Bull. Fac. Bioresources, Mie Univ.
No. 9: 89–95
January 29. 1993

Seed Germination and α-Amylase Production in Endosperm of *Avena abyssinica*, *A. sativa* and *A. fatua*

Yukiyoshi OGAWA

Faculty of Bioresources, Mie University

Abstract

Avena abyssinica seeds of 4 months old after harvest germinated well at a temperature range from 15° to 30°C. *A. sativa* seeds of the same age germinated to a lesser degree at 25° and 30°C, but *A. fatua* did not at both temperatures and that of 16 months old never germinated at 30°C. These results indicate that *A. abyssinica* seeds are not dormant, those of *A. sativa* are median dormant and those of *A. fatua* are highly dormant.

α-Amylase production in half cut seeds, embryonated endosperms termed as embryonated halves and de-embryonated endosperms as endosperm halves, was examied in the absence or the presence of gibberellin A_3 (GA_3).

The embryonated halves of *A. abyssinica,* irrespective of age, produced a certain amount of α-amylase even in the absence of GA_3. The embryonated halves as well as the endosperm halves of this species produced a great amount of GA_3-induced α-amylase. On the contrary, the embryonated halves of *A. sativa* and *A. fatua* produced a less amount of GA_3-induced α-amylase than the corresponding endosperm halves, increasing in both halves with the lengthening of after-ripening period. Among the three species, *A. abyssinica* produced the greatest amount of α-amylase, *A. sativa* did a median amount and *A. fatua* the least amount.

This evidence indicates that behaviour of seed germination in each of these species is involved with the levels and the changes in the α-amylase production in the endosperms with the advancement of after-ripening.

Key words: α-Amylase production · *Avena abyssinica* · *A. fatua* · *A. sativa* · Seed germination

I. Introduction

Nishiyama and Inamori[3] have examined seed germination in many species of *Avena* of various months old after harvest and found no dormant period in *A. abyssinica,* the longest dormant period in *A. fatua* and a medium dormant period in *A. sativa,* cv. Victory, respectively.

A. fatua seeds have a typically dormant nature so that fresh seeds, one week after harvest, can barely germinate at 5° or 10°C but cannot germinate at any higher temperatures. The seeds become to germinate at the higher temperatures with the extension of after-ripened period. However, they still cannot germinate at 30°C even after they after-ripened for two years or longer[5].

On the other hand, α-amylase production has been induced by exogenous GA_3 in half cut seeds of *A. fatua.* However, the production of α-amylase in the embryonated half endosperms of this species was much less than that in the de-embryonated half endosperms, suggesting that some factor(s) occurring in the embryo inhibits

Accepted June 17, 1992

GA_3-induced α-amylase production in the endosperm[7].

In the present study, we studied seed germination at different temperatures for *A. abyssinica, A. sativa* and *A. fatua*. α-Amylase production in the embryonated and de-embryonated endosperms for the seeds of the three species either in the absence or the presence of GA_3 was also examined.

II. Materials and Methods

The seeds of *Avena abyssinica, A. sativa,* cv. Victory, and *A. fatua* were the gift from Emeritus Professor I. Nishiyama[2], Laboratory of Plant Genetics, Kyoto University. These species of oat plants were cultivated in open field every year. Matured *A. abyssinica* seeds (caryopsis) with hulls were harvested usually at the middle of May. Seeds of *A. sativa* and *A. fatua* were harvested at the middle of June. In seed germination test, entire seeds were sown on filter paper moistened with 5 ml distilled water in a flat petri dish and exposed to various temperatures at 15° to 30°C. The number of germinating seeds was recorded daily for several days and represneted as the percentage of the total number of seeds used.

