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PROPAGATION OF ENDEMIC AND ENDANGERED *Sternbergia lutea* WITH A HIGH ORNAMENTAL VALUE BY BULB CHIPPING AND PLANT GROWTH REGULATORS

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ABSTRACT

Autumn daffodil (*Sternbergia lutea*) is a rare and endangered bulbous plant with yellow flowers grown naturally in Iran and unfortunately for different reasons this plant is at the risk of eradication. Natural multiplication rate of *S. lutea* is low, thus propagation by chipping method is one inexpensive and simple way to overcome this problem. This research was therefore performed to investigate the propagation method in two experiments. In the first experiment, the effects of some plant growth regulators (PGRs) on induction and regeneration of bulblets from bulb chips (propagules) were evaluated. The propagules were prepared by chipping method and treated with indole butyric acid (IBA), gibberellic acid (GA_3), benzyl adenine (BA), kinetin (Kin) and cycocel (CCC) at 100 and 200 mg L^{-1} concentrations for 2 h and then incubated for two months at 20 ±1°C. At the end of incubation, the results showed the highest percentage of concurrent bulblet + root regeneration (CBRR), number and fresh weight of bulblets related to 100 mg L^{-1} GA_3. The highest percentage of only bulblet regeneration (OBR), only root regeneration (ORR) and non-regeneration were obtained at 200 mg L^{-1} IBA, 100 mg L^{-1} Kin and control (distillated water: DW) treatments, respectively. In the second experiment, the bulblets that had rooted from each treatment of the first experiment were planted and the produced bulbs were compared. The results indicated that the highest percentage of bulblet sprouting, bulb number and diameter were found at 100 mg L^{-1} GA_3. Therefore, the application of GA_3 with mentioned concentration is appropriate for vegetative propagation of this plant by bulb chipping.

Key words: autumn daffodil, regeneration, bulb, chipping, plant growth regulators

INTRODUCTION

Iranian habitats host about 8000 flowering plant species, which almost 22% are endemic in nature. Iran, additionally is an affluent country in terms of distribution of bulbous plants and more than 200 species of these plants from different botanical families naturally grown and play an incredible role in the colorful exhibit of flowers in the plains, mountains, and forests [Farahmand and Nazari 2015]. *Sternbergia* sp. (*Amaryllidaceae*) is one of the delightful fabulous ornamental bulbs in western, center and northern regions of Iran with Zagros, Irano-Turanian and Hyrcanian climates, respectively [Mazhari 2004]. This genus comprises 25 species that produce yellow, golden and white flowers before, after or synchronously with leaf production in autumn to spring in the world [De Hertogh and Le Nard 1993, Conti et al. 2005]. Three species: *S. lutea* (2n = 22), *S. clusiana* and *S. fischerana* have been reported from Iran [Mazhari 2004]. The endemic and endangered spe-

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cies, *S. lutea* (Fig. 1) is one of the fascinating bulbous plants of Iran with attractive golden-yellow flowers that is at the risk of eradication primarily pertained to some factors, including climate changes, inappropriate herbivory and overgrazing, illegal bulb and flower harvesting, urban expansion and building of roads [Gurbuz et al. 2009, Farahmand and Nazari 2015, Naseri et al. 2019]. In addition to ornamental value, this plant has Amaryllidaceous-type alkaloids such as belladin, galanthamine, lycorine and tazettin which have antimicrobial, antiviral, antifungal, antitumor, antimalarial, antioxidant, antiparasitic and anti-Alzheimer properties [Sener et al. 2003, Oleyede et al. 2010, Jin et al. 2013]. It has also been reported that *S. lutea* is the most important species for bulb trade as ornamental and medicinal plant in Turkey [Zencirkiran and Tumsavas 2006].

Compared with other wild bulbous plants, *S. lutea* has several good cultural characteristics other than mentioned as ornamental and medicinal values: planting and growing easily, has glowing and attractive leaves for use as a ground cover plant, relative resistance to diseases and pests, winter cold resistance, drought tolerance and autumn flowering [Kamenetsky and Okubo 2013, Naseri et al. 2019]. Its combination with other flowering bulbs, particularly blue to violate color species, creates wonderful displays as far as landscape use is concerned. Furthermore, the natural landscapes are economically important from ecotourism perspectives [Farahmand and Nazari 2015]. Therefore, it has a high potential for cultivation in landscape and gardens or for flowering the pot plants. Considering its endangeredness, it is necessary to use easy method with high efficiency for its propagation. A ten-year natural habitat observation in Ilam province located at western Iran (from 2009 to 2018) indicated no seed formation on *S. lutea* most possibly due to low temperature during pollination of flowers. Seeds regularly become frosted before ripening and harvesting time. Vegetative or asexual propagation of bulbous plants have been achieved by many methods such as offsets, cutting, scoping, scoring, chipping, twin-scaling, scaling and leaf cutting and *in vitro* micropropagation [Bach and Sochacki 2013, Knippels 2013]. The use of tissue culture methods can be useful and efficient, but it should be noted that cost-effective methods require special facilities, but chipping technique can be used as a very useful and low-cost method for autumn daffodil propagation.

