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In Vitro Micropropagation of *Allium giganteum* R.

2. Embryoid and Plantlet Regeneration through the Anther Culture of *Allium giganteum* R.

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Abstract

Anthers in small flower buds of 2 to 5 mm in length were used for embryoid and plantlet regeneration by anther culture of *Allium giganteum*, when the bract enveloping a umbel of 4 to 5 cm in diameter, which consisted of a number of small flower buds, was tearing.

1. Better embryoid regeneration (20 to 23 %) from anther cultures was observed at the developmental stage of anther ranging from pollen mother cell to pollen tetrad on the medium containing 0.1 to 1 mg/l BA (6-benzylaminopurine) and 10mg/l NAA (α -naphthalene acetic acid). Few embryoids were regenerated from anthers at uninuclear developmental stage. Embryoids obtained under the dark condition were developed into plantlets when transferred under the light condition.

2. The cold temperature pre-treatment at 5°C for 5 to 10 days resulted in enhanced embryoid regeneration, and no embryoid regeneration in the case of one day pre-treatment.

3. Embryoids of smaller than 5 mm in length developed into normal plantlets when transferred onto the medium with 0.1 mg/l IBA, while embryoids of larger than 5 mm abnormally developed into enlarged shoot and plantlets which were of glassy and succulent.

4. Most plantlets obtained through the anther culture were diploid. It has not been detected whether the plantlets originated in generative or vegetative cell tissues.

Introduction

Giganteum (*Allium giganteum* R.) is believed to be native to Central Asia area, belonging to genus *Allium* within family Liliaceae. It has a umbel of 10 to 25 cm in diameter, including a lot of reddish violet small flowers at the top of a 1 - 1.5m-long flower stalk.

The number of flowers within a umbel of 25cm in diameter can reach over 5000.

Giganteum produces bulbs under the ground which can be grown as large as 8 cm in diameter, and be usually propagated only at the rate of 2 folds. Giganteum has recently become to be popular as a cut flower for interior decoration and flower arrangement, so the improvement in the rate of the propagation has been desired for enhancement of the consumption through the large scale production. The primary propagation has been carried out through the regeneration of lateral bulbs from a mother bulb during the growing season^{6, 7)}. Other propagation method, e.g. propagation by seedling, can be considered,

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but it is not practical because of only a small amount of seeds obtained in the normal cultivation.

In the previous report^{2, 3, 4)}, we attempted in vitro vegetative propagation (micropropagation) using common leaves and near-apical meristem within the bulbs and emerged young leaves of giganteum, and were able to obtain calluses, shoots, and plantlets from them.

In the present paper, we tried to apply anther culture to micropropagation. Anther culture has been generally carried out for the production of haploid plants from microspores (N), which have been utilized for breeding superior homogeneous plant species^{1, 5, 8, 11, 12)}. However, somaclonal plantlets derived from somatic cell tissues (connective tissue and pollen sac tissue=2N) can be frequently regenerated, which can be used for micropropagation^{9, 10)}.

Therefore we investigated the possibilities of the production of haploid plants from microspores, and of somaclonal plants from somatic cell tissues in order to apply to both of the breeding and micropropagation of giganteum.

Materials and Methods

Experiment 1. Effects of the size of flower buds and plant growth regulators on callus and embryoid formation from anthers

Umbels of *Allium giganteum* R. cultivated in the field of Kobe University, Which had a lot of small flowers (more than 2000 flowers), were provided for anther culture.

Umbels were harvested at the size of 4–5 cm in diameter when their bracts were tearing, and were stored for 2 days under the dark condition at 5°C. The umbels consisted of several sizes of flowers, and the following 3 sizes of flowers were provided for anther culture; shorter than 2mm(S), 2mm(M), and longer than 2mm(L) in diameter. Anthers in "S" were semi-transparent, white colored, and anthers in "M" and "L" were

semitransparent, yellowish green colored, and green colored, respectively.

The developmental stages of microspores within the anthers in each of "S", "M" and "L" were nearly identical, and corresponded to the pollen mother cell–pollen tetrad(S), pollen tetrad–pollen uninuclear(M), and pollen uninuclear(L) stages, respectively.

For the anther culture, the surface sterilization of flower buds was carried out throughout sinking them in 70% ethanol for 60 seconds followed by stirring them for 15 min. in 1% sodium hypochlorite solution with a few droplets of tween 20. The surface-sterilized flower buds were rinsed three times with sterilized redistilled water. The basal medium (BM) in these experiments consisted of half strength of MURASHIGE and SKOOG (MS) macro and micro nutrients, MS vitamin, 500 mg/l casamino acid, 20g/l sucrose, and 2.5g/l gellan gum as a gelling agent.

