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Effect of drought stress on male fertility restoration in A_3 CMS-inducing cytoplasm of sorghum

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ABSTRACT

Use of cytoplasmic male sterility (CMS) in hybrid breeding requires effective male fertility-restoring lines. In sorghum, very few restoring lines that can restore fertility in A_3 CMS have been reported. To identify the reasons for this deficiency, F_1 and F_2 hybrids of an A_3 CMS line crossed with the line IS1112C, a donor of fertility-restoring (*Rf*) genes for A_3 cytoplasm, and testcrosses of fertile plants to A_3 CMS lines were grown under contrasting water availability regimes in dryland and irrigated field plots. In the irrigated plots the frequency of fertile plants in testcrosses was twice that in dryland plots ($P < 0.05$). Fertile plants from the F_2 family grown in the irrigated plots showed significantly higher restoration ability than fertile plants from the same family grown in dryland plots. F_3 plants from the F_2 family grown in irrigated plots yielded on average a sixfold higher frequency of fertile plants in testcrosses than F_3 plants derived from dryland plots ($P < 0.01$). Fertility of testcross hybrids correlated negatively with air vapor pressure deficit (VPD) at flowering ($r = -0.96$; $P < 0.01$) suggesting that VPD is a trigger for downregulation of *Rf* genes for A_3 cytoplasm.

1. Introduction

In many crop species, cytoplasmic male sterility (CMS) is an efficient tool for obtaining heterotic hybrids. To extend the range of hybrid combinations and to create hybrids adapted to various environmental conditions, it is necessary to use genetically different CMS lines. Application of different types of CMS-inducing cytoplasm markedly increases the genetic diversity of CMS lines. In sorghum [*Sorghum bicolor* (L.) Moench], several groups of genetically different CMS-inducing cytoplasms have been described [1,2]. In commercial hybrid development and many sorghum breeding programs, A_1 CMS is the dominant system in A-line development. The main obstacle to the use of other CMS-inducing cytoplasms is finding sources of male restorers that can restore fertility in F_1 hybrids.

A_3 cytoplasm derived from the sorghum accession IS1112C is difficult to work with because of the rare frequency of restorer genes among sorghum accessions and low seed set in restored F_1 hybrids [3–5]. Genetic analyses of fertility restoration of crosses between A_3 CMS lines and IS1112C have suggested a two-gene gametophytic fertility restoration system in which the complementary action of restoring alleles of

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two genes, designated *Rf3* and *Rf4*, is required for pollen viability [6,7]. Restoration of male fertility correlated with enhanced transcript processing of *orf107*, a chimeric mitochondrial gene that is associated with the expression of CMS in the A_3 cytoplasm [8,9]. F_1 hybrids with restored male fertility that are heterozygous for *Rf3* and *Rf4* genes produce 25% fertile pollen, resulting in reduced seed set and limiting the use of A_3 cytoplasm in sorghum hybrid production. More recently, a sporophytic restoration system involving two complementary genes has been described in sudangrass hybrids in A_3 cytoplasm [10]. This fertility restoration did not involve enhanced transcript processing of *orf107*. In this fertility restoration system, an unusual phenomenon of poor expression of fertility restoring alleles in backcrosses of fertile hybrids to A_3 CMS lines was found, and was explained by paramutation of the *Rf* genes caused by sterility-maintaining alleles [10].

Elkonin et al. [11,12] investigated the restoration of male fertility in another CMS-inducing cytoplasm, 9E. They found an unusual inheritance pattern: *Rf* genes function in the self-pollinated progenies of F_1 hybrids but are not expressed or poorly expressed in backcrosses of these hybrids to 9E CMS lines. In addition, fertility levels of F_1 hybrids were correlated with the level of plant water availability during panicle development and anthesis [11]. They postulated that these phenomena are explained by upregulation of fertility-restoring genes under high plant water availability and suggested an epigenetic mechanism regulating restoration of male fertility in this cytoplasmic system [12]. This mechanism involved repression of nuclear fertility-restoring genes under drought stress by methylation of their nucleotide sequences, removing repression under high water availability. This hypothesis has been supported by comparative MSAP analysis (Methylation Sensitive Amplification Polymorphism) of DNA of F_1 hybrids, which revealed differences in the number and length of amplified fragments in sterile and fertile plants grown in dryland and irrigated plots [13].

