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**Abstract.** Modern biotechnologies offer the possibility to multiplication the biologic vegetal structures by shaping the morphological phenomena (organogenesis or embryogenesis), which ensure both the maintenance of their characteristic elements as well as their multiplication in a number of units as extended as possible, starting with a certain type of explant and its intrinsic capacity, according to which, under the influence of *in vitro* culture, certain cells pertaining to it manifest properties specific to stem vegetal cells.

In consequence, the aim of the experimental researches presented in this study was the innovation of *in vitro* multiplication at Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmafera*), from subvarieties *Rosella* and *Sanda*, by direct caulogenesis induced under the influence of explant type (leaf, cotyledon, epicotyl, hypocotyl and radicle) and through direct rhizogenesis induced under the influence of exogenous auxin type (NAA or IBA).

The best caulogenesis response in case of each of the two subvarieties of Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmafera*) was recorded by experimental variant that consisted of using the explants of cotyledon type. While the best direct rhizogenesis response at *Rosella* subvariety of Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmafera*) was recorded on experimental variant that consisted of using NAA exogenous auxin 0.25 mg/l, and in case of *Sanda* subvariety of Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmafera*) the best direct rhizogenesis response was recorded on experimental variant that consisted of using IBA exogenous auxin 0.75 mg/l.

**Keywords:** caulogenesis, rhizogenesis, NAA, IBA

**INTRODUCTION**

*Brassica* sp. contains many plants used in the modern food industry as vegetal raw material sources under the form of flowers (*Brassica oleracea* L. convar. *oleracea* var. *gemmafera*, *B. o.* L. convar. *italica*, *B. o.* L. convar. *botrytis*), leaves (*Brassica oleracea* L. convar. *capitata* var. *alba*, *B. o.* L. convar. *capitata* var. *rubra*, *B. o.* L. convar. *capitata* var. *sabauda*), stalks (*Brassica oleracea* L. var. *gongylodes*), roots (*Brassica rapa* L. var. *rapa*), or seeds (*Brassica juncea* L. Czern, *Brassica napus* L.). Among these plants, Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmafera*) stands out by the possibility of being included in the list of plants used in phytoremediation of Se²⁺ contaminated soils (Esringü and Turan, 2012). By using the Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmafera*) for the phytoremediation of Se²⁺ contaminated soils, both their non-invasive remedy efficient way may be achieved as well as a source of vegetal raw materials for different industries (food, pharmaceutical and animal breeding), in accordance with the principles of sustainable agriculture.
Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera*), is a biennial and allogam species (Pârvu, 1997), that may benefit, by modeling the stem properties of vegetal cells, from advantages provided by *in vitro* propagation, that is: produce of propagules in a shorter period of time since there is no need to maintain the culture in the second year of vegetation and it gives the possibility to obtain phenotypic characteristics intended from a certain variety because it does not involve the fecundation by means of insects and, implicitly, the risk that leads to variation of phenotypic characteristics.

The specialized literature, investigated for the theoretical fundamental aspects related to *in vitro* propagation researches by direct organogenesis from Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera*), presents a vast series of results that valorizes the stem properties of vegetal cells by different *in vitro* (somatic embryogenesis, somatic cell fusion, somaclonal variation and transformation; direct caulogenesis; indirect or direct caulogenesis for synthetic seeds; direct caulogenesis and direct rhizogenesis) propagation systems to *Brassica* sp. (Cardoza and Stewart, 2004; Irwin et al., 1999; Metz et al., 1995; Pavlović et al., 2010). Nevertheless, there are a few results related (Irwin et al., 1999; Pavlović et al., 2010), to *in vitro* propagation by direct caulogenesis and rhizogenesis in Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera*).

The *in vitro* propagation by direct organogenesis is a sequential regeneration system pertaining to vitroplants, which involves a separate induction and materialization of caulinar pole and radicular pole.

In consequence, our experimental researches aimed to the innovation of *in vitro* propagation by direct caulogenesis and direct rhizogenesis in Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera*), from subvarieties *Rosella* and *Sanda*.

