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Interaction of brassinosteroids and cytokinin in modulating light mediated signaling in *Arabidopsis*

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Abstract

Brassinosteroids (BRs) are a special class of plant steroid hormones that are essential for normal growth and development. Part of confusion is whether BRs are unique to plants, because they have overlapping physiological roles with other better-studied hormones and with physiological responses caused by light. In systems designed to assay for cytokinins, the effects of BRs vary. We measured hypocotyl length for testing the ability of brassinolide (BL) to rescue double mutant between *det2* and the photoreceptor null mutant phytochrome B (*phyB*). *PHYB* involved in controlling hypocotyl elongation in increased concentration of BL whereas *phyBdet2* double mutant just partially rescue to *phyB* in white and red light indicated the involvement of BRs in *PHYB* regulated cell elongation. BRs regulated hypocotyl growth was delayed by BAP, a cytokinin treatment but inhibitory effects of BAPs on hypocotyl growth was slightly recovered by BL. The result indicated that the mode of action of BR and cytokinin is independent or sequential in the downstream light-regulated response control on hypocotyl elongation and also light modulated the action of BR and cytokinin in some extent.

Key Words : brassinosteroid, phytochrome, hypocotyl elongation, plant hormone

1. Introduction

Plant perceives multiple of signals to carry out overall growth and development processes either from the cellular or extra cellular environments. The regulation of plant growth and development by light requires the action of several plant hormones (Chory and Li, 1997). But the molecular mechanisms to which light regulates the development are largely unknown except for photoreceptors themselves (Quail *et al*., 1995; Neff *et al*., 2000). The photoreceptors are most sensitive to red and far-red lights that trigger their accumulation in nucleus from cytoplasm. Genetics should help to dissect the signaling network, but it is difficult to determine whether light acts directly affects on developmental response or the metabolism or action of plant hormones is involved in sequence of events initiated by photoreceptors (Chory and Li, 1997). In *Arabidopsis* many de-etiolating events such as hypocotyl growth inhibition and cotyledon expansion are controlled by blue, red and far-red light. In genetic scenes, *Arabidopsis* seedlings with long hypocotyls and less-developed cotyledons have been identified as null mutants lacking of photoreceptors (Mathews and Sharrock, 1997; Neff *et al*., 1999). *phyB* mutants lack of *PHYB* (Reed *et al*., 1994) were identified as having long hypocotyls in continuous white light (Koornneef *et al*., 1980). Mutants *phyB* are defective in apoprotein components of *PHYB* that are mediated de-etiolation responses to red and far-red light, respectively (Nagatani *et al*., 1993; Parks and Quail, 1993; Reed *et al*., 1994; Pepper and Chory, 1997). The *phyB* mutants are impaired in numerous processes including seed germination, seedling de-etiolation in red light, shade avoidance and transition to flowering (Fankhauser, 2002). *Arabidopsis* mutant *det2* also has been isolated that resemble light-grown in dark and leads to defects in light-regulated development that can be ameliorated by application of brassinolide (BL) (Chory *et al*., 1996). The active compound of “brassin” a phytosterol brassinolide structurally resembles with the insect hormone ecdysone (Grove *et al*., 1979). BL, the end product of the brassinosteroid (BR) biosynthetic pathway, is derived from a major end product of the phytosterol

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synthesis route called campesterol (Benveniste, 1986; Yokoda, 1997). BRs were reported to act synergistically with auxin and additively with gibberellin to promote hypocotyls and stem elongation respectively; to stimulate ethylene biosynthesis by activation of ACC-synthase, and in general to stimulate protein, RNA, DNA synthesis and photosynthetic carbon-fixation in various plant species (Mandava, 1988; Yamamoto *et al.*, 1997; Clouse and Sasse, 1998; Altmann, 1999). Cytokinin can completely override the necessity for light to induce leaf and chloroplast and light induced gene expression and inhibition of hypocotyl elongation (Chory *et al.*, 1994; Cerdan and Chory, 2003). To understand how BRs and cytokinin interact in modulation of light signaling on photomorphogenesis and development, we have tried to search relationship between BRs and photoreceptor action in controlling hypocotyl elongation responses in *Arabidopsis* and also BRs action to light and cytokinin signal transduction pathways.

