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Influences of Cutting, Gibberellin A_3 and Abscisic Acid on Germination of *Avena fatua* Seeds

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Abstract

Germination of dormant *Avena fatua* seeds (caryopsis) at different temperatures was promoted partially by pricking the seed coat with pericarp or cutting intact seeds, suggesting that such treatments allow oxygen to permeate into the seeds and promote germination.

Effects of exogenous gibberellin A_3 (GA_3), 2-chloroethyl-trimethyl-ammonium chloride (CCC) and abscisic acid (ABA) on germination were examined with half cut seeds after-ripened for various months. GA_3 promoted the germination of highly dormant seeds but CCC inhibited that of slightly dormant seeds. ABA also inhibited the germination of slightly dormant seeds. The promotive effect of GA_3 decreased but the inhibitory effect of ABA increased in more highly dormant seeds or in the seeds maintained at higher temperatures. However, embryos isolated from the seeds at much highly dormant stage germinated well even at 30°C in the presence of GA_3.

These evidences suggest that the dormancy of *A. fatua* seeds occurs in combination with two regulatory events. First, an anaerobic condition around the embryo occurs within fresh seeds. The anaerobic degree is gradually reduced with the increase in after-ripened months. Second, different level of endogenous ABA and gibberellins (GAs) is involved ; high ABA level and low GAs level are in fresh seeds and/or at high temperature, whereas the converse is in seeds after-ripened for many months and/or at lower temperature.

Key words: ABA-*Avena fatua*-Cut seed-GA_3-Seed germination

Introduction

The seed coat imposes dormancy in many types of seeds due to impermeability to water and especially oxygen[1,5,11,12]. By seed coat scarification, the narrowing of the temperature range for germination of many plant seeds can often be eliminated and a wide temperature range can be established[10].

On the other hand, much evidence was accumulated concerning the effects of exogenous GA_3 in breaking the plant seed dormancy and of exogenous ABA in inducing seed dormancy in many species[2,9,13].

Only a few seeds of *Avena fatua* one week after harvest germinate at 5 and 10°C after 10 days but none of the seeds germinate at higher temperatures. Seeds that have after-ripened for longer than 4 months can germinate optimally at 15°C or higher but not at 30°C even after many months of after-ripening[7].

In the studies presented here, the germination response in *Avena fatua* seeds was examined with regard to pricking the seed coat or cutting intact seeds. The effects of exogenous GA_3, CCC as an inhibitor of GAs-biosynthesis[4] and ABA applied to half cut seeds after-ripened for various months were also examined.

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Materials and Methods

The germination of *Avena fatua* seeds (caryopsis) after-ripened for various months was examined using the same methods as reported previously[7]. However, in the present experiments, the effects of GA_3 and ABA were examined mostly in half cut seeds. Dehulled intact seeds were cut laterally into halves using a razor blade ; those with embryos are referred to as cut seeds or half seeds. In some cases, embryos with scutellum were isolated from intact seeds. They were sown on a layer of filter paper moistened with 5 ml distilled water or an aqueous solution of GA_3, CCC or ABA in a flat 9 cm petri dish, then exposed to a constant temperature specified. There were 25 to 30 seeds or embryos per each experiment. The number of germinating seeds or embryos was recorded every day for several days and are represented as the percentage of the total number of seeds.

Results

1. *Effects of pricking and cutting*

Intact seeds after-ripened for 6 months were pricked to a depth of 0.3 to 0.5 mm with a small needle at the middle of seed, either in the ventral or dorsal coat with pericarp. The germination of another group, comprising of the seeds cut into halve, at 15 to 30°C was compared with that of intact seeds. As shown in Fig. 1-a, the pricking of either coat resulted in some promotion of germination at 15 and 20°C to the same degree as the half cutting of seeds. However, neither seeds germinated at 25 and 30°C (data not shown). Furthermore, the promotive effect of half cutting on germination of 2, 14 and 26 month-seeds at 15 to 30°C was compared with the intact seeds (Fig. 1-b). Almost all of 2 month-cut seeds germinated at 15 and 20°C, campared with only half or less of the intact seeds. Neither seeds germinated at 25 and 30°C. Fourteen and 26 month-cut seeds also germinated at all the temperatures much more than intact seeds and some cut seeds germinated at 30°C but none of intact seeds.

