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Efficient Plant Regeneration from Explants of Compact Oregano (*Origanum compactum* Bentham)

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Abstract

Origanum compactum Benth., a species endemic to Morocco characterized by its biological activities, is overexploited because of its commercial value and threatened with extinction. Accordingly, measures for its conservation are needed. Micropropagation serves as a solution for the protection and the domestication of this species. In this investigation, we established a protocol for vegetative multiplication *in vitro* of *Origanum compactum* by the axillary bud technique. Six culture media (SH, SD, N30K, MS, MSm, B5) were tested to determine the most suitable mineral medium for growth and development of explants. Four cytokinins: Kinetin, Zeatin, BAP (6-Benzylaminopurine), 2ip (2-Isopentenyladenine) and three compounds with cytokinin activity: Adénine, 1,3-Diphenylurea (DPU) and Thidiazuron (TDZ) at five concentrations (0.44, 1.33, 2.22, 3.11, 4.44 µM) were tested on budding, growth, hyperhydria and rooting. Then three auxins, Indole-3-acetic acid (IAA), Naphthalene acetic acid (NAA) and Indole butyric acid (IBA) at four concentrations (1.14, 2.85, 4.56, 6.27 µM) in presence of 2.22 µM BAP were evaluated. The combination of Gibberellic acid (0.29, 1.5, 2.60, 2.89 µM GA_3) and three polyamines (Putrescine, Spermidine, Spermine) at five concentrations (1.134, 3.402, 5.67, 7.938, 11.34 µM) with cytokinins and auxins were considered. Our results show that Margara medium is the most efficient and BAP at 2.22 µM is the best cytokinin for development of the aerial parts, with a regeneration rate of 88, 90%; rhizogenesis is successful with the combination of 6.27 µM IAA and 2.22 µM BAP. Moreover, the integration of 2.89 µM GA_3 with

2.22 µM BAP and 6.27 µM IAA promotes vitroplant growth, bud and shoot multiplication and elongation of the aerial part. The addition of polyamines with 2.22 µM BAP and 6.27 µM IAA does not improve the root part, but Spermine at 5.67 µM promotes bud and shoot multiplication with a high percentage of regeneration, and spermidine at the same concentration gives long explants. Finally, plantlets with good root development were successfully acclimatized to natural conditions and served as a source to establish *in vitro* culture again.

Keywords

Auxins, Cytokinins, Gibberellic Acid, Macronutrients, Micropropagation, Polyamines, *Origanum compactum*

1. Introduction

Morocco is a huge provider of the world market in aromatic and medicinal plants, and thanks to its geographical position, it offers a varied range of Mediterranean bioclimate allowing a rich flora made up of more than 4200 species and varied vegetation. Aromatic and /or medicinal species are estimated at 500 to 600 species, many of which are endemic and among them, *Origanum compactum*.

Oreganos are aromatic and medicinal plants, dedicated to various purposes such as alimentation, drinks, and traditional medicine, used in a number of different ways due to their essential oils characterized by antioxidant, antimicrobial, cytotoxic, antitumoral, anti-corrosion activities, etc. [1]. Oreganos are native to Asia and Europe, currently distributed worldwide [2] but widespread in the Mediterranean region [3]. 80% of species are located in the western Mediterranean [4], some species being specific to a well-defined territory, such as *O. saccatum*, *O. boissieri*, *O. hypericifolium*, *O. sipyleum*, *O. acutidens*, *O. haussknechtii*, *O. srevidens* in Turkey [5], *O. syriacum* in the Middle East or *O. dictamnus* in Greece where it is very popularly known in the field of medicine.

In Morocco, there are few taxa belonging to the genus *Origanum* (*Zaatar* in Arabic): *O. elongatum* (Bonnet) Emb. & Maire (included *O. grosii* Pau & Font Quer), a Morrocan endemic species, *O. vulgare* ssp. *virens* (Hoffmanns. & Link) Ietsw. (= *Origanum virescens* Poir.) and *O. compactum* Benth. [6]. *Origanum* x *font-queri* Pau, considered a hybrid between *O. grosii* and *O. compactum,* and possibly included in *O. elongatum,* is close to *O. grosii* with larger bracts and calyx and more or less glabrous stems and leaves [7].

The geographical distribution of oreganos includes the western Rif area and the middle Atlas regions. Due to drought and mainly overexploitation, the second region is shrinking more and more, as nowadays only the Rif region offers the current wide range of exploitation of oregano [6]. *Origanum compactum* is widely exploited for marketing purposes and *O. elongatum* on a smaller

scale. Morocco exported 23 Million tons of dried oregano in 2021 [8]. *Origanum vulgare* ssp. *vulgare* could also be cultivated [9].

Origanum compactum is a native herb in the Mediterranean region belonging to the stages of thermo-Mediterranean and meso-Mediterranean vegetation with semi-arid and sub-humid bioclimate. It is the main source of production of oregano OHC in Morocco [10]. *Origanum compactum* is rich mainly in phenolic compounds and widely used in traditional Moroccan medicine to cure certain diseases, such as diarrhea, respiratory diseases, cutaneous and urinary infections [11]. Several studies have shown the biological activities of *Origanum compactum*, antioxidant and antimalarial activities [12], antibacterial activity [13] [14], antifungal activity [15], cytotoxic activity and antitumoral activity [16].

In the Rif region of Morocco, *Origanum compactum* is overexploited because of its commercial value: the species is threatened with extinction because of abusive exploitation mode (uprooting of the whole plant with its roots). Therefore, operational rationalization measures for regeneration and conservation of the species are needed. Biotechnological tools can provide solutions for the protection and domestication of the species, vegetative propagation or *in vitro* culture. Tissue culture and micropropagation have been completed in some Oregano species. Several studies use nodes from seedlings as a type of explant for micropropagation [9] [17]-[26]. In the present study, nodes with two axillary buds of *Origanum compactum* originating from Ouazzane region were used as explants with the objective to establish a protocol for vegetative multiplication *in vitro* by proceeding different tests in order to assure the farmer's needs and introducing an alternative culture of *Origanum compactum*.

2. Material and Methods

2.1. Plant Material

The explants used in this study were obtained from the apex of 3 to 4 cm of 4-week young plantlets of *Origanum compactum* Benth. preserved in the Laboratory of Plant Biotechnology, Faculty of Sciences, Tetouan.

2.2. Preparation of the Stock Material

In order to increase and to obtain a sufficient number of explants, we practiced successive subcultures of nodal segments on a medium containing Murashige and Skoog macronutrients, micronutrients and vitamins, 100 mg/L myoinositol, 3% sucrose and 0.44 µM Kin; the pH was adjusted to 5.7 with NaOH (1N). After the medium had been heating, the final solution was gradually added with agar at 7 g/L.

2.3. Effect of Mineral Nutrients

The mediums tested were MS [27], SD [28], modified MS (MSm) [29], $N_{30}K$ [30], B5 [31] and SH [32], all of them added with MS micronutrients and vitamins and 3% sucrose. The best macronutrients were used for all the following tests.

2.4. Effect of Cytokinins and Cytokinin-Like Compounds

Four cytokinins: Kinetin (Kin), Zeatin, 6-Benzylaminopurine (BAP), 2-Isopentenyladenine (2ip) and three compounds with cytokinin activity: Adénine (Ad), 1,3-Diphenylurea (DPU) and Thidiazuron (TDZ) at five concentrations (0.44, 1.33, 2.22, 3.11, 4.44 µM) were tested for their effect on growth and development of explants. Cytokinin-free medium was considered a control.

2.5. Effect of Auxins

Three auxins: indole 3-acetic acid (IAA), 1-naphtalene acetic acid (NAA) and indole-3-butyric acid (IBA) at four concentrations (1.14, 2.85, 4.56, 6.27 µM) were tested with the presence of the most suitable cytokinin determined in the previous test. Medium contain only the cytokinin served as a double control.

2.6. Effect of Cytokinins and Auxins Combined with Gibberellic Acid

Five concentrations of gibberellic acid (0.29, 1.5, 2.60, 2.89 µM) were tested with the best combination of cytokinin and auxin. The medium containing only cytokinin was considered the control medium number 1 and the medium supplemented with the best combination of cytokinin and auxin served as double control.

2.7. Effect of Cytokinins and Auxins Combined with Polyamines

Three polyamines (Putrescine, Spermidine, Spermine) at five concentrations each (1.134, 3.402, 5.67, 7.938, 11.34 µM), were tested with the best combination between cytokinin and auxin. The medium contains only cytokinin served as the control medium number 1 and the medium supplemented with the best combination of cytokinin and auxin serves as double control.

2.8. Culture Conditions

The tubes were hermetically wrapped with aluminum foil and autoclaved at 121˚C and a pressure of 1 bar for 21 minutes. The cultures were incubated under specific conditions (photoperiod: 18/6 h with 4000 lux light density, temperature: 24˚C ± 1˚C).

2.9. Acclimatization of *Origanum compactum*

The rooted explants, 1-month old and about 15 cm were removed from the tubes and their roots were freed of the agar. They were transferred to plastic pots filled with autoclaved peat and covered with transparent plastic to prevent the loss of moisture and placed in a culture room (photoperiod: 18/6h, humidity: 90% - 100%, temperature: 24˚C ± 1˚C). Leaves were sprayed with water twice a week. After four weeks, the transparent plastics were removed, later the surviving ones were transferred to large pots and placed under natural conditions of illumination and temperature. Subsequently, the number of acclimated plants and the percentage of survival were determined.

