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تكثير نباتات الخس بطريقة زراعة الأنسجة

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العراق

ان هذه الورقة لم يتم تحريرها.

89-0206

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الموصل ـ العراق

شملت الدراسة استحداث الكالس من قطع الاوراق الفتية لنبات الخس (Luctuca sativa c.v. longiflora) في وسط موراشيج وسكوك الحاوى على ٠ر٥ ملغم في اللتر من الـ 2,4 D و ٠ر١ ملغم في اللتر من الكاينيتين ٠ ووجد بان نمو الكالس يتوقف عند فصله من القطع النباتية وزراعته على الاوساط الحاوية على الكاينتين والـ 2,4 D ٠ في حين ان الكالس اظهر نموا كبيرا جدا في الاوساط الحاوية على NAA والـ IAA و الـ IBA و الـ GA_3 مع الـ BA ٠ كما لوحظ تكون للجذور والسيقان من الكسالس النامي في الوسط الحاوى على ٠ر٥ ملغم في اللتر من الـ NAA مع ٠ر١ ملغم في اللتر من الـ BA ٠ وعزلت النباتات المتكونة وتم نقلها وزراعتها في التربة٠

واظهرت الفحوصات المجهرية لمقاطع الكالس بان الجذور او السيقان او كلاهما يتكونان داخليا ونوقشت نتائج هذه الدراسة كواسطة لتكثير نباتات الخس المهمة بالنسبة لبرامج التهجين ٠

PROPAGATION OF LETTUCE (*Lactuca sativa* c.v. longiflora) BY TISSUE CULTURE

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ABSTRACT

Callus cultures were initiated from young leaves of lettuce (*Lactuca sativa* c.v. longiflora) on Murashige and Skoog medium containing 0.05 mg L^{-1} 2,4-D and 1.0 mg L^{-1} Kinetin. No callus growth was achieved on all media containing 2,4-D and Kinetin after the callus had been isolated from the explant. In contrast the isolated callus grew vigorously in all media containing NAA and BA and to a lesser extent in media containing IAA, IBA, GA_3 with BA. At 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} BA, root and shoot production were initiated and the plantlet were eventually isolated and transferred to the soil.

Microscopic examination of the sectioned callus revealed that shoot and/or root development occurred internally. The relevance of this system is an aid in the breeding programme of lettuce is discussed.

INTRODUCTION

Lettuce plant (Lactuca sativa c.v. longiflora)is an economically important crop in Iraq. Propagation of lettuce plant either as breeding material or for commercial uses based mainly on seeds production. These plants became infected with various pathogen mainly Botrytis spp. (Pink and Carter, 1987) and either oftenly die or fail to produce any seeds depending on environmental condition particularly in the field. Germination of seeds is rather slow and the seedling produced fail to grow (Zink and Yamaguchi, 1962). It was therefore decided to devise a method of propagation of healthy plants using tissue culture techniques of its great advantages, as reported by many workers (Street, 1978, Mohammad and Collin, 1979, Mohammad and Hassan 1988).

Propagation of lettuce plant from different plant parts has been reported by Koevary et al. (1978). They regenerated lettuce plants from apical segments and expanded bud but failed to get any plantlets from non-expanded bud or pith segments. Pink and Carter (1987) propagate lettuce plant for certain breeding programme selected for seed production using auxillary buds grown on Murashige and Skoog (1962) medium. The aim of the present study was to devise a more efficient method of micropropagation of lettuce plants.

MATERIAL AND METHODS

Seeds of lettuce (Lactuca sativa c.v. longiflora) were grown in the field of Horticulture Research Station, Mosul, Iraq. Mature plants (3 months old) were collected from the field before the commencement of this investigation. The internal young leaves were separated, washed several times with water and cut into 0.5 cm^2 discs avoiding the midrib region. The discs were sterilized for 10 minutes in 10% calcium hypochlorite followed by five washings in sterile distilled water. Each disc was transferred to 10 cm^3 modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) and contained in a McCartney vial. The vials were kept at 25 ± 1 °C under flourescent light in a growth chamber (Sherer Gillet Co) on a 16-h diurnal cycle. Callus were initiated from the discs on MS medium containing 0.05 mg l^{-1} 2,4-D and 1.0 mg l^{-1} Kinetin. Trials showed that this medium is the most suitable for the most rapid and sustained callus growth. The callus developed were separated aseptically and 0.5 gm were placed on media containing MS medium supplemented with different growth regulators. The growth regulators used in this investigation were benzyladenin (BA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphtylacetic acid (NAA), indole-3-acetic acid (IAA), indolebutyric acid (IBA) and gibberellic acid (GA_3) at concentrations of 0, 0.05, 0.1, 0.5, 1 and 5 mg l^{-1}. All media used were supplemented with 4%sucrose. The callus developed on particular medium were normally subcultured every 3 weeks to avoid excessive pigmentation. 0.5 gm of callus developed on particular medium were prepared for histological examination as described by Johanson (1968) with minor modification. The callus

were fixed in FAA solution, dehydrated through alcohol series, embedded in parafin and sectioned 10 um thick. They were double stained with sofranine as primary stain and fast green as counter stain (Johanson, 1968).

