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CALLUS INDUCTION AND PLANT REGENERATION FROM KURRAT (*Allium ampeloprasum* var. kurrat) AND LEEK (*Allium ampeloprasum* var. porrum)

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ABSTRACT

A procedure for plant regeneration from kurrat and leek callus derived from florets, seeds, leaf base and root explants, is described. Florets of kurrat were excised from immature inflorescences and cultured on Murashige and Skoog (MS) medium supplemented with1mg/l benzyladenine (BA) and 0.5 mg/l naphthaleneacetic acid (NAA) for five weeks prior transfer into shoot-induction medium. Seeds, leaf bases and roots of kurrat and leek were cultured on callus-induction medium composed of MS containing 300 mg/l casine hydrolyste and 200 mg/l glutamine and supplemented with 0.5, 3, 6, 10 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 3 mg/l picloram, 3 mg/l NAA, 3 mg/l naphoxyacetic acid (NOA) or 6 mg/l indoleacetic acid (IAA). 2,4-D and picloram were effective in callus induction from seeds, leaf bases and roots of kurrat and leek while IAA was inefficient. Callus was transferred into shoot-induction medium composed of MS supplemented with 0.2 mg/l BA. Produced shoots were rooted, acclimatized and transferred to soil. These procedures are efficient for improving plant characteristics through shoot regeneration from callus derived from floret, seeds and leaf bases of kurrat.

Abbreviations: AC: activated charcoal, BA: benzyladenine, IAA: indoleacetic acid, IBA: indolebutyric acid, MS: Murashige & Skoog's (1962) medium, NAA: naphthaleneacetic acid NOA: naphthoxya-

yacetic acid, picloram: 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid, 2,4-D:

Key words: *amaryllidaceae, leaf base, propagation, root, seed, tissue culture.*

1. INTRODUCTION

Kurrat (*Allium ampeloprasum* Schweinf. var. kurrat) and Leek (*Allium ampeloprasum* Schweinf. var. porrum) are popular vegetables which belong to the family Amaryllidaceae. Kurrat is grown in Egypt and eastern Mediterranean countries where it is consumed fresh and for seasoning (Tackholm and Drar, 1954; Jones and Mann, 1963; Yamaguchi, 1983; Mohamed-Yasseen *et al.*, 1995; Mohamed-Yasseen and Costanza, 1996; Mohamed-Yasseen, 2001). Kurrat is similar to leek in stature but is much smaller than leek and is completely interfertile with leek (Kadry and Kamel, 1955; 1959). Leek is an important vegetable crop and is more consumed in Europe.

Few reports were published about tissue culture of kurrat. Mohamed-Yasseen *et al.*, (1995) and Mohamed-Yasseen and Costanza (1996) used basal stem of seedlings and mature plants for clonal propagation and plant regeneration. Mohamed-Yasseen (2001) reported an efficient protocol for shoot proliferation from inflorescence and plant regeneration from callus derived from kurrat ovary using 2,4-D.

Numerous researches on tissue culture of leek were performed using stem base segments, meristem, inflorescence and ovary explants. Debergh and Standaert-Metsenaere (1976) used stem base segments for shoots and bulbils production. Dore (1988) employed meristem of leek for clonal propagation. Novak and Havel (1981); Havel and Novak (1985); Novak *et al.*, (1986) and Rauber and Grunewaldt (1988) illustrated different protocols for shoot proliferation from leek inflorescence. More attention was given by many researchers (Keller, 1990; Smith *et al.*, 1991; Schum *et al.*, 1993; Ionescu and Popandron, 1995) to the potential of leek ovary for production of dihaploid plants through gynogenesis.

Tissue culture has the potential to improve plant characteristics through somaclonal variations and recombinant DNA (Mohamed-Yasseen and Splittstoesser, 1990). In this paper a protocol for callus induction and plant regeneration from florets, seed, leaf base and root explants of kurrat and leek is presented.

