Scientia Horticulturae 242 (2018) 110–115

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

# Partial success of marker-assisted selection of 'A' and 'B' onion lines in Brazilian germplasm

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ARTICLE INFO

*Keywords:*
*Allium cepa*
Pollen viability
Male-sterile
Maintainer
Hybrid

ABSTRACT

This work aims to identify 'A' and 'B' onion lines in Brazilian germplasm, associating marker-assisted selection (MAS) of cytoplasm type and the *Ms* locus with pollen viability analysis. MAS and pollen viability tests presented a complete agreement for the lines Alfa SF, BRS 367, BRS 367, Cascuda T7, Cascuda T5, EHCEB 20102017 and EHCEB 20142027. MAS was not validated by the pollen viability tests in some plants of the lines Alfa SF, EHCEB 20142028, EHCEB 20141028, EHCEB 20141027 e EHCEB 20141017. Plants with fertile pollen within a specific 'A' line were eliminated before anthesis. The methods used to evaluate pollen viability did not present agreement in the sterile and fertile classification in five onion accessions. Six pairs of 'A' and 'B' lines identified in the present study [Alfa SF (T*msms*) x Alfa SF (N*msms*), BRS 367 (T*msms*) x BRS 367 (N*msms*), Cascuda T7 (N*msms*) x Cascuda T5 (S*msms*), EHCEB 20142027 (N*msms*) x 20141027 (T*msms*), EHCEB 20102017 (N*msms*) x EHCEB 20141017 (T*msms*), and EHCEB 20142028 (N*msms*) x EHCEB 20141028 (S*msms*)] have potential for onion hybrid development.

## 1. Introduction

Onion (*Allium cepa* L.) stands out as one of the main vegetable produced in Brazil, with an estimated production of 1.6 million tons for the year of 2015 (IBGE, 2015). The states of Bahia and Pernambuco, in the northeast region of the country, account for almost 20% of the national production, where the use of open-pollinated cultivars predominates (Santos et al., 2012). Despite the increase in the use of hybrids in the Southeast, Midwest, and part of the Northeast (Faria et al., 2012), open-pollinated cultivars account for almost 70% of the Brazilian onion production (Santos and Oliveira, 2011).

The CMS-S and CMS-T systems of nuclear-cytoplasmic sterility are employed in the production of onion hybrids seeds. The first one is the most widely used, due to the great stability in the different environments (Havey, 2000). In the CMS-S system, only one gene (*Ms*) restores fertility, while alleles of three genes are involved in fertility restoration in the CMS-T system (Kim et al., 2015). These systems require the use of male-sterile lines ('A' lines), maintainer lines ('B' lines), and lines with good combining ability ('C' lines) (Santos et al., 2008).

The obtainment of onion hybrids by the conventional method is costly and takes almost 20 years in temperate regions, including the identification of 'A' lines (male-sterile lines) and 'B' lines (maintainer lines) in the open-pollinated populations (Pike, 1986). Previous attempts to identify 'A' and 'B' lines in the Brazilian germplasm by conventional methods were not successful in the development of onion hybrids (Santos et al., 2010).

The full assisted selection of 'A' and 'B' lines by molecular markers became possible with cytoplasm identification by the markers 5'*cob* and *orf*A501 (Sato, 1998; Engelke et al., 2003) or *orf*725 (Kim et al., 2009), and with the possible identification of the male-sterility restorer nuclear locus (*Ms*) by the markers AcSKP1 (Huo et al., 2015) or AcPMS1 (Kim et al., 2015).Thus, it is expected that marker-assisted identification of 'A' and 'B' lines takes three years in tropical environments, where the seed-to-seed cycle is one year, considering at least one test cross. This result will lead to significant reduction of the time required by classic methods.

