BIOLOGIA PLANTARUM 48 (2): 167-172, 2004

Effect of radiation spectral composition on *Nicotiana* spp. seedlings grown *in vitro*

M.C. INTRIERI*, R. MULEO*[1] and M. BUIATTI**

*Department of Vegetable Production, Tuscia University, via S. Camillo de Lellis, I-10100, Viterbo, Italy**
Department of Animal Biology and Genetic, University of Florence,
*via Romana 7-25, I-50125, Florence, Italy***

Abstract

The aim of this work was to assess the responses of seedlings of five species of *Nicotiana* genus to red and far red radiation. *N. acuminata* exhibits positive photoblastic behaviour and germination was completely inhibited under far red and darkness. In *N. glauca* germination was reduced under far red and darkness, but the other species showed neutral behaviour. The hypocotyl elongation was inhibited in *N. glauca* and *N. tabacum* under white and far red radiation. In *N. langsdorffii* and *N. debneyi* hypocotyl was elongated under far red radiation. Only in *N. acuminata* red radiation promote greater hypocotyl elongation than dark condition. On the phylogenetic tree obtained from restriction analysis *N. glauca* and *N. acuminata* are grouped in one branch, while the other species, *N. langsdorffii*, *N. debneyi* and *N. tabacum*, are grouped in the other branch cluster. Moreover, the *N. debneyi* behaviours under different radiation treatments were similar to those of *N. tabacum*. These two species are allopolyploid members of the genus *Nicotiana,* as also was confirmed by this study.

Additional key words: germination, PCR-RFLP, phytochrome, plant development, tobacco.

Introduction

Plants use phytochromes, red (R) / far red (FR) photo-convertible chromoproteins, to monitor the environment surrounding them. The signal transduction cascade, originated by their activity, affects many aspects of plant development, from seed germination to flowering (Kendrick and Kronenberg 1994), as demonstrated by comparative light response studies in *Arabidopsis thaliana* mutants deficient in one of the five phytochromes (Smith 1999). Phytochromes are members of a photoreceptor family and their genes are encoded in a divergent multigene subfamily; their expression is temporally and developmentally regulated (Clark *et al.* 1994, Hauser *et al.* 1995, 1998). In *A. thaliana,* phytochrome A (*phyA*) and phytochrome B (*phyB*) are the most abundant members of the family and their role is most evident under continuous FR and R, respectively (Reed *et al.* 1994). *PhyA* and *phyB* biological activities occur under different light conditions and seem to have different transduction chains. Therefore, the relative signalling pathways might or might not converge to the same phenotypic response, *e.g*. hypocotyl growth, cotyledon unfolding, flowering (Chory and Wu 2001, Quail 2002). Thus *Phys* are involved in different phases of seedling development, from seed hydration to stem elongation and meristematic activity (Whitelam *et al.* 1998). The inhibition of hypocotyl elongation by high irradiance (R and FR) is a photomorphogenic response that has been explored in detail. Genetic studies of this process have identified unique and shared functions for *phyA* and *phyB* (Quail *et al.* 1998). The aim of this work was to assess the effects of R and FR, related to phytochrome photoreceptors, on *Nicotiana* spp. seed germination and seedling development, which are of primary importance for the understanding of the physiological mechanisms that regulate the life cycle. Five species, located in divergent branches of the *Nicotiana* evolution tree, according to botanical (Goodspeed 1954) and molecular data (Bogani *et al.*

Received 31 March 2003, *accepted* 21 January 2004.
Abbreviations: Chl - chlorophyll; D - dark; FR - far red radiation; PCR-RFLP - polymerase chain reaction-restriction fragment length polymorphism; R - red radiation; W - white light.
[1] Corresponding author; fax (+39) 0761 357531, e-mail: muleo@unitus.it

1997), were examined under white light (W), R, FR and darkness (D). Plant responses should be different and related to the specific adaptation of the species to their habitat (Botto and Smith 2002, Pepper *et al.* 2002), and to the environmental pressure on the genetic and genomic background. The *in vitro* physiological responses were also useful for pointing out the evolution of the natural species. Therefore, the *in vitro* behaviour coupled with the molecular markers can be used as a model system to stress out particular adaptations consistent with the phylogeny of the species (Bogani *et al.* 1997, Intrieri and Buiatti 2001, Piagnani *et al.* 2002).

