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Influence of Rootstocks on Sexual Compatibility of Pear Cv. 'Le Conte'

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Abstract: Successful fertilization requires deposition of pollen on stigmas, pollen germination and successful pollen tube growth resulting in fruit set. Gametophytic self-incompatibility (GI) is a natural mechanism in flowering plants, including pear that prevent inbreeding and promote outcrossing. Calcium, Boron and Zinc are essential nutrients for reproductive development. In thepresent study, influence of two rootstocks (communis and betulaefolia) on the degree of self-incompatibility of 'LeConte' pear (*Pyrus communis* L.), its cross-compatibility with 'Floridahome', 'TsuLi' and 'YaLi' cultivars and leaf mineral content (Calcium, Boron and Zinc) were investigated.Fluorescent microscopy studies showed a high level of compatibility, when flowers crossed by 'YaLi' pollen with little impact of rootstock on growth of compatible pollen tubes. Partially cross compatible was observed in styles crossed by 'TsuLi' pollen and high degree of partial cross compatibility was observed on 'communis'.While lower degree was noted on 'betulaefolia'. Conversely, 'LeConte' is considered self-incompatible with no effect of rootstock on incompatible pollination. Leaf analysis showed that 'communis'had significantly higher Calcium, Boron and Zinc mineral element concentration than 'betulaefolia'. Field data confirmed fluorescent microscopy results, fully compatible crosses had significantly fruits and seed set higher than half compatible crosses. Also, in the pistils of partially cross compatible significantly higher fruit and seed set was found in 'LeConte' trees budded on 'communis'. According to the results, the 'communis' rootstock for 'LeConte' pear cultivar, when used 'TsuLi' as a pollinizer is recommended. Moreover, the results suggest that rootstock effects depend on increase sequestration of nutrients essential for pollination and fertilization, which leads to an increase in fertilized ovule count due to growth acceleration the of fruitlets which can escape Fire blight.

Key words: Pear · Sexual compatibility · Pollination · Fertilization · Rootstocks

INTRODUCTION

Pear (*Pyrus communis* L.) is one of the major fruit in the world and grown well in temperate zones of both hemispheres. The estimated total world pear production was approximately 24 million tons in 2019. China was the largest producer, accounting for over 71% of global production and followed by the USA (2.8%), Argentina (2.5%), Turkey (2.2%) and Italy (1.8%). Egypt ranked 24th globally with a total of 62898 tons [1].

Pollination is one of the most important steps in fruit production and for almost 90% of angiosperms [2]. The first step of successful pear pollination is the transfer of pollen to the stigmatic surface followed by an adhesion of pollen grains to the papilla cells of the stigmatic surface [3, 4]. The deposited pollen hydrates and germinates and then pollen tubes penetrate downward through the style to the central part of the ovary and the ovule. Once a pollen tube reaches the ovule, sperm cells move downwards in the pollen tube to fertilize the ovule. This process is known as fertilization and under ideal conditions, it should take place 2- 4 d after pollination [5]. Fertilization is usually necessary for fruit development to begin.

Self-incompatibility (SI) is a widespread mechanism in angiosperms enforcing outcrossing [6, 7]. SI is classified into gametophytic SI (GSI) or sporophytic SI (SSI) [8-11]. Most of families including Rosaceae are known to possess (GSI) controlled by a single polymorphic locus (S-locus). In GSI, the pollen's own haploid *S. genotype* is expressed and pollen tube usually suffers from growth inhibition within the third quarter of

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the style when the S-allele of the pollen grain matches one of the S-alleles of the style [12]. In SSI, S expression in pollen is controlled by the parental S genotype. This control is believed to be accomplished by the transfer of tapetal proteins, including recognition factors, cavities in the pollen grain wall during microsporogenesis. SSI is more complex than GSI, because dominance and co-dominance of alleles can occur in pollen and the dominance hierarchy may differ in pollen and pistil so that, pollen tubes are arrested on the stigma. [13, 14].

Furthermore, the expression of self-incompatibility in Asian pear is depended on physiological plant status as well as the ecological condition. This may be due to different levels of self-incompatibility of some of the Asian pear cultivars [15]. A gradient of compatibility among compatible crosses of European pear cultivars clarified by the different genetic “strength” of interactions among S-alleles, probably mediated by ecological factors. In this manner, S-allele expression seems to be adjusted by both hereditary and ecological variables [16].

In Egypt, European pear (*Pyrus communis* L.) is grown in warm, sub-tropical climate conditions. Commercial orchards developed by "LeConte" as the most planted cultivar; Productivity varies from year to year and location to another. This variability has been attributed basically to the absence of adequate cross-pollination [17]. Likewise, another factor that can influence pear production which is fire blight. This disease is highly destructive caused by the bacterium *Erwinia amylovara* [18]. It can be especially problematic, explicitly where springtime climate is warm and wet.

Rootstocks play an essential role to determine orchard performance of fruit trees. Several studies have been documented the rootstocks can affect tree size, precocity, productivity and mineral uptake efficiencies especially in the high intensity modern orchards but rootstock effects on self and cross (in) compatibility are not well understood. Therefore, the objective of this study was to determine the impact of rootstocks on sexual compatibilityof? LeConte ? based on pollination, germination, pollen tube growth and seed number of fruits ovule fertility. In addition, to determining the influence of rootstocks on the sequestration of calcium, boron and zinc.

