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IN VITRO PROPAGATION OF ROSE CV. FOLKLORE

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Abstract : Surface sterilisation, stage of explant, media for culture establishment and multiple shoot induction were standardised for *in vitro* propagation of rose cv. Folklore. Treatment with mercuric chloride 0.08% for 12 min was found to be optimum for the surface sterilisation of axillary bud explants. Axillary buds excised four days after flower opening, having 1.0 cm length, exhibited the best response in culture establishment. The Murashige and Skoog (MS) basal medium supplemented with benzyl aminopurine (BAP) 2.5 mg l^{-1} + 2.4 dichlorophenoxy acetic acid (2,4-D) 0.5 mg l^{-1} was found to be the suitable medium for culture establishment. The best combination for multiple shoot induction was MS basal medium supplemented with kinetin 2.0 mg l^{-1} + gibberellic acid (GA_3) 1.0 mg l^{-1}.

Key words : Culture establishment, *in vitro* propagation, micropropagation, multiple shoot induction, rose.

INTRODUCTION

In vitro culture of higher plants under sterile conditions has shown spectacular development over the past three decades resulting in the production of viable plants of many species. Traditionally most of the ornamental *Rosa* species have been propagated by budding. Budding is slow and cumbersome. Tissue culture propagation offers many advantages over the conventional method. It offers a method to increase valuable genotypes rapidly and expedite the release of improved varieties. In general, three main routes of *in vitro* propagation are available, namely, enhanced release of axillary buds, somatic organogenesis and somatic embryogenesis. In the present study, attempts were made to standardise the culture conditions for the *in vitro* establishment of rose plantlets by enhanced release of axillary buds.

MATERIALS AND METHODS

Axillary bud explants were collected from pot grown plants of rose cv. Folklore. The surface sterilisation of the explant was standardised using mercuric chloride as the sterilant with different concentrations (0.06, 0.08 and 0.10) and time of treatment (6, 12 and 18 min.). The surface sterilisation was carried out under aseptic conditions in a laminar air flow cabinet. The most suitable stage of the axillary bud explant for *in vitro* culture establishment was identified by taking axillary buds at six defined physiological stages namely, vegetative stage, flower bud stage, day of flower opening and two, four and six days after flower opening.

To find out the most suitable supplements for initial culture establishment, a trial was conducted with four levels (1.0, 1.5, 2.0 and 2.5 mg l^{-1}) of cytokinin (BAP), in combination with four levels (0.25, 0.50, 0.75 and 1.00 mg l^{-1}) of auxin 2,4-D or NAA and BAP in combination with four levels (0.5, 1.0, 1.5 and 2.0 mg l^{-1}) of gibberellin (GA_3) on MS basal medium (Murashige and Skoog, 1962). Standard procedure (Gamborg and Shyluck, 1981) was followed for the preparation of the media. The pH of the media was adjusted to 5.8 before adding agar at 7.0 g l^{-1}. In order to standardise the suitable hormone supplements for early and enhanced release of axillary buds, an experiment was conducted with four levels (0.5, 1.0, 1.5 and 2.0 mg l^{-1}) of BAP/kinetin alone and in combination with four levels (0.25, 0.50, 0.75 and 1.99 mg l^{-1}) of GA_3. Microshoots measuring 1.5 to 2.0 cm,

Table 1 contd.

SI. No.	Treatments				Days to bud break	Bud break, %
	BAP, ragl^{-1}	2,4-D, ragl^{-1}	NAA, mg l^{-1}	GA mg l^{-1}		
35	1.0	-	0.75	-	8.4 cdefghijk	
36	1.0	-	1.00	-	6.0 dehijklmnopqrstu	50
37	1.5	-	-	0.50	7.0 qrtuvwxyzABCDEF	60
38	1.5	-	-	1.00	7.0 qrtvwxyzABCDEFG	50
39	1.5	-	-	1.50	8.0 dehijklmno	60
40	1.5	-	-	2.00	8.4 cdefghijkl	50
41	2.0	-	-	0.50	6.0 DEFJKLMNO	80
42	2.0	-	-	1.00	7.4 iklmnopqrstuvwxy	70
43	2.0	-	-	1.50	8.0 behijklmnop	50
44	2.0	-	-	2.00	9.2 abc	50
45	2.5	-	-	0.50	9.0 abcd	60
46	2.5	-	-	1.00	9.0 abcde	50
47	2.5	-	-	1.50	8.8 cdefgh	50
48	2.5	-	-	5.00	10.0 a	30

separated from the initial culture, were used as explants for induction of multiple shoots. The observations were recorded on days to initiate multiple shoot, percentage of cultures with multiple shoots and number of shoots / culture.

RESULTS AND DISCUSSION

The results of the experiment conducted on surface sterilisation showed that the treatment with mercuric chloride 0.08 per cent for 12 min was the best showing higher survival of 83 per cent.

