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THE EFFECT OF GIBBERELLIC ACID (GA_3) ON POST-GERMINATION MORPHOMETRIC PARAMETERS OF LISIANTHUS [*Eustoma grandiflorum* (Raf.) Shinn.] 'MARIACHI PURE WHITE (F_1)' CULTIVAR

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Abstract

Lisianthus [Eustoma grandiflorum (Raf.) Shinn.], a member of the family Gentianaceae, is native to the central and southern part of United States. Lisianthus is a relatively new floral crop to the international market, quickly rankes in the top ten cut flowers worldwide due to its garish flowers and excellent post-harvest life. Due to its importance there are several tissue culture studies working on the micropropagation of this plant by using a variety of plant growth regulators (PGRs). With the aim of contribution to these works in the present study we aimed to investigate the effect of Gibberellic acid (GA_3) at different concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 mg l^{-1}) on morphometric parameters of Lisianthus during 30 and 80-day of incubation periods. In sum, our findings revealed that according to the results, there was no significant change for shoot number, shoot length and root number but very slight increase in number of leaves (1.0 mg l^{-1}) and root length (0.5 mg l^{-1}) of GA_3 during 30-day incubation period. On the other hand, for 80-day incubation period, the highest root length and number of leaves were obtained (respectively, 2.48 ± 1.25 cm and 14.17 ± 4.59) for 2.0 mg l^{-1} concentration. In addition, shoot length to be found at the highest rate for 1.0 mg l^{-1} (3.32 ± 0.34 cm). 80-day incubation period was found to be more effective than in comparison to 30-day incubation period in terms of number of leaves, shoot and root length.

Keywords: *Eustoma grandiflorum*, Lisianthus, Mariachi Pure White (F_1), Gibberellic acid (GA_3), *In vitro*

LISIANTHUS [*Eustoma grandiflorum* (Raf.) Shinn.] 'Mariachi Pure White (F_1)' KÜLTİVARININ ÇİMLENME SONRASI MORFOMETRİK PARAMETRELERİ ÜZERİNDE GİBERELLİK ASİT (GA_3)'in ETKİSİ

Özet

Lisianthus [Eustoma grandiflorum (Raf.) Shinn.], Gentianaceae familyasının bir üyesi olup Orta ve Güney Amerika'nın yerel bitkisidir. Hasat sonrası kaliteli ömrü olması ve gösterişli çiçeklere sahip olmasından dolayı, dünya çapındaki uluslararası pazarda oldukça yeni bir çiçekli ürün olarak, kesme çiçek sektöründe hızlı bir şekilde ilk on sıralamasına girmiştir. Sahip olduğu öneminden dolayı bu bitkinin mikro çoğaltımı üzerinde çeşitli bitki büyüme düzenleyicileri (BBDler) kullanılarak yapılan birçok doku kültürü çalışması mevcuttur. Yapılan bu çalışmalara katkı sağlamak amacı ile 30 ve 80 günlük inkübasyon periyodu süresince farklı konsantrasyonlardaki (0.0, 0.5, 1.0, 2.0 and 4.0 mg l^{-1}) Giberellik asit (GA_3)'in Lisianthus'un morfometrik parametreleri üzerindeki etkisini araştırmak amaçlanmıştır. Özetle, verilen sonuçlara göre bulgularımız, 30 günlük inkübasyon periyodu süresince GA_3'in sürgün sayısı, sürgün uzunluğu ve kök sayısı üzerinde önemli bir değişikliğe neden olmadığını fakat yaprak sayısı (1.0 mg l^{-1}) ve kök uzunluğu (0.5 mg l^{-1}) parametreleri üzerinde nispeten artış gösterdiğini açıklığa kavuşturmuştur. Diğer bir taraftan 80 günlük inkübasyon periyodu süresince, en yüksek sürgün uzunluğu ve yaprak sayısı sırasıyla 2.48 ± 1.25 cm ve 14.17 ± 4.59 olarak 2.0 mg l^{-1} konsantrasyondan elde edilmiştir. Buna ek olarak, en yüksek sürgün uzunluğu 1.0 mg l^{-1} konsantrasyonda (3.32 ± 0.34 cm) bulunmuştur. Yaprak sayısı, sürgün uzunluğu ve kök uzunluğu açısından 30 günlük inkübasyon periyodu ile karşılaştırıldığında 80 günlük inkübasyon periyodu daha etkili bulunmuştur.