2.10. Evaluation of Plantlets Growth

After 30-day culture, the morphological measurements concerned:

Mean plantlets length (cm)

Mean roots length (cm)

Mean number of buds per plantlet

Mean number of shoots per plantlet

Mean number of roots per plantlet

Regeneration rate (%)

Percentage of rooting (%)

Hyperhydricity rate (%)

Data were treated by analysis of variance (ANOVA) to detect significant differences between means using logistics PSW Statistica 18 and SPSS IBM 20. Significant differences were compared using Tukey's HSD (honestly significant difference) test at the 5% probability level. Values beyond $p \leq 0.05$ were considered to be significant.

3. Results

3.1. Effect of Macronutrients

Among the six macronutrients tested, $N_{30}K$ medium gave the best elongation of the culinary part (1.91 cm) followed with SD (1.76 cm); on the other hand, the shortest stems were marked with B5 (0.42 cm).

The bud multiplication was optimum with $N_{30}K$ (14.56) followed by SD (13.38), while the minimal proliferation was noted in B5 (5.11). Regarding the roots, the multiplication was higher in $N_{30}K$ (6.27) followed by SD (6.14) and the lowest multiplication was marked in B5 (0.55).

The shoot proliferation was optimal for $N_{30}K$ medium (1.11) followed by SH (1.00) and was minimal in B5 (0.38) (Table 1, Figure 1 and Figure 2).

In addition, MSm, $N_{30}K$ and SD mediums gave the highest rate of rhizogenesis (96%) followed by SH (80%) whereas the minimal percentage was marked in B5 (21%). Hyperhydricity was absent in $N_{30}K$ and MS mediums and present in B5

Table 1. Effect of six macronutrients on the micropropagation of *Origanum compactum* Benth.

Medium	Regeneration (%)	Shoot length (cm)	Number of buds	Number of shoots	Rooting (%)	Number of roots	Hyperhydricity (%)
MS	97.22	1.23 ± 0.19^{abc}	11.33 ± 1.63^{a}	0.97 ± 0.14^{a}	75.00	4.00 ± 0.82^{b}	0.00
MS_m	100.00	1.43 ± 0.20^{abc}	12.88 ± 1.69^{a}	0.97 ± 0.13^{a}	96.00	6.14 ± 0.92^{a}	4.00
$N_{30}K$	97.22	1.91 ± 0.29^{a}	14.56 ± 2.08^{a}	1.11 ± 0.16^{a}	96.00	6.27 ± 0.94^{a}	0.00
SH	83.33	0.96 ± 0.16^{cd}	12.27 ± 2.13^{a}	1.00 ± 0.16^{a}	80.00	3.47 ± 0.64^{b}	4.00
SD	100.00	1.76 ± 0.28^{bc}	13.38 ± 2.04^{a}	0.88 ± 0.12^{a}	96.00	6.14 ± 0.92^{a}	8.00
B_5	41.60	0.42 ± 0.12^{d}	5.11 ± 1.43^{b}	0.38 ± 0.11^{b}	21.00	0.55 ± 0.25^{c}	16.00

Letters represent homogeneous groups; in each column, different letters indicate a significant difference at $p < 0.05$ using ANOVA and Tukey Post Hoc.

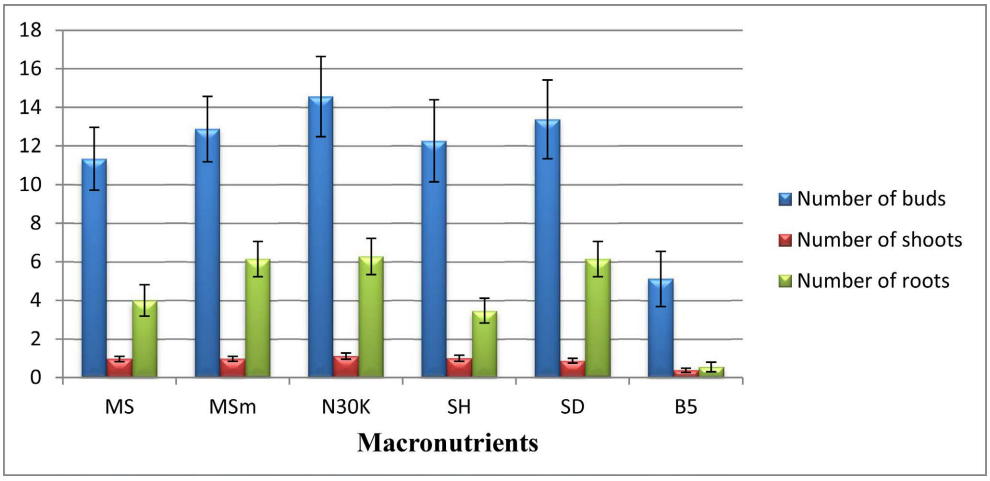

Figure 1. Effect of six macronutrients on the multiplication of buds, shoots and roots of *Origanum compactum* Benth.

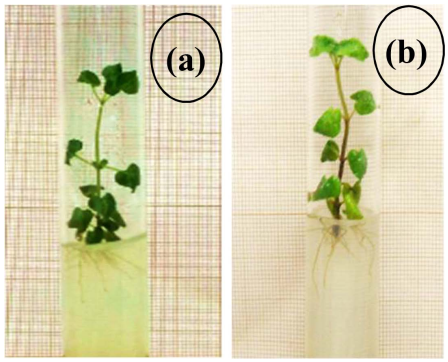

Figure 2. Effect of six macronutrients on shoot and root growth of *Origanum compactum* Benth. plantlets ((a) MSm; (b) $N_{30}K$).

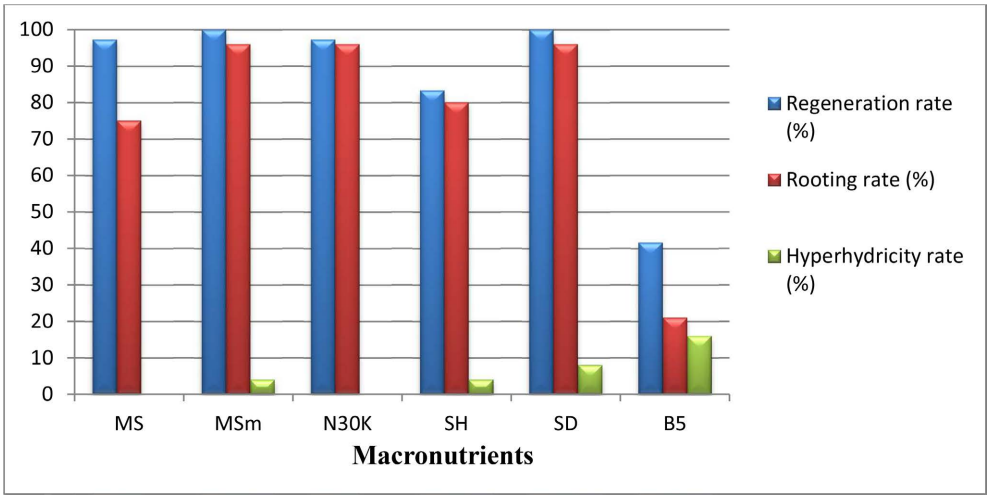

Figure 3. Effect of six macronutrients on the regeneration, rooting and hyperhydricity rates of *Origanum compactum* Benth.

(16%) (Table 1, Figure 3).

The $N_{30}K$ medium is proved the best for the growth of *Origanum compactum*

particularly the aerial part and it had chosen for further experiments.

3.2. Effect of Cytokinins and Cytokinin-Like Compounds

Differences occurred both in culinary and rooting system when cytokinins were added to $N_{30}K$ medium. Culture medium supplemented with 2.22 µM 2ip generated the longest explants in terms of the aerial part (3.06) followed by the medium containing 0.44 and 1.33 µM 2ip (2.75 and 2.66, respectively); on the other side, the shortest explants were observed in medium supplemented with 3.11 µM BAP (1.73) (**Table 2**, **Figure 4** and **Figure 5**).

Moreover, there was a significative difference between the four cytokinins and the three cytokinins-like compounds in terms of multiplication of buds and shoots; thus, the medium containing 4.44 µM BAP offered the highest number of buds (31.56) followed by 1.33 and 2.22 µM BAP (29.87 and 29.60, respectively), whereas the lowest number was marked in medium supplemented with 4.44 µM Ad (15.80). In addition, TDZ ensured a good proliferation of shoots; it was maximal in case of 3.11 µM TDZ (2.42). On the other hand, the medium containing 0.44 µM Kin marked the minimum proliferation of shoots (1.34).

With respect to root multiplication, for cytokinins, the higher concentration of Kin and 2ip gave the best results (5.62 and 5.40, respectively) followed by 3.11 µM 2ip (5.31), unlike control medium where the multiplication was the weakest (1.14), while the multiplication was absent in case of medium added by the five concentration of TDZ (**Table 2**, **Figure 4** and **Figure 5**).

