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Development of basil 'Dark Opal' from explant type and plant regulators

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ABSTRACT: Seedling abnormalities are a common problem in micropropagation and require investigation. This study aimed to assess the growth characteristics and morphoanatomy of stem apices and nodal segments of basil (*Ocimum basilicum*) 'Dark Opal' inoculated in medium containing combinations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). Presence of calluses, adventitious roots, and abnormal seedlings was quantified at 20 and 40 days after inoculation. Growth, anatomical, and morphological parameters were evaluated after 80 days. Explant type and regulators influenced the growth and morphoanatomy of basil 'Dark Opal'. Treatment of stem apices with 0.05 and 0.1 mg L^{-1} of NAA and BAP increased leaf number, fresh weight, and dry weight and decreased the incidence of calluses and abnormalities. Hyperhydric seedlings showed morphoanatomical alterations, such as disorganization and expansion of intercellular spaces with a consequent increase in parenchymal length. Thus, the results of this research allowed optimizing and understanding the important effects of growth regulators on the development of this species, which will help in *in vitro* mass culture programs and in biofactories for the production of secondary metabolites.

Key words: Auxin; cytokinin; *in vitro* culture; Lamiaceae; nodal segment; *Ocimum basilicum* L.

Desenvolvimento de manjericão 'Dark Opal' a partir do tipo de explante e reguladores vegetais

RESUMO: Anormalidades em plântulas geram dificuldades para micropropagação que precisam ser investigadas. Este trabalho possui o objetivo de avaliar as características de crescimento e a morfoanatomia de ápices e segmentos de manjericão `Dark Opal` (*Ocimum basilicum*) cultivados em combinações dos reguladores: 6-benzilaminopurina (BAP) e ácido naftalenoacético (ANA). A observação da porcentagem de calos, raízes e plântulas anormais foi realizada após 20 e 40 dias. E aos 80 dias foram feitas avaliações do crescimento, anatômica e morfológica. O tipo de explante e os reguladores utilizados influenciaram no crescimento e morfoanatomia do manjericão 'Dark Opal'. O tratamento a partir de ápice caulinar com 0,05 e 0,1 mg L^{-1} de ANA e BAP demonstrou aumento no número de folhas, na biomassa fresca e seca, menor porcentagem de calos e de anormalidades. As plântulas hiperidricas aparesentaram alteração na morfoanatomia, como desorganização e aumento de espaços intercelulares e consequente aumento do comprimento dos parênquimas. Dessa forma, os resultados desta pesquisa permitiram otimizar e conhecer importantes efeitos dos reguladores de crescimento sobre o desenvolvimento desta espécie, o que auxiliará em programas de cultivo massal *in vitro* e em biofábricas para a produção de metabólitos secundários.

Palavras-chave: Auxinas; citocinina; cultura *in vitro*; Lamiaceae; segmento nodal; *Ocimum basilicum* L.

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Introduction

Basil (*Ocimum basilicum* L.) 'Dark Opal' is a hybrid of *O. basilicum* and *O. forskolei*, with lobate leaves, clove aroma, and several compounds with pharmacological potential. Phippen & Simon (1998) reported that purple basil is a source of anthocyanins; the plant can be used to produce stable red pigments suitable for the food industry. Basil species find application in the production of foods, dental products, traditional medicinal preparations, and essential oil for use in fragrances (Omidbaigi et al., 2007).

Basil plants contain a variety of compounds with biological activities, such as fungistatic, antimicrobial, antioxidant (Stanojevic et al., 2017; Sestil et al., 2018), and anti-inflammatory (Złotek et al., 2016). Geraniol, linalool, and 1,8-cineole exert anti-depressant, sedative, and anxiolytic effects. Eugenol shows cardioprotective and antihypertensive potential, with reports of antioxidant and hypolipemic activities (Sestil et al., 2018).

Field cultivation exposes plants to factors such as soil fertility, insects, and weather conditions, which may lead to variations in the production of biomass, secondary metabolites, and essential oil (Blank et al., 2010). On the other hand, is capable of minimizing the effects of interfering factors, given the possibility of controlling growth medium composition and environmental conditions. After defining a standardized protocol for *in vitro* cultivation, it is possible to obtain homogeneous seedlings with reproducible characteristics (Shukla et al., 2021).

Culture media can be altered by adding low concentrations of plant growth regulators (PGRs). These compounds influence plant cell division, expansion, and structure, thereby acting on growth, development, and metabolism through signaling pathways (Small & Degenhardt, 2018). Auxins, for instance, are responsible for regulating cell division, elongation, and plant development (Guilfoyle et al., 1998; Saini et al., 2013). Cytokinins neutralize the apical dominance promoted by auxins and stimulate lateral growth, resulting in the production of shoots and roots (Small & Degenhardt, 2018).

Care must be taken to avoid abnormal seedling formation during micropropagation by choosing, for example, adequate explant sources, light and temperature conditions, and type of PGR (Monfort et al., 2018). Studies have reported abnormalities, such as adventitious roots, curled leaves (Trettel et al., 2018a), hyperhydric shoots (Phippen & Simon, 2000), and abnormal flowers (Manan et al., 2016). These abnormalities not only hinder seedling production by micropropagation but also decrease bioactive compound content, stemming from structural or metabolic changes, such as altered photosynthetic rate (Monfort et al., 2018).

Although widely used in micropropagation, PGRs may lead to alterations in plant physiology and growth (Shahzad et al., 2017). Thus, knowledge of the abnormalities resulting from micropropagation is a prerequisite for developing efficient protocols for successful acclimatization, large-scale production, and investigation of secondary metabolite production. This study aimed to assess the effects of auxins and cytokinins on the growth, organogenesis, physiological disorders, and abnormalities of basil 'Dark Opal' seedlings grown *in vitro* from stem apices or nodal segments.

Materials and Methods

Preparation of propagative material

The research was conducted at the Laboratory of Plant Tissue Culture of Universidade Paranaense (UNIPAR), Brazil. Seeds of purple basil (Horticeres®, lot no. 16000040, 2015/2015) of Italian origin were purchased from a local market in Umuarama, Paraná, Brazil, and used to produce seedlings that served as explant donors. Prior to inoculation in culture medium, seeds were treated to break dormancy and then disinfected. The procedure consisted of immersing seeds in distilled water under oxygenation at 25 ± 2 °C for 2 hours, followed by immersion in 70% ethanol for 2 minutes and 2% sodium hypochlorite for 15 minutes under a laminar flow hood. Finally, seeds were washed four times with autoclaved deionized water. The culture medium used was full-strength MS - Murashige & Skoog- supplemented with 30 and 6.5 g L^{-1} of sucrose and agar, respectively, and adjusted to pH 5.8. PGRs were not added at this stage.

Culture medium (50 mL) was added to 350 mL glass jars, autoclaved at 120 °C for 20 minutes, and left to cool. Under a laminar flow hood, each jar was inoculated with four seeds, sealed with plastic cap, wrapped with polyvinyl chloride (PVC) film, and incubated in a growth chamber at 25 ± 2 °C and light intensity of 72.0 µmol m^{-2} s^{-1} (LED lamp T8, 10 W, 6,000 K, 100-240 V, 50/60 Hz, ≥ 0.92 power factor; Blumenau®, Porto Alegre, Brazil) under a 24 hours photoperiod. Seeds were kept in the growth chamber until maximum growth, which occurred in 80 days.

