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Sexual Compatibility of LeConte Pear Cultivar

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Abstract: The present study concerns the evaluation of self- incompatibility (SI) of LeConte cv. (*Pyrus commuins* L.) and cross- compatibility (CC) with Hood, Flordahome and Tsuli and Yali cvs. Also, fertilization process and embryo sac development. Hood and Floridahome are European pears of Pyrus commuins while Tsuli and Yali are Asian pears of Pyrus pyrifolia. The SI and CC level was evaluated based on the observations pollen tube growth using fluorescence microscopy technique. Also, the percentage of fruit set in the orchard was recorded. The results confirmed that LeConte cultivar is self-incompatible but cross-compatible with Hood and Yali cvs. However LeConte is considered cross-incompatibility with Floridahome while, considered partially compatible with Tsuli cv.

Key words:*Pyrus commuins* · Compatibility · Incompatibility · *Pyrus pyrifolia* · Pollen tube growth · Embryo sac development · Fertilization process · Fruit set

INTRODUCTION

Many flowering plants have evolved genetically determined self-incompatibility (SI) mechanisms to prevent self- fertilization and promote out-crossing [1]. According to Linskens [2] such incompatibility may become apparent on the stigma so that the pollen does not germinate (sporophytic incompatibility), or in the style (gametophytic incompatibility). In the later case, the pollen tube usually suffers from growth inhibition within the third quarter of the style. Rosaceae families including Pears (Pyrus spp) possess gametophytic self-incompatibility (GSI) that is controlled by a single polymorphic locus(S-locus) [3]. In GSI system, Pollen tube growth is inhibited in the style when the S-allele of the pollen grain matches one of the S-alleles of the style [1].

In addition, the expression of self- incompatibility in Asian pear is depended not only on physiological plant status but also on environmental condition [4] and a difference between cultivars in fruiting after self pollination has been observed. This might be due to different degrees of self-incompatibility of some of the Asian pear cultivars. Also, self and cross incompatibility in European pear, which is estimated by the fruit set and seed formation, is yet unclear due to most cultivars exhibited parthenocarpy as well as physiological fruit drop [5], which suggests that fruit set is not a suitable criterion for distinguishing incompatibility from compatibility. However, fruit and seed set as criteria are generally accepted in other Rosaceae fruit tree that exhibit GSI such Japanese pear and apple [6]. Sanzol and Herrero [7] developed reliable *in vivo* methods to test pollen-pistil incompatibility in pear: pollen tube performance was studied along the pistil following self and cross pollination. Their results show that ovule observation at the microscope for the presence of pollen tube in the nucleus is a proper method to test incompatibility in this crop.

In Egypt, European pear (*Pyrus communis* L.) is grown in warm, sub-tropical climate conditions. Commercial orchards consist of "LeConte" as the main cultivar; the yield varies from year to year. This variability has been attributed mainly to lack of adequate cross pollination; also, other factor can affect fruit set and yield which is fire blight. LeConte pear cultivar is considered by Lee [8] a hybrid between *Pyrus serotina* x *Pyrus communis*. Atawia [9] found that LeConte and Hood pear cultivars are not pure line and were originated from different genotypes. Heinkel *et al*. [10] reported that fruit set and yield was increased significantly by cross pollination in comparison to self pollination. Also, Elbassel [11] found that the fruit set of LeConte cv following self pollination was lower than the fruit set following cross pollination with hood cv.

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The main objectives of this study were to investigate compatibility and incompatibility between Le-Conte pear cultivar as a female and Hood, Flordahome, Tsuli and Yali cvs pear cultivar under Egypt conditions.

MATERIALS AND METHODS

The experiment was set up at El-Ksasseen Research Station, Ismailia Governorate of Agriculture Research Center of Cairo. The trees were planted on *Pyrus communis* rootstock at a distance of five meters apart in 1994. It is irrigated and trained to slender spindle. Regular agricultural practices were applied to all investigated pears trees. The hand pollination was prepared at approximately on 4000 flowers of LeConte in 2007 and 2008. Two flowers in a cluster were pollinated. In order to investigate the compatibility of combinations fruit set was investigated and the number of viable and deaf seeds were also counted.

Pollen Grains Collection: Pollen grains of LeConte, Hood, Floridahome, Tsuli and Yali cultivars was collected from flowers at balloon stage and spread on a paper in the laboratory at room temperature for 24 hours to dry. Then the pollen grains were stored in small glass bottles separately and stored in desiccators at 4°C until used.

