Sterile Sorghum Mutant for 2 Line Breeding

(OTT ID 1578)

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Technology Overview

- Unique and efficient strategy to utilize a 2-line breeding system for sorghum lines
- Male sterile plants can be used for hybrid breeding and maintenance of lines
- A 3-component genetic construct is being developed to allow for male sterile plants, rescue of male fertility, ablation of transgenic pollen, and ability to sort seeds

Problem with Current Methods

- Current methods are complicated and expensive using 3 lines in cytoplasmic male sterility (CMS)
- Current lines show instability of male sterility
- The narrow germplasm resources of the lines restrict generation of hybrid vigor
- Nuclear male sterile genes are not currently exploited for hybrid breeding

New Method and Solution

- Our system is faster and cheaper using only a 2-line breeding system
- The transgenic seeds will be removed by molecular ablation and sorting to ensure male sterile plants are transgene free
- Male sterile plants can be created in diverse genetic backgrounds for hybrids

Market For Sorghum

- The global sorghum and sorghum seeds market was valued at $8 billion in 2016, and is projected to reach $10.5 billion by 2023.
- Transparency Market Research reports a growing demand for sorghum as an alternative sweetener for various alcoholic beverages is a major factor driving the global sorghum market worldwide.
- Sorghum is also used expanding markets such as floral arrangements, fencing, building material, pet food and others, which is another major driving factor for global sorghum market.
- Many sorghum producers are providing healthier product offerings based on the increasing demand for sorghum as a better substitute in a variety of food products.

Product Development

Intellectual Property

- A United States Provisional Patent was filed for this invention in January 2018.

Funding to date

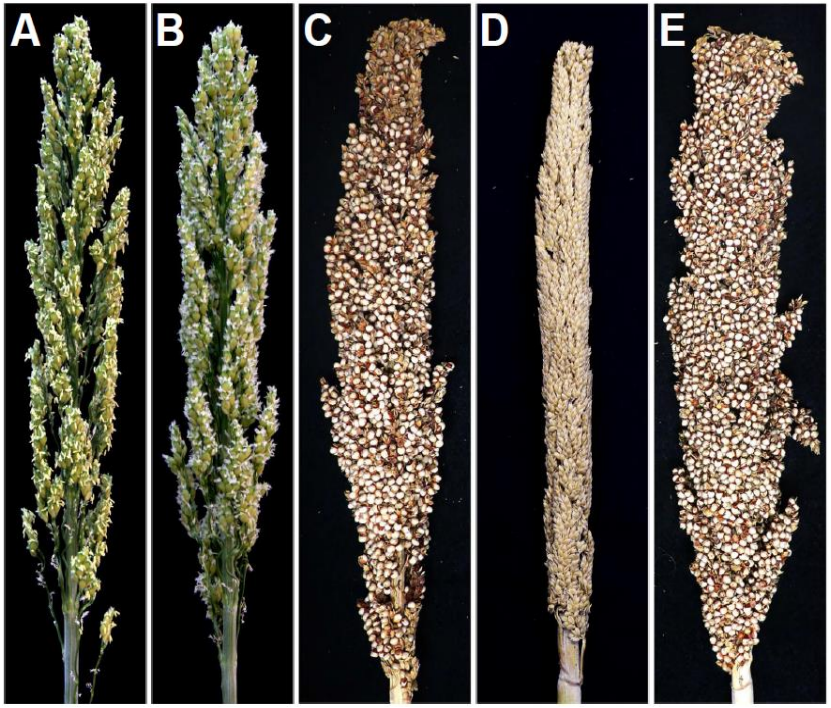
- USCP, USDA

Partnering

- This technology is part of an active and ongoing research program and is seeking partners for licensing and development of the final product.

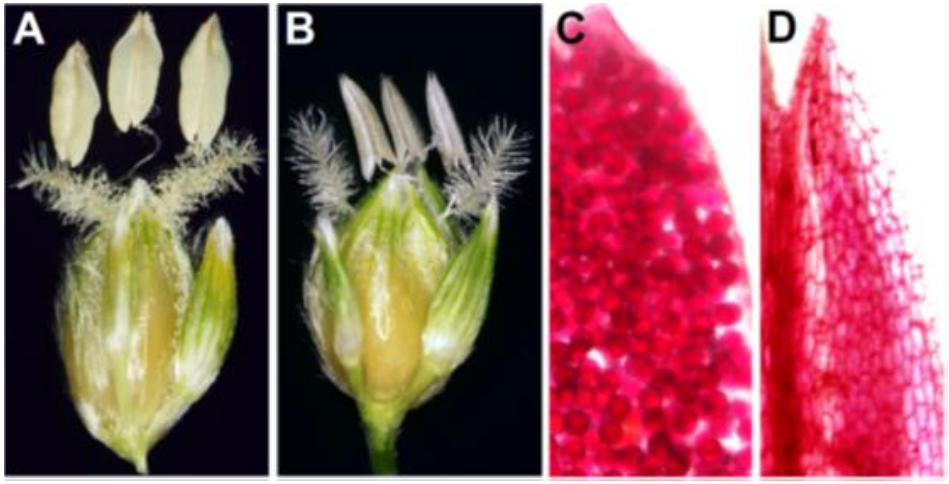
ms8 is an Easy to Recognize male sterile