2. Materials and methods

2.1. Plant material and growth conditions

Arabidopsis thaliana used in this study was ecotype Columbia. Each of the studied mutants (*det2*, *phyB* and *phyBdet2*) has a null allele and also they all are from Columbia background. Double mutant line seeds were kindly provided by J. Chory (Salk Institute, USA). Seeds were surface sterilized by washing for 20 min in 70 % (v/v) ethanol containing 0.05 % (v/v) Triton X-100, followed by a wash with 95 % (v/v) ethanol. Following the sterilization, seeds were dried on filter paper under sterile conditions and sown on 0.5 x Murashige-Skoog (MS) medium (Gibco-BRL, Cleveland) supplemented with 1 % (v/w) sucrose and 0.8 % (v/w) phytoagar (Life technologies, Grand Island, NY). BL was purchased from CID tech Research (Mississauga, Ontario, Canada). The plates were wrapped in aluminum foil and treated in dark for 4-days at 4 ℃ to synchronize germination. Seedlings were grown in environmental controlled growth room at 21 ℃ under long-day conditions (16 hours of light and 8 hours dark). Cool–white 35 W fluorescent bulbs (F24T12/CS/HO, Sylvania) and 25 W clear incandescent bulbs (25T10/CS/HO, General electric) were used to supply white light. To supply red light, 20 W fluorescent bulbs (F20T12/GRO Gro-LUX, Sylvania) filtered through red acrylic (Gavrieli no.2423, Ridout plastics, San Diego, CA) were used. Similarly, fluorescent bulbs (Sylvania) filtered through blue glass (no. 5-57 Kopp Glass, Pittsburgh, PA) were used for blue light. The far-red light source was in chambers of light emitting diodes (Percival Scientific) and measured with a portable spectrodiometer (model LI-1800, Li-cor, Lincoln, NE).

2.2. Genetic analysis

Hypocotyl lengths were measured by scanning seedlings that were sandwiched between two sheets of acetate in a flatbed scanner setting sufficient to identify the transition between hypocotyls and root. All digitized images were analyzed with imaging software from National Institute of Health (NIH).

2.3. Hormone response assays

Seeds were germinated and grown on 0.5 x MS supplied with 1 % sucrose and 0.8 % agar plates containing varying concentration of BL and BAP. Hypocotyl length of individual seedling was measured after 7 days growing in dark growth chamber at 21 ℃. For each category 50 to 80 seedlings were measured and their average hypocotyls lengths were used to compare hormone dose responses.

2.4. Data analysis

Data obtained were analyzed in sigmaplot version 9 and expressed in terms of line and bar graphs.

3. Result and discussion

Sunlight and hormones activate several photoreceptors mediating de-etiolation, however whether the hormone and light effects independent or synergistic in controlling over all growth response is still not answered. It is well established that light perceived by phytochromes strongly affect growth and development throughout the life cycle of plants (Luccioni *et al.*, 2002). It has become increasingly clear that light and hormonal signaling interact at several levels (Fankhauser, 2002). BRs may constitute a distinct class of phytohormones with an important role in light-regulated development of higher plants (Li *et al.*, 1996). To study how light interacts with BRs and cytokinin for endogenous developmental programs, we used double mutant analysis with photoreceptor null mutant, *phyB*, BR defi-

cient mutant *det2* and mutants *phyBdet2* including wild type Columbia 0 (Col-0).

Phytochrome B, a type II phytochrome stable in the light (Furuya 1993; Sarrock and Clack, 2002), is responsible for low influence responses red light and high irradiance responses (Nagy and Schafer, 2002). The ARR4, a response regulator works downstream of the cytokinin receptor, regulators found interacted to the amino-terminal extension of PHYB can modulate the light signaling (Fankhauser, 2002) and binds preferentially to and stabilizes Pfr form of PHYB (Chen *et al.*, 2004). A test of whether *PHYB* mediates light regulation in plants, we used phytochrome null mutants lacking photoreceptors owing to have long hypocotyls and less-developed cotyledons. *phyB* inhibits the hypocotyls elongation in red light (Yamaguchi *et al.*, 1999) however *PHYB* contributes redundancies in R:FR signaling (Vandenbussche *et al.*, 2005).

