Effect of Artificial Light Conditions on Local and Systemic Resistance Response of Tobacco to TMV Infection

Zoltán Á. NAGY^{1,3}, András JUNG², Zsófia VARGA²,
György KÁTAY¹, Attila L. ÁDÁM^{1*}

¹Centre for Agricultural Research, Hungarian Academy of Sciences, Plant Protection Institute, 15 Herman Ottó út, Budapest, Hungary; nagy.zoltan@agrar.mta.hu; katay.gyorgy@agrar.mta.hu; adam.attila@agrar.mta.hu (*corresponding author)

²Szent István University, Technical Department, Faculty of Horticultural Science, 29-43 Villányi út, Budapest, Hungary; jung.andras@kertk.szie.hu; varga.zsofia@kertk.szie.hu

³Present address: Mendel University in Brno, Faculty of Forestry and Wood Technology, Phytophthora Research Centre, Department of Forest Protection and Wildlife Management, Zemědělská 3, Brno, Czech Republic

Abstract

Our and literature studies indicated that systemic acquired resistance (SAR) is effectively inducible in greenhouse, and certain artificial light sources cause non-optimal growth of tobacco plants. Therefore, the morphological characteristics, local and systemic resistance response of *N. tabacum* cv. 'Xanthi' *nc* plants (harbouring NN resistance genes) to tobacco mosaic virus (TMV) infection under three artificial light sources with different spectral distribution were compared with greenhouse conditions. Statistical analysis of data was carried out by R package (R Core Team, 2015). Generally, artificial light sources (especially fluorescent tube and halogen lamp) decreased the local resistance response and caused substantial morphological and developmental differences as compared to greenhouse conditions when plants were kept during their entire life (lifelong experimental regime) under these conditions. On the contrary, no or much less differences were found when plants were transferred from greenhouse to artificial light sources only at six leaf stage (short experimental regime). While induction of SAR frequently decreased TMV lesion size by about 50-60% under greenhouse conditions, two of the three artificial light sources, fluorescent tube and halogen lamp were substantially and significantly less effective under short experimental regime conditions (25-35%). A metal halide light source with similarity to sunshine's spectral distribution, however, partially mimicked the effect of greenhouse conditions indicating the importance of light spectrum among other factors in SAR induction and prevention of distorted growth of plants. Consequently, the optimization of the effect of artificial light sources is an important factor in experimental design studying signal transduction and biochemistry of SAR.

Keywords: artificial light sources, spectral distribution of light, systemic acquired resistance (SAR), TMV, viral infection

Introduction

Light is one of the indispensable stimuli for plant growth and development. Light responses are responsible for de-etiolation (chloroplast development), flowering and phototropism (Kami *et al.*, 2010). These responses begin with signal perception by phytochromes as red-light photoreceptors and cryptochromes or phototropins as blue-light/UV-A receptors. The signalling downstream of photoreceptors regulates the biosynthesis of a number of phytohormones such as auxins, gibberellins, brassinosteroids, ethylene and cytokinins (Vandenbussche *et al.*, 2005).

Light can influence many other plant responses including resistance to pathogens. In the last decades research has revealed several biochemical, molecular and genetic factors relevant to the development of systemic acquired resistance (SAR) (Shah and Zeier, 2013). More recent efforts have focused on

identifying the mobile signal components of SAR responsible for the induction of broad-range resistance in the distant, non-infected, "systemic" leaves (Dempsey and Klessig, 2012; Shah *et al.*, 2014; Nagy *et al.*, 2017). Much less attention was paid to the characterization and optimization of environmental factors involved in the fine regulation of SAR. The first report on tobacco mosaic virus (TMV) induced SAR development and subsequent studies reported 70-80% inhibition of mean lesion size (or even symptoms invisible to the naked eyes) under greenhouse conditions (Ross, 1961; Vernooij *et al.*, 1994; Ádám and Nagy, 2016). Sometimes the accurate detection of lesion with small diameter (under 1 mm) in the plants with SAR was not possible (Pallas *et al.*, 1996). Later studies under artificial but regulated light conditions reported only 25-45% decrease in the mean of viral (TMV) lesion diameter after SAR induction (Manosalva *et al.*, 2010; Liu *et al.*, 2011a, 2011b; Nagy *et al.*, 2017).

