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ASSESSMENT OF THE ORGANOGENIC RESPONSE TO THE DIFFERENT TYPES OF EXPLANTS FROM ROMANIAN VARIETIES OF RED AND GREEN BASIL

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Abstract
Basil is a plant known since ancient times for its properties (carminative, antiseptic), being considered a sacred plant. With the passage of time and with the diversification of assortments, basil was used for decorative and aromatic purposes, being considered a spicy plant. Red basil is also used in the perfume industry due to the pleasant aroma it possesses. Multiplication procedures were developed by in vitro cultures, using varieties of Romanian origin: 4 from red basil (lines: L9, L10, L11, L12) and 9 from green basil, characterized by special aromas and leaf shapes. When evaluating the germination yield, the percentages varied between 45-80% for aseptically inoculated red basil seeds on culture medium. And in the case of green basil varieties, the yield varied between 25 -100% after sowing in universal organic substrate. For the seeds of the green basil varieties, the sowing was performed in universal organic substrate, and the germination yield varied between 25 -100%. The types of explants taken from the resulting seedlings were represented by stems (fragments of hypocotyl and epicotyl), leaves and cotyledonary node. The induction of organogenesis was evaluated differently for each type of explant under the effect of phytohormones such as cytokinins (TDZ and BAP) added in concentrations of 0.5-2 mg / L to the basal culture medium MS / 1962. After 3 subcultures of the explants on the culture medium recipes (45 days), the organogenic yields expressed in vitro were compared

Keywords: basil, in vitro culture, morphogenetic response

1. INTRODUCTION

Ocimum basilicum L., from the *Lamiaceae* family, known as the basil, is an annual plant with a straight stem, with square stems and branched at the top. (Ardelean and Mohan, 2008; Stănescu et al., 2018). As described by Roman et al., (2012) basil (*Ocimum basilicum* L.) is an herbaceous species, annual, thermophilic, sensitive to late spring frosts, with high requirements for light, moisture and soil. Prefers fertile, deep, medium to light soils with southern exposure.

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All varieties of basil plants are species belonging to the genus *Ocimum* (Basil Guide, 2003). The genus is particularly diverse and includes annuals, perennials and shrubs, originating in Africa and other tropical and subtropical regions of the old world and the new world. More than 100 species of basil are currently described, and others are hybrids.

The main substances identified by Benedec et al., (2009) in the tissues of this species are: volatile oils (0.1-1.0%), carbohydrates, lipids (3-4%), proteins (11-16%), compounds phenylpropanoids (estragol, rosmarinic acid), glycosides (esculin, esculetin), triterpenes (ursolic acid), valeric acid. The uses of basil (the aerial part entirely or only the leaves) are mainly due to the presence of volatile components and flavonic derivatives.

Thus, the plant product is frequently used for medicinal purposes as a eupeptic, stomachic, antispasmodic, antitussive and carminative. It is also used as a diuretic and mild sedative, inducing sleep. Green leaves are used to flavor various dishes, such as salads, confectionery, wines or juices.

In the breeding collection from the Research & Development Station for Vegetables Growing Buzau (SCDL Buzău) there are over 60 special varieties of basil (Vînătoru et al., 2019), among which we mention *O. basilicum* var. ‚Bulatum’ and *Ocimum basilicum* var. ‚Crispum’L., with very large and grafted leaves, with uses in gastronomy for salads, as well as many other varieties of the species *O. basilicum* L. with medium leaves, being green and red to dark garnet with distinct aromas and shapes (Luchian et al., 2017; Vînătoru et al., 2019). *Ocimum basilicum* L. var. ‚Crispum’, also popular called “Foglia di Lattuga” or “Lettuce Leaf” is recommended for culinary, ornamental, medicinal use, but also as a repellent (companion plant) for insects in organic farming, especially in tomato crops (DeBaggio, 1994; Bomford, 2009; Teodorescu et al., 2017).

Approaches to basil multiplication through modern biotechnological *in vitro* multiplication techniques have been applied by researchers worldwide to both red basil varieties (Mathew, 2011; Saha et al., 2012; Ekmekci and Aasim, 2014) as well as those of green basil (Sahoo et al., 1997; Phippen and Simon, 2000; Begum et al., 2002; Dode et al., 2003; Lee and Yi, 2003; Gopi and Ponmurugan, 2006; Daniel et al., 2010; Mathew, 2011; Livadariu, 2011).