In addition, the culture medium containing 2.22 µM 2ip and the one added with 1.33 µM Zeatin generated the highest rate of hyperhydricity (8.8 and 8.3%, respectively). Concerning the regeneration rate, it was at its maximum for 2.22 µM Kin (100%) whereas the minimal rate was noted for 1.33 µM Kin (63.80%). Additionally, the same cytokinin at 3.11 µM generated a higher percentage of rooted explants (87.80%) followed by 4.44 µM 2ip (86.60%) and 2.22 µM Kin (86.10%), unlike control medium that generated the lower percentage (21.40%) while an absence of regeneration is observed in case of TDZ (**Table 2**, **Figure 6** and **Figure 5**).

3.3. Effect of Auxins

Among the three auxins tested at different concentrations, the blank showed promising result on the elongation of the aerial part (2.57 cm), followed by the case of 1.14 µM and 6.27 µM of NAA (2.56 cm), while the shortest explants were mentioned in the case of 1.14 IAA (1.34 cm) (**Table 3**, **Figure 8**).

In addition, control medium gave inconsistent results regarding the multiplication of buds, shoots and roots (23.14; 1.36 and 1.14, respectively). Medium supplemented with 4.56 µM NAA remained opportune for bud multiplication (33.93). In the case of IAA, a high concentration in the culture medium promoted the multiplication of buds, and for IBA the multiplication was maximum at 6.27 µM (31.22) while a higher concentration had an inhibition effect on root

Table 2. Effect of 7 growth regulators with cytokinin activity on the micropropagation of *Origanum compactum* Benth.

Cytokinins (µM/L)		Regeneration (%)	Shoot length (cm)	Number of buds	Number of shoots	Rooting (%)	Number of roots	Hyperhydricity (%)
Control ($N_{30}K$)		77.70	2.50 ± 0.25^{abcd}	23.14 ± 1.33^{bcde}	1.35 ± 0.09cd	21.40	1.14 ± 0.47^{ghi}	0.00
Ad	0.44	66.70	2.40 ± 0.18^{abcdefg}	18.91 ± 1.29^{bcde}	1.66 ± 0.17^{d}	58.33	2.75 ± 0.64^{abcdefghi}	0.00
	1.33	69.44	2.26 ± 0.17^{bcdefg}	18.24 ± 0.92^{def}	1.72 ± 0.10^{bcd}	68.00	3.52 ± 0.61^{abcdefgh}	0.00
	2.22	86.11	2.43 ± 0.13^{abcdefg}	17.80 ± 0.58^{def}	1.70 ± 0.08^{cd}	83.87	4.16 ± 0.58^{abcdef}	0.00
	3.11	86.11	3.15 ± 0.26^{abcdef}	16.51 ± 0.75^{ef}	1.38 ± 0.08^{cd}	80.64	4.25 ± 0.59^{abcdef}	0.00
	4.44	83.33	2.72 ± 0.19^{abcdef}	15.80 ± 0.88^{f}	1.63 ± 0.10^{abcd}	86.70	5.06 ± 0.65^{abcd}	0.00
2ip	0.44	88.80	2.75 ± 0.16^{abcde}	20.93 ± 1.22^{cdef}	1.84 ± 0.16^{abcd}	81.20	4.28 ± 0.82^{abcdef}	0.00
	1.33	94.40	2.66 ± 0.14^{abcdef}	21.64 ± 1.30^{cdef}	2.02 ± 0.22^{abcd}	85.20	3.97 ± 0.46^{abcdefg}	0.00
	2.22	97.20	3.06 ± 1.88^{ab}	22.00 ± 1.14^{cdef}	1.74 ± 0.17^{abcd}	74.20	5.02 ± 0.74^{abc}	8.80
	3.11	92.00	2.43 ± 0.12^{cdefg}	24.05 ± 1.10^{cdef}	2.00 ± 0.15^{abcd}	80.00	5.31 ± 0.67^{abcde}	5.70
	4.44	83.30	1.92 ± 0.07^{efg}	19.53 ± 1.04^{cdef}	1.50 ± 0.13^{bcd}	86.60	5.40 ± 0.74^{abc}	0.00
Zeat	0.44	66.60	1.46 ± 0.08^{ef}	20.08 ± 1.38^{cdef}	2.05 ± 0.13^{cdefg}	83.30	3.45 ± 0.58^{abcdefh}	0.00
	1.33	86.10	1.67 ± 0.07^{ef}	20.70 ± 0.93^{cdef}	2.46 ± 0.18^{abcdefg}	74.10	4.19 ± 0.65^{abcdefh}	8.30
	2.22	80.50	1.42 ± 0.10^{ef}	19.72 ± 1.14^{cdef}	1.99 ± 0.14^{defg}	58.00	2.75 ± 0.59^{abcdefghi}	6.40
	3.11	94.40	1.24 ± 0.08^{ef}	21.67 ± 1.09^{cdef}	2.22 ± 0.16^{cdefg}	61.70	3.11 ± 0.55^{abcdefgh}	0.00
	4.44	86.10	1.32 ± 0.07^{ef}	21.87 ± 1.11^{cdef}	2.13 ± 0.13^{cdefg}	58.00	1.41 ± 0.29^{fghe}	0.00
Kin	0.44	80.50	2.19 ± 0.14^{cdefg}	22.20 ± 1.47^{cdef}	1.34 ± 0.13^{d}	48.20	2.20 ± 0.54^{defghi}	2.70
	1.33	63.80	2.65 ± 0.17^{abcdef}	24.78 ± 1.69^{abcd}	1.43 ± 0.12^{bcd}	34.70	1.34 ± 0.47^{fghi}	0.00
	2.22	100.00	1.73 ± 0.09^{g}	22.11 ± 1.04^{cdef}	1.41 ± 0.09^{cd}	86.10	5.19 ± 0.63^{abc}	5.70
	3.11	91.60	1.71 ± 0.13^{g}	18.72 ± 0.83^{def}	1.42 ± 0.09^{cd}	87.80	4.78 ± 0.64^{abcde}	0.00
	4.44	97.20	2.63 ± 0.17^{abcdef}	21.25 ± 1.13^{cdef}	1.62 ± 0.13^{abcd}	74.20	5.62 ± 0.74^{b}	6.40
BAP	0.44	86.10	1.36 ± 0.08^{fg}	26.00 ± 1.60^{abc}	1.93 ± 0.16^{abcd}	74.10	1.87 ± 0.13^{de}	0.00
	1.33	86.10	1.28 ± 0.04^{cdefg}	29.87 ± 1.81^{ab}	2.29 ± 0.24^{abcd}	80.60	2.17 ± 0.12^{bcde}	0.00
	2.22	97.20	1.27 ± 0.07^{defg}	29.60 ± 1.43^{ab}	2.17 ± 0.17^{abc}	68.50	2.09 ± 0.09^{bcde}	6.20
	3.11	94.40	1.73 ± 0.12^{g}	24.29 ± 1.15^{bcd}	1.75 ± 0.12^{abcd}	70.50	1.65 ± 0.07^{e}	0.00
	4.44	88.80	1.08 ± 0.12^{edefg}	31.56 ± 1.84^{a}	2.25 ± 0.21^{ab}	56.20	2.05 ± 0.09^{bcde}	0.00
DPU	0.44	77.77	2.15 ± 0.19^{cdefg}	18.58 ± 1.12^{def}	1.50 ± 0.10^{bcd}	96.42	5.83 ± 0.62^{ab}	0.00
	1.33	66.67	1.93 ± 0.25^{cdefg}	19.00 ± 1.15^{cdef}	1.50 ± 0.12^{abcd}	95.83	7.06 ± 0.84^{abcdefghi}	0.00
	2.22	44.44	1.95 ± 0.12^{cdefg}	17.14 ± 1.30^{def}	1.35 ± 0.13^{bcd}	10.00	4.28 ± 0.675^{abcdefgh}	0.00
	3.11	38.88	2.03 ± 0.20^{cdefg}	19.11 ± 1.43^{cdef}	1.61 ± 0.11^{abcd}	10.00	4.83 ± 0.75^{abcdef}	0.00
	4.44	50.00	2.10 ± 0.23^{cdefg}	17.33 ± 1.14^{def}	1.44 ± 0.12^{abcd}	94.44	5.72 ± 0.74^{abcde}	0.00
TDZ	0.44	44.44	3.26 ± 0.21^{a}	20.22 ± 1.51^{cdef}	2.14 ± 0.21^{abcd}	0.00	0.00 ± 0.00^{i}	0.00
	1.33	75.00	2.61 ± 0.13^{abcdef}	20.41 ± 1.30^{cdef}	2.10 ± 0.17^{abcd}	0.00	0.00 ± 0.00^{i}	3.70
	2.22	80.56	2.96 ± 0.18^{abc}	21.93 ± 1.28^{cdef}	2.26 ± 0.20^{ab}	0.00	0.00 ± 0.00^{i}	4.76
	3.11	83.33	2.86 ± 0.12^{abcd}	20.87 ± 1.24^{cdef}	2.42 ± 0.16^{a}	0.00	0.00 ± 0.00^{i}	0.00
	4.44	77.78	2.88 ± 0.20^{abcdef}	17.20 ± 1.53^{ef}	1.66 ± 0.12^{abcd}	0.00	0.00 ± 0.00^{i}	0.00

Letters represent homogeneous groups; in each column, different letters indicate a significant difference at $p < 0.05$ using ANOVA and Tukey Post Hoc.

