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Physiological characterization of grapevine rootstocks grown in soil with increasing zinc doses

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Key words:
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ABSTRACT
This study aimed to evaluate the performance of grapevine rootstocks under increasing levels of Zn in the soil and to identify physiological variables that can be used as indicators of excess of Zn in the soil. The rootstocks SO4, Paulsen1103, IAC572, IAC313 and 420A were grown in pots containing soil, which received Zn doses of 0, 20, 40, 80 or 160 mg kg^{-1} of soil. Dry matter (DM), Zn content in shoots and roots, chlorophyll index, initial fluorescence (F_o), maximum fluorescence (F_m), maximum quantum yield of photosystem II (F_v/F_m), effective quantum yield of photosystem II (Y-II) and non-photochemical quenching (NPQ) were evaluated. The increase of Zn levels in the soil decreased DM in all rootstocks, and IAC572 was superior to the others. The variation in the indices of chlorophyll a and b had little expression in relation the soil Zn levels, but allowed identifying that the rootstocks Paulsen 1103, 420A and SO4 are sensitive to Zn toxicity and that IAC572 and IAC313 were not sensitive to the tested levels. Fluorescence analysis showed a negative effect of Zn contents on the variables F_o, F_m, Y-II and NPQ in all rootstocks, which proved to be good indicators of Zn phytotoxicity.

Palavras-chave:
metal pesado
toxicidade
fluorescência da clorofila
Vitis spp.

Caracterização fisiológica de porta-enxertos de videira cultivados em solo com doses crescentes de zinco

RESUMO
Objetivou-se, neste trabalho, avaliar o crescimento de porta-enxertos (PEs) de videira cultivados em níveis crescentes de Zn no solo e identificar variáveis fisiológicas que possam ser indicadoras do excesso de Zn no solo. Os PEs SO4, Paulsen1103, IAC572, IAC313 e 420A foram cultivados em vasos contendo solo e submetidos à adição de 0, 20, 40, 80 e 160 mg kg^{-1} de Zn. Avaliaram-se a matéria seca (MS), o teor de Zn na parte aérea e raízes, o índice de clorofila, a fluorescência mínima (F_o), a fluorescência máxima (F_m), o rendimento quântico máximo do fotossistema II (F_v/F_m), o rendimento quântico efetivo (Y-II) e a dissipação não fotoquímica (NPQ). O aumento da concentração de Zn no solo reduziu a MS em todos os PEs sendo que o IAC572 foi superior aos demais. A variação dos índices de clorofilas a e b foi pouco expressiva em relação aos níveis de Zn no solo mas permitiu identificar que o Paulsen 1103, 420A e SO4 são sensíveis à toxicidade de Zn e que o IAC572 e IAC313 não apresentaram sensibilidade nos níveis testados. Com a análise de fluorescência observou-se que o teor de Zn interfere negativamente sobre as variáveis F_o, F_m, Y-II e NPQ em todos os PEs, mostrando serem boas indicadoras para caracterizar a fitotoxidez do Zn.

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Introduction

Annually, grapevines are subjected to successive application of fungicides containing copper (Cu) and zinc (Zn) in the composition, for the prevention and/or control of leaf fungal diseases (Mirlean et al., 2007). In addition, the application of organic fertilizers as sources of nutrients for grapevines can also supply Zn to the soil (Ramos & López-Acevedo, 2004). Over the years, an increase in Zn contents in vineyard soils is expected, which can cause negative effects on plants.

The effect of Zn becomes more evident in the implantation of new vines in eradicated areas of old vines, with many years of sprayings and expressive Zn accumulation. According to Nagajyoti et al. (2010), high contents of bioavailable forms of Zn have high phytotoxic potential, which can cause restrictions to plant initial growth and hamper crop development and establishment.

The first visual symptom of Zn toxicity is the chlorosis of younger leaves, evolving to necrosis and modification of the morphology of older leaves (Hermle et al., 2007; Todeschini et al., 2011). These alterations result from damages in electron transport efficiency and carbon assimilation, compromising the photosynthetic performance of leaves (Dhir et al., 2008; Cambrollé et al., 2012). In general, Zn excess mainly affects the reaction center of photosystem II (PSII), restricting the expression of the proteins D1 and D2 (Todeschini et al., 2011). From these effects, cellular alterations also occur, such as changes in membrane integrity (Radić et al., 2010) and induction of oxidative stress in root hairs, with increase in the activity of some enzymes related to the antioxidant defense (Ismail & Theodor, 2013).