(A) Wild type panicle during anthesis
(B) An *ms8* mutant panicle during anthesis
(C) A mature self pollinated wild-type panicle
(D) A mature *ms8* panicle
(E) A mature *ms8* panicle manually pollinated with WT pollen



- *ms8* is a stable male sterile mutant

ms8 is Defective in Pollen Production



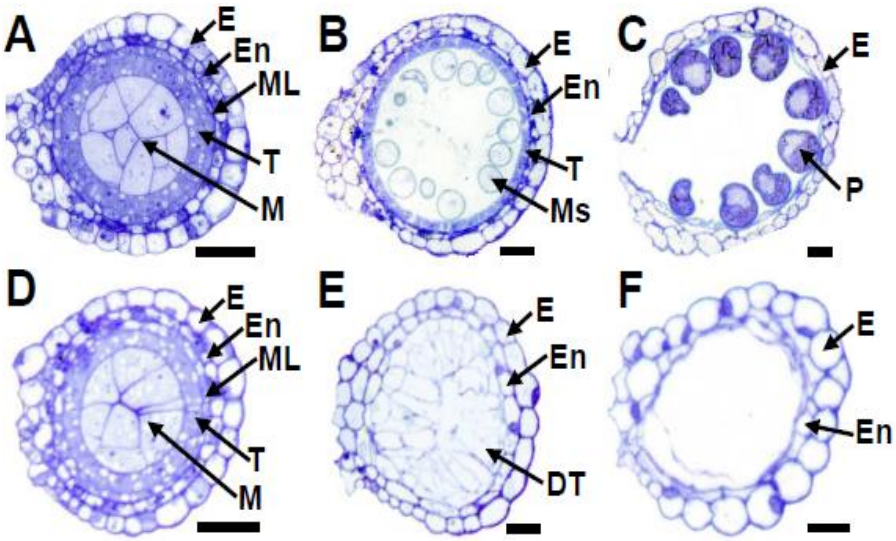
(A) A wild-type (BTx623) spikelet showing three mature anthers.

(B) An *ms8* spikelet showing three pale and flattened anthers.

(C) A part of wild-type anther displaying round pollen grains inside anther lobes.

(D) A part of *ms8* mutant anther exhibiting no pollen grains inside the anther lobe.

ms8 is Abnormal in Tapetum Development



(A-C) Wild-type (BTx623) semi-thin sections showing anthers at stage 5 (A), stage 9 (B), and stage 12 (C).

(D-F) *ms8* semi-thin sections exhibiting anthers at stage 5 (D), stage 9 (E), and stage 12 (F).

E: epidermis, En: endothecium, ML: middle layer, T: tapetu, M: microsporocyte, Ms: microspores, and DT: prematurely degenerating tapetum

- Our results suggest that the precocious degeneration of tapetum causes abnormal development of microspores, and consequently the failure of pollen production

