



Seasonal and plant growth regulators effects on bulblet regeneration in *Hippeastrum hybridum* propagated via sectioning and scoring techniques

Behzad Edrisi¹ , Asghar Hosseini nia² 

¹ Department of Postharvest Physiology and Technology, Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran.

² Department of Production Technology and Management, Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran.

Abstract

This research aimed to compare propagation methods of Amaryllis (*Hippeastrum hybridum*) bulbs in terms of propagation rate and bulblet yield. Accordingly, a split plot factorial design was conducted in a randomized complete block design with three replications. The main plot had 4 seasons and the factors included two cutting types of the bulb, sectioning and scoring, each with 4 and 8 cuts and four plant growth regulators (PGRs) treatments, including I- no PGR (control), II- indole butyric acid, III- benzyladenine, and IV- combined use of (IBA) and (BA). The best results were obtained in spring and autumn. So that the highest number of bulblets was formed in autumn and the largest size of bulblets was formed in spring. Summer was the most inappropriate time for propagation. Regardless of the season and type of PGRs, the most number of bulblets was formed in the eight-slit scoring method, but the largest size of bulblets was obtained in the four-slit scoring, and the smallest bulblets were created in the eight-piece section method. Overall, the bulb scoring method was more suitable for propagation than the bulb sectioning method. The use of PGRs increased the number of bulbs. The use of PGRs, regardless of its type, increased the size of bulblets compared to the control. The use of IBA produced relatively larger bulblets, but there was no significant difference with the BA, the smallest bulblets were formed in the control treatment without PGRs. The largest number of bulblets was also produced by benzyladenine treatment.

Keywords: Amaryllis, Benzyladenine (BA), Commercial propagation, Indole butyric acid (IBA), Protocol

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1. Introduction

Amaryllis is a genus in the Amaryllidaceae family, order Asparagales, and comprising over 75 species. Amaryllis (*Hippeastrum hybridum* Hort.), is a popular bulbous flower that can be easily cultivated both indoors and outdoors. It is native to tropical regions of South America. The large flowers and ease of flowering have made it favored around the world. The flowers display a wide range of colors including red, white, pink, fawn and orange with striped designs. Amaryllis bulbs production is valuable in international ornamental plant markets, but the production of amaryllis cut flowers is less economical (Tombolato, 2004). The Netherlands is the largest producer of this ornamental flower (Meerow et al., 2014). The greenhouse cultivation of Amaryllis in the Netherlands is more than 109 hectares, of which about one third is used for cut flower production and the rest for bulb production. More than 54 million amaryllis flowers are produced annually, most of which are exported to European countries (Meerow et al., 2014). Also, the sales of cut amaryllis flowers in 2012 were reported to be 107 million flowers in the Dutch Flora Market (Hubner, 2014; Hanks, 2015). Amaryllis is propagated by seeds, bulblet and layering (Sharifi, et al., 1994). However sexual propagation is not commercially viable and is often used for create new cultivars breeding programs (De Bruyn, 1997) and (Huang, et al., 1990).

One of the main problems with Amaryllis production is the low rate of natural propagation and the long growing period required to produce marketable flowering bulbs. Conventional propagation of Amaryllis by bulbils is very slow. Normally, each plant typically produces 2-3 bulbs per year. In addition, some Amaryllis varieties or hybrids do not produce bulbils (Dohare 1989; Smith et al., 1999). Improving the propagation method is one of the short-term ways to grow bulbs. The main problems faced by Amaryllis growers are: 1. the high labor requirement for vegetative propagation. 2. The long growing period (compared to other crops) until the bulbs are ready to flower and sell. 3. The difficulty in adjusting and controlling the flowering time of the bulbs to coincide with the best marketing season (Ephrat, et al., 2001). The main goal of Amaryllis propagation is out of season production of daughter bulbs that are completely identical to the mother plant and to obtain more bulbs in a shorter time than natural

