



Impact of Maryana and Nemagard Rootstocks on Sexual Compatibility of African Rose plum cv.

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ABSTRACT

In this study, the effects of two rootstocks (Nemagard and Maryana) on the African Rose plum cv. degree of self and cross-compatibility with Hollywood and Pioneer cultivars, as well as, the leaf mineral content (calcium, boron, and zinc) were examined. Fluorescent microscopy studies postulated that self-pollinated flowers on two studied rootstocks showed self-compatibility. Nevertheless, a high degree of self-compatibility was observed on 'Maryana' rootstock. Moreover, cross-compatibility was noted in styles crossed by 'Hollywood' pollens and high degree of cross-compatibility was observed on 'Maryana' rootstocks. Partially cross-compatible was observed in styles crossed by 'pioneer' pollen, and high level of partial cross compatibility was observed on 'Maryana'. While a lower degree was noted on 'Nemagard'. Leaf analysis showed that 'Maryana' had significantly higher calcium, boron and zinc for African Rose plum cv. mineral element concentration than 'Nemagard'. Field data confirmed fluorescent microscopy results, rootstock with the higher fruit set percentage was 'Maryana' when 'African rose' flowers crossed by 'Hollywood' pollens as well as self-pollinated flowers in comparison with 'Nemagard' rootstock. In addition, 'Maryana' was found to have significantly higher fruiting percentage than 'Nemagard' when used 'Pioneer' as a male parent. The data revealed that rootstock influences depend on increased nutrient absorption, which results in more fertilized flowers.

Key words: Plum, Sexual Compatibility, pollination, Rootstocks.

INTRODUCTION

Plum, which belongs to the *Rosaceae* family, is one of deciduous fruit trees. The European plum, *Prunus domestica* L., and Japanese plum, *Prunus salicina* L. may both be grown effectively in all irrigated areas of the world, including Egypt. According to FAO's 2019 data, 5.6 million hectares of plums were produced in the world and 3.9 million tons were obtained in this field. China was the largest producer, accounting for over 53.84% of global production and followed by Romania (6.68%), Serbia (3.41%), the United States (3.11%) and India (2.15%). In Egypt the total cultivated area of plums (i.e., mostly the Japanese cultivars) is about 1115 hectares. Egypt ranked 44th globally with a total of 14.775 tons. Cultivars

"Beauty," "Hollywood," and "Santa Rosa" are the most widely grown in Egypt.

The majority of Japanese plum (*Prunus salicina* Lindl.) cultivars feature a self-incompatible mechanism that precludes self-fertilization (Okie and Hancock 2008). In actuality, at least two loci encoding the pollen and pistil allelic determinants, which are genetically controlled by the majority of rosaceous fruit tree species, including *P. salicina*, exhibit gametophytic self-incompatibility (Tao and Iezzoni, 2010). When any of the two S-alleles expressed in the style and the S-allele expressed in the pollen is identical, this mechanism prevents pollen tube growth in the style. The pollen tube can develop through the style and fertilize the ovule, however, when the S-



allele of the haploid pollen grain is distinct from the two S-alleles expressed in the style tissue (Guerra et. al., 2009).

The biological synthesis of auxin (IAA) and gibberellin (GA₃), which play crucial roles in plant development, pollination, fertilization, and fruit set in fruit trees, is directly influenced by zinc (Zn), more than 300 plant enzymes rely on zinc as a cofactor for their structure and operation (Broadley et al., 2007). In addition to boron is a crucial component for fertilization, since it promotes pollen germination and the formation of pollen tubes, (Souza et al., 2017). Pollen germination and pollen tube growth can both be accelerated by high doses of boron (Perica et. al., 2011). The formation and germination of pollen grains, the growth of pollen tubes,

the synthesis of sugars, and the accumulation of sugars have all been demonstrated to require nutrients like calcium (Ca) and boron (B) (Stino et al., 2011).

Fruit tree performance in orchards is greatly influenced by rootstocks. Although numerous studies have shown that rootstocks can influence tree size, precocity, productivity, and mineral uptake efficiency, particularly in high intensity modern orchards, rootstock impacts on self and cross incompatibility are poorly understood. Therefore, fruit set following controlled pollinations was combined with observations of pollen tube growth under the microscope and with the impact of rootstocks on the sexual compatibility of African Rose plum cultivar.