Through this research, we developed organogenesis processes by *in vitro* cultures, using basil varieties of Romanian and international origin in the collection of improvement of the Research & Development Station for Vegetables Growing Buzau (SCDL Buzău), to analyze the morphogenetic response of explants (stems, leaves and cotyledonary node) detached from the young seedlings.

2. MATERIALS AND METHODS

For the initial biological material in the induction of organogenesis *in vitro* were used the seeds of several varieties of basil of the classic basil species *Ocimum basilicum* L., as well as from the varieties *O. basilicum* var. ‚Bulatum’ and *O. basilicum* var. ‚Crispum’.

Thus, the basil seeds purchased from the Buzau Vegetable Research and Development Station corresponded in the experimental I-Series to 4 red basil lines: L9, L10, L11, L12, in the approval process, but also from 9 varieties of green basil (experimental II-Series) from the breeding collection of the same research unit, as: mentholated green basil with red spike (B.M.S.R. symbol); the "Aromat de Buzău" basil variety approved in 2006 (B.A.Bz. symbol); basil with large lime leaf ‚Bulatum’ (B.B. symbol); basil with large lime leaf ‚Crispum’ (B.C. symbol); lemon flavored basil (B.A.L.symbol); myrrh-flavored basil (*Ocimum tenuiflorum* L./sin. *Ocimum sanctum* Linn) symbolize with B.MIR; rose-flavored basil (B.T. symbol); green basil L7 (B.-L7 symbol); and basil for lime pots ‚Minium, (*Ocimum basilicum* f. ‚Minimum’) symbolizes with B.G.

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The analyzed varieties are recommended for use for culinary, ornamental and medicinal purposes, there is the possibility of use in the perfume industry due to the strong mentholated aromas, very pleasant. The initiation of the experiments took place in March 2019 in two experimental series for the red and green basil varieties. Thus for Series I, the start of the experiment was made on 11.03.2019, by using the seeds from 4 lines of red basil: L9, L10, L11 and L12.
The working protocol consisted in the first choice of red basil seeds, followed by their surface sterilization using commercial sodium hypochlorite in a concentration of 20% for 20 minutes, after which 2-3 successive washes were performed at 5-10 minutes with sterile distilled water.
The inoculation consisted in placing the sterilized seeds, on the basal medium Murashige & Skoog reduced to half (MS ½) distributed in sterile Petri dishes, operations performed on the hood with laminar flow. After the germination of the seeds from the red basil lines and the sufficient elongation and development of the stems, which was done differently in an interval of 10-15 days, the resulting seedlings were used to inoculate the explants detached from them (leaves, stems and cotyledonary node), under aseptic *in vitro* conditions, on the complete composition MS basal culture medium (Murashige and Skoog, 1962).
In addition to the red basil lines inoculated on the MS ½ environment, we also initiated the Series II of experiments starting from 09.03.2019, by using seeds from other 9 varieties of green basil from the Research & Development Station for Vegetables Growing Buzau (SCDL Buzău), for obtaining potted seedlings, in conventional conditions, using 4 seeds / vegetation pot for sowing. Florabella ÖKO FIBER universal flower soil (producer SC Klasmann-Deilmann, Germany) was used as a substrate for seed germination, with a granular consistency, a high water storage capacity and being composed of 75% black peat, 20% blonde peat, fibers and wood, 5% clay granules and NPK fertilizers (12: 11: 18.2).
For the green basil varieties, before inoculating the explants in Petri dishes, on the MS recipe complete recipe we performed the detachment and fragmentation of seedlings into types of explants represented by stems (hypocotyl and epicotyl fragments), leaves and cotyledonary node, followed by sterilization of plant material following the protocol applied to red basil varieties.
Three recipes with various concentrations of phytohormones from the category of cytokinins like 6-Benzylaminopurine (BAP) and *Thidiazuron* (TDZ) were tested in the basal culture Murashige and Skoog (MS) medium with complete composition, pH 5.6, agar 0.8%, sucrose 3%:
-BM medium consisting of complete prescription MS (1962) + 2 mg/LTDZ;
-BM1 environment composed of complete prescription MS (1962) + 1 mg/L TDZ and 1 mg/L BAP;
-BM2 medium consisting of complete prescription MS (1962) + 0.5 mg/L TDZ and 1 mg/L BAP.