Four media were provided by combining 0.1 and 1mg/l BA with 1 and 10mg/l NAA in the basal medium. The media were autoclaved at 120°C, 1kgf/cm² for 20 min., and then diagonally left in the room for preparation of sloped media. Test tubes (20 mm in diameter × 120mm in length) with five to 6 anthers incubated on the sloped medium, which were capped with double layer-aluminium foil, were kept under the dark condition at 25°C.

Experiment 2. Effect of amino acids as nitrogen sources on callus and embryoid induction

The materials used, which were about 2 mm long flower buds between "S" and "M", were similar to those in experiment 1 except for storing for one day at 5°C before incubation.

The 8 media were prepared, consisting of BM medium without NH₄NO₃ supplemented with one of the following amino acids; L-glutamine, L-asparagine acid, L-asparagine, L-proline,

L-arginine, DL-serine, DL-methionine, and glycine as nitrogen sources. The concentration of nitrogen in the 8 media was adjusted to that in half strength of MS medium.

The kind of culture vessels and the culture condition were identical to those of experiment 1.

Experiment 3. Effect of cold temperature pre-treatment on callus and embryoid formation.

The materials used were same as that in experiment 2 except for the period of cold temperature pre-treatment. The cold pre-treatment at 5°C was applied to flower buds for 1, 5, and 10 days. The medium used was BM medium supplemented with 0.1 mg/l BA and 10 mg/l NAA. The cultures were kept at 25°C under the dark condition.

Experiment 4. Effects of the size of embryoids induced from anther cultures on the development after transferring them.

Embryoids induced by anther culture were divided into three sizes; smaller than 5mm long

embryoids(SE) and 5–10mm long embryoids (LE) which were white colored, and around 10 mm green shoot-like embryoids(SGE).

Two media were used, which were supplemented with either 0.1mg/l of IBA(IBA) or 1mg/l of BA + 5mg/l of NAA(BN). The media were adjusted to pH 5.5–5.7, and solidified with 2.5g/l of gellan gum. Culture vessels with 48 mm in caliber and 300 ml in volume containing 100 ml of the medium were provided, in which embryoids or shoots were cultured at 20°C under 16 h photoperiod with photon flux density of $33 \mu \text{mol m}^{-2} \text{s}^{-1}$.

Results and Discussion

Experiment 1. Effects of the size of flower buds and plant growth regulators on callus and embryoid formation from anthers.

Table 1 describes the results on the effects of the sizes of flower buds and growth regulators (BA and NAA) on callus and embryoid

Table 1. Effects of developmental stages of anthers and growth regulators on callus and embryoid induction through anther culture of *Allium giganteum* (cultured for 12 weeks)

Length of flower buds	Developmental stage of anther	BA	NAA	No. of incubated anthers	No. of anthers with embryoids	No. of anthers with calluses
S(<2mm)	Pollen mother cell-tetrad	0.1	1	44	7 (15.9)	2 (4.5)
		0.1	10	44	10 (22.7)	2 (4.5)
		1	1	51	5 (9.8)	0
		1	10	49	10 (20.4)	0
M(2mm)	Tetrad-early uninuclear	0.1	1	28	3 (10.7)	2 (7.1)
		0.1	10	36	0	0
		1	1	29	0	0
		1	10	35	0	0
L(>2mm)	Early-late uninclear	0.1	1	56	0	0
		0.1	10	52	0	0
		1	1	58	0	0
		1	10	59	2 (3.4)	1 (1.7)

formation at 12 weeks after incubation. Embryoid formation was observed on the cultured anthers in "S" and "M" sizes (Fig.1-B). In "M", calluses were initiated on the surface of cultured antheres (Fig.1-A), followed by embryoid induction from the anther tissues which were surrounded by the callus.

While in "S" some embryoids were produced through the process similar to that in "M", most of the other embryoids were induced from the enlarged anther cultures. In "L", callus formation was observed 9 weeks after incubation, and embryoids induced from one callus were averaged

around 10. The rate of embryoid formation was much greater in "S" than in "M" and "L", and the rate of embryoid formation in "S" showed 17% on average.

In "S" there was no difference in embryo formation between 0.1 and 1 mg/l of BA, while significant difference was observed between 1 and 10 mg/l of NAA, with higher rate of embryoid formation in 10 mg/l of NAA.

