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Full Length Research Paper

Evaluation of the resistance of few citrus rootstocks to alkalinity by applying a faste test of secreening

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Alkalinity of Moroccan soils is the major abiotic constraint on citrus production area. The best choice of citrus rootstocks adequate and resistant is a better solution to avoid this problem. The aim of this study is to develop a fast test of citrus rootstocks screening towards alkalinity. The alkaline stress was applied on ten citrus rootstocks two month old, using irrigation with a Hoagland and Arnon solution added with 1 g $CaCO_3$/L and adjusted at various pH levels 6, 7 and 9. Observations concerned symptoms incidence and severity of iron chlorosis after two months of rearing. Results permitted to classify *Poncirus trifoliata* and Flying dragon as the most sensitive to alkalinity stresses, whereas, *Citrus volkameriana* and *Citrus macrophylla* were resistant. These conclusions are equivalent with those obtained with old citrus rootstocks in field trials.

Keys words: Citrus, rootstocks, alkalinity, screening.

INTRODUCTION

It is estimated that 20 to 50% of fruit trees in the Mediterranean Basin suffer from iron deficiency (Jaegger et al., 2000). The major cause of iron deficiency in these regions is due to high concentrations of calcium and bicarbonate in soils. High concentrations of bicarbonate usually affect metabolic processes at the leaves and roots and limit the availability of iron for the plant (Mengel, 1995), which leads to the development of symptoms of iron chlorosis. Among fruits, iron chlorosis causes significant loss in performance (Pestana et al., 2003), delayed ripening of fruit affects the quality of fruits like peach (Sanz et al., 1997) and citrus (Pestana et al., 2001; Patricio et al., 2007). In citrus fruit, the problem of soil alkalinity can be overcomed by choosing a resistant rootstock (Albrigo and Davies, 1994; Pestana et al., 2001).

In Morocco, alkalinity of the soil is a serous problem for Citrus growers (Benyahia, 1998). The sour orange rootstocks, although it is adapted to the calcareous soils in Morocco, this increased sensitivity to tristeza virus, which calls into question its use. The search of rootstocks alternatives, remains a long-term strategy. The development of rapid screening tests to identify sources of resistance to this constraint are of major importance to the improvement program of rootstocks citrus from INRA Morocco and more generally, for any such project in the Mediterranean Basin.

The objective of this work is the development of a fast screening test, reliable and reproducible to alkalinity for a routine evaluation of collection of rootstocks of citrus to INRA, to identify new sources of tolerance. These rootstocks will be used later in genetic improvement programmes as well, by conventional methods as the tools of plant biotechnology.

MATERIALS AND METHODS

Planting material

Ten varieties of rootstocks were used (Table 1). We have chosen

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Table 1. List of rootstocks used.

Root-stocks	Resistance to alkalinity
Citrange Troyer (CT)	
bigaradier (Big)	
Citrus volkameriana (CV)	Rootstocks resistant (7)
Citrange Carrizo (CC)	Rootstocks sensitive (7)
Mandarnier Cléopâtre (MC)	
Citrus macrophylla (CM)	
Citrumelo (CML)	
Soh jahlia (SJ)	
Poncirus trifoliata (PT)	Porte-greffe sensitive (7)
Poncirus trifoliata flying dragon (FD)	

two rootstock references whose characteristics of sensitivity or resistance to the alkalinity are known, to estimate without excessive risk of error the degree of resistance or susceptibility of the other rootstocks. The seed came from the collection of rootstocks of citrus INRA El Menzeh.

Seedlings

The seedlings of different rootstocks were made in plastic trays with dimension of 53 x 53 cm, containing a mixture of peat and sand sterile (Blaker and MacDonald, 1986). The trays are placed in a plastic tunnel at temperatures between 25 and 30°C. Irrigation was done everyday with water, and once every two weeks with 300 ml of a nutrient solution of Hoagland and Arnon half concentrated (Zekri, 1991).

Two months after planting, young seedlings of each uniform rootstock were collected separately and transplanted into plastic pots of 15 cm deep and 8 cm in diameter, filled with a mixture of sterile sand-peat. Seedlings were irrigated daily with the nutrient solution of half concentrated Hoagland solution for 1 month at the rate of 100 ml per pot representing the field capacity of the soil used.

