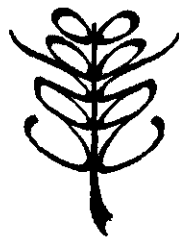




**Effects of light quality on the growth and carbon metabolism of  
internodes of *Phaseolus vulgaris* L.**

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## SAMENVATTING

Effecten van lichtkwaliteit op de groei en ontwikkeling van boneplanten werden bestudeerd in samenhang met effecten van lichtkwaliteit op het koolhydraatmetabolisme van deze planten. Boneplanten die waren opgekweekt in licht met gelijke hoeveelheden fotosynthetisch actieve straling, maar van verschillende spectrale samenstelling, vertoonden geen verschillen in gewichtsopbrengst van spruit en wortel. Licht met een relatief hoger aandeel blauwe fotonen veroorzaakte echter planten met kortere internodiën dan licht dat verrijkt was in het rode deel van het spectrum. Verondersteld wordt dat dit effect van blauw licht op de strekkingsgroei van de internodiën wordt gereguleerd door een blauwlicht receptor. Regulatie door fytochroom lijkt minder waarschijnlijk, omdat het verschil in rood: verrood licht tussen de rode en blauwe lichtbehandeling, theoretisch gezien, te klein was om een verschil in fytochroom evenwicht te veroorzaken dat dit strekkingseffect op de internodiën kan verklaren.

In dit onderzoek werd het verband tussen de activiteit van invertase in internodiën en de strekkingsgroei van de internodiën bestudeerd in planten die onder verschillende lichtkwaliteiten werden opgekweekt. Zowel in planten die in rood als in blauw verrijkt licht werden opgekweekt werd een toename van de invertase activiteit waargenomen gedurende de beginfase van de strekkingsgroei van de internodiën. De maximum invertase activiteit was iets hoger in de internodiën van de planten die werden opgekweekt onder rood verrijkt licht. In alle planten nam de invertase activiteit snel af op ongeveer hetzelfde moment als een afname in de strekkingsgroei kon worden waargenomen. De toename in invertase activiteit ging gepaard met een afname in het gehalte sucrose, maar alleen in de onder rood-verrijkt licht opgekweekte planten werd een toename in het hexose gehalte waargenomen. De mogelijke rol van invertase bij de verdeling van koolhydraten in de plant en de rol van hexoses bij de strekkingsgroei van internodiën worden bediscussieerd.

## ABSTRACT

Effects of light quality on the growth and development of bean plants were studied in relation with some parameters of carbon metabolism. Bean plants grown in light with equal irradiance levels but of different spectral composition did not show differences in fresh or dry weight of shoots or roots. However, light with a relatively higher content of blue photons resulted in plants with shorter internodes in comparison with light enriched in the red part of the spectrum. This effect of blue light on stem elongation is proposed to be controlled by a blue-light receptor, since the difference in the ratios of red to far-red light was too small to cause a difference in the phytochrome equilibrium large enough to explain the observed effect on stem elongation.

In this study the relationship between the activity of invertase and the elongation growth of internodes in plants grown in different light qualities was investigated. In both red- and blue-light treated plants the activity of invertase increased during the initial phase of elongation of an internode. The maximum activity was slightly higher in the faster elongating internodes of the red-light treated plants. In all plants the activity of invertase and the rate of internode elongation decreased about simultaneously. The increase in invertase activity was accompanied by a decrease in sucrose content in both blue- and red-light treated plants. However, the hexose content only significantly increased in the internodes of the red-light treated plants. The role of invertase activity in assimilate distribution in the plant and the function of hexoses in the growth of internodes are discussed.

## INTRODUCTION

A great many papers report on effects of different light qualities on the growth and development, i.e. photomorphogenesis, of plants (see Kendrick and Kronenberg 1986 and references therein). The majority of these papers on photomorphogenesis focusses on the photoreceptors involved in the photomorphogenetic effects of interest, and mostly describes the effects of short-term experiments with seedlings during the process of de-etiolation. Only a relatively small number of papers describes the effects of prolonged exposures to different light qualities on the development of more mature green plants (Meijer 1959, Warrington and Mitchell 1976, Smith 1986, Casal et al. 1987, Mortensen and Strømme 1987). Since photomorphogenesis involves growth, the regulation of the assimilate diversion between the different organs of a plant by light will play an important role in this developmental process.

In this paper I report on a study on the effects of a prolonged exposure of bean plants to light of different spectral quality on the morphogenesis of these plants, with special emphasis on the elongation growth and carbon metabolism of the internodes.

In several plant species internode elongation has been shown to be controlled by phytochrome. In general, a decrease in the ratio of phytochrome in the red-light absorbing form to that in the far-red-light absorbing form (Pr:Pfr) by light with a high red to far-red ratio (R:FR), inhibits stem elongation (Downs et al. 1957, McLaren and Smith 1978, Morgan and Smith 1978, Lecharny 1979, Holmes and Wagner 1981)). However, in some species stem elongation is not inhibited by light with a high R:FR in the absence of blue light, indicating that at least in these species stem elongation is not solely controlled by phytochrome, but by a blue-light receptor as well (Thomas and Dickinson 1979). Plants grown in blue or blue-enriched light were shorter than plants grown in light with a high red photon content (Warrington and Mitchell 1976, Mortensen and Strømme 1987).

Apart from the quality of the light, stem elongation may also be influenced by the amount of photosynthetic active radiation (PAR, 400-700 nm) (Ritter et al. 1981). In *Chenopodium rubrum* (Holmes and Wagner 1981) and in *Rumex obtusifolius* (McLaren and Smith 1978) stimulation of stem elongation by low R:FR ratios was less at lower levels of PAR, suggesting that the availability of assimilates also played an important role in controlling the elongation response. Not only the availability of assimilates, but also the capacity of a particular plant organ to import assimilates relative to other competing

organs (i.e., the relative sink strength) may affect the growth of a plant organ (Walker and Ho 1977, Ho 1988). E.g., the sink activity of flower buds of rose (Mor et al. 1981) and iris (Mae and Vonk 1974) was increased by the application of cytokinins to these buds, which resulted in an increased allocation of assimilates to the flower buds and in an improvement of their development at the expense of assimilate allocation to and development of non-treated buds (rose) or daughter bulbs (iris). Mor and Halevy (1980) had demonstrated before that such a stimulation of sink activity and development of rose buds could also be achieved by specifically irradiating the buds with low levels of red light. Therefore, the regulation of enzymes involved in assimilate distribution within a plant by light, may be an important process in the control of the relative growth rates of the different organs of a plant.

The involvement of the sucrose-hydrolyzing enzyme invertase in the control of sink strength and development has been proposed (Hatch and Glasziou 1963, Pryke and Bernier 1978, Morris and Arthur 1984a, 1985). Pryke and Bernier (1978) demonstrated that the activity of invertase in the apex of *Sinapis alba* increased during transition to flowering. Morris and Arthur (1984a, 1985) observed a positive relationship between the rates of internode elongation and leaf expansion in *Phaseolus vulgaris* and the activity of invertase. They suggested that the invertase activity regulates the sink strength, i.e., the capacity to import assimilates, of the internodes or leaves by controlling the gradient in sucrose concentration, and thereby the sucrose flux, between the site of production (source) and the site of utilization (sink).

Several studies have shown the effects of light quality on carbon metabolism: blue light stimulated phosphoenolpyruvate carboxylase, pyruvate kinase, and the incorporation of carbohydrates into protein; red light stimulated the accumulation of starch and various sugars of the Calvin cycle (see Voskresenskaya 1979 and references therein). Control of invertase synthesis by phytochrome was observed in radish cotyledons (Zouaghi 1976). Phytochrome control of several other enzymes involved in carbohydrate metabolism has also been demonstrated (see Schopfer 1977).



The present paper reports on a study on the effects of different light qualities on the growth and carbon metabolism of internodes of *Phaseolus vulgaris* L. This study was undertaken to investigate whether effects of light quality on elongation of internodes are related to changes in invertase activity. Levels of sucrose and hexoses in the internodes were also measured to determine if and how they relate to the activity of invertase and to growth. Finally leaf area, leaf weight, root weight and leaf pigment content were measured to determine the effects of a prolonged exposure to different light qualities on the overall development of the plants.

