CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

EFFECT OF THE VARIATION OF THE CULTURE MEDIUM COMPOSITION ON THE IN VITRO MICROPROPAGATION OF THE OLIVE TREE (OLEA EUROPEA L.SKEELS VAR. SATIVA) CULTIVAR CHEMLALI

S. El Adib [1], S. Slim [2], N. Ben Fadl [3]

[1]Université de Carthage. Laboratoire des Sciences Horticoles, INA Tunisie, 43, avenue Charles Nicolle, Tunis, 1082 Tunisie.

[2] Université de Carthage. Laboratoire des Sciences Horticoles, ESA Mateur.

[3] Laboratoire de Biotechnologie Végétale. Institut National des Sciences Appliquées et de Technologie, BP 676, 1080 Tunis Cedex, Tunisie.

Abstract:

The study of the influence of the change in the composition of the culture medium on micro propagation of olive explants has helped to highlight the importance of the culture medium on bud. Thus, cultured on MS medium (Murashige and Skoog) were noted sizable bud break rate (M11: 79.16 %) in simultaneous presence of the three growth hormones (NAA, BAP and ZEA). Whereas the binary combinations of the three hormones gave moderately high levels which did not exceed about 13.8% M14. On the other hand, OM grown on medium which is a derivative of MS medium, there was very important bud break rate reached 93.75 % on OM5. Further dilution of the culture medium did not appear effective in increasing the rate of bud OM medium with a maximum of 64.58 % on OM6. The sampling period also influenced the overall rate of bud explants. Thus in the period of vegetative rest TGDD did not exceed 6.48% while in the flowering period TGDD reached an average of 40.37 %.

Keywords: *olive- micro propagation- OM medium- growth hormones- MS medium*

Corresponding author:

S. El Adib,

Université de Carthage. Laboratoire des Sciences Horticoles,
INA Tunisie, 43, avenue Charles Nicolle,
Tunis, 1082 Tunisie.
E-Mail: Seif.eladib@gmail.com

QR code

Please cite this article in press as E L Adib et al, ***Effect of the Variation of the Culture Medium Composition on the In Vitro Micropropagation of The Olive Tree (Olea Europea L. Skeels var. Sativa) Cultivar Chemlali****, Indo Am. J. Pharm. Sci, 2015; 2(12).*

INTRODUCTION:

The olive tree occupies in the world 8.6 million hectares for a production of 17.3 million tons of olive . The first four producers are Spain , Greece and Turkey. They represent 8% of world production of olive knowing that the top 10 producing countries are all located in the Mediterranean area which has 836 million trees, with 95 % of world orchard. Tunisia is the largest olive-growing countries south of the Mediterranean with over 30 % of land devoted to olive growing (1.68 million ha). The olive orchard has more than 57 million feet over 1.5 million hectares, a third of arable land [1]. The propagation of cultivars is mainly by vegetative means, but this process is slow and does not allow further spread can meet the growing needs of farmers. Researchers and breeders have turned to biotechnological methods to spread the olive and develop the olive industry. Today, plant biotechnology based mainly on the technique of in vitro culture, are the most used. The use of biotechnology has emerged in the olive sector in the early 90s, especially as improvement instrument [2, 3]. Subsequently, several other methods have been tried [4]. For the in vitro production of new cultivars or vitroplantes, biotechnology applied to the olive aim to produce homozygous individuals from somatic haploid or hybrid fabrics through protoplast fusion [4, 5]. Among in vitro culture techniques, microprapagation, remains the most effective and least expensive method which has made it the most used since the 80s so she helped improve the capacity of rooting fragments olive [6] and led to the spread of many varieties micro [7]. However, micro propagation responses are influenced by the composition of the culture medium, the nature of the explants and the variety. This work aims to study the effect of varying the medium composition on micro propagation of olive var. Chemlali , very cultivated in central and southern Tunisia and for which the means of propagation are still limited .