MATERIALS AND METHODS

The study was done during the course of the two succeeding seasons of (2021 and 2022) to determine how rootstock affected the sexual compatibility of the African Rose (*Prunus salicina* L.). Eight years old African rose trees planted at 3 × 4 in sandy soil in El-Khatatba (Minufiya Governorate, Egypt) budded on two distinct rootstocks, Maryana and Nemagared. In addition to self-pollination, cultivars of the plums Pioneer and Hollywood were employed as male parents. Just one day prior to anthesis, during the balloon stage, the flowers from the three varieties were harvested. The flowers were placed on paper in the lab at room temperature to allow the anthers to dehisce. The pollens from each cultivar were separated after a day and placed in glass tubes to be used as pollinators for the African Rose cultivar.

In accordance with the procedures described by Operle and Watson (1953), the vitality of pollen was assessed using a 2, 3, 5-triphenyltetrazolium chloride (T.T.C.) staining solution (1 part 10% T.T.C.: 10 parts 60% sucrose).

Self and cross-compatibility and pollen tube growth.

Three trees received a total of a hundred flowers per treatment. The procedure outlined by Wertheim (1996) was followed for conducting the hand pollination. After pollination, pistils were fixed every day for seven days straight. FAA fixative, which contains 70% ethanol, glacial acetic acid, and formaldehyde in a ratio of 90:5:5, was used for the fixation process (Burgos et al., 1997). The fixed sample was refrigerated at 4°C until staining. Pistils were rinsed for 24 hours in running water prior to staining. Then, to soften their tissues, they were submerged in an 8N solution for two hours. This process was used to prepare the flowers and make it easier for the stain solution to penetrate them. Pistils were rinsed from NaOH the following day with running water. The staining process was completed by dissolving 0.1% aniline blue in 0.1 N K₃PO₄. According to the procedure described by Kho and Baer (1968), the vials containing the material were maintained at 4°C until the microscopic examination. The pollen tube can be followed through the style



tissue and the rejection site is identified thanks to the fluorescence under ultraviolet light. Martin (1959) has already provided a description of this method.

The style was dissociated from the ovary to prepare the pistils for microscopic analysis. The style was compressed, and the ovary was cut lengthwise with a razor blade to reveal the entrance of the pollen tubes to the ovules. With the use of a fluorescence microscope, pistils were examined.

Fruit set percentages.

Approximately 450 flowers per treatment were left for fruit set measurements, as was specified in the pollination treatment. At anthesis, the total number of flowers was counted. In addition, the fruits were counted 21 days following pollination, when the first fruit set occurred. The following formula was used to get the initial fruit set percentage: Initial fruit set percentage = number of set fruits/total number of flowers x 100.

Nyéki and Szabó (1996) categorized cultivars into five groups: completely self-incompatible (fruit set 0%), moderately self-fertile (fruit set 1% to 10%), very self-fertile (fruit set above 20%), and self-incompatible (fruit set 0% to 1%). based on Abd-Elaziz et al., (2017).

Leaf mineral elements content.

A total of 50 mid-terminal, newly formed leaves from current-year shoots growing 150 cm above the ground in the tree canopy made up each leaf sample. As soon as the leaves were collected, they were placed into polyethylene bags and transported to the lab in a mobile refrigerator. Miloševića and Miloševića (2016) were used to determine the mineral analyses of leaves. Atomic emission spectrometry was used to conduct the analyses. Three copies of each determination were made. % for the macro element (Ca) and ppm for the microelements (Zn and B) were used to express the results on a dry matter basis

RESULTS AND DISCUSSION

The pollen viability of the cultivars of African Rose, Pioneer, and Hollywood plum in the two seasons is shown in Table 1. After 24 hours in T.T.C., the pollens of the three plum cultivars showed a high rate of vitality. In contrast, there was no discernible difference across cultivars, with a range of (87.27 to 92-72%) over the course of the two seasons under study. While, African Rose cultivar showed more pollen grain vitality than other cultivars. In general, there were

only minor variations across cultivars, none of which were significantly different from one another. Maklad (2019) found that the pollen viability of Pioneer and Celebration plum cultivars ranged from (88.00 to 99.18%). When compared to the other cultivars Songold, Sapphire, and Santa Rosa, Pioneer's pollen had a high percentage of viability and germination (74.14%), according to Fayek et. al., (2018).

Table (1). Pollen grains viability of African Rose, Pioneer and Hollywood cultivars in 2021 and 2022 seasons.

Cultivar	Pollen grains viability %	
	1 st Season	2 nd Season
African Rose	92.72 a	90.79 a
Pioneer	90.17 a	87.55 a
Hollywood	89.41 a	87.27 a

Mean separation within columns or rows by Duncan’s multiple range test, 5% level.