## MATERIALS AND METHODS

### Plant material and growth conditions

Seeds of dwarf bean (*Phaseolus vulgaris* L. cvs Masterpiece or Prelude) were sown in vermiculite and germinated in a greenhouse with a minimum day and night temperature of 20 and 16 °C, respectively, or in darkness in an incubator at 25 °C. After 4 days (dark-grown seedlings) or 6 days (light-grown seedlings) the seedlings were planted in Levington's compost in 8.5 cm diameter plastic pots and transferred to the growth cabinets. The day and night temperatures in the cabinets were 25 and 20 °C, respectively. The plants were watered daily with tap water. The photoperiod was 12 h and the light irradiance at plant level was ca.  $115 \mu\text{mol m}^{-2}\text{s}^{-1}$  (400-700 nm). Sylvania cool white fluorescent tubes mounted in two layers were used as a light source. In one cabinet the relative amount of red light was increased by placing a red filter (Strand cinelux nr. 406) between the two layers of fluorescent tubes, whereas in another cabinet the relative amount of blue light was increased by using a blue filter (Strand cinelux nr. 419). These light conditions will be referred to as Red Enriched Light (REL) and Blue Enriched Light (BEL), respectively. The spectral composition of the light in the growth cabinets at plant level was measured by a Spectron SE-590 spectroradiometer (Fig. 1). The blue: red ratios, calculated as the ratio in photon irradiance between 400 to 600 nm and between 600 to 700 nm, were ca. 0.88 and 0.45 for the BEL- and REL-conditions. The red: far-red ratios (R:FR; photon irradiance 665-675 nm: photon irradiance 725-735 nm) in the BEL and REL were 4.4 and 3.4, respectively.

In experiment II plants were grown at 20 °C continuously at a light irradiance of  $93 \mu\text{mol m}^{-2}\text{s}^{-1}$  supplied by Philips fluorescent tubes. In this experiment BEL and REL were realized by mixing blue (TL40W/18) or red (TL40W/15) fluorescent tubes with white fluorescent (TL36W/33) tubes in a ratio of 1:1.

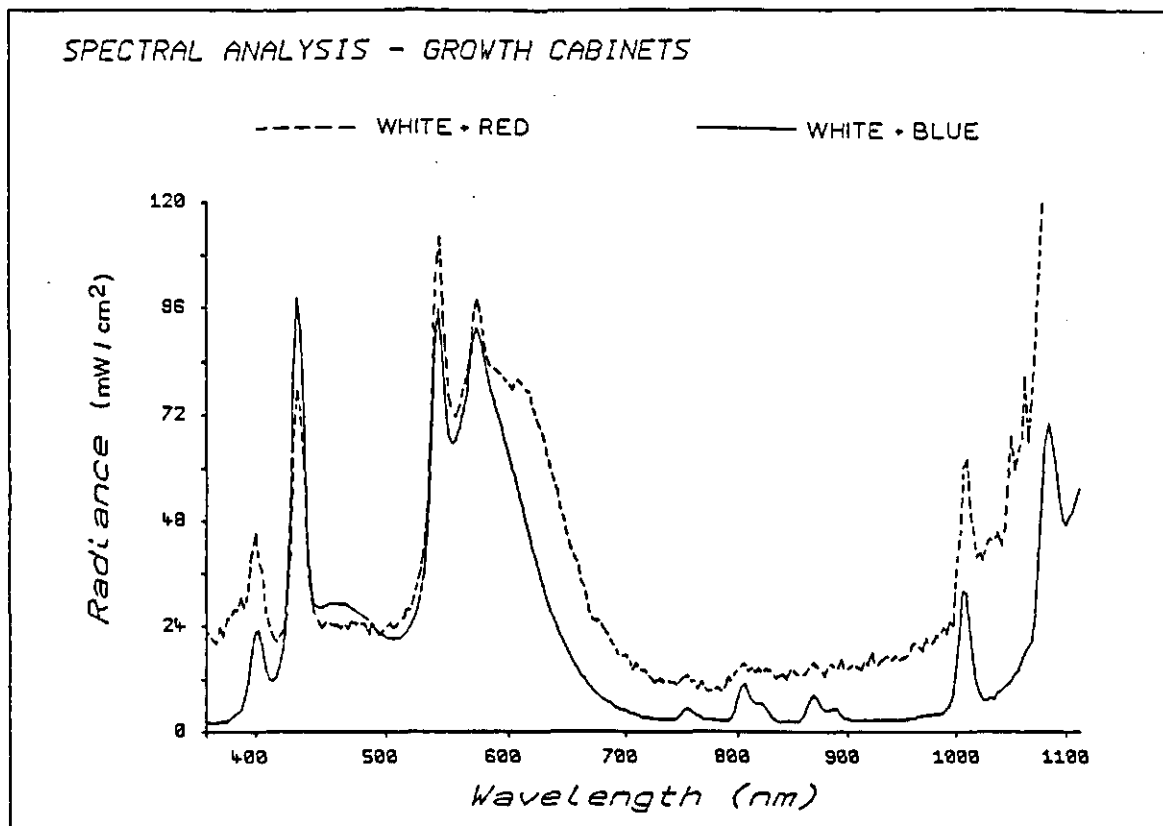


Figure 1. Spectral energy distribution of the red- and blue-enriched light. The measurements were made at plant level in the centre of the growth cabinets.

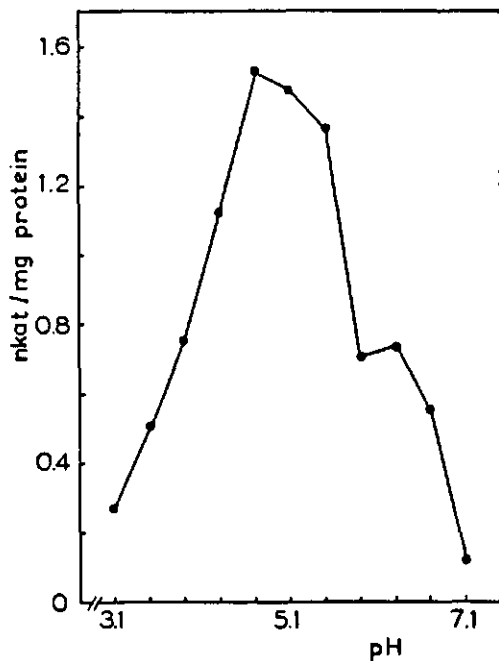
#### Growth measurements

Plants grown in the different light qualities were harvested every other day (3 plants per sample per treatment). Harvesting was started at the onset of elongation of internode 3 (ca. 15 days after sowing). The fresh weight (FW) of the shoot above the cotyledonous node, the primary leaves, the trifoliate leaves and the internodes were determined as well as the length of the internodes. Triplicate samples, each consisting of 3 randomly chosen plants, were stored at -20 °C until further use. In experiment II the plants were grown in a Steiner's nutrient solution (for composition, see De Stigter 1980) for two weeks and harvested after a light-quality treatment of 14 days. These plants were only used to determine the effects of light quality on the elongation of the internodes and on the fresh and dry weight of roots + hypocotyl, internodes and leaves.

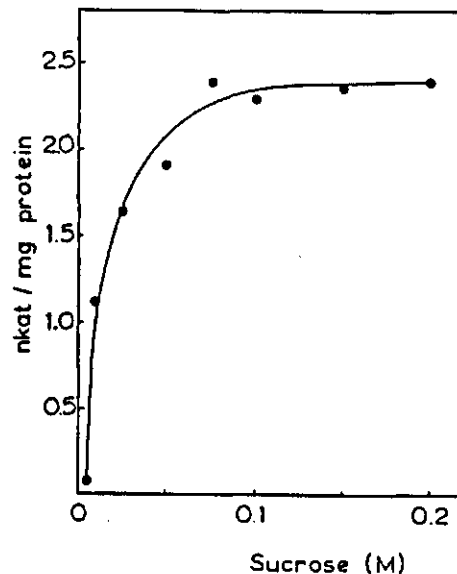
### Determination of enzyme activities

Invertase ( $\beta$ -fructofuranosidase fructohydrolase; EC 3.2.1. 26): Frozen plant tissue was ground in a mortar using a pestle and a small amount of acid washed sand in 33 mM  $\text{Na}_2\text{HPO}_4$ /16.7 mM citric acid pH 5.0 at 4 °C (20 ml/g FW). The homogenate was centrifuged at 30,000 g for 20 min (0-4 °C).

The invertase measurement was started by the addition of 0.05 ml of the 30,000 g supernatant to 0.45 ml of a solution containing 0.08 ml 100 mM sucrose, 0.1 ml 33 mM  $\text{Na}_2\text{HPO}_4$ /16.7 mM citric acid pH 5.0 and 0.27 ml  $\text{H}_2\text{O}$ . Incubation took place in 1.5 ml Eppendorf vials at 30 °C. After a period of 1 h invertase activity was stopped by the addition of 0.4 ml of a saturated  $\text{Ba}(\text{OH})_2$  solution. The  $\text{Ba}(\text{OH})_2$  was neutralized by the addition of 0.336 ml 6.5%  $\text{ZnSO}_4$ . The  $\text{BaSO}_4$  and  $\text{Zn}(\text{OH})_2$  precipitate were pelleted by centrifugation in a Beckman Microfuge B at full speed (ca. 8000 g) for 30 s. A 0.5-ml aliquot of supernatant was used for the determination of the amount of glucose released from sucrose by invertase using the glucose oxidase assay (see below). Preliminary experiments had shown that invertase activity was maximum at approx. pH 5 and at a sucrose concentration  $\geq 0.75$  M (Figs 2 and 3). This method is similar to that described by Leigh et al. (1979).

