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The effect of methyl jasmonate on selected physiological parameters of copper-treated *Phaseolus coccineus* plants

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Abstract *Phaseolus coccineus* plants in the early growth stage were preincubated with 10^{-5} M methyl jasmonate (MJ) for 1 or 24 h and subsequently transferred to a Hoagland solution or treated with 50 or 100 μM copper (Cu). After 6-day exposure to the metal, plant growth, relative water content, electrolyte leakage, the content of Cu and photosynthetic pigments, and chlorophyll fluorescence parameters were assayed. Generally, under Cu excess, MJ did not modulate growth parameters such as leaf area, root growth, shoot and root fresh weight, and the shoot/root fresh weight ratio. The content of chlorophyll *a*, *b*, and carotenoids increased with the increasing Cu content in the leaves. However, a correlation between the reduction of the leaf area and the lower content of the three photosynthetic pigments for 24-h MJ + 100-μM Cu treatment compared with metal alone was noted. The decrease in the Cu concentration was MJ-dependent only after 1-h MJ + 50-μM Cu treatment in leaves and after 24-h MJ + 100-μM Cu treatment in roots. Chlorophyll fluorescence was a weak indicator of the effect induced by MJ in Cu excess, and the most spectacular increase was observed for 1-h and 24-h MJ + 50 μM Cu in the LNU and for 1-h MJ + 50 μM Cu in the NPQ parameter. These results suggested a lack of a

clear pattern for MJ altering the Cu stress in the runner bean plants. The most important finding was that photosynthesis seemed to be quite resistant to Cu stress and slight modifications in chlorophyll fluorescence were accompanied by significant changes in growth parameters, photosynthetic pigment content, and metal content in the plant. The results obtained may have been strongly related to the plant growth stage, as the measured parameters transform greatly during plant growth and development.

Introduction

Jasmonates, a collective name for jasmonic acid (JA) and its derivatives, such as a volatile methyl jasmonate (MJ), are widespread in the plant kingdom. They originate from oxidation of linolenic acid and are naturally appearing phytohormones. JA and MJ are precursors of the bioactive molecule in JA signaling represented by the isoleucine conjugated jasmonoyl derivative (Wasternack and Hause 2013). MJ triggers reprogramming of gene expression (Jung et al. 2010), which allows plant cells to deal with stresses (Wolucka et al. 2005). Moreover, genes encoding JA biosynthesis enzymes, OPDA-reductase, lipoxygenase, and allene oxide synthase, exhibited up-regulated expression under 100-μM MJ treatment (Jung et al. 2007).

Exogenously applied MJ inhibits or activates morphological, physiological, and biochemical changes in plants in a different manner depending on the plant growth stage and on the MJ application time and concentration. At a concentration of 100 μM or higher MJ repressed germination

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and plant growth (Jubany-Marí et al. 2010; Kobayashi et al. 2010) and genes involved in photosynthesis, such as ribulose bisphosphate carboxylase/oxygenase (Rubisco), chlorophyll *a*/*b*-binding protein, light harvesting complex II, and early light-induced proteins, were down-regulated (Jung et al. 2007). At a concentration of 50 μM or lower, MJ mostly, but not always (Noir et al. 2013), stimulated plant growth, lateral root initiation and growth, dry matter accumulation, biomass production, photosynthetic pigment levels, and elevation of the net photosynthetic rate (Tung et al. 1996; Mabood et al. 2006; Piotrowska et al. 2010; Wu et al. 2012). However, the role of MJ in protecting plants from various abiotic stresses has been controversial. For instance, MJ in rice improved resistance against chilling (Lee et al. 1996); however, application thereof caused growth reduction (Kim et al. 2009).

Among different stresses, heavy metal toxicity is currently a major abiotic factor that is turning a severe risk worldwide. Even short exposition to the excess of heavy metals, such as Cu, gives visual and physiological changes in plants (Maksymiec and Krupa 2002, 2007), which suggests contribution of signaling pathways based on MJ (Maksymiec et al. 2005; Maksymiec 2007).

Photosynthetic pigments can be used with success as dependable indicators to evaluate the level of metal stress due to inactivation of photosynthesis, which is combined with loss of chlorophyll contents (Maksymiec and Baszyński 1996b; Cambrollé et al. 2013). Moreover, chlorophyll fluorescence has been employed in physiological and ecophysiological studies; since it is considered to be plant species-specific and is emitted only by fluorophores, such as chlorophyll *a* (Chl *a*), the fluorescence analysis is a useful tool for in vivo measurement of the stress level in plants (Maksymiec and Krupa 2002; Demmig-Adams et al. 2012).

To our knowledge, few studies have been carried out on MJ effects under heavy metal stress (Maksymiec and Krupa 2002; Keramat et al. 2010; Yan et al. 2013) and only single studies have been conducted on plants affected by MJ prior to Cu stress action (Maksymiec and Krupa 2002, 2007). The effect of excess Cu on *P. coccineus*, a model dicotyledonous plant, has been thoroughly examined by Maksymiec and Baszyński (1996a, b). Moreover, the literature confirms that plant reaction to Cu is the growth stage-dependent, but limited amount of research was conducted in the early growth stage (Maksymiec and Baszyński 1996b). Additionally, the way of MJ action through the octadecenoic pathway in Cu stress still needs clarification (Maksymiec and Krupa 2002). In the present work, we focused mainly on growth parameters (expressed as leaf area, root growth, plant biomass), Cu and photosynthetic pigment content, and photosynthetic apparatus activity of runner bean to evaluate the role of exogenously applied MJ

on the physiology of the Cu stressed plant in its early growth stage and to examine the effects of shorter and longer MJ incubation under two Cu concentrations. MJ and Cu doses were selected on the basis of the results achieved in earlier experiments (Hristova and Popova 2002; Maksymiec and Krupa 2002).

