

## The effect of the growing conditions on the growth and reproduction of tulip bulbs produced *in vitro*

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### ABSTRACT

The aim of the study was the shortening of the period needed to obtain flowering tulip plants derived from micropropagation. Bulbs of the tulip 'Prominence' and 'Blue Parrot' produced *in vitro* were rooted for three months in the peat growing substrates in the dark at 9°C. Then, plants were grown in a growth chamber at 15°C under light (80-100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) or in a greenhouse and fertilized every two weeks with Peters Professional 20-10-20 or calcium nitrate, both at the concentration of 0.05 or 0.1%. Bulbs were also grown in a field. It was shown that both in the growth chamber and the greenhouse the yields of daughter bulbs were poor. The daughter bulbs produced in the growth chamber were 20-40% smaller than the mother ones, with an exception of combination in which the 0.05% Peters

Professional was used, where the daughter bulbs were 13.5% larger than the mother ones. In the greenhouse, the daughter bulbs larger than the mother ones by 10-40% were noted at Peters Professional 20-10-20 fertilization. The field conditions, with the mild winters in both seasons 2000/2001 and 2001/2002, had highly beneficial effect on the efficiency of bulb reproduction. The daughter bulbs were 2-5 times larger than the mother bulbs. It is supposed that the factor limiting the growth of bulbs in the growth chamber was too low quantum irradiance and in the greenhouse too high temperature.

## INTRODUCTION

The relatively efficient method of tulip micropropagation through the cyclic shoot multiplication was developed (Podwyszyńska and Rojek 2000, Podwyszyńska 2001). In future, the method can intensify the breeding works and accelerate the commercial release of new tulip cultivars. It can also be used in the production of an elite stock plant material. However, the examination of true-to-typeness of micropropagated plants is necessary before dissemination of this *in vitro* method for tulip production. Such checking of the plant phenotype identity cannot be done earlier than 4-5 years before the bulbs from the *in vitro* culture are obtained (Le Nard et al. 1987). Such a long period is needed for flowering of tulips produced *in vitro*. In the countries where the studies on tulip micropropagation were conducted, France and the Netherlands, the bulbs produced *in vitro* were grown in a field, because the mild climate enabled such cultivation (Le Nard et al. 1987, Hulscher et al. 1992). In Poland, outdoor growing may involve a risk of freezing of the micropropagated bulbs. Therefore, it was proposed to grow bulbs in a growth chamber or in a greenhouse for one or two growing cycles. One growing cycle in artificial conditions takes seven months: three months are required for rooting of the bulbs in e.g. peat substrate at the temperature of 9°C in the dark, next three months are needed for growing at 15°C under light – for the plant growth and daughter bulb reproduction, and the last month is necessary for storage after lifting – for the root primordia development on new bulbs. Thus, introduction of the first two growing cycles in artificial conditions followed by a 2-3-year cultivation in a field could significantly shorten the period needed for checking the identity of the plant produced *in vitro*. It is worth pointing that the development of a daughter bulb in tulip is more complicated than in other bulbous plants like hyacinth or narcissus, whose bulbs persist for several years and enlarge their sizes from year to year. The mother bulb of the tulip, having formed daughter bulbs, dies itself. Therefore, tulip plants ought to be grown very carefully to obtain high effectiveness of bulb reproduction.

The aim of the study was the shortening of the period needed to obtain flowering tulip plants derived from micropropagation.