Pollen germination and pollen tube growth:

Self-pollination

Germination of pollen grains and growth of pollen tubes were observed in the style of African Rose plum cultivar growing on both Nemagard and Maryana rootstocks after self-pollination. Maryana rootstock showed the highest degree of self-compatibility after self-pollinated African Rose cultivar's flowers on the two investigated seasons. Following pollination, pollen tubes develop across the stigma surface (Fig. 1a), descend into the style (Fig.

1 b), and reach the lowest section of the style (Fig. 1c) three days later. Within 3–4 days of pollination, it entered the ovules.

When African Rose cultivar's flowers were pollinated with their own pollens on Nemagard rootstock, however, microscopic analysis revealed a reduced degree of compatibility in pollen tube growth; the majority of the pollen tubes extended to the end of 1/3 length of the style, 3 days after pollination (Fig. 2 a, b). In 4 days following pollination, they reached the lowest portion of the style (Fig. 2 c).

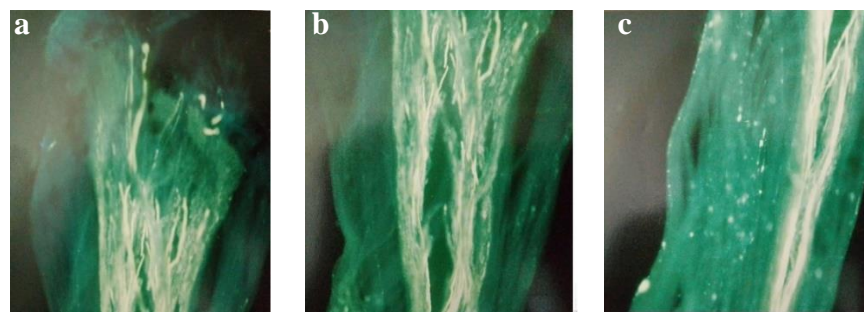


Fig. (1) Pollen tube characteristics after a high degree of compatibility.

- a) Pollen tubes grow across the stigma's surface.
- b) Pollen tubes passed down into the style after 2 days of pollination.
- c) Pollen tubes reached the lower part of the style in three days from pollination.

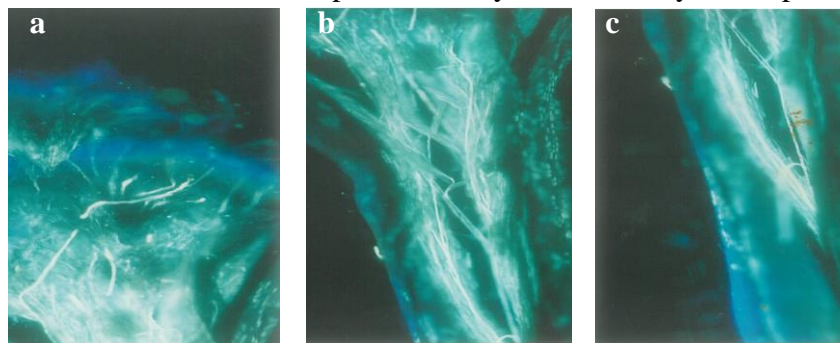


Fig. (2) Pollen tube characteristics after a lower degree of compatibility

- a) Most of the pollen tubes grew to the end of 1/3 length of the style, 3 days after pollination.
- b) Some pollen tube growth grew through the styles by spacing callus plugs along the tube.
- c) The Pollen tube reached the lower part of the style four days after pollination.

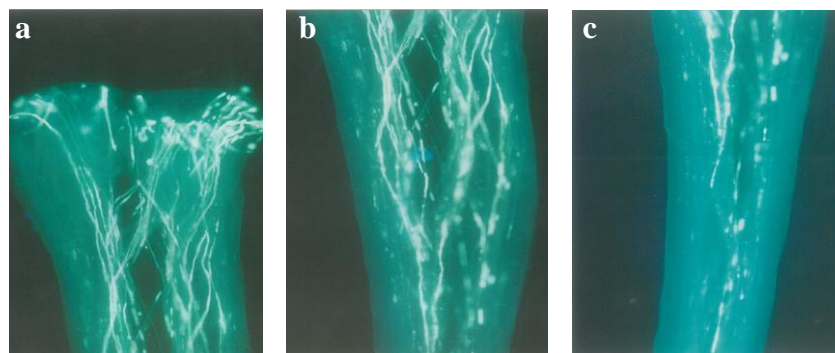


Fig. (3) Pollen tube characteristics after a high degree of partially compatible crosses

- a) Numerous pollen grains germinated on the stigma.
- b) Pollen tubes grew through the styles by spacing of callus plugs in large quantities along to the tube.
- c). A Few pollen tubes reached the lower part of the style five days after pollination.