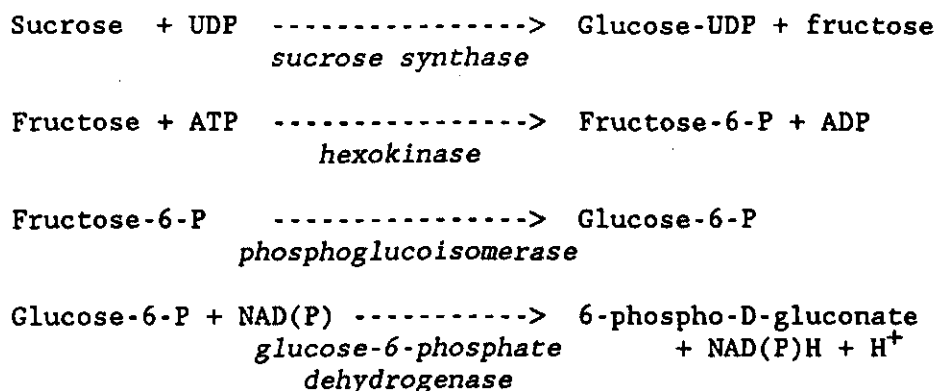


**Figure 2.** Dependence of the specific invertase activity of extracts of bean internodes on the pH of the incubation medium. The protein content of the extracts was measured by the method of Lowry et al. (1951).

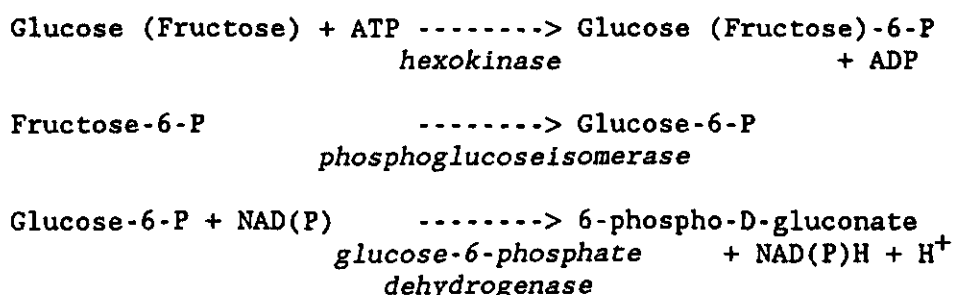


**Figure 3.** Dependence of the specific invertase activity of extracts of bean internodes on the concentration of the sucrose solution used in the invertase assay (see Materials and Methods). Otherwise as for Fig. 2.

Sucrose synthase (UDP-glucose: D-glucose 2-glucosyltransferase: EC 2.4.1.13): tissue (0.5 g FW or more) was macerated in a pestle and mortar with aid of acid-washed sand in 100 mM Tris/HCl pH 7.2 (1.5 ml/g FW) containing 20 mM EDTA, 1 mM mercaptoethanol (or dithiothreitol), and 2 mM NaF (0-4 °C). The homogenate was centrifuged at 30,000 g for 20 min. Two ml of supernatant was dialyzed overnight against extraction buffer and the dialyzed supernatant was used to assay enzyme activity. Two different enzyme assays were used: In the first assay the measurement of sucrose synthase activity was started by the addition of 0.05 ml of the dialyzed supernatant to 0.45 ml of a solution composed of 0.25 ml 50 mM Tris/HCl pH 7.2 containing 1 mM mercaptoethanol, 0.1 ml 35 mM UDP, and 0.1 ml 1 M sucrose. Samples incubated in the presence of 0.1 ml H<sub>2</sub>O instead of 0.1 ml 35 mM UDP were used as controls for non-sucrose-synthase-specific hydrolysis of sucrose (Doehlert et al. 1988). After 30 min at 30 °C the reaction was stopped by the addition of Ba(OH)<sub>2</sub> as described for the invertase assay. The fructose released by sucrose synthase was measured by the Nelson-Somogyi method (see below). In the second assay sucrose synthase activity was determined in 1.5 ml quartz cuvettes in a double beam spectrophotometer at 30 °C (Chapleo, 1987): 0.1 ml of the dialyzed supernatant was assayed in a total volume of 1.5 ml in the presence of 50 mM Tris/HCl pH 7.2, 1 mM dithiothreitol, 1 mM NaCN, 2 mM MgCl<sub>2</sub>, 2 mM ATP, 0.6 mM NAD(P), 2 mM UDP, 100 mM sucrose and 0.2 units of hexokinase (EC 2.7.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and phosphoglucosomerase (EC 5.2.1.9). The latter enzymes were desalted by micro-sample dialysis (Budowle et al. 1981) against H<sub>2</sub>O for 2 h at 4 °C. The sucrose synthase activity was measured by a coupled enzyme assay. The reaction ultimately resulted in the reduction of NAD(P) to NAD(P)H, which was followed at 340 nm. The reference cuvette contained H<sub>2</sub>O. Boiled supernatant fractions were used as controls.



Hexokinase (ATP: D-hexose-6-phosphotransferase; EC 2.7.1.1); tissue was homogenized as described for the sucrose synthase assay, except that 25 mM Hepes/KOH pH 8.0 containing 1 mM dithiothreitol, 2 mM NaF and 10 % w/v insoluble polyvinylpyrrolidone (PVP) was used as an extraction medium. The 30,000 g supernatant was dialyzed against extraction medium without PVP for 30 min before hexokinase activity was assayed. Hexokinase activity was assayed in 1.5 ml quartz cuvettes in a double beam spectrophotometer at 30 °C in 25 mM Hepes/KOH pH 8.0 containing 0.3 mM glucose or fructose, 0.3 mM NAD(P), 1 mM ATP, 1.7 mM MgCl<sub>2</sub>, 2.5 units of glucose-6-phosphate dehydrogenase and 2.5 units of phosphoglucoseisomerase. The latter enzymes were desalted by dialysis against H<sub>2</sub>O (2 h at 4 °C) prior to use. Hexokinase activity was determined by a coupled enzyme assay resulting in the reduction of NAD(P) to NAD(P)H. The reduction of NAD(P) was followed at 340 nm. The reference cuvette contained H<sub>2</sub>O. Boiled supernatant fractions were used as controls.



#### Protein determination

The protein content of the 30,000 g supernatant was measured by the method of Lowry et al. (1951) (Figs 2 and 3) or by the method of Bradford (1976), using

bovine serum albumin as a standard. Since sugars interfere with the protein determination by the Lowry method (see Bradford 1976), the Bradford method was preferred in experiments with crude plant extracts and extracts of plants of different ages and treatments. To improve the response, 0.03 % w/v sodium dodecyl sulphate was added to the Bradford reagent. The Bradford calibration curve showed two linear phases: one for protein concentrations  $\leq 60$  mg/l and one for protein concentrations from 60 to 200 mg/l. Therefore, it was decided to dilute all extracts of plant tissue to protein concentrations  $\leq 60$  mg/l with extraction buffer before the protein content was determined.

### Sugar determinations

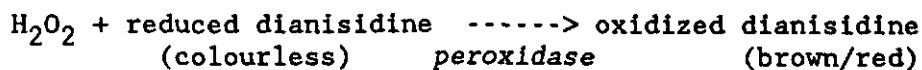
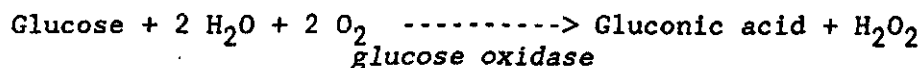
Hexoses: plant tissue was extracted as described for the invertase assay. The hexoses present in the 30,000 g supernatant were determined by one of the following methods:

1. Nelson-Somogyi method: to 0.1 ml of supernatant 0.25 extraction medium (33 mM  $\text{Na}_2\text{HPO}_4$ /16.7 mM citric acid pH 5.0) and 0.25 ml Nelson-Somogyi reagent were added. The Nelson-Somogyi reagent was made up on the day of use from 25 parts of reagent A (2.5 % w/v anhydrous  $\text{Na}_2\text{CO}_3$ , 2.5 % w/v sodium potassium tartrate (Rochelle salt), 2 % w/v  $\text{NaHCO}_3$ , and 20 % w/v anhydrous  $\text{NaSO}_4$ ) and 1 part of reagent B (15 % w/v  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  with 1 to 2 drops of concentrated  $\text{H}_2\text{SO}_4$  per 100 ml). After 20 min at 100 °C, 0.25 ml arsenomolybdate reagent (25 g ammonium molybdate dissolved in 150 ml  $\text{H}_2\text{O}$  to which 21 ml concentrated  $\text{H}_2\text{SO}_4$  and 3 g sodiumarsenate dissolved in 25 ml  $\text{H}_2\text{O}$  were added. This reagent was incubated at 37 °C for 24 h prior to use) and 2.0 ml  $\text{H}_2\text{O}$  were added. The hexose content of the sample was determined by measuring the extinction at 520 nm, using glucose or fructose as a standard.

