UDC 575.630
https://doi.org/10.2298/GENSR2101379S
Original scientific article

EVALUATION OF DIFFERENT GROWTH HORMONES MEDIATED CALLUS INDUCTION AND REGENERATION AS WELL AS THE EFFECT OF COLCHICINES, ETHYL METHANESULFONATE (EMS) AND GAMMA RADIATION ON SOME TRAITS OF *Impatiens walleriana*

Sedigheh Sadat SHARAFI[1], Pejman AZADI[2], Maryam JAFARKHANI KERMANI[3], Vahid ABDOSSI[1], Ali ESKANDRI[4]

[1]Department of Horticultural Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Genetic Engineering, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran
[3] Department of Tissue and Cell Culture, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran
[4]Department of Nuclear Agriculture, Karaj, Iran

Sharafi S. S., P. Azadi, M. Jafarkhani Kermani, V. Abdossi, A. Eskandri (2021). *Evaluation of different growth hormones mediated callus induction and regeneration as well as the effect of colchicines, ethyl methanesulfonate (EMS) and gamma radiation on some traits of Impatiens walleriana*. - Genetika, Vol 53, No.1, 379-391.

Impatiens is an ornamental member of family Balsaminaceae. This plant mostly propagated by vegetative technique, which generally time wasting process. It is often multiplicities via seed but is barricaded by F_1 seed sterility. *In vitro* culture of *Impatiens walleriana* has much significant function in fast proliferation with useful features and elicitation of healthful and disease-free plants. This experiment was conducted to investigate the impact of medium and different hormones on *in vitro* propagation of *Impatiens walleriana* by using a completely randomized design. MS medium was prepared along with various concentrations of BAP, TDZ and ZEA. Callus was induced and grew well in media supplemented with 0.5 mg/l NAA + 1 mg/l BAP. In order to indirect propagation, explants were cultured in same media containing BAP, ZEA and TDZ in combination with NAA. These treatments have ability to organogenesis. The results revealed that the control treatment had the lowest effect on traits including shoot percentage, number of shoots, number of leaves, shoot length, fresh and dry weight, and it lead to maximum proliferations in medium supplemented with 0.5 mg/l NAA + 1 mg/l BAP. The highest root length and rooting percentage was observed in 0.5 mg/l IBA + 0.5 mg/l BAP. In addition, the effect of mutation agents was studied. Aseptic samples were

Corresponding author: Pejman Azadi, Department of Genetic Engineering, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran

treated with γ- irradiation, Ethyl Methane Sulfonate and colchicines at growth chamber. Treatments with 30 and 60 grey respectively had the lower survival rate, growth rate and polyploidy while colchicines with 0.1 and 0.2 had the highest rats. Regarding to these, the present technique illustrate an effective system for *in vitro* reproduction of *Impatiens walleriana* by hypocotyls cultures. In addition, colchicines proved to be effective in induction of polyploidy in this plantlet.

Keywords: Colchicines, Ethyl methanesulfonate, Gamma radiation, *Impatiens walleriana*, Tissue culture

Abbreviation: NAA: α-Naphthalene acetic acid; BAP: 6-Benzylaminopurine; TDZ: Thidiazuron; ZEA: Zeatin; IBA: Indole-3-butyric acid; MS medium: Murashige and Skoog medium

INTRODUCTION

In last 20 years, Indian flora industry stepped into international market and the area under flower cultivation has been expanding continuously. International floriculture trade is US$ 17 billion, which is increasing 10–15 % annually and is expected to reach US$ 25 billion by 2025 (REDDY *et al*., 2007). In recent years, various strategies have been used to reproduce ornamental plant species that can help growers respond to customer needs. *Impatiens walleriana* (Busy Lizzie) belongs to the family Balsaminaceae. It is widely used in green space designing and hanging baskets and is commercially important for flowering particularly during the day at summer months. It is also valuable for its medicinal properties and has been used for gastric disorders, nausea, abdominal pain, nerve pain relief and in bronchitis (GETTER and BEHE, 2013). Therefore, propagation via sexual seeds or cutting is asexual reproduction. Virus was transmitted through vegetative propagation of *Impatiens walleriana*. These plants are particularly susceptible to infection with Impatiens Necrotic Spot Virus (INSV) and Impatiens Downy Mildew (IDM) virus. Sexual reproduction produces offspring resulting in offspring genetically different from the parent or parents, which flowers will not grow the same as their parent plants quality (LIM, 2014). Tissue culture is an important method of plant regeneration and achieving disease-free ornamental plants. Many commercial ornamental plants are being reproduced by *in vitro* culture technique in the medium with growth regulator (ROUT *et al*., 2006).