3. RESULTS AND DISCUSSIONS

A. Observations on the initiation of crops in Series I (red basil varieties)

After seed inoculation (11.03.2019) and stabilization of *in vitro* asepsis conditions, 4 determinations were performed at different time intervals on germination yields (Table 1).
From the data registered in Table 1, it resulted that the highest germination percentage is attributed to the red basil line L10 (with a germination percentage of 80%). At close values are the lines L9 and L12 with the germination percentage of 72% and 65% respectively, L11 having the lowest germination percentage, namely 45%. After germination, a rapid development was observed in the emission of root and caulinary elements of the seedlings (figure 1).

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Table 1. Dynamics of germination of inoculated red basil seeds - Series I (date of sowing in vitro conditions 11.03.2019)

Basil line variants	%. GERMINATED SEEDS			
	After 4 days	After 7 days	After 10 days	After 14 days
L 9	22	61	61	72
L 10	45	65	80	80
L 11	30	35	40	40
L 12	30	45	50	65

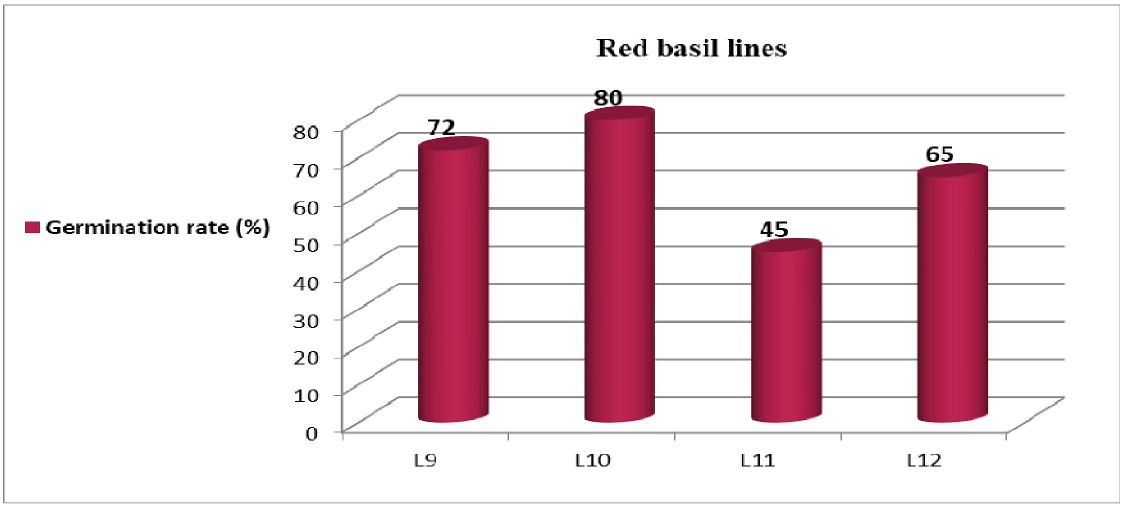

Figure 1. Germination rate (%) of seeds in the experimented lines of red basil - Series I, after 14 days

B. Observations on the initiation of crops in Series II (green basil varieties)

The seeds of Series II had the same path, germinating at different time intervals. Following the results registered, in the determinations performed on the germination of green basil seeds (Table 2) the highest percentage of germination was observed for the Buzau-flavored basil variety (100%), followed by the lemon-flavored basil variety and basil line L7 with a germination rate of 88%.

Table 2. Dynamics of green basil seed germination - Series II (date of sowing in organic substrate 09.03.2019)

Assortment of green basil		%. GERMINATED SEEDS			
Variants name	Variants Simbol	After 8 days	After 12 days	After 15 days	After 19 days
Menthol basil with red spike	**B.M.S.R.**	25	56.25	68.75	69
Basil -Aromat de Buzau’	**B.A-Bz.**	75	87.5	93.75	100
Large leaf basil -Bulatum var.	**B.B.**	25	50	62.5	63
Large leaf basil -Crispum var.	**B.C.**	31.25	50	56.25	56
Lemon flavored basil	**B.A.L**	68.75	68.75	75	88
Green basil line L7	**B.-L7**	31.25	43.75	56.25	88
Myrrh-flavored basil	**B. MIR**	12.5	37.5	43.75	50
Rose-flavored basil	**B.T.**	6.25	18.75	18.75	25
Pot basil, Minium var.	**B.G.**	37.5	37.5	43.75	50

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At close values were the varieties of mentholated green basil with a percentage of 69%, followed by basil with large lime leaf, Bulatum' with 63% and lime Crispum with 56%. At a percentage of 50% were both the myrrh-flavored basil variety and the potted basil variety, the lowest germination percentage being given by the rose-flavored basil variety (25%) (table 2). Illustration of the final registered results (after 19 days from sowing) for Series II - the germination percentage of the seeds of the 9 varieties of green basil inoculated *in vitro* is represented in figure 2.

