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ORIGINAL RESEARCH PAPER

Effect of chipping and scoring techniques on bulb production of Lachenalia cultivars

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Abstract

The genus Lachenalia (Asparagaceae) represents spectacular and botanically diverse ornamental, bulbous species originating from Southern Africa. The main aim of this study was to determine, for the first time, the possibility of propagation of lachenalia cultivars ('Namakwa', 'Rainbow Bells', 'Rupert') by two in vivo techniques: chipping and scoring, which can be applied in procedures of bulb production. In the second part of the experiment, the plant growth and the quality of bulblets after the first season of cultivation in a greenhouse were estimated. The survival ability of bulb-scale segments and scored bulbs of the tested cultivars ranged from 62% to 95%. Chipping method generated eight bulblets per mother bulb with an average weight and diameter of 0.17 g and 0.29 cm, respectively, while scoring two/three bulblets per mother bulb with an average weight and diameter of 0.28 g and 0.5 cm, respectively. In the first season of cultivation, bulblets obtained by chipping produced longer leaves than those obtained by scoring technique. Only bulblets of 'Rupert' obtained by scoring showed the capacity to flower ('Namakwa' and 'Rainbow Bells' remained in a juvenile phase). When assessing the bulb yield after the first season of cultivation, it was found that irrespective of cultivar, bulbs obtained by chipping achieved twice the weight coefficient of those obtained by scoring.

Keywords

Cape Hyacinth; bulb-scale segments; cross-cutting; bulblets; propagation

Introduction

The genus Lachenalia belongs to the Asparagaceae family and is represented by 130 endemic bulbous species native to South Africa and Namibia. The bulbs are tender to frost and thus not suitable for permanent outdoor cultivation in regions with a winter minimum temperature of less than -1°C [1]. An interesting characteristic of the genus are spots and striped markings on the leaves and a multitude of flower colors [2] - for this reason, new cultivars are becoming increasingly popular as cut flowers and are also cultivated as pot plants and for gardening [3]. Lachenalia, known internationally under the trade name "Cape Hyacinth", has a potential to become an extremely attractive ornamental bulbous plant – each cultivar has its own unique color characteristics and provides the ornamental value as a cut flower for two to four weeks [4,5].

To preserve the identical characteristics of a particular genotype, lachenalia is propagated vegetatively by bulblets (named also offsets or daughter bulbs), bulbils (aerial bulblets) [1], leaf cuttings (bulblets are formed at the base of the cutting) [6,7], or tissue culture [8]. Lachenalia does not produce offsets fast enough for commercial production and so leaf cutting technique is used to efficiently propagate new cultivars [4]. So far, there have been no studies on other vegetative techniques of lachenalia propagation, such as chipping and scoring (cross-cutting), that could be used on a larger scale in

horticultural practice. Propagation by chipping is used commercially with *Eucomis* [9], *Galanthus* [10] *Hippeastrum* and *Lilium* [11], *Narcissus* [12]. The scoring method is applicable, inter alia, for *Hyacinthus*, *Scilla*, *Fritillaria* [13], *Hymenocallis* [14], and *Ornithogalum* [15].

An experiment was conducted to compare new alternative and economic methods of producing bulblets from bulbs of three lachenalia cultivars. The results obtained show the potentiality and efficiency of lachenalia multiplication by chipping and scoring and supplement the knowledge of possible techniques of lachenalia propagation which may be helpful in the future in planning and monitoring experiments related to unknown ornamental bulbous plants.

Material and methods

Chipping and scoring

Lachenalia (Lachenalia J. Jacq. ex Murray) bulbs of similar size (2.3-2.6 cm in diameter, which corresponds to about 7.0 cm in circumference) of three cultivars: 'Namakwa' (yellow flowers with red tips, early flowering cultivar), 'Rainbow Bells' (orange-red flowers with visible stamens, early flowering cultivar), and 'Rupert' (lilac-purple flowers, late flowering cultivar), were used in the experiment. Before the beginning of the experiment, the bulbs (sourced originally from Afriflowers, Cullinan, South Africa) were cultivated in a greenhouse and on May, 2016, they were dug, cleaned, and left to slightly dry. On June 2, 2016, the bulbs were soaked in 0.25% captan suspension for 30 minutes and they were divided into two experimental groups according to the investigated techniques of propagation. First group: chipping - using a disinfected knife, each bulb was longitudinally cut (through the whole height of the bulb) into eight equal bulb-scale segments with a part of the basal plate (Fig. 1A). After treatment with fungicide (with captan again), they were placed in polyethylene bags $(23 \times 32 \text{ cm})$ (20 segments per bag) filled with perlite (fraction 2–6 mm) and water (5:1 v:v). The bags were closed with a rubber band, leaving a 2-cm space to exchange air and they were stored for a period of 4 months in a dark place at room temperature. After 4 months, survival ability (%) of segments, the number of bulblets produced on each segment, and bulblet weight and diameter were recorded. Second group: scoring - using a disinfected knife, four longitudinal cuts were made from the basal plate to a depth equal to half of



