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NATURAL PARTHENOCARPIC FRUIT PRODUCTION IN 'ANNA' APPLE CULTIVAR (*Malus domestica* cv. Anna)

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By
S. J. Owais

Department of Plant Production, Faculty of Agriculture, Mu'tah University, Karak, Jordan,

ABSTRACT

In the current study, Anna apple cv. showed parthenocarpic tendency; shriveled seeded fruits and non-seeded fruits were observed. The results showed that self-pollination and open-pollination with Anna cv. pollens released in the same field lead to parthenocarpic fruits, while artificial cross-pollination with Golden Dorset cv. gave comparably seeded fruits. Moreover, metrological data revealed that entirely seedless fruits developed under high temperatures prevailing during blooming time of Anna cv. Seedless fruits seemed to develop with stimulus pollination. The parthenocarpic fruits showed an elongated form and ring shape near the apical region. In conclusion, the results obtained in this study indicate that Anna cv. has an interesting parthenocapic property, which might addresses problems related to pollinator requirements.

Key words: *apple, cross-pollination, open-pollination, parthenocarpy, self-pollination.*

1. INTRODUCTION

Fruit development is the final stage of a continuous physiological process that begins with successful pollination and fertilization of egg and ends with fruit set and consequently maturation and ripening. Successful fertilization is usually needed for fruit set and development, otherwise no fruit is formed (Al-Joumayly *et al.*, 2010). Apples are largely self-incompatible and require cross-pollination, the self-incompatability being controlled by a multi-allelic gametophytic incompatibility system controlled in the pollen and stylar tissue (Frankel *et al*., 1977). Different apple cultivars have different S-alleles that control self-incompatibility (Sakurai *et al*., 2000). In order to produce commercial crops, most commercial cultivars must be grown with another cultivar as a pollinizer. In some cases such as those in tomato, pepper, citrus and guava, however, parthenocarpic fruits may be genetically or environmentally control (*e.g*. extreme temperatures) or might be regulated by increased levels of plant hormones such as Auxins and Gibberellins in ovaries (Gustafson, 1942; Teaotia *et al*., 1961; Nagar and Rajarao, 1981; Talon *et al*., 1990, 1992; Varoquaux *et al*., 2000; Fos *et al*., 2001; Bhardwaj *et al*., 2005; Mesejo *et al*., 2013), which can replace pollination and stimulate fruit development. On the other hand, at the genetic level, several apple (*Malus domestica*) mutants (Rae Ime, Spencer Seedless and Wellington Bloomless) are already known that lead to develop a parthenocarpic fruit and they are controlled by a single recessive gene (Yao *et al*., 2001).

So far, little information focuses on the effect of high temperature on parthenocarpic fruits in apple. Therefore, the tendency of parthenocarpic fruit formation in Anna apple was examined in relation to different pollination methods and the possible effect of high temperature during the blooming time.

2. MATERIALS AND METHODS

Twenty single productive trees of Anna apple were selected at the agricultural research station, Mu'tah University, Karak, Jordan for the purpose of this study during two successive growing seasons (2010 and 2011). Fifteen years old Anna used, were budded on MM106 (*Malus domestica* Borkh.) rootstock, semi vigorous, approximate height 2.5-3 meters in height and received the same agricultural practices. The apple trees were fertilized with 30 kg. compost/tree in winter (in January) and then compound fertilizer (20:20:20 TE) was added in four doses, each 400 g/tree. The compound fertilizer was applied in the following stages: two weeks before balloon stage, two weeks after fruit set, and two doses during fruit development. The trees were

irrigated three times (50 l /tree) using drip irrigation after compound fertilizer application. The soil of the experimental site is characterized by having a sandy loam texture soil (56 % sand, 15 % silt, 29 % clay), alkaline pH (about 8.1), 1.3 % organic matter, 640 ppm total nitrogen (N), 18 ppm available Olsen phosphorus (P), 22 % calcium carbonate, CEC of 52.7 milli-equivalent (mEq) 100 g^{-1}, and electrical conductivity (1 : 1) of 0.4 dS m^{-1}. Anna cv. can produce good crop under desert conditions due to its low chilling requirements (Rokba 1985). The experiment was consisted of three treatments: self-pollination, open-pollination and cross-pollination (*i.e.* artificial/manual pollination treatment). Cross-pollination treatment performed by crossing Anna with Golden Dorsit pollen grains, while open pollination occured only within Anna trees. Self-pollination was performed by bagging spurs with white mesh at the balloon stage to prevent any possible fertilization with foreign pollens. Four different branches for each tree and each pollination treatment were selected in different directions on the periphery of the tree. For cross-pollinated treatment, spurs were emasculated at the balloon stage and cross pollination with Golden Dorsit pollen grains was done at the full bloom stage and the spurs were then re-bagged after pollination. The pollen grains from Golden Dorsit were obtained from the National Center for Agricultural Research and Extension (NCARE), Shoubak for cross pollination treatment. The pollen grains for Golden Dorsit were collected in the same season by bagging spurs at the balloon stage, and at full pink stage, flowers were collected and subsequently dried. Thereafter, anthers were separated from other flower parts to be ready for cross-pollination and then re-bagged after pollination. Bags for self-pollination and cross-pollinated treatments were removed two weeks after full bloom. Open pollination treatment was represented by leaving four branches without bagging.

