Plant Cell, Tissue and Organ Culture 14: 41-50 (1988)
© Kluwer Academic Publishers, Dordrecht - Printed in the Netherlands

Short communication

Plant regeneration from oca (*Oxalis tuberosa* M.): the effect of explant type and culture media

M.R.I. KHAN[1], J.K. HEYES[1] & D. COHEN[2]
[1] *Botany Department, Victoria University of Wellington, Private Bag, Wellington, New Zealand;* [2] *Plant Physiology Division, DSIR, Palmerston North, New Zealand*

Received 15 September 1987; accepted in revised form 4 January 1988

Key words: Oca, *Oxalis tuberosa* M., plant regeneration, petiole, internode sections

Abstract. Shoot regeneration has been obtained from internode and petiole sections of oca on a number of culture media supplemented with 3 $mg\,l^{-1}$ naphthaleneacetic acid and 3 $mg\,l^{-1}$ of either benzylaminopurine or zeatin, the latter being more effective. A greater percentage of sections from the 4th, 5th and 6th internodes (numbered from the apex) produced shoots than sections from older or younger internodes. Of five locally available genotypes based on tuber colour, a weak-growing type 'white' showed the greatest morphogenetic potential. Out of eight nutrient media tested, a modified B_5 medium containing casein hydrolysate and L-glutamate supported the most consistent shoot regeneration. Shoot regeneration was preceded by the formation of a dark red smooth-surfaced callus. This was usually followed by the formation of a short tapering root. Swellings arose at the base of the root and developed into single or multiple shoots. These shoots were excised, rooted in basal Murashige & Skoog medium and transferred to the field.

Abbreviations: BA, 6-benzylaminopurine; 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; 2-iP, (2-isopentenyl) adenine; Kn, kinetin; NAA, α-naphthaleneacetic acid; Zn, zeatin (mixed isomers)

Introduction

Oca (*Oxalis tuberosa* M.) is a very ancient food crop of the Andes, where it is second in importance only to the potato. For the Peruvian and Bolivian Indians living between 2800 m and 4500 m it is a staple food. Selected strains are propagated vegetatively by planting tubers each year, and over 140 clones have been recognized in Peru alone [5]. Oca was introduced to New Zealand from England where for a century or more it has been grown as a minor backyard vegetable and ornamental plant.. Now, in New Zealand it is produced commercially and is sold under the name 'Yam' (a name usually used for species of *Dioscorea*). Oca tubers are served in New Zealand like potatoes: boiled, baked or fried.

Oca has the potential to become an important vegetable throughout the temperate zone. Vietmeyer [11] suggested that agronomic trials of oca should be conducted in the USA, the highlands of Asia (Nepal, for example) and Africa (Rwanda and Burundi). Reduced oxalate content, tubers with high specific gravity and increased frost tolerance are among desirable traits to be identified in selecting variants. Some work on the induction of somaclonal variability, the assessment of variants and finally the selection of superior types seemed desirable. As a first step in the use of in vitro techniques for plant improvement, it was considered essential to understand the morphogenetic potential of various tissue explants and to identify conditions for shoot regeneration.

Materials and methods

Plant materials

Tubers of five genotypes of oca maintained by D.P. Halford (Feilding, New Zealand) were kindly made available. The main genotype grown commercially in New Zealand is 'red'. Other genotypes, named after the external colour of the tuber, are 'golden yellow', 'light yellow', 'purple' and 'white'. In the field, 'light yellow' and 'purple' have more vigorous vegetative growth than 'red'. 'Golden yellow' has similar vigour to 'red' and 'white' has the weakest shoot growth. Tubers of these genotypes were grown in moist vermiculite in the dark for about three weeks, by which time shoots 50–75 mm long had been produced. These shoots were removed and surface-sterilized for 20 min in 0.6% sodium hypochlorite followed by three rinses (10 min each) in sterile water. Internodes and petiole sections ($\sim$3 mm length) were used as explants for regeneration experiments. In addition some axillary buds of the 'red' genotype were placed on a Murashige & Skoog (MS) [7] medium without hormones to produce in vitro grown shoots. These shoots were used to study the regeneration potential of internode sections. The youngest internode able to provide 4 explants 3 mm long was labelled internode 1.