## MATERIAL AND METHODS

Bulbs (Podwyszyńska and Rojek 2000, Podwyszyńska 2001) of the tulip 'Prominence' and 'Blue Parrot' produced *in vitro* were used for experiments. The latter cultivar was included only in the field experiments because the low number of bulbs was attainable. The cultivar 'Blue Parrot' has poor capacity for bulb formation *in vitro* (Podwyszyńska and Ross 2003). Before planting, bulbs were stored at 20°C for 1-2 months if derived directly from *in vitro* culture, or 2-3 months if had been already cultivated *ex vitro*. Bulbs were planted in the growing substrates composed of: (A) peat and perlite (3 : 1, v / v) and (B) peat, sand, and lime (8 : 1 : 1, v / v), supplemented with 0.5 or 1 g dm<sup>-3</sup> complete fertilizer Azofoska (13.6% N, 6.4% P<sub>2</sub>O<sub>5</sub>, 19.1% K<sub>2</sub>O, 4.5% MgO) and adjusted to pH 6.5. In such substrates, bulbs were rooted in the dark at 15°C during three months. Then plants were grown in the growth chamber at 15°C under light at 16 h photoperiod provided by high pressure sodium lamps (WLS Polam, Poland; 80-100 μmol m<sup>-2</sup> s<sup>-1</sup> Photosynthetic Photon Flux Density – PPF) or in the greenhouse from the beginning of April to the end of July at the ambient temperature of 17-26°C (occasionally temperature increased to 30°C). In all experiments, plants were fertilized once with 0.05% Peters Professional Plant Starter 10-52-10 one week after transfer to the growth chamber or greenhouse. Then, every two weeks during the three-month-cultivation period, Peters Professional 20-10-20 or calcium nitrate were applied at the concentrations of 0.05% and 0.1% or these two fertilizers were used alternately at 0.1%. The doses of the fertilizers were selected on the basis of tulip nutritional requirements (Anonymous 1984, Strojny 1993). The recommended nutrient contents in soil are as follows (mg dm<sup>-3</sup>): N-NO<sub>3</sub> – 50-100, P – 40-80, K – 100-200, Ca – 1000-2000, Mg – 45-120, and salt concentration (NaCl g dm<sup>-3</sup>) – 1-2.5. Chemical analysis of the growing substrates was performed. Soil samples were taken 3-4 times: in the first week of the culture under light and one, two, and three months later, and in the outdoor culture at the end of March, April, and May. Soil nutrients were extracted using 0.03 M acetic acid (Nowosielski 1988). The concentration of K, Ca, and Mg was measured by atomic absorption spectrophotometry, and N-NO<sub>3</sub> was measured with a nitrate specific electrode. P was determined colorimetrically using vanadium-molybdate complex.

Before planting, the bulbs were weighed. After harvest, newly formed daughter bulbs were dried for a week, then counted and weighed. The mean weight of the bulbs, percentage of reproduction (ratio of the daughter to mother bulb numbers multiplied by 100%) and the reproduction rate (ratio of the daughter to mother mean bulb weight) were counted. In each treatment 20-30 bulbs (replicates) were used. Experiments were conducted in two series. Final data were the means of two series. The data were submitted to analysis of variance and means were compared by Student's t test at p = 0.05.

In the first experiment, the effect of the growing substrate (A and B), enriched with Azofoska (0.5 and 1 g dm<sup>-3</sup>) before planting, on the bulb reproduction of 'Prominence' in the growth chamber was examined. Two classes of the *in vitro* produced mother bulbs were used: the small ones (S) weighing 45-55 mg and the large ones (L) weighing 140-150 mg. During plant cultivation at 15°C, Peters Professional 20-10-20 was used alternately with calcium nitrate, both at the concentration of 0.1%.

In the second experiment, bulbs of 'Prominence' derived directly from *in vitro* culture, of a mean weight 116.4 mg, were rooted in substrate A enriched with 1 g dm<sup>-3</sup> Azofoska. Bulb reproduction was examined depending on the cultivation conditions (in the growth chamber or the greenhouse), and fertilization given after the rooting phase. Peters Professional 20-10-20 or calcium nitrate were applied at the concentration of 0.05 and 0.1% or these two fertilizers were used alternately at 0.1%.

In the third experiment, the bulb reproduction of 'Prominence' after the second growing cycle in the growth chamber was evaluated depending on the fertilization. The fertilization and growing substrate was the same as in the second experiment. The mean weight of a mother bulb was 160 mg.