Anahtar Kelimeler: *Eustoma grandiflorum*, Lisianthus, Mariachi Pure White (F_1), Giberellik asit (GA_3), *In vitro*

1 Introduction

Lisianthus [*Eustoma grandiflorum* (Raf.) Shinn] is emerging as an important cut flower in the United States while in European and Asian markets it is already listed among the top ten cut flowers [1], its synonyms are *Eustoma andrewsii*; *E. russellianum*; *Lisianthus russellianus* [2,3], at the same time prairie gentian, prairie tulip, prairie rose and Texas bluebells are common names for this plant [4], is an ornamental, herbaceous annual plant from Gentianaceae [5] and is a native species distributed in the American prairies which ranges from South Nebraska to Louisiana and Texas [6]. The plant grows to 15-60 cm tall, with bluish green, slightly succulent leaves, mature rapidly, and produce beautiful funnel shaped flowers growing on long straight stems. The plant is well known for its long vase life, size, and different colors of its flowers [7].

In 2005, the total wholesale value of Lisianthus in the United States for operations with sales worth $ 100,000 or more was $ 4.89 million, with California accounting for 89.4 % of those sales [8]. Most cut flower cultivars of Lisianthus are multiplied by F_1 hybrid seeds and sold for cultivation. As cultivars represent a population of hybrid individuals derived from crosses between heterozygous parents, the use of a true F_1

hybrid would improve the uniformity and quality of the product [9]. However, because of small size of the Lisianthus seeds (19.000 seed/gm or 545.000 seeds/oz) it is hard to handle in field plantings [10]. The seeds are extremely small and progeny is not uniform and inconsistent, displaying rosette [11,12]. Seed-derived plants, in some cultivars, show wide variation due to their heterozygous character and take at least 4.5 months or more to reach the flowering stage [13].

If we look from the viewpoint of Turkey, it attracts attention by metropolitans dealing with cut flower trade, the relevant institutions and organizations in our country. According to TUİK data in "Cut Flower Sector Report 2015" of East Mediterranean Sea Development Agency (DOĞAKA), the royalty given the fact that plant is clearly under way %1 percentile. It is under consideration that this plant attracts considerable attention in the world markets it is also a commercially economic fund for cut flower sector in our country. Consequently, it is an unavoidable situation that for commercially large-scale production of plant need to be brought into use the new technique, method and procedure.

An extensive commercial production via conventional cut flower breeding methods is considerable time consuming and a demanding job. Besides, make production via conventional techniques merely is possible at certain times yearly. Numerous plant growth regulators have been widely used in many flowering plants and their efficacy have been demonstrated for nursery production, foliage plants and many other ornamental plants [14], Young and growing meristematic tissues, apical root cells, young fruits and germinating seeds are rich in gibberellins [15]. Additionally, The most characteristic effects of GA_3 on shoot growth are increase in inter-node extension, increase in leaf-growth, increase in diameter of plant, increase in number of flowers, induce flowering and enhanced apical dominance [16, 17]. The aims of this study are expected to associated with development of technology become a focal point in recent years of this plant that can large-scale produced commercially via plant tissue culture, desirable amounts and able to produced plants in four seasons of the year and reveal that the effects of GA_3 on post-germination period on growing plants able to contribute that commercial production in a short span of time and swiftly.

2 Material and Method

2.1 Germination and Preparation

The seeds of cultivar 'Mariachi Pure White F_1' were used in this experiment. At first, pre-germination nutrient media was supplemented with 4.44 g l^{-1} basal Murashige and Skoog's (MS) medium (1962), 30 g l^{-1} sucrose and solidified with 7 g l^{-1} of plant agar (Duchefa). Its pH was adjusted to 5.7 with 1 N NaOH or 1 N HCl prior to autoclaving at 121°C under pressure of 118 kPa for 20 min. All treatments such as disinfection and inoculations were carried out aseptically in a laminar air flow cabinet. Before the inoculation, external parts of fungicidal-coated seeds were dissolved within eppendorf tubes by dipping in 70% ethyl alcohol (EtOH) for 2 min. Subsequently, seeds were inoculated onto chilled nutrient media containing 80 ml of MS medium in the macro-dimensional petri dishes by the help of sterile forceps. After inoculation each petri dish was swathed with airproof plastic paraffin for protecting to seeds from any contamination. All cultures were maintained at 23 ± 1°C in a plant growth chamber with a 16/8 h light/dark photoperiod under of 80 µmol m^{-2} s^{-1} photosynthetic photon flux density provided by cool-white fluorescent lights.

