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Interspecific Difference in α-Amylase Production by the Endosperm in the Genus *Avena*

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Abstract

Seeds of different diploid, tetraploid and hexaploid *Avena* species, stored for eight to nine months after harvesting, were cut transversely into two pieces at three different distances from the base of embryo attachment. Embryonated and embryoless endosperm pieces of three different sizes thus prepared were compared for the ability to produce α-amylase in the absence and presence of gibberellin A_3 (GA_3). The ability of the endosperm for α-amylase production was estimated by the activity of α-amylase exuded to an agar medium from the cut surface of an endosperm during three days incubation at 30℃ in the dark.

The embryonated endosperms cut near the embryo produced a large amount of α-amylase and those cut at the middle or near the tip end produced a smaller amount of α-amylase. The same trend was observed in all species.

The embryonated endosperms of all diploid species produced a relatively large amount of α-amylase, in spite of their small size. A large amount of α-amylase was produced by the embryonated endosperms of species such as tetraploids, *A. barbata*, *A. vaviloviana* and *A. abyssinica*, hexaploids, *A. byzantina*, *A. sativa* and *A. nuda*, all of which having no dormancy or released from the secondary dormancy. While, a small or negligible amount of α-amylase was produced by the embryonated endosperms of *A. magna*, *A. murphyi*, *A. fatua* and *A. sterilis*, all of these species being still in secondary dormancy.

On the other hand, the embryoless endosperms produced no α-amylase in all species with one exception. Namely, the embryoless endosperms of *A. ventricosa* produced a lot of α-amylase regardless of their size.

Addition of GA_3 to the medium caused an increase in α-amylase production in both the embryonated and embryoless endosperms of any size. This effect of GA_3 was less pronounced in the embryonated than embryoless endosperms. The same trend was observed in all species.

These results are discussed in relation to genealogy in the genus *Avena*, secondary dormancy and the physiological role of embryos of all *Avena* species as well as that of endosperm of *A. ventricosa* in α-amylase production.

Key words: α-Amylase production • *Avena* species • Embryo • Endosperm

Introduction

Previously, OGAWA[1] reported that transversely cut embryonated half seeds of *Avena abyssinica* that

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were not dormant produced a considerable amount of α-amylase without GA_3. While, the embryonated half seeds of *A. fatua* being in a secondary dormancy at 30℃ produced only a small amount of α-amylase even in the presence of GA_3, and α-amylase production by GA_3 was lesser in the embryonated than embryoless half seeds[6,7]. We also found[8] that *A. ventricosa*, *A. abyssinica*, *A. byzantina* and *A. sativa* did not show a secondary dormancy in seed germination, while *A. hirtula*, *A. wiestii*, *A. damascena*, *A. magna*, *A. murphyi*, *A. fatua* and *A. sterilis* were characterized by a deep secondary dormancy. It has been well documented that scutellar tissues produce α-amylase in imbibed barley seeds in the initial step of their germination[1,3,9,11], and that α-amylase production is induced by GA_3 in the aleurone layers of barley endosperm[3,12,14]. Thus, it seems likely that the ability of the endosperm to produce α-amylase upon imbibition and/or the responsiveness of α-amylase producing system in the endosperm to GA_3 are implicated in the interspecific difference among the genus *Avena* in the degree of their secondary dormancy. The purpose of the present study was to investigate the difference among different diploid, tetraploid and hexaploid *Avena* species in the ability of endosperm to produce α-amylase in relation to the growth of seedlings from embryos and the response to exogenous GA_3.

Materials and Methods

Seeds (Caryopsis) of different *Avena* species (diploids; *A. hirtula*, *A. wiestii*, *A. strigosa*, *A. clauda*, *A. pilosa*, *A. ventricosa*, *A. longiglumis* and *A. damascena*, tetraploids; *A. barbata*, *A. vaviloviana*, *A. abyssinica*, *A. magna* and *A. murphyi*, hexaploids; *A. fatua*, *A. sterilis*, *A. byzantina*, *A. sativa* var. Aurora and Holdeu and *A. nuda*), stored at room temperature for eight to nine months, were cut transversely into two pieces at different distances from the base of embryo attachment, as shown in Fig. 1. Both embryonated and embryoless endosperm pieces obtained by cutting the seeds near the scutellum are designated as B pieces, those at the middle as M and those near the apical tip as A pieces, respectively. Therefore, the size of embryonated B endosperm pieces was the smallest and that of A pieces was the largest, and the reverse was the case for the embryoless endosperm pieces.