Pollination Procedure and Samples Collection:
The following crosses had been done.
LeConte selfing
LeConte x Hood
LeConte x Tsuli
LeConte x Yali
LeConte x Floridahome
LeConte open pollination as control

In each of the experimental trees, flowers at the same developmental stage (at balloon stage) in the twigs and spurs around the tree were chosen. Flowers of the female parent (LeConte) were emasculated at balloon stage using forceps in all treatments pollination. Buds at other developmental stages were eliminated. Immediately, cross pollination was performed by applying the appropriate pollen to the receptive stigmas with a fine paintbrush. Almost 500 flowers per each treatment were pollinated. Then the twigs and spurs were bagged by pergamin bag to prevent any undesirable pollination. Open pollination (control), minimum of 500 flowers at balloon stage were selected, counted and labeled and left without bagging. Self-pollination treatment was performed by bagging the shoots at balloon stage. At anthesis (75%of the flowers were open) the shoots were shacked; the shaking was repeated two days later to ensure pollination of all flowers. In open pollination, enough shoots were labeled without any treatment.

***In vivo* Pollen Germination and Pollen Tube Growth:** For each treatment a minimum of 70 flowers were used. These flowers were emasculated and then pollinated at the balloon stage as mentioned above. Pistils of the treated flowers were collected from all treatments to investigate pollen germination, pollen tube growth and fertilization process. These collections were conducted after pollination in cross-pollination and after anthesis in open-and self-pollination. These were collected daily for seven successive days after pollination as well as after anthesis. All samples were fixed in 70% F.P.A (5:5:90 by volume, formalin: propionic acid: 70% ethanol) immediately after the collection time [12]. The fixed flowers were washed in running water to eliminate the FPA for 24h and softened for 2h in 8N NaOH. This procedure was done to facilitate the penetration of stain solution and the preparation of flowers. After that, all samples were washed from the NaOH overnight in running tap water. Finally, the flowers were stained with 0.1% aniline blue (W/S), dissolved in 0.1N K_3 PO_4. The vials containing the material were kept at 4°C until the microscopic examination [13, 14]. To follow the pollen germination and pollen tube growth, the styles and the stigmas were separated from the ovary, squashed gently under pressing coverslip. The slides were investigated under a leica fluorescence microscope.

Fruit Set: As mentioned, in the pollination treatments, about 450 flowers per each treatment were left for fruit set measurements. The numbers of total flower were counted at anthesis. Moreover, initial and final fruit set were determined According to Westwood [15] as follows:

Initial fruit set% = Number of fruit set (21 days after pollination) / total number of flowers x 100
Final fruit set % = Number of final fruits (Before the date of yield harvest) / total number of flowers x 100

Histological Study: For histological study 10-20 samples of different pollination treatments from newly setted fruits were fixed in F.P.A. embedded in paraffin. Samples were dehydrated in tertiary butyl alcohol (T.B.A) series and embedded in paraplast plus barafin. Sections were cut at 8-10 ìm and stained with hematoxylin according to Delafield [12] in order to observe and detect the fertilization process.

Experimental Design: The experiments followed the Completely Randomized Block Design (CRBD) on 15 trees as 5 treatments were applied, each treatment comprised of three trees, each tree was considered as replicate.

The obtained data of fruit set were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [16]. Mstat-c program was used to compare between means of combinations according to Waller and Duncan [17] at probability of 5%.

RESULTS AND DISCUSSION

The Compatibility And/or Incompatibility Characteristics Pollen Tube Characteristics in Leconte Style Pollination with Different Pollen Donors

LeConte x Yali: The germinated pollen was observed on the stigma surface of LeConte after 24h from pollination. All the pollen tubes were visible in the upper part of the style two days after pollination (Fig. 1-a) and they reached the lower part of the style after 3 days from pollination (Fig. 1-b, c. and Fig. 3). The appearance and behavior of pollen tubes were detected in the observations mad by Sanzol and Herrero [7] and Abou El-Nasr and Stösser [18] they found that the tubes from compatible cross pollination, grew rapidly down the style and were characterized by small widely spaced intermittent callose plugs and also, absences of terminal plugs (Fig. 1-a, b).

LeConte x Hood: The germinated large number of pollen grains were observed on the stigmatic surface of LeConte after 24h from cross pollination and pollen tube reached the base of style after 4 days of pollination and absences of plugs along the pollen tube (Fig. 1-a, b,c and Fig. 2) [19].