In order to further characterize and optimize the effect of light quality (spectral distribution) on local and systemic (SAR development) response to TMV infection, we compared in this experiment the effects of three different artificial light sources with greenhouse conditions. Preliminary data suggested that cool white or daylight fluorescent tubes (FT) with shortage of red and far red light constituents, result in dark green and very “compact” plants with short internodes. Therefore, we have chosen an FT light source planned for plant growth. In addition, halogen lamp (HL) and metal-halide lamp (MHL) were also investigated.

Materials and Methods

Biological material and cultivation conditions

Nicotiana tabacum cv. ‘Xanthi’ *nc* plants grown in a 50-50% mixture of peat and soil were used in all experiments. Seedlings were planted in their final pot when the first two true leaves reached about 5 mm in diameter. Induction of SAR started when tobacco plants developed 5-6 fully expanded true leaves in greenhouse (but plants under FT and HL lights produced only four leaves by this time) (Fig. 1). Distance between plants and light sources was set to obtain approximately the same photosynthetically active radiation (PAR) (about 70-80 $\mu\text{mol m}^{-2} \text{s}^{-1}$). PAR intensity was measured with a BQM-SUM quantum meter (Apogee Instruments, UT, USA). However, the light intensity at summer in the greenhouse was inevitably higher (regardless the shade applied) with daily fluctuation of temperature between minimum (18 °C) and maximum (28 °C). The orientation of the long axis of the greenhouse was SE-NW, in Budapest.

Experimental design

Two kind of experimental regimes were applied: *i*) One part of potted plants was permanently irradiated with different artificial light sources in growth chambers with 10/14 h dark/light photoperiod (20 °C and 23 °C, respectively) while

other part of plants remained in the greenhouse during the whole experiment (lifelong experimental regime, LER); *ii*) in other type of experiment one part of plants was moved from greenhouse to different light sources only at the start of SAR induction (5-6 fully expanded leaves, short experimental regime, SER). During both experimental regimes resistance responses were evaluated. In order to induce SAR, the four bottom-most leaves were inoculated with the U1 strain of TMV as described earlier (Ádám *et al.*, 1990). After one week incubation the development of SAR was detected and evaluated on the leaf level 5-6 by challenge inoculation with TMV. The artificial light sources were as follows: 36 W fluorescent tubes (FT, Fluora, Osram, Germany), 70 W halogen lamps (HL, Tungstam, Hungary), 250 W metal halide lamp (MHL, Arcstream, General Electrics, Hungary). Light spectral measurements were carried out by using a hand-held spectrometer, Qmini (RGB Lasersystems, Kelheim, Germany). The measurement principle of mobile spectrometers is based on diffuse reflectance spectroscopy. It has been widely used in terrestrial spectroscopy and spectral proximal sensing for decades (Milton, 1987). The spectral range from 200 nm to 1015 nm with a spectral resolution of 0.327 nm was measured.

Statistical analysis

TMV lesion development was evaluated with ImageJ 1.48v analysis software as described earlier (Nagy *et al.*, 2016; Nagy *et al.*, 2017). Statistical calculations were carried out with R (R Core Team, 2015). For comparison of morphological and developmental characteristics, *t*-test was applied. If the data were not normally distributed, *t*-test was replaced with Mann-Whitney rank sum test (Supplementary Table 1). The Shapiro-Wilk *w*-test for normal distribution of lesion size was calculated using function ‘shapiro.test’ with its default settings. For comparison of sample means, a multiple comparison procedure was used with the R package multcomp (Hothorn *et al.*, 2008). This method tolerates well the heteroscedasticity of samples (unequal variances, non-normal distribution of data, and unbalanced group sizes often present in biological datasets) (Herberich *et al.*, 2010). In order to analyse the difference in decrease of TMV lesion size due to different light sources during SAR we compared the mean lesion size on SAR induced plants to their corresponding controls. As a standardized effect size, the proportion of lesion size on SAR induced and control plants was calculated. This calculation makes the effect sizes comparable even if the lesion size on control plants grown under different light sources differ. Confidence intervals of these proportions were determined with 5000 bootstrap resampling of the datasets with custom R scripts (Efron, 1979). TMV lesion size distributions of treatments were also calculated with function ‘density’ of R with its default settings.