Level of alkalinity

For the level of alkalinity we used the method of Abbadia (1998), which is to use the solution of Hoagland half concentrated with $CaCO_3$ added to 1 g/L and adjusted to pH 7, 8 and 9. The plants control were irrigated with half concentrated Hoagland solution without $CaCO_3$ and whose pH was adjusted to the value of 6. The values of pH used were chosen, taking into account the pH of soil and water for irrigation in citrus orchards in Morocco. In parts of the Gharb and Berkane of pH, in the range of 8 is observed by Benyahia (1998). pH 9 represents extreme alkalinity while pH 6 represents the optimum conditions for the growth of citrus fruits.

Application of alkaline stress

After the breeding period of 1 month, 18 seedlings each of the rootstock, according returned an experimental split-plot with three replications and on the basis of two plants by repetition. The pH factor was placed in large parcels and the rootstock factor in small parcels. The plants were irrigated three times a week with alkaline solutions. The test was carried out for two months in a greenhouse, whose temperature ranges between 25 and 35°C.

Determination of resistance to rootstocks to alkalinity

Incidence of iron chlorosis

This is the number of plants showing iron chlorosis on the total number of plants subjected to stress.

Severity of symptoms of iron chlorosis

To estimate the severity of chlorosis, we used a rating scale from 0 to 5 (Romera et al., 1991; Sanz et al., 1997). With 0 plants showing no sign of iron chlorosis with 5 plants showing total chlorosis with falling leaves.

RESULTS

Incidence of iron chlorosis

The symptoms of chlorosis have emerged after the second week of treatment (Figure 1). The incidence or frequency of plants showing symptoms of chlorosis varies from a rootstock to another and depending on the pH of the solution for irrigation. Statistical analyses of the variable incidence of iron chlorosis shows significant effects of the factors door rootstocks and pH. By contrast, the interaction between the rootstock and the pH is not significant. The comparison of the average incidence of iron chlorosis for different rootstocks can distinguish 4 groups of rootstocks statistically different (Figure 2).

Group 1. Includes flying dragon, *Poncirus trifoliata* and *Carrizo citrange* for which the frequencies of plants affected are the highest, ranging from 60 to 80%.
Group 2. Consisting of Citrumelo and Soh jahlia affecting chlorosis of the order of 50%.
Group 3. Contains door rootstocks that have an impact on the order of 35%. They are *Citrus volkameriana*, citrange Troyer, the mandarin Cleopatra and *Citrus macrophylla*.
Group 4. Contains only the orange as the least affected with an incidence of 5%.

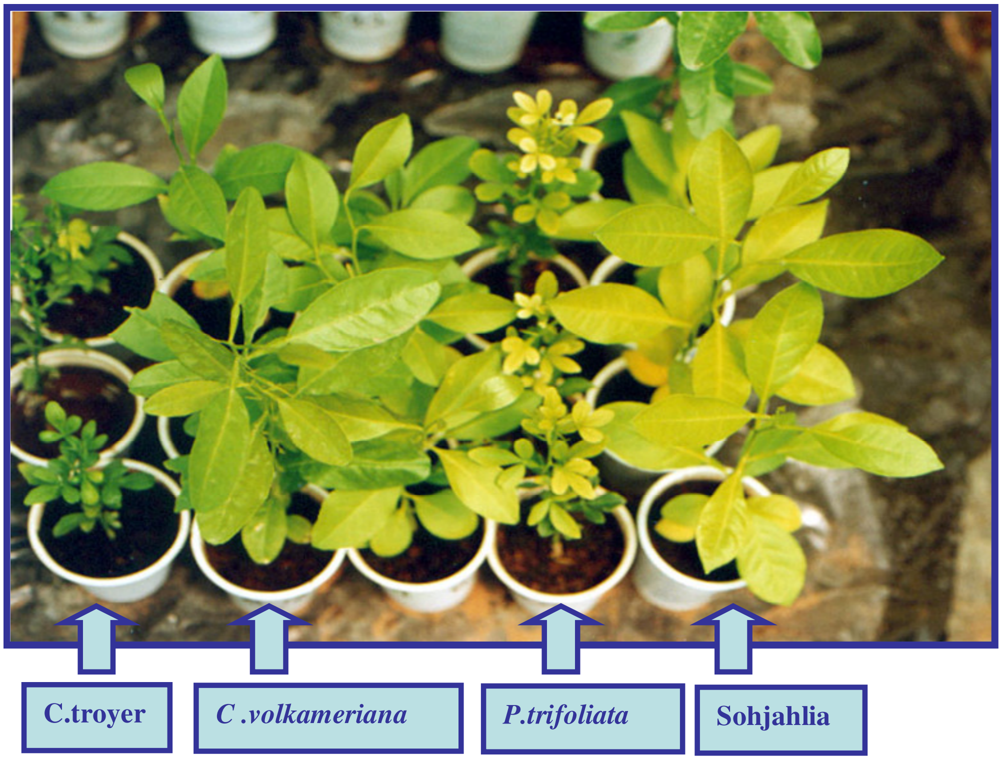

Figure 1. Symptoms of iron chlorosis on leaves of rootstocks citrus irrigated by irrigation solution with a pH = 8.

