Control of Bulb Dormancy in Hyacinth - a Molecular Biological Approach

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Key words: abscisic acid, bulb formation, dormancy, hyacinth, low temperature

Abstract

Studies were conducted to investigate the hypothesis that the induction of bulb formation and the induction of bulb dormancy are the same phenomenon. Hyacinth plants cultured in vitro at 23°C developed leaves without forming bulbs, and formed bulbs with treatment of eight weeks at 4°C (Bach, 1992). Using this difference of growth response, the effects of temperature and growth regulators on bulb formation and the changes in endogenous ABA content were investigated. Shoot explants were taken from in vitro immature leaves of hyacinth (Hyacinthus orientalis L. cv. Delft Blue) and cultured at 25°C on MS medium containing NAA and BA. The explants treated for eight weeks at 5°C formed bulbs four weeks after the completion of the cold treatment at 25°C, whereas bulb formation was suppressed without cold treatment. Addition of ABA to the medium promoted bulb formation without cold treatment. By adding fluridone to the medium, bulb formation of the cold-treated explants was inhibited. Under bulb-forming conditions, endogenous ABA content increased and it was correlated with the increase of bulb-forming rate. These results suggest that bulb formation is induced by low temperature and regulated by ABA. Thus, the controlling methods are; bulb-forming conditions are obtainable by cold treatment or ABA without cold treatment and non-bulb-forming conditions are by non-cold treatment or fluridone with cold treatment.

INTRODUCTION

Since the induction of dormancy in bulbous plants is accompanied by bulb formation, the relationship between the induction of bulb formation and the induction of bulb dormancy is unclear. Okubo and Uemoto (1981) showed that bulblets of Dutch iris (Iris hollandica), 3 - 4 g, stored at 20°C and then grown at 15°C developed only 3 to 4 leaves and the vegetative apex produced new bulbs; whereas, bulbs grown at 25°C continued to develop leaves and did not form new bulbs. The level of endogenous abscisic acid (ABA) increased only under the bulb-forming condition. They proposed that the induction of bulb formation and bulb dormancy are the same phenomenon. The results are; (i) bulbous iris does not have a dormant period without bulb formation and (ii) ABA increased when plants received low temperatures. In addition (i) low temperatures induced bulb formation in tulips (Le Nard and Cohat, 1968), garlic (Aoba, 1971), bulbous oxalis (Aoba, 1972) and Dutch iris (Aoba, 1974); (ii) dormancy is induced by low temperatures (Le Nard, 1983); (iii) ABA is assumed to control dormancy in various plant organs and it declines with dormancy release in iris bulbs (Tsukamoto and Ando, 1973) and gladioli (Tsukamoto, 1974).

Recently, with Lilium speciosum plantlets regenerated in vitro, ABA added to the medium completely blocked leaf formation and induced dormancy; whereas, bulb formation and induction of dormancy were developed by ABA and inhibited by fluridone, an inhibitor of ABA biosynthesis (De Klerk, 1992; Gerrits and De Klerk, 1992). These results support the hypothesis of Okubo and Uemoto (1981).

Molecular biological studies are another approach to clarify this relationship. However, it is necessary to establish the controlling system of bulb formation and dormancy to conduct the studies. Hyacinth (Hyacinthus orientalis L.) plants cultured in vitro at 23°C developed leaves without forming bulbs, and formed bulbs after eight weeks at 4°C (Bach, 1992). Using this difference of growth response, we examined the effects of temperature and growth regulators on
bulb formation and the changes in endogenous ABA content, to establish the control mechanisms of bulb formation.

MATERIALS AND METHODS

Plant Material and Culture

Hyacinth (Hyacinthus orientalis L. cv. Delft Blue) bulbs, 16cm in circumference, were obtained from a commercial grower in Japan. They were stored at 5°C for 3-4 months until used.

Explants were cultured as described by Bach (1992). For the initial explants, immature leaves were removed from inside the bulbs and explants sterilized with a sodium hypochlorite solution. The explants (3-5 and 8-10 mm) were prepared and cultured on MS medium (Murashige and Skoog, 1962) with 1 mg/l indolebutyric acid (IBA), 0.7 % agar and 3 % sucrose at pH 5.8. Culture condition was 25°C in continuous light.

Shoot explants (2-3 and 6-8 mm) were taken from the initial explants and cultured on MS medium containing 0.01 mg/l α-naphthaleneacetic acid (NAA) and 1 mg/l 6-benzylaminopurine (BA). This MS medium was used in the following experiments.

1. Experiment 1. Effects of low temperatures on bulb formation. The initial explants were treated for eight weeks at 5°C in the dark. After the cold treatment, the shoot explants were isolated and cultured on MS medium at 25°C in light. The control explants were kept at 25°C.