2.2 Shoot Elongation and Foliation

In the following experiment, MS medium and different concentrations of Gibberellic acid (GA_3) were used to test influence of GA_3 on shooting, foliation and growth.

The germinated plantlets were prepared for the later stage. Plantlets obtained from germination phase were inoculated vertically in culture vessels containing 40 ml of medium undermentioned. Shooting media were supplemented with 30 g l^{-1} sucrose, different concentrations (0, 0.5, 1.0, 2.0 and 4.0 mg l^{-1}) of Gibberellic acid (GA_3), respectively and all of the media were solidified with 7 g l^{-1} of plant agar (Duchefa). Their pH was adjusted to 5.7 with 1 N NaOH or 1 N HCl prior to autoclaving at 121°C under pressure of 118 kPa for 20 min. All treatments such as disinfection and inoculations were carried out aseptically in a laminar air flow cabinet. The aims of this application were the effects of GA_3 on shooting, foliation, growth and compare with two different incubation periods (30 and 80-day) of GA_3 on morphological changes of inoculated plantlets.

Cultures were then incubated at 23 ± 1°C in a plant growth chamber with a 16/8 h light/dark photoperiod under of 80 µmol m^{-2} s^{-1} photosynthetic photon flux density provided by cool-white fluorescent lights. A factorial experiment was arranged in a completely randomized design with six replications. Each treatment consisted of five culture vessels and each vessel contained five plantlets. Data processing of the results was carried out by an EXCEL. Analysis of variance (ANOVA) was done using the software IBM SPSS Statistics 22 was used for statistical analysis and means were compared using Duncan's Multiple Range Test (DMRT). After 30-day incubation period, first results were measured and the second one was measured after 80-day incubation period. Morphometric parameters observed in the experiment that including number of leaves, number of shoots, length of shoots, number of roots and length of roots.

3 Results

3.1 Germination

Plantlets germinated approximately between 11 and 14 days after seed inoculations and gradually showed a bifoliate structure. Totally 151 seeds germinated from 190 inoculated seeds and some of those germinated laboredly. Germinated plantlets of the cultivar that we studied were considerable small and not available for tissue culture applications (Figure 1). Therefore, we performed an application for becoming a plant to the core and we decided to use Gibberellic acid (GA_3) at different concentrations for stem elongation, foliation and growth.

3.2 The Measurements of Morphometric Parameters

After 30-day incubation period, measurements were done according to determined parameters above. The highest average number of leaves was found to be 8.37 ± 1.02 and 8.47 ± 0.77 that media containing 0.5 mg l^{-1} and 1.0 mg l^{-1} GA_3 and increased as compare to control group, respectively while the lowest average number of leaves was found to be 7.07 ± 3.36 and 5.68 ± 2.17 that media containing 2.0 mg l^{-1} and 4.0 mg l^{-1}, respectively. GA_3 was not effective significantly on the number of shoots. The average length number of shoots was found to be 0.79 ± 0.10 cm in control group while decreasing with GA_3 treatments (0.70 ± 0.05 and 0.54 ± 0.11 cm). The average number of roots was found to be 1.87 ± 0.56 in control group while decreasing with GA_3 treatments (0.5, 1.0, 2.0 and 4.0 mg l^{-1}) (1.27 ± 0.27, 1.23 ± 0.32, 1.20 ± 0.54 and 1.13 ± 0.39, respectively). The average length number of roots increased with 0.5 mg l^{-1} GA_3 treatment (3.00 ± 0.53 cm) and was found

to be 2.66 ± 0.52 cm in control group while decreased with other GA_3 treatments (2.47 ± 0.17, 2.47 ± 0.85 and 1.90 ± 0.96 cm, respectively) (Table 1).