LeConte x Tsuli: Partial cross incompatibility was observed when LeConte cv. flowers were pollinated with Tsuli pollens. Some of the styles were observed with normal pollen tube growth. However, most of the pollen tubes, which grew through the other styles revealed abnormal development and they reached the base of the style in 5 days after pollination (Fig. 3).

LeConte (Self): Although most of pollen grains germinated on the stigma surface but its growth reached only to 1/3 length of style 5 days after pollination (Fig. 2-a, b and Fig. 3). In addition, microscopic examination revealed vary degrees of self-incompatibility, where different rates of pollen tube growth were detected after self-pollination. Terminal plugs were present in most of selfing pollen tubes, which indicated pollen tube incompatibility. In this respect, Stösser and Anvari [20] and Abd El-Aziz [21] reported that, incompatible tubes contained frequent large callose plugs which, sometimes continuous deposition along the tubes (Fig. 2 a, b). Moreover, pollen tube growth of LeConte cv. was slow in its own style. This lag in self pollen tube growth delayed its arrival to the base of the style. The slow growing pollen tubes was found with genetically incompatible pollen tube and often thickly callused for considerable lengths [22]. Moreover Fig. 2-b, c, d illustrated the presence of distorted tubes, short tubes,

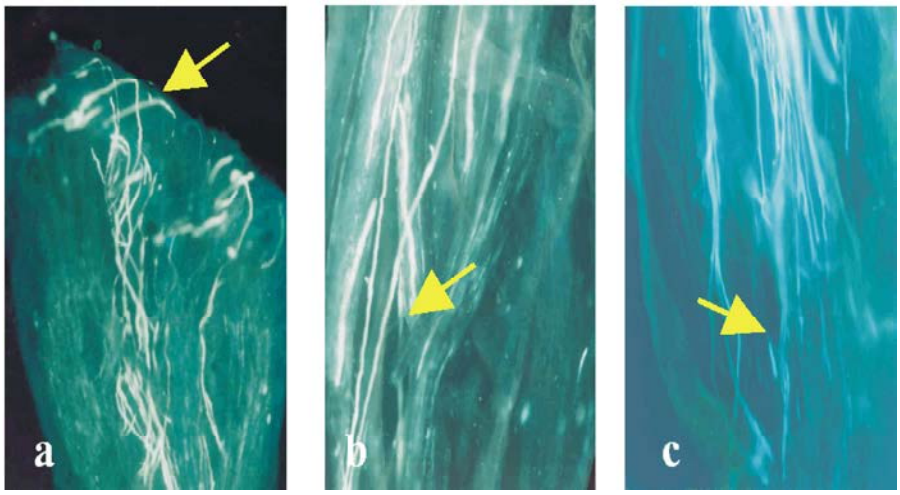

Fig. 1: Pollen tube characteristics after compatible pollination
a) Pollen tubes were visible in the upper part of the style (100X)
b) and grew to the 1/2 of style length two days after pollination (100X)
c) Pollen tubes reached the end of style after 4 days from pollination (100X)

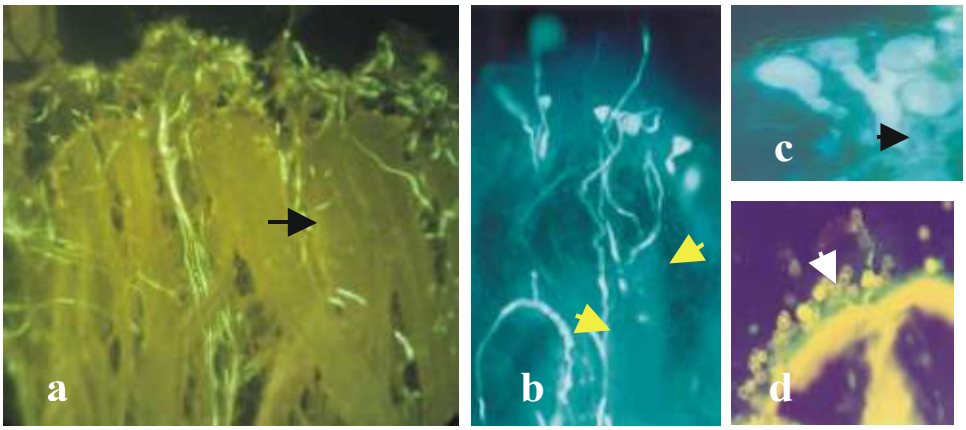

Fig. 2: Pollen tube characteristics after incompatible pollination

a) Callose in large quantities and plugs (100 X)
b) Decomposition of Callose along the tube which stopped to 1\2 of style length after 7 days after pollination (100 X)
c) Short tube with plugs, which was unable to penetrate the stigma (X400)
d) Poor pollen germinated on the stigma (100 X)

