J Plant Growth Regul (1994) 13:143–146

Journal of
Plant
Growth
Regulation

Morphogenesis of Potato Plants In Vitro. I. Effect of Light Quality and Hormones

Nina P. Aksenova,[1,*] Tatyana N. Konstantinova,[1] Lydiya I. Sergeeva,[1] Ivana Macháčková,[2] and Svetlana A. Golyanovskaya[1]

[1]Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia; and [2]Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Praha, Czech Republic

Received January 20, 1994; accepted April 27, 1994

Abstract. Stem cuttings of potato plants (*Solanum tuberosum* L., cv. Miranda) were cultured in vitro on MS medium with sucrose either without or with addition of indole-3-acetic acid (IAA) or kinetin (K) under red light (R) or blue light (B). Plants on medium without hormones under R were thin, long, with very small leaves, and produced no or only a few microtubers (after longer-lasting cultivations). In B, plants remained short, thick, with large, well-developed leaves and produced a significant amount of microtubers. Darkening of both roots and shoots strongly promoted tuber formation; the tubers were formed on the darkened part of the plant. IAA had no pronounced effect on plant development in B except for slight lengthening of the stem, and, in longer cultivations, slightly enhanced tuber formation as well. In R, IAA brought about several significant effects: stem reduction and induction of tuber formation being the most significant. Kinetin in R increased tuber formation slightly. In B, kinetin not only strongly stimulated tuber formation, but also increased the total fresh weight and root (+stolons)/shoot ratio. Results are discussed with regard to the possible role of auxins and/or cytokinins in mediating the morphogenetic effects of light.

Growth and morphogenetic effects of light (quality, intensity, and duration) and phytohormones are well documented (e.g., Voskresenskaya 1979, Vince-Prue 1985, Pharis and Reid 1985), but their modes of action and mutual interactions are far from clear. One of the important questions is whether at least some of the morphogenetic effects of light are mediated by changes in phytohormone levels. Some evidence in the literature shows that red light (R) decreases the IAA level, as for example, in either etiolated maize mesocotyl (Iino 1982) or etiolated oat coleoptiles (Briggs 1963) and reduces growth rate as well. Recent studies showed that red light decreased the IAA level in epidermal cells of maize mesocotyl (Jones et al. 1991, Behringer et al. 1992). Also blue light (B) was reported to decrease elongation growth of hypocotyls of, for example, *Sinapis alba* and *Lactuca sativa* (Thomas 1980). Endogenous cytokinin levels were increased in B in hypocotyls of *Amaranthus caudatus* (Obrenovic 1980).

Comparing the conditions leading to flowering in tobacco explants and to tuber formation in potato plants in vitro, Aksenova et al. (1986) observed a pronounced effect of light quality (R or B) on morphogenesis and especially on tuber formation in in vitro cultured potato plants. Also, IAA or kinetin applied to the medium affected morphogenesis at different light treatments. The aim of this study was to determine in detail the effect of light quality and hormones on morphogenesis, especially tuber formation, in in vitro potato plants. In a subsequent study, morphogenesis will be related to endogenous levels of hormones.

Material and Methods

Potato (*Solanum tuberosum* L., cv. Miranda) stem cuttings were cultured in vitro for 1–4 months on MS medium (Murashige and Skoog 1962) supplemented with 60 mg · L^{-1} inositol, 0.4 mg · L^{-1} thiamine, 1 mg · L^{-1} pyridoxine, and 6–10% sucrose, which is necessary for tuber initiation and formation (Aksenova et al. 1986). In some experiments, the medium was supplemented with one of the following substances: 1 mg · L^{-1} kinetin, 1

* Author for correspondence.

