**Journal of Applied Molecular Biology (JAMB)**

**MolecularBiology Research & Studies Institute, Assiut University, Egypt.**

**ISSN 2974-4008**

**Vol. 2(1).JAN.2024**

**Journal of Applied Molecular Biology (ekb.eg)**

# Effect of Genotype and Plant Growth Regulators on Callus Formation of Sweet Basil

Israa Mansour[1], Mohamed I. Hassan[2] and Muhammad Youssef*[2]

1Applied Biotechnology Department, Molecular Biology Research and Studies Institute, Assiut University.

2Genetics Department, Faculty of Agriculture, Assiut University.

*Corresponding Author: mkhirshy@aun.edu.eg

## ARTICLE INFO

**Article History:**
Received:09-10-2023
Accepted:14-11-2023
Online: 16-01-2024

**Keywords**:
Ocimum basilicum, In vitro, Biotechnology, Tissue culture, Plant hormones .

## ABSTRACT

Plant tissue culture is one of the most efficient biotechnological techniques, which reduces time, effort, space, and finance needed for plant improvement. Here, the effect of genotype and different combinations of plant growth regulators (PGR) on the callus formation of sweet basil (*Ocimum basilicum* L.) was investigated. Twelve callus induction media, supplemented by different combinations of 6-Benzylaminoburine (BAP) and Naphthalene acetic acid (NAA), were tested with two basil landraces (i.e., Balady and French). Results showed highly significant differences among the PGR and basil landraces, as well as their interaction. Balady was superior in all evaluated traits to the French landrace. The percentage of callus formation ranged from 37.47 to 100%. The callus fresh weight per explant (CFW/exp) in Balady ranged from 159.49 to 1275.00 mg obtained by 20 and 10 µM BAP, respectively. Meanwhile, the CFW/exp for the French landrace ranged from 115.74 to 446.29 mg obtained by 10 µM NAA and 40 µM BAP + 5 µM NAA, respectively. The callus dry weight per explant (CDW/exp) for Balady ranged from 17.94 to 82.08 mg formed by 20 µM BAP and 10 µM + 10 µM NAA, respectively. On the other hand, the highest CDW/exp for the French landrace was 42.78 mg obtained by 20 µM BAP + 10 µM NAA, while the lowest CDW/exp was 4.04 mg resulted by 10 µM NAA. These results indicate that callus formation in basil is significantly influenced by both genotype and PGR. Thus, using the appropriate type and combination of PGR for each genotype can effectively enhance the callus formation rate in basil.

## INTRODUCTION

The family Lamiaceae incorporates almost 200 species of the genus *Ocimum* [1]. Basil (*Ocimum basilicum* L.) is one of this genus, which is aromatic perennial herb native to the semi-tropical and tropical regions of America, Asia and Africa [2]. Due to strong odor and sharp taste of basil it was used in food seasoning, perfumery, cosmetics industry [3]. In addition, it is being used in traditional medicine, in the treatment of migraine, dysentery, alimentary stoppage, renal failure, cough, verruca, worms [4], cold, fever, abdominal pain, tension, rheumatoid, snake and insect bites and heat stroke [5]. The plant has integral part in pharmaceutical industry, thus it was used in synthetic drugs and antibiotics [6]. Basil is a good source of secondary metabolites [7]. Its leaf surface has glandular trichomes [8], which produce medicinal-valued compounds like terpenoids and phenylpropanoids such as anthocyanins, caffeic acid, chicoric acid and rosmarinic acid [9]. The most vital compounds are linalool, 1,8-cineole, methylchavicol (estragole) and eugenol. Moreover, basil is a good source of essential oil which has anticancer, hypoglycemic, tuberculosis [10], antifungal [11], antibacterial [12], and antioxidant activities [4].

The conventional method for propagation of basil is by seeds. However, it is extensive, has unregulated collection, some species may be threatened or endangered [13]. The poor germination potential limits its multiplication, and the cross-pollination produces seed progenies not true to type [14]. Recently, plant tissue culture has become an efficient replacement of conventional culture, because the possibility of control and improve the quality and quantity of the related compounds, and the rapid increase of the plant scale propagation [15]. It produces identical offspring to the parent [16], in thus plant tissue culture produces metabolities free from environmental limitations [17]. Media with plant growth regulators are used for callus induction. Auxins have a biovital role in cell elongation and cell division [18]. Also, auxins in combination with cytokinins produce a huge number of small and undifferentiated cells [16]. Therefore, the objective of the current study was to investigate the effect of genotype and plant growth regulators on the callus formation in sweet basil.