RESULTS

Callus initiation and growth:

Callus initiation occurred on medium containing 0.05 mg l^{-1} 2,4-D and 1.0 mg l^{-1} Kinetin. The callus developed on such medium was isolated and used for further investigation. Despite the fact that different combinations of various concentrations of 2,4-D and Kinetin supplemented to MS media, the medium which gave suitable callus growth was the medium containing 0.05 mg l^{-1} 2,4-D and 1.0 mg l^{-1} Kinetin.

No callus growth was achieved in all media containing different concentrations of 2,4-D and Kinetin after the callus being isolated from the explant. The callus growth was more vigorous in all media containing NAA and BA thanin the other media used. The absence of NAA in the media inhibited completely callus growth (Table 1). Callus fresh weight was 18.39 gm on medium containing both NAA and BA at 1.0 mg l^{-1} concentration. The callus developed on such medium was soft, compact and light green in colour (Fig.1). Increasing the concentrations of both NAA and BA in the media leads to a substantial increase in callus fresh weight. Moreover, shoots and roots were produced on media containing a relatively high level of both NAA and BA (Figs. 2 and 3). After 10 weeks the

plantlets had grown to a height of about 12 cm and ready to transfer to the soil. The plantlets were hardened by normal procedure and transferred to the soil. The plants appeared to have normal morphology as those developed from the seeds (Fig. 4).

In contrast, replacing IAA instead of NAA affected greatly callus growth. Callus growth occurred only in a few combinations of IAA and BA (Table 2). Maximum growth was on medium containing 1.0 mg l^{-1} BA and 0.5 mg l^{-1} IAA with no indication of shoot or root development. However, callus growth was achieved on media containing different concentrations of IBA and BA. Even though callus fresh weight was less than in a media containing NAA and BA, maximum growth (5.53 gm) was obtained on medium containing 0.5 mg l^{-1} IBA and 1.0 mg l^{-1} BA (Table 3). Nevertheless, addition of GA_3 instead of other auxins stimulated callus growth to a certain extent (Table 4). Also there was no indication of shoot or root development in all combinations of GA_3 and BA used.

Roots and shoot formation:

The roots and shoot production noted during the period of callus growth occurred on different media containing the auxins at various concentration. The roots and shoot formation was apparent on media containing IBA, NAA and BA at particular concentrations (Table 5). However, callus growth was vigorous in medium containing both NAA and BA at 1.0 mg l^{-1}. The number of shoots developed on medium containing NAA was far more better than other media. Microscopic examination of the sectioned callus revealed that shoot or

root development occurred internally (Fig. 5). The emberyonic callus consisted of two types of tissue, a loose friable tissue and compact clumps. The compact clumps represent the early development of growth region while the loose tissue may indicate the part of callus which developed into initiating growth region tissue with subsequent growth period.

DISCUSSION

The lettuce callus could be initiated from explant tissue and maintained either in undifferentiated form with a high growth rate or in differentiated form for plantlet production. Koevary et al. (1978) propagated lettuce (crisp cultivar) using buds but with limited success in producing plants for breeding lines. Pink and Carter (1987) successfully propagated lettuce from both expanded and non-expanded buds in both capitan and butterhead cultivars. The plantlet produced failed to heart but started to bolt 45 days after they were transferred to compost. In the present study, lettuce c.v. longiflora plant could be propagated by the system used with high rate of plantlet production. The major differences between the present work and other works were in the culture media and the explant source. Lettuce callus could be initiated very readily from sterilized leaf discs on Murashige and Skoog (1962) media containing 0.05 mg l^{-1} 2,4-D and 1.0 mg l^{-1} Kinetin. The isolated callus showed no growth when subcultured on media containing 2,4-D and Kinetin. This could be due to explant in supplying certain growth factor as in the case of other plants (Street 1978). However,

rapid callus growth was achieved in most of the media containing BA as cytokinin source and NAA, IAA, IBA as an auxxins. Increasing of both BA and auxins level leads to an increase in the callus fresh weight. Optimal callus growth was achieved in media containing BA and NAA at 1.0 mg l^{-1} (Table 1). Higher concentrations of BA and all auxins and GA_3 used retarded callus growth to a certain degree depending on types of auxin used (Tables 2,3, and 4). This was the same as in other plants reported by Takayama and Misawa (1979), Mohammad and Collin 1979, and Dodds and Roberts (1985). Therefore, it would seem likely that lettuce callus growth was directly related to the type of auxxins used as in the case of other systems used for callus and plantlet formation (Pink and Carter 1987).

