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The initiation of the *in vitro* cultures of some *Lachenalia* cultivars

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Abstract: *The initiation of the in vitro cultures of some Lachenalia cultivars*. The aim of the study was to determine the effect of the cultivar, explant type and medium composition on the initiation of the *in vitro* cultures of *Lachenalia* 'Rosabeth', 'Rupert', 'Namakwa' and 'Ronina'. An examination of the effectiveness of disinfection of the starting material revealed that the fewest infections (12%) occurred in *Lachenalia* 'Rupert'. The cultivar that suffered the highest rate of infection was 'Rosabeth' (78.8%). As to regeneration, it was found that this process proceeded most intensely in the cultivar 'Rupert', and most poorly in the cultivar 'Rosabeth'. Mid-range values were obtained for *Lachenalia* 'Namakwa' and 'Ronina'. Another factor considered was the type of explant. There were no significant differences between the percentage of infection and regeneration in apical buds, top scale parts or basal scale parts in the four cultivars tested. The culture media used in the experiment contained different amounts, combinations and ratios of growth substances. The lowest percentage of infected material was associated with the medium containing 1 μM BA + 10 μM 2,4-D (the most effective callus formation). The opposite results were obtained on the media supplemented with 5 μM BA + 1 μM 2,4-D or 5 μM BA + 1 μM NAA.

Key words: cape hyacinth, *Lachenalia*, tissue culture, contamination, regeneration

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INTRODUCTION

When analysing the production of flower bulbs in the European area it is clearly visible that tulips occupy a leading position, but it is worth noting that this structure is changing as the production area devoted to little-known bulbous plants increases [Wróblewska, 2009]. This tendency may be propitious for beginning to commercialise new ornamentals and for supplementing the flower-bulbs group with new products. In this connection lachenalia (*Lachenalia* J. Jacq. ex Murray) would appear to be the perfect plant to satisfy the demands of the international flower market. A *Lachenalia* breeding programme was begun in South Africa [Kleynhans, 2006] in the 1960s to obtain superior pot plant hybrids [Kleynhans and Hancke, 2002, Niederwieser et al., 2002]; and *Lachenalia* were registered under the trade name 'Cape Hyacinth' in the 1990s [Kleynhans et al., 2002]. It is worth recalling that a large share of the bulbous species introduced into Europe originated from South Africa, but despite its great floral diversity this country has made little contribution to the number of commercial crops in the international flower bulb industry [Niederwieser et al., 2002]. The commercialisation of a new flower bulb crop usually

requires a comprehensive approach utilizing trade strategies, cultivation methods and propagation techniques. A major issue in this process is how to multiply sufficient material both quickly and economically [Sage, 2005]. Research efforts should therefore focus on establishing a precise knowledge of the propagation physiology of individual species and even of cultivars. Generally, *Lachenalia* can be propagated through seeds, offsets and leaf cuttings [Duncan, 1988] but, as Kleynhans [2006] emphasizes, offsets are too slow for commercial production. Propagation by seeds cannot be the basic method as there are species which produce them in limited numbers [Kleynhans and Hancke, 2002]. For this reason, modern breeding requires rapid *in vitro* propagation methods. The objective of this study was to evaluate the effect of the genotype, medium composition and explant type on the initiation process of the micropropagation of *Lachenalia* under *in vitro* conditions.