Materials and methods

Plants and growth conditions: Seeds of analysed *Nicotiana* spp. were kindly provided by the Istituto Sperimentale per il Tabacco (Scafati, Salerno, Italy). The *Nicotiana* species used were: *N. glauca* Graham (2n=24) belonging to subgenus *Rustica* section *Paniculatae*; *N. tabacum* L. (2n=48) belonging to subgenus *Tabacum* section *Genuinae*; *N. langsdorffii* Weinmann (2n=18) belonging to subgenus *Petunioides* section *Alatae*; *N. acuminata* Hooker (2n=24) belonging to subgenus *Petunioides* section *Acuminatae* and *N. debneyi* Dimin (2n=48) belonging to subgenus *Petunioides* section *Suaveolentes*.

The seeds were sterilised using 16 % (v/v) NaClO for 20 min, subsequently they were washed three times, 10 min each time, with sterile distilled water. For each treatment, from 100 to 200 seeds were plated on 90 mm Petri plates containing 25 cm^3 of Linsmaier and Skoog (1965) culture medium, supplemented with 50 mM of sucrose and pH 5.8. The medium was sterilised at 120 °C for 20 min after the addition of 6 g dm^{-3} of *Bacto Agar* (*Difco*). All experiments were performed in growth cabinets at a temperature of 24 ± 1 °C under continuous irradiance.

Radiation of various spectral composition was provided by *Philips* (Eindhoven, The Netherland) fluorescent lamps with the following characteristics. Red radiation (R): TLD18W/15 lamps in combination with primary red polyester filter (No. 106, 0.2 mm, *Lee Filters Ltd.*, Andover, Hants, UK). FR radiation: 40 W incandescent lamps in combination with one primary red polyester filter, one light blue polyester filter (No. 118, 0.2 mm) and a congo blue polyester filter (No. 181, 0.2 mm). White light TLD18W/33 cool-white fluorescent lamps. The spectral outputs from the various lamp/filter combinations were recorded using a calibrated spectroradiometer (*LI-COR 1800*, Lincoln, USA) placed horizontally in the cabinets used for the experiments. Phytochrome photoequilibrium ($\varphi = P_{fr}/P_{tot}$) and spectral relative photon fluence rate were previously reported (Muleo *et al.* 2001). Photon fluence rates for the different treatments were 40 ± 1 μmol m^{-2} s^{-1} for W, 38.5 ± 1 μmol m^{-2} s^{-1} for R and 41 ± 1 μmol m^{-2} s^{-1} for FR. Phytochrome photoequilibrium values were the following: φ of W = 0.78, φ of R = 0.86, φ of FR = 0.05.

Parameters and statistical analysis: Three replicate plates, repeated twice, were used for each treatment. Seed germination data were detected at 7 and 14 d after sowing. Measurements of hypocotyl and root growth were performed after 14 d. The hypocotyl and root lengths were measured on 30 seedlings chosen randomly. The mean value of all repeated independent experiments and the double value of the standard error of the mean are shown.

Chlorophyll determination: Ten seedlings of each species for each treatment were placed in 2 cm^3 of N,N-dimethylformammide and kept for 24 h in the dark at a temperature of 4 °C. Chlorophylls and protochlorophyll were determined following the Moran's protocol (1982), by spectrophotometric measurements of absorbance (*Uvicon860*, *Konotron Instruments*, Walford, UK) at 625, 647, 664 nm.