Materials and methods

Plant material and growth conditions

Runner bean plants (*P. coccineus* L. cv. Piękny Jaś) were germinated for 3 days and then cultivated hydroponically for 8 days in a Hoagland nutrient solution continuously aerated by air pumps. Hoagland's solution was changed after 2 days, shortly before supplementation with the metal. The plants were grown in a growth chamber in Maria Curie-Skłodowska University in Lublin, Poland, at 24/18 °C day/night temperature, relative humidity 60–70 %, and photosynthetic photon flux density of 120 μmol m^{-2} s^{-1} under a 16 h photoperiod. The plants were divided into nine groups: control plants—cultivated in the Hoagland nutrient solution only (MJ0Cu0), treated with 50 (MJ0Cu50) or 100 μM (MJ0Cu100) copper as CuSO$_4$ × 5 H$_2$O (after 2 days of acclimation in hydroponic culture), preincubated with 10^{-5} M MJ for 1 or 24 h (after 2 days or 1 day of acclimation in hydroponic culture, respectively), and then transferred to the pure nutrient solution (MJ1Cu0 or MJ24Cu0), or to the solution with addition of 50 μM Cu^{2+} (MJ1Cu50 or MJ24Cu50) or 100 μM Cu^{2+} (MJ1Cu100 or MJ24Cu100). After 6 days of metal treatment, the leaves and roots were examined or harvested for analysis.

Determination of growth parameters

Net root growth was calculated as the difference between the initial (shortly before the time of metal addition) and final (at the end of the experiment) lengths.

Measurements of the relative water content and electrolyte leakage

The relative water content (RWC) in shoots and roots was determined by measuring fresh weight (FW) and dry weight (DW) of shoots and roots, respectively, and calculated according to the equation: RWC (%) = (FW − DW)/FW × 100.

Leaf disks (0.2 g; 6 mm of diameter each) from fresh leaves of control and treated plants were washed with 40 mL of ultrapure water (MilliQ, Millipore, USA) for 3 min to remove surface-adhered electrolytes and dried with filter paper. The samples were placed in test tubes

containing 10 mL of ultrapure water and gently shaken for 3 h at 25 °C, then conductivity (C1) was measured using an MPC227 meter (Mettler Toledo, Switzerland). Next, the samples were heated in a thermoblock at 95 °C for 20 min, and the conductivity (C2) was measured. Relative electrolyte leakage (REL) was defined as REL (%) = (C1/C2) × 100.

Determination of the copper content

The leaves and roots were rinsed in distilled water and dried at 105 °C to constant dry weight. Samples of 0.2–0.3 g DW were wet digested in the mixture of concentrated $HNO_3 + HClO_4$ (4:1, v/v) using a Velp Scientica digester, DK20 (Milan, Italy). The Cu content was determined by atomic absorption spectrophotometry (Polarized Zeeman Z-8200, Hitachi, Japan).

Bioaccumulation (BAF) and translocation factors (TF) were defined as follows: the ratio of the metal concentration in the plant organ to that in the solution and the ratio of the metal concentration in leaves to that of roots, respectively. TF allows measuring the effectiveness of the plant in translocating the metal from roots to leaves.

Measurements of the photosynthetic pigment content

Chl a and chlorophyll b (Chl b) and total carotenoids (Car) were extracted using 80 % acetone and their concentrations were determined spectrophotometrically (UV-160A Shimadzu, Japan) according to Wellburn (1994).

Measurements of the chlorophyll fluorescence

Chlorophyll a fluorescence was measured at 20 °C using a PAM 101 Chlorophyll Fluorometer (H. Waltz, Effeltrich, Germany) equipped with a PDA-100 system and Schott KL 1500 lamps. Prior to the measurements of fluorescence intensity, leaf sections were dark-adapted for at least 30 min in order to relax the reaction center. The minimal fluorescence level in the dark-adapted state (F_0) was measured using a photon fluence rate of 10 nmol m^{-2} s^{-1} at 1.6 kHz. The maximal fluorescence level at the dark-adapted state (F_m) was measured after applying a saturating light pulse of 8100 μmol m^{-2} s^{-1}. Then, two other parameters were determined: the maximal fluorescence level at the light-adapted state (F_m') and the minimal fluorescence level in the light-adapted state (F_0') after turning on the actinic light of 190 μmol m^{-2} s^{-1} or applying a far-red light of 6 μmol m^{-2} s^{-1}, respectively.

Using fluorescence parameters determined in both light- and dark-adapted states, the following parameters were calculated: maximum quantum efficiency of PSII photochemistry in the dark adapted state (F_v/F_m), photochemical quenching of fluorescence (qP), non-photochemical quenching (NPQ), fraction of the absorbed light energy not used for photochemistry (LNU), and variable chlorophyll fluorescence decrease ratio/vitality index (R_{fd}). The two latter parameters are calculated according to the following equations: LNU = 1 − (qP F_v' F_m)/(F_m' F_v), R_{fd} = (F_m − F_s)/F_s.

Statistical analysis

All analyses in this work were repeated at least four times with five replicates, except for the data of the Cu content which were obtained from three independent experiments with two replicates each, and presented as the mean values ± SE. Statistical analyses were carried out using two-way ANOVA and Tukey's post hoc analysis for determination of the interaction significance at $p < 0.05$. The results of the principal component analysis (PCA) were obtained from three independent replications. The data were analyzed using Statistica ver. 6 (StatSoft, Inc. 2004).

