Plant Regeneration and Bulblet Formation of *Allium wakegi* Araki

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ABSTRACT

***Allium wakegi* was cultured shoot tip in the condition of light culture. The *Allium wakegi* added plant growth regulator was observed of plant regeneration and bulblet formation. Callus Induction and growing rate was the best of 78% when added alone 2,4-D 0.5mg/L. In the formation of shoot, its regeneration rate was 96% when added BA 0.5mg/L in the light culture condition. When BA 0.5mg/L and NAA 0.5mg/L mixed and BA 0.5 mg/L and NAA 1.0mg/L mixed, the rates were 99% and 97% respectively, and these conditions were suitable for forming shoot. In the formation of roots, when added NAA 2.0mg/L in the light culture condition, the regeneration rate was 90.6% and the roots were abnormal. When added NAA 1.0mg/L, the rate was 82% and the highest. In the formation of bulbs, when BA 0.5mg/L and NAA 1.0mg/L mixed, the root generantion and its size in the bulbs was the best compare to other treatment experiments.**

Key words **:** *Allium wakegi*, plant regeneration, bulblet formation, plant growth regulator

INTRODUCTION

As *Allium wakegi*, which belongs to Liliaceae, is a perennial plant, it is a seasoning vegetable that generally cultures with vegetable propagation through its bulb(Yoo, 2000). It is made by crossing *Allium fistulosum* with *Allium ascalonicum*. Its leaves and bulb has nicotinic acid, gluatamic acid, fatty oil, and phlegmatic temperament as well as vitamin B, B2, and C of good quality as a leek does(Ryu and Song, 2004). The main ingredients of its phlegmatic temperament are cellulose, hemicellulose, and pectin.

In traditional oriental medicine, it has been used of removing extravasated blood, detoxicating, and exterminating insects. It is also effective on wounds, chills by wind and headaches as well as nasal congestion, fever, and not sweating symptom. It has used of food in tradition and gets contaminated with virus easily because it cultures with vegetable propagation through its bulb. The result of the contamination reduces the product and needs to buy new bulblet annually to increase its productivity. This has farmer's expenses and efforts, so it is seriously required to multiplicate artificially through making tissue culture. Bulb production of it through tissue culture establishes year-round production system of its multiplication and can reduce the production expenses (Song, 2004). This can increase the period of use *Allium*

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wakegi. This can also enlarge its usage up to medical use through extracting physiological activation materials from the callus.

Until now, the study of it has not mean activated compare to other plants which belong to *Allium*, Since the study about its chromosome was reported, it has been reported about its krayologic variation through callus culture, the isozyme of its genetic variants through tissue culture, and the analysis of changing the protein types(Ryu, 1988 ; Seo and Kim, 1975 ; Park, 1989). On the foundation of these studies, the possibility of growing *Allium wakegi* through tissue culture is presented(Paek *et al.*, 1990). Also, since selective examination of superior quality of *Allium wakegi*, the results of many tests have been reported such as its seedtime test, the system test of year-round product, the study of its bulb enlargement, and the physiological study of breaking its resting(Yoo, 2000). The studies of its cultivating methods are cutting bulblet and the influences of plant growth regulator on its growing.

Recently, many studies about *Allium wakegi* have been reported. For examples, the study of Korean *Allium wakegi's* genealogic classification, the study of Korean traditional *Allium wakegi's* growth characteristics and selection of superior quality of it, and the influences of the temperature treatment of Korean *Allium wakegi's* bulblet on dormancy breaking(Ashalatha and Seo, 1992; Cho *et al.*, 1992, 1993; Kim *et al.*, 1996; Kim and Soh, 1993; Lee, 2001; Yoon *et al.*, 1999).

This report is about basic experiments on being able of establishing mass plant production system of *Allium wakegi* used widely of a seasoning vegetable through its tissue culture.