The use of ornamental plants in the landscape design projects and the increasing demand long-term action for new varieties have enabled the breeders to plan new varieties with attractive color, fragrance, longevity, marketability of horticulture crops and durable resistance against bio stress. In general, diversity is a principal principle in any breeding score strategy program, and plant breeding relies upon collection, generation of new varieties, selection, evaluation and reproduction. Induction of polyploidy was widely recognized as a useful technique for plant improvement with less time and labor consuming. Each plant may respond differently to polyploidy stimulation, associated with the determinate plant's basic ploidy rate, genome combination, multiplication state, propagation, perennial or annual types habit, or plant organ (MASON, 2016). For *I. walleriana*, the normal ploidy level of diploid cells has been shown to be x= 8 and 2n = 2x = 16 (ZINOV'EVA-STAHEVITCH and GRANT, 2014). Chemical mutagenic agents such as colchicine and Ethyl Methane Sulfonate are more readily

available than physical and may be less costly but due to their low permeability and mutation rate, their use in increasing species diversity and production of mutant cultivars was less extended (DEFIANI *et al*., 2014). Seeds treated with 0.1% aqueous colchicine solution for 10 min proved to be effective in induction of polyploidy in *Impatiens balsamina* (MANAWADU and NILANTHI DAHANAYAKE, 2014). Gamma irradiation stimulated polyploidization in (*Impatiens balsamina* L.) seedling (DEFIANI *et al*., 2017). Morphological features indicated that improved plants treated by 60 Gy irradiation possessed the greater level in fresh weigh, leaf area, and stem length in comparison with control and 30 Gy ray treated seedling of *Catharanthus roseus* L (NOORMOHAMMADI *et al*., 2020).

The seed germination of two French bean cultivars reduces with increment of concentration of EMS from 0.15 to 0.35%. Various useful mutants were chosen in M2 generation for early flowering, pods number and length of pod length in both the cultivars at several concentrations of EMS but highest rate of desirable mutants was seen in EMS at 0.15%. It improved number of pods per plant which may be directly linked to yield and it will finally raise the total yield. The mutant population in M2 generation can be better applied to survey its performance in next generation (RAMANDEEP *et al*., 2018).

This research could be used as plant material to improve *Impatiens walleriana* production in order to achieve a protocol for obtaining virus-free plants and to develop new varieties through mutagenic agents to increase marketability and customer attraction.

MATERIALS AND METHODS

This study has been performed in the tissue culture laboratories of the Islamic Azad University, Tehran Sciences and research Branch, with the following treatments in two stages of propagation and induction of mutation.

First experiment (Plant regeneration, callus induction and rooting via in vitro culture)
Sterilization of shoot explants: The hypocotyls explants were surfaces disinfection with ethanol 70% (40 seconds) and then sodium hypochlorite 2% (10 minutes), followed by rinsing three times with sterile distilled water.

Media: Media in this experiment were Murashige and Skoog medium, which were improved with 30 g/l sucrose and 7 g/l agar. Growth hormones regulator depending on each experiment was added to the medium. Glass jars contain each 20 ml media and pH was adjusted with NaOH 0.1 N or HCL 0.1 N before autoclaving. Autoclaving was carried out in 1.05 kg/cm and 121°C (15 minutes).