It is well-established fact that Cytokinin has been implicated in many developmental processes and environmental responses of plants and controls cell division in combination with auxin. Cytokinin has been reported to mimic some of the effects of light on de-etiolation responses (Jain *et al.*, 2006) in dark-grown *Arabidopsis* seedlings (Su and Howell, 1995) and is a negative regulator of hypocotyls elongation (Cary *et. al.*, 1995). The interrelationship between cytokinin with BR is still unknown. The ARR4 regulators found interacted to PHYB can modulate the light signaling and also ARR4 transcript levels are increased in response to cytokinin, the ARR4 expression is regulated post-transcriptionally in light response regulator (Huchinson and Kieber, 2002). The report of light-dependent ARR4 protein accumulation and interaction between the red light photoreceptor PHYB provides the direct link between light and cytokinin signaling (Sweere *et al.*, 2001; Hutchison and Kieber, 2002).

The question of whether BRs are involved in light regulated development or in the *PHYB* light signaling pathway is still open. Double mutant plants have a wild type appearance in most responses. If BRs act independently to the photoreceptors, BRs treated double mutant will have the appearance of photoreceptor mutant parent under appropriate light condition. We measured the ability of BL to rescue double mutants between *det2* and *phyB*. *PHYB* control the cell elongation when concentration of BL is increased, but

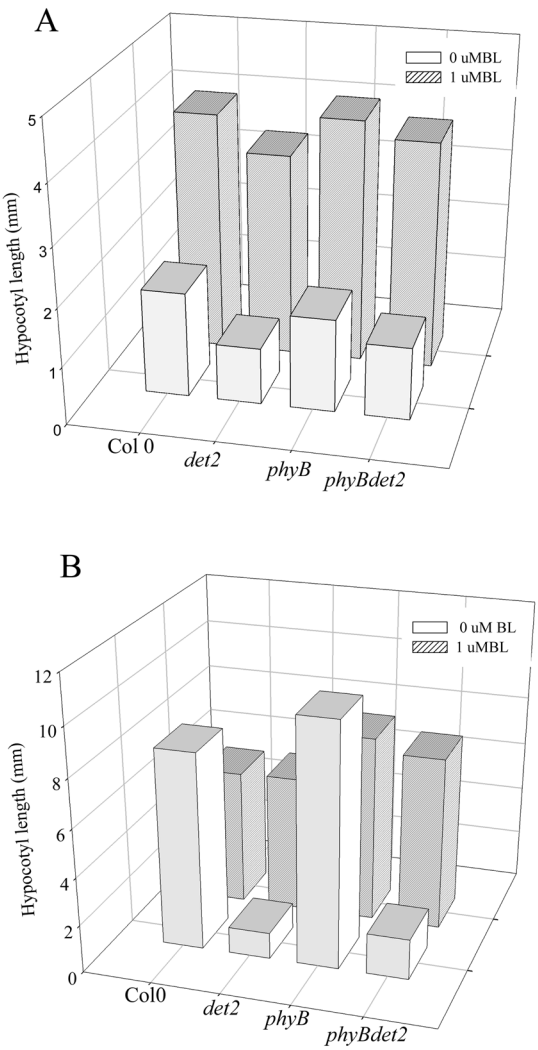

Fig. 1. Relationship of brassinosteroids action in mutants. BL (1 μM) dose response *det2*, *phyB* and *phyBdet2* under blue, and red. light conditions, **A.** BL dose response under 6mEi blue light. *phyBdet2* hypocotyl length was shorter than wild type in very low blue light (6Ei). **B.** BL dose response under Red light. *phyBdet2* hypocotyls were taller than wild type but does not fully rescue to *phyB* hypocotyl length under 10 $\mu m^{-2}S^{-1}$ of red light.

phyBdet2 was not fully rescued to *phyB* in white, red and blue lights (Fig. 1A, 1B and Fig.3B) indicating BRs involved in *PHYB* regulated hypocotyl elongation. Hypocotyl growth inhibition was primarily driven by *PHYB* and *phyB* mutant had hypocotyls approximately 1.5 fold longer than wild type (Neff and Chory, 1998).