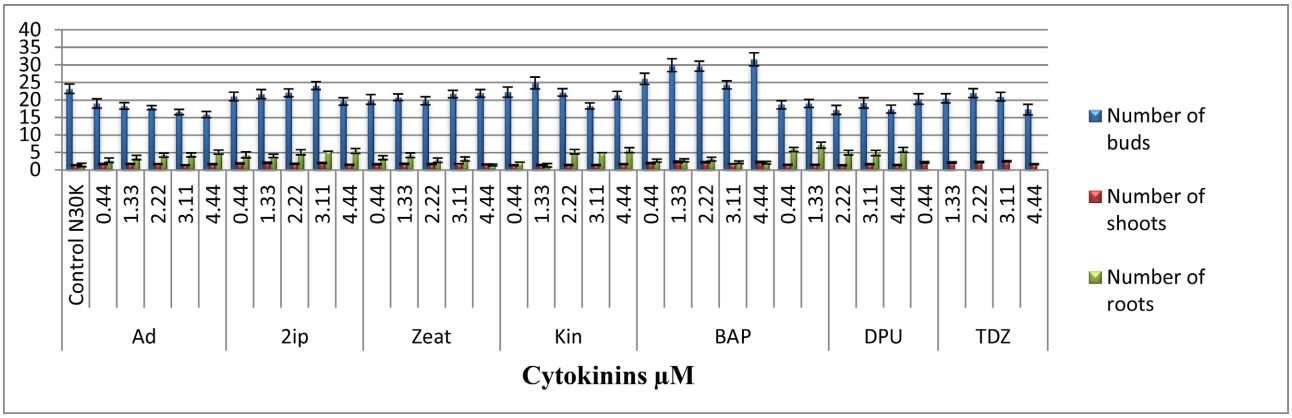

Figure 4. Effect of 7 growth regulators with cytokinin activity at 5 concentrations on the multiplication of buds, shoots and roots of *Origanum compactum* Benth.

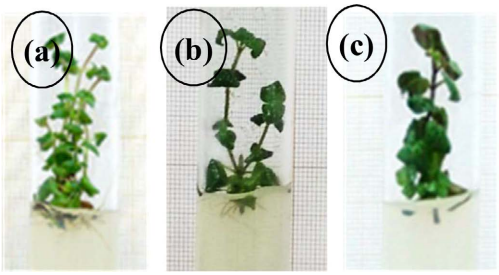

Figure 5. Effect of growth regulators with cytokinin activity on micropropagation of *Origanum compactum* Benth. ((a) 0.44 µM BAP; (b) 4.44 µM BAP; (c) 2.22 µM TDZ).

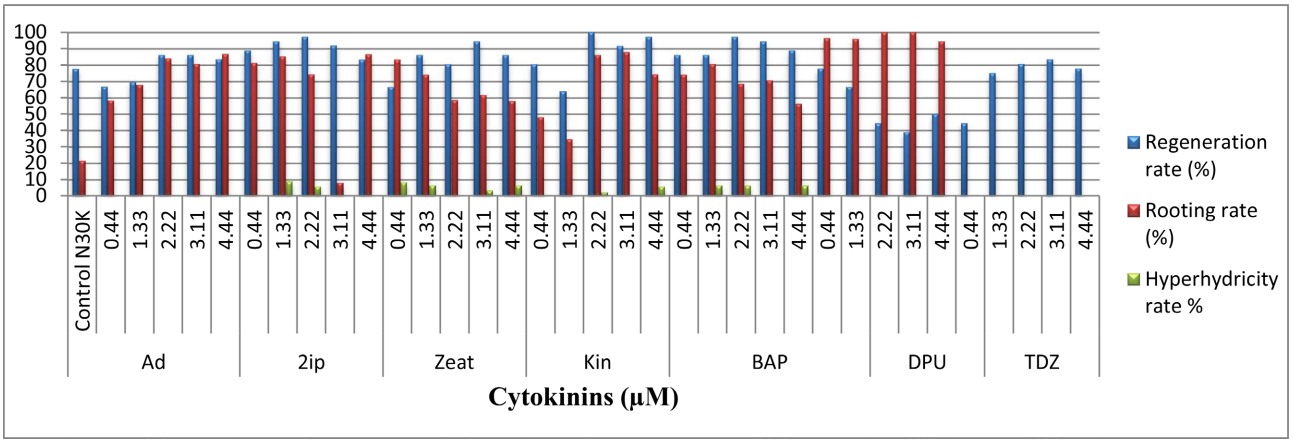

Figure 6. Effect of 7 growth regulators with cytokinin activity at 5 concentrations on the regeneration, rooting and hyperhydricity rates of *Origanum compactum* Benth.

development. For the multiplication of shoots, the medium containing only the BAP was the most suitable (2.17) while the PGR free medium was not favourable (1.36); For medium supplemented by different concentration of IBA, the maximum value was mentioned with 4.56 µM (2.14) (**Table 3**, **Figure 7** and **Figure 8**).

Regarding the proliferation of roots, it was maximum for 6.27 µM IAA (5.61) and minimum for the medium free of PGR (1.14) and for the one containing

Table 3. Effect of three auxins at five concentrations combined with 2.22 µM BAP on the micropropagation of *Origanum compactum* Benth.

Auxins (µM/L)		Regeneration (%)	Shoot length (cm)	Number of buds	Number of shoots	Rooting (%)	Number of roots
Control 1 $N_{30}K$		77.70	2.57 ± 0.25^{a}	23.14 ± 1.33^{d}	1.36 ± 0.09^{b}	21.40	1.14 ± 0.47^{d}
Control 2 (2.22 µM BAP)		97.20	2.09 ± 0.09^{abc}	29.60 ± 1.43^{abcd}	2.17 ± 0.17^{a}	68.50	3.08 ± 0.52^{bcd}
IAA	1.14	91.66	1.34 ± 0.07^{d}	24.36 ± 1.21^{cd}	1.78 ± 0.10^{ab}	81.81	4.81 ± 0.58^{ab}
	2.85	94.44	1.52 ± 0.08^{cd}	24.23 ± 1.04^{cd}	1.82 ± 0.08^{ab}	88.23	4.70 ± 0.51^{ab}
	4.56	83.33	1.51 ± 0.10^{cd}	26.33 ± 1.61^{bcd}	1.86 ± 0.11^{ab}	80.00	5.06 ± 0.73^{ab}
	6.27	94.44	1.40 ± 0.08^{d}	27.41 ± 0.99^{abcd}	2.15 ± 0.12^{a}	91.17	5.61 ± 0.58^{a}
IBA	1.14	91.66	1.57 ± 0.07^{bcd}	30.32 ± 1.28^{abc}	1.93 ± 0.08^{ab}	87.09	5.41 ± 0.64^{ab}
	2.85	86.11	1.51 ± 0.08^{cd}	26.26 ± 1.22^{bcd}	1.84 ± 0.11^{ab}	93.54	4.67 ± 0.50^{ab}
	4.56	66.70	1.58 ± 0.08^{bcd}	28.4 ± 1.39^{abcd}	2.14 ± 0.12^{a}	92.85	4.92 ± 0.50^{ab}
	6.27	77.41	1.77 ± 0.01^{bcd}	31.22 ± 1.32^{abc}	2.03 ± 0.08^{ab}	87.09	4.25 ± 0.46^{abc}
NAA	1.14	94.44	2.56 ± 0.19^{a}	32.47 ± 2.19^{ab}	2.29 ± 0.22^{a}	82.35	3.03 ± 0.41^{bcd}
	2.85	88.89	2.06 ± 0.13^{abc}	32.17 ± 2.04^{ab}	2.20 ± 0.15^{a}	58.82	1.88 ± 0.33^{cd}
	4.56	88.89	2.15 ± 0.13^{ab}	33.93 ± 1.85^{a}	2.15 ± 0.17^{a}	78.12	2.97 ± 0.38^{bcd}
	6.27	97.22	2.56 ± 0.17^{a}	32.80 ± 1.90^{ab}	2.05 ± 0.13^{a}	82.85	3.68 ± 0.38^{abc}

Letters represent homogeneous groups; in each column, different letters indicate a significant difference at $p < 0.05$ using ANOVA and Tukey Post Hoc.

