Studies on Vegetative Propagation of Tulips

IV. Regeneration of Bulblets in Bulb Scale Segments Culture¹ *in vitro*

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Summary

Excised tulip bulb scale segments were able to generate adventitious buds when cultured on a modified Murashige-Skoog medium fortified with auxin and kinetin. Transferring the buds to a medium low in auxin and kinetin induced further development. Bulb formation occurred at the proximal end of buds receiving a chilling treatment at 5°C, but failed to occur in unchilled buds. The optimal duration of the chilling period was found to be about 80–100 days. Bulb formation was enhanced by the application of NAA together with kinetin at low concentrations, although at high concentrations formation was delayed. Sucrose was also necessary for bulb formation, and its optimum concentration was found to be range between 4 and 6%. Culture temperature for the bulb formation was optimal at 25°C.

Introduction

Many investigators are in general agreement that tissue culture is a potentially useful tool for studying growth and differentiation in higher plants. Neo-formation of bulblets in such plants as amaryllis, hyacinth and lily (5, 10, 11, 12, 13, 14, 15) has been successfully induced through *in vitro* culture of excised bulb scales. It was reported in a previous paper that thickening-type growth of the bulblets around the proximal part of the growing buds was enhanced when the buds were transferred for subculture into a medium with low concentrations of growth substances; failure of growth under other hormonal conditions seems to be directly attributable to an unfavorable hormonal environment. Generally, bulb crops planted in autumn, such as tulip, bulbous iris, and freesia, appear also to have a special requirement for low temperature if they are to form bulblets (1, 2, 3, 6, 7). The following experiments were performed in an attempt to investigate variables in culture conditions involved in bulb formation in tulip bulb scale explants.

Materials and Methods

Tulip bulbs (*Tulipa gesneriana* cv. Apeldoorn) which had been harvested at full maturation were used as the source for the tissue culture. The experimental design for the cultures was essentially the same as described previously (8). Adventitious buds regenerated from cultured bulb scale segments were developed under subculture on a medium supplemented with 0.3 mg/l NAA and 0.03 mg/l kinetin. The resulting tissue fragments together with many growing buds, were further cultured on a modified Murashige-Skoog medium (8) adjusted to pH 5.1. Each culture included 20 tissue fragments, experiments were repeated at least twice with similar results.

Results

- 1) Effects of chilling treatment on bulb formation with respect to time and at varying pH.

Preliminary observations indicated that adventitious buds on cultured scale fragments seldom result in bulb formation unless the buds are chilled, even when exogenous growth substances are present at any concentration. As shown in Table 1, it was found that

Received for publication January 5, 1980.

Fig. 1. Adventitious buds on the cultured scale segments.

the amount of bulb formation varies with the duration of exposure to cold at 5°C: the longer chilling treatment, the more bulbs formed, up to 80 days of treatment. Without this treatment, bulb initials attached to the proximal part of the buds soon started to elongate as shoot-like bodies instead of bulb. Thus, the chilling treatment appears to be requisite for bulb formation *in vitro*;

the results indicate that at least 80 days of treatment is required for optimal induction. Furthermore, there appears to be a close association between the effectiveness of the chilling treatment and the pH value of the medium, as is indicated in Table 2. The effectiveness of the treatment at pH 6.5 is apparently less than that at pH 4.8, especially when the duration of the chilling period is 40 days.

2) Effects of various other factors on bulb formation.

In the experiments with hormones, auxin and cytokinin were tested for their ability to induce bulb formation both singly and in combination. With NAA alone, the rate of bulb formation rose gradually with increasing concentration, up to a limit of 3.0 mg/l. There was no visible enhancement of formation in the presence of kinetin alone at any concentration, although a considerable increase in the weight of the individual bulblets was noticed; Bulb formation was drastically inhibited at higher concentration (Table 3). With NAA held constant at 0.3 mg/l, bulb formation was stimulated

Table 1. Effect of chilling on the bulb formation of cultured buds (21 week-old cultures).

Period of chilling at 5°C (days)	Number of bulblets	Weight of bulblet (mg)	Total weight of bulblets (g)
0	0	0	0
40	11	196	2.16
60	13	211	2.75
80	35	200	7.0
100	26	193	5.02

Explants of tissue fragments with buds originated from excised bulb scale of tulip were cultured on the medium with 0.3 mg/l NAA and 0.03 mg/l kinetin. Culture started at Jan. 30, 1978. After the chilling, each was cultured in laboratory under prevailing natural light.

Table 2. Effect of chilling on the bulb formation at different pH values (17 week-old cultures).

Period of chilling at 5°C (days)	pH	Number of bulblets	Weight of bulblet (mg)	Total weight of bulblets (g)
40	4.8	12	242	2.90
	6.5	2	60	0.12
80	4.8	49	281	13.77
	6.5	24	125	3.0

Chilling treatment started on Mar. 3, 1979. After the chilling, those were cultured under continuous illumination of fluorescent light ca. 1000 lx at 25°C.