MATERIALS AND METHODS

The present investigation was set upat El-Kssaseen Experimental Station (Ismailia Governorate), Horticultural research Institute, Agriculture Research center, Egypt. The experiment extended for two successive seasons 2017 and 2018. This experiment was conducted on mature trees of LeConte pear cultivar. Trees was grafted on two rootstocks (*Pyrus communis and Pyrus betulaefolia*). Trees were healthy and nearly uniform in vigour and free from any pathogenic symptom. The experiment was performed on six trees for each rootstock, cultivated at 4x4 m, grown in a sandy soil and randomly selected. Florida home, TsuLi and YaLi pear cultivars were used as male parents as well as self-pollination.

Pollen Collection: As flowering date differ from one cultivar to another;‘Florida home’ and ‘TsuLi’flowers were collected earlier than ‘LeConte’, however, flowers of ‘YaLi’ were collectedfrom shoots subjected to low temperatures at 5 ?C in refrigerator for 750 h.The flowersof each cultivarwere collected at the balloon stage just one day before anthesis. The flowers were placed on paper in the laboratory at room temperature. Pollens were extracted from the anthers which were dried on a piece of paper for 24 h at 24 ?C. The dried pollens were then collected in a test tube and stored at 4–6 ?C in a desiccator until pollination.

Field Controlled Pollination: In each of the experimented LeConte trees, self-pollinated flowers were bagged at balloon stage using pergamin bags to prevent cross-pollination in the field. The flowers were actively self-pollinated with their own pollen, when the stigmata were receptive.Cross-pollinated flowers were chosen around the tree at balloon stage and controlled cross-pollination was made according to the method described by Wertheim [19]. Then flowers enclosed in isolation bags of pergamin to prevent pollination by honey-bees.

Pollen Germination, Pollen Tube Growth and Fertilization: From each pollination treatment, 7-10 pistils were harvested at intervals of 1, 2, 3, 4, 5, 6and 7 days after pollination. The pistils were placed in FAA fixative consisting of 70% ethanol, glacial acetic acid and formaldehyde at 90:5:5 for 24 h and stored at 4ºC.Pollen tubes growth were examined using aniline blue staining protocols[20-22].The tissueswereobserved under the fluorescent microscope (Carl Zeiss, Jena) and photographed (Fujifilm Digital Camera finePixA900).

Fruit Set Percentages and Seed Set: For self and cross pollination on two studied rootstocks flower clusters were thinned, leaving two to three flower buds of similar size in each cluster. The remaining flowers were pollinated as mentioned in the pollination treatments. Fruit set percentage and seed set was calculated as follows:

Table 1: Physical and chemical properties of the experimental soil

Mechanical analysis	Value	Chemical analysis	Value	Cations (mg/l)	Value	Anions (mg/l)	Value
Sand	63.5	SP	20	Ca^{++}	3.29	$CO_3^{=}$	--
Silt	32.5	pH	7.9	Mg^{++}	1.60	HCO_3^{-}	2.36
Clay	4.0	EC ds/m	0.66	Na^{++}	1.88	Cl^{-}	3.39
				K^{+}	0.45	$SO_4^{=}$	1.47
Soil texture				Loamy sand			

Table 2: Available macro and micro nutrients of the experimental soil.

Macro nutrients (mg/Kg)	Value	Micro nutrients (mg/Kg)	Value
N	143	Fe	0.622
K	76	Mn	0.244
P	9.78	Zn	0.132
----	----	Cu	0.11
----	----	B	0.02

Fruit set % = number of set fruits/total number of flowers x 100.

Seed set (average number of viable seedsper fruit)= number of viable seeds/ number of fruit set.

Leaf Mineral Elements Content: Leaf sampling was done at 0, 30 and 60 days after full bloom (DAFB). Each leaf sample consisted of 50 new but fully developed midterminal leaves from current-year shoots at 150 cm above the ground in the tree canopy. The collected leaves were immediately packed into polyethylene bags and taken to the laboratory in a portable refrigerator. Leaf mineral analyses were determined according to the method described by Milosevic [23]. Analyses were performed by Atomic Emission Spectrometry. Each determination was replicated three times. The results were expressed on a dry matter basis: % for macro (Ca) and mg 100 g^{-1} for microelements (Zn and B).

Embryological Studies: In order to examine embryo and endosperm development five samples of fruitlets originated from self and cross pollination were collected at 7 and 12 days after pollination (DAP) and fixed in FAA. Samples were dehydrated, infiltrated and embedded through a tertiary butyl alcohol paraffin- wax series according to the procedure of Johannsen [24]. Sections were cut 10 ìm thick with a rotary microtome and stained with hematoxylin and then enclosed with Kandapalsm.

Statistical Analysis: Six adult trees of 'LeConte' pear cultivars budded on 'communis' rootstock were compared with six trees grafted on 'betulaefolia' rootstock growing in the same area. All pollination treatments per tree have been done. Data obtained from pollination experiments were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [25]. Significant differences were analysed using *t*-test for data obtained from experiment of leaf mineral content. M. Static program was used to compare between means according to Waller and Duncan [26] at probability of 5 %.

RESULTS AND DISCUSSION

Pollen Germination and Pollen Tube Growth: Germination of pollens and growth of pollen tubes were observed in the style of LeConte pear cultivar growing on bothcommunis and betulaefolia rootstocks after self and cross-pollination. In the case of pollinating flowers with the pollen grains of 'YaLi', the results showed that numerous pollen grains germinated normally followed by the formation of pollen tubes. There was no difference in the growth characteristics of pollen tubes between rootstocks along the longitudinal axis of the style. Pollen tubes grew across the stigma surface andpassed down into the style after two days of pollination (Fig. 1a). Pollen tubes also reached the lower part of the style after three days of pollination (Fig. 1b). It penetrated the ovules within 3-4 days after pollination (Fig. 1c). It is obvious that 'LeConte' x 'Yali' cross showed a high level of compatibility.