METERIALS AND METHODS

Material and Sterilization

Allium wakegi from Bo-sung is announced publically as public material and the shoot tip of it was used for culture material. For callus induction and plant regeneration after its bulb was cleaned the surface of the bulb was disinfected once for 15 minutes with 20% sodium hypochlorite. Then, it was disinfected again for 15 minutes with 20% sodium hypochlorite and one or two drops of Tween 20 liquid. After that, it was cleaned thoroughly with distilled water until not coming out its bobbles. Then it was cleaned again in sterilizing machine for 3 or 4 minutes with sterilized water disinfected in a high pressure disinfector at 120℃ for 30 minutes. After that, it was soaked in 95% alcohol for 3 or 4 seconds.

Plant growth regulators and the condition of culture

Shoot tip culture added 3% sucrose through using MS medium(Murashinge and Skoog, 1962). For investigate to effects of callus induction and plant regeneration, shoot tips were excised and inoculated with the adaxial side down on MS basal medium supplemented with different plant growth regulators(2,4-D, NAA, BA) at the concentration of 0.5, 1.0 and 2.0mg/L, BA 0.5mg/L and 2,4-D (0.5, 1.0mg/L) combination treatment, BA 0.5mg/L and NAA(0.5, 1.0mg/L) combination treatment. Every treatment was from pH5.7 to 5.8 in high pressure disinfection and was added to 8g/L of Agar. 70mL of MS medium put into 200mL triangle flask and this was kept inside high pressure distilling disinfector at 119℃ for 10minutes. Then, the shoot tip of *Allium wakegi* in the cleanbench was cut. And, in the disinfection machine, the shoot tip of the *Allium wakegi* was put into 200mL triangle flask included 70mL of MS medium. Inside the flask, 10 tissues of the shoot tip of the *Allium wakegi* were cut with 0.3±1cm size and inoculated. It was repeated 10 times and investigated every 5 day. For observation of the influences the culture condition of callus induction

and plant regeneration, 24 hour dark-culture and 16 hour light-culture under 2,000Lux fluorescent lights were proceeded. The temperature of culture room was 24±1℃. It was investigated of callus induction and regeneration ratio of its tissues in every 5 day. After inoculation, subculture was proceeded in every 30 day.

RESULTS AND DISCUSSION

The effect of 2,4-D alone treatment

To investigate callus induction and regenerated ratio of shoot and root, 0.5mg/L, 1.0mg/L, and 2.0mg/L of 2,4-D liquid were experimented respectively(Fig. 1, 2).

The callus didn't occur well at the beginning of its development.

On the other hand, when 0.5mg/L, 1.0mg/L, and 2.0mg/L of the 2,4-D liquid were put respectively, the callus occurred at the 25th day, the 25th day, and the 15th day respectively but didn't develop well. By the way, in the case of the control groups, the callus didn't occur until the 60th day of the culture. At the 60th day, the induction ratio of 0.5mg/L, 1.0mg/L, 2.0mg/L of 2,4-D liquid showed 77.4%, 77.7%, and 65.7% respectively. The induction of callus started with the development of the shoot tip of it and inducted gradually. The induction of *Allium wakegi* is seemed to be led by 2,4-D as other vegetables belonging to *Allium* do. About the regeneration ratio of shoot when 0.5mg/L, 1.0mg/L, and 2,0mg/L of 2,4-D liquid adding, in the case of the control group, the specialized ratio was rapid at the beginning of the culture. On the other hand, in the case of the treatment group, the development of shoot was restricted. In the case of the control group, the ratio was 85% at 15th day of the culture, however, in the case of the treatment group, the ratio was 15.4%, 6.1%, and 3.3% in 0.5mg/L, 1.0mg/L, and 2.0mg/L of 2,4-D liquid respectively. The shoot of the control group occurred after the 10th day of the culture, and the regeneration ratio of shoot was up to 56.3%, 28.3%, and 14.2% respectively at the 60th day of the culture. Therefore, it is showed that high concentration of 2,4-D was not effective on occurrence shoot of *Allium wakegi*. Also, the regeneration of roots didn't happen by additing 2,4-D. Another study on organ regeneration of leek showed the similar results that 2,4-D addition was not suitable to regenerate vegetables(Yoon *et al.*, 1999).