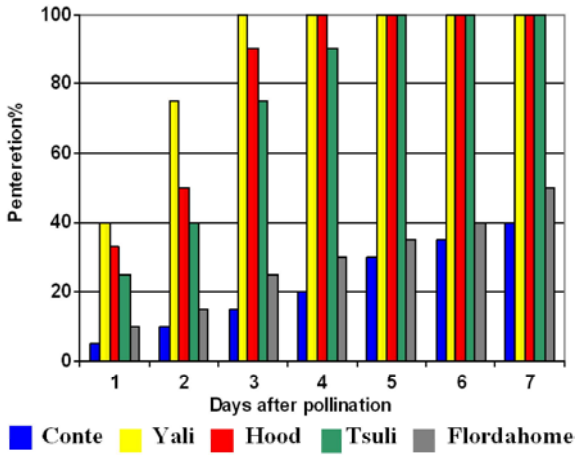

Fig. 3: Penetration %of pollen tube of different cultivars in LeConte style, [100% = total style length]

which were unable to penetrate the stigma as well as poor germination. The slight penetration of tubes, were observed in LeConte selfing. These abnormalities usually observed in pollen stigma interaction [1].

Pollen-stigma interaction is important in the biology of sexual reproduction because the vital function of selection of the male gametes in flowering plants is performed not by the egg, but by paistillate [23]. From the overall data, it is clear that if pollination is incompatible, the pistil will effectively prevent fertilization either through inhibiting pollen germination on the stigma or growth of the pollen tube through the style. Pollen tube growth was consistently more rapid after cross-pollination than after selfing.

LeConte x Floridahome: The germination of pollen was poor and the pollen tubes grew very slowly and stopped its growth after 7days from pollination (Fig. 2-d and Fig. 3). In additions, pollen tube contained frequent large callose plugs.

Fertilization, Embryo and Endosperm Development: Fertilization occurred after 4 and 5 days in cross pollination by Yali and Hood respectively. The ovule developed and increased in size and formed primary endosperm and developed (Fig. 4, a-b-c) compared to incompatible combination the unfertilized ovules didn't increased in size (Fig. 5, a-b). The zygote formation of LeConte was showed 7 days after pollination, (Fig. 4, a-b). The ovule length was increased and embryo begins to develop. Its development began extensive elongation of the zygote and transverse division to form along narrow suspensor with primer embryo (Fig. 4c). The embryo sacs have polar nuclei which are already fused after cross pollination. Sato and Kanbe [24] found that the growth of ovules in pollinated Mutsu and Golden Delicious apple cultivars increased in size during the period from 3-35days after pollination and growth was slow during 60 days period after pollination, while, the ovules in non pollinated flowers stopped increasing by around 5 days after anthesis. Moreover, Herrero and Gascon [25] reported that in the untreated unpollinated flowers of pear, ovules degenerate between 12 and 21 days after anthesis, while in cross pollinated flowers this degeneration is postponed by about 10 days.

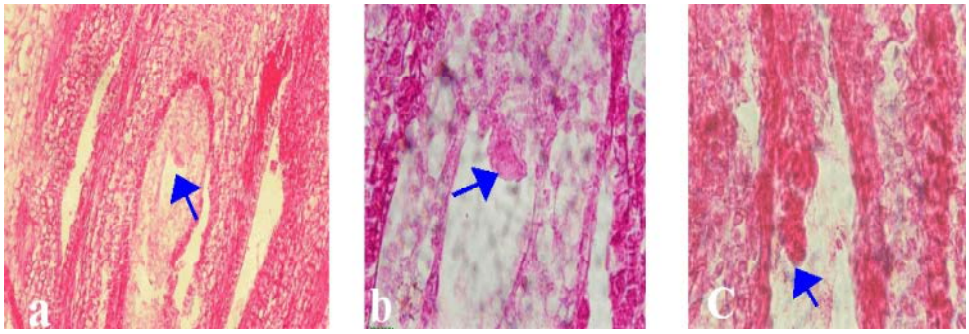

Fig. 4: Fertilization process after compatible pollination.
a) Fertilized ovule with zygote 7 days after pollination (X 63)
b) Magnification from a, (X 200)
c) Increasing zygote in size and primary endosperm nucleus 15 days after pollination (X 200)