The embeyonated and embryoless endosperm pieces prepared as above were sterilized with 70% ethanol and 1% sodium hypochlorite, and washed thoroughly with sterilized water. Ten ml of a hot solution containing 0.5% starch, 1% agar and 1 ppm GA_3 was poured into a flat petri dish of 9 cm in diameter to create a solidified gel medium of 0.5 cm in depth. The control medium without GA_3 was also prepared. Five or six embryonated and embryoless endosperm pieces were placed on the medium so as to keep their cut surface closely contact to the medium. They were then incubated at 30℃ in the dark for three days, after which the activity of α-amylase exuded from the endosperm pieces to the medium was measured. Iodine solution of 10^{-3} N was poured to the surface of the medium to stain

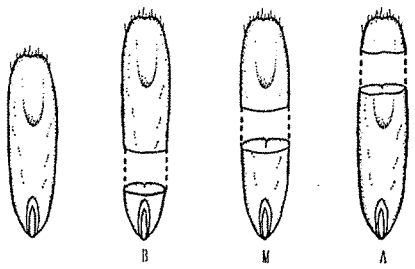

Fig. 1. Schematic presentation of cutting site of *Avena* seed. Intact seeds (lefmost) were cut at various sites to make embryonated and embryoless B, M and A pieces of different size.

unhydrolized starch, and the diameter of unstained circular areas around the endosperm piece placement was measured with milimeter section paper laid beneath the dish. The mean diameter of five to six endosperm pieces per dish was presented as an index of α-amylase production per endosperm piece. The length of roots and coleoptiles developed from embryos was also measured, and the sum of roots and coleoptile lengths was used as a growth parameter of seedlings.

Results

Diploids

The amount of α-amylase produced in the embryonated and embryoless endosperm pieces of diploid species cut at various sites in the absence and presence of GA_3 is shown in Fig. 2. Growth of seedlings is also shown in the same figures.

Without GA_3, the embryonated B endosperm pieces produced the largest amount of α-amylase, while the A pieces produced the least amount of α-amylase in most diploid species. In *A. pilosa* and *A. ventricosa*, however, the amount of α-amylase produced in the M and A pieces was not different from that in the B pieces. Seedlings were larger in the M and A pieces than those in the B pieces in all diploid species. The embryoless endosperm pieces in general did not produce α-amylase without added