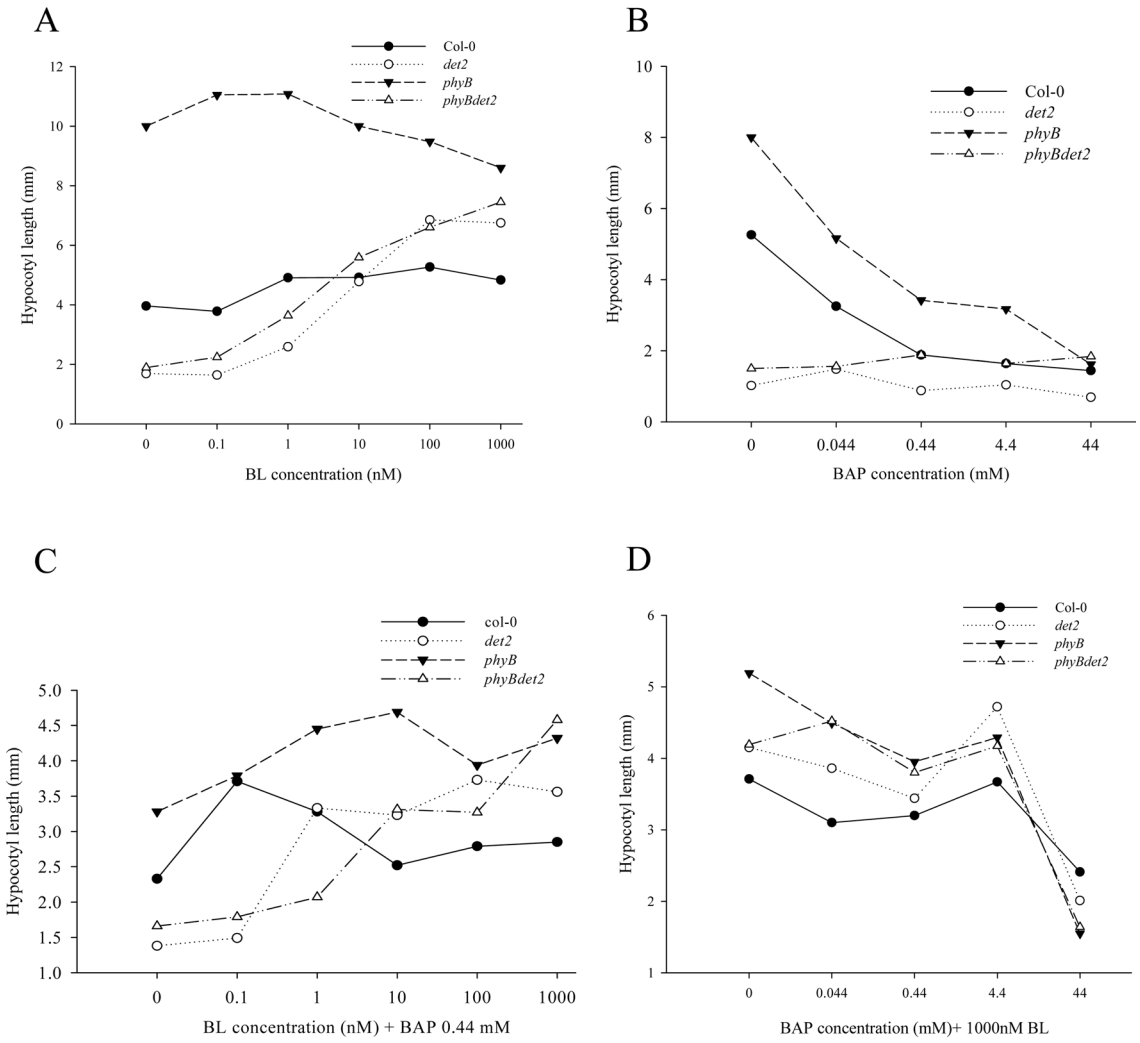

Fig. 2. Effects of BAP and BL on hypocotyl lengths of dark grown *Arabidopsis* seedling. Seedlings were grown for 5 days in the dark on 0.5 X MS medium supplemented with BAP and BL. **A.** Dose response varied from 0 to 1 uM of BL. **B.** Dose response varied from 0 to 44 mM of BAP. **C.** BAP dose response varied from 0 to 44 mM with 1 μM BL. **D.** BL dose response from 0 to 1 μM with 0.44 mM BAP.