To test α-amylase production, the intact seeds were cut laterally into two halves. The embryonated half endosperms were referred to embryonated halves and the de-embryonated half endosperms were as endosperm halves. α-Amylase production of these seed halves was examined according to the procedure previously reported by Ogawa[7]; five to 7 embryonated halves or endosperm halves sterilized were placed separately with the cut face on the agar gel containing 0.5% starch with or without 1 ppm GA_3 in flat petri dish of 9 cm in diameter, and incubated at 30°C for 3 days. The plates were flooded with iodine solution and starch was visualized. The mean diameter values of circular zones of starch digested by α-amylase produced by these seed halves was taken as an index of the α-amylase production. Two dishes with a total of 10 to 14 seed halves were used for each experiment. Also, the number of embryonated halves germinated during the α-amylase test was represented as a percentage of the total number of embryonated ones tested.

III. Results

1. *Seed germination*

The percentage of seed germination in *A. abyssinica, A. sativa* and *A. fatua* of 4 months old and *A. fatua* of 16 months old at various temperatures is represented in Fig. 1. At 15° and 20°C, seeds of *A. abyssinica, A. sativa* and the older *A. fatua* germinated to a considerable extent, but the younger seeds of *A. fatua* germinated to a much lesser extent. At 25° and 30°C, seeds of *A. abyssinica* germinated to a similar extent to those at 15° and 20°C, whereas seeds of *A. sativa* germinated to a lesser extent than those of *A. abyssinica*. The older seeds of *A. fatua* germinated to a lesser extent than *A. sativa* at 15° and 20°C and did not germinate at 30°C. The younger seeds of *A. fatua* seeds never germinated at all at 25° and 30°C.

2. *α-Amylase production*

Embryonated halves and endosperm halves prepared from various month-old seeds of *A. abyssinica, A. sativa* and *A. fatua* were subjected to the α-amylase test either in the absence or the presence of GA_3. The experimental results for the three species are represented in Fig. 2-a, b and c, respectively. The embryonated halves of *A. abyssinica,* irrespective of age, produced a certain amount of α-amylase even in the absence of GA_3,

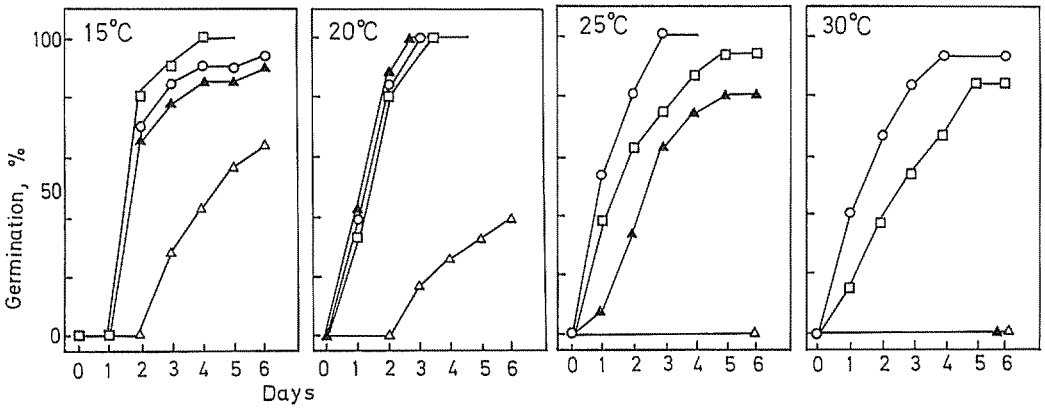

Fig. 1. Germination of 4 month-old seeds of *Avena abyssinica* (○), *A. sativa* (□), *A. fatua* (△) and 16 month-old seeds of *A. fatua* (▲) at 15°, 20°, 25° and 30°C.

but the endosperm halves did not. In the presence of GA_3, both the embryonated and the endosperm halves produced a great amount of α-amylase independently of their after-ripening period.

The embryonated halves of all ages germinated well in both the absence and the presence of GA_3.

