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Inhibitory Influence of the Embryo on Gibberellin A_3-Induced α-Amylase Production in Endosperm of *Avena fatua* Seed

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Abstract

Gibberellin A_3 (GA_3)-induced α-amylase production in embryonated endosperms and de-embryonated endosperms of *Avena fatua* seeds was examined. The seeds cut into halves were termed as embryonated halves and endosperm halves, respectively. The embryonated halves produced less α-amylase than the endosperm halves. α-Amylase production of both halves increased with lengthening after-ripening period, being far less in the embryonated halves.

The α-amylase production of the endosperm halves decreased remarkably when intact seeds were imbibed at temperatures of 20° to 30°C before removal of the embryo. The inhibitory effect caused by the presence of the embryo disappeared by notching, pricking or burnt-needling the embryo or its near around. Exogenous abscisic acid (ABA) inhibited α-amylase production even when the embryo was absent.

The implications of these experimental evidences are discussed in relation to the mechanism of the inhibitory influence of the embryo on the GA_3-induced α-amylase production in the endosperm of *A. fatua* seeds.

Key words: α-Amylase production · *Avena fatua* · Embryo · Endosperm · GA_3.

I. Introduction

Yomo and Iinuma[14] and Paleg[10] have found that GA_3 stimulates the production of α-amylase in the embryoless endosperm of barley seeds. Varner[13] has reported that α-amylase is produced in response to exogenous GA_3 in the aleurone cells by *de novo* synthesis. This evidence has been confirmed by many other investigators[1,3]. GA_3 is also produced in the barley embryo at the early germinating stage,[11,15,17] suggesting that the endogenous gibberellins (GAs) secreted from the embryo induce α-amylase production in the endosperm. In contrast, exogenous ABA inhibited GA_3-induced α-amylase synthesis in barley endosperm tissue[2].

On the other hand, *Avena fatua* seeds, one week after harvest, can scarcely germinate at 5° or 10°C, but not at any temperatures above 10°C. With lengthening of after-ripened period the seeds become capable of germinating even at higher temperatures, but not at all at 30°C[7].

In the present study the effect of the embryo on GA_3-induced α-amylase production in the endosperms of *Avena fatua* seeds was examined comparing embryonated endosperms with de-embryonated endosperms. We studied also the α-amylase production in both the endosperms in relation to after-ripening periods and the

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exposure to different temperatures during imbibition.

II. Materials and Methods

In the first experiments, seeds of *Avena fatua* were cut latitudinally into two parts at the different sites (Fig. 1), but in most later experiments seeds cut at the middle site were used. Thus, the embryonated and de-embryonated endosperms were obtained. α-Amylase production in both endosperms with embryo and without was estimated by the gel method reported by Ogawa previously[6] with some modifications. These cut seeds were sterilized with 70% ethanol and 1% sodium hypochlorite, then washed thoroughly with sterile water. Ten ml of a heated solution containing 0.5% starch, 1% agar and 1 ppm GA_3 unless stated otherwise, was poured into a flat petri dish of 9 cm in diameter so that the solidified gel layer was 0.5 cm deep. Five to 7 embryonated or de-embryonated endosperms were placed separately with the cut face in contact with the gel. Dishes were inverted to prevent water drip onto the gel surface and maintained at 30°C for 3 days unless otherwise specified. α-Amylase produced in the endosperms in response to GA_3 diffused into the gel. The plates were flooded with 2.5×10^{-2} N iodine solution for 5 minutes and starch undigested was visualized. The diameter of circular stained zones digested by α-amylase was measured in two directions, being at right angles to each other, by 1-mm section-paper laid beneath the plates. Two dishes with a total of 10 to 14 embryonated or de-embryonated endosperms were used for each experiment. Index of α-amylase production in figures and tables shows mean of diameters with standard errors.

The number of embryonated endosperms germinating on the gel during the amylase test is represented as a percentage of the total number of embryonated ones.

III. Results

1. *Seeds cut at various sites*

Six, 18 and 30 month-old seeds were used. Fig. 1 shows the cut sites of the seed. In the first group, seeds were cut slantwise along the scutellum (E), so that the embryos with the scutellum were separated from the endosperms. The formers are referred tentatively to as embryos and the others as endosperms removed embryo (E), respectively. The second group was cut laterally at the tip of the scutellum (B), which was about 2 mm apart from basal tip of the seed and referred to as the basal embryonated endosperms and basal endosperms (B). The third group was cut at the midline into two halves which was termed embryonated halves and endosperm halves (H). The fourth group was cut 2 mm below the seed apex as apical embryonated endosperms and apical endosperms (A). These cut seeds at different sites with and without embryo were examined for their α-amylase production either at 20° or at 30°C. These results are shown in Fig. 2. Production of α-amylase of the embryonated endosperms differed depending on the cutting site, but their amount was reduced compared with that of the corresponding embryoless endosperms. The basal embryonated endosperms (B) produced the most amount of α-amylase among the different embryonated endosperms, whereas the embryonated halves (H) produced the smallest amount of α-amylase. The embryos (E) and the apical embryonated endosperms (A) produced a small amount of α-amylase, the formers being slightly more than the latters. Thus, there was a zigzag pattern of amylase production according to the cutting site of the seeds from the embryo (E) to the apical part (A). On the other hand, among the embryoless endosperms with