MATERIALS AND METHODS:

1. The Plant Material

To study the effect of the change in the composition of media on the in vitro micro propagation of olive trees , we have cultivated fragments of stems with nodes taken from different parts of mature trees of the variety " Chemleli " grown in full fields.

The explants used for micro propagation are taken from branches of the year that emerge on the branches mainly during vegetative growth (fall and spring) and twigs at the base of the trunk at the beginning of their growth . These light green twigs have a juvenile character and are made up of still young tissue. The explants are taken from the same tree branches in three successive periods in nine months

P1: from September until November;
P2: from December until February
P3: from March until May

The length of these branches node is between 1 and 2 cm. The explant is formed by a node with two leaves with part of the upper node and between some of the lower node between

2. Sterilization Plant Material

After rinsing with tap water, the explants were soaked in alcohol 70 ° and subsequently transferred in a solution of HgCl 2 concentration of 1 gl- 1 for 30 min. A few drops of Tween 80 are added to the jar sterilization. After disinfection, explants were rinsed four or five times with sterile distilled water under a laminar flow hood.

3 .Crops Media

Several culture media were tested according to the phases of the culture. These media are the MS medium [8] and OM middle containing different hormonal combinations and doses.

3.1 To Induce the Multiplication

The basic Murashige and Skoog [8] is the one we tested the first (Table 1). To this medium we added separately or in combination on 6 -benzyl-aminopurine (BAP), zeatin (ZEA) and naphthalene acetic acid (NAA) , sucrose (30g / l) and agar (5 g / l) . For each environment the explants were tested 6 times, wherein each experiment contains 12 explants thus 72 explants tested medium (Table 2).

Further that the MS medium, we tested the OM medium [6] (Table 1) , which is a specific medium for the Oliver and which differs from the MS medium by mineral composition . OM medium (Table 3) has a positive effect on shoot multiplication leafy olive knowing that it is rich in minerals [9, 10]. Disinfection of thermolabile phytohormones used is using autoclaved syringe (120 ° C for 20 min's and a pressure of 0.8 bar) with a circular Millipore filter whose size does not exceed its micropores 0.2µm. Each OM medium was tested 4 times with middle 12explant in each experiment (a total of 48 explants per medium)

3.2 Rooting Phase

The rooting media used are the MS / 2 medium (MS diluted by half for minerals) supplemented with 1 mg / l NAA and IAA in root induction phase during the first 20 days. The shoots are cultured in the dark then they will be transferred into test tubes of 20 x 200 containing OM medium without growth regulators and a photoperiod 16h / day.

4. Culturing Explants

Transplanting on different culture media is performed under laminar hood. After disinfection, explants are stripped of their leaves and dried between two sterile filter paper. Subsequently, the explants were cut from both ends and placed vertically on the autoclaved media (for 20min at 121 ° C and pressure of 1 atm) after cooling in the host to a temperature of 40 ° C.

5. Culture Conditions

After inoculation, the cultures were placed in a climatically controlled chamber under the following conditions:

• Temperature: 22 ° C ± 1 ° C during the day and 19 ° ± 1 ° C at night

• photoperiod: 16 hours of light, 8 hours of darkness (16/8).

• The light provided by fluorescent lamps placed at 60 cm above the cultures.

• The illuminance is about 6000 lux

• The relative humidity is about 80% in the tubes

6. Statistical Data Processing

To assess the potential of morphogens olive explants cultured, we evaluated:

- The percentage of bud in different MS environments

- The percentage of bud breaks in mid OM

The various parameters were subjected to analysis of variance factor (medium effect) classification [11]. A comparison test medium by Duncan's test was performed to classify the different groups averages

RESULTS:

1. Explants Cultured On the Medium and Skoog Muraschige

I.1 Effect of Naphtlylacétique acid (NAA) on the Rate of Sprouting of Buds

The only ANA has no effect on the bud. Both medium M4 (0.1mg / l NAA) and M24 (0.5 mg / l NAA) gave no start axillary buds (Table 4). The in vitro cultured tissue in the presence of auxin generally induces histogenesis and production of somatic embryos, but inhibit the neoformation of buds [12]. These results at least for the concentrations used are consistent with the results of our predecessors [13].

