Efficient Plant Regeneration System for New Guinea Impatiens (*Impatiens Hawkeri W. Bull*) CV. 'Violet'and 'Scarlet Bronze Leaf'

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Research Article

Keywords: New Guinea Impatiens, High efficiency Regeneration system, Vitrification overcomes, PIC

Posted Date: December 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1140050/v1

Abstract

New Guinea Impatiens (*Impatiens hawkeri W. Bull*) is an eye-popping landscaping plant which is of bright and colorful blooms. A highly efficient in vitro plant regeneration system through direct shoot organogenesis was established for the first time from hypocotyl with partial cotyledons of New Guinea Impatiens. The results showed that Explant sterilization method, basic medium type,$AgNO_3$,sucrose and plant growth regulators (PGRs) have greatly influences on in vitro morphogenesis.The regeneration rate in regeneration media that MS supplemented with 0.5mg·L^{-1} TDZ and 0.1mg·L^{-1} NAA was acceptable ,the induction rate of 'Violet' was 86.67%, and its proliferation coefficient was 5.27, while the induction rate of 'Scarlet Bronze Leaf' was 83.33%, and its proliferation coefficient was 5.13.PIC was unable to induce clumped sprouts, but it had a better effect on callus induction.We also included a shoot multiplication stage using regeneration New Guinea Impatiens medium that MS supplemented with 0.8mg·L^{-1} 6-BA,0.5mg·L^{-1} TDZ and 0.05mg ·L^{-1} NAA.Reducing sucrose concentration to 20g·L^{-1} or adding 1mg·$L^{-1}$$AgNO_3$ could alleviate the vitrification phenomenon in the process of tufted bud proliferation.The optimal root culture medium for the regenerated seedlings of 'violet' and 'scarlet bronze leaf' of New Guinea Impatiens was MS supplemented with 0.05mg·L^{-1}IBA, the rooting rate reached 100%.The study examined the micropropagation responses of New Guinea Impatiens in the presence of various growth regulators and provided a simple and more suitable protocol adapted for the mass propagation of clones.

Introduction

New Guinea Impatiens (*Impatiens hawkeri W. Bull*) is a perennial evergreen herb, which has the characteristics of long flowering period, rich flower color and high ornamental value, so it has become an important flower in pots and flower beds in the world(Fu et al.2007). Its discovery originated in 1884 when Kew garden in England received an impatiens herb specimen from Dr. Schomburgk, curator of the Adelaide Botanical Garden in Australia, and marked that the specimen had been collected by Lieutenant hawker (Stafleu et al.1976). It was introduced into Europe and the United States after 1886. Since the launch of the 'Circus' (circus) series in 1972, more than one hundred series have been successfully launched and distributed all over the world, and most of the varieties have obtained the right to breed (Ling et al.2007).

The main breeding forms of New Guinea Impatiens were seed, rootless cuttage and cuttings. There are few literatures about tissue culture and transformation of New Guinea Impatiens. At present, the available articles on New Guinea Impatiens tissue culture are mainly involve in vitro propagation (Han et al.1987;Stephens et al.1985;Witomskaet al.2003), in vitro germination of immature ovules (Han K, Stephens LC, 1992), in vitro regeneration (Taha A, et al, 2009), callus culture (Josekutty et al.1998; He et al.1989), growth of cotyledon sections(Han.1994), embryo and ovule culture in vitro (Arisumi et al.1980), formation of secondary products in cell culture(Panichayupakaranant et al.2001) and so on. To our knowledge, there is no report on the establishment of an efficient regeneration system of New Guinea Impatiens. Therefore, the establishment of an efficient regeneration system for New Guinea Impatiens is important

Taking the seeds of New Guinea Impatiens ‘violet’ and ‘scarlet bronze leaf’ with good ornamental characteristics and widely used in landscape in recent years as materials, this study used single factor and multi factor orthogonal experiments to explore the suitable conditions for seed disinfection and sterilization, induction of cluster buds, proliferation of cluster buds and rooting and strengthening seedlings of cluster buds. In order to establish an efficient and stable regeneration system of New Guinea Impatiens, which not only provided a certain degree of technical support for solving the defects of traditional breeding methods, but also laid a foundation for the follow-up research of transgenic genetic transformation and molecular breeding.

Materials And Methods

Plant materials and explants preparation

In this case, two varieties ‘violet’ and ‘scarlet bronze leaf’ of New Guinea Impatiens divine series were used as explants(Fig. 1). The seeds used in this experiment were purchased uniformly from Bauer (ball seed) Company in the United States .

Wrapping the seeds of New Guinea Impatiens in gauze, fixed them with a rubber band and rinsed for 30-40min with clean water. Then put the seeds after the above treatment into the ultra-clean worktable for sterilization operation.A total of 9 different disinfection treatments were set up and the disinfection scheme is shown in table 1. Finally, the sterilized seeds were dried on the sterilized filter paper and then put into the pre-prepared MS medium for culture. Each disinfection treatment was inoculated with 10 bottles, each bottle was inoculated with two seeds, repeated 3 times. Two weeks later, the pollution rate and germination rate were counted. In order to establish an efficient regeneration system, enough clustered buds must be obtained, according to the results of previous induction of clustered buds of African impatiens (*Impatiens walleriana* Hook.f).

The hypocotyls with partial cotyledons of the germinated New Guinea impatiens 'Violet' and 'Scarlet Bronze Leaf' after disinfection were used as explants and cultured in MS medium with hormone concentration of 0.4mg ·L^{-1}6-BA and 0.5mg ·L^{-1}TDZ(Dan Y, et al，2010) .

Culture medium and culture conditions

Medium: MS (Murashige and Skoog,1962), 1/2MS , B5 and N6 were used as the basic medium in this experiment. Various concentrations of plant growth regulators,0.7% Agar and 3% sucrose were added to the culture medium before autoclaving,.The pH value was adjusted to 5.8-6.0 by using 1mol ·L^{-1}NaOH/1mol ·L^{-1}HCl and autoclaved at 121°C for 20 min. PGRs was added to the culture medium after the bacteria were removed by 0.22um microporous membrane filtration. The inoculated cultures were maintained at 25±2°C with 16h photoperiod under white fluorescent light and 8h dark.

Induction of clustered buds of New Guinea Impatiens

To examine the effects of PGRs on in vitro morphogenesis, the concentrations of 0.1mg ·L^{-1} NAA and different concentrations of 6-BA, TDZ, and PIC(a novel synthetic hormone with high cytokinin activity) were added to basic medium. Hypocotyls of 'violet' and 'scarlet bronze leaf' explants with partial cotyledons were inoculated in basic medium (I1-I12) , as shown in Table 2.

Fifteen bottles were inoculated per treatment and two explants were placed per bottle.The sprouting of clumped buds was continuously observed every week, and the growth of clumped buds, induction rate and proliferation coefficient of different treatments were recorded after 4-5 weeks.

Proliferation of cluster buds of New Guinea Impatiens

In order to explore a better proliferation of cluster buds, MS (P1-P4), 1/2MS (P5-P8), B5 (P9-P12) and N6 (P13-P16) were used as the basic medium. The orthogonal test of L16 (44) was designed by adding different concentrations of 6-BA (0.4mg ·L^{-1}, 0.8mg ·L^{-1}, 1.2mg ·L^{-1}, 1.6mg ·L^{-1}), TDZ (0.25mg ·L^{-1}, 0.5mg ·L^{-1}, 0.75mg ·L^{-1}, 1mg ·L^{-1}), NAA (0.01mg ·L^{-1}, 0.05mg ·L^{-1}, 0.1mg ·L^{-1}, 0.2mg ·L^{-1}). The tufted buds of two varieties of New Guinea impatiens with good growth and the same subculture times were selected,cut into small pieces of 0.5cm × 0.5cm. They were randomly inoculated in different multiplication medium combinations (P1-P16). Each treatment was inoculated with 15 bottles, and each bottle was inoculated with 2 pieces. After 4 weeks of culture, the proliferation of tufted buds was photographed , the proliferation coefficient and seedling height was calculated.

Rooting of regenerated seedlings of New Guinea Impatiens

When the height of the stem segment of the single seedling with clustered buds reached more than 3cm, the well-growing clump buds were divided into individual seedlings and inoculated in the MS medium respective supplemented with NAA, IBA and 6-BA. Each hormone was set with four gradients (R2~R13) of 0.025mg ·L^{-1}, 0.050mg ·L^{-1}, 0.100mg ·L^{-1} and 0.200mg ·L^{-1}, and the MS medium without any hormone was used as the control (R1), as shown in Table 4. The suitable rooting medium for 'Violet' and 'Scarlet Bronze Leaf' of New Guinea Impatiens was screened. Thirty regenerated seedlings were inoculated in each treatment. Two weeks later, photos were taken and the test results were recorded, and the rooting rate, root length and number of roots were counted.