In "M" and "L" few embryoids were obtained on the medium with 0.1mg/l BA + 1mg/l NAA, and with 1mg/l BA + 10mg/l NAA, respectively. Two processes to embryoid formation, which were through calluses, and

Table 2. Effect of cold temperature pre-treatment on embryoid induction through anther culture of *Allium giganteum* (cultured for 15 weeks)

Days for cold temperature pre-treatment	No. of incubated anthers	No. of embryoids induced(%)
1	42	0
5	60	5 (8.3)
10	59	3 (5.1)

Table 3. Shoot, and plantlet regeneration from embryoid obtained by anther culture *Allium giganteum* (cultured for 13 weeks)

Type of explants	Growth regulators	No. of explants	Shoot growth(%)	Root growth (%)	No. of plantlets(%)
Embryoid(SE)	Free	14	14 (100)	10 (71)	10 (71)
	IBA*	14	14 (100)	14 (100)	14 (100)
	BN**	10	10 (100)	2 (20)	2 (20)
Embryoid(LE)	IBM	6	6 (100)	6 (100)	6 (100)
	BM	10	10 (100)	1 (10)	1 (10)
Green shoot-like embryoid (GSE)	IBA	10	10 (100)	10 (100)	10 (100)
	BN	7	7 (100)	4 (57)	4 (57)

* : IBA contains 0.1mg/l of IBA

** : BM contains 1mg/l of BA and 5mg/l of NAA

through enlarged anther cultures with no callus, were observed, and the latter case was more frequently observed.

Transferring the embryoid obtained under the dark condition to the light condition with 16 hr photoperiod of $33 \mu\text{mol m}^{-2}\text{s}^{-1}$ at 25°C showed better development than non-transferring condition under the dark condition.

Experiment 2. Effect of amino acids as nitrogen sources on callus and embryoid induction.

Sixty anthers were incubated in each treatment, and only one embryoid was induced on the medium supplemented with glycine as nitrogen source. The embryoid developed into the normal shoot when transferred to the light condition described in experiment 1.

Experiment 3. Effect of cold temperature pre-treatment on callus and embryoid formation.

Table 3 represents the number of embryoids induced in 15 weeks of incubation after cold temperature pre-treatment at 5°C for 1, 5, and 10 days. Embryoid formation was observed at the rates of 0, 8.3, and 5.1% for 1, 5, and 10 days cold temperature pre-treatment, respectively. Embryoids obtained were activated to grow into shoots when transferred to the light condition of 16h photoeriod with $33 \mu\text{mol m}^{-2}\text{s}^{-1}$ photon flux density at 25°C .

Experiment 4. Effects of the size of explants(embryoids induced from anther cultures)on the development of the embryoids after transferring.

Table 3 represents the results of experiment 4 after 13 weeks of incubation. Root formation in the shoots growing from embryoid(SE)was observed at the rates of 100, 20, and 71% on the medium with 0.1mg/ℓ IBA, 1mg/ℓ IBA +5mg/ℓ NAA, and no growth regulator respectively. Less root formation on the medium with 1mg/1 BA + 5mg/1 NAA might result from suppressinon of the

growth of root primordia in the embryoids. Embryoids(SE) transferred to the medium with 0.1mg/1 IBA developed upright into shoots (Fig.2-A), and from which rooting was observed 7 weeks after transferring. The embryoids (LE) transferred to the medium with 0.1mg/1 IBA developed abnormally into the enlarged shoots (Fig.2-B), and from which little rooting was observed. Green shoot-like embryoids(GSE) transferred to the medium with 0.1mg/1 IBA developed into abnormally enlarged plantlets with roots.

Embryoids(SE) transferred to the medium with 1mg/1 IBA + 5mg/1 NAA developed into shoots which elongated like creeping on the medium and enlarged a little. Secondary shoot induction was frequently observed on the shoots from embryoids. Embryoids(LE) transferred to the medium with 1mg/1 BA + 5 mg/1 NAA developed into the shoots through the same process to that of embryoids(SE). There was no normal development in embryoids(GSE) which were transferred to the medium with 1mg/1 BA + 5mg/1 NAA, and vitrification, which was the phenomenon that leaves or plantlets looked glassy and succulent, was observed in embryoids (LE) and (GSE), but not in embryoids (SE).

The present studies revealed the effective procedure for production of embryoids or plantlets through the anther culture of giganteum was as follows; firstly to store the umbel for 2 to 5 days at 5°C of which the pract began to tear, and then to be followed by cultureing anthers at the developmental stage of younger than pollen tetrad on BM medium supplemented with 1mg/1 of BA + 1 0mg/1 of NAA. The suitable range of the developmental stage of anther for anther culture was estimated to be narrower in giganteum than in other plant species.