## MATERIALS AND METHODS

### 1. Plant materials

This study was done at the Plant Biotechnology Laboratory, Molecular Biology Research and Studies Institute, Assiut University. Seeds of the two landraces of basil (i.e., Balady and French) were donated from Department of Ornamental Plants, Faculty of Agriculture, Assiut University.

### 2. Explant preparation

Seeds from the two basil landraces were washed under tap water for half an hour. Then, they were transferred to laminar flow hood for sterilization using 30% commercial bleach (5% NaOCl) for 20 minutes. Seeds were then washed two times by sterilized distilled water, followed by a treatment with 70% ethanol for one minute. Subsequently, seeds were washed four times with sterilized distilled water to be cultured upon germination

medium. For callus induction, leaves of one month old seedlings were separated and resized to 0.5 cm × 0.5 cm and placed upon callus induction media.

## 3. Culture media

Germination medium was consisted of free growth regulators full strength Murashige and Skoog (MS) (Lab Egypt Company). For callus induction full strength MS medium was used supplemented with different combinations of NAA (naphthalene acetic acid) and BAP (6-benzylaminopurine) (Table 1). For all media, 30g sucrose and 8 g agar were added. pH was adjusted to 5.8 using NaOH and HCl, media were then autoclaved at 121°C for 20 minutes. Cultures were incubated under 25± 1°C and 16/8 hours fluorescent light regime.

## 4. Effect of plant growth regulators on basil callus induction

After 45 days of callus induction, some parameters were recorded, including percentage of callus formation (%CF, as the number of explants gave callus divided by the total number of explants), callus fresh (FW) and dry (DW) weight and total water content using this formula: TWC = (FW - DW) / FW × 100.

## 5. Experimental design and statistics

Callus induction experiment was designed using a complete randomized design (CRD). Four explants of leave segments were placed in each 200 ml culture jar. Three replicates were used for each growth regulators combination, each replicate was consisted of four jars. The total number of experimental units was 1152 (2 landraces × 12 combinations × 3 replicates × 4 jars × 4 explants = 1152). Analysis of variance (factorial analysis) was performed using MSTATC software. Duncan's least range test was used for mean comparison.

## RESULTS

Two sweet basil landraces (i.e., Balady and French) were used in this study to investigate the effect of PGR on callus induction. MS media were utilized supplemented with various combinations of BAP and NAA. After 45 days the data was registered.

## 1.Seed germination:

Seeds of the two landraces were germinated on PGR-free MS solid media. After 30 days the percentage of germination was 80% in the two landraces (**Fig. 1**).


**Figure (1):** Seed germination of *Ocimum basilicum*, **(a)** Balady and **(b)** French landraces.

## 2.Callus induction:

Leaves of *in vitro* grown plants were separated, resized, and cultured on MS medium supplemented with various concentrations of BAP in combination with various concentrations of NAA to induce callus. Analysis of variance showed highly significant differences among the PGR combinations and between the two landraces, as well as the interaction between them in all traits (data not shown). After 45 days it was noted that MS media without PGR didn't lead to any callus formation. However, the combinations of BAP and NAA produced callus formation different in callus fresh weight (CFW), callus dry weight (CDW), and total water content (TWC) (**Fig. 2 and 3**).