In this context, it is essential the selection of diagnostic techniques for the effects of Zn excess on plants to be employed in the monitoring of new crops, which must be fast, non-destructive, low-cost and of early detection of the negative metabolic effects of Zn, allowing the intervention when convenient.

Since Zn toxicity can alter leaf pigmentation, one of the fast, non-destructive techniques that can be considered in plant analysis is the estimation of chlorophyll content. However, the chlorophyll index provides a static analysis of leaf tissue and does not show how Zn interferes with the activity of the photosystems, which can restrict the sensitiveness of this technique when plants grow under different Zn contents in the soil.

The functioning of the photosystems in relation to the stress conditions has been analyzed by the chlorophyll fluorescence. This technique characterizes the photosynthetic performance and already has great advances with respect to portable analysis devices (Maxwell & Johnson, 2000). The fluorescence technique has been refined and, with the evolution of modulated devices, a great combination of measurements (light and dark) for obtaining photochemical variables in situ. Among these variables, initial fluorescence (F_o), maximum fluorescence (F_m), effective quantum yield of photosystem II (Y-II) and non-photochemical quenching (NPQ) stand out, among others that have been used as tools in the characterization of biotic or abiotic stresses in grapevines (Flexas et al., 2000). In spite of that, this technique has not been used in the characterization of Zn toxicity in young grapevines, and can be of great value for the early diagnosis of the effects of high Zn levels on vineyards.

This study aimed to evaluate the productive growth of grapevine rootstocks cultivated under increasing levels of Zn in the soil and identify physiological variables that can be used as indicators of Zn excess in the soil.

Material and Methods

The experiment was carried out in a greenhouse at the Embrapa Grape & Wine, in the conty of Bento Gonçalves, in the Serra Gaúcha region, in the state of Rio Grande do Sul, Brazil. The soil in the area is a Humic Cambisol with the following attributes: 290 g kg^{-1} of clay; 16 g kg^{-1} of organic matter; pH in water = 5.5; exchangeable Ca = 35.3 $mmol_c$ dm^{-3}; exchangeable Mg = 8.4 $mmol_c$ dm^{-3}; exchangeable Al = 5.7 $mmol_c$ dm^{-3} (KCl 1 mol L^{-1} extractant); available P = 3.6 mg dm^{-3}, exchangeable K = 70 mg dm^{-3} and Available Zn = 4.2 mg kg^{-1} (Mehlich-1 extractant). After sampling, the soil was air-dried and sieved through a 4.0-mm grid. Then, the soil received 2.0 g kg^{-1} of limestone (RNV 75%) in order to raise soil pH to 6.0; 100 mg kg^{-1} of P_2O_5 (source: H_3PO_4) and 50 mg kg^{-1} of N (source: urea), according to CQFS (2004). The sources of N and P_2O_5 were applied as a solution on the soil, which was then homogenized and incubated for 30 days, with water content at field capacity. Later, the soil was put into pots with capacity for 7 L.

The experiment was set in a completely randomized factorial design (factor A = five young grapevine rootstocks and factor B = five Zn doses), with three replicates. The following rootstocks were used: SO4 (*Vitis berlandieri* x *Vitis riparia*), Paulsen1103 (*Vitis berlandieri* x *Vitis ruprestis*) (P1103), IAC572 (*Vitis tiliifolia* x 101-14 Mgt), IAC313 (*Golia* x *Vitis caribaea*) and 420A (*Vitis berlandieri* x *Vitis riparia*).

The seedlings were derived from explants of tissue cultures, multiplied in vitro, with acclimation and rooting in substrate in a greenhouse. Before transplantation (Dec/2012), plant shoots were pruned and three buds were maintained. Roots were washed and pruned at 3 cm of length; after 45 days of transplantation, the first pruning was performed, removing the shoots. Then, after a new sprout, only one main branch per plant was maintained, which was conducted using a stake, for the evaluations at 50 days after pruning (DAP). The following Zn doses were used: 0, 20, 40, 80 and 160 mg kg^{-1}. The Zn source used was $ZnSO_4.7H_2O$, which was applied in the soil in the form of a solution together with the sources of P and N, before planting the grapevines. After the experiment, at 50 DAP, one soil sample was collected in each pot, air-dried, sieved through a 2.0-mm grid and subjected to analysis using the extractant Mehlich-1 to quantify/estimate the available contents of Zn.