Statistical analysis

Contamination rate (%) = (total number of contaminated seeds / total number of seeds inoculated) × 100%

Germination rate (%) = (total number of germinated seeds / total number of seeds inoculated) × 100%

Induction rate of clustered buds (%) = (total number of germinated explants / total number of inoculated explants) × 100%

Multiplication coefficient = total number of buds produced after multiplication / number of buds inoculated

Rooting rate (%) = (total number of rooting explants / total number of inoculated explants) × 100%

The variance analysis of the data was carried out by using SPSSS tatistics software, and multiple comparisons were made by LSD method to test the significant differences of each factor ($P < 0.05$).

Results And Discussion

Effects of different sterilization treatments on seed germination of New Guinea Impatiens

By controlling 70% alcohol sterilization for 15 seconds, the study demonstrates that different 2% NaClO disinfection length of time have significant impacts on both germination rate and contamination rate, these results also further highlight a decreasing tendency in contamination rate with an additional period of disinfection time, while seed germination rate exhibits an upward trend and followed by a downward trend at some point of time. Moreover, various plant seeds tend to have different tolerances to different disinfection reagents, this is consistent with the statement proposed by Jones, who suggesting that the seed germination rate would decrease significantly despite an improvement in sterilization effect given an additional period of disinfection time (Jones, L. H, 1983).Also, under 2% NaClO treatment time, the seed germination rate of 'Violet' reaches to the maximum value of 88.33% in 12 minutes (S5), while the seed germination rate of 'Scarlet Bronze Leaf' reaches to the maximum value of 86.67% in 10 minutes (S4), meanwhile, the seed contamination rate reaches to the minimum value in 15 minutes (S6). Under the premise of 2% NaClO sterilization treatment for 10 minutes, both germination rate increases first and then decrease as 70% alcohol sterilization time goes by, in contrast, the contamination rate showed a downward trend over time, which indicates that 70% alcohol has a good effect on seed sterilization, however, takes disadvantage in seed germination. Therefore, regard to this finding, the treatment time of alcohol should be controlled. The similar results obtained from Ye Wei yan further imply a certain degree of agreement in this experiment, where Ye Wei yan conclude that the contamination rate of grapefruit seeds would not decrease significantly even if that an extra sterilization time using 75% alcohol was provided(Ye et al.2015), however, with a strikingly reduce in seed germination rate. Hence, the best disinfection scheme in this experiment is as follows: usage of 70% alcohol 15s and 12min disinfection time of 2%NaClO for the seeds of New Guinea Impatiens' Violet', the contamination rate of which is 10% and a maximum 88.33% of germination rate. For the seeds of New Guinea Impatiens' Scarlet Bronze Leaf', 70% alcohol 15s and 10min disinfection time of 2%NaClO are suggested, the pollution rate of which is 10% and the germination rate is 86.67%. They both present significant differences in this experiment. However, results here are inconsistent with Yun gui Guo's conclusion, which concerns that 70% alcohol and 15-20min of 2%NaClO as the best disinfection scheme (Guo et al.2012), the reasons for the differences in outcomes may be related to the variety, seed quality and pretreatment time of Impatiens *balsamina*.

Effects of different concentrations of PGR on tufted bud induction of 'Violet" and 'Scarlet Bronze Leaf" of New Guinea Impatiens

6-BA and TDZ are two types of cytokinins which are commonly used in the processes of tissue culture and plant regeneration of Impatiens. They can promote cell division, differentiation, elongation or regulation of endogenous hormones and metabolic synthesis of CTK by removing apical dominance(Wang Dong mei, Huang Shang zhi, 1996). In this way,Both of them can contribute to the induction of tufted buds (Miloševic, et al, 2011). In this experiment, the effects of 0.1mg ·L-1NAA and different concentrations of 6-BA, TDZ and PIC on cluster bud induction of New Guinea Impatiens were compared. As shown in Table 2, for New Guinea Impatiens' Violet' and 'Scarlet Bronze Leaf', no matter 6-BA or TDZ is used, the induction rate and increment coefficient of regenerated seedlings both show a trend from rise to decline as rising in hormone concentration. For the growth medium that only contains 6-BA, the induction rate and increment coefficient reached to the maximum value when the concentration of 6-BA is 0.8mg $\cdot L^{-1}$. Similarly, for the growth medium with only TDZ, the induction rate and increment coefficient reached their maximum value when the concentration of TDZ approaches to 0.5mg $\cdot L^{-1}$, which reveals a significant difference when comparing the results with the other three media with only TDZ concentration. In conclusion, the experiment shows that combination of TDZ and NAA have a better induction ability of cluster bud than 6-BA and NAA, this outcome corroborates with the previous study on the induction of African impatiens (*I.walleriana*)(Dan Y, et al, 2010). Besides,PavaniChirumamilla et al also find that TDZ had a good effect on cluster bud induction in Kashi eggplant regeneration system (Pavani chirumamilla, et al, 2020). This is probably because that cytokinin activity of TDZ is more active than other cytokinins (Bhattacharyya P, et al,2016), while other scholars conjecture that TDZ can induce explants to produce endogenous IAA (Guang,et al,2010).In this experiment, although the proliferation coefficient of tufted buds induced by 6-BA and NAA is low, the combination of both could induce root formation as well as helping buds form callus, this finding in my paper lends support previous results of Taha A and Han who focus on in vitro regeneration of impatiens cotyledons and cucurbit cotyledons. No matter the combination of 6-BA and NAA or the combination of TDZ and NAA, both induction rate and proliferation coefficient of 'Violet' are always slightly better than' Scarlet Bronze Leaf', this proves that genotypes have a certain effect on plant regeneration. Among all, the combination of MS with 0.5mg $\cdot L^{-1}$ TDZ and 0.1mg $\cdot L^{-1}$NAA have the best effect on the induction of clustered buds for the two varieties and exhibits a significant difference. Likewise, the results denote the 86.67% induction rate of 'Violet" and a value of 5.27 for its proliferation coefficient, while the induction rate of 'Scarlet Bronze Leaf' is 83.33%, and its proliferation coefficient is 5.13. No matter what concentration of PIC is added to 'Violet" and 'Scarlet Bronze Leaf" of New Guinea Impatiens, the buds cannot be induced, yet with more culture time, the explants not only gradually turns yellow from the base to the leaves, but also forms callus, meanwhile, the degree of callus increase along with the additional increase in PIC concentration. In the study of tissue culture of Gasteria verrucosa haw and Haworthia fasciata haw (Beyl CA, Sharma GC, 1983), Beyl and Sharma also discover that pic was a good callus inducer. which is

similar to our results. Zhou Yin et al(Yin et al.,2013) found that the proper increase of PIC concentration was beneficial to the induction of clump buds of Cymbidium, it contradicts with experimental results. Hence, we suspect that the role of PIC in plant tissue culture depends on its ratio to cytokinins.

Effects of different hormone concentrations and medium types on the proliferation of rosette buds of New Guinea Impatiens

In the process of proliferation of tufted buds, the type and concentration ratio of cytokinin and auxin in culture medium is usually considered to play a key role in regulating the growth and differentiation of clustered buds (Mu et al.2011). Skoog and Miller (1957) proposed that plant organ differentiation is regulated by two kinds of hormones (auxin and cytokinin) (Xu et al.1996).All the treatments in this experiment can differentiate into clustered buds(Table 3), in which treatment P2 is the best in terms of proliferation coefficient, seedling height and vitrification rate, and there is a significant difference between treatment P2 and other treatments. 'Violet' proliferation coefficient reached 13.18, Scarlet Bronze Leaf' multiplication coefficient reached 11.52, the average plant height of ‘Scarlet Bronze Leaf’ reached 1.46 cm, and the average plant height of Scarlet Bronze Leaf' reached 1.19cm. The vitrification rate of 'Violet" is 13.33%. The vitrification rate of 'Scarlet Bronze Leaf' is 20.00%Fig. 3e. At the same time, the cluster buds proliferated by P2 were treated, the buds were dense and the color was bright green. The effects of basic medium, 6-BA, TDZ and NAA on the proliferation, seedling height and vitrification rate of 'Violet" and 'Scarlet Bronze Leaf' clustered buds were compared.