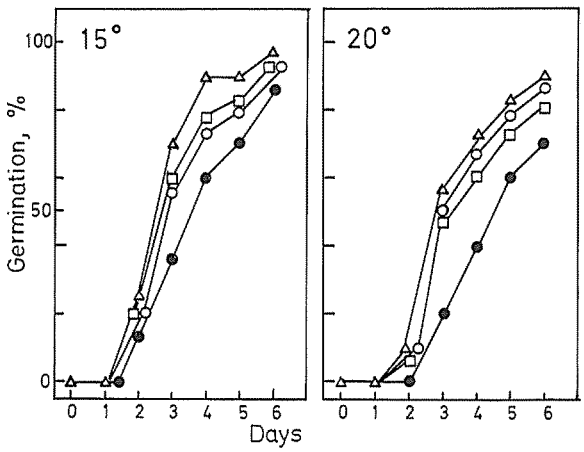

Fig. 1-a. Germination at 15 (15°) and 20°C (20°) of 6 month-*Avena fatua* seeds with ventral seed coat pricked (□), with dorsal seed coat pricked (△), cut laterally into halves (○) and intact seeds (●).

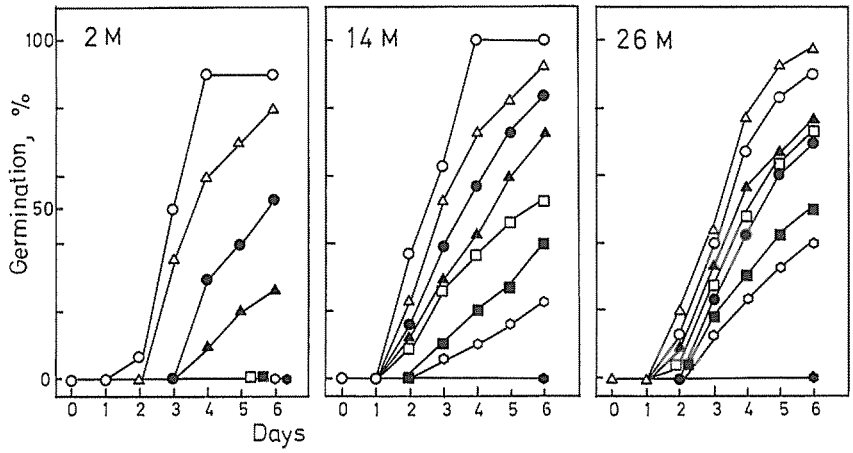

Fig. 1-b. Germination of 2 (2 M), 14 (14 M) and 26 month (26 M)-*Avena fatua* half seeds (○, △, □, ○) and intact seeds (●, ▲, ■, ●) at 15 (○, ●), 20 (△, ▲), 25 (□, ■) and 30°C (○, ●).

2. *Effect of GA_3*

The effects of 0, 0.01, 0.1 and 1 ppm GA_3 on germination at 15 to 30°C was examined with cut seeds after-ripened for 2.5 months (Fig. 2-a). Germination at 15 and 20°C was evidently promoted by GA_3 at concentrations as low as 0.01 or 0.1 ppm but the promotive effect of GA_3 at 25°C decreased and no seeds germinated at 30°C. However, the germination of 11 month-seeds at higher temperatures was promoted by GA_3 and some seeds germinated at 1 ppm GA_3 even at 30°C (data not shown). Embryos were isolated from 2.5 month-seeds as described above and germination at 15 to 30°C was examined in the presence or absence of 1 ppm GA_3 as compared with the half seeds (Fig. 2-b). The embryos germinated much more than the half seeds at all temperatures including 30°C in the presence of GA_3 but did not in its absence. The embryos isolated from 11 month-seeds all germinated at higher rate at 30°C in the presence of GA_3 (data not shown).

