STUDIES REGARDING THE MICROPROPAGATION OF *ORIGANUM VULGARE* L. (OREGANO, WILD MARJORAN) THROUGH „IN VITRO" TISSUE CULTURE

STUDII PRIVIND MICROPOPAGAREA SPECIEI *ORIGANUM VULGARE* L.(ARIGAN, MAGHERAN, SOVÂRF) PRIN CULTURI DE ȚESUTURI *„IN VITRO"*

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Abstract: *The economical importance of „in vitro" tissue culture at ornamental plants is a actual problem,the existing results underlying the fact that over 500 milion of plants are micro propagated annually, the biggest part of them being ornamental plants.*

The authors aimed at the identification of explant type, as well as the cultivation media and environmental factors that are optimal for the regeneration of plants with the same genetic inheritance.

The accomplished researches demonstrate the fact that, the mainatining of the cultures in photoperiod of 16 hours light/ 8 hours dark, with a light intensity of 3000 lx and a temperature of 25ºC +/- 1ºC represents the best condition for the initiation, development and regeneration. Starting with a standard media,there were established many hormonal combinations. The results demonstrate the fact that the presence of BAP in association with NAA, as well as with IAA induce the initiation and sustain the regeneration processes.

In what concern the utilised explant and its morphogenetic expressions we underline the fact that even if both of the explant allow the regeneration of plants, the reactivity of the explant – apexes was higher (69%) comparing with the uninodal explants (60.00%).

Origanum vulgare L. – oregano, is a perennial plant of 0.6 - 0.8 m, that belongs to the *Lamiaceae* family. The flowers are small witha red flowers. It blossom from July till September, it prefers light places but can also grow in shadow. It have many utilities, being an ornamental plant but is more utilised as a condimentary plant (the leaves are recommanded for fresh or cooked consumption). It is also used as a medicinal plant in respiratory diseases, aromoterapy etc.

In the biologic agriculture is extremely useful because it has a repellent effect for the insects and is recommended to be associated with numerous vegetable species. In the same time it covers very well the soil with importance in weed control.

A protocol to regenerate shoots from in vitro cultured tissues is important for future programs of genetic transformation of this species.

Conventional techniques of vegetative propagation of *O. vulgare* based on cuttings are difficult because of the low rates of rooting. The cells and tissues cultures "in vitro" assure a unique opportunity to manipulate the morphogenesis in a perfectly controlled medium, thus offering a powerful complementary instrument that can help in overcoming such problems. In the same time, now-a-days the micropropagation (in vitro propagation of axillary and/or adventitious buds as well as somatic embryos) is presently used as an advanced biotechnological system for the production of identical pathogen-free plants for agriculture.

This paper describes the first results of experiments carried out to induce organogenesis in tissue culture of *Origanum vulgare* L. – oregano under influence of different combinations of growth regulators. It also aims toward the identification of explant type, as well as the cultivation media and environmental factors that are optimal for the regeneration of plants with the same genetic inheritance.

MATERIAL AND METHODS

The explants were collected from selected mother plants maintained at Vegetable Research Station Bacau in controlled conditions. Young shoots of 2 cm length were excised from actively growing plants.

The defoliated shoots were first washed in tap water and the sterilized in 0.1% HgCl2 for 15 minutes, and 3 rinses in sterile distilled water.

The shoots were then utilized as donor source for explants. The uninodal segments and the apexes of ~ 1,5 cm were excised and inoculated on to Murashige Skoog, 1962 culture medium supplemented with combination of 1.0 - 5.0 mg/L BAP and 0.05 – 1.0 mg/L NAA and 0.1 mg/L IAA.

Cultures were incubated at 25±1°C under 16 hr photoperiod of 3000-lux light intensity.

The cultures were transferred at a 2 weeks interval on fresh media, for a period of 90 days.

Observation of shoot multiplication and growth were recorded at weekly intervals. After two weeks, shoots of above 3 cm length were harvested and subcultured on the same medium containing BAP and mg/L NAA. A part of the newly formed shoots that demonstrated a good development of leafs were transferred to rooting medium containing different concentration of NAA.

After 2 weeks, the rooted plants were acclimatized and planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for 2 weeks before transfer to a green house.

RESULTS AND DISCUSSIONS

Shoot buds got initiated on nodal segments after 6 days of culture. Immediately after the inoculation, the explants raise their volume and the peripheral parts presented a slight necrosis. The higher frequency (85%) formation of maximum number of shoots was observed in the media variant that contained BAP in combination with NAA. Initially 1 or 2 buds developed, later up to 12 shoots of above 3 cm length were formed in node in two weeks.