The stability of fertile and sterile pollen grains in 'B' and 'A' lines, respectively, which is the basis of the production of onion hybrids, can be proven with several reagents, such as acetic carmine and Alexander's staining solutions, or *in vitro* germination of the pollen grain (Abdelgadir et al., 2012). Khar and Saini (2016) reported marker-assisted selection of 'A' and 'B' lines in an Indian onion germplasm, associating pollen viability with acetic carmine staining. For the Brazilian germplasm, no similar studies associating marker selection for 'A' and

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https://doi.org/10.1016/j.scienta.2018.08.002
Received 15 January 2018; Received in revised form 25 June 2018; Accepted 1 August 2018

**Table 1**
Cytoplasm type identified with the marker *orf725* and genotyping of the male-fertility restorer locus by the marker AcSKP1 in plants (n) of onion accessions with potential for 'A' and 'B' lines.

| Accession | Cytoplasm | | | | Genotyping | | | |
|---|---|---|---|---|---|---|---|---|
| | N | N | S | T | n | *MsMs* | *Msms* | *msms* |
| Alfa SF linha 'A' | 12 | – | – | 12 | 12 | – | – | 12 |
| Alfa SF linha 'B' | 5 | 5 | – | – | 5 | – | – | 5 |
| BRS 367 | 9 | 6 | 1 | 2 | 9 | 3 | 2 | 4 |
| Cascuda T7 ('B') | 13 | 13 | – | – | 13 | 7 | 4 | 2 |
| Cascuda T5 ('A') | 23 | – | 23 | – | 23 | 11 | 10 | 2 |
| EHCEB 20142028 | 13 | 13 | – | – | 13 | – | – | 13 |
| EHCEB 20141028 | 42 | – | 42 | – | 42 | – | – | 42 |
| EHCEB 20142027 | 3 | 3 | – | – | 3 | – | – | 3 |
| EHCEB 20141027 | 14 | – | – | 14 | 14 | – | – | 14 |
| EHCEB 20101017 | 10 | – | – | 10 | 10 | – | – | 10 |
| EHCEB 20102017 | 12 | 12 | – | – | 12 | – | – | 12 |

'B' lines with pollen viability analysis have been reported yet.

The objective of this work was to identify 'A' and 'B' lines in Brazilian onion germplasm, associating marker-assisted selection of the cytoplasm type and the *Ms* locus with pollen viability analysis as an initial stage for the development of onion hybrids.

## 2. Material and methods

### 2.1. Plant material

Eleven accessions (Table 1) from the onion germplasm bank of EMBRAPA (Brazilian Agricultural Research Corporation) were evaluated in this study. The accessions had been previously identified (Ferreira et al., 2017) with potential for selection of 'A' and 'B' lines, as they presented either mixed cytoplasm and recessive *ms* alleles or 100% of plants at N*msms* and S*msms* or T*msms* condition. Onion seedlings were transplanted in May/2016, in the experimental field of Embrapa Semiárido, Petrolina, PE, and harvested in September/2016.

Bulbs were stored and vernalized in a cold chamber for 120 days, at 8 °C, and approximately 60% air humidity, for further planting and emission of floral scape, aiming at the evaluation of pollen viability and analyses with cytoplasmic markers and male-fertility restorer locus.

### 2.2. Evaluation of cytoplasm type and male-fertility restorer locus (Ms)

Genomic DNA was extracted using the 2x CTAB protocol (Doyle and Doyle, 1990), as described by Ferreira et al. (2017).

The cytoplasm type was identified with the marker *orf*725 (Kim et al., 2009), for a final volume of 10 µl: 0.2 µM of each primer, 0.2 mM of each dNTP, 1x of PCR buffer, 2.0 mM $MgCl_2$, 1.0 unit of Taq DNA polymerase, and 50 ng total DNA. The thermocycler was programmed for the amplification of this marker, as follows: initial denaturation cycle of 5 min at 94 °C, 40 cycles of 30 s at 94 °C, 1 min at 60 °C, and 90 s at 72 °C, and a final cycle of 5 min at 72 °C. PCR products were visualized and classified on 1.5% agarose gels: 833 bp fragment = 'N' cytoplasm; 833 bp and 628 bp fragments = 'T' cytoplasm, and 628 bp fragment = 'S' cytoplasm.

