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REGISTRATION

Germplasm

Registration of three *Gossypium barbadense* L. American pima-like germplasm lines (PSSJ-FRP01, PSSJ-FRP02, and PSSJ-FRP03) with improved resistance to Fusarium wilt race 4 and good fiber quality

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Registration by CSSA.

Abstract

Over the past 18 yr, Fusarium wilt race 4 (FOV4) has affected cotton (*Gossypium* spp.) production in the San Joaquin Valley of California, and more recently this pathogen was formally identified in 2017 in the El Paso, TX, region and in 2019 in New Mexico. The primary purpose for the germplasm release is to provide cotton breeders with needed alternative sources for continuing to improve FOV4 resistance in pima cotton. PSSJ-FRP01 (Reg. no. GP-1107, PI 699963) (PS-592) originated from Pima S-6 × *G. barbadense* pool accessions GB1368/k-7616. Pima S-6 possesses a major gene(s) for FOV4 resistance. GB1368/k-7616 is Uzbekistan accession introduction with different plant structure, large leaves, columnar growth, and a more clustered fruiting habit when compared to most American pima. PSSJ-FRP02 (Reg. no. GP-1108, PI 699964) and PSSJ-FRP03 (Reg. no. GP-1109, PI 699965) originated from PS-592 × SA-3208 (LIAO MIAN 7 HAO). SA-3208 is moderately resistant to FOV4. Multiple selections and recurrent cycles of selections were applied to these new germplasm lines based on an asymptomatic single plant after greenhouse FOV4 inoculations and/or infested field evaluations from F_1 to F_4. In subsequent field and greenhouse tests in 2019–2021, the germplasm lines showed improved resistance to FOV4 based on low percentage mortality and vascular staining and fiber quality when compared to commercial cultivars. PSSJ-FRP01, PSSJ-FRP02, and PSSJ-FRP03 lines will help to continue reducing the vulnerability of the pima cotton industry to this fungal pathogen and to advance efforts to broaden the genetic base of FOV4 resistance, which is critical to the pima cotton industry in the United States and Uzbekistan.

Abbreviations: FOV, Fusarium wilt; FOV4, FOV race 4; PS, plant stand; SJV, San Joaquin Valley; VRS, vascular root staining.

1 | INTRODUCTION

Diseases such as Fusarium wilt (FOV; caused by *Fusarium oxysporum* f. sp. *vasinfectum* W.C. Snyder & H.N. Hansen) represent expanding threats to cotton (*Gossypium* spp.) production worldwide by causing plant wilt and death (Egamberdiev et al., 2013; Hutmacher et al., 2013; Kochman et al., 2002; Ulloa et al., 2006, 2011, 2013, 2020; Ulloa, Hutmacher, et al., 2016; Ulloa, Wang, et al., 2016; Wang et al., 2018; Zhang et al., 2015). Over the past 18 yr, FOV race 4 (FOV4) has affected the cotton crop in the San Joaquin Valley (SJV) of California (Diaz et al., 2021; Kim et al., 2005), and more recently this pathogen was formally identified in 2019 in New Mexico (Zhu et al., 2020) and in 2017 in El Paso, TX (Halpern et al., 2018), causing disruption to production in affected fields similar to what was observed previously in California. In the 1960s and 1970s cotton production suffered significant losses in yield and quality in the country of Uzbekistan because of *Verticillium* and *Fusarium* fungi epidemics (Abdullaev et al., 2010, 2013; Abdurakhmonov, 2007; Abdurakhmonov et al., 2012; Marupov et al., 2010). It had been reported that *Fusarium* primarily affected *G. barbadense*; however, recently Upland (*G. hirsutum* L.) cultivars have also been increasingly affected by *Fusarium* in different cotton growing regions of Uzbekistan (Yuldashev et al., 2000). Pima cultivars planted in field soils with relatively high levels of FOV4 have expressed extensive disease symptoms and, in some cases, large reductions in plant survival in clay loam and loam soils even when root knot nematode (*Meloidogyne incognita*) populations and root damage from nematodes have been absent and/or extremely low (Holmes et al., 2009; Kim et al., 2005; Ulloa et al., 2006, 2009; Ulloa, Hutmacher, et al., 2016). Host-plant resistance has been an effective and economical approach for dealing with disease threats such as FOV and root knot nematode and for limiting yield loss in cotton (Hutmacher et al., 2011, 2013; Ulloa et al., 2011, 2013; Ulloa, Wang, et al., 2016).