Tubers could not be successfully surface-sterilized by the above method. However, by cutting the surface of a tuber superficially towards each end it was possible to break the tuber twice and a sterile cylinder could be removed from the middle portion using a sterile cork borer. The technique avoided contact between sterile implements and contaminated epidermis and buds.

Culture media

Unless otherwise stated all media contained MS minerals [7] with B_5 vitamins [4], 30 $g\,l^{-1}$ sucrose and 8 $g\,l^{-1}$ Difco Noble agar and pH was adjusted to 5.8 before autoclaving. Initially all combinations of the cytokinins BA (0.2 and 1.0 $mg\,l^{-1}$), 2-iP (1.0 $mg\,l^{-1}$), Kn (1.0 $mg\,l^{-1}$) and Zn (1.0 $mg\,l^{-1}$) with the auxins IAA (0.2 and 1.0 $mg\,l^{-1}$), IBA (1.0 $mg\,l^{-1}$), NAA (0.2 and 1.0 $mg\,l^{-1}$) and 2,4-D (1.0 $mg\,l^{-1}$) were tested.

Subsequently the cytokinins were tested alone at the above concentrations, using fresh explants. The auxins were also tested alone at 1, 3, 5 and 10 $mg\,l^{-1}$ concentrations. In further experiments various combinations of BA, Zn and 2-iP with NAA were tested as described in the 'Results and discussion' section.

To study the effects of the non-growth regulator components of the medium, eight different nutrient media were tested: B_5 [4], E_1 [3], MS [7], modified B_5 [2], N_6 [1], Nitsch [8], SH [9] and White [12]. Each of these was supplemented with Zn (3 $mg\,l^{-1}$) and NAA (3 $mg\,l^{-1}$).

Culture conditions

All cultures were maintained at 25 °C under a 16 h photoperiod of fluorescent light (30 $\mu mol\,m^{-2}\,s^{-1}$). Shoot regeneration was recorded as the number of visible shoots after two months in culture. Regenerated shoots (2–4 cm long) were excised and rooted in MS basal medium. They were then transferred to pots of vermiculite in the glasshouse, fertilized and kept covered for 3–4 days. After new roots had formed, the plants were transferred to a potting mix containing peat:vermiculute:sand (5:5:1) and a balanced fertilizer.

Scanning electron microscopy

Samples were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 3 h at room temperature and washed three times (10 min each) with fresh phosphate buffer. The samples were critical-point dried and coated with gold. Observations and photographs were made on a Philips SEM 505 at 30 KV.

Results and discussion

Plant regeneration

Initially leaf, petiole and internode sections (randomly selected) from 3-weekld oca shoots of 'red' genotype (grown from tubers in the dark) were placed

on MS media supplemented with a range of growth regulator as indicated in 'Materials and methods'. No regeneration was obtained from leaf and petiole sections. Internode sections gave a low percentage of shoot regeneration only on a medium supplemented with BA and NAA, each at 1 mg l^{-1}.

Media with 2,4-D (0.2 mg l^{-1}), NAA (1.0 mg l^{-1}) and IAA (1.0 mg l^{-1}) produced more callus than media containing NAA (0.2 mg l^{-1}), IAA (0.2 mg l^{-1}) and IBA (1.0 mg l^{-1}). This indicated that increasing auxin concentrations generally stimulated callus growth.

Auxins and cytokinins tested individually over a wide range of concentrations also failed to induce shoot regeneration from internode, petiole or leaf sections. Some callus formed, but callus growth was generally less than on media containing both an auxin and a cytokinin at 1 mg l^{-1} or more. It was expected that with higher concentrations of 2,4-D or NAA, explants might form friable callus leading to embryogenesis but this was never observed. The calli from media containing either auxins or cytokinins alone, when transferred to basal MS medium or the regeneration medium (3 mg l^{-1} BA and NAA) (see below), failed to regenerate any shoots.

