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# Callus Induction and Maintenance in Seven Local Olive (*Olea Europaea* L.) Cultivars

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**(Research Note)**


## ABSTRACT

Seven olive cultivars 'Nabali', 'Rasiei', 'Nasuhi', 'Shami', 'Kudari', 'Surani' and 'Kaisi' were used to investigate their ability for *in vitro* callus induction using nodal cuttings.

To induce callus from the above mentioned seven olive cultivars, nodal cuttings were used as explants and placed either on gelled Olive Medium (OM) or laid over still liquid OM in the dark. Callus was induced from all olive cultivars both in gelled and liquid media. The callus was initiated and detected at three sites: the proximal ends of explants (most frequent site for all tested cultivars) in both gelled and liquid OM, at the bases of petioles (second most frequent site for all tested cultivars) in both gelled and liquid OM with better growth in liquid OM, and at the distal ends of explants (very rare occurrence) in liquid OM only. 'Rasei' showed the highest callus induction (19%) in gelled medium, while 'Nabali' showed the highest callus induction (16%) in liquid medium. To find out the potential of callus growth, it was subcultured from small callus fragment every 3-4 weeks. 'Kudari' showed the maximum increase in callus weight (4.05 fold) on gelled medium. However, in liquid medium, 0.42 fold increase was recorded in the cultivar 'Shami'.


**KEYWORDS**: Olive cultivars, callus, *in vitro.*

## INTRODUCTION

Olive (*Olea europaea* L.) is a long-lived evergreen tree and one of the major fruit trees in the Mediterranean Basin (Canas *et al.*, 1992). Olive seedlings have a long juvenile phase. Therefore, vegetative propagation is practiced by cuttings, suckers, or by grafting (Canas, 1988). However, there is a high variability among olive cultivars for rooting potential (Mencuccini and Rugini, 1993; Charri-Rkhis *et al.*, 1999). Some important cultivars are very hard to root as in 'Nabali', where spontaneous rooting, without any external treatment, may take very long time (Ezzain, 1980) and may not exceed 6% (Qrunfleh, *et al.*, 1994).

The olive tree has rarely been genetically improved due to its long juvenile phase of 10-15 years (Canas *et al.*, 1992; Garcia *et al.*, 2002), very slow development, and prevalent self sterility which make conventional breeding difficult (Rugini, 1988). Therefore, *in vitro* culture establishment followed by organ regeneration of genetically transformed tissues can support classical plant breeding for olives.

Tissue culture is defined as the growth or maintenance of plant cells, organs, or whole plants *in vitro*, narrowly, the culture *in vitro* of either organized or unorganized tissues, most frequently used synonymously with callus culture (Schaeffer, 1990). Micropropagation has significant advantages over traditional clonal propagation techniques, e.g. propagation is more rapid than *in vivo* (Pierik, 1987), propagation of some species which are difficult to regenerate or cannot be propagated by conventional methods as the 'Kalmon' olive cultivar (Dimass, 1999), production of disease free plants, production of genetically identical plants, saving production cost, space, land and labor and achieving year-round production independent from seasonal effects (Pierik, 1987).

The Murashige and Skoog's (MS) medium (1962) is usually used for micropropagation of most plants species (Pierk, 1987), however, for propagating olives, many of the tested media gave neither satisfactory growth rates

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nor good quality shoots. Therefore, a new medium was derived by Rugini (1984) for olive micropropagation, the medium was developed by comparing data from analysis of the main mineral elements found in the apical shoots (4-5 mm long) and in mature embryos in olive and almond. Olive tissue was characterized by a high content of Ca, Mg, S, Cu and Zn compared to almond. In this newly derived medium which was called Olive Medium (OM) and has a high content of these elements, olive multiplication rate was higher and shoots were more tender when compared with growth on other media (Rugini, 1984).

