowered phenotype in cyclamen is recessive (2).

For reducing of breeding time, vegetative propagation is expected to be useful. In cyclamen, micropropagation should be an effective propagation methods because the daughter tuber is not formed, and because division and splitting are difficult.

Although many reports on micropropagation through organogenesis (3, 4, 5, 6, 7, 8, 9, 10, 11, 12) or embryogenesis (13, 14, 15, 16, 17, 18, 19) of cyclamen are available and the varietal difference or organogenesis and embryogenesis have been reported in some papers (11, 17, 20, 21, 22), the success rate for regeneration through organogenesis or embryogenesis was low in some cultivars especially in the yellow-flowered cultivars.

The aim of the present study is to establish a new effective micropropagation method for the breeding of yellow-flowered cyclamen. In our present study, the regeneration of yellow-flowered cyclamen through adventitious organogenesis in medium containing 2, 4-D was examined.

Materials and methods

Explants

The dry seeds of a yellow-flowered cyclamen cultivar 'Kage Yellow' were soaked in 20% sodium

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hypochlorite solution containing about 2% available chlorine with a few drops of detergent (Tween 20) for 10 minutes, and rinsed three times with sterile distilled water. The disinfected seeds were sown on 1/3 MS medium [23] with 3% sucrose and 0.3% gellan gum. The incubation conditions were same as in the earlier report [12]. The 7 week-old aseptic seedlings were then divided into cotyledons, petioles, tubers and roots. The tubers were sectioned into eight segments and used as explants.

Culture conditions for the adventitious organogenesis by the use of medium with 2, 4-D

1/3 MS medium containing 3% sucrose and 0.3% gellan gum was used as the basal medium. To investigate the effects of plant growth regulators on the shoot formation, 2,4-D at 0, 0.05, 0.5, 5.0 and 50.0 μM were tested in each basal medium, and kinetin at 0, 0.05 and 0.5 μM were tested in each basal medium supplemented with 0.5 μM 2,4-D. Cultures were incubated at 20℃ in the dark. The cultures were transferred to the basal medium 8 weeks later, and they were maintained at 20℃ in the dark.

The cultures, which were cultured on the basal medium with 0.5 μM 2,4-D in the dark at 20℃ for 8 weeks, were transferred to the basal medium with 1.0 μM N^6-benzyladenin (BA) or the medium without plant growth regulators so as to investigate the effects of subculture medium on organogenesis. Incubation conditions in the subculture were 20℃ in the dark. Shoot formation on the basal medium containing 0.5 μM 2,4-D without transferring served a control.

For investigations of the effects of light on the adventitious organogenesis, the explants were cultured on the basal medium with 0.5 μM 2,4-D. Cultures were maintained in the dark or 16 h photoperiod (day light, about 30 μmol $m^{-2}s^{-1}$) at 20℃. The cultures were transferred to the basal medium without plant growth regulators at 8 weeks after culture, and they were maintained at 20℃ in the dark or 16 h photoperiod.

Eighty explants were used in each experiment and more than two replication of experiment was carried out. In all experiments, the media were adjusted to pH 5.8 and autoclaved at 121℃ for 20 minutes. The number of explants forming shoots and the number of shoots were recorded after 8 weeks of subculture. Shoots were transferred to the basal medium for their rooting in 16 h photoperiod (day light, about 30 μ mol $m^{-2}s^{-1}$) and regenerated plantlets were grown in the greenhouse after transplanting to the soil.

Culture conditions for the adventitious organogenesis on the medium containing BA and somatic embryogenesis

For the adventitious organogenesis the explants were cultured on the 1/3 MS medium with 1.0 μM BA, 3% sucrose and 0.3% gellan gum and the cultures were incubated at 20℃ in the dark for 8 weeks. The shoots were transferred to the basal medium for their rooting and regenerated plantlets were grown in the greenhouse after planting to the soil.