Cotton is an economically important crop and provides the world's leading natural fiber for the textile industry (Smith et al., 1999). Because FOV4 is a soil-inhabiting fungus and can survive for long periods in soils, even in the absence of a host, it is nearly impossible to eradicate from a field. First identified in India on Asiatic cottons, FOV4 was not identified in the United States until 2003 (Kim et al., 2005). Many studies (Holmes et al., 2009; Kim et al., 2005; Kochman et al., 2002; Ulloa et al., 2006; Wang et al., 2004) have confirmed that FOV4 is becoming a recurring and potentially expanding threat to cotton production (Diaz et al., 2021).

In response to the identification of FOV4 as a potential production issue in California in the early 2000s, USDA-ARS and the University of California, with the support of the California Cotton Ginners and Growers Association and Cotton Incorporated, initiated efforts to identify sources of FOV4 resistance and to field test these materials, taking the necessary research approaches, and later developing germplasm with improved FOV4 resistance (Ulloa et al., 2006, 2009, 2016; Ulloa, Hutmacher, et al., 2013). In the years since 2003, both private and public breeders in California have expanded efforts to develop FOV4-resistant pima germplasm and commercially suitable pima cultivars with workable levels of resistance to FOV4. The California pima cotton industry and growers throughout the SJV adopted the more FOV4-resistant cultivars as they were identified and released and gradually reduced planted acreage of more FOV4-susceptible cultivars. These approaches by breeders and growers have helped the California pima cotton industry to survive and even expand in some years. In the SJV, Upland cotton production has radically declined over the past two decades, whereas pima production has increased, partly due to the development of FOV4-resistant/tolerant pima cultivars (https://www.cottoninfo.ucdavis.edu; https://www.ccgga.org; Ulloa et al., 2020). However, FOV4 seems to be evolving and still represents a threat, especially since its identification in New Mexico and Texas near one of the most important Upland cotton–producing regions (High Plains, TX) in the United States (Diaz et al., 2021; Ulloa et al., 2020).

The primary purpose for releasing these three *Gossypium barbadense* L. American pima-like cotton germplasm lines (PSSJ-FRP01 [Reg. no. GP-1107, PI 699963], PSSJ-FRP02 [Reg. no. GP-1108, PI 699964], and PSSJ-FRP03 [Reg. no. GP-1109, PI 699965]) is to continue providing needed alternative sources for improving FOV4 resistance to cotton breeders and to advance efforts to broaden the genetic

Core Ideas

- Alternative germplasm sources are needed to improve FOV4 resistance in pima cotton.
- Introgression of American pima and Uzbek *G. barbadense* breeding-pool material increases diversity.
- Lines will continue to reduce the vulnerability of the pima industry to FOV4 fungal pathogen.
- Lines possess improved resistance to Fusarium wilt race 4 when compared to commercial cultivars.
- This is the first time that the United States and Uzbekistan publicly released *G. barbadense* germplasm.

base, which is critical to the future of the cotton industry in the United States and Uzbekistan. These three new and improved pima cotton germplasm lines were developed by the USDA-ARS and jointly released with the University of California and Academy of Sciences of Uzbekistan, Center of Genomics and Bioinformatics, Tashkent, Uzbekistan, in 2021 under research grant no. UZB2-31016-09, funded by the Office of International Research Programs of USDA-ARS, through the Civilian Research Foundation. The lines possess good levels of resistance to FOV4 and good fiber quality characteristics, such as fiber fineness, strength, and uniformity. This is the first time that both countries publicly released *G. barbadense* germplasm, with introgression of American pima and Uzbek *G. barbadense* breeding gene-pool material, thereby increasing genetic diversity. The PSSJ-FRP01-03 lines will help to continue reducing the vulnerability of the pima cotton industry to this fungal pathogen.

2 | METHODS

2.1 | Parental background and selection

The parental line sources with different genetic backgrounds used to derive germplasm lines pima PSSJ-FRP01, PSSJ-FRP02, and PSSJ-FRP03 were obtained from the USDA-ARS cotton germplasm collection at College Station, TX, and the USDA-ARS cotton breeding program formerly in Shafter, CA, and currently at Lubbock, TX.