OM and MS were tested on cultivar 'Maurino' to improve *in vitro* establishment (Bartolini, *et al.*, 1990), MS medium was more efficient than OM, where OM was not suitable for good establishment and good shoot quality for cultivar 'Maurino'. In another occasion, 'Chondrolia Chalkidikis' olive micro-shoots displayed better morphological appearance on woody plant medium compared to OM (Grigoriadou *et al.*, 2002). These reports indicate that there is no general medium for all olive cultivars and various applications as proposed by Rugini (1984). Biricolti and Romagnoli (2002) confirmed the low morphogenic capacity of olive petiole and leaf explants, and considered the regeneration process to be a sporadic event not strictly connected with bioregulators concentration. Mencuccini and Rugini (1993) used different explant origins as petiole, leaf discs and midribs to test the callus induction and morphogenic capacity in olives; petioles from *in vitro* grown shoots have the best regeneration percentage on OM or MS in dark.

The herein described study was conducted to achieve callus induction and maintenance of seven Jordanian cultivars of olive (*O. europaea* L.). Both cultures of nodal cuttings over liquid and gelled media were assessed.

## MATERIALS AND METHODS

Young olive trees, around 10 years old, of the cultivars ('Nabali', 'Rasei', 'Nasuhi', 'Shami', 'Kudari', 'Surani', 'Kaisei') growing in a mother-stock orchard (Mushagar) which belongs to the National Center for Agricultural Research and Technology Transfer (NCARTT), Jordan, were used as a source of explants for the herein described work. Healthy soft wood cuttings (30-40 cm long) of current growths were collected (from April 2003 to April 2004). Cuttings were transferred to the laboratory in a cooled ice box. In the laboratory, leaves were removed away. Only the distal two thirds nodes (not exceeding ten nodes) were considered for nodal cuttings, the rest proximal nodes and the apex were not included (Sadder, 2002). Cuttings were washed well with tap water for 10 minutes then they were soaked temporarily in an anti-oxidant solution (100 mg/l ascorbic acid). Nodal cuttings (1 cm long) containing two opposite axillary buds were excised. Cuttings were soaked in fungicide solution (Carbendazim 50% (w/w), VAPCO) for 15 minutes then they were rinsed with distilled water. Cuttings were surface sterilized with continuous stirring in 75% ethanol for 1 min, followed by 7 minutes in 20% commercial bleach (containing 6.5% Na hypochlorite) and 0.1% Tween 20. Thereafter, they were rinsed three times in sterilized distilled water, each for 5 minutes.

For all *in vitro* culture stages, plantlets or callus were cultured using 10 ml OM (Rugini, 1984) medium in 25x150 mm test tubes with polypropylene cover and wrapped with parafilm. Cultures were incubated in growth chamber at 22±2°C. Callus was induced from the explants on still liquid and gelled OM in the dark. For liquid medium, nodal cuttings were air-dried for 5 minutes over sterile paper tissues under the laminar hood. Afterwards, each nodal cutting was laid carefully over the surface of the liquid medium in a separate tube (Sadder, 2002). The produced callus was subcultured every 3-4 weeks over gelled OM by cutting the callus fragments (0.5-1 cm in diameter) into 4-6 equal portions and culturing them separately in new glass tubes over fresh medium.

Test tubes containing callus were weighted then the callus was cultured over new media and test tube weight without callus was recorded. This process was done twice at the beginning and at the end of the experiment in new tubes to avoid evaporation influence. Therefore, callus weight equals:

Callus weight = Weight of test tube with callus - Weight of test tube without callus

The fold increase in callus weight was measured as follows:

$$\text{Fold} = \frac{[\text{Callus weight after culture (gm)} - \text{Callus weight before culture (gm)}]}{\text{Callus weight before culture (gm)}}$$

Callus induction experiment was arranged in completely randomized design, each treatment was comprised of 27-52 replicates, each replicate is composed of one nodal cutting. All recorded values (percentage)

were transformed by adding 0.5. Chi Square $X^2$ was used to analyze data and analysis of variance (ANOVA) was conducted using SAS program (SAS Institute, 1998).