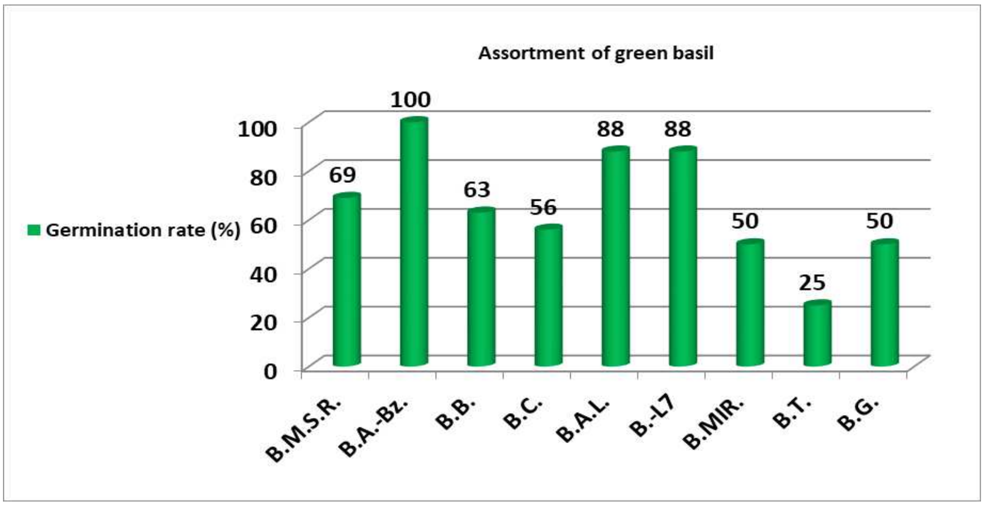

Figure 2. Germination rate (%) of seeds from the experimented assortment of green basil - Series II, after 19 days from sowing

C. Observations on the influence of the culture medium in the induction of organogenesis processes **-** <u>Series I (red basil varieties)</u>

After obtaining in aseptic conditions the seedlings from the red basil lines (L9, L10, L11 and L12) for 15 days, we proceeded to inoculate the fragments of explants (on 28.03.2019) detached from them and continued the development organogenic processes at their level, at different time intervals. Thus, 6 days after inoculation, the first changes were observed in the development of explants manifested by elongation of the stems and slight hypertrophy of the nodes. (Figure 3)

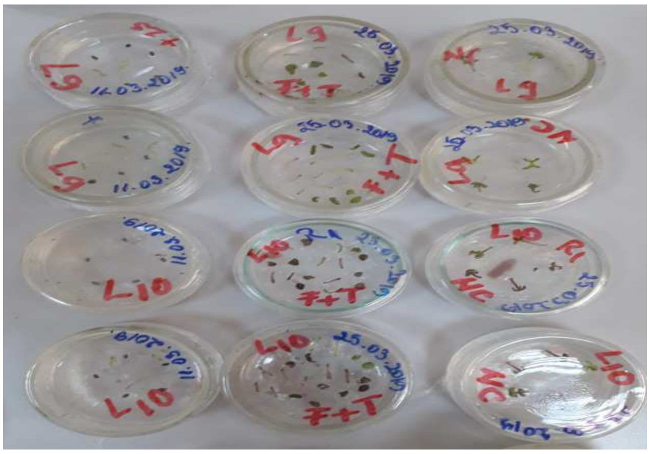

Figure 3. Stem explants and leaf fragments from the red basil lines L9 and L10, 6 days after inoculation

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In the case of the experimental Series I, after 20 days from the inoculation of explants of red basil varieties, it was found that all explants from lines L9 and L10 were maintained viable, with the exception of stem explants (hypocotyl and epicotyl fragments) and leaves from line L11, as well as those of nodes in line L12, where developmental stagnation or browning was found. After this period of time, differences in the expression of organogenesis were observed depending on the type of inoculated explant, on the same MS culture medium with complete composition. Thus, in the case of cotyledonary node (NC) explants, the elongation of the epicotyl and the development of leaf primordia were observed; in leaf explants (F) mild hypertrophy and in stem explants (T) mild hypertrophy and their elongation. (Figure 4)