propagation (Jamil, et al., 2014). According to old reports, the standard method for propagating Amaryllis is to use twin scales, however, small daughter bulbs produced from twin scales require about three years to produce mature bulbs (Okubo, 1993). Amaryllis bulbs are produced in both the Northern and Southern Hemispheres. Temperature and storage duration of bulbs may cause variation in germination or flowering. Evaluation of the effects of storage temperatures of 5, 9, 13, 21 and 29°C for 6, 9, 12 and 15 weeks on dry bulb life, germination, flowering and time to completion of forcing of three Amaryllis cultivars 'Apple Blossom', 'Red Lion' and 'Minerva' showed that leaf and bud emergence of bulbs treated at 21°C decreased with increasing storage temperature. In storage at lower temperatures, bulbs are more likely to become unusable during storage. For forced bulbs, days to leaf emergence increased with increasing storage temperature but decreased with increasing storage duration. Days to bud emergence decreased with increasing storage time. The storage temperature of the bulbs that produced a high-quality product after planting was 5, 9 or 13°C for Minerva and Red Lion and 5 or 9°C for Apple Blossom. (Kuehny and Miller. 2006)

The results of the study (Witomska, et al., 2005) showed that for the propagation of scaly cuttings of hippeastrum, the use of perlite growing medium gave much more favorable results than perlite growing medium with peat. However (El-Naggar and El-Nasharty, 2009) reported that leaf soil growing medium or leaf soil growing medium with sand (in equal volume ratio) was the best growing medium for the propagation of Amaryllis with desirable quality. Jayshree et al (2020) carried out an experiment to study the effects of benzyl adenine and gibberellic acid (GA) on the morphological behavior of Asiatic lilies. In this study, a total of 16 treatments were administered with varying amounts of GA (100, 150, and 200 ppm) and BA (50, 100, and 150 ppm). The investigation confirmed that the plants treated with BA at 150 ppm took the longest (12.12 days) to sprout bulbs, had the largest basal stem diameter (18.06 mm), the highest level of total chlorophyll, and the shortest plant height (36.72 cm), leaf length (7.11 cm), and leaf number (29.33).

Currently, almost all of the amaryllis bulbs needed in Iran are imported, so supplying them domestically is of great importance. On the other hand, studies

conducted in reliable databases show that few reports on the propagation of this ornamental plant in Iran have been published so far. Therefore, providing quick and cost-effective propagation protocols for mass production of this plant appears both necessary and essential. This research aimed to compare methods of artificial propagation of *Amaryllis* bulb in terms of propagation rate and bulblet production, in order to determine the best propagation season, the effects of PGRs on bulblet formation.

2. Materials and Methods

2.1. Location and plant Materials

This research was conducted in 2021 in the research greenhouses of Ornamental Plants Research Center located in Mahallat (33°88' N, 50°48' E, altitude 1714 m). *Amaryllis* bulbs of selected genotype were prepared from the Ornamental Plants Research center. For this purpose, cutting treatments were performed on selected bulbs during the first ten days of each season. All equipment was disinfected with alcohol and the bulbs were surface-sterilized using a fungicide solution containing benomyl, captan and sodium hypochlorite. The bulb's incisions were made with a sharp, sterilized knife.

2.2. Performing treatments

A split plot factorial experiment was carried out and analyzed as a randomized complete block design with three replications. The main plot had 4 times including the seasons of spring to winter and the factors included propagation method (complete cutting of the bulb and scoring (each with 4 and 8 cuts) and a total of four types of cuts. Another factor were four treatments including I- control (no PGR), II- indole butyric acid treatment (IBA) 250 mgL⁻¹, III- benzyladenine (BA) 250 mgL⁻¹, and IV- simultaneous use of IBA and BA.

For PGR treatment, the bulbs were immersion in PGR solutions at a concentration of 250 mg L⁻¹ for 10 minutes. Then, to allow drying and healing of the incisions, bulbs were placed in a mixture of 30% perlite and 70% cocopeat, and transferred disposable plastic containers and stored in a dark place at 25°C. The bulb formation and growth period was divided into three stages; stage I: 2 weeks for wound healing and induction of bulb formation, stage II: 10 to 11 weeks for growth of the formed bulbs and completion of possible dormancy effects at 20 °C and 80% relative humidity.