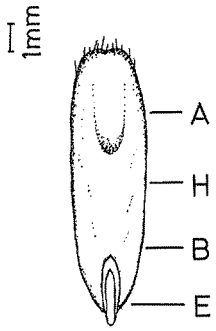

Fig. 1. Front face of *Avena fatua* seed. Lateral lines indicate cut sites of the seeds; embryo (E), basal (B), half (H), and apical part (A).

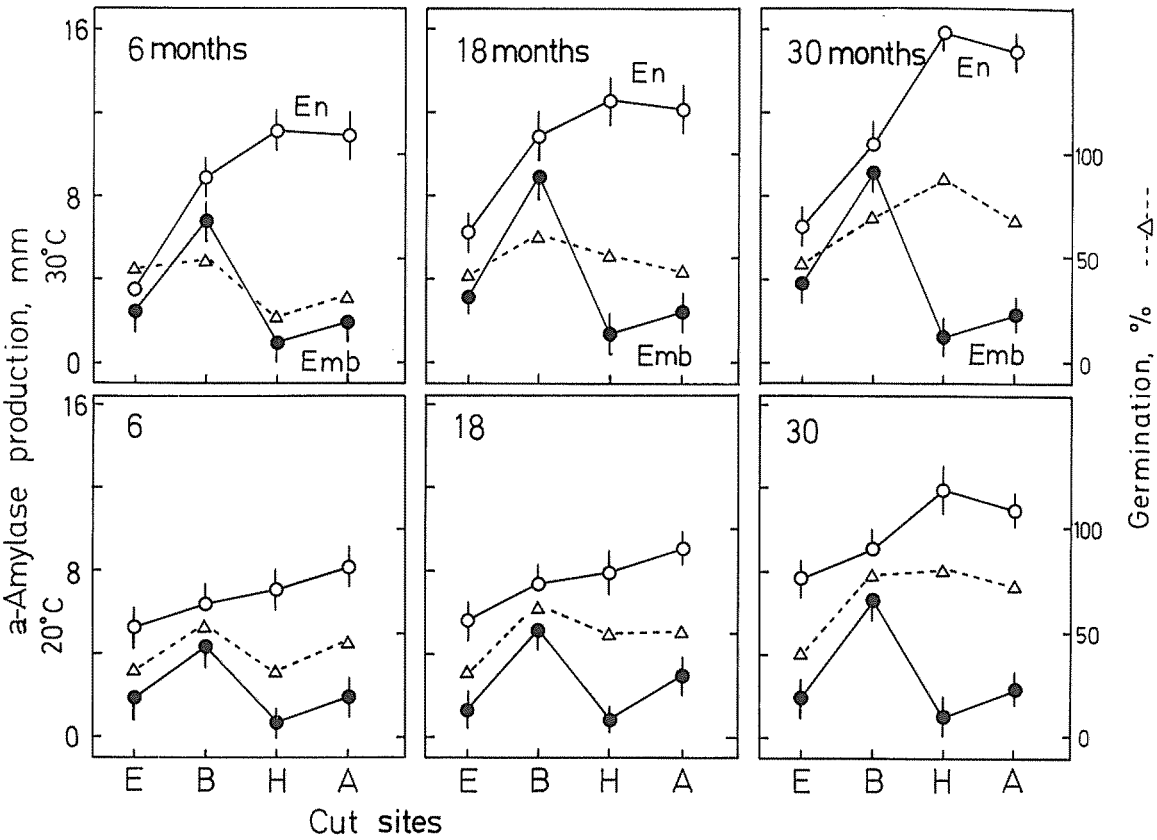

Fig. 2. α-Amylase production in various embryonated endosperms (●, Emb) and embryoless endosperms (○, En) of 6, 18 and 30 month-old *Avena fatua* seeds in the presence of 1 ppm GA_3 at 20° or 30°C. The embryonated and embryoless endosperms are obtained by cutting at the sites represented in Fig. 1. Germination percentage of the embryonated endosperms is shown by symbol (△).

different size of endosperm, the endosperms removed embryo (E) produced the smallest amount of amylase, while the endosperm halves (H) and the apical endosperms (A) produced a large amount of α-amylase. Thus, there was a linear increase in amylase production of the endosperms from the embryo (E) to the apical part (A),

showing their production of the amylase exceeded the production of the embryonated endosperms, especially at the endosperm halves (H) and apical endosperms (A).