High degree of partial cross compatibility was observed when flowers pollinated with 'TsuLi' on 'communis. rootstock. Pollen tube morphologies was similar to those of 'LeConte' x 'YaLi' cross however, the growth speed of some pollen tubes was slower and other tubes stopped growing before they reached the end of the style. Therefore, the numbers of pollen tubes forming in the style progressively decreased along the length of the style as they approached the ovary until eventually only four or five reached the lower part of style and penetrated the ovules after 5 days from pollination. In contrast, lower degree of partial cross compatibility was observed in the

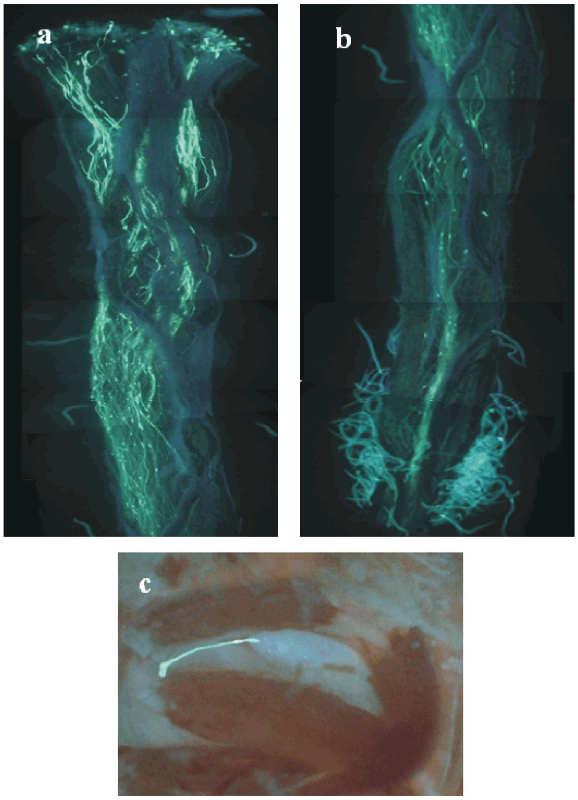

Fig. 1: Pollen tube behavior in the style of LeConte after compatible cross (‘LeConte’ x ‘YaLi’ cross on both‘communis’ and ‘betulaefolia’).
(a) Pollen tubes grew across the stigma surface and passed down into the style(X90).(b)Pollen tube reached the lower part of the style 3 days of pollination (X90). (c) Pollen tube entering the nucellus and fertilized embryo sac (X350)

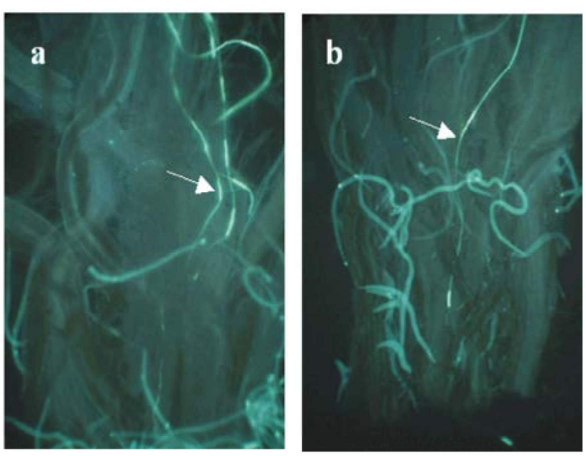

Fig. 2: Pollen tube growth after partially compatible of ‘ LeConte’ x ‘TsuLi’ cross on ‘betulaefolia’.
(a, b). Few numbersof pollentubes reached the lower part of style 6 days after pollination (X100)

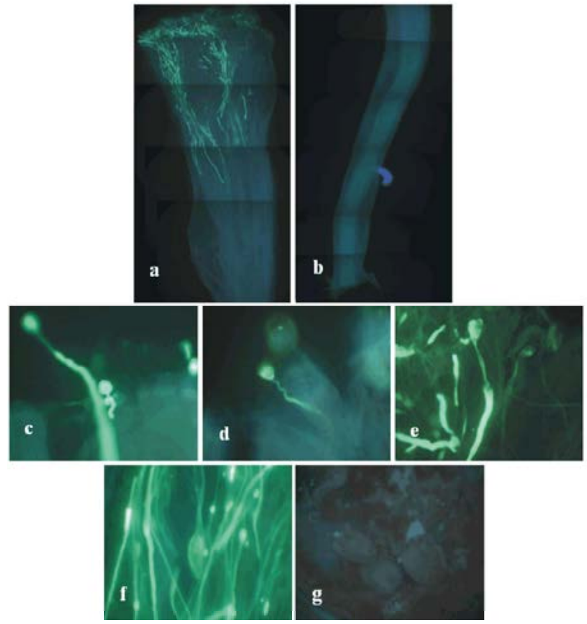

Fig. 3: Pollen tube characteristics after incompatible crosses (‘LeConte’ x ‘Florida home’ cross and self-pollination on ‘communis’ and ‘betulaefolia’)
(a) Pollen tubes growth stopped upper part of style 5 days after pollination(X90). (b) No pollen tube was visible in the lower part of the style 5 days after pollination(X90).(c) Short, twisted tube(X350).(d) Twisted tube(X350).(e) Distorted tube (spiraling) (X350).(f)Balloon-like swelling (X350).(g)Unfertilized ovules(X350).