NAA and TDZ on the proliferation coefficient of New Guinea impatiens clustered buds reached a very significant level(Table 3-1), while the effect of 6-BA on the proliferation of clustered buds had no significant difference. The results showed that the basic medium, TDZ and NAA played a decisive role in the proliferation of rosette buds of New Guinea Impatiens, while the concentration of 6-BA did not play a decisive role in the proliferation of tufted buds. Among them, TDZ had a very significant effect on the proliferation of 'Violet" clustered buds, while it had a significant effect on the proliferation of 'Scarlet Bronze Leaf' clustered buds', indicating that Violet' was more sensitive to TDZ than 'Scarlet Bronze Leaf' in the proliferation of clustered buds. This may have a relationship with the plant's genes.

Among the four selected media, MS medium is significantly better than the other three media(Table 3-2), and the average proliferation coefficient of 'Violet' can reach 9.74. The average increment coefficient of ‘ScarletBronze Leaf 'can reach 8.91. Therefore, MS medium had a good effect on the proliferation of tufted buds, and similar results were also found in the proliferation culture of legumes ((Perveen S, Anis M, Aref IM, 2012;Siddique I, Anis M, 2007). It shows that inorganic salts, large amounts and trace elements are indispensable hard nutrients in the tissue culture of Impatiens *balsamina* in New Guinea. Once there is a lack of nutrition, it is extremely disadvantageous to the proliferation and growth of clustered buds. The proliferation coefficient of clustered buds increased at first and then decreased with the increase of TDZ concentration and NAA concentration. The average value-added coefficient of both New Guinea Impatiens reached the best when the concentration of TDZ was 0.5mg $\cdot L^{-1}$. The average value-added coefficient of 'Violet' can reach 5.83, the average value-added coefficient of ‘Scarlet Bronze Leaf’ can reach 4.5, and the average proliferation coefficient of ‘Violet’can reach 5.43. when the concentration of NAA is 0.05mg $\cdot L^{-1}$, the proliferation coefficient of clustered buds can reach the highest. The average value-added coefficient of ‘Scarlet Bronze Leaf’ can reach 4.58. And it was significantly different from the other three concentration levels. The results showed that in the process of proliferation of clustered buds, the concentration of auxin and cytokinin should be appropriate, and too high or too low would inhibit its differentiation and be disadvantageous to its growth.

For 'Violet', there is a very significant difference between basic medium and TDZ on the seedling height of clustered buds(Table <link rid="tb9">3</link>-3). For 'Scarlet Bronze Leaf', the basic medium has extremely significant difference on the seedling height of clustered buds, while 6-BA and NAA have significant difference on the seedling height of clustered buds, indicating that the choice of basic medium plays an important role in the seedling height of clustered buds, while cytokinin and auxin may be different because of different varieties.

There are significant differences between MS medium and 1/2MS, B5, N6 for two kinds of New Guinea Impatiens(Table 3-4). The tufted buds treated with MS medium grow best. The average seedling height of 'Violet" and 'Scarlet Bronze Leaf' are 1.23 cm and 1.01 cm(Fig. 3i-l), respectively. For 'Violet' among the four concentrations of TDZ, the growth of clustered buds of 0.5mg $\cdot L^{-1}$TDZ was the best, and there was significant difference between 0.5mg $\cdot L^{-1}$TDZ and the other three concentrations. For 'Scarlet Bronze Leaf', among the four concentrations of 6-BA, 0.4 mg$\cdot L^{-1}$ and 0.8 mg$\cdot L^{-1}$ were significantly different from 1.6 mg$\cdot L^{-1}$ and 3.2 mg$\cdot L^{-1}$, and the value-added coefficient increased at first and then decreased. For the concentration of NAA, the average seedling height of 0.005 mg $\cdot L^{-1}$ 'Scarlet Bronze Leaf' was the best, which was 0.94 cm, which was significantly higher than that of the other three levels, so the suitable concentration of NAA was beneficial to the increase of 'Scarlet Bronze Leaf' of New Guinea Impatiens.

The type of basic medium has a significant effect on the vitrification rate of 'Violet' tufted buds (Table 3-5), indicating that the basic medium has an important effect on the vitrification rate of 'Violet' clustered buds. However, the concentration of 6-BA, TDZ and NAA had no significant difference on the vitrification rate of clustered buds. For 'Scarlet bronze leaf',the type of basic medium and the concentrations of

6-BA, TDZ and NAA showed no significant difference in the vitrification rate of clumped sprouts. Kevers,Pagues and Debergh pointed out that(Maene L et al.1986;M pâques.1991) ,Cytokinins in the culture medium were important for vitrification, which is different from the results of this study, which may be due to different plant materials.

Most studies have shown that MS media are less prone to yield vitrified seedlings(Wang et al.1990;Wang et al.2009) ,which is consistent with the results of this experiment.Among the four media used in this experiment,MS medium has significant difference in vitrification rate compared with the other two types of media,and the vitrification rate is the lowest,the vitrification rate is only 17.50%, and the growth condition of clustered buds is the best(Table 3-6).

Overcoming vitrification seedlings

The effects of sucrose on the vitrification rate and proliferation coefficient of New Guinea Impatiens 'violet' are significantly different. Among them, treatment O1 reduced the sucrose concentration to 20 g L^{-1}, significantly improved the vitrification rate, its vitrification rate reached the lowest value, only 21.67%, At the same time, the proliferation coefficient was the highest among all treatments. However, with the continuous increase of sucrose concentration, the vitrification rate increased, the color became light green, the growth was slow, and the proliferation coefficient decreased obviously(Fig. 4a,b,c). Numerous studies have shown that increasing sucrose or other particle concentrations to increase media osmolality can reduce vitrification rates(He et al.2008;Liu et al.2013;Xiao et al.1997), which is not consistent with the results of this paper.We speculate that maybe because different plants have an optimum value for the tolerance of sucrose concentration, the excessive sucrose concentration may increase the metabolic burden of plants.

$AgNO_3$ is an ethylene inhibitor in plant tissue culture, which acts on ethylene sites to promote organogenesis, bud proliferation and plant regeneration frequency (Akasaka-kennedy Y et al.2005;Ozudogru EA et al.2005). Vinoth and other studies have shown that the addition of silver ions can reduce the occurrence of vitrification(Vinoth et al.2015). In this paper, we also found that the effects of $AgNO_3$ on the vitrification rate and proliferation coefficient of ‘Violet’ were significantly different(Table 5). Among them, the O4 treatment with 1mg $L^{-1}AgNO_3$ added to the culture medium had the best inhibitory effect on vitrification(Fig. 4c), the vitrification rate was only 23.67%, the growth condition of the seedlings was better, the leaf color was dark green, and the proliferation coefficient was reaching 9.70. However, with the increase of concentration, the vitrification rate gradually increased and the proliferation coefficient decreased continuously. Wu Li fang found that when $AgNO_3$ increased to a certain concentration, the proliferation rate of buds began to decrease(Wu et al.2020), which was similar to the results in this paper.

Effects of different kinds and concentrations of hormones on rooting of regenerated seedlings of New Guinea Impatiens

IBA and 6-BA have different effects on the rooting efficiency of New Guinea Impatiens 'Scarlet Bronze Leaf'. In all treatments, although the rooting rate of 'Violet' and 'Scarlet Bronze Leaf' of New Guinea Impatiens in MS medium R1 without any hormone in the control group reached 100%, the roots induced by them were so thin and weak that there were only a few hairy roots, which was not suitable for rooting of regenerated seedlings of New Guinea Impatiens. The rooting effect of adding IBA (R6-R9) and 6-BA (R10-R13) alone was better than that of adding NAA (R2-R5) alone, especially in terms of rooting rate, the rooting rates of 'Violet' and 'Scarlet Bronze Leaf' of New Guinea Impatiens 'Violet' and 'Scarlet Bronze Leaf' were all 100% in the four different treatments added IBA and 6-BA respectively. This is consistent with the result of Pavani Chirumamilla et al that IBA is a better rooting inducing hormone in rooting induction of Kashi eggplant (Chirumamilla P et al.2021). The high frequency of IBA rooting in vitro is due to its fine structure, stability and easy migration into tissue (Hussain SA et al.2018). It was also found that 6-BA and IBA had different emphasis on root induction. As for the root length of rooting, there were significant differences between treatments R12 and R13 with 6-BA and other treatments. As for the rooting number, the treatment with different concentrations of IBA was better than the treatment with other hormones. According to the comprehensive comparison of the average root length, the average number of roots and the growth status of roots, it was found that the best rooting medium for New Guinea Impatiens 'Violet' and 'Scarlet Bronze Leaf' was R7 supplemented with 0.05mg L^{-1}IBA alone, which was consistent with the best rooting induction in the establishment of Ying hui Dan regeneration system of African Impatiens (*I.walleriana*) (Dan Y, et al, 2010). Although the mean root length of R7 was not the longest among all treatments, all were stout hairy roots, and the number of roots was also greatest, 'violet' at 14.17, 'Scarlet Bronze Leaf' at 12.37(Fig. 4e), and the rooting rates of both are 100%.