Fig. 1. Natural rocky habitat of *S. lutea* in Ilam province (Chardavol region) in flowering time (a – November, 2009) and after flowering (b – January, 2011) under Zagros climate

In bulb chipping method, bulbs are cut into more than four segments depending on bulb size and then placed in wet media. Bulb chipping is simpler to twin-scaling for vegetative propagation of some bulbous plants. The benefits of bulb chipping compared to other vegetative methods such as twin-scaling, are simplicity of propagules preparation, doing faster, producing larger bulblets, more mechanized, low cost because it makes possible to plant directly in soil, and achieve high sprouting percentage after transfer to the soil [Flint 1981, Vreeburgh 1986, Rees 1992, Harvey and Selby 1997]. Vegetative propagation using bulb chips (propagules) with or without application of PGRs, have been reported previously for many bulbous plants such as *Narcissus* [Flint and Anderson 1986, Fenlon et al. 1990, Farahmand and Khosh-Khui 2006], *Galanthus* [Aksu and Celikel 2003], *Hippeastrum* [Zhu and Liu 2003], *Lycoris* [Li et al. 2005] and *Hymenocallis* [Knippels 2013]. In this regard, Flint and Anderson [1986] studied the effects of some of growth retardants on propagation of *Narcissus* 'Carlton' through chipping method. They showed that ethephon, triiodobenzoic acid (TIBA), CCC, ancymidol and paclobutrazol increased the number of bulblets per chip, but individual bulblet size was greatly reduced.

The propagation ratio of *S. lutea* is not satisfactory with 1–3 offset bulblets per year [Arslan et al. 2002]. Up to the moment, there is only one report on propagation of *S. lutea* by bulb chipping that has been carried out by Seyidoglu and Zencirkiran [2008] without PGRs application.

This low propagation ratio hinders large-scale cultivation of this plant for ornamental and medicinal purposes. Therefore, a simple, efficient and applicable method for vegetative propagation, large-scale cultivation and conservation must be optimized. This putative method is very crucial to prevent its natural harvest and subsequent eradication. Consequently, the scope of the study was to evaluate the effects of some PGRs on induction and regeneration of bulblets from bulb chip propagules of *S. lutea* and subsequent growth of produced bulbs from planting of these bulblets.

MATERIAL AND METHODS

This study was conducted as two experiments based on completely randomized design from June 2015 to January 2016 on *S. lutea*. In the first experiment, flowering size bulbs of *S. lutea* (Fig. 2a) were collected from natural habitat of Ilam province (Chardavol region) on 15 June 2015 and transferred to laboratory of Department of Horticultural Science, College of Agriculture, University of Kurdistan. Uniform bulbs with similar size and weight were picked out for the experiment. The average length, diameter and weight of one hundred bulbs were 3.35 cm, 3.82 cm and 19.83 g, respectively. In the bulbs, outer brown tunics and dead roots along with part of a basal plate were removed followed by removal of one-third of the bulbs, so that, the remainder had a smooth surface for cut. Afterwards, the bulbs were cut into 5 chips. The propagules were dipped in 100 and 200 mg L^{-1} solutions of BA, GA_3, IBA, CCC and Kin for 2 h along with control that consisted of distilled water (DW). Therefore, in this experiment the effects of 11 mentioned treatments with 6 replications were investigated. Each replication included a plastic bag containing 10 propagules. After these treatments, propagules were mixed with vermiculite and cocopeat (1 : 1 v/v) that was moistened with 2 g L^{-1} of Mancozeb fungicide as medium, then placed in polythene bags (Fig. 2b) and incubated at 20 ±1°C for 2 months under dark conditions. There were also 4 holes in each bag for ventilation. During incubator period of propagules, 4 events occurred in them: (1) concurrent bulblet + root regeneration (CBRR), (2) only bulblet regeneration (OBR), (3) only root regeneration (ORR) and (4) non-regeneration. Thus, at the end of experiment (16 August 2015), the percentage of these traits as well as the number, length and fresh weight of regenerated bulblets and root were measured.