2. Dinitrosalicylic acid method: to 0.4 ml of the 30,000 g supernatant, 0.5 ml 500 mM  $\text{Na}_2\text{HPO}_4$ , and 1.0 ml dinitrosalicylic acid reagent (1 % w/v 3-5, dinitrosalicylic acid, 1 % w/v NaOH, 2 % w/v phenol, 0.05 % w/v  $\text{Na}_2\text{SO}_3$ ) were added. After 15 min at 100 °C the samples were allowed to cool before 1 ml of 40 % w/v sodium-potassium tartrate was added. The hexose content of the samples was determined by measuring the absorption of the sample at 575 nm, using a glucose or fructose solution as a standard.

3. Glucose-oxidase method: this method is specific for the determination of D-glucose and can therefore not be used to determine total hexose content. To 0.1 ml of the 30,000 g supernatant, 0.25 ml extraction medium, 0.15 ml  $\text{H}_2\text{O}$ , and 1.0 ml chromogen solution were added. The chromogen solution was composed of 0.1 % w/v glucose oxidase ( $\beta$ -D-glucose:oxygen 1-oxidoreductase; EC 1.1.3.4; 75 units/ml) and 0.1 % w/v peroxidase (donor: hydrogen-peroxide

oxidoreductase; EC 1.11.1.7; 95 units/ml) in a glycerol buffer (40 % v/v glycerol, 362 mM  $\text{NaH}_2\text{PO}_4$  and 200 mM Tris, pH 7.0), 0.5 % w/v 5-dianisidine-2HCl in  $\text{H}_2\text{O}$ , and glycerol buffer in a ratio of 30:3:4:63. The sample was incubated at 37 °C for 1 h. The reaction was stopped by the addition of 1 ml of 9 M  $\text{H}_2\text{SO}_4$ . In this method the glucose is determined by a coupled enzyme reaction yielding oxidized dianisidine:



Addition of  $\text{H}_2\text{SO}_4$  results in a bright pink colour

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The oxidized dianisidine is a measure for the amount of glucose present in the sample and was spectrophotometrically determined at 540 nm. A glucose solution was used as a standard. (see also Leigh et al. 1979).

Methods 1 and 2 are non-specific and can therefore only be used to determine the total content of reducing sugars, whereas method 3 is specific for the determination of D-glucose only. Apart from its specificity for D-glucose, this method has a much higher sensitivity for glucose (Fig. 4).

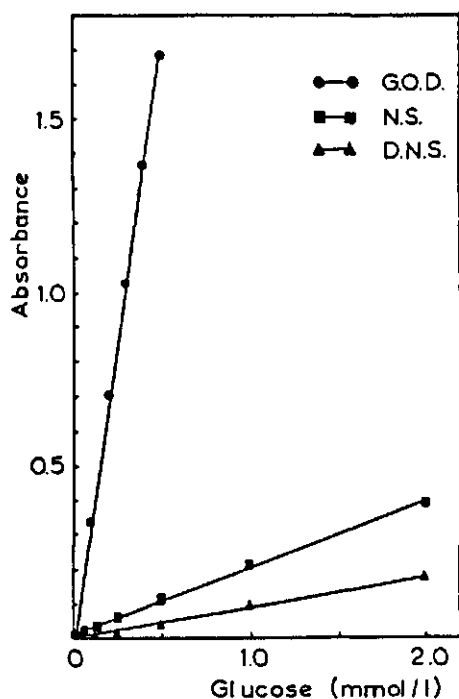


Figure 4. Calibration curves for the determination of glucose by the glucose oxidase method (G.O.D.), the Nelson-Somogyi method (N.S.), and the dinitrosalicylic acid method (D.N.S.). Absorbance was measured against  $\text{H}_2\text{O}$  at 540 nm (G.O.D.), 520 nm (N.S.), or 575 nm (D.N.S.).



Sucrose: plant tissue was extracted as described for the invertase assay. The sucrose content of the 30,000 g supernatant was measured as the difference in the content of hexoses or glucose after and before hydrolysis of the sucrose into fructose and glucose. Sucrose was hydrolyzed either by acid or enzymatically. In the first method 0.1 ml supernatant was added to 0.125 ml 0.3 M HCl and incubated at 100 °C for 40 min, after which the sample was neutralized by the addition of 0.125 ml 0.3 M KOH. This method resulted in a 100 % hydrolysis of 0.3 ml 0.25 mM sucrose. However, the hydrolysis is non-specific and in crude plant extracts acid hydrolysis may liberate hexoses from other oligo- and polysaccharides and result in an overestimation of the true sucrose content. In the second method 0.1 ml supernatant was added to 0.25 ml of yeast invertase (dialyzed against 33 mM Na<sub>2</sub>HPO<sub>4</sub>/16.7 mM citric acid pH 5.0 for 2 h at 4 °C and diluted to approx. 20 units/ml) and incubated at 30 °C for 15 min. Preliminary experiments had shown that this amount of invertase was sufficient to hydrolyze 0.1 ml 1 mM sucrose within 5 min.

In comparison with the enzymatic hydrolysis of sucrose, hydrolysis of a crude plant extract by acid indeed resulted in a higher sucrose content (Table 1), but only if the Nelson-Somogyi method was used to measure hexoses before and after hydrolysis. This indicates that besides sucrose, HCl also hydrolyzed a non-glucose containing oligo- or polysaccharide, e.g. a fructan.

Table 1. Sucrose content in extracts of bean internodes based on the difference in the level of glucose or hexoses after and before hydrolysis by HCl or yeast invertase, as measured by the glucose oxidase (G.O.D.) or the Nelson-Somogyi assay (N.S.), respectively.

| Treatment | Sucrose (mg / g FW) |      |
|-----------|---------------------|------|
|           | G.O.D.              | N.S. |
| Invertase | 2.3                 | 2.6  |
| HCl       | 2.6                 | 3.8  |

Starch: (essentially as described by Pharr and Sox, 1984) Tissue samples (ca. 1.0 g FW) were chopped into small pieces (ca. 5 mm for internodes and 0.25 cm<sup>2</sup> for leaves) and extracted with 10 ml 80 % v/v ethanol at 60 °C. This extraction procedure was repeated 5 times over a 3-h period. Between each

extraction step the tissue was pelleted by centrifugation at 3000 g for 5 min. The supernatant was discarded and, after the final extraction step, the pellet was dried at 60 °C for 16 h. The dried material was ground to powder using a glass rod and a whirlmixer. One ml of 200 mM KOH was added and the sample was boiled for 30 min (gelatinization). After gelatinization of the sample, 0.19 ml 1 M acetic acid was added to neutralize the sample, followed by the addition of 1 ml of 50 mM acetate buffer pH 4.5 and centrifugation at 3000 g for 5 min. An aliquot of 0.05 ml was taken from the supernatant to determine the glucose content in the sample before starch hydrolysis by amyloglucosidase (1,4- $\alpha$ -D-glucan glucohydrolase; EC 3.2.1.3) was started. The pellet was resuspended and 0.1 ml of amyloglucosidase (dialyzed against H<sub>2</sub>O for ca. 4 h and diluted to 7 units/ml with 50 mM acetate buffer pH 4.5) was added. The samples were incubated at 55 °C for 4 h. This incubation period was sufficient to hydrolyze all the starch present in a leaf-tissue sample (Fig. 5). At the end of the incubation period enzyme activity was stopped by boiling the sample for 5 min and the glucose content was determined by the glucose oxidase method as described above.

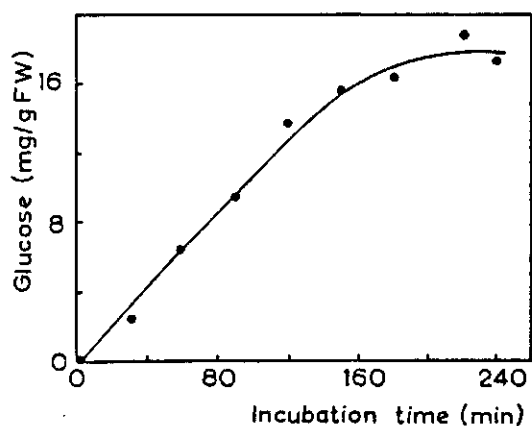


Figure 5. Time dependence of starch hydrolysis by amyloglucosidase in a leaf sample of bean. The hydrolysis of starch was measured as the amount of glucose released in time, which was assayed by the glucose oxidase assay.

Chlorophyll and carotenoid content: plant tissue was homogenized in 100 % acetone at 4 °C in dim light (10 ml/g FW). The homogenate was centrifuged at 3000 g for 5 min and the supernatant was diluted 10 times with 100 % acetone followed by dilution to 80 % v/v acetone with H<sub>2</sub>O. The absorbance of the diluted supernatant was measured against water in a double beam spectrophotometer at 470, 646 and 663 nm, and the chlorophyll and carotenoid content were calculated according to the formulas of Lichtenthaler and Wellburn (1983).

