Vitis **43** (2), 97–98 (2004)

Research Note

Physiological responses of native Tunisian grapevines and some rootstocks to direct iron deficiency

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Introduction: In Tunisia, grapevine culture area is mainly localised in the north and north-east regions, characterised by calcareous soils. Iron chlorosis symptoms associated with low chlorophyll and bivalent iron contents have been already identified in these native vineyards (KSOURI *et al.* 2001). Iron is an essential micronutrient for plant development since its active form is involved in several biochemical processes, *e.g.* chlorophyll synthesis (FERRARO *et al*. 2003). Resistance to iron chlorosis is genotype-dependent; moreover it seems to be correlated with the ability of grape to achieve rhizosphere acidification and an enhanced root Fe(III)-reductase activity (BRANCADORO *et al.* 1995). The present study investigates the variability of physiological responses of grapevine genotypes exposed to a direct iron deficiency.

Material and Methods: Seven Tunisian *Vitis vinifera* L. varieties (Saouadi, Arich Dressé, Mahdaoui, Blanc3, Balta4, Beldi and Khamri), previously characterised by biochemical or molecular markers (BEN ABDALLAH *et al.* 1998), were studied. The introduced cv. Cardinal and two rootstocks (140Ru and SO4) were also assessed.

Experiments were carried out in a glasshouse under controlled climatic conditions (16/8 h light/darkness, 25 ± 5 °C and 70 % relative humidity). One month-old woody cuttings were cultivated for 75 d in inert sand and irrigated with a complete nutrient solution (BRANCADORO *et al.* 1995) either supplied with 1 µM ('iron-deficient') or 20 µM Fe(III)-EDTA. pH of the nutrient solution was weekly adjusted at 6.1 with 0.1 M NaOH.

After two months of treatment, the chlorosis status of young leaves was evaluated either using a non-destructive method based on a visually appreciated score (POUGET and OTTENWAELTER 1978), or by determining the chlorophyll (TORRECILLAS *et al.* 1984) and bivalent iron contents (LLORENTE *et al.* 1976) of the 4th leaf (beginning from the shoot tip). Afterwards, two genotypes differing in their iron-deficiency tolerance (Khamri and Balta4) and the rootstock (140Ru) were transferred to a liquid medium and submitted for two weeks to the treatments described above. During this period, acidification and iron reduction capacities were assessed. Acidification was determined by measuring the nutrient solution pH during one week, while the FeIII-reductase activity was assayed *in vivo* on excised apical root segments, according to BRANCADORO *et al.* (1995). ANOVA statistical analysis was performed to show genotype-treatment interaction.

Results: Leaf chlorosis parameters: The leaf chlorosis score varied from 0.6 to 2 (Table), with lower values for the rootstock 140Ru and cv. Khamri, while Cardinal, Beldi and Balta4 showed highest values, indicating severe leaf chlorosis symptoms in the latter varieties. Iron deficiency led to a significant decrease of the leaf chlorophyll content for all the genotypes, though there were genotypic differences: Balta4 was the most severely affected variety (-40 %), whereas the negative tendency was less pronounced in 140Ru and Khamri (-14 %).

Under conditions of iron-deficiency, the bivalent iron contents of the 4th leaf showed fluctuations which were simi-

Table

Effects of direct deficiency (DD) on leaf chlorosis score and chlorophyll and iron content of 10 genotypes. Means (7 replicates) followed by the same letters are not statistically different according to Newman-Keuls test performed at $P < 0.05$

Varieties	Chlorosis score control	Chlorosis score DD	Chlorophyll content control	Chlorophyll content DD	Iron content control	Iron content DD
140Ru	0f	0.57ef	1.21bc	1.10cd	55.69bcd	47.97ef
Khamri	0f	0.86de	1.45a	1.30b	63.35a	54.46bcd
Mahdaoui	0f	1.14cd	1.12cd	0.96ef	60.73ab	50.56de
Saouadi	0f	1.29bc	1.12cd	0.80g	58.19abc	44.34fgh
SO4	0f	1.43abc	1.03de	0.79g	53.22cd	39.67h
Blanc3	0f	1.43abc	1.11cd	0.90f	62.10a	46.96efg
A. Dressé	0f	1.43abc	1.12cd	0.96ef	58.59abc	42.48fgh
Cardinal	0f	1.71ab	1.20bc	0.91f	57.29abc	42.77fgh
Beldi	0f	1.86a	1.10cd	0.76g	57.29abc	41.87gh
Balta 4	0f	1.86a	1.16cd	0.66h	59.36abc	32.33i

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lar to those of the chlorophyll (Table). At optimal iron availability the accumulation levels were close in the majority of the genotypes. This was not the case for plants subjected to iron-deficiency which showed a great genotypic variability (-14 % of leaf iron content for Khamri and -46 % in Balta4).