Explant preparation and experimental procedures

Seedlings without abnormalities or signs of oxidation were used for excision of stem apices and nodal segments. Each apex or segment measured about 1.5 cm in length and had two buds. Explants (apices and segments) were inoculated one per jar. The culture medium was the same as that used for seed inoculation, with the exception that PGRs were added according to an experimental design. Conditions for apex (coded A) and segment (coded S) explants are shown in Table 1. Numbers ranging from 0 to 6 indicate phytohormone treatments (Table 1).

Growth medium and phytohormone concentrations were defined on the basis of preliminary assays and previous studies (Monfort et al., 2018; Trettel et al., 2018b). First, 350 mL jars containing 50 mL of medium were autoclaved and allowed to cool until solidification. Then, explants were inoculated one per jar and incubated in a growth chamber under the same conditions described above for 80 days.

Table 1. Concentrations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) used in stem apex and nodal segment cultures of purple basil (*Ocimum basilicum* L. 'Dark Opal').

Explant		BAP	NAA
Apex	Nodal segment	(mg L^{-1})	
A0	S0	0.0	0.0
A1	S1	0.05	0.1
A2	S2	0.1	0.2
A3	S3	0.1	0.05
A4	S4	0.3	0.2
A5	S5	0.5	0.3
A6	S6	1.0	0.5

Abnormalities during *in vitro* development

Calluses, abnormal roots, and abnormal seedlings were quantified at 20 and 40 days after inoculation. Seedlings showing hyperhydricity, adventitious roots, curled leaves, curled stems, leaf narrowing, or color alterations were considered abnormal (Trettel et al., 2018b). Six replications of five jars each were used per treatment.

Growth assessment

At the end of the culture period (80 days), plants were evaluated for leaf number, shoot length, root length, shoot fresh and dry weights, root fresh and dry weights, callus fresh and dry weights, and chlorophyll index. Hyperhydricity and presence of adventitious roots were also recorded. Analyses were performed in six replications of three jars per treatment.

For dry weight determination, fresh samples were oven-dried at 65 °C for 3 days and weighed on a precision scale. Length measurements were taken with a digital caliper. Determination of chlorophyll index was performed on a leaf at the middle third of each plant by using a chlorophyll meter (ChlorofiLOG®, model CFL 1030), according to the manufacturer's instructions FALKER®, 2008.

Hyperhydricity and presence of adventitious roots

The presence or absence of hyperhydricity and adventitious roots was recorded in binary format (1 = presence, 0 = absence) at 80 days after explant inoculation. These data were used to calculate the percentage of hyperhydricity and adventitious roots.

Anatomical and morphometric analysis of leaves

Leaf samples were collected from three replications per treatment at the end of the culture period (80 days). The material was fixed in solution of formaldeide with alcohol FAA_{50} for 24 hours according to Antoniazzi et al. (2016), dehydrated through a butyl alcohol series (50, 70, 85, 95, and 100%), and embedded in paraplast. Then, 10 µm thick anatomical sections were cut transversely to the median region of the leaf surface and petiole using a manual rotary microtome (Leica Biosystems®, model RM2125 RT). Subsequently, sections were transferred to glass slides, dewaxed, dehydrated through an ethanol series (90, 80, 70, and 50%), and stained with Safrablau solution diluted to 0.5%. Subsequently, samples were washed in water and passed through an ethanol series (50, 70, 80, and 90%). Coverslips were fixed with nail varnish.

Images were captured by using a digital camera attached to an Olympus BX-60 microscope at 40× magnification. Histological sections were measured using MoticImages Plus 3.0 software. Three images were selected per treatment, and 10 measurements were performed per image. The following tissues were analyzed: adaxial (upper) epidermis, palisade parenchyma, spongy parenchyma, abaxial (lower) epidermis, and distance between adaxial and abaxial surfaces (blade thickness).

Statistical design and analysis

In vitro cultivation and plant abnormalities

The experiment was carried out in a completely randomized design. For each explant type (stem apex and nodal segment), seven PGR treatments were performed, with six replications, five jars per replication, and one explant per jar.

At 40 days, the incidence (%) of calluses, adventitious roots, and abnormal seedlings was assessed in a 7 (treatments) × 2 (explant type) factorial design. Data were subjected to analysis of variance ($p \leq 0.05$), and means were compared by Tukey test ($p \leq 0.05$) using SISVAR version 5.6 (Ferreira, 2011).

Plant growth and morphoanatomy

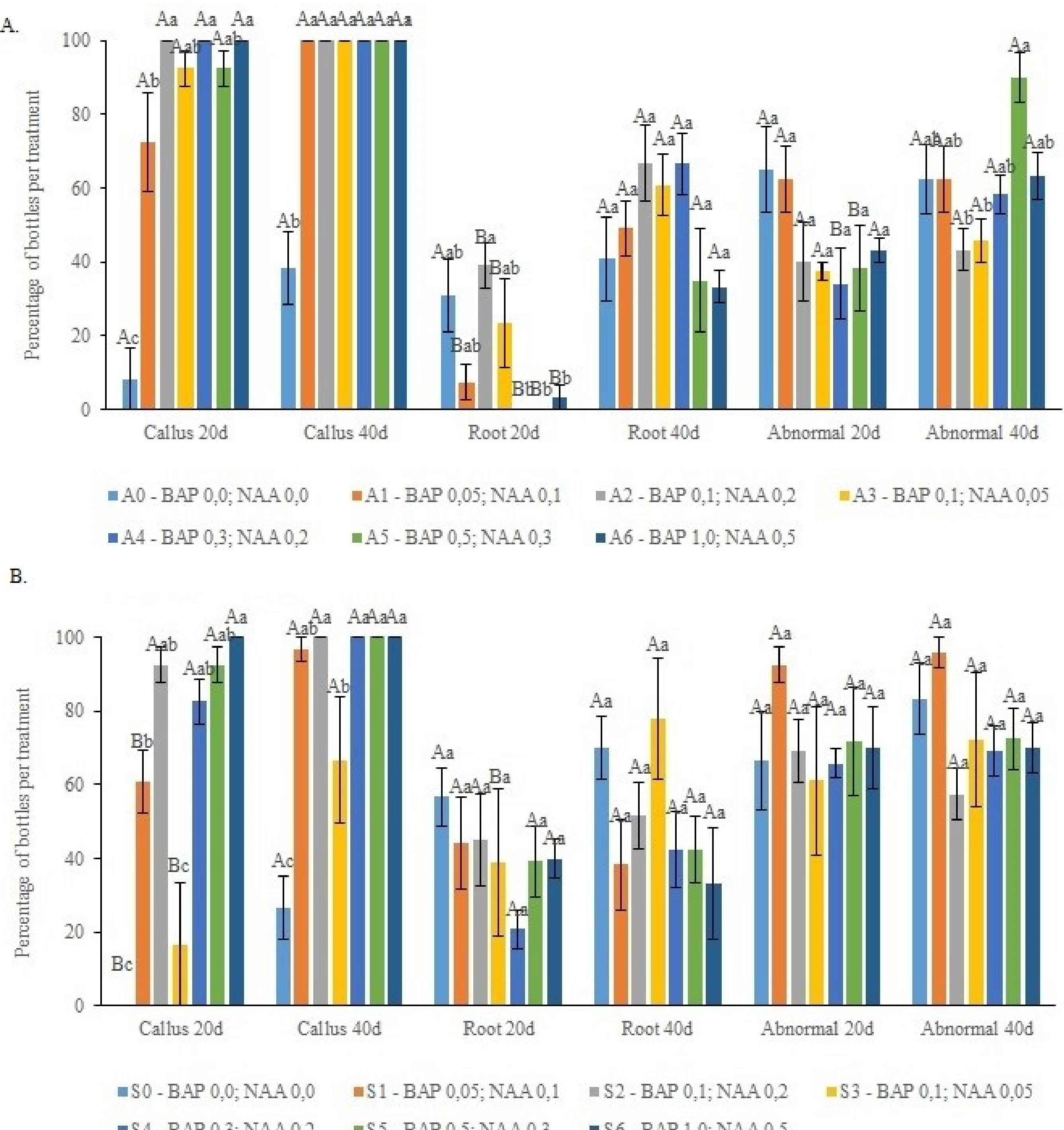
Plant growth, physiology, and leaf morphometry were evaluated at the end of the experimental period (80 days) in a 7 (treatments) × 2 (explant type) factorial design. Data were subjected to analysis of variance ($p \leq 0.05$), and means were compared by Tukey test ($p \leq 0.05$) using SISVAR version 5.6 (Ferreira, 2011).