The chlorophyll index a (ICa) and b (ICb) were measured in the lateral position of the 4ª basal leaf, from 9 h to 16 h, using the portable measuring device Falker ClorofiLOG. In the same leaf and time, the variables of chlorophyll fluorescence were determined using a pulse-amplitude modulated fluorometer (Junior-PAM, Walz).

Before each measurement of fluorescence, leaves were maintained in the dark for 30 min for the opening of the

reaction centers (PSII). For the determination of initial fluorescence (F_o), a modulated light pulse (< 0.1 µmol m^{-2} s^{-1}) was used; while a pulse of 0.6 s of saturating white light (10000 µmol m^{-2} s^{-1}) was used for the maximum fluorescence (F_m). From these variables, the variable fluorescence (F_v=F_m-F_o) and the maximum quantum yield of photosystem II (F_v/F_m) were calculated. Immediately after this initial condition, a light curve was performed with pulses of 125, 190, 285, 420, 625, 820, 1150 and 1500 µmol m^{-2} s^{-1}, interspersed with actinic light of 285 µmol m^{-2} s^{-1} and using the activated mode of extreme red radiation for the measurement of F_o'. From this curve, the effective quantum yield of PSII [Y-II = (F_m' - F')/F_m; where F' = fluorescence recorded just before the beginning of a strong light pulse and F_m' = maximum fluorescence yield when the reaction centers of PSII are closed by a strong light pulse] and the non-photochemical quenching of the fluorescence [NPQ = (F_m/ F_m') - 1, Kooten & Snel, 1990] were obtained.

At 50 DAP, the rootstocks shoots were cut close to the soil surface and stored. Then, the roots were removed from the pots, washed using HCl (0.5 mol L^{-1}) and distilled water, and stored. Immediately after that, shoots and roots were dried in a forced-air oven at 65 ºC until constant weight, weighed for the determination of dry matter (DM), ground and subjected to the analysis of the total content of Zn (Tedesco et al., 1995).

The data were subjected to analysis of variance and, when significant interaction between factors was observed, the regression equations were fitted. The differences between rootstocks, inside each Zn dose, were compared using Tukey test at $p < 0.05$. The response curves of the fluorescence variables to the light intensity were estimated and compared by F test, using the R program (R Development Core Team, 2014).

Results and Discussion

The addition of Zn doses of 0, 20, 40, 80 and 160 mg kg^{-1} in the soil promoted Zn availability of 4, 13, 20, 31 and 60 mg dm^{-3}, respectively, at the end of the experiment. Applied Zn not extracted by the method may have remained complexed by the organic matter, adsorbed to the surface functional groups of the clay minerals (McBride et al., 1997) or uptake by the grapevines.

The analysis of variance did not show significant interaction between the factors Rootstocks and Zn doses, for the variables Zn contents and dry matter (DM) of shoots and roots. The mean shoot Zn content ranged from 17 mg kg^{-1} (zero dose) to 46 mg kg^{-1} (dose of 160 mg kg^{-1}) and was similar for all rootstocks (Table 1). A quadratic relationship with $R^2 = 0.95$ was observed between Zn doses and shoot Zn contents. Through the equation, the maximum content was estimated as 95.5 mg kg^{-1}, obtained with the Zn dose of 821 mg kg^{-1}. It is observed that, in order to reach the maximum shoot Zn content, it would be necessary to add 3.5 times more Zn to the soil than the necessary to reach the maximum in the roots.

The rootstocks 420A and IAC572 showed the highest mean root Zn content, which coincided with the highest DM reduction in these tissues (Table 1). DM reduction with the addition of the highest Zn dose, in comparison to no addition, was equal to 47 and 52% in the roots, while the DM reduction

Table 1. Dry matter and Zn contents in shoots and roots of grapevines rootstocks cultivated in soil with increasing levels of zinc (Zn)