2. MATERIALS AND METHODS

2.1. Source of explants

Immature inflorescences were collected from kurrat plants, cultivar Baladi, growing in a shadehouse at the Genetic Engineering and Biotechnology Research Institute.All experiments were conducted from 2001 to 2002 at the Genetic Engineering and Biotechnology Research Institute at Sadat City, University of Minufiya.

2.2. Explant preparation

Immature inflorescences of kurrat *cv* Baladi were collected during April and May. Florets were excised and surface sterilized as described earlier (Mohamed-Yasseen, 2001). Seeds of kurrat (cultivar Baladi) and leek (local cultivar), were surface sterilized with 0.1% $HgCl_2$ for 10 min and rinsed three times in sterile distilled water. Seeds were subsequently sterilized with 0.79% (v/v) sodium hypochlorite, with two drops of Tween x100, for 20-25 min then rinsed three times in sterile distilled water.

2.3. Culture of florets of kurrat *in vitro*

Florets of kurrat were cultured on MS supplemented with 30 g/l sucrose, 2 g/l gelrite and supplemented with 1mg/l BA and 0.5 mg/l NAA. Florets were maintained for five weeks. Floret explants were then transferred into shoot-induction medium composed of MS supplemented with 30 g/l sucrose, 8 g/l agar and 0.2 mg/l BA.

2.4. Culture of seeds of kurrat and leek *in vitro*

Seeds of kurrat and leek were cultured on callus-induction medium. Callus-induction medium was composed of MS medium containing 30g/l sucrose, 2 g/l gelrite, 300 mg/l casine hydrolyste and 200 mg/l glutamine and supplemented with 0.5, 3, 6or 10 mg/l 2,4-D, 3 mg/l picloram, 3 mg/l NAA, 3 mg/l NOA or 6 mg/l IAA. Seeds of kurrat and leek were germinated on MS medium containing 30 g/l sucrose and 8 g/l agar contained in 55-ml culture tubes and sealed with plastic polypropylene lids (Sigma, Saint Louis, MO).

2.5. Culture of leaf base and root explants of kurrat and leek *in vitro*

Seedlings of kurrat and leek produced on MS medium were used as source of leaf base and root explants. Explants were excised from seedlings reaching 30-70 mm in length. Leaf base (approximately 4-10 mm in length) and root explants (approximately 12-20 mm in length) were removed from seedling and cultured on callus-induction medium.

2.6. Shoot regeneration from callus derived from seeds, leaf base and root explants

Callus was maintained for 12-14 weeks in the dark before transfer to shoot-induction medium. Shoot-induction medium was composed of MS supplemented with 30 g/l sucrose, 8 g/l agar and 0.2 mg/l BA.

2.7. Root-induction medium

Regenerated shoots were transferred into root-induction medium. Root-induction medium was composed of MS supplemented with 30 g/l sucrose, 8 g/l agar and containing 1 mg/l IAA. In some instances some shoots were transferred into bulb-induction medium. Bulb-induction medium consisted of MS supplemented with 90 g/l sucrose, 8 g/l agar and 1g/ AC.

2.8. Media and culture conditions

Media pH was adjusted to 5.7 with 1N KOH after adding growth regulators but before adding agar. Growth regulators were added before sterilization in an autoclave at 121 °C and 98 KPa for 20 min. Cultures were maintained under an 18 hr photoperiod (cool white fluorescent light, 40 $\mu mol.m^{-2}.s^{-1}$) and 28 °C except for callus cultures which were maintained in the dark.

2.9. Rooting, bulb induction and transfer to soil

Produced shoots were separated and transferred into root-induction medium. Rooted shoots were planted in black polyethylene bags containing autoclaved mixture of soil, peat moss and vermiculite (1:1:1, v:v:v) and covered with a transparent polyethylene bag for acclimatization in a glasshouse. In some instances some produced shoots were transferred into bulb-induction medium. Bulb induction can be operated with shoot prior root-induction or after root-formation. Produced bulbs can be transferred directly to soil without need for acclimatization or stored under 4 °C for several months until needed.