Sweere *et al.*, (2001) suggested that PHYB might be the target of a hormone-modulated signaling system rather than the sensor kinase itself. DET2 may play a negative role in the temporal elaboration of light responses during *Arabidopsis* development (Chory *et al.*, 1989a/b, 1991; 1994) noted that cytokinin could mimic some of the de-etiolation effects of light and produce a *det2* mutant phenotype in wild-type seedlings. Hypocotyl examined on 7 days dark-grown seedlings showed shorter length with increased concentration of BAP (0 to 44 mM) (Fig. 2B) and taller with increased the concentration of BL (Fig. 2A). BL treatments slightly recovered the inhibition action of BAP on hypocotyl growth (Fig. 2C and 2D) and BAP treatments also delayed the BL action that regulates the hypocotyl elongation (Fig. 2D). Addition of BL only could not fully rescue the *phyBdet2* to *phyB* phenotype but it was achieved with addition of BAP (Fig. 2B, 2C). Chory (1993) showed that neither functional phytochrome nor blue-light photoreceptors are needed for the cytokinin

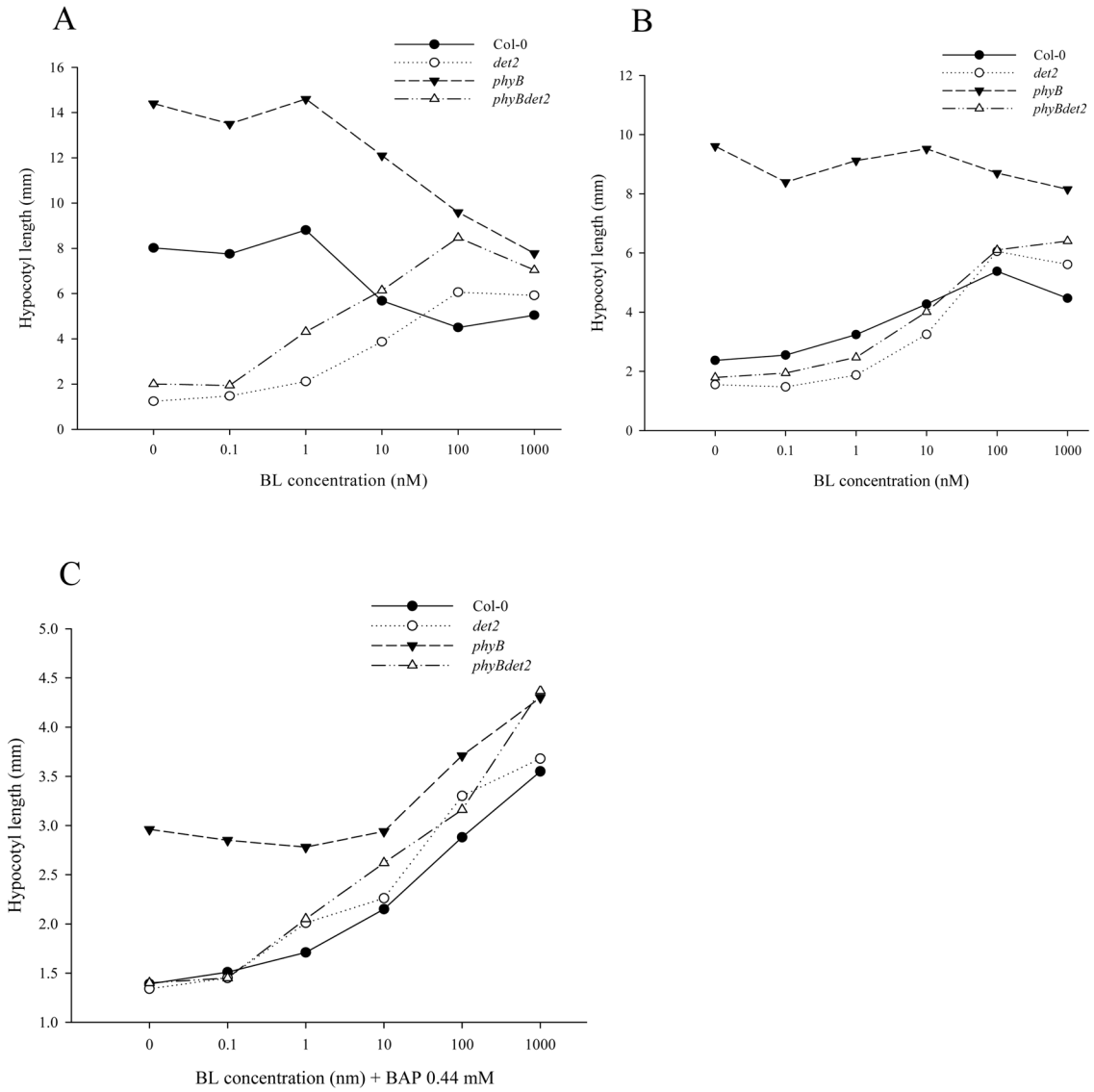

Fig. 3. Effect of various concentration of BL and addition of BAP under white, and red light condition. **A.** Effect of various concentration of BL under red light **B.** Under white light and **C.** Effects of different concentration of BL and 0.44 mM BAP under white light.

response. However, the cytokinin resistance in *phyB* might be construed to represent a direct involvement of the PHYB apoprotein in cytokinin responses (Su and Howell, 1995). BL treatment to *phyB* under red light and white light showed similar trend of hypocotyls growth retardation, which in treatment with BAP regularized