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EFFECT OF MEDIUM COMPOSITION ON *IN VITRO* PROPAGATION OF *Oxalis deppei*.

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ABSTRACT

The present research was carried out to study the effect of 4 types of culture media containing various mineral salts of 4 media types on the *in vitro* micropropagation of *Oxalis plant*. Each medium was supplemented with vitamins as described by Murashige & Skoog (1962) casein hydrolysate(1.0 g/l), sucrose (30 g/l), Difco agar (7.0 g/l), GA_3 (0.1 mg/l) and certain growth regulators at different concentrations on proliferation and multiplication of bulb explants. Bulbs at a small size (0.8 – 1.5 cm long and 0.5 – 0.8 thickness) excised from mother plants grown in the greenhouse, were surface sterilized and cultured on the proliferation medium. The developed bulblets were excised and cultured on multiplication medium to produce small plantlets.

The obtained results indicated that the mineral salts of Murashige & Skoog (1962) which supplemented with vitamins as described by M & S (1962), casein hydrolysate (1.0 g/l) sucrose (30.0 g/l), Difco agar (7.0 g/l), casein hydrolysate (1.0 g/l), GA_3 (0.1 g/l), 6-benzylaminopurine (BAP) at 0.3 mg/l and Indole-3-acetic acid (IAA) at 0.3 mg/l showed significant positive effect on leaves and bulblets proliferation expressed as highest survival percentage of cultured explants, leaves initiation was occurred within a few days, highest percentage of explants which formed leaves and highest number of leaves and bulblets per explant.

The obtained results showed also that culture medium containing the mineral salts of M & S (1962) and vitamins as described by M & S (1962) at full strength which supplemented with casein hydrolysate (1.0 g/l), sucrose (30.0 g/l), Difco agar (7.0 g/l), GA_3 (0.1 mg/l), BAP at 0.3 mg/l and IAA at 0.3 mg/l yielded better results of the growth vigor of bulb explants expressed as highest survival percentage, leaves initiation was occurred within a few days and highest number of leaves as well as bulblets per explants than vitamins free medium and those containing M & S vitamins at half strength.

In addition, Murashige & Skoog (1962) medium enriched with casein hydrolysate (1.0 g/l), sucrose (30.0 g/l), Difco agar (7.0 g/l), GA_3 (0.1 mg/l), kinetin (1.0 mg/l) and BAP (0.1 mg/l) was the most favourable medium for leaves and bulblets proliferation as well as the highest survival percentage. Leaves initiation was occurred within a few days, the highest percentages of explants which formed leaves and the number of leaves and bulblets per explant.

Moreover, the multiplication medium was the Murashige & Skoog (1962) medium containing casein hydrolysate (1.0 g/l), sucrose (30.0 g/l), Difco agar (7.0 g/l), GA_3 (0.1 mg/l), kinetin at 0.5 mg/l and BAP at 0.05 mg/l which produced the highest survival percentage; leaves initiation was occurred within a few days, the highest percentage of explants which formed leaves; the highest number of leaves and bulblets and the highest number of roots as well as the longest roots per explant. Rooted bulblets were successfully transferred to a mixture of peat moss and perlite (3 : 1 v/v) and kept under intermittent mist for one week in the greenhouse to complete their normal growth.

INTRODUCTION

The genus *Oxalis* belonging to the family Oxalidaceae includes several American species ranked as a very important ornamental plant. *Oxalis*

deppei is one of these species along with *Oxalis erosa*, *Oxalis dispar* and *Oxalis hedysaroides*. They are usually used in different medical purposes and as ornamental herbaceous plants and along with as root crops (Sunderland, 1966 & 1967; Sunderland and Wells, 1968; Brücher, 1977; Rea and Morales, 1980; Arenas, 1981; Maene and Debergh, 1981 and Vietmeyer, 1983).

Very Little informations about *Oxalis* species are available with respect to the convenient *in vitro* propagation method. The same is true about the effects of various macro. and microelements as well as various concentrations of vitamins and cytokinins on the *in vitro* formation of multiple leaves and bulblets of *Oxalis* explants. Therefore, the main objectives of the present study was to find out the appropriate nutrient medium for *in vitro* propagation of *Oxalis* plants.

MATERIALS AND METHODS

This investigation was carried out during the period from June to December 1994 at the Laboratory of Plant Tissue Culture, University of Horticulture and Óbuda Farm for Floriculture, Budapest, Hungary.