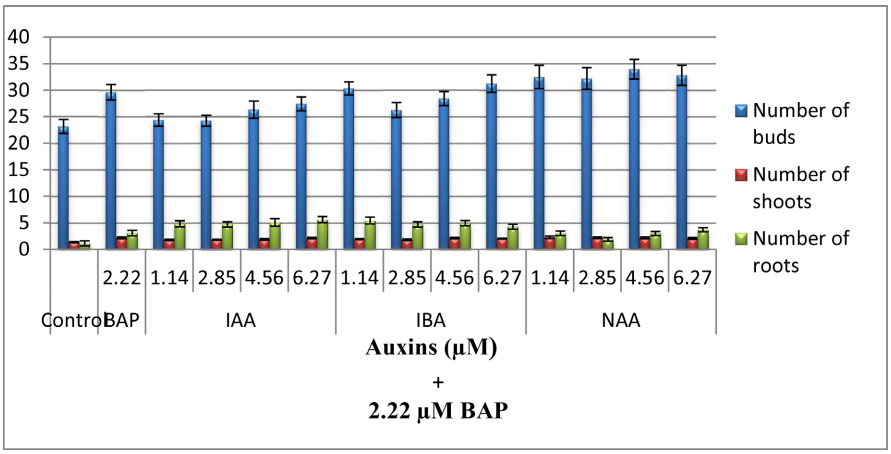

Figure 7. Effect of three auxins at four concentrations combined with 2.22 µM BAP on the multiplication of buds, nodes and roots of *Origanum compactum* Benth.

2.85 µM NAA (1.88); the integration of NAA at its different concentrations was not suitable for root multiplication (Table 3, Figure 7 and Figure 8).

The regeneration varied according to the auxins and their concentrations. The highest percentage of rooted plantlets was observed in the medium supplemented with 2.85 µM IBA (93.54%) while control medium 1 showed the lower percentage (21.40%). For IAA-BAP and NAA-BAP, the highest rate of rhizogenesis

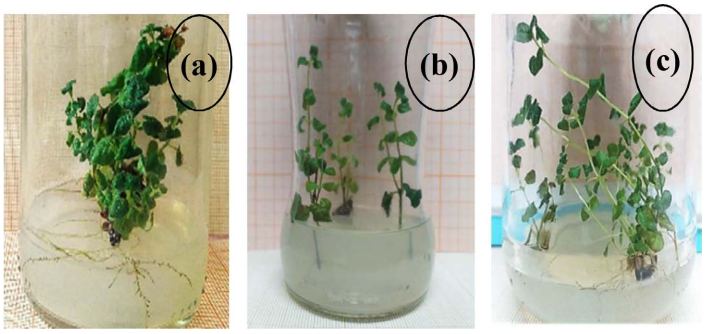

Figure 8. Effect of three auxins combined with 2.22 µM BAP on the micropropagation of *Origanum compactum* Benth. ((a) 1.14 µM NAA; (b) 6.27 µM IAA; (c) 6.27 µM IBA).

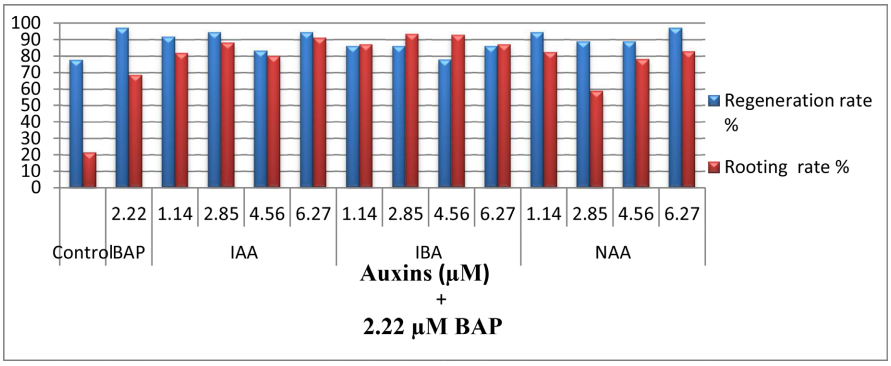

Figure 9. Effect of three auxins at four concentrations combined with 2.22 µM BAP on the regeneration, rooting and hyperhydricity rates of *Origanum compactum* Benth.

was observed at 6.27 µM (91.17 and 82.85%, respectively). Regarding the regeneration rate the highest percentage was marked in the medium which contains 6.27 µM NAA (97.22%). The presence of hyperhydric explants was not reported (**Table 3**, **Figure 9**).

3.4. Effect of Cytokinins and Auxins Combined with GA_3

Addition of different concentrations of GA_3 resulted in some changes in the *in vitro* growth of *Origanum compactum* explants.

The integration of GA_3 into $N_{30}K$ medium supplemented with 2.22 µM BAP and 6.27 µM IAA had a positive impact on bud multiplication and was at its maximum for 2.89 µM GA_3 (19.12) followed by 2.60 µM GA_3 (18.70) and control medium 2 (17.75) and was at its minimum for 0.29 µM GA_3 (15.70). Shoot proliferation was highest at 2.89 µM GA_3 (2.28) followed by control medium 1 (2.00) and 2.60 µM GA_3 (1.90), and lowest at 0.29 µM GA_3 (1.55). Moreover, compared with the combinations of GA_3 with 2.22 µM BAP and 6.27 µM IAA, control medium 2 was the most favorable for root multiplication (5.03) followed by 2.89 µM GA_3 (3.44) and 2.60 GA_3 (2.75), and the minimal value was noted in the case of 1.5 µM GA_3 (2.45) (**Table 4**, **Figure 10** and **Figure 11**).

Furthermore, the highest concentration of GA_3 (2.89 µM) was efficient for the elongation of the aerial part (2.93 cm) followed by control medium 2 (2.90), and 1.5 µM GA_3 (2.55); however, the lowest value was marked for 2.60 µM GA_3

(2.13) (Table 4, Figure 11).

The highest rate of regeneration was observed in the case of 1.5 µM GA_3 (86.11%) followed by control medium 2 (75.00) and 2.89 µM GA_3 (69.44). In addition, control medium 2 generated a high percentage of rooted explants (88.89%) followed by 2.89 (80.00) and 2.60 µM GA_3 (70.00) (Table 4). No hyperhydricity was noted for the different combinations of GA_3 with 2.22 µM BAP and 6.27 µM IAA (Table 4, Figure 12).

Table 4. Effect of gibberellic acid combined with 2.22 µM BAP and 6.27 µM IAA on the micropropagation of *Origanum compactum* Benth.

AG_3 (µM/L)	Regeneration (%)	Shoot length (cm)	Number of buds	Number of shoots	Rooting (%)	Number of roots
Control 1 (SD)	75.00	2.90 ± 0.20^a	16.14 ± 1.04^a	1.66 ± 0.09^{ab}	88.88	5.03 ± 0.55^a
Control 2 (2.22 µM BAP + 6.27 µM IAA)	44.44	2.53 ± 0.23^a	17.75 ± 0.85^a	2.00 ± 0.09^{ab}	56.25	2.93 ± 0.98^{ab}
0.29	55.56	2.43 ± 0.26^a	15.70 ± 1.08^a	1.55 ± 0.11^b	50.00	2.55 ± 0.67^b
1.50	86.11	2.55 ± 0.17^a	16.32 ± 0.66^a	1.74 ± 0.09^{ab}	50.00	2.45 ± 0.49^b
2.60	55.56	2.13 ± 0.17^a	18.70 ± 0.93^a	1.90 ± 0.06^{ab}	70.00	2.75 ± 0.53^{ab}
2.89	69.44	2.93 ± 0.15^a	19.12 ± 1.20^a	2.28 ± 0.34^a	80.00	3.44 ± 0.49^{ab}

Letters represent homogeneous groups; in each column, different letters indicate a significant difference at $p < 0.05$ using ANOVA and Tukey Post Hoc.

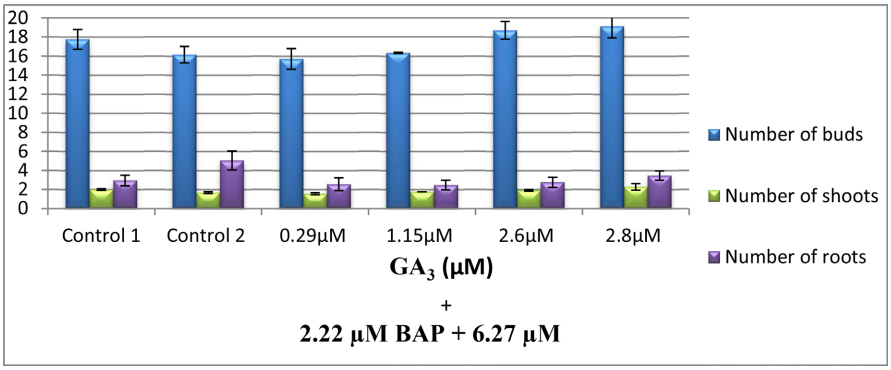

Figure 10. Effect of GA_3 at four concentrations combined with 2.22 µM BAP and 6.27 µM IAA on the multiplication of buds, nodes and roots of *Origanum compactum* Benth.

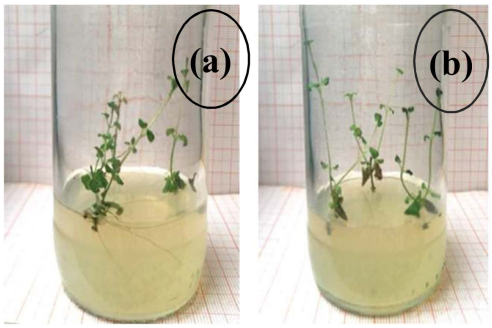

Figure 11. Effect of GA_3 combined with 2.22 µM BAP and 6.27 µM IAA on micropropagation of *Origanum compactum* Benth. ((a) 2.60 µM GA_3; (b) 2.89 µM GA_3).