The bulbs were also grown in a field for two seasons. In the first season, in autumn 2000, 'Prominence' and 'Blue Parrot' bulbs, derived directly from the *in vitro* culture and the bulbs of 'Prominence' grown previously for one cycle in the growth chamber, were planted into plastic boxes filled with growing substrate A and placed in a gauze house. The bulbs were protected against frost with a bark layer (10 cm). In spring, the bark was partly removed and the plants were fertilized three times every two weeks with 0.1% Peters Professional 20-10-20 and watered, as needed. If the frost below -5°C was forecast, the plants were covered with an unwoven fabric.

In the second season (2001/2002), the bulbs of both cultivars, either derived directly from the *in vitro* culture or previously grown for one cycle in the growth chamber or in the field, were grown, as above, and fertilized weekly with 1% complete fertilizer Nowokont (1.4% N, 0.6% P<sub>2</sub>O<sub>5</sub>, 2.2% K<sub>2</sub>O, 1.1% CaO, 0.4% MgO), from mid-April until the end of May. Some 'Prominence' bulbs (after one cultivation cycle in the growth chamber) were rooted in the cooling chamber at 9°C from mid-February to mid-May 2002, then cultivated in the gauze house and fertilized with Nowokont to the end of July.

The following mean month temperatures were noted in Skierniewice from October to June in season 2000/2001 respectively: 11.6, 6.2, 1.8, -0.3, -0.4, 2.3, 6.1, 14.2, 15.2°C and the minimal month temperatures: -1.2, -0.3, -10.8, -8.3, -20.1, -11.6, -2.3, -0.8, 4.5°C; in season 2001/2002 – the mean month temperatures: 11.1, 2.7, -3.5, 0.3, 4.0, 4.9, 8.6, 17.2, 17.6°C and the minimal month temperatures: -4.4, -6.3, -19.9, -21.5, -5.8, -3.6, -5.9, 1.9, 6.6°C.

In the field experiments, 25-120 bulbs were planted in each treatment. After lifting, the bulbs were dried, counted and the total weight of all bulbs (harvest) from each treatment was noted. Reproduction rate was counted as the ratio of daughter to mother

weight of total number of bulbs. Only bulbs weighing more than 100 mg were included.

## RESULTS AND DISCUSSION

The results showed that the character of the growth and development of bulbs derived from the *in vitro* culture was similar in the growth chamber, greenhouse and during outdoor cultivation. In the first and second growing cycle, the mother bulbs developed a single leaf and usually produced a stolon (“dropper”) followed by the formation of the daughter bulbs at its extremity (Figs 1 and 2).