The embryonated halves of 6 month-old *A. sativa* produced a small amount of α-amylase in the absence of GA_3, which increased slightly with the lengthening of after-ripening period. In the presence of GA_3, both the embryonated and endosperm halves produced a large amount of α-amylase, though the former's production was less than the latter's. The GA_3-induced α-amylase production in both the embryonated and endosperm halves also increased slightly with the lengthening of after-ripening period.

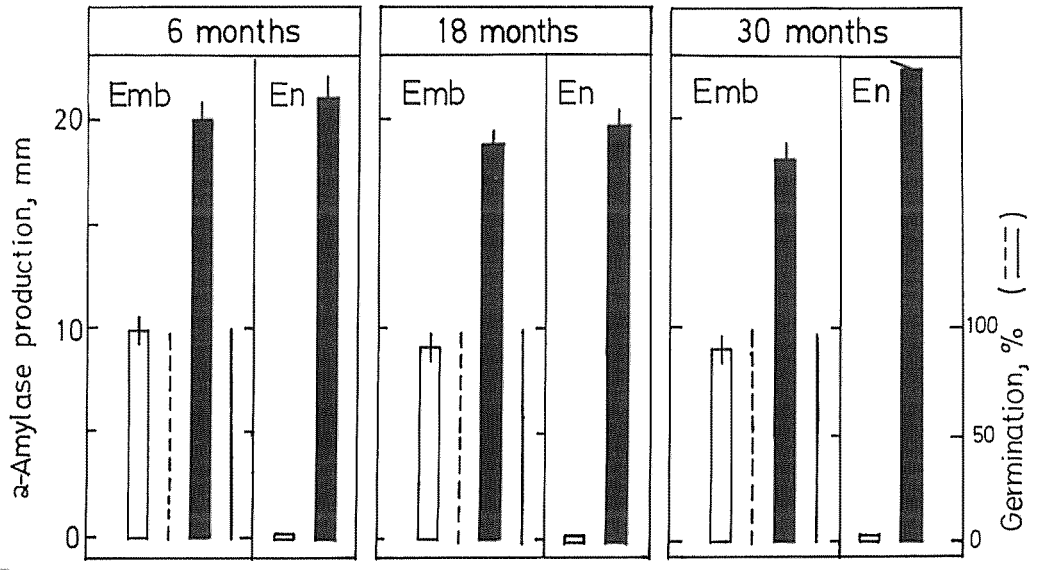

Fig. 2-a. α-Amylase production in 6, 18 and 30 month-old embryonated endosperm halves (Emb) and embryoless endosperm halves (En) of *Avena abyssinica* in the absence (□) and in the presence of GA_3 (■). Vertical bars on the histograms indicate standard errors. Germination percentages of the embryonated halves in the absence of GA_3 and those in the presence of GA_3 are shown by brocken and solid lines, respectively.

