ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LX 12 Number 8, 2012

EFFECT OF EXOGENOUSLY APPLICATION SELECTED PHYTOHORMONAL SUBSTANCES ON THE PHYSIOLOGICAL AND MORPHOLOGICAL INDICATORS OF *PHILADELPHUS* X HYBRID IN CONTAINERS

M. Jonáš, P. Salaš, T. Baltazár

Received: September 13, 2012

Abstract

JONÁŠ, M., SALAŠ, P., BALTAZÁR, T.: *Effect of exogenously application selected phytohormonal substances on the physiological and morphological indicators of Philadelphus x hybrid in containers.* Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 8, pp. 109–118

The experiment was established in order to eliminate the effect of stress factors acting on woody plants cultivated in containers. The timber *Philadelphus* x hybrid 'Mont Blanc'. The timber was in 1.5 l containers. The experiment was evaluated effect of exogenouse application of phytohormonal substances on select physiological indicators (stomatal conductance, chlorophyll fluorescence and chlorophyll content), were measured three times during the vegetation. Morphological indicators (diameter of root neck, total length of shoots and number of shoots), were evaluated at the end of vegetation. Phytohormones affecting the impact of stress on plants were used for the purpose. Abscisic acid, 24- epibrassinolid, kinetin and spermine were applied by spraying the leaf in three concentrations (0.01 mg.l^{-1}, 0.1 mg.l^{-1} a 1 mg.l^{-1}). In the results were found highly significant differences compared to controls with other variants, especially in the evaluation of physiological parameters. The most significant influence on the stomatal conductance was observed in the variants treated with abscisic acid. Application 24- epibrassinolid significantly increased the chlorophyll content in comparison with control variant. Morphological parameters reached the best results in variants treated with 24- epibrassinolid and spermine.

abiotic stress, phytohormones, *Philadelphus* x hybrid, physiological indicators, morphological indicators

Plants respond to abiotic factors in environment. These include drought, heavy metals, high salt and changes in temperature and light. Abiotic stress leads to morphological, physiological, biochemical and molecular changes (Behnamnia *et al.*, 2009a).

Drought, salinity, extreme temperature, and oxidative stress are often interconnected and may induce similar cellular damage (Bajguz and Hayat, 2009).

Drought stress is defined as a condition in which water available to plants is so low that it is unfavourable for the growth of a plant species (Behnamnia *et al.*, 2009a). Drought stress has been considered as one of the serious environmental stresses on plant growth and development as well as crop production (Fariduddin *et al.*, 2009). Drought is one of the major environmental conditions that adversely affect plant growth and crop yield (Larcher, 2003). In the face of a global shortage of water resources, water stress has already become a primary factor in limiting crop production. Plants respond to water deficiency and adapt to drought stress through various physiological and biochemical changes, including changes of the endogenous phytohormone levels, especially that of abscisic acid (Wang *et al.*, 2008).

Phytohormones are well known to be involved in plant adaptation to water stress and may play an important role in growth and development (Wang *et al.*, 2008). The exogenous application of plant hormones has been found to counteract toxic effects of various abiotic stresses (Fariduddin *et al.*, 2009).

Abscisic acid (ABA) is an important phytohormone and plays a critical role in response to various stress signals. As many abiotic stresses ultimately results in desiccation of the cell and osmotic imbalance. ABA levels are induced as a reaction to various stress signals. Main function of ABA seems to be the regulation of plant water balance and osmotic stress tolerance (Peleg, 2011). ABA is involved in the signal transduction pathway regulating several genes that are expressed at specific developmental stages or as a result of an environmental stress. ABA accumulates in vegetative cells in response to water deficit, salinity, cold temperature and light variations and is thought to act as a signal for the initiation of acclimation to these stresses (Hassanein *et al.*, 2009).

Brassinosteroids (BRs) is a relatively new class of plant hormone showing a wide occurrence in the plant kingdom with unique biological effects on growth and development (Zhang *et al.*, 2008). BRs stimulate metabolic processes such as photosynthesis, nucleic acid, protein synthesis, vascular differentiation and activation of enzymes. BRs have an anti-stress effect on plants helping overcome low and high temperatures, drought and heavy metals (Fariduddin *et al.*, 2009; Zhang *et al.*, 2008). Exogenous application of BRs may influence a range of diverse processes in plant growth and development (Ali *et al.*, 2006; Behnamnia *et al.*, 2009; Fariduddin *et al.*, 2009). A number of studies show that exogenously applied BRs are effective in controlling plant growth under stressful rather than optimal conditions. For example: increase photosynthesis rate (Shahbaz *et al.*, 2008), shoot dry biomass and seed yield of Brassica juncea were considerably increased by the exogenous application of BRs (Ali *et al.*, 2006). It is clear that BRs provide protection against a number of abiotic stresses.