MATERIAL AND METHODS

The research was carried out between March 2009 and October 2009 in the laboratory of the Ornamental Plants Department at the University of Agriculture in Kraków. The study was performed to find out whether factors such as the genotype, medium composition and explants influence the initiation process of *Lachenalia* micropropagation. The research material consisted of four cultivars, all of which were from the African Beauty series. The 'Rupert' and 'Ronina' bulbs (approx. 6 cm in circumference) were imported from the company Afriflowers of South Africa, while the 'Namakwa' and 'Rosabeth' bulbs (approx. 6 cm in circumference) were reproduced in Polish conditions. The bulbs were disinfected prior to explant isolation: first they were washed with tap water and detergent, and then soaked in a 20-percentage-solution of Domestos for twenty minutes. Next, the plant material was rinsed three times in distilled water, before finally being immersed for two minutes in a 2-percentage-solution of Domestos with one drop of Ludwik detergent. Following disinfection, an apical bud and two of the innermost scales from each bulb were excised. Each bud was then cut lengthwise and each scale was divided into a basal part and a top part. The explants were randomized so that each Petri dish contained six explants: two parts of a bud, two top scale parts and two basal scale parts. The basal medium used consisted of MS [Murashige and Skoog, 1962] containing 30 $g \cdot dm^{-3}$ sucrose and 7 $g \cdot dm^{-3}$ agar, pH 5.8. The first group of tested media (for organogenesis) was supplemented with 5 μM BA[1] + 1 μM NAA[2] or 1 μM BA + + 5 μM NAA or 5 μM BA + 1 μM 2,4-D[3], while the second group (for somatic embryogenesis) contained 1 μM BA + + 10 μM 2,4-D or 5 μM BA + 5 μM 2,4-D. The cultures were maintained for six months in the dark in a growth room at a temperature of 20°C (passages every 4–6 weeks). To study the rate of contamination and regeneration, the cultures were initially evaluated at two-week intervals and thereafter once a month.

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[1] Benzylaminopurine.
[2] α-naphthaleneacetic acid.
[3] 2,4-dichlorophenoxyacetic acid.

For each cultivar, the experiment was performed in five replicates for each medium separately, with 30 explants in each. With the aid of Statistica 8 software, the results were subjected to an analysis of variance for a three-factorial experiment and the significance of the differences was estimated by the Duncan test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The percentage of explants that had been decontaminated in the process of disinfection by the adopted method and their regeneration rate were studied. In most of the *Lachenalia* cultivars, and for most combinations of nutrient media, the *in vitro* regeneration process at the initiation stage proceeded indirectly through the production of large amounts of morphologically and physiologically diverse callus. For example, a delicate callus of a light, pearly colour and crystalline habit formed in the presence of NAA, while the callus forming in the presence of 2,4-D was yellowish, hard and compact.

The first factor that had an impact on the rates of infection and regeneration was the genotype. The data presented in Table 1 show that the lowest percentage of infections occurred in *Lachenalia* 'Rupert', and that this result is not significantly different from the infection rate of cultivar 'Ronina', which also suffered a small degree of infection. The cultivar 'Namakwa', for which the infection rate was determined at 20%, was not significantly different in this respect from *Lachenalia* 'Ronina', but there was a significant difference when comparing the cultivar 'Namakwa' with the cultivar 'Rupert'. The cultivar that had the highest infection rate was 'Rosabeth'. The rate of infection in this cultivar was nearly 79%, which was significantly different from that in the cultivars 'Rupert', 'Ronina' and 'Namakwa'. High infection rates have also been reported by Sochacki and Orlikowska [2004] in *Narcissus* cultures. In that case the contamination of initial explants varied from 40 to 100% depending on the cultivar. Further results showed that it was possible to improve the *in vitro* propagation protocol of *Narcissus* by using a more intensive bulb-disinfection procedure [Sochacki and Orlikowska, 2005]. In the present study, the high level of infection of 'Rosabeth' explants could have been caused by the origin of the bulbs, which had been reproduced in Polish conditions. On the other hand, *Lachenalia* 'Namakwa' shared the same reproduction conditions but had a significantly lower infection rate. It is possible that the particularly high level of infection in the 'Rosabeth' culture could

TABLE 1. The effect of the genotype on the contamination and *in vitro* regeneration of *Lachenalia* (irrespective of explant type and composition of medium)

Cultivar	Contamination rate (%)	Regeneration rate (%)
'Rupert'	12.0 a*	88.0 c
'Ronina'	16.0 ab	82.0 b
'Namakwa'	20.0 b	80.0 b
'Rosabeth'	78.8 c	19.3 a