2.3. Planting and evaluating the generated bulbs

At the end of the propagation period, mother bulbs or pieces of divided bulbs were planted together with the newly formed bulbs in pots containing the same mixture of perlite-cocopeat (30:70) and maintained in a greenhouse at 18-20 °C. During this period, the number of diameters and weights of the resulting bulbs per mother bulb were recorded. After harvesting, the yield per mother bulb was determined based on the number, diameter and weight of the bulbs produced. Irrigation and fertilization were also carried out according to existing practical recommendations.

2.4. Statistical Analysis

The data were analyzed using SAS software, and means were compared with Duncan's test at a 5% level.

3. Results and Discussion

Based on the analysis of variance results presented in Table 1, the effects of season, cutting type, and PGR treatment were all significant ($P < 0.01$) on the number, weight, and diameter of formed bulblets. Propagation outcomes varied significantly across seasons (Tables 1 and 2), with the best results observed in spring and autumn. The highest number of bulbs formed in autumn while the largest bulbs developed in spring.

Table 1.

The ANOVA analysis of PGR, scoring or sectioning (cutting type) and season on bulblets from bulb mothers

SOV	DF	Mean square		
		Bulb number	Bulb weight (gr)	Bulb diameter (mm)
Block	2	2.976 ^{ns}	2394.194**	147.63**
Season	3	47.978***	23665.39***	5000.98***
Block × Season	6	2.503 ^{ns}	935.70*	37.31 ^{ns}
Cutting type	3	25.811***	15080.44***	3633.37***
PGR	3	24.623***	3244.12***	290.6***
Season× Cutting type	9	28.633***	689.02*	179.66***
Season × PGRs	9	4.561*	1043.54**	52.05*
Cutting type × PGRs	9	4.289*	1004.18**	47.48*
Season× PGRs × Cutting type	27	1.911 ^{ns}	434.115 ^{ns}	56.646***
Error	120	1.906	330.79	23.475

ns, *, **, *** means non-significant and significant using Duncan test at 0.05 and 0.01, 0.001 respectively

Table 2.

The effects of different seasons on bulblets from bulb mothers

Season	Bulb number		Bulb weight (gr)		Bulb diameter (mm)	
Spring	4.46	b	67.61	a	28.27	a
Summer	3.05	c	14.28	c	6.61	c
Autumn	5.46	a	48.87	b	27.58	a
Winter	4.04	b	39.04	b	17.26	b

Summer was the least suitable season for propagation. It was reported that the best results for scale propagation of *Hippeastrum vittatum* were achieved when the scale sections weight was not less than 1.0 g. Vermiculite and perlite were identified as suitable culture media. The best (V/V) water content was 3-18% for vermiculite and 9% for perlite. Significant difference in propagation were also observed among cultivars. The growth stage of the bulb affected the production of bulblets, and round bulbs had a higher propagation efficiency than the main bulb. And December was the best time for scale propagation of *Hippeastrum vittatum* (Ya-ling et al., 2008).

By using the bulb sectioning method, the size of the daughter bulbs increases and as a result, a shorter time is required to produce marketable bulbs (Ephrath et al.,

2001; Sandler-Ziv et al., 1997). Ephrath et al (2001), regarding the effect of cutting types on the propagation of the mother bulb, reported that when the mother bulb is divided into smaller pieces, the number of daughter bulbs increases. In a study for propagation of bulbs (8 cm in diameter) 9 cutting methods were applied. The methods including 1. wholebulb, 2. Only the basal plate of the bulb, 3. making 8. Cuts in the basal plate, 4. Basal plate without a central bud, 5. Cutting the basal plate into two halves, 6. Cutting the basal plate into four parts, 7. Longitudinal cutting into four parts, 8. Longitudinal cutting into eight parts, and 9. Cutting the basal plate into four sections and then separating each into five two-scale pieces. When the main bud was maintained, no bulblet were formed; when using the basal plate: by removing the central bud, cutting into two parts and cutting into four parts, 5-6 bulbs were obtained; when the whole bulb was used and cut

lengthwise into four and eight parts, 10 and 13 bulbs were obtained, respectively. In contrast, when the basal section was divided into four parts and each part into five two-scale groups, a total of 25 bulbs were obtained. The results showed that *Amaryllis* propagation was higher when the basal plate of the mother bulb was divided into four parts and each part into five two-scale groups (Andrade-Rodríguez, et al., 2015).

