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ORIGINAL PAPER

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PROPAGATION OF *Lachenalia* CULTIVARS FROM LEAF CUTTINGS

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ABSTRACT

Lachenalia is a poorly known genus of ornamental bulbous plants from South Africa with a huge floricultural potential. The paper discusses *in vivo* multiplication with leaf cuttings of *Lachenalia* cv. 'Namakwa', 'Romaud', 'Ronina' and 'Rupert'. Mature leaves were collected from non-flowering donor plants. Each leaf was halved to make proximal and distal leaf cuttings. 'Ronina' distal leaf cuttings produced the lowest number of bulblets (5.7 per cutting), while 'Namakwa' proximal leaf cuttings turned out the most productive (10.5 bulblets per cutting). The bulblets produced by cv. 'Namakwa' distal leaf cuttings were the lightest (0.3 g per bulblet), and those formed on cv. 'Ronina' proximal leaf cuttings were the heaviest (0.9 g per bulblet). In general, the weight of individual bulblets ranged from 0.1 to 1.2 g. Irrespective of the cutting type, the leaves of cv. 'Namakwa' produced the greatest number of bulblets, and the leaves of 'Ronina' yielded the heaviest bulblets. With no dependence on their genotype, proximal leaf cuttings were advantageous in terms of total number of generated bulblets and their quality (weight, diameter).

Key words: bulblet formation, reproduction, Cape Hyacinth, 'Namakwa', 'Romaud', 'Ronina', 'Rupert'

INTRODUCTION

Species of *Lachenalia* genus (Asparagaceae, previously Hyacinthaceae) are endemic to Namibia and South Africa. Most of them are half-hardy plants and withstand temperatures around 0°C only for a few days [Duncan 2012]. *Lachenalia* is a well known bulbous ornamental plant in Africa, still treated as a novelty in the international flower market due to lack of knowledge on the technology of growing new cultivars [Kapczyńska and Kidawska 2016, Kapczyńska and Malik 2016]. The genus comprises a wide range of flower color combinations. Most species have attractively spotted and dappled foliage and long-lasting blooms [Duncan 2012]. The botanical species and new cultivars thrive as container [Kapczyńska 2014a] or garden plants [Kapczyńska 2013, 2014b] and are perfect cut flowers for floristic compositions.

Lachenalia propagation methods include: seeds, offsets, i.e. side bulbs formed out of a mother bulb, bulbils (aerial bulblets), leaf cuttings (bulblets are formed on the basis of severed leaves), and tissue cultures [Kleynhans 2006, Duncan 2012]. The efficiency of offsets developing in *Lachenalia* cultivars is low [Niederwieser and Ndou 2002], and thus leaf cuttings are typically used in commercial production to yield large number of plants [Kleynhans et al. 2002]. This basic propagation technique takes advantage of adventitious buds forming on adaxial

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and abaxial surface of leaf tissue [Niedervieser and Ndou 2002] and is used to obtain a large number of adventitious bulblets. Another efficient method is micropropagation but its costs considerably exceed those of traditional vegetative propagation [Singh 2015]. Perrignon [1992] claimed that propagation strategies based on leaf cuttings should be elaborated for each cultivar of *Lachenalia*. This is because the marketed cultivars may respond differently to the cultivation and propagation methods [Kleynhans 2006]. Therefore, the aim of this study was to quantitatively and qualitatively assess the bulblets obtained from leaf cuttings depending on cultivar and the way of the cutting preparation.