α-Amylase production of cut seeds with different size of endosperm and with or without the embryo increased with increase in after-ripening period. α-Amylase production at 30°C was far superior in its amount than that at 20°C.

The germination rate of the different embryonated endosperms was similar to the profile of their α-amylase production. However, some difference was found between the two events: a considerably high germination percentage took place despite a less α-amylase production in the embryonated halves and apical embryonated endosperms. Also, the germination percentage of these embryonated endosperms gradually increased with the lengthening of after-ripening period.

2. *GA_3 at various concentrations*

Seven, 19 and 31 month-old seeds were cut into halves. The embryonated halves and embryoless halves termed as endosperm halves were subjected to the α-amylase test with various concentrations of GA_3 either at 20° or 30°C (Fig. 3). Neither embryonated halves nor endosperm halves of any age produced α-amylase in the

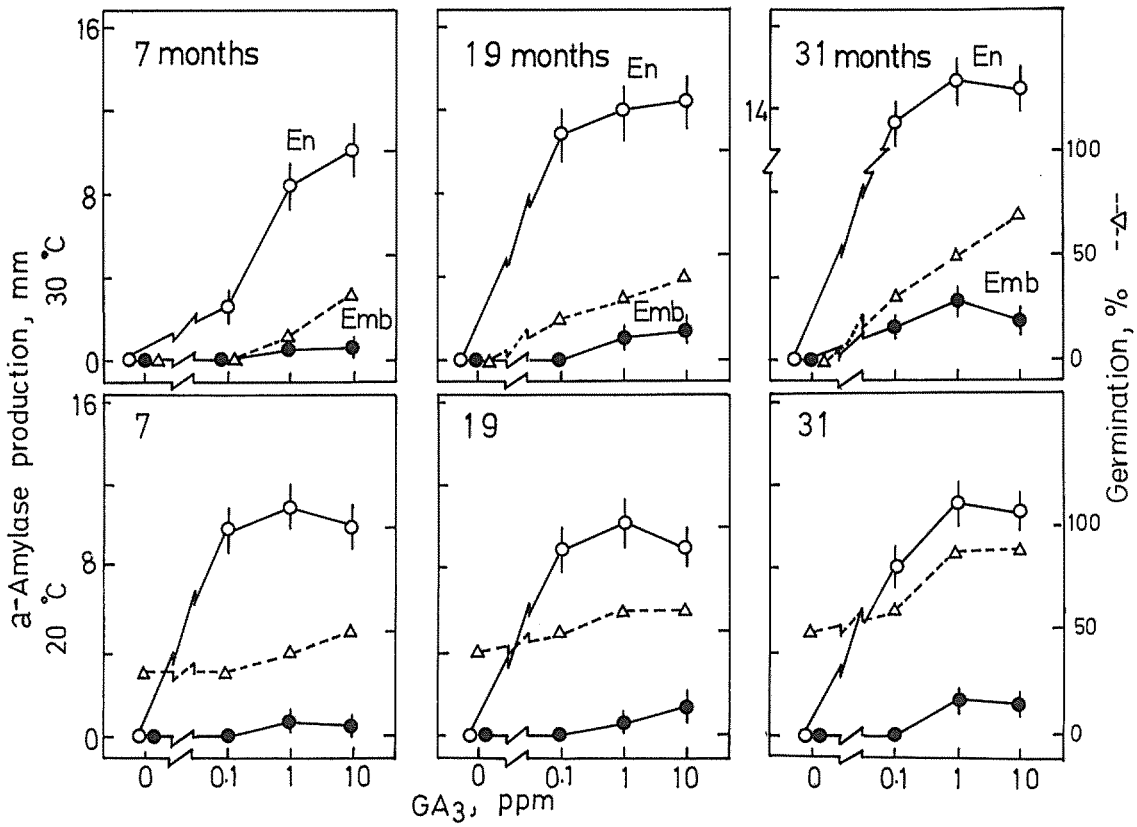

Fig. 3. α-Amylase production in embryonated endosperm halves (●, Emb) and embryoless endosperm halves (○, En) of 7, 19 and 31 month-old *Avena fatua* seeds in the presence of various concentrations of GA_3 at 20° or 30°C. Germination percentage of the embryonated halves is shown by symbol (△).

absence of GA_3. The embryonated halves produced only a minor amount of α-amylase at 1 and 10 ppm, whereas the endosperm halves produced a considerable amount even at 0.1 ppm. The increase in α-amylase production with the lengthening of after-ripening was more remarkable in the endosperm halves than that in the embryonated halves.