Regardless of the season and type of PGRs used, the largest number of bulbs was formed in the eight-section cutting method, but the largest bulbs were obtained in the four-slit method, and the smallest bulbs were formed in the eight-section method, but there was no significant difference with the four-slit method. Overall, the bulb scoring method was more suitable for propagation than the bulb division method (Table 3).

Table 3.

The effects cutting type and season on bulblets from bulb mothers

Cutting types	Bulb number		Bulb weight (gr)		Bulb diameter (mm)	
Four-scored	3.96	bc	64.172	a	28.55	a
Eight-scored	5.28	a	49.575	b	26.27	b
Four-section	4.18	b	29.427	c	12.95	c
Eight-section	3.56	c	26.628	c	11.94	c

Means with different letters at each column were significant using Duncan test (P value<0.05)

Previous studies have shown that if the mother bulb is cut into undivided parts, it produces fewer bulbs compared to the separated parts of the bulb. Increasing the number of parts into which the bulb was divided produces more bulbs. Sectioning method for cutting the mother bulb is a suitable method for creating 12-16 pieces depending on the size of the mother bulb (Jamil, et al., 2014). Increasing the number of sections into which the bulb was cut resulted in a larger number of bulblets, but the diameter of bulblets produced decreased (Kharrazi et al., 2017). They reported that that sectioning the bulb into 8 segments is the best method for vegetative propagation of *Amaryllis*.

Study the effect of bulb cutting and planting medium composition (Jamil, et al., 2014), showed that bulb cutting significantly affected all parameters except the days required for the emergence of the first leaf and leaf width on the 60th day after planting. The highest number of plants (2.20) per piece and bulbs (2.20) per piece of bulb were obtained from the 4-sections bulb cutting treatment, and the maximum diameter (20.74 mm) and the weight (57.65 g) of the bulblet and plant were obtained from the 2-sections bulb cutting treatment. The planting medium also had a significant effect on all the parameters studied. The maximum number (2.04) of plants and bulbs per piece

of onion was obtained in the medium containing only compost, while the medium containing equal proportions of sand, soil and compost produced the largest bulb size (20.07 mm) and the maximum combined weight (44.75 g) of the bulb and plant. However, the combined effect of 4-sections bulb cutting and compost substrate produced the maximum number (2.60) of plants and bulbs per bulb piece, while the largest bulb size (23.05 mm) and the highest yield (68.66 g) of bulbs and plants were obtained in the 4-sections bulb cutting treatment and substrate containing equal proportions of sand, soil, and compost (Jamil, et al., 2014).

The use of PGRs increased the number of bulbs. Regardless of their type, the PGRs caused the bulbs to be larger than the control. Although there was no significant difference in bulb size between the PGRs, the use of IBA produced relatively larger bulbs, but there was no significant difference with BA. The smallest bulbs were formed in the control treatment without PGRs. The largest number of bulbs was also produced by the use of BA (Table 4).

Table 4.

The effect of different PGRs on bulblets from bulb mothers

Treatment	Bulb number	Bulb weight (gr)	Bulb diameter (mm)
Control	4.25 b	31.41 b	17.46 b
BA	5.17 a	46.05 a	21.40 a
IBA	4.16 b	50.65 a	22.6 a
BA+IBA	3.42 c	41.69 a	18.26 b

Means with different letters at each column were significant using Duncan test (P value<0.05)

To compare the effects of treatments in different seasons, their effects were analyzed separately in each season. Accordingly, in the spring, there was a completely significant difference between the types of bulb cutting in all traits (P value<0.01). The type of PGRs was significant on the number of bulbs and their

diameter (P value<0.01 and 0.05), respectively, but it had no effect on the weight of the bulb. The interaction effects of PGRs and cutting were also significant only on the fresh weight of the bulb (P value<0.05) (Table 5-6).

Table 5.