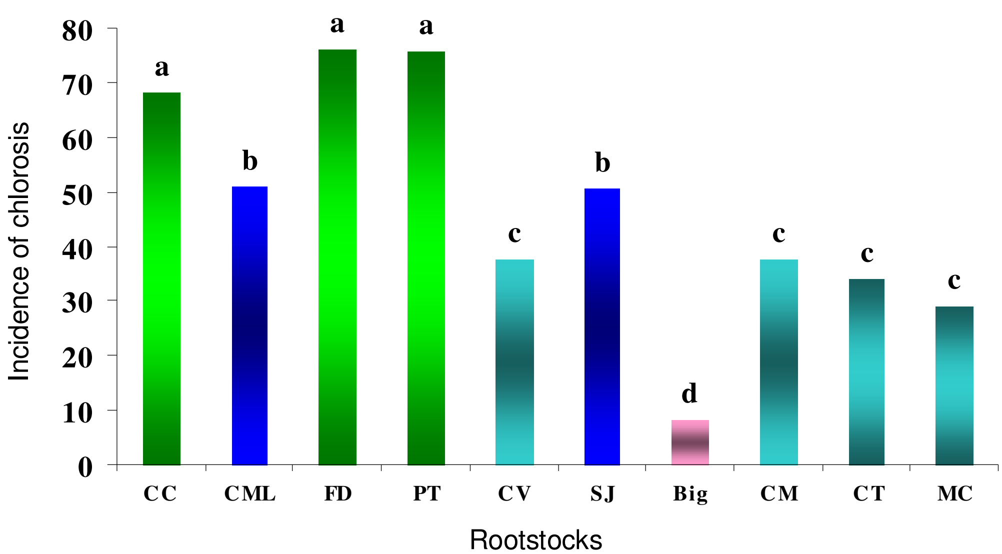

Figure 2. Average effect of iron chlorosis among rootstocks. All pH confused. The rootstocks followed by the same letter did not differ significantly at the 5% threshold (Test Duncan).

Severity of iron chlorosis

The analysis of the variance of severities symptoms of chlorosis shows a very highly significant factor rootstocks and pH. In addition, a highly significant interaction between factors pH and the rootstock was observed. Thus, the increase in the pH of the solution leads to an increase in the severity of iron chlorosis, but with a differential behavior of rootstocks (Table2).

According to Table 2, flying dragon and *P. trifoliata*

Table 2. Effect of pH of the solution on irrigation. Severities of iron chlorosis recorded on different rootstocks.

Porte-greffes	pH of the irrigation solution		
	7	8	9
Citrange Carrizo	1 b	1.66 b	1.93 b
Citrumelo	0.8 b	1.33 b	1.5 b
Flying dragon	2.8 a	3 a	3.5 a
Poncirus trifoliata	3 a	3.33 a	3.66 a
Citrus volkameriana	1.16 b	1.16 b	1.33 b
Soh jahlia	0.33 b	0.93 b	2 b
Bigaradier	0 c	0 d	0.33 c
C. macrophylla	0.5 b	0.5 c	0.66 c
Citrange Troyer	0.16 b	0.33 c	0.66 c
M. Cleopatre	0.16 b	0.33 c	0.5 c

The rootstocks followed by the same letter did not differ significantly at the 5% threshold (Test Duncan).

Table 3. Resistance (R) and sensitivity (S) of rootstocks to alkalinity.

Root-stocks	F	SEV	Classification according to Albrigo and Davies (1994)
Flying dragon	S	S	?
C. volkameriana	RM	RM	R
bigaradier	R	R	R
Citrange Troyer	R	R	R
Citrange Carrizo	S	MR	S
M. Cléopâtre	R	R	R
Soh jahlia	RM	RM	?
C. macrophylla	R	R	R
Poncirus trifoliata	S	S	S
Citrumelo	S	RM	?