2. Experiment 2. Effects of ABA in the culture medium on bulb formation of non-cold-treated explants. The shoot explants without cold treatment were cultured at 25°C on MS medium containing ABA at 0, 0.1, 1.0 or 10.0 mg/l.

3. Experiment 3. Effects of fluridone in the culture medium on bulb formation of 5°C explants. The shoot explants from 5°C were cultured on MS medium with fluridone in the medium at 0, 0.03, 0.3 or 3.0 mg/l.

4. Experiment 4. Effects of 5°C on the changes in endogenous ABA content in cold-treated explants. Endogenous ABA in the shoot explants with or without cold treatment was extracted and estimated by an enzyme-linked immunosorbent assay (ELISA).

RESULTS

Experiment 1. Effects of Cold Treatment on Bulb Formation

After four weeks of culture at 25°C following eight weeks at 5°C over 70% of the cold-treated explants formed bulbs; whereas, less than 20 % of the non-cold-treated explants formed bulbs. The bulb formation rate reached 100 % in the cold-treated explants after 28 weeks in culture. However, it remained low in the controls after 22 weeks.

Shoots were formed in the cold-treated explants four weeks after the start of bulb formation. Shoot formation started six weeks earlier in the control explants than in cold-treated explants.

Experiment 2. Effects of ABA in the Culture Medium on Bulb Formation of Non-Cold-Treated Explants

After 14 weeks, the application of ABA to the culture medium promoted bulb formation in non-cold-treated bulbs; whereas, the bulb formation rate in the control explants was low. The highest rate of bulb formation was 75% after 22 weeks. It was obtained at 1 mg/l ABA followed by the treatment at 0.1 mg/l in the second position.

Shoot formation was delayed at 1 mg/l and 10 mg/l. After 22 weeks culture, the shoot formation rate at 10 mg/l ABA was 30%; whereas, it reached 80% at 1 mg/l. Control explants and explants treated with 0.1 mg/l ABA exhibited a high shoot formation rate.
Experiment 3. Effects of fluridone in the culture medium on bulb formation of cold-treated explants

All the 5°C treated explants formed bulbs without fluridone (Fig. 3). By adding fluridone, the bulb-forming rates of the explants with cold treatment were suppressed to 0 - 45% depending on its concentration. The higher the concentration of fluridone, the lower the bulb-forming rate.

Shoot formation was also suppressed depending on the concentration of fluridone. With high concentrations, the survival rate of the explants was low. This was probably due to toxicity.

Experiment 4. Effects of 5°C on changes in endogenous ABA content in cold-treated explants

Under the bulb-forming condition, the endogenous ABA content increased four weeks after the completion of the cold treatment. It did not increase in non-cold-treated explants. The changes of endogenous ABA content were synchronized with the increase of bulb-forming rate.

DISCUSSION

Bulb formation in hyacinths was promoted by 5°C and suppressed by a non-cold treatment. Bach (1992) showed that explants of hyacinths transferred to 23°C after 16 weeks cold treatment did not form shoots. These results clearly prove that low temperature induces bulb formation.

An increase of the bulb formation rate by addition of ABA and suppression of bulb formation by fluridone have also been reported in in vitro explants of *Lilium speciosum* (Gerrits and De Klerk, 1992). They found that endogenous ABA increased from the start of bulb formation which is in agreement with the present study. It also agrees with the previous report that ABA activity was observed only under bulb-forming conditions in Dutch iris (Okubo and Uemoto, 1981). These results indicate that bulb formation is controlled by ABA.

Okubo et al. (1988a, b) investigated the effect of scaling date on leaf emergence and endogenous plant hormone levels in hyacinths and *L. longiflorum*. They showed that the dormancy in these species was induced by low temperature and ABA activity increased at the induction of dormancy. Our results and these suggest that the induction of bulb formation and the induction of bulb dormancy are the related phenomena and regulated by ABA. Delvallee et al. (1990) also concluded that the induction of dormancy in lily bulblets corresponds to a switch in the developmental pattern of new primordia. Thus, they become incapable of forming leaves and can only form scales.

Molecular biological studies are another approach to clarify the relationship. Thus we have obtained the methods of controlling bulb dormancy to be used for future molecular biological analysis; bulb-forming conditions are obtainable by cold treatment or ABA without cold treatment and non-bulb-forming conditions are by non-cold treatment or fluridone with cold treatment. Characterizing the genes that are expressed in bulb-forming condition, and not in non-bulb-forming condition, can give further understanding of the relationship.

Literature Cited


Gerrits, M.M. and de Klerk G.J. 1992. Dry-matter partitioning between bulbs and leaves in


Figures

Fig. 1. Effect of temperature on the growth of hyacinth explants.

Fig. 2. Effect of ABA on the growth of hyacinth explant under nonbulb-forming condition.
Fig. 3. Effect of fluridone on the growth of hyacinth explants under bulb-forming condition.

Fig. 4. Changes in ABA content in explants of hyacinth with cold treatment.