Fig. 1. Effect of spectral distribution of different artificial light sources on growth and development of tobacco (*Nicotiana tabacum* cv. ‘Xanthi’ *nc*). Plants were grown under different light sources, fluorescent tubes (FT), halogen lamp (HL), metal halide lamp (MHL) and greenhouse (GH) conditions during their entire lifetime. Statistical analysis is given in Supplementary Table 1. LN: leaf number (pcs, pieces); RM: root mass (g); RL: root length (cm); SM: shoot mass (g); SL: shoot length (cm)

Results and Discussion

Comparison of the effect of different light sources on the development of plants and local resistance response to TMV infection

First the effect of the different light sources with different spectral distribution and greenhouse (GH) conditions on plant growth and development were compared (Fig. 1 and Supplementary Table 1). In these experiments plants

Fig. 2. Plants grown under various light sources in the lifelong experimental regime. Photographs were taken at the end of the experiment. Light sources from a to d: fluorescent tube, halogen lamp, metal halide lamp, greenhouse. Inserts show symptoms of TMV infection on the corresponding plants (3 dpi). White bar: 5 cm, black bar for inserts: 3 mm

developed during their entire lifetime (LER) under different artificial light sources or GH conditions. Greenhouse conditions, however, were not considered as a “control” treatment because other conditions than the light quality and quantity (exposition time, intensity and spectral distribution) may also have been different (see ‘Materials and Methods’). When plants were kept under LER, a number of growth and developmental characteristics showed substantial differences (Figs. 1 and 2, Supplementary Table 1). Especially the shoot mass (SM), shoot length (SL) and root mass (RM) were significantly higher and SM/RM ratio was low (2.32 calculated from Supplementary Table 1.) under greenhouse conditions as compared to two artificial light sources (HL and FT). FT resulted in serious stunting effect, dark green leaves with shortened internodes and reduced number of leaves (Figs. 1 and 2). HL light caused retarded growth, smaller root and shoot mass, high SM/RM ratio (24.75), long internodes and decreased number of leaves. HL and FT light showed very different spectral distribution, abundance or shortage in red and far red light, respectively (Supplementary Fig. 1). The abundance of red light putatively acts as stimulant of stem elongation (Tsuchida-Mayama *et al.*, 2010). Far red light, however, blocks germination by promoting abscisic acid synthesis (Piskurewicz *et al.*, 2009). MHL light source compensated well most of the developmental characteristics (especially number of leaves, shoot mass and length) as compared to GH conditions (Figs. 1 and 2, Supplementary Table 1). However, the highest root mass was found under GH conditions (Fig. 1, Supplementary Table 1). Among spectral distributions of light sources used, MHL was the most similar to sunshine in the greenhouse (Supplementary Fig. 1). These metal halide lamps were successfully applied either in greenhouses or phytotrones (Duke *et al.*, 1975; Tischner and Vida, 1981).