Polyamines are nitrogen compounds present in all living cells. The three main Polyamines putrescine, spermidine and spermine, participate in different cellular processes ranging from growth promotion and cell division to inhibition of ethylene production and senescence. Many papers report changes in Polyamines levels in relation to several types of environmental stresses (Groppa *et al.*, 2007). During the abiotic stress Polyamines serve as a stabilizer of cell membranes, alleviate effects of various kinds of stresses in different organs (Mulas *et al.*, 1998) and have a protective function in the plants under the stress conditions (Nieddu *et al.*, 1997).

Cytokinins are a class of phytohormones that stimulate water uptake, increase cell division and promote organ development. Kinetin is used to alleviate severe effects of stress. Kinetin is thought to be promising in that respect (Al-Hakimi, 2007). The effect of kinetin on chlorophyll content, growth and on some metabolic processes was demonstrated by Kaur *et al.* (1998) Exogenous cytokinins applied to the leaf can positively affect stomatal conductance and stimulate transpiration (Huang *et al.*, 2006).

The present paper reports the impact of exogenous application of abscisic acid, 24-epibrassinolide, kinetin and spermine on *Philadelphus* x hybrid 'Mont Blanc'. The aim of this work was alleviating the effect of stress factors acting on woody plants cultivated in containers.

MATERIALS AND METHODS

The experiment was established in 2011 at a scientific experimental workplace of the Faculty of Horticulture in Lednice. Woody species *Philadelphus* x hybrid 'Mont Blanc' was selected as a model plant. Already rooted herbal cuttings were planted on May the 8th into 1.5 l containers. Growing medium was used in the market standard substrate RKS II. Woody plants were irrigated using a sprinkler controlled by sensors VIRIB.

The experiment was divided into twelve variants according to phytohormones treatments and a controlled variant without treatment. The following were chosen for the experiment: abscisic acid, 24-epibrassinolide, kinetin and spermine. Each of these phytohomones was applied in three concentration 0.01 $mg.l^{-1}$, 0.1 $mg.l^{-1}$ a 1 $mg.l^{-1}$ of the spray liquid. Each variation has been divided into four repetitions with 50 pcs woods. The first spraying with phytohormones was carried out two weeks after the initial start of the experiment. The plants were sprayed another three times with two weekly spacing.

I: *Distribution of experimental variants*

variants	phytohormon	concentration
control	without treatment	0 $mg.l^{-1}$
variant 1	abscisic acid	0.01 $mg.l^{-1}$
variant 2		0.1 $mg.l^{-1}$
variant 3		1 $mg.l^{-1}$
variant 4	24-epibrassinolide	0.01 $mg.l^{-1}$
variant 5		0.1 $mg.l^{-1}$
variant 6		1 $mg.l^{-1}$
variant 7	kinetin	0.01 $mg.l^{-1}$
variant 8		0.1 $mg.l^{-1}$
variant 9		1 $mg.l^{-1}$
variant 10	spermine	0.01 $mg.l^{-1}$
variant 11		0.1 $mg.l^{-1}$
variant 12		1 $mg.l^{-1}$

Measurement of physiological parameters:

Measurements were performed on the leaves of second to third node at a height of 100–150 mm from the root neck.

Determination of chlorophyll content

Chlorophyll Content Meter (CCM-200) was used to determine the chlorophyll content. Chlorophyll Content Meter is a hand-held, battery operated instrument designed for rapid, non-destructive determination of chlorophyll content in intact leaf samples. Chlorophyll content is a direct indication of plant health, condition, evaluation of plant stress and detection of plant physiology. 200 plants of each variant were measured in the experiment three times during the season.

Determination of chlorophyll fluorescence

Measurements of chlorophyll fluorescence were performed non-destructively using the apparatus Chlorophyll Fluorometer OS–30. Chlorophyll fluorescence records enable us to study photosynthetic processes in plants. 40 average plants from each variant were selected for evaluation, measured three times during the season.

Determination of stomatal conductance

A non-destructive cycling porometer AP4 Porometer was employed to determine the stomatal conductance. This device is used for measuring stomatal resistance in plant leaves. This is a measure of the resistance t loss of water vapour through the stomata and is an indicator of the physiological state of the plant. Twenty plants from each variant were selected for measuring, again three times during the season.

Data analysis

The data processing was carried out in Microsoft Office Excel 2010 and all statistical analyses were performed using freely available statistical program R version 2.15.1. (R Development Core Team, 2012), Tinn-R code editor was used for editing R scripts (Faria, 2011) and for graph plotting package "gplots" (Morales, 2011) respectively "sciplot" (Warnes, 2012).

