Plant Physiol. (1977) 60, 360-362

# Dormancy in *Dioscorea*

## GIBBERELLIN-INDUCED INHIBITION OR PROMOTION IN SEED GERMINATION OF *D. TOKORO* AND *D. TENUIPES* IN RELATION TO LIGHT QUALITY[1,2]

Received for publication September 13, 1976 and in revised form March 2, 1977

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### ABSTRACT

**Effects of light and gibberellic acid ($GA_3$) application on the germination of *Dioscorea tokoro* Makino and *Dioscorea tenuipes* Franch. et Savat. were observed. For complete germination, seeds of both species required prechilling in moist condition before incubation at a higher temperature. Red light irradiation during the incubation after the prechilling promoted germination; blue, green, or far red light markedly inhibited the germination of both species.**

**Application of $GA_3$ induced complicated changes in the germination of both species in relation to light quality. In the germination of *D. tokoro*, $GA_3$ inhibited in the dark and red; however, it promoted germination in blue and far red light. $GA_3$ promoted germination of *D. tenuipes* in the dark and in blue, green, or far red light. These phenomena are explainable by assuming two counteractive reactions (germination-promoting and germination-inhibiting) which are both activated by applied $GA_3$.**

Gibberellin is well known to break dormancy of seeds and buds in many plants (3, 11). However, in bulbils of *Begonia evansiana* (2, 4-7, 9) and also bulbils and subterranean dormant organs of some species in the genus *Dioscorea* (8, 10), dormancy is induced by endogenous and exogenous gibberellin. Sprouting in winter buds of some woody plants is also retarded by applied gibberellin (1, 12). In seeds, however, no information about the germination-inhibiting effect of gibberellin seems to have been published.

We have studied comparative physiology in dormancy of seeds and buds of many species of the genus *Dioscorea*, perennial monocotyledonous herbaceous plants, which range from the tropics to the cold temperate zones. In the present paper, we tested light and temperature effect on germination and $GA_3$-induced germination inhibition in seeds of *Dioscorea tokoro* and *Dioscorea tenuipes*.

### MATERIALS AND METHODS

Seeds of *D. tokoro* Makino and *D. tenuipes* Franch. et Savat. were harvested from the plants growing spontaneously in the middle part (Shizuoka Prefecture) or south part (Kagoshima Prefecture) of Japan, dried at room temperature, and then stored in a desiccator containing silica gel until used. In germination experiments, 40 to 70 seeds were placed in a 9-cm Petri dish on a thin layer of absorbent cotton moistened with distilled $H_2O$ or aqueous solutions of $GA_3$ (gift from Kyowa Fermentation Industries, Tokyo) and allowed to stand under various light conditions at 25 or 26 C in *D. tokoro* and 20 C in *D. tenuipes*. In some experiments, prior to incubation at the above conditions, the seeds were placed under 5 C in the dark in a Petri dish on a thin layer of absorbent cotton moistened with distilled $H_2O$ (prechilling).

White light of 1,500 lux was obtained from real daylight (40-D-SDL, Toshiba, Tokyo) fluorescent lamps. Blue, green, and red light were obtained by filtering the radiations from colored fluorescent lamps (FL-20BF, FL-20GF, and FL-20RF, respectively, Mitsubishi, Tokyo) through a 1-mm thickness of blue, green, and red vinyl resin plates, respectively. For far red irradiation, light from medical IR incandescent lamps (Toshiba) was filtered through 10-cm of distilled $H_2O$ and a 1-mm thickness of red and blue vinyl resin plates. Approximate wavelength of peak and half-bandwidth of colored light are as follows; blue, peak 460 nm (half-bandwidth 35 nm); green, 540 nm (20 nm); red, 650 nm (13 nm); far red, 770 nm (100 nm). All of the colored light exposures were given at the intensity of 300 $ergs \cdot cm^{-2} \cdot sec^{-1}$. Experiments were repeated at least twice using the seeds harvested in different places or years. Consistent trends were observed in the results of the replicate experiments.