Pollen grain germination test was conducted using a germination medium consisted of 15% sucrose and two grams of agar placed in Petri-dishes, then pollen grains were spread on the medium. Thereafter, the Petri-dishes were incubated at 20 ºC for two days (Pinney and Polito, 1990). For each cultivar, three fields from each of the three Petri-dishes were chosen in order to determine the number of germinated pollen grains under light microscope and were presented as a percentage.

Fruit set percentage for open and cross pollination was recorded three weeks after full bloom drop according to the following formula (Westwood, 1979):

$$Fruit\ set\ \% = \frac{Number\ of\ fruit\ set}{Number\ of\ fruit\ clusters} X100$$

Five fruits from each replicate from four directions for each treatment (i.e. each experimental unit was represented by 20 fruits) were selected from each direction to record the number of seeds per fruit and pomological traits such as fruit weight (g), fruit length (L) and width (D) (cm), and fruit shape (L/D). Moreover, some chemical related traits were recorded: total soluble solids and acidity. The mean of fruit weight (g) was recorded using electrical balance and average fruits' length and width (cm) were taken with a caliper to determine the fruit shape (LD). Total soluble solids (TSS) were recorded by using hand refractometer and total acidity was measured by titrating 5 ml of the fruit juice sample with 0.1 N NaOH.

The experimental design was a Randomized Complete Block Design (RCBD). The experiment was replicated twenty times (*i.e*. data were taken from 20 trees from 4 branches selected at four directions). Data were analyzed by one way analysis of variance using the SAS program, and the differences between the means were compared using Fisher's least significant difference (LSD) at $P \leq 0.05$ (Steel and Torrie, 1980).

3. RESULTS AND DISCUSSION

3.1. Pollen grain germination

The germination percentage for Anna and Golden Dorsit was significantly ($P<0.01$) different, the highest value was 77.6% for Anna cultivar and was lower for Golden Dorsit (64.2%). These findings might indicate the genetic variation in this trait among apple cultivars.

3.2. Fruit set

Pollinating Anna with Golden Dorsit gave high percentage of fruit set indicating that the cross-pollination was more efficient to improve fruit set than the open and self-pollination treatment in the two successive growing seasons. Fruit se t% in the two successive seasons for cross-pollination was in average 54.45% and 52.83% in 2010 and 2011, respectively (Table 1). These values were significantly higher

compared to fruit set% of both self- (fruit set=18.07% and 9.75% in the two successive growing seasons, respectively) and open-pollination fruit set = 4.89% and 13.53% in the two successive seasons, respectively) treatments (Table 1). The high percentage of fruit set is normal for a crop like apple, where apple trees frequently set and retain excessive numbers of fruits in relation to tree size and leaf area (http://apples.hdc.org.uk/pdfs/section-4.html.pdf?id=16/10/2013%2016:30:51). This also is in accordance with other studies which showed high level of fruit set in apple grown with a pollinizer. For example different treatments (Zuniga-Guevara and Fischer,, 1992) including manual pollination, self- pollination, and natural (open) pollination showed that the numbers of fruits/tree were the best when Anna cultivar was crossed with Noyle or Quintanilla pollen. Wide range of genotypic variations in the percentages of fruit set with non-pollination have been reported as 72% in 'Ohrin' (Saito *et al*., 2007) and 48% in 'Spencer Seedless' (Tanaka *et al*., 2004); the percentage of fruit set obtained in self- and cross-pollinated treatments in this study were much lower (range = 9.75-18.07%), which might be due to complete self incompatibility if we take into account the seedless fruits obtained by both self- and cross-pollination.