General linear models (LM) were used for modelling of this relationship for comparison the group means between control and phytohormones one-way analysis of variance (ANOVA) type I (sequential) sum of squares was used. Also was used in case of comparison of group means between the control and other variants. Treatment contrast was used to estimate factor level means and to determine significance difference between control and phytohormones respectively between control and all variants (at significance level 0.05). 95% confidence intervals (CI) were also calculated for the mean in all cases.

After the analysis all accepted statistical models were checked. Kolmogorov-Smirnov test and Anderson-Darling test from package "nortest" (Gross *et al.*, 2011) was used for testing the normality (at significance level 0.01). Bartlett's test and Levene's test from package "car" (Fox and Weisberg 2011) was used for testing the homoscedasticity (also at significance level 0.01), the model checking was also performed by diagnostics plots. The following were used for the purpose: plot of residuals versus fitted values, normal Q-Q plot of standardized residuals, scale-location plot, plot of residuals versus leverage, Cook's distance plot and plot of Cook's distance versus leverage.

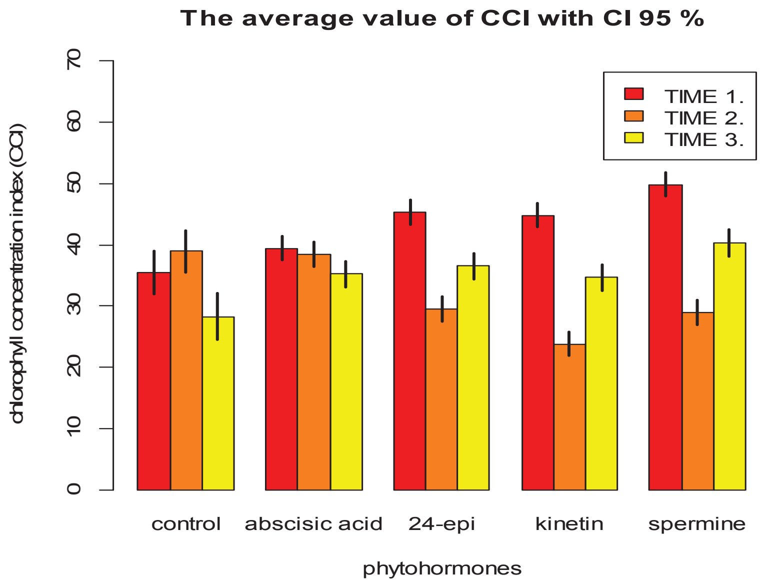
RESULTS AND DISCUSSION

Because the data were not normally distributed, variance was not constant and data contained a lot of outliers, the all data analysis was also repeated with non-parametric statistical methods. For this purpose Kruskal-Wallis chi-square test was used. Two-tailed comparison (variants vs. control) was used to detect the difference after Kruskal Wallis chi-square test. For this purpose was used package "pgirmess" (Giraudoux, 2012).

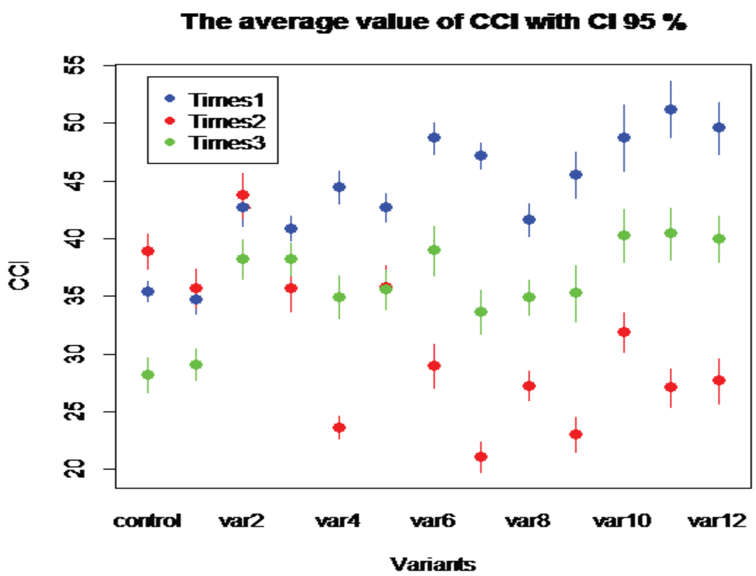
Plant hormones effecting every aspect of plant growth and development (Peleg *et al.*, 2011). Effect of plant hormones have been already observed in many cases of crops e.g. wheat (Bano and Yasmeen, 2010), tomatoes (Behnamnia *et al.*, 2009), peas (Al Hakimi, 2007), sunflowers (Hussain *et al.*, 2010), spider lily (Franks and Farquhar, 2001), lodine bush (Gul and Khan, 2008) and mulberry (Sengupta *et al.*, 2002).