‘LeConte’ x ‘TsuLi. cross on ‘betulaefolia’ rootstock. Numerous pollen grains germinated on the stigma and their growing pollen tubes decreased in number as they approached the ovary until eventually only three (sometimes one) (Fig. 2a and b) reached the lower part of style and penetrated the ovules after 6 days from pollination.

In self-pollinated flowers on two studied rootstocks, although most of pollen grains germinated on the stigma surface, its growth was slowlyand finally stopped in the upper part of the style after 5 days of pollination (Fig. 3 a and b). Arrested pollen tubes may show one or more of characteristic morphological abnormalities such as slow growth rates, branching, short tube (Fig. 3 c), twisting (Fig. 3c and d), variable diameters of tube (Fig. 3 e), balloon-like swelling (Fig. 3 f), formation of callose plugs at irregular intervals and irregular deposition of callose on the walls. Therefore, pollen tubes unable fertilized the ovules (Fig. 3 g). Similar result was found in flowers pollinated with ‘Florida Home’ pollen.

The morphology of incompatible pollen tubes differs from that of compatible tubes [27, 28]. In incompatible tubes, the pattern of growth is similar to that initially seen in a compatible pollination but at further stages, growth becomes irregular, the pollen tube walls become thicker and the tip may burst [28]. Dramatic morphological changes accompany the arrest of pollen tube growth in SI interaction [29]. Williams *et al*. [30] also found different abnormalities of arrested pollen tube tips within incompatible crosses of Rhododendron (*Ericaceae*) including burst, tapered, swollen, coiled, spiraling, spiky and variable diameter syndromes and consequently defined a series of errors of callose deposition within incompatible tubes.

Consequently, microscopic examination showed rootstock had no effect on incompatible pollen tube growth while it had little impact on growth of compatible pollen tubes. On the contrary, degree of partial compatibility was more variable and growth of pollen tubes greatly influenced by rootstock. Abd Elaziz [31] evaluated Florida home, YaLi and TsuLi pear cvs. as pollen donors for commercial cultivar (LeConte) growing on communis rootstock in terms of bloom time, pollen compatibility, fruit and seed set. He found that YaLi cultivar performed well as pollen source for LeConte cultivar. Meanwhile, Elbassel [32] reported that Hood was found to be an effective pollinizer for LeConte pear trees growing on communis rootstock. The present results were generally consistent with these findings.

Embryological Studies: Pear produces a core normally consisting of five locules, each with two crassinucellar ovules. In the anatropic ovule an embryo sac is formed. The mature embryo sac contains the egg apparatus (egg cell and two synergids), the central cell with two polar nuclei and three antipodes. Sometimes the polar nuclei fuse and form a secondary nucleus with a distinct nucleolus. At 2-7 days after pollination the tubes from ‘YaLi’ pollen appeared to be capable of entering and fertilizing the most embryo sacs as a result of the high degree of compatibility between the two parents on two rootstocks. At this time, the first endosperm cell and the zygote at the micropylar region were observed (Fig.4 a, b), showing evidence of double fertilization. In the period 11 to 12 days after pollination the ovules grew considerably. In the embryo sac nuclear endosperm was rapidly formed by numerous divisions of the central cell nucleus. The center of the embryo sac contained a large vacuole surrounded by the endosperm as a layer of cytoplasm with many nuclei (Fig.4 c). The zygote just started to divide and developed quite slowly; in some ovules the embryo consisted of 2-3 cells (Fig.4 c. and d.). Whereas, other ovules the embryo having only formed 3-6 cells (Fig. 4 e.). Other having also formed 6-12 cells (Fig. 4 f.)

In many embryo sacs of ‘LeConte’ x ‘TsuLi. cross on ‘communis’ the synergids was dark and shrunken, the egg cell was sometimes enlarged and secondary nuclei were beginning to form in the central cell (Fig. 5 a). Such a structure was typical for just fertilized embryo sacs. This occurred between five and six days after pollination. In the period 11 to 12 days after pollination some nucleus having pro embryo with 2-3 cells (Fig. 5 b) whereas, other fertilized embryo sacs had pro embryo consisted of 12 or more cells (Fig.5 c). Embryos were in globular stage (Fig. 5 d). Regardless of embryo sacs development in ‘LeConte’ x ‘TsuLi’ cross on ‘betulaefolia’ embryological studies showed that although fertilization occurred late the embryo development was faster. Most embryos had developed to the globular stage at 12 DAP (Fig.6 a, b and c) whereas, other heart-shaped embryos were found by this period (Fig. 6 d).

The above results show that embryo development was markedly influenced by pollinations and rootstocks. Some differences were observed concerning the rate of embryo development and the number of fertilized ovules per fruit; dataclarify that the embryo developed slower and the number of fertilized ovules was higher. In the fruitlets of self-pollinated ‘LeConte’ flowers and cross-pollinated with ‘Florida home’ pollen on two studied rootstocks the embryo sacs started to degenerate (data not shown). Many investigators reported that the zygote formation was showed 7 days after compatible pollination and formed primary endosperm after 10 days from pollination, compared to incompatible combinations where, the ovules were unfertilized Herrero and Gascon [33]; Medeira and Maia [34]; Elbassel [32]; Abd Elaziz [31] on pear; Oukabli *et al*. [35] on almond and Abd Elaziz [36] on plum.