The ANOVA analysis of PGRs and cutting type on bulblets from bulb mothers

SOV	DF	Mean square		
		Bulb number	Bulb weight (gr)	Bulb diameter (mm)
Block	2	8.52 ^{ns}	4587.13*	153.928**
Cutting type	3	32.80***	7731.77***	1222.27***
PGRs	3	11.25*	1599.26 ^{ns}	132.369**
Cutting type × PGRs	9	1.97 ^{ns}	2319.66 ^{ns}	55.55*
Error	30	3.899	958.15	22.362

ns, *, **, *** means non-significant and significant using Duncan test at 0.05 and 0.01, 0.001 respectively

In the summer, there was a completely significant difference between the types of bulb cutting in all traits (P Value<0.01). The effect of PGRs type was significant only on the number of bulbs at the 5% level, but it had no effect on the weight and diameter

of the bulb. The interaction effects of PGRs and cutting were also significant on the number and fresh diameter of the bulb (P value<0.01 and 0.05), respectively (Table 7-8).

Table 7.

The ANOVA analysis of different PGRs and cutting type and season on bulblets from bulb mothers in summer season

SOV	DF	Mean square		
		Bulb number	Bulb weight (gr)	Bulb diameter (mm)
Block	2	0.5058 ^{ns}	3.902 ^{ns}	2.405 ^{ns}
Cutting type	3	30.719***	1678.20***	337.014***
PGRs	3	1.1275*	71.089 ^{ns}	22.249 ^{ns}
Cutting type × PGRs	9	3.1984***	80.445	25.26**
Error	30	0.304	5.13	7.683

ns, *, **, *** means non-significant and significant using Duncan test at 0.05 and 0.01, 0.001 respectively

Table 6-The effects of different PGRs and cutting type and season on bulblets from bulb mothers in spring season

Treatment	Bulb number		Bulb weight (gr)		Bulb diameter (mm)	
Four-scored	2.00	de	71.70	bcd	31.20	cd
Four-scored+BA	2.33	c-e	115.17	ab	47.68	a
Four-scored+IBA	3.00	b-e	78.93	bcd	38.71	bc
Four-scored+BA+IBA	1.33	e	147.58	a	41.46	ab
Eight-scored	3.00	b-e	38.10	d	27.45	de
Eight-scored +BA	6.33	ab	53.15	cd	31.43	cd
Eight-scored+IBA	3.67	a-e	109.14	abc	42.94	ab
Eight-scored+BA+IBA	4.33	a-e	71.59	bcd	32.71	cd
Four-section	4.67	a-e	39.46	d	18.47	f
Four-section+BA	7.33	a	52.60	cd	21.05	ef
Four-section+IBA	5.67	a-d	66.40	bcd	24.53	def
Four-section+BA+IBA	4.67	a-e	44.31	d	20.14	ef
Eight-section	6.00	a-c	40.37	d	16.82	f
Eight-section+BA	7.33	a	56.84	bcd	19.50	ef
Eight-section+IBA	5.33	a-d	53.19	cd	17.69	f
Eight-section+BA+IBA	4.33	a-e	43.15	d	20.60	ef

Means with different letters at each column were significant using Duncan test (P value<0.05)

Table 8-The effects of different PGRs and cutting type and season on bulblets from bulb mothers in summer season

Treatment	Bulb number		Bulb weight (gr)		Bulb diameter (mm)	
Four-scored	3.83	bc	23.42	b	10.19	bc
Four-scored+BA	4.27	bc	25.51	b	11.88	bc
Four-scored+IBA	6.37	a	21.72	b	14.90	ab
Four-scored+BA+IBA	4.43	bc	25.05	b	7.70	cde
Eight-scored	4.67	b	21.49	b	10.23	bc
Eight-scored +BA	2.40	d	38.39	a	17.90	a
Eight-scored+IBA	3.70	bc	22.44	b	8.97	cd
Eight-scored+BA+IBA	3.43	c	16.89	c	7.46	cde
Four-section	4.33	bc	2.47	fg	1.51	f
Four-section+BA	3.60	c	5.36	ef	2.34	f
Four-section+IBA	2.17	de	8.93	de	3.58	ef
Four-section+BA+IBA	1.93	f	10.15	d	4.56	def
Eight-section	0.80	f	1.18	fg	0.96	f
Eight-section+BA	0.90	de	1.85	fg	1.18	f
Eight-section+IBA	0.53	f	0.67	g	0.80	f
Eight-section+BA+IBA	1.37	f	3.02	fg	1.63	f

Means with different letters at each column were significant using Duncan test (P value<0.05)

Table 10- The effects of different PGRs and cutting type and season on bulblets from bulb mothers in autumn season