### RESULTS

**Effect of Various Periods of Prechilling on Germination of *D. tokoro* and *D. tenuipes* Seeds.** *D. tokoro* seeds which had experienced no storage period after harvest were chilled at 5 C in the dark for 0, 30, 50, or 80 days before incubation at 25 C in the light or in the dark (Fig. 1). With an increasing period of prechilling at 5 C, the capacity to germinate increased; however, light inhibited germination. Similar results but more scarce requirements of prechilling were observed in germination of *D. tenuipes* seeds which experienced no storage period after harvest (Fig. 1). These prechilling requirements for germination of both species gradually decreased with increasing the storage of seeds in a desiccator.

**Effects of $GA_3$ in the Light or Dark on Germination of *D. tokoro*.** *D. tokoro* seeds were incubated with $GA_3$ in the light or in the dark at 25 C after various periods of prechilling at 5 C in the dark (Fig. 2). In the seeds which had experienced no prechilling, germination was induced, though slightly, by 30 and 300 $\mu M$ $GA_3$ in the light; but no germination occurred in the dark. In the seeds prechilled for 50 days, application of $GA_3$ obviously promoted the germination in the light; however, it inhibited germination in the dark. $GA_3$ had no major effect on

[1] This work was supported in part by a research grant from the Ministry of Education of Japan.

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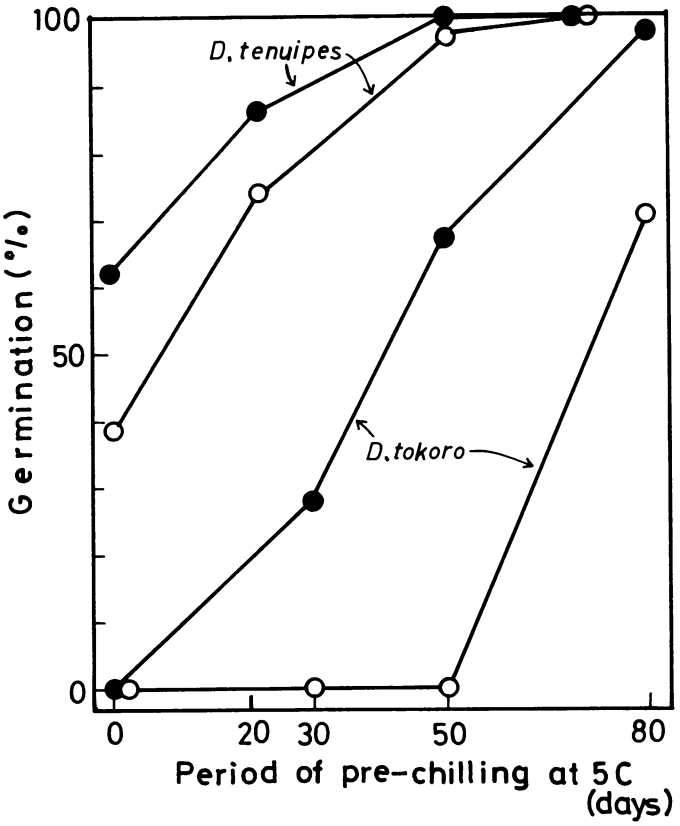


FIG. 1. Effect of the light (○) and the dark (●) on germination of *D. tokoro* and *D. tenuipes* seeds after various periods of prechilling at 5 C in the dark. Seeds which had experienced no storage period after harvest were exposed to 5 C in the dark for 0, 30, 50, and 80 days in *D. tokoro* and for 0, 20, 50, and 70 days in *D. tenuipes*, then incubated in the dark or in white light at 25 C in *D. tokoro* and 20 C in *D. tenuipes*. Germination was counted 100 days after the start of prechilling in both species.

germination in either light or dark in the seeds which had been prechilled for 80 days. Thus in the dark germination was inhibited by application of $GA_3$, but in the light $GA_3$ promoted germination.