The embryoids obtained by the above procedure could be developed successfully into plantles when transferred on the

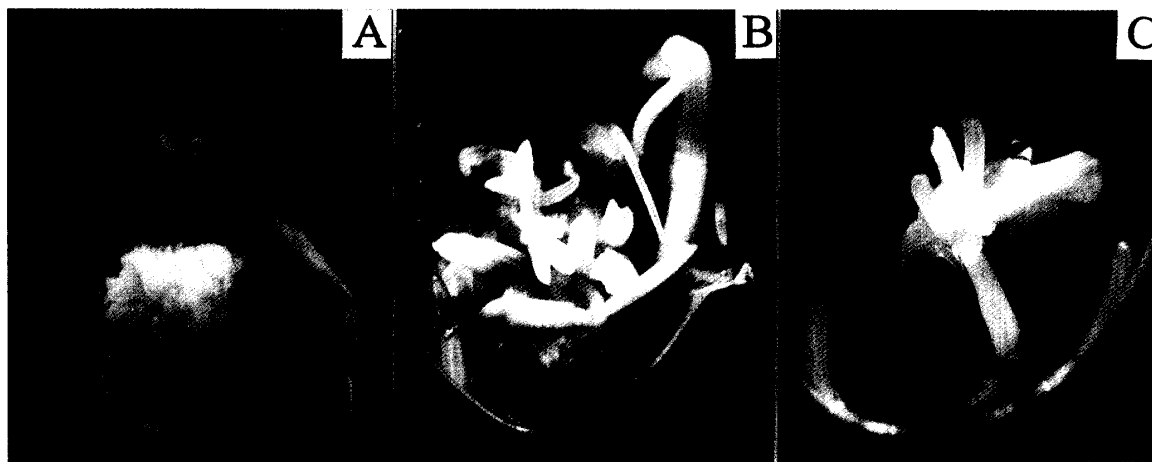


Fig. 1. Callus formation and embryoid regeneration from anther cultures in *Allium giganteum* R.

A: Callus induced from anthers at the developmental stage of pollen tetrad.

B: Embryoid regenerated directly from anther cultures.

C: Secondary embryoid regeneration from direct-embryoid from anther cultures.

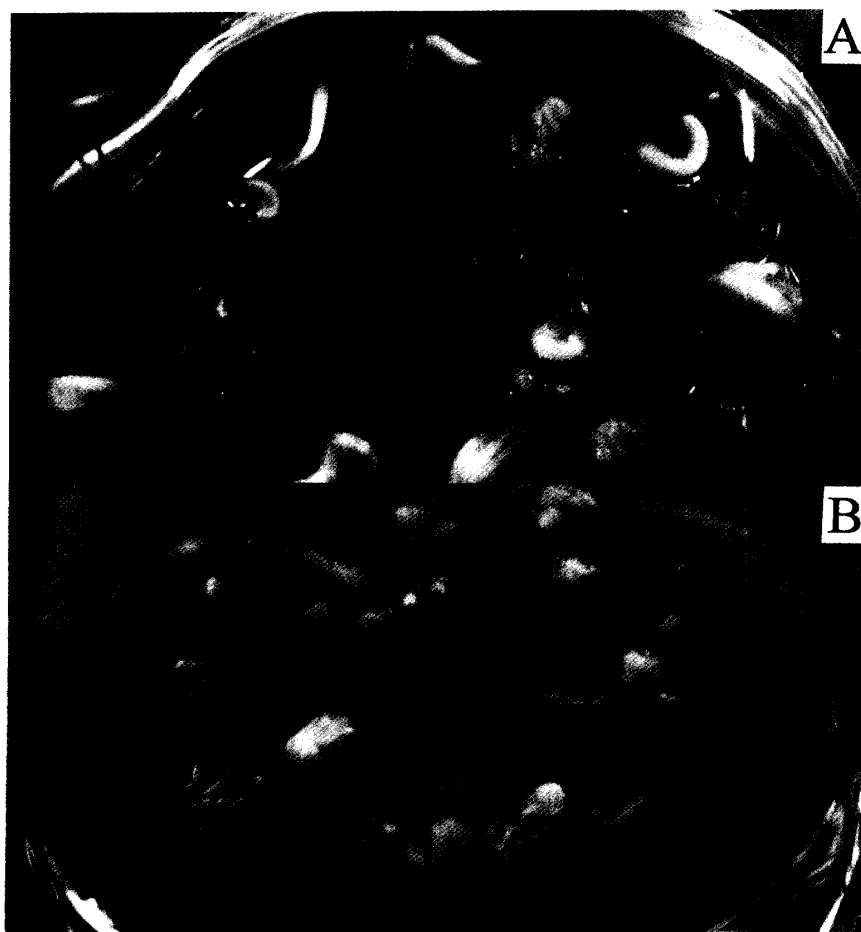


Fig. 2. Development of embryoids after transferring to the medium supplemented with 0.1mg/l of IBA(A), and with 1mg/l of BA and 5mg/l of NAA(B).

medium with 0.1mg/1 of IBA.