In the second experiment, some of rooted bulblets from each treatment randomly selected of the first experiment as 4 replications (each replication consisted of one pot with 3 bulblets) and were planted in 4 clay pots (2 L) with soil and leaf mold mixtures (1 : 1 v/v) and then incubated in greenhouse at 24/17

±2°C (day/night) temperature, 50 ±5% relative humidity, 800–1000 µmol photon m^{-2} s^{-1} and 16/8 h light/dark regime. After two months, the percentage of sprouted bulblets (Fig. 2d) was recorded and these plants were fertilized with complete commercial nutrient solution (Rosasol Even, containing 20-20-20 combination of NPK) at concentration of 2 g L^{-1} every month. When the plant leaves turned yellow on 15 January 2016, the traits such as number, length, diameter and fresh weight of produced bulbs (Fig. 2e) were measured. Data analysis was performed using MSTATC software and the means were compared at 5% level of probability using Duncan's New Multiple Range Test (DNMRT).

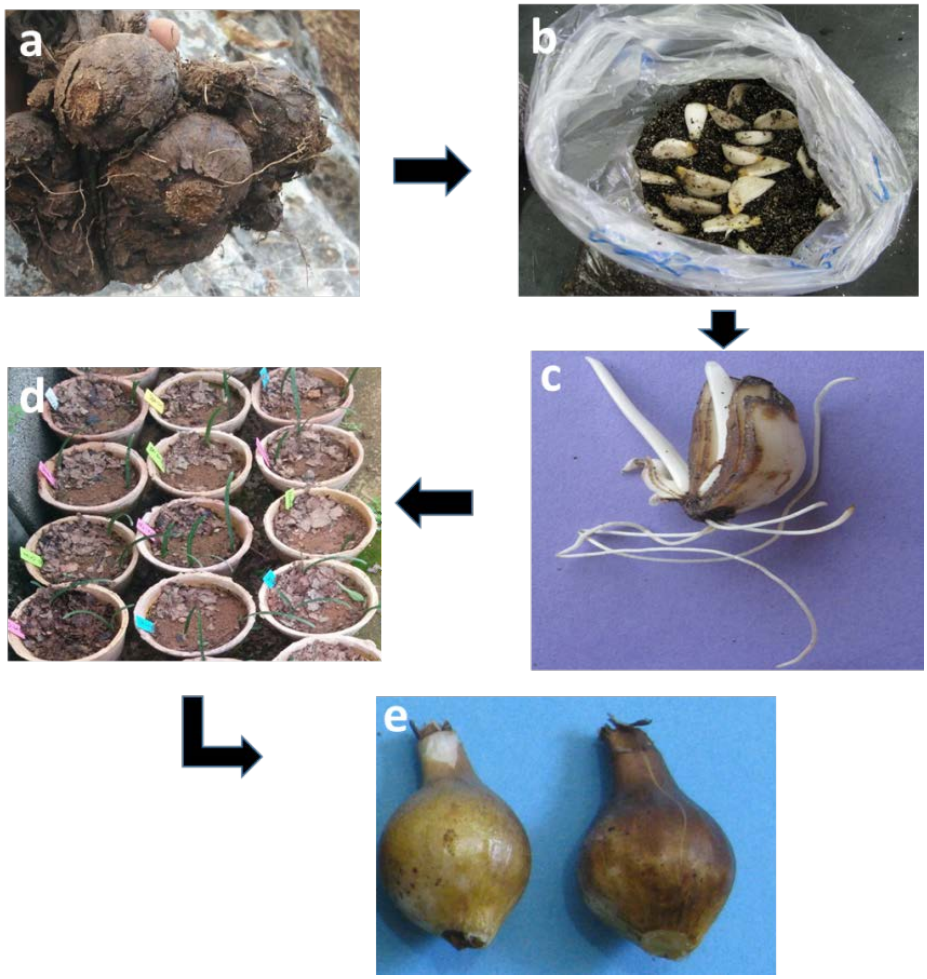

Fig. 2. Flow diagram indicating the concurrent bulblet + root regeneration (CBRR) and bulb production of *S. lutea* by chipping method: a) the mother bulb harvested from natural habitat, b) preparing propagules and incubating with moist vermiculite and cocopeat (1 : 1 v/v) after treated with PGRs, c) bulblets produced from propagules after two months of treated with PGRs (here was 100 mg L^{-1} GA_3) at the end of incubation, d) sprouted plants from bulblets that have root after two months of planting, e) bulbs obtained from sprouted bulblets after three months