**Effect of $GA_3$ in Various Colored Lights on Germination of *D. tokoro* and *D. tenuipes*.** Seeds stored in desiccator for about 3 months were incubated with various concentrations of $GA_3$ in various colored lights at 26 C in *D. tokoro* and 20 C in *D. tenuipes*. Prior to the above incubation, *D. tokoro* seeds were prechilled at 5 C in the dark for 20 days. On the other hand, *D. tenuipes* seeds were exposed to no prechilling, since in incubation at 20 C without prechilling (Fig. 1), these seeds are capable of germination to a suitable extent for testing effects of $GA_3$. In the absence of $GA_3$, colored lights produced the same effect on germination of both species: red light promoted, and blue, green, or far red lights inhibited germination (Figs. 3 and 4).

In the incubation of *D. tokoro* with $GA_3$, $GA_3$-induced inhibition in the germination was observed in the dark or red light (Fig. 3). Germination was increasingly promoted by increasing concentrations of $GA_3$ in blue or far red light as it had been under white light (Fig. 2). In green light, significant promotion was not observed. Thus in *D. tokoro* seeds, whether applied $GA_3$ promotes or inhibits germination depends on light quality.

In *D. tenuipes*, $GA_3$-induced promotion in germination was observed in the dark and in green, far red, or blue light. On the contrary, in white and red light, germination was inhibited by 3 $\mu$M $GA_3$, but in higher concentrations, germination percentages increased. The same tendency of these combined effects of $GA_3$ and colored lights in *D. tenuipes* seeds was also obtained in incubation at 26 C, but the germination percentages of all lots are very low.

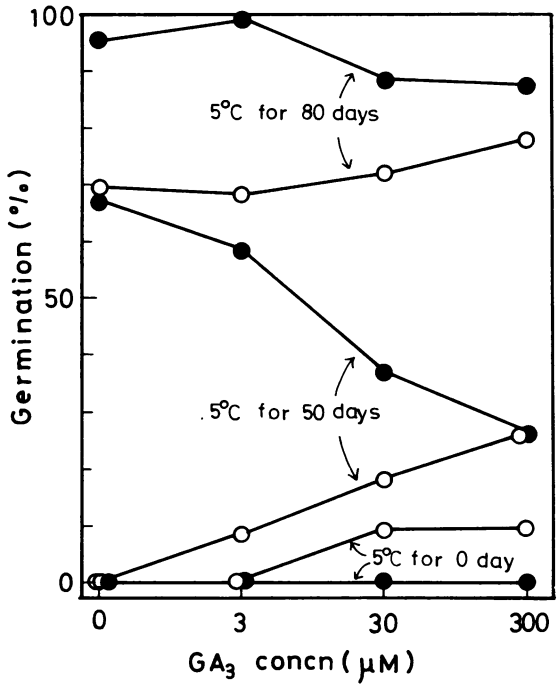


FIG. 2. Effect of $GA_3$ on germination in the light or dark after various periods of prechilling in *D. tokoro* seeds. Seeds which had experienced no storage period after harvest were chilled at 5 C in the dark for 0, 50, and 80 days, then incubated with various concentrations of $GA_3$ in the dark (●) or in white light (○) at 25 C. Germination was scored 100 days after the start of prechilling.