Callus induction and plant regeneration: In order to callus formation, hypocotyls explants were transferred the MS medium 0.5 mg/l NAA containing (0, 0.1, 0.5, 1 mg/l) BAP, TDZ and ZEA, in (1200 Lux), 8/16 h condition. Callus induction percentage and the volume callus induction were noted. The model used to estimate the volume of callus is according to (GANJI DASTJERDI *et al*., (2009). Then explants were planted to the MS medium with same hormones. The number, length and percentage of shoot proliferation, number of leaves, and fresh/dry weight of shoot (with digital scale) were recorded.

Rooting: Shoots were cultured to the MS medium supplemented with (0, 0.1, 0.5, 1 mg/l) IBA and (0.1, 0.5, 1 mg/l) IAA and roots percentage induction, fresh/dry weight of roots were recorded. Root length (with Collis) and rooting percentage were measured.

1. *Second Experiment (Mutation treatment):*

Ethyl Methane Sulfonatex (EMS) treatment: The explants were treated with EMS (in concentrations (0, 0.005, 0.05, and 0.1 %). Aseptic samples were transferred to the growth chamber under the laminar hood. Sterilize leaves samples scratched by pence so EMS penetrated through the wounds. The tube door was quickly closed with a parafilm. After the 48 h, they were placed under laminar hood and rinsing three times with sterile distilled water.

Colchicines treatment: Colchicines stock solution sterilized with the 0.2-micron 4-mm diameter filter were added in concentration of (0, 0.05, 0.1, and 0.2 %). Mutation agent used to promote polyploidy induction. After 24 hours, they were sterilized with distilled water and transferred to MS culture medium without colchicines.

Gamma radiation treatment: The selected explants are placed in a wet bag for 48 hours at 20-22 °C to stimulate the cell proliferation and meristematic growth. They were packed and irradiated with cobalt 60 with doses (0, 30 and 60 grey) at the Atomic Energy Center of Iran.

Plants adaptations: After optimizing sterilization, explants were cultured on MS medium. Plants were transferred in growth chambers to provide constant, reproducible conditions for further studies. pots contained (75% peat moss + 25% cocopeat), with controlled temperature and 75% relative humidity at 25 °C and 3000 Lux for 20 days. In the next step, the adapted plants were placed in the greenhouse, exposed to 12°C, 16 / 8 hours. Plants growth rate, polyploidy percentage, and survival rate were measured.

Statistical analysis: This research was done as completely randomized design with three replicates. The data were analyzed to Analysis of variance (ANOVA), Duncan's test was calculated for mean comparison at 1% by using SPSS 19 software, and the charts were drawn in Microsoft Office Excel 2013.

RESULTS

1. *Plant regeneration, callus induction and rooting via in vitro culture*

The effect of differences hormonal treatments on callus induction of hypocotyls explants:

Significant differences were found between the treatment callus percentage parameter ($p \leq 0.01$) (Table 1). According to our results (Table 2), the maximum callus induction frequency (100%) was obtained on Murashige and Skoog (MS) medium supplemented with 0.5 mg/l NAA plus 1 mg/l BAP. Based on our results, the minimum callus formation frequency (79.4%) was obtained from MS medium supplemented with 0.1 mg/l TDZ along with 0. 5 mg/l NAA. The degree of callus percentage was increased as BAP, ZEA and TDZ concentrations increased. The

explants did not promote any callus in control treatment. According to the results, between the plants growth hormones concentration applied in this experiment, it illustrated that the callus induction did not appear until 3 weeks after transplantation. Browning was observed around callus in (0.1 ZEA + 0.5 NAA mg/l) and (0.5 TDZ + 0.5 NAA mg/l). These necrosis calluses were completely destroyed by the end of the second week. Based on coding the lowest callus volume (code: 1) was observed in (0.1 ZEA + 0.5 NAA mg/l) and maximum (code: 5) was in (1 BAP+ 0.5 NAA mg/l) (Table 2) (Pic 1).

