THE ELIMINATION OF SOME VIRUSES AFFECTING PINKS (*DIANTHUS* SP.) BY MERISTEM TIP CULTURE AND HEAT TREATMENT

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Abstract

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A nutrient medium for successful *in vitro* growth of pinks (carnation) meristem tips was developed. Clones of 6 pinks varieties were freed from Carnation etched ring virus (CERV), Carnation ring spot virus (CRSV) and Carnation vein mottle virus (CarVMV). CERV and CRSV were eliminated by meristem tip culture alone, but more easily when prior heat treatment was applied. CarVMV was eliminated only when the meristem tip excision was preceeded by heat treatment. None of these therapeutic methods deactivated Carnation latent virs (CarLV).

Introduction

Aseptic culture of excised meristem tips is used largely in curing vegetatively propagated crops species from virus diseases (8, 10).

Four viruses were identified in pinks (Abdulmagid and Robb, in press). These were carnation etched ring virus (CERV), carnation latent virus (CarLV), carnation ring spot virus (CRSV) and carnation vein mottle virus (CarVMV). This paper investigates methods for the elimination of some or all of these viruses.

Materials and Methods

1. Meristem tip source. These were the pinks varieties, Doris Diane, Haytor and Joy, all of which were naturally infected with CERV, CarLV and CarVMV, in addition Joy was also infected with CRSV.
2. Nutrient media. Four nutrient media were tried.
   I) Buys' medium (1) for carnation meristem (2), II) Davis' medium (2) for shoot initiation of carnation meristem tips, III) Kowalska medium (5) for carnation meristem tip culture, IV) Neergaard's modification of Morel's medium (NER) as was described by Stone (8) for carnation. Each of the four media (Buys, Davis, Kowalska and NER) was tested in liquid form using filter paper bridges (6) and in solid form by addition of 6 gm / 1 Ion agar. Usually 8 - 10 mls of medium were poured into McCartiney bottles. For each treatment there were 20 replicates. Meristem tips (0.2 - 0.9 mm long) were excised from Doris and Joy varieties. Methods of excision and subsequent culture of the meristem tips were as described by Stone (8). Cultures were inspected regularly over 12 weeks.
3. Heat treatment. The application of prior heat treatment was tested using the 4 varieties of pinks. The parent plants were maintained at 38°C inside a growth cabinet below 1850 lux fluorescent light for 16 hours. Meristem tips (0.3 - 1 mm long) were excised at 4 week intervals over a 20 week exposure period. They were cultured on the improved Buys' medium.
4. Transfer to potting media. Plantlets 1.5 - 3 cm long were aseptically and carefully transfered to pots containing sand / peat / loam (1:1:1) mixture and arranged in trays lined with water moistened capillary matting.
5. Indexing for virus. Regenerated plants were stringently tested for virus infection as described by Abdulmagid and Robb (in press) when about 10 cm long and then every 2 - - 3 weeks for over several months.

Results

1. Performance of the meristem tips in different media.

   I) Buys' medium. There was 100% rooting on either form of medium with both varieties. All explants in liquid culture had rooted in 3 weeks; on agar, this took 9 weeks. Root produced in liquid medium were well developed. Callus growth on liquid was relatively greater. Shooting was extensive on both types with all varieties, approaching 100% on liquid form (Table 1), and shoots remained healthy. The number of plants successfully potted was 75% from liquid form, and 50% and 55% for Doris and Joy, respectively, on agar (Table 3). The number of survivors from liquid form was 60% for Doris and 55% for Joy, compared with 30% and 50%, respectively, on agar.

   II) Davis medium. Root initiation in this medium liquid or solid was very slow. Only 15% of explants had roots 7 weeks after excision. Most rooting took place between the 10th and 12th week and some of the transplanted cultures were rootless. Rooting was substantially higher in liquid than on solid form (Table 2). Shoot initiation was better on liquid than on agar, varying with variety (Table 1). However, on both form the explants displayed malformed leaves. Huge calluses as wide as the culture were produced with in 3 - 5 weeks on both forms of media (Fig. 1) making it very difficult to transfer the plantlets to the pots. Also the plantlets on agar had firm anchorage through burying their roots at the bottom of the bottle, so it was found impossible to remove them without leaving behind or damaging the roots.