Callus fresh weight after 6 weeks was 18.39 gm in media containing BA and NAA at 1.0 mg l^{-1} as compared with 5.53 gm in media containing BA at 1.0 mg l^{-1} and IAA at 0.5 mg l^{-1}. In contrast lower levels of BA (0.05 mg l^{-1}) in combination of 0.1 mg l^{-1} GA_3 was required for optimal callus growth.

The callus produced on the selected medium was soft and compact (Fig. 1) as in the case of other plant callus (Sandra et al. 1977, Amin 1985 and Mohammad et al., 1986).

Somaclonal variants have been found among lettuce plants regenerated from leaves and cotyledons (Sibi 1976, Brown et al., 1986). In the present study there was no evidence of somaclonal variation (Fig. 4), and in general clones derived from meristematic

tissue are more likely to be genetically uniform (Murashige 1974). The lettuce plants regenerated in this investigation derived mainly from meristematic tissues as evident from callus section (Fig. 3).

It can be concluded from the results that lettuce plant could be propagated easily and the cultures grown on MS media were easy to grow and tolerated a range of BA and auxins concentrations. This system may be useful for commercial uses and to rescue certain genotypes needed for special breeding programme.

Table 1: Fresh weight of lettuce callus (g) after three subcultures (6 weeks) on media containing varying concentrations of NAA and BA. Each value represents the mean of 10 replicates ± (SE) Standard error of the mean. (X) No callus growth (*) All callus contaminated.

NAA mgl^{-1}	BA mg l^{-1}					
	0.0	0.05	0.1	0 .5	1.0	5.0
0.0	X	X	X	X	X	X
0.05	5.16 ± 0.11	11.43 ± 0.21	7.14 ± 0.02	7.17 ± 0.32	6.58 ± 0.07	6.55 ± 0.211
0.1	6.93 ± 1.35	9.18 ± 1.02	11.35 ± 0.92	8.53 ± 0.92	11.24 ± 0.23	8.49 ± 3.106
0.5	8.31 ± 0.72	9.98 ± 1.22	9.59 ± 1.12	8.60 ± 1.14	12.24 ± 0.86	14.59 ± 0.023
1.0	6.83 ± 0.37	6.84 ± 1.39	15.87 ± 3.56	8.32 ± 0.89	18.41 ± 2.19	7.22 ± 0.231
5.0	*	9.68 ± 2.49	13.12 ± 2.03	13.56 ± 0.64	9.11 ± 1.90	8.38 ± 8.89

Table 2: Fresh weight of lettuce callus (g) after three subcultures (6 weeks) on media containing varying concentrations of IAA and BA. Each value represents the mean of 10 replicates ± (SE) Standard error of the mean. (X) No callus growth. (*) All callus contaminated.

IAA mg l^{-1}	BA mg l^{-1}					
	0.0	0.05	0.1	0.5	1.0	5.0
0.0	X	X	X	X	X	X
0.05	X	X	X	2.44 ± 0.45	1.53 ± 0.36	X
0.1	X	*	3.02 ± 0.065	3.09 ± 0.2	2.98 ± 0.06	X
0.5	X	X		2.627 ± 0.45	3.92 ± 0.04	X
1.0	X	X	X	X	X	X
5.0	X	X	X	X	X	X

Table 3: Fresh weight of lettuce callus (g) after three subcultures (6 weeks) on media containing varying concentrations of IBA and BA. Each value represents the mean of 10 replicates. ± Standard error of the mean. (X) No callus growth. (*) All callus contaminated.