Pima germplasm line PSSJ-FRP01 originated from a cross of germplasm lines Pima S-6 (PI 560140) and *G. barbadense* accession pool GB1368/k-7616. Uzbek genotype GB1368/k-7616 was obtained from Uzbekistan germplasm resources under germplasm exchange program of research grant no. UZB2-31016-09. Pima S-6 was released in 1983 as an F_4 selection from a cross of two experimental lines (5934-23-2-6 and 5903-98-4-4). At the time of release, the major advantages of Pima S-6 were early maturity and high yield. We now know that Pima S-6 also possesses a major gene(s) for FOV4 resistance (Ulloa et al., 2006, 2013, 2020; Ulloa, Wang, et al., 2016). Since its identification as a source of FOV4 resistance in pima cotton, we have performed several cycles of evaluations and selections to increase its uniformity and the level of FOV4 resistance in the S-6 source that we used as a parent and for use in further evaluations under FOV4 pressure. We reference our selection source sometimes a 'Pima-S6' in several publications and herein Pima-S6 (Ulloa et al. 2013, 2020). GB1368/k-7616 is Uzbekistan accession introduction with different plant structure, large leaves, columnar growth, and more clustered fruiting habit (Figure 1a,b) compared with most American pima cotton commercial cultivars (Figure 1d). In greenhouse and FOV4-infested field tests, this accession showed to be moderately resistant to FOV4, with vascular root necrosis or vascular root staining (VRS) values ranging from 1.5 to 2.2. PSSJ-FRP01 was experimentally known as PS-592 (Figure 1c) and was developed, advanced, and maintained as a mixed population. The percentage of segregant genotypes varied between American pima (~30%) and Uzbek pima-like (~70% with large leaves, columnar growth, and fruiting cluster habit). The breeding pedigree of this line before the ID designation of PS-592 is obscure because a large seed increase was accomplished in an isolated, noninfested FOV4 field at the University of California Kearney Research and Extension Center, Parlier, CA, in which Pima S-6 and Pima S-7 germplasm were included in the same field. Outcrossing in cotton ranged from 0.5 to 10%, depending on insect pressure and bee activity for the most part (Loureiro et al., 2016; Van Deynze et al., 2011). Mass selection was applied toward selection of columnar growth and fruiting cluster habit genotypes, and open pollinated bolls were harvested from single plants in 2009. However, starting in 2014 four new cycles of evaluations and single plant selections were performed on PS-592 (now PSSJ-FRP01) to increase its uniformity and level of FOV4 resistance. The general evaluation and selection procedures are outlined in a recent published article by Ulloa et al. (2020), and additional information about FOV4 greenhouse and FOV4-infested field evaluations is provided below.

Pima germplasm lines PSSJ-FRP02 and PSSJ-FRP03 originated from a cross of germplasm lines PS-592 and SA-3208 (LIAO MIAN 7 HAO). There is no additional information about germplasm line LIAO MIAN 7 HAO in the USDA-ARS collection. SA-3208 contained plants that we found to be moderately resistant to FOV4 in evaluations done in both greenhouse FOV4 inoculations and in field evaluations done on plants grown in naturally FOV4-infested fields (Ulloa et al., 2020). Briefly, starting in 2015 parental lines were selected after FOV4 evaluation under naturally infested field conditions, typically 6–8 wk after planting. Selected entries were arranged in one row plot in a randomized complete block design or incomplete block designs with susceptible and resistant checks (information in the Data Analysis section) using three replications. Susceptible and resistant cultivar checks were spatially placed at the site. After initial FOV4 evaluation and during the flowering period, healthy or asymptomatic plants from parental lines from each plot were selected as female or male to make crosses and produce the F_1 seed. After boll maturity, F_1 seed cotton was harvested from individual bolls of each selected plant-female and planted in the greenhouse for further evaluation and to produce seed to evaluate selections in the infested field the following growing season. After selecting within parental lines for improved FOV4 response and making crosses, F_2 seed from a single plant were grown and evaluated again under natural FOV4 pressure in the field and were arranged in one row plot in a randomized complete block design or incomplete block designs with susceptible and resistant checks using three or