The effects of CCC on the germination of the half seeds at 20°C were examined with 9, 21 and 33 month-seeds (Fig. 2-c). The higher the concentration of CCC, then the stronger the inhibition. A higher CCC concentration was necessary for decreasing the germination of older seeds ; One hundred ppm CCC was enough in 9 month-seeds to decrease germination by 50% or less at 4 days in contract with 10^3 and 10^4 ppm in 21 and 33 month-seeds, respectively. The inhibitory effect of CCC was completely overcome by the simultaneous application of GA_3 (data not shown).

3. *Effect of ABA*

The effects of 0, 0.1, 1 and 10 ppm ABA on the germination of half seeds at 15 to 25°C were examined with seeds after-ripened for 11 months (Fig. 3-a). Germination at 15 and 20°C was inhibited by 1 and 0.1 ppm ABA, respectively. The lesser germination at 25°C was further inhibited by 0.1 ppm ABA. Furthermore, the interaction of the inhibitory effects of 0, 0.1, 1 and 10 ppm ABA and the promotive effects of 0, 0.1, 1 and 10 ppm GA_3 was examined with 12 month-half seeds at 30°C, at which temperature none of them germinated in the absence of GA_3 (Fig. 3-b). The inhibitory action of ABA was reduced by higher concentrations of GA_3. The action of ABA was reduced or overcome by lower concentrations of GA_3 in older seeds (data not shown).

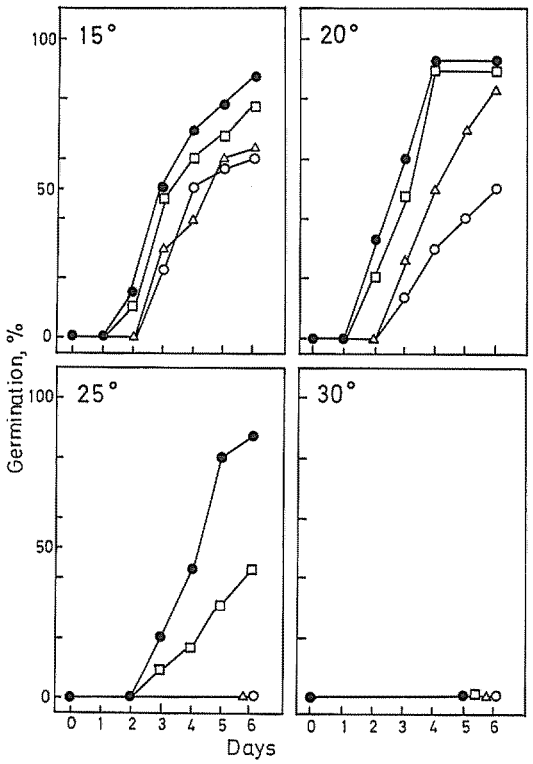

Fig. 2-a. Germination of 2.5 month-*Avena fatua* half seeds at 15 (15°), 20 (20°), 25 (25°) and 30°C (30°) in the presence of 0, (○), 0.01 (△), 0.1 (□) and 1 ppm GA_3 (●).

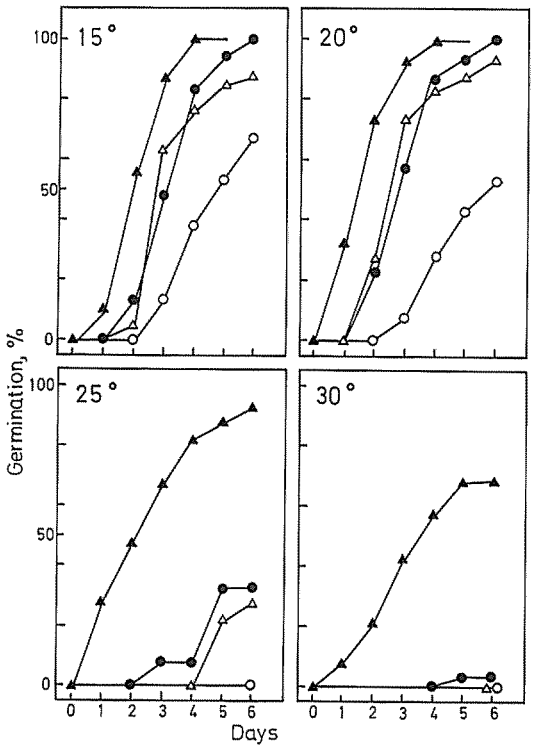

Fig. 2-b. Germination of isolated *Avena fatua* embryos from 2.5 month-seeds (▲, △) and half seeds (○, ●) at 15 (15°), 20 (20°), 25 (25°) and 30°C (30°). In the presence of 1 ppm GA_3 (▲,●) and the absence (△, ○).