Treatment	Bulb number		Bulb weight (gr)		Bulb diameter (mm)	
Four-scored	6.67	bc	46.83	b-f	35.48	b
Four-scored+BA	5.33	cd	94.05	a	42.67	ab
Four-scored+IBA	5.67	cd	86.45	a	45.22	a
Four-scored+BA+IBA	3.00	d	56.29	bc	34.41	bc
Eight-scored	8.67	ab	39.50	b-g	36.87	ab
Eight-scored +BA	11.00	a	61.61	b	39.92	ab
Eight-scored+IBA	6.67	bc	90.90	a	42.31	ab
Eight-scored+BA+IBA	6.33	bcd	50.90	bcd	36.04	ab
Four-section	3.00	cd	21.07	gh	14.62	de
Four-section+BA	6.00	bcd	36.06	c-h	16.19	D
Four-section+IBA	4.00	cd	58.74	bc	26.77	C
Four-section+BA+IBA	3.67	cd	13.75	h	7.00	E
Eight-section	4.33	cd	24.91	e-h	15.97	d
Eight-section+BA	6.00	bcd	28.73	e-h	16.93	d
Eight-section+IBA	4.00	cd	48.49	b-e	18.20	d
Eight-section+BA+IBA	3.00	cd	23.71	fgh	12.62	de

Means with different letters at each column were significant using Duncan test (P value<0.05)

Table 12-The effects of different PGRs and cutting type and season on bulblets from bulb mothers in winter season

Treatment	Bulb number		Bulb weight (gr)		Bulb diameter (mm)	
Four-scored	3.87	b-e	46.71	bc	19.65	bcd
Four-scored+BA	3.73	b-e	56.19	ab	18.99	bcd
Four-scored+IBA	4.93	a-d	52.98	b	24.49	abc
Four-scored+BA+IBA	2.67	e	78.18	a	32.23	a
Eight-scored	5.17	abc	37.71	b-e	19.78	bcd
Eight-scored +BA	6.30	a	41.07	b-e	18.50	bcd
Eight-scored+IBA	4.40	a-e	54.48	ab	28.87	ab
Eight-scored+BA+IBA	4.43	a-e	45.84	bcd	18.98	bcd
Four-section	3.73	b-e	21.69	cde	9.90	de
Four-section+BA	5.37	ab	31.89	b-e	13.39	cde
Four-section+IBA	3.67	b-e	38.75	b-e	14.84	cde
Four-section+BA+IBA	3.17	de	19.20	de	8.33	de
Eight-section	3.20	cde	25.94	cde	10.32	de
Eight-section+BA	4.47	a-e	38.35	b-e	22.87	abc
Eight-section+IBA	2.87	e	18.25	e	8.75	de
Eight-section+BA+IBA	2.63	e	17.41	e	6.25	e

Means with different letters at each column were significant using Duncan test (P value<0.05)

However, in the autumn season, there was a completely significant difference between bulb cutting types and PGRs type in all traits (P Value<0.01). The interaction effect of PGRs type and cutting type was not significant (Table 9-10).

Table 9.

The ANOVA analysis of different PGRs and cutting type and season on bulblets from bulb mothers in autumn season

SOV	DF	Mean square		
		Bulb number	Bulb weight (gr)	Bulb diameter (mm)
Block	2	0.771 ^{ns}	148.72 ^{ns}	26.58 ^{ns}
Cutting type	3	41.417***	4801.15***	2130.85***
PGRs	3	19.805***	3784.34***	246.23***
Cutting type× PGRs	9	3.657 ^{ns}	276.26 ^{ns}	26.43 ^{ns}
Error	30	2.393	169.54	24.143

ns, *** means non-significant and significant using Duncan test at 0.001 respectively

In the winter season, the effect of bulb cutting type was also completely significant and there was a difference in all traits (P Value<0.01). The effect of PGRs type was only significant on the number of bulbs

and (P Value<0.01), but had no effect on bulb weight and diameter. The interaction effects of PGRs and cutting were also not significant (Table 11-12).

