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The effect of polyamines and SICS on the compatibility, fertility and yield indices of apple cv. Golden Delicious

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All relevant data are within the paper and its Supporting Information files.

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Abstract: The most critical problems of temperate fruit trees are poor fruit set and low yield. To solve the problem, some major chemical compounds such as polyamines and SICS (self-incompatibility control substance, Mn+B) can be used. Popular polyamines including Putrescine (0.1 and 0.25 mM), both Spermine and Spermidine (0.05 and 0.25 mM), and SICS (1 and 2 mgL^{-1}) were used alone or with cotton coverage bags to cover branches in order to investigate self-incompatibility in *Malus domestica*. Results showed that Spermidine (0.25 mM) led to higher yields in comparison with that of the control. SICS (2 mgL^{-1}), also, demonstrated the highest yield compared with that of the control. At June fruit set, treatment with Spermidine (0.25 mM) led to the highest percentage of fruit set and also the highest index of self-incompatibility and percentage of final fruit set among treatments.

1. Introduction

Alternate bearing and self-incompatibility are the main issues in apple. Alternate bearing is vigorous in some apple cultivars like 'Golden Delicious'. Most apple cultivars are self-incompatible and self-unfruitful; therefore, they need another cultivar for pollination and fertilization. Gametophytic self-incompatibility (GSI) occurs when the S allele of the pollen grain matches either of the S alleles of the stigma. In such a case, the pollen tube begins to develop but stops before reaching the micropyle (Asatryan and Tel-Zur, 2013). Gametophytic self-incompatibility is controlled by glycoprotein with RNase (S-RNase) activity expressed in the pistil. S-RNase (Qing-qing *et al.*, 2009; Duca *et al.*, 2010; Uchida *et al.*, 2012) encoded by the *S*-locus gene is named *SFB* (*S*-haplotype-specific F-box gene) in the Rosaceae. To date, in Rosaceae family, SI has mainly been studied in Japanese pear (*Pyrus pyrifolia*) and almond (*Prunus dulcis*) (Qing-qing *et al.*, 2009; Uchida *et al.*, 2012). However, some requirements are necessary for suitable cross pollination: having (1) compatible pollen grains with enough quantity and high quality (in fact, pollination with semi-compatible pollen resulted in lower fruit-set than that with fully compatible pollen) (Sapir *et al.*, 2008), (2) an overlap period of pollination between pollinizer and pollinator (pollinizer trees must be cultivated along with the pollinator trees) (3) proper time of flowering and blooming, and finally, (4) having attractive flowers in order to reduce the number of bees visiting weeds on the orchard floor (the pollen grain of fruit trees is sticky and heavy causing not to be carried by the wind) (Bekey and Burgett, 1981).