   III) Kowalska's medium. The percentage of explants grew and further transfered to and survived in compost was relatively higher on agar than in liquid (Table 1 and 3).

Table 1. Shooting of meristem tips on different culture media.

جدول ١ ـ تطور نمو القمات النامية عند زرعها في بيئات اصطناعية مختلفة .

Medium البيئة المستعملة	Type of medium نوع البيئة	Shooting[a] نسبة نجاح النموات		
			Doris variety الصنف دوريس	Joy variety الصنف جوي
Buys' medium بيئة باي	Liquid سائل	No.	20	20
		%	100	100
	Agar اجار	No.	18	18
		%	90	90
Davis' medium بيئة دينس	Liquid سائل	No.	20	20
		%	100	100
	Agar اجار	No.	13	15
		%	65	75
Kowalska's medium بيئة كوالاسكا	Liquid سائل	No.	15	15
		%	75	75
	Agar اجار	No.	18	17
		%	90	85
NER medium بيئة نير	Liquid سائل	No.	15	12
		%	75	60
	Agar اجار	No.	12	9
		%	60	45

a. Percentage of successful meristem tips excised (20).

أ ـ النسبة المئوية للقمم النامية التي قطعت ونمت بنجاح بعد نقلها (٢٠).

There was no difference in rooting (Table 2). All cultures developped moderate calluses i.e 5 mm diameter. A few plants developped sufficiently inside culture bottles to be transfered to compost and survive (Table 3).

IV) NER. As noted in table 2, rooting on liquid form (70%) was nearly 3 times higher than on agar (25%) in case of Doris, and twice as high (6% to 30%) in case of Joy. Shooting although followed a similar trend, yet the difference between the 2 forms of media was not as dramatic as in rooting (Tables 1 and 2). No culture on agar grew well enough to be transfered to compost. Even those cultured in liquid did not grew well so that only one plant survived in compost.

The conclusion from the results obtained with the 4 media is that Buy's medium in the liquid form was the best. With further improvement to speed up the rate of shoot growth and reduce the tendancy of callus formation in can be considered satisfactory for pinks meristem tip culture.

V) Improvement of Buys' medium. In the light of these results, subsequent investigations were aimed at improving this medium by adding or omitting organic constituents. Tentative trials indicated that a supplement of 2mg / 1 of Gibberellic acid (GA3) induced a considerable (2 - 3 times) increase in the rate of growth of the cultures (Fig. 2).

Based on the improved performance of meristem tips on Buys' medium + GA3 observed in preliminary trials, an experiment was set up where 60 meristem tips (0.3 - 0.9 mm long) were excised from each of Doris, Diane and Joy varieties. Thirty meristem tips from each variety were cultured in liquids Buys' medium + 2mg / 1 of GA3, whereas the other 30 were similarly cultured in the same medium without GA3. The different sizes of the excized meristem tips were randomized between the treatments. Data on rooting, shooting and number of plants transfered to pots were collected for each variety in each treatment 6 weeks after excision. Only plantlets that attained a minimum shoot length of 15 mm were transfered to pots. The results (Table 4) indicated that by the end of the 6th week over 80% of the plantlets grown on this medium + GA3 were transfered to pots, whereas none of their counter-

Table 2. Rooting of meristem tips on different culture media.

جدول ٢ ـ تجذر القمات النامية عند زرعها في بيئات اصطناعية مختلفة .

Medium البيئة المستعملة	Type of medium نوع البيئة		Rooting[a] تجذر[أ] Doris variety الصنف دوريس	Joy variety الصنف جوي
Buys' medium بيئة باي	Liquid سائل	No.	20	20
		%	100	100
	Agar اجار	No.	20	20
		%	100	100
Davis' medium بيئة ديفيس	Liquid سائل	No.	15	13
		%	75	67
	Agar اجار	No.	5	8
		%	25	40
Kowalska's medium بيئة كوالاسكا	Liquid سائل	No.	18	17
		%	90	85
	Agar اجار	No.	18	17
		%	90	85
NER medium بيئة نير	Liquid سائل	No.	14	12
		%	70	60
	Agar اجار	No.	5	6
		%	25	30

a. Percentage of rooted meristem tips excised (20).

