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Production of Cucumber Mosaic Virus (CMV) free gladiolus shoots by meristem tip culture

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The gladiolus cultivar Morala was selected based on specific symptoms of infection (leaf streaking, colour breaking etc.). On serological indexing, Bean Yellow Mosaic Virus (BYMV) and Cucumber Mosaic Virus (CMV) were found to be the infecting agents. Meristems were excised and cultured on different media. Medium containing half strength MS medium supplemented with 0.5 mg^{-1} l and 1,0 mg^{-1} l IAA was found to be best for meristem survival. The size of meristem was directly proportional to their survival. BYMV was detected in shoots raised from 0,3-0.4 mm size of meristem but CMV was eliminated from even 0,9-1,0 mm meristem.

Key words: Cucumber mosaic virus, gladiolus, meristem, serological indexing

INTRODUCTION

Among different viruses infecting Gladiolus, Cucumber Mosaic (CMV) and Bean Yellow Mosaic (BYMV) viruses are of utmost importance. Infection by these viruses results in stunted growth, less number of florets per spike, leaf streaking, colour breaking of petals and small sized and few number of corm. Meristem tip culture is a standard procedure for production of virus free planting material, however the technique is not easy to follow in gladioli, The detail procedure for getting gladioli free of CMV is being studied in this communication

MATERIALS AND METHODS

Maintenance of diseased plants in the field

Out of 54-germplasm collections, the diseased plants were selected, on the basis of common viral symptoms, which included leaf streaking, colour breaking, general poor health, poor growth and scanty flowering during the months of April-May, Out of these collections of promising cultivars like American beauty, Jackson villa, Friendship, Jester, Morallo, Pink flamingo etc, prominent virus symptoms were shown by Morallo. Plants showing viral symptoms were tagged/marked and maintained in the field itself till the time of corm harvest.

Pre-treatment serological indexing and maintenance of virus culture

The plants, which were marked on the basis of visual symptoms, were subjected to serological indexing using Double Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) and plants infected by Cucumber Mosaic (CMV) and Bean Yellow Mosaic (BYMV) viruses were then maintained in the field as such and from these infected plants the viruses were mechanically transmitted to other healthy plants. Further corms and cormels from infected plants werd collected as a source of viral culture.

Meristem tip culture

Meristem was excised from just sprouted cormels under Stereoscopic microscope The outer layers

of the cormels were removed with sterile forceps and first cut was given vertically just ahead of the half of the cormel to remove the surrounding leaf primordia. Second cut was given below the primordia and third and fourth cuts were given on the two sides of the primordia respectively and the two sides were then lifted up to further expose the meristem, Cuts to the three sides of the meristem except the lower sides were given to remove the primordia and a final cut on the lower side was given to free the meristem from the cormel.Each time the cut was given, a new blade was used in order to prevent mechanical transmission of the viruses. Meristems were then lifted up and cultured on MS medium supplemented with different combinations of growth hormones. Meristems of four different size ranges viz: 0,3-0.4; 0.5-0.6; 0.7-0.8 and 0,9-1,0 mm sizes were cultured, Size of meristems was measured by using ocular micrometer, calibrated with a stage micrometer.

Post-treatment serological indexing

Shoots retrieved from meristem culture were again subjected to DAS- ELISA to monitor virus status of newly regenerated shoots

RESULTS AND DISCUSSION

Pre-treatment serological indexing

Out of twenty plans selected for serological indexing eighteen plants showed presence of both Cucumber Mosaic (CMV) and Bean Yellow Mosaic (BYMV) viruses (Table1)

Effects of different combinations of BAP, Kinetin, NAA and IAA on establishment of gladiolus meristems

Out of twenty-four different combinations of BAP, Kinetin, NAA and IAA used, medium containing half strength MS salts supplemented with 0.5 mg l^{-1} and 1.0 mg1^{-1}IAA was found to be best medium for meristem survival (Table 2).

Effect of size of meristems on their survival

It was evident from the investigations that increasing size of meristem increased survibility of meristems (Table 3), and meristems survived only when meristems of size more than 0.3 mm were cultured.

Effect of meristem size on virus elimination

Shoots obtained from meristem of different sizes were subjected to DAS-ELISA protocol for testing their virus status. The results are presented in Table 4. From the visual observation of microplates it was evident that BYMV could not be eliminated even when meristem of size 0.3-0.4 was cultured meaning thereby that the shoots developed from all sizes of meristems tried here under still carried the virus. However CMV was eliminated in shoots produced from all sizes of meristems.

It is found that meristem culture to be a successful technique for producing CMV free gladiolus plants. The technique has been most frequently used to obtain virus-eliminated plants because, concentration of infectious viruses has been observed to be low in meristematic region (Hollings, 1965). However in a large number of plant species this cannot be claimed as a rule (Appiano and Pennazio,1972). As in case of gladiolus under present investigations for meristem survival the treatment containing half strength MS medium supplemented with 0.5 mg^{-1}l Kinetin and 1.0 mg^{-1} l IAA was found to be the best medium for

meristem survival which is also supported by the work of Sangwan and his co- workers in 1987 who reported 100 per cent bud formation in *Chrysanthemum morifolium* when MS medium was supplemented with 2,0 mg l^{-1} NAA and 0,5 mg l^{-1} kinetin. Further only 27.20 per cent survival of cultures was recorded when the size of meristem was 0.3-0.4 mm. However, highest percentage of establishment was observed when meristem of size 0.9-1.0 mm was used for establishment. This suggests that the size of meristem tip explant is one of the most important factors governing its regenerative capacity and the probability of recovering virus free plants. Results obtained here were similar with those reported by Manisha *et al.* (2003) Though it was logical to find the number of virus free plants obtained to be inversely proportional to the size of meristem as virus concentration is restricted in the meristematic region, yet during present investigations no such relation with respect to elimination of BYMV was observed as the shoots obtained from all sizes of meristems were still carrying viruses in them. However plants free from CMV could be retrieved through meristem tip culture and virus free shoots were obtained from all sizes of meristem cultured. Since BYMV is a member Potyviridae it is very difficult virus to eliminate.