Chlorophyll content is according Madhava *et al.* (2006) one of the indicators of stress on the plants. Due to our results we can conclude that in case of chlorophyll concentration index (CCI) there are significance difference between the control and phytohormones in all cases: 1. measurement ($F_{4,1295} = 19.82$, $p < 0.001$; $\chi^2 = 104.30$, df = 4, $p < 0.001$, n = 100 in control, n = 300 in phytohormones), 2. measurement ($F_{4,1295} = 32.93$, $p < 0.001$; $\chi^2 = 137.11$, df = 4, $p < 0.001$, n = 100 in control, n = 300 in phytohormones) and 3. measurement ($F_{4,1295} = 8.14$, $p < 0.001$; $\chi^2 = 41.21$, df = 4, $p < 0.001$, n = 100 in control, n = 300 in phytohormones). Second measure shows totally diferent results (fig. 1), which could be caused by change of weathers during the measuring. However in first and third measured was found the simillar results. There were found the highly significant diferences between control and 24- epiBL, kinetin and spermine. Plants with foliar spray of 24-epiBL had significantly enhanced of chlorophyll content (Fig. 1). It confirms the findings of Das (2002) and Bajguz and Hayat (2009). Effect of kinetin and spermine on CCI were significantly increased in compare with control. The same influence was observed by Ha *et al.* (2012) and Das *et al.* (2002) after the foliar spraying with kinetin and spermine. For details of average and confidence intervals (95%) of variants see the Fig. 2.

The result is same (Fig. 2) (in case of chlorophyll concentration index – CCI) there are significance difference between control and variants in all cases: 1. measurement ($F_{12,1287} = 8.69$, $p < 0.001$; $\chi^2 = 140.86$, df = 12, $p < 0.001$, n = 100 in each group), 2. measurement ($F_{12,1287} = 15.62$, $p < 0.001$; $\chi^2 = 193.47$,



1: *The average value of CCI*

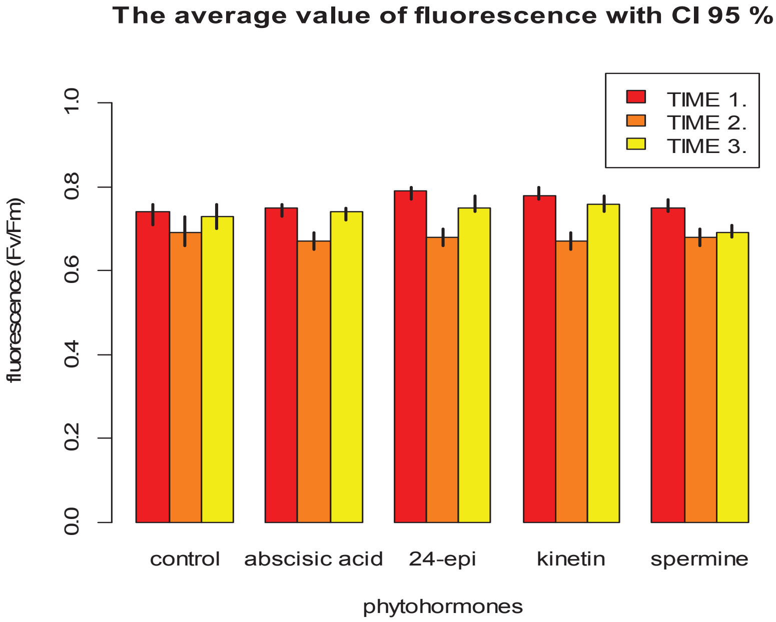


2: *Details of average and confidence intervals (95%) of CCI*

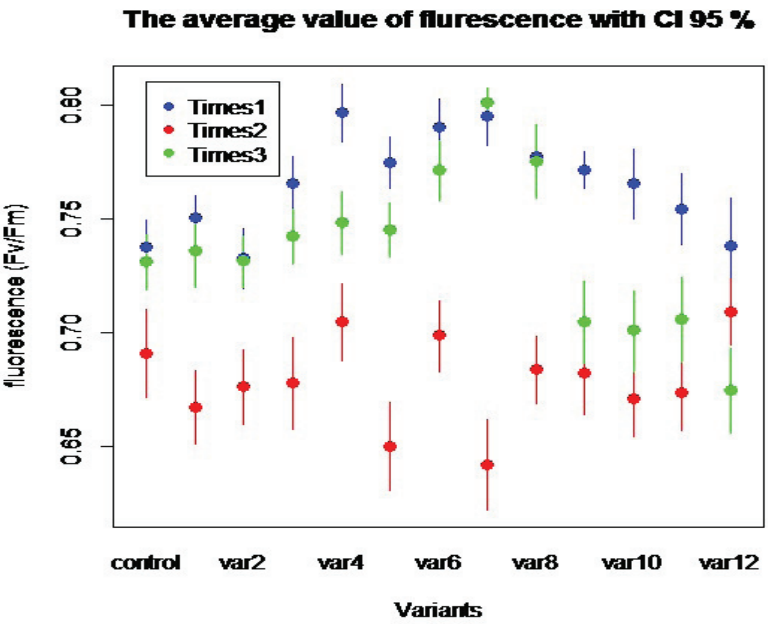
df = 12, $p < 0.001$, n = 100 in each group) and 3. measurement ($F_{12,1287} = 4.21$, $p < 0.001$; $\chi^2 = 69.25$, df = 12, $p < 0.001$, n = 100 in each group).