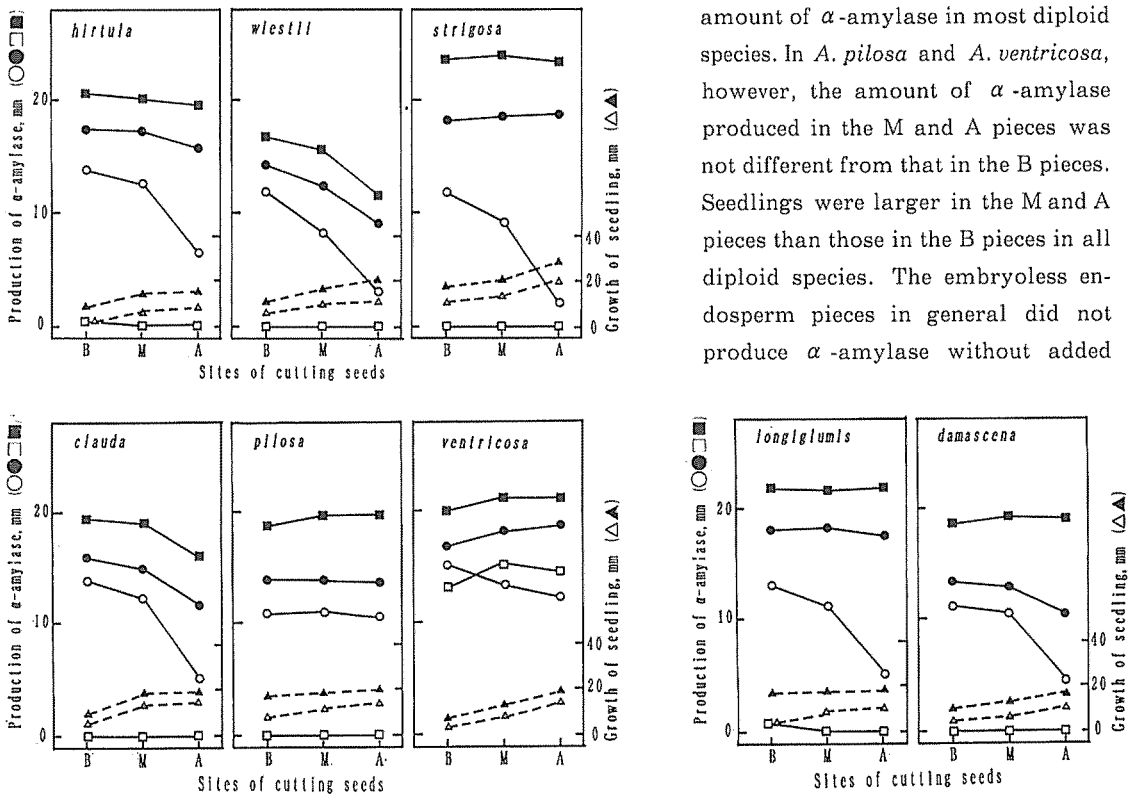

Fig. 2. Production of α-amylase in embryonated (circle) and embryoless endosperms (square) cut at basal (B), middle (M) and apical site (A) and growth of seedling (triangle) in different diploid *Avena* species, *A. hirtula*, *A. wiestii*, *A. strigosa*, *A. clauda*, *A. pilosa*, *A. ventricosa*, *A. longiglumis* and *A. damascena*. Open and closed symbols show the absence and presence of GA_3, respectively.

GA_3, regardless of the site of endosperm cut. However, any piece of embryoless endosperms of *A. ventricosa* was able to produce a lot of α-amylase.

GA_3 stimulated production of α-amylase in both the embryonated and embryoless endosperms of any size, and seedling growth as well in all diploid species. The stimulatory effect of GA_3 was lesser in the embryonated than in the embryoless endosperms.

Tetraploids

The amount of α-amylase produced in the embryonated and embryoless endosperm pieces of tetraploid species cut at various sites in the absence and presence of GA_3 is shown in Fig. 3. Growth of seedlings is also shown in the same figures.

Without GA_3, the embryonated B endosperm pieces produced a larger amount of α-amylase than the M and A pieces in all tetraploid species. Conversely, growth of seedlings was greatest in the A pieces and least in the B pieces. The embryonated endosperm pieces of *A. abyssinica* produced a comparatively large amount of α-amylase, while those of *A. magna* and *A. murphyi* produced a very small amount of α-amylase and a negligible amount in the M and A pieces. The embryoless endosperm pieces of any size did not produce α-amylase without added GA_3 in all tetraploid species, although the B pieces of *A. abyssinica* produced a small amount of α-amylase.

Inclusion of GA_3 in the medium resulted in an increase in α-amylase production in both the embryonated and embryoless endosperm pieces and in seedling growth as well in all tetraploid species. However, in *A. magna* and *A. murphyi* the stimulatory effect of GA_3 was very small in α-amylase production by the embryonated M and A pieces.

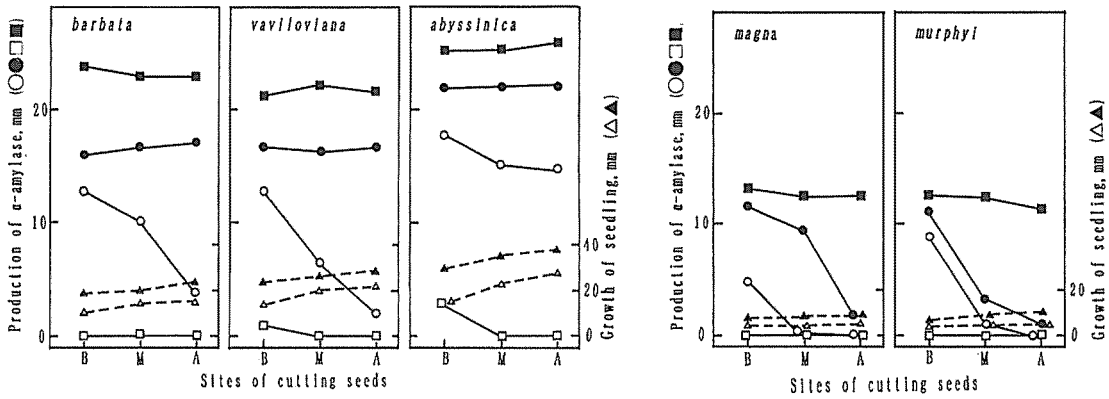

Fig. 3. Production of α-amylase in embryonated (circle) and embryoless endosperms (square) cut at basal (B), middle (M) and apical site (A) and growth of seedling (triangle) in different tetraploid *Avena* species, *A. barbata*, *A. vaviloviana*, *A. abyssinica*, *A. magna* and *A. murphyi*. Open and closed symbols show the absence and presence of GA_3, respectively.