The morphogenetic reaction was more pregnant in the apexes cases; it showed a longitudinal growth that was associated with the increase in leaves number and surfaces and with the apparition of the regenerative structures. The uninodal explants presented a slight increase of its volume followed by the apparition of 1-2 explants generated at internodes level. The transfers of the cultures were accomplished at a 2 weeks interval for a period of 90 days.

The reaction of the explants on the 6 variants of induction media utilised in our experiments are shortly presented in table 1.

Tabelul 1

The frequency of "in vitro" morphogenetic answer of the explants on the induction media variants

Nr crt	No. expl. inoculated	Reactive explants	V1	V2	V3	V4	V5	V6	Freq.%
1.	100 apexes	69	26	3	6	-	24	10	69%
2.	100 uninodal explants	61	16	-	9	-	19	17	61%

From the 100 explants inoculated 69, respectively 61 presented a strong morphogenetic reaction with the proliferation of shoots, while a part of them just continued to show the longitudinal growth without bud proliferation. A part of them degenerated in necrosis or were eliminated because of the secondary infections.

The media variants that allowed the induction of the regenerative processes were characterized through the presence of BAP in association with NAA or IAA. The replacement of BAP with other cytokinine (for example the kinetin) doesn't allow the regeneration. The results obtained by us underline once again the benefic effect that BAP has when comparing to other cytokinins.

After almost 17-18 days the shoots were transferred on fresh media that should support the regenerative processes. The media variants were characterized through a lower rate of BAP and NAA and determined a good proliferation of the shoots (fig. 1).

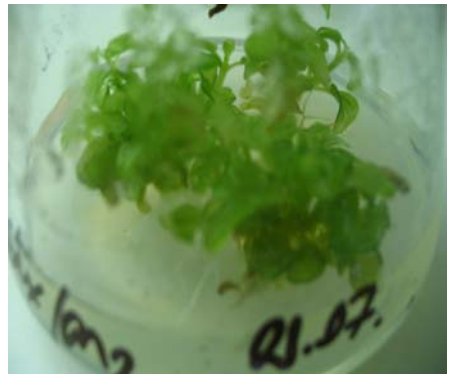

Fig. 1 - Neo-formation of the plantlets at the basis of the initial shoot

Table 2

Subculture of plant on BAP and NAA combination media - plantlets production

Subcultures period	No. of shoot harvested	No. of plantlets successfully planted	% of plantlets production
15	25	**19**	76
30	31	**26**	83.9
45	34	**30**	88.2
60	38	**33**	86.8
75	38	**33**	86.8
90	43	**35**	81.4

This association between BAP and NAA also determined the apparition and developments of roots inside the media but also airing roots. This is extremely important because allow us to obtain plantlets more quickly by skipping the rooting period (figure 2).

The presence in the media of GA3 determined a reaction of vitrification, a part of the shoots becoming improper to run through the next stages.

Fig. 2 - The apparition of the airing roots at the plantlets regenerated on BAP and NAA combination media

Depending on the way that plantlets evolved, they were transferred either on a rooting media or directly to hygroponic conditions. The rooting media containing NAA determine a good development of roots, in the same time allowing also the development of foliar system.

The plants that presented a well developed rooting and foliar system were transferred directly in hydroponic conditions for their acclimatization (figure 3).

Due to the fact that the humidity must be gradually reduced over time because tissue-cultured plants are extremely susceptible to wilting, the plants were covered with plastic folia for three days and then gradually discovered.

Fig. 3 - The acclimatization stage

After the plants were fully adapted to the environmental conditions (almost 10 days), we passed them to soil substrate in plastic recipients (figure 4) and then utilized it in the breeding activity in open-field or greenhouses.

Fig. 4 - Fully adapted plants, in plastic recipients

CONCLUSIONS

- the results obtained in the present work showed that the micropropagation of *Origanum vulgare* L. „in vitro" is a viable tool for the production of identical pathogen-free plants for agriculture;

- the higher frequency (85%) formation of maximum number of shoots was observed in the media variant that contained BAP in combination with NAA or IAA. The replacement of BAP with other cytokinine (for example the kinetin) doesn't allow the regeneration of plants;

- in what concern the utilised explant and its morphogenetic expressions we underline the fact that even if both of the explant allow the regeneration of plants, the reactivity of the explant – apexes was higher (69%) comparing with the uninodal explants (60.00%).

- the mainatining of the cultures in photoperiod of 16 hours light/ 8 hours dark, with a light intensity of 3000 lx and a temperature of 25ºC +/- 1ºC represents the best condition for the initiation, development and regeneration of plants.

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