Results

Growth parameters

Leaf area

After MJ preincubation, Cu addition yielded a significantly smaller leaf area in all the experimental combinations without considerable differences between MJ + Cu supplementation or Cu doses only (from 7.8 to 18.1 % of the control) (Fig. 1a). Moreover, in MJ24Cu100 a significantly smaller leaf area compared with the metal alone was achieved. Plants preincubated with MJ for 24 h exhibited the biggest leaf area among all the plant groups tested (17.8 % higher than in the control) (Fig. 1a).

Net root growth

No influence of MJ on plants exposed to Cu was detected, but MJ alone strongly reduced the NGR parameter (Fig. 1b). A significant difference was detected between plants growing with and without Cu supplementation.

Plant biomass

MJ had no significant influence on Cu stress in the fresh weight of shoots and roots. The fresh weight of shoots was gradually lower; first for the 24 h MJ preincubated plants, then in the plants treated with 50 μM Cu, and it was the lowest in the plants treated with 100 μM Cu (Fig. 1c). The highest fresh weight of roots was obtained after preincubation of plants with MJ and the lowest for all metal
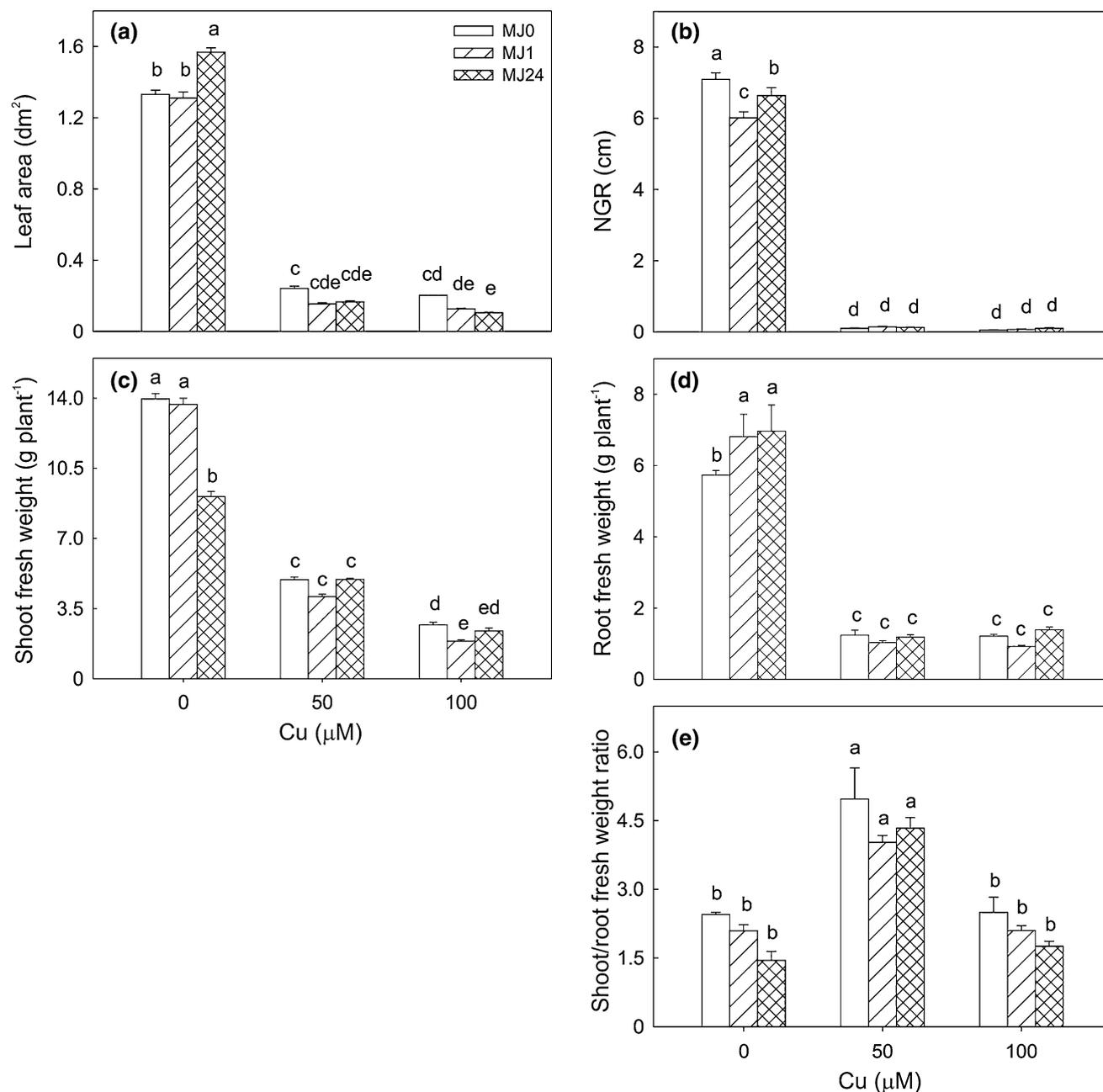
Fig. 1 Primary leaf area (**a**), relative root growth (**b**), fresh weight of shoots (**c**) and roots (**d**), and the shoot/root weight ratio (**e**) in runner bean plants. The control and copper-treated plants, after MJ pre-treatments (Fig. 1d). The highest values of the shoot/root ratios were obtained only for the lower Cu doses without any difference in the other cases (Fig. 1e).

exposure for 1 h (MJ1) or 24 h (MJ24), were analyzed 6 days after metal supplementation. The data represent the mean ± SE (n = 20, at least), the *different letters* indicated the interactions significant

The reverse relationships between the leaf area and shoot fresh weight for the 24-h MJ incubation and between NGR and root fresh weight after 1-h and 24-h MJ action were detected. Moreover, the reverse relationships were observed between the leaf area and NGR, and shoot and root fresh weight for the 24-h MJ incubation.

