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ARTIGO

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Incompatibility of 'Cleopatra' mandarin rootstock for grafting citrus in Central Amazon, State of Amazonas, Brazil

Incompatibilidade de enxertia de base com tangerineira 'Cleópatra' em citros na Amazônia Central, Estado do Amazonas

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ABSTRACT: In sweet orange 'Pêra-Rio' and acid lime 'Tahiti' citrus plants (*Citrus* spp.) stalked on "Cleopatra" mandarin, a necrosis in the grafting point was observed, causing plant death and dry fruits. Aiming to identify the causes of changes in the rootstock, which lead to grafting incompatibility, an anatomical study comparing plants with and without symptoms, stalked on Rangpur lime, was performed. In addition, the plant nutritional conditions and soil fertility of orchards were evaluated. Necrotic processes caused by vascular discontinuity between the crown and the rootstock were the main causes of loss in productivity and fruit quality. The incompatibility between the rootstock with the crown of 'Pêra-Rio' orange or 'Tahiti' lime causes plant nutritional imbalance that is not related to low natural fertility of the soil in the region. The results indicated problems in the use of 'Cleopatra' mandarin rootstock with the soil and climatic conditions of the Central Amazon region.

RESUMO: Em plantas de laranjeira 'Pêra-Rio' e de lima ácida 'Tahiti' (*Citrus* spp.) enxertadas sobre tangerineira 'Cleópatra', observaram-se uma necrose do ponto de enxertia, a morte das plantas e os frutos secos. Com o objetivo de detectar as causas das alterações no porta-enxerto que levaram à incompatibilidade de enxertia, realizou-se um estudo anatômico comparativo entre plantas com sintomas e plantas sem sintomas enxertadas sobre limão 'Cravo', avaliando-se também o estado nutricional e a fertilidade do solo dos pomares. Processos necróticos ocasionados por descontinuidade vascular entre a copa e o porta-enxerto foram os principais fatores que levaram a perdas da produtividade e da qualidade dos frutos. A incompatibilidade entre o porta-enxerto e a copa de laranjeira 'Pêra-Rio' ou a lima ácida 'Tahiti' acarreta distúrbio nutricional que não tem relação com a baixa fertilidade do solo da região. Os resultados indicaram problemas no uso de tangerineira 'Cleópatra' como porta-enxerto nas condições edafoclimáticas da Amazônia Central.

PALAVRAS-CHAVE:
Citrus sinensis
Soil fertility
Nutritional status
'Tahiti' lime
'Pêra-Rio' orange

KEY-WORDS:
Citrus sinensis
Fertilidade do solo
Estado nutricional
Lima ácida 'Tahiti'
Laranjeira 'Pêra-Rio'

1 Introduction

The production of citrus is one of the main agricultural activities in the State of Amazonas. In 2011 (INSTITUTO, 2011), there were 3,382 ha of citrus orchards comprising mandarin, orange and lime trees with average yield of 4.7 t ha^{-1}, mostly grafted on Rangpur lime (*Citrus limonia* Osbeck). In the central region of the state, 95% of the orchards are located in the municipalities of Iranduba, Itacoatiara, Manaus, Presidente Figueiredo and Rio Preto da Eva.

In the production system, the seedling is the most important input for the formation of an orchard. The main characteristics of citrus seedlings are the origin of the graft and rootstock, the root system quality and its health (SCHÄFER; BASTIANEL; DORNELLES, 2001). In Brazil, although there are countless rootstocks, nearly 80% of citrus are stalked on Rangpur lime (SCHÄFER et al., 2001; STUCHI; DONADIO; SEMPIONATO, 2002).

However, other rootstocks – such as 'Swingle' citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.], 'Cleopatra' (*Citrus reshni* Hort. ex. Tanaka) and 'Sunki' (*Citrus sunki* Tan.) mandarins, 'Troyer' citrange [*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.] and 'Caipira' orange [*Citrus sinensis* (L.) Osbeck] – may provide better quality than Rangpur lime (LIMA, 1993; SCHÄFER; BASTIANEL; DORNELLES, 2001; NASCIMENTO et al., 2003); what has not yet been noticed in the State of Amazonas, despite the increasing use of these rootstocks in the region.