Hand pollination is a type of cross pollination that can be effective on producing crop in adverse weather conditions. When the king flower opens, it is necessary to place bee colonies in order to do cross pollination by bees and then remove them at petal fall in fruit orchards: however, this method is also time-consuming and needs a big number of workers (Bekey and Burgett, 1981; Maib *et al.*, 1996). Despite the presence of hives, pollen transfer limitation and subsequent seed set reductionwas observed in orchards (Quinet *et al.*, 2016). The other suitable technique applied to do pollination involves chemical methods; in fact, some chemical compounds can be used in order to achieve enough fruit set and high yield. Plant bio regulators (PBRs) had significant effects on increasing pollen germination and pollen tube length in almond pollen: the action of these PBRs significantly increased the percentage of fruit set at both the bud pink and petal fall phenological stages (Maita and Sotomayor, 2015). As an alternative, the use of polyamines (putrescine, spermine, spermidine), that are natural compounds involved in plant growth and development process, has been suggested reduce flowers and fruits drop; polyamines competes with ethylene synthesis with whose they share the same precursor called s-adenosyle methionine (Crisosto *et al.*, 1988; Khezri *et al.,* 2010). Polyamines as plant bio regulator revealed to increase pollen tube growth and fruit set by stimulating pollen germination (Crisosto *et al.*, 1988; Liu *et al.,* 2006) and were effective on pollen tube elongation (Aloisi *et al.*, 2015). Also, self-incompatibility control substance (SICS), which is a mixture of manganese and boron (Son *et al.*, 2009), could be useful for increasing fruit set. Polyamines and SICS were effective on increasing yield and fruit set in crops such as pear (Crisosto *et al.*, 1988; Son *et al.*, 2009), apple and apricot (Asadi *et al.*, 2013), olive (Costa *et al.*, 1986), sweet cherry (Grant Sheard, 2008), sweet orange (Saleem *et al.*, 2008), pistachio (Khezri *et al.*, 2010), mango (Malik *et al.*, 2005), and date palm (Tavakoli and Rahemi, 2014). With regard to the effect of these compounds on pollen tube growth, Spermidine (Spd) and Spermine (Spm) influence the promotion of the pollen tube elongation at Polyamines concentrations up to 50 mM in Rosaceae family, -whereas higher concentration of Spd and Spm resulted inhibitory for pollen tube elongation in Rosaceae family and correlate with male sterility in *Actinidia deliciosa* (Aloisi *et al.,* 2015). They are compounds useful in enhancing ovule longevity (Crisosto *et al.*, 1988; Liu *et al.*, 2006), without having any deleterious and toxic effects on human life (Azh *et al.*, 2014). In the initial stage of fruit development, an active cell division takes place, which possibly needs sufficient polyamines. At the later stage of fruit development, polyamine synthesis is reduced. As biosynthesis of polyamines takes place before pollen tube emergence, low level of free polyamines in cytoplasmic male sterile plants influences cell division and its enlargement, leading to abnormal development and low viability of pollens (Liu *et al.*, 2006). Polyamines increase pollen tube growth and fruit set by stimulating pollen germination. As well, they play a role in carbohydrates and nitrogen regeneration, followed by increasing chlorophyll content and leaf area (Baninasab and Rahemi, 2008). Use of polyamines (Put, Spm, Spd) at bloom improves ovule longevity in fruit crops such as apricot and pear. Higher endogenous polyamine contents have been correlated with improved ovule viability in apricot and sour cherry (Grant Sheard, 2008).

Boron increased pollination activity (Nyomora *et al.*, 1997). In boron deficiency, phenolic compounds aggregates on stigma. The accumulation of these compounds due to the activation of dehydrogenase enzyme led to the pollen grain not to be germinated. Boron increases pollen grain viability by increasing the flavenoids content of pollen grain (Marschener, 1995).

The percentage of fruit set as a vital factor is as important as other quantitative and qualitative traits to achieve an acceptable yield in apple (*Malus domestica*). It seems the contemporary application of polyamine and SICS together can enhance their effects. Although there are numerous researches on the individual application of polyamine and SICS, there was not found any literature comparing the effect of polyamines and SICS on yield indices, especially along with cotton coverage bags on the index of fertility (IF) and the index of self-incompatibility (ISI). The aim of this study was to increase yield indices in apple cultivar ‘Golden Delicious’, accompanying the decrease of fruit drop using polyamines and SICS.

2. Materials and Methods

The study was carried out on thirteen year-old apple trees cv. Golden Delicious in an orchard located in Mashhad (latitude of 36º20'and altitude 59° 34'). It is an area with arid and semiarid climate and annual average participation of 255 mm. The foliar application was done with 5 l sprayer on four selected branches between bud swollen and flower opening pheological stages at early morning. The compounds included polyamines (putrescine [Put] 0.1 or 0.25 mM; spermine [Spm] 0.05 or 0.25 mM; spermidine [Spd] 0.05 or 0.25 mM) and SICS (1 mg/L [3.5 mg boric acid, 6.8 mg manganase sulphate] and 2 mg/L [6.8 mg boric acid, 13.6 mg manganese sulphate]) (Son *et al.*, 2009) with (+) or without (-) cotton coverage bags (ccb), and control (untreated) plants. Cotton coverage bags were used in order to prevent cross and open pollination.

In this study, the following traits were measured: yield , percentage of fruit set (initial [2 weeks after petal fall]; June [fruit drop in June]; final [at harvest]) and fruit drop. Index of fertility (IF) was measured based on the percentage of initial fruit set ratio in each treatment compared to control. Index of self-incompatibility (ISI) was evaluated based on the percentage of final fruit set ratio in each treatment in comparison with control; in this regard, the ratios of 0.2, between 0.2-1, and higher than 1 represent incompatibility, semi-compatibility and full self-compatibility, respectively (Zeinanlo *et al.*, 2001; Azimi *et al.*, 2008; Seifi 2008; Taslimpour and Aslmoshtaghi, 2013).