I.2 Simultaneous Effect of the ANA and Zeatin (ZEA) in the Bud buds Rate

The rate of bud explants varies from combinations of both growth regulators. The maximum percentage is 13.8 % on M14 (MS + 1mg / l ZEA; 0.1mg / l ANA) . It is 11.11% on M17 (MS + 1mg / l ZEA; 0.5mg / l ANA) . In the presence of 1mg / l ZEA, ANA is more cost effective in low doses (0.1mg / l). The minimum rate of bud break was 8.3 % (MS + 2mg / l ZEA + 0.5mg / L NAA) (Table 5). Increasing the concentration of ZEA on environments for the same concentration of NAA (0.1 or 0.5 mg / l) reduces the rate of bud break. A percentage decline on M14 (1 mg / l ZEA; 0.1mg / l NAA) on M16 (3mg / l ZEA; 0.1mg / l NAA) on M17 (1 mg / l ZEA; 0.5mg / l NAA) and M19 (3mg / l ZEA; 0.5mg / l ANA) is recognized.

I.3 Simultaneous Effect of BAP and ANASUR Bud Buds Rates

In the presence of BAP and NAA, bud rate is very low. The maximum rate is 4.16 % on M21 (1 mg / l BAP , 0.5 mg / l NAA) and M23 (1 mg / l BAP , 0.1 mg / l NAA) . But by comparing the response of all backgrounds, we note that 1 mg / l BAP, the rate of bud burst has moderately increased. On the other hand, the variation of the concentration of NAA (0.1 to 0.5mg / l) showed no influence on the rate of bud explants (Table 6).

I.4 Simultaneous Effect of BAP, ANA and Zeatin on Bud Buds Rates

The combination of ZEA + BAP + NAA is generally beneficial to bud. However, the percentage of buds developed depends on the variation of concentration of ZEA allowing classifying the 12 media tested into 4 groups (Table 7). For the first group of explants cultured on M1 (0.5mg / l BAP + 0.1mg / l ANA + 1mg / l ZEA) , M2 (0.5mg / l BAP + 0.1mg / l ANA + 2mg / l ZEA) and M3 (0.5mg / l BAP + 0.1mg / l ANA + 3mg / l ZEA) , there is a decrease in the bud rate of 75 % (for M1) to 27.77 % (M2) and 15.12 % (M3) knowing that the concentration of zeatin on these three media spent respectively , 1 mg / l to 2 mg / l and 3 mg / l. For the second group, the buds of growth rates in the 3 circles M5, M6 and M7 is average. We observed a maximum rate which has not exceeded 23.61 % on M5 (0.5mg / l BAP + 0.5mg / l ANA + 1mg / l ZEA). But this reduction is influenced by the increase of the concentration of zeatin. For the third group , cuttings developed on the M8 backgrounds, M9 and M10 , the maximum percentage of bud break (61.11 %) is noted on M8 (1mg / l BAP + 0.1mg / l ANA + 1mg / l ZEA) but when increasing the dose of ZEA 2 and 3 mg / l , bud rate on M9 and M10 decreases to 45.83 %. For the last group of media (M11, M12, and M13) bud rates are important and the maximum is noted on M11 (1 mg / l BAP + 0.5mg / l ANA + 1mg / l ZEA) with 79.16 %. But like other media groups, increasing the concentration of ZEA causes decrease development percentages in M12 and M13. It is apparent in all media tested a complete lack of proliferation of preexisting leafy shoots but a developing sheets primary axillary buds . The secondary buds are inhibited.