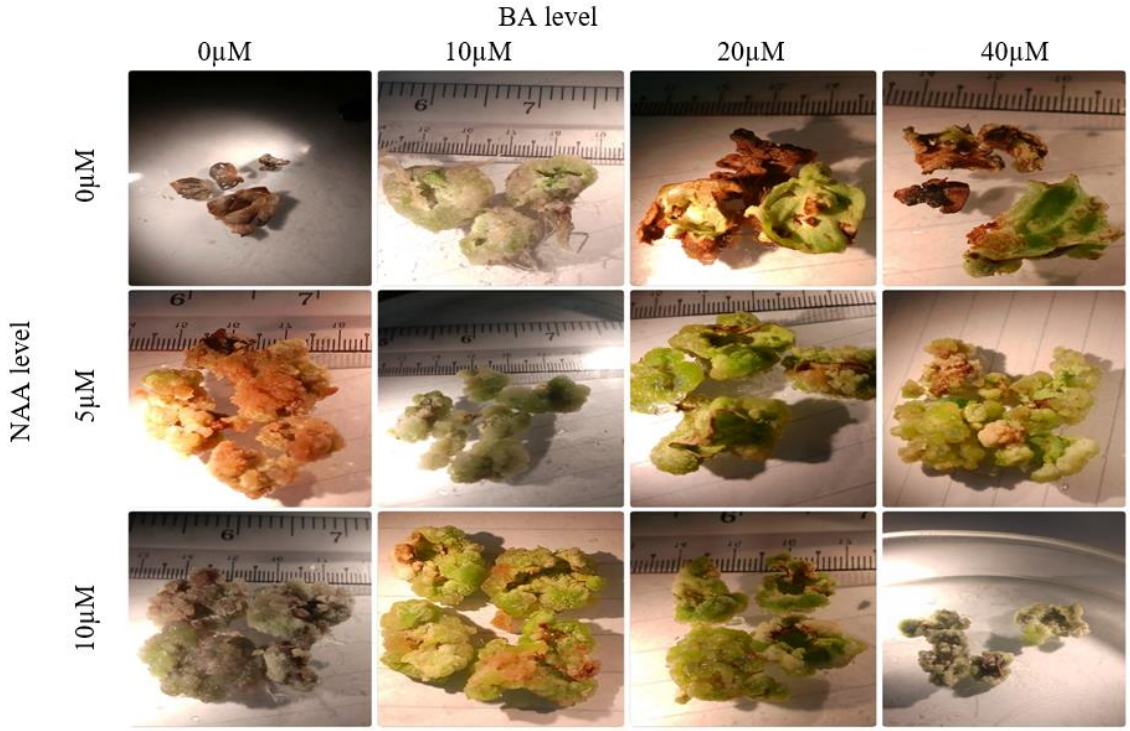


**Figure (2):** Effect of various combinations of BAP and NAA on callus induction of Balady landrace.

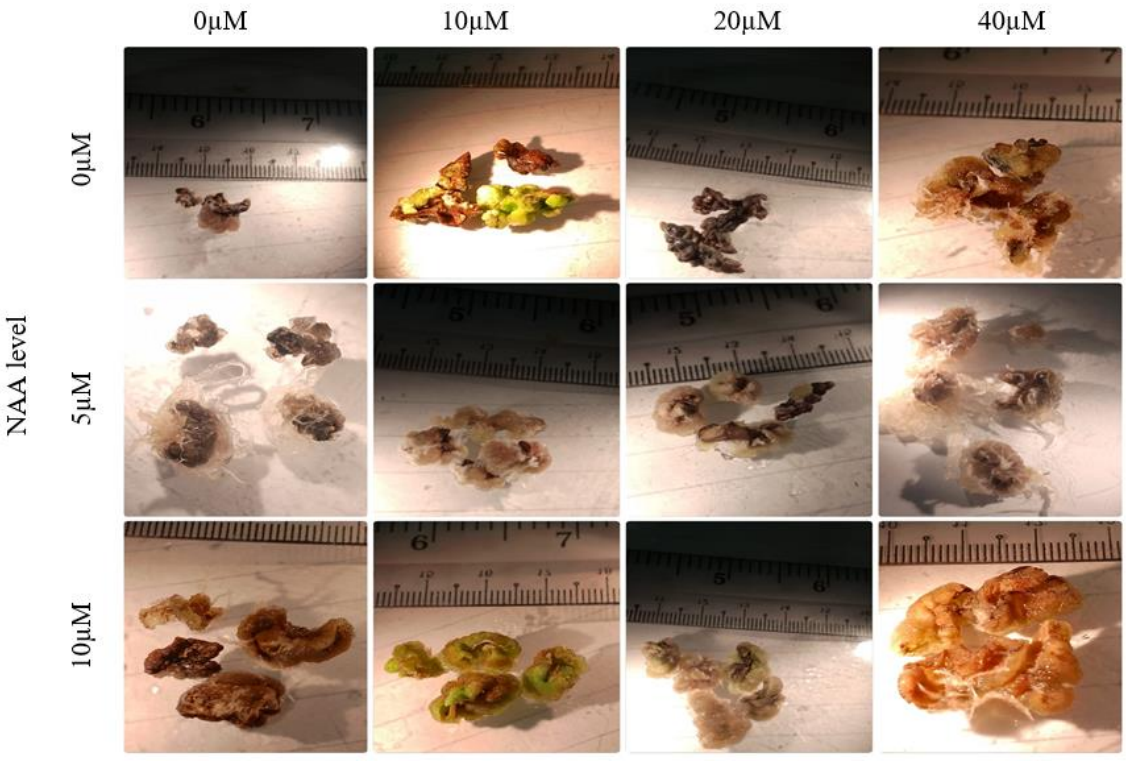


**Figure (3):** Effect of various combinations of BAP and NAA on callus induction of French landrace.

## 3.The effect of PGRs on percentage of callus formation (CF%) of the two basil landraces

Different combinations of PGRs widely affected the percentage of callus formation. Also, there was a difference in response of the two landraces on the same media as shown in figures 2 and 3. In this regard, no callus formation was observed in the PGR-free medium for both landraces. However, among media containing different PGR combinations the lowest CF% for Balady landrace was 37.5% obtained from M7 (20 μM BAP), followed by 50 and 82% obtained from M10 (40 μM BAP) and M11 (40 μM BAP + 5 μM NAA). All other media were able to give 100% callus formation. On the other hand, for French landrace the medium M11 gave the lowest CF% (60%), followed by M7 (67%), M12 (75%), M3, M8 (86%) and M10 (87%), while the percentage 100% was obtained from other media (M2, M4, M6 and M9) (**Table 2**).