Embryonated halves germinated more at 20° and 30°C with increasing of GA_3 concentration, the rate of which also slightly enhanced further with lengthening after-ripening period. Embryonated halves at 20°C were able to germinate even in the absence of GA_3 but no production of α-amylase did occur.

3. *Different temperatures during seed imbibition*

The effect of temperatures during seed imbibition prior to the α-amylase test was examined. In one group of 4 month-old seeds, the embryonated halves and endosperm halves were prepared, then placed on filter paper moistened with sterilized water in petri dishes and incubated for 3 days at 5°, 10°, 15°, 20°, 25° or 30°C. In the other group, intact seeds were first incubated at the different temperatures described above, then cut into halves. They were examined for the α-amylase production with or without GA_3 at 30°C for 2 days. As seen in Fig. 4-a, the embryonated halves produced a small amount of GA_3-induced α-amylase when imbibed at 5° to 15°C, but did not at the higher temperatures, being independent on timing of the cut. In contrast, the endosperm halves produced a great amount of GA_3-induced α-amylase at 5° to 15°C, but a smaller amount at 20° to 30°C. Especially remarkable was the decrease at 20° to 30°C at which temperatures they were imbibed before separation from the embryonated halves (Fig. 4-a, I).

Germination of the embryonated halves, regardless of excision time, decreased with the rise in the temperatures of imbibition.

A dramatic contrast in GA_3-induced α-amylase production in the endosperm halves prepared after imbibition was found between the low and high temperature, as seen in Fig. 4-a, I. Intact seeds of 6, 18 or 30 months old were imbibed either at 5° or 30°C for 0 to 5 days, then cut into two halves, and production of GA_3-induced α-amylase in the embryonated halves and endosperm halves was tested. The results are shown in Fig. 4-b. The endosperm halves of any age, imbibed at 5°C, produced α-amylase rapidly, reaching to some level in a few days. Six or 18 month-old endosperm halves, thereafter, produced a more amount of amylase, whereas 30 month-old endosperm halves produced a constant amount. In contrast, 6 and 18 month-old endosperm halves, imbibed at 30°C, produced a smaller amount of amylase with increasing imbibition period, whereas 30 month-old halves produced a constant amount of amylase. On the other hand, embryonated halves of any age produced only a small amount of amylase with minor change over the imbibition period, showing a slight increase with after-ripening period and the imbibition period at 5°C.

4. *Various surgical treatments to embryo and its around*

The effect of embryo on the GA_3-induced α-amylase was examined by surgical treatments given to the embryo in 6 month-old embryonated halves. In one group, the basal part of endosperm with the embryo, the scutellum with the embryo or the embryo alone was removed, respectively. In the other group, those excised tissues were placed again on the same cut site so that cut faces closely attached. These halves with embryo treated were subjected to the amylase test. As seen in Table 1, the embryonated halves excised embryo or its around region, and those placed again the excised embryo or its around region produced almost the same amount of α-amylase as the corresponding embryoless endosperm halves.