Following the demonstration of shoot regeneration with BA and NAA at 1 mg l^{-1}, another experiment was set up using four concentrations (0.2, 0.5, 1.0 and 3.0 mg l^{-1}) each of BA and NAA. This was done in order to try to improve regeneration. However, shoot regeneration was obtained in only two combinations, viz. 1.0 mg l^{-1} each of BA and NAA, and 3.0 mg l^{-1} each of BA and NAA, from internode sections of genotype 'red'. Also, the proportion of explants regenerating shoots was low. Table 1 is a summary

Table 1. Some growth regulator combinations that supported shoot regeneration from different explants of oca ('red' genotype).

Source of explant	Growth regulator combination in MS medium (mg l^{-1})	Total number of explants cultured	Proportion of explants forming shoots (%)
Internode	BA 1 NAA 1	164	12.6 ± 4.2
Internode	BA 3 NAA 3	192	17.0 ± 3.2
Internode	Zn 3 NAA 3	554	28.8 ± 4.1
Petiole	BA 3 NAA 3	84	14.0 ± 6.4
Petiole	Zn 3 NAA 3	276	8.8 ± 1.0
Petiole	2-iP 4 NAA 2	48	17.8 ± 4.1

Table 2. Percentage of sections from different internodes of in vitro grown shoots of the 'red' genotype[1] producing shoots in 3 media combinations.

MS medium +		Sections from internode number									Medium mean[2]
Zn ($mg\,l^{-1}$)	NAA ($mg\,l^{-1}$)	1	2	3	4	5	6	7	8	9	
3.0	2.0	9.4	10.4	18.8	14.6	22.9	29.2	12.5	4.2	2.1	13.8
3.0	3.0	25.0	33.3	27.1	41.7	52.1	37.5	18.8	14.6	14.6	29.4
3.0	4.0	12.5	11.5	13.5	18.8	22.9	15.6	5.2	4.2	2.1	11.8
Internode mean[3]		15.6	18.4	19.8	25.0	32.6	27.4	12.2	7.6	6.3	

[1] The youngest internode used was numbered 1. Results are the mean of 2 independent experiments using 24 and 16 explants per treatment, respectively.
[2] LSD 1% for medium means = 6.6.
[3] LSD 1% for internode means = 11.4.

of several experiments and lists the growth regulator combinations that supported shoot regeneration.

When petiole sections were used, regeneration was only obtained with BA and NAA both at $3\,mg\,l^{-1}$. Replacement of $3\,mg\,l^{-1}$ BA with $3\,mg\,l^{-1}$ Zn (keeping NAA at $3\,mg\,l^{-1}$) enhanced shoot regeneration from internode sections to 28.8% (Table 1). Media containing $2\,mg\,l^{-1}$ NAA with 2, 4, 6 or $8\,mg\,l^{-1}$ of 2-iP were tested using leaf, petiole and internode sections. Regeneration was obtained from petiole sections on one medium (2-iP at $4\,mg\,l^{-1}$, NAA at $2\,mg\,l^{-1}$). No regeneration was obtained with higher or lower concentrations of 2-iP nor from any cultures of internode and leaf sections.

In a further attempt to improve the regeneration, internode sections of different physiological age (internodes numbered 1–9 from the apex) were placed on media containing $3\,mg\,l^{-1}$ Zn with 2, 3 or $4\,mg\,l^{-1}$ NAA (Table 2). In one experiment the medium with $3\,mg\,l^{-1}$ Zn and $3\,mg\,l^{-1}$ NAA gave significantly higher regeneration, but no difference was found between the media in a second experiment. However, the results for regeneration from the different internode sections were consistent between the two experiments. The greatest number of shoots was produced from the internodes 4–6. Good regeneration was also obtained from the first three internodes with the least regeneration from the basal internodes.

Eight different nutrient media, each supplemented with $3\,mg\,l^{-1}$ Zn + $3\,mg\,l^{-1}$ NAA, were compared using randomly selected internode sections of the five oca genotypes grown from tubers in the dark. The effects of media, genotype and their interaction were highly significant (Table 3).