## 4.The effect of PGRs on callus fresh weight (CFW):

There was a difference in response of the two landraces on callus induction medium. It was noted that the response of Balady was more than French. The high concentration of BAP produced Balady callus with low FW. The lowest CFW of Balady was 159.47 mg obtained from M7 followed by M10 which have CFW 272.2 mg. While the highest mass accumulation (1274.9 mg) was obtained from M4. Other media, i.e., M12, M9, M11, M2, M8, M3, M5, and M6 have 288.8, 460.4, 640.2, 819.4, 888.8, 1104.1, 1212.5, 1218 mg, respectively for one callus. But in French landrace, the high concentration of BAP in combination with NAA produced high CFW mass. In this regard, high CFW was obtained from M11 which gave 446.2mg, followed by M4, M12, M8, M6, M5 and M9 with 340, 321.4, 304.1, 295.8, 291.6 and 286.1mg, respectively. However, the high concentration of BAP without addition of NAA produced slightly low CFW (M7 and M10 with 161.1 and 200mg, respectively). The lowest CFW obtained from NAA without BAP (M2 and M3) produced 136.1 and 115.7 mg, respectively (**Figure 4**).

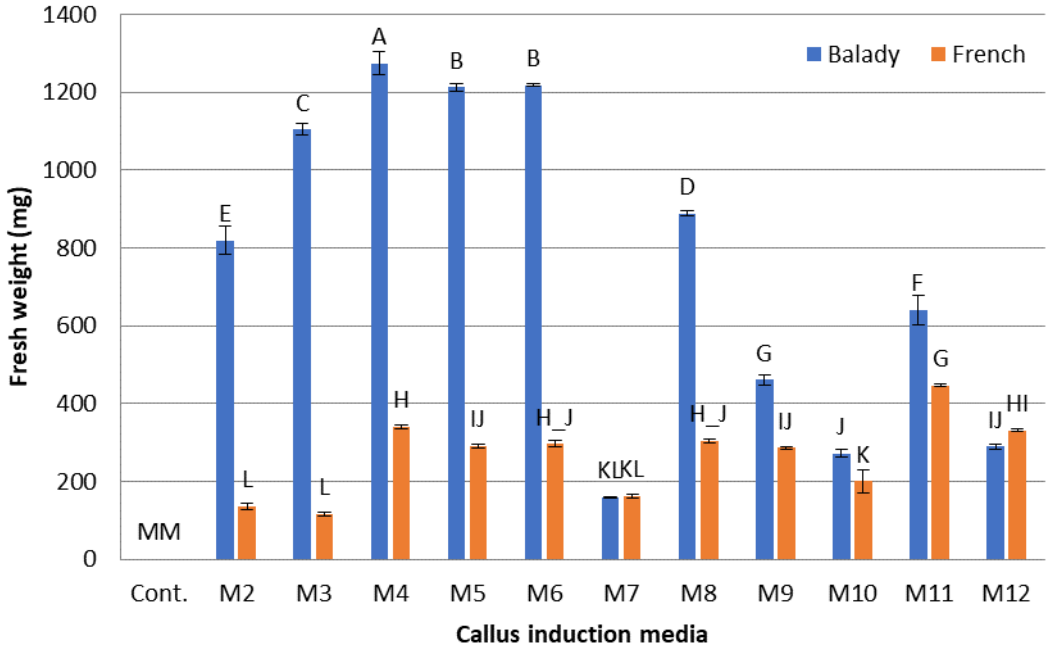


**Figure (4):** callus fresh weight (CFW) of two basil landraces on different medium. Different letters indicate significant differences, Duncan's test (n=3, $\alpha$=0.05).

## 5.The Effect of PGRs on Callus Dry weight CDW of two Landraces

It was noted that the combination of BAP and NAA widely affected callus dry weight. In this regard, Balady landrace was superior in all evaluated traits than the french landraces. The highest Balady CDW was 82.00 mg which obtained from M6 followed by M4, M5, M3, M8, M2, M11, M9, M10 and M12 which have 77.4, 76.8, 69.9, 67.7, 62.6, 55.8, 36.0, 29.9, and 28.8 mg, respectively for one callus. The lowest dry weight mass obtained from M7 showed 17.9 mg for one callus. But in the French genotype the highest dry weight is obtained from M9 which have 42.77 mg for one callus. While the lowest dry weight was obtained from M3 which have 4.0 mg CDW. The other media, i.e., M11, M8, M6, M5, M12, M4, M10, M7 and M2 gave values of 36.4, 33.4, 28.54, 28.52, 28.4, 23.4, 21.6, 13.3 and 6.0 mg, respectively (**Figure 5**).