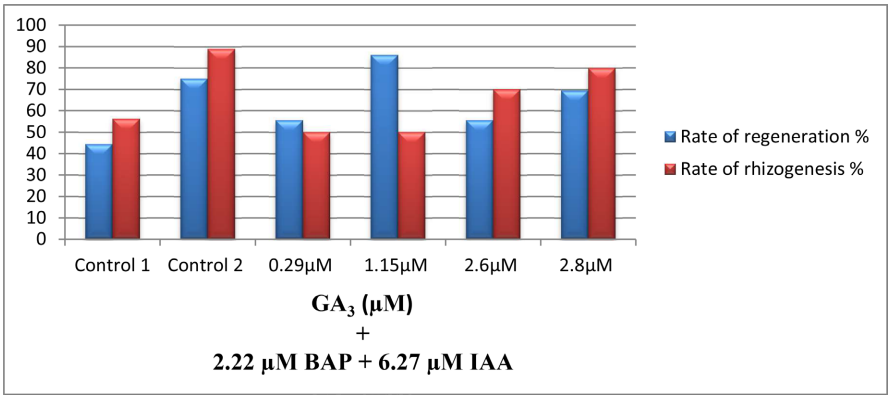

Figure 12. Effect of GA_3 at four concentrations combined with 2.22 µM BAP and 6.27 µM IAA on the regeneration, rooting and hyperhydricity rates of *Origanum compactum* Benth. vitroplants.

3.5. Effect of Cytokinins and Auxins Combined with Polyamines

Integration of three polyamines at different concentrations with 2.22 µM BAP and 6.27 µM IAA resulted in changes in the micropropagation of vitroplants of *Origanum compactum*.

Thus, an improvement was noted in the multiplication of buds in the case of 5.67 µM Spermine (18.82) followed by 7.938 µM Putrescine and control medium 1 (18.57), in contrast of the medium supplemented with 7.938 µM Spermidine where a minimum number of buds has been reported (12.80) (**Table 5**).

In addition, propagation of shoots was maximal for 5.67 µM Spermine (2.17) followed by 5.67 and 7.938 µM Putrescine (1.85) followed by control medium 2 (1.80), and minimal for 3.402 µM Spermine (1.38).

Root multiplication was at its maximum for control medium 1 (1.61) followed by 7.938 µM Putrescine (1.42) and 5.67 µM Spermine (1.37) (**Table 5**, **Figure 14**), at its minimum for 1.134 µM Spermine (0.13) and absent for 1.134, 3.402, 5.67 and 7.938 µM Spermidine and 11.34 µM Spermine (**Table 5**, **Figure 13** and **Figure 15**).

On the other hand, the longest explants were noted in the medium supplemented with 5.67 µM Spermidine (4.22 cm) followed by 11.34 µM Spermine (4.12) and 5.67 Spermine (3.84), and the shortest for 1.134 µM Spermidine (2.13) (**Table 5**, **Figure 15**).

The medium supplemented with 5.67 µM Spermine allowed a relatively high rate of regeneration (80.55%) followed by 5.67 µM Spermidine (69.44%) and 1.134 µM and 7.938 µM Spermine (63.89). The highest percentage of rhizogenesis was observed with 3.402 µM Putrescine (52.63%) followed by control medium 2 (52.38) and 1.134 µM Putrescine (47.62). The absence of hyperhydric explants was noted in the different combinations (**Table 5**, **Figure 14**).

3.5. Acclimatation of *Origanum compactum* Vitroplant

Explants grown in the medium supplemented with 2.22 µM BAP and 6.27 µM IAA (best medium for rooting) showed good root and foliar development, and the survival percentage of seedlings acclimatized in the culture chamber and

Table 5. Effect of polyamines combined with 2.22 µM BAP and 6.27 µM IAA on the micropropagation of *Origanum compactum* Benth.

Polyamines (µM/L)		Regeneration (%)	Shoot length (cm)	Number of buds	Number of shoots	Rooting (%)	Number of roots
Control 1 (2.22 µM BAP)		52.80	3.42 ± 0.29^{ab}	18.57 ± 0.80^{ab}	1.57 ± 0.13^{ab}	42.10	1.61 ± 0.45^{a}
Control 2 (2.22 µM BAP + 6.27 µM IAA)		58.33	3.23 ± 0.26^{ab}	17.05 ± 0.92^{abc}	1.80 ± 0.11^{ab}	52.38	1.31 ± 0.40^{ab}
Putrescine	1.134	58.33	3.43 ± 0.28^{ab}	16.85 ± 1.02^{abc}	1.71 ± 0.10^{ab}	47.62	1.00 ± 0.31^{ab}
	3.402	52.80	3.24 ± 0.25^{ab}	16.84 ± 1.12^{abc}	1.73 ± 0.10^{ab}	52.63	1.21 ± 0.32^{ab}
	5.670	58.33	2.56 ± 0.20^{b}	15.80 ± 1.03^{abc}	1.85 ± 0.10^{ab}	28.57	0.95 ± 0.38^{ab}
	7.938	58.33	2.43 ± 0.21^{b}	18.57 ± 1.19^{abc}	1.85 ± 0.07^{ab}	33.33	1.42 ± 0.49^{ab}
	11.34	42.10	2.53 ± 0.19^{b}	15.50 ± 0.92^{ab}	1.68 ± 0.11^{ab}	31.25	0.87 ± 0.46^{ab}
Spermidine	1.134	22.22	2.13 ± 0.22^{b}	12.50 ± 0.73^{abc}	1.25 ± 0.16^{ab}	0.00	0.00 ± 0.00^{b}
	3.402	41.66	3.36 ± 0.25^{ab}	16.13 ± 1.17^{abc}	1.60 ± 0.13^{ab}	0.00	0.00 ± 0.00^{b}
	5.670	69.44	4.22 ± 0.19^{a}	15.12 ± 0.73^{abc}	1.76 ± 0.08^{ab}	0.00	0.00 ± 0.00^{b}
	7.938	55.55	3.38 ± 0.26^{ab}	12.80 ± 0.65^{c}	1.45 ± 0.11^{ab}	0.00	0.00 ± 0.00^{b}
	11.34	50.00	2.61 ± 0.21^{ab}	13.55 ± 0.78^{bc}	1.55 ± 0.12^{ab}	16.70	0.44 ± 0.03^{ab}
Spermine	1.134	63.89	3.13 ± 0.33^{ab}	15.39 ± 0.87^{abc}	1.56 ± 0.10^{ab}	8.70	0.13 ± 0.09^{ab}
	3.402	58.33	3.31 ± 0.25^{ab}	14.76 ± 0.98^{abc}	1.38 ± 0.10^{b}	23.80	0.76 ± 0.37^{ab}
	5.670	80.55	3.84 ± 0.25^{a}	18.82 ± 1.40^{a}	2.17 ± 0.23^{a}	44.82	1.37 ± 0.41^{ab}
	7.938	63.89	3.14 ± 0.20^{ab}	16.43 ± 0.98^{abc}	1.60 ± 0.12^{ab}	30.34	0.56 ± 0.27^{ab}
	11.34	52.78	4.12 ± 0.23^{a}	13.89 ± 0.83^{bc}	1.47 ± 0.11^{ab}	0.00	0.00 ± 0.00^{ab}

Letters represent homogeneous groups; in each column, different letters indicate a significant difference at $p < 0.05$ using ANOVA and Tukey Post Hoc.

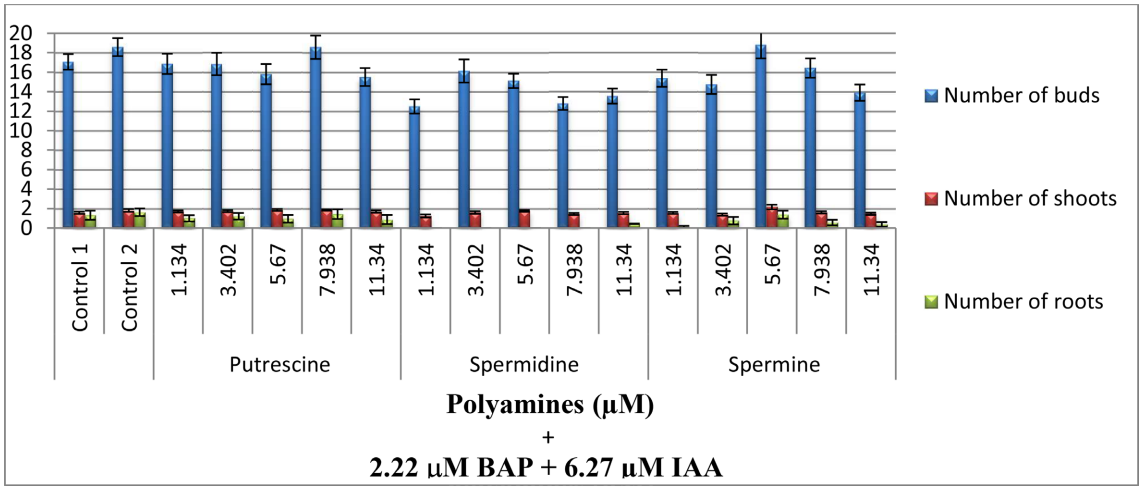

Figure 13. Effect of 3 polyamines at 5 concentrations combined with 2.22 µM BAP and 6.27 µM IAA on the multiplication of buds, shoots and roots of *Origanum compactum* Benth. vitroplants.