The low level of fruit set with parthenocarpic fruits with self- and open-pollination treatment and the high level of fruit set with seeded fruits when Anna was pollinated with Golden Dorsit is an evidence of the high tendency of self-incompatibility in Anna. Self- incompatability in apple is genetically regulated by self-incompatibility alleles at the *S*-locus carrying distinct specificity genes, one expressed in the pollen, and the other is expressed in the stylar tissue. Haploid carrying S-allele identical to those in the pistil will be rejected and consequently no fertilization will take place, however, any combination of different S-alleles will be compatible (Tassinari and Sansavini, 2005).

3.3. Seed set

Fruit development on normal apple flowers requires pollination for seed development. Normal apple fruit contains up to 10 seeds (Pratt, 1988), depending on the level of pollination. With manual pollination (cross-pollination), the number of seeds obtained in this study per fruit was 3.8 and 3.1 in the two successive seasons, respectively (Table 1). With self- and open-pollination, fruits readily bear parthenocarpic fruits without seeds and/or shriveled seeds. The low number of seeds per fruit in the cross-pollination treatment and the parthenocapic tendency in self- or cross-pollinated treatments might be due to partial or complete self-incompatibility system in Anna.

3.4. Effect of pollination method on fruit characteristics

The parthenocarpic fruits showed an elongated form and ring shape near the apical region similar to the characteristics described by Sampaio *et al*. (1983) for parthenocarpic fruits (Table 2). The mature seedless fruit was with high length/width ratio as compared with fruits obtained by cross pollination. The ratio of fruit length to fruit width (*i.e*. fruit shape) obtained from self- and open-pollination, was 1.2 compared to <1.0 for fruits harvested from cross-pollinated treatment (Table 2). The self- and open-pollination treatments exhibited

Table (1): Effect of different pollination methods on fruit and seed setting of Anna fruits.

Pollination method	Total number of seeds	Average Number spores p	Number of fruits	% of fruit set
Open	**0^{a}**	**23.8^{a}**	**1.3^{b}**	**4.9^{c}**
Self	**0^{a}**	**21.1^{a}**	**3.3^{b}**	**18.1^{b}**
Cross	**3.8^{b}**	**21.1^{a}**	**10.8^{a}**	**54.5^{a}**
LSD	**0.7851**	**5.4491**	**2.465**	**10.5**
Pollination method	**Total number of seeds**	**Average Number spores p**	**Number of fruits**	**% of fruit set**
Open	**0^{b}**	**24.8^{a}**	**2.3^{b}**	**9.750^{b}**
Self	**0^{b}**	**25.4^{a}**	**3.3^{b}**	**13.538^{b}**
Cross	**3.1^{a}**	**23.1^{a}**	**11.6^{a}**	**52.830^{a}**
LSD	**0.7433**	**8.412**	**2.742**	**10.158**

somewhat smaller average fruit weight as compared with those obtained by cross-pollination indicating that cross-pollination has advantageous effect on the fruit size (Table 2). A comparative study of natural and artificial pollination of apple cv. 'Anna' in high regions of Costa Rica showed that in all treatments the average fruit weight was similar (Zuniga Guevara, 1992). Other studies showed that the parthenocarpic fruits obtained either by exogenous application of growth regulators or by preventing fertilization due to temperature fluctuation showed a deformed fruit shape (Bosland and Votava, 1999) and a reduced fruit size (Rylski, 1974**).** Seeds are usually formed, and the ovary and receptacle enlarge if pollination and fertilization occur normally in apples (Watanabe *et al*., 2008). There were no significant differences in fruit firmness and chemical characteristics between parthenocarpic and seeded fruits (Table 2).

increase in day temperature during the blooming time; day/night temperature was 23-26/8-12 °C and 27-30/10-18 °C in 2010 and 2011 growing seasons, respectively (Fig. 1). While some authors showed that high temperature can stimulate parthenocarpy and lead to almost complete seedless fruits in tomato (Lin *et al*., 2014) and pepino (*Solanum muricatum* Aiton) (Ruiz and Nuez, 2000). In contrary, low temperature showed the ability of producing seedless fruits including apple (Dennis, 1967), tomato (Vardy *et al*., 1989 a and b) and pepper (Tiwari *et al*., 2011). Changing the endogenous level and external applications of plant growth regulators were found to be a likely cause for seedless fruits and development of parthenocarpic fruits in fruit crops. A close relation was observed between Gibberellins parthenocarpy in apples (Bukovac, 1963; Bukovac and Nakagawa, 1967; Nakagawa *et al*., 1968). Also gibberellins applied in a

Table (2): Effect of different pollination methods on some morphological traits and chemical characteristics of Anna fruits.