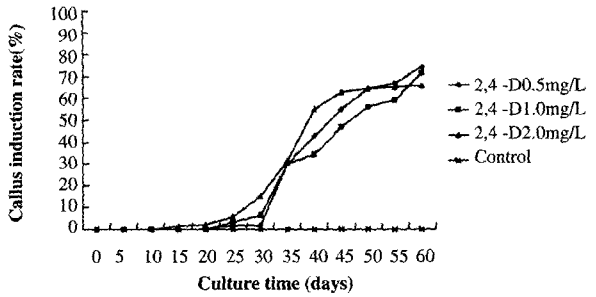

Fig. 1. Effect of 2,4-D concentration on callus induction of *Allium wakegi* Araki.

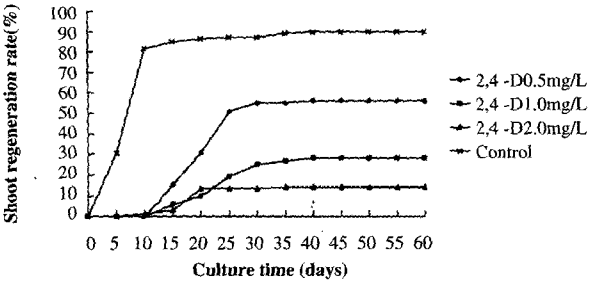

Fig. 2. Effect of 2,4-D concentration on shoot regeneration of *Allium wakegi* Araki.

The effect of NAA alone treatment

This experiment was proceeded to know the regenerated ratio of roots by the concentration changes of 0.5mg/L, 1.0mg/L, and 2.0mg/L of NAA in the light culture condition(Fig. 3, 4). It also was for knowing callus induction, shoot regeneration, and bulb formation. The Roots began to be formed at the 10th day of the culture at the beginning of the growth, and showed rapid regenerated ratio. At the 10th day of the culture, the regenerated ratio of the control group,

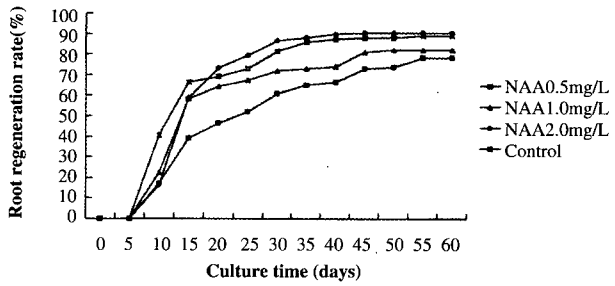

Fig. 3. Effect of NAA concentration on root regeneration of *Allium wakegi* Araki.

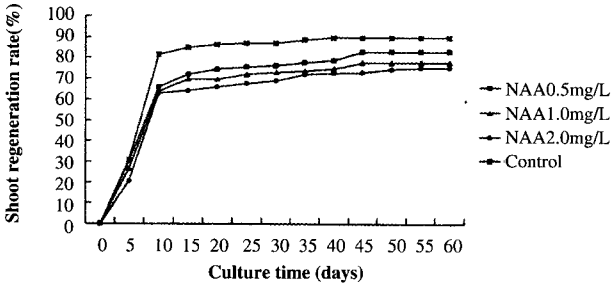

Fig. 4. Effect of NAA concentration on shoot regeneration of *Allium wakegi* Araki.

0.5mg/L, 1.0mg/L, and 2.0mg/L of NAA showed 17.3%, 22.3%, and 16.6% respectively. In the case of the control group, the ratio was the highest of 40.7%, and in the case of 1.0mg/L, and 2.0mg/L of NAA, it showed rapid regenerated ratio at the from 10th to 15th day of the culture. Regenerated ratio of root was good in all treatment groups. At the 60th day of the culture, 20mg/L of NAA was treated and the ratio was the highest of 90.6%, and the similar result showed in the control group as the ratio was 89%. Adding NAA was beneficial to regenerate root, but when NAA 2.0mg/L adding, abnormal root was regenerated. Adding NAA 1.0mg/L was beneficial to form bulbs but through doing this, callus didn't induction and also it was not effective on regenerating callus. On the other hand, in the callus culture of *Allium sativum* L., NAA addition was helpful to induct callus(Choi *et al.*, 1993), in the micropropagation of *Rhodiola sachalinensis*, the NAA addition was helpful for callus to induct entirely, 100%; however, in the case of *Allium wakegi's* callus induction, NAA addition was not effective on its regeneration(Song, 2004). Therefore, it was showed that according to plant growth regulators effected differently. The regenerated ratio of shoot was rapid at the beginning of the growth when treated NAA 0.5mg/L, 1.0mg/L, and 2.0mg/L respectively. At the 10th day of the culture, the regenerated ratio of NAA 0.5mg/L, 1.0mg/L, and 2.0mg/L was 66%, 63.8%, and 62.9% respectively. On the other hand, in the case of the control group, the ratio was 81.5% at the 10th day of the culture. The regeneration ratio of every treatment group was similar. The NAA 2.0mg/L treatment group showed the lowest ratio of 75.4% at the 60th day of the culture, and the control group showed the highest ratio of 90% at the 60th day of the culture. In the case of NAA treatment, shoot regeneration occurred and compared to the control group, the ratio was low.