Mature plants of *Oxalis* Fam. Oxalidaceae examined in this study, were as pot plant with red purple leaves and pink flowers grown in the greenhouse at temperature varying between 18 and 24^{o}C and 85% relative humidity at the Óbuda Farm for Floriculture. Fifteen very small bulbs apparently in uniform (0.8 – 1.5 cm long and 0.5 – 0.8 cm thickness) excised from a single pot plant, leaves were shortened as well as partial dissection of scale enclosed vegetative dormant buds of small bulbs, were soaked in running tap water for 3 hours to remove all impurities. Explants were surface sterilized by rinsing in a distilled water for 10 min. and immersed in calcium hypochlorite at 7.0% containing a few drops of Tween-20 as a wetting agent for 10 min., followed by rinsing four times in changed sterile distilled water for 2 min. each.

Uniform size bulb explants were subjected to the first test concerned with culturing explants on the various mineral salts of 4 media types can be abbreviated as follows:

1- $BM_{1/1}$: The first culture medium which contained the mineral salts of Murashige & Skoog (1962) medium.
2- $BM_{2/1}$: The second culture medium which contained the macroelements as described by Murashige & Skoog (1962) combined with microelements as described by Heller (1953).
3- $BM_{3/1}$: The third culture medium which contained the macro. and microelements of Gamborg *et al*. (1968) medium.
4- The fourth culture medium which contained the macro. and microelements of Quoirin & Lepoivre (1977) medium.

Each medium was supplemented with vitamins as described by Murashige & Skoog (1962), casein hydrolysate (1.0 g/l), sucrose (30.0 g/l), Difco agar (7.0 g/l), gibberellic acid (GA_3) (0.1 mg/l), 0.3 mg/l BAP and 0.3 mg/l IAA. The pH was adjusted to 5.7 and the media were autoclaved for 20 min. at 121^{o}C.

The second test was concerned with evaluating the effect of different concentrations of M & S (1962) vitamins on explants initiation. The culture medium containing macro. and microelements of M & S (1962) medium was supplemented with, Murashige & Skoog (1962) vitamins at half and full strength as well as vitamins-free medium to evaluate their effect on proliferation rate of explants in the presence of casein hydrolysate (1 g/l), sucrose (30.0 g/l), Difco agar (7.0 g/l), gibberellic acid (GA_3) (0.1 mg/l), BAP (0.3 mg/l) and IAA (0.3 mg/l).

The third test was concerned with evaluating the effect of various cytokinins at different concentrations on proliferation rate of oxalis bulb explants. Explants were cultured in the proliferation media which consisted of Murashige & Skoog (1962) medium supplemented with casein hydrolysate (1.0 g/l), sucrose (30.0 g/l), Difco agar (7.0 g/l), gibberellic acid (GA_3) (0.1 mg/l) and 6-furfuryladenine (kin.) at 0.3, 1.0, 2.5 and 10.0 mg/l combined with 6-benzylaminopurine (BAP) at 0.03, 0.10, 0.25 and 0.50 mg/l were added. In the fourth test, the well developed bulblets were excised and cultured at 7 weeks-interval on multiplication media as in the third test and each medium contained 0.5 mg/l kinetin combined with BAP at the concentrations of 0.00, 0.05, 0.10 and 0.50 mg/l to indicate their effect on explants multiplication.

In all above treatments, media 50 ml were dispensed into 220 ml jars for explant culture, 1 - 5 explants were cultured per jar. They were maintained at 23^{o}C under a constant fluorescent light (day light F-29) of 3000 Lux for 16/8 h. day/night. Rooted plantlets were transferred to the greenhouse and were potted in 5.7 cm plastic pots in a mixture of peatmoss and perlite (3 : 1 v/v). They were kept under an intermittent mist for one week and repotted into 10 cm pots when it was necessary.

Throughout the different tests of the present study, the following characters were recorded: Contamination percentage of cultured explants and number of days until appearance of visible formed leaves, seven weeks later, survival percentages of cultured explants, percentage of explants which formed leaves per explant and number of leaves and bulblets per explant were determindes. As for the fourth test where culturing media containing certain concentrations of plant growth regulators, number and length of roots (cm) per explant were recorded after incubation for seven weeks in culture medium.

Treatments were arranged in a complete randomized block design with four replicates. Data were examined statistically using the computerized analysis of variance and Duncan's multiple range test procedures within the statistical analysis system, SAS (2000).