DNA isolation, PCR amplification and restriction analyses: Total DNA was isolated from fresh leaf tissue by Doyle and Doyle (1989) methods. After RNAse treatment, it was purified once with phenol:chloroform extraction and ethanol precipitation; finally DNA was maintained in standard Tris-EDTA buffer. PCR forward and reverse primer sequences used, designed on published *N. tabacum phyA* sequence (Adam *et al.* 1993), were GTGACACTATGGTTCAGGAG and GAGCTACTGGCATCAGCATA, respectively. Gene amplifications were performed by mixing in a total volume of 0.05 cm^3, 100 ng of total DNA, 25 ng of each of the forward and reverse primers, 200 μM of deoxynucleotide mixture, 0.5 U of Taq polymerase (*Amersham Biosciences*, Little Chalfond, UK) and 1X of provided Taq-reaction buffer. After an initial step of 5 min at 94 °C, PCR was carried out for 30 cycles of 1 min at 94 °C, 1.5 min at 57 °C and 1.85 min at 72 °C. A final incubation of 5 min at 72 °C was performed to ensure complete extension. PCR product direct restriction analyses were performed overnight by mixing 0.02 cm^3 of amplification reactions, 2 U of suitable enzyme (*Boehringer Mannheim*, Milano, Italy), in a total reaction volume of 0.025 cm^3, also containing 1X of enzyme buffer. Amplification products and restriction fragments were visualised on agarose or acrylammide gel, ethidium bromide stained, prepared at suitable concentration (Sambrook *et al.* 1989). The data were processed by means of *NTSYS-pc2.02i* (*Exeter Software*, New York, USA) for matrix computation and to obtain phylogenetic grouping.

Results

Germination was detected at days 7 and 14 after sowing. At day 7, roots and cotyledons were completely developed in all of the seedlings under W (Table 1). No more germinated seedlings were observed at day 14. Seed germination of *N. acuminata* was completely inhibited under FR and dropped down to 3.6 % under D in *N. glauca*, in which the number of germinated seeds was lower under D than under W, and the higher germination rate was under R (83.6 %) than under FR (56.6 %). The germination ratio of *N. acuminata* and *N. glauca* seeds was similar under W and R. The other three species roughly displayed similar germination patterns even though some differences were observed. A reduction in germination was detected for *N. tabacum* when seeds were kept under FR, as well as for *N. debneyi* and *N. langsdorffii*; this latter species also displayed inhibition under D (80.6 %).

Table 1. Germinated seeds [%] after 7 d from the beginning of culture. Different letters along the row means statistically significant differences, as determined by χ^2 for $P = 0.05$.

Species	White	Red	Far-Red	Dark
N. glauca	71.5^{b}	83.6^{a}	56.6^{c}	33.6^{d}
N. tabacum	94.9^{a}	98.3^{a}	88.3^{b}	99.2^{a}
N. langsdorffii	90.9^{a}	90.5^{a}	72.8^{b}	80.6^{b}
N. acuminata	71.6^{a}	80.4^{a}	0	3.6^{b}
N. debneyi	71.4^{b}	81.6^{a}	65.6^{b}	83.6^{a}

W and R induced the highest chlorophyll (Chl) synthesis, irrespectively of the species studied (Table 2). Among the species, *N. langsdorffii* showed the highest amount of Chl under all treatments compared to the other species. In general, under R, the seedlings synthesised twice the amount observed under W. *N. langsdorffii* was also partially reversed by FR, while in *N. tabacum*, the opposite behaviour was detected, mainly due to the reduction in Chl *a* synthesis. In *N. debneyi*, the Chl *a*/*b* ratios under W and R were similar; on the contrary, an R/FR action was clearly evident on Chl *a* synthesis. Protochlorophyll is accumulated differently between species and between different radiation treatments. Only in *N. tabacum* is a clear accumulation due to R and FR condition evident.

Hypocotyl lengths of seedlings (Table 3) grown under W were similar, except for *N. acuminata*, which showed longer hypocotyl (almost 48 %). Seedlings grown in D exhibited increased hypocotyl elongation for all species, except for *N. glauca*, which showed the smallest hypocotyl elongation (not detectable). Under R, seedlings of all species exhibited increased hypocotyl elongation compared to seedlings grown under W (Table 3). This enhanced hypocotyl elongation was higher for seedlings of *N. langsdorffii* (536 %), *N. debneyi* (391 %), *N. acuminata* (345 %) than for *N. glauca* (194 %) and *N. tabacum* (147 %). Seedlings of *N. glauca* and *N. tabacum* displayed inhibition of hypocotyl elongation under FR, as it has previously observed under W.