The Randomized complete block design with four replicates was applied in this study. At final, the data were analyzed by SAS software ver. 9.1 and the means were compared using LSD test at 0.05.

Fruit diameter and length were measured with non destructive method during the fruit growth period until the harvest time. This method was better than the destructive technique. Non destructive method are less time consuming and no need to laboratory space, and without harvesting According to this method, fruit length and diameter were measured every 2 weeks without harvest, whereas in destructive method, these parameters are measured in fruits harvested every 2 weeks and their length and diameter were measured. Therefore, non destructive method was used in the present study (Arzani *et al.*, 1999; Dehghani *et al.*, 2012).

3. Results

The effects of polyamines and SICS on yield and fruit set

Without cotton coverage bags. Data showed that Spd treatment (0.25 mM) led to a higher yield in comparison to the control ($P <0.05$). SICS (2 mgL^{-1}) demonstrated the highest yield among all of the treatments ($P <0.05$). . It was also found that Put (0.1 mM) and Spm (0.05 mM) showed increase in yield compared with the control, but it was statistically non-significant at 0.05 level.

SICS (1 mgL^{-1}) increased initial fruit set when compared to control.

The highest June and fruit set related to Spd (0.25 mM) among all of the treatments (P Value<0.05).

With cotton coverage bags. There was significant increase in percentage of initial fruit set by application of SICS (2 mgL^{-1}), and Spm (0.25 mM) (Table 1) in comparison to the control. The results also demonstrated the higher percentage of initial fruit set under treatment with Spd (0.05 mM) than the control, but it was statistically non-significant at 0.05 level.

Percentage of final fruit set showed statistically significant difference ($P <0.05$) in most of the treatments in comparison with the control, except for Put (0.1 mM), SICS (2 mgL^{-1}), Spm (0.25 mM), Spm (0.05 mM) and Spd (0.25 mM).

The effects of polyamines and SICS on IF and ISI

IF increased in treatments with Spm (0.25 mM + ccb) and SICS (2 mgL^{-1} + ccb).

All of the treatments could led to semi or full fertility and compatibility, due to the percentage of fruit set ratio in each treatment compared to the control was larger than 0.2.

The effects of polyamines and SICS on fruit drop

In general, final fruit drop decreased significantly in treatments with Put (0.1 mM) and Spd (0.05, 0.25 mM).

Without coverage. SICS (1, 2 mgL) decreased percentage of initial fruit drop in comparison with the control of 12 and 10%, respectively ($P <0.05$). Final fruit drop decreased significantly in treatments with Put (0.1 mM) and Spd (0.05, 0.25 mM).

With cotton coverage. Spm (0.25 mM) demonstrated the significantly ($P <0.05$) decrease of 14% in percentage of initial fruit drop in comparison with the control.

Table 1 - The effect of polyamines (putrescine, spermine and spermidine) and SICS on yield, fruit set and first drop