The plantlets obtained through anther culture were diploid and partly tetraploid, and no haploid plant could be observed. Hence we would reveal whether the calluses, embryoids, and plantlets obtained by anther culture were derived from generative or vegetative cell tissues.

References

- 1) BAJAI, Y. P. S. : In *Handbook of Plant Cell Culture* 3, pp228-287, McGraw-Hill Publishing Company, New York, 1983.
- 2) HU H. and J. Z. ZENG : In *Handbook of Plant Cell Culture* 3, by ZHENGHUA C., D. A. EVANS, W. R. SHARP, P. V. AMMIRATO and M. H. SONDAHI(Ed.), pp65-90, McGraw-Hill Publishing Company, New York, 1984.
- 3) INAGAKI, N., H. MATSUNAGA, T. KAWANO, S. MAEKAWA and M. TERABUN: *Sci. Rep. Fac. Agr. Kobe Univ.*, 20, 47-53. 1992.
- 4) INAGAKI, N., H. MATSUNAGA, S. MAEKAWA and M. TERABUN : *Jour. Japan. Soc. Hort. Sci.*, 58(SUPPL.2), 520-521. 1989.
- 5) INAGAKI, N., T. KAWANO, S. MAEKAWA and M. TERABUN : *Abstr. Japan. Soc. Hort. Sci. Autumn Meet.*, 518-519. 1988.
- 6) INAGAKI, N. : *Sci. Rept. Fac. Agr. Kobe Univ.* 20, 37-45. 1992.
- 7) KAWABATA, T. : *Garden Life*, 8, 23-25, 1988.
- 8) KOYAMA, Y : *Garden Life*, 8, 25, 1988.
- 9) NISHI, S., K. OSAWA and T. TOYOTA : *Bull. Natl. Res. Inst. Veg., Ornam. Plants. Japan*, A-1, 1-40. 1974.
- 10) OSAWA, K., M. TODA and S. NISHI: *Bull. Natl. Res. Inst. Veg., Ornam. Plants. Japan*, Ser. A-1, 41-57. 1974.
- 11) SANGWAN, R. S. and B. S. SANGWAN-NORREEL : In *Plant Tissue Culture : Application and Limitations*, S. S. BHOJIWANI(Ed.) Elsevier, Amsterdam, 1990.
- 12) ZIV, M.: In *Micropropagation*, DEBERGH and H. ZIMMERMAN (Ed.), pp45-69, Kluwer Academic Publishers, Dordrecht, 1991.

アリウムギガンチウムの大量増殖に関する研究

第2報 薬培養による胚様体誘導と幼植物の再生

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要 約

直径4～5 cmの花球（散形花序）の苞が裂け出した頃の小花蕾を薬培養の材料に用い、薬からの不定胚および幼植物の再生について試みた。

1. 胚様体形成は、小孢子母細胞期から小孢子4分子期にわたる時期の薬で最も良好で、BA 0.1～1 mg/lおよびNAA 10mg/l添加区で20～23%の形成率を示した。小孢子1核期の薬においては、胚様体形成は殆ど認められなかった。胚様体は、明所条件下に移すことにより幼植物に成育した。
2. 培地中の窒素源として、各種のアミノ酸を添加しその効果を検討したが、グリシン添加区で1個体の胚様体を得られたのみであった。
3. 薬置床前に、花蕾の5℃における低温前処理を試みた。低温処理が1日では、胚様体形成は認められなかったが、5および10日では8.3および5.1%と低率ながら低温前処理の効果が認められた。
4. 得られた胚様体の中で、5 mm以下の胚様体を0.1mg/l IBA添加培地へ移植した場合には正常な幼植物に成育したが、5 mm以上では生長したシュートが肥大し、水浸状化する傾向を示した。
5. 得られた幼植物は殆ど2倍体で、一部4倍体が認められた。これらの幼植物の起源は現在のところ不明である。