Fig. 1 (A) Bulb-scale segments obtained by chipping. (B) Scoring technique – longitudinal cuts made on mother bulbs. (C) Bulb-scale segments with newly formed bulblets. (D) Scored bulbs with newly formed bulblets. (E) Flowering bulblet of 'Rupert'.

the bulb; each cut passed through the center of the basal plate (Fig. 1B). After treatment with fungicide (with captan again), the scored bulbs were placed upside down in trays (in a single layer) in darkness, at room temperature, for 4 months. From July, every 2 weeks observations were made regarding the number of scored bulbs on which new bulblets were formed (Fig. 1D). After 4 months, survival ability (%) of scored bulbs, the number of bulblets produced on each scored bulb, bulblet weight and diameter were recorded.

For each cultivar and technique of propagation, the experiment was designed with four replicates. Each replication included 20 segments in chipping method and 20 scored bulbs.

Bulblets in the first year of cultivation

On October 2016, the obtained bulblets (which did not go dormant but started sprouting) were planted at a depth equal to twice the height of the bulblet in propagation trays (54 cells per tray, single cell $55 \times 55 \times 65$ mm) containing a universal gardening substrate Biovita (pH of 5.5-6.5) to determine the growth of bulblets obtained by two different techniques of propagation - scoring and chipping. The experiment was conducted in the greenhouse of the Faculty of Biotechnology and Horticulture (University of Agriculture in Krakow, Poland) with a temperature of 18°C/15°C (day/night) and under natural light conditions. For each cultivar and technique of propagation, the experiment was designed with four replicates. Each replication included 10 bulblets. After 3 months of cultivation, number of leaves per bulblet and first leaf length and width were estimated. Additionally, if the bulblet produced the inflorescence stem, its quality (inflorescence stem height, inflorescence length, the number of florets per inflorescence, the inflorescence stem diameter, the length of single floret) was evaluated. At the end of April 2016, the leaves began to dry out. Then, the bulbs (described earlier as bulblets) were manually cleaned, and their weight, diameter, and weight coefficient (the ratio of the initial weight of a bulb and its final weight) were estimated.

Statistical analysis

All data were analyzed using STATISTICA 10.0 data analysis software (StatSoft, USA). Experimental data were subjected to variance analysis, and Tukey's multiple range test was used to separate mean values at a significance level of $p \le 0.05$.

Results

The study revealed that scored bulbs of *Lachenalia* 'Rupert' started to form bulblets with greater dynamics in comparison to 'Namakwa' and 'Rainbow Bells'; as a result, after 2 months of incubation half of scored bulbs produced bulblets (Fig. 2). Such a result was noticed for 'Rainbow Bells' and 'Namakwa' after a period of 2.5 months and 3 months, respectively. The presented experiment showed that survival ability of bulb-scale segments and scored bulbs of the tested cultivars was high – it ranged from 85% to 95%. The exception were only scored bulbs of 'Rainbow Bells', which survived in 62% (Tab. 1). Irrespective of cultivar, each segment formed one bulblet (Fig. 1C) with a similar weight (0.15–1.19 g) but with a different diameter – the highest value in this group was noted for 'Rupert' (0.35 cm) (Tab. 1). In the case of scoring, on average from two ('Namakwa', 'Rupert) to three ('Rainbow Bells') bulblets were obtained from one bulb. They had a larger weight and diameter in comparison to those obtained from bulb-scale segments.

In the first season of growth, the bulblets of 'Rainbow Bells' and 'Rupert' obtained by two propagation techniques presented higher survival ability (97–100%) compared to 'Namakwa' (84–95%) (Tab. 2). Bulblets of 'Rainbow Bells' and 'Rupert' obtained by scoring formed more leaves than bulblets of these cultivars but obtained by chipping. In *Lachenalia* 'Namakwa' propagation technique did not affect this parameter. Bulblets



Fig. 2 The dynamics of bulblet formation by scored bulbs.