Relative water content and electrolyte leakage

Preincubation with MJ did not modify RWC in the adverse Cu conditions in shoots or in roots. Generally, a lower RWC was found in the roots (from 85.2 % ± 0.91 to 86.7 % ± 0.54 and from 89.1 % ± 0.31 to 89.9 % ± 0.51 for the Cu-treated and non-metal-treated plants, respectively) (data not shown) than in the leaves (from 88.9 % ± 0.91 to 91.1 % ± 0.28 and from 94.9 % ± 0.10

to 95 % $\pm$ 0.14 for the Cu-treated and non-metal treated plants, respectively) (data not shown). In the leaves and roots, a significantly highest RWC was found in the non-Cu-treated plants (data not shown).

Addition of MJ for 1 or 24 h before application of 50 μM Cu (9.2 % $\pm$ 0.18 e and 9.6 % $\pm$ 0.17 de, respectively) significantly diminished REL induced by 50 μM Cu (13.6 % $\pm$ 0.24 c), but for 100 μM Cu lower REL was obtained only after 24-h MJ incubation (12.1 % $\pm$ 0.09 cd) than after 1-h MJ incubation or 100 μM Cu alone (23.9 % $\pm$ 0.13 a and 16.9 % $\pm$ 0.14 b, respectively) (data not shown). Moreover, MJ applied for 24 h gave significantly higher REL (15.9 % $\pm$ 0.38 b) than the 1-h MJ action or control (11.8 % $\pm$ 0.21 cde and 11.6 % $\pm$ 0.38 cde, respectively) (data not shown).

Copper content

Overall, the tissue Cu content was greater in the roots than in the leaves (Fig. 2a, b). The level of the Cu content in leaves was correlated with Cu supplementation, but not with its concentration or MJ addition (Fig. 2a). The only difference was found between MJ0Cu50 and MJ1Cu50 being higher for metal supplementation alone. In the roots, a gradual increase in the Cu concentration was observed with its rising dose (Fig. 2b). The 24-h MJ preincubation lowered the Cu concentration in roots supplemented with 100 μM Cu in comparison with the metal alone. At MJ1Cu50, the Cu passage from roots to leaves was reduced compared with the control, but such an effect was not seen at the higher metal doses; therefore, the observed effect of MJ was dependent on the Cu dose. MJ in combination with 50 μM Cu gave higher disparity between the leaves and roots (85–117-fold difference) than the metal alone (71-fold difference), but this was reverse at the higher metal concentration (141–145 and 170-fold difference for the treatments with MJ + Cu and Cu alone, respectively). Relatively small amounts of Cu were transported from the roots to leaves, especially when 100 μM Cu was added.

The BAF values were significantly higher in the roots than in the leaves. In the leaves, MJ action, to some extent, was more pronounced in plants under 50-μM than 100-μM Cu stress, and the relation between the BAF values and the Cu content was as follows: MJ0Cu50 (3.52) > MJ24Cu50 (2.94) > MJ1Cu50 (2.63) > MJ1Cu100 (1.77) > MJ0Cu100 (1.73) > MJ24Cu100 (1.53). In turn, the BAF values in the roots were mainly dependent on the length of the preincubation with MJ and were as follows: MJ1Cu50 (307.9) > MJ0Cu100 (295.2) > MJ1Cu100 (256.8) > MJ24Cu50 (251.1) > MJ0Cu50 (248.5) > MJ24Cu100 (215.1). The sequence of TF values detected in the runner bean plants being mostly dependent on the length of MJ incubation in 50-μM Cu treated plants was as follows: MJ0Cu50

(0.014) > MJ24Cu50 (0.012) > MJ1Cu50 (0.009) > MJ24Cu100 (0.007) $\geq$ MJ1Cu100 (0.007) > MJ0Cu100 (0.006). The higher Cu concentration applied was accompanied by the lower TF.

Photosynthetic pigments

For the three photosynthetic pigments, Chl a, Chl b and Car, the same pattern of statistical results was revealed. Regardless of the MJ preincubation, the higher the Cu dose, the higher the Chl a, Chl b, and Car contents were (Fig. 2c–e). Only the MJ preincubation for 24 h with the subsequent 100-μM Cu application decreased the concentration of all the three pigments, which was still significantly higher than for the control and MJ-treated plants. MJ did not alter the Chl a/chl b ratio and Chl $(a + b)$/car ratio in the Cu-treated plants (Fig. 2f, g). In plants preincubated with MJ for 24 h, the highest Chl a/chl b ratio and, by contrast, the lowest Chl $(a + b)$/car ratio of all treatments without Cu were detected (Fig. 2f, g). For all combinations with 50 μM Cu, only the metal alone gave a lower Chl a/chl b ratio and MJ0Cu50 and MJ1Cu50 gave a lower Chl $(a + b)$/car ratio than in the analogical experimental combinations without metal supplementation (Fig. 2f, g). Moreover, the higher Cu dose gave significantly lower Chl a/chl b and Chl $(a + b)$/car ratios, compared with the control, except for MJ24Cu100 for the latter ratio (Fig. 2f, g).