2.10. Experimental design

All experiments were conducted using a completely randomized design. Twenty replicates were used in each treatment and each experiment was repeated at least twice. Data were evaluated by analysis of variance (Duncan, 1955).

3. RESULTS AND DISCUSSION

3.1. Morphogenesis of kurrat florets

Florets of kurrat produced green callus on MS supplemented with 1mg/l BA and 0.5 mg/l NAA. This callus produced shoots upon transfer into shoot-induction medium (Fig.1a). About sixty percent of the florets produced regenerable callus. Each floret produced multiple shoots (average 2.5 ± 0.4 SD shoots/explant). Mohamed-Yasseen (2001) illustrated the potential of kurrat florets for producing regenerable callus through gynogenesis using media containing 3 mg/l 2,4-D. The present work displayed another method for shoot regeneration from kurrat florets.

3.2. Morphogenesis of kurrat seeds

Seeds of kurrat cultured on callus-induction medium produced granular friable callus (Fig. 1b) on MS supplemented with 2,4-D, picloram and NOA (Table, 1).

Table (1): Effect of different auxins on callus formation from kurrat seeds after 12 weeks from culture.

Supplement	Callus formation %	Callus weight (mg/seed)	Shoot/seed
0.5 mg/l 2,4-D	92	226 b[Z]	5.2a
3 mg/l 2,4-D	84	131 c	4.4 ab
6 mg/l 2,4-D	32	52 d	3.2 b
10 mg/l 2,4-D	11	207 b	0.0 d
3 mg/l Picloram	94	0.0 e	3.8 b
3 mg/l NAA	0.0	292 a	1.3c[Y]
3 mg/l NOA	52	0.0 e	3.3 b
6 mg/l IAA	0.0	0.0 e	0.0 d

[Z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05. [Y] Media containing 3 mg/l NAA did not produce callus, however, seedlings produced multiple shoots.

The percentage of callus formation and callus weight decreased with increasing 2,4-D concentration from 0.5 mg/l to 10 mg/l. Shoot regeneration occurred after transfer of callus into shoot-induction medium (Fig. 2a). Shoot regeneration exhibited the same