**MATERIALS AND METHODS**

**The biologic material** used was procured from a commercial source and it consisted of *Rosella* and *Sanda* subvarieties of Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera*). The two subvarieties are included in the category of vegetable plants cultivated to be commercialized both fresh and frozen, and their distinctive element is the vegetation period, that is: semilate for *Rosella* subvariety and semi-early for *Sanda* subvariety pertaining to *Brassica oleracea* L. convar. *oleracea* var. *gemmifera*.

The primary source of explants (leaf, cotyledon, epicotyl, hypocotyl and radicle) (Irwin et al., 1999; Pavlović et al., 2010) consisted of those plantlets founded in the stage with two real leaves and obtained by germination of seeds given *in vitro* conditions.

**Methods for the surface aseptisation of the biologic material.** The surface aseptisation of the Brussels cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera*), seeds was achieved by a treatment which consisted in their immersion in a solution of ethylic alcohol (C\(_6\)H\(_{12}\)O\(_6\)) 70% for 1', rinse with distilled three times sterilized H\(_2\)O and their re-immersing in a solution of sodium hypochlorite (NaC\(_{1}O\)) 8% for 20' (Pavlović, 2010). After applying the chemical sterilization agent there were necessary 3 successive rinses of 10' each, in aseptized distilled water (Badea and Săndulescu, 2001; Cachiţă–Cosma et al., 2004), under continuous agitation.

**The artificial nutritive mediums** (Tab. 1), has as basis the nutritive formula of the MS medium (Murashige and Skoog, 1962), supplemented with agar 0.7% and sucrose 4.0% (Pavlović, 2010). Starting from this basic medium, there were chosen artificial nutritive medium variants, whose composition was different from the point of view of the content of exogenous phytohormones (BAP, TDZ, NAA and IBA), (Badea and Săndulescu, 2001;
The pH values of the artificial nutritive mediums were adjusted to 5.8 before sterilization (Pavlović, 2010), by autoclaving at 121°C, 1 atm for 15′.

Tab. 1

<table>
<thead>
<tr>
<th>Variants of artificial nutritive mediums</th>
<th>Exogenous phytohormones</th>
<th>Exogenous cytokinins (mg/l)</th>
<th>Exogenous auxins (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS DC 0</td>
<td>BAP</td>
<td>0.00 mg/l</td>
<td>0.00 mg/l</td>
</tr>
<tr>
<td>MS DC 1</td>
<td>TDZ</td>
<td>0.00 mg/l</td>
<td>0.00 mg/l</td>
</tr>
<tr>
<td>MS DC 2</td>
<td>NAA</td>
<td>0.00 mg/l</td>
<td>0.00 mg/l</td>
</tr>
<tr>
<td>MS DC 3</td>
<td>IBA</td>
<td>0.00 mg/l</td>
<td>0.00 mg/l</td>
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<tr>
<td>MS DC 4</td>
<td></td>
<td>0.25 mg/l</td>
<td>0.00 mg/l</td>
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<tr>
<td>MS DC 5</td>
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<td>0.50 mg/l</td>
<td>0.00 mg/l</td>
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<td>MS DC 6</td>
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<td>0.75 mg/l</td>
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<td>MS DR 7</td>
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<td>0.75 mg/l</td>
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<tr>
<td>MS DR 8</td>
<td></td>
<td>0.00 mg/l</td>
<td>0.25 mg/l</td>
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<td>MS DR 9</td>
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<td>MS DR 10</td>
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<td>MS DR 11</td>
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<td>0.25 mg/l</td>
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<tr>
<td>MS DR 12</td>
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<td>0.00 mg/l</td>
<td>0.50 mg/l</td>
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</tbody>
</table>

Abbreviations: MS = Murashige T. and F. Skoog (1962); DC = direct caulogenesis; DR = direct rhizogenesis; BAP = 6-benzylaminopurine; TDZ = 1-phenyl-3-(1,2,3 thiazol-5-yl) urea; NAA = α-naphthaleneacetic acid; IBA = indole-3-butyric acid.