## RESULTS

### Effect of light quality on plant growth

Bean plants germinated in the dark and grown for 2 to 3 weeks in BEL or REL showed no differences in fresh weight of the shoots or roots (Table 2, Fig. 6). The developmental stage of the plants was also not affected by the

Table 2. Effect of a 2-week exposure of bean plants to red- or blue-enriched light on the fresh and dry weight of different parts of the plants. The data represent the mean of 10 measurements  $\pm$ SD. Significant differences ( $p < 0.01$ ) between light treatments are indicated by an asterisk.

| Exp. II    | Fresh weight (g) |                  | Dry weight (g)  |                  |
|------------|------------------|------------------|-----------------|------------------|
|            | RED              | BLUE             | RED             | BLUE             |
| SHOOT      | 5.98 $\pm$ 1.11  | 5.63 $\pm$ 1.01  | -               | -                |
| Internodes | 0.85 $\pm$ 0.18  | 0.59 $\pm$ 0.11* | 0.08 $\pm$ 0.02 | 0.05 $\pm$ 0.01* |
| Leaves     | 5.13 $\pm$ 0.18  | 5.04 $\pm$ 0.11  | 0.47 $\pm$ 0.09 | 0.46 $\pm$ 0.08  |
| Apex       | 0.04 $\pm$ 0.01  | 0.04 $\pm$ 0.02  | -               | -                |

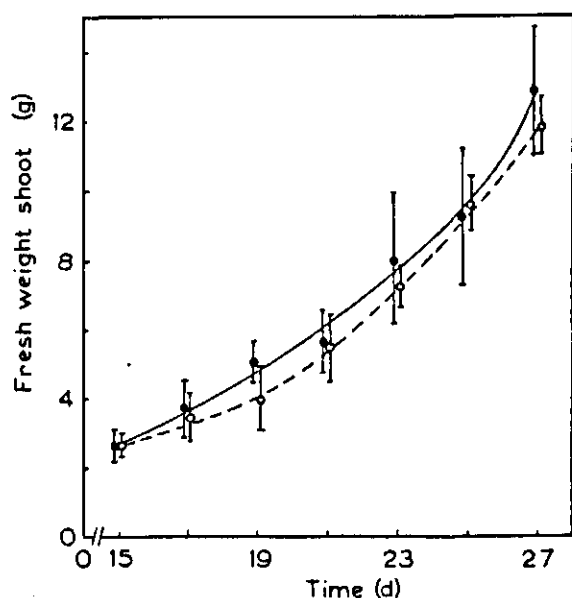


Figure 6. Effect of red-enriched light (REL, 0---0) or blue-enriched light (BEL, ●—●) on the growth of the shoots of bean. The data represent the mean of the fresh weight of 9 randomly chosen shoots  $\pm$ SD.

difference in light quality. However, there was a significant difference in internode elongation: REL resulted in longer internodes than BEL. This effect on internode length was observed in all internodes developed during the experiment (Fig. 7).

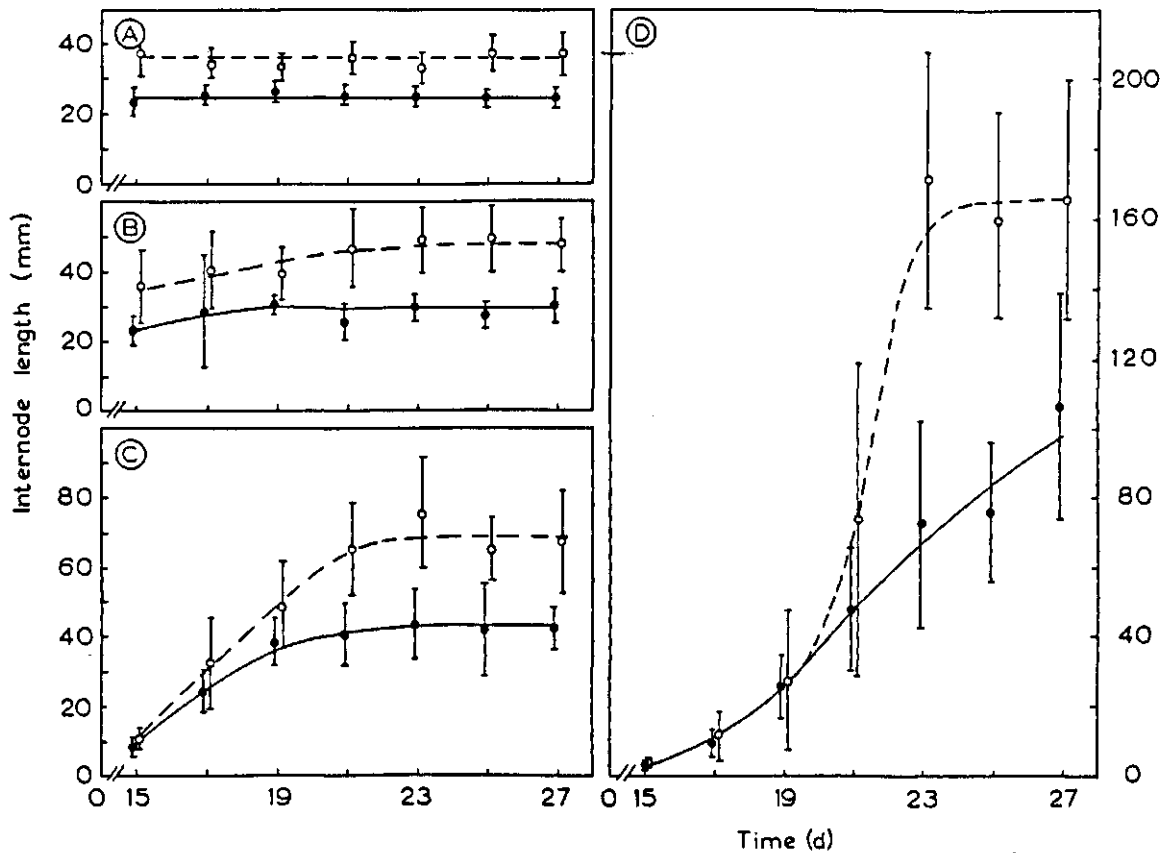


Figure 7. Effect of REL (0---0) or BEL (●—●) on the elongation growth of the first (A), the second (B), the third (C), and the fourth (D) internode of bean. The data represent the mean of the length of 9 internodes  $\pm$ SD.

Fresh and dry weight determinations of plants grown in BEL or REL for two weeks (exp. II) showed that red light did not only stimulate internode elongation, but also increased the fresh and dry weight of the internodes as compared with the internodes of the BEL-grown plants, whereas the total dry weight of both shoots and roots remained the same (Table 2). This indicates that more assimilates were used for internode growth in REL than in BEL. Bean plants germinated in the light and transferred to the REL- or BEL-environment of the growth cabinets 6 days after sowing, showed only minor differences in internode growth as compared with beans germinated in the dark and transferred directly from darkness to BEL or REL, which indicates that the growth pattern of light-grown seedlings is not as easily affected by a change in light quality as that of etiolated seedlings not yet adapted to any light condition (results not shown).

The fresh weights of primary and trifoliate leaves were not significantly different in BEL- and REL-grown plants (Fig 8A, B). The leaf area of the primary leaves was also not affected by the difference in light quality. However, the area of the trifoliate leaf was significantly greater in the BEL-grown plants on day 19 (Table 3).

**Table 3.** Effects of red-enriched and blue-enriched light on the fresh weight (FW) and area of the primary leaves (PL) and first trifoliate leaf (TF) of bean. The fresh weights of PL and TF, and the area of the PL were determined 9 and 13 days after the start of light treatment. The leaf area of the TF was measured on day 13 only. Leaf areas represent the mean of 18 (PL) and 9 (TF) leaves  $\pm$ SD. Statistically significant differences ( $P < 0.01$ ) between light treatments are indicated by an asterisk.

| Treatment |     | Primary leaf  |                         | Trifoliate leaf |                         |
|-----------|-----|---------------|-------------------------|-----------------|-------------------------|
|           |     | FW (g)        | Area (cm <sup>2</sup> ) | FW (g)          | Area (cm <sup>2</sup> ) |
| Red       | 9d  | 2.0 $\pm$ 0.5 | 56.6 $\pm$ 1.9          | -               | -                       |
|           | 13d | 2.2 $\pm$ 0.5 | 60.9 $\pm$ 9.5          | 0.85 $\pm$ 0.23 | 63.9 $\pm$ 3.3          |
| Blue      | 9d  | 2.0 $\pm$ 0.4 | 56.0 $\pm$ 1.9          | -               | -                       |
|           | 13d | 2.7 $\pm$ 0.3 | 69.2 $\pm$ 9.3          | 1.17 $\pm$ 0.18 | 84.1 $\pm$ 4.1*         |