In spite of a few studies, the introduction of the 'Cleopatra' mandarin as a rootstock is due to the positive results obtained in other locations, such as the northeastern states of the country and the State of Sao Paulo; having the following as main characteristics: moderate tolerance to the dry season and gummosis; tolerance to sudden death and decline, which Rangpur 'Volkamer' lime (*Citrus volkameriana* Pasq.) and 'Rugoso Nacional' lime (*Citrus limon* Burn f.x) do not present; besides good yield induction to the crowns grafted on it, characterized by the late onset of fruit production (DONADIO; CABRITA; SEMPIONATO, 1993; NASCIMENTO et al., 2003).

In these locations, no occurrence of incompatibility have been recorded in citrus, whose symptoms are characterized by nutritional disorders, exaggeration of sprouts, leaves fall, dry pointers and dry fruits. In extreme cases, with the removal of the bark, it enters the trunk, almost always causing necrosis and gummosis (NAURIYAL; SHANNON; FROLICH, 1958; KIRKPATRICK; BITTERS; FOOTE, 1962; POMPEU JUNIOR; DONADIO; FIGUEIREDO, 1972; MATTOS JUNIOR et al., 2005).

However, due to these symptoms, before producers utilize new citrus rootstocks in central Amazon, more comprehensive studies will be necessary on the compatibility with the crown, adaptability to climate and soil conditions – such as roots growth and vegetation vitality, peculiar to the region – and resistance or susceptibility to attacks by pests and diseases. Nevertheless, these assessments may take several years because of the perennial characteristics of the crop.

Confirming the descriptions of incompatibility in orangeries located in Iranduba (3° 17' 6" S and 60° 11' 9" W), Presidente Figueiredo (2° 1' 2" S and 60° 1' 3" W), and Rio Preto da Eva (2° 41' 56" S and 59° 42' 0" W), State of Amazonas; all citrus plants with the following characteristics were observed: approximately five years old, in the beginning of fruit production, with necrosis at the grafting point, yellowish leaves, oversprouting of the rootstock, drying of pointers, and dry fruits with thick peel. These plants had 'Cleopatra' mandarin as rootstock and the crowns with sweet orange 'Pêra-Rio' [*Citrus sinensis* (L.) Osbeck] and acid lime 'Tahiti' (*Citrus latifolia* Tan.).

The purpose of this research was to diagnose the problems of incompatibility of sweet orange 'Pêra-Rio' and acid lime 'Tahiti' grafted on 'Cleopatra' mandarin with necrosis at the grafting point, through the internal anatomy of plants, nutritional status and assessment of soil fertility of the orchards located in the central region of the State of Amazonas.

2 Materials and Methods

In view of the similarity of symptoms in all visited locations, anatomic studies were carried out only in diseased and healthy plants in orchards in Iranduba and Presidente Figueiredo, where samples were

collected from the trunk of 'Cleopatra' mandarins with incompatibility symptoms and without symptoms originating from Rangpur lime rootstocks.

The trunk samples were obtained from the region of the tissue union of plants with sweet orange 'Pêra-Rio' and acid lime 'Tahiti' crowns. In these regions, parts with 10.0 cm in length and 5.0 cm in width were removed; later, 5.0 mm of wood, including the bark, was removed making use of a grafting knife. After sample collection, the hard bark layer was discarded; keeping the rest adhered to the wood. The samples were fixed in FAA 50 containing 90 mL of ethanol in aqueous solution at 70%, 5.0 mL of acetic acid and 5.0 mL of formalin (JOHANSEN, 1940; BERLYN; MIKSCHE, 1976), softened by cooking in glycerin and water at 3:1 ratio, for a time period of three days, and kept in ethanol 70% until the final processing.

After being fixed in FAA 50, these samples were cut in the radial longitudinal and tangential longitudinal directions with the use of a freezing microtome, obtaining 30 μm-thick sections. The lignified cell walls were colored with toluidine blue at 0.05% and pH 6.2 for 5 min (O'BRIEN; FEDER; MCCULLY, 1965).