MATERIALS AND METHODS

The study was conducted in the years 2013−2014 in a glass-glazed Venlotype greenhouse (equipped with Integro 724 process computers, Priva) at the Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Poland. Four cultivars (genotypes) of lachenalia (*Lachenalia* J. Jacq. ex Murray) were investigated: 'Namakwa' (yellow with red tips), 'Romaud' (yellowy-green), 'Ronina' (yellow), and 'Rupert' (lilac-purple). For the propagation by leaf cutting the bulbs of donor plants (sourced from Afriflowers, Cullinan, South Africa) were planted, on October 15, 2013, into three-liter plastic pots (five bulbs per pot) to a depth equal to twice the height of the bulb, containing a peat substrate (Botanica Professional; Comeco, Poland) of pH 5.5−6.5. The pots with bulbs were placed in a chamber under natural illumination, but due to light deficit during the winter months, additional photosynthetic irradiation was provided (from 6.00 a.m. to 9.00 a.m. and from 3.00 p.m. to 6.00 p.m) by high-pressure sodium (HPS) lamps of 400 W (Philips SON-T Agro, USA). The greenhouse day/night temperature was set at 20/15°C. Donor plants were irrigated two to three times a week (depending on the substrate moisture level). On 5 December, 2013, leaves of the donor plants in the vegetative phase were randomly cut off at the plant base to get 40 leaves per genotype with an average length of 20−25 cm and an average width of 3−4 cm. Next, to test the effect of leaf section position on the bulblet production, each leaf was cut into two parts of equal length to obtain two types of cuttings: a proximal leaf cutting and a distal leaf cutting (Fig. 1A, 1B), as a consequence, cuttings with an average length of 10−12.5 cm were obtained. The experiment consisted of four replications of 10 cuttings each – 40 cuttings per treatment combination: two types of leaf cuttings and four genotypes, which gave a total number of 320 leaf cuttings. The leaf cuttings were placed vertically (with the base of the cutting about 2 cm below the medium surface) in plastic boxes with mesh bottom (32 cm × 45 cm × 8 cm). Proximal and distal parts of the cuttings were placed in separate boxes (forty cuttings per box), containing a mixture of a peat substrate (Botanica Professional) and perlite with a fraction 2−6 mm (1 : 1, v : v). They were kept under the same greenhouse conditions (temperature, light) as the donor plants and were irrigated two to three times a week (depending on the moisture level). For the first four weeks the boxes were covered with perforated plastic film (125 holes/m^2) to provide optimal moisture conditions. After this time, the film was gradually opened and finally completely removed. Than the percentage of cuttings that survived was calculated. In the beginning of May (five months after planting), the original leaf cuttings began to dry out (Fig. 1 C), and the irrigation ceased. Then, the bulblets were manually cleaned and their number per individual cutting was estimated together with weight and diameter of individual bulblets. Using the electronic weight (Radwag WPX 1500), the share of bulblets of a given weight in total yield (%) was assessed and four weight categories were specified: 0.10−0.40 g, 0.41−0.80 g, 0.81−1.20 g, and 1.21−1.60 g.

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

Fig. 1. A – a leaf cut into distal and proximal cutting, B – adaxial surface of distal and proximal leaf cutting showing bulblet formation, C – senescence stage of the original leaf cutting

RESULTS AND DISCUSSION

Apart from *Lachenalia*, only a few other genera of bulbous plants are capable of regenerating from leaf cuttings, including *Allium* [Gorelick 2015], *Eucomis* [Knippels 2012], *Hyacinthus* [Śmigielska and Jerzy 2013], *Ornithogalum* [Blomerus and Schreuder 2002], and *Scilla* [Gorelick 2015]. The presented experiment proved that survival percentage of the tested cultivars was extremely high – from 90% ('Romaud' – distal cuttings) to 100% ('Namakwa', 'Ronina' – distal and proximal cuttings and 'Romaud' – proximal cuttings) of leaf cuttings survived (data not shown) and formed bulblets (mostly on the adaxial leaf surface, Fig. 1B). Proximal leaf cuttings of 'Namakwa' produced the greatest number of bulblets (on average 10 per cutting) (Tab. 1). Distal leaf cuttings of 'Ronina' and 'Romaud' generated nearly two times fewer bulblets, which made them the least productive experimental variants. Proximal leaf cuttings of 'Namakwa', 'Romaud' and 'Ronina' formed more bulblets than distal cuttings of these cultivars. In cv. 'Rupert' the type of cutting did not affect propagation efficiency. Proximal cuttings of all genotypes yielded heavier bulblets than the distal ones. Differences in weight ranged from 0.1 to 0.2 g in favor of the proximal bulblets, which, depending on genotype, made up 22–33% of the weight of a single bulblet. The heaviest bulblets (0.9 g) originated from the proximal cuttings of cv. 'Ro nina', and the lightest (0.3 g) from distal cuttings of cv. 'Namakwa'. The results of this experiment are in line with the findings of Perrignon [1992], Suh et al. [1997] or Ndou et al. [2002], who proved that bulblets obtained from proximal leaf cuttings were heavier than those from distal parts, and with a report by Krause [1980] and by Nndwambi et al. [2013], who stated that in *Hyacinthus* (a genus closely related to *Lachenalia*) and in *Eucomis* the number and size of bulblets depended on a genotype. Genetical differences in *Lachenalia* are also considered to be an important factor responsible for bulblet induction *in vitro* [Bach et al. 2015]. In this study, greater bulblet weight resulted in its greater diameter except for cv. 'Rupert', where the type of the leaf cutting did not affect bulblet quality as assessed by its diameter.