Hexaploids

The amount of α-amylase produced in the embryonated and embryoless endosperm pieces of hexaploid species cut at various sites in the absence and presence of GA_3 is shown in Fig. 4. Growth of seedlings is also shown in the same figures.

Without GA_3, the embryonated endosperm of *A. fatua* and *A. sterilis* produced a small amount of α-amylase in the B pieces and only a trace amount in the M and A pieces. In contrast, the embryonated endosperms of *A. byzantina* and the two varieties of *A. sativa* produced a large amount of α-amylase regardless of the piece size, although the embryonated pieces of *A. nuda* produced a somewhat smaller amount. Seedlings of these species of any size also showed substantial growth. The embryoless endosperm pieces produced only a small or negligible amount of α-amylase in all hexaploid species.

GA_3 stimulated α-amylase production in both the embryonated and embryoless endosperm pieces of all hexaploid species; to a lesser extent in the former endosperms. Particularly, in the embryonated M and A pieces of *A. fatua* and *A. sterilis* the stimulatory effect of GA_3 was very small or negligible in both α-amylase production and seedling growth.

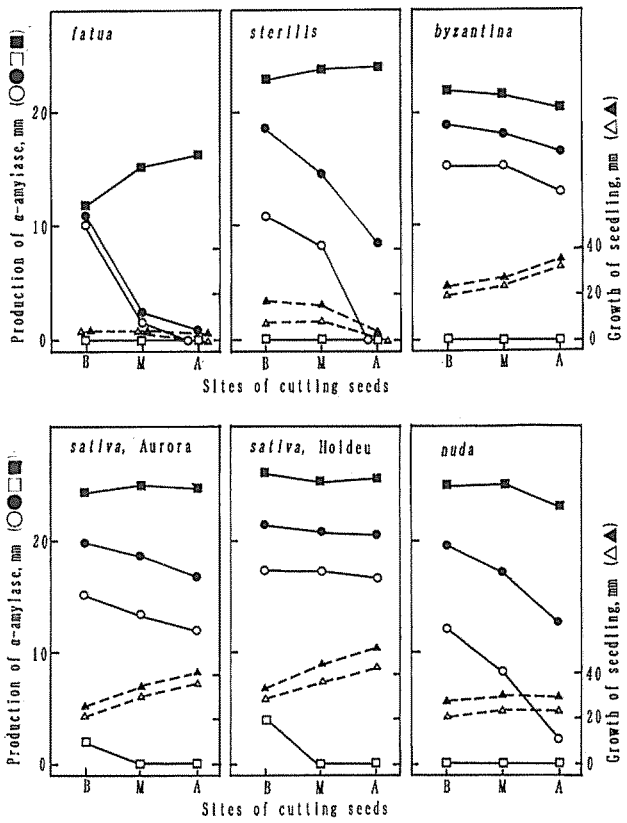

Fig. 4.
Production of α-amylase in embryonated (circle) and embryoless endosperms (square) cut at basal (B), middle (M) and apical site (A) and growth of seedling (triangle) of different hexaploid *Avena* species, *A. fatua*, *A. sterilis*, *A. byzantina*, *A. sativa*, Aurora, *A. sativa*, Holdeu and *A. nuda*. Open and closed symbols show the absence and presence of GA_3, respectively.

Discussion

The present study showed that the embryonated endosperms cut near the embryo (B pieces) produced a larger amount of α-amylase compared to those cut more distantly from the embryo attachment (M and A pieces) in all *Avena* species tested. This difference may be attributable to the distance from the embryo to the site of endosperm cut; in the smallest B pieces α-amylase produced in the scutellum tissue adjacent to embryos can be most readily exuded out of the endosperm pieces. A possibility that water influx from the cut surface to near embryos in the A pieces was insufficient to induce the enzyme production seems most unlikely, because the growth of seedlings in this pieces was always greater than that in the B pieces. The difference of seedling growth in the different piece size was probably caused by the difference in mobilizable amount of carbohydrate reserves to the embryo.

Generally, α-amylase production by the embryonated endosperms seems to be associated with the degree of the secondary dormancy of embryos, which is estimated, in most cases, by the extent of seed germination as well as the development of seedlings. All diploid species seeds released from the secondary dormancy were quite active in α-amylase production, irrespective of their small size[8]. A greater amount of α-amylase was produced in the species that were non-dormant or released from the secondary dormancy such as *A. barbata*, *A. vaviloviana*, *A. abyssinica*, *A. byzantina*, *A. sativa* and *A. nuda*; while the species that seemed to remain still dormant produced a smaller amount of α-amylase, as seen in *A. magna*, *A. murphyi*, *A. fatua* and *A. sterilis*. The genome AC, which *A. magna* and *A. murphyi* carry, seems to contribute to the low ability of α-amylase production in *A. fatua* and *A. sterilis* that carry the ACD genome. A close relationship between the above two groups of the species has also been proposed in the degree of secondary dormancy of the seeds[8].