Fruit Set Percentage and Seed Set: The data pertaining to the effect of pollen source and rootstocks on fruit setpercentage of LeConte cv. are presented in Table (3) fruit set percentage wasdiffered significantly with in the testedpollinizers and rootstocks. In the first year of the study, rootstockwith the highest fruit set percentage was ‘betulaefolia’in which was significantly at per with ‘communis’ when LeConte flowers pollinated with ‘YaLi’ pollen. However, ‘communis’ was found to have significantly higher fruitset than ‘betulaefolia’ when used ‘Yali’ as a mal parent during second seasonof study.

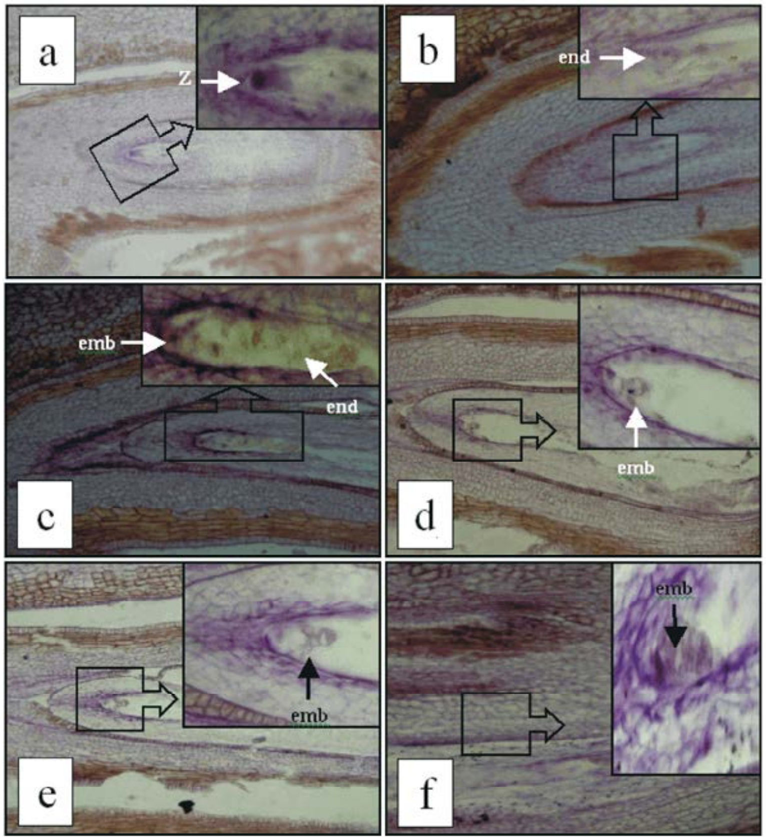

Fig. 4: The development of embryo and endosperm in the 'LeConte' x 'YaLi' cross on two rootstocks.
(a) Longitudinal section showing the zygote 5 days after pollination.(b) Longitudinal section showing the first endosperm cell 5 days after pollination. (c) Pro embryo and endosperm with many nuclei. (d) 3-6 celled embryo. e and f) 6-12 celled embryo. (X. 100 and magnification X400). (*z)* zygot, (*emb)* embryo, (*end)* endosperm.

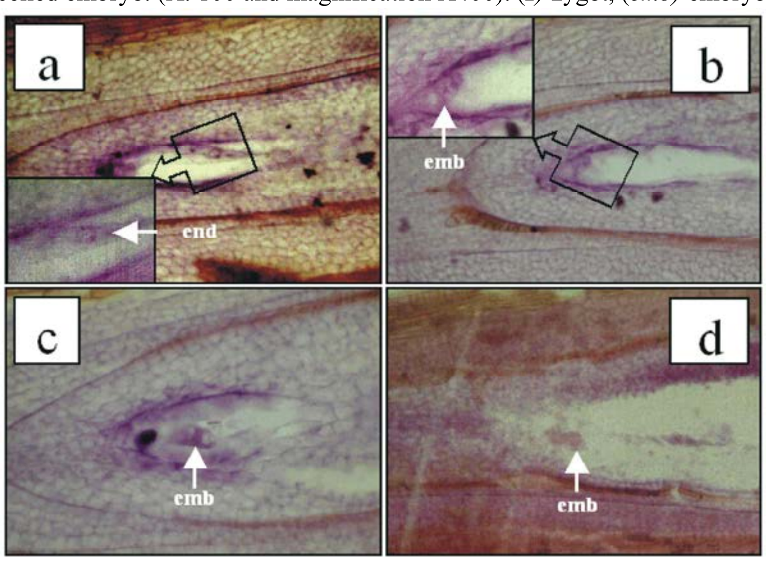

Fig. 5: The development of embryo and endosperm in the 'LeConte' x 'TsuLi' crosson 'communis'.
(a) Longitudinal section showing secondary nuclei were beginning to form in the central cell. (b) Pro embryo with 2-3 cells. (c)Pro embryo consisted of 12 or more cells(d) Embryo was in globular stage (X100 and magnification X400). *(emb)* embryo, (*end)* endosperm