Chlorophyll fluorescence

In the 50-μM Cu treated plants, MJ did not change the F_v/F_m value, but when MJ + Cu was compared with MJ alone, it reached a similar level, simultaneously higher than for the 50-μM Cu treated plants (Fig. 3a). Furthermore, the values of F_v/F_m were statistically lower for MJ0Cu50 and all the treatments with 100 μM Cu compared with the control. After MJ supplementation, the LNU value rose significantly under 50-μM Cu application, simultaneously not having an influence on 100-μM Cu treatment (Fig. 3b). Generally, the LNU values were significantly higher for all MJ + Cu treatments and for MJ0Cu100 compared with the control (Fig. 3b). MJ did not exert any effect on the qP parameter in plants exposed to Cu, but MJ1Cu100 exhibited the lowest value when compared with MJ alone (Fig. 3c). MJ caused elevation of the NPQ value under 50-μM Cu excess compared with the metal alone, but had no effect on 100-μM Cu addition (Fig. 3d). The highest NPQ values were found after metal treatment, except for MJ0Cu50 and MJ1Cu100. MJ did not cause any effect on the R_{fd} values under Cu treatment (data not shown). The only significant difference in this parameter was found between the control (R_{fd} = 3.33—the highest value) and 100 μM Cu (R_{fd} = 3.14—the lowest value).

Two principal factors of PCA explained 60.1 % of the variation, with PC1 and PC2 variability of 36.8 and 23.3 %,

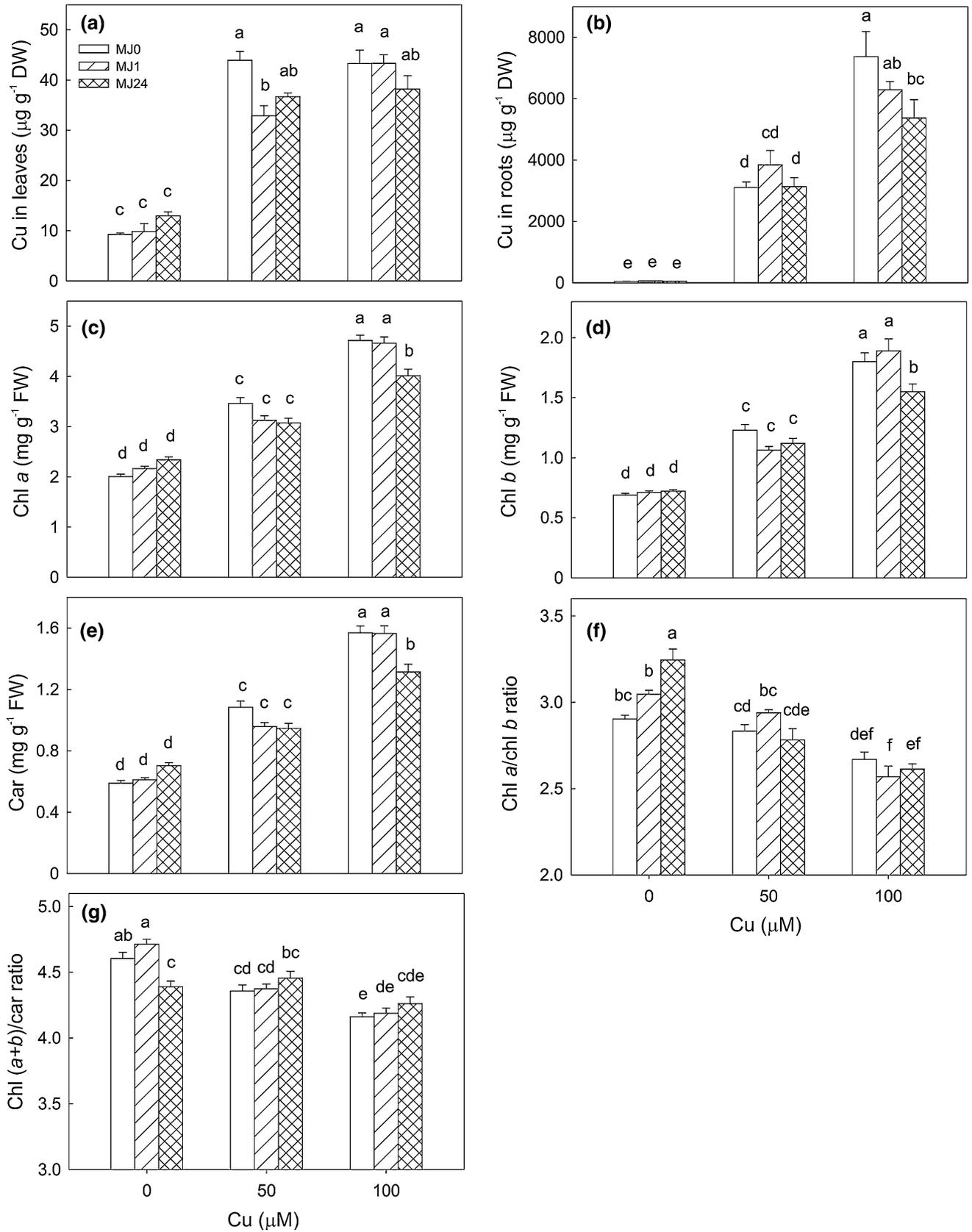◀**Fig. 2** Content of copper in leaves (**a**), roots (**b**), content of chlorophyll *a* (**c**), chlorophyll *b* (**d**), carotenoids (**e**), ratio of chlorophyll *a*/chlorophyll *b* (**f**), chlorophyll (*a* + *b*)/carotenoids (**g**) in the leaves of runner bean plants. The control and copper-treated plants, after MJ pre-exposure for 1 h (MJ1) or 24 h (MJ24), were analyzed 6 days after metal supplementation. The data represent the mean ± SE (n = 6 for the Cu content and n = 20, at least, for the pigment content), the *different letters* indicate significant interactions

respectively (Supplementary Fig. 1). A majority of samples had negative PC1 scores and the distribution of the samples along the PC2 axes was less clear. PC1 was positively correlated with LNU and NPQ, and negatively with qP, R_{fd} and F_v/F_m. The strongest negative correlation of PC2 was found with NPQ. MJ was not a considerably separating agent, but all data concerning 100 μM Cu were well separated. Significantly worse separated data concerned the treatments with 50 μM Cu and without the metal.