As a whole, it can be concluded that the endosperms with embryos produced a larger amount of α-amylase, while those without embryos were able to produce a small or negligible amount of α-amylase. These results suggest that the embryo has a role in α-amylase production in the endosperm.

Exogenous GA_3 stimulated α-amylase production in both the embryonated and embryoless endosperms. It seems possible that endogenous gibberellins (GAs) in embryos is implicated in stimulating effect on α-amylase production in the endosperm[10,13]. Certainly, α-amylase production in the embryonated pieces of *A. abyssinica* and *A. sativa* was blocked by GAs biosynthesis inhibitor, uniconazole (data not shown).

A. ventricosa was unique in α-amylase production, apparently depending neither on the embryo nor on exogenous GA_3. α-Amylase was produced even in the embryoless endosperms separated from starchy reserves without added GA_3. Production of α-amylase by the embryoless ones was not reduced by uniconazole so much as in the embryonated ones (data not shown). These evidences strongly suggest the possibility that endogenous GAs accumulated in the aleurone cells during seed development have remained to be active in dried seeds.

GA_3-induced α-amylase production was always lesser in the embryonated endosperms than in embryoless ones in all *Avena* species tested. The reduction of response to GA_3 in α-amylase production was most marked in the embryonated endosperms of the species that seemed to be still secondary dormant, as previously reported for *A. fatua*[6,7]. These evidence suggests that GA_3-induced α-amylase production in the endosperm is inhibited by the presence of embryos, and the extent of this effect of

embryos varies with the species. OGAWA found[6] that the inhibitory influence of embryos in *A. fatua* was eliminated by heat killing the embryo or scutellum, and suggested that the intact embryo part generates substances that cause inhibition of α-amylase production induced by GA_3 in the endosperm.

There is much evidence that endogenous abscisic acid (ABA) is involved in the induction and maintenance of seed dormancy in different plant species[4] and that ABA is a potent inhibitor of α-amylase production by GA_3 in barley aleurone layer cells[2]. In *A. fatua* also, ABA inhibited germination of embryonated half seeds[5], and also α-amylase production by GA_3 in the embryoless endosperm halves[6]. These evidence suggests that embryos of *Avena* seeds exert dual functions, promotion or inhibition, on the production of α-amylase in the endosperm via the actions and/or the interactions of endogenous GAs and ABA. Presumably, the relative strength of these dual functions of the embryo could control a turning point of seeds in all *Avena* species to the germination or the maintenance in a dormant state.

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エンバク属の種子胚乳におけるα－アミラーゼ生産の種間差異について

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収穫後8－9ヶ月経過した2倍種，4倍種および6倍種のエンバク属の各種の種子（えい果）を胚に近い基部，中央部あるいは先端部で横に切断して，胚が在る胚乳片と胚の無い胚乳片に分けた．この胚乳片によるα-アミラーゼの生産を種間差異および胚の休眠との関連について調査した．

胚が在る胚乳片は，一般に基部で切断した胚乳片は最も多量のアミラーゼを生産し，先端部からの胚乳は最少の生産であった．

2倍種の各種は休眠が終っていて，小さい胚乳片にもかかわらず多量のアミラーゼを生産した．休眠がないか，終っている4倍種の *A. barbata*, *A. vaviloviana*, *A. abyssinica*, 6倍種の *A. byzantina*, *A. sativa*, *A. nuda* は多量のアミラーゼを生産した．他方，休眠がまだ残っている4倍種の *A. magna* と *A. murphyi*, 6倍種の *A. fatua* と *A. sterilis* は少量しか生産しなかった．

胚が無い胚乳片は，いずれの種もアミラーゼを殆ど生産しなかった．しかし，*A. ventricosa* は多量のアミラーゼを生産した．

GA_3の供与は，胚の存否に関係なくアミラーゼ生産を著しく促進した．しかし，胚が存る胚乳片における促進作用は胚が無い胚乳片よりも常に劣った．

これらの実験結果から，エンバク属の種の系統，休眠性，胚乳のアミラーゼ生産に及ぼす胚の促進と抑制の二面的な作用，*A. ventricosa* の胚乳のアミラーゼ生産の特性について考察した．

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