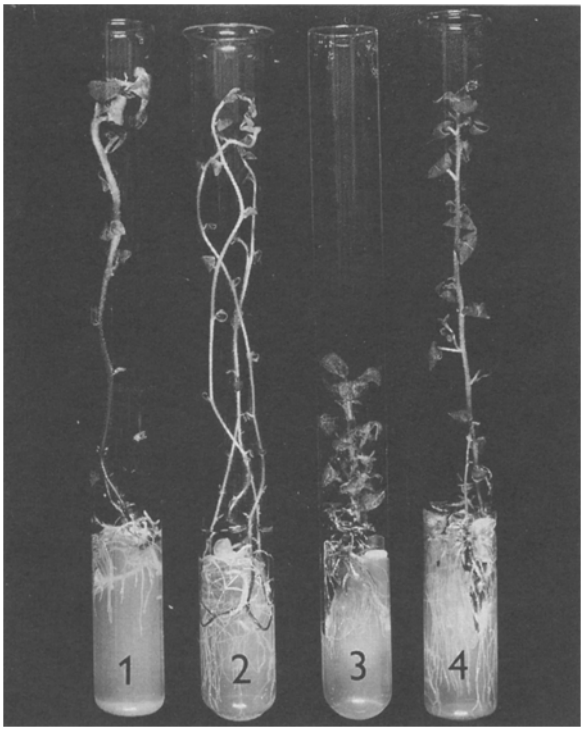

Fig. 1. Potato plants grown in vitro for 40 days under red (1, 2) and blue (3, 4) light, and short (10 h light) (2, 4) or long (18 h light) (1, 3) days.

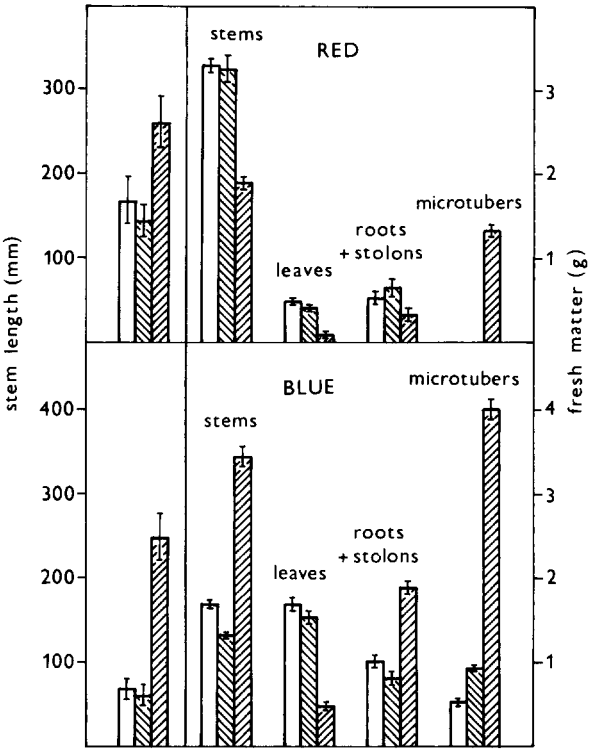

Fig. 2. Growth parameters of potato plants cultivated in vitro on MS medium without hormones under red or blue light during an 18-h photoperiod for 3 months. Whole plants or only roots or shoots were illuminated. Results represent mean values with SE (bars) from three experiments expressed as fresh matter of organs of 10 plants and that of stem length, which is the mean value with SE of one plant. (□) Whole plants illuminated; (▨) shoots illuminated; and (▨) roots illuminated. The values to the left of the dividing line should be viewed from the left *y* axis, and the values to the right of that line should be viewed from right *y* axis.

mg · L^{-1} IAA, 0.5 mg · L^{-1} benzylaminopurine (BAP), or 1 mg · L^{-1} α-naphthylacetic acid (NAA), respectively. Single plants were grown in test tubes at 20°C and either 18 h (LD) or 10 h (SD) photoperiod, light being provided in red (600–700 nm, maximum at 660 nm) or blue (400–500 nm, maximum at 480 nm) regions by means of luminiscent lamps with appropriate filters. Both light sources had the same photon flux of 160 μmol · m^{-2} · s^{-1} after filtering. In some experiments only aboveground or underground parts were illuminated. All experiments were repeated three to five times with similar results. Each experimental treatment included 15–30 plants. The results in the tables are means of all experimental series with standard errors, and the results shown in the photos come from one representative experiment.