Discussion

Our research proved that MJ was a potent molecule in changing growth parameters mostly in an incubation time-

dependent manner. It is known that the leaf area and the number of cells are negatively correlated with the cell density (Kobayashi et al. 2010); hence, the greatest leaf area after the 24-h exposure to MJ may have been caused by the elevated number of cells. However, our results concerning the MJ influence on leaf area were opposed to these reported by Kobayashi et al. (2010) mainly because of the prominent differences in the MJ concentration. Furthermore, MJ synthesis takes place mainly in leaves; therefore, only leaves, but not the whole shoots, could be particularly sensitive to exogenous MJ. This statement is in agreement with our results—the highest leaf area after the 24 h MJ preincubation was correlated with lower shoot fresh weight, probably dependent on lower cell density.

Moreover, since MJ was applied directly to the nutrient solution where roots were immersed, the MJ signal could arrive straight at the root growth zone, where it was sensed. Next, it led to the reduction of cell growth (Schmidt et al. 2010) and inhibited cell proliferation and elongation (Noir et al. 2013). Additionally, MJ can directly or indirectly modulate the influence of auxin on root growth and development (Wasternack and Hause 2013). Consequently,
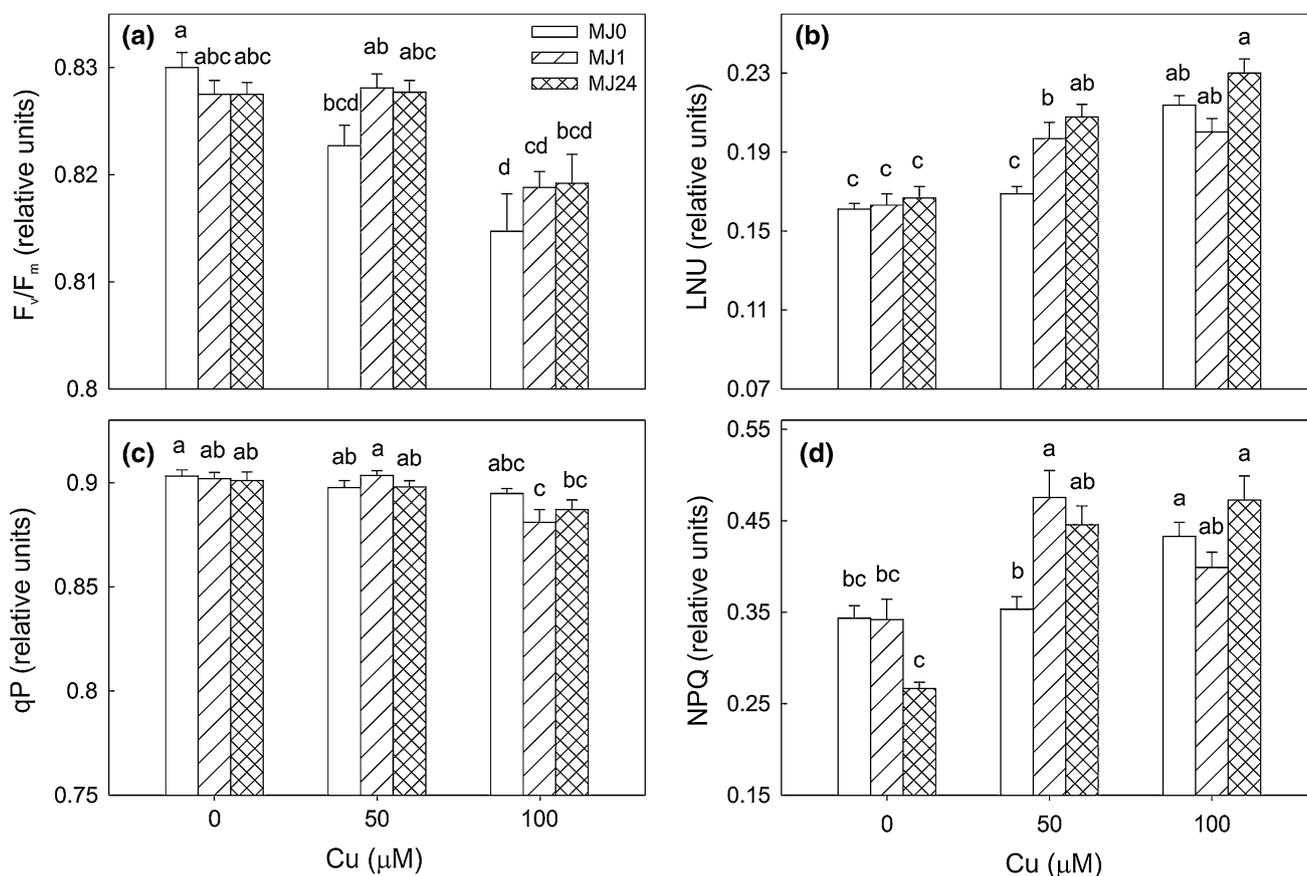

Fig. 3 Chlorophyll fluorescence parameters: F_v/F_m (**a**), LNU (**b**), qP (**c**), NPQ (**d**) in the leaves of runner bean plants. The control and copper-treated plants, after MJ pre-exposure for 1 h (MJ1) or 24 h (MJ24), were analyzed 6 days after metal supplementation. The data represent the mean ± SE (n = 20, at least), the *different letters* indicate significant interactions

the cell size and number were reduced in our experiment resulting in decreased NGR, which could stimulate higher root density and, hence, increased root fresh weight.