أ ـ النسبة المئوية لتجذر القمات النامية (٢٠).

parts from the other treatment was yet ready for transfer.

2. Virus indexing. The overall indexing results indicated that CERV and CRSV appeared to be eliminated from a few of the regenerated plants. However meristem tip culture alone failed to produce any CarLV – free or CarVMV – free plants from any of the varieties tested. Therefore the application of prior heat treatment as mentioned before was tested using 4 varities of pinks.
3. Heat treatment. The effect of prior heat treatment on virus status was as follows:

   CarLV: None of the regenarated plants were freed from this virus even when the meristem tips excision was preceeded by heat therapy for 20 weeks (Table 5).

   CarVMV: Prior heat treatment of 8 – 12 weeks was necessary to obtain CarVMV – free plants (Table 5).

   CERV and CRSV: Heat treatment greatly enhanced the elimination of these viruses. It was also observed that none of the CRSV – infected parent plants developed symptoms of this virus on return to the glasshouse bench at the end of the experiment, nor did they react positively when indexed for CRSV. This suggested that they were therapeutically cured of CRSV.

Discussion

Four media (Buys', Davis, Kowalska and NER) were compared for their suitability to support growth of pinks meristem tips. The quantified data as well as observation on general growth, all indicated performance on liquid Buys, Davis and NER media exceeded that on solid agar. NER media was particularly bad in the solid form, so that not a single culture grew to the stage of rooting up.

Of all 4 media, Buys' liquid form proved most satisfactory. Modification of Buys' medium showed that a supplement of 2 mg / 1 of gibberellic acid stimulated very rapid and healthy growth of the pinks meristem tips so that the period of *in vitro* culture was considerably shortened, with a high expectancy (c. 80%) of survival.

It can be conjenctured that addition of GA3 brought about a favourable balance in the medium to make it nearly ideal for pinks. The role of GA3 in regulating the growth of cultured meristem tips has for long been realized (4).

Table 3. Survival of plantlets cultured on 4 different media.

جدول ٣ ـ نسبة نجاح الشتلات على أربع بيئات مختلفة .

Media البيئة	Doris variety الصنف دوريس				Jor variety الصنف جوي			
	Liquid سائل				Agar اغار			
	No. of plants transferred to potting medium عدد النباتات التي نقلت الى وسط ترابي	Survivors عدد النباتات الناجحة	% Survival out of النسبة المئوية للنباتات		No. of Plants transferred to potting medium	Survivors عدد النباتات الناجحة	% Survival out of النسبة المئوية للنباتات	
			No. transfered التي نقلت	No. excised التي قطعت			No. transfered التي نقلت	No. excised التي قطعت
Buys' medium بيئة باي	15	12	80	60	10	6	60	30
Davis' medium بيئة ديفس	12	9	75	45	8	4	50	20
Kowalska's medium بيئة كوالاسكا	2	0	0	0	7	3	43	15
NER medium بيئة نير	3	0	0	0	0	0	0	0
Buys' medium بيئة باي	15	11	73	55	11	7	64	35
Davis' medium بيئة ديفس	13	8	62	40	9	4	44	20
Kowalska medium بيئة كوالاسكا	3	0	0	0	6	3	50	15
NER medium بيئة نير	4	1	25	5	0	0	0	0

The results of virus tets indicated that CarLV is very difficult to dislodge from infected pinks. It eluded all the various therapeutic methods tried in the present investigations It is difficult to understand how CarLV has been readily eliminated solely by meristem tip culture by Stone (9) from both Carnation and *Dianthus barbatus*. It is perhaps possible that the pinks CarLV isolate is better able to invade and / or survive in meristem tips, even under adverse conditions such as long exposure to heat, than the carnation isolate. Quak (7) noted that strains of virus may respond differently to heat treatment. Additionally, it is the view of the author that the results of Stone (9) cannot be truely compared with the present study on the thesis that Stone has excided meristem tips from artificially inoculated plant material which was far from being as fully invaded by the virus as naturally infected stock of the type used in the present work.