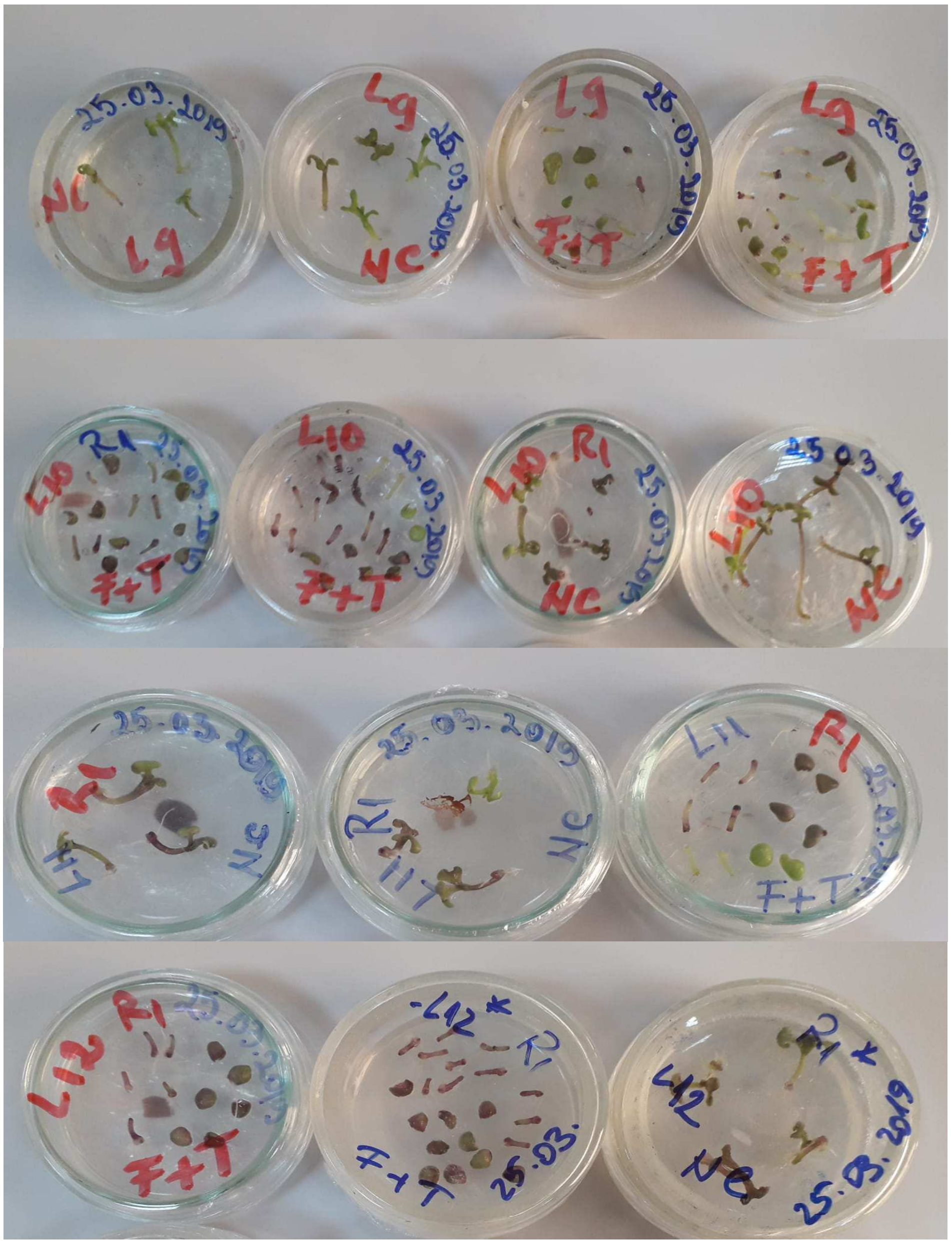

Figure 4. Expression of organogenesis in red basil lines L9, L10, L11 and L12 explants after 20 days from the date of inoculation