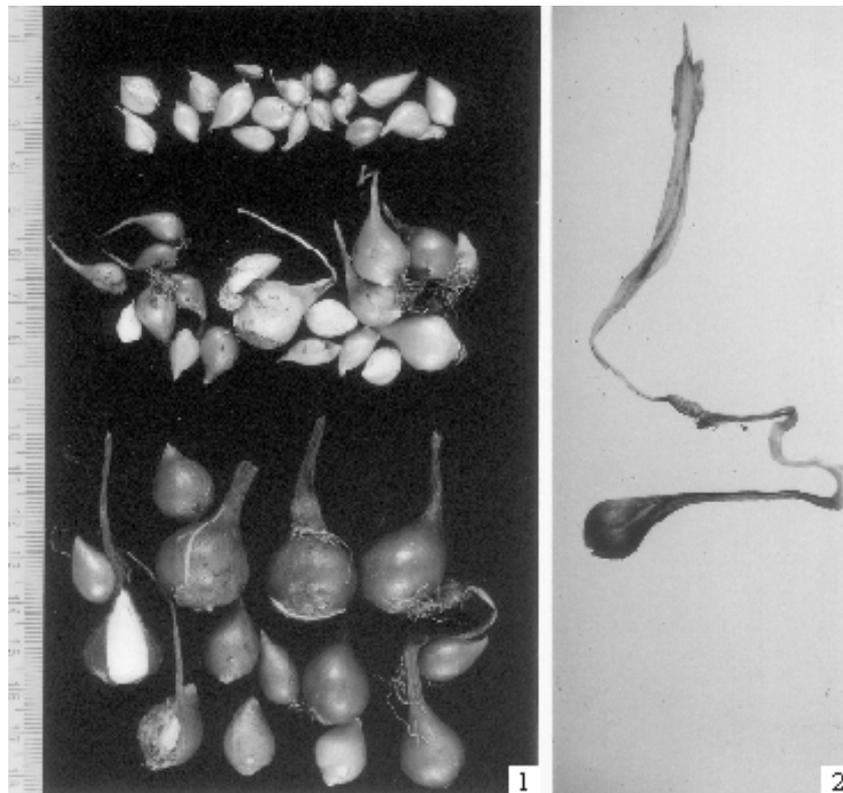


Figure 1. Bulbs of tulip ‘Prominence’, from the top: bulbs derived directly from *in vitro* culture, after one and two growing seasons in a field

Figure 2. Formation of daughter bulb on a mother bulb derived directly from *in vitro* culture. A dried leaf, remains of the mother bulb and stolon and daughter bulb covered with a tunic

At the time of harvest, after the leaves dried, the bulbs had already been covered with tunic. The similar development of the *in vitro* derived bulbs in the *ex vitro* conditions, analogical to that observed in young tulip seedlings, was also reported by Le Nard et al. (1987) and Hulscher et al. (1992). It is worth noting that the emergence of a single leaf is characteristic for the small non-flowering bulbs being in a juvenile phase that lasts until a critical flowering bulb size of the 6-9 cm in circumference is obtained (Rees 1992).

In the first experiment, in the growth chamber, the bulbs were grown in two different substrates enriched with Azofoska at two concentrations given prior to planting. The reproduction of bulbs was generally very poor in the growth chamber (Table 1). The daughter bulbs were smaller than the mother ones in all treatments. The large bulbs were obtained in the substrate A at the higher dose of Azofoska and therefore this substrate was used in the further experiments. It was also found that small mother bulbs produced daughter bulbs in 30-65%, while the large ones in 90-100%. A similar positive correlation between the size of mother bulbs and the effectiveness of reproduction was noted in earlier studies (Le Nard et al. 1987, Hulscher et al. 1992, Podwyszyńska 2001). Chemical analysis showed that the nutrient contents remained in the range of the recommended optimum in all treatments.

Table 1. Effects of different substrates and the fertilization applied before planting on the growth and reproduction of tulip 'Prominence' bulbs grown in the growth chamber, in the first growing cycle, depending on the size of mother bulbs; (S) small mother bulbs, (L) large mother bulbs

Substrate, fertilization before planting	Reproduction (%)		Mean weight of daughter bulb (mg)		Reproduction rate	
	S	L	S	L	S	L
Substrate A, Azofoska 0.5 g dm <sup>-3</sup>	60.0	100.0	47.3 ab*	115.0 a	0.69	0.68
Substrate A, Azofoska 1.0 g dm <sup>-3</sup>	65.0	90.0	60.3 bc	129.0 a	0.86	0.93
Substrate B, Azofoska 0.5 g dm <sup>-3</sup>	30.0	100.0	49.3 ab	105.8 a	0.77	0.74
Substrate B, Azofoska 1.0 g dm <sup>-3</sup>	45.0	100.0	44.3 a	113.4 a	0.69	0.78

\* Means in one column marked with the same letter do not differ significantly at  $p = 0.05$

In the second experiment, the bulb reproduction was examined at the first growing cycle in the growth chamber and the greenhouse, depending on the fertilization applied during the growing period. Both in the growth chamber and the greenhouse the yields of the daughter bulbs were poor, lower in the growth chamber (Fig. 3). The daughter bulbs produced in the growth chamber were 20-40% smaller than the mother ones, with an exception of combination in which the 0.05% Peters Professional was used, where the daughter bulbs were 13.5% larger than the mother ones.