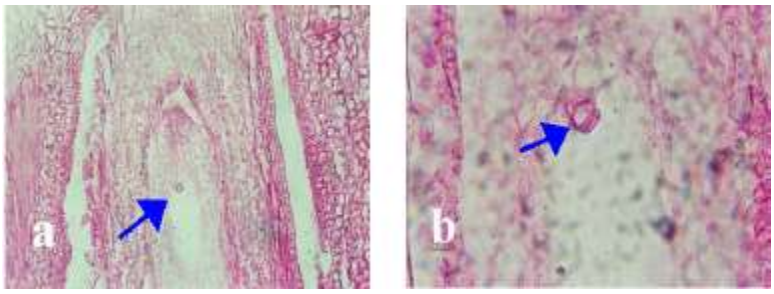

Fig. 5: Fertilization process after incompatible pollination.
a) Unfertilized ovule 10 days after pollination (X 63)
b) Magnification from a, ovule infused degeneration (200)

Table 1: Effect of different pollination treatment on percentage of initial fruit set and fruiting of LeConte cultivar during 2007, 2008 and 2009 seasons

	Initial Fruit set			Fruiting		
Pollination treatment	1st season	2nd season	3rd season	1st season	2nd season	3rd season
LeConte open	27.86 d	***	34.63 d	9.62 d	***	10.61 d
LeConte self	12.70 f	***	25.10 f	0.00 e	***	3.77 f
LeConte x Flordahome	22.32 e	***	29.00 e	0.00 e	***	7.55 e
LeConte x Yali	61.22 a	60.03 a	77.39 a	59.16 a	55.15 a	72.30 a
LeConte x Hood	40.38 b	19.83 b	48.87 b	36.73 b	16.21 b	42.22 b
LeConte x Tsuli	36.36 c	6.872 c	42.79 c	34.01 c	5.038 c	34.47 c

* Means having the same letter(s) in each column are statistically insignificant at 5%level of Duncan's multiple range tests

*** Treatment which were not completed

Thus, in a cross pollinated flower this extends the period over which a successful fertilization can take place. This increased period of viability is accompanied by an elongation of the embryo sac itself. Elongation takes place two weeks prior to fertilization in cross pollinated flowers.

Table 1 showed the initial fruit set and fruiting percentage in LeConte by self- pollination, open pollination and cross pollination with four different cultivars. Open pollination gave initial fruit set percentages ranged between 27.86 to 34.63, whereas, fruiting percentages ranged between 9.62 to 10.61.Concerning fruit set for the different pollination combinations of the five pear cultivars, data in the same table indicated that the initial fruit set in LeConte give the highest significant difference when cross pollinated with Yali followed by Hood and Tsuli, respectively. The fruiting percentage in LeConte at harvest also followed the same order for the different cultivars as observed for initial fruit set.

It registered 36.73 and 34.01% fruiting when cross-pollinated with Hood and Tsuli, respectively during the first season. Similar response was observed during second and third seasons. However, the lowest initial fruit set and fruiting percentage was obtained in self-pollination during all seasons of study. A marked improvement in initial fruit set in LeConte was observed when cross-pollinated with pollens of Foridahome cultivar while fruiting percentage significantly decreased. It gave 0.0 and 7.55 per cent, respectively during first and third seasons.

Finally, results of the present study showed that there were high initial fruit set percentage resulted from all combinations. Nevertheless, histological studies observed that premature fruits resulted from self and cross incompatibility combinations had degenerate ovule therefore, fruit abscission were observed through three week after pollination. In addition, the good result of the fruiting percentage of LeConte x Yali and LeConte x Hood combinations could be taken as a reflection for the high degree of sex compatibility between these combinations. On the contrary, low fruiting percentage was achieved by following combinations: LeConte (Selfing), LeConte x Flordhome, whereas this percentage much too low to consider as commercial production. While, LeConte x Tsuli combination is considered as partially compatible. This may be due to the pollen-pistil incompatibility. Such results are in harmony with the findings obtained by Elbassel *et al.* [11], who stated that LeConte cv. considered self-unfruitful and cross-unfruitful with Flordhome as well as LeConte cv. appeared to be cross-fruitful with Hood cv. under Egyptian condition.

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