RESULTS

Effect of mineral salt formulations on explant proliferation:

The data in Table (1) showed that calcium hypochlorite solution at 7.0% for 10 min. is a satisfactory sterilizing agent for bulb explants, since 66.0% of these explants were healthy. The significantly highest survival percentage of cultured explants was observed with explants grown on $BM_{1/1}$ (100%) than those grown on $BM_{2/1}$, $BM_{3/1}$ and $BM_{4/1}$ (58.0, 33.0 and 41.6%, respectively). The visible formed leaves were produced from the bulb

explants within 0.0 – 11.5 days from culturing date on different culture media. The explants cultured on $MB_{1/1}$ produced significantly the visible formed leaves within a shorter time (8 days). While, those cultured on BM 4/1 produced the visible formed leaves after 11.5 days from being placed in culture medium. Not all explants formed leaves. The bulb explants cultured on BM 1/1 produced significantly the highest percentage of explants which formed leaves (100%) than those grown on $BM_{2/1}$, $BM_{3/1}$ and $BM_{4/1}$ (50.0, 0.0 and 40.0%, respectively). Whereas, the explants grown on $BM_{3/1}$ remained green without leaves formation. The explants grown on $BM_{1/1}$ produced significantly the highest number of leaves per explant (3.80 leaf) than those grown on the $BM_{2/1}$, $BM_{3/1}$ and $BM_{4/1}$ (2.10, 0.00 and 1.87 leaf/explant, respectively). Explants grown on $BM_{1/1}$ formed significantly the highest number of bulblet (3.80 bulblet), while those grown on $BM_{3/1}$ remained green and no bulblets formation was observed.

Effect of vitamins concentration on explant proliferation:

Data in Table (2) indicated that, the highest survival percentage was observed with explants grown on medium containing M & S (1962) vitamins at full strength (100%) comparing to those grown on vitamins-free medium and medium containing M & S (1962) vitamins at half strength (67.0 and 75.0%, respectively). The visible formed leaves produced from the explants within 9 – 10 days from culturing date on different culture media. The explants cultured on medium containing the M & S (1962) vitamins at full strength significantly produced the visible formed leaves earlier few days (9.0 day), while explants cultured on vitamins-free medium as well as medium containing M & S (1962) vitamins at half strength produced the leaves at very low frequency within 10 days. The explants cultured on medium containing M & S (1962) vitamins at full strength produced significantly the highest percentage of explants which formed leaves (100%) than those grown on medium supplemented with the M & S (1962) vitamins at half strength and vitamins-free medium (66.0 and 50.0%), respectively. Explants grown on medium containing M & S (1962) vitamins at full strength produced significantly the highest number of leaves (3.5 leaves) than those grown on vitamins-free medium (1.8 leaf). Moreover, explants grown on medium containing M & S (1962) vitamins at full strength produced the highest number of bulblets (2.1 bulblet), while those grown on vitamins free medium produced the lowest ones (1.7 bulblet).

Effect of combinations of kinetin + BAP on explant proliferation:

Data reported in Table (3) revealed that, the significantly highest survival percentage of cultured explants (100%) was observed with bulb explants grown on medium containing 1.0 mg/l kin. + 0.1 mg/l BAP than those grown on media containing 0.3 mg/l kin. + 0.03 mg/l BAP, 2.5 mg/l kin. + 0.25 mg/l BAP and 10.0 mg/l kin. + 0.5 mg/l BAP (81.0, 72.0 and 18.0%, respectively). The visible formed leaves were produced from bulb explants within 0.0 – 11.0 days from culturing date on different culture media. Explants grown on medium containing 1.0 mg/l kin. + 0.1 mg/l BAP produced significantly visible formed leaves earlier within few days (8 days).

T1-2

As the concentration of kin. + BAP increased the percentage of explants which started to form leaves gradually decreased. In this concern, the significantly highest percentage of explants, which formed leaves, was obtained on medium containing 1.0 mg/l kin. + 0.1 mg/l BAP (100%) when compared with those grown on media containing 0.3 mg/l kin. + 0.03 mg/l BAP, 2.5 mg/l kin. + 0.25 mg/l BAP and 10.0 mg/l kin. + 0.5 mg/l BAP (81.0, 63.6 and 0.0%, respectively). The explants grown on medium containing 1.0 mg/l kin. + 0.1 mg/l BAP produced significantly the greatest number of leaves per explant (3.3 leaf), while the explants grown on medium containing 2.5 mg/l kin. + 0.25 mg/l BAP produced the lowest number of leaves per explant (2.5 leaf). The explants grown on medium containing 10.0 mg/l kin. + 0.5 mg/l BAP remained green without leaves formation. Moreover, explants grown on medium containing 1.0 mg/l kin. + 0.1 mg/l BAP produced significantly higher number of bulblets per explant (2.8 bulblet), while those grown on medium containing 2.5 mg/l kin. + 0.25 mg/l BAP produced the lowest number of bulblets per explant (1.8 bulblet).