RESULTS

The effect of PGRs on concurrent bulblet + root regeneration (CBRR)

The results in Table 1 show that the application of PGRs significantly increased the percentage of CBRR compared to control (DW) treatment. The highest percentage of CBRR was obtained in the propagules treated with 100 mg L^{-1} GA_3, although there was not significantly different with some treatments (Tab. 1). The lowest percentage of CBRR was observed in control and IBA 200 mg L^{-1} treatments. In this propagules with CBRR (Fig. 2c), the highest record for bulblet number per 10 propagules was obtained in the treatment of 100 mg L^{-1} GA_3, although no significant difference was found between this treatment with 100 mg L^{-1} IBA and 200 mg L^{-1} CCC. The use of Kin increased the length of regenerated bulblets and propagules treated with 200 mg L^{-1} Kin had the highest bulblet length, although there was not significant difference with another concentration of Kin. The use of 100 mg L^{-1} Kin significantly reduced bulblet fresh weight, but two concentrations of GA_3 were improved this trait. Higher root fresh weight was obtained of propagules treated with 100 mg L^{-1} GA_3 compared to other treatments (Tab. 1).

Table 1. Effect of two concentrations (100 and 200 mg L^{-1}) of Kin, IBA, BA, GA_3 and CCC along with control (DW) on the percentage of concurrent bulblet + root regeneration (CBRR), number, length and fresh weight of bulblets as well as root fresh weight in theses regenerated bulblets from propagules of *S. lutea* after two months incubated at 20 ±1°C under dark conditions

Treatments	mg L^{-1}	CBRR (%)	Bulblet number[a]	Bulblet Length (cm)[b]	Bulblet fresh weight (g)[b]	Root fresh Weight (g)[b]
Control (DW)	–	28.9 e[*]	5.0 c	2.5 bc	0.43 bc	0.01 de
Kin	100	62.2 a–c	9.7 b	2.8 ab	0.14 e	0.04 f
	200	60.0 bc	10.0 b	3.0 a	0.37 cd	0.12 bc
IBA	100	66.7 ab	10.7ab	2.3 c	0.35 d	0.11 cd
	200	42.2 de	6.3 c	2.6 bc	0.38 cd	0.12 c
BA	100	55.6 b–d	9.0 b	1.9 d	0.32 d	0.04 f
	200	57.8 bc	9.3 b	2.5 c	0.53 a	0.13 bc
GA_3	100	75.0 a	12.7 a	2.5 c	0.56 a	0.38 a
	200	64.4 a–c	10.0 b	2.4 c	0.53 a	0.14 b
CCC	100	51.1 cd	9.0 b	2.3 c	0.32 d	0.05 f
	200	64.4 a–c	11.0 ab	2.5 bc	0.51 ab	0.09 e

[*] In each column, means with the same letter (s) are not significantly different at 5% level of probability using DNMRT
[a] per 10 propagules
[b] per bulblet

The effect of PGRs on only bulblet regeneration (OBR), only root regeneration (ORR) and non-regeneration. The use of IBA at 200 mg L^{-1} led to the highest percentage of OBR than other applied PGRs. The highest bulblet number in these propagules (which regenerated only bulblet) was obtained at 200 mg L^{-1} IBA and 100 mg L^{-1} CCC treatments, although there was no significant difference with control treatment. Bulblet length and fresh weight were affected by application of PGRs (Tab. 2). The use of 100 mg L^{-1} IBA and CCC reduced the bulblet length, although they were not significantly different with some other treatments. The highest bulblet fresh weight was obtained in treatments of 200 mg L^{-1} Kin and two concentrations of BA. The use of Kin caused a greater regeneration of only root and the highest percentage of ORR was observed in propagules treated with 100 mg L^{-1} Kin. Some propagules did not regenerate any organs and the highest of this trait was found in the control propagules. The treatments of 100 mg L^{-1} Kin, 100 mg L^{-1} GA and 200 mg L^{-1} CCC were the best to reduce the percentage of non-regeneration (Tab. 2).

