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Altered callose deposition during embryo sac formation of multi-pistil mutant (*mp1*) in *Medicago sativa*

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ABSTRACT. Whether callose deposition is the cause or result of ovule sterility in *Medicago sativa* remains controversial, because it is unclear when and where changes in callose deposition and dissolution occur during fertile and sterile embryo sac formation. Here, alfalfa spontaneous multi-pistil mutant (*mp1*) and wild-type plants were used to compare the dynamics of callose deposition during embryo sac formation using microscopy. The results showed that both mutant and wild-type plants experienced megasporogenesis and megagametogenesis, and there was no significant difference during megasporogenesis. In contrast to the wild-type plants, in which the mature embryo sac was observed after three continuous cycles of mitosis, functional megaspores of mutant plants developed abnormally after the second round of mitosis, leading to degeneration of synergid, central, and antipodal cells. Callose deposition in both mutant and wild-type plants was first observed in the walls of megasporocytes, and then in the megaspore tetrad walls. After meiosis, the callose wall began to degrade as the functional

megaspore underwent mitosis, and almost no callose was observed in the mature embryo sac in wild-type plants. However, callose deposition was observed in *mp1* plants around the synergid, and increased with the development of the embryo sac, and was mainly deposited at the micropylar end. Our results indicate that synergid, central, and antipodal cells, which are surrounded by callose, may degrade owing to lack of nutrition. Callose accumulation around the synergid and at the micropylar end may hinder signals required for the pollen tube to enter the embryo sac, leading to abortion.

Key words: *Medicago sativa*; Multi-pistil mutant; Embryo sac; Callose; Abortion