In case of fluorescence also was found significance difference between control and phytohormones: 1. measurement ($F_{4,515} = 5.75$, $p < 0.001$; $\chi^2 = 20.93$, df = 4, $p < 0.001$, n = 40 in control, n = 120 in phytohormones). There is no statistical significance difference in case of 2. Measurement ($F_{4,515} = 0.55$, $p = 0.70$; $\chi^2 = 2.13$, df = 4, $p = 0.71$, n = 40 in control, n = 120 in phytohormones), but also was found statistical significance difference in case of 3. Measurement ($F_{4,515} = 8.78$, $p < 0.001$; $\chi^2 = 29.94$, df = 4, p = < 0.001, n = 40 in control, n = 120 in phytohormones).

Chlorophyll fluorescence is sensitive indicators of photosynthetic processes. Fluorescence can reached the value of 0.83 for plants under optimal conditions. Impairment is caused by the effect of stress (Krausch *et al.*, 1991). There were found the highly significant diferences in average and confidence interval (95%) between control and first measure of 24-epiBL and kinetin (Fig. 3). The slightly increased of PS II (Fv/Fm) were observed at the treated variants. Variants treated by 24-epiBL and kinetin had the highest value of fluorescence in compare with control (Fig. 3). This results confirms Shahbaz *et al.*, (2008) at the wheat plants treated by 24-epiBL under saline stress. For details of average and confidence intervals (95%) of variants see the Fig. 4.



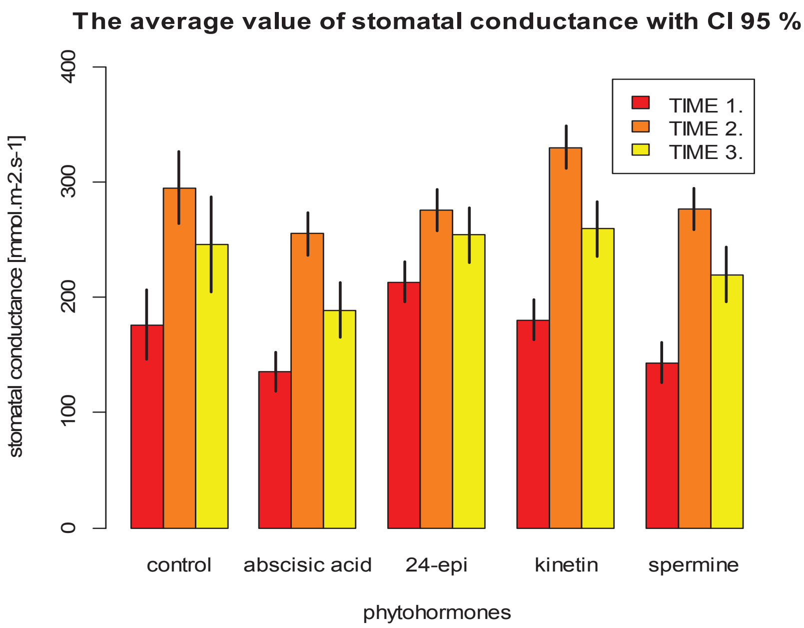
3: *The average value of fluorescence (Fv/Fm) with CI 95 %*