The effect of PGRs on bulblet sprouting and the growth of produced bulbs. After two months of planting, already rooted bulblets from first experiment in soil mixture, the bulblet sprouting percentage (Fig. 2d) was measured and also after three months of emerging of bulblets, some of traits about produced bulbs (Fig. 2e) were evaluated. All of planted bulblets had high sprouting percentage with the exception of bulblets obtained from 200 mg L^{-1} BA treatment (Tab. 3). The bulblets produced from first concentration of all treatments (100 mg L^{-1}) had higher percentage of sprouting and bulb number, although no significant difference was observed between the two concentrations. Bulb length, diameter and weight were significantly affected by PGRs that was applied during bulblets production. The highest bulb length and diameter were obtained from bulblets that regenerated with 200 mg L^{-1} BA and 100 mg L^{-1} GA_3. The highest bulb fresh weight was observed in bulblets that regenerated with 200 mg L^{-1} BA (Tab. 3).

Table 2. Effect of two concentrations (100 and 200 mg L^{-1}) of Kin, IBA, BA, GA_3 and CCC along with control (DW) on the percentage of OBR, number, length and fresh weight of regenerated bulblets, percentage of ORR and non-regeneration from propagules of *S. lutea* after two months incubation at 20 ±1°C under dark conditions

Treatments	mg L^{-1}	OBR (%)	Bulblet number[a]	Bulblet length (cm)[b]	Bulblet fresh weight (g)[b]	ORR (%)	Non-regeneration (%)
Control (DW)	–	28.9 b[*]	5.0 a	1.5 a–c	0.12 bc	6.7 d	42.22 a
Kin	100	22.2 c	3.7 b	1.7 ab	0.10 de	17.8 a	15.6 fg
	200	13.3 d	2.0 c	1.9 a	0.18 a	8.9 cd	26.7 cd
IBA	100	11.1 d	1.7 c	1.1 c	0.07 f	6.7 d	22.2 de
	200	33.3 a	5.7 a	1.5 a–c	0.11 cd	13.3 b	24.4 de
BA	100	11.1 d	1.7 c	1.6 a–c	0.17 a	8.9 cd	33.3 b
	200	11.1 d	2.0 c	1.6 a–c	0.17 a	11.1 bc	31.1 bc
GA_3	100	13.3 d	3.7 b	1.4 bc	0.13 b	6.7 d	11.7 g
	200	13.0 d	2.0 c	1.3 bc	0.09 e	8.9 cd	22.6 de
CCC	100	28.9 b	5.7 a	1.1 c	0.06 f	8.9 cd	20.0 ef
	200	20.0 c	3.0 b	1.6 a–c	0.10 de	8.9 cd	15.6 fg

[*] In each column, means with the same letter (s) are not significantly different at 5% level of probability using DNMRT
[a] per 10 propagules
[b] per bulblet

Table 3. Percentage of bulblet sprouting, number, length, diameter and weight of bulbs of *S. lutea* after planting of already rooted bulblets that were produced in the first experiment with applying two concentrations (100 and 200 mg L^{-1}) of Kin, IBA, BA, GA_3 and CCC along with control (DW)

Treatments	mg L^{-1}	Bulblet sprouting (%)	Bulb number[a]	Bulb length (mm)[b]	Bulb diameter (mm)[b]	Bulb fresh weight (g)[b]
Control (DW)	–	88.9 a[*]	2.7 a	16.7 cd	11.27 bc	1.3 b
Kin	100	100.0 a	3.0 a	17.2 b–d	10.2 b–d	0.1 b–e
	200	88.9 a	2.7 a	16.3 cd	9.6 b–e	0.1 b–e
IBA	100	100.0 a	3.0 a	13.9 cd	9.2 c–e	0.7 cde
	200	77.8 ab	2.3 ab	13.7 d	9.1 c–e	0.6 de
BA	100	77.8 ab	2.3 ab	13.6 d	7.1 e	0.5 e
	200	55.6 b	1.7 b	22.5 a	12.3 ab	1.9 a
GA_3	100	100.0 a	3.0 a	21.4 ab	14.1 a	1.2 bc
	200	77.8 ab	2.3 ab	13.3 d	8.1 de	0.6 de
CCC	100	100.0 a	3.0 a	18.3 bc	9.1 b–d	0.1 b–e
	200	88.9 a	2.7 a	16.7 cd	9.1 b–e	1.1 b–d

[*] In each column, means with the same letter (s) are not significantly different at 5% level of probability using DNMRT
[a] per a pot with three bulblets cultivated in it
[b] per one bulb

DISCUSSION

Wild geophyte plants have a tremendous diversity paving the grounds for the entrance of some other species into flower markets in the future. Autumn daffodil is one of the endangered fabulous species of geophytes, suffering from the lack of knowledge about the physiology of flowering, bulb dormancy, bulb cold requirement and propagation compared to other known bulbous plants such as *Hyacinthus*, *Gladiolus*, *Lilium*, *Narcissus* and *Tulipa* [Gurbuz et al. 2009].