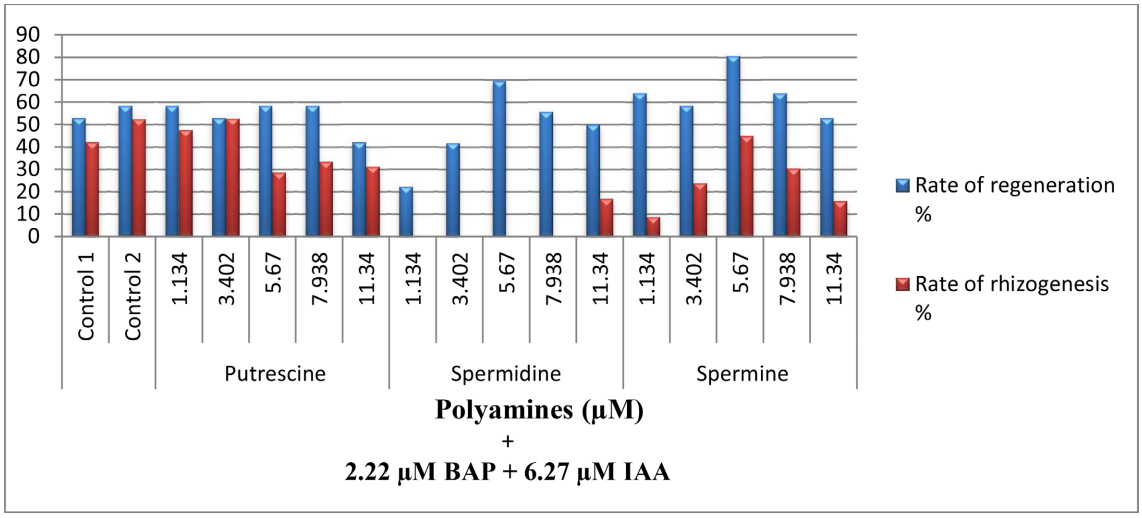

Figure 14. Effect of 3 polyamines at 5 concentrations combined with 2.22 µM BAP and 6.27 µM IAA on the regeneration, rooting and hyperhydricity rates of *Origanum compactum* Benth. vitroplants.

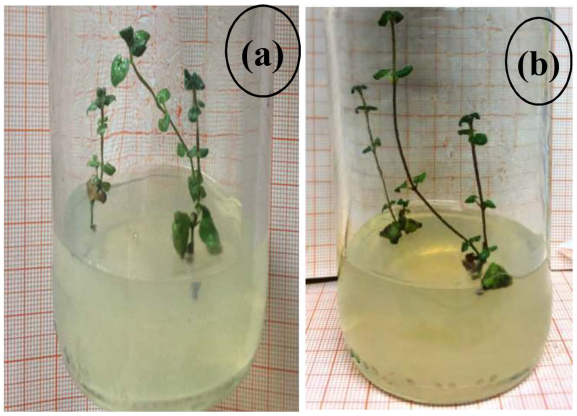

Figure 15. Effect of polyamines combined with 2.22 µM BAP and 6.27 µM IAA on micropropagation of *Origanum compactum* Benth. ((a) 7.938 µM Putr; (b) 5.67 µM Sp).

after their transfer under natural conditions was 96% (**Figure 16**).

4. Discussion

Testing the effects of macronutrients on the micropropagation of *Origanum compactum* allows us to choose $N_{30}K$ macronutrients for the experiments that follow. This possibility is made by the complete absence of hyperhydric plants as well as the successful development of the culinary and rooting part. However, in many cases with other species of oregano and plants belonging to Lamiaceae family, MS medium has been the basal one in most researches. Studies that reflect this include the micropropagation in *Origanum minutiflorum [33]*, *O. vulgare* [34], *O. majorana* [35], *O. sipyleum [36]*, *O. acutidens* [26], *O. syriacum* and *O. ehrenbergii* [37], *Lavandula angustifoli*a [38], *Salvia rosmarinus* [39], *Thymus vulgaris* [40], *Salvia officinalis*, *Melissa officinalis* and *Mentha longifolia* [41]. Furthermore, Morone-Fortunato and Avato (2008) [42] opted for the micropropagation of *Origanum vulgare* in the (BM) Buffered Minimum medium

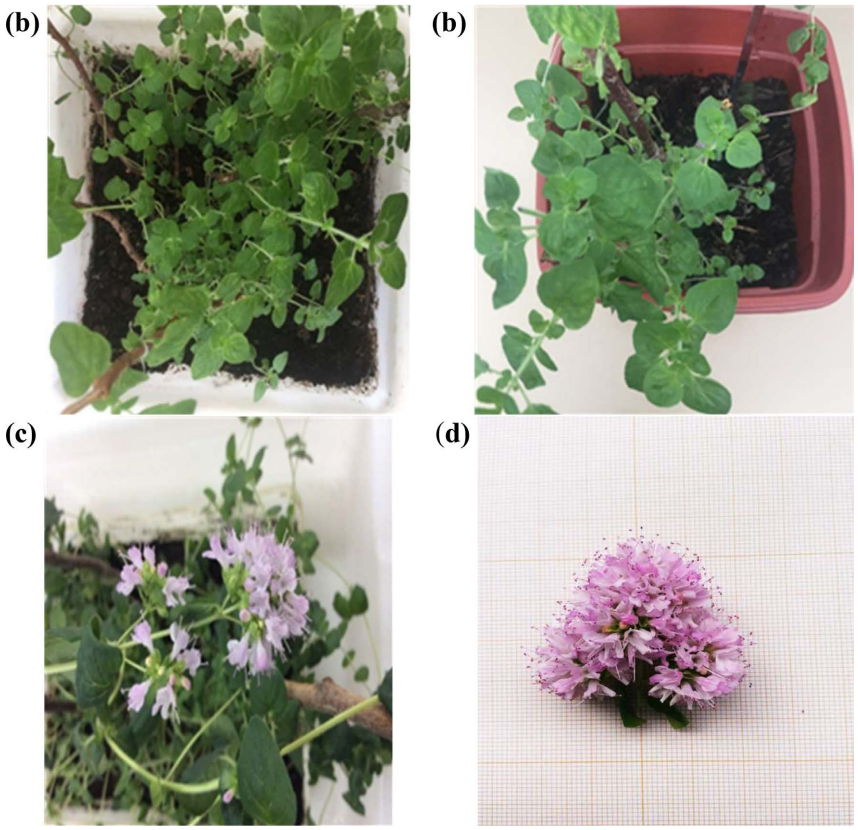

Figure 16. Acclimatization of *Origanum compactum* Benth. plantlets ((a) and (b) Acclimatization after 5 months; (c) inflorescences of acclimatized plants; (d) inflorescences).

containing the macronutrients of MS and the micronutrients of Nitsh and Nitsh [43]. On the other hand, few studies used modified MS as a less concentrated media as in the case of *Thymus mastichina* [44]. Basal medium $N_{30}K$ is demonstrated to be chosen by less authors, such as Nobre (1996) [45], for the micropropagation of *Lavandula stoechas* from single nodes.

Moreover, the explant response varies according to the concentration and the type of cytokinins, as, indeed, a higher concentration of BAP, Zeatin and 2ip promoted the best multiplication of buds and nodes. However, less concentration of Kin (1.33 µM) remained the best, not only for the bud and node proliferation, but also for the elongation of the culinary part. This result is similar to BAP and Zeatin, as well as in the case of 2ip. Additionally, elevated concentrations of Kin, DPU and TDZ improved the multiplication of shoots, unlike Adenine and Zeatin, BAP and 2IP where lower concentrations remained efficient. Furthermore, the highest concentration of Kin and the lowest concentration of Zeatin, 2ip, and BAP provided best root development and the optimal rate of rhizogenesis is mentioned in case of 3.11 µM Kin. Moreover, Zeatin and adenine was more effective at concentration of 3.11 µM in terms of all the parameters evaluated.