The explants were cultured on MS medium with 5.0 μM 2,4-D, 0.5 μ M kinetin, 6% sucrose and 0.2% gellan gum, and they were incubated at 25℃ in the dark for the somatic embryogenesis. The cultures were transferred to the MS medium without plant growth regulators at 6 weeks after culturing as did in the earlier report [18].

Results and discussion

Effects of 2, 4-D and kinetin on shoot formation in medium with 2, 4-D

In the organogenesis of cyclamen from the tuber segments of aseptic seedling in medium with 2,4-D, few shoots were formed in 8 weeks of primary culture. Most shoots were formed in subculture.

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No shoot formation was observed in the basal media with 50.0 μM 2,4-D after subculture and only one shoot was obtained from 80 explants in the media with 5.0 μM 2,4-D (Table 1). In the media with 0.05 μM 2,4-D, 4.6 shoots per explant were obtained, but many of the shoots did not form the leaf. Most shoots without leaf did not form leaf even after being transferred to the basal medium for rooting. The shoots without leaf, except infant shoots which can form leaf, were of no use since all of the rooted shoots without leaf died after transplanting to the soil. The number of shoots per explants was 6.2 in the media with 0.5 μM 2,4-D, and more than 50% of the shoots formed the leaf. These results suggest that the optimal concentration of 2,4-D in the primary culture for the plant regeneration was 0.5 μM.

Table 1. Effects of 2,4-D in primary culture on shoot formation.

Conc. of 2,4-D[z] (μM)	No. of explants examined	No. of explants forming shoots	No. of shoots per explant	Percent shoots with leaves
0	80	7 (9)[y]	1.1 ± 0.1	63
0.05	80	64 (80)	4.6 ± 0.4	14
0.5	80	74 (93)	6.2 ± 0.4	57
5.0	80	1 (1)	1	0
50.0	80	0 (0)	—	—

[z] In primary culture
[y] Percent explants forming shoots

The presence of 2,4-D and cytokinin-like substance in the medium, and the relative ratio of auxin to cytokinin in primary culture were important factors in embryogenesis of cyclamen[15,16,18]. On the other hand, the best response in organogenesis in medium with 0.5 μM 2,4-D appeared to be at cytokinin-free medium and no explant formed shoots in the medium with 0.5 μM kinetin (Table 2). It is, therefore, suggested that cytokinin was not necessary in the primary culture and high concentration of kinetin, which is one of the cytokinin, definitely prevented the shoot formation in organogenesis in medium with 2,4-D.

Table 2. Effects of kinetin in primary culture on shoot formation.

Conc. of kinetin[z] (μM)	No. of explants examined	No. of explants forming shoots	No. of shoots per explant	Percent shoots with leaves
0	80	72 (90)[y]	6.5 ± 0.3	61
0.05	80	52 (65)	4.0 ± 0.2	49
0.5	80	0 (0)	—	—

[z] In 1/3 MS medium with 0.5 μM 2,4-D in primary culture
[y] Percent explants forming shoots

Effects of subculture medium

For the three methods shown in Table 3, transferring of cultures to the hormone-free medium was the best method to obtain higher number of shoots. Continuation of culture without transferring to new medium prevent the shoot formation. The supplement of 1.0 μM BA to subculture medium was useless for the shoot formation in the organogenesis in medium with 2,4-D, although it had been reported that BA was effective for the shoot formation from various explants in many reports on the organogenesis of cyclamen[5,7,8,9,12].

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Table 3. Effects of subculture medium on shoot formation.

Subculture[z]	No. of explants examined	No. of explants forming shoots	No. of shoots per explant	Percent shoots with leaves
Without transferring	80	68 (85)[y]	5.1 ± 0.4	48
Hormone free	80	72 (90)	7.4 ± 0.5	54
With 1.0 μM BA	80	71 (89)	6.0 ± 0.7	36

[z] The basal medium of subculture was 1/3 MS medium containing 3 % sucrose and 0.3 % gellan gum.
[y] Percent explants forming shoots

Effects of light on the adventitious organogenesis by the use of the medium with 2, 4-D

It had been reported that in vitro culture in the dark was effective for shoot formation in the organogenesis of cyclamen from etiolated-petiole segments[6], and from tuber and petiole segments[11]. In the embryogenesis of cyclamen, in vitro culture in the dark was also useful for callus formation, embryo induction and embryo development[12, 16, 19].

