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CALLUS CULTURES OF *ROSA HYBRIDA* CVS., DIAMOND JUBLY AND LANS FRANCE

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Abstract

Callus cultures were initiated from flower cups of cultivars of *Rosa hybrida*. Copious callus formation and growth was observed in cv. Diamond Jubly with 0.5 mg/l 2,4-D + 0.1 mg/l kn and in cv. Lans France with 0.5 mg/l 2,4-D + 0.5 mg/l kn. Sodium diethyldithiocarbamate (SDC) at 250 mg/l effectively controlled browning in cultures of both cultivars. SDC effect was related to concentration of phytohormones required by cultures at every 24 day cycle.

Introduction

Callus and suspension culture studies have been reported for different *Rosa* species which include *R. glauca* (Hustache *et al.*, 1975), Paul's Scarlet rose (Nesius *et al.*, 1972; Nash & Davies, 1972; Nash & Boll, 1975; Cladas & Cladas, 1976; Fosket, 1981) and Damask Rose (Kireeva *et al.*, 1977, 1978).

R. hybrida is an extensively cultivated rose. Tissue culture studies of various cultivars viz., Super Star (Jacobs *et al.*, 1969, 1970), Improved Blaze (Hasegawa, 1980); Bridal Pink (Khosh-Khui & Sink 1982a,b); Crimson Glory and Glenfiditch (Barve *et al.*, 1984) have been reported. No reports are available on *in vitro* cultures of *R. hybrida* cvs., Diamond Jubly and Lans France. The present studies were therefore undertaken to establish cultures of these cultivars.

Materials and Method

R. hybrida cvs., Diamond Jubly and Lans France were obtained from the PCSIR Laboratories, Complex, Lahore. Flower cups, after removal of all floral parts, were used as explants for initiating callus cultures. The plant material was surface sterilized in 0.1% mercuric chloride and transferred on sterilized Murashige & Skoog's (MS) (1962) medium containing 3% sucrose and 0.7% agar in 100 ml flasks containing 25 ml medium. 2,4-Dichlorophenoxyacetic acid (2,4-D) alone and in combination with kinetin (Kn) were used as plant hormones for initiation and proliferation of callus in both cultivars. Sodium diethyldithiocarbamate (SDC) was used as an antioxidant and added into culture medium at 250 mg/l. pH of the medium was adjusted at 5.7. Cultures were incubated at $26 + 1^{\circ}C$ under 16h cycled fluorescent light/total darkness. Other procedures were the same as reported earlier (Akram & Ilahi, 1985). Callus index (CI) was calculated as follows:

$$CI = \frac{100n \times G}{N}$$

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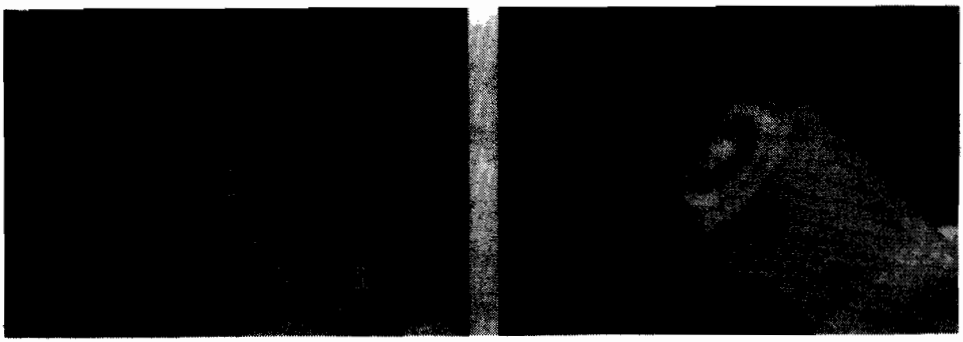

Fig.1. Callus formation on flower peduncles of *Rosa hybrida* on MS medium supplemented with 2,4-D and Kinetin. A) Copious callus cv. Diamond Jubly 2,4-D 0.5 + Kn 0.1 mg/l.; B) A good quantity cv. Lans France treatment as (A) above.

Where n = Number of explants forming callus
G = Average callus rating
N = Total number of replicates.

A visual rating of 1 to 4 from the smallest to largest was assigned to each callus respectively (Khosh-Khui & Sink, 1982 b). Five to six replicates were used to calculate CI.