Cross-pollination

African Rose x Pioneer

Likewise, when flowers were pollinated with Pioneer on Maryana rootstock, a high level of partial cross compatibility was seen. Some pollen tubes developed more slowly than others, and some grew through the spaces between the callus plugs that were positioned along the tube. Following five days of pollination, only five or four pollen tubes remained in the style's lower portion and penetrating the ovules. This is because the number of pollen tubes formed in the style gradually decreased along its length as they approached the ovary. African Rose x Pioneer on 'Nemagard' rootstock was found to have a lower degree of partial cross-compatibility.

African Rose x Hollywood

The crosses African Rose x Hollywood on 'Maryana' rootstock behaved with high compatibility where the results showed that the germinated pollen were observed on the stigma surface of African Rose after 24 h of pollination. All the pollen tubes were visible in the upper part of the style two days after pollination, and they reached the lower part of the style after 3 days of pollination. While the degree of cross-compatibility was lower for the same cross on 'Nemagard' rootstock.

These findings concur with those of Abdelaziz, (2021), who focused on pears. When 'Le-Conte' flowers were pollinated by 'YaLi' pollen, he discovered a high level of compatibility with little effect from the rootstock on the development of suitable pollen tubes. When 'TsuLi' pollen was crossed, it was found to be somewhat compatible, and 'Communis' was found to have a high degree of partial cross compatibility. While 'Betulaefolia' was reported to be of lower degree. 'Le-Conte', on the other hand, is regarded as self-incompatible, with no effect of rootstock on incompatible pollination.

Fruit set percentage.

The data pertaining to the effect of pollen source and rootstocks on the fruit set percentage of African rose cv. are presented in Table (2). Fruit set percentage differed significantly among the tested pollinizers and rootstocks. Fruit set percentage was differed significantly within the tested pollinizers and rootstocks. In the first year of the study, the rootstock with the highest fruit set percentage (36.11%) was 'Maryana' following self-pollination, while 'Nemagard' was found to have a significantly lower fruit set percentage after self-pollination (7.95 %). Moreover, 'Maryana' showed a higher fruit set percentage (31.25%) than 'Nemagard' when



used 'Hollywood' as a male parent (8.00%). Meanwhile, the fruit set percentage of African Rose plum cultivar after cross pollination with 'Pioneer' pollens was (6.12%) on 'Maryana', whereas on 'Nemagard' was less than 5.0%.

In the second year of the study, the rootstock with the higher fruit set percentage was 'Maryana' when 'African rose' flowers were

crossed by 'Hollywood' pollens as well as self-pollinated flowers, in comparison with 'Nemagard' rootstock. In addition, 'Maryana' was found to have a significantly higher fruit set percentage than 'Nemagard' when used 'Pioneer' as a male parent. Generally, fruit set percentage results were a real reflection of observations of pollen tube growth.

Table (2). Fruit set percentage after self and cross-pollination in African Rose cultivar during the 2021 and 2022 seasons.

Rootstocks	Pollination	Fruit set (%)	
		1 st Season	2 nd Season
Maryana	Self-pollination	36.11a	34.73 b
	African Rose X Pioneer	6.12 c	7.29 cd
	African Rose X Hollywood	31.25 b	37.63 a
Nemagard	Self-pollination	7.95 c	9.45 c
	African Rose X Pioneer	3.66 d	5.40 d
	African Rose X Hollywood	8.00 c	9.81 c

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

According to a considerable interaction between cultivar and rootstock that was discovered, yield is not influenced by either factor alone (cultivar or rootstock), but rather by how they work together (Miloevia and Miloevib, 2012). Additionally, the same authors hypothesized that a comparison of Stanley and Myrobalan rootstocks showed significant differences in yield and fruit weight. This finding is consistent with earlier studies on plum (Vangdal et. al., 2007, Rato et. al., 2008), which found that trees on the vigorous Myrobalan had restricted vigour and larger fruit size and yield than those on the trees on Stanley. Furthermore, these results are consistent with those of Hrotkó, et. al., (2002), who found that "Stanley" produced the highest yield efficiency on vigorous Marianna GF 8-1, "St Julien" GF 655/2, "My-BO-1," "MY-KL-A," and "My-KL-A" seedlings, while "Fehérbesztercei" produced the lowest yield efficiency.