Results and Discussion

Induction of abnormalities

At 20 days of *in vitro* cultivation, 93 and 74% of apices and nodal segments treated with PGRs, respectively, developed calluses, mainly at the explant base. By contrast, treatments grown without PGRs showed the lowest percentages of callus formation: calluses were observed in 38% of seedlings obtained from control stem apex cultures (A0) and in 26% of seedlings obtained from control nodal segment cultures (S0). At 40 days, there was a 7 and 20% increase in callus formation in A and S treatments, respectively (Figure 1).

Explants treated with PGRs showed callus formation at the base, whereas control explants did not. Therefore, it can be inferred that callus formation is directly influenced by the use of PGRs, in particular, by the combination of auxins and cytokinins. Calluses are disorganized cell masses originating from different biotic and abiotic stimuli. Ikeuchi et al. (2013), in elucidating some of the mechanisms of callus induction, discussed how PRGs signal and modulate the expression of transcription factors responsible for callus formation. Similar characteristics were reported by Dode et al. (2003), who observed calluses in 90-100% of basil seeds grown *in vitro*



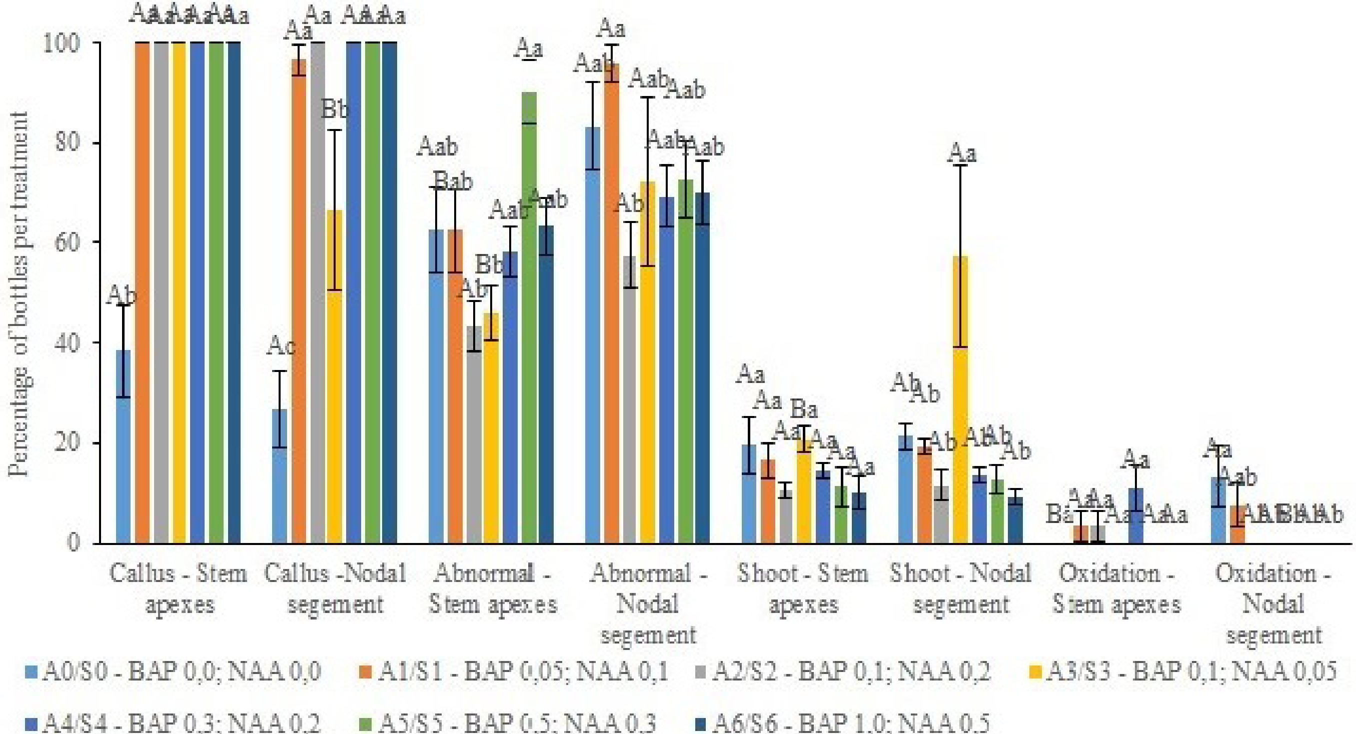
(A) Percentage for treatments that used stem apexes as an explant. (B) Percentage for treatments that used nodal segments as explants. * Uppercase letter compares the treatment according to the micropropagation days (20 and 40 days). Lowercase compares the treatments in the same period of time. Tukey ($p \leq 0.05$). 20d = 20 days after inoculation of the explant. 40d = 40 days after inoculation of the explant.

Figure 1. Incidence of callus, adventitious roots, and abnormal seedlings in stem apex and nodal segment cultures of purple basil (*Ocimum basilicum* L. ‘Dark Opal’) treated with 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) at 20 and 40 days after inoculation.

with low concentrations of auxins (0.2 mg L^{-1} NAA) cytokinins (1-5 mg L^{-1} BAP).

In the current study, the main abnormalities observed were hyperhydricity and adventitious root formation. In the second evaluation, 90% of A5 seedlings showed abnormalities; this treatment led to the highest number of abnormal seedlings among A treatments (Figure 1A). Abnormality incidence did not differ between S0-S6 treatments, with 74% of seedlings from nodal segment cultures exhibiting abnormalities (Figure 1B).

Hyperhydric leaves have higher water content and translucent appearance and are generally wrinkled, brittle, and deficient in chlorophyll. The hyperhydricity can be triggered by numerous factors, including pH, type of explant,



Different uppercase letters indicate significant differences between explants (Tukey test, $p \leq 0.05$). Different lowercase letters indicate significant differences between phytohormone treatments (Tukey test, $p \leq 0.05$).

Figure 2. Callus formation, abnormal seedlings, shoots per seedling, and oxidation in stem apex and nodal segment cultures of purple basil (*Ocimum basilicum* L. 'Dark Opal') treated with 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) at 40 days after inoculation.

growth medium composition, light intensity, and ventilation. In the current experiment, explant type and PGR use were found to influence the occurrence of hyperhydricity (Liu et al., 2017).