Table 11

The ANOVA analysis of different PGRs and cutting type and season on bulblets from bulb mothers in winter season

SOV	DF	Mean square		
		Bulb number	Bulb weight (gr)	Bulb diameter (mm)
Block	2	0.687 ^{ns}	461.55 ^{ns}	76.62 ^{ns}
Cutting type	3	6.768**	2936.37***	482.209***
PGRs	3	6.122**	199.64 ^{ns}	45.901 ^{ns}
Cutting type × PGRs	9	1.193 ^{ns}	350.56 ^{ns}	110.17*
Error	30	1.028	190.358	39.71

ns, *, **, *** means non-significant and significant using Duncan test at 0.05 and 0.01, 0.001 respectively

Zhuang et al. reported that sawdust was the most optimal substrate for the propagation of twin scales, which caused the least amount of scale decay and the production of the best quality bulbs. In addition, the temperature of 25 °C was more suitable than 30 or 20 °C. Gibberellic acid (GA3), Indole-3-butyric acid (IBA) or 1-naphthyleneacetic acid (NAA) had no effect on scale propagation and bulb growth. Treatment with NAA in particular significantly increased the abundance of decayed scales. They were

reported that, during the formation and growth of the bulblets, the starch content in the mother scales, especially in the outer scales of the twin scales, decreased earlier and faster (Zhang, et al., 2013).

Four different sizes of *Hippeastrum hybridum* 'Appleblossom' bulbs were soaked in 0, 0.01, 0.1 and 0.5 gL⁻¹ benzyladenine (BA) and incubated at 15, 20, 25°C with variable temperatures of 31°C during the day and 21°C at night. The number of bulbs after incubation and the number of leaves after cultivation were recorded. Eight weeks after planting, bulb diameter and leaf number were assessed. BA application had no effect on bulb induction. However, bulb formation increased with both increased temperature (above 25°C) and mother bulb diameter. Bulb diameter and survival increased with increasing incubator temperature to 20°C. After planting, leaf emergence rate was inversely related to bulb size. (Gushing and Klingaman, 1995). In bulb production from *Fritillaria imperialis* bulbs, mother bulbs were compared in solutions containing equal doses of NAA plus BA at four levels (0, 100, 200 and 300 mgL⁻¹) and four vegetative propagation methods (scoring, 4-sections cutting, 8-sections cutting). The results showed that there were significant differences between the different vegetative propagation methods and levels of growth regulators (NAA BA). The highest average number of bulb production was obtained from the 8-sections cutting method, and the lowest average was obtained from the bottom-slit method. Bulbs treated with 300 mgL⁻¹ NAA BA showed the highest and lowest bulblet regeneration in the non-PGR treatment. The use of 300 mgL⁻¹ NAA along with 300 mgL⁻¹ BA in combination with horizontal cutting of the bulbs showed the highest bulblet formation rate in the mother bulb (31.3), which was significantly higher than the control (Solgi, et al., 2015). BA have participated in influencing the morphology of plant, flower induction process, sex expression, breaking dormancy, synthesis of proteins, cell elongation and enlargement (Thakur et al., 2023)

3.1. Multivariate analysis

A "hierarchical agglomerative cluster assessment" was used to group the treatments based on increasing dissimilarity. Cluster I included four-section and eight-scored, which had low to very low values for bulb number (Figure 1). Cluster II included four-section and eight-scored. Summer and autumn had medium to low values for bulb weight and diameter and highest bulb number. Control had lowest bulb diameter and weight. IBA+BA had lowest bulb number.