Pollination method	2010 growing season					
	Fruit length (cm)	**Fruit witdth (cm)**	**Fruit shape**	**Firmness**	**Total soluble solids**	**Acidity**
Open	**6.52^{b}**	**5.38^{b}**	**1.212^{a}**	**10.9760^{a}**	**10.2600^{b}**	**3.780^{a}**
Self	**6.75^{ab}**	**5.478^{b}**	**1.232^{a}**	**11.0920^{a}**	**11.7360^{c}**	**6.156^{a}**
Cross	**6.9^{a}**	**7.43^{a}**	**0.928^{b}**	**10.4480^{b}**	**11.9200^{a}**	**4.342^{a}**
LSD	**0.226**	**0.21**	**0.0364**	**ns**	**ns**	**ns**
Pollination method	**2011 growing season**					
	Fruit length (cm)	**Fruit witdth (cm)**	**Fruit shape**	**Firmness**	**Total soluble solids**	**Acidity**
Open	**7.186^{a}**	**6.497^{b}**	**1.106^{a}**	**9.87^{a}**	**13.00^{a}**	**3.925^{a}**
Self	**6.698^{a}**	**6.41^{b}**	**1.04^{ab}**	**9.65^{a}**	**12.98^{a}**	**4.53^{a}**
Cross	**6.858^{a}**	**7.130^{a}**	**0.972^{b}**	**9.63^{a}**	**13.03^{a}**	**4.21^{a}**
LSD	**0.577**	**0.544**	**0.0992**	**ns**	**ns**	**ns**

3.5. Effect of high temperature on parthenocarpy

The present results revealed that a sudden increase in temperature during the blooming time was the likely cause for parthenocarpy, and consequently it could be concluded that the increase of temperature during the blooming time had a favorable effect on the production of parthenocarpic fruits. The blooming extended from the 8th to the 15th and from the 17th to the 21th of March in 2010 and 2011 cropping season, respectively. At the experimental site (Rabba Agricultural Station), there was an obvious

combination with cytokinins (Bangerth and Schröder, 1994;) can induce seedless fruit production. Exogenous plant hormone application to unfertilized ovaries might serve as a switch that starts the continuing autonomous development of the fruit (Bangerth and Schröder, 1994), and controls the continuity of the flow of assimilates and nutrients required for fruit growth (Treharne *et al*., 1985). Parthenocarpy might also be cultivar dependent (Tassinari and Sansavini, 2005). This might also have switch-induced autonomous development of the fruit although the time at which this