Treatment 3 led to different results depending on the type of explant used. S3 resulted in 33% fewer seedlings with calluses and 37% more shoots per seedling. A3, however, led to 27% fewer abnormalities than S3. At 40 days, abnormalities were observed in 53% of seedlings grown from stem apex cultures and in 74% of seedlings from nodal segment cultures (Figure 2).

Table 2 shows that hyperhydricity incidence was similar among seedlings grown from stem apex cultures (A0-A6). S0 and S1 treatments were the greatest inducers of hyperhydricity among S treatments. On average, 72% of seedlings grown from segment cultures exhibited hyperhydricity, whereas half as much of seedlings from apex cultures showed this abnormality. Adventitious roots occurred at a higher rate (64%) in stem apex treatments. The number of adventitious roots was 14% higher in stem apex treatments than in S treatments. A3 led to the lowest incidence of hyperhydricity, with only 11% of hyperhydric seedlings. Stem apex treatments afforded the highest means of vitreous seedlings.

In this study, nodal segments exhibited greater hyperhydricity development than stem apices, in agreement with the results of Toma et al. (2004), who also observed plants with a vitreous aspect. The authors used combinations of cytokinins and auxins for the cultivation of nodal segments of *Hyssopus officinalis*. The seedlings showed calluses and different degrees of hyperhydricity in stems and leaves (Toma et al., 2004).

Table 2. Incidence of hyperhydricity and adventitious roots in purple basil (*Ocimum basilicum* L. 'Dark Opal') seedlings produced from stem apex and nodal segment cultures treated with 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) at 80 days after inoculation.

Treatments		Hyperidricity	Adventice roots
		(%)	
Stem apexes	A0	$50.00 \pm 16.67^{Ba*}$	55.56 ± 9.62^{Aab}
	A1	38.89 ± 9.62^{Ba}	88.89 ± 9.62^{Aa}
	A2	16.67 ± 16.67^{Ba}	88.89 ± 9.62^{Aa}
	A3	11.11 ± 16.67^{Ba}	88.89 ± 9.62^{Aa}
	A4	22.22 ± 25.46^{Ba}	61.11 ± 19.25^{Aab}
	A5	44.44 ± 19.25^{Aa}	22.22 ± 25.46^{Ab}
	A6	44.44 ± 25.46^{Aa}	44.44 ± 16.67^{Aab}
Nodal segments	S0	100.00 ± 28.87^{Aa}	44.44 ± 16.67^{Aab}
	S1	100.00 ± 0.00^{Aa}	50.00 ± 9.62^{Bab}
	S2	55.56 ± 25.46^{Aab}	77.78 ± 9.62^{Aa}
	S3	66.66 ± 41.94^{Aab}	66.67 ± 44.10^{Aab}
	S4	61.11 ± 38.49^{Aab}	44.44 ± 44.10^{Aab}
	S5	72.22 ± 19.25^{Aab}	22.22 ± 16.67^{Ab}
	S6	50.00 ± 9.62^{Ab}	44.44 ± 9.62^{Aab}

A0/S0 - control - without the addition of phytohormones; A1/S1 with an increase of 0.05 and 0.1 mg L^{-1} of BAP and NAA; A2/S2 - 0.1 and 0.2 mg L^{-1} of BAP and NAA; A3/S3 - 0.1 and 0.05 mg L^{-1} of BAP and NAA; A4/S4 - 0.3 and 0.2 mg L^{-1} of BAP and NAA; A5/S5 - 0.5 and 0.3 mg L^{-1} of BAP and NAA; A6/S6 - 1.0 and 0.5 mg L^{-1} of BAP and NAA
* Uppercase letter compares the treatments of the different explants in the column. Lowercase compares the treatments of the same explanta in the column. Same letters do not differ from each other. Tukey ($p \geq 0.05$).

A1, A2, A3, and S2 afforded the highest incidence of adventitious roots, with 78-89% of seedlings affected by this abnormality. Many compounds and PGRs have been associated with adventitious root development. Guilfoyle et al. (1998) genetically screened plants with increased resistance to exogenous auxin and were able to produce auxin-responsive mutants. The authors found that AXR3, an auxin signaling gene, increased apical dominance and adventitious rooting.

The findings show that cytokinins, when acting synergistically and responsively to auxins, may induce the formation of adventitious roots by promoting the expression of genes associated with this characteristic, which was believed to occur in the current study. It is important to emphasize that A treatments resulted in a higher percentage of plants with adventitious roots, possibly because stem apices exhibit apical meristems, a region where auxin signaling occurs (Rademacher, 2015).

Influence of PGRs and explant type on purple basil growth

Plant growth and development are supported by meristems. Meristematic activity is mainly controlled by the PGRs auxin and cytokinin, as they antagonistically affect the organogenesis of shoots and roots (Ioio et al., 2008).

Table 3 shows that A treatments afforded superior results to S treatments. Of note, A3 resulted in 45 leaves per seedling, about 14 leaves more than the other A treatments and 36 leaves more than S treatments. Zuzarte et al. (2010) indicated that PGRs influence not only plant growth but also essential oil production. This is because glandular trichomes, which store essential oil, are distributed on abaxial and adaxial leaf surfaces. Therefore, the number and size of leaves are directly associated with essential oil production capacity.

The treatments did not differ significantly in most of the analyzed variables. S0 and S3 had the highest leaf numbers; especially S3, which resulted in 40 leaves per seedling. Shoot length, shoot fresh and dry weights, and root fresh weight were three times lower in S3 than in grown from the stem apexes (Tables 3 and 4).

The only advantage of using nodal segments compared with stem apices was that segments resulted in lower callus biomass. Callus fresh and dry biomass were eight and four times lower, respectively, in S3 than in A3. For the majority of variables, apices performed better than segments (Table 4).

The mean shoot length (126.24 mm) and root length (97.94 mm) of A3 were 34 and 31% higher than those of the other A treatments and 81 and 69% higher than those of S treatments. Furthermore, A3 resulted in the highest shoot and root fresh and dry biomass. Weight values were 2-fold those of the other A treatments and 4-fold those of S treatments. Root fresh biomass was 10 times higher. Callus fresh (4.67 g) and dry (0.23 g) biomass of A3 were lower than those of the other A treatments but higher than those of S treatments (Tables 3 and 4).

As shown in Figure 1, rooting was evident at the first evaluation, and rooting time was influenced by explant type. Root growth continued to increase throughout the experiment. Seedlings grown from apex cultures began rooting mainly after 40 days: 31% of seedlings had rooted at 20 days and 41% at 40 days (Figure 1A). On the other hand, 57 and 70% of seedlings from nodal segment cultures had rooted at 20 and 40 days, respectively (Figure 1B), totaling 30% more rooted seedlings than those grown from apices. The results show that rooting occurred faster in seedlings grown from nodal segments.

Seedlings from stem apex cultures are assumed to have a greater capacity to control meristematic activation and inactivation, which may improve growth performance by reducing energy requirements. This hypothesis is supported by the results of Ioio et al. (2008), who investigated the activity of PIN genes and cell division when SHY2 is degraded for gene activity in *Arabidopsis thaliana*. PIN gene activity

Table 3. Leaf number, shoot length, root length, and chlorophyll index of purple basil (*Ocimum basilicum* L. 'Dark Opal') seedlings produced from stem apex and nodal segment cultures treated with 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA).