I.Budbreak Buds OM Environments

Tests on MS media showed that the increase in the concentration of cytokinin relative to auxin that is effective in the bud explants. To induce bud break followed by a development leafy shoots of higher order (secondary, tertiary , ..) , we tested the most profitable combination hormone in MS circles and adjust the middle OM (medium olive) specific for the olive tree. This is the concentration of 1mg / l ZEA, 1mg / l BAP and 0.5 mg / l NAA on the medium OM1 . Bud rate is 12.5% and the increases of leafy shoots are low. For this we have tested 5 different environments to maximize OM bud and the development of leafy shoots. These

environments contain different doses of BAP , ZEA and ANA .

II.1 Effect of Naphtalylacétique acid on Bud Explants Grown On Media OM

To highlight the effect of auxin and knowing that the concentration ratio of cytokinin / auxin should be always greater than 1, we tried an OM medium without auxin and concentrations of both cyokinines was fixed (ZEA and BAP) 1 mg / L (Table 8) . We found that the elimination of the ANA on OM3 medium (1mg / l ZEA ; 1mg / l BAP) allowed to have a performance four times greater than on OM1 (1mg / l ZEA + 1mg / l BAP + 0.5mg / l ANA) the addition of 0.5 mg / l of ANA appears to inhibit bud and propagation of leafy shoots .

II.2 Effect of Lowering the Concentration of Cytokinins

Based on the basis that the elimination of auxin is critical to improve the performance of media , and knowing that cytokinins doses above 1 mg / l are inhibiting the growth we have varied cytokinin dose of 1mg / l to 0.5 mg / l and culturing the explants in the absence of NAA .

We have noticed an increase in rates in the bud OM5 medium (0.5mg / l ZEA + 0.5mg / l BAP) by 37 % compared to OM3 (1mg / l ZEA ; 1mg / l BAP) (Table 9) . The combination of hormonal OM5 is best for bud and the development of leafy shoots with 93.75 % energy efficient

II.3 The effect of Dilution of the Medium OM on Developing Leafy Shoots

To investigate the effect of diluting the culture medium on the yield of shoots, we diluted by half the mineral composition of media OM1, OM3 and OM5 for three other defined media respectively OM2, and OM4 OM6. We found that the dilution has not favored the bud for the profitability of the media. As bud burst rates on the OM2 media (OM 1/2 + 1 mg / l ZEA + 1mg / l BAP + 0.5mg / l ANA) is lower than the middle OM1. For OM4 environment (OM3 / 2 + 1mg / l ZEA + 1mg / l BAP), we noticed a decrease of 36% compared to OM3. (Table 10). The same for the middle OM6 (OM5 / 2 + 0.5mg / l ZEA + 0.5mg / l BAP). The fall of one also noticed a budding rate drop by 30% compared to that observed on OM5. But the quality of the shoots is similar to other environments OM .So we noticed that dilution has influée the quantity and not the quality of leafy shoots

III. Effect of the Sampling Period of the Explant on the Rate of Bud

We tested the effect of the withdrawal date explants on the mother tree of September 2007 until the month of May 2008. The buds are harvested at levels comparable to the shaft and culture was tested on the first 12 MS community on the bud. The results are shown in Figure 1.