Since the lifelong experimental regime (LER) in two of the three light sources studied was not fully satisfactory in growth and development of tobacco plants (Figs. 1 and 2, Supplementary Table 1), the two growth regimes, LER and SER were compared in the next experiments. Generally, both

growth regimes, LER and SER under artificial light sources decreased the local resistance of plants to TMV infection as compared to GH conditions (Fig. 3a, b). As indicated in lesion size distribution curves (Fig. 3a), when plants developed under LER, artificial light sources significantly decreased the local resistance response to TMV infection as compared to GH conditions (Fig. 2 inserts, Fig. 3c, Supplementary Table 2). HL light source caused the highest increase in mean diameter of TMV lesions (2.784 ± 0.072 mm as compared to 1.197 ± 0.019 mm of greenhouse plants) and margins of lesions were not well characterized (Fig. 2b insert, Fig. 3a, c and Supplementary Table 2). Less but significant differences were found between GH and artificial light sources when plants were transferred from GH to different artificial light sources only after TMV infection for SAR induction (SER) (Fig. 3b and d). There are some fluctuations in lesion size distributions of GH plants of SER and LER experiments (Fig. 3a, b), but the fact that under SER conditions significant differences among different artificial light sources were not detectable (Fig. 3d) suggests the role of the length of irradiation with different light sources. Thus, the differential effect of the two growth regimes on local resistance response indicates the role of putative preformed long-lasting factors in resistance regulated at least in part by different spectral distribution of light sources. For SAR experiments, however, we chose SER conditions. This is explained among others by two main factors: *i*) under SER conditions developmental differences with different light sources were not detectable or less pronounced (data not shown) and *ii*) the difference in local resistance response to TMV infection under different light sources was also less pronounced (Fig. 3b and d).

The effect of different light sources on SAR induction capacity of plants

As compared to GH conditions, light sources with different spectral distribution influenced not only local resistance response but SAR induction capacity of plants as well (Fig. 4). After induction of SAR, the effect size (proportion of

Fig. 3. Effects of different artificial light sources and greenhouse conditions on local resistance response of tobacco plants (*Nicotiana tabacum* cv. 'Xanthi' nc) to TMV infection. Kernel density estimation of TMV lesion size distribution (a,b) and multiple comparison of means (c,d) on leaf level 5 of plants kept under various light sources for their entire lifetime (a,c) or moved from greenhouse to different light sources only after TMV inoculation (b,d). Multiple comparisons of mean TMV lesion size were also computed on leaf 5. Dots represent the difference of the estimated means between treatments. Horizontal segments flank the 95% confidence intervals. The difference is considered significant if the confidence interval does not contain the 0, represented by a vertical dashed line. GH: greenhouse; MHL: metal halide lamp; HL: halogen lamp; FT: fluorescent tube

Fig. 4. Comparison of the effect size of systemic acquired resistance (SAR) of *Nicotiana tabacum* cv. 'Xanthi' nc plants to tobacco mosaic virus (TMV) infection on leaf 5 (a) and 6 (b). Plants were incubated under various light sources during induction of SAR only. Dots: proportion of average TMV lesion size of plants after SAR induction to control. Horizontal segments represent the 95% confidence interval obtained with 5000 bootstrap resampling. The effect is considered significantly different if the respective confidence intervals do not overlap. GH: greenhouse; MHL: metal halide lamp; HL: halogen lamp; FT: fluorescent tube

mean lesion diameter of SAR induced and corresponding control plants) was evaluated in two different leaf level under different light sources. MHL and GH conditions induced higher degree of SAR response on fifth and sixth leaves (about 40-50% reduction in lesion size, Supplementary Table 2). As it was predictable from the spectral analysis, GH and MHL lights were relatively close to each other and so was their effect on SAR induction capacity (Fig. 4a and b). In addition, HL and FT lights had deficiencies in their spectra compared to natural light (GH), and their effects were also reduced (i.e. manifestation of SAR decreased). Thus, bootstrap confidence intervals in Fig. 4a and b clearly indicate that GH conditions and MHL light source significantly differ from FT and HL light sources. Despite the fact that under GH conditions light-dependent factors (for example seasonal fluctuation in photoperiod) and other environmental factors than light also can influence SAR induction, the results presented and the spectral similarity of MHL to sunshine (Supplementary Fig. 1) suggest a specific spectral distribution of light that promotes SAR induction.