## RESULTS

Callus was detected in gelled (Figure 1.b and c) and liquid (Figure 1.a) media at three sites. The first one was at the bases of petioles for both liquid medium (Figure 1.a) and gelled medium (Figure 1.b and c); the majority of callus appeared at this site for all tested cultivars. The second most detected site of callus induction was at the bases of explants (proximal ends), which was recorded for both liquid medium (Figure 1.a) and gelled medium (Figure 1.b); however, callus growth at proximal ends was much larger and healthy in liquid medium (Figure 1.a). Furthermore, callus was also detected at explant distal ends only for liquid medium but in rare cases (Figure 1.a).

A comparison of the percentages of callus induction for all tested cultivars for both gelled and liquid media are shown in Table (1). High percentage of callus induction was obtained in olive cultivars 'Rasei' (19% and 8%), 'Nabali' (8% and 16%) and Kudari (10% and 13%) in both gelled and liquid medium, respectively. However, low callus induction was recorded in olive cultivars Shami (0%-3%) and 'Kaisei', 'Nasuhi', and 'Surani' (1% and 0%) in both gelled and liquid medium, respectively. For the 'Rasei' olive cultivar, callus induction in gelled medium (19%) was significantly higher than in liquid medium (8%) at $P \leq 0.003$. However, the opposite effect was noticed for 'Shami' with no callus induction in gelled medium, where callus induction in liquid medium was 3% (Table 1). For all other olive cultivars, no significant differences were detected for callus induction using either media (Table 1).

The induced calli were subcultured in a fresh gelled OM (Figure 1.d). The callus weight was measured before and after subculturing (as described in experimental procedure). The different cultivars showed different average callus weight increase for both tested media. Table (2) illustrates the average increase in callus weight induced in gelled medium. Cultivar 'Kudari' presented the highest increase in callus average weight with 4.05 folds, followed by 'Surani' with 3.3 folds. On the other hand, 'Shami' olive cultivar did not show any increase in callus weight. However, in liquid medium (Table 3), the olive cultivar 'Shami' showed the highest increase in callus average weight with 0.42 fold, followed by 'Nabali' with 0.35 fold, whereas when using the same liquid medium, 'Kudari' showed a slight decrease in average callus weight (-0.073 fold), while, olive cultivars 'Kaisei', 'Nasuhi' and 'Surani' showed no increase in callus average weight.

## DISCUSSION

*In vitro* establishment and callus culture are highly valuable for several purposes. It is a prerequisite for genetic transformation as a tool in improving olives (Mencuccini *et al*., 1992), where induced callus forms adventitious shoots and roots. Callus could further be used in suspension cultures to study plant cell wall composition and analyze induced defensive secondary metabolites (Stella and Braga, 2001). Furthermore, while callus tissue is often heterogenous in cell composition, it is sometimes desirable in studying of somaclonal variations (Zacchini and De agazio, 2004).

Callus induction can be generated from different olive explants, e.g. shoot, internodes, young mesocarp, and apical twigs (Canas, 1988). Callus could be induced under dark or light conditions (Pretto and Santarem, 2000). However, many other factors can influence callus induction, e.g. subspecies, cultivar, basal medium, sucrose concentration, propagation method and subsequent plant regeneration (Zheng *et al*., 1998). Callus was reported to be induced from petiole explants in green velvet cultured on MS medium (Thao *et al.,* 2003). Mencuccini and Rugini (1993) reported successful adventitious shoot regeneration from callus induced from olive leaf petioles over modified OM. Furthermore, specific hormones may be needed for callus induction as influenced by olive explant source (Mencuccini and Rugini, 1993).