CarVMV which resisted eradication by meristem tip culture alone was successfully eliminated in this work when the technique was used in conjuction with prior heat treatment of the parent plant. Success of elimination CarVMV by meristem tip culture alone has been reported (3, 9).

CERV and CESV in the present work were eliminated by meristem tip culture alone. However, they were more readily eliminated when meristem tip excision was preceeded by hot air treatment. Evidence is presented here that CRSV – infected parent plants were cured from CRSV after 20 weeks exposure to hot air at 30°C. There is no previous report on similar finding.

Table 4. Performance of meristem tips excised from 3 varieties of pinks 6 weeks after culture on Buys' medium with and without giberellic acid (GA_3).

جدول ٤ ـ نمو وتجذر القمات النامية المؤخوذة من ثلاث أصناف من القرنفل وذلك بعد ستة اسابيع من وجودها على بيئة باي الذي أضيف إليها حامض الجبريليك بالمقارنة مع الشاهد.

Treatment المعاملة	Variety of pinks اصناف القرنفل								
	Doris دوريس			Diane ديان			Joy جوي		
	Shooting تجذر	Rooting نموات	Plants transferred to pots عدد النباتات التي نقلت إلى الأحواض	Rooting تجذر	Shooting نموات	Plants transferred to pots عدد النباتات التي نقلت إلى الأحواض	Rooting تجذر	Shooting نموات	Plants transferred to pots عدد النباتات التي نقلت إلى الأحواض
+ GA_3 مع حامض الجبريليك	No. 30	29	25	30	30	25	30	30	24
	% 100	97	83	100	100	83	100	100	80
− GA_3 الشاهد	No. 30	24	0	30	23	0	30	25	0
	% 100	80	0	100	77	0	100	83	0

Table 5. Survival and virus status of plants obtained from pinks by heat treatment plus meristem tip culture.

جدول ٥ ـ مدى نجاح وخلو نباتات القرنفل من الفيروس التي انتجت من القمم النامية بعد معالجتها حرارياً.

Source variety الأصناف	Heat treatment in weeks المعاملة الحرارية (اسابيع)	No. of tips excised عدد القمم الموجود	Survivors النباتات الناجحة		Virus presence in survivors[a] وجود الفيروس في الشتلات النامية							
					CERV		CarLV		CRSV		CarVMV	
			No.	%	No.	%	No.	%	No.	%	No.	%
Doris دوريس	4	25	23	92	14	61	23	100	N.P.	N.P.	23	100
	8	25	22	88	10	43	22	100	N.P.	N.P.	22	100
	12	25	22	88	6	27	22	100	N.P.	N.P.	16	73
	16	25	21	84	5	24	21	100	N.P.	N.P.	11	52
	20	25	22	88	4	18	22	100	N.P.	N.P.	8	36
Diane ديان	4	25	23	92	13	57	23	100	N.P.	N.P.	20	100
	8	26	23	88	10	43	23	100	N.P.	N.P.	14	64
	12	25	21	84	4	19	21	100	N.P.	N.P.	7	37
	16	24	21	88	3	14	21	100	N.P.	N.P.	4	19
	20	25	22	88	2	9	22	100	N.P.	N.P.	2	11
Joy جوي	4	25	21	84	13	62	21	100	0	0	21	100
	8	24	21	88	11	52	21	100	0	0	18	86
	12	24	20	83	7	35	20	100	0	0	14	70
	15	21	17	85	4	24	17	100	0	0	7	41
	20	20	17	85	2	12	17	100	0	0	5	29
Haytor هيثور	4	18	16	89	10	63	16	100	N.P.	N.P.	20	100
	8	14	12	86	4	33	12	100	N.P.	N.P.	8	53
	12	14	12	86	2	17	12	100	N.P.	N.P.	4	36