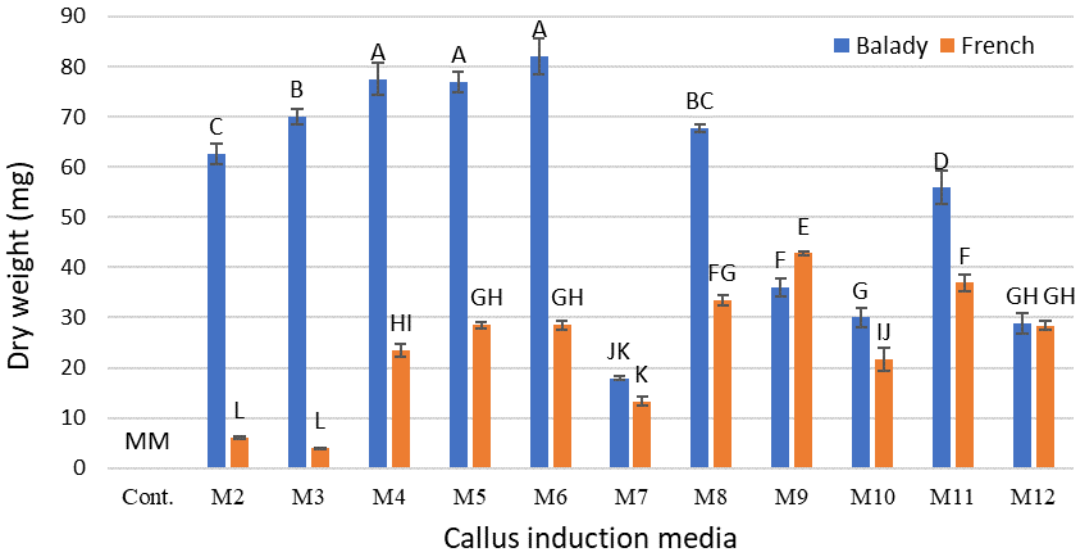


**Figure (5):** callus dry weight of the two basil landraces on different media. Different letters indicate significant differences, Duncan's test (n=3, $\alpha$=0.05).

## 6.The Effect of PGRs on Total water content TWC of Callus of two Landraces

The percentage of water on one callus of balady genotype ranged from 88.7 to 93.9% and were obtained from M7 and M4, respectively. While the other media, i.e., M3, M5, M6, M8, M2, M9, M11, M12 and M10 gave percentages of total water content of 93.66, 93.65, 93.26, 92.37, 92.34, 92.14, 91.28, 90.00 and 88.9%, respectively. But in the French landrace, the highest percentage of TWC was 96.4% obtained from M3, and the lowest percentage was 85% produced by M9. While the other media have the percentages of 95.5, 93.1, 91.7, 91.6, 91.4, 90.3, 90.2, 89.00 and 88.90% obtained by M2, M4, M11, M7, M12, M6, M5, M10 and M8, respectively (**Figure 6**).