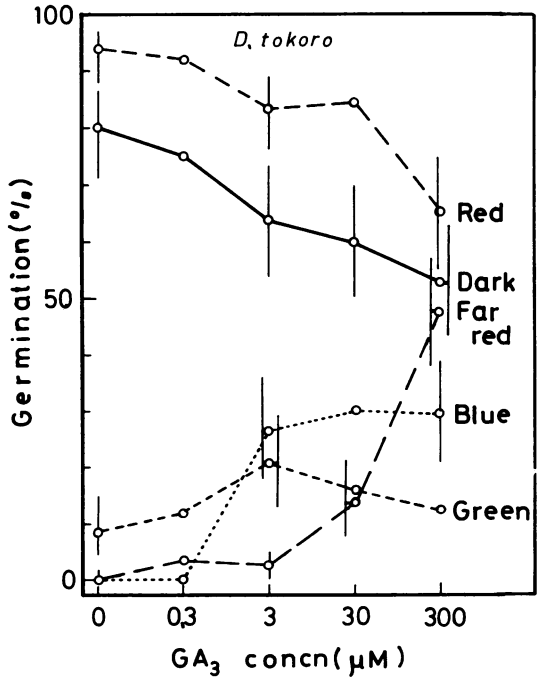


FIG. 3. Effect of colored lights on $GA_3$ action on germination of *D. tokoro* seeds. Seeds prechilled at 5 C in the dark for 20 days were then incubated at 26 C with various concentrations of $GA_3$ for 10 days in the dark or in red light and for 70 days in blue, green, or far red lights. Vertical bars indicate confidence limits at 90% level.

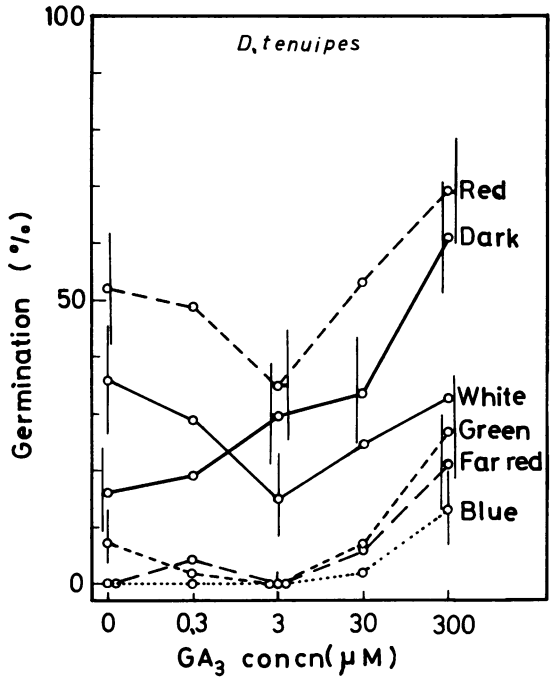


FIG. 4. Effect of colored lights on $GA_3$ action on germination of *D. tenuipes* seeds. Seeds stored for about 3 months after harvest were incubated with various concentrations of $GA_3$ in the dark and in white or light of various colors for 45 days at 20 C. Vertical bars indicate confidence limits at 90% level.

## DISCUSSION

The germination of seeds of *D. tokoro* and *D. tenuipes* was inhibited by $GA_3$ application (Figs. 2-4). These cases are probably the first examples of $GA_3$-induced inhibition in seed germination. However, conditions for occurrence of $GA_3$-induced germination inhibition varied with species (Figs. 3 and 4). In the previous reports, existence of two counteractive reactions (sprouting-inhibiting and sprouting-promoting) which are both activated by $GA_3$ was assumed in bulbils of *Begonia evansiana* (6) and some species in the genus *Dioscorea* (10). In the present study, similar to the case of bulbils, existence of two counteractive reactions is observed in clearly separated states in the seed germination of *D. tokoro* (Figs. 2 and 3), namely, $GA_3$-activated germination-inhibiting reaction worked in the dark or in red light, and $GA_3$-activated germination-inducing reaction worked in blue, far red, or white light. In the seeds of *D. tenuipes*, it is recognizable that the $GA_3$-activated germination-inducing reaction worked in the dark and in green, far red, or blue light. In the incubation in white or green light 3 $\mu$M $GA_3$ induced statistically significant (confidence limit at 90% level) inhibition, and the similar tendency, but statistically insignificant, was caused by 3 $\mu$M $GA_3$ in red light (Fig. 4). These concentration effects of $GA_3$ are explainable by a difference of $GA_3$ concentration dependency of activity of the two counteractive reactions, similar to the assumption in bulbils of some species in the genus *Dioscorea* (10). That is to say, the germination-inhibiting reaction was markedly activated even by diluted $GA_3$ (0.3 and 3 $\mu$M), but the higher concentrations (30 and 300 $\mu$M) of $GA_3$ strongly activated the germination-inducing reaction. In the incubation in far red or blue light, whether or not $GA_3$ activates the germination-inhibiting system could not be judged from the results in Figure 4.