For the 24-h MJ incubation, a reverse relationship between the leaf area and NGR and shoot and root fresh weight was observed, which revealed different susceptibility of shoots and roots to the MJ exposure. Regardless of its systemic distribution along the phloem, MJ is differently accumulated in various plant organs (Schmidt et al. 2010) and can provide better protection to leaves than to roots, which was evidenced by the significant decrease in NGR.

Despite the MJ stimulation of the leaf area and root fresh weight, in our research MJ did not serve a protective role in the case of the examined growth parameters in Cu stress. Other results demonstrated that at 10 μM or a lower concentration, MJ protected plants growth from the negative influence of Cu and Cd, but had no positive effect at a higher concentration (Maksymiec and Krupa 2002; Yan et al. 2013). Besides, the depressed Cu action on the leaf area is stronger than the protective role of 24-h MJ preincubation. Therefore, the combined influence of MJ and Cu may result in an inhibitory effect of Cu not sustained by the too weak protective role of MJ. It seems that the results obtained could be dependent on the MJ incubation time and its concentration, plant species, and the strength of the stress agent. Moreover, our results demonstrated that MJ could not play an effective role of a protective barrier for Cu passage from roots to leaves.

In our experiment, runner bean seedlings exposed to Cu exhibited visible morphological changes, such as a significant decrease in the leaf area caused by decreasing cell wall elasticity (Maksymiec 2007), root length, and plant height, compared with the control plants. Moreover, the growth reduction was more pronounced in roots than in leaves at the lower Cu dose, which was reflected by considerably reduced values of shoot fresh weight and elevated values of the shoot/root fresh weight ratio. This observation is consistent with the experiment with 75 μM Cu conducted by Gajewska and Skłodowska (2010).

Furthermore, Cu exhibited low translocation to the above-ground parts of the plant, since it was mostly retained by the roots (Gajewska and Skłodowska 2010; Cambrollé et al. 2013), which was confirmed by the BAF and TF values (higher and lower than 1, respectively) calculated in the present research. The difference in the distribution of the uptaken metal between leaves and roots was caused by efficiently working mechanisms of blocking Cu translocation (plant excluders) and the fact that the growth inhibition was organ-dependent (Gajewska and Skłodowska 2010) and, in our experiment, it was more considerable in roots than in leaves. It is known that the translocation barrier works efficiently in some range of the

metal dose; the tested Cu doses fit in this range and the above-ground part of the plant is protected from toxicity and destruction.

Our experiments showed that MJ was not a protector of the dehydration process under metal stress. Moreover, similarly to the results reported by other scientists (Wu et al. 2012; Mahmood et al. 2012), in the present study RWC showed no significant difference between the control and the 10 μM MJ-treated plants, suggesting that the MJ treatment did not induce osmotic stress (Wu et al. 2012). On the other hand, excess of Cu is known to decrease RWC (Marschner 1995), and in our research a significantly lower RWC was detected after Cu supplementation.

We affirmed quite successfully maintenance of membrane stability by MJ in Cu stress conditions, although the higher the Cu concentration, the higher the REL. Furthermore, the level of permeability of leaf cellular membranes presented as REL was elevated under the 24-h MJ incubation, compared to the control, which is in accordance with other papers (Hristova and Popova 2002).

In our experiment, a less significant increase in the Chl content was detected for MJ24Cu100, but this could be explained as a possible effect of Fe and Mg deficiency and chlorophyllase activity stimulation by MJ, but not by Cu, which was confirmed by the increased levels of Fe^{3+} and Mg^{2+} after 100-μM Cu treatment (Martínez-Peñalver et al. 2012). Furthermore, the responses of accumulation of pigments, amino acids, and mineral nutrients were dependent on both MJ and metal concentration (Kováčik et al. 2011). The increase in the Chl a/b ratio in leaves incubated for 24 h with MJ and the decrease in the Chl $(a + b)$/car ratio could be explained by a greater inhibitory effect on Chl b than Chl a and induction of the senescence process after the longer MJ incubation, respectively. In general, photosynthetic pigments either decrease significantly after exposure to Cu (Maksymiec and Baszyński 1996b) or no effect is observed (Burzyński and Żurek 2007); however, we indicated that Cu increased Chl accumulation, which is in agreement with the results obtained at 80-μM Cu supplementation (Maksymiec and Baszyński 1996b) and 100-μM Cu treatment (Fatima et al. 2011). In our research, MJ without metal supplementation did not alter the pigment content, although JA treatment can result not only in Chl reduction as a consequence of induction of the chlorophyllase gene (Tsuchiya et al. 1999), but also in Rubisco subunit reduction (Rakwal and Komatsu 2000).