Results

Callus formation in cv. Diamond Jubly was observed in 20% of the cultures after 15 days where 2,4-D @ 0.1 to 2.0 mg/l was used. The quantity of callus improved noticeably in another 10 days, but callus initiation percentage remained the same. 2,4-D with Kn gave varying results (Fig. 2A). 2,4-D @ 0.1 mg/l + Kn @ 1.5 mg/l showed 40% callus formation which increased to 60% after 10 days. Significant results were observed when

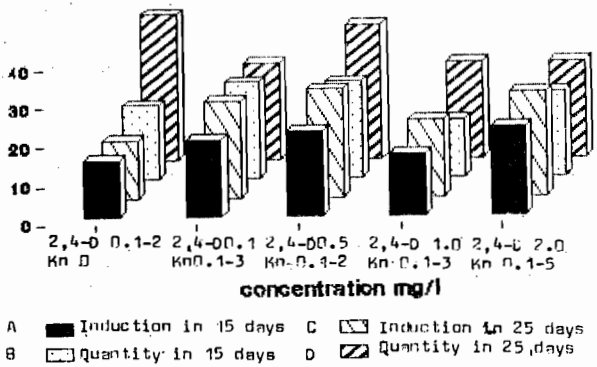

Fig.2A. Callus formation on flower - peduncles of *Rosa hybrida* cv. Diamond Jubly. A) Induction in 15 days; B) Quantity in 15 days; C) Induction in 25 days; D) Quantity in 25 days.

2,4-D @ 0.5/1.0 - 2.0 mg/l with Kn @ 0.1 to 5.0 mg/l was used. With 2,4-D @ 0.5 + Kn @ 0.1 mg/l, callus was formed in 60% cultures. Similarly a good amount of callus was observed with 2,4-D @ 2.0 mg/l + Kn @ 0.1 mg/l, while copious callus formation and growth was observed with 2,4-D @ 0.5 mg/l + 0.1 mg/l Kn (Fig.1A).

Callus formation in cv. Lans France was observed in 50% cultures after 15 days, where 2,4-D @ 0.5 mg/l was used alone. Further improvement was not seen in the quantity of callus in these cultures in another 10 days. Good callusing was observed with different combinations of 2,4-D and Kn (Fig.2B). Copious callus formation was observed with 2,4-D @ 0.5 mg/l + 1.0/0.1 mg/l Kn, in 20-25 days (Fig. 1B). Growth of callus was copious in further 10 days in cultures having 2,4-D @ 0.5/1.0 + 0.1, 1.0 and 1.5 mg/l Kn.

Callus formation in the two cultivars occurred between 15-20 days. During callus growth periods (15-25th days) the calli were observed to turn brown by 20th day. Total browning of callus occurred in 25-30 days. Browning was not produced when the calli were subcultured to media containing 250 mg/l SDC, in addition to other constituents. The browning control action of SDC on calli of both cultivars was observed to be influenced also by the concentration and combinations of growth regulators given in a treatment (Figs. 3A,B). Approximately 50-60% browning was con trolled in calli of cv. Diamond Jubly and Lans France with SDC treated media, in 12 days, containing 2,4-D @ 0.1 to 2.0 mg/l. The browning control percentage (BCP) then dropped to 30 by the 24th day. All such cultures were then subcultured to fresh media containing SDC and growth regulators. BCP was regained by cul tures to original values in 12-13 days, except in calli grown with 0.1 and 0.5 mg/l 2,4-D in cv. Diamond Jubly.

Flower-cups of both cultivars grown in dark, with 2,4-D + Kn combinations gave marked improvement in callus index (CI) values (Table 1). Cultures of c.v. Diamond Jubly containing 1.0 mg/l 2,4-D + 3.0 mg/l Kn produced more than double Cl, compared to similar cultures grown in 16 h light regime. Similarly callus index values of cv. Lans France cultures containing 0.1 mg/l 2,4-D + 1.5 mg/l Kn was found much higher to values observed for the cultures placed in 16h light cycle (Table 1).

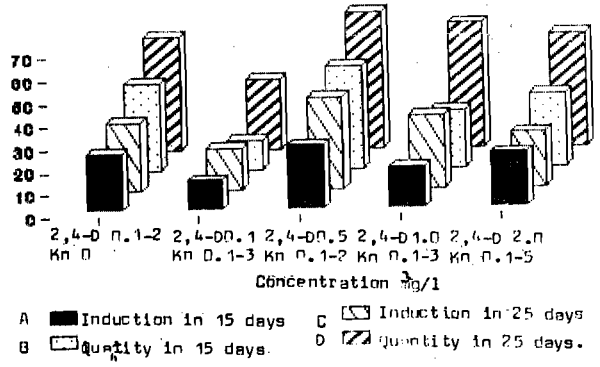

Fig.2B. Callus formation on flower - peduncles of *Rosa hybrida* cv. Lans France. A) Induction in 15 days; B) Quantity in 15 days; C) Induction in 25 days; D) Quantity in 25 days.