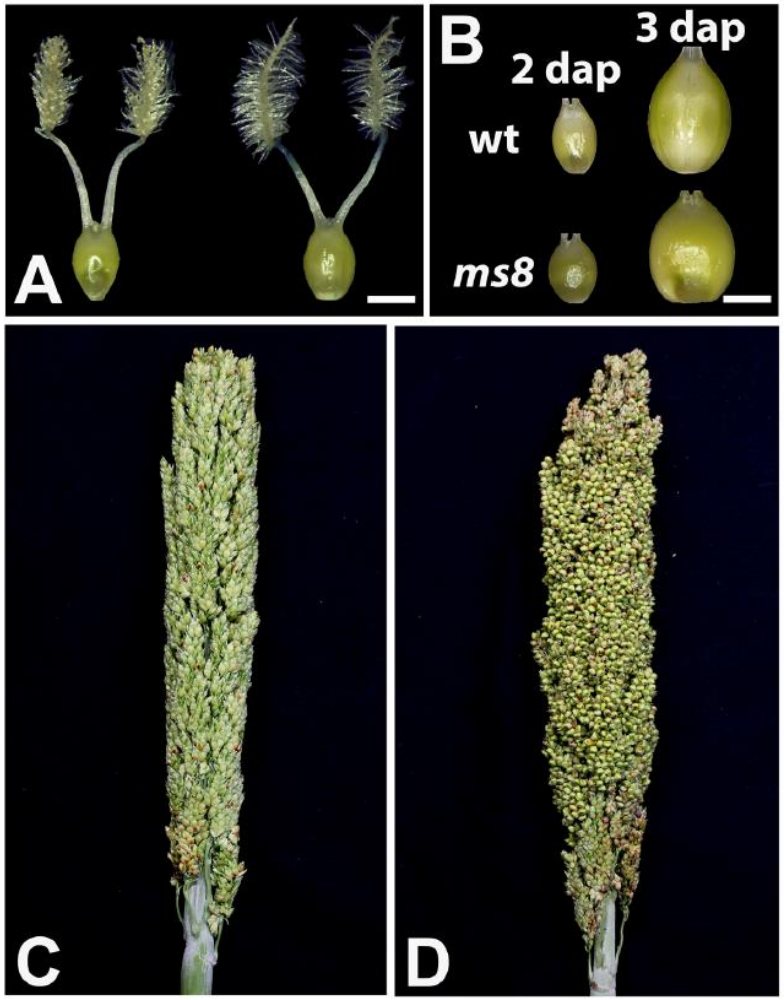
Female Fertility is Unaffected in *ms8*

(A) The BTx623 wild-type ovary (left) was the same as that of the *ms8* mutant (right) without manual pollination.

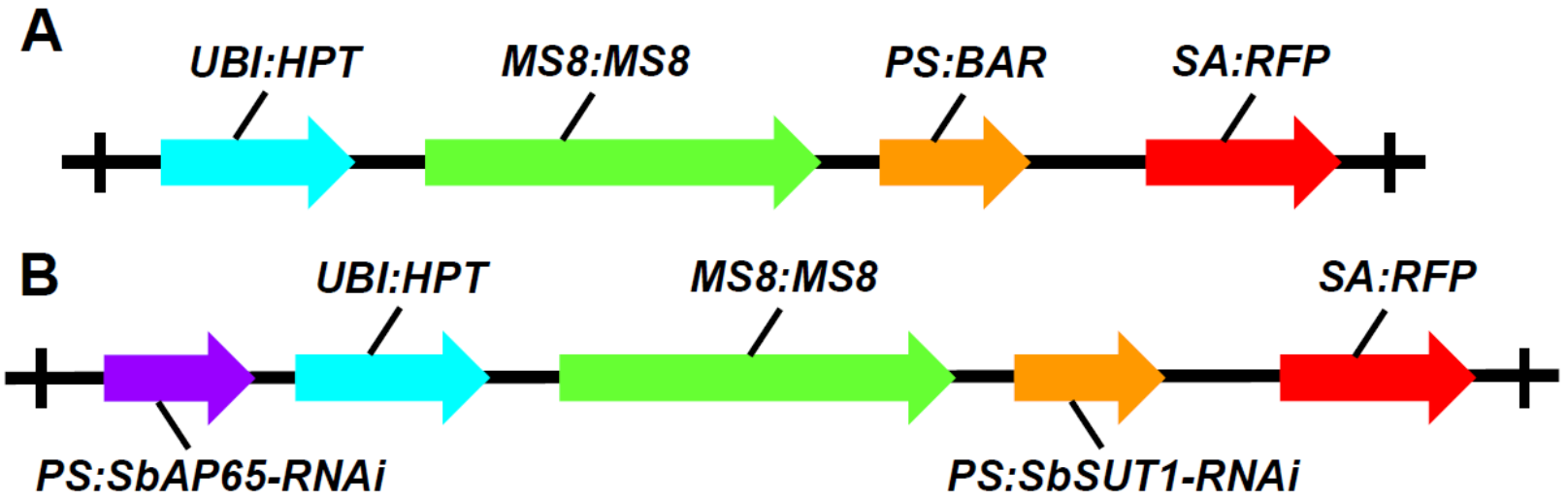
(B) There was no difference of ovary development between BTx623 and the *ms8* mutant after manual pollination. dap: days after pollination. Bars = 1 mm in A and B.

(C) An *ms8* panicle bagged before anthesis showing no developing seeds.