Effect of combinations of kinetin + BAP on explant multiplication:

Data in Table (4) showed that, the significantly highest survival percentages of cultured explants (100%) were observed with explants grown on medium containing 0.5 mg/l kin. + 0.05 mg/l BAP than those grown on media containing 0.5 mg/l kin. + 0.0 mg/l BAP, 0.5 mg/l kin. + 0.1 mg/l BAP and 0.5 mg/l kin. + 0.5 mg/l BAP (77.2, 90.0 and 63.6%), respectively. Explants grown on medium containing 0.5 mg/l kin. + 0.05 mg/l BAP significantly produced the visible formed leaves within a shorter time (7.0 days). Otherwise, the explants cultured on medium containing 0.5 mg/l kin. + 0.5 mg/l BAP produced the visible formed leaves at very low frequency within 12.0 days. The explants cultured on medium containing 0.5 mg/l kin. + 0.05 mg/l BAP produced significantly the highest percentage of explants which formed leaves (100%) than those grown on media containing 0.5 mg/l kin. + 0.0 mg/l BAP, 0.5 mg/l kin. + 0.1 mg/l BAP and 0.5 mg/l kin. + 0.5 mg/l BAP (72.0, 86.0 and 50.0%, respectively). The explants grown on medium containing 0.5 mg/l kin. + 0.05 mg/l BAP produced significantly the highest number of leaves (5.5 leaf) than those grown on media containing 0.5 mg/l kin. + 0.0 mg/l BAP, 0.5 mg/l kin. + 0.1 mg/l BAP and 0.5 mg/l kin. + 0.5 mg/l BAP (4.3, 5.2 and 4.0 leaves, respectively). Furthermore, explants grown on medium containing 0.5 mg/l kin. + 0.05 mg/l BAP, produced significantly the highest number of bulblets (3.3 bulblet), while those grown on medium containing 0.5 mg/l kin. + 0.5 mg/l BAP produced the lowest ones (1.3 bulblet). Data revealed also that the significantly highest number of roots were observed with explants grown on medium containing 0.5 mg/l kin. + 0.05 mg/l BAP per explant (7.0 roots), while those grown on medium containing 0.5 mg/l kin. + 0.5 mg/l BAP produced the lowest ones (1.6 roots). Moreover, explants grown on medium containing 0.5 mg/l kin. + 0.05 mg/l BAP formed the tallest roots (6.1 cm) than those grown on media containing 0.5 mg/l kin. + 0.0 mg/l BAP, 0.5 mg/l kin. + 0.1 mg/l BAP and 0.5 mg/l kin. + 0.5 mg/l BAP (2.9, 3.6 and 1.1 cm, respectively).

T3-4

DISCUSSION

An increasing number of reports indicate that tissue culture methods are promising for propagating species or cultivars in which other vegetative propagation are difficult or impossible (Rugini, 1984).

Although early tissue-culture formulations (White, 1934 and Gautheret, 1939) were suitable for the culture of callus cells, later ones, such as those of Murashige & Skoog (1962), Heller (1953), Gamborg *et al*. (1968) and Quoirin & Lepoivre (1977) are more suitable for a wider range of plants and for the promotion of organogenesis in cultures. A formulation has to be determined that supports the growth of cultured cells for each species, and sometimes each variety within a species. In the present work data suggested that the development of a truly optimal mineral formulation may be practically impossible. Nevertheless, the mineral salt formulation of the Murashige & Skoog (1962) medium represents an approximation to this, and has proved to be superior over all other mineral formulations recorded by Heller (1953); Gamborg *et al*. (1968) and Quoirin & Lepoivre (1977) *in vitro* culture of *Oxalis tissues*. These results were supported by the finding of Seabrook (1980) who reported that, the Murashige & Skoog (MS) salt solution will support the growth of most plant cells in culture, and this is in part due to its high salt concentration. Some ions, the NH_4 ion in particular, may be in too high a concentration for all plants and tissue at all stages of development *in vitro*. Moreover, Huang and Murashige (1977) reported that there are trace impurities of minor elements in supplies of major elements such as N, K and P, additional quantities of these microelements are almost always necessary. The M & S salt solution in particularly high in microelements when compared to other media formulations. The addition of cheating agents such as Fe-EDTA ensures that iron is available over a wide range of pH. In addition, these results agreed with those reported by Ochatt and De Azkue (1984) who found that the most suitable media for a successful micropropagation methods for *Oxalis erosa* was observed by using mineral salts of Murashige & Skoog (1962) medium.