Table 1. Analysis of Variance of traits in Impatiens walleriana

SOV	DF	Shoot dry weight (mg)	Shoot fresh weight (mg)	Shoot percentage	Shoot number	Shoot length	Callus percentage	Root percentage	Root length
Rep.	2	0.004	0.036	0.124	0.001	0.215	0.211	0.205	0.223
treatment	9	0.094*	0.971*	0.395*	0.003*	0.028*	0.027*	0.099*	0.128*
CV	-	5.23	11.23	11	18.8	11.13	19.7	10.03	8.7

ns, * and ** indicates non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 2. Effect of hormones in callus formation in Impatiens walleriana

treatment	Callus volume	Callus percentage
-	0	0[e]
0.1 BAP + 0.5 NAA	4	93.66[ab]
0.2 BAP + 0.5 NAA	5	98[a]
0.5 BAP + 0.5 NAA	5	100[a]
0.1 TDZ + 0.5 NAA	2	83.06[c]
0.2 TDZ+ 0.5 NAA	3	88[b]
0.5 TDZ + 0.5 NAA	4	90[b]
0.1 ZEA + 0.5 NAA	1	76.33[d]
0.2 ZEA + 0.5 NAA	2	79.4[c]
0.5 ZEA + 0.5 NAA	2	80.33[c]

† Means within each column followed by the same letter are not different according to the Duncan test.

Photo 1. Effect of hormones on callus induction in *Impatiens walleriana*

The effect of differences hormonal treatments on plant regeneration of explants:

As shown in Table 1, the hormonal treatments significantly affected organogenesis ($p \leq 0.01$). The finding obtained from indirect regeneration was through following:

In vitro effect of different cytokinin types (BAP, TDZ, and ZEA) individually was studied on plant regeneration. Organogenic and plant regeneration have been achieved using in this way (Pic 2). Therefore, growth hormones at different combinations brought out remarkable variations in organogenesis where according to (Fig 1); (1BAP+ 0.5 NAA mg/l) had the highest percentage of shooting (55%), and the lowest at (0.1 ZEA + 0.5 NAA mg/l) (16.67%) (Table 3).

Photo 2. Effect of hormones on organogenesis in *Impatiens walleriana*

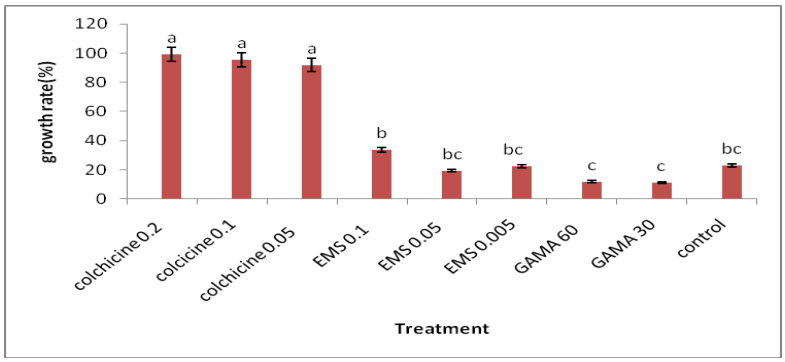

Fig 1. Effect of hormones on growth rate in *Impatiens walleriana*

Vertical bars indicate standard error. Means followed by the same letter are not different according to the Duncan test

Table 3. Effect of hormones in organogenesis in Impatiens walleriana

Treatment	Shoot fresh weight (mg)	Shoot dry weight (mg)	Shoot percentage	Shoot number	Shoot length	Root percentage	Root length
BAP1-NAA0.5	6.8[a]	2.4[a]	55[a]	5.67[a]	4.12[a]	75[a]	8.92[a]
BAP0.5-NAA0.5	6.72[a]	2.32[a]	53.33[a]	4.67[ab]	3.6[b]	61.67[b]	7.52[b]
BAP0.1-NAA0.5	6.42[b]	2.18[bc]	53.33[a]	4.67[ab]	3.6[b]	60[b]	6.97[b]
TDZ1-NAA0.5	5.7[c]	1.63[bcd]	53[a]	4.33[abc]	2.62[c]	56.67[bc]	6.97[b]
TDZ0.5-NAA0.5	5.17[d]	1.53[cd]	48.33[ab]	3.67[bcd]	2.05[c]	55[bc]	5.33[c]
TDZ0.1-NAA0.5	5.1[d]	1.31[cd]	41.67[ab]	3.67[bcd]	1.78[d]	53.33[bc]	4.68[cd]
ZEA1-NAA0.5	4.57[e]	0.88[e]	36.67[b]	3.33[cd]	1.76[d]	48.33[cd]	4.67[cd]
ZEA0.5-NAA0.5	3.68[f]	0.78[f]	28.33[c]	2.67[cd]	1.76[d]	38.33[de]	3.9[e]
ZEA0.1-NAA0.5	2.55[g]	0.67[f]	16.67[d]	2.33[d]	1.37[e]	35[e]	3.58[e]
control	0.1[h]	0.2[g]	0.3[e]	0.1[e]	0.1[f]	2[f]	0.4[f]