* Means in columns followed by the same letters do not differ significantly at $\alpha = 0.05$.

be explained by endogenous contamination, but further analysis is required to confirm this argument. In general terms, it may be that field-grown bulbs as the source of explants are difficult to free from pathogens because they are underground parts of plants. Renau-Morata et al. [2013] have nevertheless claimed that storing non-planted mother corms of saffron at low temperatures (1–3°C) for nine months results in a reduction in the contamination level during micropropagation. In turn, Uranbey [2011] has suggested using the immature embryos of *Muscari* as the starting material to eliminate the infection of explants isolated from underground storage organs.

As far as regeneration is concerned, it progressed the fastest and the most intensely in the cultivar 'Rupert', and at the lowest rate in the cultivar 'Rosabeth'. Meanwhile, mid-range values were obtained for *Lachenalia* 'Namakwa' and 'Ronina'. Genotypic differences in the regeneration potential of *Lachenalia* have been observed during *in vitro* bud formation from leaf explants [Niederwieser and Van Staden, 1990]. A relationship between the genotype and regeneration rate during *in vivo* propagation by leaf cuttings was also noticed by Perrignon [1992], who suggested that individual propagation strategies should be formulated for each cultivar.

The explant type did not affect the parameters evaluated (Table 2). There were no significant differences between the infection rate and regeneration in the apical buds, the basal scale parts and top scale parts of the four cultivars tested. Despite these results, the explant type

TABLE 2. The effect of explant type on the contamination and *in vitro* regeneration of *Lachenalia* (irrespective of genotype and composition of medium)

Explant type	Contamination rate (%)	Regeneration rate (%)
Apical bud	31.0 a*	65.5 a
Basal scale parts	32.0 a	67.5 a
Top scale parts	32.0 a	69.0 a

* Means in columns followed by the same letters do not differ significantly at $\alpha = 0.05$.

can certainly be a decisive factor in the later stages of micropropagation. In their analysis of *Muscari mirum*, Nasirciral et al. [2011] found that explant type was one of the factors influencing the number of bulblets per explant. Regeneration can be significantly affected not only by the type of explants, but also by their age. It was found in one study, for example, that *Lachenalia* formed significantly more buds on young leaf tissue in comparison to old explants [Niederwieser and Van Staden, 1990]. The size of explants is a further factor that can play an important role in the regeneration process of ornamental bulbs in micropropagation. In this connection, Cardona Suárez et al. [2011] reported significant responses of lily on explants differing in scale size: the bulblets formed on large explants were found to be larger when compared to those formed on small explants.

An investigation of the impact of the next factor, the medium, on the infection rate and on regeneration in *Lachenalia* (Table 3), found that the lowest percentage of infected plant material was associated with a medium containing 1 μM BA + 10 μM 2,4-D. This value, however, did not differ significantly from that

TABLE 3. The effect of the medium on the contamination and *in vitro* regeneration of *Lachenalia* (irrespective of genotype and explant type)

Composition of medium	Contamination rate (%)	Regeneration rate (%)
1 μM BA + 10 μM 2,4-D	15.0 a*	82.5 c
5 μM BA + 5 μM 2,4-D	25.0 ab	72.5 bc
1 μM BA + 5 μM NAA	35.0 bc	65.0 ab
5 μM BA + 1 μM 2,4-D	40.0 c	60.0 a
5 μM BA + 1 μM NAA	43.3 c	56.7 a

* Means in columns followed by the same letters do not differ significantly at $\alpha = 0.05$.

obtained for the medium supplemented with 5 μM BA + 5 μM 2,4-D. The highest proportion of explants – as high as 40% – were infected on the media supplemented with 5 μM BA + 1 μM 2,4-D and 5 μM BA + 1 μM NAA. The percentage of infections obtained on these media was not significantly different from the value recorded on the medium supplemented with 1 μM BA + 5 μM NAA.