Besides the present studies on spectrum of light sources, other light dependent factors were studied during SAR induction. For example duration of light radiation after bacterial infiltration influences the quality of signal transduction pathway(s) activation and the strength of SAR development afterwards (Attaran *et al.*, 2009; Liu *et al.*, 2011a). Moreover, lack of light (darkness) can completely inhibit bacterium-induced hypersensitive response and development of SAR and accumulation of salicylic acid (SA) in distant leaves in *Arabidopsis* (Zeier *et al.*, 2004). In addition, phytochrome signalling also modulates salicylic acid dependent SAR activation in *Arabidopsis*

(Genoud *et al.*, 2002). However, it is unclear at present and needs further studies whether spectral distribution of light influences SAR development via phytochrome signaling.

Conclusions

Our results clearly indicate that spectral distribution of light sources influences *i*) plant growth and development, *ii*) local resistance response to TMV infection and *iii*) SAR inducing capacity of tobacco plants. Certain light sources (FT and HL) with unbalanced light spectrum had negative impact on plant growth and development, local resistance response (especially under LER conditions) and SAR induction capacity of plants under SER conditions. However, the most optimal conditions for either plant development or SAR induction capacity were still detectable in the greenhouse. Strong plant responses are especially important features if phenotypes of SAR minus mutant plants or signalling processes are tested. On the other hand, experiments with tobacco draw the attention to optimized growth of plants that could be a more difficult problem with rosette-shaped plants. Therefore, in addition to exact detection and comparison of symptoms' expression after SAR induction (Nagy *et al.*, 2016, 2017), optimization of the effect of artificial light sources is also an important factor in experimental design studying SAR.

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Supplementary Materials

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Zoltán Á. NAGY^{1,3}, András JUNG², Zsófia VARGA²,
György KÁTAY¹, Attila L. ÁDÁM^{1*}

¹Hungarian Academy of Sciences, Plant Protection Institute, Centre for Agricultural Research, 15 Herman Ottó út, Budapest, Hungary;
nagy.zoltan@agrar.mta.hu; katay.gyorgy@agrar.mta.hu; adam.attila@agrar.mta.hu (*corresponding author)

²Szent István University, Technical Department, Faculty of Horticultural Science, 29-43 Villányi út, Budapest, Hungary; jung.andras@kertk.szie.hu; varga.zsofia@kertk.szie.hu

³Present address: Mendel University in Brno, Faculty of Forestry and Wood Technology, Phytophthora Research Centre, Department of Forest Protection and Wildlife Management, Zemědělská 3, Brno, Czech Republic

Supplementary Fig. 1. Spectral composition of light sources. For better visualisation of the curves, different constants were added to certain spectra, hence the ordinates are in arbitrary units. GH: greenhouse; HL: halogen lamp; MHL: metal halide lamp; FT: fluorescent tube

Supplementary Table 1. Morphometric data of experimental plants grown under different artificial light sources and in greenhouse during their entire lifetime

Character ^a	Light source ^b	Mean	SD ^c	n ^d	t ^e	w ^f	p ^g
LN	GH	6.06	0.18	8		–	–
LN	MHL	5.75	0.45	12		67.5	0.084
LN	HL	4.12	0.35	8		64.0	< 0.001***
LN	FT	4.31	0.46	8		64.0	< 0.001***
RL	GH	19.00	4.00	3	–		–
RL	MHL	20.67	10.40	3	0.259		0.815
RL	HL	7.00	1.00	3	5.041		0.029*
RL	FT	18.88	4.37	4	0.039		0.970
RM	GH	7.60	3.60	3	–		–
RM	MHL	1.37	0.23	3	4.222		0.050*
RM	HL	0.12	0.07	3	5.088		0.036*
RM	FT	0.63	0.22	4	4.733		0.041*
SL	GH	24.50	0.50	3	–		–
SL	MHL	25.75	3.52	4	0.700		0.532
SL	HL	12.50	3.28	3	6.267		0.022*
SL	FT	7.62	1.44	4	21.804		< 0.001***
SM	GH	17.67	1.77	3	–		–
SM	MHL	20.88	6.88	3	0.784		0.507
SM	HL	2.97	1.22	3	11.841		< 0.001***
SM	FT	10.29	2.93	4	4.138		0.009**