the growth increase (Fig. 3A,3B and 3C). Growth retarded win BL under white light alone treatment reversed with the treatment of BAP (Fig. 3B,3C). The *phyBdet2* double mutant shows the partial similarities with wild type phenotype in BL-BAP treatment under white light (Fig. 3C) indicating the involvement of cytokinin in *phyB* related hypocotyls growth. The question is whether BRs are acted to link photoreceptors and cytokinin signal transduction pathway. Based on results, we propose a model for interaction among pho-

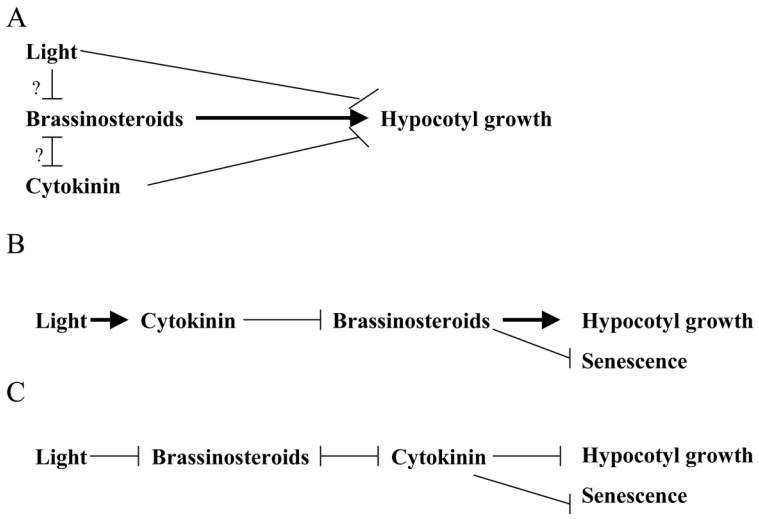

Fig. 4. Model for interaction among photoreceptors, bra- ssinosteroids (BRs) and cytokinins (BAP). **A.** Light and BAP are negatively and BL is positively acting components on hypocotyl growth that is modulated independently by light and BRs and BAP. **B.** BAP are postulated to act through BRs signal transduction pathway to down regulated the hypocotyl length. **C.** BRs act to link a BAP signal transduction pathway they regulated the down- stream BRs-regulated responses.

toreceptors, BRs and cytokinins (Fig. 4). The action of BRs and cytokinin is independent to each other however it is sequential in controlling hypocotyls elongation (Fig. 2C, 2D) and could be accounted by the similar interaction between BR and light. Therefore, light, BRs and cytokinins may control downstream light-regulated response independently, while light balances the action of BR and cytokinin in some extent.

References

[1] Altmann T, "Molecular physiology of brassinosteroids revealed by the analysis of mutants", *Planta.*, vol. 208, pp. 1-11, 1999.

[2] Benveniste P, "Sterol biosynthesis inhibitors", *Trends Plant Sci.*, vol. 4, pp. 348-353, 1986.