Table 1. Effect of light/dark conditions on callus formation in cultivars of *Rosa hybrida*.

Cultivars	Treatment (mg/l)		Callus index	
Diamond Jubly	0.5	2.0	100	150
	1.0	3.0	100	210
	2.0	0.1	140	210
Lans France	0.1	1.5	040	100
	0.5	2.0	100	180
	2.0	0.1	120	210

Discussion

In the two cvs. studied successful results on callus formation and its progressive growth were achieved. Best callus was formed with 2.0 mg/l 2,4-D + 0.1 mg/l Kn in cv. Diamond Jubly while 0.5 mg/l each of 2,4-D and Kn in cv. Lans France.

Browning in cultures is a common occurence in phenols exudating plants viz., coffee (Monaco *et al.*, 1977), pistachio (Barghchi, 1986) and rose (Davies, 1972; Khosh-Khui, 1982b). According to Davies (1972) the duration of polyphenol accumulation was largely determined by the availability of carbohydrate in Paul's Scarlet rose, while the initiation and initial rate of synthesis were influenced by a complex of factors, of which auxin concentration and light were the most important. The browning of calli was effectively controlled in another woody plant, *Rauwolfia* by the addition of sodium diethyldithiocarbamate (SDC) @ 250 mg/l (Akram & Ilahi, 1985). SDC used in the present study showed

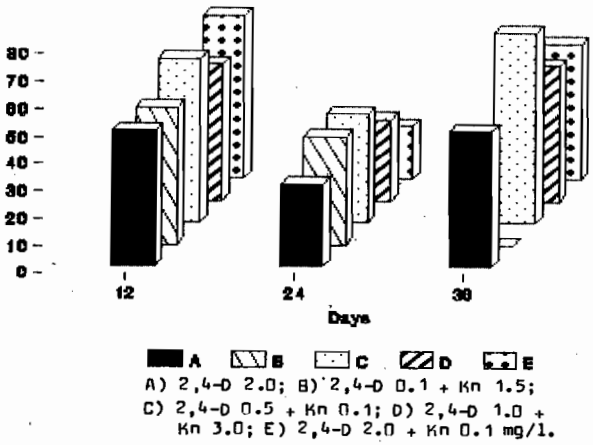

Fig.3A. Effect of 250 mg/l sodium diethyldithiocarbamate (SDC) on control of browning in *Rosa hybrida* cv. Diamond Jubly. A) 2,4-D 2.0; B) 2,4-D 0.1 + Kn 1.5; C) 2,4-D 0.5 + Kn 0.1; 2,4-D 1.0 + Kn 3.0; E) 2,4-D 3.0 + Kn 0.1 mg/l.

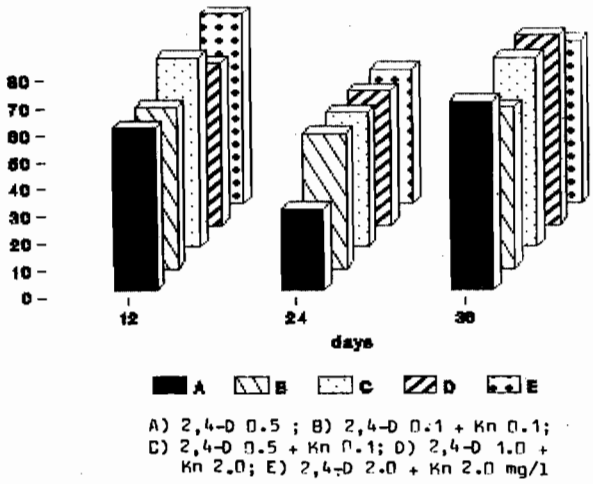

Fig.3B. Effect of 250 mg/l sodium diethyldithiocarbamate (SDC) on control of browning in *Rosa hybrida* cv. Lans Franc. A) 2,4-D 0.5; B) 2,4-D 0.1 + Kn 0.1; C) 2,4-D 0.5 + Kn 0.1; D) 2,4-D 1.0 + Kn 2,0; E) 2,4-D 2.0 + Kn 2.0 mg/l.

interesting results. Our observation for *R. hybrida* cvs. Diamond Jubly and Lans France were similar in giving high callus index for dark grown cultures as reported for Tropicana (Khosh-Khui & Sink, 1982b), Macgredy Yellow (Rasheed, 1989) and for Queen Elizabeth (Khan *et al.*, 1990).

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