The alleles of the male-sterility restorer locus were identified by the marker AcSKP1 (Huo et al., 2015), for a total volume of 25 µl: 50 ng of total DNA, 0.4 µM of FU898, 0.4 µM of FD898, 0.2 µM of SU628, 0.2 µM of SD628, 0.15 mM of each dNTP, 1x of PCR buffer, 2.0 mM $MgCl_2$, and 2.0 units of Taq DNA polymerase. The amplification protocol adopted for AcSKP1 followed the description of Huo et al. (2015). Amplified products were separated and classified on 1.5% agarose gels. The evaluation for the fertility restorer locus of the marker AcSKP1 considered: 898 bp fragment = dominant homozygote (*MsMs*), 898 and 628 bp fragment = heterozygote (*Msms*), and 628 bp fragment = recessive homozygote (*msms*).

### 2.3. Analysis of pollen viability

Plants identified with cytoplasm *Smsms* or *Tmsms* and *Nmsms* were evaluated for pollen grain fertility or sterility. Four freshly opened flowers were randomly collected from the umbel of each plant for the analysis of pollen viability using 2% acetic carmine and modified Alexander's solution (Peterson et al., 2010) and *in vitro* germination.

#### 2.3.1. Staining with 2% acetic Carmine and modified alexander's solution

Freshly opened flower buds were individually collected from plants of each accession. For the preparation of the slides, pollen grain was removed by crushing the anthers immersed in a drop of dye deposited on a histological slide covered with coverslips. Four slides per inflorescence were prepared for each dye, and in each slide, 100 pollen grains were counted, using a light microscope at 100× magnification, totaling 400 pollen grains for the four replications per dye.

For 2% acetic carmine, pollen grains were classified as fertile when they presented 100% reddish coloration, and as sterile when they showed 100% brownish coloration, no coloration, or partial coloration. For the modified Alexander's solution, pollen grains were considered as viable when they presented purple coloration and as non-viable when they presented green or diffuse coloration.

#### 2.3.2. In vitro germination

Pollen grains were collected during the anthesis of the inflorescence flowers and inoculated on excavated slides containing 200 g $L^{-1}$ of sucrose, 50 mg $L^{-1}$ of boric acid, and 1 g of agar, after being heated to total agar dilution (Gomes et al., 2000). The medium was distributed in the wells of the excavated slides after being heated to total agar dilution.

Pollen grain was spread on the culture medium for a homogeneous distribution of the material. Slides containing pollen grain and culture medium were kept in Petri dishes with moistened paper, simulating a moist chamber, to avoid the drying of the medium, and stored at room at 25 °C temperature with exposure to bright light for a minimum period to start the evaluation of 4 h. Germination was evaluated by measuring the pollen tube length, with the aid of an optical microscope with 100x magnification (Karak and Hazra, 2012). To facilitate the counting of pollen grains, each well of the slide was divided into two vision fields, corresponding to two replications. For each plant, two wells were prepared, totaling four replications. A hundred pollen grains was counted in each replication. Pollen grains were considered as fertile when they presented a tube length equal to or greater than the diameter of the pollen grain itself.

## 3. Results

Gels were easily identified, producing fragments of the expected sizes, both for the cytoplasmic marker (*orf*725) (Kim et al., 2009) (Fig. 1) and for the marker of the restorer locus (AcSKP1) (Huo et al., 2015) (Fig. 2).

'A' and 'B' pure lines were observed among the evaluated accessions, which presented 100% *Smsms* or T*msms* and 100% N*msms*, respectively, for the accessions selected for this allele-cytoplasmic combination: Alfa SF line 'A'/Alfa SF line 'B', EHCEB 20141028/EHCEB 20142028, EHCEB 20141027/EHCEB 20142027, and EHCEB 20101017/EHCEB 20102017 (Table 1). The accession 'BRS 367' presented a mixture for cytoplasmic type, as well as genotypic mixture for the fertility restorer locus, which may allow the identification of 'A' and 'B' lines (Table 1). The accessions Cascuda T7 ('B') and Cascuda T5 ('A') presented a single type of cytoplasm and genotypic mixture for fertility restoration (Table 1), indicating the possibility of using Cascuda T7 plants as maintainers of Cascuda T5, considering that both accessions belong to the Valencian onion group.