Table 1. Effect of genotype and type of leaf cutting on the number and quality of bulblets

Genotype	Type of cutting	Number of bulblets per cutting	Bulblet weight (g)	Bulblet diameter (cm)
'Namakwa'	proximal	10.5 ±0.6 d*	0.4 ±0.1 b	0.8 ±0.1 c
	distal	8.9 ±0.6 c	0.3 ±0.0 a	0.6 ±0.0 a
'Romaud'	proximal	6.7 ±0.7 b	0.6 ±0.1 c	0.9 ±0.1 d
	distal	5.5 ±0.2 a	0.4 ±0.1 b	0.7 ±0.1 b
'Ronina'	proximal	8.6 ±0.5 c	0.9 ±0.1 d	1.0 ±0.0 e
	distal	5.7 ±0.8 a	0.7 ±0.1 c	0.9 ±0.1 d
'Rupert'	proximal	8.5 ±0.6 c	0.6 ±0.1 c	0.7 ±0.1 b
	distal	8.0 ±0.4 c	0.4 ±0.1 b	0.7 ±0.1 b
Main effects**				
Genotype		<0.0001	<0.0001	<0.0001
Type of cutting		<0.0001	<0.0001	<0.0001
Genotype × Type of cutting		0.0059	NS	0.0008

*Mean values ±SD in columns followed by different letter are significantly different at $p \leq 0.05$ according to Tukey's multiple range test
**Significant effects ($p \leq 0.05$), NS – not significant

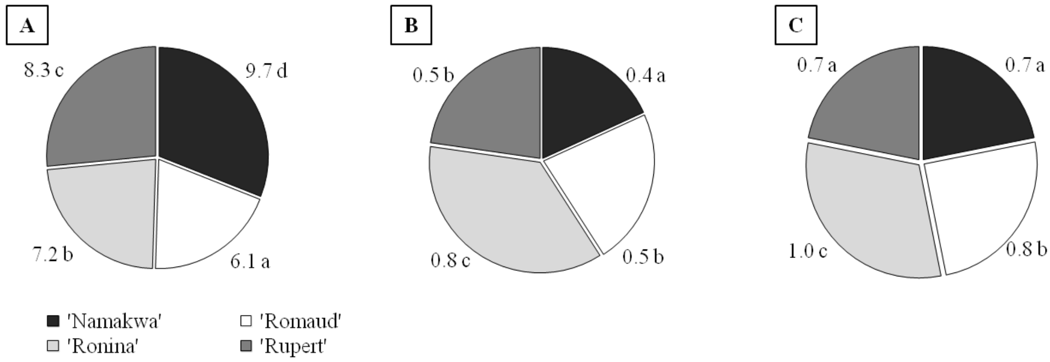

Fig. 2. Effect of genotype on: A – the number of bulblets, B – bulblet weight (g), C – bulblet diameter (cm) irrespective of the leaf cutting type

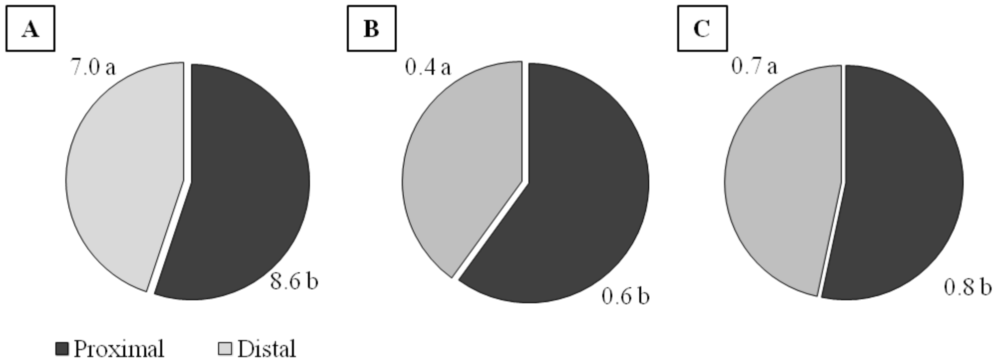

Fig. 3. Effect of the leaf cutting type on: A – the number of bulblets, B – bulblet weight (g), C – bulblet diameter (cm) irrespective of genotype