Results

The Effect of Red Light and Blue Light in the Absence of Phytohormones

Both light quality and photoperiod affected very pronouncedly the appearance of in vitro potato plants. Plants grown under long day (LD) in R were elongated, thin, with very small leaves, whereas in B, plants remained short with thick stems and large, well-developed leaves (Fig. 1). Culture under the same conditions but a short photoperiod of 10 h brought about only slight changes in R, while in B a significant lengthening of plants was observed (Fig. 1). Growth parameters of plants grown under LD in R and B are given in Figs. 2 and 3. It is evident that great differences occurred in stem length, which was higher in R, and in leaf fresh weight, which was much higher in B. Furthermore, differences were observed in microtuber formation: no tubers in R (some may appear in longer-lasting experiments), but some tubers were always found in B (Figs. 2 and 3). The appearance of the plants depended also on which part of the plant was exposed to light. The effect of darkening either shoots or roots of plants cultured in B is shown in Figs. 2 and 4. Darkening of the shoot-induced etiolation phenomena, while darkening of the roots had no significant effect on plant growth. However, darkening significantly promoted tuber formation; the tubers were formed

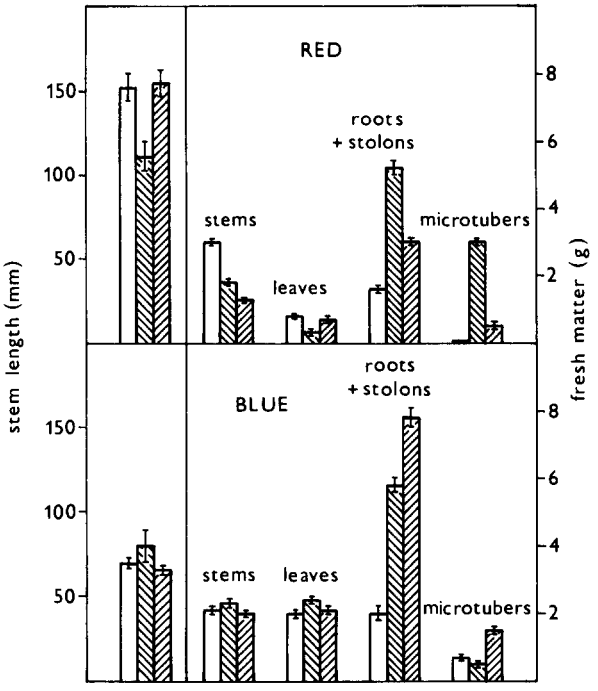

Fig. 3. Growth parameters of potato plants cultivated in vitro on MS medium without hormones or with IAA or kinetin (1 mg · L^{-1}) under red or blue light during an 18-h photoperiod for 3 months. Results represent mean values with SE (bars) from three experiments expressed as fresh matter of organs of 10 plants and that of stem length, which is the mean value with SE of 1 plant. (□) Control plants; (▨) IAA; and (▨) kinetin. The values to the left of the dividing line should be viewed from the left *y* axis, and the values to the right of that line should be viewed from the right *y* axis.

on that part of the plant, which was darkened (Figs. 2 and 4). In R, darkening of roots had no pronounced effect, darkening of shoots increased stem length, decreased stem, leaf and root fresh weight, but strongly increased tuber formation. In both R and B, darkening of shoots increased the ratio of fresh weight of underground/aboveground parts (Fig. 2).

