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ADVENTITIOUS ROOTS AND BULBS FORMATION ON *Hyacinthus orientalis* LEAF CUTTINGS UNDER DIFFERENT COLOURS OF ARTIFICIAL LIGHT

Małgorzata Śmigielska, Marek Jerzy
University of Life Sciences in Poznań

Abstract. Using fluorescent lamps with different colours of light for improvement *in vivo* propagation of hyacinths by leaf cuttings, was the aim of this experiment. Leaves of three hyacinth cultivars were taken from plants forced in glasshouse, they were rooted in peat in growing room under fluorescent lamps TLD 36W emitting different colours of light: white, blue, green, yellow and red. Light with quantum irradiance of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was applied for 12 hours per day. Under lamps with white, blue or red colour of light leaf cuttings formed adventitious roots in large number and the longest; their weight was the greatest under lamps with white colour of light. There was no effect of the colour of light on number of adventitious bulbs developed on the leaf cuttings of cultivars 'Anna Marie' and 'Blue Star'. In the cultivar 'White Pearl' the greatest number of bulbs was created under white, blue and green colour of light. However, a favourable effect on quality of bulbs was observed. The biggest bulbs with the greatest weight were formed under lamps emitting white and blue colour of light.

Key words: fluorescent lamps, hyacinth cultivars, quality of adventitious organs

INTRODUCTION

In natural conditions, adventitious bulbs develop at the base of the mother bulb. They are small and they start to bloom first after 2–3 years of cultivation. They are formed in small numbers (2–4). More adventitious bulbs can be obtained by incision of the mother bulb (10–12), or by boring the mother bulb (40–60). Hyacinth can be also reproduced by planting of scales cut off together with a piece of mother bulb [Pierik and Woets 1971, Krause 1986]. However, each of the above mentioned methods results in a complete destruction of the mother bulb. This fact can be avoided by rooting and planting of leaves which will create adventitious bulbs [Krause 1980 a, b, Krause 1981], or by reproduction of hyacinth by *in vitro* cultures [Pierik and Post 1975]. Primarily, we

Corresponding author – Adres do korespondencji: Małgorzata Śmigielska, Marek Jerzy, Departament of Ornamental Plants, University of Life Science in Poznań, Dąbrowskiego 159 Street, 60-594 Poznań, Poland, e-mail: ozdobne 4@up.poznan.pl

can use as explants all juicy bulb scales, leaves, but also flower fragments [Lu et al. 1988] and even cells of leaf epidermis and mesophyll tissues, as well as cells of adventitious bulb storage tissues [Bach 1989].

Studies referrig to the effect of light on the *in vitro* reproduction of hyacinth are already far advanced [Bach and Świderski 2000, Bach et al. 2000, Bach and Król 2001]. The role of light and its colour in the reproduction process of hyacinth bulbs *in vivo* has not been sufficiently recognized yet [Gude and Dijkema 1992a].

MATERIAL AND METHODS

The presented studies have been carried out on *Hyacinthus orienalis* L.: 'Anna Marie', 'Blue Star' and 'White Pearl' cultivars. Bulbs with 18–19 cm diameter were planted on the 26th of October 2009 into 11 cm diameter pots filled with limed sphagnum with an addition of sand (in 3:1 proportion). The pots with bulbs were wet cooled at +9°C for 12 weeks, i.e. until January 2010. Then, the pots were transferred to the greenhouse and plant forcing was started. Air temperature in the greenhouse was 12°C, air humidity was 58%.