Total yield of the bulblets was highly heterogeneous, as their weight varied from 0.1 g to 1.2 g (Tab. 2). Detailed analysis of the yield structure showed that a greater number of the smallest (0.10–0.40 g) bulblets formed on the distal leaf cuttings of cv. 'Namakwa', 'Romaud' and 'Ronina' vs. their proximal leaf cuttings. No such tendency occurred for cv. 'Rupert'. In the next weight category, lachenalia 'Namakwa' and 'Romaud' produced more bulblets weighting 0.41–0.80 g from proximal than distal leaf cuttings. A reverse trend appeared in cv. 'Rupert', and in cv. 'Ronina' the leaf cutting type did not affect the results in this weight category. In all cultivars, the bulblets weighting from 0.81 to 1.20 g formed more often on proximal than distal leaf cuttings. The heaviest bulblets (1.21–1.60 g) originated only from proximal leaf cuttings of cv. 'Rupert' and proximal and distal cuttings of cv. 'Ronina'. In the latter cultivar, the proximal surface produced more bulblets than the distal one. The study findings confirm differential response of the investigated lachenalia genotypes to given propagation method, as reported before by Perrignon [1992] or Ndou et al. [2002], who also recommended development of individual propagation protocols for each cultivar. To arrive at more synthetic conclusions, the results were analyzed irrespective of the leaf cutting type and irrespective of the genotype. This analysis showed that the greatest number of bulblets originated in cv. 'Namakwa', while those produced by cv. 'Ronina' had the greatest weight and diameter (Fig. 2). Also, the proximal leaf cuttings formed more bulblets and of greater weight and diameter than the distal leaf cuttings (Fig. 3). According to Ndou [2000], bulblets formed on lachenalia leaf cuttings originate from epidermal and sub epidermal cell division. Tissues of the same organ have different developmental potential that depends e.g. on the tissue age. Proximal sections of lachenalia leaves represent the youngest tissue, while distal ones are the oldest [Niederwieser and Vcelar 1990]. Tissue aging occurs in the tissues distant from the leaf basis and cell wall extensibility decreases as the tissue matures [Bouchabké et al. 2006]. It may be therefore assumed than in older leaf tissues the cell walls are less elastic and cells are not so closely packed, which disrupts water transport and decreases cell turgor. Water deficit disturbs plant physiological and biochemical processes [Meng et al. 2014]. This abiotic stress might affect regeneration potential in different parts of lachenalia cuttings originating from the same organ. Kleynhans [2006] states that lachenalia commercial size bulbs suitable for pot plant production is 6 cm in circumference, which corresponds to about 2 cm in diameter [Kapczyńska and Kidawska 2016]. It means that bulblets obtained from leaf cuttings should enlarge their size two or three times (depending on genotypes or type of cutting, Fig. 2 and 3) to achieve the marketable value and according to Kapczyńska [2014b] to obtain plants of the highest quality (larger inflorescence length, stem diameter and the number of florets), it is recommended to cultivate larger bulbs of lachenalia (5.1–6.0 cm in circumference) rather than smaller ones (4.1–5.0 cm in circumference).

Table 2. Effect of genotype and type of leaf cutting on the share of bulblets from individual weight category in total yield (%)

Genotype	Leaf cutting	Bulblet weight (g)			
		0.10–0.40	0.41–0.80	0.81–1.20	1.21–1.60
'Namakwa'	proximal	56.1 ±5.6 d*	36.3 ±5.9 bc	7.7 ±2.3 b	0.0 ±0.0 a
	distal	79.9 ±3.1 e	17.0 ±3.8 a	3.5 ±0.9 a	0.0 ±0.0 a
'Romaud'	proximal	33.4 ±3.6 bc	47.2 ±3.4 d	19.5 ±0.6 d	0.0 ±0.0 a
	distal	60.7 ±13.0 d	32.1 ±10.5 b	7.0 ±2.5 ab	0.0 ±0.0 a
'Ronina'	proximal	12.2 ±2.5 a	41.5 ±3.4 abc	29.8 ± 3.5 e	16.5 ±2.1 c
	distal	31.9 ±4.2 b	41.7 ±6.7 abc	19.5 ±4.2 d	7.0 ±3.4 b
'Rupert'	proximal	47.9 ±7.7 cd	31.3 ±5.9 b	11.8 ±1.2 c	9.3 ±3.5 b
	distal	47.7 ±7.2 cd	46.2 ±8.6 cd	6.4 ±2.9 ab	0.0 ±0.0 a
Main effects**					
Genotype		<0.0001	0.0004	<0.0001	<0.0001
Type of cutting		<0.0001	0.0451	<0.0001	<0.0001
Genotype × Type of cutting		0.0018	<0.0001	0.0093	<0.0001

*Mean values ±SD in columns followed by different letter(s) are significantly different at $p \leq 0.05$ according to Tukey's multiple range test
**Significant effects ($p \leq 0.05$)

CONCLUSIONS

1. Survival percentage of leaf cuttings of the tested cultivars is extremely high (90%–100%).

2. Each leaf cutting produces about 6–11 bulblets depending on genotype and type of leaf cutting, with the following parameters: 0.4–0.9 g (weight) and 0.6–1.0 cm (diameter).

3. The results show differential reaction of individual cultivars to tested method of propagation.

4. Proximal leaf cuttings produce more bulblets with higher quality than those obtained from distal parts of the leaves.

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