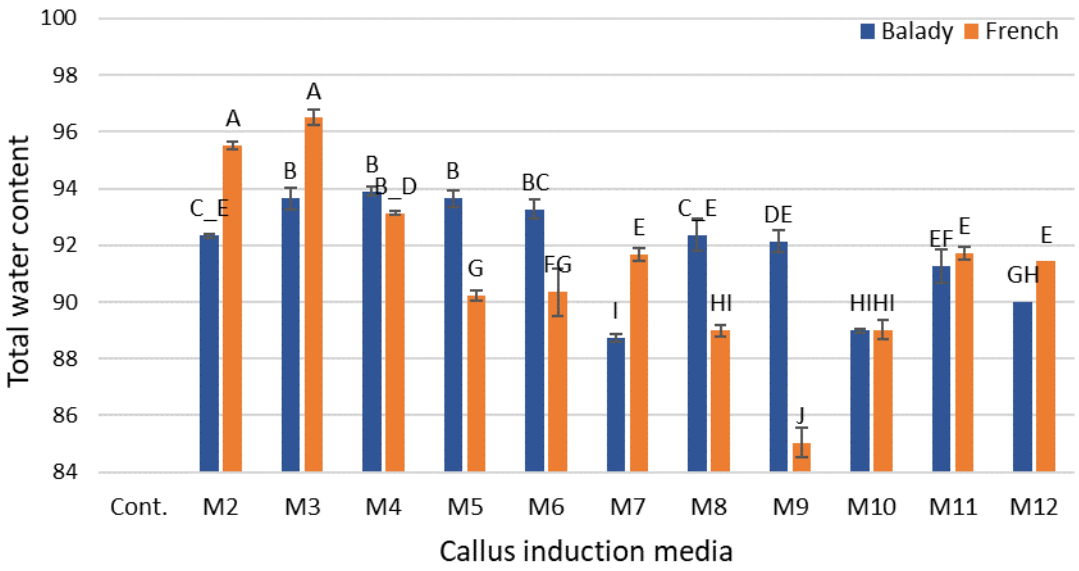


**Figure (6):** Total water content (%) of the two basil landraces on different media. Different letters indicate significant differences, Duncan's test (n=3, $\alpha$=0.05).

## DISCUSSION

Field production always has several associate problems mainly related to environmental uncontrolled conditions which cause yield loss and affect its quality. Also, plant cell and tissue culture technologies provide other technique to overcome such problems and promotes their conservation [19]. These technologies can be considered as a vital, maintainable, and affordable biomass source to produce high-value phytochemicals [20]. Biotechnologically valuable plant characteristics, as secondary metabolites can be easily obtained with high content and in a short time [21]. In the present study, leaf segments of two basil landraces were used to induce callus. Various combinations of growth regulators were evaluated. Results indicated that the callus formation depended mainly on genotype and types and concentration of plant growth regulator.

The action of plant growth regulators (e.g., cytokinin and auxin) is required for plant growth and developmental processes [22]. Benzyl aminopurine (BAP) is one of the important cytokinin-like growth regulators, affects *in vitro* shoot length and multiplication [23]. Also, naphthalene acetic acid (NAA) is a source of auxin, which has an important role in regulating processes on all plant levels, such as tropisms, apical dominance and root initiation, and have a role on cellular level like cell enlargement, division, and differentiation [24]. In the present study, there was a difference in response of the two genotypes (Balady and French) on the same medium, and concentration of BAP and NAA effected widely on callus fresh weight (CFW) and callus dry weight (CDW). However, Both genotypes didn't expose any callus formation in PGRs free media. These findings are in agreement with those of Wongsen *et al*. [25]. In addition, Mendoza and Kaeppler [26] reported that media supplemented with PGRs (auxin and cytokinin) would enhance callus induction effectively. Combining cytokinin with auxin is

vital for callus induction, and this was proved by the results of the current study, whereas the lowest percentage for callus induction (0.0%) was obtained by free PGRs media, but the percentage reached the maximum (100%) by the supplementation of BAP and NAA. Accordingly, Ibrahim *et al*. [27] reported that, combination of BAP and NAA is the best to induce callus. In this regard, they stated that medium with 5mg/l BAP and 1mg/l NAA was found to enhance callus production [28]. Mishra [29] found that 2,4-D with concentrations of 1, 3 and 5 mg/l produced white, light greenish, compact callus. Also, Osman *et al*. [16] found that medium supplemented with BA and 2,4-D produced yellowish, friable callus, easy to separate from the leaf explant. Furthermore, Asghari *et al*. [18] proved that the source explants have been played an effective factor for callus induction.

In the present study the high concentration of BAP without combination with NAA leads to low callus formation with low fresh and dry weight for Balady genotype. However, combination of cytokinin with auxin increased the callus fresh and dry weight. On the other hand, for French genotype, the high concentration of NAA alone had low FW and DW, but a high concentration of BAP combined with NAA caused high CFW and CDW. These results indicated that genotype affected widely on CFW and CDW, whereas the Balady genotype responded more than Frensh genotype. Other studies reported the same indication with different types of growth regulators [30].

## CONCLUSION

In conclusion, the protocol established here presents an effective tool for callus induction for basil. The callus formation affected mainly by genotype and plant growth regulators. The good combined phytohormones for suitable genotype led to the best callus formation. This biotechnological tool can help in improving basil production as well as the bio-production of its secondary metabolites, which could enhance the yield and quality of the product.

## REFERENCES

[1] J.E. Simon, M.R. Morales, W.B. Phippen, R.F. Vieira, Z. Hao, Basil: A source of aroma compounds and a popular culinary and ornamental herb, Perspectives on new crops and new uses 16 (1999) 499-505.
[2] Y. Sahoo, S. Pattnaik, P. Chand, In vitro clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L.(sweet basil) by axillary shoot proliferation, In Vitro Cellular & Developmental Biology-Plant 33 (1997) 293-296.
[3] I. Siddique, M. Anis, Rapid micropropagation of *Ocimum basilicum* using shoot tip explants pre-cultured in thidiazuron supplemented liquid medium, Biologia Plantarum 51 (2007) 787-790.
[4] S.-J. Lee, K. Umano, T. Shibamoto, K.-G. Lee, Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties, Food chemistry 91 (2005) 131-137.
[5] M. Kasem, Micropropagation and In Vitro Secondary Metabolites Production of Ocimum Species. Review Article, Journal of Plant Production 8 (2017) 473-484.
[6] M. Fowler, Commercial applications and economic aspects of mass plant cell culture, Seminar series-Society for Experimental Biology, 1983.