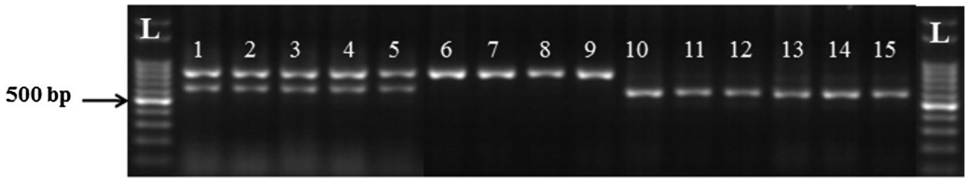


**Fig. 1.** DNA fragments from 15 onion samples (*Allium cepa* L.), amplified with the primers of the marker *orf725*. Alfa SF 'A' (cytoplasm 'T', 628 and 833 bp fragments) = columns 1, 2, 3, 4 and 5. Alpha SF 'B' (cytoplasm 'N', 833 bp fragments) = columns 6, 7, 8, and 9. EHCEB 20142028 ('S' cytoplasm, 628 bp fragments) = columns 10, 11, 12, 13, 14, and 15. L = 100 bp ladder.

The differentiation between viable and non-viable pollen grains was easily detectable for CMS-S (Fig. 3) and CMS-T (Fig. 4). The analyses of pollen grain viability of plants with S*msms* or T*msms* and N*msms* cytoplasm presented identical classification for fertile or sterile plants for the accessions Alfa SF (T*msms*), Alfa SF (N*msms*), BRS 367 (T*msms*), BRS 367 (N*msms*), Cascuda T7 (N*msms*), Cascuda T5 (S*msms*), EHCEB 20142027 (N*msms*), and EHCEB 20102017 (N*msms*) in the three methods used in this study (Table 2). Small differences in classification were observed between the two staining methods and *in vitro* germination for the accessions EHCEB 20142028 (N*msms*) and EHCEB 20141028 (S*msms*); small differences in classification were observed between the three methods for the accession EHCEB 20141027 (T*msms*); and small differences in classification were observed between acetic carmine and modified Alexander's solution for the accession EHCEB 20141017 (T*msms*) (Table2).

In the sterile line Alfa SF (*Tmsms*), only two out of the 12 plants analyzed presented the expected sterility, while all the fertile plants of Alfa SF (*Nmsms*) presented fertility by the three methods of pollen viability (Tables 1 and 2). For the accessions BRS 367 (T*msms*), BRS 367 (N*msms*), Cascuda T5 (S*msms*), Cascuda T7 (N*msms*), EHCEB 20142027 (N*msms*), and EHCEB 20102017 (N*msms*), full agreement was observed between the nuclear-cytoplasmic classification with sterility and fertility using the three methods of pollen viability (Tables 1 and 2).

Differences of 8% and 20% were observed for EHCEB 20142028 (N*msms*) and EHCEB 20141017 (T*msms*), respectively (Table 1), considering the marker-assisted allele-cytoplasmic classification with the pollen viability by the three methods (Table 2). For the accession EHCEB 20141028 (S*msms*), a classification difference of 17% was observed between molecular identification (Table 1) and the staining tests; and of 2.3% with *in vitro* germination (Table 2), whereas for accession EHCEB 20141027 (T*msms*), the discrepancies were higher (32% and 14.3%) for the two staining methods and *in vitro* germination, respectively (Table 2).

## 4. DISCUSSION

The phenomenon of heterosis or hybrid vigor has benefited agriculture and fascinated geneticists for over 100 years. Heterosis is defined as the situation in which hybrids exhibit agronomic performance superior to that of their parents (Schnable and Springer, 2013). Santos et al. (2012) reported heterobeltiosis of 34.0% and 30.2% in experimental hybrids of Baia Periforme, with 'A' and 'B' lines identified in BRS Alfa São Francisco, evaluated in Petrolina, PE. The identification of several 'A' and 'B' lines, associating marker-assisted selection for cytoplasmic type and *Ms* locus with pollen viability, as demonstrated in this work, could substantially reduce the prices of hybrids available in the local market, making them affordable to producers.