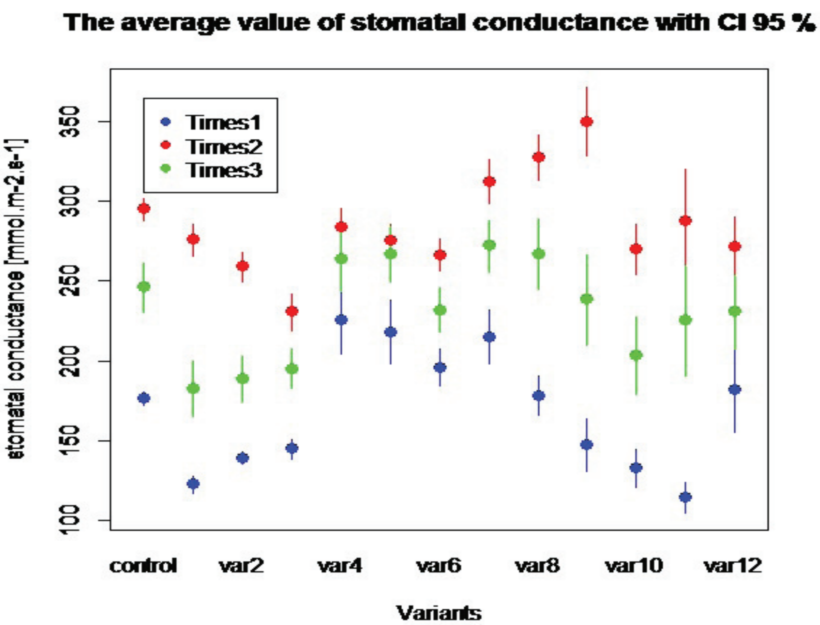
4: *Details of average and confidence intervals (95%) of fluorescence*

The result is same (Fig. 4) (in case of fluorescence), there are significance difference between control and variants in 1. measurement ($F_{12,507}$ = 2.60, p = 0.0023;χ^2 = 28.41, df = 12, p = 0.0048, n = 40 in each group). There is no statistical significance difference in 2. measurement ($F_{12,507}$ = 1.23, p = 0.259; χ^2 = 12.68, df = 12, p = 0.39, n = 40 in each group), but also was found statistical significance difference in 3. measurement ($F_{12,507}$ = 5.25, p < 0.001; χ^2 = 57.06, df = 12, p < 0.001, n = 40 in each group).

In case of stomatal conductance also was found significance difference between control and phytohormones in all cases (Fig. 5): 1. measurement ($F_{4,255}$ = 12.36, p < 0.001; χ^2 = 49.39, df = 4, p < 0.001, n = 20 in control, n = 60 in phytohormones), 2. measurement($F_{4,25\ 5}$= 8.97, p < 0.001; χ^2 = 32.17, df = 4, p < 0.001, n = 20 in control, n = 60 in phytohormones) and 3. measurement ($F_{4,255}$ = 5.63, p < 0.001; χ^2 = 24.71, df = 4, p < 0.001, n = 20 in control, n = 60 in phytohormones). The regulation of stomatal conductance is the main mechanism by which plants control gas exchange (Pospíšilová, 2003). Application of exogenous citokinins to plants can increase stomatal apertures and transpiration in