Table 2. Total chlorophyll (Chl) and protochlorophyll (Protochl) content [μg seedling^{-1}]. Values represent means ± 2 SE.

Species	Radiation	Chl *a*+*b*	*a*/*b*	Protochl
N. glauca	W	0.687 ± 0.167	1.0	0.211 ± 0.078
	D	n.d.	n.d.	n.d.
	R	n.d	n.d.	n.d.
	FR	n.d	n.d.	n.d.
N. tabacum	W	0.633 ± 0.088	3.0	0.047 ± 0.016
	D	0.201 ± 0.042	0.6	0.084 ± 0.016
	R	1.657 ± 0.015	3.4	0.142 ± 0.024
	FR	0.461 ± 0.138	2.6	0.156 ± 0.067
N. langsdorffii	W	2.450 ± 0.311	2.8	0.184 ± 0.044
	D	0.384 ± 0.035	0.8	0.166 ± 0.019
	R	4.659 ± 0.327	2.8	0.164 ± 0.031
	FR	0.818 ± 0.081	3.5	0.121 ± 0.040
N. acuminata	W	0.633 ± 0.078	2.4	0.140 ± 0.040
	D	n.d.	n.d.	n.d.
	R	1.058 ± 0.084	2.7	0.174 ± 0.030
	FR	n.d.	n.d.	n.d.
N. debneyi	W	1.533 ± 0.067	2.8	0.136 ± 0.033
	D	0.394 ± 0.198	1.1	0.184 ± 0.097
	R	1.476 ± 0.075	3.2	0.148 ± 0.043
	FR	0.391 ± 0.015	3.2	0.099 ± 0.009

Under D and R, *N. glauca* root development was blocked, after the extrusion of the primary root from the coating (Table 3), while the root elongated under both FR and W. Root elongation of *N. tabacum* seedlings appeared similar under all treatments, with an increased elongation in D and inhibited elongation under FR. In this species, the root elongation might be related to hypocotyl growth. D, FR and R increased the elongation of primary roots of *N. debneyi* and *N. langsdorffii*. Primary root growth of *N. acuminata* was strongly inhibited under D but not under R. The hypocotyl/root length ratio was higher in seedlings grown in D. *N. glauca* shows the highest value under R. For R and FR, the values were similar for all the other species analysed and were larger than under W except for *N. glauca*. *N. langsdorffii* showed a higher growth rate under FR than under R.

PCR primers (*phyA*) amplified the expected fragment in all of the species. Four restriction enzymes of the twelve used were able to evidence site polymorphisms. As results of all restriction experiments, the restriction profiles of all enzymes and species are reported (Table 4).

Table 3. Hypocotyl and root length of seedling grown under W, D, R and FR (ng - not germinated, nd - not detected; means ± 2 SE) and hypocotyl length/root length ratio (HY/RO). The values of hypocotyl and root elongation normalised to the corresponding length in W are indicated as HEI and REI.

Species	Radiation	Hypocotyl length	Root length	HY/RO	HEI	REI
N. glauca	W	0.214 ± 0.010	0.123 ± 0.012	1.740	1.000	1.000
	D	nd	nd			
	R	0.416 ± 0.015	0.052 ± 0.006	8.000	1.944	0.423
	FR	0.212 ± 0.022	0.130 ± 0.022	1.631	0.991	1.057
N. tabacum	W	0.243 ± 0.019	0.212 ± 0.023	1.146	1.000	1.000
	D	2.189 ± 0.106	0.309 ± 0.026	7.084	9.008	1.457
	R	0.358 ± 0.027	0.227 ± 0.021	1.578	1.473	1.071
	FR	0.197 ± 0.014	0.126 ± 0.008	1.563	0.811	0.594
N. langsdorffii	W	0.188 ± 0.012	0.321 ± 0.022	0.586	1.000	1.000
	D	1.990 ± 0.116	0.338 ± 0.031	5.887	10.585	1.053
	R	1.009 ± 0.146	0.545 ± 0.042	1.851	5.367	1.698
	FR	1.199 ± 0.084	0.545 ± 0.050	2.200	6.378	1.698
N. acuminata	W	0.505 ± 0.039	0.406 ± 0.078	1.244	1.000	1.000
	D	1.270 ± 0.384	0.190 ± 0.011	6.684	2.515	0.468
	R	1.743 ± 0.226	0.739 ± 0.076	2.358	3.451	1.820
	FR	ng	ng			
N. debneyi	W	0.298 ± 0.024	0.442 ± 0.078	0.674	1.000	1.000
	D	1.542 ± 0.124	0.630 ± 0.058	2.448	5.174	1.425
	R	1.167 ± 0.090	1.046 ± 0.176	1.116	3.916	2.366
	FR	0.554 ± 0.072	0.850 ± 0.114	0.652	1.859	1.923