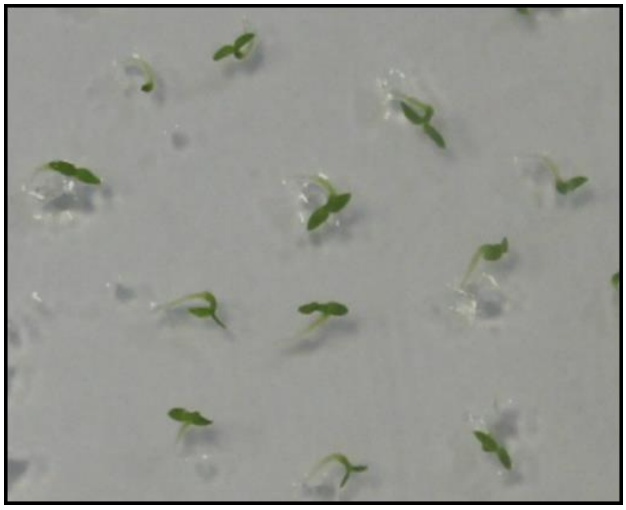

Figure 1. A bifoliate appearance of germinated plantlets 11 days after seed inoculation on MS medium.

After 80-day incubation period, morphological changes turned out to be clear as compare to 30-day incubation period. Length of shoots and improvements of leaves became suitable for tissue culture applications (Figure 2). In addition to this, root number and formation were differ greatly as compare to 30-day incubation period noticeably (Figure 3).

The highest average number of leaves was found to be 14.03 ± 1.85 and 14.17 ± 4.59 that media containing 1.0 mg l^{-1} and 2.0 mg l^{-1} GA_3 and increased as compare to control group, respectively while the lowest average number of leaves was found to be 13.67 ± 1.12 and 12.17 ± 2.81 that media containing 0.5 mg l^{-1} and 4.0 mg l^{-1}, respectively. GA_3 was not effective significantly on the number of shoots the similar with the results of 30-day incubation period. The average length number of shoots was found to be 2.35 ± 0.90 cm in control group while decreasing with 0.5 mg l^{-1} and 4.0 mg l^{-1} GA_3 (2.06 ± 0.44 and 1.90 ± 0.51 cm) and increasing with 1.0 mg l^{-1} and 2.0 mg l^{-1} GA_3 (3.32 ± 0.34 and 2.99 ± 0.89 cm, respectively). The average number of roots was found to be 10.60 ± 1.6 in control group while decreasing with GA_3 treatments (0.5, 1.0, 2.0 and 4.0 mg l^{-1}) (9.67 ± 0.65, 7.80 ± 2.01, 8.77 ± 3.58 and 6.17 ± 2.64, respectively). The average length number of roots increased with 1.0 mg l^{-1} and 2.0 mg l^{-1} GA_3 treatments (2.42 ± 0.77 and 2.48 ± 1.25 cm) and was found to be 2.14 ± 1.22 cm in control group while decreased with 0.5 mg l^{-1} and 4.0 mg l^{-1} GA_3 treatments (1.98 ± 0.72 and 1.45 ± 0.80 cm, respectively) (Table 2). In our study, two important issues caught our attention. The first of these after 80-day incubation period, indication of tip-burned leaves were observed almost in half of plants in MS media containing 4.0 mg l^{-1} GA_3 (Figure 4). The second of these, after 80-day incubation period, indication of nigrescence was observed on root-tips almost all of plants in MS media containing 4.0 mg l^{-1} GA_3 (Figure 5). The reason is that maybe accumulating of toxic compounds within the body of these plants. By virtue of stayed at the same vessels for 80 days running that colour and odour of nutrient media showed an alteration from transparent appearance to dark-smoky.