† Means within each column followed by the same letter are not different according to the Duncan test.

It was found that all treatments significantly increased shoots' numbers. The results indicated that the maximum shoots number was gain from explants in MS medium containing (1BAP+ 0.5 NAA mg/l). Highest number of leaves was induced I from hypocotyls in MS media with same hormone (4.67) as the lowest amount was found in control plants (Table 3).

Application of growth hormones significantly affected the length of shoots. The highest shoots length was relieved for the explants treated with concentrations of (1BAP+ 0.5 NAA mg/l) (4.12 cm) and the lowest was recorded in control (0.1 cm) (Table 3).

Significant difference was observed between treated and untreated plants in fresh weight. The highest observed fresh weight belonged to treatment of (1BAP+ 0.5 NAA mg/l) followed by (0.5 BAP+ 0.5 NAA mg/l) (6.8 and 6.72 mg, respectively) and its lowest measurement recorded in (0.1 ZEA + 0.5 NAA mg/l) treatments (2.55 mg) (Table 3).

Different concentrations of BAP, TDZ, and ZEA influenced significantly on the dry weight. The weight change was minimum in (0.1 ZEA + 0.5 NAA mg/l) treatment whereas combined treatment application of (1BAP+ 0.5 NAA mg/l) (2.4 mg) (Table 3).

Effect of hormone application ratio became significant in rooting. Most rooting ratio percentage was observed (1BAP+ 0.5 NAA mg/l) (75%), while the (0.1 ZEA + 0.5 NAA mg/l) had the lowest ratio (35%) (Table 3).

As compared with control, all hormones treatments significantly enhanced length of roots, as (1BAP+ 0.5 NAA mg/l) and (0.5 BAP+ 0.5 NAA mg/l) had the highest root length (8.92 and 7.52 cm), respectively.

2. The effect of mutation treatment on explants

Analysis of variance revealed significant effect doses of gamma radiation, colchicines and EMS treatment evaluation for growth rate, polyploidy percentage and survival rate (Table 4).

Growth rate significantly increased from 91.67 to 99.22 % with colchicines treatment. Moreover, all 0.1 % EMS treatments (33.82%) significantly enhanced this trait as compared with control plants. Growth rate in gamma doses treatment was 11.08% in 60 grey and 11.99% in 30 grey. It revealed that organ alter in irradiation of different Gama ray amounts was arise after 6 week, control treatment necrosesed (Fig 1) (Pic 3).

Table 4. Analysis of Variance of traits in Impatiens walleriana

SOV	DF	polyploidy	Survival percentage	Growth percentage
Rep.	2	12	11	9.2
treatment	2	19.8*	4.46*	5.35*
CV	-	5.23	11.23	11

ns, * and ** indicates non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

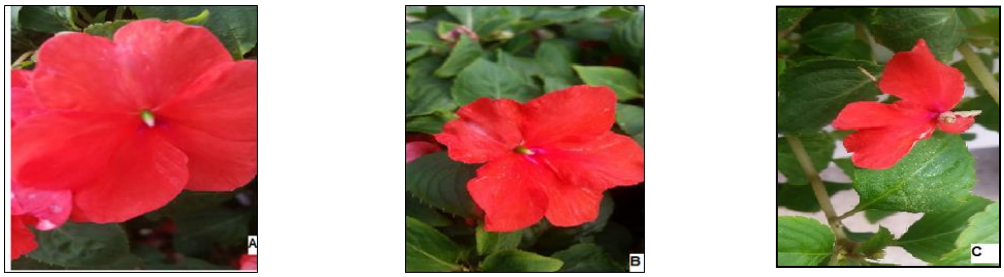

Photo 3. Effect of colchicine (A), EMS (B), gamma radiation (C) on organogenesis in *Impatiens walleriana*