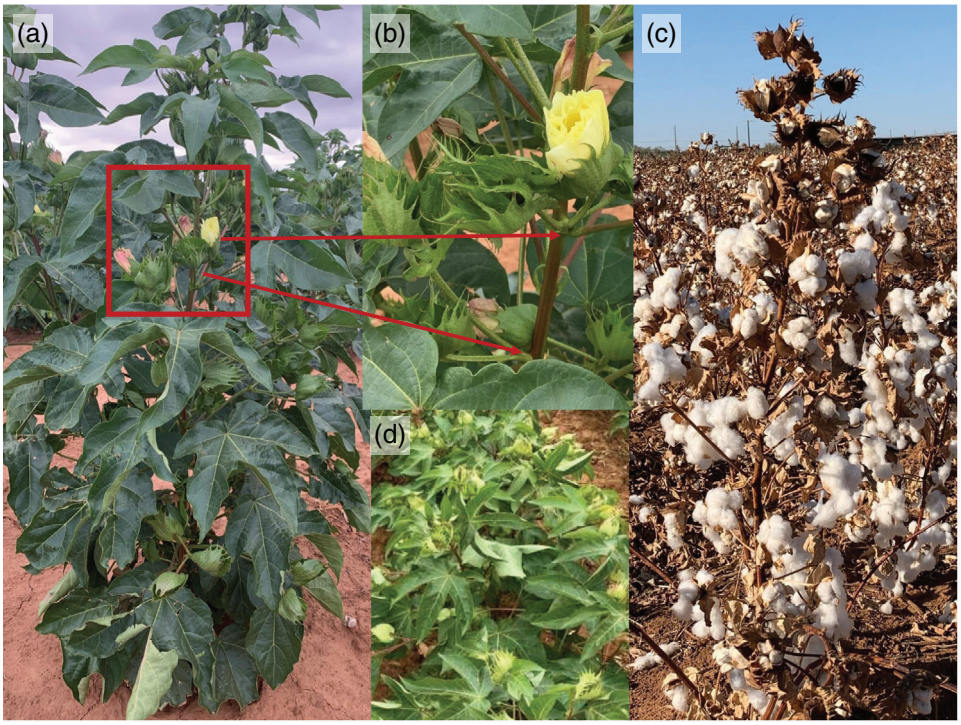

FIGURE 1 (a, b) *Gossypium barbadense*, GB1368/k-7616, an Uzbekistan accession introduction with different plant structure, large leaves, columnar growth, and fruiting cluster habit. (c) Full irrigated plant from PSSJ-FRP01 germplasm line. (d) American pima *Gossypium barbadense* cotton

two replications, depending on seed availability. Susceptible and resistant cultivar checks were also spatially placed at the site. After FOV4 evaluation, healthy or asymptomatic plants were selected from each of these selections from the plot or progeny-row at the infested FOV4 field up to F_4. At each generation from these asymptomatic single plants, seed was advanced for the following year cycle of evaluation and selection by self-pollination and caging using nets to cover the single plant for each selection every year. Three recurrent cycles of selection were performed under infested FOV4 pressure on these newly developed germplasm lines. To obtain nontransgenic progeny, single parental plants and F_1 and/or F_2 plants were tested for the adventitious presence of transgenes in every cross-combination using Envirologix available cotton kit-tests, and plants that tested negative for adventitious presence were used for further advancement.

To obtain enough seed for multiple FOV4 testing sites (California and Texas) in 2018 and 2020, seed increases were performed that also assisted in increasing uniformity within the pima PSSJ-FRP01, PSSJ-FRP02, and PSSJ-FRP03 germplasm lines. The 2020 increase provided the seed for the germplasm release distribution to private and public breeders. These seed increases were performed in an uninfested field at the University of California, West Side Research and Extension Center at Five Points, CA. Net-cages were placed on a single or multiple plots for each line to obtain "pure" seed. Because there is no soil FOV4 quantification assay available, the field was determined to be below the FOV4 infection threshold (noninfested) based on scouting and the growth and development of susceptible lines, which showed no symptoms to FOV4 during the growing season.

2.2 | FOV4 evaluation and fiber quality

For field evaluations, the lines were planted in fields known to be infested with FOV4 and exhibiting severe *Fusarium* wilt symptoms (inoculum levels were unknown and varied from year to year). Each line was grown in one-row plots that were 5 m long with 1-m row spacing in a randomized complete block design or incomplete block designs with three replications. Because of seed availability and/or limitation of infested FOV4 field space/area showing better distribution and infestation of this pathogen, two replications were occasionally used. Five plants were assayed per plot from 2014 through 2021. To evaluate lines for FOV4 resistance, responses from field test evaluations were examined using percentage plant survival or percentage mortality, foliar symptoms, and vascular necrosis or staining of roots. Responses were examined using ANOVA or nonparametric analyses as appropriate. Greenhouse evaluations for infection of FOV4 plant selections and advanced generations were conducted at the University of California Kearney Research and Extension Center (near Parlier, CA). No supplemental lighting was used to modify photoperiod