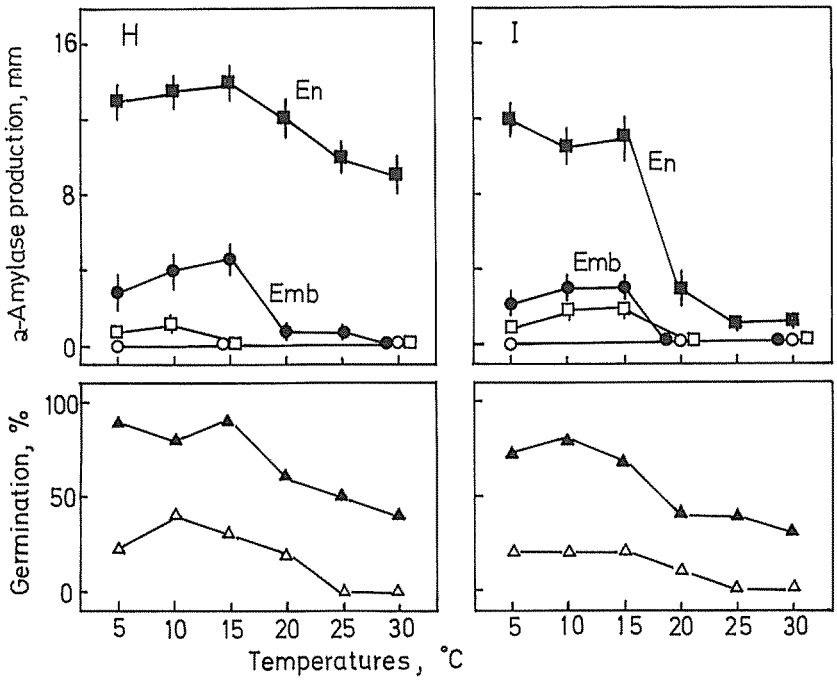

Fig. 4-a. α-Amylase production as affected by the imbibition temperature in embryonated endosperm halves (○, ●, Emb) and embryoless endosperm halves (□, ■, En) of 4 month-old *Avena fatua* seeds in the presence (●, ■, ▲) and in the absence of 1 ppm GA_3 (○, □, △). Germination percentage of the embryonated halves is shown by symbols (△, ▲). H; embryonated halves and embryoless endosperm halves were imbibed at different temperatures (5° to 30°C) for 3 days prior to the amylase test. I; intact seeds were imbibed, then the embryonated halves and the embryoless endosperm halves were prepared for the amylase test.

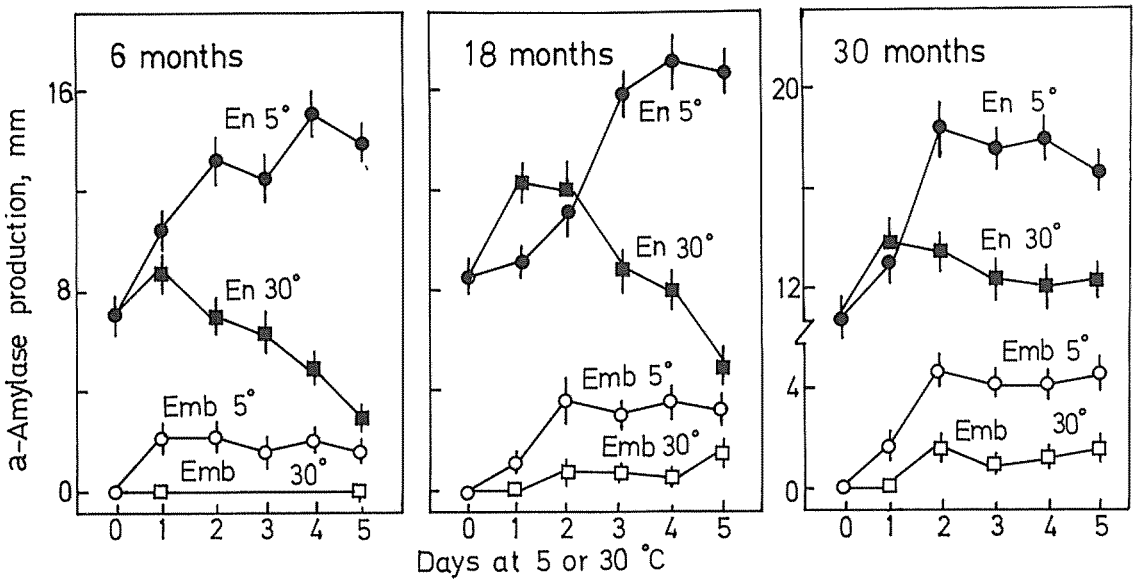

Fig. 4-b. α-Amylase production as affected by the imbibition temperature in embryonated endosperm halves (○, □, Emb) and embryoless endosperm halves (●, ■, En) of 6, 18 and 30 month-old *Avena fatua* seeds in the presence of 1 ppm GA_3. Embryonated halves and embryoless endosperm halves were prepared from intact seeds imbibed either at 5°C (○, ●) or 30°C (□, ■) for various days (0 to 5 days).

Further, the effects of embryos on the α-amylase production of notching with a razor blade about 0.4 mm in depth and pricking by needle to the same depth given at various sites of the embryo or its around were examined using 6 month-old embryonated halves. As seen in Table 2, these treatments both at the mid point of the embryo (Embryo) and at the tip of scutellum (Front) resulted in the same level of α-amylase production as that of the embryoless endosperm halves. The same treatments at the flanks (Flanks) and the midpoint between the scutellum tip and the cut line (Middle) had no effect on amylase production. Furthermore, various sites of the embryo or its around were touched with a burned needle. As seen in Table 3, this treatment to the embryo itself or the tip of scutellum resulted in α-amylase production, but the treatments at both sides of scutellum did not. Thus, the various surgical treatments applied to the embryo itself resulted in the α-amylase production, even though the germination did not occur.