5: *The average value of stomatal conductance (mmol.m^{-2}.s^{-1}) with CI 95 %*

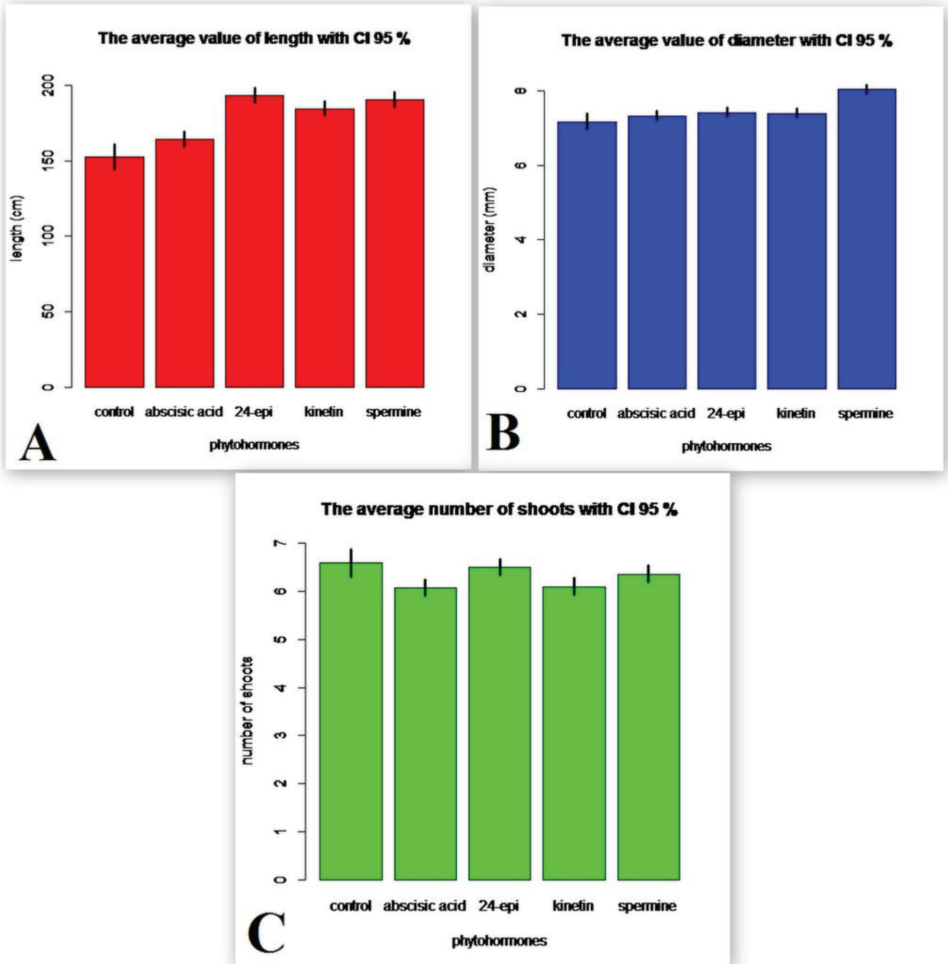


6: *Details of average and confidence intervals (95%) of stomatal conductance*

many plants (Ha *et al.*, 2012). Significance differences between ABA treated variants and control were found (Fig. 5). In Fig. 5 we can see involvement of ABA in the regulation of stomatal conductance which is generally accepted by (Pospíšilová, 2003). At the other hand, slightly stimulated effect of kinetin was found. The additional role of kinetin as a stimulator of stomatal opening were confirms (Pospíšilová, 2003). For details of average and confidence intervals (95%) of variants see the Fig. 5.

The result is same (Fig. 6) (in case of stomatal conductance) there are significance difference between control and variants in all cases: 1. measurement ($F_{12,247}$ = 6.47, p < 0.001; χ^2 = 70.04, df = 12, p < 0.001, n = 20 in each group), 2. measurement ($F_{12,247}$ = 3.67, p < 0.001; χ^2 = 39.05, df = 12, p < 0.001, n = 20 in each group) and 3. measurement ($F_{12,247}$ = 2.21, p = 0.01; χ^2 = 31.67, df = 12, p = 0.002, n = 20 in each group).

Also was found significance difference between control and phytohormones in case of total length of shoot (Fig. 7). ($F_{4,1035}$ = 32.91, p < 0.001; χ^2 = 116.67, df = 4, p < 0.001, n = 80 in control, n = 240 in phytohormones), in case of diameter of root neck ($F_{4,1295}$ = 23.27, p < 0.001; χ^2 = 71.90, df = 4, p < 0.001, n = 100 in control, n = 300 in phytohormones), and



7: *Morphological parameters, A – The average value of total length of shoots, B –The average value of root neck diameter, C – The average number of shoots*

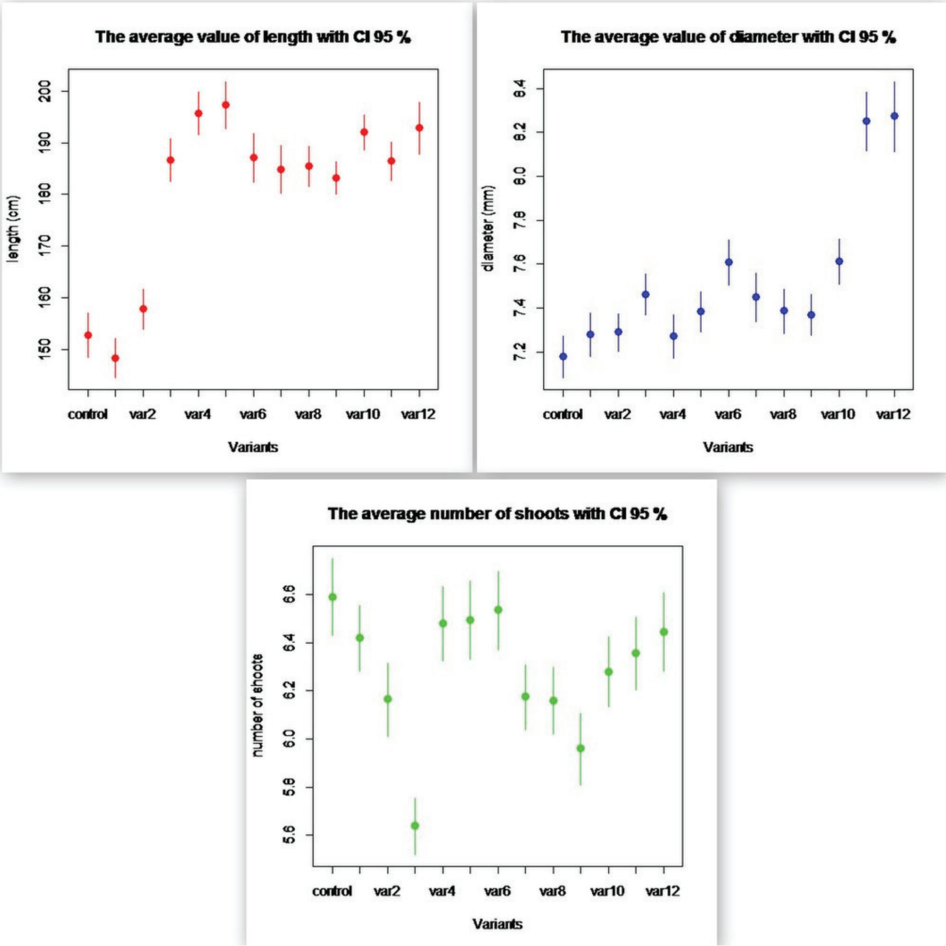
in case of shoot numbers ($F_{4,2595}$= 5.44, p < 0.001; χ^2 = 18.82, df = 4, p < 0.001, n = 200 in control, n = 600 in phytohormones).