The effect of BA alone treatment

This experiment was proceeded to know the influences of the regenerated ratio of callus induction and shoot, root, and bulb when 0.5mg/L, 1.0mg/L, and 2.0mg/L of BA was treated alone(Fig. 5, 6). The regeneration of shoot showed rapid reaction at the beginning of the growth. The control tools of the beginning of the growth showed 81.5% of regeneration ratio of shoot at the 10th day of the experiment. The ratio was high compare to the control tool and the regeneration ratio are 64.1%, 65%, and 50% of BA 0.5mg/L, 1.0mg/L, and 2.0mg/L respectively. As the culture was proceeded, the regeneration ratio of BA 2.0mg/L became a little slow, and other treatment tools showed the similar regeneration ratio by 15th day. In the case of BA 0.5mg/L, the regeneration ratio of shoot was the highest, and at the 60th day the ratio was 96%. At this time, the ratio of BA 2.0mg/L was 87% which was the lowest and in the control tool, the ratio was 90%. In

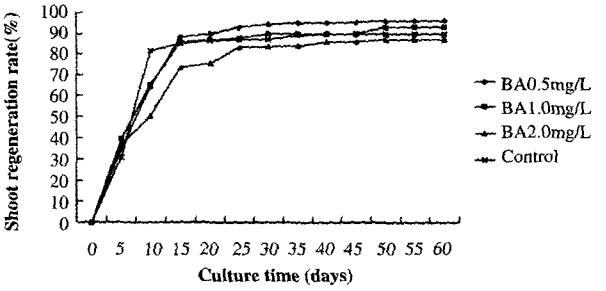

Fig. 5. Effect of BA concentration on shoot regeneration of Allium wakegi Araki.

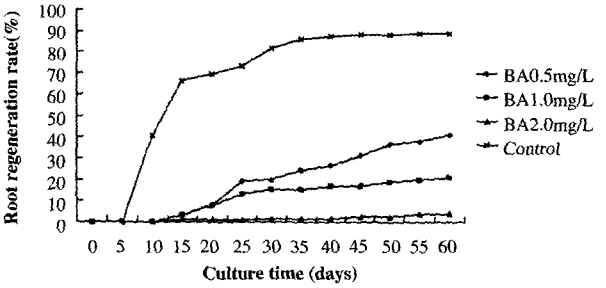

Fig. 6. Effect of BA concentration on root regeneration of *Allium wakegi* Araki.

the case of meristem culture of *Wasavia japonica*(Eun *et al.*, 1997), when BA, kinetin, and zeatin were added alone, the report was presented that the regeneration of shoot was good. It was reported that in the case of shoot tip culture of *Gypsophila paniculata*, the regeneration of shoot was effective when BA was added(Han *et al.*, 1991). In the case of shoot tip culture of a leek, the regeneration of shoot was effective when BAP and kinetin were treated. Similary, in the case of *Allium wakegi*, BA alone addition was good for shoot formation. When the growth regulator of 0.5mg/L, 1.0mg/L, and 2.0mg/L of BA, the regeneration ratio of root was low for 60 days of the culture. Especially, in the case of BA 2.0mg/L, the ratio showed 4% for 60days of the culture, and in the case of the control tool the ratio was the highest of 89%. In the case of the control tool, the root generated rapidly 5days later of the culture. In other cases of the treatment of BA 0.5mg/L, 1.0mg/L, and 2.0mg/L, it was observed that the generation of the root was not influenced at the beginning of the culture, but the root regenerated from the 15th day of the culture. However, compare to the control tool of the 15th day of the culture, the regeneration rate was seriously low. BA of the plant growth regulator was not effective in generating roots, and as the density of BA was high, the generation of the root became poor. In the case of the alone treatment of BA, callus wasn't induct, and when BA was added, from bulblet of *Allium senescens*, it was reported that the callus wasn't induct(Ashalatha and Seo, 1992).

