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## Abstract

Grain amaranth (*Amaranthus* spp.) seed stocks are customarily from self-pollinating plants that open pollinate and are not $F_1$ hybrids. However, cytoplasmic male sterility (CMS) traits are available for hybrid seed production. DB 199313 (Reg. No. GS-10, PI 686465) grain amaranth (*A. hypochondriacus* L.) is the first publicly available CMS line in *Amaranthus*. It is an $F_1$ hybrid from a cross between male sterile plants of DB 921 (PI 568125) and male fertile PI 568179. Fertility can be restored by crossing DB 199313 with K432 (PI 538323). DB 199313 is adapted for seed production in Ames, IA, from direct seeding in the field, but it has not been selected for competitive agronomic performance. It provides a new publicly available genetic resource for improved grain amaranth crossing.


## Disciplines

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## Comments


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GENETIC STOCK

# Registration of DB 199313, Cytoplasmic Male Sterile Grain Amaranth Genetic Stock

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## Abstract

**Grain amaranth (*Amaranthus* spp.) seed stocks are customarily from self-pollinating plants that open pollinate and are not $F_1$ hybrids. However, cytoplasmic male sterility (CMS) traits are available for hybrid seed production. DB 199313 (Reg. No. GS-10, PI 686465) grain amaranth (*A. hypochondriacus* L.) is the first publicly available CMS line in *Amaranthus*. It is an $F_1$ hybrid from a cross between male sterile plants of DB 921 (PI 568125) and male fertile PI 568179. Fertility can be restored by crossing DB 199313 with K432 (PI 538323). DB 199313 is adapted for seed production in Ames, IA, from direct seeding in the field, but it has not been selected for competitive agronomic performance. It provides a new publicly available genetic resource for improved grain amaranth crossing.**



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Amaranth (*Amaranthus* spp.) is a drought-tolerant crop with excellent nutritional protein in both the grain and foliage harvested from a broad-leafed summer annual plant (Alemayehu et al., 2015; Assad et al., 2017). It is included in international development plans as it is advantageous for small farmers in the developing world (Alemayehu et al., 2015). The grain is readily available in the developed world, where it is valued as a health-promoting, gluten-free food (Moya Cortazar et al., 2017) and is included in the new health-promoting and commercial category "ancient grains" (Hinterthuer, 2017). A collection of 3341 *Amaranthus* accessions is maintained and distributed by the US National Plant Germplasm System (NPGS) at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA (US National Plant Germplasm System, 2018), and there are other collections worldwide (Brenner et al., 2000). However, amaranth plant breeding lags behind the major crops, and access to cytoplasmic male sterility (CMS) could help improve progress.

Because amaranth flowers are so small, making emasculation difficult, CMS grain amaranths will be useful for amaranth breeding (Pandey, 1984; Peters and Jain 1987; Gudu and Gupta, 1988; Brenner, 1993; Brenner et al., 2000; Stetter et al., 2016). Both nuclear-gene male sterility (Peters and Jain, 1987; Gudu and Gupta, 1988) and CMS (Peters and Jain, 1987) are known in the grain amaranths. In other crops, CMS is an established conventional plant breeding method that generally facilitates plant crossing (Bohra et al., 2016; Kim and Zhang, 2018), making $F_1$ hybrid cultivars practical and thus contributing to crop yield increase. In the grain amaranths, heterosis is known for biomass (Murray, 1940; Lehmann et al., 1991), grain yield, seed weight, and grain protein content (Pandey, 1980; Pandey, 1984; Prajapati et al., 2009; Hooda et al., 2015). This heterosis research was accomplished without the use of male sterility to facilitate crossing, but with CMS, crossing will be more efficient.

The NPGS has a substantial, but little used, collection of male sterile *Amaranthus* germplasm. Male sterility is documented for 175 NPGS amaranth accessions observed from 1990 through 2017, and these observations are accessible via the US Genetic Resources Information Network (GRIN) database (US

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**Abbreviations:** CMS, cytoplasmic male sterility; NCRPIS, North Central Regional Plant Introduction Station; NPGS, National Plant Germplasm System.

National Plant Germplasm System, 2018). Most of these male sterile accessions were discovered by the author as spontaneous mutations with distinctive male sterile anthers (Peters and Jain, 1987) observed during routine gene-bank seed regenerations in plastic pollen-controlling tents in a greenhouse at the NCRPIS in Ames (Brenner and Widrlechner, 1998). The Peters and Jain (1987) male sterility research populations were donated by Ingrid Peters and are accessioned in the NPGS as PI 576485 to PI 576489 (US National Plant Germplasm System, 2018). The collection also includes seven accessions, PI 568125 to PI 568131, that were segregated from 'Plainsman' (PI 558499) (Baltensperger et al., 1992) for male sterility. The Plainsman segregates were identified in a 1992 experimental planting of seeds with black or brown seed coats, or translucent-vitreous perisperm, sorted from normal, predominantly opaque-white Plainsman seeds (Brenner et al., 2000). Seeds with these infrequent black or brown seed coats are thought to descend from earlier spontaneous hybridization with crop wild relatives that also have these seed traits (Hauptli and Jain, 1984; Brenner et al., 2013; Jimenez et al., 2013; Stetter et al., 2017), including translucent-vitreous perisperm (Jimenez et al., 2013), and the associated male sterility may originate in genetic incompatibility within spontaneous cross-species hybrids with wild relatives.