The percentage of recorded infections was inversely related to the regeneration rate of the initial explants (callus formation). Therefore, the highest percentage of regenerating explants was obtained for the medium containing 1 μM BA + + 10 μM 2,4-D. The resulting value, however, did not differ significantly from the one recorded on the medium containing 5 μM BA + 5 μM 2,4-D. The explants laid out on the media with the following proportions of growth substances: 5 μM BA + + 1 μM NAA and 5 μM BA + 1 μM 2,4-D, regenerated most poorly, but the resulting percentage did not differ significantly from that obtained for the medium supplemented with 1 μM BA + + 5 μM NAA.

The combination of growth regulators is frequently the most critical feature in many aspects of plant tissue cultures [Sharma and Nautiyal, 2009, Cardona Suárez et al., 2011, El Tahchy et al., 2011]. In the case of *Lachenalia*, authors will be able to give more precise information about the media used in the present experiment once the quality of the regenerated material has been analysed.

Relevant statistical calculations were performed to examine the interaction of cultivar, explant type and medium composition. It was observed that, with the interaction of these three factors, the highest percentage of infections occurred in the cultivar 'Rosabeth' (Table 4). In the case of the cultivars 'Namakwa' and 'Rupert', it can be stated that all explants reached the highest level of disinfection in the media containing 1 μM BA + the higher dose of auxin, NAA or 2,4-D. In the cultivar 'Ronina', however, the lowest infection rate of 0–20% was obtained on the media with a predominance of the cytokinin BA over the auxin NAA or 2,4-D, and on the medium with the same amounts of cytokinin and auxin.

A similar analysis was performed for regenerating explants (Table 5). The lowest percentage of regenerating *Lachenalia* explants was obtained in the cultivar 'Rosabeth'. This was the case particularly on the media containing NAA in their composition, where that percentage was 0–20%. It should be noted that all of the three remaining cultivars, that is 'Namakwa', 'Ronina' and 'Rupert', achieved a high percentage of regenerating explants (70–100%) on the medium with equal amounts of cyto-

TABLE 4. The effect of the genotype, explant type and composition of medium on the *in vitro* contamination of *Lachenalia* explants

Cultivar	Explant type	Composition of medium (%)				
		5 μM BA 1 μM NAA	5 μM BA 1 μM 2,4-D	1 μM BA 5 μM NAA	1 μM BA 10 μM 2,4-D	5 μM BA 5 μM 2,4-D
‘Rosabeth’	Apical bud	80 e*	80 e	100 f	40 c	80 e
	Basal scale parts	100 f	80 e	100 f	40 c	80 e
	Top scale parts	100f	80 e	100 f	40 c	80 e
‘Namakwa’	Apical bud	60 d	20 b	0 a	0 a	20 b
	Basal scale parts	60 d	20 b	0 a	0 a	20 b
	Top scale parts	60 d	20 b	0 a	0 a	20 b
‘Ronina’	Apical bud	0 a	20 b	40 c	20 b	0 a
	Basal scale parts	0 a	20 b	40 c	20 b	0 a
	Top scale parts	0 a	20 b	40 c	20 b	0 a
‘Rupert’	Apical bud	20 b	40 c	0 a	0 a	0 a
	Basal scale parts	20 b	40 c	0 a	0 a	0 a
	Top scale parts	20 b	40 c	0 a	0 a	0 a

* Means in columns followed by the same letters do not differ significantly at $\alpha = 0.05$.