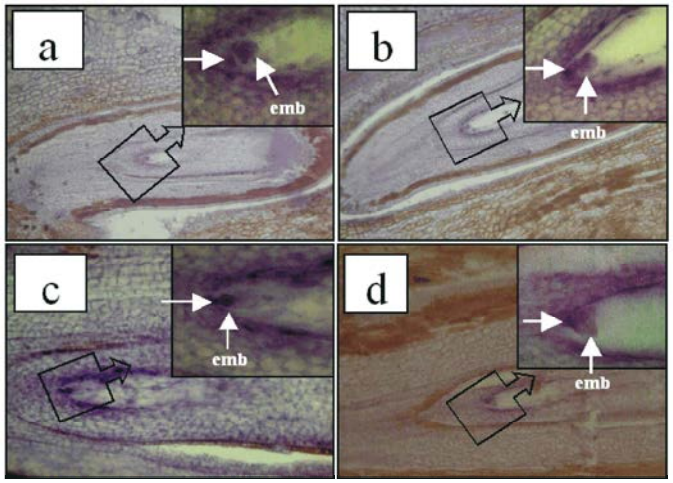

Fig. 6: The development of embryo and endosperm in the 'LeConte' x'TsuLi' cross on 'betulaefolia'.
(a, b and c) Embryos had developed to the globular stage. (d) heart-shaped embryos (X100 and magnification X400) (*emb)* embryo, (*s)* suspensor

No fruits were arising following self-pollination within both of tested rootstocks. However, betulaefolia showed a significantly higher fruit set percentage in comparison with open pollinated flowers of trees budded on 'betulaefolia'. In addition, 'communis' had no fruits following cross-pollination by 'Florida home' pollen during the two studied seasons. While, the same parent gave significantly higher fruit set than open pollinated flowers of trees grafted on 'betulaefolia'.

Ontwo studied rootstocks fruit setpercentage following cross pollination with 'TsuLi' pollen was significantly lower than the obtained from cross pollination with 'YaLi' pollen.Nevertheless, LeConte trees grafted on 'communis' rootstock gave significantly higherfruit setpercentagefrom cross pollination with 'TsuLi' pollen than ones resulted in trees grafted on'betulaefolia'pollinated by the same male parent during the two seasons of study. Trees on 'communis' rootstock tended to produce higher fruit setpercentage than trees on 'betulaefolia' rootstock when pollenated with 'TsuLi' pollen while 'YaLi' pollen showed almost similar fruit set except in the second season, 'communis' showed a significantly high fruit set percentage.

Concerning seed set, forthe two studied rootstocks, fruits of cross-pollination with 'Florida home' pollen and self-pollinatedfruits produced no seeds (parthenocarpic fruit). In addition, According to statistical analysis fruits resulted from cross pollination with 'YaLi' and 'TsuLi' showed significantly higher seed set compared to open pollinated fruits which had low seed set. As well as cross pollination with 'YaLi' pollen produced always higher seed set over cross-pollination by 'TsuLli' pollen during both of studied seasons. Generally, when flowers crossed by 'TsuLi' pollen the most effective rootstock on seed set was 'communis' than 'betulaefolia'. Whereas, effect of the two rootstocks was similar when flowers crossed by 'YaLi' pollen during first season, except 'communis. showed a significantly higher level during the second one.

These results are convergent with those obtained by Moriya *et al*. [37] they found that comparison of the number of viable seeds per fruit after self- and cross-pollination can determine the SI or SC phenotype in pear cultivar with low parthenocarpic potential. Also, in the weakly SC cultivars of European pear, 'Bartlett', Doyenné du Comice' and 'Agua de Aranjuez' and of apple 'Golden Delicious', 0.2 – 2 viable seeds per fruit were formed via self-pollination [38-40]. In addition, Ali [41] reported that cross pollination increased number of completely developed seeds compared to open and self-pollination. Also, Moriya *et al*. [42] reported that the fruit of 'Grand Champion' pear produced by self-pollination contained an average of 4.8 viable seeds per fruit, which was significantly lower than in cross pollinated fruit (an average of 5.8 viable seeds per fruit). Moreover Jahed and Hirst [43] reported that pollen tubes of apple cvs. ('Ralph Shay' and Malus floribunda) had the lowest germination and slowest growth in stigmas and styles of 'Honeycrisp' and comparatively, fewer pollen tubes reached the style base. This may have been a result of semi-incompatibility of 'Ralph Shay' and 'Malus floribunda' crabapples with 'Honeycrisp', which resulted in lower fruit set. 'Honeycrisp' fruit also had lower seed numbers when pollinated with 'Ralph Shay' in comparison with other pollen sources [44].

Table 3: Fruit set percentage and seed set formed by self and cross-pollination in LeConte pear growing in two different rootstocks during 2017 and 2018 seasons

		Fruit set %		Seed set	
Rootstocks	Parent cultivars	1stSeason	2ndSeason	1stSeason	2ndSeason
Pyrus communis	Openpollination	10.02 d	6.25 ef	1.3 d	1.2 d
	LY	57.88 a	59.97 a	7.1 a	6.8 a
	LT	28.03 b	31.42 c	5.1 b	4.9 b
	LF	---	---	---	---
	LL	---	---	---	---
Pyrus betulaefolia	Openpollination	5.67 e	4.58 f	1.0 d	1.0 d
	LY	59.18 a	42.87 b	7.6 a	5.4 b
	LT	17.32 c	27.55 d	2.3 c	2.6 c
	LF	7.91 d	7.56 e	---	---
	LL	---	7.50 e	---	---

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

**, LY (Le-Conte x YaLi), LT (Le-Conte x TsuLi), LF (Le-Conte x Florida home)and LL (self-pollination). --- Treatment which were not completed.