The use of vitamins in plant tissue culture has been a matter of custom rather than proved necessity. Thiamine-HCL is the only vitamin for which there seems to be a consistent requirement for growth of plant tissues *in vitro* at levels between 0.1 mg/l and 1.0 mg/l. Pyridoxine and nicotinic acid could be deleted from the medium without loss on growth. Ascorbic acid increased growth only when levels of thiamine were suboptimal. In the present work, data suggested that the M & S (1962) vitamins at full strength is more effective than such vitamins at low concentrations or medium vitamins free. These results agreed with those reported by Huang and Murashige (1977) who stated that vitamins should be added to media formulations where enhancement of growth or morphogenesis indicate they are necessary.

Shoot formation is strongly dependent on the presence of cytokinin. The cytokinins that are used in plant tissue culture are the naturally occurring compounds 2 ip and zeatin together with N^6-benzyladenine (BA or BAP) and kinetin. These compounds have physiological effects on the plants and plant cells. The differences in the need for cytokinins may reflect endogenous

differences in growth substances contents. In evaluating the requirement for cytokinin by a specific cell line, concentrations in the range of 0.01 to 10.0 mg/l are often tested (Dougall, 1980).

The results of this study suggest that leaves and bulblets proliferation and multiplication were satisfactory when oxalis explants were cultured on M & S medium containing kinetin at 1.0 mg/l + BAP at 0.1 mg/l and kinetin at 0.5 mg/l + BAP at 0.05 mg/l, respectively. The explants growing on these media had the highest number of leaves and bulblets as well as their growth was almost normal but at higher kinetin + BAP concentrations explants remained green without leaves formation. Similar results were obtained by Singh and Sehgal (1999) who noticed a favorable influence on adventitious shoot formation of Holy Basil on the medium containing BAP at 0.1 mg/l in the presence small quantities of NAA. While, Shabde and Murashige (1977) analysed the influence of different kinetin concentrations on adventitious shoot formation of carnation and they obtained the highest number of shoots on the medium containing 1.0 mg/l kinetin and small quantities of NAA. Finally, Price *et al*. (1977) concluded that kinetin at 1.0 mg/l was preferred for callus initiation on cotton hypocotyl tissue.

In this concern, the author of this study observed the multiplication and growth of leaves and bulblets on multiplication media. The most favorable medium in this respect, was the one with 0.5 mg/l kin. + 0.05 mg/l BAP. Similar results were obtained by Ziv *et al*. (1970) studying the influence of different kinetin concentrations on shoot formation of Gladiolus and found the highest number of shoots was resulted on the medium containing 0.5 mg/l kinetin. In addition, Hussey (1977) observed multiplied shoot formation on Gladiolus explants grown on medium containing 0.03 – 1.0 mg/l BA. Thus, different cytokinins showed different responses in the different tests. It is possible also that different cytokinins may give different responses, at least quantitatively, in the same test.

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تـأثير تركيـب البيئـة الغذائيـة علـى الإكثـار المعملـى الـدقيق لنبـات الأوكسـالس (*Oxalis deppei*)

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أجرى هذا البحث بغرض الوصول إلى أفضل بيئة غذائية يمكن استخدامها لإكثار نبات الأوكسالس بنظام زراعة الأنسجة. ولهذا فقد تم اختبار ٤ أنواع من البيئات المحتوية على ٤ أنواع مختلفة من الأملاح المعدنية كل منهم على حده. وأضيف إلى الأملاح المعدنية المختبرة الفيتامينات الخاصة ببيئة Murashige & Skoog (1962) وcasein hydrolysate بتركيز ١ جرام/لتر وسكروز بتركيز ٣٠ جرام/لتر وDifco agar بتركيز ٧ جرام/لتر وحمض الجبريليك (GA_3) بتركيز ٠,١ ميللجرام/لتر وبعض منظمات النمو بتركيزات مختلفة لمعرفة تأثيرها على تكشف وتكوين الأوراق والبصيلات.