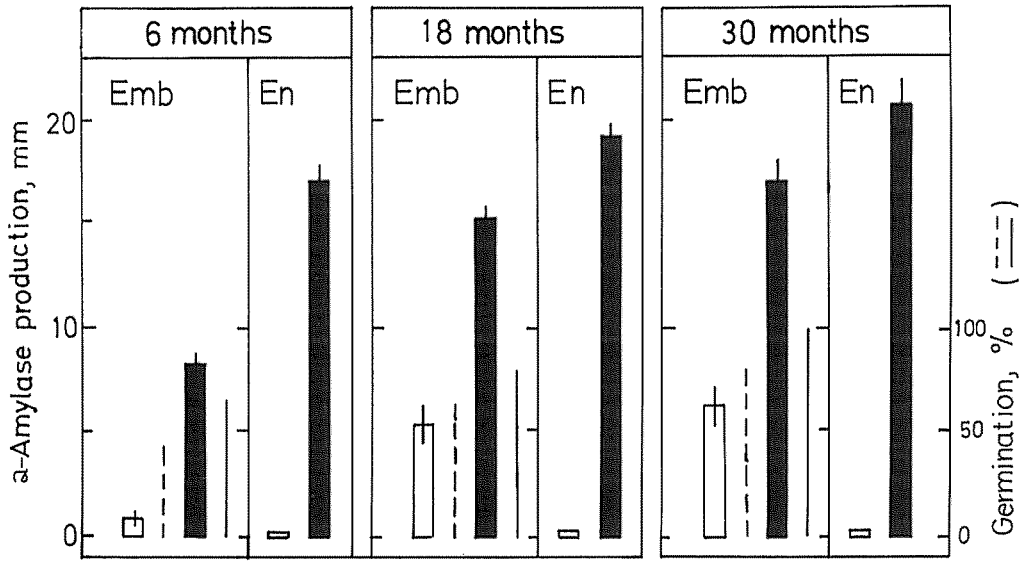

Fig. 2-b. α-Amylase production in 6, 18 and 30 month-old embryonated endosperm halves (Emb) and embryoless endosperm halves (En) of *Avena sativa* in the absence (□) and in the presence of GA_3 (■). Vertical bars on the histograms indicate standard errors. Germination percentages of the embryonated halves in the absence of GA_3 and those in the presence of GA_3 are shown by brocken and solid lines, respectively.

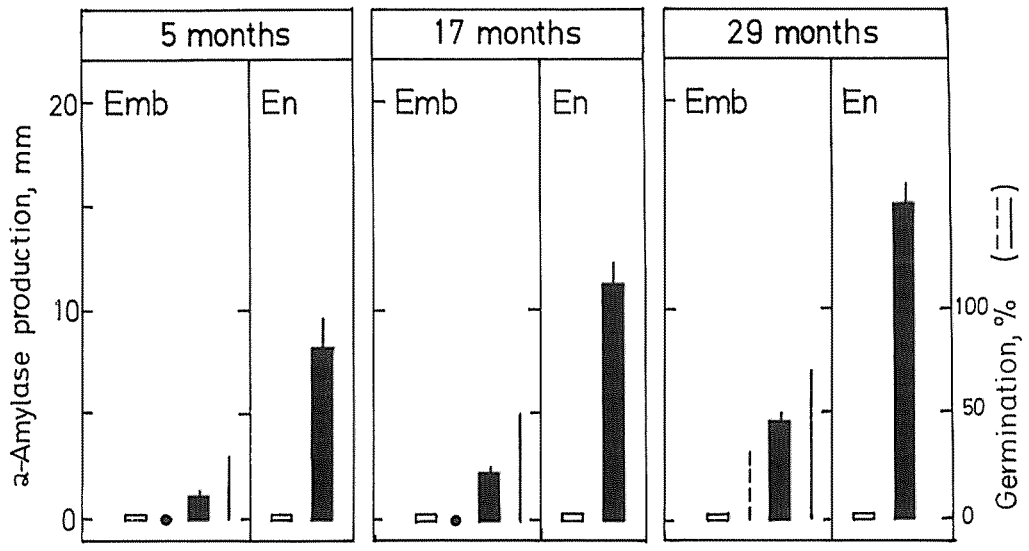

Fig. 2-c. α-Amylase production in 5, 17 and 29 month-old embryonated endosperm halves (Emb) and embryoless endosperm halves (En) of *Avena fatua* in the absence (□) and in the presence of GA_3 (■). Vertical bars on the histograms indicate standard errors. Germination percentages of the embryonated halves in the absence of GA_3 and those in the presence of GA_3 are shown by closed circles or brocken lines and solid lines, respectively.

The older the embryonated halves, then the more the germination in the absence of GA_3 as well as the presence of GA_3.