5. *GA_3 or ABA through the endosperm*

Both the basal and the apical portions of 2 mm in length were excised from the intact seeds. The embryoless endosperm columns of 5 mm in length obtained were placed at the originally apical end of seeds on the gel. A small filter paper, 2 mm×2 mm, wetted with one μl of aqueous solution of GA_3 or ABA was placed on the top of the endosperm columns. The α-amylase producing action of GA_3 placed on the top was tested by the gel in the absence of GA_3. A dose of 10^{-3} μg GA_3 or more induced α-amylase production. The inhibitory action of ABA on the top for the GA_3-induced α-amylase production was examined by the gel in the presence of 1 ppm GA_3. A dose of 10^{-3} μg ABA or more inhibited GA_3-induced α-amylase production. The older the endosperms, the more the action of GA_3 or the less the action of ABA (these data not shown). Furthermore,

Table 1. Effects of removal and replacement of embryo regions or the embryo itself on GA_3-induced α-amylase production and germination of 6 month-old *Avena fatua* embryonated halves. The scutellum and embryo are symbolized by larger and smaller hexagons, respectively. Cut sites are indicated by dotted figures.

Treatment sites	α-Amylase production in mm	Germination in %
Embryonated halves	0	60
Removed basal part with embryo	11.0±1.3	—
Replaced the basal part	11.3±1.2	50
Removed scutellum with embryo	10.9±1.2	—
Replaced scutellum with embryo	10.5±1.4	40
Removed embryo alone	11.3±1.0	—
Replaced embryo alone	11.0±1.3	30
Embryoless endosperm halves	12.0±1.2	—

Table 2. Effects of notching and pricking the embryo and its around on GA_3-induced α-amylase production and germination of 6 month-old *Avena fatua* embryonated halves. The scutellum and embryo are symbolized by larger and smaller hexagones, respectively. Notched and pricked sites are indicated by short lateral lines and small open circles, respectively.

Treatment sites			α-Amylase production in mm	Germination in %
Embryonated hlaves			0	30
Notching	Embryo		11.2±0.93	—
	Front		10.9±0.75	30
	Flanks		0	25
	Middle		0	35
Pricking	Embryo		10.4±0.57	—
	Front		11.7±0.85	70
	Flanks		0	10
	Middle		0	20
Embryoless endosperm halves			12.5±0.53	—

Table 3. Effects of killing small areas of the embryo and its around on GA_3-induced α-amylase production and germination of 6 month-old *Avena fatua* embryonated halves. The scutellum and embryo are symbolized by larger and smaller hexagons, respectively. Killed by touching with a burned needle and the sites are indicated by small closed circles.

Treatment sites		α-Amylase production in mm	Germination in %
Embryonated halves.		0	20
Entire embryo		11.3±1.0	—
Prumule		11.7±1.0	—
Radicle		10.3±0.8	—
Tip of scutellum		11.6±1.2	10
Both sides of scutellum		0	20
Embryoless endosperm halves		11.0±0.9	—

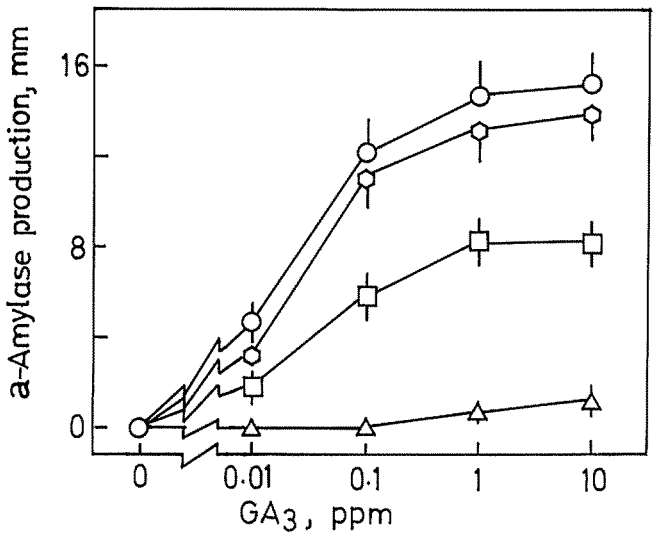

Fig. 5. Interaction between inhibitory action of 0 (○), 10^{-4} (○), 10^{-3} (□) and 10^{-2} μg ABA (△) applied to the top of embryoless endosperm coloumns prepared from 12 month-old *Avena fatua* seeds and α-amylase producing action of GA_3 at various concentrations applied to the base of the endosperm coloumns at the gel.

the interaction between the inhibitory action of ABA applied to the top of endosperm columns prepared from 12 month-old seeds and the inducing action of GA_3 present at the gel was examined. The experimental results are shown in Fig. 5. The α-amylase production by the GA_3 was inhibited by ABA at 10^{-3} μg or more.