Table 4. For each enzyme, the corresponding sizes of the restricted fragments are shown. *N.g.* - *N. glauca*, *N.t.* - *N. tabacum*, *N.l.* - *N. langsdorffii*, *N.a.* - *N. acuminata*, *N.d.* - *N. debneyi*.

	[bp]	*N.g.*	*N.t .*	*N.l.*	*N.a.*	*N.d.*
TaqI	515	0	0	0	0	1
	371	0	1	0	0	0
	261	1	1	1	1	1
	220	1	1	1	1	1
	110	1	1	1	1	1
	100	0	0	0	0	1
	24	1	1	1	1	1
AluI	246	0	0	1	0	0
	227	1	1	1	1	1
	160	1	1	0	1	1
	142	1	1	1	1	1
	86	1	1	0	1	1
Tru91	615	1	0	0	0	0
	355	0	1	1	1	1
	260	0	1	1	1	1
HindIII	370	1	1	1	0	1
	245	1	1	1	1	1
	200	0	0	0	1	0
	170	0	0	0	1	0

In particular, the enzyme HindIII shows two restriction sites in *N. acumimata* and one in all the others, originating different numbers of fragments. The position of the Tru91 site is common for all species, and it is not present in *N. glauca*. The enzyme TaqI shows polymorphic sites and the presence of two copies of the *phyA* gene in *N. tabacum* and *N. debneyi*, known as allopolyploid species. In fact, when the PCR products were digested with TaqI, the two copies of the gene were distinguishable by the presence of two different and overlapping restriction patterns. For example, in *N. tabacum*, one of the copies has 2 sites (originating the fragments: 24 + 371 + 220 bp) for TaqI, while the other copy shows 3 sites (24 + 261 + 115 + 220 bp). The AluI enzyme also permits detecting site polymorphisms in *N. langsdorffii* but not the two copies of *phyA* in *N. tabacum* and *N. debneyi*, the restriction sites analysed being similar for the two copies derived from the two ancestral subgenomes. The other three diploid species do not show the presence of two copies with the 12 enzymes tested. The restriction profiles of all enzymes and species (Table 4) were reported in a 0/1 matrix of site absence/presence. The data were processed by *NTSYSpc 2.02i* to obtain a Simple Matching matrix. A Neighbour Joining tree was found and reported in Fig. 1. Despite the low number of species, one cluster groups *N. langsdorffii* and *N. debneyi*, from the *Petunioides* subgenus, and *N. tabacum*, an allopolyploid derived from a species of the same subgenus (*N. sylvestris*). The second cluster groups *N. acuminata* and *N. glauca*, far from a phylogenetical point of view.

Discussion

From germination and hypocotyl growth patterns, photoblastic positive behaviour was found in *N. acuminata* and *N. glauca,* while the other three species showed neutral behaviour in regard to the light condition. This is in accordance with botanical data (Goodspeed 1954). In particular, *N. acuminata* (herbaceous species) and *N. glauca* (shrub species), under natural conditions, are heliophylous rapidly growing plants, producing several branches, and the germination under shade conditions could be related to the subsequent reduction of plant fitness.