DB 199313 *Amaranthus hypochondriacus* L. (Reg. No. GS-10, PI 686465), a CMS grain amaranth, is the first $F_1$ hybrid *Amaranthus* 100% male sterile line to be publicly available. The taxonomic identity of this variant is ambiguous because its parents may be derived from interspecific hybrid events, but the diagnostic traits conform to those of *A. hypochondriacus* (Sauer, 1967). The full range of species or cultivars that will cross with or restore fertility in crosses with DB 199313 is not known and is a potential area of research. However, male fertility is restored by crossing with K432 (PI 538323), a temperate-adapted grain amaranth breeding line.

# Methods

Male sterile plants of DB 921 (PI 568125) and male fertile plants of PI 568179 were crossed in the winter 1993 greenhouse using methods in Brenner et al. (2000). The $F_1$ progeny designated DB 199313 were evaluated for the presence of male sterility in the 2001 field and were 100% male sterile. The 100% male sterility of DB 199313 was replicated by repeating the cross in the fall 2016 greenhouse and was evaluated in the winter 2017 greenhouse. Male sterility was restored in 2017 by crossing DB 199313 with K432, in the winter 2017 greenhouse, and evaluating the progeny, designated as DB 2017008 in the fall 2017 greenhouse. These plantings were either in the Iowa State University Agronomy Department greenhouse or field plantings at the NCRPIS, both in Ames, IA. In the greenhouse, populations were isolated in plastic 0.8-$m^2$ tents as described by Brenner and Widrlechner (1998) or in 13-$m^2$ isolation rooms. The 2001 and 2017 field plantings were seeded directly in the field in the first week of June and observed through September.

Production of DB 199313 required distinguishing male sterile plants from their male fertile siblings in order to use only male sterile plants as female parents. Male sterile DB 921 plants growing in a greenhouse were identified by examining the anthers. Anthers of male sterile plants are shrunken and pale green, rather than swollen and yellow for fertile plants (Peters and Jain, 1987). Early flowers that may have been pollinated by siblings were removed, and the plants were taken to an isolated greenhouse room for crossing. PI 568179 plants were used as male parents and at maturity seeds of DB 199313 were harvested from the DB 921 female parents.

Starting in 2016, pollination methods were improved. Pollination was accomplished with a cordless hand vacuum cleaner to improve pollen movement between plants. A small compact lithium vacuum cleaner (Black and Decker), marketed for home use, was acquired and the dust filter was removed. Pollen was vacuumed from the male fertile parents during pollen release, at about 10 AM, and immediately vented about 20 cm away onto the male sterile plants. Without a vacuum cleaner, pollination is more difficult since the male fertile flowers must be positioned above the male sterile flowers to allow pollination by falling pollen.

# Characteristics

DB 199313 is a CMS line with 100% male sterility. It reached seed maturity 101 d after direct seeding in the field in Ames on 2 June 2017. The plants are 150 cm tall at maturity. The stem has red vertical striping, petioles are faintly red, leaf blades are reddish and have red midveins, inflorescences are red, and the seed is white. DB 199313 and DB 2017008 root lodged in small-plot field conditions in Ames in 2017, an important grain production weakness.

The parents of DB 199313 are male sterile plants of DB 921 used as females and PI 568179 plants used as males. Since DB 199313 is a male sterile $F_1$ hybrid, the cross must be repeated to increase seed stocks. The female parent, DB 921, was selected from Plainsman in 1992 as a male sterile open-pollinated field plant, grown from a black seed in a Plainsman seed lot (Brenner et al., 2000). Populations of DB 921 segregate for male sterility, which is maintained by sib mating. A plant population grown from the 2015-produced seed lot that the NPGS distributes was 34% male sterile in an April 2018 greenhouse evaluation ($n$ = 123). The male parent, PI 568179, was selected from PI 608791 because of its unusual, daylength-neutral rapid flowering and was later found to have CMS maintainer ability, as first reported here. It appears to be a spontaneous hybrid between amaranth species since it has intermediate diagnostic flower traits. The line that restores male fertility in the progeny of crosses with DB 199313, K432, is from the amaranth plant breeding project previously conducted at the Rodale Institute (US National Plant Germplasm System, 2018). K432 performed well in grain amaranth variety trials (Sooby et al., 1998).

# Conclusions

The CMS research by Peters and Jain (1987) was replicated with temperate-adapted germplasm, and the genetic stocks are now available to researchers from the NPGS. Cytoplasmic male sterility was demonstrated by producing an $F_1$ population with 100% male sterility. DB 199313 should be useful for *Amaranthus* research. However, DB 199313 and DB 2017008 are prone to lodging and have not been evaluated for yield. The parents, and especially PI 568179, which is poor agronomically, can be supplanted by better-adapted lines for use in commercial hybrids. Molecular markers for male sterility traits could assist a backcrossing program to include these traits in well-adapted heterotic parents, as is already done in other crops (Bohra et al., 2016).

# Availability

Small seed quantities of DB 199313, the parents DB 921 (PI 568125) and PI 568179, and the fertility restoring K432 (PI 538323) are available for research and development from the USDA-ARS National Plant Germplasm System. There are no intellectual property claims restricting use or distribution other than requesting that the source of these plant materials is acknowledged by citing this registration and PI accession numbers when it contributes to research publications or germplasm development.

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