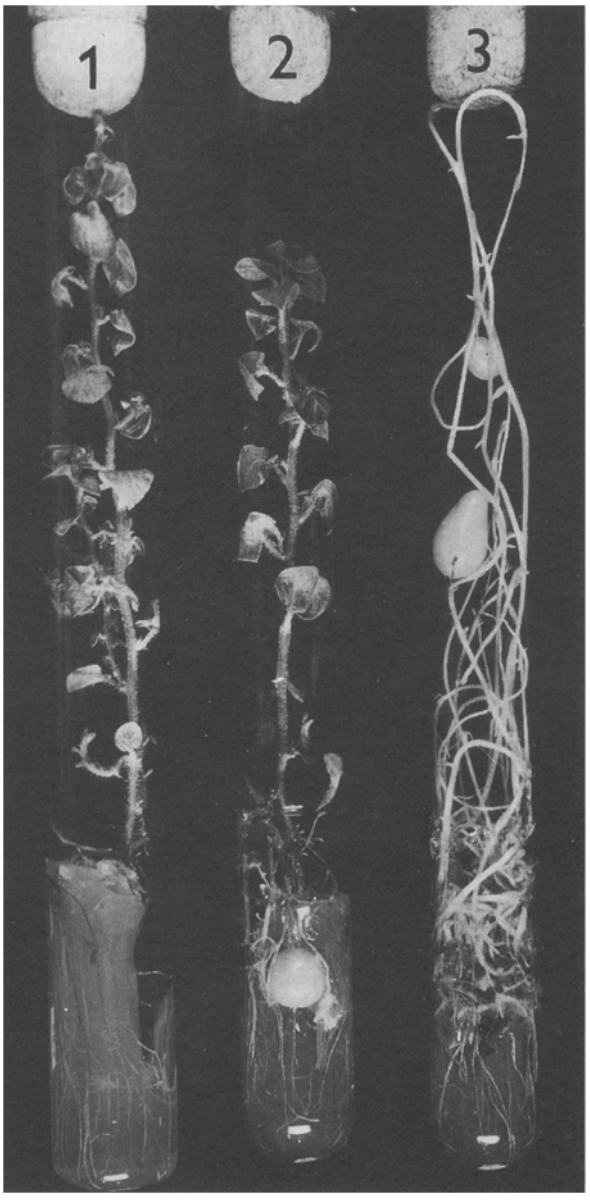

Fig. 4. Potato plants grown in vitro for 3 months under blue light and during an 18-h photoperiod with various parts of the plants covered: (1) Whole plants illuminated; (2) roots covered; and (3) shoots covered.

The Effect of Auxins and Cytokinins in R and B

The effects of IAA and kinetin on growth and morphogenesis of potato plants cultured in R and B are summarized in Fig. 3. IAA had no pronounced effect in B, with the exception of a slight lengthening of the stem and a slight increase in tuber formation in longer-lasting experiments, whereas in R, IAA brought about many significant changes: stem reduction, a decrease in total leaf fresh weight, significant increase of the root/shoot ratio (this stands for the ratio of fresh weight of underground/aboveground parts of the plants), root fresh matter, and induction of tuber formation. Kinetin had two effects in R: an increase of the root/shoot ratio and induction of tuber formation, but these effects were much weaker than that of IAA. In B, kinetin increased fresh weight of roots and stolons, root/shoot ratio, and tuber formation (Fig. 3). The ef-

fects of NAA (1 mg · L^{-1}) and BAP (0.5 mg · L^{-1}) were comparable with those of IAA and kinetin (data not shown).