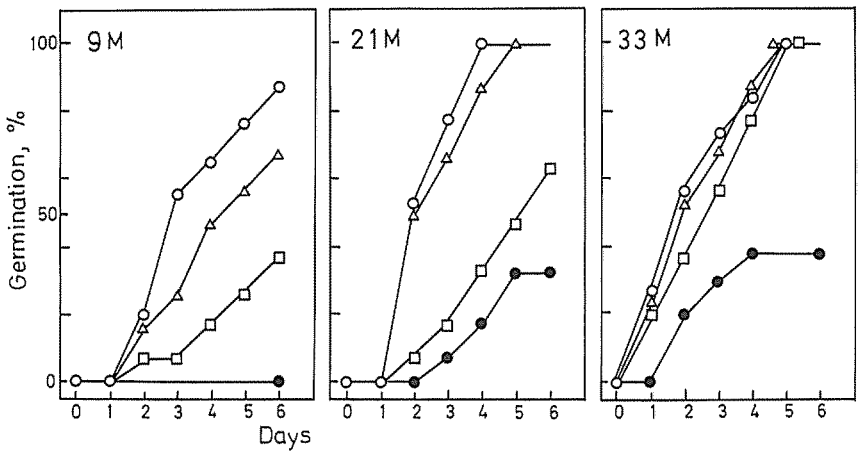

Fig. 2-c. Germination of 9 (9 M), 21 (21 M) and 33 month (33 M)-*Avena fatua* half seeds at 20°C in the presence of 0 (○), 10^2 (△), 10^3 (□) and 10^4 ppm CCC (●).

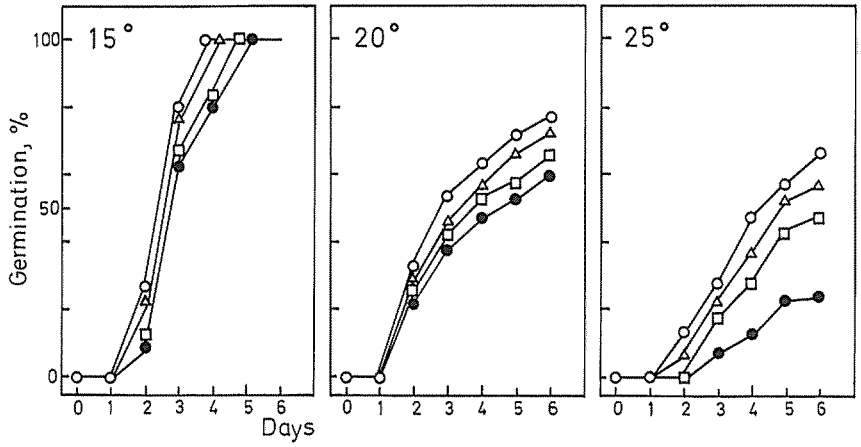

Fig. 3-a. Germination of 11 month-*Avena fatua* half seeds at 15 (15°), 20 (20°) and 25°C (25°) in the presence of 0 (○), 0.1 (△), 1 (□) and 10 ppm ABA (●).