Rootstocks	Zn doses applied (mg kg^{-1})					Mean	Equation
	0	20	40	80	160		
Shoot Zn contents (mg kg^{-1})							
IAC572	19.8	29.1	30.4	40.3	47.9	33.5 a	
IAC313	18.5	29.0	30.1	38.2	51.4	33.8 a	
P1103	14.1	18.4	23.9	32.3	39.6	26.5 a	
SO4	17.5	20.8	29.0	34.3	48.5	30.0 a	
420A	16.2	20.4	23.2	32.1	43.6	27.1 a	
Mean	17.2	23.5	27.3	35.5	46.2	CV= 11%	$\hat{y} = 18.0 + 0.23x - 0.00014x^2$ (R^2=0.95**)
Root Zn contents (mg kg^{-1})							
IAC572	37	129	297	397	634	299 a	$\hat{y} = 39.2 + 5.85x - 0.013x^2$ (R^2=0.94***)
IAC313	34	69	163	263	474	205 bc	$\hat{y} = 25.7 + 3.10x - 0.0018x^2$ (R^2=0.99***)
P1103	27	48	103	201	356	158 c	$\hat{y} = 22.4 + 2.11x$ (R^2=0.77**)
SO4	29	75	130	288	275	159 c	$\hat{y} = 6.8 + 4.56x - 0.018x^2$ (R^2=0.82**)
420A	30	104	184	385	595	259 ab	$\hat{y} = 15.5 + 5.08x - 0.009x^2$ (R^2=0.97***)
Mean	31	85	175	307	467	CV= 9%	$\hat{y} = 23.2 + 4.21x - 0.0089x^2$ (R^2=0.99***)
Shoot dry matter (g $plant^{-1}$)							
IAC572	26.8	31.1	29.7	32.3	23.3	28.6 a	ns
IAC313	16.1	15.4	11.2	10.8	7.2	12.2 b	$\hat{y} = 15.7 - 0.056x$ (R^2=0.55*)
P1103	11.0	12.0	10.7	9.8	9.1	10.3 b	ns
SO4	11.4	15.3	13.2	11.1	8.8	11.9 b	ns
420A	13.4	13.2	15.5	10.4	11.0	12.7 b	ns
Mean	15.7	17.4	16.0	14.9	11.8	CV= 13%	$\hat{y} = 16.7 + 0.003x - 0.0002x^2$ (R^2=0.58*)
Root dry matter (g $planta^{-1}$)							
IAC572	14.1	14.6	13.2	13.8	6.7	12.5 a	$\hat{y} = 13.9 + 0.025x - 0.0004x^2$ (R^2=0.75**)
IAC313	4.1	3.6	1.9	1.2	1.6	2.6 c	$\hat{y} = 4.3 - 0.060x + 0.00027x^2$ (R^2=0.68*)
P1103	2.8	3.2	2.2	1.3	2.1	2.2 c	ns
SO4	4.3	9.2	6.2	4.4	2.6	5.3 b	ns
420A	4.9	5.4	5.5	2.9	2.6	4.3 bc	$\hat{y} = 5.4 - 0.019x$ (R^2=0.39*)
Mean	6.1	7.2	5.8	4.7	3.1	CV= 23%	$\hat{y} = 6.9 - 0.0142x - 0.00006x^2$ (R^2=0.59*)

Equal letters in the column do not differ by Tukey test at 0.05 probability level; ns – not significant; *, ** and *** Equation significant at 0.05, 0.01 and 0.1 probability level, respectively, by F test

in the shoots was only of 18 and 13% for 420A and IAC572, respectively, indicating that both rootstocks absorbed higher amount of Zn, with also higher DM reduction in the roots. However, the Zn translocation to the shoots and the DM reduction in the shoots were not as intense as in the roots. This might have occurred because 420A and IAC572 show some internal mechanisms of Zn accumulation/immobilization while, on the other hand, the other rootstocks have mechanisms restricting Zn absorption. According to Reichman (2002), these are the two main strategies that plants have for the tolerance to heavy metals.

The mean production of shoot and root DM of the rootstocks varied quadratically with the increase in Zn contents in the soil (Table 1), i.e., rootstocks cultivated in soil with Zn addition of 20 and 40 mg kg^{-1} increased or did not restrict DM production. In spite of that, from the Zn dose of 80 mg kg^{-1} on, and especially at 160 mg kg^{-1}, the highest restrictions in DM production were observed, particularly in the roots, which showed mean reduction for all rootstocks was of 49%, against 25% reduction in shoot DM.