Using leaf chlorosis as discriminative criterion, it can be noted that the rootstock 140Ru, Khamri and Mahdaoui were the most tolerant varieties, while cvs Beldi and Balta4 were particularly sensitive to iron-deficiency. After this first exploration step, three genotypes with contrasting behaviour (Khamri, Balta4 and the rootstock 140Ru) were chosen to better understand their physiological responses to iron-deficiency, *i.e.* the processes of root acidification and iron reduction were compared.

Rhizosphere acidification: Decreasing iron availability resulted in progressive genotype-dependent acidification of the culture medium (Figure, A). Indeed, the stronger pH reductions were observed in the most tolerant cvs Khamri and 140Ru, contrasting with the sensitive cv. Balta4.

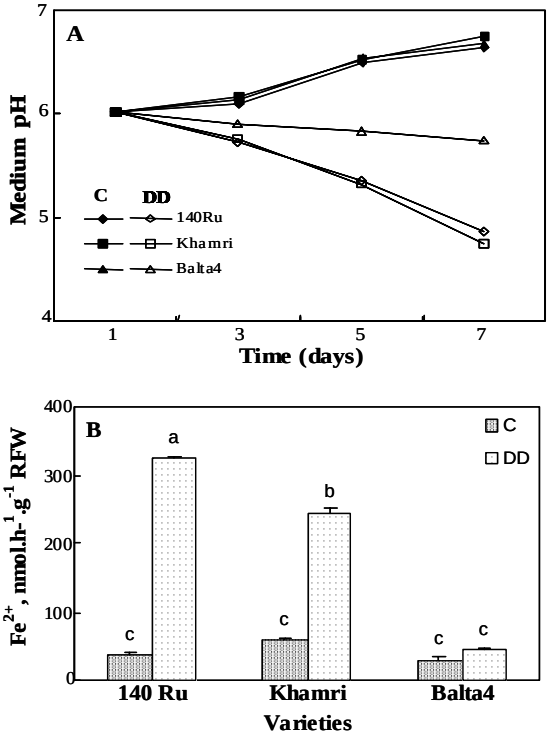

Figure: **A**. The medium culture pH with the grapevine genotypes Khamri, Balta4 and rootstock 140 Ru supplied for 67 d with a nutrient solution containing 20 µM (C) or 1 µM iron (DD), measurements were performed every second day for one week. **B.** Iron reduction capacity of the same grapevine genotypes. Means of 7 replicates ± SE at $P < 5$ %. Means followed by the same letters are not statistically different according to Newman-Keuls test performed at $P < 5$ %.

Iron reduction capacity: Control plants showed weak Fe(III)-reductase activity (30-60 $nmol \cdot h^{-1} \cdot g^{-1}$ root FW) (Figure, B). However, this parameter was strongly stimulated by iron deficiency in tolerant grapevines (266 $nmol \cdot h^{-1} \cdot g^{-1}$ FW in Khamri and 350 $nmol \cdot h^{-1} \cdot g^{-1}$ FW in 140Ru), whereas no significant effect was found in the iron-deficiency sensitive variety Balta4.

Discussion and Conclusion: Our results show that the physiological response of grapevine to iron-deficiency is strongly genotype-dependent. Indeed, native varieties used in this study showed a significant variability in their leaf chlorosis status when subjected to lowered iron availability in their culture medium. These differences seem to be correlated with the iron status of the youngest leaves, which constitutes a reliable criterion for the selection of iron-deficiency tolerant grapevine rootstocks, especially those cultivated on calcareous soils (BAVARESCO *et al.* 1992). A close positive correlation was also found between the chlorophyll content of young leaves and their bivalent iron content ($R = 0.86$), confirming that chlorosis results from the lack of bivalent iron, the latter being essential for chlorophyll pigment biosynthesis (NIKOLIC and KASTORI 2000).

The plant response to iron-deficiency is associated with morpho-physiological and biochemical mechanisms, in order to improve the iron mobilisation capacity in the external medium (PIAGNANI and ZOCCHI 1997). ELLSWORTH *et al.* (1997) proposed the utilisation of the iron reduction capacity as a criterion to iron chlorosis tolerant grapevines. The most tolerant genotypes (Khamri and 140Ru) were able to reduce the pH of the culture medium to a higher extent, and exhibited a much higher root Fe(III)-reductase activity. In contrast, Balta4 displayed only a non-significant fluctuation.

This study allows to distinguish between three groups of genotypes: the first one includes the tolerant varieties Khamri and Mahdaoui and the rootstock 140Ru; the second includes the moderately tolerant varieties Blanc3, Arich Dressé, Saouadi and SO4, while Balta4, Beldi, and Cardinal represent the most sensitive ones.

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Received February 18, 2004