[3] Cary AJ, Liu W, and Howell SH, "Cytokinin action is coupled to ethylene in its effects on the inhibition of root and hypocotyl elongation in *Arabidopsis thaliana* seedlings", *Plant Physiol.*, vol. 107, no. 4, pp. 1075-1082, 1995.

[4] Cerdan PD and Chory J, "Regulation of flowering time by light quality", *Nature.*, vol. 19, 423, no. 6942, pp. 881-885, 2003.

[5] Chen M, Chory J, and Fankhauser C, "Light signal transduction in higher plants", *Annu. Rev. Genet.*, vol. 38, pp. 87-117, 2004.

[6] Chory J, "Out of darkness: mutants reveal pathways controlling light-regulated development in plants", *Trends Genet.*, vol. 9, no. 5, pp.167-72, 1993.

[7] Chory J and Li J, "Gibberellins, brassinosteroids and light-regulated development", *Plant Cell and Environment*, vol. 20, pp. 801-806, 1997.

[8] Chory J, Chatterjee M, Cook RK, Elich T, Fankhauser C, Li J, Nagpal P, Neff M, Pepper A, Poole D, Reed J, and Vitart V, "From seed germination to flowering, light controls plant development via the pigment phytochrome", *Proc. Natl. Acad. Sci.*, vol. 29, 93, no. 22, pp.12066-71, 1996.

[9] Chory J, Nagpal P, and Peto CA, "Phenotypic and Genetic Analysis of *det2*, a New Mutant That Affects Light-Regulated Seedling Development in *Arabidopsis*", *Plant Cell.*, vol. 3, no. 5, pp. 445-459, 1991.

[10] Chory J, Peto C, Feinbaum R, Pratt L, and Ausubel F, "*Arabidopsis thaliana* mutant that develops as a light-growth plant in the absence of light", *Cell.*, vol. 58, pp. 991-999, 1989a.

[11] Chory J, Peto CA, Ashbaugh M, Saganich R, Pratt L, and Ausubel F, "Different Roles for Phytochrome in Etiolated and Green Plants Deduced from Characterization of *Arabidopsis thaliana* Mutants", *Plant Cell.*, vol. 1, no. 9, pp. 867-880, 1989b.

[12] Chory J, Reinecke D, Sim S, Washburn T, and Brenner M, "A Role for cytokinins in de-etiolation in *Arabidopsis*: det mutants have an altered response to cytokinins", *Plant Physiol.*, vol. 104, no. 2, pp. 339-347, 1994.

[13] Clouse SD and Sasse JM, "Brassinosteroids: essential regulators of plant growth and development", *Annu. Rev. Plant Physiol. Plant Mol Biol.*, vol. 49, pp. 427-451, 1998.

[14] Fankhauser C, "Light perception in plants: cytokinins and red light join forces to keep phytochrome B active", *Trends Plant Sci.*, vol. 7, no. 4, pp.143-145, 2002.

[15] Furuya M, "Phytochromes: their molecular species, gene family and function", *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, vol. 44, pp.617-645, 1993.

[16] Grove M.D, Spencer GF, and Rohwedder WK, "Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen", *Nature*, vol. 281, pp. 216-217, 1979.

[17] Hutchison CE and Kieber JJ, (2002) Cytokinin Signaling in *Arabidopsis*. *The Plant Cell* S47-S59. The supplement.

[18] Jain M, Tyagi AK, and Khurana JP, (2006) Molecular characterization and differential expression of cytokinin-responsive type-A response regulators in rice (*Oryza sativa*). *BMC Plant Biology* BioMed central.

[19] Koornneef M, Rolff E, and Spruit CJP, "Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana*", *Heynh Z Pflanzenphysiol.*, vol. 100, pp. 147-160, 1980.

[20] Li J, Nagpal P, Vitart V, McMorris TC, and Chory J, "A role for Brassinosteroids in light-dependent development of *Arabidopsis*", *Science*, vol. 19, 272, no. 5260, pp. 398-401, 1996.

[21] Luccioni LG, Oliverio KA, Yanovsky MJ, Boccalandro HE, and Casal JJ, "Brassinosteroid Mutants Uncover Fine Tuning of Phytochrome Signaling", *Plant Physiol.*, vol. 128, no. 1, pp. 173-181, 2002.