The effect of combination treatment of BA and 2,4-D

In the condition of light culture, when the plant growth regulator was mixed and treated, to investigate the callus induction, the regeneration ratio of root and shoot, the bulb formation, BA and 2,4-D were mixed and experimented(Fig. 7, 8). When BA and 2,4-D were mixed and experimented, the regeneration rate of shoot showed that the ratio when mixed with BA 0.5mg/L and 2,4-D 0.5mg/L was higher than the ratio when mixed with BA 0.5mg/L and 2,4-D 1.0mg/L. However, compare to the control tool, the ratio was low. Therefore, mixed treatment showed low rate of the regeneration of shoot, and the mixed addition was not suitable for regeneration. In two of BA 0.5mg/L mixed with 2,4-D 0.5mg/L and 1.0mg/L respectively, the induction rate of callus were investigated in light culture. Auxin of 2,4-D and NAA was not used alone, and cytokinin of BAP, kinetin and zeatin were often mixed and used(Yoo *et al.*, 1991). When BA 0.5mg/L and 2,4-D 0.5mg/L were added at the 15th day of the culture, the callus generated and induction rate of callus was poor of 2.5% each. In all treatment tools, from the 35th day of the culture, the induction rate of callus become increased. In the case of BA 0.5mg/L and 2,4-D 0.5mg/L, the rate became increased at the 40th day of the

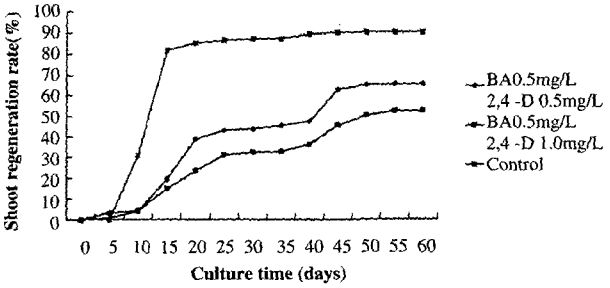

Fig. 7. Effect of BA concentration(0.5mg/L) and different concentration of 2,4-D on root regeneration of *Allium wakegi* Araki.

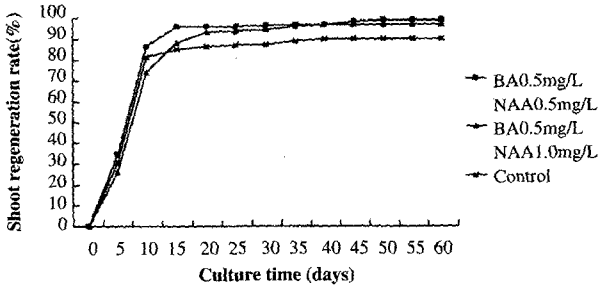

Fig. 9. Effect of BA concentration(0.5mg/L) and different concentration of NAA on shoot regeneration of *Allium wakegi* Araki.

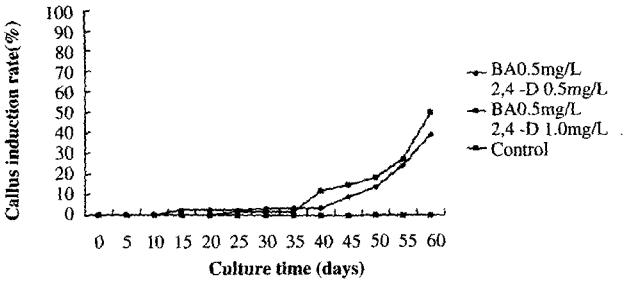

Fig. 8. Effect of BA concentration(0.5mg/L) and different concentration of 2,4-D on callus induction of *Allium wakegi* Araki.