The culture conditions. The inoculated seeds and explants were incubated in growth chambers, at temperatures of 23°C±2°C and a luminous intensity of 1700 luxes during the photoperiod (16 h), and respectively 20°C±2°C during the period of darkness (8 h). The subcultivations were performed periodically, at 2-4 week intervals (Takayama, 1990).

The acclimatization of the vitroplants of Brussels cabbage (Brassica oleracea L. convar. oleracea var. gemmifera) in ex vitro conditions was made by gradually increasing the relative air humidity inside the culture vessels for a period of 3 days. After the 8 days as from the beginning of the acclimatization, the Brassica oleracea L. convar. oleracea var. gemmifera subvar. Rosella and subvar. Sanda vitroplants were transferred in plastic containers (glasses) containing a nutritive mixture consisting of peat and sand in a proportion of 3:1. The nutritive substrate was aseptized by 90’ autoclaving at t°=121°C before being used.

The statistical procedures. Each experimental variant consisted in 3 repetitions and the experiments were repeated 2 times. The measurements were made for each individual inoculate. There were determined the average value and the standard deviation for parameter analyzed, at variants as compared to the control found in the same conditions.

RESULTS AND DISCUSSIONS

This study presents the experimental results recorded as effect of experimental researches performed to innovate in vitro propagation by direct organogenesis in Brussels
cabbage (*Brassica oleracea* L. convar. *oleracea* var. *gemmifera*), by direct caulogenesis induced under the influence of explants (leaf, cotyledon, epicotyl, hypocotyl and radicle) and by direct rhizogenesis induced under the influence of exogenous auxin type (NAA or IBA).

The prelevation of explants used to shape *in vitro* propagation by direct organogenesis was achieved from the plantlets obtained by *in vitro* germination of *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* seeds, from Rosella and Sanda subvarieties.

The induction and achievement of necessary competences to acquire the dedifferentiation and redifferentiation processes specific to direct organogenesis normally involve the materialization of direct caulogenesis in the first step and of direct rhizogenesis in the second step.

In the first stage, the direct caulogenesis consisted of the induction and achievement of necessary competences to acquire the following processes: of return of some of somatic cells from the level of explants to the state of stem cells (dedifferentiation) and of redifferentiation (Fig. 1), of some of the cells in caulinary cells. In the second stage of direct caulogenesis, we obtained morphogenetic cultures that developed caulinary shoots (Fig. 2) and in its third stage, morphogenetic cultures were obtained to elongate the caulinary shoots (Fig. 3).

![Fig. 1. Explants of *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Rosella* (a, b) and of subvar. *Sanda* (c, d) at the level of which there were induced and achieved the *in vitro* dedifferentiation (a, c) and redifferentiation (b, d) processes of the direct caulogenesis](image)

![Fig. 2. Morphogenetic cultures of *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Rosella* (a) and of subvar. *Sanda* (b), in the development stage of caulinary shoots (direct caulogenesis)](image)
For *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Rosella*, the frequency of phenomena related to induction and generation of direct caulogenesis from cotyledon explants had the following values: 0.00±0.00/MC DC0, 0.00±0.00/MC DC1, 0.00±0.00/MC DC2, 20.00±5.42/MC DC3, 0.00±0.00/MC DC4, 0.00±0.00/MC DC5 and 0.00±0.00/MC DC6. While the frequency of phenomena related to induction and generation of direct caulogenesis from leaf, epicotyl, hypocotyl and radicle explants was 0.00±0.00 irrespective of the artificial nutritive medium used (Tab. 1). In addition for *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Sanda* as well, the frequency of phenomena related to induction and generation of direct caulogenesis from leaf, epicotyl, hypocotyl and radicle explants was 0.00±0.00. Nevertheless, for *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Sanda* the frequency of phenomena related to induction and generation of direct caulogenesis from cotyledon explants recorded values that were also influenced by the artificial nutritive medium used (Tab. 1), that is: 0.00±0.00/MC DC0, 0.00±0.00/MC DC1, 11.00±2.98/MC DC2, 33.00±8.94/MC DC3, 0.00±0.00/MC DC4, 0.00±0.00/MC DC5 and 6.00±1.62/MC DC6.