Leaf mineral analysis.

Nutrient levels of African Rose leaves at full bloom appeared to be influenced by rootstocks (Table 3). In the case of Ca content, 'Maryana' had statistically higher values (4.06 and 4.2%) than Nemagard (3.91 and 3.88%). Regardless of Zn content, significantly highest values were shown by 'Maryana' (174 and 150 ppm) in comparison with Nemagard (150 and 158 ppm). Moreover, statistical analysis showed that boron (B) content was significantly higher in 'Maryana' (17 and 18.5 ppm) than Nemagard' (13 and 15 ppm) during the two seasons, respectively.

According to our findings, the 'Nemagard' rootstock has a much lower capacity for absorbing Ca, Zn, and B than the 'Maryana' rootstock. Numerous angiosperms depend on these elements for successful reproductive growth; in particular, nitrogen, calcium, boron, and zinc must be present in sufficient amounts on both the macro and micro scales (Brewbaker



and Kwack 1963; Chen et. al., (1998), Kaiser et. al., (2005). It has been shown that calcium plays many significant roles in plant growth. According to Brewbaker and Kwack (1963), calcium binds to pectate carboxyl groups throughout the pollen wall, improving pollen germination and growth. As a result, calcium helps to maintain the rigidity and straightness of the pollen tube. As a result of sugar borate complexes that improve sugar absorption, sugar translocation, and the metabolism of sugars in the pollen, iron helps with stigma receptivity, pollen germination, and pollen tube expansion (Chen et. al.,1998; Lee et. al.,2009). Zinc participates in numerous

enzymatic processes and is necessary for the development of both pollen and fruit. It is essential for protein synthesis because it is a cofactor for RNA-polmerase (Faust 1989). As a result of variations in xylem dimension and morphological and physiological aspects of root morphology, which can directly affect ion absorption, translocation, and redistribution (Hell and Stephan, 2003), different genotypes of rootstocks may limit nutrient translocation (Tombesi et. al., 2011; Nawaz et. al., 2016). By doing so, it is able to choose the rootstocks that will sequester nutrients the most effectively, which will affect reproductive success and therefore influence the degree of compatibility.

Table (3). Levels of Ca, Zn, and B in of African Rose cultivar's leaves during two seasons.

Rootstocks	Ca (%)		Zn (ppm)		B (ppm)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Maryana	4.60	4.20	174	150	17	18.5
Nemagard	3.91	3.88	150	158	13	15.0
Significance	S*	S	S	S	S	S

*S (significant)

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تأثير أصلي مارينا ونيماجارد على التوافق الجنسي في صنف برقوق الافريكان روز

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تمت دراسة تأثير الاصول على درجة التوافق الذاتي لصنف برقوق افريكان روز المطعوم على أصليين مختلفين (ماريانا و نيماجارد)، وكذلك على درجة التوافق الخلطي مع صنف هوليوود وبايونير. وكذلك دراسة محتوى الأوراق من (الكالسيوم والبورون والزنك).

كانت نتائج الفحص الفلوريسنسي للأزهار صنف برقوق افريكان روز المطعوم على كلى الأصلين متوافق ذاتيا إلا أن درجة التوافق الذاتي كانت مرتفعة للأشجار المطعومة على أصل الماريانا. كما أن صنف برقوق افريكان روز متوافق خلطيا

مع صنف برقوق هوليوود إلا أن درجة التوافق الخلطي كانت مرتفعة للأشجار المطعومة على أصل الماريانا. بينما لوحظ أن صنف افريكان روز متوافق جزئياً مع صنف بايونير إلا أن درجة التوافق الجزئي كانت مرتفعة للأشجار المطعومة على أصل الماريانا مقارنة بالأشجار المطعومة على أصل النيماجارد. كما أشارت الدراسة أن محتوى الأوراق من الكاسيوم والبورن والزنك كان مرتفعاً للأشجار المطعومة على أصل الماريانا بالمقارنة بأصل النيماجارد.

النتائج الحقلية أكدت نتائج الفحص الفلوروسنسي حيث وجد أن الأزهار الملقحة ذاتياً وكذلك الأزهار الملقحة خلطياً بحبوب لقاح صنف هوليوود وبايونير على أصل الماريانا أعطت نسبة عقد مرتفعة معنوياً بالمقارنة بأصل النيماجارد. أشارت النتائج إلى أن تأثيرات الأصول تعتمد على توفير العناصر الغذائية الضرورية للتلقيح والخصاب، مما يؤدي إلى زيادة نسبة الأزهار المخصبة.