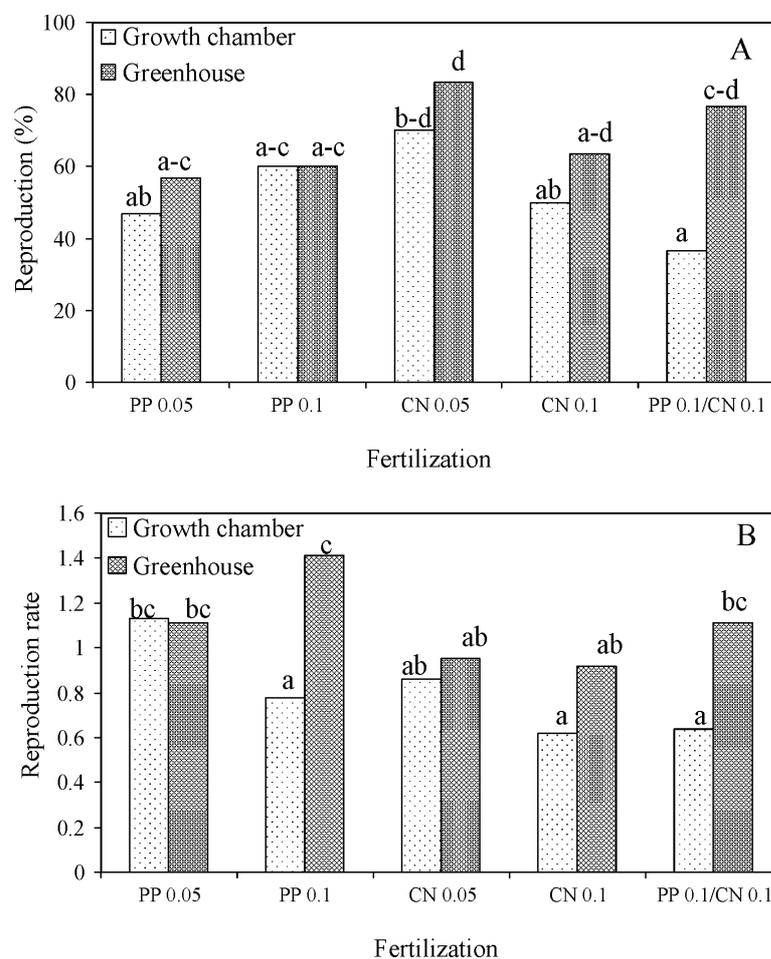


Figure 3. Effects of growing conditions (in a growth chamber or in a greenhouse) and fertilization given after the rooting phase on the percentage of reproduction (A) and reproduction rate (B) of tulip 'Prominence'. The mother bulbs derived directly from *in vitro* culture. The following fertilization was given every two weeks for two months: 0.05% and 0.1% Peters Professional 20-10-20 (PP) or 0.05% and 0.1% calcium nitrate (CN) or 0.1% Peters Professional 20-10-20 (PP) and 0.1% calcium nitrate alternately (PP/CN)

In the greenhouse, the daughter bulbs larger than the mother ones by 10-40% were noted in all treatments in which Peters Professional 20-10-20 was used. The chemical analysis of growing substrates showed that after the starting of fertilization and then during the whole cultivation period, the level of  $\text{N-NO}_3$  was 2-4 times higher ( $200\text{-}400\text{ mg dm}^{-3}$ ) in all treatments than the recommended one. The level of other nutrients (P, K, Ca, and Mg) remained within the range of

recommended optimum, with an exception of the Peters Professional 20-10-20 application at 0.1%, where the super optimal level of P by 30% and K by 20-30% was found.