Table 3. Effect of NAA and kinetin on the bulb formation of the cultured bud (9 week-old cultures).

Growth regulator		Number of bulblets	Weight of bulblet (mg)	Total weight of bulblets (g)
NAA (mg/l)	Kinetin (mg/l)			
0	0	14	114	1.60
0.3	0	40	160	6.40
3.0	0	31	330	10.23
12.0	0	6	252	1.51
0	2.0	10	166	1.66
0	6.0	13	132	1.72
0	12.0	0	0	0

Cultures were treated with 5°C for 90 days since Jan. 20, 1979. Culture started on Apr. 20, under continuous illumination at 25°C.

Table 4. Effect of kinetin in combination with NAA on the bulb formation of cultured buds (9 week-old cultures).

Growth regulators		Number of bulblets	Weight of bulblet (mg)	Total weight of bulblets (g)
Kinetin (mg/l)	NAA(mg/l)			
0	0.3	40	160	6.40
0.003	0.3	60	148	8.88
0.03	0.3	63	153	9.64
0.3	0.3	38	242	9.20
3.0	0.3	23	229	5.27
6.0	0.3	16	205	3.28

Explants were treated with 5°C from Jan. 30 for 90 days. After the chilling, culture started on Apr. 30, 1979 under continuous illumination with 25°C.

Table 5. Effect of sucrose concentrations on the bulb formation of cultured buds (17 week-old cultures).

Sucrose concentrations (%)	Number of bulblets	Weight of bulblet (mg)	Total weight of bulblets (g)
0	0	0	0
0.5	1	55	0.05
1.0	6	68	0.41
2.0	19	66	1.25
4.0	30	103	3.09
6.0	32	142	4.54
10.0	24	91	2.18

Explant were cultured on the medium with 0.3 mg/l NAA plus 0.03 mg/l kinetin. 5°C treatment started on Mar. 3, 1979 for 80 days. After the chilling, those were cultured under continuous illumination with 20°C.

Table 6. Effect of culture temperatures in combination with sucrose concentrations on the bulb formation of cultured buds (9 week-old cultures).

Culture temperatures	Sucrose concentrations (%)	Number of bulblets	Weight of bulblet (mg)	Total weight of bulblets (g)
15°C	2	8	60	0.48
	6	19	131	2.49
20	2	9	58	0.52
	6	17	153	2.60
25	2	10	123	1.23
	6	27	130	3.51

Treatment at 5°C started from Feb. 5, 1979 for 90 days. After the chilling, those were cultured at 15, 20 and 25°C in the dark, separately.

Fig. 2. Formed bulblets on the buds cultured *in vitro*.

by kinetin at both 0.003 and 0.03 mg/l, and the development of the bulblets was enhanced from 0.3 to 6.0 mg/l (Table 4). Thus, it appears that it is possible to obtain both a high frequency of bulb formation and a vigorous further development using suitable combinations of kinetin and NAA.

In the experiment pertaining to the requirement for sucrose in establishing a culture, tulip buds cultured *in vitro* appeared to require the presence of sucrose if bulb formation was to proceed. Sucrose was supplied in serial concentrations, and marked enhancement of bulb formation and development was found at the level of 4~6% sucrose (Table 5). Experiments to see the effect of ambient temperature on bulb formation and development were performed at 15°, 20° and 25°C, respectively, at two different concentrations of sucrose, 2% and 6%. (Table 6).

Comparing the total weight of the bulblets obtained, a 6% sucrose medium was found

to be superior to a 2% medium, regardless of the culture temperature. A temperature of 25°C was found to be most effective in terms of total yield at both concentrations. However, in comparing effects on bulb formation and development separately, the interaction between temperature and sucrose concentration was found to be somewhat more complex: Although culture temperature exerted no significant effect on bulb formation in a 2% medium, a temperature of 25°C was found to be especially effective in promoting development. Contrarily, using 6% medium, a noticeable increase in the bulb formation rate was found at 25%.

Discussion

It is well established that scale fragments of tulip bulbs are able to spontaneously generate adventitious buds when cultured on the M. S. medium supplemented with a favorable combination of auxin and cytokinin (8). The present investigation offered evidence that chilling treatment of the cultured tissue fragments at 5°C induces neo-formation of bulblets on the proximal part of the newly generated bud. Although the chilling was requisite for bulb induction, an aberrant organogenesis leading to the formation of immature bulblets without stored starch grains occurred occasionally, even though the cultures had been adequately chilled. Thus, it may be said with certainty that chilling is essential for induction of bulb formation; however, it may not always satisfy the requirements for further development.