The plants of the accessions analyzed in this study presented a classification of 100% S*msms* or T*msms* and 100% N*msms*, confirming previous evaluation performed with markers for cytoplasm type and *Ms* locus (Ferreira et al., 2017), and indicating their potential for the development of hybrids. The absence of segregation within 'A' and 'B' lines is essential for onion hybrid production, considering that the existence of segregants may decrease heterosis vigor and bulb uniformity, as well as the expected maturation in commercial hybrids.

Marker-assisted selection in onions has been used to differentiate 'N', 'S', and 'T' cytoplasm (Khar and Saini, 2016; Patil et al., 2016; Ragassi et al., 2012, 2010; Santos et al., 2010), contributing to reduce the number of test crosses required to identify 'A' and 'B' lines. Markers for the *Ms* locus, developed by Huo et al. (2015) and Kim et al. (2015), enabled the full assisted identification of 'A' and 'B' lines, and have been applied in Indian germplasm (Khar and Saini, 2016) and in Brazilian germplasm (Ferreira et al., 2017) for the simultaneous identification of the cytoplasm type and recessive condition of the *Ms* allele.

The use of markers may be associated with other methods that allow their validation, such as pollen viability tests. Studies on molecular association and staining and *in vitro* germination have been reported for onion materials from India (Khar and Saini, 2016; Patil et al., 2016; Saini et al., 2015).

The results of the analyses of the pollen viability with acetic carmine, modified Alexander's solution, and *in vitro* germination showed high agreement in the present study, indicating the usefulness of these methods to evaluate onion fertility. For Ockendon and Gates (1976), staining methods overestimate the fertility of the pollen grain; this is because pollen grains with cytoplasm are not necessarily fertile. However, the same authors point out that non-viable pollen grains are identified with acetic carmine due to the absence of cytoplasm. The same principle can be applied to the modified Alexander's solution, which also considers the filling of the pollen grain by the protoplasm as a viability evidence.

Staining with acetic carmine has been used in studies with 'A' and 'B' onion lines (Lorenzon and Almeida, 1997) and coconut lines (Machado et al., 2014) associating with *in vitro* germination of the pollen grain. For Alexander (1980), the dye developed by him presents

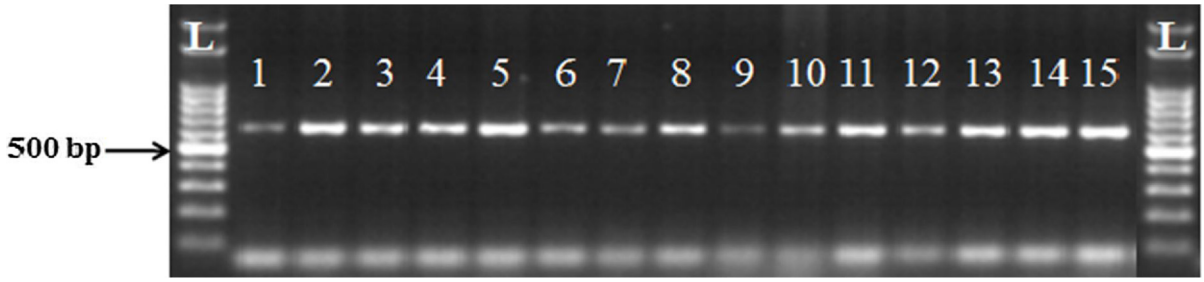


**Fig. 2.** DNA fragments from 15 onion samples (*Allium cepa* L.), amplified with the primers of the marker AcSKP1. EHCEB 20142028 (recessive homozygote, 628 bp fragments) = columns 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. EHCEB 20102017 (recessive homozygote, 628 bp fragments) = columns 11, 12, 13, 14, and 15. L = 100 bp ladder.