The direct rhizogenesis consisted, in the first stage, of induction and achievement of necessary competences to acquire the return processes of some of the cells from caulinary shoots condition to stem cells (dedifferentiation) and the redifferentiation of some of the cells in radicular cells (Fig. 4). In the second stage of direct rhizogenesis, bipolar vegetal biological structures were obtained, made of caulinary shoots and roots (Fig. 5).
Fig. 5. Bipolar vegetal biological structures were obtained, made of caulinary shoot and roots of *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Rosella* (a) and of subvar. *Sanda* (b).

For *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Rosella*, the frequency of phenomena related to induction and generation of direct rhizogenesis from caulinary shoots under the influence of NAA exogenous auxin recorded the following values: 4.00±1.08 / MS DR1, 1.20±0.32 / MS DR2, 0.00±0.00 / MS DR3, values that demonstrated the existence of a critical level of NAA concentration, above which its influence became inhibitory, while the frequency of phenomena related to induction and generation of direct adventitious rhizogenesis from the level of adventitious sprouts under the influence of IBA exogenous auxin recorded values of 0.00±0.00, irrespective of its concentration, values that emphasized the dependence regarding the materialization of direct rhizogenesis not only by the concentration of exogenous auxin but also by the type of exogenous auxin.

For *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Sanda*, the frequency of phenomena related to induction and generation of direct rhizogenesis from caulinary shoots under the influence of NAA exogenous auxin recorded the following values: 0.00±0.00 / MS DR1, 0.00±0.00 / MS DR2, 2.00±0.54 / MS DR3. In addition, the frequency of phenomena related to induction and generation of direct rhizogenesis from caulinary shoots under the influence of IBA exogenous auxin recorded the following values: 0.00±0.00 / MS DR4, 0.00±0.00 / MS DR5 and 3.50±0.95 / MS DR6, values that emphasized the correlation between the increase of auxin concentration in the artificial nutritive medium and the increase of frequency pertaining to induction and generation phenomena regarding direct rhizogenesis.

The vitroplants pertaining to *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Rosella* and subvar. *Sanda*, obtained by means of direct caulogenesis and direct rhizogenesis were acclimatized in *ex vitro* conditions (Fig. 6), in percentage of 76% and 80%.

Fig. 6. Vitroplants of *Brassica oleracea* L. convar. *oleracea* var. *gemmifera* subvar. *Rosella* (a) and of subvar. *Sanda* (b), acclimatized in *ex vitro* conditions.
CONCLUSION

The experimental researches made for the innovation of in vitro propagation by direct organogenesis at Brussels cabbage (Brassica oleracea L. convar. oleracea var. gemmifera), from Rosella and Sanda, subvarieties, through direct caulogenesis induced under the influence of explants (leaf, cotyledon, epicotyl, hypocotyl and radicle) and through direct rhizogenesis induced under the influence of exogenous auxin (NAA or IBA), generated the following experimental results that reveal the following aspects:

- the achievement of direct caulogenesis is correlated with both the explant type as well as with the type of exogenous cytokinine and its concentration;
- the best response in achieving direct caulogenesis from Brussels cabbage (Brassica oleracea L. convar. oleracea var. gemmifera), both for subvar. Rosella as well as for subvar. Sanda was recorded in the experimental variant that consisted of using cotyledon explants;
- the most efficient materialization of direct rhizogenesis depends upon both the type of exogenous auxin and its concentration;
- the best response regarding the materialization of direct rhizogenesis in Brussels cabbage (Brassica oleracea L. convar. oleracea var. gemmifera), for subvariety Rosella was recorded in the experiment that consisted of NAA exogenous auxin in quantity of 0.25 mg/l, and for Sanda subvariety, it was obtained in the experiment that implied the use of IBA exogenous auxin in quantity of 0.75 mg/l.

The experimental researches in this study innovate the propagation by direct organogenesis in Brussels cabbage (Brassica oleracea L. convar. oleracea var. gemmifera), since they demonstrated that in vitro propagation by caulogenesis and direct rhizogenesis in Brassica oleracea L. convar. oleracea var. gemmifera, subvarieties Rosella and Sanda, is feasible.

REFERENCES