[7] W.B. Phippen, J.E. Simon, Anthocyanins in basil (*Ocimum basilicum* L.), Journal of Agricultural and Food Chemistry 46 (1998) 1734-1738.
[8] Y. Iijima, R. Davidovich-Rikanati, E. Fridman, D.R. Gang, E. Bar, E. Lewinsohn, E. Pichersky, The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil, Plant physiology 136 (2004) 3724-3736.
[9] D.R. Gang, J. Wang, N. Dudareva, K.H. Nam, J.E. Simon, E. Lewinsohn, E. Pichersky, An investigation of the storage and biosynthesis of phenylpropenes in sweet basil, Plant physiology 125 (2001) 539-555.
[10] K. Poonkodi, Chemical composition of essential oil of *Ocimum basilicum* L.(Basil) and its biological activities-an overview, Journal of Critical Reviews 3 (2016) 56-62.
[11] J.-W. Zhang, S.-K. Li, W.-J. Wu, The main chemical composition and *in vitro* antifungal activity of the essential oils of *Ocimum basilicum* Linn. var. pilosum (Willd.) Benth, Molecules 14 (2009) 273-278.
[12] R.C. Padalia, R.S. Verma, A. Chauhan, P. Goswami, C.S. Chanotiya, A. Saroj, A. Samad, A. Khaliq, Compositional Variability and Antifungal Potentials of *Ocimum basilicum*, *O. tenuiflorum*, *O. gratissimum* and *O. kilimandscharicum* Essential Oils against *Rhizoctonia solani* and *Choanephora cucurbitarum*, Natural Product Communications 9 (2014) 1934578X1400901026.
[13] R. Arora, S.S. Bhojwani, *In vitro* propagation and low temperature storage of Saussurea lappa CB Clarke—an endangered, medicinal plant, Plant Cell Reports 8 (1989) 44-47.
[14] V.H. Heywood, Flowering plants of the world, BT Batsford Ltd., 1993.
[15] Y. Bajaj, M. Furmanowa, O. Olszowska, Biotechnology of the micropropagation of medicinal and aromatic plants, Medicinal and aromatic plants I (1988) 60-103.
[16] A. Osman, A.I. El-Kadafy, E. Sewedan, M. Moubarak, M. Abdel-Rahman, The effect of polyethylene glycol (PEG) on calluses of sweet basil (*Ocimum basilicum* L.), Scientific Journal of Flowers and Ornamental Plants 7 (2020) 447-459.
[17] R. Khurshid, T. Khan, A. Zaeem, L. Garros, C. Hano, B.H. Abbasi, Biosynthesis of precious metabolites in callus cultures of *Eclipta alba*, Plant Cell, Tissue and Organ Culture (PCTOC) 135 (2018) 287-298.
[18] F. Asghari, B. Hossieni, A. Hassani, H. Shirzad, Effect of explants source and different hormonal combinations on direct regeneration of basil plants (*Ocimum basilicum* L.), Australian Journal of Agricultural Engineering 3 (2012) 12-17.
[19] N. Nabi, S. Singh, P. Saffeullah, Responses of in vitro cell cultures to elicitation: Regulatory role of jasmonic acid and methyl jasmonate: A review, In Vitro Cellular & Developmental Biology-Plant 57 (2021) 341-355.
[20] M. Nazir, M. Asad Ullah, S. Mumtaz, A. Siddiquah, M. Shah, S. Drouet, C. Hano, B.H. Abbasi, Interactive effect of melatonin and UV-C on phenylpropanoid metabolite production and antioxidant potential in callus cultures of purple basil (*Ocimum basilicum* L. var purpurascens), Molecules 25 (2020) 1072.
[21] D. Jakovljević, M. Stanković, M. Warchoł, E. Skrzypek, Basil (*Ocimum* L.) cell and organ culture for the secondary metabolites production: A review, Plant Cell, Tissue and Organ Culture (PCTOC) 149 (2022) 61-79.
[22] A. Bajguz, A. Piotrowska, Conjugates of auxin and cytokinin, Phytochemistry 70 (2009) 957-969.