Although there was no interaction between rootstocks and Zn doses, the reduction in shoot DM for the application of 160 mg kg^{-1} of Zn, in comparison to the non-application, was more intense for IAC313 (55%), intermediate for P1103 (17%), 420A (18%), SO4 (23%) and lower for IAC572 (13%). However, for root DM between the highest Zn dose and the non-application, higher reductions were observed in the rootstocks IAC313 (61%), IAC572 (52%) and 420A (47%). According to Reichman (2002), Zn toxicity can significantly reduce root growth, especially lateral roots, with little interference in the shoots, as observed in the rootstocks 420A and IAC572.

It should be pointed out that IAC572 showed the highest growth vigor in the shoots among the other rootstocks, reaching DM production 2.4 times higher than the mean value for the others (Table 1). Considering Zn contents in the shoots (overall mean of 30 mg kg^{-1}), IAC572 can accumulate 2.4 times more, thus having higher tolerance to Zn, compared with the other rootstocks evaluated. The restriction in growth imposed by Zn can be associated with its effects on the normal ionic homeostasis, interfering with absorption, transport and regulation of essential nutrients and, negatively, with processes like photosynthesis (Reichman, 2002; Sagardoy et al., 2009).

According to the estimate of chlorophyll content, the effects of Zn contents on the chlorophyll indices were of little significance (Table 2). Considering the variation in Zn contents in the leaves, from 17.2 (zero dose) to 46.2 mg kg^{-1} (dose of 160 mg kg^{-1}), there were mean variations of -10.8% (ICa) and -17.1% (ICb), respectively between both doses. In spite of that, there was no significant interaction between the factors Rootstocks and Zn doses, which reveals a more specific response for each rootstock. While IAC572 and IAC313 were very insensitive to Zn doses with respect to ICa and ICb, the rootstock 420A showed a slight variation (α = -0.011 and -0.009, respectively for ICa and ICb) and SO4 (α = -0.041 and -0.016) and P1103 (α = -0.046 and -0.030) showed a moderate variation (Table 2).

The highest variation of ICa and ICb for P1103 and SO4 occurred from the Zn dose of 80 mg kg^{-1}. Therefore, the decrease in ICa and ICb and, consequently, in the chlorophyll content, although not very expressive, can be directly related to the increment in shoot Zn content (Table 1). High doses of Zn in the soil and, consequently, in the tissues, can interfere with the integrity and metabolism of chloroplasts (Cherif et al., 2010; Todeschini et al., 2011). The excess of Zn in the leaves acts mainly on the transport of electrons between photosystems and increases the oxidative stress in chloroplasts, resulting in the degradation of chlorophylls and in the reduction of photosynthesis (Reichman, 2002; Cherif et al., 2010; Cambrollé et al., 2012).

The absence of variation in the indices of chlorophyll for the rootstock IAC572 deserves attention. Considering the amount of Zn accumulated in the shoots, at the highest dose applied, the amounts in IAC572 (1.12 mg of Zn) are higher than the mean value for the others (0.41 mg of Zn). Therefore, the rootstock IAC572 seems to have a biochemical mechanism of tolerance to Zn in the shoots, such as the storage of this metal in the vacuoles or specific Zn complexation by proteins (ex.: metallothionein), peptides (ex.: phytochelatins), organic acids or other binding agents (Küpper et al., 1999; Reichman, 2002), which can protect leaf metabolism from the effects of Zn.

Table 2. Indices of chlorophyll a (ICa) and b (ICb) in grapevine rootstocks cultivated in soils with increasing levels of zinc (Zn)

Rootstocks	Zn doses applied (mg kg^{-1})					Equation
	0	20	40	80	160	
			ICa			
IAC572	28.8 ab	31.8 a	29.7 a	27.3 ab	30.3 a	ns
IAC313	30.5 ab	27.6 a	28.8 a	28.6 ab	27.7 a	ns
P1103	27.1 b	24.3 a	25.6 a	23.5 b	21.6 b	$\hat{y} = 26.8 - 0.046x$ (R^2=0.80**)
SO4	30.4 ab	27.1 a	29.4 a	27.4 ab	22.9 b	$\hat{y} = 29.9 - 0.041x$ (R^2=0.76**)
420A	32.2 a	32.1 a	31.4 a	31.8 a	30.4 a	$\hat{y} = 32.2 - 0.011x$ (R^2=0.63**)
					CV= 7%	
			ICb			
IAC572	7.5 c	9.4 a	8.4 a	7.3 b	8.2 ab	ns
IAC313	8.3 bc	7.4 a	7.6 a	7.5 b	6.9 bc	ns
P1103	7.6 bc	6.5 a	6.7 a	6.0 b	5.6 c	$\hat{y} = 7.5 - 0.03x + 0.00009x^2$ (R^2=0.86**)
SO4	9.2 ab	7.7 a	8.8 a	8.0 ab	6.2 c	$\hat{y} = 8.9 - 0.016x$ (R^2=0.67**)
420A	10.6 a	10.1 a	9.8 a	9.9 a	8.9 a	$\hat{y} = 10.4 - 0.009x$ (R^2=0.81***)
					CV= 8%	

