

## Abortive seed development in common bean (*Phaseolus vulgaris* L.)

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**Introduction.** Embryo defective mutants in *Phaseolus vulgaris* obtained by Ethyl methanesulfonate (EMS) induction were studied to determine the structural and molecular basis of seed abortion in this species. In the wild-type, the classical pattern of seed development showed coordinated differentiation of the embryo proper, suspensor, endosperm tissue, and seed coat. On the contrary, EMS mutant showed disruption in the normal seed development leading to embryo abortion.

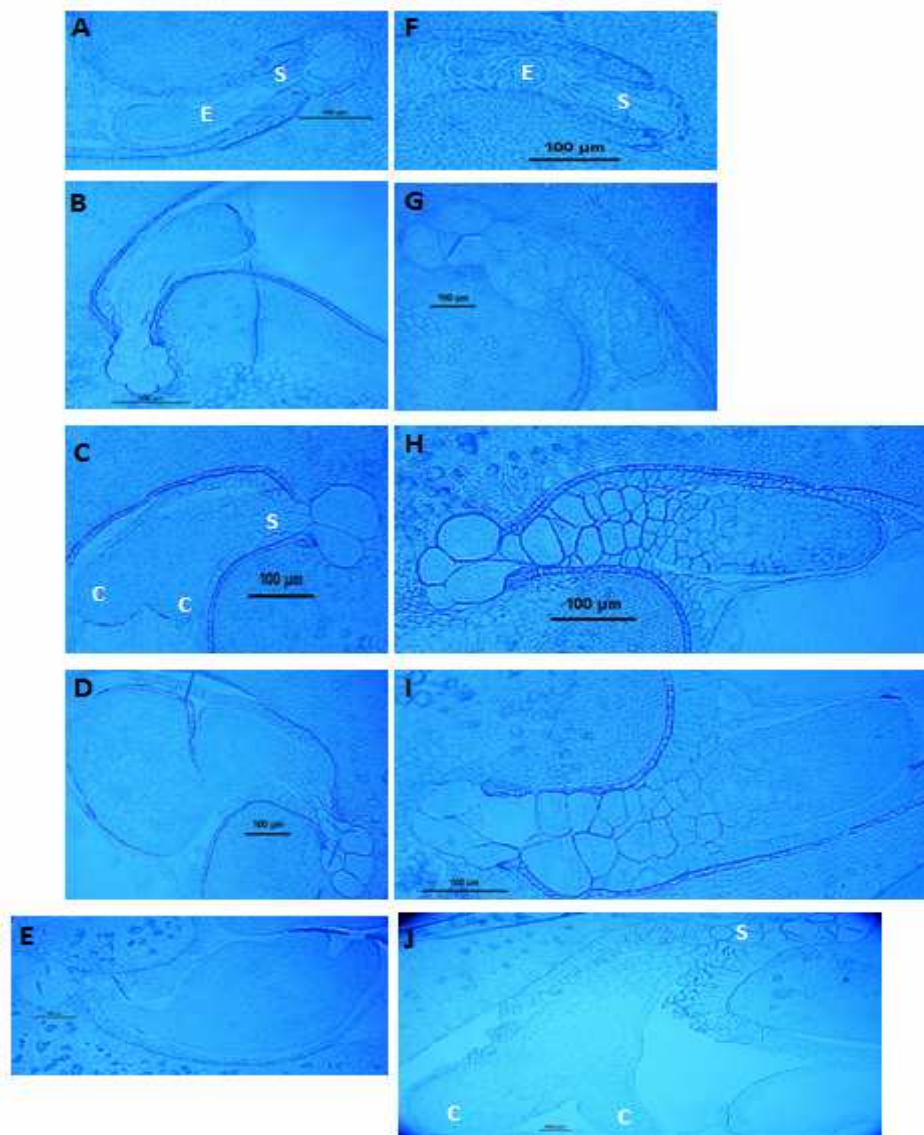
Most studies are limited to the early stages of embryo development (1). Little is known concerning the maturation process of the embryo or seed development. The present study was therefore undertaken firstly to detail the structural pattern of embryo development and secondly to investigate the structural basis of embryo abortion in common bean.

**Material and methods.** The wild-type and mutant EMS (genotype BAT93) seeds were freshly harvested and eventually nicked with a scalpel to facilitate penetration of fixing solutions. Seeds were then embedded in Technovit 8100 resin for two days at 4°C. Sections 3 µm thick were cut on a Zeiss HM 360 microtome fitted with a tungsten-carbide knife. They were stained with an adapted Toluidine blue O and viewed with a Nikon Eclipse E800 fluorescence microscope.

**Results and discussion.** An embryo-defective mutation was identified by screening plant lines treated with EMS (2). EMS mutant embryos development was considerably delayed compared with wild-type embryos. Shortly after fertilization in wild-type, the embryo begins to develop. Approximately 3 days after fertilization (DAF), the embryo reaches the early globular stage of development (Fig 1A). Approximately 8 DAF, the embryo transform into a heart-shaped embryo with the formation of cotyledons (Fig 1C). Cotyledons expand rapidly by vacuolation and gradually extend to produce a torpedo-shaped embryo (Fig 1D). About 12 days after fertilization, the cotyledons continue to expand with a well-defined embryo axis (Fig1E). In EMS mutant (F2 B52 1.1.14), the first stages of seed development are similar to the wild-type genotype. At 3 DAF, embryos are at the early globular stage (Fig 1F). The tissue organization of the EMS mutant embryo appeared to be normal at this early stage of embryogenesis. At 8 DAF EMS mutant embryos did not exhibit the characteristic heart shape and appeared elongated (Fig 1H). Abnormalities begin to appear in the late heart or early torpedo stage. Embryo development appears to be delayed or arrested (Fig 1J). The typical wild-type bent-cotyledon or mature embryo stages were not observed for mature embryos. In EMS mutant, embryo progresses normally until the late globular stage (Fig 1G). Abnormal suspensor development could be observed in some aborted seeds (Fig 1G, H, I, J). In all cases the suspensor appeared to develop but showed

dramatic evolution. The early globular stage contains a morphologically normal suspensor and embryo (Fig 1F). Abnormal divisions in the suspensor first appear at the late globular stage (Fig 1G) and are pursued during subsequent growth (Fig 1G, H, I, J). Cell divisions continue in both the embryo and the suspensor through the torpedo stage, resulting in an elongated embryo and a suspensor often the size of the embryo (Fig 1I).

With often the size we demonstrate that in every case, morphological defects in the suspensor precede visible defects in the embryo. This is consistent with that abnormal growth of embryo in mutant EMS is an indirect consequence of a defect in the suspensor. These results suggest that disruption of development in the suspensor can lead to embryo disruption and partial transformation of the embryo.



**Figure 1.** Developmental anatomy of wild-type and EMS mutant embryos. A-F, images of cleared phenotypically wild-type seeds at early globular (A, 3DAF), late globular (B, 7 DAF), heart stage (C, 8 DAF), torpedo (D, 9 DAF), and cotyledonary stage (E, 12 DAF) of embryo development. F-J, images of cleared aborted seed (F, 3 DAF), (G, 7 DAF), (H, 8DAF), (I, 9 DAF), (J, 12 DAF). C, cotyledon; E, embryo ; S, suspensor. Scale bars: in A, B, C, D, E ,F ,G, H, I, J, 100  $\mu$ m.

## References

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2. Silue *et al.* (2006). *BIC* **49**, 149-150