In the third experiment, the bulb reproduction after the second growing cycle in the growth chamber was evaluated depending on the fertilization applied during the growing period (Table 2). Similarly as in the former experiment, the application of Peters Professional 20-10-20 was generally more beneficial compared with the application of calcium nitrate alone. The daughter bulbs, obtained at Peters Professional, were 30-50% larger than the mother ones. When this fertilizer was used at a lower concentration or alternately with calcium nitrate, also the percentage of reproduction was high – 90-100%.

Table 2. Effect of fertilization applied during vegetation period on the growth and reproduction of bulbs of tulip 'Prominence' grown in the second cycle in the growth chamber

Fertilization	Reproduction (%)	Mean weight of daughter bulb (mg)	Reproduction rate
Peters Professional 0.05%	100	205.4 b*	1.28
Peters Professional 0.1 %	65	232.8 b	1.46
Calcium nitrate 0.05%	95	150.1 a	0.94
Calcium nitrate 0.1%	75	106.1 a	0.66
Peters Professional 0.1% / Calcium nitrate 0.1%	90	221.6 b	1.39

\* Explanation as in Table 1

The field conditions, with the mild winters in both seasons, had a very positive effect on the bulb growth. A sporadic night drop of temperature below -20°C, noted in December, January or February, did not damage the bulbs. Table 3 demonstrates the results of the bulb reproduction obtained in the second season 2001/2002. Thus, the high reproduction percentages were noted, from 85.7% to 108.9% in 'Prominence' and about 100% to 153.2% in 'Blue Parrot' (Table 3). In 'Prominence' for the mother bulbs derived directly from the *in vitro* culture, the reproduction rate was only 1.35, but for the bulbs grown during the first cycle in the growth chamber followed by one season of the outdoor growing was very high – 5.15. The latter was over 2-times higher compared with that noted for the bulbs cultivated during the 1<sup>st</sup> cycle in the growth chamber and in the 2<sup>nd</sup> cycle – rooted in the cooling chamber at 9°C and subsequently grown outdoor (2.4). In general, the mean weight of the daughter bulbs after one outdoor growing season ranged in 'Prominence' from 393 to 1052 mg, and in 'Blue Parrot' it reached about 600 mg (Table 3). In the latter cultivar, the outdoor grown daughter bulbs were about 3-times larger than their mother bulbs irrespectively to the previous culture conditions, whether they derived directly from *in vitro* culture or had been already

cultivated in the growth chamber. In both cultivars, the formation of two to five daughter bulbs from one mother bulb was often observed.

In the case of plants grown outdoors for the first and second cycles, the daughter bulbs of 'Prominence' were about 3.3-times larger and those of 'Blue Parrot' were even 4-times larger than their mother bulbs (Table 3 and Fig. 1). The mean bulb weight of 'Prominence' reached 1238 mg and in 'Blue Parrot' 2119 mg. The weight of some bulbs reached 6000-9000 mg (about 6-8 cm in circumference). Such bulbs formed flowers, which was confirmed by their development in the spring 2003.

Table 3. Bulb reproduction of tulip 'Prominence' and 'Blue Parrot' grown outdoors in the season 2001/2002 depending on the number of growing cycles and previous growing mode of the mother bulbs derived directly from *in vitro* culture or had been already cultivated during the first growing cycle in the growth chamber or outdoors

Cultivar, number of growing cycles (GC), growing mode of mother bulbs	Reproduction (%)	Mean bulb weight (mg)		Reproduction rate
		Mother bulb	Daughter bulb	
<b>'Prominence'</b>				
1 GC outdoors (directly from <i>in vitro</i> )	86.7	291.0	393.0	1.35
2 GC: 1 <sup>st</sup> – growth chamber, 2 <sup>nd</sup> – rooting at 9C <sup>o</sup> and further cultivation outdoors	85.7	183.8	440.9	2.40
2 GC: 1 <sup>st</sup> – growth chamber, 2 <sup>nd</sup> – outdoors	108.9	204.2	1052.0	5.15
2 GC: 1 <sup>st</sup> and 2 <sup>nd</sup> – outdoors	108.0	376.8	1238.0	3.29
<b>'Blue Parrot'</b>				
1 GC (directly from <i>in vitro</i> )	104.2	210.2	594.8	2.83
2 GC: 1 <sup>st</sup> – growth chamber, 2 <sup>nd</sup> – outdoors	100.0	186.0	531.0	2.85
2 GC: 1 <sup>st</sup> and 2 <sup>nd</sup> – outdoors	153.2	525.6	2119.0	4.03