Tab. 1	Survival ability of bulb-scale segments and scored bulbs, the quality and number of bulblets obtained by
chippin	ng and scoring techniques.

Propagation technique	Cultivar	Survival ability (%)	No. of bulblets	Bulblet weight (g)	Bulblet diameter (cm)
Chipping	'Namakwa'	95.0 ±4.1 ^{b*}	1.0 ± 0.0 ^a	0.15 ±0.05 ª	0.23 ±0.01 ª
	'Rainbow Bells'	91.3 ±8.6 ^b	1.0 ±0.0 ª	0.16 ±0.02 ^{ab}	0.28 ±0.03 ^b
	'Rupert'	85.3 ±13.8 ^b	1.0 ±0.0 ª	0.19 ±0.02 ^b	0.35 ±0.04 °
Scoring	'Namakwa'	87.5 ±12.6 ^b	2.4 ±0.2 ^b	0.28 ±0.01 °	0.51 ±0.02 ^d
	'Rainbow Bells'	62.5 ±5.0 ª	3.1 ±0.6 °	0.29 ±0.01 °	0.50 ± 0.02 ^d
	'Rupert'	89.8 ±6.5 ^b	2.4 ±0.3 ^b	0.28 ±0.01 °	0.50 ± 0.02 ^d
		Main e	ffects**		
Cultivar		0.0157	0.0441	NS	0.0023
Propagation technique		0.0112	<0.0001	< 0.0001	<0.0001
Cultivar × Propagation Technique		0.0066	0.0441	NS	0.0001

* Mean values ±*SD* in columns followed by different letter(s) are significantly different according to Tukey's least significant difference test at $p \le 0.05$. ** Significant effects ($p \le 0.05$); NS – not significant.

Tab. 2 Survival ability of bulblets obtained by chipping and scoring techniques, the number and quality of leaves formed by these bulblets in the first season of cultivation.

Propagation technique Cultivar		Survival ability (%)	No. of leaves per bulblet	Leaf length (cm)	Leaf width (cm)	
Chipping	'Namakwa'	95 ±10.0 ^{a*}	1.4 ± 0.1 ^{ab}	26.7 ±3.3 °	1.1 ±0.2 ^b	
	'Rainbow Bells'	100 ±0.0 ^b	1.3 ±0.2 ^{ab}	25.2 ±1.7 °	0.7 ±0.1 ª	
	'Rupert'	100 ±0.0 ^b	1.0 ±0.1 ª	26.4 ±3.8 °	1.0 ± 0.1 ab	
Scoring	'Namakwa'	84 ±11.9 ª	1.5 ±0.2 ^{bc}	9.6 ±1.9 ª	0.8 ± 0.1 ^{ab}	
	'Rainbow Bells'	97 ±6.0 ^b	1.8 ±0.2 °	16.9 ±3.2 ^b	1.0 ±0.2 ^{ab}	
	'Rupert'	97 ±0.6 ^b	1.8 ±0.2 °	16.9 ±3.3 ^b	1.9 ±0.1 °	
		Main ef	fects**			
Cultivar		0.0330	NS	NS	< 0.0001	
Propagation technique		NS	<0.0001	<0.0001	<0.0001	
Cultivar × Propa	gation Technique	NS	0.0077	0.0207	< 0.0001	

* Mean values $\pm SD$ in columns followed by different letter(s) are significantly different according to Tukey's least significant difference test at $p \le 0.05$. ** Significant effects ($p \le 0.05$); NS – not significant.

Propagation technique	Cultivar	Bulb weight (g)	Bulb diameter (cm)	Weight coefficient
Chipping	'Namakwa'	1.9 ±0.1 ^{c*}	1.5 ±0.1 ^b	13.9 ±3.8 °
	'Rainbow Bells'	1.1 ±0.1 ^b	1.2 ±0.1 ª	6.9 ±1.0 ^b
	'Rupert'	2.0 ±0.1 °	1.5 ±0.1 ^b	10.8 ±0.9 °
Scoring	'Namakwa'	0.7 ±0.1 ª	1.1 ±0.1 ª	2.4 ±0.4 ª
	'Rainbow Bells'	0.9 ±0.1 ^b	1.2 ±0.1 ª	3.1 ±0.3 ^{ab}
	'Rupert'	3.1 ±0.1 ^d	1.9 ±0.1 °	11.3 ±0.3 °
		Main effects**		
Cultivar		< 0.0001	< 0.0001	< 0.0001
Propagation technique		0.0010	NS	<0.0001
Cultivar × Propagation Technique		< 0.0001	<0.0001	< 0.0001

Tab. 3	The qualit	y of bulbs obtained after the first season of cultivation.	