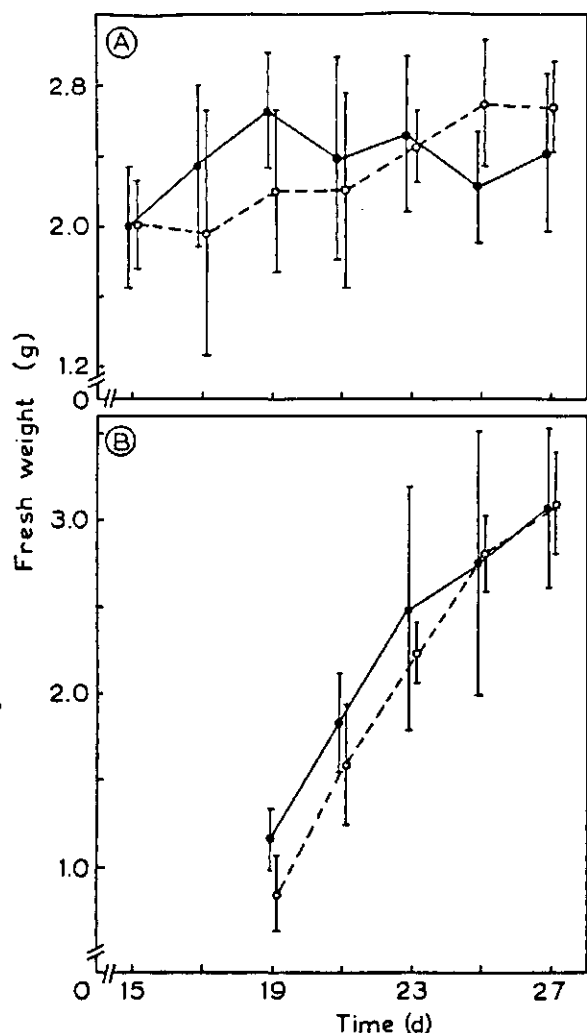


Figure 8. Effect of REL (0---0) or BEL (●—●) on the growth of the primary leaves (A) and the first trifoliate leaf (B) of bean. The data represent the mean of the fresh weight of 18 (A) or 9 (B) leaves  $\pm$ SD.

#### Light quality and carbon metabolism

Invertase activity, sucrose and hexose content of internodes 3 and 4 were studied during the development of the internodes in BEL or REL. Invertase activity was already high at the onset of internode elongation and increased further as elongation rate increased (Fig. 9A, B). Maximum invertase activity was higher in faster elongating REL-grown plants, both in internode-3 and -4 extracts. Since no differences in soluble protein content were observed in the internodes of BEL- or REL-grown plants (Fig. 10A, B), the specific invertase activity showed a pattern similar to that of the invertase activity expressed on a fresh weight basis (Fig. 11A, B). Invertase activity started to decrease at about the same time as a decrease in the rate of internode elongation was observed. Half-maximum activity was reached in about 3 days in both BEL- and REL-grown internodes. At the end of the experiment the invertase activities in internode extracts of BEL- and REL-grown plants were similar. Expressed on a fresh weight basis, invertase activity measured 27 days after sowing was about

8 and 10 % of the maximum activity in both internode 3 and 4 of the REL- and BEL-grown plants, respectively.

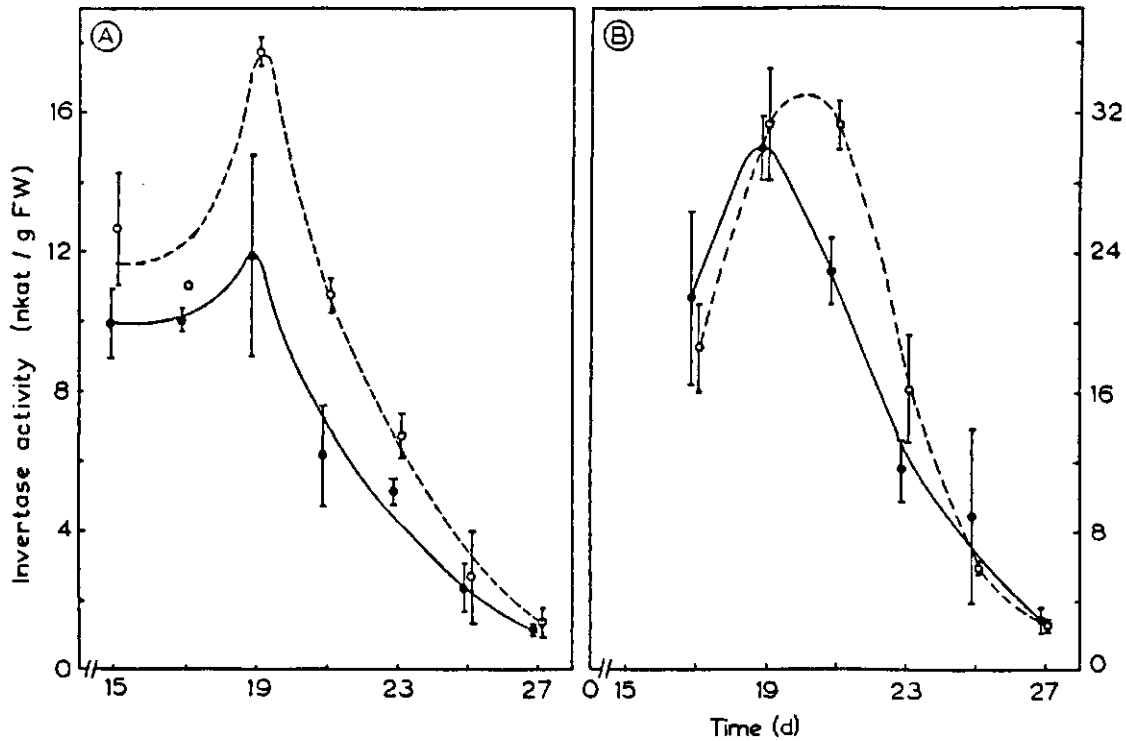


Figure 9. Invertase activity in extracts of the third (A) and fourth (B) internode of beans grown from day 6 in REL (0---0) or BEL (●—●). The data represent the mean activities of 3 extracts made from 3 internodes each  $\pm$ SD.

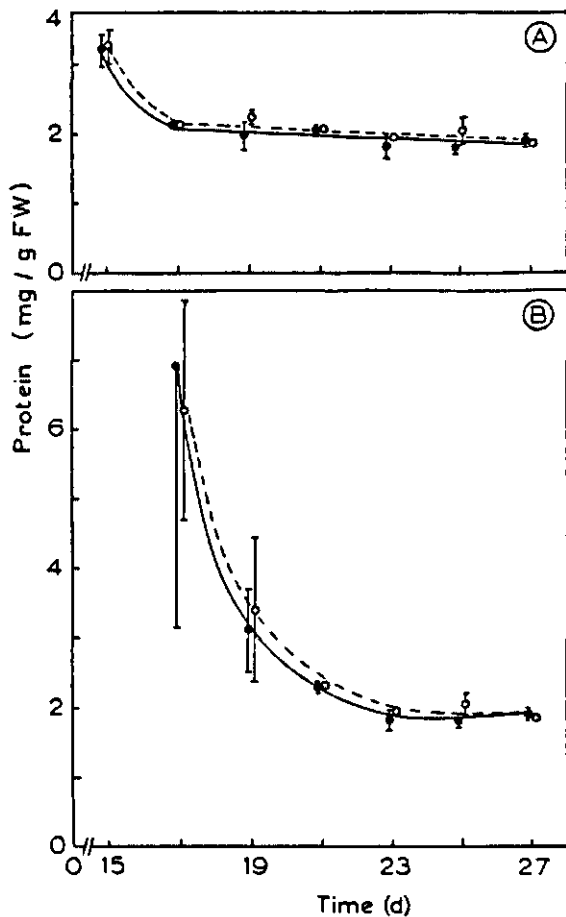


Figure 10. Protein content of the third (A) and fourth (B) internode of beans grown from day 6 in REL (0---0) or BEL (●—●). Protein content was measured by the method of Bradford (1976) and represents the mean of 3 extracts made from 3 internodes each  $\pm$ SD.