The reproduction efficiency of 'Prominence' can be compared between the two seasons, the 2000/2001 and 2001/2002 with the similar mild winters but differing in fertilization (Table 4). If the fertilization was more intensive during the growing period (1% Nowokont, 7 times), the reproduction rate was very high, over 5. But with the lower fertilization (0.1% Peters Professional 20-10-20, 3 times) applied in the first season, the daughter bulbs were only 2-times larger than the mother ones. The nutrient contents in the substrates in the both seasons remained at the optimum level recommended for tulip reproduction. It seems that the continuation of the study on tulip fertilization can result in a further improvement of the growth efficiency of the micropropagated bulbs grown in a field.

Table 4. Reproduction efficiency of tulip 'Prominence' bulbs after one outdoor growing season (previously grown in the growth chamber) evaluated in two seasons differing in fertilization

Growing season, fertilization	Reproduction (%)	Mean bulb weight (mg)		Reproduction rate
		Mother bulb	Daughter bulb	
2000/2001, Peters Professional	100.0	145.6	290.9	1.99
2001/2002, Nowokont	108.9	204.2	1052.0	5.15

Considering all the data obtained, it seems that the effects of growing substrate and fertilization was markedly limited in artificial conditions (growth chamber or greenhouse) due to the action of the factors decreasing the bulb growth potential. Our results showed that the nutrient contents in the substrates during the cultivation either outdoors and in the growth chamber, as in the first experiment, remained about optimum. Also, in both cases, the temperatures (15°C in a growth chamber and 15-17°C as the mean month temperatures in a field) maintained during the cultivation as well as the irrigation of the crops were generally proper. But the daughter bulbs formed in the growth chamber were usually smaller than their mother bulbs (Table 1) and those obtained outdoors – markedly larger (Tables 3 and 4). The factor in which these conditions of the cultivation considerably differed was the light intensity (quantum irradiance). In the field, PPFD reached 1000-1300  $\mu\text{mol m}^{-2}\text{s}^{-1}$  but in the growth chamber under the sodium lamps it was 80-100  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . The study on tulip photosynthesis showed that the light saturation point in 'Apeldoorn' grown outdoor in April was 200  $\text{W m}^{-2}$  (Benschop 1980), i.e. about 920  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and the light compensatory point in tulip (with a removed flower) was 60  $\text{W m}^{-2}$  (Rees 1992), approximately 250  $\mu\text{mol m}^{-2}\text{s}^{-1}$  (Czarnowski 1993). That indicates that the light requirements of mature tulip plants are relatively high. The light requirements of the young plants, derived from *in vitro* propagation, are probably lower. But the PPFD (80-100  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) kept in our experiment in the growth chamber was presumably too low, being the factor limiting the growth of the bulbs. It seems that the quantum irradiance ought to be much higher for the tulip reproduction to enable an intensive production of assimilates in the leaves, as well as carbohydrate storage in the developing bulbs. In order to verify such assumption, further study on photosynthetic activity of juvenile tulip plants produced *in vitro* is required.