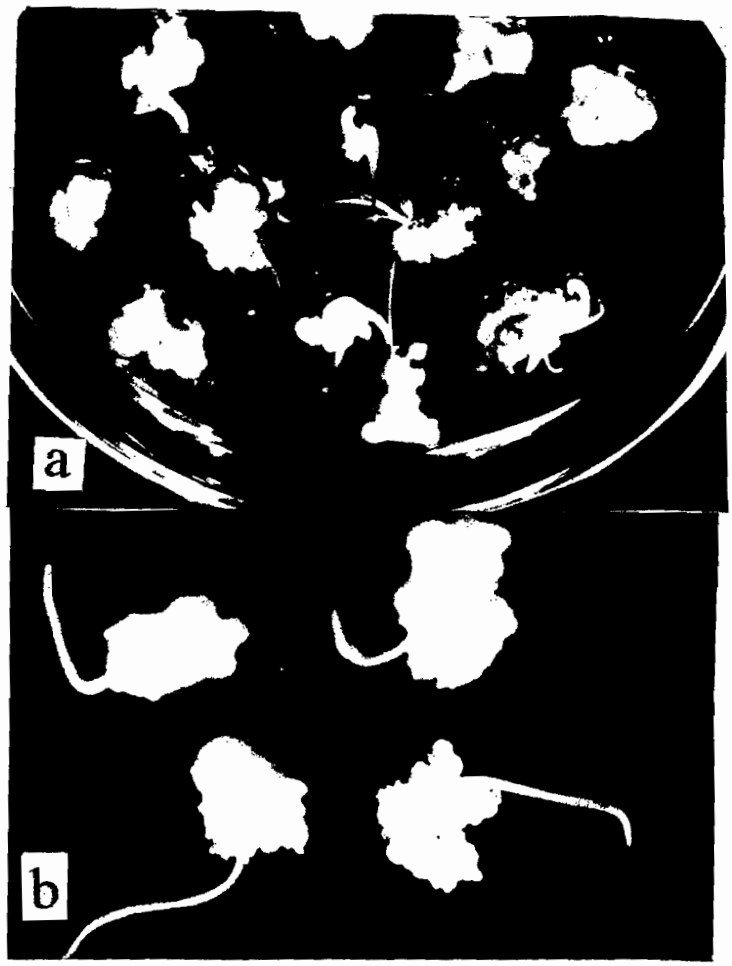

Fig. (1): Callus formation from florets and seeds. (a) Callus and shoot initiation from kurrat florets. (b) Nodular friable callus produced from kurrat seeds.

trend of callus production. Callus produced on MS supplemented with 10 mg/l 2.4-D was brownish and did not regenerate shoots. Some shoots were produced on MS supplemented with NAA without callus formation.

3.3. Morphogenesis of leek seeds

Callus was produced from leek seed cultured on callus-induction medium containing 2,4-D, picloram and NOA, but there was no callus formation on media supplemented with NAA or IAA (Table, 2).

Table (2): Effect of different auxins on callus formation from leek seeds cultured on callus induction medium for 12 weeks.

Supplement	Callus formation %	Callus weight (mg/seed)	Shoot/seed
0.5 mg/l 2,4-D	31	16.0 e[z]	1.3 c
3 mg/l 2,4-D	30	20.2 b	2.8 bc
6 mg/l 2,4-D	28	29.8 a	4.7 a
10 mg/l 2,4-D	10	14.0 cd	0.9 d
3 mg/l Picloram	6	11.3 d	0.4 d
3 mg/l NAA	65	31.3 a	2.2 c
3 mg/l NOA	12	16.2c	3.2 b

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

Percentage of callus formation increased with increasing 2,4-D concentration from 0.5 mg/l to 6 mg/l. However, callus weight decreased drastically with increasing 2,4-D concentration to attain its lowest value at 10 mg/l. Shoot regeneration per seed of leek was clearly lower than shoot regeneration from kurrat seed.

3.4. Morphogenesis of kurrat leaf base

Callus was produced from leaf base of kurrat cultured on MS supplemented with 2,4-D, picloram and NAA (Table, 3).

Callus formation increased with increasing 2,4-D concentration from 0.5 mg/l to 3 mg/l and decreased when 2,4-D concentration reached 6 mg/l. Percentage of callus formation and callus weight was high on MS supplemented with picloram. However, shoot regeneration from callus produced on picloram was lower (2.2 shoot/explant) than shoot regeneration from callus produced on 3