The study of both the concentration and type of cytokinin effects on the organogenesis is reported in several species belonging to *Origanum* genus. The

findings reveal the importance of these chemicals in the culture's micropropagation and reproduction. Thus, the investigation of Özkum (2007) [33] showed that high concentration of BAP (2 µM) was efficient for the shoot regeneration; in addition, Cristea *et al.* (2008) [34] revealed that BAP at 2.97-5.94 µM gave the best shoot proliferation and regeneration of *Origanum majorana* and its replacement with Kin did not promote explants regeneration, unlike Abdallah *et al.* (2017) [22] who demonstrated that 2 µM Kin gave the highest number of shoots, shoot length, number of leaves and number of leaves per shoot. Zayova *et al.* (2016) [46] proved that 2.28 and 4.56 µM Zeatin remained the best for multiple shoot formation of Greek oregano (*Origanum heracleoticum*) with 70% shoot formation. On the other hand, Korkor *et al.* (2017) [35] demonstrated that 5.94 µM BAP had negative effect on shoot elongation of *Origanum majorana* explants but favorise their multiplication. Moreover, the study of Baricevic and Padulosi [47] indicated that the micropropagation of *Origanum vulgare* is independent of cytokinins and the integration of Kin in the culture medium had no effect on proliferation unlike Borovec (1988) [48] who revealed that a very high concentration of BAP (23.76 µM) exhibited good development of cultures. In addition, Morone-Fortunato and Avato (2008) [42] reported that 5.94 µM BAP was suitable for the production of multiple shoots of *Origanum vulgare* ssp. *hirtum*. Furthermore, Socorro *et al.* (1998) [49] noted a maximum elongation of main and axillary stems, an increasement of leaves number and axillary stems also a good development of root system of *Origanum bastetanum* (=*O. vulgare* ssp. *virens*) vitroplants when the MS media is added with 2.22 µM BAP. The effect of cytokinins is evaluated in other species belonging to Lamiaceae family as *Lavandula angustifolia* where lower concentration growth regulators was efficient for induction of buds, elongation and multiplication of shoots [38]; for the case of *Salvia fructicosa*, the multiplication of shoots, buds and nodes, and the elongation of stems were optimum in medium containing 0.75 µM BAP [50]. The integration of cytokinins on media culture had a positive effect on development of aerial parts more specifically. 2.22 µM BAP that gave the best results in terms of budding, growth, regeneration rate and absence of hyperhydric vitroplants but on the other hand the growth of the roots was not good enough to succeed the acclimatization.

The combination of different auxins at different concentrations with 2.22 µM BAP ensured a better development of roots similarly for the culinary part, except NAA that had a negative response on root development, unlike the investigation of Cristea *et al.* (2008) [34] who showed that the rooting medium containing lower concentration of NAA gave a good development of foliar and root system of *Origanum vulgare* explants, and those who are exposed to highest concentration of NAA 10.74 µM or more have become necrotic and have lost their leaves with shoot fragments dying gradually. Therefore, the choice of the best cytokinin/auxin balance ensures not only a better development of roots, but a better proliferation of all parts of the plant and the response to growth regulators could

change for species to an another. Indeed, in case of *Origanum acutidens* [26], the regeneration of roots was successful in case of medium containing a high concentration of BAP (10.69 µM) and a low concentration of NAA (1.07 µM) and shoots regenerated in the medium containing different concentrations of BAP and 1.07 µM NAA easily developed roots, in agreement with Socorro *et al.* (1998) [49] and Goleniowski *et al.* (2003) [26]. On the other side, for the micropropagation of *Lippia graveolens* performed on media containing a combination of BAP and IAA, no significant differences in shoot numbers were found, and addition of IAA allowed only the stimulation of the roots [51]. Contrariwise, in our study, the combination between BAP and IAA stimulates higher rate of root formation with satisfactory shoot formation. Besides, the protocol established for the micropropagation of *O. sipyleum* showed that the best rooting medium contained higher concentrations of IBA (7.38 or 12 µM) (Sevindik *et al.*, 2017) [23], whereas in our case lower concentration of IBA (1.14 µM) was sufficient, result similar to that reported in the study of Oluk and Çakýr (2009) [36] which demonstrated that 96% of *Origanum sipyleum* explants developed roots in the medium supplemented with 246 µM IBA with a very important root length. It has also been shown that the rooting of *O. minutiflorum* was successful in the MS medium supplemented with 14.76 µM IBA [33]. Contrariwise, in our case, higher concentration of IBA had a negative influence on root development. Arafeh *et al.* (2003) [52] also demonstrate that lower concentration of IBA (1.96 µM) gave acceptable length of roots and shoots of *Origanum syriacum* L. but higher percentage of rhizogenesis (90%) was mentioned in MS medium supplemented with 4.56 µM IAA in agreement with our study where the addition of high IAA concentration generate an important percentage of rooted explants.

The combination of GA_3 with 2.22 µM BAP and 6.27 µM IAA results in some changes in the growth of vitroplants of *Origanum compactum*, particularly in the aerial part. Control medium 2, compared with the different combinations, is the best for root growth and development, and generates a high percentage of rooted vitroplants. Few studies rely on the effect of GA_3 alone or in combination with cytokinins and auxins. Goleniowski *et al.* (2003) [26] showed that combining 0,25 µM GA_3 with BAP had a positive effect on the elongation of *O. vulgare* x *applici* shoots but had a negative effect on the multiplication of shoots of Origanum *sipyleum*. In addition, Harfi *et al.* (2019) [53] reported that the addition of 2.22 µM BAP to the medium supplemented with 0.58 µM GA_3 allowed maximum elongation of the vitroplants and that the combination of 1,44 µM GA_3 with 2.22 µM BAP and 1.425 µM IAA induced callus formation. Moreover, they showed that the absence of cytokinins in the medium supplemented with GA_3 and IAA promoted root development and regeneration of a significant percentage of rooted vitroplants of *Origanum compactum*. In addition, El-Antably *et al.* (1975) [54] demonstrated that the addition of 100 µM GA_3 to the culture medium containing or not 116 µM Kin allowed the increase of the length of *Origanum majorana* vitroplants.

The effect of GA_3 alone or in combination with cytokinins or auxins have been explored in other Lamiaceae species, such as the study by Mandal (2013) [55] which showed that the addition of 4.44 µM BAP and 1.44 µM GA_3 to the culture medium is favorable for the multiplication of *Hyptis suaveolens* and allows a total regeneration of the explants. In addition, Samantaray *et al.* (2013) [56] obtained a maximum regeneration rate of *Vitex trifolia* explants in the medium containing 1.14 µM GA_3, 0.54 µM NAA, and 271.5 µM Adenine. On the other hand, Aicha *et al.* (2013) [57] reported that the presence of 1 µM GA_3 alone in MS 1/2 medium improves the elongation of *Thymus saturejoides* vitroplants. Similarly, Paul *et al.* (2010) [58] demonstrated that the incorporation of 1 µM GA_3 into MS medium has a positive effect on the elongation of *Pogostemon cablin*.

Furthermore, the *in vitro* growth of *Origanum compactum* explants is optimized in the case of a combination of 2.22 µM BAP and 6.27 µM IAA and the integration of three polyamines at different concentrations in the $N_{30}K$ medium supplemented with 2.22 µM BAP and 6.27 µM IAA leads to some modifications in the micropropagation of *Origanum compactum*, especially the aerial part. In fact, compared with the two controls media and other combinations, 5.67 µM Spermine is the most suitable for bud and shoot propagation and provides a high percentage of regeneration. On the other hand, Spermidine at the same concentration produced the longest explants. However, the addition of polyamines has no improving effect on root multiplication, and Spermidine at four concentrations has an inhibitory effect on root development. As well for 11.34 µM Spermine. In contrast, root multiplication is optimal for control medium 2.

Our study (9) is the one which has examined the effect of polyamines on the *in vitro* culture of *Origanum vulgare* L. ssp. *vulgare*; we have found that 1.304 µM Putrescine provides better multiplication of buds and roots and gives a high percentage of regeneration and rhizogenesis. However, investigations into the influence of these growth regulators have been undertaken on other Lamiaceae. Thus, Bajaj (2013) [59] showed that the presence of Spermidine alone in the culture medium did not induce the morphogenesis of *Sideritis angustifolia* but had an effect on growth when combined with NAA, IAA or BAP. In addition, the combination of 0.01 mM Spermidine with 27 µM NAA had a remarkable effect on rhizogenesis and reversed the 27 µM NAA inhibitory response on root formation.

5. Conclusions

The protocol established in this study for the micropropagation of *Origanum compactum* Benth. by the technique of axillary bud is reliable and original. $N_{30}K$ medium was chosen because it ensures good development of the stem part, provides a relatively high rate of regeneration and rhizogenesis, and guarantees a total absence of hyperhydric explants.

Subsequently, the evaluation of the effect of seven cytokinins at five concen-

trations showed that 2.22 µM BAP gave the best results in terms of growth, regeneration and absence of hyperhydric plants.

Root-part development was optimized only after the 2.22 µM BAP was combined with auxins, more specifically 6.27 µM IAA, which ensured not only root-part development but also whole plant growth.

Subsequently, the incorporation of GA_3 into the culture medium in the presence of 2.22 µM BAP and 6.27 µM IAA resulted in some changes in the growth of vitroplants, particularly in the aerial part. Bud and shoot multiplication and aerial elongation were at their maximum for a combination with 2.89 µM GA_3. However, control medium 2 (2.22 µM BAP + 6.27 µM IAA) remained the best for root growth and development, generating a high percentage of rooted vitroplants.

The integration of polyamines at 2.22 µM BAP and 6.27 µM IAA showed that Spermine at 5.67 µM was the most efficient for bud and shoot propagation and provided a high percentage of regeneration. On the other hand, Spermidine at the same concentration produced the longest explants. However, the addition of polyamines did not improve the root part.

Finally, acclimatization of rooted seedlings from 12 to 16 weeks 3 was successfully established. These seedlings allow a re-initiation of micropropagation after sterilization and the proliferation of a large number of the vitroplants. The protocol followed in this study thus makes it possible to ensure the conservation of this species, to avoid its disappearance and to introduce plants on the market with a high added value.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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