Conclusion

In this study, the hypocotyls with some cotyledons of 'Violet' and 'Scarlet Bronze Leaf' of New Guinea Impatiens were used as explants to study the disinfection and sterilization of seeds, the induction of clustered buds, the proliferation of clustered buds, the rooting of regenerated seedlings, and the effects of sucrose and $AgNO_3$ on the vitrification of regenerated seedlings. All above in this paper enable us to establish efficient and stable regeneration systems of 'Violet' and 'Scarlet Bronze Leaf' of New Guinea Impatiens.

Abbreviations

MS Murashige and Skoogs medium

PGRs Plant growth regulators

TDZ Thidiazuron

IAA Indole acetic acid

IBA Indole butyric acid

2,4-D 2,4-Dichlorophenoxy acetic acid

PIC Picloram

NAA 1- Naphthalene acetic acid

ANOVA Analysis of variance

Declarations

Acknowledgments

Thanks to Southwest Forestry University for providing experimental instruments and equipment for this experiment, and also thanks to Teacher Huang Haiquan for his patient guidance during the experiment.

Funding

This work was supported by Major Projects of Yunnan Province（202102AE090052）. All author has received research support from Southwest Forestry University.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Di Zhang ,Jie Wei ，Yang Li， Xin Yi Hai Li，Quan Huang，Mei Juan Huang and Yong Hui Wen.The first draft of the manuscript was written by Di Zhang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statements

1. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

2.All data generated or analysed during this study are included in this published article (and its supplementary information files).

3.The datasets generated during and/or analysed during the current study are not publicly available due to [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

4.Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

1. Akasaka-kennedy Y,Yoshida H,Takahata Y. Efficient Plant Regeneration From Leaves of Rapeseed (brassica Napus L.): the Influence of Agno3 and Genotype[J]. Plant Cell Reports, 2005, 24(11): 649-654.https://doi.org/10.1007/s00299-005-0010-8
2. Arisumi T. In Vitro Culture of Embryos and Ovules Certain Incompatible Self and Crosses Among Impatiens Species.[J]. J.amer.soc.hort, 1980, 105.

3. Beyl CA,Sharma GC. Picloram Induced Somatic Embryogenesis in Gasteria and Haworthia[J]. Plant Cell, Tissue and Organ Culture, 1983, 2(2): 123-132.https://doi.org/10.1007/BF00043357
4. Bhattacharyya P,Kumaria S,Job N, et al. En-masse Production of Elite Clones of Dendrobium Crepidatum: a Threatened, Medicinal Orchid Used in Traditional Chinese Medicine (tcm) - Sciencedirect[J]. Journal of Applied Research on Medicinal & Aromatic Plants, 2016, 3(4): 168-176.https://doi.org/10.1016/j.jarmap.2016.04.001
5. Chirumamilla P,Gopu C,Jogam P, et al. Highly Efficient Rapid Micropropagation and Assessment of Genetic Fidelity of Regenerants By Issr and Scot Markers of Solanum Khasianum Clarke[J]. Plant Cell, Tissue and Organ Culture (pctoc), 2021, 144(2): 397-407.https://doi.org/10.1007/s11240-020-01964-6
6. Dan Y,Baxter A,Zhang S, et al. Development of Efficient Plant Regeneration and Transformation System for Impatiens Using Agrobacterium Tumefaciens and Multiple Bud Cultures as Explants[J]. Bmc Plant Biology, 2010, 10(1): 165-165.https://doi.org/10.1186/1471-2229-10-165
7. Guang M A , Guo J P . Effects of thidiazuron and N~6-benzylaminopurine on endogenous hormone levels during regeneration of Brassica campestris ssp. Rapifera[J]. Journal of Zhejiang University, 2010.https://doi.org/10.3724/SP.J.1142.2010.40521
8. Han K. In Vitro Shoot Regeneration From Cotyledons of Immature Ovules of Impatiens Platypetala Lindl[J]. In Vitro Cellular & Developmental Biology. Plant, 1994, 30P(2): 108-112.https://doi.org/10.1007/bf02632138
9. Han K,Stephens LC. Growth Regulators Affect in Vitro Propagation of Two Interspecific I mpatients Hybrids[J]. Scientia Horticulturae, 1987, 32(3): 307-313.https://doi.org/10.1016/0304-4238(87)90096-3
10. Han K,Stephens LC. Carbohydrate and Nitrogen Sources Affect Respectively in Vitro Germination of Immature Ovules and Early Seedling Growth of Impatiens Platypetala Lindl[J]. Plant Cell, Tissue and Organ Culture, 1992, 31(3): 211-214.https://doi.org/10.1007/BF00036226
11. Hussain SA,Ahmad N,Anis M. Synergetic Effect of Tdz and Ba on Minimizing the Post-exposure Effects on Axillary Shoot Proliferation and Assessment of Genetic Fidelity in Rauvolfia Tetraphylla (l.)[J]. Rendiconti Lincei. Scienze Fisiche E Naturali, 2018.https://doi.org/10.1007/s12210-012-0210-4
12. Josekutty PC,Mpikeleli P,Nikolova RV. Calcium Mediated Callus Induction in Impatiens Flanaganiae (vol 62, Pg 199, 1998)[J]. Phyton, 1998, 63(1): 0-0.
13. Kevers C,Gaspar T. Vitrification of Carnation in Vitro: Changes in Water Content, Extracellular Space, Air Volume, and Ion Levels[J], 1986.
14. Maene L,Debergh P. Liquid Medium Additions to Established Tissue Cultures to Improve Elongation and Rooting. Plant Cell Tissue Organ Cult 5: 23-33[J]. Plant Cell Tissue & Organ Culture, 1984, 5(1): 23-33.https://doi.org/10.1007/BF00033566
15. Ozudogru EA,Akcin OT. Effect of Silver Nitrate on Multiple Shoot Formation of Virginia-type Peanut Through Shoot Tip Culture[J]. Vitro Cellular & Developmental Biology Plant, 2005, 41(2): 151-156.https://doi.org/10.2307/4293833
16. Perveen S,Anis M,Aref IM. In Vitro Morphogenic Response and Metal Accumulation in Albizia Lebbeck (l.) Cultures Grown Under Metal Stress[J]. European Journal of Forest Research, 2012, 131(3): 669-681.https://doi.org10.1007/s10342-011-0540-2
17. Siddique I,Anis M. In Vitro Shoot Multiplication and Plantlet Regeneration From Nodal Explants of Cassia Angustifolia (vahl.): a Medicinal Plant[J]. Acta Physiologiae Plantarum, 2007, 29(3): 233-238.https://doi.org/10.1007/s11738-007-0029-2
18. Stafleu FA,Cowan RS,Taxonomy UNIAFP. Taxonomic Literature. a Selective Guide to Botanical Publications and Collections with Dates, Commentaries and Types[J]. Bohn, Scheltema & Holkema, 1976.
19. Stephens LC,Krell SL,Weigle JL. In Vitro Propagation of Java, New Guinea, and Java X New Guinea Impatiens[J]. Hortscience (usa), 1985.
20. Taha A,Wagiran A,Ghazali H, et al. In Vitro Regeneration of Garden Balsam, Impatiens Balsamina Using Cotyledons Derived From Seedlings[J]. Biotechnology(faisalabad), 2009, 8(1).https://doi.org/10.3923/biotech.2009.44.52
21. Witomska M,Lukaszewska .A Effect of Cytokinin Concentration and Explant Type on Micropropagation of Impatiens X Walleriana Hook[J]. Ieee, 2003.
22. Yi-kun HE. Relationship Between the Accumulation of Anthocyanin and Phenylalanine Ammonia Lyase in Impatiens Balsamina[J]. Plant Physiology Communications, 1989.https://doi.org/10.13592/j.cnki.ppj.1989.02.012
23. Jones,L. H. Experiments in Plant Tissue Culture: By J. H. Dodds and L. W. Roberts, Cambridge University Press, 1982. 5.95 (xiii + 178 Pages) Isbn 0521299659. Also Available in Hard Cover. a Complementary Series of Videotapes Are Available From Dr J. H. Dodds, Dept[J]. Trends in Biochemical Sciences, 1983, 8(9): 343.https://doi.org/10.1016/0968-0004(83)90117-2
24. Pâques M. Vitrification and Micropropagation: Causes, Remedies and Prospects[C], 1991.