Cuttings were taken four weeks after the start of forcing, i.e. on the 18th of February 2010. In that time, the blooming of plants started and the first lower buds developed. Leaf cuttings (the whole leaf blades) were taken from the external whorl by tearing them off directly above the bulb in such a way as not to deprive the cuttings of their etiolated leaf base. From one plant, three cuttings were taken. Depending on the cultivar, the cuttings had a different length and weight: 'Anna Marie' – was on the average 19.6 cm long and 4.68 g weight; 'Blue Star' – about 9.4 cm and 1.79 g; 'White Pearl' – about 11.9 cm and 1.96 g. The cuttings were planted into boxes filled with a two-layer substrate. The lower layer was 5 cm thick and it consisted of a limed sphagnum peat. The upper layer was 2 cm thick and consisted of fine grained river sand. The depth of planting was 2 cm. Plants were distributed in 3 × 3 cm density. Substrate moisture was 75%. Boxes with leaf cuttings were placed on shelvings provided with fluorescent lamps (Philips TLD 36W): emitting white colour 33 (370–700 nm), blue colour 18 (400–580 nm), green colour 17 (360–630 nm), yellow colour 16 (500–650 nm) and red colour 15 (600–700 nm). Quantum irradiance (PPFD) was 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the day length lasted 12 hours. Air temperature in the growing room was maintained at the level of 18–20°C, while the relative air moisture was within 64–70%.

One combination of experiment (colour of light × cultivar) included 80 leaf cuttings (8 replications with 10 cuttings in each one). The experiment was terminated in three terms after complete wilting of all leaf cuttings within one cultivar: on the 10th of May ('Blue Star'), 30th of May ('White Pearl') and 7th of June ('Anna Marie'). On the mentioned dates, the cutting developing adventitious roots were counted, their length was measured and the fresh weight of roots grown from one cutting was determined. Cuttings forming adventitious bulbs were counted as well and their diameter and fresh weight were measured. The number of rooted cuttings and the number of cuttings forming adventitious bulbs were additionally expressed in percentages. All results were statistically elaborated by the analysis of variance for one-factorial experiments, separately for each cultivar. The significance of differences was evaluated by Duncan's test at the level of α = 0.05. Values expressed in percentages were subject to Bliss' transformation.

RESULTS

It has been observed that the colour of light exerts a significant effect on the number of cuttings which create adventitious roots and bulbs in all studied cultivars. Under white, blue and green colours of light, the greatest number of cuttings was rooted, independent of their cultivar. The white, blue and red colours of light also are favourable for the creation of a great number of adventitious roots on leaf cuttings. The least number of bulbs was developed by leaf cuttings rooted under yellow colour of light (tab. 1).

Table 1. Effect of light colour on leaf cuttings of three hyacinth cultivars forming adventitious roots (%) and adventitious bulbs (%)
Tabela 1. Wpływ barwy światła na sadzonki liściowe tworzące korzenie przybyszowe (%) i cebule przybyszowe (%) trzech odmian hiacynta

Cultivar Odmiana	Leaf cuttings Sadzonki liściowe	Light colour – Barwa światła				
		white biała	blue niebieska	green zielona	yellow żółta	red czerwona
'Anna Marie'	forming adventitious roots tworzące korzenie przybyszowe	58.8 c	47.5 b	23.8 a	21.3 a	56.0 c
	forming adventitious bulbs tworzące cebule przybyszowe	72.5 c	80.0 c	73.8 c	40.0 a	61.3 b
'Blue Star'	forming adventitious roots tworzące korzenie przybyszowe	55.0 c	46.3 b	17.5 a	20.0 a	60.0 c
	forming adventitious bulbs tworzące cebule przybyszowe	82.5 b	92.5 c	87.5 bc	62.5 a	85.0 b
'White Pearl'	forming adventitious roots tworzące korzenie przybyszowe	52.8 c	55.0 c	31.3 b	23.8 a	48.8 c
	forming adventitious bulbs tworzące cebule przybyszowe	71.3 b	75.0 b	85.0 c	52.5 a	76.3 b

Means followed by the same letters do not differ significantly at α = 0.05
Średnie oznaczone tymi samymi literami nie różnią się istotnie przy α = 0,05