Treatments	yield (g)	Initial fruit set (%)	June fruit set (%)	final fruit set (%)	IF	ISI	first drop (%)	final drop (%)
Control	1230 cde	55.893 d-g	40.395 b-e	11.02 f	1 b-e	1 bcd	30.7 cde	68.25 a-e
Without cotton coverage bag								
Put (0.1 mM)	1416.7 c	50.179 e-h	39.046 c-f	28.636 bc	0.8625 def	1.4872 b	34.091 cd	42.424 g
Put (0.25 mM)	1101 def	15.152 j	37.231 c-f	27.778 bc	0.5565 f	0.3715 e	31.548 cde	55.357 efg
Spd (0.05 mM)	728.5 ghi	34.953 hi	32.749 c-g	22.281 de	0.6352 ef	1.2683 bcd	25.417 d-g	51.25 fg
Spd (0.25 mM)	1990.8 b	41.692 ghi	61.722 a	61.722 a	0.7621 def	2.4166 a	18.75 gh	20.313 h
Spm (0.05 mM)	1274.5 cd	74.605 abc	43.367 bcd	29.592 bc	1.3588 ab	1.0409 bcd	24.107 efg	76.259 ab
Spm (0.25 mM)	1130 def	69.86 bcd	23.077 g	12.268 f	1.2552 abc	1.529 b	64.245 a	68.593 a-e
SICS (1 mgL^{-1})	452 ij	75.63 abc	52.241 ab	13.26 f	1.3693 ab	1.2772 bcd	19.063 fg	56.818 d-g
SICS (2 mgL^{-1})	2349 a	59.127 c-e	40.857 b-e	32.241 b	1.0773 bcd	1.4317 bc	9.43 h	58.772 c-f
With cotton coverage bag								
Put (0.1 mM)	556 ij	42.361 f-i	28.03 fg	12.374 f	0.8869 c-f	1.4954 b	35.714 c	64.249 b-e
Put (0.25 mM)	1116 def	33.145 i	27.083 fg	22.917 de	0.7276 def	1.2771 bcd	48.889 b	72.222 a-d
Spd (0.05 mM)	696 hi	60.348 cde	30.925 efg	20.049 c	1.0839 bcd	1.4104 bc	50.379 b	63.258 b-f
Spd (0.25 mM)	984 efg	56.25 d-g	43.75 bc	12.5 f	1.015 b-e	0.7663 de	16.193 gh	77.399 ab
Spm (0.05 mM)	355 j	51.667 e-h	43.089 bcd	13.365 f	0.9567 cde	0.8757 cde	56.034 ab	74.353 abc
Spm (0.25 mM)	599 ij	84.858 ab	31.579 efg	10.526 f	1.5371 a	1.2116 bcd	22.5 efg	55 efg
SICS (1 mgL^{-1})	900.7 fgh	57.143 defg	32.712 c-g	25.173 cd	1.054 bcd	1.3247 bcd	28.333 c-f	56.667 d-g
SICS (2 mgL^{-1})	720 ghi	87.326 a	44.355 bc	12.903 f	1.5973 a	1.1082 bcd	49.958 b	83.953 a

Means with the same letters were not significantly different according to LSD (0.05).
Spermine=Spm, Spermidine=Spd, Putrescine=Put, IF=index of fertility, ISI=index of incompatibility.

The effects of polyamines and SICS on fruit growth habit

Based on figure 1, fruit growth totally increased in all of the treatments; in fact, fruit length and diameter increased gradually from July 9 to harvest (Fig. 1 b and d) whereas Spm (0.25 mM + ccb) showed a different trend.

4. Discussion and Conclusions

The effects of polyamines and SICS on yield and fruit set

In the present study, application of polyamines Put (0.1, 0.25 mM), Put (0.25 mM + ccb), Spd (0.05, 0.25mM) and Spd (0.05 mM + ccb) between periods