25. Miloševic,Snežana,Subotic, et al. Elimination of Tswv From Impatiens Hawkerii Bull. and Regeneration of Virus-free Plant.[J]. Electronic Journal of Biotechnology, 2011.https://doi.org/10.2225/vol14-issue1-fulltext-5
26. Panichayupakaranant,P.Naphthoquinone Formation in Cell Cultures of Impatiens Balsamina.[J]. Pharmaceutical Biology, 2001.https://doi.org/10.1076/phbi.39.4.293.5909
27. Chirumamilla P,Gopu C,Jogam P, et al. Highly Efficient Rapid Micropropagation and Assessment of Genetic Fidelity of Regenerants By Issr and Scot Markers of Solanum Khasianum Clarke[J]. Plant Cell, Tissue and Organ Culture (pctoc), 2020(prepublish).https://doi.org/10.1007/s11240-020-01964-6
28. Vinoth,A,Ravindhran, et al.Reduced Hyperhydricity in Watermelon Shoot Cultures Using Silver Ions[J]. In Vitro Cellular and Development Biology. Plant: Journal of the Tissue Culture Association, 2015..https://doi.org/10.1007/s11627-015-9698-5
29. Fu Guofang. Tissue culture and improvement of plant quality of Impatiens officinalis in New Guinea [D]: Beijing Forestry University, 2007.
30. Guo Yungui, Yang Ying, You Linhong. Tissue culture and in vitro flowering test of Impatiens from New Guinea [J]. Green science and technology, 2012 (5): 1-2
31. He Fanglan, Li Yi, Zhao Ming, et al. Study on several factors affecting vitrification of test tube seedlings of Alpine Rhododendron [J]. Journal of Northwest Forestry University, 2008, 023 (1): 104-107.
32. Linzi. Greenhouse scale production of African violet [J]. China Flower horticulture, 2007, 000 (2): 10-13.
33. Liu Xia, sun Chong. Research progress on Causes and control of vitrification of test tube seedlings of woody plants [J]. Journal of Southwest Forestry University, 2013, 33 (5): 93-97
34. Mu Yane, Wang shuanfu, Ji Qingyun, et al. Potato germplasm health testing and regeneration of virus free plantlets [J]. Seed technology, 2011, 029 (1): 29-30.
35. Wang Aizhi, Shen Hailong, Zhang Peng, et al. Occurrence and prevention of vitrification in tissue culture of Sorbus mandshurica [J]. Journal of Northeast Forestry University, 2009, 37 (10): 18-22
36. Wang Dongmei, Huang Shangzhi. Mechanism of seed osmotic regulation and selection of optimal osmotic regulation conditions [J]. Seed, 1996 (5): 7-9
37. Wang Jiawang, Luo Peng, Liao Yanhui. MS and B_ Effect of 5 medium on Vitrification in rape shoot tip culture [J]. Plant physiology communication, 1990 (3): 37-38.
38. Wu Lifang, Tian Xuelian, Wei Xiaomei, et al. Study on vitrification control of test tube plantlets of pigment marigold [J]. Journal of tropical crops, 2020, 41 (1): 110-115
39. Xiao Yulan, Qiu Minghua, Zhou Yonghe. Study on overcoming vitrification of carnation test tube seedlings [J]. Journal of Yunnan Agricultural University, 1997, V12 (3): 188-193
40. Xu Huasong, Xu Jiulong, Huang Xuelin. The role of TDZ in plant tissue culture [J]. Guangxi plants, 1996 (1): 77-80
41. Ye Weiyan, Guo Xiaoyue, Liu Huimin, et al. Establishment of tissue culture rapid propagation system of aseptic seedlings of grapefruit seeds [J]. Guangxi plants, 2015, 35 (6): 891-898
42. Yin Liqing, Zhou Yin, Hu Yonghong, et al. Effects of atrazine on callus induction and somatic embryogenesis of peony [J]. Journal of Nuclear Agriculture, 2013, 27 (8): 1106-1110

Tables

Table 1 Effects of different sterilization treatments on seed germination of Impatiens hawkeri cv.'Violet' and'Scarlet Bronze Leaf'

Contamination method	Sterilization method	V		S	
		Pollution frequency	Germination frequency	Pollution frequency	Germination frequency
S1	70%alcohol 15s+2%Naclo0min	70.00±2.89^{d}	10.00±2.89^{d}	100.00^{d}	0.00^{e}
S2	70%alcohol 15s+2%Naclo5min	30.00±1.67^{c}	60.00±1.93^{c}	26.67±2.89^{b}	60.00±1.67^{c}
S3	70%alcohol 15s+2%Naclo8min	25.00±2.89^{bc}	66.67±4.19^{c}	25.00±2.89^{b}	65.00±1.93^{bc}
S4	70%alcohol 15s+2%Naclo10min	10.00±0.96^{a}	80.00±1.93^{b}	10.00±1.93^{a}	86.67±1.93^{a}
S5	70%alcohol 15s+2%Naclo12min	10.00±0.96^{a}	88.33±0.96^{a}	10.00±2.89^{a}	81.67±1.67^{a}
S6	70%alcohol 15s+2%Naclo15min	6.67±0.96^{a}	76.67±1.93^{b}	5.00±2.89^{a}	70.00±2.89^{bc}
S7	70%alcohol 0s+2%Naclo10min	80.00^{d}	10.00±0.96^{d}	70.00±2.89^{c}	25.00±2.89^{d}
S8	70%alcohol 30s+2Naclo10min	15.00±0.96^{b}	81.67±1.67^{a}	10.00±1.93^{a}	81.67±1.67^{a}
S9	70%alcohol 60s+2%Naclo10min	6.67±1.93^{a}	78.33±0.96^{b}	5.00±2.89^{a}	75.00±1.93^{b}

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance (P < 0.05).V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 2 Effects of different concentrations of 6-BA, TDZ and PIC on adventitious Bud Induction of 'Violet" and 'Scarlet Bronze Leaf" of Impatiens balsamina

Culture medium	PGR(mg·L^{-1})				Induction frequency%%		proliferation coefficient	
	NAA	BA	TDZ	PIC	V	S	Violet	S
I1	0.1	0.4			76.67	70	1.53±0.20^{b}	1.50±0.21^{b}
I2	0.1	0.8			83.33	80	2.73±0.30^{a}	2.67±0.30^{a}
I3	0.1	1.6			80	73.33	2.57±0.29^{a}	2.53±0.34^{a}
I4	0.1	3.2			63.33	66.67	1.47±0.27^{b}	2.23±0.37^{ab}
I5	0.1		0.3		80	70	2.17±0.25^{c}	2.07±0.30^{b}
I6	0.1		0.5		86.67	83.33	5.27±0.59^{a}	5.13±0.60^{a}
I7	0.1		1		80	90	3.60±0.50^{b}	3.97±0.43
I8	0.1		2		56.67	66.67	1.63±0.31^{c}	1.87±0.30^{b}
I9	0.1			0.5	-	-	-	-
I10	0.1			1	-	-	-	-
I11	0.1			2	-	-	-	-
I12	0.1			4	-	-	-	-

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance (P < 0.05).V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 3. Effects of different hormone concentrations and medium types on the proliferation of clustered buds of 'Violet' and 'Scarlet Bronze Leaf' of New Guinea Impatiens