TABLE 1 Fiber quality properties of the pima germplasm releases PSSJ-FRP01, PSSJ-FRP02, PSSJ-FRP03 from the seed increase at the University of California, West Side Research and Extension Center, Five Points, CA, in 2020

Entry	Micronaire	Strength	Length	Uniformity
		kN m kg^{-1}	mm	%
PSSJ_FRP01	4.6	426	34.5	87.1
PSSJ_FRP02	4.1	421	35.0	85.4
PSSJ_FRP03	4.5	411	35.0	86.2
Pima cotton checks	3.5–4.9	385–425	35.0–36.8	83.0–85.0

during these greenhouse tests. In addition, greenhouse tests were performed at University of California Riverside, CA, and California State University Fresno, CA, to confirm FOV4 infection response on selected lines. The root-dip method was used for evaluation of parents and selections. Briefly, germplasm lines were seeded into a moist composite medium of 1:1 rate soil of vermiculite and peat moss prior to inoculation. Roots of 2-to-4-wk-old seedlings were dipped in water or a spore suspension of 1×10^6 conidia ml^{-1} for 2 min (data not shown). Additional information can be found in Ulloa et al. (2006, 2020).

To determine the level of resistance for each line, plants were assayed from each replicated plot for VRS and plant stand (PS). Individual plants from each line were rated for disease severity based on VRS. A scale of 0–5 was used, where VRS = 0 indicated no vascular root staining evident and VRS 5 = indicated severe plant damage or death with staining evident throughout the root tissue. Plant stand in each plot was recorded first after planting (12–14 d) and later at ~90–100 d and/or at final evaluation. Plant stand was calculated by dividing the total number of surviving plants on each sample date by the initial plant count made 12–14 d after planting and multiplying by 100. Percentage plant mortality (Mortality %) was calculated by Mortality % = 100% – PS. Additionally, fiber samples were collected in 2020 from the noninfested seed increase field at Five Points, CA, and analyzed for quality using a high-volume instrument at the USDA Cotton Program in Visalia, CA (Table 1). Compared with Upland cotton, foliar symptoms, VRS, and Mortality % diagnostic symptoms in pima tend to be more consistent during the entire season and as disease progressed through the season on susceptible pima (Figure 2).

2.3 | Data analyses

Differences among observed VRS, PS, and Mortality % phenotypes within study entries and among study entries were evaluated for each experiment using PROC GLM (ver. 9.2 or 9.4, SAS Institute). After significant differences ($p < .05$) were observed from the ANOVA, mean separation examinations of main effects were conducted using the Waller–Duncan *k*-ratio procedure (Ott, 1988) and LSD. The pima PSSJ-FRP01-FRP03 lines were included in all FOV4 evaluations and field-replicated trials. In addition, FOV4-susceptible Pima S-7 (Feaster & Turcotte, 1984), susceptible pima Deltapine 357 (DP357), resistant Pima S-6 (Turcotte et al., 1992) and Phytogen PHY 881 RF (PHY881 RF) (Corteva AgriScience), susceptible Phytogen PHY 764 WRF (PHY764), and tolerant FOV4 FiberMax FM 2334GLT (FM2334 GLT) (BASF Co.) cultivar checks were included in these evaluations at the different sites in California and Texas. Analyses of variance are presented herein for the germplasm lines (PSSJ-FRP01, PSSJ-FRP02, and PSSJ-FRP03) with the above cultivars checks that were performed from data of one FOV4 field evaluation from California (Tipton 2021) and two evaluations (2020 and 2021) from one FOV4 field site in the El Paso, TX, region. In the figures, different letters define significant differences at $p > .05$.