Equal letters in the column do not differ by Tukey test at 0.05 probability level; ns – not significant; * ,** and *** Equation significant at 0.05, 0.01 and 0.1% probability level, respectively, by F test

The mean values of initial fluorescence (F_o) and maximum fluorescence (F_m) increased linearly with the increase of Zn doses in the soil (Table 3). IAC572, IAC313 and 420A were the rootstocks with the highest contributions to the variation of F_o and F_m. According to Torres Netto et al. (2005), the variations in F_o, and especially in F_m, depend on the chlorophyll content in the tissue, which was higher in these rootstocks and at the highest Zn doses (Table 2). F_o reflects the level of basal fluorescence after a dark period and corresponds to the maximum oxidation state of quinone A (Q_A) (Open PSII), while F_m represents the maximum state of reduction in Q_A (Closed PSII) (Baker & Rosenqvist, 2004). Therefore, the increase in F_o with the variations in Zn doses is associated with the higher reduction in Q_A, restricting the capacity of PSII to receive electrons and conditioning it to a 'closed' state. At the same time, an increase in F_m was observed with the variations in Zn, representing higher proportion of reduced Q_A.

Based on the mean data of F_o and F_m, both showed an increment of 20% at the highest Zn dose, in comparison to the zero dose (Table 3). With this variation balance, there were no significant differences in the F_v/F_m ratio in the different rootstocks and Zn doses, considering that the change in this ratio depends on a discrepancy of values between F_o and F_m (Krause & Weis, 1991). In addition, it should be pointed out that the overall mean value for F_v/F_m (0.79 ± 0.02) remained within the range of non-stressed plants (0.75 to 0.85), according to Bolhàr-Nordenkampf et al. (1989). Therefore, it can be inferred that the Zn doses used in this study did not cause irreversible damages on PSII for the different rootstocks. However, the highest Zn doses promoted a reduction in the flow of photochemical energy in the chloroplasts, since the increase of fluorescence (F_m) is directly associated with the reduction in the quenching of photochemical energy or in the form of heat (Baker & Rosenqvist, 2004).

The effect of Zn doses on the dissipation of energy and electron flow in the photosystems was best explained bythe effective quantum yield of PSII (Y-II) and the non-photochemical quenching (NPQ), with different behavior among the rootstocks (Figure 1). According to Baker & Rosenqvist (2004), the variable Y-II represents the dissipation of energy of PSII for the photochemical step, while the variable NPQ represents the loss of energy in the form of heat. In general, the variations were not very expressive and remained within the normal levels for a plant under no stress or very slight stress conditions (Maxwell & Johnson, 2000), which reflects the previously mentioned response for the indices of chlorophyll, F_o and F_m. However, different response groups can be observed among the rootstocks, in relation to the Zn doses: reduction only for Y-II and without variation of NPQ (420A); with reduction of Y-II and increase in NPQ (IAC313); without significant variation in Y-II and with increase in NPQ (IAC572 and P1103) and without any significant variation in Y-II and NPQ (SO4).