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In the rapid multiplication research conducted by Sahoo et al., (1997), in *Ocimum basilicum* L. (sweet basil), nodal fragments were used as a type of initial explants, for the development of axillary shoots, on the Murashige and Skoog (1962) culture medium supplemented with 1.0 mg / L N6-benzyladenine (BA). However, as the subsequent stages of growth and proliferation of adventitious shoots went through, it was found that a decrease in its concentration of up to 0.25 mg / L N6-benzyladenine (BA) was favorable to the multiplication yield. Other researchers such as Saha et al., 2012, tested the effect of cytokinins (BA / KN / 2-iP) at different concentrations, in order to obtain morphogenetic responses of caulogenesis in nodal segment explants in basil.
After sufficient stabilization and development on the MS medium (complete recipe) of the explants obtained from the red basil lines, their transfer was performed, in aseptic conditions on the freshly symbolized BM environment, to which in addition to the inorganic and organic salts elements corresponding to the basal culture recipe MS, the phytohormone thidiazuron (TDZ) was added in a concentration of 2 mg / L in culture medium.
Approximately 21 days after inoculation of the explants on the BM variant, the following morphogenetic responses due to cytokinin thidiazuron (TDZ) introduced into the culture medium were observed: at the cotyledonary node (NC) explants, the elongation of the epicotyl and the appearance of 2-3 layers of leaves are observed; in the case of leaf explants (F) a strong hypertrophy and the appearance of adventitious roots developed by direct organogenesis; at stem explants (T) are observed hypertrophies, elongations, the appearance of caulinary centers and adventitious roots developed by direct organogenesis. (Figure 5)

Figure 5. Evolution of cotyledonary node explants in inoculated red basil lines (lines L9, L10, L11 and L12), 21 days after inoculation, on BM culture medium recipe (MS +2 mg / L TDZ)

The explants obtained from the red basil lines responded favorably to the artificial conditions provided for the induction of morphogenesis, most obtaining a maximum viability percentage, except for the stems and leaves obtained from the L11 line (54% and 50% respectively) and in the case of cotyledonary node explants belonging to the L12 line (50 %).

D. Observations on the influence of the culture medium in the induction of organogenesis processes - Series II (green basil varieties)
After the germination of the seeds from the green basil varieties (Series II) and the sufficient development of the seedlings, followed the stage of obtaining and inoculation of the explants in aseptic conditions on the MS recipe complete recipe. The inoculation of the explants was performed on two different dates, namely: 01.04.-05.04.2019.

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After the stabilization of the asepsis conditions at the level of the explants obtained from the green basil varieties, changes were observed after 21 days from the date of inoculation, on their development (Figure 6.a -g.), as follows: in the cotyledonary node (NC) explants, the elongation of the epicotyl and the appearance of 1-2 nodal levels provided with leaves were observed; respectively at leaf explants (F) hypertrophies were observed as well as the appearance of caulinary centers developed by direct organogenesis; hypertrophies, elongations and the appearance of 1-2 primordia of adventitious roots developed by direct organogenesis were observed in fragments of stems explants (T).

a.) ***b.)*** ***c.)*** d.) ***e.)*** ***f.)*** **g.)**

Figure 6. Evolution of green basil explants after 21 days from the date of inoculation on the BM culture medium recipe (MS +2 mg / L TDZ): a.) "Aromat de Buzău" basil (B.A.Bz. symbol); b.) basil with large lime leaf ‚Bulatum' (B.B. symbol); c.) basil with large lime leaf Crispum (B.C. symbol); d.) rose-flavored basil (B.T. symbol); e.) green basil L7 (B.-L7 symbol); f.) lemon flavored basil (B.A.L.symbol); g.) myrrh-flavored basil (B.MIR symbol).

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At 15 days after the transfer of the green basil explants (with the age of 35 days from the initiation of the cultures) on the variant BM1 (MS + 1 mg / L TDZ and 1 mg / L BAP) and BM2 (MS + 0.5 mg / L TDZ and 1 mg / L BAP), of culture medium, evolutions of the morphogenetic processes were observed as follows (figure 7): at cotyledonary node (NC) explants was observed: the development of the root system; (figure 7. a.-c.), elongation of the epicotyl and the appearance of 2-3 layers of leaves (figure 7.b.); on leaf explants (F) it was observed: strong hypertrophy and the appearance of caulinary centers developed by direct organogenesis; and in the case of stem explants (T) were observed: hypertrophies, elongations as well as the appearance of 4-5 adventitious roots / each explant developed by direct organogenesis;