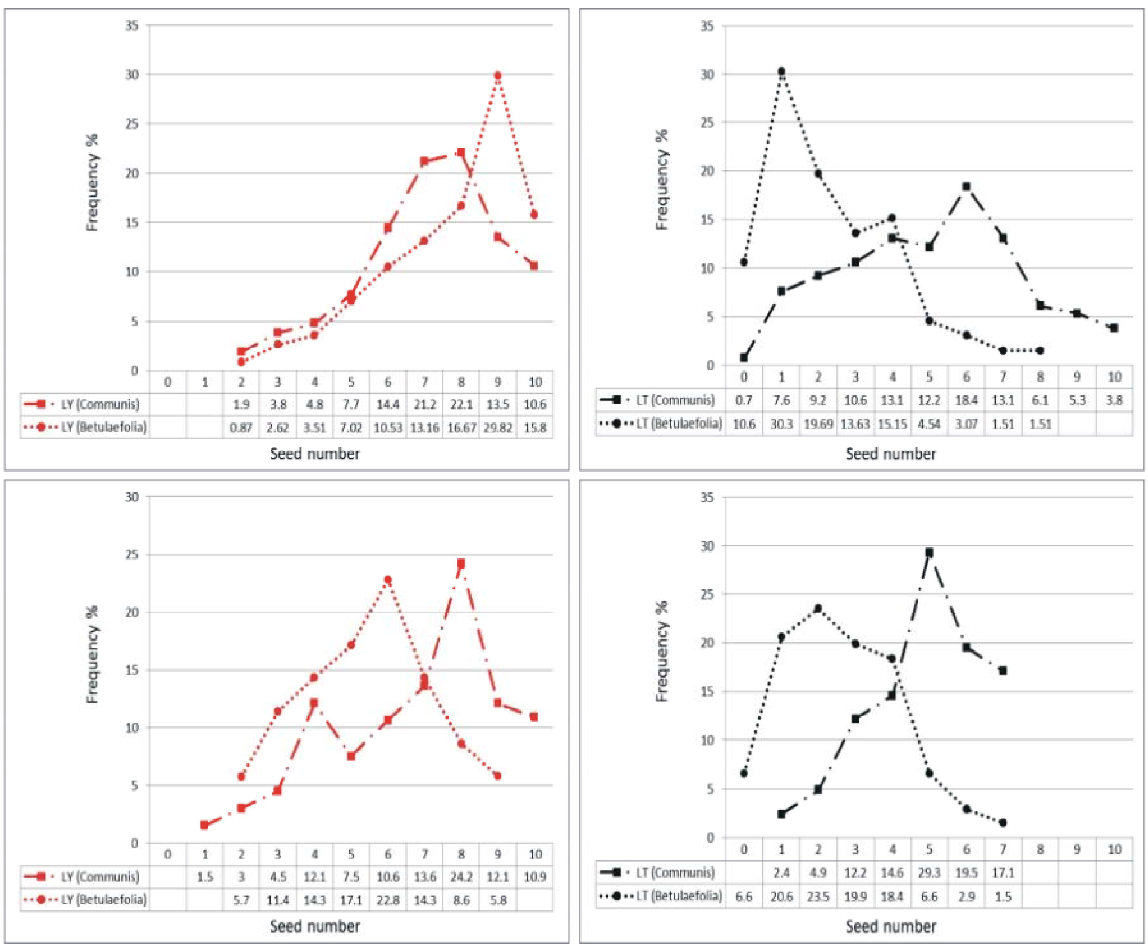

Fig. 7: Frequency distribution for seed number/fruit

Frequency Distribution of Full Seed Number per Fruits: Pollen source and its interaction with rootstocks had strong effects on frequency distribution of seed number per fruit (Fig.7). Both fruits of 'LeConte' X 'YaLi' cross on 'betulaefolia' and 'communis. had almost the same curve of frequency distribution of seed number per fruit. Consequently, fruits had mostly number of seeds, ranging from 6 to 10 seeds during first season while, in the second one, the curve of 'betulaefolia' was shifted a little further northward. So that, most fruits on 'betulaefolia' contain number of seeds, ranging from 3 to 7 seeds while, fruits on 'communis' had almost number of seeds, ranging from 4 to 10 seeds. With regard to fruits of 'LeConte' X 'TsuLi' cross on 'betulaefolia', the curve was shifted further northward. Consequently, a high percentage of fruits had 1 or 2 seeds during two seasons of study. On the contrary, a high percentage of fruits on 'communis' contain 5 and 6 seeds during two studied seasons respectively.

It has long been known that fruit weight and size were apparently related to the presence of hormone. Dennis [45] reported that hormone produced first by pollen tubes and later by the developing endosperm and embryo especially auxin and gibberellin. Also, Keulemans *et al*. [46] hypothesized that seeds affect in a quantitative and a qualitative way the sink strength of individual fruits, probably through hormones. Moriya *et al*. [37] found that seedless fruits of Barteltt, Flemish, Beauty, Conference and La France pear cultivars tend to be smaller than the seeded ones.Weinbaum *et al*. [47] found that the presence of seeds increased the weight of 'Bartlett' pear fruit by 13% under natural crop loads and by 20% under reduced crop loads. Thus, the "seed effect" on fruit growth, expressed as a percentage of fruit enlargement. Bla_ek and Hlušièková [48] reported that better pollinated fruits contained more seeds and larger. Moreover, Abd Elaziz [31] reported that the use of 'YaLi' as a mal parent for 'LeConte' budded on communis rootstock led to fruits with the highest seed number followed by 'TsuLi' pollen. Conversely self-pollinated fruits and fruits crossed by 'Florida home' had no viable seeds.