Among the different olive explant sources (leaf blades, leaf petioles, hypocotyls of germinated seeds and roots of germinated seeds) roots gave the highest callus induction on media containing 5µM 2.4.D, 0.5 µM kinetin and 5µM NAA in darkness (Shibli, *et al,*. 2001). Moreover, petioles induced more callus when they shifted to cytokinin (Mencuccini and Rugini 1993). However, from the results described here, both (gelled and liquid) media were able to produce callus in all the tested seven olive cultivars. This achievement is significant to establish callus culture for these cultivars that can be used for further studies, e.g. gene transfer, somaclonal variation, cell culture. However, Sadder (2002) reported that dark incubation over still liquid OM induced callus

without the use of plant bioregulators as reported for other olive cultivars. However, the low BA concentration (0.5 mg/l) in OM used in this study should be tested and modulated for organ regeneration.

Results shown in Tables (2 and 3) showed a higher potential of gelled OM for callus induction than liquid OM. There was a huge difference in the increase of average callus weight between the tested cultivars in this study. This could be directly affected through the heterogeneous content of endogenous explant hormones both qualitatively and quantitatively.

In conclusion, culture establishment with liquid medium is an attractive method, and should be further improved to avoid explants death after sinking in the medium. Gelled OM proved to be effective to induce callus growth. Both 'Kudari' and 'Surani' olive cultivars produced high callus weight fold increase when they were cultured in gelled medium. The most important olive cultivar in Jordan the 'Nabali' produced callus in both gelled and liquid medium; however, relatively much weaker than 'Kudari' induced callus over gelled medium. Therefore, further studies should be conducted to improve callus induction for this cultivar followed by organogenesis for future genetic transformation experiments.

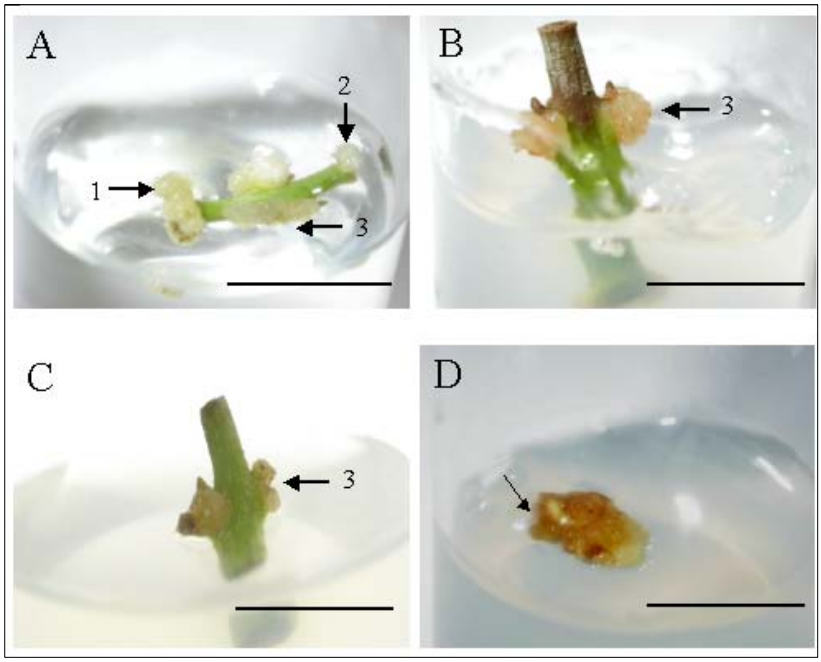


**Figure (1):** Callus induction for different olive cultivars. (a) 'Rasei' over liquid OM; (b) 'Shami' over gelled OM; (c) 'Kudari' over gelled OM. Arrows are pointing to site of callus induction (1: at proximal end; 2: at distal end; 3: at petiole base). (d) Subcultured callus of 'Rasei', 2 weeks under dark conditions. Scale bar =1 cm.