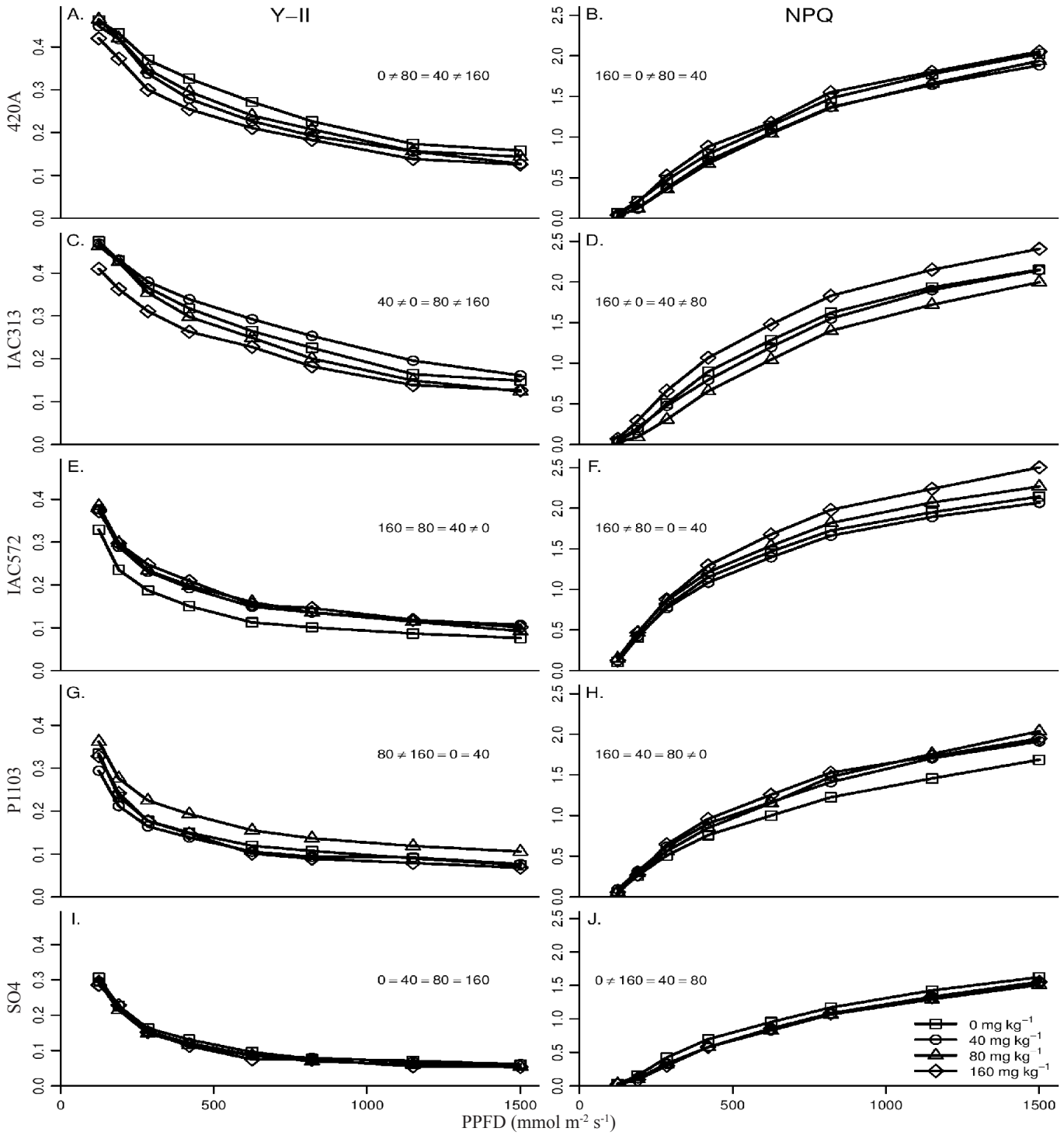
Considering that 420A and IAC313 caused the greatest alteration in Y-II, they also showed the highest Y-II values, compared with the other rootstocks, and the highest F_m values at the Zn dose of 160 mg kg^{-1} (Table 3). Therefore, for these two rootstocks, Zn mainly interfered with the photochemical dissipation of PSII, possibly by the interference that this metal has on the electron flow from the breakdown of the water molecule (Reichman, 2002).

Considering the rootstocks that showed increases in NPQ at the highest Zn dose, IAC572 and IAC313 stand out with the highest energy dissipation in the form of heat (Figures 1D and 1F). This comparison between rootstocks was only possible because of the similarity of F_v/F_m, according to the requirements for the use of NPQ highlighted by Maxwell & Johnson (2000). At the highest Zn dose, IAC313 already showed increments in NPQ from the first radiation levels (125 µmol m^{-2} s^{-1}), while IAC572 showed NPQ increments only from 625 µmol m^{-2} s^{-1}. As to P1103, the substantial increases also

Table 3. Initial fluorescence (F_o), maximum fluorescence (F_m) and maximum quantum yield (F_v/F_m) in different grapevine rootstocks cultivated in soil with increasing levels of zinc (Zn)