Leaf Mineral Content: Leaf mineral contentof 'LeConte' pearwas affected by rootstocks (Table 4). Also, the results indicated that Ca contentshowed an increase from full bloom to 60 days after full bloom (DAFB), while Zn and B content showed a decrease.In the case of Ca content, 'communis' had the statistically higher values (0.74, 0.97 and 1.27) from full bloom to 60 (DAFB) when compared with 'betulaefolia' (0.55, 0.61 and 0.94) respectively. Regardless of Zn content a significantly higher values was shown by 'communis' (7.79 and 7.12) from full bloom to 30 (DAFB) compared to 'betulaefolia' respectively while, 'betulaefolia' had higher value but not significant at 60 DAFB.Moreover, statistical analysis showed that boron (B) content was significantly higher in 'communis' from full bloom to 60 (DAFB) (4.29, 4.08 and 1.73) respectively. The effect of rootstocks on Leaf Ca, Zn and B contents behaved the same trend in second season while, rootstocks revealed a positive effect on Ca uptake with no significant effectat full bloom and 30 DAFB (Table 5). These results were similar to those of several investigators such as Ikinci *et al*. [49] they found the highest concentration of mineral elements (N, P, K, Ca, Mg, Fe and Cu) on the pear seedling and BA 29 rootstocks. Moreover, Kidman *et al*. [50] reported that calcium, zinc, boron and molybdenum are nutrients essential for pollination and fertilization in grapevines and rootstocks were found to affect the sequestration of nutrients which affected reproductive performanceand thus influence the degree of compatibility.

It can be concluded that 'communis' was more effective than 'betulaefolia' in terms of uptake Ca, Zn and B from root medium. These elements plays essential role in successful reproductive development of many angiosperms, there is a requirement for adequate levels of both macro and micro elements, in particular, nitrogen, calcium, boron and zinc [51-53]. Many important roles of calcium in plant growth have been demonstrated. Among these, the improvement in germination and growth of pollen due to calcium relates primarily to the binding of calcium to pectate carboxyl groups along the pollen wall thence calcium aids in the rigidity and straightness of the pollen tube [51]. Boron aids in stigma receptivity, pollen germination and pollen tube growth as a result of sugar borate complexes that promote sugar absorption, sugar translocation and metabolism of sugars in the pollen [52, 54]. Zinc is required for both pollen and fruit development, being involved in many enzymatic reactions. It is a cofactor for RNA-polmerase thus it is critical for protein synthesis and is involved in regulating the protein as well as carbohydrate metabolism [55, 56]. Zinc is also involved in the formation of tryptophan, a precursor to IAA which can be a limiting factor in vascular tissue differentiation [57].

Based on previous results, crossing 'LeConte' on 'communis' with pollen from YaLi cultivar showed high level of cross compatibility in terms of pollen tube characteristics, seed set and fruit set .Similar levels of cross compatibility was found also in 'LeConte' on 'betulaefolia' when flowers crossed by the same pollen donor. In addition, crossing 'LeConte' on 'communis' with pollen from TsuLi cultivar showed high level of partial cross compatibility. On the contrary, 'LeConte' x 'TsuLi' cross showed low level of partial cross compatibility. Therefore, the present work suggested that 'YaLi' and 'TsuLi' seemed to be an effective pollinizer for 'LeConte' trees grafted on commuinus rootstock.

Table 4: Seasonal variation in Ca, Zn and B foliar concentration of LeConte pear cultivar grown on two different rootstocks during 2017 season

	Leaf Ca (g /100 g)			Leaf Zn (mg /100 g)			Leaf B (mg /100 g)		
Rootstock	0 DAFB	30 DAFB	60 DAFB	0 DAFB	30 DAFB	60 DAFB	0 DAFB	30 DAFB	60 DAFB
Pyrus communis	0.74	0.97	1.27	7.79	7.12	4.03	4.29	4.08	1.73
Pyrus betulaefolia	0.55	0.61	0.94	5.01	4.28	4.13	1.70	1.68	1.37
Significance	S.	S.	S.	S.	S.	N.S.	S.	S.	S.

N.S.(non-significant) S.(significant)

Table 5: Seasonal variation in Ca, Zn and B foliar concentration of Le-Conte pear cultivar grown on two different rootstocks during 2018 season.

	Leaf Ca (g /100 g)			Leaf Zn (mg /100 g)			Leaf B (mg /100 g)		
Rootstock	0 DAFB	30 DAFB	60 DAFB	0 DAFB	30 DAFB	60 DAFB	0 DAFB	30 DAFB	60 DAFB
Pyrus communis	0.23	0.27	0.34	2.51	1.39	1.32	3.57	3.10	2.51
Pyrus betulaefolia	0.21	0.25	0.28	2.26	1.18	1.09	1.81	1.59	1.46
Significance	N.S.	N.S.	S.	S.	S.	S.	S.	S.	S.

N.S.(non-significant) S.(significant)

While, ‘TsuLi’ was not suitable pollinizer for ‘LeConte’ trees buded on ‘betulaefolia’. On the other hand, Abd Elaziz, [31] found that poor overlap occurred in the blooming periods between ‘LeConte’ and both ‘Tsu Li’ and ‘Ya Li’. So, in case of using ‘Tsu Li’ as a pollinizer, ‘Le Conte’ trees must be break endodormancy artificially by applying chemicals. While, in case of using ‘Ya Li’ as a pollinizer, ‘Ya Li’ trees must be break endodormancy artificially.

CONCLUSION

Results highlight the importance of cross-pollination of ‘LeConte’ cultivar for obtaining satisfactory fruit set in the environmental conditions of Egypt and the beneficial effect of rootstocks on reproductive, possibly by affecting positively the fertilization process and the sequestration of nutrients which affected reproductive performance and thus influence the degree of compatibility.One can say that high fertilized ovule count seems to accelerate growth of fruitlets which can escape Fire blight.

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