In turn, the factor limiting the reproduction growth of tulip in the greenhouse could be the super-optimal temperature. The light intensity in the greenhouse was relatively high, as in the field, but the day temperatures noted during the growing in July were usually high (20-26°C) and occasionally the growing substrate heated to 30°C. It is worth pointing that the best conditions for bulb reproduction are in the regions where the mean month temperatures in December fluctuate about 0°C, in

the spring between from 12 to 15°C and do not exceed 20°C (Le Nard and De Hertogh 1993 according to Schenk 1969, Szlachetka and Drozd 1990, Hetman 1996). In our experiments, the conditions closest to those mentioned above occurred in the field. Moreover, the bark layer, used in the outdoor experiment, protected the soil against the excessive heating and moisture loss. Such mulching was applied in our experiments according to the recommendations of Krause (1986) for the reproduction of tulips grown in Polish climatic conditions.

In conclusion, our data and the above mentioned information about tulip climatic requirements indicate that the bulb cultivation in the growth chamber, due to the insufficient light intensity or in the greenhouse because of the difficulties to maintain the proper temperature did not provide the proper conditions for bulb reproduction and would not lead to shortening the period needed to obtain flower size bulbs. The optimization of the bulb growing system in the artificial conditions has to involve high costs and seems to be not feasible. Due to the application of the proper fertilization and protection of the bulbs against frost, relatively high yields of the bulbs were obtained in the field, even in the case of mother bulbs derived directly from *in vitro* culture (Table 3). The reduction of the period from 5 to 3.5 years needed to obtain flowering of the plants produced *in vitro* may be achieved by the introduction of the two growing cycles in one season. The first cycle is 'winter rooting' in cooling chambers at 9°C of bulbs deriving directly from *in vitro*, starting from January, February or March and subsequently, the growing of plants in a field for 2-3 months, beginning from April, May or June. Thus, the bulb harvesting occurs from July to August. That enables the start of a second growing cycle in the same year by planting the bulbs in a field in autumn. Such cultivation system was successfully used in the season 2002/2003 (data not presented). Moreover, this method rationalizes the *in vitro* production. It allows to prepare the bulbs produced *in vitro* for the two terms of transplanting.

Further experiments are in progress. It is necessary to examine the bulb reproduction efficiency in the successive seasons, in which more frosty winters can occur. After checking a true-to-typeness of the *in vitro*-produced plants during their flowering, and showing that the level of somaclonal variation is low, the method of tulip *in vitro* propagation can be widespread in breeding and horticultural production.

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## WPŁYW RÓŻNYCH WARUNKÓW UPRAWOWYCH NA WZROST I ROZWÓJ CEBUL TULIPANÓW POCHODZĄCYCH Z MIKROROZMNAŻANIA

Streszczenie: Celem badań było skrócenie okresu uprawy potrzebnego do uzyskania kwitnienia roślin tulipanów pochodzących z mikrorozmnażania. Cebule tulipanów odmian 'Prominence' i 'Blue Parrot' wytworzone *in vitro* ukorzeniano w ciemności w podłożach torfowych, w temperaturze 9°C przez 3 miesiące, a następnie uprawiano w fitotronie w 15°C przy sztucznym świetle (80-100  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) lub w szklarni i nawożono pogłównie nawozem Peters Professional 20-10-20 lub saletrą wapniową (0,05-1%). Cebule uprawiano także w polu. Zarówno w fitotronie, jak i w szklarni plony cebul potomnych były niskie. W fitotronie masy cebul potomnych były mniejsze od matecznych o 20-40%, z wyjątkiem kombinacji, w której stosowano 0,05% Peters Professional 20-10-20 - cebule potomne były większe od matecznych o 13,5%. W szklarni przy zastosowaniu tego nawozu cebule potomne były większe od matecznych o 10-40%. Warunki polowe, z łagodnymi zimami w obu sezonach, wpłynęły bardzo korzystnie na wzrost cebul. Wyprodukowane w polu cebule potomne były 2-5-krotnie większe od matecznych. Przypuszcza się, iż czynnikiem ograniczającym wzrost cebul w fitotronie było zbyt niskie natężenie napromieniowania kwantowego, a w szklarni - zbyt wysoka temperatura.

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