Measurements of chlorophyll fluorescence provide valuable information about the efficiency of photochemical processes taking place inside the leaf. MJ did not exert any effect on Cu exposed plants in the F_v/F_m ratio. The unchanged F_v/F_m ratio in our experiments with MJ1Cu50, MJ24Cu50 and MJ supplementation is in agreement with

the results obtained in the initial growth stage in other research papers (Krupa et al. 1993; Maksymiec and Baszyński 1996a; Ananieva et al. 2007; Fedina et al. 2009) and suggests that the maximal photochemical yield of PSII was insensitive to MJ + 50 μM Cu and to MJ alone. Besides, the level of F_v/F_m ratio could be strongly dependent on the length of the MJ treatment (Jung 2004). In our experiment, the decrease in the F_v/F_m for MJ1Cu100, 50 μM Cu and 100 μM Cu could indicate damage to chloroplasts, especially thylakoid membranes (Yamane et al. 2003), induction of photoinhibition processes (Maxwell and Johnson 2000), or reversible inactivation rather than photodamage of PSII (Demmig-Adams et al. 2012).

The decline in F_v/F_m may have also been correlated with the increase in NPQ caused by the conversion of violaxanthin to zeaxanthin (Jahns and Holzwarth 2012). A significant role in the development of NPQ is played by the PsbS subunit of PSII, the xanthophyll cycle, and a decrease in pH, which operates as a signal for enhancement of NPQ (Jahns and Holzwarth 2012). In the present study, MJ alone did not alter the NPQ value, which agrees well with other findings (Jung 2004), but alleviated NPQ only in one case under Cu stress, i.e. MJ1Cu50. An increase in the NPQ parameter, as evidence of protection against photodamage, was observed for most treatments concerning the presence of the metal. Similarly to the research of Jung (2004) and Burzyński and Żurek (2007), we have not detected any significant change in the qP value, also in the MJ influence under the Cu treatment; therefore, the utilization of excitation energy to drive the photosynthetic electron transport was not a stress diversifying parameter in our research.

In our experimental conditions, MJ in the 50-μM Cu environment caused a rise in LNU, similarly to the most metal-treated plants when compared to the control. This could be interpreted as lower efficiency in utilization of the energy absorbed by the antenna complexes in photochemical processes of photosynthesis. Most R_{fd} values were similar to the control, being insensitive to the MJ and metal doses, except for the highest metal content, which reassured us that the photosynthetic apparatus and Calvin cycle worked correctly, even where simultaneous reduction of fresh weight of shoots, leaf area, and water content was observed. Our results are in agreement with other findings (Maksymiec and Baszyński 1996b).

It is well known that fluorescence parameters change severely during the plant growth and development and can differ significantly in the intensive, intermediate, and final growth stages (Maksymiec and Baszyński 1996a). The photosynthesis in the leaves exhibited age-dependent susceptibility to Cu, i.e. young leaves were less sensitive to Cu than mature ones (Vinit-Dunand et al. 2002). Furthermore, the level of JA was also age-dependent, i.e. higher for

younger than older leaves (Maksymiec et al. 2005), which suggests that JA may be involved in the cellular responses at the early stage of metal stress (Yan et al. 2013), and this may be the cause of the lower susceptibility to MJ detected in the early growth stage of runner bean plants. Besides, Maksymiec and Krupa (2002) reported an MJ dose-dependent influence on chlorophyll fluorescence parameters measured under Cu or Cd stress; MJ only up to 10 μM provided protection and lost its positive effect at higher concentrations. Moreover, membrane stability measured as REL seemed to be fundamental for the maintenance of photosynthetic functions (Hristova and Popova 2002).

Despite the considerable and evident changes in the growth parameters accompanying the MJ and Cu supplementation, the photosynthetic apparatus remained partially untouched in our experimental conditions, which was in agreement with the literature data (Maksymiec and Baszyński 1996a, b). Moreover, the good condition of chloroplasts (membranes and granal structures) and the Cu supplementation-induced higher Chl content also detected by other scientists (Maksymiec and Baszyński 1996b; Fatima et al. 2011) could be the explanation for the not severely affected chlorophyll fluorescence parameters.

Conclusions

In conclusion, MJ did not demonstrate an obvious way of action in altering Cu stress in the early growth stage of *P. coccineus* plants. The growth parameters examined were mostly insensitive to MJ application under Cu treatment, but significantly higher growth parameters were reported in the control and MJ-treated plants. In the case of the MJ24Cu100 treatment, reduction of the leaf area and a lower content of three photosynthetic pigments compared with the metal alone were found. However, the 24-h MJ preincubation alone increased the leaf area and reduced the fresh weight of shoots, which corresponded with the elevated Chl *a*/chl *b* ratio and the decreased Chl $(a + b)$/car ratio. Moreover, the increase in the Cu content corresponded with an increase in the Chl *a*, Chl *b*, and Car concentrations. Reduction of the Cu concentration was barely MJ-dependent (only after MJ1Cu50 treatment in leaves and after MJ24Cu100 treatment in roots). From all the parameters measured in this study, chlorophyll fluorescence was not a strong indicator of the effects caused by MJ, Cu, or the combination of both, but the most pronounced rise was found in LNU for MJ1Cu50, MJ24Cu50 and NPQ for MJ1Cu50. The PCA of the fluorescence parameters proved no MJ-dependent separation, but quite considerable separation for all treatments with 100 μM Cu. The results suggested a lack of an unequivocal pattern for the MJ role in modification of the Cu stress in the

runner bean plants. In summary, from the physiological point of view, the future research should inevitably be expanded to include enzymatic and non-enzymatic antioxidants for holistic interpretation of the results, explanation of possible interactions between MJ and Cu, and elucidation of the mechanism of the MJ action in metal stress.

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