The transformation of anthers in the \textit{msca1} mutant of maize

Abstract In normal anther development in maize (\textit{Zea mays} L), large hypodermal cells in anther primordia undergo a series of proscribed cell divisions to form an anther containing microsporogenous cells and three distinctive anther wall layers: the tapetum, the middle layer and the endothecium. In homozygous \textit{msca1} mutants of maize, stamen primordia are initiated normally and large hypodermal cells can be detected in developing anthers. However, the normal series of cell division and differentiation events does not occur in \textit{msca1} mutant plants. Rather, structures containing parenchymal cells and ectopic, nonfunctional vascular strands are formed. The epidermal surfaces of these structures contain stomata, which are normally absent in maize anthers. Thus, all of the cell layers of the "anther" have been transformed in mutant plants. The filaments of the mutant structures are normal, and all other flower parts are normal. The \textit{msca1} mutation does not affect female fertility, but transformed "stamen" structures remain associated with mature ovules rather than aborting as in normal ear development. The \textit{msca1} mutation is distinctive in that only one part of a single (male) reproductive organ is transformed. The resulting structure has general vegetative features, but cannot be conclusively identified as a particular vegetative organ.

Keywords Anther development · Male sterility · Mutant maize · \textit{Zea mays}

Introduction

Sexual reproduction in higher plants depends upon the elaboration of floral structures that produce both the male and female gametophytes, which in turn produce the male and female gametes. In maize, the development of male floral structures begins with the initiation of pairs of spikelet primordia on the apical tassel (Kiesselebach 1949). The meristem of each spikelet initiates outer glumes and inner florets, which in turn produce primordia for lemma, palea, lodicules, stamen and gynoecium. The florets on the maize tassel are thus initially bisexual, becoming unisexual after abortion of the gynoecium (Cheng et al. 1983). Stamen primordia, once initiated, become somewhat rectangular in shape. In maize, large hypodermal archesporial cells in each of the four corners of the stamen primordia undergo a series of proscribed cell divisions, followed by the differentiation of each cell layer, to produce an anther locule (Davis 1966; Greyson 1994). First, a periclinal division of the archesporial cell forms a large interior primary sporogenous cell and a smaller hypodermal primary parietal cell. The \textit{msca1} mutation is distinctive in that only one part of a single (male) reproductive organ is transformed. The resulting structure has general vegetative features, but cannot be conclusively identified as a particular vegetative organ.

Mutations that result in male sterility (lack of functional pollen) have been identified in many plant species (Gottschalk and Kaul 1974; Kaul 1988), including maize (Beadle 1932; Cheng et al. 1979; Greyson et al. 1980; Albertsen and Phillips 1981; Morton et al. 1989; Loukides et al. 1995; Chaubal et al. 2000), tomato (Rick 1948; Gorman and McCormick 1997), soybean (Graybosch and...
Palmer 1988), pea (Gottschalk 1971; Nirmala and Kaul 1991; Myers et al. 1992) and Arabidopsis thaliana (Chaudhury 1993; Dawson et al. 1993; Chaudhury et al. 1994; Peirson et al. 1996; Taylor et al. 1998; Sanders et al. 1999). By comparing development in mutant plants with that in normal fertile plants, much can be learned about the genetically regulated cellular events involved in anther and pollen development.

Materials and methods

Change of mutant designation

The ms22 mutation was originally described by (West and Albertsen 1985) as a typical male-sterile mutant, in which anthers were not exserted from the tassel, but in which the female is fully fertilized. Examination of the male sterility phenotype was conducted to determine the cause of male sterility, but also that the phenotype is unique in comparison with other male-sterile mutant phenotypes described (Neuffer et al. 1997), since the mutation results in a transformed “anther” lacking sporogenous tissue. For this reason, msca1 (male sterile converted anther) was deemed a more descriptive gene name for this locus.