Discussion

The effect of R seems to be contradictory to reports in the literature, which describe a decreased elongation in R in correlation with a decreased IAA level (Briggs 1963, Iino 1982, Jones et al. 1991, Behringer et al. 1992). But in all these cases, plants were etiolated, and the action of R was relatively short. In vitro potato plants grown in R or B for the whole cultivation period produced longer stems in R than in B, which was not due to higher fresh weight, as this was comparable in both light types (Fig. 2). The difference lies in the root/shoot ratio, which was higher in B than in R. Moreover, IAA application in R showed the opposite effect on stem elongation: a reduction instead of stem elongation which is usually reported in the literature. Thus, it can be argued that plants in R have sufficient IAA to sustain elongation growth, and as the effect of kinetin in R was very weak, the same might be expected for cytokinins. But then the question arises, why were no or only very few tubers initiated in R and why did both hormones, especially IAA, induce their formation? Our results provide evidence that the root/shoot ratio together with the actual elongation rate are determining factors in tuber formation. It is well known that tuber initiation is usually coupled with cessation of vegetative growth and start of senescence (Mokronosov 1964). In all our experiments, all conditions where tubers were initiated and formed, a relatively high root/shoot ratio was observed, that is, in R after IAA and K application, in B after K application and in R and B, when the shoots are darkened. Thus, light quality and hormone application may affect morphogenesis of in vitro plants probably in part due to changes in sink strength, and, as a consequence, to redistribution of active growth. The question remains whether hormones do mediate morphogenetic effects of light. This question was addressed in the following article where the relationship between morphogenesis in potato is related to levels of endogenous hormones and uptake, distribution and metabolism of applied hormones.

Acknowledgments. The authors wish to thank Prof. G. W. M. Barendse of the University of Nijmegen a critical reading of the manuscript and Mrs. B. Čermáková for preparing the manuscript.

References

Aksenova NP, Konstantinova TN, Bavrina TV, Chailakhyan MKh (1986) Interaction of light and phytohormones in regulation of morphogenetic processes in the in vitro culture (In Russian). DAN SSSR 290:508–512

Behringer FJ, Davies PJ, Yang P, Law DH (1992) The role of indoleacetic acid in mediating changes in stem elongation of etiolated *Pisum* seedlings following exposure to light. In: Karssen CM, vanLoon LC, Vreugdenhil D (eds) Progress in Plant Growth Regulation. Kluwer Acad. Publ. House, Holland, pp 437–445

Briggs WR (1963) Red light, auxin relationships and the phototropic responses of corn and oat coleoptiles. Am J Bot 50:196–207

Iino M (1982) Inhibitory action of red light on the growth of the maize mesocotyl: evaluation of the auxin hypothesis. Planta 156:388–395

Jones AM, Cochran DS, Lamerson PM, Evans ML, Cohen JD (1991) Red-light regulated growth. I. Changes in the abundance of indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize mesocotyl. Plant Physiol 97: 352–358

Mokronosov AT (1964) Photoperiodic reaction of potato (In Russian). Bot Zhurnal 49:935–946

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Obrenovic S (1980) Study of the blue and red light action in *Amaranthus caudatus* sections. In: DeGreef J (ed) Photoreceptors and plant development, Antwerpen Univ Press, Belgium, pp 383–392

O'Brien T, Beall FD, Smith H (1985) De-etiolation and plant hormones. In: Pharis RP, Reid DM (eds) Hormonal regulation of development. III. Role of environmental factors. Encycl Plant Physiol, New Ser, Vol 11, Springer Verlag, Berlin Heidelberg New York Tokyo, pp 282–307

Pharis RP, Reid DM (eds) (1985) Hormonal regulation of development. III. Role of environmental factors. Encycl Plant Physiol, New Ser, Vol 11, Springer Verlag, Berlin Heidelberg New York Tokyo

Thomas B (1980) The control of hypocotyl extension by blue light. In: DeGreef J (ed) Photoreceptors and plant development. Antwerpen Univ Press, Belgium, pp 531–535

Vince-Prue D (1985) Photoperiod and hormones. In: Pharis RP, Reid DM (eds) Hormonal regulation of development. III. Role of environmental factors. Encycl Plant Physiol, New Ser, Vol 11, Springer Verlag, Berlin Heidelberg New York Tokyo, pp 308–364

Voskresenskaya NP (1979) Photoregulatory aspects of plant metabolism (In Russian). Nauka, Moscow