Treatment		LN	SL (mm)	RL (mm)	CI
Stem apexes	A0	47.22 ± 12.58^{Aa*}	89.55 ± 18.11^{Ab}	78.79 ± 19.87^{Aa}	22.26 ± 1.56^{Aab}
	A1	29.55 ± 10.27^{Aab}	87.59 ± 16.36^{Ab}	71.60 ± 12.56^{Aa}	32.32 ± 4.44^{Aa}
	A2	51.95 ± 19.38^{Aa}	114.47 ± 10.01^{Aa}	71.79 ± 10.24^{Aa}	22.68 ± 4.69^{Aab}
	A3	45.72 ± 15.33^{Aab}	126.24 ± 13.24^{Aa}	97.94 ± 14.88^{Aa}	19.35 ± 1.09^{Aab}
	A4	27.31 ± 9.21^{Ab}	105.40 ± 16.12^{Aab}	60.61 ± 21.23^{Aa}	24.34 ± 3.75^{Aab}
	A5	18.67 ± 8.68^{Ab}	54.22 ± 13.50^{Ac}	75.70 ± 21.58^{Aa}	16.26 ± 1.95^{Ab}
	A6	17.22 ± 8.29^{Ab}	45.43 ± 11.84^{Ac}	47.11 ± 15.84^{Aa}	18.87 ± 2.84^{Aab}
Nodal segment	S0	21.97 ± 7.24^{Bab}	29.61 ± 3.12^{Ba}	28.33 ± 5.59^{Ba}	10.94 ± 1.00^{Ba}
	S1	16.13 ± 5.13^{Ab}	28.87 ± 7.68^{Ba}	29.26 ± 10.88^{Ba}	9.37 ± 1.03^{Ba}
	S2	5.70 ± 3.07^{Bb}	16.71 ± 3.03^{Ba}	25.83 ± 8.94^{Ba}	11.63 ± 1.66^{Ba}
	S3	40.00 ± 1.58^{Aa}	31.99 ± 19.37^{Ba}	52.97 ± 45.69^{Ba}	22.57 ± 1.81^{Aa}
	S4	6.07 ± 1.69^{Bb}	18.52 ± 4.16^{Ba}	18.29 ± 31.07^{Ba}	18.57 ± 2.63^{Aa}
	S5	4.83 ± 1.97^{Ab}	15.13 ± 3.31^{Ba}	41.79 ± 87.60^{Aa}	13.07 ± 0.67^{Aa}
	S6	3.63 ± 1.36^{Ab}	22.29 ± 5.25^{Ba}	14.16 ± 14.85^{Aa}	14.20 ± 2.04^{Aa}

* Within a column, different uppercase letters indicate significant differences between explants and different lowercase letters indicate significant differences between phytohormone treatments (Tukey test, $p \leq 0.05$).
Legend: A - apex explant; S - segment explant; A0/S0 - without growth regulator (control); A1 and S1 - 0.05 and 0.1 mg L^{-1} of BAP and NAA; A2 and S2 - 0.1 and 0.2 mg L^{-1} of BAP and NAA; A3 and S3 - 0.1 and 0.05 mg L^{-1} of BAP and NAA; A4 and S4 - 0.3 and 0.2 mg L^{-1} of BAP and NAA; A5 and S5 - 0.5 and 0.3 mg L^{-1} of BAP and NAA; A6 and S6 - 1.0 and 0.5 mg L^{-1} of BAP and NAA; LN - leaf number; SL - shoot length; RL - root length; CI - chlorophyll index.

Table 4. Fresh and dry shoot, root and callus biomass of purple basil (*Ocimum basilicum* L. 'Dark Opal') seedlings produced from stem apex and nodal segment cultures treated with 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA).

Treatment		SFB	RFB	CFB	SDB	RDB	CDB
		(g)					
Stem apexes	A0	3.74 ± 0.57^{Abc}	2.89 ± 1.58^{Aab}	0.00 ± 0.00^{Ad}	0.29 ± 0.05^{Aab}	0.16 ± 0.08^{Aa}	0.00 ± 0.00^{Ab}
	A1	2.32 ± 0.46^{Ade}	1.69 ± 1.01^{Abc}	5.61 ± 1.16^{Ac}	0.18 ± 0.05^{Abc}	0.08 ± 0.05^{Aab}	0.38 ± 0.13^{Aa}
	A2	4.32 ± 1.19^{Aab}	2.92 ± 0.97^{Aab}	7.14 ± 1.39^{Bbc}	0.31 ± 0.10^{Aab}	0.12 ± 0.05^{Aab}	0.41 ± 0.15^{Ba}
	A3	5.21 ± 1.52^{Aa}	3.86 ± 1.59^{Aa}	4.67 ± 0.92^{Ac}	0.37 ± 0.11^{Aa}	0.18 ± 0.06^{Aa}	0.23 ± 0.03^{Aa}
	A4	2.84 ± 1.03^{Acd}	1.21 ± 0.94^{Abc}	8.98 ± 2.03^{Aab}	0.18 ± 0.09^{Abc}	0.06 ± 0.06^{Aab}	0.36 ± 0.11^{Aa}
	A5	1.48 ± 0.72^{Ae}	1.83 ± 2.83^{Abc}	11.14 ± 2.23^{Aa}	0.10 ± 0.04^{Ac}	0.05 ± 0.08^{Aab}	0.42 ± 0.04^{Aa}
	A6	1.32 ± 0.37^{Ae}	0.57 ± 0.52^{Ac}	10.80 ± 1.47^{Aa}	0.06 ± 0.01^{Ac}	0.02 ± 0.02^{Aa}	0.42 ± 0.07^{Ba}
Nodal segment	S0	2.42 ± 0.22^{Ba*}	0.08 ± 0.05^{Ba}	0.00 ± 0.00^{Ac}	0.23 ± 0.04^{Aa}	0.01 ± 0.01^{Ba}	0.00 ± 0.00^{Ac}
	S1	1.34 ± 0.46^{Bab}	0.05 ± 0.05^{Ba}	0.57 ± 0.07^{Bc}	0.12 ± 0.04^{Aab}	0.01 ± 0.01^{Aa}	0.06 ± 0.01^{Bc}
	S2	0.51 ± 0.21^{Bb}	0.57 ± 0.56^{Ba}	12.35 ± 3.70^{Aa}	0.07 ± 0.08^{Bab}	0.04 ± 0.04^{Aa}	0.61 ± 0.27^{Ab}
	S3	1.61 ± 0.01^{Bab}	1.31 ± 0.01^{Ba}	0.55 ± 0.05^{Bc}	0.10 ± 0.00^{Bab}	0.10 ± 0.00^{Aa}	0.06 ± 0.00^{Bc}
	S4	1.07 ± 0.18^{Bab}	0.40 ± 0.39^{Aa}	6.25 ± 0.75^{Bb}	0.08 ± 0.02^{Bb}	0.11 ± 0.21^{Aa}	0.46 ± 0.04^{Ab}
	S5	0.80 ± 0.16^{Ab}	0.16 ± 0.28^{Ba}	8.73 ± 1.99^{Bb}	0.05 ± 0.01^{Ab}	0.01 ± 0.02^{Aa}	0.47 ± 0.10^{Ab}
	S6	0.97 ± 0.67^{Ab}	0.12 ± 0.13^{Aa}	8.39 ± 1.08^{Bb}	0.04 ± 0.03^{Ab}	0.05 ± 0.06^{Aa}	0.88 ± 0.26^{Aa}