At the end of period, treated plants with 0.2% of colchicines had the highest polyploidy (32.27%). Treated plants with 0.1 % EMS showed the 9.3% in evaluated times. Polyploidy percentage in 60 grey (0.5 %) was less than 30 grey (0.7 %). Control plants were planted in substrates had no polyploidy rate (Fig 2).

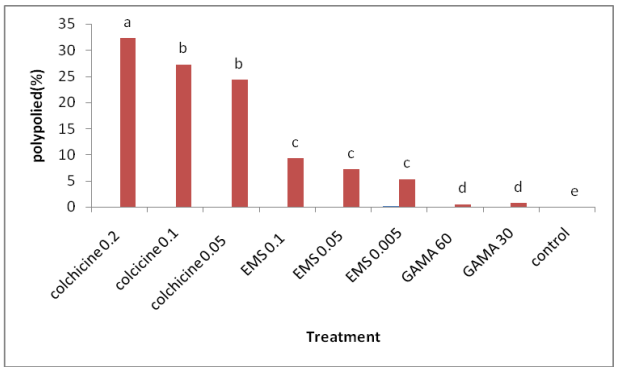

Fig 2. Effect of hormones on polyploidy in *Impatiens walleriana*

Vertical bars indicate standard error. Means followed by the same letter are not different according to the Duncan test

Survival rate on plants grown in 0.2% of colchicines (98.57%) is higher than plants in the other treatments. It was for plants grown in 0.1 EMS (60.3 %) and 30 grey gamma radiation (23.5%), respectively (Fig 3).

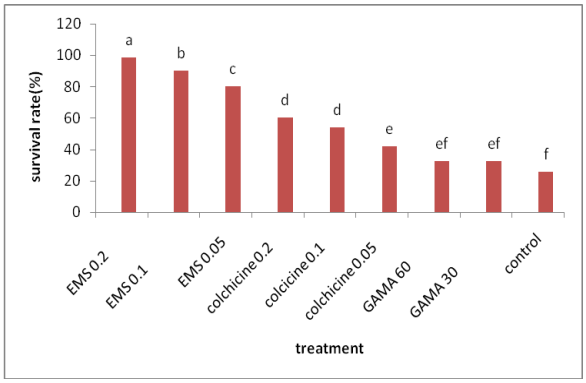

Fig 3. Effect of hormones on survival rate in *Impatiens walleriana*

Vertical bars indicate standard error. Means followed by the same letter are not different according to the Duncan test

DISCUSSION

Impatiens walleriana were evaluated in response to different hormones for callus induction and regeneration. Cytokinin and auxin were provided in disparate quantities to initiate shoot and root development from explants. The optimum level of callus formation was induced in NAA+BAP. Similarly, maximum level of regeneration and organogenesis was also observed in NAA+BAP. IBA in combination with BAP was largely effective for root induction.

Callus induction often varies depending on various other factors such as hormones composition, specimens, and genotype. Auxin and cytokinin have been widely used to generate callus while gibberellins and other hormones are used only in special cases (FARSI and ZOLALI, 2001). Various amount and compositions of NAA and BAP, ZEA, TDZ elicit the desired effects on callus induction. The same results were also observed for *Lagenaria siceraria* Standl and Helianthus by combining these two hormones from small specimens of hypocotyls (HAN *et al.*, 2004). Callus induction were initiated using the MS medium supplemented with NAA and BAP which showed that high concentrations of cytokinin induced greenish and greater callus in cotyledon and hypocotyls segments in two bell pepper cultivars (ASHRAFUZZAMAN *et al*., 2009). ZHANG *et al*. (2011) developed callus from cotyledon and hypocotyls bell pepper explants planted on MS with several concentrations of BAP and NAA. The highest percentage of callus induction was found in the same media. There are numerous reports on auxins and cytokinins play a fundamental role in callus formation in many ornamental plants such as *Anthurium andreanum* Lind cv Rubrun, Begonia, *Ranunculus asiaticus* L. which is consistent with our results (JAIN and OCHATT, 2010; PATI *et al*.,2010).