The sections were selected in variable number from ten to eight for coloration and mounting. Entellan® synthetic resin was used for the mounting. For coloration, the sections were quickly wiped dry in paper towel before immersion in dye. The slide study was performed in a clear field microscope with 260-time increase.

In the orchard located in Iranduba, soil samples in triplicate at 20 cm depth were also collected between the planting lines and at the tree crown projection in sweet orange 'Pêra-Rio' and at another adjacent area with acid lime 'Tahiti' crown, both with Rangpur and 'Cleopatra' mandarin rootstocks. At the same crown and in a 'Cleopatra' mandarin ungrafted plant, leaves from the middle third of the plant in the four quadrants were collected, as described by Oliveira Junior et al. (1994).

After being air dried and sieved in 2.0 mm mesh, the soil samples were taken to the laboratory at 'Embrapa' Western Amazon for determination of soil chemical attributes such as:, pH in water, P, K and Na available, Ca, Mg, H+Al and Al exchangeable, Cu, Fe, Mn and Zn available, according to the methodologies described by Claessen (1997) and Moreira et al. (2004).

The leaf content of N was determined by semi-micro Kjeldahl distillation after sulfuric acid digestion and the P and B contents were determined by colorimetry with ammonium molybdate and azomethine H, respectively, after nitric-perchloric digestion. The content of S was quantified by turbidimetry in barium chloride, while the leaf contents of K, Ca, Mg, Cu, Fe, Mg and Zn were obtained by atomic absorption spectrophotometry, according to the description provided by Malavolta, Vitti and Oliveira (1997).

The results of the soil and foliar studies were analyzed in completely randomized design with three replicates. The comparison of results was made by variance analysis (ANOVA) with the application of F test and by the comparison of contrasts between means through Tukey's test at 5% significance level (PIMENTEL GOMES; GARCIA, 2002).

3 Results and Discussion

The sweet orange 'Pêra-Rio' plants grafted on 'Cleopatra' mandarin presented pointers death (Figure 1a), low yield with less than 10 kg/plant year^{-1}, dry and thick peel fruits with ± 10.0 mm - similar to the symptoms of B deficiency (MALAVOLTA et al., 1994; MOREIRA et al., 2008), not viable for commercialization. Similar symptoms were also observed in acid lime 'Tahiti' plants. In the plants with such symptoms, there was a necrotic line on the grafting spot almost always accompanied by gum exudation, both in the bark and wood; oversprouting of the rootstock; 'Cleopatra' mandarin ungrafted sprouting (Figure 1b); rootstock sprouting after death of plants (Figure 1c); and penetration of the bark in the wood, in part or all around the circumference of the trunk (Figure 2a) – fact that was not verified in plants with Rangpur rootstock (Figure 2b) with crown from the same orange ('Pêra-Rio'). Such results confirm the descriptions by Nauriyal, Shannon and Frolich (1958) and Kirkpatrick, Bitters and Foote (1962) and Pompeu Junior, Donadio and Figueiredo (1972) and Mattos Junior et al. (2005) on the incompatibility symptoms in citrus grafting.

Figure 1. Sweet orange 'Pêra-Rio' plants with: a) drying of the pointer and posterior death of plants; b) necrotic line on the grafting spot with 'Cleopatra' mandarin; c) sprouting of the rootstock of 'Cleopatra' mandarin after rootstock death.

In addition, this incompatibility in grafted plants, between crowns and rootstocks, can also be defined as a phenomenon of premature aging caused by anatomical and biochemical processes, which may be intensified by stress conditions (FEUCHT, 1988). In general, incompatible genotypes normally develop in the first years after grafting, what was noticed in all evaluated orchards that were between four and six years old.

In the rootstock of 'Cleopatra' mandarin located in the orchard in Iranduba, it was possible to verify that there were plants with sub-grafting with the same rootstock; in the expectation of recovery, there was an oversprouting of the rootstock (Figure 2c) and the symptoms on the crown kept on evolving until their complete drying. One possible explanation for this incompatibility is the rootstock origin – zygotic and not nuclear; but the general occurrence in all plants, regardless of the orchards assessed, rules this hypothesis out.

