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Effect of some growth regulators on chlorophyll fluorescence in *Viola* × *wittrockiana* 'Wesel Ice'

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The objective of this study was to determine the influence of daminozide, paclobutrazol and daminozide in combination with CCC on chlorophyll fluorescence and morphological characteristics in *Viola* × *wittrockiana* 'Wesel Ice'. Chlorophyll fluorescence was measured on the abaxial side of the leaves three weeks after treatment. Measurements of chlorophyll fluorescence were done using a fluorescence monitoring system (Model FMS-1, Hansatech, Kings Lynn, UK). Before measurements, the leaves were dark-adapted for 30 min. The parameters of maximal fluorescence (Fm), variable fluorescence (Fv) and Fv / Fm ratio under the influence of growth regulators increased by 1.6–8.3%, 4.7–10.6% and 2.4–3.9%, respectively. PBZ had the strongest retarding effect in all aspects, while B9 + CCC proved to be the least effective. At the concentrations used, PGR did not provide control of plant and flower diameter and significant differences in the number of flowers. There was a significant reduction in fresh weight (0.05–0.20 g) and area (0.6–2.8 cm^2) of leaves. PGR significantly increased total foliar chlorophyll (33.7–55.4 mg, 100 g^{-1} 25.6–42.0%) and carotenoid (15.3–28.2 mg 100 g^{-1}, 29.9–55.3%) concentration. Chlorophyll fluorescence parameters showed that photochemical activity was greater in leaves of the treated plants.

Key words: chlorophyll fluorescence, growth regulators, pigments, *Viola* × *wittrockiana*

Abbreviations: Fo, Fv, Fm – minimal, variable and maximal fluorescence of dark-adapted leaves; Fs, Fm – steady state and maximal fluorescence of light-adapted leaves; qP – photochemical quenching; qNP – non-photochemical quenching; FPSII – quantum efficiency of PSII; PGR – plant growth regulators; PBZ – paclobutrazol; B9 – daminozide, CCC – chlormequat

INTRODUCTION

The germination of cool season annuals as *Viola* × *wittrockiana*, scheduled for fall sales, may occur as early as July. This often necessitates applications of plant growth regulators (PGR) to control excessive stem elongation. Applications of PGR for this purpose are applied to many annuals at plug stage, greenhouse stage, or both [1–3]. Most of the commercially available PGRs are antigibberellins which work by inhibiting gibberellin synthesis in the plant. Typical growth retardants in horticulture are B-Nine, Cycocel, A-Rest, Bonzi, Sumagic, etc. Daminozide (B9) (Alar, B-Nine [butanedioic acid mono(2,2dimethylhydrazide)]) shows a low phytotoxicity, is easy to use and may delay flowering, but it needs multiple applications. It is a growth retardant with a proven effect and practical application on many plant species [4]. However, its use in recent years has been limited because of data about its toxic effect [5]. It is actively transported within the plant and only used as a foliar spray. B9 has a shorter residual effect, so multiple applications are required. Its efficacy is lower in warm climates. Because it has a lower residual effect, phytotoxicity rarely occurs, but late applications can delay flowering. PBZ (Bonzi, paclobutrazol)[(2RS,3RS)-1-(4-chlorophenyl)-4,4dimethyl-2-(1,2,4-triazolyl)-pentan-3-ol], a commercial triazole derivative, shows a low phytotoxicity, is mobile (root, stem, shoot absorbed), affects flower size, leaf size and has been recommended for use as either a fungicide or a plant growth regulator [6]. Triazoles are the largest and most important group of systemic compounds developed in the 1960s for controlling fungal diseases in plants and animals. The characteristic of the effects of triazole include reduced shoot elongation and trichome length, increased epicuticular wax, larger chloroplasts and accelerated root growth, detoxification of active oxygen, higher levels of chlorophyll [7–12]. Triazole compounds have been found to protect plants from environmental stress such as drought, extreme temperature, gaseous sulphur dioxide and fungal infections [13, 14]. CCC (Chlormequat Chloride, chlorcholinchlorid (2-chloroethyltrimethyl-ammonium ion) shows a temporary high phytotoxicity, is less active under high temperatures, easy to use, promotes flowering, also is actively transported within the plant. It can be applied as a foliar spray or drench. It has a

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shorter residual effect, so multiple applications are required. Phytotoxicity can occur with rates greater than 1,500 mg l^{-1}.