Therefore, strategies for conservation and propagation of this multipurpose plant are essential. In the present study considering the results, it seems that bulb endogenous substances of the *S. lutea* are enough to stimulate bulblet induction and regeneration, but the application of some PGRs could improve regeneration percentage and quality of bulblets. With consideration of 5 propagules per bulb and also by calculating the percentage of bulblet sprouting after planting, for each mother bulb, it can be produced 16.3 daughter bulbs compared to the control (8.8 daughter bulbs were produced) with application of 100 mg L^{-1} GA_3 by bulb chipping method. It may be one of the reasons for better results with GA_3 for CBRR and root fresh weight. It has an effect on eliminating the partial dormancy [Naseri et al. 2019] of the bulb propagules and also with accumulating some carbohydrates such as hexoses finally resulted in energy generation for regeneration and growth of bulblets [Khodorova and Boitel-Conti 2013]. Another reason for this increase may be associated with GA_3 mode of action, as this substance increases the cell division and particularly cell enlargement [Heuvel et al. 2001]. Moreover, it is assumed that increasing in bulblet number and fresh weight using 100 mg L^{-1} GA_3 could be related to the increase in respiration rate and cell division [Farahmand and Khosh-Khui 2006]. Respiration rate and cell division were already reported as the main reasons for reduction in bulblet number in *Narcissus* after the application of maleic hydrazide (anti-GA_3) in chipping method [Flint and Alderson 1986]. Our results for bulblet fresh weight

corresponds to Farahmand and Khosh-Khui [2006], who obtained the highest bulblet fresh weight in Meskin' population of *Narcissus tazetta* with GA_3 compared to other PGRs in bulb chipping.

Our findings also confirm the positive role of GA_3 on *in vitro* corm formation in *Watsonia vanderspuyiae* [Ascough et al. 2008] and *Gladiolus hybrida* [Dharmasena et al. 2011]. Conversely, according to Hanks and Rees [1977], GA_3 application in twin-scaling method of *Narcissus* reduced the number and weight of bulblets. The differences may be related to different methods of propagation, various concentrations of PGRs and the genetic variations, as well. Of course it can be acknowledged that GA_3 with positive effect on the growth of propagules roots and consequently, the absorption of nutrients from the vermiculite and cocopeat mixture by roots, has resulted in better growth of bulblets.

In the present study, application of 200 mg L^{-1} IBA increased the percentage of only bulblet regeneration and following bulblet number in these propagules. Therefore, based on these findings it can be concluded that autumn daffodil bulbs do not have strong apical dominance or auxin PGRs with its self-regulating mechanism decreasing its endogenous concentration. In this regard, Flint and Alderson [1986] applied TIBA as an anti-auxin transport substance on *Narcissus* cv. 'Carlton' propagules and obtained the highest bulblet number. They reported that positive effect of TIBA could possibly be related to weakening the apical dominance, as TIBA interferes with polar transport of auxins. Contrary to our results, also Farahmand and Khosh-Khui [2006] reported that treating the bulb propagules of two populations of *Narcissus tazetta* ('Meskin' and 'Shahla') with IBA, significantly decreased bulblet number in comparison with control and concluded that *Narcissus* has strong apical dominance and application of IBA increases the apical dominance leading to lower bulblet induction. The highest bulblet or bulb length in Tables 1, 2 and 3 was obtained in propagules treated with cytokine sources such as Kin and BA, which may be related to their mechanisms of action including cell division and mobilization [Flint and Alderson 1986].

CONCLUSIONS

1. Natural multiplication rate of *S. lutea* is low and use of bulb chipping method can overcome this problem for large-scale propagation.

2. Bulb endogenous substances of the *S. lutea* are enough to stimulate bulblet induction and regeneration, but the application of some PGRs, particularly GA_3 could improve regeneration percentage and quality of bulblets.

3. The GA_3 has positive effect on the percentage of CBRR from bulb chips propagules, number and fresh weight of the bulblets and bulbs.

4. The application of 100 mg L^{-1} IBA and Kin resulted in the greatest percentage of OBR and ORR, respectively.

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