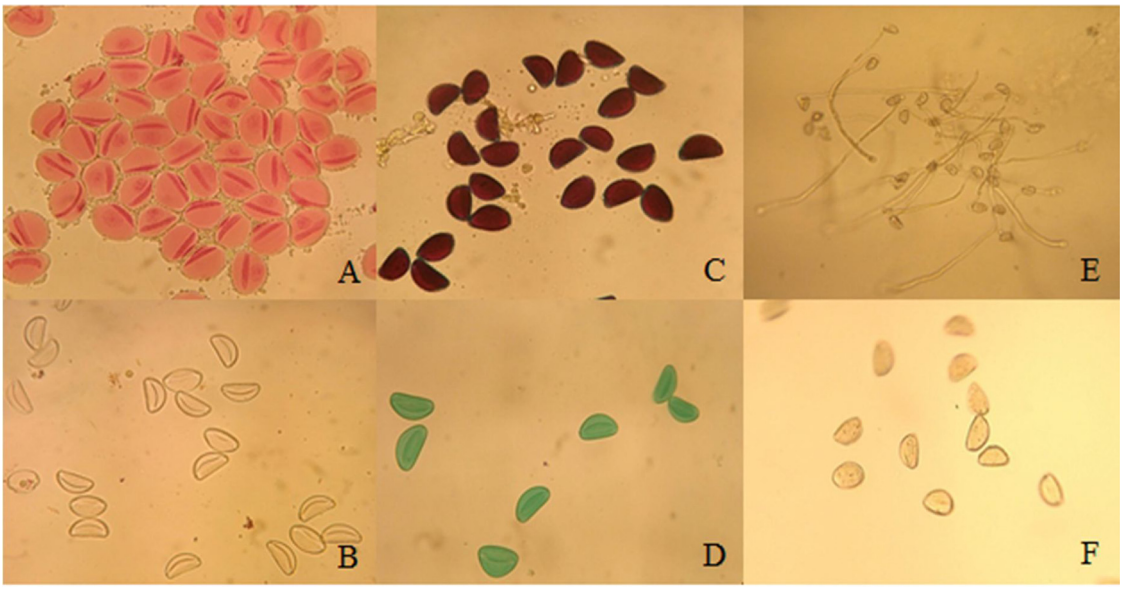


**Fig. 3.** Viability of onion pollen grains with acetic carmine, modified Alexander's solution, and *in vitro* germination in CMS-S. Acetic carmine: (A) viable pollen and (B) non-viable pollen; modified Alexander's solution: (C) viable pollen and (D) non-viable pollen; *in vitro* germination: (E) germinated pollen (viable) and (F) non-germinated pollen (non-viable).