Processing number	PGR(mg·L-1)			Proliferation coefficient		Mean Multiple shoots height	
	6-BA	TDZ	NAA	V	S	V	S
P1	0.40	0.25	0.01	10.50±0.86^{b}	9.22±0.78^{b}	1.27±0.04^{b}	1.05±0.04^{b}
P2	0.80	0.50	0.05	13.18±0.91^{a}	11.52±0.78^{a}	1.46±0.04^{a}	1.19±0.05^{a}
P3	1.20	0.75	0.10	8.48±0.69^{c}	6.37±0.61^{c}	1.14±0.03^{cd}	0.90±0.03^{cde}
P4	1.60	1.00	0.20	6.80±0.59^{de}	7.27±0.65^{c}	1.04±0.03^{de}	0.94±0.12^{bcd}
P5	0.80	0.25	0.10	2.23±0.47^{g}	2.23±0.37^{f}	0.96±0.03^{efg}	0.78±0.02^{efg}
P6	0.40	0.50	0.20	2.53±0.48^{f}	2.53±0.48^{f}	1.04±0.10^{de}	0.71±0.03^{g}
P7	1.60	0.75	0.01	1.82±0.36^{gh}	2.33±0.40^{f}	0.97±0.03^{efg}	0.74±0.02^{fg}
P8	1.20	1.00	0.05	2.28±0.43^{g}	2.57±0.39^{f}	0.89±0.02^{gh}	0.70±0.02^{g}
P9	1.20	0.25	0.20	5.95±0.61^{ef}	4.22±0.43^{de}	1.17±0.04^{bc}	0.88±0.04^{cde}
P10	1.60	0.50	0.10	7.52±0.52^{cd}	3.60±0.34^{ef}	1.16±0.04^{bc}	0.87±0.04^{cdef}
P11	0.40	0.75	0.05	4.70±0.50^{f}	5.05±0.55^{d}	1.02±0.03^{def}	0.96±0.04^{bc}
P12	0.80	1.00	0.01	5.12±0.53^{f}	3.38±0.37^{ef}	1.03±0.03^{de}	0.90±0.04^{cde}
P13	1.60	0.25	0.05	0.42±0.14^{hi}	0.62±0.18^{g}	0.90±0.03^{gh}	0.90±0.03^{cde}
P14	1.20	0.50	0.01	0.37±0.12^{hi}	0.53±0.16^{g}	1.03±0.03^{de}	0.81±0.03^{defg}
P15	0.80	0.75	0.20	0.20±0.09^{i}	0.63±0.17^{g}	0.89±0.03^{gh}	0.84±0.03^{cdefg}
P16	0.40	1.00	0.10	0.75±0.19g^{hi}	0.83±0.21^{g}	0.96±0.02^{efg}	0.88±0.03^{cde}

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance (P < 0.05).V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 3-1. Analysis of variance of different factors on the proliferation of cluster buds of New Guinea impatiens

Variance source	Degree of freedom		Sum of squares		Mean square		F value		Significance	
	V	S	V	S	V	S	V	S	V	S
Basic medium	3	3	12637.14	9095.792	4212.379	3031.931	235.646	210.279	**	**
6-BA	3	3	189.486	174.945	63.162	58.315	2.044	2.615		
TDZ	3	3	625.42	246.428	211.87	82.143	6.96	3.696	**	*
NAA	3	3	336.511	218.586	112.17	72.862	3.649	3.274	*	*

Note：* stands for significant.** stands for very significant.V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 3-2. Multiple comparative test of the effects of different treatments on the multiplication coefficient of rosette buds of impatiens in New Guinea

Basic medium	Multiplication coefficient		TDZ		Multiplication coefficient		NAA		Multiplication coefficient	
	V	S	V	S	V	S	V	S	V	S
MS	9.74±0.41^{a}	8.91±0.39^{a}	0.25	0.25	4.76±0.38^{b}	4.07±0.32^{ab}	0.01	0.01	4.35±0.37^{b}	3.87±0.32^{ab}
1/2MS	2.03±0.21^{c}	2.42±0.20^{c}	0.5	0.5	5.83±0.43^{a}	4.55±0.36^{a}	0.05	0.05	5.43±0.43^{a}	4.58±0.36^{a}
B5	6.10±0.28^{b}	3.70±0.20^{b}	0.75	0.75	3.98±0.32^{bc}	3.23±0.25^{b}	0.1	0.1	4.73±0.32^{ab}	3.26±0.24^{b}
N6	0.43±0.07^{d}	0.65±0.09^{d}	1	1	3.74±0.28^{c}	3.51±0.27^{ab}	0.2	0.2	3.80±0.30^{b}	3.66±0.28^{ab}

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance (P < 0.05).V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 3-3. Analysis of variance of different factors on the height of clustered shoots of New Guinea impatiens

Variance source	Degree of freedom		Sum of squares		Mean square		F value		Significance	
	V	S	V	S	'Violet'	S	V	S	V	S
Basic medium	3	3	14.495	10.143	4.832	3.381	45.673	27.513	**	**
6-BA	3	3	0.722	1.436	0.241	0.479	2.002	3.627		*
TDZ	3	3	6.692	0.423	2.231	0.141	19.577	1.059	**	
NAA	3	3	0.493	1.242	0.164	0.414	1.365	3.131		*

Note* stands for significant.** stands for very significant.V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 3-4 Multiple comparative tests of the effects of different factors on the height of clustered shoots of New Guinea impatiens

Basic medium	Average seedling height		TDZ		Average seedling height		6-BA		Average seedling height		NAA		Average seedling height	
	V	S	V	S	V	S	V	S	V	S	V	S	V	S
MS	1.23±0.02a	1.01±0.04a	0.25		1.04±0.02b		0.4		0.90±0.02a		0.01		0.87±0.02ab	
1/2MS	0.96±0.03c	0.73±0.01c	0.5		1.17±0.03a		0.8		0.93±0.02a		0.05		0.94±0.02a	
B5	1.09±0.02b	0.90±0.02b	0.75		1.01±0.02b		1.6		0.82±0.02b		0.1		0.86±0.02b	
N6	0.91±0.02c	0.85±0.02b	1		0.95±0.01c		3.2		0.86±0.03ab		0.2		0.84±0.03b	

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance (P < 0.05).V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 3-5. Analysis of variance of different factors on the vitrification rate of cluster buds of New Guinea impatiens

Variance source	Degree of freedom		Sum of squares		Mean square		F value		Significance	
	V	S	V	S	V	S	V	S	V	S
Basic medium	3	3	24113.536	15134.786	8037.845	5044.929	286.361	172.414	*	
6-BA	3	3	75.003	12.517	25.001	4.172	0.028	0.007		
TDZ	3	3	163.761	179.092	54.587	59.697	0.062	0.106		
NAA	3	3	249.9	12.483	83.3	4.161	0.095	0.07		

Note：* stands for significant.** stands for very significant.V means New Guinea impatiens'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 3-6. Multiple comparative tests of the effects of different treatments on the vitrification rate of Violet' tufted buds of New Guinea Impatiens'

Basic medium	Vitrification rate (%)
MS	17.50 ± 1.34^{a}
1/2MS	65.42 ± 1.12^{c}
B5	26.25 ± 2.33^{b}
N6	84.17 ± 2.36^{d}

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance ($P < 0.05$).V means New Guinea impatiens'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 4：Effects of different sucrose concentrations on vitrification of Violet' tufted buds

Treatment	Sucrose concentration (g/L)	Vitrification rate (%)	Multiplication coefficient
CK	30	33.33 ± 0.03^{b}	9.35 ± 0.51^{ab}
O1	20	21.67 ± 0.02^{a}	10.17 ± 0.49^{a}
O2	50	45.00 ± 0.02^{c}	8.03 ± 0.36^{b}
O3	70	55.00 ± 0.02^{d}	6.37 ± 0.60^{c}

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance ($P < 0.05$).V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 5. Effects of different AgNO3 concentrations on vitrification of 'Violet' tufted buds

Treatment	AgNO3（mg·L^{-1}）	Vitrification rate (%)	Multiplication coefficient
CK	0	33.33 ± 0.03^{b}	9.35 ± 0.51^{a}
O4	1	23.67 ± 0.02^{a}	9.74 ± 0.63^{a}
O5	2	31.67 ± 0.00^{b}	9.40 ± 0.49^{a}
O6	5	36.67 ± 0.00^{c}	8.55 ± 0.48^{b}

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance ($P < 0.05$).V means New Guinea impatiens 'Violet'.S means New Guinea impatiens 'Scarlet Bronze Leaf'.