* Within a column, different uppercase letters indicate significant differences between explants and different lowercase letters indicate significant differences between phytohormone treatments (Tukey test, $p \leq 0.05$).
Legend: A - apex explant; S - segment explant; A0/S0 - without growth regulator (control); A1 and S1 - 0.05 and 0.1 mg L^{-1} of BAP and NAA; A2 and S2 - 0.1 and 0.2 mg L^{-1} of BAP and NAA; A3 and S3 - 0.1 and 0.05 mg L^{-1} of BAP and NAA; A4 and S4 - 0.3 and 0.2 mg L^{-1} of BAP and NAA; A5 and S5 - 0.5 and 0.3 mg L^{-1} of BAP and NAA; A6 and S6 - 1.0 and 0.5 mg L^{-1} of BAP and NAA; SFB - shoot fresh biomass; RFB - root fresh biomass; CFB - callus fresh biomass; SDB - shoot dry biomass; RDB - root dry biomass; CDB - callus dry biomass.

is maintained by AHK3/ARR1 signaling, which decreases according to the auxin-cytokinin balance. Müller & Sheen (2008) observed that direct transcriptional activation of *Arabidopsis* response regulator (ARR) genes ARR7 and ARR15 is associated with cytokinin antagonization in auxin signaling pathways. Such alterations in hormonal balance lead to changes in the gradient of PLT proteins. These proteins are responsible for directing responses in different regions of the meristem.

In addition to substrate type, temperature, and other exogenous characteristics, roots play a fundamental role in plant acclimatization (Arrigoni-Blank et al., 2011). Thus, good root development is essential for successful seedling production. Apices showed a competitive advantage over nodal segments as explants because of the intrinsic growth potential of apices, resulting from the fact that auxin signaling occurs in the apical meristem.

Influence of PGRs and explant type on morphology and proliferation capacity of purple basil leaf cells

Basil leaf is smooth, bifacial, with upper and lower uniseriate epidermis covered by a thin cuticle. The mesophyll is divided into palisade and spongy parenchyma; the former is located on the adaxial surface and the latter on the abaxial surface. The vascular bundle is located at the center of the leaf, protruding on the abaxial face. Leaf morphology and organ size are regulated by cell division, PGRs, meristematic competence, and genes not yet fully elucidated. In most plant species, the flat structure of leaves is caused by coordinated growth of epidermal, palisade, and spongy mesophilic cells as well as of vascular tissue (Horiguchi et al., 2006).

The number of epidermal layers varied between treatments. Adaxial and abaxial epidermis thicknesses did not differ significantly between A treatments, with means of 14.48 and 15.21 µm, respectively. Palisade parenchyma (175.87 µm), spongy parenchyma (492.88 µm), and blade (671.12 µm) thicknesses were largest in A5, almost two times larger compared with the control (A0) (Table 5).

Figure 3C shows that the palisade parenchyma in A5 is formed by a single layer of cells; the increase in palisade parenchyma thickness was due to an increase in cell length. By contrast, A0 leaves had smaller cells arranged in more than one layer (Figure 3A). The spongy parenchyma of A5 seedlings was composed of about 7 layers of irregular. disorganized cells. with many intercellular spaces. These characteristics might have contributed to the fragility of cell walls. which were visibly brittle (Figure 3C). In the control, spongy parenchyma cells were oval-shaped, with well-defined edges, and distributed in three to four layers with fewer intercellular spaces (Figure 3A).

The blade section of A5 leaves showed that blade and petiole thicknesses were the same. suggesting that layer number and cell number and size influenced blade thickness (Figure 3C). Palisade parenchyma and blade thicknesses were 45 and 37% greater in S5 than in the other S treatments, respectively (Table 5 and Figure 3).

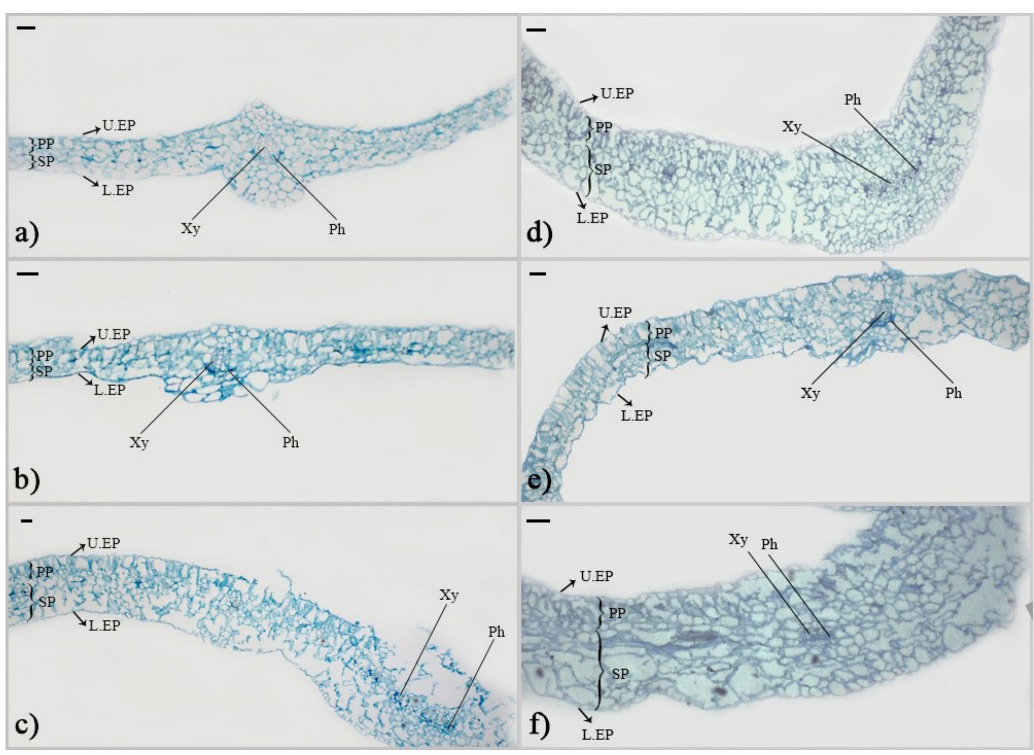
Explant type influenced palisade parenchyma thickness, which was 24% higher in seedlings grown from nodal segment cultures. Some treatments differed in adaxial epidermis, abaxial epidermis, palisade parenchyma, and blade thicknesses. A3 afforded a 9.67 µm increase in adaxial epidermis thickness compared with S3. Abaxial epidermis thickness was 44% higher in S0 than in A0. Palisade parenchyma and blade thicknesses were 54 and 50% higher, respectively, in S0 than in A0 (Table 5 and Figure 3). Such increases in cell size can be explained by the results of Horiguchi et al. (2006) who found that when the number of cells decreases. A compensatory effect is observed on leaf area resulting in enlarged leaf cells.

Table 5. Upper and lower epidermis (UE, LE), palisade and spongy parenchyma (PP, SP), and blade thicknesses (BT) of leaves of purple basil (*Ocimum basilicum* L. ‘Dark Opal’) seedlings produced from explant cultures treated with 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA).