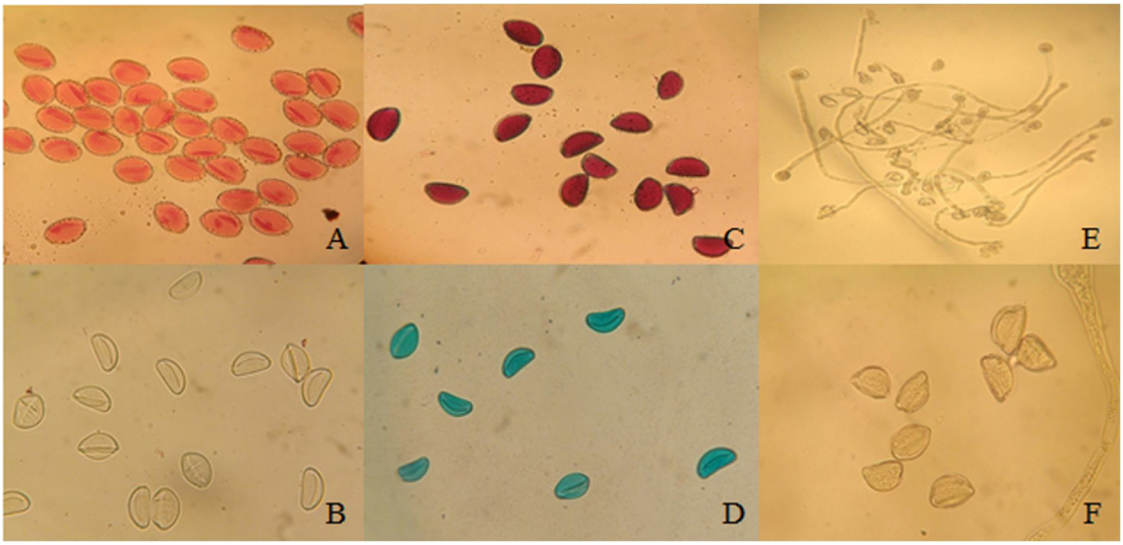


**Fig. 4.** Viability of onion pollen grains with acetic carmine, modified Alexander's solution, and *in vitro* germination in CMS-T. Acetic carmine: (A) viable pollen and (B) non-viable pollen; modified Alexander's solution: (C) viable pollen and (D) non-viable pollen; *in vitro* germination: (E) germinated pollen (viable) and (F) non-germinated pollen (non-viable).

greater precision than acetic carmine, which has also been confirmed by Auler et al. (2006) in pollen viability estimates in *Baccharis trimera*. Tome et al. (2007) and Vieira et al. (2012) evaluated pollen viability of species of the genus *Solanum* and *Manihot*, respectively, and found discrepancies between the staining test and the *in vitro* germination test, suggesting that, in these cases, only the staining tests are not adequate to estimate pollen grain viability.

The association of allele-cytoplasmic molecular selection with pollen viability was not effective for the plants Alfa SF (T*msms*), EHCEB 20142028 (N*msms*), EHCEB 20141017 (T*msms*), EHCEB 20141028 (S*msms*), and EHCEB 20141027 (T*msms*). This is because the plants which were supposed to have presented full sterility showed fertile pollen grains or *vice versa*. A full agreement of the molecular classification of 'A' and 'B' lines with pollen viability occurred in the present study for plants of the accessions Alfa SF (N*msms*), BRS 367 (T*msms*), BRS 367 (N*msms*), Cascuda T5 (S*msms*), Cascuda T7 (N*msms*), EHCEB 20142027, and EHCEB 20102017, indicating that this approach is adequate for the development of onion hybrids.

Saini et al. (2015) verified concordant results of pollen viability with marker-assisted selection for 'A' and 'B' lines in three Indian open-pollinated onion populations. In another study with Indian germplasm, Khar and Saini (2016) reported a low agreement between the marker-assisted selection of 'A' and 'B' lines with viability test with 0.5% acetic carmine. The low agreement reported by the authors mentioned above may be related to the reduced penetrance of the *Ms* allele reported by Melgar and Havey (2010), in which plants homozygous dominant at *Ms* may not present 100% fertility restoration, suggesting not a failure of the marker-assisted selection. In the present study, agreement was observed for the classification by the pollen viability methods employed with assisted selection for the plants of seven accessions and absence of agreement for plants of five accessions, indicating reasonable accuracy of the assisted selection. *Smsms* or *Tmsms* plants with pollen viability or

**Table 2**
Pollen viability according to the staining methods with 2% acetic carmine (AC) and modified Alexander's solution (MAS) and *in vitro* germination (IVG) of N*msms*, S*msms* and T*msms* plants (n), identified by cytoplasm marker and male-sterility restorer locus in onion accessions.