IV. Discussion

Embryoless endosperms of many cereals, such as barley,[10,14] oats[9,12] and rice[5] produce α-amylase in response to exogenous GA_3. This is also true for those of *Avena fatua* (Figs. 2 and 3). However, α-amylase production in embryonated endosperms of *A. fatua* is always much lower than that in embryoless endosperms (Figs. 2 and 3). This lower production of α-amylase in the embryonated halves has not been found in other cereals, such as barley, wheat and rice, whose seeds are not dormant to germination (Ogawa, unpublished).

α-Amylase production in both the embryonated halves and embryoless endosperm halves increased gradually when after-ripening period was lengthened, but the increase of the amylase production in the embryonated halves was much lesser than that in the endosperm halves (Figs. 2 and 3). α-Amylase production by GA_3 in barley endosperm occurs in the aleurone layer cells[4,16]. Despite of the larger amounts of the aleurone layer in the embryonated endosperms such as the embryonated halves and the apical embryonated endosperms in the present *A. fatua,* comparing with that of corresponding embryoless endosperm, α-amylase production was always considerably lower in these embryonated endosperms (Figs. 2 and 3). This trend was independent on the time of cutting seeds, before or after imbibition (Figs. 4-a and b). These facts suggest that inhibitory factor(s) from the embryo exerts an inhibitory influence on the α-amylase production in the aleurone cells of endosperm.

Production of GA_3-induced α-amylase in both embryonated and embryoless halves increased at 5°C but

decreased at 30°C, at which temperatures intact seeds or seed halves were imbibed (Figs. 4-a and b). The inhibition of α-amylase production at the high temperature reduced when after-ripened period was increased (Fig. 4-b, 6 mon. vs 30 mon.). This suggests the occurrence of a promoting factor(s) in the embryo imbibed at low temperature and that of an inhibitory factor(s) at high temperature, the former(s) being increased and the latter(s) being decreased with the length of after-ripening period. The promotive factor(s) was replaced by GA_3 appliced to the top of embryoless endosperm column and the inhibitory factor(s) was by ABA. The ABA applied to the top of the endosperm column interacted with GA_3 applied to the base (Fig. 5). This evidence reemphasizes our idea stated previously that the dominant action of endogenous ABA in seeds after-ripened for only a few months is reduced by the interaction of endogenous GAs occurred in seeds after-ripened for many months[8].

So far, however, no apparent evidence of inhibitory factor(s) can be found by replacing the excised embryo region or the embryo itself on the cut site of embryoless endosperm (Table 1). The superficial scarification of the embryo (Table 2) as well as heat killing of the embryo itself (Table 3) could release the inhibitory effect from the embryo. We cannot explain exactly why these surgical treatments can completely release the inhibitory influence from the embryo, particularly, the treatments applied to the tip of the scutellum (Tables 2 and 3). These traumas might bring about an aerobic condition, which could then activate the amylase production by GA_3. Also, an embryo attenuated by such treatment would be unable to produce the unfavorable condition or factor(s) generated by the living embryo which inhibits GA_3-induced α-amylase production. Ogawa[8] has found that GA_3 can stimulate the germination of the isolated embryo *in vitro* even at 30°C, but cannot for the embryo enclosed in the seed tissue. This indicates a certain similarity in the action of GA_3 "at an aerobic state" between two events; 1) germination of the isolated embryos, and 2) α-amylase production in the endosperms with embryo removed or in those with embryo wounded. However, further studies on mechanism of inhibitory effect of the embryo on α-amylase production must be performed with regard to the actions of different external stimuli in relation to the actions of endogenous GAs and ABA in the embryo. Perhaps, the inhibitory influence of the embryo could be adaptable to produce not only the α-amylase but also other enzymes such as protease, ribonuclease and others in the seeds.

Incidentally, we usually observed that some embryonated endosperms germinated and produced the α-amylase, but sometimes others only germinated but did not produce the amylase (ref. Fig. 2; 18, 30 months, H, and Fig. 3; 20°C, 0, 0.1 ppm GA_3), or *vice versa* (ref. Tables 1–3, embryo treated). However, the GA_3-induced α-amylase production in both embryonated and embryoless endosperms increased more or less with lengthening after-ripening period (Figs. 2 and 3). Seed germination at the higher temperature also increased with lengthening after-ripening period[7]. Thus, the increase in the seed germination of *A. fatua* acconpanied with the after-ripening period must be involved with the increase in the α-amylase production in the endosperm.