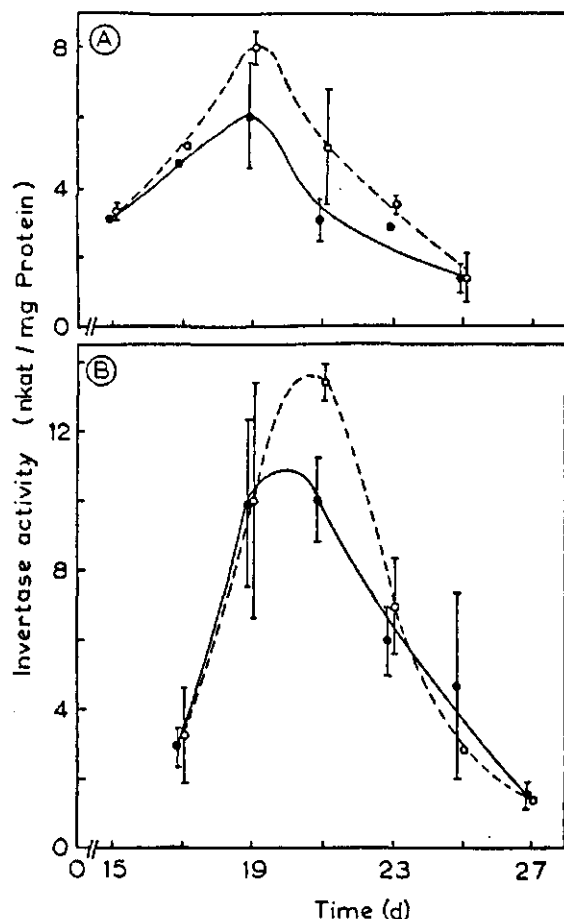


Figure 11. Specific activity of invertase in extract of the third (A) and fourth (B) internode of beans grown from day 6 in REL (O---O) or BEL (●—●). Otherwise as for Figs 9 and 10.

The pattern of increase and decrease in invertase activity during internode development was accompanied by an increase and decrease in the hexose: sucrose ratio (Fig. 12A, B). During the initial increase in internode length the level of sucrose decreased rapidly (Fig. 13A, B) and hexose content increased (Fig. 14A, B). This increase in hexose content was much more pronounced in the internodes of the REL-grown plants. About one day after the invertase activity started to decrease a decrease in hexose content was observed in the third internode (Figs 11A and 14A). However, the sequence of these events was less obvious in the fourth internode; in this internode both invertase activity and hexose content decreased at about the same moment (Figs 11 and 14B). Sucrose content increased at about the same time as invertase activity decreased. These changes in hexose and sucrose content resulted in a decrease in hexose: sucrose ratio which accompanied the decrease in invertase activity (Figs 9 and 12).



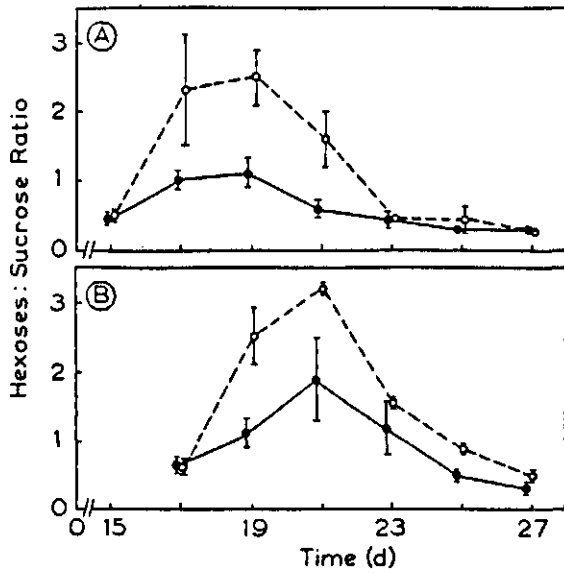


Figure 12. Ratio of hexoses to sucrose in extracts of the third (A) or fourth (B) internode of beans grown from day 6 in REL (O---O) or BEL (●---●). The data represent the mean of 3 measurements with three plants in each  $\pm$ SD.

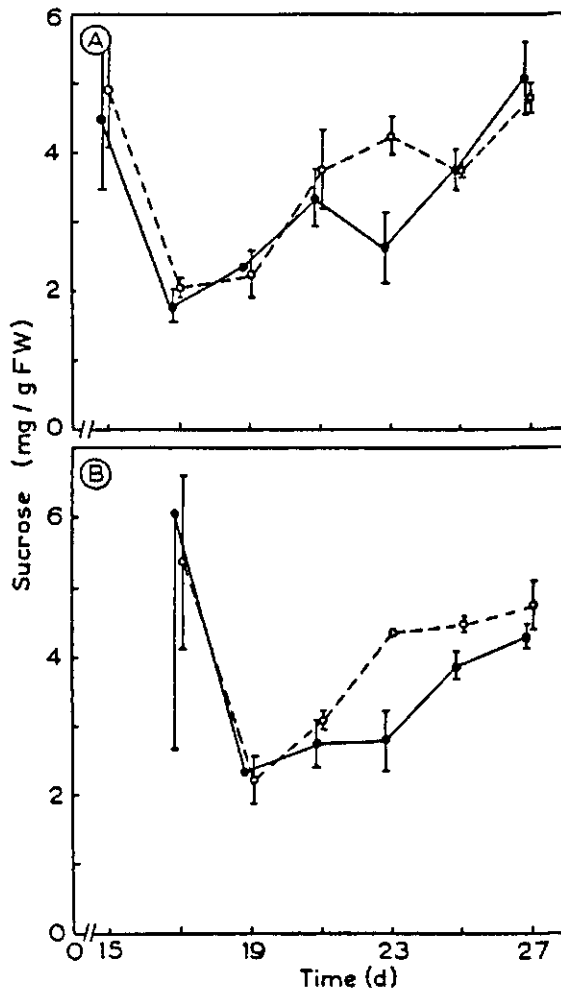


Figure 13. Sucrose content of the third (A) and fourth (B) internode of beans grown from day 6 in REL (O---O) or BEL (●---●). Otherwise as for Fig. 12.

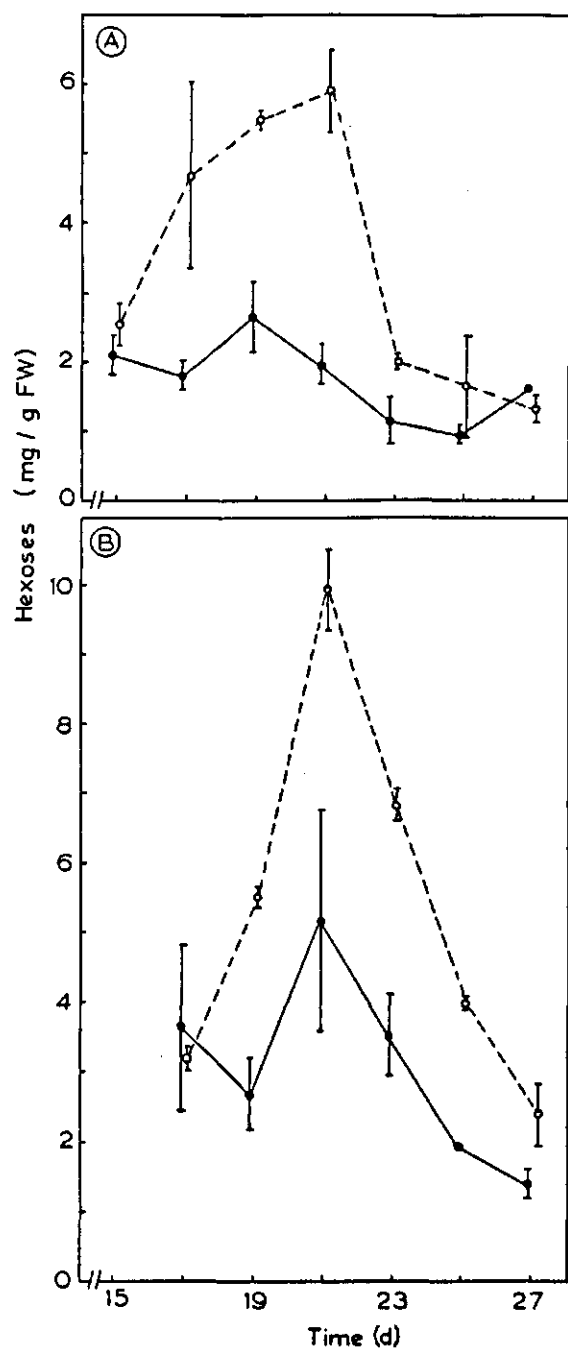


Figure 14. Hexose content of the third (A) and fourth (B) internode of beans grown from day 6 in REL (O---O) or BEL (●—●). Otherwise as for Fig. 12.

The starch content of internode-3 samples from bean plants grown for 19 days in REL or BEL was about equal; expressed as glucose units it was  $0.77 \pm 0.03$  and  $0.70 \pm 0.4$  for the REL- and BEL-grown plants, respectively.

Sucrose-synthase and hexokinase activities were only measured incidentally to gain some experience in the methodology of their determination. The results of the measurements of their activities are presented in Table 4. The measured activity of sucrose synthase in bean roots was similar to that reported for roots of *Ricinus communis* (Chapleo 1987). Since both enzymes were not studied in relation to the development of the plants in different light qualities, the results of their determination will not be further discussed in this paper.