The number and length of adventitious roots grown from one leaf cutting was in all cultivars the highest under lamps emitting white, blue and red colours. In the green and yellow colour of light, the least number of adventitious roots was formed and at the same time, they were also significantly shorter. The influence of the colour of light on the weight of adventitious roots was found in all cultivars. Under the lamps with white light, the root weight was the highest, while the blue and green light caused a medium root weight. The lowest weight was shown under the lamps emitting yellow and red lights (tab. 2)

Table 2. Features of adventitious roots formed on one leaf cutting of three cultivars of hyacinth under fluorescent lamps with different colours of light

Tabela 2. Cechy korzeni przybyszowych wyrosłych z jednej sadzonki liściowej trzech odmian hiacynta pod lampami fluorescencyjnymi o różnej barwie

Cultivar Odmiana	Adventitious roots Korzenie przybyszowe	Light colour – Barwa światła				
		white biała	blue niebieska	green zielona	yellow żółta	red czerwona
'Anna Marie'	number liczba	4.5 c	4.0 bc	1.0 a	1.5 a	4.1 bc
	lentgth (cm) długość (cm)	2.2 b	2.5 b	1.5 a	1.9 ab	2.3 b
	weight (mg) masa (mg)	363.0 c	267.3 b	275.8 b	158.6 a	160.8 a
'Blue Star'	number liczba	2.3 b	2.8 b	0.4 a	0.6 a	2.2 b
	lentgth (cm) długość (cm)	2.2 b	2.4 b	1.3 a	1.4 a	1.8 b
	weight (mg) masa (mg)	61.2 c	34.9 b	36.2 b	22.4 a	26.9 a
'White Pearl'	number liczba	2.7 b	3.4 c	1.6 a	1.4 a	2.4 b
	lentgth (cm) długość (cm)	1.7 b	1.6 b	1.9 a	1.0 a	2.0 b
	weight (mg) masa (mg)	71.3 c	43.2 b	40.1 b	31.6 a	33.8 a

Explanations – see table nr 1; Objaśnienia – patrz tabela 1

Table 3. Features of adventitious bulbs formed on one leaf cutting of three cultivars of hyacinth under fluorescent lamps with different colours of light

Tabela 3. Cechy cebul przybyszowych wyrosłych z jednej sadzonki liściowej trzech odmian hiacynta pod lampami fluorescencyjnymi o różnej barwie

Cultivar Odmiana	Adventitious bulbs Cebule przybyszowe	Light colour – Barwa światła				
		white biała	blue niebieska	green zielona	yellow żółta	red czerwona
'Anna Marie'	number liczba	2.6 a	2.8 a	2.9 a	2.6 a	2.0 a
	circumference (cm) obwód (cm)	1.9 b	2.0 b	1.5 a	1.9 b	1.3 a
	weight (mg) masa (mg)	282.6 c	295.5 c	172.5 b	171.7 b	98.8 a
'Blue Star'	number liczba	2.8 a	2.6 a	2.8 a	2.3 a	2.8 a
	circumference (cm) obwód (cm)	1.7 bc	2.0 c	1.5 b	1.3 b	0.8 a
	weight (mg) masa (mg)	209.2 c	243.2 d	143.7 b	84.9 a	80.4 a
'White Pearl'	number liczba	3.0 b	2.6 b	3.1 b	1.8 a	1.8 a
	circumference (cm) obwód (cm)	1.6 b	1.6 b	1.0 a	1.2 a	1.1 a
	weight (mg) masa (mg)	149.7 c	158.0 c	129.1 bc	101.4 b	47.2 a

Explanations – see table nr 1; Objaśnienia – patrz tabela 1

The number of adventitious bulbs which were developed by one leaf cutting of hyacinth did not depend on the colour of light in the cultivars 'Anna Marie' and 'Blue Star'. The greatest number of adventitious bulbs of the 'White Pearl' cultivar was obtained in the light with white, blue and green colours. The colour of light exerted an influence on the diameter of bulbs and on their weight in all studied cultivars. The biggest bulbs with the highest weight were developed by the cuttings of 'Anna Marie' cultivar under the light with white and blue colours. In case of the 'Blue Star' cultivar, the biggest and the heaviest bulbs were formed under blue colour of light. Under the action of the white and blue colour light, the cuttings of 'White Pearl' cultivar formed the biggest and the heaviest adventitious bulbs (tab. 3).

