EFFECT OF LIGHT QUALITY ON GROWTH OF *IN VITRO* CULTURED ORGANS AND TISSUES

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It has been widely shown that *in vitro* microenvironmental culture conditions such as light, temperature, and moisture, may alter tissue response. A literature search showed several studies on light intensity and photoperiod but light quality has not attracted much research attention.

Photomorphogenetic effects of certain light spectral bands are mediated by pigments such as phytochrome, blue, and near UV photoreceptors. The physiological processes that underlie *in vitro* photomorphogenetic effect expression may be various but strictly connected to the degree of tissue differentiation. When microcuttings, shoot apexes, and leaves are concerned these processes involve mainly apical dominance physiology, dormancy induction, and/or bud opening and root induction and formation. With undifferentiated tissue such as callus, cells, or protoplasts we may obtain induction and formation of organs such as roots and shoots and somatic embryos.

Because of different protocols and methodologies among *in vitro* systems, the effects observed under various light conditions are often contradictory and difficult to compare. The purpose of this review is to indicate, on the bases of knowledge available today, the most important physiological and technical aspects related to quality of light applied to *in vitro* cultures.

EFFECTS OF LIGHT QUALITY ON DIFFERENTIATED TISSUES

Shoot Proliferation and Shoot Quality. Research has recently shown that light quality can be considered a means for influencing morphogenesis of *in vitro* cultured tissues. Light quality may work by modifying the efficacy of added growth regulators as well as affecting the endogenous hormonal balance of the tissue. Therefore it could perhaps be manipulated to induce a physiological balance favorable for a desired growth response and it may be possible to maintain it as long as wanted.

Among the various growth responses, shoot proliferation is particularly interesting for micropropagation; it is based on cytokinin induced release from apical dominance of axillary buds. That apical dominance might be affected by light conditions has been accepted for many years (8). Two light qualities, red and

blue, are able to stimulate shoot proliferation; their efficacy seems to be related to the presence in the tissue of specific pigments. Various species have been shown to respond to red light and others to blue. Finally, there are plants which give similar responses to the same light quality but probably, as already suggested, it mainly depends on the experimental procedures followed.

Red Light. Red light was the first shown to stimulate shoot proliferation. Phytochrome is the active photoreceptor sensitive to red and far-red light. In its active form it seems to alter the endogenous hormonal balance in favour of reducing apical dominance and increasing lateral shoot development. The earliest work on this subject was done by Tucker (23) who showed that five minutes of far-red light after 16 h of fluorescent light inhibited the opening of axillary buds in *in vivo* tomato plants. The formation of abscisic acid in or near the buds, as a consequence of increased auxin synthesis in the apex and young leaves, was indicated as the cause of reduced buds development.

Similar results were obtained *in vitro* by continuous irradiation with far-red light. Trials were carried out by Baraldi et al. (1) to study the effect of phytochrome on GF 655/2 plum shoot proliferation *in vitro*. The authors did not obtain any increase of proliferation rate (Figure 1) with or without BA in far-red light conditions and the result was similar to that detected in darkness. However, white, red and blue light treatments displayed higher and very similar promoting effects.