4 Discussion

We studied the effect of different concentrations of GA_3 on post-germination morphometric parameters of Lisianthus 'Mariachi Pure White F_1' cultivar. Studied characteristics were number of leaves, number of shoots, length of shoots, number of roots and length of roots. Our data revealed that there are differences in the effect of the different concentrations of GA_3 between incubation periods (30 and 80-day). 80-day incubation period was found to be more effective than in comparison to 30-day incubation period that in terms of measurements of number of leaves, shoot and root length. But, the effect of different concentrations of GA_3 on post-germination morphometric parameters were not significant (Tab. 1 and Tab. 2). F_1 hybrid seeds were inoculated on MS medium. Subsequently within the next 11 days, germination was observed. Germinated plantlets of the cultivar that we studied were considerable small and not available for tissue culture applications (Fig. 1). Therefore, we performed an application for becoming a plant to the core and we decided to use Gibberellic acid (GA_3) at different concentrations for stem elongation, foliation and growth. Totally 151 seeds germinated from 190 inoculated seeds and some of those germinated laboredly. The reason is that may F_1 hybrid seeds were used in current study. [18], reported that hybridization is a maladaptive event. In addition, hybrids can have intermediate fitness to their parental species [19, 20], have equal or higher fitness than parental species [21], or have lower fitness [22]. However, in the study of [23] on *Helianthus annuus* (sunflower), they reported that hybrids involving wild plants form different parts of the species' range vary in their fecundity and phenology relative to purely wild plants and part of this variation could be due to different histories of past hybridization and argued over fecundity differences between wild plants and their hybrids that wild-crop hybrids have disadvantage in some cases. For example, F_1 generation may act as a weak and temporary barrier to spread of crop genes after episodes of hybridization and they added more that crop-to-wild introgression will occur most quickly in situations when F_1 fitness is similar to that of wild plants, but even when F_1 plants are at a strong disadvantage, crop-specific alleles will be able to persist when they recombine into a wild-type genetic background.

We were proceed to the next step. Germinated seeds were transferred on MS medium containing different concentrations of GA_3 (0.0, 0.5, 1.0, 2.0 and 4.0 mg l^{-1}). Within the next 3-4 weeks, differences were observed and measurements of 30-day incubation period were done. The supplemented with 1.0 mg l^{-1} GA_3 resulted in the best average number of leaves (8.47 ± 0.77) and supplemented with 0.5 mg l^{-1} GA_3 resulted in the best average root length (2.99 ± 0.53 cm)(Tab.1).

The effects of other treatments (2.0 and 4.0 mg l^{-1} GA_3) were not significant on morphometric parameters in comparison with the control group. Subsequently, cultures incubated in a plant growth chamber again until the 80 days are complete. In the fullness of time that measurements were done. The supplemented with 2.0 mg l^{-1} GA_3 resulted in the best average number of leaves (14.17 ± 4.59), supplemented with 1.0 mg l^{-1} GA_3 resulted in the best average shoot length (3.32 ± 0.34 cm) and supplemented with 2.0 mg l^{-1} GA_3 resulted in the best average root length (2.48 ± 1.25) (Tab.2). The effects of other treatments (0.5 and 4.0 mg l^{-1} GA_3) were not significant on morphometric parameters in comparison with the control group.

Table 1. After 30-day incubation period that the effect of GA_3 at different concentrations on post-germination morphometric parameters of ´Mariachi Pure White (F_1)´ Cultivar.

Hormonal Concentrations (mg l^{-1})	Number of Leaves	Shoot Number	Shoot Length(cm)	Root Number	Root Length(cm)
0.0	7.47 ± 0.82^{ab}	1.07 ± 0.16^{a}	0.79 ± 0.10^{c}	1.87 ± 0.56^{b}	2.66 ± 0.52^{ab}
0.5	8.37 ± 1.02^{b}	1.00 ± 0.00^{a}	0.70 ± 0.05^{bc}	1.27 ± 0.27^{a}	3.00 ± 0.53^{b}
1.0	8.47 ± 0.77^{b}	1.00 ± 0.00^{a}	0.72 ± 0.12^{c}	1.23 ± 0.32^{a}	2.47 ± 0.17^{ab}
2.0	7.07 ± 3.36^{ab}	1.00 ± 0.00^{a}	0.59 ± 0.12^{ab}	1.20 ± 0.54^{a}	2.47 ± 0.85^{ab}
4.0	5.68 ± 2.17^{a}	1.00 ± 0.00^{a}	0.54 ± 0.11^{a}	1.13 ± 0.39^{a}	1.90 ± 0.96^{a}

Values represent means ±S.D. and mean values followed by the same letter are not significantly different based on DMRT at $P<0.05$