Table 4. Sucrose synthase and hexokinase activities in extracts of bean tissue. TF1 and TF2 are the first (mature) and second (expanding) trifoliolate leaves, respectively.

| Tissue    | Sucrose synthase<br>(nkatal/mg protein) | Hexokinase<br>(pkatal/mg protein) |
|-----------|-----------------------------------------|-----------------------------------|
| Root      | 0.22                                    | 11 to 20                          |
| Internode | 0.22                                    | -                                 |
| TF1       | 0.01                                    | 2.9                               |
| TF2       | 0.04                                    | -                                 |

#### Chlorophyll and carotenoid content

The chlorophyll and carotenoid content of the primary leaves and the first trifoliolate leaf of bean were determined 9 and 13 days after the start of the REL- and BEL-treatment. No or only very small differences in pigment content were observed in the leaves after 9 days, but after 13 days the chlorophyll *b* of the primary leaves of the BEL-grown plants was slightly lower and the carotenoid content slightly higher. Thirteen days of REL resulted only in a small increase in the chlorophyll *a* content of the first trifoliolate leaf (Table 5).

Table 5. Effect of a prolonged exposure of bean plants to red- or blue-enriched light on the pigment content of the leaves. Chlorophyll *a*, chlorophyll *b*, and carotenoid contents were measured 9 days (primary leaves) or 13 days (primary leaves and first trifoliate leaf) after the start of the light treatment and represent the mean of 3 measurements with leaves from 3 plants in each  $\pm$ SD.

| Tissue<br>and<br>light treatment | Pigment content (mg / g FW) |                      |                 |
|----------------------------------|-----------------------------|----------------------|-----------------|
|                                  | Chlorophyll <i>a</i>        | Chlorophyll <i>b</i> | Carotenoids     |
| <u>Primary leaf</u>              |                             |                      |                 |
| Red 9d                           | 1.29 $\pm$ 0.01             | 0.32 $\pm$ 0.01      | 0.28 $\pm$ 0.01 |
| Blue 9d                          | 1.29 $\pm$ 0.05             | 0.31 $\pm$ 0.02      | 0.29 $\pm$ 0.01 |
| Red 13d                          | 1.38 $\pm$ 0.09             | 0.36 $\pm$ 0.01      | 0.29 $\pm$ 0.03 |
| Blue 13d                         | 1.41 $\pm$ 0.08             | 0.40 $\pm$ 0.01      | 0.32 $\pm$ 0.01 |
| <u>Trifoliate leaf</u>           |                             |                      |                 |
| Red 13d                          | 1.66 $\pm$ 0.04             | 0.39 $\pm$ 0.02      | 0.39 $\pm$ 0.01 |
| Blue 13d                         | 1.56 $\pm$ 0.02             | 0.36 $\pm$ 0.03      | 0.37 $\pm$ 0.01 |

## DISCUSSION

The present study demonstrates the possibility to affect the distribution of assimilates and the relative growth rates of different parts of a plant by changing the quality of the light in which the plants are grown: REL resulted in an increase of internode growth in comparison with BEL without affecting the growth of the plant as a whole (Table 2). This suggests that the photosynthesis of the plants grown under the two light conditions differing in spectral quality, but supplying the plants with equal amounts of PAR, had been similar. This suggestion is further supported by the observations that the leaf areas and the contents in chlorophyll and carotenoids of the leaves were hardly affected by the difference in light quality. However, since effects of light quality on the quantum efficiency of CO<sub>2</sub> fixation (McCree 1972) and on respiration (Voskresenskaya 1979) have been observed in plants, it would be better to validate the assumption of equal rates of photosynthesis by determining the rates of respiration and CO<sub>2</sub> fixation under the conditions at which the plants were grown in this experiment.

It seems a logical assumption that faster growing internodes have a higher demand for assimilates. However, the mechanisms that regulate the distribution of assimilates within plants are only poorly understood. Morris and Arthur (1984a, 1985) have proposed that an increase in the activity of invertase in the cells of the internodes increases their sink strength for sucrose. Increased hydrolysis of sucrose may facilitate the unloading of sucrose from the phloem to the growing cells of the internode by maintaining a gradient in the concentrations of sucrose in the vascular tissue and the elongating cells. Further, Morris and Arthur suggested that the increased supply of hexoses by the stimulated invertase accelerates the growth rate of the internodes by enhancing the availability of sugars for the synthesis of cell walls and other cellular constituents. In my opinion, the observation that the faster rate of internode elongation in REL-treated plants is accompanied by an increase in the level of hexoses indicates that the supply of hexoses is greater than needed for structural growth alone. Apart from providing more substrates for growth, an increase in sucrose hydrolysis by invertase might well function as a means to lower the osmotic potential of the cells, which would subsequently result in a stimulation of the water uptake and elongation of the cells. A more elaborate study on the involvement of hexoses in the regulation of the osmotic properties of the expanding cells is needed to test the role of hexoses in internode elongation just referred to.

Although the levels of sucrose decreased at about the same rate and the differences in invertase activity in the internodes of REL- and BEL-grown

plants were small, only the REL-grown plants showed an increase in internode elongation and an increase in hexose content. Since only the level of sucrose was measured and not the influx of sucrose into the internode, similar decreases in sucrose content do not necessarily imply equal rates of hexose production. Therefore, without measuring sucrose and hexose fluxes, it is not possible to attribute the lower level of hexoses in BEL-grown plants to a higher rate of conversion of hexoses into other metabolites, to an increase in respiration, or to a lower rate of sucrose transport into the internode.

Blue light has been reported to stimulate the incorporation of photosynthates into proteins and to increase respiration (Voskresenskaya 1979). However, it seems unlikely that these effects of blue light are responsible for the observed lower levels of hexoses in internodes of BEL-grown bean plants. Firstly, because no differences in the protein content of the internodes of REL- and BEL-grown plants were observed (Fig. 6) and secondly, because the dry weights of both groups of plants after a 2-week light treatment (Table 2) did not indicate any difference in the amount of photosynthates respired (assuming equal rates of photosynthesis).

Another question that remains to be investigated is whether an increase in invertase activity stimulates internode elongation by supplying more substrates for growth and/or by lowering the osmotic potential of the cells as discussed above, or whether the increase in growth causes depletion of sucrose in the cells which then stimulates invertase synthesis. Indirect evidence for the first possibility is suggested by experiments of Morris and Arthur (1984b) in which they demonstrated that stimulation of invertase activity by auxin was not a consequence of the increase in internode elongation normally caused by the auxin application, since it was also observed in auxin-treated plants in which the auxin-induced elongation response was inhibited by growth inhibitory concentrations of  $\text{Ca}^{2+}$  ions or mannitol.

In general, light-quality effects on internode elongation are attributed to differences in R:FR ratios in the light received by the plant and are believed to be mediated by the plant photoreceptor phytochrome. It seems unlikely that the differences in internode elongation in bean plants grown in BEL or REL observed in this study, were caused by a purely phytochrome-controlled reaction, as the R:FR ratios in BEL and REL of 4.4 and 3.4, respectively, should, theoretically, have resulted in the same phytochrome equilibrium in the plants of about 0.68 (Smith 1986). Therefore, it is unlikely that the internode elongation in the BEL- and REL-grown plants was solely controlled by phytochrome. Thomas and Dickinson (1979) demonstrated that both phytochrome and a blue-light receptor were involved in the control

of hypocotyl extension in lettuce, cucumber and tomato seedlings, but that the relative importance of the two photoreceptors varied greatly between species. Mohr (1986, 1987) has proposed a mechanism of photoreceptor coaction, in which blue light regulates the sensitivity of the plant for changes in the phytochrome equilibrium brought about by changes in R:FR of the light. Ritter et al. (1981) demonstrated that in neutral shade, blue light is the most important light quality factor regulating internode-extension growth in *Chenopodium rubrum*. Although the quality of the light is a very important factor in the regulation of internode elongation, this elongation growth is not totally independent of the level of PAR. Several studies have demonstrated interactions between effects of light quality and light quantity on the growth of internodes. E.g., in *Rumex obtusifolius* petiole elongation was stimulated by a decrease in R:FR, but only if the plants had been grown at a high irradiance level (McLaren and Smith 1978). Similarly, the extent of stimulation of internode elongation in *Chenopodium album* as a response to a decrease in R:FR was less in plants grown at lower irradiances (Morgan and Smith 1981). Morgan and Smith suggested that in canopy shade the level of blue light controls the extent to which the internodes elongate as a response to a decrease in R:FR, thereby suggesting a role for the blue-light receptor as an irradiance counter. This suggests that in the present experiment the growth of the internodia may have been primarily controlled by the amount of blue light and not by the R:FR ratio of the light.

Finally, the observations presented and discussed in this paper clearly demonstrate the necessity for combined studies on the effects of light (quantity and quality) on photosynthesis as the assimilate-generating process and on the processes involved in the distribution of the available assimilates within the plant and in their metabolization, in order to explain the complex series of events that ultimately determine photomorphogenesis.

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