The development of accurate, non-intrusive, portable modulated fluorometers and imaging techniques have opened new vistas in rapid monitoring of photosynthetic functioning in response to a variety of abiotic and biotic stresses [15–17].

Therefore, the objective of this study was to determine the influence of B9, PBZ, and B9 in combination with CCC on chlorophyll fluorescence and morphological characteristics of *Viola × wittrockiana* 'Wesel Ice'.

MATERIALS AND METHODS

Seeds of *Viola × wittrockiana* 'Wesel Ice' (Ernst Benary Samenzucht GmbH) were sown at the end of June on polystyrene plug trays filled with commercial peatmoss medium and transplanted to 9 cm plastic pots when second leaves were fully expanded. Each pot was filled with commercial peat-moss medium mixed with sand (3 : 1). The medium was enriched with 2 g of a slow-release mixed fertiliser Osmocote 1 dm^3. Leaf area was estimated by taking a sample of 20 leaves from each experimental unit and measuring with a Digital Image Analysis System.

The growth regulators B9 (1000, 5000 mg l^{-1}), PBZ (10, 15 mg^{-1} l) and B9 (1000, 2000, 3000 mg l^{-1}) + CCC (1000 mg l^{-1}) and water (control) foliar sprays were applied on 25 August 2005, i. e. when the leaves had expanded to the edge of the pot. The experiment was a completely randomized design with eight single-plant replications of the eight treatments.

Chlorophyll fluorescence was measured on the abaxial side of leaves in September 2005 (three weeks after treatment). Measurements of chlorophyll fluorescence were done using a fluorescence monitoring system (Model FMS-1, Hansatech, Kings Lynn, UK). Before measurements the leaves were dark-adapted for 30 min in special leaf clips designed for use with the FMS-1. Dark-adapted ground fluorescence (Fo) and maximum fluorescence (Fm) were determined by a weak modulated light (0.02 µmol m^{-2} s^{-1}) and a saturating 700 ms flash of 14,400 µmol photons m^{-2} s^{-1}. Actinic light pulse (1800 µmol m^{-2} s^{-1}) was given to obtain Fm'. The following parameters were assessed: Fo – minimal fluorescence when all PSII reaction centres are opened in dark-adapted leaves; Fm – maximal chlorophyll fluorescence when all PSII reaction centres are closed in dark-adapted leaves; Fv –variable fluorescence after dark acclimation; apparent quantum yield of PSII (Fv / Fm) – (where Fm is the maximal fluorescence value and Fv = Fm – Fo); the ratio of Fv / Fo – a useful measure of photosynthetic capacity associated with a disruption of the water-splitting system [18] estimates the maximum primary yield of photochemistry of photosystem II to provide an estimation of leaf photosynthetic capacity [19]. Fm / Fo is used as an indicator of the physiological state of plants and is called the state change index. The value of the latter parameter increases with the adaptive process [20]; Fo / Fm is an indicator of the physiological state of the photosynthetic apparatus with Fo / Fm values of 1, i. e., Fo = Fm corresponding to destabilization of the photosynthetic apparatus; Fm' – maximum fluorescence in the light-adapted state, Fs – steady-state fluorescence in the light-adapted state; qNP – the non-photochemical quenching coefficient of chlorophyll fluorescence; qP – photochemical quenching, and FPSII – quantum efficiency of PSII. Rfd were calculated as the Fm-Fs / Fs ratio (Fs – the intensity of stationary fluorescence) according to [21]. Chlorophylls a and b and total carotenoids were determined in the same leaf pigment extract in 80% water acetone spectrophotometrically using the redetermined extinction coefficients and Lichtenthaler equations [22]. Data were analysed as a factorial experiment using SAS ANOVA, with mean separations, where appropriate, by LSD, p = 0.05.