Table 6 Effects of different hormone types and concentration ratios on rooting of 'Violet" and 'Scarlet Bronze Leaf" regenerated seedlings of New Guinea Impatiens

Processing number	Rooting frequency		Mean rooting number		Mean rooting height	
	V	S	V	S	V	S
R1	100	100	9.87 ± 0.53^{cd}	11.63 ± 0.85^{a}	1.08 ± 0.06^{d}	1.11 ± 0.05^{de}
R2	96. 67	93.33	7.43 ± 0.92^{fg}	7.56 ± 0.76^{def}	0.68 ± 0.05^{fg}	0.72 ± 0.05^{fg}
R3	100	96.67	9.73 ± 0.92^{d}	8.73 ± 0.75^{d}	0.58 ± 0.03^{g}	0.63 ± 0.04^{h}
R4	73.33	70	4.43 ± 0.87^{h}	6.17 ± 0.90^{f}	0.65 ± 0.05^{fg}	0.66 ± 0.05^{fh}
R5	100	100	1.30 ± 0.53^{i}	3.40 ± 0.78^{g}	0.49 ± 0.04^{g}	0.60 ± 0.03^{hi}
R6	100	100	13.33 ± 1.1^{a}	11.07 ± 0.70^{ab}	1.08 ± 0.06^{d}	1.08 ± 0.06^{e}
R7	100	100	14.17 ± 1.05^{a}	12.37 ± 0.71^{a}	1.01 ± 0.07^{de}	1.14 ± 0.06^{cde}
R8	100	100	10.37 ± 0.73^{bcd}	9.33 ± 0.42^{bcd}	0.80 ± 0.05^{ef}	0.96 ± 0.09^{ef}
R9	100	100	14.53 ± 1.05^{a}	10.57 ± 0.93^{ab}	0.70 ± 0.03^{efg}	0.75 ± 0.08^{fg}
R10	100	100	6.90 ± 0.58^{g}	6.57 ± 0.57^{ef}	1.08 ± 0.06^{d}	1.42 ± 0.10^{b}
R11	100	100	7.47 ± 0.64^{efg}	7.37 ± 0.68^{def}	1.44 ± 0.11^{c}	1.60 ± 0.11^{ab}
R12	100	100	8.10 ± 0.79^{defg}	7.67 ± 0.74^{cde}	1.91 ± 0.16^{a}	1.74 ± 0.14^{a}
R13	100	100	8.63 ± 0.50^{defg}	9.10 ± 0.52^{bcd}	1.74 ± 0.12^{b}	1.55 ± 0.11^{ab}

Note: different lowercase letters in the table indicate significant differences in the results of analysis of variance ($P < 0.05$).V means New Guinea impatiens ‘Violet’.S means New Guinea impatiens ‘Scarlet Bronze Leaf’.

Figures

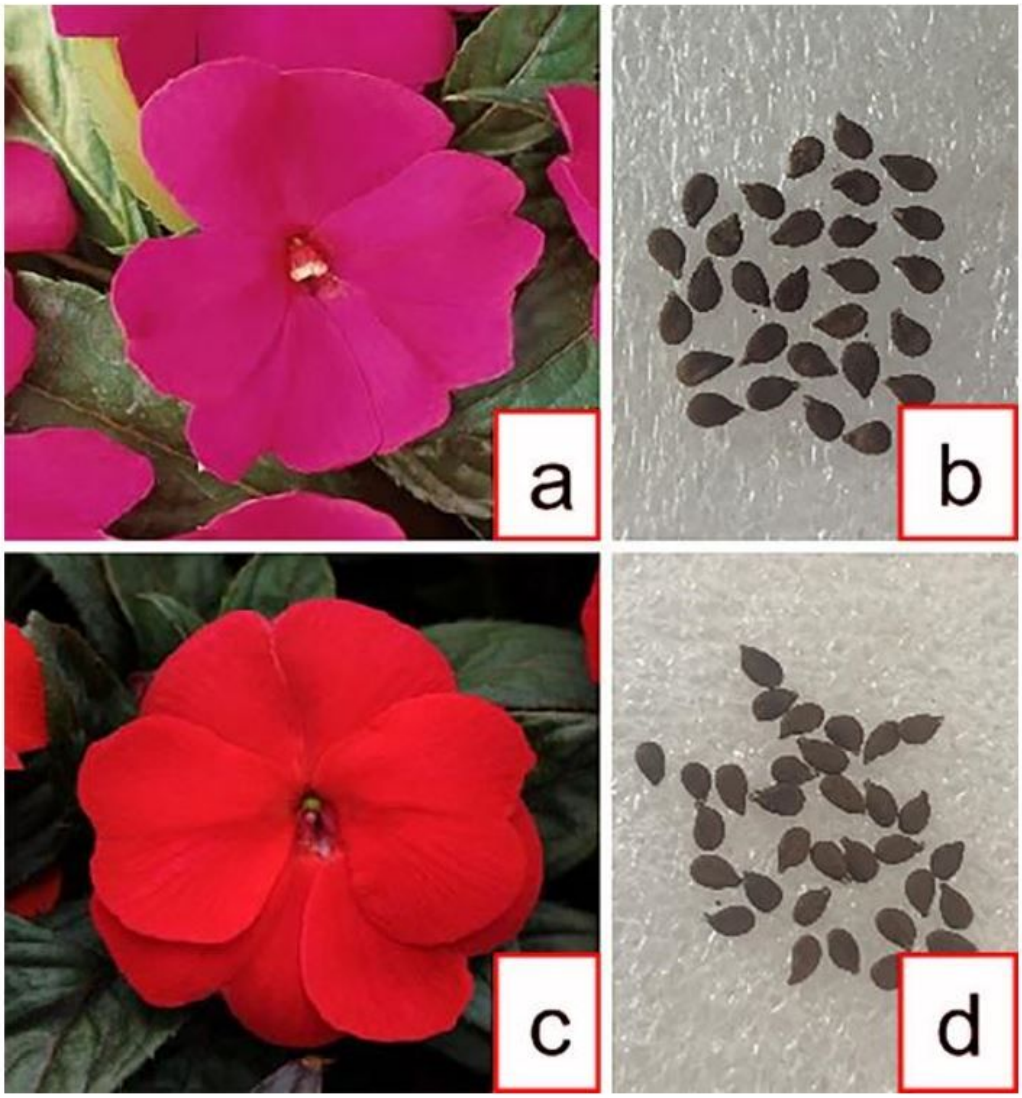

Figure 1

Pictures of plant materials used in this experiment. a-b ‘Violet’ flowers and seeds.c-d ‘Scarlet Bronze Leaf’ flowers and Seeds

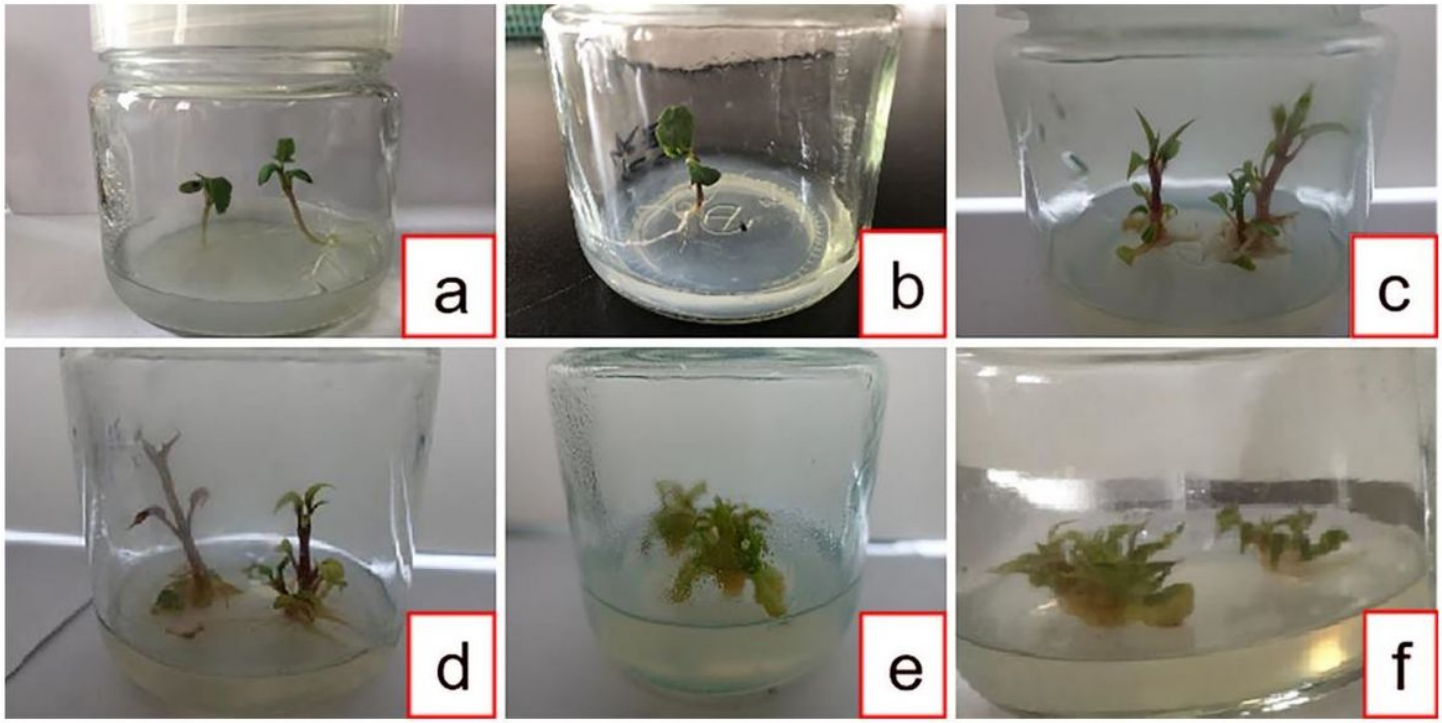

Figure 2

Effects of different sterilization treatments on seed germination and various PGR on tufted bud induction of 'Violet' and 'Scarlet Bronze Leaf'. a The seeds germinated on the culture medium after S4 disinfection treatment of 'Violet' . b The seeds germinated on the culture medium after S7 disinfection treatment of 'Scarlet Bronze Leaf'. c–d The hypocotyls with partial cotyledons of 'violet' and 'scarlet bronze leaf' of Impatiens New Guinea were inoculated in I2 medium for cluster bud induction.e–f The hypocotyls with partial cotyledons of 'violet' and 'scarlet bronze leaf' of Impatiens New Guinea were inoculated in I6 medium for cluster bud induction.