Granting that endogenous gibberellins have the same effect as the exogenously applied $GA_3$, the working of two $GA_3$-activated counteractive reactions probably gives the seeds a delicate regulation of the germination process to respond to temperature and light conditions.

In a previous paper (10), $GA_3$-induced dormancy in asexual dormant organs in the genus *Dioscorea* was reported. With addition of the results in the present work on seed germination it is apparent that $GA_3$-induced modification in inhibitive tendency of sprouting and germination of buds and seeds is a common property in all dormant organs in *Dioscorea*. $GA_3$-induced dormancy, namely, relatively stronger working of sprouting- or germination-inhibition reactions in the genus *Dioscorea*, presents a very interesting problem on the origin, distribution, and adaptation of this genus related to the role of plant growth regulators in morphogenesis.

*Acknowledgments* – We wish to thank T. Iwao and Y. Takeuchi for very kind arrangement in experiment of *D. tenuipes*.

### LITERATURE CITED

1. BRIAN, PW, JHP PETTY, PT RICHMOND 1959 Extended dormancy of deciduous woody plants treated in autumun with gibberellic acid. Nature 183: 1198-1199
2. CHO S 1970 Response to gibberellic acid of the sterile-cultured buds of *Begonia* aerial tubers. Sci Rep Tohoku Univ 4th Ser (Biol) 35: 139-148
3. JONES RL 1973 Gibberellins: their physiological role. Annu Rev Plant Physiol 24: 571-598
4. NAGAO M, E MITSUI 1959 Studies on the formation and sprouting of aerial tubers in *Begonia evansiana* Andr. III. Effect of gibberellin on the dormancy of aerial tubers. Sci Rep Tohoku Univ. 4th Ser. (Biol.) 25: 199-205
5. NAGAO, M, N OKAGAMI 1966 Effect of (2-chloroethyl)-trimethylammonium chloride on the formation and dormancy of aerial tubers of *Begonia evansiana*. Bot Mag (Tokyo) 79: 687-692
6. OKAGAMI N 1972 The nature of gibberellin-induced dormancy in aerial tubers of *Begonia evansiana*. Plant Cell Physiol 13: 763-771
7. OKAGAMI N, Y ESASHI 1972 Dormancy regulation by morphactin in aerial tubers of *Begonia evansiana*. Planta 104: 195-200
8. OKAGAMI N, M NAGAO 1971 Gibberellin-induced dormancy in bulbils of *Dioscorea*. Planta 101: 91-94
9. OKAGAMI N, M NAGAO 1973 Gibberellin-induced dormancy in *Begonia* aerial tubers. Increase of the growth inhibitors content by gibberellin treatment. Plant Cell Physiol 14: 1063-1072
10. OKAGAMI N, N TANNO 1977 Dormancy in *Dioscorea:* generality of gibberellin-induced dormancy in asexual dormant organs. Plant Cell Physiol 18: 309-316
11. STUART NW, HM CATHEY 1961 Applied aspect of gibberellins. Annu Rev Plant Physiol 12: 369-394
12. WEAVER RJ 1959 Prolonging dormancy in *Vitis vinifera* with gibberellin. Nature 183: 1198-1199