Table 1 : Pre treatment indexing of gladiolus bulblets

Ag/Ab	1	2	3	4	5	6	7	8	9	10	11
A		BYMV	BYMV	BYMV	BYMV	CMV	CMV	CMV	CMV	+C	–C
B		1	6	11	16	1	6	11	16	+C	–C
C		2	7	12	17	2	7	12	17	+C	–C
D		3	8	13	18	3	8	13	18	+C	–C
E		4	9	14	19	4	9	14	19	+C	–C
F		5	10	15	20	5	10	15	20	+C	–C

Planner

Ag/Ab	1	2	3	4	5	6	7	8	9	10	11
A		BYMV	BYMV	BYMV	BYMV	CMV	CMV	CMV	CMV	+C	–C
B		+	+	–	+	+	+	–	+	+	–
C		+	+	+	+	+	+	+	+	+	–
D		+	+	+	–	+	+	+	–	+	–
E		+	+	+	+	+	+	+	+	+	–
F		+	+	+	+	+	+	+	+	+	–

Coating antiserum	:	BYMV and CMV	Conc : 5x 10^{-2}	Time : 4 h / Temp : 37°C
Antigen	:	Sap from infected plants of Gladiolus	Conc: 10^{-1}	Time : Overnight / Temp : 4°C
Conjugates	:	BYMV and CMV antiserum Conjugates	Conc :5 x10^{-2}	Time : 2.5 h / Temp : 37°C
Substrate type	:	ALP	Conc: 1 ppm	TIme : 30 min-1 h / Temp : 27°C

1,2,3: Are the numerical codes given to the infected plants collected from the field
+C : sap from infected plants
–C: sap from healthy plant

Table 2 : Combination of growth regulators on survival of meristem cultures

Treatments	MS salt strength	BAP (mgl^{-1})	Kinetin (mgl^{-1})	NAA (mgl^{-1})	IAA (mgl^{-1})	% Survival
T1	Full	0.25	00	00	00	79.50 (62.87)
T2	Full	0.5	00	00	00	74.80 (59.81)
T3	Full	1.0	00	00	00	74.82 (60.07)
T4	Full	2.0	00	00	00	69.37 (56.28)
T5	Full	00	0.25	00	00	81.22 (64.18)
T6	Full	00	0.5	00	00	83.06 (65.59)
T7	Full	00	1.0	00	00	79.29 (62.49)
T8	Full	00	2.0	00	00	65.07 (53.69)
T9	Half	0.5	00	0.05	00	92.23 (73.87)
T10	Half	1.0	00	0.05	00	76.05 (60.53)
T11	Half	1.5	00	0.05	00	69.81 (56.66)
T12	Half	2.0	00	0.05	00	69.81 (56.66)
T13	Half	00	0.5	0.1	00	85.04 (67.24)
T14	Half	00	1.0	0.1	00	62.41 (52.25)
T15	Half	00	1.5	0.1	00	84.11 (66.48)
T16	Half	00	2.0	0.1	00	64.61 (53.21)
T17	Half	0.5	00	00	1.0	74.12 (59.30)
T18	Half	0.5	00	00	2.0	83.10 (65.73)
T19	Half	1.0	00	00	1.0	69.13 (56.25)
T20	Half	1.0	00	00	2.0	86.61 (68.53)
T21	Half	00	0.5	00	1.0	98.22 (82.54)
T22	Half	00	1.0	00	1.0	69.70 (56.30)
T23	Half	00	0.5	00	2.0	91.43 (72.99)
T24	Half	00	1.0	00	2.0	89.76 (71.36)
CD 0.05						2.34 (1.63)

Figures in parentheses are arcsine transformed values

Table 3 : Effects of size of meristem of on their survival

Treatments	Size of meristem (mm)	Survival (%)
T_1	0.3-0.4	27.20 (31.43)
T_2	0.5-0.6	42.70 (40.80)
T_3	0.7-0.8	68.73 (56.00)
T_4	0.9-1.0	82.71 (65.43)
CD 0.05		1.31(0.85)

Figures in parentheses are arcsine-transformed values

Table 4 : Effect of size of meristem (mm) on elimination of viruses from gladiolus

	1	2	3	4	5	6	7	8	9
A		BYMV	BYMV	BYMV	BYMV	CMV	CMV	CMV	CMV
		0.3-0.4	0.5-0.6	0.7-0.8	0.9-1.0	0.30.4	0.5-0.6	0.7-0.8	0.9-1.0
B		T1	T2	T3	T4	T1	T2	T3	T4
C		T1	T2	T3	T4	T1	T2	T3	T4
D									
E		+C	−C			+C	−C		
F		+C	−C			+C	−C		

PLANNER

	1	2	3	4	5	6	7	8	9
A		BYMV	BYMV	BYMV	BYMV	CMV	CMV	CMV	CMV
B		+	+	+	+	−	−	−	−
C									
D		+	+	+	+	−	−	−	−
E		+	−			+	−		
F		+	−			+	−		

Coating antiserum	:	BYMV and CMV	Conc : 5x 10^{-2}	Time : 4 h	Temp : 37°C
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+C : sap from infected plants
−C: sap from healthy plant

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