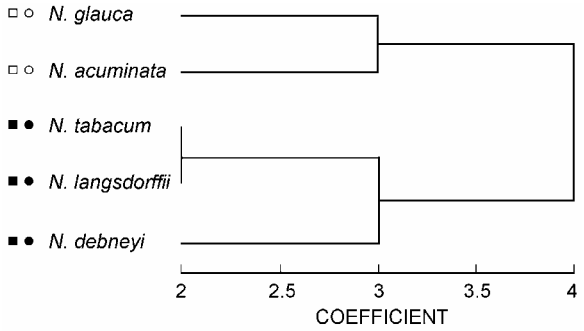

Fig. 1. Dendrogram of restriction site obtained by *NTSYS-pc*. Symbols located on the left show the germination behaviour and the elongation of hypocotyl under the different radiation quality (□ - photoblastic positive, ■ - photoblastic neutral, ● - long hypocotyl in D, ○ - long hypocotyl in R).

Hypocotyl and root elongation were used to differentiate *Arabidopsis* accessions using different R/FR ratios (Botto and Smith 2002) or W, R, FR and blue enriched radiation (Pepper *et al.* 2002), and to study the action of CRY1 and PHYA on hypocotyl growth inhibition (Ahmad *et al.* 2002). The regulation of hypocotyl growth is modulated by the best fitness strategy for obtaining an apical structure of the plant to receive the correct radiation. Under R, the *N. glauca* hypocotyl is 8 times longer than the rootlet, while under FR and W, growth was reduced. Under R, only PHYB exists in the active form, while PHYA and CRY1 are not activated under FR and W, respectively. This indicates that PHYB counteracts the negative action on hypocotyl growth of the two former photoreceptors, as already reported for *A. thaliana* (Folta and Splanding 2001). Similar behaviour is also found in *N. acuminata*. Shade avoidance behaviour is found in all species: *N. tabacum* (herbaceous), *N. langsdorffii* (herbaceous), *N. debneyi* and *N. acuminata* (herbaceous). In this scenario, PHYA seems to have the function of perceiving the irradiance level. With the exception of *N. langsdorffii*, under FR the hypocotyl is shorter than under R. It is a typical FR-HIR due to the inhibition of PHYA action.

Although we cannot exclude natural variation among accessions of the single species studied, as found in *A. thaliana* (Maloof *et al.* 2001), the *in vitro* condition used in this work is indicative of the genetic background of the peculiar species response. The different behaviour can be partially explained by the phylogenetic position of the species analysed. In fact, if in the same genus the photobiological behaviour is different in different species, a species like *N. debneyi* close to *N. langsdorffii* and *N. acuminata* shows quite independent behaviour.

Further indications on species differentiation also came from the restriction analyses of the *phyA* DNA portion. Although the region analysed is a relatively conserved one, there is a sufficient number of informative sites able to distinguish between the species. In *A. thaliana*, a similar relationship between phenotype and genotype was found by Maloof *et al.* (2001). The dendrogram, based on molecular analyses, shows a clusterization that is consistent with the photomorphogenic behaviour of hypocotyl growth under R and D, clustering the *N. glauca* and *N. acuminata* in one branch, different than that of the other species. It should also be noted that the *N. debneyi* and *N. tabacum* responses to different radiation treatments are similar, and both of these species are allopolyploid members of the genus *Nicotiana*; further studies will be necessary to understand if the presence of two different copies of *phyA*, or of all the other photoreceptors, could condition the photobiological responses. It can be hypothesised that the presence of two copies of the genes confers greater physiological plasticity to the hybrid plant.

References

Adam, E., Deak, M., Kay, S., Chua, N.H., Nagy, F.: Sequence of a tobacco (*Nicotiana tabacum*) gene coding for type A phytochrome. - Plant Physiol. **101**: 1407-1408, 1993.

Ahmad, M., Grancher, N., Heil, M., Black, R.C., Giovani, B., Galland, P., Lardemer, D.: Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in *Arabidopsis*. - Plant Physiol. **129**: 774-85, 2002.

Bogani, P., Liò, P., Intrieri, M.C., Buiatti, M.: A physiological and molecular analysis of the Genus *Nicotiana.* - Mol. Phylogen. Evol. **1**: 62-69, 1997.