References

1) CHING, T. M. Metabolism of germinating seeds. *In* Seed Biology 2. Ed. by T. T. Koslowski. pp. 103–218. Academic Press Inc. New York (1972).
2) CHRISPEELS, M. J. and J. E. VARNER. Inhibition of gibberellic acid induced formation of α-amylase by abscisin II. Nature, **212**: 1066–1067 (1966).
3) KAHN, A. A. and K. L. TAO. Phytohormones, seed dormancy and germination. *In* Phytohormones and Related

Compounds-A. Comprehensive Treatise, Vol. II. Ed. by Letham, Goodwin and Higgins, pp. 371–422. Elsevier, North-Holland Biomedical Press (1978).

4) MACLEOD, A. M. and A. S. MILLAR. Effects of gibberellic acid on barley endosperm. J. Inst. Brewing 68: 322–332 (1962).
5) OGAWA, Y. Amylase activity in rice endosperms as influenced by gibberellins, gibberellin-derivatives, kaurenoic acid and mevalonic acid with special reference to their growth promoting activity. Bot. Mag. Tokyo, 80: 27–32 (1967).
6) OGAWA, Y. New barley endosperm tests for gibberellin, paper disc methods. Bull. Fac. Agr. Mie Univ. 49: 315–320 (1975).
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) OGAWA, Y. Influences of cutting, gebberellin A_3 and abscisic acid on germination of *Avena fatua* seeds. Bull. Fac. Bioresources, Mie Univ. 8: 73–79 (1992).
9) OGAWA, Y. and K. KOSHIMIZU. Activities of 3, 13-dihydroxy-C_{20} gibberellins in endosperm tests of cereals. *In* Plant Growth Substances 1973, pp. 267–274. (Proc. 8th Inter. Con. Plant Growth Substances), Hirokawa Pub. Co. Tokyo (1974).
10) PALEG, L. G. Physiological effects of gibberellic acid. I. On carbohydrate metabolism and amylase activity of barley endosperm. Plant Physiol. 35: 293–299 (1960).
11) RADLEY, M. Site of production of gibberellin-like substances in germinating barley embryos. Planta, 75: 164–171 (1967).
12) SIMPSON, G. M. and J. M. NAYLOR. Dormancy studies in seed of *Avena fatua*. 3. A relationship between maltase, amylase and gibberellin. Can. J. Bot. 40: 1659–1673 (1962).
13) VARNER, J. E. Gibberellic acid control synthesis of α-amylase in barley endosperm. Plant Physiol. 39: 413–415 (1964).
14) YOMO, H. and H. IINUMA. Studies on the amylase activating substance. IV. On the amylase activating action of gibberellin. Hakko Kyokaishi, 18: 600–602 (1960) (In Japanese).
15) YOMO, H. and H. IINUMA. Studies on the amylase activating substance. V. Purification of the amylase activating substance in the barley malt and its properties. Hakko Kyokaishi. 18: 603–606 (1960) (In Japanese).
16) YOMO, H. and H. IINUMA. The enzymes of the aleurone layer of barley endosperm. Proc. Amer. Soc. Brew. Chem. 97–102 (1964).
17) YOMO, H. and IINUMA. Production of gibberellin-like substances in the embryo of barley during germination. Planta, 71: 113–118 (1966).

野性エンバク（*Avena fatua*）種子胚の胚乳におけるジベレリン A_3 誘導 α-アミラーゼ生成の抑制作用

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野性エンバク（*Avena fatua*）の種子（えい果）を横半分に切断して，胚のある側と胚のない側に分けて，ジベレリン A_3 (GA_3) 誘導 α-アミラーゼ生成を比較した。

1. 胚のある側の α-アミラーゼ生成は，胚のない側のアミラーゼ生成に比べて，著しく少なかった。
2. 胚のある側および胚のない側，それぞれの α-アミラーゼ生成は，後熟の進行にともない増加した。しかし，胚のある側の増加程度は，胚のない側に比べ著しく少なかった。
3. 完全種子を，5°C から 15°C で吸水した後，切断した胚のない側の α-アミラーゼ生成は多かった。これに反して，25°C あるいは 30°C で吸水した後，切断した胚のない側の α-アミラーゼ生成は，著しく減少した。
4. 胚のある側の胚直接に，あるいは胚盤の先きの部分に，小さい穴，または浅い切り込みをつけるか，または熱処理をすると，胚による α-アミラーゼ生成の抑制作用は完全に消失した。
5. 胚の抑制作用は，外生アブシジン酸 (ABA) の作用によって代替された。

上記の実験結果は，*A. fatua* 種子の胚は，胚乳における GA_3 誘導 α-アミラーゼ生成を抑制することを示している。胚による抑制作用，および胚あるいはその周辺の外科的処理によって，胚の抑制作用が消失する現象の生化学的機構をさらに研究する必要がある。