Behnamnia *et al.* (2009) found the significance increased morphological parameters of tomatoes under drought stress after application of 24-epiBL. BRs increased rate of stem elongation (Pospíšilová, 2003). There was found highly significant diference of total length of shoots between control and 24-epiBL, kinetin and spermine treated plants (Fig. 7 A). The highly significant diference in root neck diameter was found between control and spermine (Fig. 7 B). The growth was also increased as a result of foliar spray of kinetin and spermine in salinized and non-salinized mulberry platns (Das, 2002). Our results about improving of growth parameters of 24-epiBL treated plants were reported. The similar results were reported by Shahbaz *et al.* (2008).

The result is same (Fig. 8), there are significance differencebetween control and all variants: total length of shoots ($F_{12,1026}$ = 15.62, p < 0.001; χ^2 = 160.47, df = 12, p < 0.001, n = 80 in each group), diameter of root neck ($F_{12,1287}$ = 10.498, p < 0.001; χ^2 = 92.448, df = 12, p < 0.001, n = 100 in each group) and number of shoots ($F_{12,2587}$ = 3.16, p < 0.001; χ^2 = 38.44, df = 12, p < 0.001, n =200 in each group).

CONCLUSION

In the investigated of CCI were found the highly significant diferences between control and 24-epiBL, kinetin and spermine. Plants with foliar spray of 24-epiBL had significantly enhanced of chlorophyll content. The slightly increased of fluorescence (Fv/Fm) were observed at the treated variants. There were found involvement of ABA treated plantas in the regulation of stomatal conductance. There was found highly significant diference of total length of shoots between control and 24-epiBL, kinetin and spermine treated plants. The highly significant diference in root neck diameter was found between control and spermine. The best improving of morphological parameters were found for 24-epiBL, kinetin and spermine treated plants.



8: *Details of measuring morphological parameters*

SUMMARY

The present paper reports the impact of exogenous application of abscisic acid, 24-epibrassinolide, kinetin and spermine on *Philadelphus* x hybrid 'Mont Blanc'. The aim of this work was alleviating the effect of stress factors acting on woody plants cultivated in containers.

As a model plants was selected *Philadelphus* x hybrid 'Mont Blanc'. The experiments was divided into twelve variants by phytohormones treatments and without treatment control variant. On experiment were chosen: abscisic acid, 24-epibrassinolide, kinetin and spermine. Each of these phytohomones was applied in three concentration 0.01 $mg.l^{-1}$, 0.1 $mg.l^{-1}$ a 1 $mg.l^{-1}$ spray liquid.

In my opinion the results confirm the report of Peleg *et al.* (2011) that plant hormones effecting every aspect of plant growth and development.

Due to our results we can conclude that in case of chlorophyll concentration index (CCI) there are significance difference between the control and in all of phytohormones. Highly significant diferences between control and 24- epiBL, kinetin and spermine were found. There were found the highly significant diferences in average and confidence interval (95%) between control and first measure of 24-epiBL and kinetin. 24-epiBL treated and kinetin treated variants had the highest value of fluorescence in compare with control. The involvement of ABA in the regulation of stomatal conductance which is generally accepted was reported. The slightly improved of morphological parameters were found on phytohormonals treated plants, but on the the other hand the effect of this treated were in some cases irrelevant.

Acknowledgement

This publication was supported by Project No. 4/2012/519 provided by the Internal Grant Agency of MENDELU.

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Address

Ing. Martin Jonáš, Ústav šlechtění a množení zahradnických rostlin, Mendelova univerzita v Brně, Valtická 337, 691 44 Lednice, Česká republika, e-mail: jonas.ma@seznam.cz