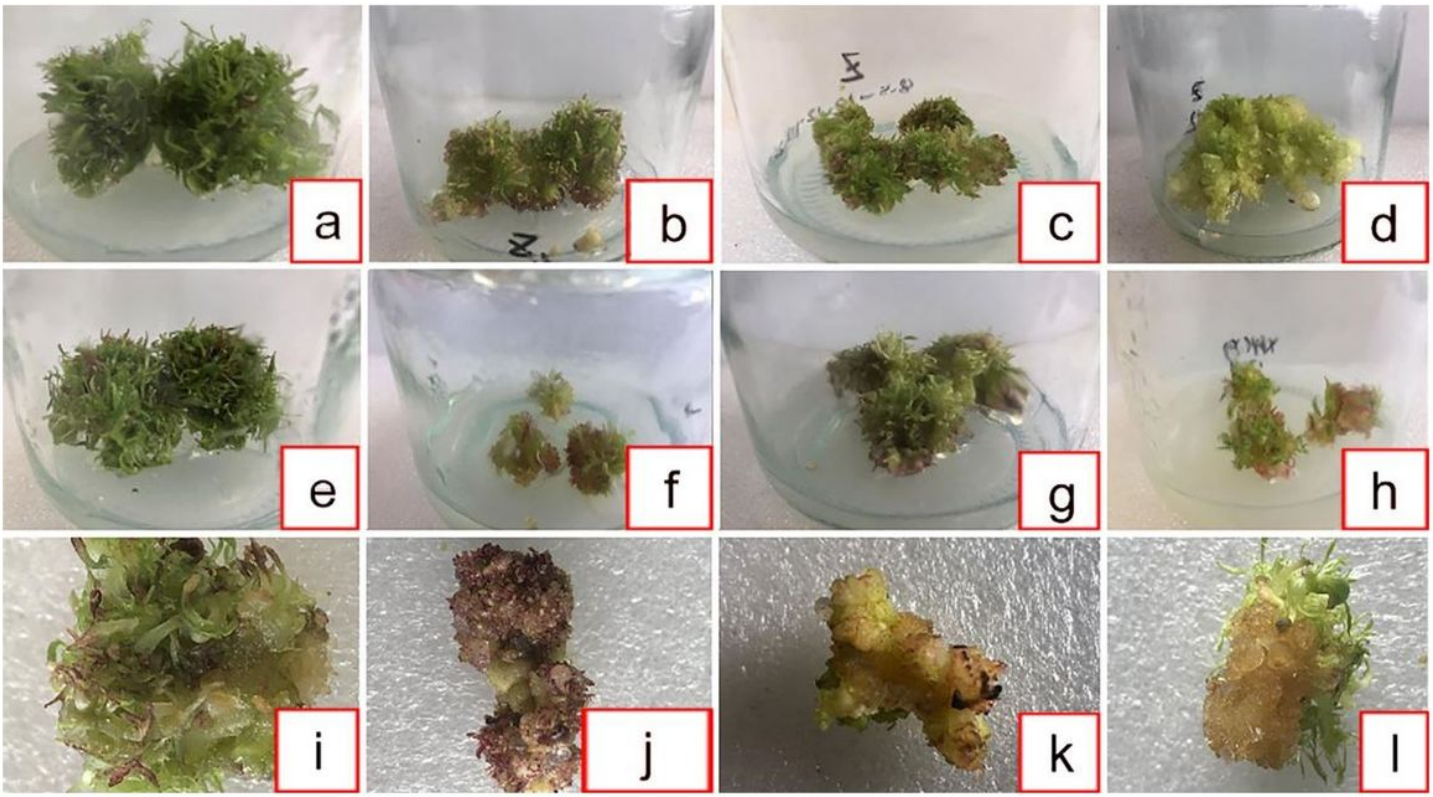

Figure 3

Effects of various PGRs and types of basic media on bud induction and callus induction of Impatiens New Guinea.a-h New Guinea Impatiens ‘violet’（a-d） and ‘Scarlet Bronze Leaf’（e-h） grows on various PGRs and basic media(P2、P5、P10、P13) for cluster bud appreciation..i-l New Guinea Impatiens' Scarlett bronze leaf 'was grown on four different basic media（MS、1/2MS、B5、N6) for callus induction.

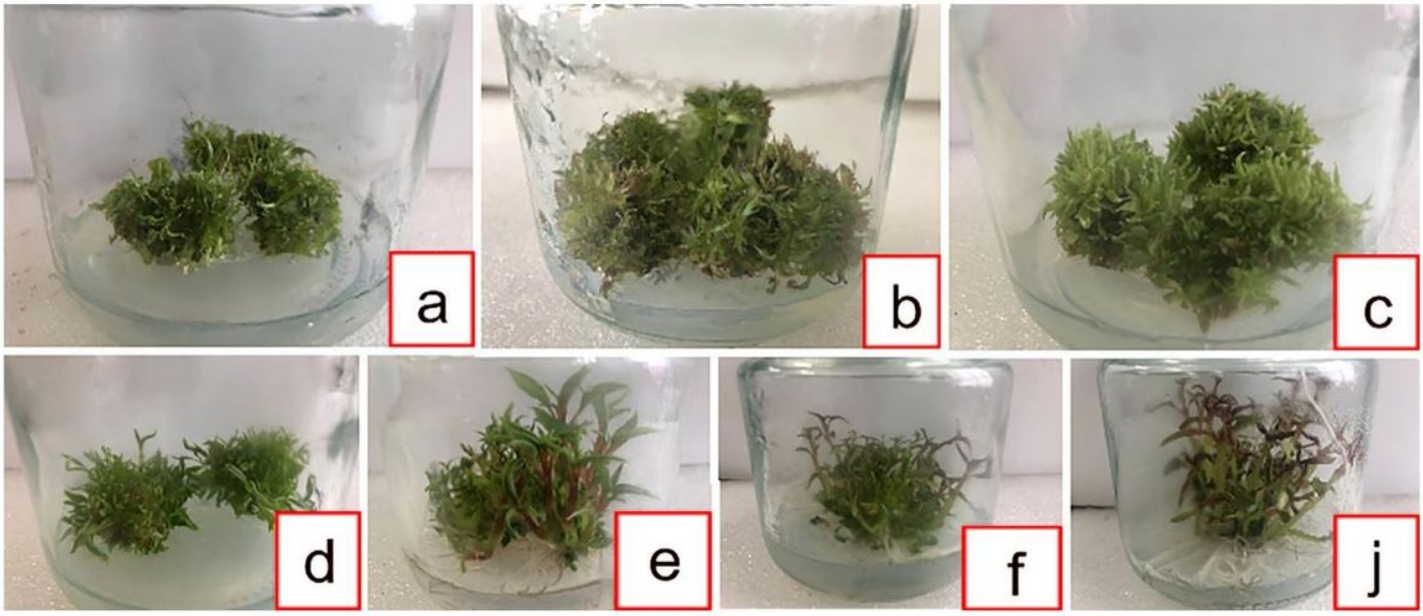

Figure 4

Impatiens New Guinea grew on the medium containing different sucrose and AgNO3 content for vitrification and the medium containing different PGRs for seedling strengthening. a-c Impatiens New Guinea of ‘Violet’ grew on medium containing different sucrose and AgNO3 （CK、O2、O4）content for vitrification. d-j(R6-R9) Impatiens New Guinea of ‘Violet’ and ‘Scarlet Bronze Leaf’ grew on medium containing different PGRs for rooting regenerated.