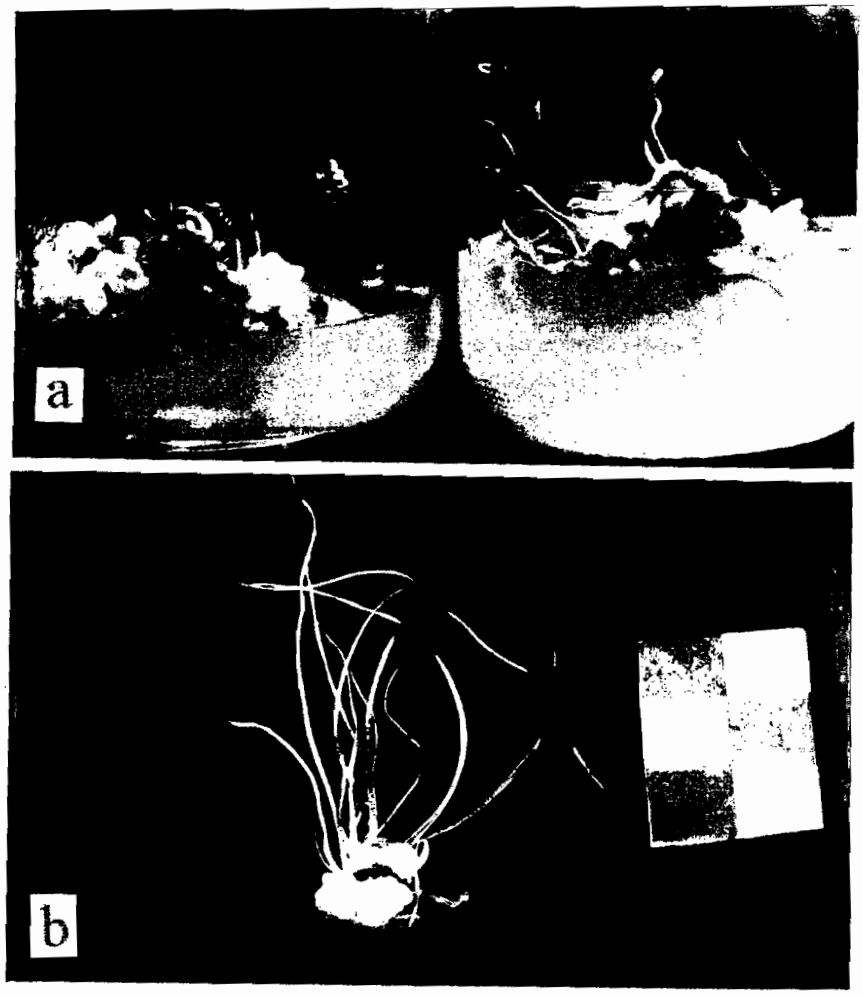

Fig. (2): Typical shoot regeneration from callus. (a) Shoot regeneration from kurrat seed callus. (b) Shoot regeneration from kurrat leaf base callus (each square corresponds to 10mm).

mg/l 2,4-D (4.7 shoot/explant) or NAA (3.2 shoot/explant) (Table 3; Fig. 2b).

Table (3): Effect of different auxins on callus formation from leaf base explants of kurrat after 12 weeks from culture on callus induction medium.

Supplement	Callus formation %	Callus weight (mg/10 explant)	Shoot explant
3 mg/l 2,4-D	32	45 c[z]	0.07 b
3 mg/picloram	56	234 a	0.15 a
3 mg/l NAA	46	115 b	0.16 a
3 mg/l NOA	29	87 b	0.08 b

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

3.5. Morphogenesis of leek leaf base

Callus was produced from leaf base of leek cultured on MS supplemented with 2,4-D, picloram, NAA and NOA but callus was not produced on MS supplemented with IAA (Table, 4). Callus formation decreased with increasing concentration of 2,4-D from 0.5 mg/l to 6 mg/l. Frequency of shoot regeneration from callus produced from leek leaf base on 2.4-D, picloram, NAA and NOA was very low compared to shoot regeneration from leaf base callus of kurrat.

Table (4): Effect of different auxins on callus formation from leaf base explants of leek after 12 weeks from culture on callus induction medium.

Supplement	Callus formation %	Callus weight (mg/explant)	Shoot/explant
0.5 mg/l 2,4-D	62	42.9 bc[z]	0.4 a
3 mg/l 2,4-D	57	28.3 c	0.0 c
6 mg/l 2,4-D	23	23.2 d	0.0 c
10 mg/l 2,4-D	12	15.3 d	0.0 c
3 mg/l Picloram	94	81.2 a	0.2 b
3 mg/l NAA	34	26.5 cd	0.0
3 mg/l NOA	46	44.1b	0.1 b
6 mg/l IAA	0.0	0.0 e	0.0 c

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

3.6. Morphogenesis of kurrat root explants

Kurrat root explants produced callus on MS supplemented with 2,4-D, picloram, NAA and NOA (Table, 5). Percentage of callus formation and callus weight were high in the presence of picloram and NAA. Produced callus was nodular with all auxins, nevertheless, the frequency of shoot regeneration was low.