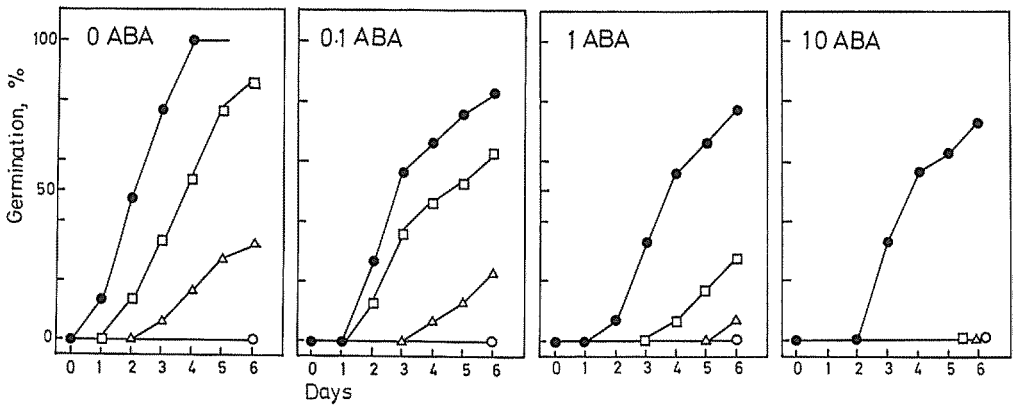

Fig. 3-b. Germination of 12 month-*Avena fatua* half seeds at 30°C in the presence of 0 (0 ABA), 0.1 (0.1 ABA), 1 (1 ABA) and 10 ppm ABA (10 ABA) with 0 (○), 0.1 (△), 1 (□) and 10 ppm GA_3 (●).

Discussion

Germination of *A. fatua* seeds was promoted by pricking the seed coat and also by cutting into half seeds at wider temperature ranges for the older seeds. Some of 14 month-half seeds were capable of germinating even at 30°C, at which temperature intact seeds were not (Figs. 1-b). Cutting facilitates the entry of water or air into the seeds after the sowing. A comparison of the amount of water imbibed by seeds either at 15 or 30°C revealed that it was always greater at 30°C than at 15°C in intact seeds as well as in half seeds (data not shown). Therefore, the failure of intact seeds to germinate at 30°C could not be caused by depression of water imbibition. Conversely, the promotive effect of seed cutting on germination could not be caused by enhancement of water imbibition. It could be mostly due to better oxygen permeation into the seed tissue bringing about an aerobic condition favorable for germination of the seeds. Perhaps, the embryos within fresh seeds would be in an anaerobic state that becomes less anaerobic in the after-ripened period.

Effects of exogenous GA_3, CCC and ABA were examined with half cut seeds, in which inhibitory effect arised from the seed coat could be lesser than that in intact seeds. Germination of all ages of seeds was promoted by GA_3 as Naylor and Sympson reported[6]. However, the higher the temperature, the lesser was the effect of GA_3 (Fig. 2-a). This suggests that germination of the half seeds is still depressed by the anaerobic situation of the embryo itself enclosed with the half seed tissue. The unfavorable condition is furthered at higher temperatures as 30°C and the action of GA_3 will be reduced. This idea can be emphasized from the evidence that GA_3 can stimulate the germination of the isolated embryos even at 30°C when they are kept in an aerobic state *in vitro* (see Fig. 2-b). CCC decreased germination. The minimum dosage of CCC required for germination inhibition increased as the seeds after-ripened (Fig. 2-c). Simpson[8] reported that CCC prevented the synthesis of GA-like substances in non-dormant seeds of *A. fatua*. CCC inhibited α-amylase production in barley seeds by inhibiting the synthesis of GA-like substances during germination[3]. The present evidence and the previous reports indicate that the capacity for biosynthesis of endogenous GAs in *A. fatua* embryo increases with the period of after-ripening. In contrast with GA_3, the higher the temperature, the stronger was the inhibitory effect of ABA (Fig. 3-a), suggesting that the action of ABA is synergistic with inhibitory action of the higher temperature. However, the action of ABA was reduced by that of GA_3 (Fig. 3-b). Preliminary examination of ethanol extracts obtained from the basal portions with the embryos of 6 month-seeds revealed that the extract from seeds incubated at 10°C contained larger amounts of GAs and less growth inhibitors than that incubated at 25°C. It can be inferred that the action of endogenous ABA is predominant in seeds after-ripened for only a few months at high temperatures and that the action of ABA is reduced by endogenous GAs in seeds after-ripened for many months at lower temperatures.