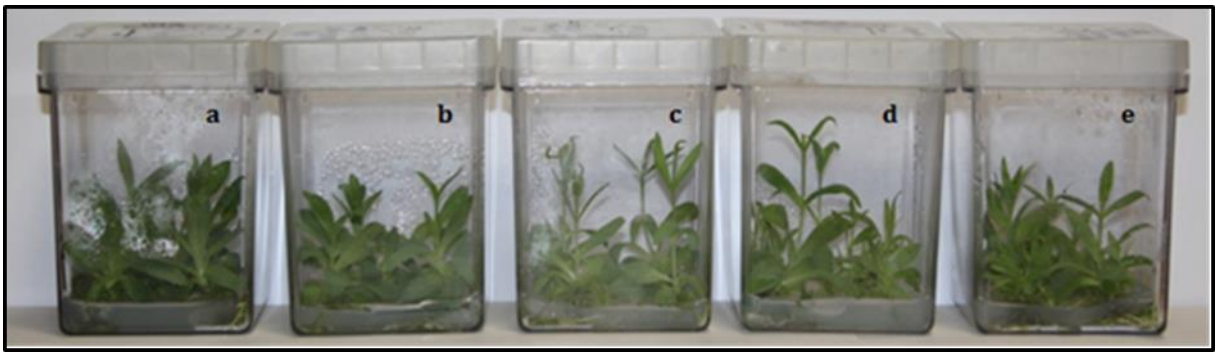

Figure 2. The appearance of 'Mariachi Pure White (F_1)' cultivar after 80-day incubation. **a.** 0 mg l^{-1} GA_3 (Control group), **b.** Shoot elongation and foliation on MS medium with 0.5 mg l^{-1} GA_3, **c.** Shoot elongation and foliation on MS medium with 1.0 mg l^{-1} GA_3, **d.** Shoot elongation and foliation on MS medium with 2.0 mg l^{-1} GA_3, **e.** Shoot elongation and foliation on MS medium with 4.0 mg l^{-1} GA_3.

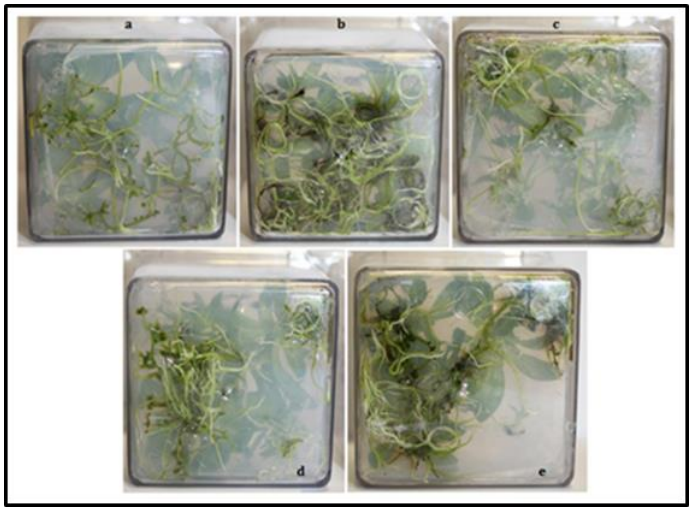

Figure 3. The attitude of root-tips after 80-day incubation. **a.** 0 mg l^{-1} GA_3 (Control group), **b.** Rooting on MS medium with 0.5 mg l^{-1} GA_3, **c.** Rooting on MS medium with 1.0 mg l^{-1} GA_3, **d.** Rooting on MS medium with 2.0 mg l^{-1} GA_3, **e.** Rooting on MS medium with 4.0 mg l^{-1} GA_3.

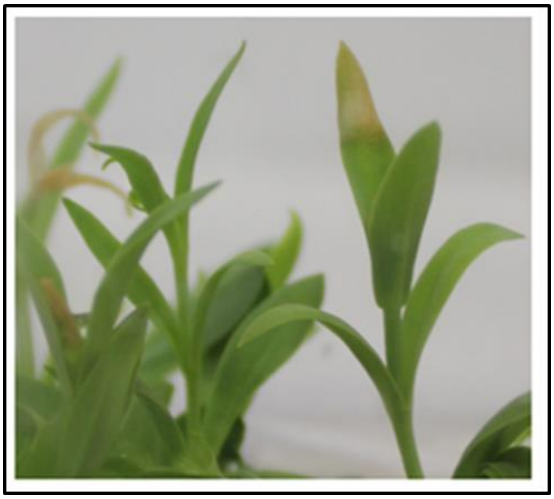

Figure 4. Tip-burned leaves of 'Mariachi Pure White (F_1)' cultivar in MS media containing 4.0 mg l^{-1} GA_3 after 80-day incubation period.

Figure 5. Nigrescence on root-tip of 'Mariachi Pure White (F_1)' cultivar in MS media containing 4.0 mg l^{-1} GA_3 after 80-day incubation period.