Within the incompatibility studies, the anatomic sections showed that, in plants without symptoms with Rangpur lime as rootstock, the vase elements that constitute the vases of the secondary xylem, located near the grafting union tissue, showed intact cell walls and transparent lumen (Figure 3a). In plants with symptoms with 'Cleopatra' mandarin rootstock, the vase elements had degraded aspect and lumen filled with substances of dark color and phenolic aspect (Figure 3b), typical of necrotic processes, causing vascular discontinuity between the crown and the rootstock (ERREA, 1997).

Such anomaly in the xylem vases has, as main consequence, the restriction to the translocation of water and nutrients absorbed by the rootstock roots to the rootstock crown (APEPEZZATO-DA-GLÓRIA; CARMELLO-GUERREIRO, 2006). This restriction reduces the nutrient contents in the crown tissues, mainly in the leaves, altering a series of physiological processes, including those that are directly involved in the expression of the genetic potential in terms of fruits yield and quality (TAIZ; ZEIGER, 2004).

These symptoms can be intensified in the studied region, since plants are under drought conditions from June to October, when the crop evapotranspiration is greater than the rainfall and the temperatures are high, above 27 °C (ANTÔNIO, 2010). There are also the following aspects: the region soil is extremely poor, with H+Al predominance in the cation exchange complex (MOREIRA; FAGERIA, 2009), and roots are restricted to soil surface due to topdressing and the presence of organic matter – important source of nutrients, with only a small part in the pit. Insofar the fruit production period occurs exactly in this period, the plants cannot provide their physiological necessities and start aging.

The analysis of the chemical attributes of the soil in the orchards with and without problems shows that, because of the symptoms observed and the similarity between the areas, there has been an increase in the application of fertilizers by the producers on the crown of sweet orange 'Pêra-Rio' and acid lime 'Tahiti' plants with 'Cleopatra'

Figure 2. Plants with sweet orange 'Pêra-Rio' crowns and 'Cleopatra' mandarin rootstock, union with penetration of bark in the tissue (a) and Rangpur lime rootstock, normal aspect of rootstock union (b). Oversprouting of the 'Cleopatra' mandarin rootstock (c).

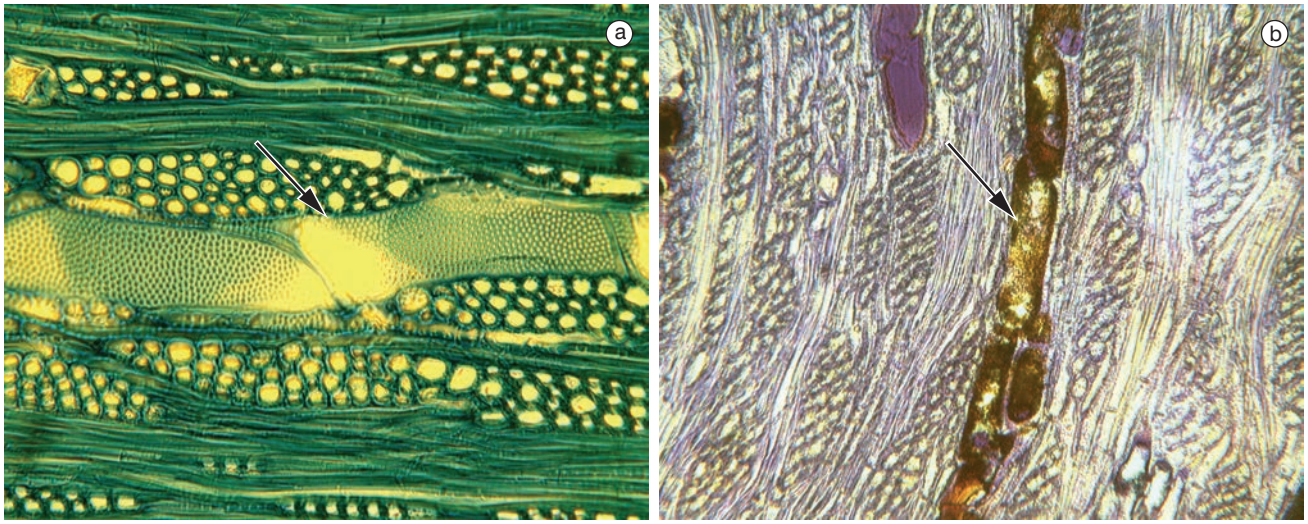

Figure 3. Elements of the vases that constitute the secondary xylem vases, tissue near the rootstock union: a) cells with intact walls and vases with transparent lumen (arrow), b) cells with degraded walls and vases with lumen filled with substances of dark color and phenolic aspect (arrow).