The embryonated halves of any old *A. fatua* did not produce α-amylase in the absence of GA_3, but did only a small amount of α-amylase in the presence of GA_3, being much less than the endosperm halves. The GA_3-induced α-amylase production in both embryonated and endosperm halves also increased with the length of after-ripening period, though being much less than that of *A. sativa.*

Both 5 and 17 month-old embryonated halves of *A. fatua* did not germinate in the absence of GA_3, and in the presence of GA_3 the older the embryonated halves, the more the germination, though being much less than those of *A. sativa.*

IV. Discussion

A. abyssinica seeds germinated well at temperatures ranging from 15° to 30°C. Both *A. sativa* and the older *A. fatua* seeds germinated well at 15° and 20°C as *A. abyssinica* did, but *A. sativa* seeds hardly germinated at 25° and 30°C. Neither the older seeds nor the younger seeds of *A. fatua* germinated at 30°C (Fig. 1). On occasion, some viviparous seeds were found on mature panicles of *A. abyssinica* grown in fields at rainy season. They have never been found in both *A. sativa* and *A. fatua.* The above evidence confirms the early report of Nishiyama and Inamori[3] that *A. abyssinica* seeds are not dormant, *A. sativa* seeds are median dormant and *A. fatua* seeds are highly dormant.

Incidentally, the germination of *A. abyssinica* seed was not inhibited by the application of a gibberellin-biosynthesis inhibitor, CCC, which potentially inhibited the germination of *A. fatua* seeds[6] (data not shown). This suggests the pre-existence of endogenous gibberellins (GAs) in the embryo of *A. abyssinica* and/or the much higher capacity of the embryo of this species for synthesizing endogenous GAs, which may result in the minimum inhibition of germination by CCC.

On the other hand, the embryonated halves of *A. abyssinica* produced a certain amount of α-amylase with high germination rate even in the absence of exogenous GA_3 (Fig. 2-a). The embryoless endosperm cut at near the embryo produced α-amylase considerably (data not shown). This evidence suggests that this species produces much more α-amylase near around the embryo, which might be induced by the endogenous GAs occurred in the embryo. The embryonated halves as well as the endosperm halves of *A. abyssinica* produced also a great amount of GA_3-induced α-amylase (Fig. 2-a), suggesting that the embryo of this species does not inhibit α-amylase production in the endosperm but rather promotes it. In contrast, the embryonated halves of *A. sativa* and *A. fatua* produced a smaller amount of GA_3-induced α-amylase than the corresponding embryoless endosperm halves, in consistence with Ogawa's report for *A. fatua*[7] (Figs. 2-b and c). This suggests that the embryo of these species inhibits the α-amylase production in the endosperm.

GA_3-induced α-amylase production in *A. abyssinica* was at a high level in both the young and old seed halves (Fig. 2-a), whereas that in *A. sativa* and *A. fatua* was at a low level in young seed halves, increasing with the lengthening of after-ripening period (Fig. 2-b and c). Thus, the degree of seed dormancy in germination of these species parallels the level of their α-amylase production in the endosperm (Fig. 1 vs Figs. 2-a, b and c). In *A. sativa* and *A. fatua,* the germination rate and α-amylase production in the embryonated halves was also stimulated by exogenous GA_3 (Figs. 2-b and c). The characteristics of seed germination and α-amylase production in the three species might be a reflection of the interaction between the induction by GAs and

inhibition by abscisic acid (ABA), both occurring in the embryo[6,7].

The germination ability of *A. abyssinica* seeds at higher temperatures, which indicates the lack of seed dormancy, may be a consequence of an ecological adaptation of this species to the native, tropical drier steppes in Ethiopia[1,4,9]. *A. abyssinica,* a tetraploid (4x, AsB) would be evolved from *A. barbata* (4x, AsB) which would be a combination of *A. hirtula* (2x, As) and an undetermined species (2x, B) (I. Nishiyama, personal communication). On the other hand, many hexaploid *Avena* species including *A. sativa* and *A. fatua* are variously dormant[3]. *A. sativa* (6x, ACD) would have evolved from *A. fatua* (6x, ACD)[4]. *A. fatua* grows as a weed in the Pamirs[1,4,9] and in Canada[8] which are cool lands. It is of interest to study further the behavior of seed germination in many native *Avena* species in relation to the ability to produce α-amylase in the endosperm, and to the amounts of endogenous GAs and ABA in the embryo.