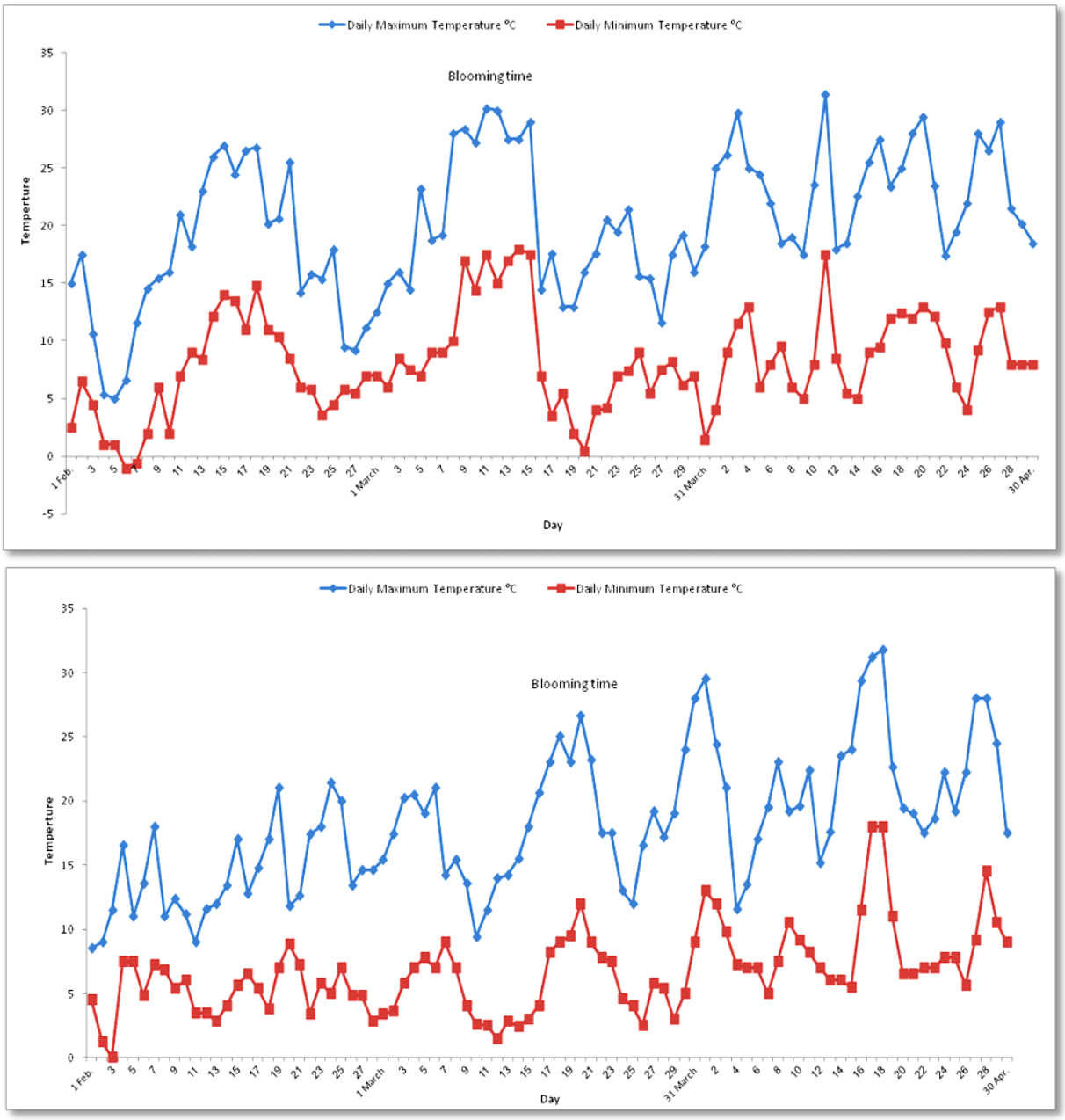

Fig. (1): Daily temperature from February to April during the two successive seasons, 2010 and 2011.

switch acts in naturally occurring parthenocarpic fruits remains unclear.

In some crop species such as tomato, high temperature stress reduces pollen grain number and germination (Aloni *et al.*, 2001). In pepper, additional morphological changes are associated with high temperatures. Day/night temperatures of 28/23°C decreased ovary diameter and increased style length compared to a day/night temperatures of 23/18°C (Polowick and Sawhney, 1985). High temperature after anthesis also reduces fruit and seed growth and seed quality in chili pepper (Pagamas and Nawata, 2008).

Even though the parthenocarpic property could be an interesting property, which might address problems related to pollinator requirements, the initial fruit set and final commercial yield in apple trees are closely correlated in Anna cultivar. In our study, relying on self-pollination will decrease the proportion of fruit set per tree and in consequence the commercial yield per tree. Different pollination methods did not show any significant differences

in firmness and total soluble solids and consequently might keep the qualities of apple fruits. In conclusion, Anna cv. displayed strong tendency for natural parthenocarpy where shriveled seeded fruits and non-seeded fruits were observed on trees handled with self and cross-pollination treatments. Our results indicate that high temperatures can induce parthenocarpic formation of Anna apple cv. when temperature is elevated during the blooming time. Anna cv. is self-incompatible but can be self-fruitful through the production of parthenocarpic fruits.

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العقد البكري في انتاج الثمار في صنف التفاح Anna
(*Malus domestica* cv. Anna)

سائد جوزيف عويس

قسم الانتاج النباتي - كلية الزراعة - جامعة مؤتة - الكرك- الأردن

ملخص

اظهر صنف Anna ميولا للعقد البكري حيث تم ملاحظة ثمار ببذور ضامرة واخرى دون بذور. اظهرت النتائج ان التلقيح الذاتي والتلقيح المفتوح لصنف Anna مع حبوب لقاح من نفس الصنف في الحقل أدت الى ظهور ثمار عاقدة بكريا . بينما اظهرت نتائج التلقيح الخلطي مع الصنف Golden Dorsit ثمارا تحتوي بذورا. اظهرت معلومات الارصاد الجوية ظهور ثمار عديمة البذور تحت ظروف الحرارة العالية السائدة في مرحلة الازهار في صنف Anna. كانت الثمار الخالية من البذور مستطيلة الشكل وشكلها حلقي عند منطقة القمة . كملخص لنتائج التجربة اظهرت النتائج ان لدى صنف Anna صفة انتاج الثمار البكرية التي قد تستخدم كبديل للملقحات.

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