mandarin rootstocks (Table 1), aiming the recovery of plants. Still, the nutrient contents were below the ones indicated by Moreira et al. (2008) for the growth of citrus in the region.

Taking into account the low fertility characteristics observed between the planting lines (Table 1), the application of large amounts of fertilizers and soil acidity correctives would be necessary in order to have an increase in nutrient levels for reaching appropriate baselines to obtain productivity. In these areas, even with the fertilization at crown projection done by the producers, the soil contents of K available and Ca and Mg exchangeable were below the levels interpreted as good or very good soil fertility (ALVAREZ VENEGAS et al., 1999).

In both orchards, the low mobility nutrients (MARSCHNER, 1995; FAGERIA, 2009) in plants, such as Ca, B, Mn and Zn, were the most influenced by the incompatibility between 'Cleopatra' mandarin rootstock and citrus crowns (Table 2). Except for Mn, which is predominantly absorbed through roots interception, Ca, B and Zn absorption occurs mainly through mass flow (BARBER, 1995; MARSCHNER, 1995). With the obstruction of the xylem vases (Figure 3b), these elements are not translocated and remobilized in the plant, leading to the symptoms shown in Figure 1a. Among the main functions of these nutrients, the following can be highlighted: Ca – formation of middle lamella; B – synthesis of sugar; Mn – photolysis of water, and Zn – synthesis of tryptophan, precursor of indole acetic acid (IAA),

responsible for cellular elongation (LOUÉ, 1993; MARSCHNER, 1995). According to the data in Table 2, the negative effect of the rootstock was more distinct at the acid lime 'Tahiti' crown, also with significant reduction in the leaf contents of N, P, K, S and Cu and the increase in the content of Fe, even being within the range - 50 to 129 mg kg^{-1}, indicated as low (MALAVOLTA et al., 1994).

Regardless of the rootstock, in 'Pêra-Rio' and 'Tahiti' crowns, and in "Cleopatra' mandarin ungrafted, the contents of P and Mg were diagnosed as high, ranging from 1.8 to 2.9 g kg^{-1} and from 3.1 to 5.0 g kg^{-1}, respectively; while the contents of Ca, Cu, Mn and Zn remained within the range considered as deficient and low: <34 g kg^{-1} of Ca, <9.9 mg kg^{-1} of Cu and <24 mg kg^{-1} of Mn or Zn (MALAVOLTA et al., 1994; ROBINSON; TREEBY; STEPHENSON, 1997), with plants presenting symptoms that are typical of lack of these nutrients (MALAVOLTA et al., 1994; MOREIRA et al., 2008). Regarding the leaf levels of N, K and S, the leaf contents in sweet orange 'Pêra-Rio', with and without symptoms, in acid lime 'Tahiti' without symptoms, and in 'Cleopatra' mandarin, they were within the range considered appropriate by Malavolta et al. (1994).

In the case of B (Table 2), the contents were considered low, with 45 ± 11 mg kg^{-1}, except in acid

Table 1. Chemical attributes of soil in the crown projection and between the planting lines of the orchards with and without rootstock incompatibility.