* Mean values $\pm SD$ in columns followed by different letter(s) are significantly different according to Tukey's least significant difference test at $p \le 0.05$. ** Significant effects ($p \le 0.05$); NS – not significant.

of all tested cultivars obtained by chipping produced longer leaves than those obtained by scoring method. This difference was notable especially in the case of 'Namakwa' – the leaves of bulbs obtained by scoring were 17 cm shorter in comparison to leaves of bulblets obtained by chipping. The widest leaves were recorded for 'Rupert' bulblets obtained by scoring (they were almost twice as wide as the rest of the objects). 4% of these bulblets produced inflorescence stems (Fig. 1E) already in the first season of cultivation (the average inflorescence stem height – 20.5 cm, inflorescence length – 5.0 cm, the number of florets per inflorescence – 8.5, the inflorescence stem diameter – 0.6 cm, the length of single floret – 2.9 cm). Other planted bulblets did not demonstrate the ability to flower.

It was noted that the quality of bulblets, named from this moment bulbs, after the first cultivation season depended on cultivar and propagation technique (Tab. 3). Bulbs of 'Rupert' obtained by scoring achieved a larger weight and diameter among all treatments tested but their weight coefficient was on the same level as for 'Rupert' and 'Namakwa' bulbs obtained by chipping. Evaluating the yield of bulbs, it was noticed that only every fifth 'Namakwa' bulb and every tenth 'Rainbow Bells' bulb, propagated initially by chipping, produced an additional adventitious bulb, bulbs from the other treatments did not evince such an ability (data not shown). To arrive at more synthetic conclusions, the weight coefficient was analyzed irrespective of cultivar and irrespective of propagation technique (Fig. 3). This analysis showed that the highest weight



Fig. 3 The weight coefficient analyzed irrespective of cultivar and irrespective of propagation technique. Mean values followed by different letter are significantly different according to Tukey's least significant difference test at $p \le 0.05$.

coefficient was found for 'Rupert' (11.1), next for 'Namakwa' (8.1), and the lowest one for 'Rainbow Bells' (5.0). For bulbs obtained by chipping, after the first season of cultivation a nearly two times higher weight coefficient (10.5) was recorded than for bulbs obtained by scoring (5.6).

Discussion

Irrespective of lachenalia cultivar, the average survival ability of bulb-scale segments and scored bulbs was 90% and 80%, respectively. Aksu and Çelikel [10] or Yanagawa [11], testing the chipping method of propagation in several plant species from Liliaceae and Amaryllidaceae, showed the differential potential in regeneration – it ranged from 40% to 100% depending on species. In the case of scoring, cross-cut mother bulbs of *Nerine* showed 100% of bulblet formation [16] but scoring technique is not always applicable for other species of geophytes – Mofokeng et al. [17] showed that in the case of *Hypoxis hemerocallidea*, the scoring method did not lead to the formation of daughter corms. Also, Knippels [14] reports that *Eucomis autumnalis* did not form any bulblets after scoring.

Noteworthy is the fact that newly formed bulblets of lachenalia did not go dormant but started to form leaves immediately. This could be explained by the fact that damaged bulbs in reaction to stress produced ethylene, which could affect the dormancy break [18].

The results showed that the number and size of bulblets depended more on technique of propagation than on the genotype. Heavier bulblets with a bigger diameter originated from the scored bulbs than from bulb-scale segments but the tested methods differed in efficiency measured by the number of bulblets obtained from one mother bulb – using chipping method, eight bulblets from one mother bulb were obtained, while using scoring only one or two (depending on cultivar). These results are in agreement with findings of Solgi et al. [19] for *Fitillaria imperialis* who proved that compared with chipping, fewer bulblets resulted from scoring, but they were larger. The number of bulblets obtained by mechanical treatments depends on mother bulb size and genotype and may range from a few to several dozen of bulblets per one mother bulb [14,20,21].