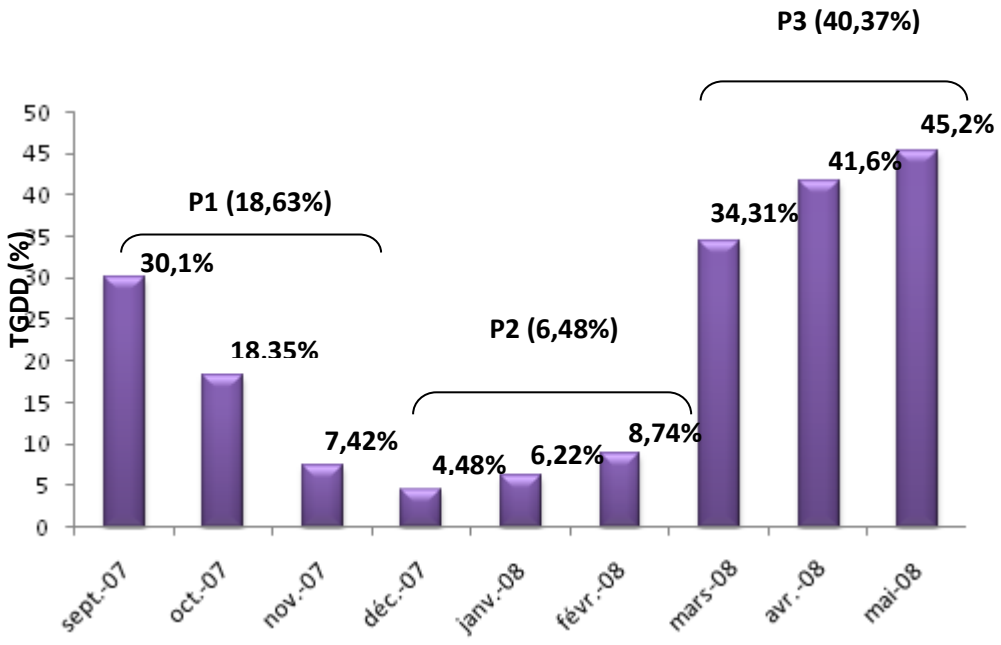

Fig 1: Influence of the Sampling Period on the General Rate of Bud

The first period P1 from September to November, the fruit ripening period, the first 12 tested environments MS, average bud percentages of the three months did not exceed 18.63% with a TGDD (general rate of bud) up to 30% in September. P2 for the second period from December to February, dormancy period, we noticed that the general rate of bud buds TGDD, fell to 4.5% in December and the average of the percentages of three months has not exceeded 6.48%. For the period P3, from March to May, flowering time, the TGDD rose again from March to reach its maximum of 45.2% in May, which resulted in a fairly high average TGDD of 40.37%. So we can say that the explants taken out of the dormancy period, are generally more responsive to the bud.

DISCUSSION:

The study of micropropagation Chemlali cultivar has shown that for optimum bud rate on MS environments , the combination between the two cytokinins BAP and zeatin with the ANA is necessary to induce bud break while the association of NAA with BAP alone or only zeatin has not given satisfactory results and this can be explained by the simultaneous positive effect of hormones on three axillary buds and its importance on the physiology of the explant [14]. Furthermore, we noticed that even in this combination, the variation of cytokinins rates compared to auxin has a direct influence on the response of explants at bud .

Indeed, increased concentraion (≤1mg / l) of both cytokinins used relative to that of auxin is essential to have a better performance in bud on MS medium [10,14]. But at the same time, it was noted that the response of explants on MS media in the presence of the three regulators of growth was limited at bud with total absence of multiplication leafy shoots which is consistent with the work of Rugini in 1986 [15] and Walali work in 2001[16] which was explained by the influence of the mineral composition of budbreak and more precisely the absence of calcium nitrate and potassium chloride. For a multiplication of leafy shoots after bud break , we we are facing a culture medium OM (Olive Medium) which is rich in macronutrients and organic elements mainly glutamine while keeping a ratio of concentrations of cytokinin / auxin greater than 1 which induced the proliferation of leafy shoots but with a low rate that did not exceed 12, 5%. The decrease of the concentration of the auxin (NAA) to 0mg / l, based on the concentration of cytokinin (BAP + ZEA), improved budbreak rate followed by a good multiplication of leafy shoots, by compared to the first OM media tested in the presence of ANA. After the elimination of auxin, the only issue to optimize the bud and the proliferation of leafy shoots was the decrease in the concentration of cytokinins knowing that its increase has shown inhibitory to bud on MS medium in our work and after Bradha and Aboussalim work in 2003, Trabelsi and Rugini Bouzid in 2006 and in 1984 the OM environment, which allowed to have optimum performance that exceeded 90%.