^aLN: leaf number (pieces); RL: root length (cm); RM: root mass (g); SL: shoot length (cm); SM: shoot mass (g).^bGH: greenhouse; MHL: metal halide lamp; HL: halogen lamp; FT: fluorescent tube. Because of the delayed development of HL and FT plants, LN was determined 10 days earlier (at 5-6 fully expanded leaves of GH plants) than other characters.^cStandard deviation.^dNumber of plants.^eData were compared to GH values in each character in a t test.^fLeaf number comparison was done with a Mann-Whitney rank sum test statistic (*w*) due to non normal data distribution.^gCorresponding *p* values for Mann-Whitney and t tests.Supplementary Table 2. Lesion sizes in TMV inoculated *Nicotiana tabacum* cv. 'Xanthi' nc plants after lifelong or short term experimental regimes under various light sources

Light type ^a	Treat-ment ^b	Leaf level	n ^c	Lesion diameter (mm)		Norm test <i>p</i> ^e	Multiple comparison ^f			
				Mean	SE ^d		Diff.	Lower CI	Upper CI	<i>p</i>
Lifelong exposition regime (LER)										
MHL	Cont.	5	472	1.718	0.021	0.0002	-0.521	-0.592	-0.449	<0.001*
FT	Cont.	5	499	1.920	0.019	0.0041	-0.723	-0.790	-0.655	<0.001*
HL	Cont.	5	78	2.784	0.072	0.0001	-1.553	-1.744	-1.362	<0.001*
GH	Cont.	5	475	1.197	0.019	0.0001	–	–	–	–
Short term exposition regime (SER)										
MHL	Cont.	5	195	1.839	0.04	0.5182	-0.282	-0.399	-0.165	<0.001*
FT	Cont.	5	408	1.842	0.021	<0.0001	-0.285	-0.364	-0.205	<0.001*
HL	Cont.	5	573	1.844	0.021	<0.0001	-0.287	-0.366	-0.208	<0.001*
GH	Cont.	5	404	1.557	0.023	0.0028	–	–	–	–
MHL	SAR	5	353	1.103	0.028	<0.0001				
FT	SAR	5	232	1.309	0.031	0.0043				
HL	SAR	5	615	1.365	0.022	<0.0001				
GH	SAR	5	519	0.773	0.018	<0.0001				
MHL	Cont.	6	57	1.714	0.055	0.3858				
FT	Cont.	6	325	1.642	0.023	<0.0001				
HL	Cont.	6	400	1.884	0.022	<0.0001				
GH	Cont.	6	403	1.556	0.023	0.1981				
MHL	SAR	6	193	0.830	0.029	<0.0001				
FT	SAR	6	245	1.139	0.024	0.1375				
HL	SAR	6	506	1.210	0.023	<0.0001				
GH	SAR	6	410	0.776	0.016	<0.0001				

^aHL: halogen lamp; MHL: metal halide lamp; FT: fluorescent tube; GH: greenhouse.^bSAR (Systemic Acquired Resistance) was induced by TMV inoculation of lower leaves as described in 'Materials and Methods'. Cont: control plants.^cn: number of lesions.^dSE: standard error of mean lesion diameters.^eShapiro-Wilk normality test. The *p* value refers to the probability of the hypothesis that the data indeed originate from normal distribution.^fResults of the test for multiple comparison of means. Data in all these four columns refer to a comparison of experimental light treatments (both lifelong and short exposition regimes) and plants kept in greenhouse as a reference. Diff: difference of mean TMV lesion diameter between the reference (GH) plants and those kept in various light sources. Lower and Upper CI: 95% confidence intervals for each difference of means. An associated *p* value labelled with * indicates a significant difference of the group means on the level of $\alpha = 0.001$.