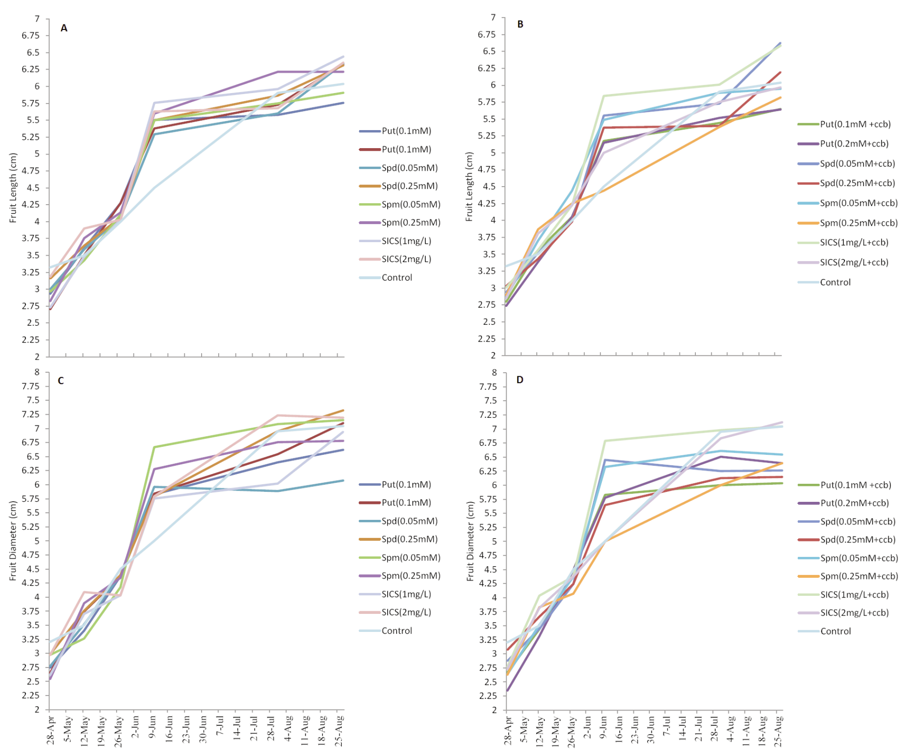

Fig. 1 - Effects of foliar application of polyamines and SICS on fruit length without ccb (a), with ccb (b) and fruit diameter without ccb (c) and with ccb (d) of apple (cv. 'Golden Delicious'). Put1= Putrescine (0.1 mM), Put2= Putrescine (0.25 mM), Spm or Spd=1 [Spermidine or Spermine (0.05 mM), Spm or Spd=2 Spermidine or Spermine (0.25 mM)], SICS 1= SICS 1 mg/L), SICS 2= SICS 2 mg/L and cotton coverage bags (ccb).

of swollen buds and the start of flowering phenological stages could increase fruit set at final fruit set stage. Several researchers studied about the effects of polyamines on yield indices. Polyamines (Put, Spm, Spd) enhanced ovule longevity at bloom in fruit crops such as apricot, pear and sour cherry (Grant Sheard, 2008). Polyamine synthesis had also positive influence on development and viability of pollen and it occurred before pollen tube emergence. Therefore, lower content of free polyamines caused male sterility in flowers (Liu *et al.*, 2006). Exogenous application of different polyamines at full bloom had influence on increasing fruit set and total yield in apples, olive, litchi and mango. Increase in fruit set and yield by polyamines was due to raising pollination, fertilization and fruit retention (Costa *et al.*, 1986). Saleem *et al.* (2008) stated that polyamines significantly increased initial fruit set and yield and maximum fruit set was observed in Spd, Spm and Put, respectively. Put had positive effect on increasing ovule longevity, EPP (Effective pollination Period), N and B and might raise the pollen tube growth rate in the styles of pears (Crisosto *et al.*, 1988), but their role on raising ovule longevity might be due to the improved nutritional status of the flower (Grant Sheard, 2008). Application of Put raised fruit set and yield of 'Comice' pear at the start of flowering (Crisosto *et al.*, 1988). Fruit set, crop density and yield efficiency under low fruit set conditions were improved by Put application at flower opening stage in pear (Crisosto *et al.*, 1988).

According to our results, the effects of polyamines on fruit set and yield were in agreement with Crisosto *et al.* (1988) on pear, Malik *et al.* (2005) on mango, Grant Sheard (2008) on sweet cherry, Saleem *et al.* (2008), Khezri *et al.* (2010) and Asadi *et al.* (2013) on pistachio.

In present study, SICS (2 mgL^{-1}) had also a positive influence on fruit set and yield. In boron deficiency, phenolic compounds aggregate on stigma. Accumulation of these compounds due to activation of dehydrogenase enzyme did not lead to germination of pollen grain. Boron increased pollen grain viability by increasing the flavenoids content of pollen grain (Marschener, 1995). Boron increased pollination activity (Nyomora *et al.*, 1997). Foliar application of boron increased yield (Mashayekhi and Atashi, 2008) and fruit set in comparison with control and fruit abscission was lower than control (Khoshghalb *et al.*, 2011). Maz Ardalan and Savaghebi FiroozAbadi (1997) reported that foliar application of Mn increased fruit yield. High levels of boron in floral organs such as the stigma and style, may aid pollen germination and make faster pollen tube growth down the style and into the ovary. Application of SICS a day before full bloom at 1 or 2 mgL^{-1} on three pear cultivars increased fruit set especially at 1mgL^{-1} SICS. Furthermore, SICS at 2 mgL^{-1} ascended the number of seeds (Son *et al.*, 2009). In addition, regarding fruit yield, foliar application of Mn alone showed significant increase in fruit yield of sweet oranges due to its effect on increasing the number of fruit/tree as well as fruit average weight (Hasani *et al.*, 2012).

Our results for SICS were in accordance with Maz Ardalan and Savaghebi Firooz Abadi (1997), Khoshghalb *et al.* (2011) on pear, Mashayekhi and Atashi (2008) on strawberry, Son *et al.* (2009) on pear, Hasani *et al*. (2012) on pomegranate.