DISCUSSION

Adventitious roots are formed above the place where hyacinth leaf cutting had been cut off in the time from the 7th to the 30th day after planting in the substrate [Krause 1980b]. On the basis of our own observations, it follows that adventitious roots are visible after four weeks from the date of cutting taking. However, not all cuttings produce roots, but, in spite of that, at the base of the cutting, there develop adventitious bulbs. The greatest number of cuttings create adventitious roots under the action of the white, blue and red colour of light. Also the number and the length of adventitious roots is the highest under these three colours of light.

Adventitious bulblets appear in a similar time as the roots, i.e. already 15–20 days after the plantation of leaves. Their development lasts until the leaf cuttings get wilted. For this purpose, the most adequate are the complete leaves. Cuttings prepared from the upper part of leaf blade wilt quickly without the formation of any adventitious roots or bulbs. On the other hand, the cuttings from the lower part of the leaf blade root poorly and create a small number of adventitious bulbs. Krause [1980b] argues that the base of leaf cutting should be etiolated (deprived of chlorophyll). In the *in vitro* cultures of hyacinth, a good regeneration takes place at the base of a scale [Pierik and Post 1975]. The above mentioned authors have found that there exists a correlation between the surface of the explant and the regeneration. The number of bulbs from an explant increases with the increase of its surface and there also takes place an increase of the fresh weight of adventitious bulbs.

Many factors exert an influence on the regeneration process in the *in vitro* cultures. One of the most important factors is the composition and number of exogenous growth regulators. An unfavourable effect is exerted by cytokinins, gibberelic acid, abscisic acid and ethephon [Pierik and Steegmans 1975]. The initiation of the root regeneration *in vitro* takes place only on nutrient medium without any cytokinins addition [Bach and Cecot 1988], or on nutrients enriched with naphthalene-1-acetic acid (NAA) [Bach and Cecot 1989]. The content of indole-3-acetic acid (IAA) or the indole-3-butyric acid (IBA) in the amount of 10^{-5} $g \cdot ml^{-1}$ in the nutrient solution exerts the best effect on the number of adventitious bulbs formed on explants from scales [Bach and Cecot 1989].

An unusually important physical factor is the temperature. Low temperature of hyacinth bulbs storaging (4°C for 4 months) increases the number of adventitious bulbs created on explants from scales [Chung et al. 2006]. Explants from not cooled bulbs regenerate most intensively at the temperature of 13°C, while the range of 21–25°C is the optimal one for the growth of adventitious bulbs. On the other hand, no differences have been found during the regeneration of explants from hyacinth scales in the darkness, or in conditions of continuous light [Pierik and Ruibing 1973], our own studies indicate that the colour of light exerts a significant influence on the production and quality of adventitious bulbs. The average number of bulbs from one leaf cutting did not differ in the cultivars 'Anna Marie' and 'Blue Star'. In the 'White Pearl' cultivar, the greatest bulb was found under the white, blue and green colour lights. Gude and Dijkema [1992 b] confirmed that the colour of light does not exert any effect on the number of descendant bulbs developed *in vivo* in bored hyacinth bulbs. A major problem with the "scooping" technique for the propagation of hyacinth is that the young bulbs remain dormant after planting. Gude i Dijkema [1992 b] studied whether light quality in the propagation room and/or low temperature after planting could enhance the number of non-dormant bulbs of cultivar 'Viking'. Light treatment had no effect on the number of newly formed bulbs. The appearance of these adventitious bulbs, however, was markedly affected by the light treatment. Under blue light the bulbs were smaller but more differentiatied than in darkness. Pigmentation (anthocyanin and chlorophyll) was also enhanced by blue light. Under red light some differentiation occurred, but without pigmentation. The light treatments effectively prevented the young bulbs from becoming dormant, as appeared from the number of emerged leaves. This effect was saturated at a quantum irradiance 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, irrespective of light quality. The cold treatment accelerated leaf emergence, but no time was gained if the moment of planting was taken into account. The positive effect of the light treatment on the number of leaves could even be observed with the bulbs that were planted outdoors (after a natural cold treatment of several months). A positive correlation was found between the number of leaves emerged and the yield of new bulbs after the growing season. Consequently a 30–45% increase in yield was accomplished by the light treatment.