Treatment		UE	LE	PP	SP	BT
		(µm)				
Stem apexes	A0	13.95 ± 1.72^{Aa}	18.31 ± 6.81^{Ba}	85.43 ± 6.59^{Bab}	190.70 ± 85.64^{Aab}	284.51 ± 107.43^{Bb}
	A1	15.04 ± 4.85^{Aa}	16.62 ± 6.25^{Aa}	103.14 ± 28.95^{Bab}	235.45 ± 188.45^{Aab}	354.03 ± 214.91^{Aab}
	A2	15.41 ± 3.47^{Aa}	16.66 ± 6.30^{Aa}	62.03 ± 3.32^{Ab}	149.79 ± 30.61^{Ab}	223.41 ± 24.79^{Ab}
	A3	16.94 ± 3.76^{Aa}	14.85 ± 0.35^{Aa}	73.08 ± 7.74^{Ab}	152.87 ± 61.08^{Ab}	229.41 ± 55.13^{Ab}
	A4	13.88 ± 3.19^{Aa}	14.06 ± 1.52^{Aa}	102.84 ± 27.37^{Aab}	294.88 ± 79.76^{Aab}	407.79 ± 104.56^{Aab}
	A5	15.53 ± 6.13^{Aa}	14.88 ± 2.34^{Aa}	175.87 ± 60.52^{Aa}	492.88 ± 244.82^{Aa}	671.12 ± 249.38^{Aa}
	A6	10.60 ± 3.93^{Aa}	11.11 ± 2.95^{Aa}	69.97 ± 14.03^{Ab}	143.01 ± 29.84^{Ab}	228.48 ± 38.24^{Ab}
Nodal segment	S0	15.86 ± 3.43^{Aab}	32.79 ± 11.04^{Aa}	183.97 ± 27.50^{Aa}	383.97 ± 77.85^{Aab}	554.59 ± 123.54^{Aa}
	S1	20.22 ± 2.13^{Aa}	22.05 ± 5.34^{Aab}	174.76 ± 19.27^{Aa}	368.72 ± 9.35^{Aab}	540.62 ± 34.74^{Aa}
	S2	14.85 ± 0.70^{Aab}	16.55 ± 3.58^{Abc}	110.04 ± 11.50^{Aabc}	296.35 ± 55.83^{Aab}	428.79 ± 13.93^{Aab}
	S3	7.27 ± 1.30^{Bb}	7.13 ± 1.76^{Ac}	55.56 ± 23.85^{Ac}	81.54 ± 14.35^{Ab}	147.74 ± 31.38^{Ab}
	S5	15.43 ± 6.11^{Aab}	14.46 ± 2.55^{Abc}	154.48 ± 72.09^{Aab}	446.22 ± 263.01^{Aa}	589.30 ± 275.24^{Aa}
	S6	9.46 ± 2.42^{Ab}	9.44 ± 1.36^{Ac}	75.44 ± 8.90^{Abc}	87.93 ± 26.27^{Ab}	173.09 ± 30.76^{Ab}

* Within a column different uppercase letters indicate significant differences between explants and different lowercase letters indicate significant differences between phytohormone treatments (Tukey test, $p \leq 0.05$).

Legend: A - apex explant; S - segment explant; A0 and S0 - without growth regulators (control); A1 and S1 - 0.05 and 0.1 mg L^{-1} of BAP and NAA; A2 and S2 - 0.1 and 0.2 mg L^{-1} of BAP and NAA; A3 and S3 - 0.1 and 0.05 mg L^{-1} of BAP and NAA; A4 and S4 - 0.3 and 0.2 mg L^{-1} of BAP and NAA; A5 and S5 - 0.5 and 0.3 mg L^{-1} of BAP and NAA; A6 and S6 - 1.0 and 0.5 mg L^{-1} of BAP and NAA; UEP - upper epidermis; LEP - lower epidermis; PP - palisade parenchyma; SP - spongy parenchyma; E-E - distance between upper and lower epidermis.



Legend: PP - palisade parenchyma; SP - spongy parenchyma; UEP - upper epidermis; LEP - lower epidermis; Xy - xylem; Ph - phloem. Scale - 100 µM.

Figure 3. Cross-section of leaves and different types of leaves in purple basil (*Ocimum basilicum* L. ‘Dark Opal’) seedlings produced from explant cultures treated with 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). (A) A0 - apex culture without growth regulator (control); (B) A3 - apex culture treated with 0.1 and 0.05 mg L^{-1} of BAP and NAA; (C) A5 - apex culture treated with 0.5 and 0.3 mg L^{-1} of BAP and NAA; (D) S0 - segment culture without growth regulator (control); (E) S1 - segment culture treated with 0.05 and 0.1 mg L^{-1} of BAP and NAA; and (F) S5 - segment culture treated with 0.5 and 0.3 mg L^{-1} of BAP and NAA.

Conclusions

Explant type and PGR concentration influenced the morphoanatomical characteristics of basil ‘Dark Opal’ seedlings grown *in vitro*.

The use of nodal segments as explant enhanced hyperhydricity compared with the use of stem apices, 74% more than abnormality.

Shoot length, shoot fresh and dry weight and root fresh weight were three times lower in S3 compared to the same

treatment when applied at the apexLow concentrations of PGRs afforded fewer morphometric changes.

A3 treatment (stem apex treated with 0.05 and 0.1 mg L^{-1} of NAA and BAP) favored basil growth, as evidenced by the higher leaf number, shoot and root lengths. fresh weights- and dry weights. A3 also resulted in a lower incidence of abnormalities.

Insufficient or toxic concentrations of PGRs may lead to the formation of abnormal seedlings, with adventitious roots, hyperhydric leaves, and altered leaf cell structure.

Compliance with Ethical Standards

Author contributions: Conceptualization: HMM; Data curation: JRT, MSQ, MMA; Formal analysis: JRT, HMM; Funding acquisition: HMM; Investigation: JRT, MSQ, MMA; Methodology: HMM; Project administration: HMM; Resources: HMM; Supervision: HMM; Validation; HMM, JRT; Visualization: HMM, JRT; Writing – original draft: HMM, JRT; Writing – review & editing: HMM, JRT.

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Literature Cited

Antoniazzi, D.; Souza Ferrari, M.P.; Nascimento, A.B.; Silveira, F.A.; Pio, L.A.S.; Pasqual, M.; Magalhães, H. M. Growth regulators, DNA content and anatomy *in vitro*-cultivated *Curcuma longa* seedlings. African Journal of Biotechnology, v. 15, n. 32, p. 1711-1725, 2016. https://doi.org/10.5897/AJB2016.15445.

Arrigoni-Blank, M.F.; Aline V. S.; Arie, F.B. Direct organogenesis and acclimatization of patchouli. Horticultura Brasileira, v. 29, n. 2, p. 145-150, 2011. https://doi.org/10.1590/S0102-05362011000200002.

Blank, A.F.; Soares, E.M.D.S.; Paula, J.W.A.D.; Alves, P.B. Comportamento fenotípico e genotípico de populações de manjericão. Horticultura Brasileira, v. 28, n.3, p. 305-310, 2010. https://doi.org/10.1590/S0102-05362010000300011.

Dode, L.C.; Bobrowski, V. L.; Braga, E. J. B.; Seixas, F. K. Schunch, W. *in vitro* propagation of *Ocimum basilicum* L. (Lamiaceae). Acta Scientiarum. Biological Sciences, v. 25, n. 2, p. 435-437, 2003. https://doi.org/10.4025/actascibiolsci.v25i2.2034.

Ferreira, D.F. Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia, v. 35, n.6, p. 1039-1042, 2011. https://doi.org/10.1590/S1413-70542011000600001.

Guilfoyle, T.; Hagen, G.; Ulmasov, T.; Murfett, J. How does auxin turn on genes? Plant physiology, v. 118, n. 2, p. 341-347, 1998. https://doi.org/10.1104/pp.118.2.341.