[23] C.K. Yew, B. Balakrishnan, J. Sundasekaran, S. Subramaniam, The effect of cytokinins on *in vitro* shoot length and multiplication of Hymenocallis littoralis, J Med Plants Res 4 (2010) 2641-2646.
[24] G. Hagen, T. Guilfoyle, Auxin-responsive gene expression: genes, promoters and regulatory factors, Plant molecular biology 49 (2002) 373-385.
[25] W. Wongsen, K. Bodhipadma, S. Noichinda, D. Leung, Influence of different 2, 4-D concentrations on antioxidant contents and activities in sweet basil leaf-derived callus during proliferation, International Food Research Journal 22 (2015).
[26] M.G. Mendoza, H.F. Kaeppler, Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of wheat (*Triticum aestivum* L.), In Vitro Cellular & Developmental Biology-Plant 38 (2002) 39-45.
[27] M.M. Ibrahim, N. Danial, M.K. El-Bahr, Ethanolic extract of sweet basil callus cultures as a source of antioxidant and sun-protective agents, Egyptian Pharmaceutical Journal 22 (2023) 78.
[28] A. Elsaadany, In-vitro production of rosmarinic acid from basil (*Ocimum basilicum* L.) and Lemon Balm (Melissa officinalis L.), Sciences 5 (2015) 47-51.
[29] S. Mishra, Family environment and achievement motivation of school going adolescents: An intervention report, Lulu. com, 2015.
[30] E. Enkhbileg, M. Fári, E. Kurucz, In vitro effect of different cytokinin types (BAP, TDZ) on two different *Ocimum basilicum* cultivars explants, International Journal of Horticultural Science 25 (2019) 15-20.

Table (1): Composition of different combinations of growth regulators used for callus induction.

| Medium | BAP (μM) | NAA (μM) |
|---|---|---|
| Control | 0 | 0 |
| M2 | 0 | 5 |
| M3 | 0 | 10 |
| M4 | 10 | 0 |
| M5 | 10 | 5 |
| M6 | 10 | 10 |
| M7 | 20 | 0 |
| M8 | 20 | 5 |
| M9 | 20 | 10 |
| M10 | 40 | 0 |
| M11 | 40 | 5 |
| M12 | 40 | 10 |

**Table (2):** Effect of different concentrations of BAP and NAA on percentage of callus formation CF% of the two basil landraces.

| Cultivars | Medium | PGR (μM) | CF% |
|---|---|---|---|
| Balady | Control | 0 BAP + 0 NAA | $0.00^{G} \pm 0.00$ |
| | M2 | 0 BAP + 5 NAA | $100^{A} \pm 0.00$ |
| | M3 | 0 BAP + 10 NAA | $100^{A} \pm 0.00$ |
| | M4 | 10 BAP + 0 NAA | $100^{A} \pm 0.00$ |
| | M5 | 10 BAP + 5 NAA | $100^{A} \pm 0.00$ |
| | M6 | 10 BAP + 10 NAA | $100^{A} \pm 0.00$ |
| | M7 | 20 BAP + 0 NAA | $37.5^{F} \pm 0.23$ |
| | M8 | 20 BAP + 5 NAA | $100^{A} \pm 0.00$ |
| | M9 | 20 BAP + 10 NAA | $100^{A} \pm 0.00$ |
| | M10 | 40 BAP + 0 NAA | $50^{E} \pm 0.00$ |
| | M11 | 40 BAP + 5 NAA | $82^{BC} \pm 0.03$ |
| | M12 | 40 BAP + 10 NAA | $100^{A} \pm 0.00$ |
| French | Control | 0 BAP + 0 NAA | $0.00^{E} \pm 0.00$ |
| | M2 | 0 BAP + 5 NAA | $100^{A} \pm 0.00$ |
| | M3 | 0 BAP + 10 NAA | $86^{B} \pm 0.01$ |
| | M4 | 10 BAP + 0 NAA | $100^{A} \pm 0.00$ |
| | M5 | 10 BAP + 5 NAA | $96^{A} \pm 0.02$ |
| | M6 | 10 BAP + 10 NAA | $100^{A} \pm 0.00$ |
| | M7 | 20 BAP + 0 NAA | $67^{D} \pm 0.08$ |
| | M8 | 20 BAP + 5 NAA | $86^{B} \pm 0.01$ |
| | M9 | 20 BAP + 10 NAA | $100^{A} \pm 0.00$ |
| | M10 | 40 BAP + 0 NAA | $87^{B} \pm 0.07$ |
| | M11 | 40 BAP + 5 NAA | $60^{D} \pm 0.04$ |
| | M12 | 40 BAP + 10 NAA | $75^{C} \pm 0.00$ |

Different letters indicate significant differences, Duncan's test (n=3, $\alpha$=0.05)