3 | CHARACTERISTICS

In 2019–2021, the pima PSSJ-FRP01-FRP03 germplasm lines were planted in fields known to be infested with FOV4 and to produce severe Fusarium symptoms (inoculum levels were unknown) under replicated trials in California and Texas. Soil textures in the FOV4-infested field sites were clay loam in California; soils at the Texas site had similar texture. During evaluations in 2019–2021 in the SJV of California and El Paso, TX, region, significant differences ($p < .05$) were found between the germplasm lines and susceptible cultivar checks Pima S-7 and DP357. Depending on the infested field site (California or Texas) and analyzed data sets (Figure 3), significant differences were observed for VRS between evaluated sites in some of these developed FOV4-resistant pima PSSJ-FRP01, PSSJ-FRP02, and PSSJ-FRP03 germplasm lines. Some VRS differences were also observed among lines based on sites and analyzed data sets. No significant differences were observed among developed FOV4 resistance lines for Mortality %, showing <10% and levels of FOV4 VRS infection value ratings that were generally below 1.2, with many lines rated below VRS <1.0. Importantly, even though FOV4 VRS was 0.0 or some evaluated plants did not show infection at a replicated test site and/or using an analyzed data set from a site, lines could not be considered to be immune to FOV4 because at a different site at least one plant showed infection, averaging VRS <0.5 (Figure 3).

For comparison of the lines described below, resistant Pima-S6 from 2019 to 2021 averaged 0.82 for VRS and 16 for Mortality %, and in the 2021 test, Pima-S6 averaged 0.4 for VRS and 7 for Mortality %. Susceptible Pima S-7 from 2019 to 2021 averaged 3.5 for VRS and 72 for

FIGURE 2 Images of different Fusarium wilt race 4 (FOV4) disease. (a) Mortality %. (b and d) Typical leaf foliar symptoms. (c) Typical FOV4 root infection and vascular root staining

Mortality % and in the 2021 test Pima S-7 averaged 2.3 for VRS and 60 for Mortality %. In 2021, susceptible Upland PHY764 averaged 1.8 for VRS and 12 Mortality % in California FOV4-infested field sites (Figure 3a,c). Under infested field conditions, seed germination was variable, and, although the seed of the germplasm lines was not treated with fungicide or pesticide, the lines performed as well or better for VRS and Mortality % compared with known FOV4-resistant commercial cultivars. In the El Paso, TX, region in 2020 and 2021, susceptible pima DP357 averaged 4.2 for VRS and 80 for Mortality %. Upland tolerant FM2334 GLT averaged 0.85 for VRS and 20 for Mortality %. The resistant commercial pima cultivar PHY 881 RF averaged 0.72 for VRS and 20.0 for Mortality % (Figure 3b,d).

To compare fiber quality from fiber samples collected in 2020 from the seed increase field at Five Points, CA, and analyzed for quality using a high-volume instrument at the USDA Cotton Program in, Visalia, CA, measured estimates for pima commercial cultivars grown under irrigated conditions normally range in fiber quality from 3.5 to 4.9 for micronaire, from 385 to 425 kN m kg^{-1} for strength, from 34.5 to 36.8 mm for length, and from 83.0 to 85.0% for uniformity. Pima germplasm line PSSJ-FRP01 averaged 4.6 for micronaire, 426 kN m kg^{-1} for strength, 34.5 mm for length, and 87.0% for uniformity. Pima germplasm line PSSJ-FRP02 averaged 4.1 for micronaire, 421 kN m kg^{-1} for strength, 35.0 mm for length, and 85.4% for uniformity. The pima germplasm line PSSJ-FRP03 averaged 4.5 for micronaire, 411 kN m kg^{-1} for strength, 35.0 mm for length, and 86.2% for uniformity (Table 1).

4 | AVAILABILITY

Small quantities of seed (10–25 g) are available to cotton breeders, geneticists, and other research personnel upon written request to M. Ulloa (mauricio.ulloa@usda.gov), USDA-ARS, PA, CSRL, Plant Stress and Germplasm Development Research, 3810 4th Street, Lubbock, TX 79415. It is requested that appropriate recognition of the source be given when these germplasm lines contribute to the development of a new breeding line, hybrid, or cultivar. Genetic material of this