[22] Mandava NB, "Plant growth promoting brassinosteroids", *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, vol. 39, pp. 23-52, 1988.

[23] Mathews S and Sharrock RA, "Phytochrome gene diversity", *Plant Cell Environ*, vol. 20, pp. 666-671, 1997.

[24] Nagatani A, Reed JW, and Chory J, "Isolation and Initial Characterization of *Arabidopsis* Mutants That Are Deficient in Phytochrome A", *Plant Physiol.*, vol. 102, no. 1, pp. 269-277, 1993.

[25] Nagy F and Schafer E, "Phytochromes control photomorphogenenis by differentially regulated, interacting signaling pathways in higher plants", *Annu. Rev. Plant Biol.*, vol. 53, pp. 329-355, 2002.

[26] Neff MM, and Chory J, "Genetic interactions between Phytochrome A, Phytochrome B, and Cryptochrome 1 during *Arabidopsis* development", *Plant Physiol.*, vol. 118, pp. 27-36, 1998.

[27] Neff MM, Fankhauser C, and Chory J, "Light: an indicator of time and place", *Genes Dev.*, vol. 14, no. 3, pp. 257-571, 2000.

[28] Neff MM, Nguyen SM, Malancharuvil EJ, Fujioka S, Noguchi T, Seto H, Tsubuki M, Honda T, Tadatsuto S, Yoshida S, and Chory J, "BAS1: A gene regulating brassinosteroid levels and light responsiveness in *Arabidopsis*", *Proc. Natl. Acad. Sci.*, vol. 96, no. 26, pp. 15316-15323, 1999.

[29] Parks BM and Quail PH, "*hy8*, a new class of long hypocotyl mutants deficient in phytochrome A", *Plant Cell.*, vol. 5, pp. 39-48, 1993.

[30] Pepper AE and Chory J, "Extragenic suppressors of the *Arabidopsis det1* mutant identify elements of flowering-time and light-response regulatory pathways", *Genetics.*, vol. 145, pp. 1125-1137, 1997.

[31] Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, and Wagner D, "Phytochromes: photosensory perception and signal transduction", *Science.*, vol. 5, 268, no. 5211, pp. 675-80, 1995.

[32] Reed JW, Nagatani A, Elich TD, Fagan M, and Chory J, "Phytochrome A and Phytochrome B Have overlapping but Distinct Functions in *Arabidopsis* Development", *Plant Physiol.*, vol. 104, no. 4, pp. 1139-1149, 1994.

[33] Sarrock RA and Clack T, "Patterns of expression and normalized levels of the five *Arabidopsis* phytochromes", *Plant Physiol.*, vol. 130, pp. 442-4456, 2002.

[34] Su W and Howell SH, "The Effects of Cytokinin and Light on Hypocotyl Elongation in *Arabidopsis* Seedlings Are Independent and Additive", *Plant Physiol.*, vol. 108, no. 4, pp. 1423-1430, 1995.

[35] Sweere U, Eichenberg K, Lohrmann J, Mira-Rodado V, Baurle I, Kudla J, Nagy F, Ser E, and Harter K, "Interaction of the response regulator ARR4 with phytochrome B in modulating red light signaling", *Science.*, vol. 294, no. 5544, pp. 1108-11, 2001.

[36] Vandenbussche F, Pierik R, Millenaar FF, Voesenek LACJ, and Straeten DVD, "Reaching out of the shade", *Current Opinion in Plant Biology*, vol. 8, pp. 462-468, 2005.

[37] Yamaguchi R, Nakamura M, Mochizuki N, Kay SA,

and Nagatani A, "Light-dependant Translocation of a Phytochrome B-GFP fusion protein to the nucleus in transgenic Arabidopsis", *J. cell Biol.*, vol. 145, no. 3, pp. 437-445, 1999.

[38] Yamamoto YY, Kondo Y, Kato A, Tsuji H, and Obokata J, "Light-responsive elements of the tobacco PSI-D gene are located both upstream and within the transcribed region", *Plant J.*, vol. 12, pp. 255-265, 1997.

[39] Yokoda T, "The structure, biosynthesis and function of brassinosteroids", *Trends in plant science.*, vol. 2, no. 4, pp. 23-52, 1997.