Table 3. Percentage of internode explants of 5 genotypes forming shoots in 8 different nutrient media. (Growth regulator combination was $3\,mg\,l^{-1}$ each of Zn and NAA; percentage calculated from 48 explants in each treatment, from 2 independent repetitions.)

Genotype	MS	B_5	MB_5	N_6	SH	White	Nitsch	E_1	Genotype mean[1,3]
Golden yellow	7.8	1.7	13.9	12.8	10.6	0	0	0	5.8
Light yellow	4.4	0	7.8	0	0	0	0	0	1.5
Purple	3.3	6.1	10.6	0	0	0	0	0	2.5
Red	20.0	12.2	36.1	12.8	10.6	0	0	26.6	14.0
White	37.2	22.7	46.7	18.9	16.7	0	2.8	21.1	20.7
Medium mean[2,3]	14.6	8.4	23.0	8.9	7.6	0	0.6	8.3	

[1] LSD 1% for genotype means = 3.73. Genotype - significant at $p = 0.001$.
[2] LSD 1% for medium means = 4.72. Medium - significant at $p = 0.001$.
[3] LSD 1% for medium × genotype interaction = 10.55. Genotype × medium - significant at $p = 0.001$.

A modified B_5 medium [2], which contained casein hydrolysate and L-glutamate, supported the greatest amount of shoot regeneration. All genotypes were able to regenerate shoots on this medium, with the greatest number of shoots being obtained from the 'white' and 'red' genotypes. Whereas on MS or B_5 medium only one shoot was normally formed on an explant, with the modified B_5 medium some of the explants produced two or three shoots on independent sites. It is worth noting that the genotypes 'light yellow' and 'purple' are more vigorous in field conditions but showed least regeneration, whereas the less vigorous genotype 'white' showed greater regeneration potential. This was followed by intermediate vigorous type 'red'. 'Golden yellow', although similar in vigour to 'red', showed much less shoot regeneration.

Callus initiation from tuber sections

Sections, 2–3 mm thick, of the cores from the internal part of the tuber (see 'Materials and methods') were cultured in $3\,mg\,l^{-1}$ each of BA and NAA or $3\,mg\,l^{-1}$ each of Zn and NAA. Calli were found to initiate quickly from the vascular cambium in 3–4 days. The other cells of the tuber explant started callusing after 3–4 weeks. Callus cells developed from vascular cambium were smaller compared to callus cells from other tuber tissues. However, no shoot regeneration has yet been observed from tuber explants using these media.

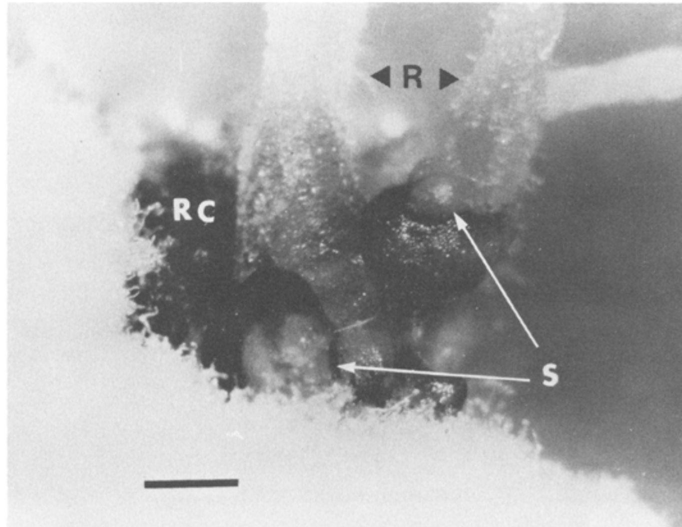


Fig. 1. Light macrophotograph of part of a callus with two tapering roots (R), each having shoot primordia (S) which originated at the base. (RC) indicates the red smooth-surfaced callus which gave rise to tapering root surrounded by white filamentous callus. Bar represents 1.0 mm.