Reproduction of hyacinth *in vivo* using leaf cuttings in production of adventitious bulbs represents a simple method, but not a very efficient one and it is rarely utilized in production practice. In natural light conditions (e.g. in a greenhouse), from one leaf, one can obtain, on the average, 5–7 small bulbs [Krause 1986]. In conditions of artificial light, one can obtain from one leaf only 2–3 bulbs, as it was shown in our own studies.

However, there is one reason why the latter method of reproduction has a particular importance. The fact is that this method is using in radiomutation breeding of new cultivated varieties of hyacinth. According to this method, leaves torn off from the mother plant are treated with ionizing radiation X, or gamma radiation and from the created adventitious bulbs, there frequently develop mutated plants with a changed colour of inflorescences.

CONCLUSIONS

1. A significant effect of the colour of light has been shown to be exerted on the creation of adventitious roots and bulbs on leaf cuttings of three cultivars of *Hyacinthus orientalis* L.

2. In all cultivars, the number and length of adventitious roots grown from one leaf cutting was the highest under lamps emitting light of white, blue and red colour. The highest weight was shown by roots developed under lamps emitting white colour of light, while the lowest weight was shown under lamps with yellow and red colour of light.

3. The colour of light did not exert any effect on the number of adventitious bulbs developed on the leaf cuttings of cultivars: 'Anna Marie' and 'Blue Star'. In the cultivar 'White Pearl', the greatest number of bulbs was created under the lamps with white, blue and green colour of light

4. The colour of light exerted a significant influence on the size and weight of adventitious bulbs (with the widest diameter and the highest weight) in all hyacinth cultivars under the lamps emitting white and blue colours.

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TWORZENIE SIĘ KORZENI I CEBUL PRZYBYSZOWYCH NA SADZONKACH LIŚCIOWYCH HIACYNTA WSCHODNIEGO PRZY ŚWIETLE SZTUCZNYM O RÓŻNEJ BARWIE

Streszczenie. Celem pracy było udoskonalenie technologii rozmnażania hiacynta wschodniego *in vivo* przez zastosowanie sztucznego światła fluorescencyjnego o barwie sprzyjającej tworzeniu się cebul przybyszowych na ukorzenionych sadzonkach liściowych. Liście trzech odmian hiacyntów pobrane z roślin pędzonych w szklarni, ukorzeniano w torfie wysokim w pokoju wzrostowym pod lampami fluorescencyjnymi TLD 36W, emitującymi światło o barwie białej, niebieskiej, zielonej, żółtej i czerwonej. Światło o natężeniu 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ stosowano przez 12 godzin na dobę. Pod lampami o barwie białej, niebieskiej i czerwonej sadzonki liściowe tworzyły liczne i najdłuższe korzenie przybyszowe; ich masa była największa pod lampami o barwie białej. Barwa światła nie miała wpływu na liczbę tworzących się cebul przybyszowych u odmian 'Anna Marie' i 'Blue Star'. U odmiany 'White Pearl' najwięcej cebul tworzyło się pod światłem o barwie białej, niebieskiej i zielonej. Wywarła natomiast korzystny wpływ na jakość cebul. Cebule największe o największej masie tworzyły się pod lampami o świetle białym i niebieskim.

Słowa kluczowe: lampy fluorescencyjne, odmiany hiacynta, jakość organów przybyszowych

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