Rootstocks	Zn doses applied (mg kg^{-1})				Mean	Equation
	0	40	80	160		
					F_o	
IAC572	103	97	106	120	106 a	ns
IAC313	97	94	108	120	105 ab	ns
P1103	76	87	79	80	80 bc	ns
SO4	68	80	80	79	76 c	ns
420A	102	95	91	140	107 a	ns
Mean	89	90	93	108	CV= 18%	$\hat{y}$ =86 + 0.121x (R^2=0.62*)
					F_m	
IAC572	457	418	420	521	454 a	
IAC313	468	424	543	582	504 a	
P1103	341	432	389	413	394 a	
SO4	347	421	422	406	399 a	
420A	477	458	456	600	498 a	
Mean	418	430	446	504	CV= 13%	$\hat{y}$ =411 + 0.548x (R^2=0.67**)
					F_v/F_m	
IAC572	0.771	0.769	0.749	0.771	0.765 b	ns
IAC313	0.793	0.776	0.802	0.791	0.790 ab	ns
P1103	0.779	0.799	0.798	0.808	0.796 a	ns
SO4	0.805	0.811	0.812	0.807	0.808 a	ns
420A	0.787	0.790	0.800	0.768	0.786 ab	ns
Mean	0.787	0.789	0.792	0.789	CV= 23%	ns

Equal letters in the column do not differ by Tukey test at 0.05 probability level; ns – not significant; * and ** Equation significant at 0.05 and 0.1 probability level, respectively, by F test



Zn doses separated by ≠ differ by F test ($P < 0.05$), considering the behavior of all the points/doses

Figure 1. Effective quantum yield of PSII (Y-II) (A, C, E, G, I) and non-photochemical quenching (NPQ) (B, D, F, H, J) as a function of the photosynthetic photon flux density (PPFD) in the grapevine rootstocks 420A (A, B), IAC313 (C, D), IAC572 (E, F), P1103 (G, H) and SO4 (I, J), cultivated in soil with increasing levels of zinc (Zn)

occurred from 625 μmol m^{-2} s^{-1}, but the effects were observed for all the levels of Zn (Figure 1H).

The increase in NPQ is especially associated with the component of energy dissipation in PSII or activation of a protection mechanism with the acidification of the lumen of thylakoids and, in contrast, activation of the violaxanthin de-epoxidase enzyme and protonation of the PsbS protein (Baker, 2008). These alterations result in higher synthesis of zeaxanthin and conformational change of antennae for the dissipation of incident energy in the form of heat, protecting the photosystems from more severe damages under high radiations (Maxwell & Johnson, 2000; Baker, 2008). Therefore, in the rootstocks that showed the highest NPQ increments as Zn doses increased, there is a greater activation of protecting mechanisms, reducing the impacts on the photosynthetic metabolism. In addition, the rootstocks IAC313 and IAC572, which showed the highest levels of NPQ at the highest dose (160 mg kg^{-1}), were the ones that showed the greatest relative reduction in root DM (-61 and -52%, respectively), in comparison to plants without Zn. However, for P1103, SO4

and 420A, this inverse relationship between NPQ increase and DM reduction with the increase in Zn does not follow the same tendency. P1103, which showed the lowest relative variation of root and shoot DM, showed intermediate variation in NPQ; in contrast, for SO4 and 420A, there was no variation in NPQ, even at the higher Zn doses, but they showed relative reductions 39 and 47% in root DM, respectively, compared with the zero dose. Therefore, the inverse relationship between NPQ and growth was dependent on the genotype and was more favored in cultivars that are more vigorous.

Conclusions

1. The negative effect of Zn on dry matter production occurred in both shoots and roots of grapevine rootstocks.

2. The indices of chlorophyll a and b allowed identifying that the rootstocks Paulsen 1103 and SO4 are sensitive to Zn toxicity in the soil and that IAC572 and IAC313 were not sensitive to the tested Zn levels.

3. The increase in Zn contents in the soil cause negative effects on initial fluorescence (F_o), maximum fluorescence (F_m), effective quantum yield of photosystem II (Y-II) and non-photochemical quenching (NPQ) of grapevine rootstocks, and these chlorophyll photochemical variables proved to be good indicators of Zn phytotoxicity.

4. The maximum quantum yield of photosystem II (F_v/F_m) did not prove to be a good indicator to evaluate the interference of soils with high Zn content on grapevines.

5. The effect of Zn on physiological alterations is conditioned by grapevine rootstock and IAC572 proved to be more tolerant to the applied levels.

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