In our study, organogenesis development was provided by medium MS with BAP and NAA. Like further plant species the ages of Impatiens cotyledon explants have a considerable role in shoot regeneration. Tissue culture methods for Impatiens plants are shown from segment of cotyledon obtained from 7 days old seedlings on MS medium supplemented with BAP. In addition, there was not any other information on shoot propagation from Impatiens' hypocotyls (HUYOP *et al*., 2009). Our results have confirmed previous reports of *Arabis gunnisoniana* and nyger (*Guazotia abysinica)* (MURTHY *et al*., 2003). Remarkable differences were observed in shoot regeneration with BAP compared to other cytokines sources in *Impatiens platypetala* Lind, Impatiens L. interspesific hybrids (KYUNGKUL and STEPHENS, 1987). Non-purine chemical synthesis such as TDZ and ZEA also takes place with cytokines where cell divisions are occurring. Results revealed that there was shoot branching in medium contain TDZ and ZEA with staminate plants less than BAP. Our findings are in agreement with other data of HUYOP *et al*. (2009). This type of branching is present in control plants (without hormones) which may be due to effect of exogenous cytokines present inside the plants (SAKIKABARA .*et al*., 2004).

This study concludes that hormone application promoted adventitious roots in 4 days. Hairy roots were recognized in all treatment. Growth of hairy roots in the MS medium is less than the others. These results are consistent with the findings of HAN *et al*. (2004) in squash; YANG *et al*. (2001) in *Swainsola salsula*., *Prunus persica*; ALI *et al*. (2009) in Olive; WYNNE and MCDONALD (2002) in Avocado. IBA initially described as a synthetic auxin that induces rooting but root formation was inhibited by at supra-optimal concentrations (KRIEKEN *et al*., 1993).

Our results indicated that change in ploidy level of plants, survival, growth rate and morphological changes through colchicine has been proven to be more effective than EMS and gamma ray.

Cell mutagenesis is an important genetic method to enhance the amount of mutants achieved. Even when mutants occurred naturally via genetic selection, chemical agent mutagen treatment (colchicines and EMS) or radiation may change the kinds of mutants gain (PRAKASH and SHERMAN, 1973).

Investigated traits significantly increased in response to colchicines application. Colchicines block the cell division by inhibiting the synthesis of microtubules, which results in chromosome doubling (ALISHA and OMIDI, 2008). Previous studies in *Arabidopsis thaliana* have demonstrated that increased ploidy increases growth rate. Enhancing gene expression was directly associated with an increase in its transcriptional activity which in turn promotes metabolic process so, it is able to speed up the growth (BREUER *et al*., 2007).

There were the different doses of Ethyl Methane Sulfonatex (EMS) showed medium influence on trait increase for explants. In the evaluation, it was found that trait presented greater increase with the dosage of 0.1 %, however, the growth rate, survival and polyploidy expressed positive response only up to a dose 0.05%. Ethyl methan sulfanate was noted to stimulate firstly base pair Guanine/Citozin to Adenine/Timin transversions (PRAKASH and SHERMAN, 1973).

While gamma radiation levels ranged from 30 to 60 grey, there was no increase for trait depending on the radiation dose. For all trait doses 60 gray revealed decrease for characters. Radiation could cause genetic, several morpho-physiological and biochemical trait changes, which may stimulate plant growth and induce plant characteristics depending on the dose rate (SHERIF *et al*., 2011). Gamma rays were able to breaks in the DNA double and nucleotide

changes lead to the dysfunction which mutation is hard or impossible to prepare or analyze (PENMETSA and COOK, 2000). The impacts of gamma ray on *Pisum sativum* are achieved by KHAN (1999) and barley (SUBHAN *et al*., 2004), that shows the relative low dose of gamma irradiation have positive effects on various, biological procedure.