وفصلت الأبصال الصغيرة الحجم من نباتات الأمهات النامية فى الصوب الزجاجية حيث كان يتراوح طولها بين ٠,٨ – ١.٥ سم، وسمكها بين ٠,٥ – ٠,٨ سم وزرعت بعد تطهيرها فى البيئات المختبرة للحصول على نموات الأوراق والبصيلات الجديدة والتى نقلت بعد ذلك إلى البيئات المشجعة على عملية التضاعف والتجذير لتكوين النبيتات الصغيرة.

وتشير النتائج المتحصل عليها إلى أن البيئة الأولى التى تحتوى على العناصر الكبرى والصغرى لبيئة Murashige & Skoog (1962) والفيتامينات الخاصة بها علاوة على الإضافات السابق ذكرها، والمضاف إليها أيضا بنزيل أمينوبيورين (BAP) بتركيز ٠,٣ ميللجرام/لتر وأندول حمض الخليك (IAA) بتركيز ٠,٣ ميللجرام/لتر هى أفضل الأملاح المعدنية ذات التأثير الإيجابى على تكشف وتكوين الأوراق المخلقة من الأبصال المزروعة فيها حيث أعطت أعلى نسبة مئوية للأنسجة التى نجحت فى تكوين النموات الورقية وأعلى متوسط لعدد الأوراق وعدد البصيلات الجديدة من كل بصلة مزروعة.

وأظهرت النتائج أيضا أن استخدام الفيتامينات الخاصة ببيئة Murashige & Skoog (1962) عند تركيزها الأساسى الكامل (قوة كاملة) فى البيئة الأولى السابق ذكرها، والمضاف إليها أيضا بنزيل أمينوبيورين (BAP) بتركيز ٠,٣ ميللجرام/لتر وأندول حمض الخليك (IAA) بتركيز ٠,٣ ميللجرام/لتر قد أعطت نتائج إيجابية نحو تحسين حالة النسيج النباتى المزروع وأعطت أعلى متوسط لعدد الأوراق وذلك عند مقارنتها بنتائج نفس البيئة التى تحتوى على الفيتامينات الخاصة ببيئة Murashige & Skoog (1962) ولكن عند نصف التركيز الأساسى (نصف قوة) أو الخالية من هذه الفيتامينات.

وبينت النتائج أيضاً أن البيئة الأولى والمضاف إليها الكينتين بتركيز ١ ميللجرام/لتر، وبنزيل أمينوبيورين (BAP) بتركيز ٠,١ ميللجرام/لتر هى بيئة التكشف الأكثر فاعلية لتخليق النموات الورقية والبصيلات الجديدة حيث أعطت أعلى نسبة مئوية لنجاح الأنسجة على البقاء والتبكير فى تكوين الأوراق كما أعطت أعلى نسبة مئوية للأنسجة التى نجحت فى تكوين الأوراق وأعلى متوسط لعدد الأوراق والبصيلات الجديدة من كل بصله مزروعة.

بالإضافة إلى ذلك فقد أظهرت النتائج إلى أن البيئة الغذائية المشجعة على تضاعف الأوراق وتجذير البصيلات والتى تتكون من بيئة Murashige & Skoog (1962) الأولى السابق ذكرها، والمضاف إليها الكينتين بتركيز ٠,٥ ميللجرام/لتر وبنزيل أمينوبيورين (BAP) بتركيز ٠,٠٥ ميللجرام/لتر كانت البيئة الأكثر فاعلية لإعطاء أعلى معدل تضاعف للأوراق وتجذير للبصيلات المزروعة حيث أعطت أعلى نسبة مئوية لنجاح نسيج المزروع على البقاء والتبكير فى تكوين الأوراق وأعلى نسبة مئوية للأنسجة التى نجحت فى تكوين النموات الورقية والبصيلات بالإضافة إلى أنها أعطت أكبر متوسط لعدد وطول الجذور على البصيلات المتكونة فى البيئة المشجعة على التضاعف.