Secondly, dilution of the inorganic composition of the medium was not beneficial for the response of the tested OM media. Thus, it works by decreasing the percentage of bud break but keeping the same quality of leafy shoots obtained before diluting the mineral composition of OM media tested initially which is consistent with the results of Trabelsi and Bouzid in 2006 which showed that dilution of the mineral composition of the culture medium and MS medium OM is beneficial for the response of the explants in multiplication phase protecting the quality of leafy shoots. So with this work, the MS medium was effective for bud break in the presence of low doses of auxin but the OM environment was more effective for the growth of leafy shoots in total absence of auxin in the presence of low doses of cytokinins. Olive cultivars have different reactions depending on the nature of culture medium[14] . The importance of the basic medium for the in vitro propagation of the olive is often discussed. Grigoriadou et al [15] showed that the WPM medium (Woody Plant Medium) is more favorable than the OM medium for the in vitro cultivation of olive trees, while in other works, Bartolini, Leva and Benelli [11] showed that the MS medium allowed a better proliferation OM medium. The analysis of the influence of the sampling period of the explant on the tree showed good responsiveness explants taken at P1 (September, October and November 2007) and especially in P3 (March, April and May 2008) , this could be explained by the pre-training in winter and summer buds [16] (Aiâchi et al , 2007). So , spring and fall are then shoots from a winter and summer preforming . Growth regulators are also involved in the development of the olive tree in leafy shoots in vitro culture. However , the explants have low reactivity to cytokinins[6,7]. So according to our works and those of our predecessors, the most appropriate cytokinin for micropropagation of olive trees is zeatin with 1 or 2mg / l maximum.

CONCLUSION

Finally, after testing the effect of both MS media [8] and OM[6] on the in vitro propagation of the olive tree, we can say and review the results of several studies made on the olive, the MS environment is not favorable enough for the in vitro propagation of olive explants. While the medium OM showed great efficiency, which is the result of extreme physiological complicity with olive explants even outside of any influence of growth regulators added to both types of environments.

Moreover, the variation of the response of the first 12 MS media of the time of year, concluded that the profitability of the entire MS community is outside of the other factors under the direct influence of the time sampling the explant. This response can be explained, firstly, in that the fabrics have a high cell activity during this period is characterized by a high growth and thus better response to culturing (Leva et al, 2000) and secondly, by the remarkable reduction in fungal and bacterial contamination [14].

REFERENCES:

1. Msallem M. Etude de la juvénilité chez l'Olivier (*Olea europaea*). Aspects morphologiques, anatomiques, physiologiques et moléculaires. Thèse Doct. Sci. Agronomiques. INAT,2002; 219 pp.
2. Fontanazza G, Aspects génétiques et techniques de la propagation pour une plantation intensive. In Encyclopédie Mondiale de l'olivier. Conseil Oléicole international. Espagne 1997. pp : 113-144
3.Mekuria G., Collins G. et Sedgley M. Genetic variability between different accessions of some common commercial olive cultivars.*Jour.hort.Sci.Bioteh*., 1999;74 (3) : 309-314.
4.Fiorino P. Amélioration génétique dans l'Olivier (Olea europaea L.).In Ressources Génétiques. Seminaire International sur les Innovations Scientifiques et leur application en Oléiculture et oléiotechnie, 1999.
5. Trujillo I. et Barrnco D. Identification variétale de l'Olivier. In Ressources Génétiques. Séminaire International sur les Innovations Scientifiques et leurs application en Oléiculture et oléiotechnie, Florence, 1999 ;10-12 .
6. Rugini E. *In vitro* propagation of some olive (*Olea europaea sativa* L.) cultivars with different root ability and medium development using analytical data from developing shoots and embryos.*Sci.Hort.1984;* 24: 123-134.
7. Walali L. Techniques de micropropagation in vitro. Outils pour la multiplication et l'assainissement des variétés d'Olivier. P 64-72. In Multiplication et certification des plants d'Olivier. Actes du Séminaire International.ENA. Meknès-Maroc, 2001 ;2-7, Page-231.
8. Muraschige T. et Skoog F. A revised medium for rapid growth and bioassay with Tobacco tissue cultures. Physiol. Plant., 1962; 15:473-497.
9. Rugini E. Olive (*Olea europaea sativa* L.). Biotechnology in agriculture and forestry. Trees I, edited by YPS bajaj Springer Verlag Berlin.1986;pp.253-267.
10. Abousalim A., Brhadda N., Walai L. Essais de prolifération et d'enracinement de matériel issude rajeunissement par bouturage d'oliviers adultes (*Olea europaea* L.) et de germination *in vitro* : effets de cytokinine et d'auxines 2005.
11. Diamantoglou S. and Mitrakos K., 1979. Sur la culture in vitro de l'embryon d'Olivier.
12. Bartoloni G, Leva A. R. et Benelli A. Advances in *in vitro* culture of the olive: propagation of cv. "Maurino", *Acta Hort*,1990 ; 286: 41-44
13.Bouzid S. et Trabelsi EB. Etude de la micropropagation *in vitro* de l'olivier (*Olea eurpea* L.) : *Fac.Sci.Tunis.Thèse* 2006 ;:92-117.
14.Trabelsi E.B. et Bouzid S. Essai de micropropagation de l'Olivier (Olea eurpaea L.) : Action des régulateurs de croissance. Bull.Soci.Sci.Nat. Tunisie. 2002 ;29 : 186-196.
15. Rugini E. et Fontanazza G. In vitro propagation of «Dolce Agogia» Olive. *Hort.Sci*., 1981;16 (4) : 492-492.
16.Brhadda N., Abousalim A., Walali LD. Effets du milieu de culture et de la lumière sur l'embryogenèse somatique de l'olivier (*Olea europaea* L.) cv. Picholine marocaine. *Fruits,2003 ;* **58** (3), p. 167–174.

Table 1: Composition of MS Media (Murashige and Skoog) and OM (Olive Medium)

Elements	Murashige et Skoog	OM (Olive Medium).
Macro- elements		
Ca (NO_3), $4H_2O$	-	0,6
$MgSO_4$. $7H_2O$	0,37	1,5
KH_2PO_4	0,17	0,34
KNO_3	1,90	1,1
$CaCl_2$,$2H_2O$	0,44	0,44
NH_4NO_3	1,65	0,412
• **Micro- elements (mg/l)**		
$MnSO_4$, $7H_2O$	22,30	22,30
ZnSO4, $7H_2O$	8,60	14,3
H_3Bo_3	6,200	6,200
KI	0,83	0,83
Na_2MOO_4, $2H_2O$	0,35	0,25
$CuSO_4$, $5H_2O$	0,025	0,25
$CoCl_2$, $6H_2O$	0,025	0,25
• **Organic elements (mg/l)**		
Myoinositol	100	100
Acide nicotinique	0,50	5
Thiamine-HCl	0,10	0,5
Glycine	2,00	2194
Glutamine	200	0,50
Pyridoxine-HCl	0,50	0,05
$FeSO_4 7H_2O$	5,57 g/l	0,5
Na_2EDTA	7,45 g/l	5,57 g/l

Table 2: 24 MS Tested Mediums

	Growth Regulators		
Medium	**BAP (mg/l)**	**ANA (mg/l)**	**ZEA (mg/l)**
M1	0.5	0.1	1
M2	0.5	0.1	2
M3	0.5	0.1	3
M4	0	0.1	0
M5	0.5	0.5	1
M6	0.5	0.5	2
M7	0.5	0.5	3
M8	1	0.1	1
M9	1	0.1	2
M10	1	0.1	3
M11	1	0.5	1
M12	1	0.5	2
M13	1	0.5	3
M14	0	0.1	1
M15	0	0.1	2
M16	0	0.1	3
M17	0	0.5	1
M18	0	0.5	2
M19	0	0.5	3
M20	0.5	0.5	0
M21	1	0.5	0
M22	0.5	0.1	0
M23	1	0.1	0
M24	0	0.5	0