Botto, J.F., Smith, H.: Differential genetic variation in adaptive strategies to a common environmental signal in *Arabidopsis* accessions: phytochrome-mediated shade avoidance. - Plant Cell Environ. **25**: 53-63, 2002.

Chory, J., Wu, D.: Weaving the complex web of signal transduction. - Plant Physiol. **125**: 77-80, 2001.

Clark, T., Mathews, S, Sharrock, R.A.: The phytochrome apoprotein family in *Arabidopsis* is encoded by five genes:

the sequences and expression of PHYD and PHYE. - Plant mol. Biol. **25**: 413-27, 1994.

Doyle, J.J., Doyle, J.I.: Isolation of plant DNA from fresh tissue. - Focus **12**: 13-15, 1989.

Folta, K.M., Spalding, E.P.: Opposing roles of phytochrome A and phytochrome B in early cryptochrome-mediated growth inhibition. - Plant J. **28**: 333-340, 2001.

Goodspeed, T.H.: The Genus *Nicotiana.* - Chronica Botanica, Walthum 1954.

Hauser, B.A., Cordonnier-Pratt, M.M., Daniel-Vedele, F., Pratt, L.H.: The phytochrome gene family in tomato includes a novel subfamily. - Plant mol. Biol. **29**: 1143-55, 1995.

Hauser, B.A., Cordonnier-Pratt, M.M., Pratt, LH.: Temporal and photoregulated expression of five tomato phytochrome genes. - Plant J. **14**: 431-439, 1998.

Intrieri, M.C., Buiatti, M.: The horizontal transfer of *Agrobacterium rhizogenes* genes and the evolution of the genus *Nicotiana*. - Mol. Phylogen. Evol. **20**: 100-110, 2001.

Kendrick, R.E., Kronenberg, G.H.M.: Photomorphogenesis in Plants. 2nd Edition. - Kluwer Academic Publishers, Dordrecht 1994.

Linsmaier, E.M., Skoog, F.: Organic growth factor requirements of tobacco tissue cultures. - Physiol. Plant. **18**: 100-127, 1965.

Maloof, J.N., Borevitz, J.O., Dabi, T., Lutes, J., Nehring, R.B., Redfern, J.L., Trainer, G.T., Wilson, J.M., Asami, T., Berry, C.C., Weigel, D., Chory, J.: Natural variation in light sensitivity of *Arabidopsis*. - Nat. Genet. **29**: 441-446, 2001.

Moran, R.: Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. - Plant Physiol. **69**: 1376-1381, 1982.

Muleo, R., Morini, S., Casano, S.: Photoregulation of growth and branching of plum shoots: physiological action of two photosystems. - *In Vitro* cell. dev. Biol. Plant **37**: 609-617, 2001.

Pepper, A.E., Corbett, R.W., Kang, N.: Natural variation in *Arabidopsis* seedling photomorphogenesis reveals a likely role for TED1 in phytochrome signalling. - Plant Cell Environ. **25**: 591-600, 2002.

Piagnani, C., Iacona, C., Intrieri, M.C., Muleo, R.: A new somaclone of *Prunus avium* shows diverse growth pattern under different spectral quality of radiation. - Biol. Plant. **45**: 11-17, 2002.

Quail, P.H.: The phytochrome family: dissection of functional roles and signalling pathways among. - Phil. Trans. roy. Soc. London B **353**: 1399-1403, 1998.

Quail, P.H.: Photosensory perception and signalling in plant cells: new paradigms? - Curr. Opinion Cell Biol. **14**: 180-188, 2002.

Reed, J.W., Chory, J.: Mutational analyses of light-controlled seedling development in *Arabidopsis*. - Semin. Cell Biol. **5**: 327-334, 1994.

Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning: a Laboratory Manual. - Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989.

Smith, H.: Phytochromes. Tripping the light fantastic. - Nature **400**: 710-713, 1999.

Whitelam, G.C., Patel, S., Devlin, P.F.: Phytochromes and photomorphogenesis in *Arabidopsis*. - Phil. Trans. roy. Soc. London B **353**: 1445-1453, 1998.