وقد تم نقل النبيتات الصغيرة المتكونة إلى ظروف الصوب الزجاجية حيث زرعت فى أصص بلاستيك تحتوى على مخلوط من البيتموس والبيرليت بنسبة ٣ : ١ وحفظت تحت ظروف الرى الرذاذى لمدة أسبوع واحد فى الصوبة الزجاجية وتركت لتنمو إلى نباتات طبيعية كاملة.

Table (1): Effect of different mineral salts formulation on explants proliferation of *Oxalis deppei* after incubation for 7 weeks on various proliferation media.

Characters / Treatments	Contamination percentage of cultured explants (%)	Survival percentage of cultured explants (%)	Number of days until visible leaves formation per explant	Percentage of explants which formed leaves (%)	Average number of leaves per explant	Average number of bulblets per explant (bulblet)
$BM_{1/1}$	34.0	100.0	8.0	100.0	3.80	3.80
$BM_{2/1}$	34.0	58.0	11.0	50.0	2.10	3.50
$BM_{3/1}$	34.0	33.0	*	*	*	**
$BM_{4/1}$	34.0	41.6	11.5	40.0	1.87	2.80
L.S.D. at 5%	0.00	2.40	0.51	1.88	0.50	0.57

$BM_{1/1}$: medium containing mineral salts of Murashige & Skoog (1962) medium
$BM_{2/1}$: medium containing microelements of M & S (1962) + microelements of Heller (1953) medium
$BM_{3/1}$: medium containing mineral salts of Gamborg *et al*. (1968) medium
$BM_{4/1}$: medium containing mineral salts of Quoirin & Lepoivre (1977) medium
***: Explants remained green without leaves formation, **: Explants remained green without bulblets formation.**

Table (2): Effect of vitamins concentration on explants proliferation of *Oxalis deppei* after incubation for 7 weeks on proliferation media.

Characters / Treatments	Survival percentage of cultured explants (%)	Number of days until visible leaves formation per explant (day)	Percentage of explants which formed leaves (%)	Average number of leaves per explant	Average number of bulblets per explant
Medium vitamins-free	67.0	10.0	50.0	1.8	1.7
Medium containing MS (1962) Vitamins at half strength	75.0	10.0	66.0	2.1	1.8
Medium containing MS (1962) Vitamins at full strength	100.0	9.0	100.0	3.5	2.1
L.S.D. at 5%	0.015	0.86	0.01	0.67	0.52

Table (3): Effect of different concentration of 6-furfuryladenine (kin.) + 6-benzylaminopurine (BAP) combinations on explants proliferation of *Oxalis deppei* after incubation for 7 weeks on various proliferation media.

Characters / Treatments	Survival percentage of cultured explants (%)	Number of days until visible leaves formation per explant	Percentage of explants which formed leaves (%)	Average number of leaves per explant	Average number of bulblets per explant
0.3 mg/l Kin. + 0.03 mg/l BAP	81.0	10.0	81.0	2.8	2.0
1.0 mg/l Kin. + 0.10 mg/l BAP	100.0	8.0	100.0	3.3	2.8
2.5 mg/l Kin. + 0.25 mg/l BAP	72.0	11.0	63.6	2.5	1.8
10.0 mg/l Kin. + 0.50 mg/l BAP	18.0	*	*	*	**
L.S.D. at 5%	2.01	0.97	2.49	0.40	0.46

***: Explant remained green without leaves formation, **: Explant remained green without bulblets formation.**

Table (4): Effect of different concentration of 6-furfuryladenine (kin.) + 6-benzylaminopurine (BAP) combinations on explants multiplication of *Oxalis deppei* after incubation for 7 weeks on various multiplication media.

Characters / Treatments	Survival percentage of cultured explants (%)	Number of days until visible leaves formation per explant	Percentage of explants which formed leaves (%)	Average number of leaves per explant	Average number of bulblets per explant	Average number of roots per explant	Average length of roots per explant (cm)
0.5 mg/l Kin. + 0.00 mg/l BAP	77.2	8.0	72.0	4.3	1.9	5.0	2.9
0.5 mg/l Kin. + 0.05 mg/l BAP	100.0	7.0	100.0	5.5	3.3	7.0	6.1
0.5 mg/l Kin. + 0.10 mg/l BAP	90.0	9.0	86.0	5.2	2.1	5.7	3.6
0.5 mg/l Kin. + 0.50 mg/l BAP	63.6	12.0	50.0	4.0	1.3	1.6	1.1
L.S.D. at 5%	0.02	0.64	0.03	0.71	0.33	0.86	0.66