The physiological stage of axillary bud was found to have significant role in the initial culture establishment in terms of days to bud break, percentage of bud break and length of shoot, three weeks after culture. Buds collected four days after flower opening showed early bud break (4.44 days), highest bud break (83%) and maximum shoot length (1.95 cm) three weeks after culture. This may be due to the fact that the buds below the apex that are suppressed due to apical dominance get stimulated with the harvest of flower and attained the maximum physiological activity on the fourth day. Poor response was shown by the explants collected from vegetative shoot. However, Mederos and Rodriguez (1987) reported early shoot growth in buds excised from vegetative shoot and shoots of flower bud stage.

Among the different treatment combinations tried to find out the most suitable hormone supplements for initial culture establishment, the best response was observed in MS medium supplemented with BAP 2.5 mg l^{-1} + 2,4-D 0.5 mg l^{-1} (Table 1). In this combination, early bud break (four days) and higher percentage of bud break (80) was observed. The stimulatory effect of 2,4-D and cytokinin had been reported in *Dactylils glomerata* and *Festuca* spp. (Dale, 1977a) and *Lolium multiflorium* (Dale, 1977b). Although in a number of plants it has been established that cytokinin can induce shoot formation and auxin can

Table 2. Effect of BAP, kinetin and in combination with GA_3 on shoot proliferation (cv. Folklore)

Sl. No.	Treatments, mg l^{-1}			Days to initiate multiple shoot	Cultures with multiple shoot, %	No. of shoots per culture	No. of elongated shoots per culture
	BAP	Kinetin	GA_3				
1	0.5	-	-	43.0 ab	58.3	1.6 jklm	0.0 pqrstuvwxyzABCDE FGHIJKL
2	1.0	-	-	39.1 ghijkl	66.7	2.0 BCDEFGHIJK	0.0 pqrstuvwxyzABCDE FGHIJKLM
3	1.5	-	-	37.1 mm	58.3	3.0 nopqrstuvwxyzABCD	0.6 LMNOPQRSTUV WXYZab
4	2.0	-	-	35.2 pq	41.7	3.4 lmnopqrstuvwxyz	0.4 opqrstuvwxyzABCD
5	0.5	-	0.25	34.8 pqrs	33.3	3.0 nopqrstuvwxyzABCDEFG	0.3 pqrstu vwxyzABCDEFG
6	0.5	-	0.50	34.3 pqrstuvwx	33.3	3.5 jklmnopqrstuvw	0.8 hlmnopqrstuvwxy
7	0.5	-	0.75	34.4 prstuvw	41.7	3.0 nopqrstuvwxyzABCD	0.2 pqrstuvwxyzABCD EFGHI
8	0.5	-	1.00	34.5 pqrstuv	33.3	3.0 nopqrstuvwxyzABCDE	0.8 hlmnopqrstuvwxy
9	1.0	-	0.25	33.4 rstuvwxy	41.7	2.0 DEFGHIJK	0.4 opqrstuvwxyzANCDE
10	1.0	-	0.50	31.6 yABCD	41.7	3.4 lmnopqrstuvwxy	1.0 efghijklnop
11	1.0	-	0.75	31.7 yz ABC	50.0	3.8 fghijklmnopqrst	0.8 efghijklmnopqrstu
12	1.0	-	1.00	31.6 yzABCDE	58.3	4.0 efghijklmn	1.8 defghij
13	1.5	-	0.25	35.0 pqr	33.3	2.8 tuvwxyzABCDEFGHI	0.8 hlmnopqrstuvw
14	1.5	-	0.50	34.6 pqrstu	41.7	4.0 efghijklmnop	1.6 defghijkl
15	1.5	-	0.75	32.3 xyzAB	66.7	4.8 cdef	2.0 cdef
16	1.5	-	1.00	29.7 CEFGHI	58.3	5.0 cde	2.4 bcd
17	2.0	-	0.25	31.3 BCDEF	58.3	4.6 defghj	1.0 efghijklmnop
18	2.0	-	0.50	29.0 GHU	58.3	4.8 cdefgh	1.8 delghi
19	2.0	-	0.75	23.5 LM	91.7	6.2 ab	3.6 a
20	2.0	-	1.00	24.8 L	75.0	5.8 abc	3.2ab
21	-	0.5	-	44.3 a	25.0	1.3 KLM	0.0 pqrstuvwxyzABCD EFGHIJ
22	-	1.0	-	43.0 ab	33.3	2.5 vwxyzABCDEFGHIJ	
23	-	1.5	-	42.6 abcd	41.7	3.2 mnopqrstuvwxyzAB	0.0 pqrstuvwxyzABCD EFGHIJ
24	-	2.0	-	39.3 ghjk	33.3	3.5jklmnopqrstuvw	0.0 pqrstuvwxyzABCD EFGHIJK
25	-	0.5	0.25	40.2 fghi	41.7	3.2 mnopqrstuvwxyzAB	0.5 mnopqrstuvwxyz ABC
26	-	0.5	0.50	41.8 bcdef	50.0	4.0 efghijklmnopqr	1.0 efghijklmnopqr
27	-	0.5	0.75	40.5 efgh	50.0	3.6 fghijklmnopqrstuv	1.0 efghijklmnopqrs
28	-	0.5	1.00	42.3 bcde	33.3	3.3 mnopqrstuvwxyzA	0.8 hlmnopqrstuvwx