In this paper, we report a strong correlation between restoration of male fertility in A_3 cytoplasm of sorghum and plant water availability, specifically as it relates to vapor pressure deficit, and on the effect of water availability on selection of fertility restorers for this cytoplasm.

2. Material and methods

2.1. Plant material

Three grain sorghum lines: A_3Karlik-4 (A_3K-4), A_3Topaz, and A_3KP-70, were used as maternal parents. These lines have A_3 CMS-inducing cytoplasm derived from the line A_3Tx398, which was generously provided by the late Dr. K. F. Schertz (USDA-ARS, College Station, Texas, USA). A_3Karlik-4, A_3Topaz, and A_3KP-70 were developed using a nonrecurrent backcrossing program using A_3Tx398 as the non-recurrent parent. Karlik-4, Topaz and KP-70 are from the collection of the All-Russian Research Institute for sorghum and maize "Rossorgo" (Saratov, Russia). Euplasmic A_3 line IS1112C was used as a donor of fertility-restoring genes. This line was obtained from ICRISAT (Patancheru, India). All CMS lines, the paternal lines, the F_1 and F_2, and the testcross hybrids were grown in experimental fields located at the Agricultural Research Institute of the South-East Region (Saratov, Russia).

2.2. Experimental procedures

The F_1 hybrids A_3Karlik-4/IS1112C were grown under field conditions in 2011 and 2012 (Fig. 1) in 4–5 m rows. In 2012, these F_1 hybrids were also grown in irrigated plots, in which irrigation started at booting (twice per week, each time at 5–8 L m^{-2}). F_2 progeny from the same F_1 plant were grown in 2012 in two plots (Fig. 1), a dryland plot in which a roof made of translucent polycarbonate was constructed to prevent irrigation of plants by rain (the roof was placed over the plot at the beginning of the boot stage), and an irrigated plot, starting from the same developmental stage. These plots were located in the same field, with 50 m between plots.

To investigate the restoration ability of the F_2 plants, plants shedding the most pollen in irrigation and dryland plots were selected and crossed to line A_3K-4. Testcross hybrids A_3K-4/F_2 (A_3K-4/IS1112C) and the self-pollinated progeny of paternal plants (F_3 A_3Topaz/(A_3K-4/IS1112C)) were grown under field conditions in 2013. All testcross hybrids and the progenies of paternal plants were grown in the same field plot.

To investigate the restoration ability of plants from the F_3 families grown in irrigated and dryland plots, plants shedding the most pollen were selected and crossed to A_3Topaz. These testcross hybrids, A_3Topaz/F_3 (A_3K-4/IS1112C), were grown in replicated irrigated and dryland trials. Each testcross was grown in two replications, randomly distributed among other sorghum lines. The progeny of self-pollinated paternal plants, F_4 A_3K-4/IS1112C, were grown in the same field plots and were used in subsequent testcrosses with the A_3Topaz. To evaluate the level of fertility, the first panicle of each plant was bagged before anthesis. The level of male fertility was estimated at maturity by percent seed set. Depending on the percent seed set, the panicles were classified as sterile (*s* = 0% or one to two seed), partially sterile (*ps* = 1%–40%; usually no more than basal ⅓ of the panicle), partially fertile (*pf* = 40%–75%; usually ⅔ of the panicle) and fertile (*f* > 75%).

To assess the effect of environment on fertility restoration, average daily temperature, rainfall, relative humidity, and atmospheric vapor pressure deficit (VPD) were recorded. Daily indexes of these parameters were kindly provided by the meteorological station of the Agricultural Research Institute of the South-East Region (Saratov, Russia).

2.3. Statistical methods

The numbers of *f* and *pf* plants in different progenies was evaluated by a comparison of the proportions according to the Fisher method using the *F* criterion, which is applied in the case of small samples or samples differing in size [14]. A two-factor variance analysis with Duncan's multiple range test was used for statistical analysis of the influence of irrigation on frequency of fertility-restored plants in testcrosses. In this analysis, the effect of growing conditions on testcrosses of the F_3 plants (irrigated or dryland) (factor A) and growing conditions of F_2 families (dryland or irrigated plots)