**Table (1): The effect of liquid and gelled media on callus induction percentage in some olive (*Olea europaea* L.) cultivars.**

| | Percent induced calli from nodal cuttings | | |
|---|---|---|---|
| **Cultivar** | **Gelled medium** | **Liquid medium** | **X2** |
| 'Rasei' | 19 | 8 | 8.63** |
| 'Shami' | 0 | 3 | 6.33** |
| 'Kaisei' | 1 | 0 | $1.63^{ns}$ |
| 'Nabali' | 8 | 16 | $1.21^{ns}$ |
| 'Nasuhi' | 1 | 0 | $0.9^{ns}$ |
| 'Surani' | 1 | 0 | $0.87^{ns}$ |
| 'Kudari' | 10 | 13 | $0.02^{ns}$ |

* Significant according to Chi Square analytical method at ($P \leq 0.01$).

**Table (2): The average increase in callus weight on gelled medium of some olive (*Olea europaea* L.).**

| | Average callus weight | | | |
|---|---|---|---|---|
| **Cultivar** | **Number of tubes** | **Before culture (gm)** | **After culture (gm)** | **Fold increase** |
| 'Rasei' | 19 | 0.41 | 0.62 | 0.51 |
| 'Kaisei' | 1 | 0.4 | 0.64 | 0.65 |
| 'Nabali' | 8 | 0.75 | 0.85 | 0.13 |
| 'Nasuhi' | 1 | 0.91 | 0.94 | 0.03 |
| 'Surani' | 1 | 1.2 | 5.21 | 3.3 |
| 'Kudari' | 10 | 0.38 | 1.92 | 4.05 |

**Table (3): The average increase in callus weight in liquid medium of some olive (*Olea europaea* L.).**

| | Average callus weight | | | |
|---|---|---|---|---|
| **Cultivar** | **Number of tubes** | **Before culture (gm)** | **After culture (gm)** | **Fold increase** |
| 'Rasei' | 8 | 0.65 | 0.69 | 0.06 |
| 'Shami' | 3 | 0.38 | 0.22 | 0.42 |
| 'Nabali' | 16 | 0.63 | 0.85 | 0.35 |
| 'Kudari' | 13 | 0.26 | 0.07 | -0.073 * |

* Decrease in callus weight.

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# تحفيز وتضاعف الكلّس لسبعة أصناف محلية من الزيتون (*Olea Europaea* L.)

*وفاء رشدي اسليم ومنذر طه الصدر**

## ملخـص

تم تأسيس زراعة لسبعة أصناف من الزيتون المحلية و هي "النبالي"، "الرصيعي"، "النصوحي"، "الشامي"، "الخضري"، "الصوراني" و "القيسي" لتحديد مقدرتها على تحفيز الكلّس باستخدام العقل العقدية داخل الأنابيب.

لقد تم تحفيز الكلّس من الاصناف المذكورة اعلاه باستخدام البراعم الطرفية في الظلام على بيئة زيتون(OM) سائلة و صلبة، و تم التعرف على الكلّس في ثلاثة مواقع: القواعد اوالنهايات القريبة من الأجزاء النباتية (وهو الموقع الاكثر لجميع الاصناف المدروسة) و ذلك في بيئتي الزيتون السائلة و الصلبة، وقواعد الانصال (وهو ثاني اكثر موقع لجميع الاصناف المدروسة) و ذلك في بيئتي الزيتون السائلة و الصلبة مع انه كان افضل في بيئة الزيتون السائلة، و كذلك في القمم او النهايات البعيدة من الأجزاء النباتية (و هو موقع نادر الحدوث) و ذلك في بيئة الزيتون السائلة فقط.

و لقد حقق الصنف "الرصيعي" أعلى نسبة لتحفيز الكلّس (19%) في البيئة الصلبة في حين ان 16% كانت اعلى نسبة حققها الصنف "النبالي" في البيئة السائلة.

تم تكثير الكلس كل 3-4 أسابيع، حيث كانت أعلى زيادة في الوزن هي 4.05 ضعف في البيئة الصلبة للصنف "الخضري" في حين سجل الصنف "الشامي" زيادة تقدر بـ 0.42 ضعف في البيئة السائلة.

الكلمات الدالة:اصناف الزيتون، الكلس، داخل الانابيب.

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