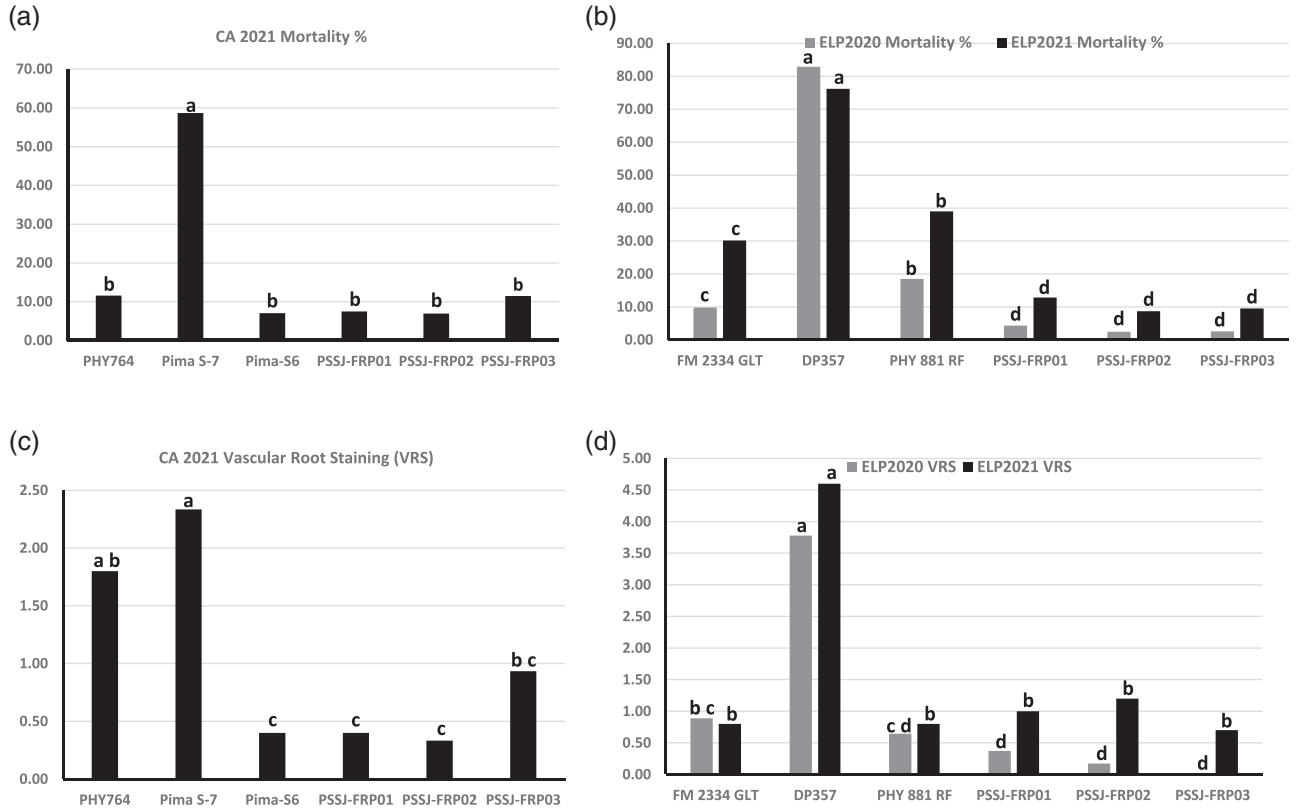

FIGURE 3 Mean percentage mortality (Mortality %) and vascular root staining (VRS) of PSSJ-FRP01-PSSJ-FRP03 germplasm lines and susceptible and resistant known control checks evaluated under Fusarium wilt race 4 (FOV4)-infested field at two sites, California (CA) and Texas (ELP) for pathogen infection response in replicated trials. Included FOV4 known control susceptible checks: Pima S-7 and pima Deltapine 357 (DP357); resistant Pima S-6 (Pima-S6), Phytogen PHY 881 RF (PHY881 RF), and Phytogen PHY 764 WRF (PHY764); and tolerant FiberMax FM 2334 GLT (FM2334 GLT). Different letters represent significant differences at $p > .05$. VRS scale = 1 (no symptoms) to 5 (plant death)

release has been deposited in the USDA-ARS National Plant Germplasm System where it will be available after five years for research and breeding purposes, including development and commercialization of new cultivars.

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AUTHOR CONTRIBUTIONS

Mauricio Ulloa: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Visualization; Writing-original draft; Writing-review & editing. Ibrokhim Y Abdurakhmonov: Conceptualization; Funding acquisition; Investigation; Resources; Validation; Writing-review & editing. Robert Hutmacher: Data curation; Investigation; Methodology; Project administration; Validation; Writing-review & editing. TariLee Schramm: Data curation; Validation; Writing-review & editing. Shukhrat Shermatov: Resources; Writing-review & editing. Zabardast Buriev: Resources; Writing-review & editing. Philip Roberts: Funding acquisition; Investigation; Methodology; Project administration; Writing-review & editing. Margaret L. Ellis: Funding acquisition; Investigation; Methodology; Project administration; Writing-review & editing. Paxton Payton: Funding acquisition; Validation; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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