N.P. = Virus not present in parent plant = الفيروس غير موجود في النبات الأم

a

CERV =carnation etched ring virus = فيروس التبقع المحفور للقرنفل

CarLV = carnation latent virus = فيروس القرنفل المستتر

CRSV = carnation ring spot virus = فيروس التبقع الحلقي للقرنفل

CarVMV = carnation vein mottle virus. = فيروس تبرقش عروقه القرنفل

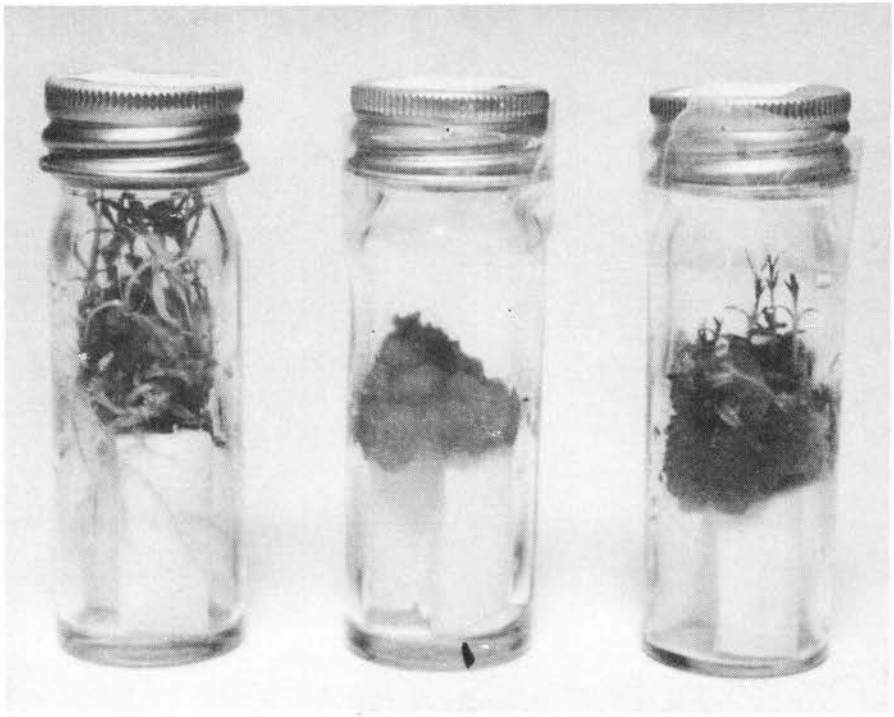

Figure 1. Callus growth and development on Davis' medium

شكل ١ ـ نمو الندبة وتطورها على وسط ديفس الغذائي .

Figure 2. Growing meristem tips 4 weeks after culture on improved Buys' medium.

شكل ٢ ـ قمم مرستيم نامية على وسط باي الغذائي بعد ٤ أسابيع من زراعتها .

الملخص

عبد الماجد، عبد القادر وشيلا روب. ١٩٨٥. القضاء على بعض الأمراض الفيروسية التي تصيب زهرة القرنفل بواسطة زراعة القمة النباتية والمعالجة الحرارية. مجلة وقاية النبات العربية ٣: ١٠٤ - ١١٠.

وجد أن أربعة أمراض فيروسية تصيب زهرة القرنفل في انجلترا هي: Carnation etched ring virus (CERV), Carnation ring spot virus (CRSV), Carnation vein mottle virus (CarVMV) and Carnation latent virus (CarLV). الغرض من هذا البحث هو العمل على استخراج سلالات من زهرة القرنفل خالية من هذه الأمراض. أمكن التوصل إلى استعمال وسط غذائي لزراعة القمة النامية لزهرة القرنفل لانتاج عدة سلالات لأربعة أصناف من هذه الزهرة خالية من الثلاثة أمراض الفيروسية الأولى. تم التخلص من الفيروس الأول والثاني، عن طريق زراعة هذه القمم في الوسط الغذائي فقط ولكن يسهل ذلك كثيراً مع استعمال المعالجة الحرارية. أما الفيروس الثالث فقد تم التخلص منه فقط بعد أن عرضت الأمهات المصابة لهذه القمم لعدة أسابيع قبل قطع وزرع هذه القمم في الوسط الغذائي. لم تنجح أي من هذه الطرق العلاجية في القضاء على المرض الفيروسي الرابع.

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