RESULTS AND DISCUSSION

All variants of retardant treatment turned out to inhibit growth in comparison with control. PBZ had the strongest retarding effect in all aspects, while B9 + CCC proved to be the least effective (Table 1). Significant differences in the height of control plants and those treated are recorded. Paclobutrazol was found better than other treatments for production of compact pansies. Plants treated with 15 mg l^{-1} PBZ were 36.8% shorter than the nontreated plants. Ball [1] states that *Petunia × hybrida* growth may be severely reduced by 15 mg l^{-1} paclobutrazol applications during production, but growth spurts of plants after initial suppression with paclobutrazol at low doses (5 mg l^{-1}) during container production on the subsequent flowering of *Plumbago auriculata* Lam. have been reported. At the PGR concentrations used did not provide control of plant and flower diameter (data not shown) or significant differences in the number of flowers. There was a significant reduction in fresh weight (0.05–0.20 g) and area (0.6–2.8 cm^2) of leaves. Plants treated with PGR showed darker green leaves in comparison to control. This is a common effect in PGR-treated plants as a result of either increasing chlorophyll biosynthesis and / or reduction of leaf expansion accompanied by normal rates of chlorophyll biosynthesis [23, 24]. PGR significantly increased the total foliar chlorophyll (33.7–55.4 mg 100 g^{-1}, 25.6–42.0%) and carotenoid (15.3–28.2 mg 100 g^{-1}, 29.9–55.3%) concentration of pansy 'Wesel Ice' after three weeks of application (Table 2), in agreement with other reports [25]. Treatment with PGR mostly effected chlorophyll a but not chlorophyll b concentration.

Minimal fluorescence (Fo) in dark-adapted leaves when all PSII reaction centres are opened under the influence of PGR

Table 1. **Morfological characteristics of *Viola × wittrockiana* 'Wesel Ice' after exposure to PGR for 3 weeks, September 2005**

Treatment mg l^{-1}	Plant height, cm	Leaf area, cm^2	Leaf mass, g	Number of flowers
Control	9.5	9.5	1.02	2.3
B9 1000	8.3	8.6	0.94	2.2
B9 5000	7.7	8.0	0.88	2.4
PBZ 10	7.4	7.6	0.84	2.4
PBZ 15	6.0	6.7	0.82	2.1
B9 1000 + CCC 1000	8.5	8.9	0.97	2.3
B9 2000 + CCC 1000	8.1	8.5	0.89	2.3
B9 3000 + CCC 1000	7.9	8.9	0.88	2.1
LSD_{05}	0.87	0.5	0.07	0.5

Table 2. **Content of pigments in *Viola × wittrockiana* 'Wesel Ice' leaves after exposure to PGR for 3 weeks, mg 100 g^{-1}**

Treatment mg l^{-1}	Chlorophyll a	Chlorophyll b	Chlorophylls a + b	Carotenoids
Control	97.97	33.88	131.85	50.97
B9 1000	124.98	40.61	165.59	66.23
B9 5000	136.82	43.70	180.52	73.70
PBZ 10	124.99	44.23	169.22	68.87
PBZ 15	140.57	46.70	187.27	77.25
B9 1000 + CCC 1000	129.85	41.15	171.00	76.62
B9 2000 + CCC 1000	133.41	43.39	176.8	77.57
B9 3000 + CCC 1000	135.26	42.65	177.91	79.17
LSD_{05}	12.7	4.2	19.4	9.8

Table 3. **Effect of various PGR on chlorophyll fluorescence parameters of dark-adapted and light-saturated leaves of control and treated plants of *Viola × wittrockiana* 'Wesel Ice'**

Parameters	Treatment, mg l^{-1}							
	Control	B9 1000	B9 5000	PBZ 10	PBZ 15	B9 1000 + CCC 1000	B9 2000 + CCC 1000	B9 3000 + CCC 1000
Fo	266.4	248.2	247.4	250.4	244	265.6	255.6	233.8
Fm	1295.2	1379.8	1358.2	1332.1	1321.2	1402.4	1393.8	1316.8
Fv	1028.8	1131.6	1110.8	1081.6	1077.2	1136.8	1138.2	1083
Fv / Fm	0.80	0.82	0.82	0.81	0.82	0.81	0.82	0.82
Fo / Fm	0.21	0.18	0.18	0.19	0.18	0.19	0.18	0.18
Fv / Fo	3.9	4.6	4.5	4.3	4.4	4.3	4.4	4.6
Fm / Fo	4.9	5.6	5.5	5.3	5.4	5.3	5.4	5.6
Fs	347.6	296.6	317.4	284	305.2	281.8	281.0	281.2
Fm'	436.2	380	390.4	380	383.4	369.6	371.2	367.6
ΦPSII	0.20	0.22	0.19	0.25	0.20	0.24	0.24	0.23
qP	0.52	0.63	0.51	0.74	0.56	0.84	0.78	0.64
qNP	0.83	0.88	0.87	0.88	0.87	0.91	0.89	0.88
qP / qN	0.63	0.72	0.59	0.84	0.64	0.92	0.87	0.73
Rfd	3.69	3.65	3.28	3.69	3.33	3.98	3.96	3.68