Plant growth and sampling

Plants were grown either in glasshouses at Colorado State University or in fields at the Agricultural Research, Development and Education Center in Fort Collins, Colorado. Young spikelets were collected from immature tassels at 2- to 3-d intervals from the mid-portion of the tassel from plant families segregating 1:1 for male-sterile/male-fertile plants. Samples were placed on ice in polypropylene tubes containing 0.5 ml 0.15 M NaCl. Multiple plants were sampled to assure collection of a series of developmental stages of both heterozygous fertile and homozygous male-sterile florets. Anthers from each spikelet were squashed on glass slides and observed with a microscope to assess the pollen developmental stage. Selected anthers were processed for anther exsertion at anthesis. Materials and methods

Genetic mapping

An F2 family, segregating for the msca1 mutation, was grown and classified for male fertility. DNA was isolated from 20 male-sterile plants and 17 male-fertile siblings. Equal amounts of DNA from each plant were pooled, based on their male-fertility classification, to create two bulk DNA samples. Each DNA pool was digested with BamHI, EcoRV, HindIII, and EcoRV, electrophoresed, and blotted onto nylon membrane. The DNA blots were sequentially hybridized with approximately 50 restriction fragment length polymorphism (RFLP) markers that were evenly dispersed throughout the genome. Each chromosome arm was represented by at least two RFLP markers. A polymorphism between the two pools was detected with marker php20-S81, which maps to the short arm of chromosome 7 and has a second locus on the long arm of chromosome 2. An additional DNA blot that contained individual DNA samples was made and hybridized with php20-S81. All male-sterile plants were homozygous for a 9.4-kb BamHI fragment whereas the male-fertile plants were either homozygous for a 12-kb BamHI fragment or were heterozygotes. The RFLP marker umc36, which is closely linked to php20-S81 on chromosome 2, was hybridized to the individual DNA blot. No linkage was found between any umc36-hybridizing RFLP fragment and male sterility. Thus, these data indicate that the mscA gene is linked to php20-S81 on the short arm of chromosome 7.

Microscopy

For light and scanning electron microscopy (SEM), samples were fixed for 1 h at room temperature and overnight at 4 °C with a mixture of 2% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). For light microscopy, young anthers were fixed whole and older anthers were cut transversely into two to three pieces while submerged in the fixative. For SEM, whole spikelets, whole anthers or transverse pieces of young ears were fixed. Samples were rinsed (3×5 min) with 0.1 M sodium cacodylate buffer (pH 7.2) post-fixed with 2% OsO4 (in the same buffer) for 2 h at room temperature, rinsed again with buffer and dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 3×100%; 15 min each). Light-microscopy samples were infiltrated and embedded in Spurr’s resin (medium hardness).

For detection of callose using decolorized aniline blue fluorescence microscopy, unfixed anthers were immersed in a 0.005–0.01% solution of aniline blue WS in 0.15 M potassium phosphate buffer (pH 9), for 24 h at room temperature (Currier and Shih 1968). Samples were mounted on clean glass slides in the decolorized aniline blue solution, covered with coverslips, gently pressed, and examined and photographed using blue light (590–430 nm wavelength) using an Olympus Provis AX70 fluorescence microscope. For cellulose detection, thick sections were incubated for 2 h in 0.1% aqueous Calcofluor White MR2 (now called Fluorescent Brightener 28; Sigma–Aldrich) and rinsed with water. Slides were examined for fluorescence using a 360- to 370-nm excitation filter, a 400-nm dichroic mirror, and a 420-nm barrier filter.

Reverse transcription–polymerase chain reaction (RT–PCR) of wild-type and msca1 tissues

DNA from wild-type B73 fertile tassels and msca1 tassels was purified using TRIzol reagent (Life Technologies) based on the protocol of Chomczynski and Sacchi (1987), using the manufacturer’s instructions. A 5-μg sample of total RNA was used to synthesize first-strand cDNA using oligo dT primers and Superscript II RT reverse transcriptase (Life Technologies), following the manufacturer’s instructions. Approximately 0.05 μg of cDNA or 0.025 μg of B73 genomic DNA was used in each PCR reaction. Primers were synthesized for the specific amplification of Ms45 (GenBank Accession #AF360356), SB200 (GenBank Accession #AF366297) and maize polyubiquitin (GenBank Accession #U29158) genes. Primer sequences were:

- Ms45 forward: 5′ cgagggctgtgctgctgtgc 3′;
- Ms45 reverse: 5′ ggtgcctctcttacagcagaagc 3′;
- SB200 forward: 5′ atgcacgactggctgttggc 3′;
- SB200 reverse: 5′ cgtggaagctctgctgctgtgc 3′;
- maize polyubiquitin forward: 5′ atgcagatcttcgtcaagactctcact 3′;
- maize polyubiquitin reverse: 5′ gcctcccctgaggcggagaacaaggg 3′.

PCR conditions were: an initial 95 °C for 2 min, then each cycle 95 °C 1 min, 68 °C 1 min, and 72 °C 2 min for 35 cycles, followed by 25 °C for 30 s.