TABLE 5. The effect of the genotype, explant type and composition of medium on the *in vitro* regeneration of *Lachenalia* explants

Cultivar	Explant type	Composition of medium (%)				
		5 μM BA 1 μM NAA	5 μM BA 1 μM 2,4-D	1 μM BA 5 μM NAA	1 μM BA 10 μM 2,4-D	5 μM BA 5 μM 2,4-D
‘Rosabeth	Apical bud	20 b*	20 b	0 a	60 e	20 b
	Basal scale parts	0 a	20 b	0 a	40 c	20 b
	Top scale parts	0 a	20 b	0 a	50 d	20 b
‘Namakwa’	Apical bud	40 c	80 g	100 h	100 h	80 g
	Basal scale parts	40 c	80 g	100 h	100 h	80 g
	Top scale parts	40 c	80 g	100 h	100 h	80 g
‘Ronina’	Apical bud	100 h	80 g	60 e	80 g	100 h
	Basal scale parts	100 h	80 g	60 e	80 g	70 f
	Top scale parts	100 h	80 g	60 e	80 g	100 h
‘Rupert’	Apical bud	80 g	60 e	100 h	100 h	100 h
	Basal scale parts	80 g	60 e	100 h	100 h	100 h
	Top scale parts	80 g	60 e	100 h	100 h	100 h

* Means in columns followed by the same letters do not differ significantly at $\alpha = 0.05$.

kinin and auxin. It was also found that the ‘Namakwa’ and ‘Rupert’ explants of *Lachenalia* laid out on the medium containing 1 μM BA + 5 μM NAA or 10 μM 2,4-D regenerated at a rate of 100%.

The results of the present investigation show the existence of a large inter-species variability in the *in vitro* culture of *Lachenalia* even at the initiation stage. The infection and regeneration rates seem to depend mostly on the genotype, but also on the composition of the medium.

CONCLUSIONS

1. The regeneration of *Lachenalia* at the initiation stage of *in vitro* cultures proceeds indirectly through the production of large amounts of morphologically diverse callus.
2. The rates of infection and regeneration depend on the genotype: the highest percentage of infection was observed in the cultivar 'Rosabeth', and the most intensely regenerating cultivar was 'Rupert'.
3. The type of explant affected neither the percentage of infection nor the regeneration of the *Lachenalia* cultivars tested.
4. A significantly lower percentage of infected material (the best regeneration) was obtained on the medium supplemented with 1 μM BA + 10 μM 2,4-D when compared with the infection rates recorded on the media enriched with 5 μM BA + 1 μM 2,4-D and 5 μM BA + 1 μM NAA (the worst regeneration).

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Streszczenie: *Inicjacja kultur in vitro wybranych odmiana z rodzaju Lachenalia.* Celem niniejszej pracy było określenie wpływu odmiany, rodzaju eksplantatu oraz pożywki na inicjację kultur *in vitro* lachenalii 'Rosabeth', 'Rupert', 'Namakwa' oraz 'Ronina'. Badając skuteczność dezynfekcji materiału wyjściowego, stwierdzono, iż najmniej zakażeń (12%) zanotowano u lachenalii 'Rupert'. Jako odmianę, która uległa najbardziej zakażeniom, opisuje się 'Rosabeth' – poziom zakażeń aż 78,8%. Analizując regenerację, uznano, iż najszybciej i najintensywniej proces ten przebiegł u odmiany 'Rupert', a najsłabiej u odmiany 'Rosabeth'. Średnie wartości zanotowano zaś dla lachenalii 'Namakwa' i 'Ronina'. Następnym badanym czynnikiem był rodzaj eksplantatu. Nie wykazano istotnych różnic pomiędzy procentem zakażeń oraz regeneracją u pąków wierzchołkowych, górnych i dolnych części łusek czterech badanych odmian. W doświadczeniu wykorzystano pożywki o różnej zawartości, kombinacji i proporcji między substancjami wzrostowymi. Najmniejszy procent zakażonego materiału, a jednocześnie największy procent regeneracji eksplantatów wyjściowych (formowanie tkanki kalusowej), dotyczył pożywki zawierającej 1 μM BA oraz 10 μM 2,4-D. Odwrotne rezultaty uzyskano na pożywkach z dodatkiem 5 μM BA i 1 μM 2,4-D oraz 5 μM BA i 1 μM NAA.