decreased by 0.3–12.2% (the Fo value increases as plant stress increases), maximal chlorophyll fluorescence increased by 1.7–8.3%, and variable fluorescence (Fv) after dark acclimation increased by 4.7–10.6%. The parameters of maximal fluorescence, variable fluorescence (Fv) and the Fv / Fm ratio characterize the functional state of PSII in dark-adapted leaves. The higher the variable fluorescence, the higher the photosynthetic capacity of the leaf [26]. Our experiment shows that photosynthetic capacity after PGRs increases (Table 3).The chlorophyll fluorescence Fv / Fm ratio is considered to be a measure of PSII effectiveness in the primary photochemical reactions [27, 28] and is correlated with the efficiency of leaf photosynthesis, and a decline in this ratio is a good indicator of photoinhibitory damage caused by incident PFD when plants are subjected to a wide range of environmental stresses [29]. Fv / Fm has been widely used to detect stress-induced perturbations in the photosynthetic apparatus, since a decreases in Fv / Fm may be caused by the development of slowly relaxing quenching processes and photodamage to PSII reaction centres, both of them reducing the maximum quantum efficiency of PSII photochemistry. In contrast to the variable fluorescence (Fv), this ratio was found to be highly constant in unstressed leaves of various species. The observed values are as an accepted norm for healthy, non-stressed leaves – 07.75–0.85 [30, 31], and obtained data show that after treatment with PGR the PSII effectiveness increases, B9, PBZ, and B-9 in combination with CCC change the Fv / Fm (2.4–4.0 %), Fm / Fo (8.6–15.8%) and especially the Fv / Fo (10.8–20.0%) ratios (Table 3). The potential of the use of the fluorescence induction parameter ratios has been demonstrated by examining the effects of a number of herbicides that are known not to impact directly the photosynthetic metabolism [32]. Experiments with *Alopecurus myosuroides, Avena fatua, Phaseolus vulgaris, Sinapis alba, Triticum aestivum* and *Zea mays* have demonstrated a wide applicability of these fluorescence parameters for the detection of herbicide-induced perturbations of metabolism [33–35]. The relationship between photochemical and non-photochemical quenching parameters expressed as the ratio of qP and qNP showed clearly that in a PGR-treated plant most of light energy is utilized in photochemistry (Table 3).

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KAI KURIŲ AUGIMO REGULIATORIŲ POVEIKIS *VIOLA* × *WITTROCKIANA* 'WESEL ICE' CHLOROFILO FLUORESCENCIJAI

Santrauka

Darbo tikslas – nustatyti alaro (1000, 5000 mg l^{-1}), paklobutrazolio (10, 15 mg l^{-1}), alaro (1000, 2000, 3000 mg l^{-1}) ir CCC (1000 mg l^{-1}) poveikį darželinių našlaičių (*Viola* × *wittrockiana*) 'Wesel Ice' lapų chlorofilo fluorescencijai, chlorofilo a, chlorofilo b ir karotinoidų kiekiui lapuose, taip pat morfologijai. Našlaičių daigai purkšti augimo reguliatorių tirpalais 2005 m. rugpjūčio mėn. (kontrolė – vanduo). Fluorescencija matuota fluorometru FMS-1 (Hansatech, Kings Lynn, Anglija). Tirti Fo, Fm, Fv, Fv / Fm ir kiti parametrai. Dėl augimo reguliatorių poveikio fluorescencijos kvantų našumas (Fm, Fv ir Fv / Fm) buvo aukštesnis 1,6–8,3%, 4,7–10,6% ir 2,4–3,9%. Augimo reguliatorių tirpalais purkštų našlaičių lapų fotocheminis aktyvumas buvo didesnis rudenį, po žiemojimo esminio skirtumo nenustatyta.