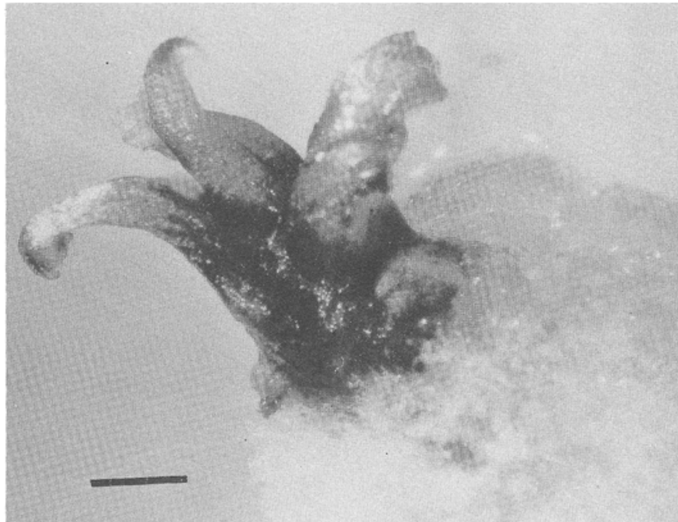
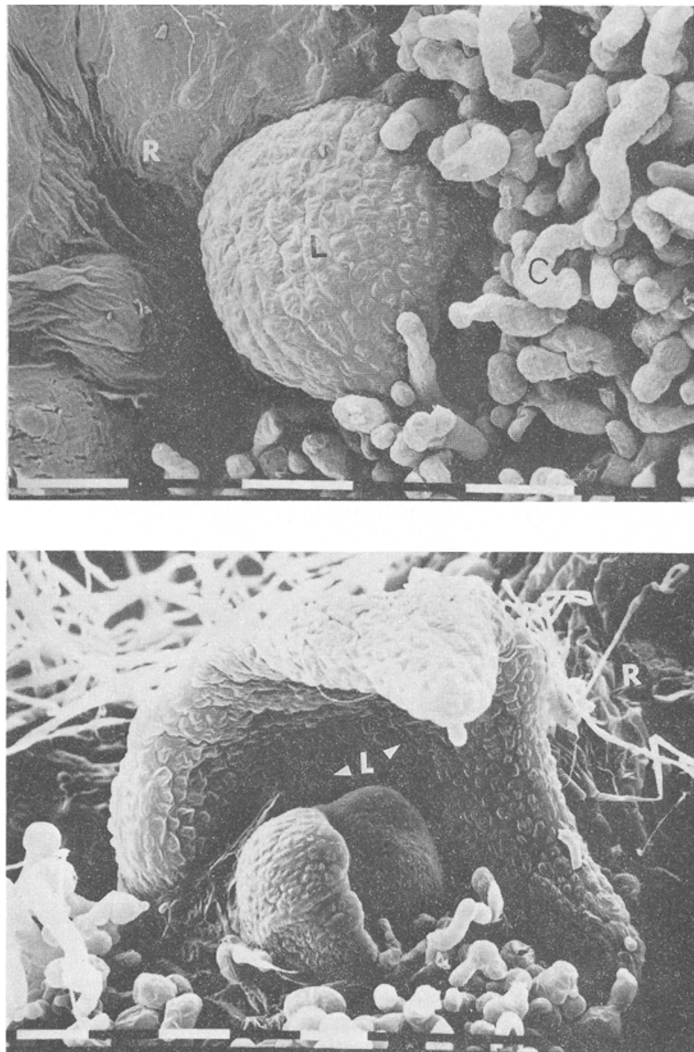


Fig. 2. Less common development of a shoot directly from red smooth-surfaced callus region without prior formation of a tapering root. Bar represents 1.0 mm.



Figs. 3, 4. SEM photographs of leaf primordia (L) arising from the base of the root (R) during the process of shoot regeneration. Most of the adjacent surface remains covered with white filamentous callus (C). Bar represents 0.1 mm.