IBA mg l^{-1}	BA mg l^{-1}					
	0.0	0.05	0.1	0.5	1.0	5.0
0.0	X	X	X	X	X	X
0.05	1.85 ± 0.06	2.45 ± 0.21	3.12 ± 0.10	2.18 ± 0.17	3.11 ± 0.06	1.16 ± 0.27
0.1	2.08 ± 0.15	2.09 ± 0.18	2.54 ± 0.06	3.39 ± 0.09	2.01 ± 0.13	1.61 ± 0.07
0.5	1.50 ± 0.28	1.80 ± 0.09	2.15 ± 0.10	4.12 ± 0.37	5.53 ± 0.27	0.91 ± 0.16
1.0	1.36 ± 0.06	1.97 ± 0.23	3.32 ± 0.12	1.64 ± 0.15	*	X
5.0	X	X	X	X	X	X

Table 4: Fresh weight of lettuce callus (g) after three subcultures (6 weeks) on media containing varying concentrations of GA_3 and BA. Each value represents the mean of 10 replicates. ± Standard error of the mean. (X) No callus growth. (*) All callus contaminated.

GA_3 mg l^{-1}	BA mg l^{-1}					
	0.0	0.05	0.1	0.5	1.0	5.0
0.0	X	X	X	X	X	X
0.05	3.33 ± 0.43	3.97 ± 0.49	3.81 ± 0.12	4.03 ± 0.77	1.44 ± 0.07	X
0.1	2.16 ± 0.08	5.07 ± 0.35	4.53 ± 0.36	4.67 ± 0.36	3.37 ± 0.20	3.04 ± 0.49
0.5	2.57 ± 0.22	2.11 ± 0.08	2.59 ± 0.18	2.59 ± 0.37	3.07 ± 0.23	3.30 ± 0.40
1.0	2.30 ± 0.24	2.24 ± 0.16	2.11 ± 0.23	2.13 ± 0.13	2.19 ± 0.21	X
5.0	1.46 ± 0.33	1.46 ± 0.26	1.09 ± 0.12	*	X	X

Table 5: Effect on concentration of BA and NAA, IBA, IAA ,2,4 - D and GA_3 on callus growth, shoot and root formation of lettuce.

(-) No callus growth.

Auxins mg l^{-1}		BA mg l^{-1}				
		0	0.05	0.1	0.5	1.0
NAA	0.05	C	C & R	S & R	C	N
	0.1	N	C & R	S	R & C	N
	0.5	R	S	S	S & C	N
	1.0	R	C	C	C	N
IBA	0.05	-	C	C & R	S	R
	0.05	-	-	C	R & S	C
	1.0	C	C	C & R	C	-
IAA	0.05	-	-	-	C	-
	0.1	-	-	C	C	C
	0.5	-	-	-	C	-
GA_3	0.05	-	C	C	C & S	C
	0.1	C	C	C	R & S	R & S
	0.5	C	C	C	C	C
	1.0	C	C	C	C	C
2,4-D	-	-	-	-	-	-

N = normal callus growth

C = callus only after prolonged growth period

R = roots formation occurred

S = shoots formation occurred

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LIST OF FIGURE CAPTIONS

Fig. 1. Lettuce callus grown on medium containing both NAA and BA at 1.0 mgl^{-1} concentrations after 6 weeks.

Fig. 2. Lettuce callus showing roots and supporting a number of shoots after 8 weeks on medium containing NAA and BA.

Fig. 3. Lettuce callus showing plantlets redy for transfer to the soil after 10 weeks on medium containing NAA and BA.

Fig. 4. Mature plant derived from seeds (A) and from callus (B).

Fig. 5. Transverse section through lettuce emberyonic callus grown on medium containing both NAA and BA consisting of loose fraible tissue (L) and compact clumps (C). X20.

Figure 1

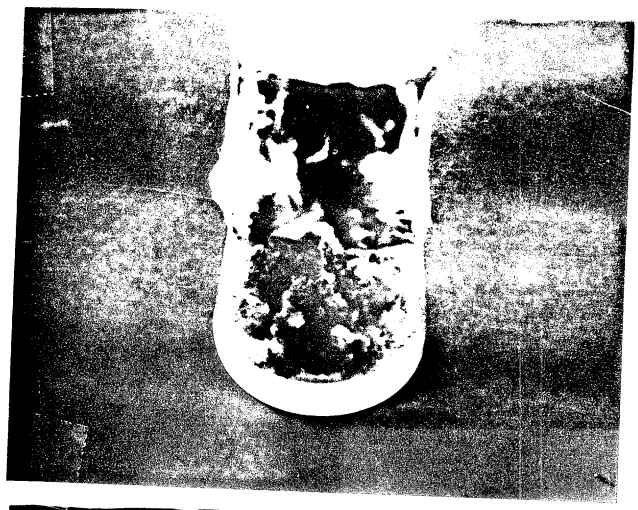

Figure 2

Figure 3

Figure 4

Figure 5