(D) Seeds are being normally developed in a manually pollinated *ms8* panicle.

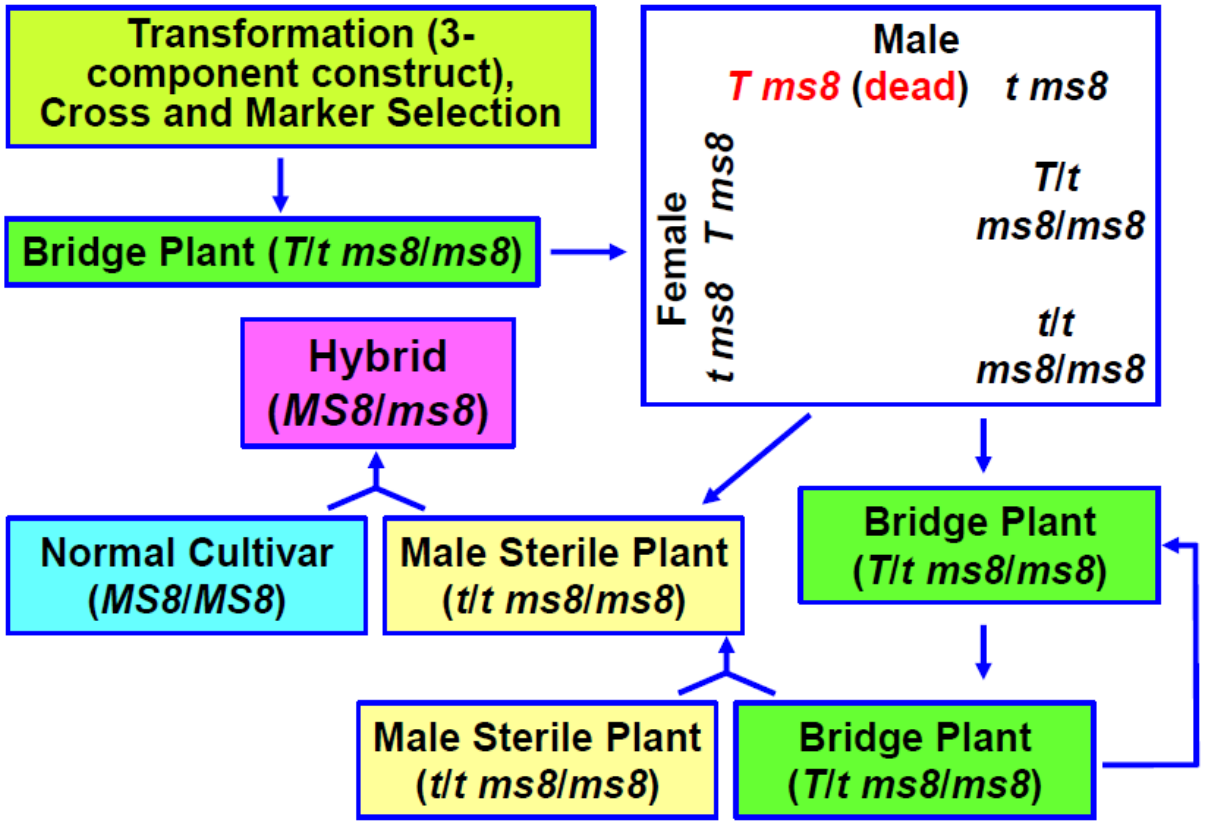


Construct Design



UBI: the constitutive promoter, *HPT*: the selection maker gene conferring hygromycin, *MS8*:*MS8*: genes used for rescuing the male sterility, *PS*: pollen-specific promoter, *BAR*: the toxic *BARNASE* gene, *SbSUT1*: the gene of sorghum *SUCROSE TRANSPORTER1*, *SbAP65*: the gene of *ASPARTIC PROTEASE 65*, *SA*: seed active promoter to drive RFP (Red Fluorescent Protein) to be expressed in seeds.

Breeding Schematic



Schematic diagram showing how our two-line nuclear male sterility (NMS) hybrid breeding system works for hybrid breeding in sorghum

Next Steps

Experimental

- **Year 1**: Building construct for easier sorghum breeding with the *ms8* mutant
- **Year 2**: Hybrid breeding test in field using the bridge plant
- **Year 3**: Establish a panel of diverse bridge plants for creating a broad spectrum of hybrids

Patents and Commercialization

- Looking for partners to support the next stages of research
- File utility/plant patents
- Develop the final product for farmers worldwide
- Commercialize the new 2-line breeding system

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