In the case of 'Namakwa', after the first season of cultivation the initially smaller bulblets obtained by chipping were characterized by a higher final weight in comparison to the initially bigger bulblets obtained by scoring. The opposite tendency was noted for lachenalia 'Rupert'. In the case of 'Rainbow Bells', the size of bulbs after the first season of cultivation was the same and did not depend on the initial propagation method. Only bulblets of 'Rupert' obtained by scoring showed the capacity to flower (a few percent of the whole group). The bulblets of 'Namakwa' and ' Rainbow Bells' remained in the juvenile phase during the first season of cultivation. The capacity to flower is directly related to bulb size, and the critical size of bulb which determines flowering varies with the genus, species, and even cultivar; for example, the juvenile phase (a nonflowering bulb) can last from 1 to 6 years [22]. The presented results showed that the bulbs of lachenalia 'Rupert' (initially obtained by scoring) finally achieved the biggest weight and diameter among all tested treatments - that confirms the importance of the internal genetic factor. According to Kleynhans [4], the commercial size of lachenalia bulbs is 6 cm in circumference, which corresponds to about 2 cm in diameter. Thus, we can conclude that after the first season of cultivation only bulbs of 'Rupert', obtained initially by scoring, achieved the required marketable size. In the first season of cultivation, nonflowering bulbs of lachenalia produced from 1.0 to 1.8 leaves depending on the cultivar and method of propagation. In contrast, flowering bulbs of lachenalia produce even more than three leaves [5]; it can therefore be concluded that leaf number is also related to plant juvenility, which agrees with earlier findings of de Hertogh and Le Nard [22] in relation to the *Tulipa* cycle of development where the apical bud of a small nonflowering bulb produces only one leaf, while flowering tulips form two or more.

Conclusion

Lachenalia, still treated as a novelty in the international flower market, deserves more attention due to the unusual decorative values and high quality of flowers. In commercial production, leaf cuttings are typically used to yield large number of plants. The presented results show that other techniques of propagation, such as chipping and scoring, can also stimulate bulblet induction and regeneration, but the marketed cultivars may respond differently to the propagation methods. The presented techniques can be recommended for in vivo propagation of lachenalia because they may provide additional possibilities of plant propagation, which may be applied in periods different from those used for leaf cutting propagation and will not require greenhouse cultivation. The presented methods of propagation can be successfully used by professional gardeners and amateurs for the commercialization and popularization of new lachenalia cultivars.

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Rozmnażanie odmian *Lachenalia* (Asparagaceae) technikami nacinania cebul oraz cięcia na sadzonki łuskowe

Streszczenie

Rodzaj Lachenalia (Asparagaceae) obejmuje botanicznie zróżnicowane ozdobne gatunki roślin cebulowych pochodzących z południowej Afryki. Głównym celem badań była ocena, po raz pierwszy, możliwości rozmnażania odmian lachenalii ('Namakwa', 'Rainbow Bells', 'Rupert') dwoma technikami in vivo: nacinania cebul oraz cięcia na sadzonki łuskowe, które mogłyby być stosowane w produkcji materiału nasadzeniowego. W drugiej części eksperymentu oceniano wzrost oraz jakość cebul po pierwszym roku uprawy w warunkach szklarniowych. W zależności od zastosowanej techniki rozmnażania przetrwało od 62% do 95% naciętych cebul lub sadzonek łuskowych. Stosując technikę cięcia cebul uzyskano z jednej cebuli matecznej osiem cebul przybyszowych o masie 0,17 g i średnicy 0,29 cm, natomiast technikę nacinania dwie/trzy cebule przybyszowe o masie 0,28 g i średnicy 0,5 cm. W pierwszym sezonie wegetacji cebule uzyskane z sadzonek łuskowych wytworzyły dłuższe liście od uzyskanych z naciętych cebul. Bez względu na zastosowaną technikę rozmnażania cebule Lachenalia 'Namakwa' oraz 'Rainbow Bells' wytworzyły tylko liście, natomiast pojedyncze cebule lachenalii 'Rupert' uzyskane techniką nacinania zakwitły. Oceniając przyrost cebul po pierwszym sezonie wegetacji stwierdzono, że niezależnie od odmiany, cebule uzyskane techniką cięcia na sadzonki łuskowe osiągnęły dwukrotnie wyższy współczynnik wagowy od tych uzyskanych z nacinania cebul.