Table 2. After 80-day incubation period that the effect of GA_3 at different concentrations on post-germination morphometric parameters of 'Mariachi Pure White (F_1)' Cultivar.

Hormonal Concentrations (mg l^{-1})	Number of Leaves	Shoot Number	Shoot Length(cm)	Root Number	Root Length(cm)
0.0	13.97 ± 1.56^{a}	1.03 ± 0.0^{a}	2.35 ± 0.90^{ab}	10.60 ± 1.6^{b}	2.14 ± 1.22^{a}
0.5	13.67 ± 1.12^{a}	1.00 ± 0.0^{a}	2.06 ± 0.44^{a}	9.67 ± 0.65^{b}	1.98 ± 0.72^{a}
1.0	14.03 ± 1.85^{a}	1.03 ± 0.0^{a}	3.32 ± 0.34^{c}	7.80 ± 2.01^{ab}	2.42 ± 0.77^{a}
2.0	14.17 ± 4.59^{a}	1.00 ± 0.0^{a}	2.99 ± 0.89^{bc}	8.77 ± 3.58^{ab}	2.48 ± 1.25^{a}
4.0	12.17 ± 2.81^{a}	1.00 ± 0.0^{a}	1.90 ± 0.51^{a}	6.17 ± 2.64^{a}	1.45 ± 0.80^{a}
Values represent means ±S.D. and mean values followed by the same letter are not significantly different based on DMRT at P<0.05					

Our results indicated that there are differences in the effect of the different concentrations of GA_3 for number of leaves, shoot length and root length in view of 80-day incubation period while number of leaves and root length parameters were significant in view of 30-day incubation period. Various gibberellins are available and are associated with several plant growth and development processes, such as seed germination, stem elongation, flowering, and fruit development [24,25]. In general, *in vitro* use of gibberellic acid in plant tissue culture takes part in literature in the way that combination with other plant growth regulators or in the way that exogenous foliar spray applications for stem elongation of Lisianthus (*Eustoma grandiflorum*) and usually was tested on shoot elongation or shoot regeneration. Similar to our findings many researchers showed that GA_3 induced shoot length for tissue culture applications on shoot elongation of Lisianthus [26], on *Momordica charantia* L. shoots [27], stimulating on shoot elongation of *Atriplex canescens* [28], on shoots of *Acacia mearnsii* [29], on shoots of *Eucalyptus tereticornis* Smith. [30]. Notwithstanding many researchers showed that GA_3 inhibited

shoot length on shoot elongation of *Vaccinium macrocarpon* Ait. [31, 32] reported that supplemented with GA_3 to media, shoots of *Artemisia dracunculus* L. were browning and trailed off. We observed chlorosis and tip-burned shoots on MS media containing higher concentrations of GA_3 (Fig.4). In accordance with our finding, [33] showed that on *Ficus carica* L. GA_3 induced excessive elongation associated with tip-burned shoots. However, studies of [34] on shoot elongation and rooting of *Prunus institia* showed that GA_3 was deleterious to shoots, causing chlorosis and apical die-back. Over and above this we observed nigrescences on root-tips on MS media containing 0.5 and 4.0 mg l^{-1} of GA_3 (Fig. 5). In accordance with our finding, [35] showed that 0.5 mg l^{-1} GA_3 always finally caused the nigrescence on roots of *Rosa canina*. Contrary to our results, studies of [36] *in vitro* plant regeneration on *Jatropha curcas* showed that lower concentration of GA_3 was found essential for elongation of shoots. Our findings demonstrated that supplemented with 1.0 and 2.0 mg l^{-1} GA_3 resulted in the best shoot lengths after 80-day incubation period.

5 Conclusion

The conducted study reveals that the effect of GA_3 on post-germination morphometric parameters of Lisianthus [*Eustoma grandiflorum* (Raf.) Shinn.] 'Mariachi Pure White (F_1)' cultivar. The use of 1.0 and 2.0 mg l^{-1} GA_3 gave the best results (number of leaves, shoot length and root length) in the long-term (80-day incubation period). But, this period is time consuming, this protocol cannot suitable for *in vitro* large-scale production. Consequently, different concentrations of GA_3 can be attempted in the way that combination with the other plant growth regulators on the behalf of speeding up the process for commercial production.

6 Acknowledgement

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