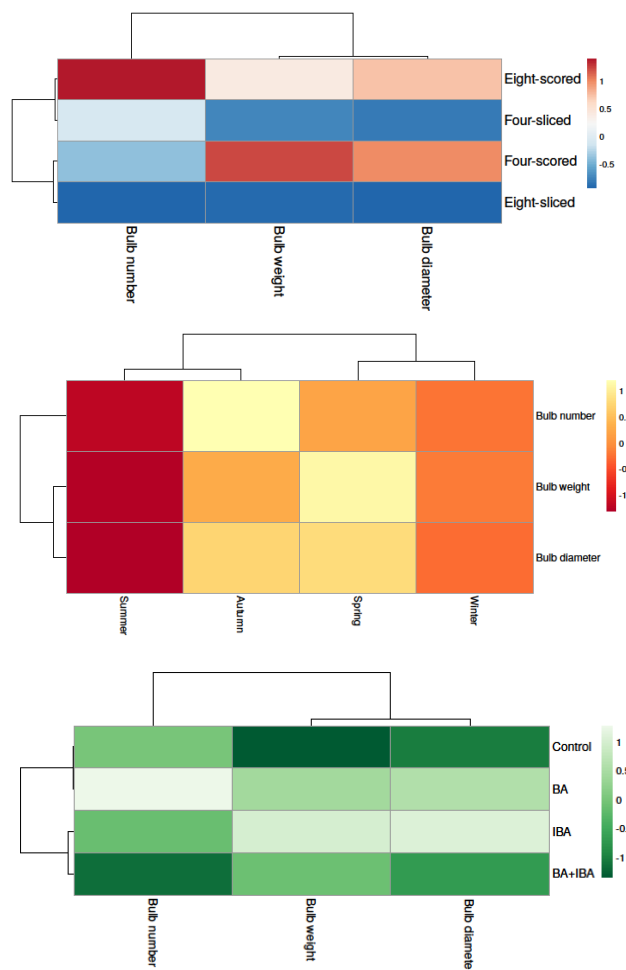


Figure 1. Cluster analysis (a) of PGRs, cutting type and season on bulblets induction from mother bulbs.

Principle components analysis (PCA) was performed to determine the dispersion of treatments. The variances explained by the first two components were 99.4 and 0.6%, respectively (Figure. 2). The PC1 showed the positive correlations with bulb diameter and number.

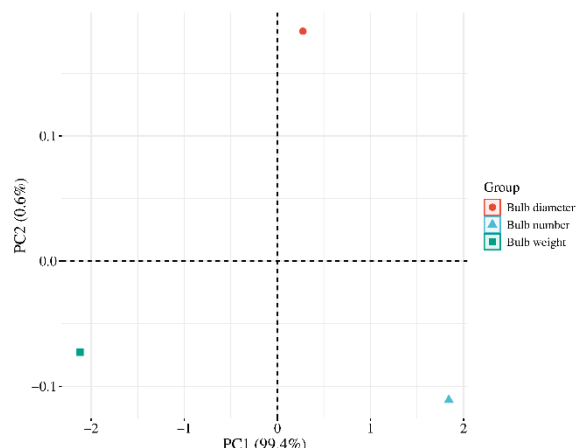
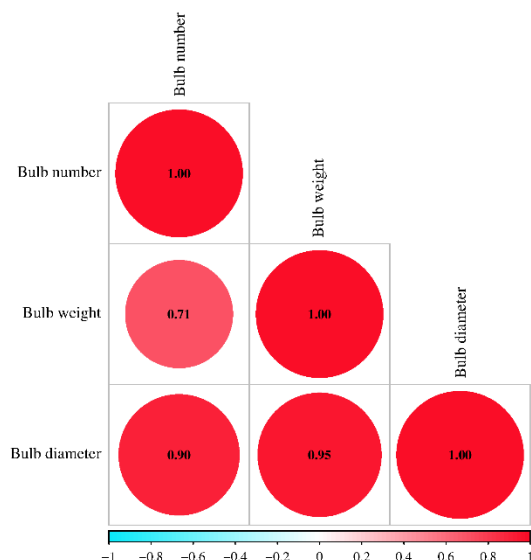


Figure 2.
The dispersion of PGRs, cutting type and season on bulblets induction from mother bulbs.

The PC2 was negatively correlated with bulb weight (Figure. 2). According to the correlation between traits, Bulb number had positive correlation with, bulb weight (0.71) and diameter (0.90) (Figure.



3).

Figure 3.
Correlation plots of PGR, cutting type and season on bulblets induction from mother bulbs.

4. Conclusion

Based on the findings of this study, both scoring and bulb division techniques can be effectively

used to propagate Amaryllis bulbs, but in general, the scoring technique yield better results than bulb sectioning. As a recommended commercial protocol, the following guidelines are suggested: For achieving the largest number of propagated bulbs, it is recommended to use the 8- scoring method in the fall and use BA treatment. For producing larger bulbs with greater weight and diameter, the 4-sectening method in the spring and IBA treatment is recommended.

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