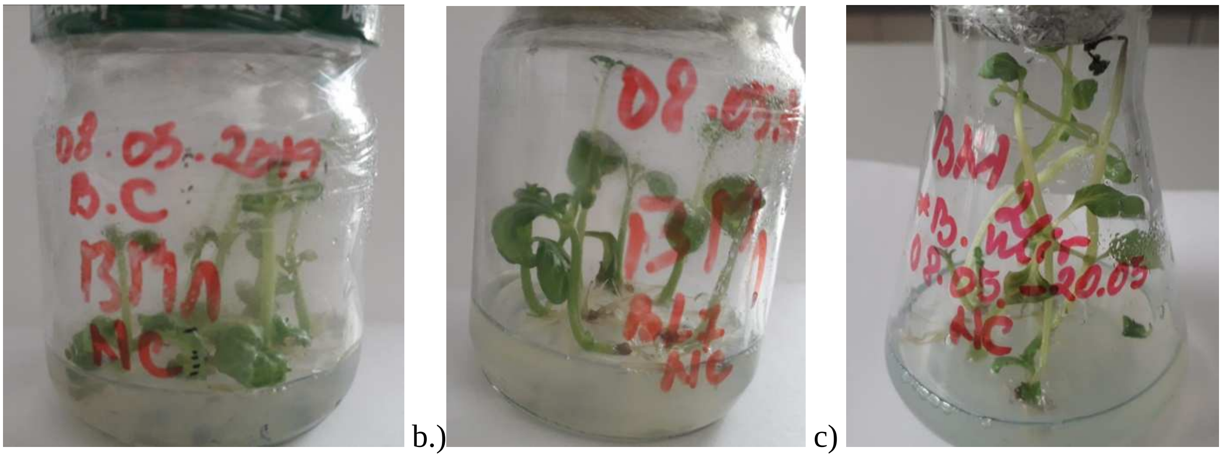
a.) b.) c)

Figure 7. Evolution of cotyledonary node (NC) explants from green basil varieties: a.) basil with large lime leaf Crispum (B.C. symbol); b.) green basil L7 (B.-L7 symbol); c.) myrrh-flavored basil (B.MIR symbol).

In the analysis, the influence of the culture medium in the expression of organogenesis processes *in vitro* conditions, significant direct caulogenesis responses were reported 15 days after inoculation on the BM1 variant (MS + 1 mg / L TDZ and 1 mg / L BAP) of culture medium in the case of 4 of the green basil varieties, at the level of two of the inoculated explants: leaves and cotyledonary node at "Aromat de Buzău" basil (BABz. symbol), stem fragments and cotyledonary node explants at varietyes basil with large lime leaf ‚Bulatum' (B.B. symbol), basil with large lime leaf ‚Crispum' (B.C. symbol) and green basil L7 (B.-L7 symbol).

4. CONCLUSIONS

For the red basil lines ((Series I),), all the explants provided a favorable response in relation to over 50% to the artificial conditions provided for the induction of morphogenesis. The appearance of caulinary centers developed by direct organogenesis from the leaves was more pronounced on the combination of 0.5 mg/L TDZ and 1 mg/L BAP (BM2). For, the cotyledonary node explants ensured both the elongation of the vegetative elements from the stems and the strong development of the root system by *in vitro* cultivation, for 45 days on the culture medium variant supplemented with 2mg/L TDZ (BM).

Regarding the green basil varieties (Series II), the biological material was easily obtained, the germination percentage of the seeds being different from one variety to another (25% - rose-flavored basil, 50% - myrrh-flavored basil and potted basil Minium lime, 56% - large-leaved basil

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‚Crispum' lime, 63% - large-leaved basil, Bulatum'lime, 69% - mentholated green basil with red spike, 88% - lemon-flavored basil and green basil L7, 100% - "Aromat de Buzău" basil), but the expression of morphogenesis in inoculated explants was lower, as long as they were not placed on culture media with the addition of phytohormones.

As a result, the transfer of explants from the analyzed varieties of red and green basil on the basal medium MS supplemented with hormonal TDZ (Thidiazuron) in a concentration of 2 mg / L and 1 mg / L and 0.5 mg / L, but also 1 mg / L BAP (6-Benzylaminopurine), led to superior results in elongation of stems, their hypertrophy, the presence of caulinary centers, but also the development of adventitious roots in leaf explants, influencing the development of cotyledonary nodes by lengthening the epicotyl, floor appearance and the development of the root system.

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