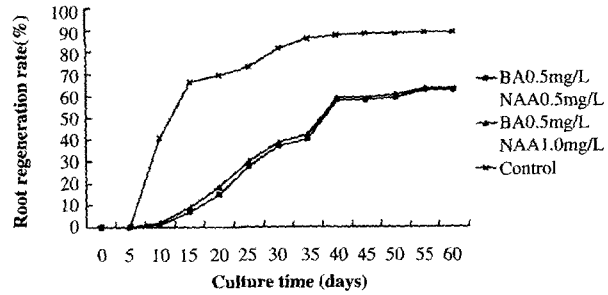

Fig. 10. Effect of BA concentration(0.5mg/L) and different concentration of NAA on root regeneration of *Allium wakegi* Araki.

culture. However, in the case of the control tool, the callus not induced by the 60th day of the culture. In the case of the treatment tools mixed BA 0.5mg/L and 2,4-D0.5 mg/L, 1.0mg/L respectively, the root did not generate. The mixed treatment of BA and 2,4-D was not suitable for generating the root.

The effect of combination treatment of BA and NAA

This experiment was proceeded to investigate callus induction and the regeneration of shoot and roots when BA 0.5mg/L mixed into NAA 0.1mg/L, 0.5mg/L and 1.0mg/L respectively were cultured(Fig. 9, 10). The regeneration rate of shoot was rapidly at the beginning of the culture. At the 10th day of the culture, when BA 0.5mg/L was mixed into NAA 0.5mg/L and 1.0mg/L, the rate was 86.3% and 74.2% respectively. At the 60th day of the culture, the rate was 99% and 97% respectively, and the rate of the control tool was 90% which was high. When BA 0.5mg/L was mixed with NAA 0.5mg/L, 1.0mg/L, the experiment showed that it was suitable for generating shoot of *Allium wakegi*. When NAA was mixed with BA, in the case of *Allium sativum*, the regeneration of shoot was good(Choi *et al.*, 1993). In the case of the peduncle culture of *Allium sativum* when BA and NAA were added, it was reported that the shoot formation was good(Kim *et al.*, 1996). This experiment showed the similar result of the regeneration of shoot, however, callus not induced in any mixed treatment tools. When vegetable body was regenerated from the bulb of *Allium victorialis* var. *platyphyllum*, its callus formation was observed 4 or 5 weeks later of the culture in almost cases. In the case of when BA and NAA were mixed and treated, it was not

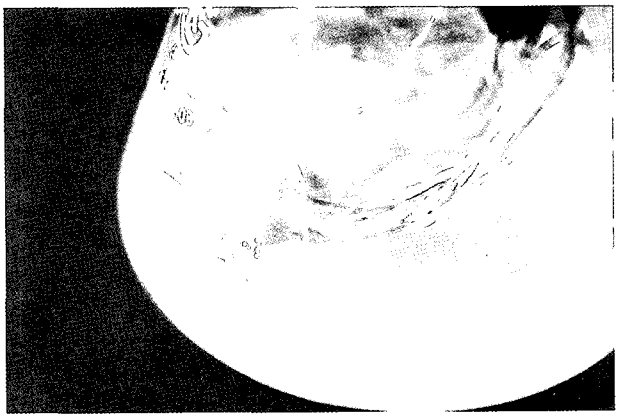

Photo. 1. Regeneration of plants and bulblets from shoot tip explants of *Allium wakegi* in light culture.

treated, it was not suitable for induction of callus. When BA 0.5mg/L was mixed into NAA 0.5mg/L and 1.0mg/L, the regeneration rate of roots was poor compare to the control tool. When BA 0.5mg/L mixed into NAA 0.5mg/L and 1.0mg/L was cultured, the rate showed the similar trend. At the 60th day of the culture, the rate was 62.5% and 62.9% respectively and the control treatment was good. In the case of bulb information, in every medium the bulb was formed. Particularly, in the medium of BA 0.5mg/L mixed with NAA 1.0mg/L, the bulb formation was good.

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