Table (5): Effect of different auxins on callus formation from root explants of kurrat after 12 weeks from culture.

Supplement	Callus formation %	Callus weight (mg/10 explant)	Shoot explant
3 mg/l 2,4-D	32	45 c[z]	0.07 b
3 mg/ l picloram	56	234 a	0.15 a
3 mg/l NAA	46	115 b	0.16 a
3 mg/l NOA	29	87 b	0.08 b

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

3.7. Morphogenesis of leek root explants

Leek root explants produced nodular callus on roots cultured on MS supplemented with 2,4-D, picloram, NAA and NOA (Table, 6). Percentage of callus formation and callus weight were higher on the presence of picloram and NAA. Shoot regeneration from leek root callus was low.

Table (6): Effect of different auxins on callus formation from root explants of leek after 12 weeks from culture.

Supplement	Callus formation %	Callus weight (mg/10 explant)	Shoot explant
3 mg/l 2,4-D	67	75 d[z]	0.20 a
3 mg/ l picloram	85	303 a	0.10 b
3 mg/l NAA	62	207 b	0.15 a
3 mg/l NOA	44	119 c	0.05 c

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

Conclusions

Several explants from kurrat and leek were investigated for callus-induction and plant regeneration using four different auxins. Type of auxin, plant genotype and type of explant affected callus

formation and plant regeneration from callus. Kurrat and leek explants were generally responsive in callus formation however, shoot regeneration was higher from kurrat callus. Kurrat seeds produced regenerable callus with high frequency suggesting that kurrat seed would be an excellent explant for plant propagation and production of plants with improved traits. Kurrat leaf base showed good response in producing regenerable callus. On the other hand, roots of kurrat as well as seeds, leaf bases and roots of leek produced callus but the frequency of shoot regeneration from produced callus was fairly low.

This paper described a protocol for callus formation and plant regeneration from florets of kurrat as well as seed, leaf base and root explants of kurrat and leek. Shoot regeneration from kurrat florets, seed and leaf base is an excellent tool for plant regeneration and offered the basis for improving plant characteristics.

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إنتاج نباتات من الكالوس المتكون من أجزاء مختلفة من الكرات البلدى والكرات أبو شوشة فى مزارع الأنسجة

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ملخص

يتناول هذا البحث استخدام تقنية زراعة الأنسجة في إنتاج نباتات من الكالوس المتكون من أزهار و بذور وقواعد الأوراق والجذور لكل من الكرات البلدى والكرات أبو شوشة في مزارع الأنسجة. تم زراعة الأجزاء النباتية على بيئة موراشيج وسكوج المحتوية على ٠,٥ أو٣ أو٦ أو ١٠ مليجرام/لتر من التو فوردى أو٣ مليجرام/لتر من نفثالين حمض الخليك أو نفثوكسى حمض الخليك. وقد تم الحصول على النباتات من الكالوس بعد نقله الى بيئة موراشيج وسكوج المحتوية على ٢,. مليجرام/لتر بنزيل ادينين وتكونت العديد من الفروع بعد عدة أسابيع من الزراعة. وتم نقل الأفرع المتكونة الى بيئة موراشيج وسكوج المحتوية على ١مليجرا م من إندول حمض الخليك لتكوين الجذور وتم أقلمة النباتات وزراعتها في الصوبة. نقلت بعض الأفرع المتكونة إلي بيئة موراشيج وسكوج محتوية على .٩جرام/لتر سكروز وذلك لتشجيع تكوين الأبصال التى يمكن تخزينها لحين الحاجة إليها. وقد وجد ان الكالوس المتكون من أزهار وبذور وقواعد الأوراق للكرات البلدى لها القدرة على انتاج عدد كبير من النباتات.

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