Table 3: Composition of Growth Regulators used OM Medium

Medium	BAP (mg/l)	ZEA (mg/l)	ANA (mg/l)	Dilution n=2
OM1	1	1	0.5	**no**
OM2	1	1	0.5	**yes**
OM3	1	1	0	**no**
OM4	1	1	0	**yes**
OM5	0.5	0.5	0	**no**
OM6	0.5	0.5	0	**yes**

Table 4: Effect of Two Concentrations of ANA Bud Break Rates

Medium	ANA (mg/l)	Rates of budbreak (%)
M4	0,1	**0**
M24	0,5	**0**

Table 5: Effect of Varying the Concentration of the Naphtalylacétique Acid and Zeatin on the Rate of Bud Breaking of Buds

Medium	ANA (mg/l)	ZEA (mg/l)	Rates of budbreak (%)
M14	0,1	1	**13,8gijh**
M15	0,1	2	**11,11gijh**
M16	0,1	3	**9,72ijh**
M17	0,5	1	**11,11gijh**
M18	0,5	2	**8,33ijh**
M19	0,5	3	**9,72ijh**

Table 6: Effect of Varying the Concentration of 6 - Benzylaminopurine and Naphtalylacétique acid on the Rate of Bud Breaking of Buds

Medium	BAP (mg/l)	ANA (mg/l)	Rates of budbreak (%)
M20	0,5	0,5	**2,77ij**
M21	1	0,5	**4,16ij**
M22	0,5	0,1	**2,77ij**
M23	1	0,1	**4,16ij**

Table 7: Effect Of Varying The Concentration of 6 - Benzylaminopurine , the Naphtalylacétique Acid and Zeatin on the Rate of Bud Breaking of Buds

Medium	BAP (mg/l)	l'ANA (mg/l)	ZEA (mg/l)	Taux (%)
M1	0,5	0,1	1	**75**
M2	0,5	0,1	2	**27,77**
M5	0,5	0,5	1	**23,61**
M 3	0,5	0,1	3	**15,12**
M6	0,5	0,5	2	**20,83**
M7	0,5	0,5	3	**12,15**
M8	1	0,1	1	**61,11**
M9	1	0,1	2	**56,94**
M10	1	0,1	3	**45,83**
M11	1	0,5	1	**79,16**
M12	1	0,5	2	**66,66**
M13	1	0,5	3	**51,38**

Table 8: ANA Effect on Bud Breaking Axillary Bud Explants Cultured on Medium OM

Medium	BAP (mg/l)	ZEA (mg/l)	ANA (mg/l)	Taux de débourrement (%)
OM1	1	1	0.5	**12.5**
OM3	1	1	0	**56.25**

Table 9: Effect of Varying The Concentration of Cytokinins on Bud Breaking Axillary Bud Explants in a Medium OM

Medium	BAP (mg/l)	ZEA (mg/l)	rates
OM3	1	1	**56.25**
OM5	0.5	0;5	**93.75**

Table 10: Effect of Dilution of the Mineral Composition of the Axillary Buds of the Explants Cultured On Medium OM

Medium	BAP	ZEA	ANA	Dilution	rates
OM1	1	1	0.5	Non	**12.5c**
OM2	1	1	0.5	Oui	**8,33c**
OM3	1	1	0	Non	**56,25b**
OM4	1	1	0	Oui	**20,83c**
OM5	0.5	0.5	0	Non	**93,75a**
OM6	0.5	0.5	0	Oui	**64,58b**