In the present study, subculture in the dark enhanced shoot formation but few effects of light condition in the primary culture on the number of shoots were observed (Table 4). The optimal light condition was darkness. On the other hands, percent shoots with leaf was higher in the explants cultured in the light (16 h photoperiod) in subculture than those cultured in the dark though few effects of light condition in the primary culture on percent shoots with leaf were observed. These findings suggest that light condition in subculture, in which shoots were formed, is an important factor for the shoot formation in the organogenesis of cyclamen grown in medium with 2, 4-D.

Table 4. Effects of light on shoot formation.

Light condition[z]		No. of explants examined	No. of explants forming shoots	No. of shoots per explant	Percent shoots with leaves
PC	SC				
Dark	Dark	80	74 (93)	7.2 ± 0.4	45
Dark	Light	80	76 (95)	4.3 ± 0.3	58
Light	Dark	80	71 (89)	6.7 ± 0.5	37
Light	Light	80	72 (90)[y]	3.9 ± 0.4	67
Significance					
Effects of light in primary culture (P)			Not significant	Not significant	Not significant
Effects of light in subculture (S)			Not significant	$P < 0.05$	$P < 0.05$
P × S			Not significant	Not significant	Not significant

Comparison of plant regeneration through the organogenesis by the use of the medium with 2, 4-D and other methods

The results of plant regeneration from seedling tuber in three different plant regeneration methods were shown in Table 5. No explant formed embryoids even on the media for embryogenesis, on which 'Anneke' explants had formed many somatic embryos[12]. Much higher number of shoots and plantlets were obtained by organogenesis in medium with 2, 4-D than by organogenesis in the medium with BA.

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These results suggest that plant regeneration through organogenesis in medium with 2,4-D is very useful for the micropropagation of yellow-flowered cyclamen.

The plant regeneration through organogenesis in medium with BA and through embryogenesis are useful for the micropropagation of cyclamen. However, as mentioned earlier it is difficult to regenerate plants through the organogenesis in medium with cytokinin and embryogenesis in some cultivars like 'Kage Yellow'. For such cultivars, the organogenesis in medium with 2,4-D may be one of the effective micropropagation method.

Table 5. Comparison of plant regeneration from 'Kage Yellow' seedling tuber in three different plant-regeneration methods.

Plant-regeneration method[z]	No. of explants	Total no. of shoots	Total no. of embryoids	Total no. of plantlets
Organogenesis (BA)	80	49	0	16
Embryogenesis	80	0	0	0
Organogenesis (2.4-D)	80	486	0	288

[z] Organogenesis (BA); Organogenesis in medium containing BA, Organogenesis (2,4-D); Organogenesis in medium containing 2,4-D.

Acknowledgement

Gratitude is expressed to Prof. Dr. C. S. Hew of National University of Singapore for his help in preparing the manuscript.

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2,4ージクロロフェノキシ酢酸を添加した培地を用いた不定器官形成による黄色花シクラメンのマイクロプロパゲーション

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2,4ージクロロフェノキシ酢酸(2,4ーD)を添加した培地を用いて,黄色花シクラメン'カゲイエロー'の無菌実生塊茎からの植物体再生を試みた.

0.5μM 2,4ーDを添加した1/3MS培地で8週間培養した後,2,4ーDを含まない1/3MS培地で継代培養した場合に最も多くのシュートが得られた.また,暗黒条件下で培養することによりシュート形成が促進された.2,4ーDを添加した培地を用いた不定器官形成においては,体細胞胚形成やBAを用いた不定器官形成より,より多くの植物体が再生された.したがって,2,4ーDを添加した培地を用いた不定器官形成は,黄色花シクラメンのマイクロプロパゲーションの有効な手段の一つになると考えられた.