F : Frequency of symptoms of chlorosis; SEV : Symptom severity of chlorosis; R= Resistant; RM= Moderately resistant; S= sensitive.

carriers are transplants who showed high levels of chlorosis used at different pH. The degree of chlorosis is respectively 3.5 and 3.66 at pH 9. The sour orange has shown good resistance to pH 8 and 9, followed by *C. macrophylla* whose index chlorosis at pH 9 is only 0.66. The mandariner Cleopatra too, with hints of 0.33 and 0.5 respectively at pH 8 and 9, has been resistant to alkalinity. *C. volkameriana* showed resistance acceptable to alkalinity, compared to *P. trifoliata* and flying dragon. Citrumelo has engaged in conduct similar to that of Carrizo citrange. The citrange Troyer showed a high level of tolerance, much higher than Carrizo citrange at pH 8 and 9.

Evaluation of resistance to rootstocks to alkalinity

The classification of rootstocks in terms of tolerance or susceptibility to iron chlorosis varies depending on the criterion used for some rootstocks, while it is the same for others (Table 3). Thus, flying dragon ranked as sensitive to the frequency of the severity of chlorosis. For the majority of rootstocks, our results are similar to those of Albrigo and Davis (1994).

DISCUSSION

The addition of 1 g/L of a solution $CaCO_3$ irrigation adjusted at different pH causes iron chlorosis on leaves of rootstocks citrus. Abbadia (1998) measured the activity of the chelate reductase and showed that alkalinity in the presence of $CaCO_3$ in small quantities induced decrease in activity of this enzyme in the susceptible rootstocks and consequently a reduction of supplied iron. Thus, the onset of symptoms of chlorosis is probably the result of an iron deficiency. The intensity of the iron chlorosis on the rootstocks used varies in frequency and severity. Flying dragon and *P. trifoliata* were very sensitive to alkalinity. Albrigo and Davies (1994) have also classified these two rootstocks as sensitive. *P. trifoliata* is sensitive to alkalinity, and it affects the yield and quality of fruit varieties grafted (Durón, 1999; Zekri, 1995; Alva and Tucker, 1999).

The sour orange showed a high tolerance in terms of intensity of the symptoms of iron chlorosis. This result is well correlated with observations in orchards. Indeed, it is renowned for its good behaviour in calcareous soils in the region of Souss in Morocco. *C. volkameriana* has shown intermediate tolerance. This root-stock has shown promise in the region of Souss, in terms of tolerance and performance (Ait et al., 2004). In addition, Castel and Gmitter, (1999) ranked this rootstock as moderately tolerant to alkalinity. In Venezuéla, *C. volkameriana* was widely used in calcareous soils as the rootstock for replacement to sour orange (Jackson, 1999). In Florida, *C. volkameriana* presented good behaviour in terms of resistance to alkalinity (Castle et al., 1993; Castle et al., 1997).

The mandarnier Cleopatra presented a good level of tolerance to alkalinity in our study. This root-stock was also ranked by Albrigo and Davies (1994) as tolerant to alkalinity but Castle et al. (1993) sees it as moderate and that in Florida, Cleopatra mandarin presents an intermediate tolerance (Hutchison, 1982). It is possible that these differences in outcomes are related to the use of different genotypes of Mandarins Cleopatra. Studies on the molecular markings indeed identified a genetic polymorphism between different accessions of Mandarins Cleopatra in collections (Luro, com; pers).

The citrange Troyer, although it is a hybride of Poncirus, it demonstrated in this study a high degree of tolerance for alkalinity. Recupero and Russo (1988) ranked this rootstock as an intermediary between the sour orange orange and *P. trifoliata*. In the end, Maribela et al. (2005) ranked these rootstocks as resistant to chlorosis. The change in behavior of citrange in the various studies could be connected with the conditions of experimentation and the homogeneity of the plant material used. *C. macrophylla* has shown good resistance to iron chlorosis, which is consistent with the classification of Albrigo and Davies (1994).

The results from our test seedlings aged three months are consistent with the results obtained in patients to rootstocks older. This proves the effectiveness of our test, which is faster. It can therefore be generalized to assess, in a limited space, many genotypes or collection of hybrid breeding programs. In the longer term, it will be necessary to seek other physiological criteria, to retrieve the genes responsible for tolerance to rootstocks. In this sense, the study of the activity and regulation of certain enzymes involved in iron metabolism could be a promising approach for the identification of rootstocks with degrees of tolerance best meet the shortfall in iron.

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