Shoot initiation process

The origin of in vitro formed shoots is of interest. After about one week in culture, some internode and petiole explants produced one or more red, globular, smooth-surfaced regions from callus which was in contact with the nutrient medium. Some of these red smooth-surfaced calli gave rise to thick

roots with root hairs and a typical root cap. These roots rarely exceeded 20 mm in length. Swellings arose at the base of the roots which developed into single or multiple shoots (Fig. 1). In a few cases, shoots have apparently arisen directly from the red callus without the prior formation of a tapering root (Fig. 2). Stages in the development of a shoot (via root) are shown in Figs. 3 and 4. Leaf primordia (L) can be seen arising from the base of the root (R) and the callus surface remains covered with filamentous cells. Hand sections of the smooth-surfaced red regions of a callus showed cells with deep red vacuoles. These red cells contained some starch grains in their cytoplasms. Embedded in the red callus were groups of cells which contained no pigment. These non-red regions were made up of smaller cells filled with starch and also contained xylem vessels. It was from these non-red zones that the roots developed. Similar dense depositions of starch bodies have been recorded in the organ-forming cells of tobacco callus [10].

Rooting

Regenerated shoots were excised and 100% rooting was achieved on MS basal medium. After 2–3 weeks, when the roots were 2–5 cm long, the plants were transferred to a glasshouse and subsequently to the field.

Fig. 5. Two regenerated plants from an internode explant of 'red' genotype, one normal and a variant with shorter, twisted internodes and thicker leaves. Bar represents 5.0 cm.

Variation among the regenerants

A number of plants regenerated in this study have shown variation in morphological characters such as vigour, leaf shape, phyllotaxy, internode length, shoot and tuber pigmentation, tuber shape and size. Two regenerants from the same explant, one normal and the other a variant with shorter, twisted internodes and thicker leaves are shown in Fig. 5. Variation in chromosome number, sugar and oxalic acid content has also been noticed among the regenerated plants [6, and unpublished data]. Since the regenerated plants showed morphological as well as compositional variation, improvement of the existing genotypes by tissue culture may be possible.

Acknowledgements

M.R.I. Khan acknowledges receipt of a Commonwealth Scholarship from the University Grants Committee, New Zealand, and thanks the Bangladesh Institute of Nuclear Agriculture for granting study leave. This research was also supported by a grant from the New Zealand Lotteries Grants Board.

References

1. Chu C-C (1978) The N_6 medium and its applications to anther culture of cereal crops. In: Proc Symp on Plant Tissue Culture, pp 43–50. Science Press, Peking
2. Furner K, King J, Gamborg OL (1978) Plant regeneration from protoplasts isolated from a predominantly haploid suspension culture of *Datura innoxia*. Plant Sci Lett 11: 169–176
3. Gamborg OL, Davies BD, Stahlhut RW (1983) Somatic embryogenesis in cell cultures of *Glycine* species. Plant Cell Rep 2: 209–212
4. Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50: 151–158
5. Kay DE (1973) Root crops. TPI Crop and Product Digest No. 2, pp 96–99. Trop Prod Inst, London
6. Khan MRI, Heyes JK, Cohen D (1987) Regeneration and induction of somaclonal variability in oca (*Oxalis tuberosa*). In: Zerda AA (Ed) Proc Intern Congress Plant Tissue Culture (Tropical Species), Bogota, Colombia, 21–26 September 1987 (in press)
7. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497
8. Nitsch JP, Nitsch C (1969) Haploid plants from pollen grains. Science 163: 85–87
9. Schenk RV, Hildebrandt AC (1977) Nutrient media for plant organ, tissue and cell culture. In: Rechcigl M (Ed) CRC Handbook in Nutrition and Food, Vol IV, pp 305–359. CRC Press, Boca Raton, Florida
10. Thorpe TA, Murashige T (1970) Some histochemical changes underlying shoot initiation in tobacco callus cultures. Can J Bot 48: 277–285
11. Vietmeyer N (1984) The lost crops of the Incas. Ceres 17(3): 37–40
12. White PR (1963) The Cultivation of Animal and Plant Cells. Ronald Press, New York