Horiguchi, G.; Ferjani, A.; Fujikura, U.; Tsukaya, H. Coordination of cell proliferation and cell expansion in the control of leaf size in *Arabidopsis thaliana*. Journal of Plant Research, v. 119, n. 1, p. 37-42, 2006. https://doi.org/10.1007/s10265-005-0232-4.

Ikeuchi, M.; Sugimoto, K.; Iwase, A. Plant callus: mechanisms of induction and repression. The Plant Cell, v. 25, n. 9, p. 3159-3173, 2013. https://doi.org/10.1105/tpc.113.116053.

Ioio, R.D.; Nakamura, K.; Moubayidin, L.; Perilli, S.; Taniguchi, M.; Morita, M.T.; Sabatini, S.A genetic framework for the control of cell division and differentiation in the root meristem. Science, v. 322, n. 5906, p. 1380-1384, 2008. https://doi.org/10.1126/science.1164147.

Liu, M.; Jiang, F.; Kong, X.; Tian, J.; Wu, Z.; Wu, Z. Effects of multiple factors on hyperhydricity of *Allium sativum* L. Scientia Horticulturae, v. 217, p. 285-296, 2017. https://doi.org/10.1016/j.scienta.2017.02.010.

Manan, A.A.; Taha, R.M.; Mubarak, E.E.; Elias, H. In vitro flowering, glandular trichomes ultrastructure, and essential oil accumulation in micropropagated *Ocimum basilicum* L. In vitro Cellular & Developmental Biology-Plant, v. 52, n. 3, p. 303-314, 2016. https://doi.org/10.1007/s11627-016-9755-8.

Monfort, L.E.F.; Bertolucci, S.K.V.; Lima, A.F.; de Carvalho, A.A.; Mohammed, A.; Blank, A. F.; Pinto, J.E.B.P. Effects of plant growth regulators, different culture media and strength MS on production of volatile fraction composition in shoot cultures of *Ocimum basilicum*. Industrial Crops and Products, v. 116, p. 231-239, 2018. https://doi.org/10.1016/j.indcrop.2018.02.075.

Müller, B.; Sheen, J. Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. Nature, v. 453, n. 7198, p. 1094-1097, 2008. https://doi.org/10.1038/nature06943.

Omidbaigi, R.; Mirzai, M.; Moghadam, M.S. Chemical investigation of a new cultivar of purple basil (*Ocimum basillcum* cv. Opal) from Iran. Journal of Essential Oil Bearing Plants, v. 10, n. 3, p. 209-214, 2007. https://doi.org/10.1080/0972060X.2007.10643544.

Phippen, W. B.; Simon, J. E. Anthocyanins in basil (*Ocimum basilicum* L.). Journal of Agricultural and Food Chemistry, v. 46, n. 5, p. 1734-1738, 1998. https://doi.org/10.1021/jf970887r.

Phippen, W.B.; Simon, J.E. Shoot regeneration of young leaf explants from basil (*Ocimum basilicum* L.). In vitro Cellular & Developmental Biology-Plant, v. 36, n. 4, p. 250-254, 2000. https://doi.org/10.1007/s11627-000-0046-y.

Rademacher, W. Plant growth regulators: backgrounds and uses in plant production. Journal of Plant Growth Regulation, v.34, p.845-872, 2015. https://doi.org/10.1007/s00344-015-9541-6.

Saini, S.; Sharma, I.; Kaur, N.; Pati, P.K. Auxin: a master regulator in plant root development. Plant Cell Reports, v. 32, n. 6, p. 741-757, 2013. https://doi.org/10.1007/s00299-013-1430-5.

Sestili, P.; Ismail, T.; Calcabrini, C.; Guescini, M.; Catanzaro, E.; Turrini, E.; Fimognari. C. The potential effects of *Ocimum basilicum* on health: a review of pharmacological and toxicological studies. Expert Opinion on Drug Metabolism & Toxicology, v. 14, n. 7, p. 679-692, 2018. https://doi.org/10.1080/17425255.2018.1484450.

Shahzad, A.; Parveen, S.; Sharma, S.; Shaheen, A.; Saeed, T.; Yadav, V.; Upadhyay, A. Plant tissue culture: applications in plant improvement and conservation. In: Abdin, M.Z.; Kiran, U.; Kamaluddin, Ali, A. (Eds.). Plant Biotechnology: principles and applications. Springer, Singapore, 2017. Chap. 2, p. 37-72. https://doi.org/10.1007/978-981-10-2961-5_2.

Shukla, M.R.; Kibler, A.; Turi, C.E.; Erland, L.A.; Sullivan, J.A.; Murch, S.J.; Saxena, P.K. Selection and micropropagation of an elite melatonin rich tulsi (*Ocimum sanctum* L.) germplasm line. Agronomy, v. 11, n. 2, p. 207, 2021. https://doi.org/10.3390/agronomy11020207.

Small, C.C.; Degenhardt, D. Plant growth regulators for enhancing revegetation success in reclamation: A review. Ecological Engineering, v. 118, p. 43-51, 2018. https://doi.org/10.1016/j.ecoleng.2018.04.010.

Stanojevic, L. P.; Marjanovic-Balaban, Z. R.; Kalaba, V. D.; Stanojevic, J. S.; Cvetkovic, D. J.; Cakic, M.D. Chemical composition, antioxidant and antimicrobial activity of basil (*Ocimum basilicum* L.) essential oil. Journal of Essential Oil Bearing Plants, v. 20, n. 6, p. 1557-1569, 2017. https://doi.org/10.1080/0972060X.2017.1401963.

Toma, I.; Constantin, I.; Ghiorghita. G. Histo-anatomy and in vitro morphogenesis in *Hyssopus officinalis* L. (Lamiaceae). Acta Botanica Croatica, v. 63, n. 1, p. 59-68, 2004. https://hrcak.srce.hr/index.php?id_clanak_jezik=5619&show=clanak. 22 Aug. 2021.

Trettel, J.R.; Gazim, Z.C.; Gonçalves, J.E.; Stracieri, J.; Magalhães, H.M. Effects of copper sulphate ($CuSO_4$) elicitation on the chemical constitution of volatile compounds and the in vitro development of Basil. Scientia Horticulturae, v. 234, p. 19-26, 2018a. https://doi.org/10.1016/j.scienta.2018.01.062.

Trettel, J.R.; Nascimento, A.B.; Barbosa, L.N.; Magalhães, H.M. *In vitro* growth of genovese basil in response to different concentrations of salts and interaction of sucrose and activated carbon. Journal of Agricultural Science, v. 10, n. 9, p. 142-152, 2018b. https://10.5539/JAS.V10N9P142.

Złotek, U.; Michalak-Majewska, M.; Szymanowska, U. Effect of jasmonic acid elicitation on the yield, chemical composition, and antioxidant and anti-inflammatory properties of essential oil of lettuce leaf basil (*Ocimum basilicum* L.). Food Chemistry, v. 213, p. 1-7, 2016. https://doi.org/10.1016/j.foodchem.2016.06.052.

Zuzarte, M.R.; Dinis, A.M.; Cavaleiro, C.; Salgueiro, L.R.; Canhoto, J.M. Trichomes, essential oils and in vitro propagation of *Lavandula pedunculata* (Lamiaceae). Industrial Crops and Products, v. 32, n. 3, p. 580-587, 2010. https://doi.org/10.1016/j.indcrop.2010.07.010.