References

1) BLACK, M. Dormancy study in seed of *Avena fatua*. 1. The possible role of germination inhibitors. Can. J. Bot. 37: 393–402 (1959).
2) KAHN, A. A. and K. L. TAO. Phytohormones, seed dormancy and germination. *In* Phytohormones and Related Compounds A Comprehensive Treatise, Vol. II. Ed. Letham, Goodwin and Higgins, pp. 371–422. Elsevier. (1978).
3) KAHN, A. A. and M. A. FAUST. Effect of growth retardants on α-amylase production in germinating barley seed. Physiol. Plant. 20: 673–681 (1967).
4) KENDE, H., H. NINNEMANN and A. LANG. Inhibition of gibberellic acid biosynthesis in *Fusarium moniliforme* by Amo-1618 and CCC. Naturwiss. 50: 599–600 (1963).
5) MAYER, A. M and Y. SHAIN. Control of seed germination. Ann. Rev. Plant. Physiol. 25: 167–193 (1974).
6) NAYLOR, J. M. and G. M. SIMPSON. Dormancy studies in seed of *Avena fatua*. 2. A gibberellin-sensitive inhibitory mechanism in the embryo. Can. J. Bot. 39: 281–295 (1961).
7) OGAWA, Y. Thermo-control of germination of *Avena fatua* seeds in relation to after-ripened period. Bull. Fac. Bioresources, Mie Univ. 8: 65–72 (1992).
8) SIMPSON, G. M. The suppression by (2-chloroethyl) trimethyl-ammoniumchloride of synthesis of a gibberellin-like substances by embryos of *Avena fatua*. Can. J. Bot. 44: 115–117 (1966).
9) TAYLORSON, R. B. and S. B. HENDRICKS. Dormancy in seeds. Ann. Rev. Plant Physiol. 28: 331–354 (1977).
10) VEGIE, A. Dormancy in higher plants. Ann. Rev. Plant Physiol. 15: 185–224 (1964).
11) WAREING, P. E. and A. FODA. Growth inhibitors and dormancy in *Xanthium* seeds. Physiol. Plant. 10: 266–280 (1957).

12) WAREING, P. E. and I. D. J. PHILLIPS. Dormancy. *In* The Control of Growth and Differentiation in Plants, pp. 223–254 Pergamon Press (1970).
13) WAREING, P. E. and P. E. SAUNDERS. Hormones and dormancy. Ann. Rev. Plant Physiol. 22: 261–288 (1971).

野生エンバク種子の発芽に及ぼす種子切断，ジベレリン A_3 およびアブシジン酸の作用

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野生エンバク（*Avena fatua*）の休眠中の種子（えい果）の種皮（果皮と種皮）に小さい穴をあけるか，横半分に切断すると，各種温度における発芽を促進された。この処理によって酸素の種皮透過が容易になり，発芽が促進されることを示唆している。

収穫後の各種の期間（後熟期間）の種子，即ち休眠の程度が異なる種子の発芽に及ぼす外生ジベレリン A_3 (GA_3)，ジベレリン生合成阻害剤の CCC およびアブシジン酸（ABA）の作用を，半分に切断した種子を用いて調べた。GA_3 は，後熟期間が短い，深い休眠種子の発芽を促進し，CCC は，後熟期間が長い，浅い休眠種子の発芽を抑制した。ABA もまた浅い休眠種子の発芽を抑制した。しかし，GA_3 の促進作用は休眠の深い種子，または高温では減少し，ABA の抑制作用は増加した。しかし，分離した胚の発芽は，GA_3 によって 著しく促進され 30°C の高温でも発芽した。

上記の実験結果は，野生エンバク種子の休眠の原因は二つの事柄が関係していることを示唆している。第1は，短い後熟期間の種子の胚の周辺は嫌気的な状態にあり，この程度は，後熟期間の経過につれて減少する。第2は，内生植物ホルモンの量的レベルあるいはその作用的変化である。短い後熟期間の種子あるいは高温では，ABA 抑制の作用が優勢的である。しかし，長い後熟期間の種子あるいは低温では，ジベレリンの促進作用と拮抗して ABA の抑制が減少する。