CONCLUSION

In conclusion, said the present research suggests that treating *I. walleriana* with NAA and BAP can produce regenerated plants. Regarding to these, the present technique illustrate an effective system for *in vitro* reproduction of *Impatiens walleriana* by hypocotyls cultures. By using mutant agent especially colchicines compared to their diploid counterparts, the polyploidy plants have significant differences in their survival rate, growth rate and polyploidy level. These approaches will ultimately contribute to the future of breeding programs aimed at adding diversity to this important ornamental species.

Received,February 06th, 2020
Accepted October 29th, 2020

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UTICAJ RAZLIČITIH HORMONA RASTA, INDUKCIJE I REGENERACIJE KALUSA I UTICAJA KOLHICINA, ETIL METANESULFONATA (EMS) I GAMA ZRAČENJA NA NEKE OSOBINE Impatiens *walleriana*

Sedigheh Sadat SHARAFI[1], Pejman AZADI[2], Maryam JAFARKHANI KERMANI[3*], Vahid ABDOSSI[1], Ali ESKANDRI [4]

[1]Departman za hortikulturu, Ogranak za naučna istraživanja, Islamski Azad Univerzitet, Teheran, Iran
[2] Departman za genetički inžinjering, Poljoprivredni institut za biotehnološka istraživanja, Karaj, Iran
[3] Departman za kulturu tkiva i gensku transformaciju, Poljoprivredni institut za biotehnološka istraživanja (ABRII), Karaj, Iran
[4]Departman za nuklearnu poljoprivredu, Karaj, Iran

Izvod

Dupla pištika je ukrasni član porodice *Balsaminaceae*. Ova biljka se uglavnom razmnožava vegetativnim putem, koji je uglavnom vremenski zahtevan. Često se razmnožava semenom, ali je to ograničeno sterilnošću semena F1. *In vitro* kultura duple pištike ima značajnu funkciju u brzom razmnožavanju sa korisnim karakteristikama i stvaranju zdravih biljaka bez bolesti. Ovaj eksperiment je sproveden da bi se istražio uticaj medijuma i različitih hormona na *in vitro* propagaciju duple pištike korišćenjem potpuno slučajnog dizajna. MS medijum je pripremljen zajedno sa različitim koncentracijama BAP, TDZ i ZEA. Kalus je indukovan i dobro je rastao u podlogama dopunjenim sa 0,5 mg / l NAA + 1 mg / l BAP. Da bi se došlo do indirektne propagacije, eksplantati su kultivisani u istim medijumima koji sadrže BAP, ZEA i TDZ u kombinaciji sa NAA. Ovi tretmani imaju sposobnost organogeneze. Rezultati su pokazali da je kontrolni tretman imao najmanji efekat na osobine, uključujući procenat izdanaka, broj izdanaka, broj listova, dužinu izdanaka, svežu i suvu masu, i doveo je do maksimalne proliferacije u medijumu dopunjenom sa 0,5 mg / l NAA + 1 mg / l BAP. Najveća dužina korena i procenat korenja zabeleženi su kod 0,5 mg / l IBA + 0,5 mg / l BAP. Pored toga, proučavan je efekat mutagenih agenasa. Aseptični uzorci su tretirani γ -zračenjem, etil metan sulfonatom i kolhicinima u komori za rast. Tretmani sa 30, odnosno 60 greja imali su nižu stopu preživljavanja, stopu rasta i poliploidiju, dok su tretmani kolhicinom od 0,1 i 0,2 imali najvišu stopu rasta. U vezi sa njima, postojeća tehnika ilustruje efikasan sistem za *in vitro* reprodukciju duple pištike kulturama hipokotila. Pored toga, kolhicin se pokazao efikasnim u indukciji poliploidije kod ove biljke.

Primljeno 06. II.2020.
Odobreno 29. X 2020.