| Accession | n | AC | | MAS | | IVG | |
|---|---|---|---|---|---|---|---|
| | | Fertile | Sterile | Fertile | Sterile | Fertile | Sterile |
| Alfa SF (T*msms*) | 12 | 10 | 2 | 10 | 2 | 10 | 2 |
| Alfa SF (N*msms*) | 5 | 5 | 0 | 5 | 0 | 5 | 0 |
| BRS 367 (T*msms*) | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| BRS 367 (N*msms*) | 3 | 3 | 0 | 3 | 0 | 3 | 0 |
| Cascuda T7 (N*msms*) | 1 | 1 | 0 | 1 | 0 | 1 | 0 |
| Cascuda T5 (S*msms*) | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| EHCEB 20142028 (N*msms*) | 13 | 13 | 0 | 13 | 0 | 12 | 1 |
| EHCEB 20141028 (S*msms*) | 42 | 7 | 35 | 7 | 35 | 1 | 41 |
| EHCEB 20142027 (N*msms*) | 3 | 3 | 0 | 3 | 0 | 3 | 0 |
| EHCEB 20141027 (T*msms*) | 14 | 5 | 9 | 4 | 10 | 2 | 12 |
| EHCEB 20141017 (T*msms*) | 10 | 2 | 8 | 3 | 7 | 2 | 8 |
| EHCEB 20102017 (N*msms*) | 12 | 12 | 0 | 12 | 0 | 12 | 0 |

*Nmsms* sterile plants were eliminated before pollination, and seeds of tested 'A' plants were produced with tested 'B' plants to overcome the limitation of assisted selection.

The line Alfa SF (T*msms*), carrying the 'T' cytoplasm, presented a significant percentage of viable pollen by the methods employed, which may be associated with the limitations of the 'T' system for hybrids production (Havey, 2000), or even with contamination that occurred during seeds production. This was confirmed by Santos et al. (2012), who reported 100% of purple bulbs for seeds produced in Alfa SF 'A' line plants paired with plants of the purple bulb population.

The presence of viable pollen in male-sterile lines was also reported by Barham and Munger (1950) in CMS-S, and by Meer Van Der and Van Bennekom (1969) in CMS-T, for temperature variation from 21.1 to 26.6 °C and from 20 to 23 °C, respectively. In both works, the authors mention the influence of temperature as the main factor influencing the production of viable pollen in 'A' lines. In the present study, the temperature variation during flowering was from 25 to 35 °C and might have influenced the production of viable pollen grains in reduced quantity in male-sterile lines.

The six pairs of 'A' and 'B' lines identified in the present study [Alfa SF (T*msms*) x Alfa SF (N*msms*), BRS 367 (T*msms*) x BRS 367 (N*msms*), Cascuda T7 (N*msms*) x Cascuda T5 (S*msms*), EHCEB 20142027 (N*msms*) x 20141027 (T*msms*), EHCEB 20102017 (N*msms*) x EHCEB 20141017 (T*msms*), and EHCEB 20142028 (N*msms*) x EHCEB 20141028 (S*msms*)] are potential accessions for the development of hybrids adapted to the conditions of São Francisco Valley, and may significantly contribute to their expansion in the region.

## 5. Conclusions

1 Molecular-assisted selection and pollen viability tests presented complete agreement for the lines Alfa SF (N*msms*), BRS 367 (T*msms*), BRS 367 (N*msms*), Cascuda T7 (N*msms*), Cascuda T5 (S*msms*), EHCEB 20102017 (N*msms*), and EHCEB 20142027 (N*msms*).
2 Molecular-assisted selection was not validated by pollen viability tests in some individuals of the lines Alfa SF (T*msms*), EHCEB 20142028 (N*msms*), EHCEB 20141028 (S*msms*), EHCEB 20141027 (T*msms*), and EHCEB 20141017 (T*msms*).
3 The evaluation methods of pollen viability did not present sterile and fertile classification agreement for accessions EHCEB 20142028, EHCEB 20141028, EHCEB 20141027, and EHCEB 20141017.
4 The six pairs of 'A' and 'B' lines identified in the present study [Alfa SF (T*msms*) x Alfa SF (N*msms*), BRS 367 (T*msms*) x BRS 367 (N*msms*), Cascuda T7 (N*msms*) x Cascuda T5 (S*msms*), EHCEB 20142027 (N*msms*) x 20141027 (T*msms*), EHCEB 20102017 (N*msms*) x EHCEB 20141017 (T*msms*), and EHCEB 20142028 (N*msms*) x EHCEB 20141028 (S*msms*)] are potential materials for the development of hybrids.

## Acknowledgements

We thank CAPES for granting a scholarshipto R. R. Ferreira. C. A. F. Santos is a CNPq researcher.

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