Acknowledgments

The author thanks Emeritus Professor I. Nishiyama of Kyoto University for his gift of *Avena* seeds and his helpful discussion on this manuscript.

References

1) KIHARA, H. and M. NAGAO. Oats. *In* Cytogenetics of Cereals, pp. 89–93. Yokendo, Tokyo (1933) (In Japanese).
2) NISHIYAMA, I. Interspecific cross-incompatibility system in the genus *Avena.* Bot. Mag. Tokyo 97: 219–231 (1984).
3) NISHIYAMA, I. and Y. INAMORI. Length of dormant period in seeds of *Avena* species. Japan. J. Breeding, **16**: 1–4 (1966). (In Japanese with English summary)
4) MELZEW, A. T. Wild and cultivated oats Sectio *Euavena* GRISEB. Bul. Appl. Bot. Genetics and Plant Breeding. Suppl. 38 (1930). Cited from Kihara, H. and M. Nagao (1).
5) 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).
6) OGAWA, Y. Influences of cutting, gibberellin A_3 and abscisic acid on germination of *Avena fatua* seeds. Bull. Fac. Bioresources, Mie Univ. 8: 73–79 (1992).
7) OGAWA, Y. Inhibitory influence of the embryo on gibberellin A_3-induced α-amylase production in endosperm of *Avena fatua* seed. Bull. Fac. Bioresources, Mie Univ. 9: 77–88 (1993).
8) POEHLMAN, J. M. Breeding oats. *In* Breeding Field Crops, pp. 398–401. AVI Publishing Comp. INC. Westport, Connecticut (1987).
9) ZEVEN, A. C. and P. M. ZHUKOVSKY. Dictionary of cultivated plants and their centres of diversity. Centre. Agr. Pub. Doc. Wageningen (1975).

Avena abyssinica, A. sativa, および *A. fatua* の種子発芽と胚乳における α-アミラーゼ生成

小 川 幸 持
三重大学生物資源学部

エンバク属，*Avena abyssinica* (4x, AsB)，*A. sativa* cv. Victory (6x, ACD)，および *A. fatua* (6x, ACD) の収穫後4か月の種子（えい果）の各種温度における発芽を比較した。

A. abyssinica は，15°C から 30°C の温度でよく発芽した。*A. sativa* の発芽は，15°C と 20°C でよく発芽したが，25°C と 30°C では減少した。また，*A. fatua* は 25°C と 30°C で発芽しなかった。この実験結果は，*A. abyssinica* の種子は休眠性がなく，*A. sativa* は中程度の休眠性を，*A. fatua* は深い休眠性を示している。

この3種の種子を横半分に切断して胚のある側と胚のない側に分けて，外生ジベレリン A_3 (GA_3) の有る無しのそれぞれの場合の α-アミラーゼ生成を，後熟期間との関連で調べた。

A. abyssinica の胚のある側は，GA_3 がなくとも α-アミラーゼを生成した。胚のある側も胚のない側も，GA_3 誘導 α-アミラーゼ生成は，後熟期間に関係なく多かった。これに反して，*A. sativa* と *A. fatua* の胚のある側の GA_3 誘導 α-アミラーゼ生成は，胚のない側に比べ少なかった，それぞれの側の GA_3 誘導 α-アミラーゼ生成は，後熟期間に伴い増加した。この3種の間で，*A. abyssinica* のアミラーゼ生成が最も多く，*A. fatua* が最も少なく，*A. sativa* は両者の中間程度であった。

上記の実験結果は，*A. abyssinica* の胚は，胚乳の GA_3 誘導 α-アミラーゼ生成に影響しないことを示している。これに対して，*A. sativa* の胚は，*A. fatua* の場合と同様に，胚乳の 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。上の各種エンバク属のの種子の発芽休眠性の違いは，胚乳における α-アミラーゼ生成の多少，あるいは後熟に伴う α-アミラーゼ生成の変化と関係があることを示唆している。