Concerning the effect of pH on bulb formation, the results obtained here appear to be similar as those reported for immature lily embryos (4). The stimulating influence of low pH on bulb formation may indicate that low pH values promote the absorption of NAA as has been hypothesised in the case of the pea (9). Several studies have reported growth factors play a significant physiological role in bulblet or plantlet formation in hyacinth, amaryllis, and lily (5, 10, 11, 13, 14). The most plausible explanation for the regeneration of the tulip bulblet

is that both auxin and cytokinin are indispensable for bulb regeneration, as is the case with hyacinth (11).

Acknowledgement

The author thanks Professor Hiroshi Myodo and Professor Yozo Okazawa of Hokkaido University for their advice and encouragement during the course of this study and, also for criticizing the manuscript.

Literature Cited

1. AOBA, T. 1972. Effect of temperature on bulb- and tuber-formation in bulbous and tuberous crops. I. On formation of pupa (nikaitama) in freesia. J. Japan. Soc. Hort. Sci. 41(3): 290—296 (Japanese with English summary).
2. AOBA, T. 1974. Effect of temperature on bulb- and tuber-formation in bulbous and tuberous crops. IV. On the pupa formation in freesia corms at various states of dormancy. J. Japan. Soc. Hort. Sci. 43(1): 69—76. (Japanese with English summary)
3. AOBA, T. 1974. Effect of temperature on bulb- and tuber-formation in bulbous and tuberous crops. VI. On the bulb formation in bulbous iris. J. Japan. Soc. Hort. Sci. 43(3): 273—280. (Japanese with English summary)
4. ASANO, Y. and H. MYODO. 1977. Studies on crosses between distantly related species of lilies. II. The culture of immature hybrid embryos. J. Japan. Soc. Hort. Sci. 46(2): 267—273. (Japanese with English summary)
5. MII, M., T. MORI and N. IWASE. 1974. Organ formation from the excised bulb scales of *Hippeastrum hybridum* *in vitro* (Research note). J. hor. Sci. 49: 241—244.
6. NARD, M. LE and COHAT, J. 1968. Influence des températures de conservation sur l'élongation, la floraison et la bulbification de la tulipe (*Tulipa gesneriana* L.). Annls Amel. Pl. 18: 181—215.
7. NARD, M. LE. 1973. Effect of bulb storage temperature on the differentiation and elongation of the aerial organs and bulbing in the Dutch bulbous iris cv. 'Wedgwood'. Annls Amel. Pl. 23(3): 265—278.
8. NISHIUCHI, Y. 1979. Studies on vegetative propagation of tulip. II. Formation and development of adventitious buds in the excised bulb scale cultured *in vitro*. J. Japan. Soc. Hort. Sci. 48(1): 99—105.
9. NORRIS, R. F. and M. J. BUKOVAC. 1972. Effect of pH on penetration of naphthaleneacetic acid and naphthaleneacetamide through isolated pea leaf cuticle. Plant Physiol. 49: 615—618.
10. PIERIK, R. L. M. and J. WOETS. 1971. Regeneration of isolated bulb scale segments of hyacinth. Acta Hort. 23—II: 423—428.
11. PIERIK, R. L. M. and H. H. M. STEEGMANS. 1975. Effect of auxin, cytokinins, gibberellins, abscisic acid and ethephon on regeneration and growth of bulblets on excised bulb scale segments of hyacinth. Physiol. Plant. 34: 14—17.
12. STIMART, D. P. and P. D. ASCHER. 1978. Tissue culture of bulb scale sections for asexual propagation of *Lilium longiflorum* Thumb. J. Amer. Soc. Hort. Sci. 103(2): 182—184.
13. TAKAYAMA, S. and M. MISAWA. 1979. Differentiation in *Lilium* bulb scales grown *in vitro*. Effect of various cultural conditions. Physiol. Plant. 46: 184—190.
14. TAMURA, S. and H. KABE. 1971. Tissue culture of hyacinth (II) Agr. and Hor. 46: 784—788. (Japanese)
15. YANAGAWA, T. and Y. SAKANISHI. 1977. Regeneration of bulblets on *Hippeastrum* bulb segments excised from various parts of a parent bulb. J. Japan. Soc. Hort. Sci. 46(2): 250—260. (Japanese with English summary)

チューリップの栄養繁殖に関する研究（第4報）

培養リン片における球根形成について

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摘 要

アペルドーンのリソ片をオーキシソとサイトカイニンを加えたM・S修正培地上で培養すると不定芽が形成される。この不定芽を低濃度のオーキシソとサイトカイニンを含む培地に移植したところ、顕著な生育がみられた。そこで伸長生長した不定芽を5℃の低温下で処理した後、室温に移し培養を続けたところ、その基部に小さな球根が形成された。この5℃処理の最適な期間は80～

100日間であり、低温を与えない場合には、球根は形成されなかった。更に、球根形成は低温条件に加えて、低濃度のオーキシソとサイトカイニンを同時に与えることにより促進されたが、高濃度の場合には抑制された。また球根形成にはしょ糖が必要であり、4～6%を加えた場合に最も良好であった。培養温度は25℃が適していた。