	Crown projection		Between planting lines	
	Normal	With symptoms	Normal	With symptoms
pH_{water}	4.8b	5.5a	4.9A	4.3A
N. g kg^{-1}	1.2a	1.2a	0.9B	1.5A
MO. g kg^{-1}	17.3a	19.0a	25.3A	13.0B
C/N	9.4a	8.4a	8.2A	10.1A
P. mg dm^{-3}	24.0a	26.0a	2.0A	2.0A
K. mg dm^{-3}	29.0a	19.0b	18.0A	15.0A
Na. mg dm^{-3}	1.0a	1.0a	2.0A	1.0A
Ca. $cmol_c$ dm^{-3}	0.7b	1.2a	0.4A	0.2A
Mg. $cmol_c$ dm^{-3}	0.5a	0.8a	0.2A	0.1A
Al. $cmol_c$ dm^{-3}	0.3a	0.1a	0.8A	1.2A
H+Al. $cmol_c$ dm^{-3}	3.4a	3.6a	4.3A	4.3A
CTC. $cmol_c$ dm^{-3}	1.7b	2.6a	1.6A	1.6A
V. %	29.2b	42.3ª	15.9A	6.6B
Cu. mg dm^{-3}	4.4b	8.1ª	0.1A	0.2A
Fe. mg dm^{-3}	244a	162b	169A	155A
Mn. mg dm^{-3}	10.0a	10.6a	1.0A	2.2A
Zn. mg dm^{-3}	13.3b	27.3a	0.4A	0.5A

[1]Normal plants grafted on Rangpur lime; plants with symptoms grafted on 'Cleopatra' mandarin. Means followed by distinct lowercase and uppercase letters in the same line, within each location. differ from each other at 5% probability Tukey's test.

Table 2. Leaf contents of macronutrients and some micronutrients in sweet orange 'Pêra-Rio', acid lime 'Tahiti' with and without rootstock incompatibility, and 'Cleopatra' mandarin ungrafted.

Nutrientes	Sweet orange 'Pêra-Rio'		Acid lime 'Tahiti'		'Cleopatra' mandarin ungrafted
	Normal	With symptoms	Normal	With symptoms	
N. g kg^{-1}	26.85a	27.62a	25.07A	20.18B	32.25
P. g kg^{-1}	1.82a	1.93a	3.15A	1.07B	1.82
K. g kg^{-1}	12.45a	16.43a	13.23A	8.79B	20.34
Ca. g kg^{-1}	20.31a	11.26b	33.62A	21.21B	13.30
Mg. g kg^{-1}	3.88a	3.24a	5.38A	4.13A	3.17
S. g kg^{-1}	2.46a	2.23a	3.70A	2.19B	3.26
B. mg kg^{-1}	57.95a	36.96b	183.86A	51.04B	34.42
Cu. mg kg^{-1}	5.52a	6.20a	9.12A	4.49B	11.20
Fe. mg kg^{-1}	114.08a	123.31a	96.32B	128.14A	217.93
Mn. mg kg^{-1}	17.16a	13.01b	23.31A	9.35B	20.06
Zn. mg kg^{-1}	14.50a	14.71a	17.66A	12.67B	19.42

[1]Normal plants grafted on Rangpur lime; plants with symptoms grafted on 'Cleopatra' mandarin. Means followed by distinct lowercase and uppercase letters in the same line, within each sweet orange crown. differ from each other at 5% probability Tukey's test.

lime 'Tahiti' with Rangpur lime rootstock, whose content of B was considered high (ROBINSON; TREEBY; STEPHENSON, 1997). These results demonstrate that, besides the rootstock and crown combination, the presence of low fertility soils (MOREIRA; FAGERIA, 2009) and the regional climatic conditions characterized by high temperatures and rainfall (ANTÔNIO, 2010), hamper the maintenance of citrus plants nutritional status at levels considered appropriate by Moreira et al. (2008) for growing citrus in the region.

4 Conclusions

Confirming the visual results, in the climatic conditions of the central region of the State of Amazonas, the growing of sweet orange 'Pêra-Rio' and acid lime 'Tahiti' grafted on 'Cleopatra' mandarin rootstock presents the elements of the vase with degraded aspect and the lumen filled with substances of dark color and phenolic aspect, causing vascular discontinuity between crown and rootstock.

The incompatibility between the rootstock and the crown of sweet orange or acid lime is not influenced by the low fertility of soil.

Regardless of the nutrients, the nutritional status of plants is strongly influenced by the incompatibility between the rootstock and the crowns of sweet orange 'Pêra-Rio' and acid lime 'Tahiti'.

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