The effects of polyamines and SICS on If and ISI

In our experiment, all of the treatments could led to semi or full fertility and compatibilty. So, this result was in line with Duca *et al.* (2010) on pear. If the ISI is lower than 0.2 means (self or cross) incompatibility, if it is between 0.2 to 1 interpret as semi (self or cross) compatibility, and if it is higher than 1, reveals full (self or cross) compatibility (Zeinanlo *et al.*, 2001; Azimi *et al.*, 2008.; Seifi, 2008).

Duca *et al.* (2010) reported that, in compatible pollinated styles, the levels of Put and Spm were similar and higher than Spd, whereas in self-incompatible pollinated styles, Put was the highest. In the compatible pollinated styles, these three polyamines showed higher content when compared to self-incompatible pollinated styles (Duca *et al.*, 2010).

Pollen germination and pollen tube development are important for fertilization (Koruki *et al.*, 2017). Extremely low pollen germination rates may cause fruit setting failure because of ovule degradation before the pollen tube reaches the ovary (Koruki *et al.*, 2017). Full compatibility is superior to semi-compatibility for ensuring high fruit set, even when environmental conditions are favorable for growth and pollination (Sapir *et al.*, 2008). Among the natural polyamines, Spm showed strongest effects on tube growth (Aloisi *et al.*, 2015).

The effects of polyamines and SICS on fruit drop

Put treatments significantly reduced secondary fruit drop on date palm, apple, pear, mango, sweet orange and avocado (Asadi *et al.*, 2013). Reduce in secondary fruit drop was observed in date palm, apple, pear,mango, sweet orange and avocado (Tavakoli and Rahemi, 2014).

Due to preventing enzymatic conversion of 1-

aminocyclopropane-l-carboxylic acid (ACC) to ethylene by polyamines, the ethylene production reduced and is followed by fruit drop (Tavakoli and Rahemi, 2014). Decreased fruit drop one week before full bloom in the "on" year and increased yield per shoot two weeks before full bloom in the "off" year were observed by application of Spm (0.1 and 1 mM), but Spd (1mM) just lowered fruit abscission one week before full bloom in the "on" year (Khezri *et al.*, 2010).

In our study, Spd (0.25 mM) was the best treatment on fruit set (June and final), yield, ISI and fruit drop. Asadi *et al*. (2013) reported that the most effective treatment on raising fruit set were Spm and Spd in apricot, respectively (Asadi *et al.*, 2013).

The content of Spd was the highest at four development stages, followed by Put and Spm, respectively (Valero, 2010). Tavakoli and Rahemi (2014) stated that treatment with Spd 1 mM led to the highest fruit yield.

The effects of polyamines and SICS on fruit growth habit

Regarding the effects of polyamines on fruit length and diameter, fruit growth totally increased because of increasing fruit length and diameter in all of the treatments in our study. In the case of fruit growth, Malik *et al.* (2005) demonstrated that the amount of polyamines increased during initial fruit growth period of apple, pear, apricot and strawberry followed by gradually decrease near maturity. Polyamine content of pericarp declined from fruit set to maturity. Spd and Spm were higher than Put during initial fruit growth compared to later during fruit development (Malik *et al.*, 2005). Put application had a positive effect on fruit size and weight, which might due to its role in cell division leading to improved weight and diameter of fruit (Saleem *et al.*, 2008).

Mn application in SICS increased fruit diameter and length but only the 0.6% rate of manganese was significant on fruit diameter (Hasani *et al.*, 2012) and influenced fruit growth habit.

Control trees produced significantly lower fruit yield, so naturally the fruit size was greater compared to fruit from polyamines-treated trees (Saleem *et al.*, 2008).

Spermidine in concentration of 0.25 mM, with/without cotton coverage bags, was the best polyamine treatment to increase yield, fruit set and to decrease fruit drop. Put in concentration of 0.1 mM, with/without cotton coverage bags, was effective on improving percentage of final fruit set and IS. Although, SICS in two applied concentrations in this study had suitable influence on raising percentage of fruit set and lowering percentage of fruit drop. Also, SICS in concentration of 2 mgL^{-1} was the best on traits such as yield, index of fertility (IF) and index of compatibility (IS). In general, all treatments were useful to induce semi and full fertility and compatibility.

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