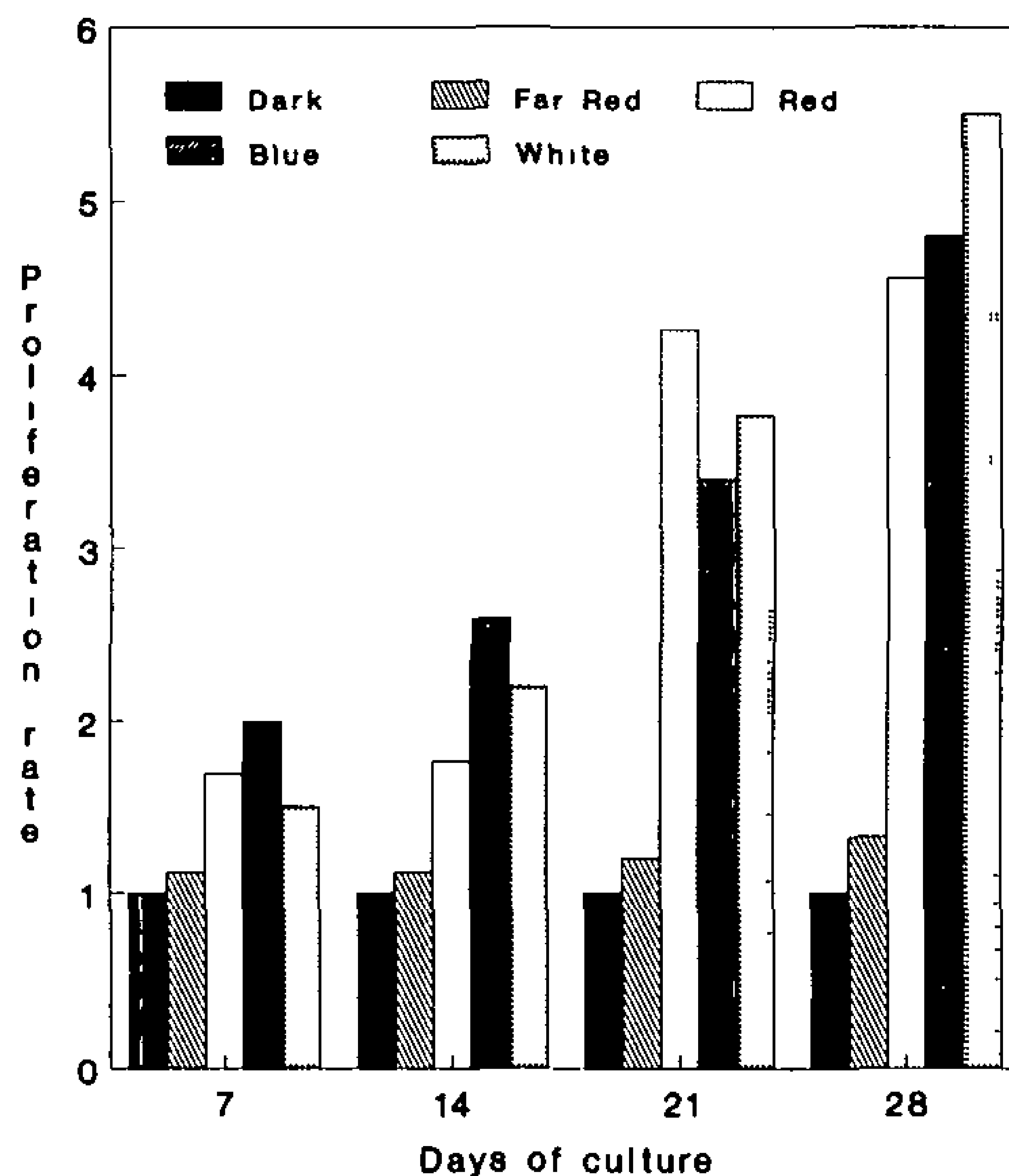


Figure 1. Effect of light quality on proliferation rate of GF 655/2 plum (modified from Baraldi et al 1988)

A similar response was induced on spirea (15) by red light while blue induced a lower proliferation rate than white light (control). The authors (14, 15) observed an interaction between cytokinin and red light. Subculturing over a long period under white light at both high and low cytokinin concentrations caused a reduction in proliferation rate. This negative trend was reversed when red light was applied which also reduced cytokinin requirement.

The interaction between cytokinin and radiation has not yet been definitively demonstrated. Neither blue, red or white light had any enhancing effect on plum rootstock GF 655/2 proliferation in the absence of cytokinin in the culture medium (1); a similar response to that induced by far-red light was seen. With BA in the medium the three above mentioned radiations caused considerable enhancement of proliferation. With red light the new shoots were more numerous and of higher quality compared to those produced under far-red light. However, in other research incorporation of 2iP into the medium eliminated the promotive effect of red light (18).

It is worth noting that phytochrome seemed to react to HIR and LIR in a trial carried out on microcuttings of peach rootstock GF 677; white light induced a higher production of shorter shoots compared to those produced in blue and red light (Table 1). When red light was applied at two levels (15 and 40 μmol m^2 sec^{-1}), the number of shoots produced at the lower one tended to be greater but the performance of the explants did not change (13).

Table 1. Effect of different light qualities on some parameters of GF 677 microcuttings

Light treatment	Proliferation rate	Shoots longer than 1 cm (%)	Internode length (mm)
White (a)	7.1 b	28 0 a	1 54 a
Blue (a)	2 4 a	40 0 b	2 37 b
Red (a)	3 5 a	40 1 b	2 71 b
Red (b)	5 2 ab	43 2 b	2 69 b

(a) = 40 μmol m^{-2} sec^{-1}, (b) = 15 μmol m^{-2} sec^{-1}

Finally, attempts to modify phytochrome activity by 15 min treatments of low-intensity red or far-red light at the end of 16 h of white light had no influence on the proliferation rate of azalea (5) and MrS 2/5 plum rootstock (6). Nevertheless two interruptions of the dark period with red light caused an increase in the dry matter in the latter species (Table 2).

Table 2. Fresh and dry weight of MrS 2/5 shoot cluster as affected by one or two red light interruptions of dark period in a photoperiod of 16 h light and 8 h dark

Light treatment	Fresh weight (g)	Dry matter (%)
16/8	2.4 ns	5 7 a
16/8 (1 red light)	2 1 ns	6.3 ab
16/8 (2 red light)	2.3 ns	7 1 b

Blue light. Not every species responds to red light with proliferation. Chee (2, 3) observed that as well as enhancing shoot size, blue light also increased proliferation rate in grape cultivars by about 50% more than red light. These results led the author to suggest that a blue photoreceptor, not phytochrome, was involved, hence determining a blue light-induced inhibition of apical dominance.

The use of high pressure sodium vapor lamps with *Potentilla* and *Spiraea* (15) raised the proliferation rate and increased shoot length; the effect of these lamps on grape was to diminish proliferation (16). Red light had the same effect on *Spiraea*. Blue light induced shorter shoots when BA concentration in the medium was low (0.25, 0.5 mg/l), whereas at 1 mg/l, shoot length was similar to that of the fluorescent light of the control (16). The opposite response in grape was found by Chee (3) and may depend on a specific response to morphogenetic induction.

The difference between results from sodium and fluorescent lamps may be explained by the differences in their spectra since light from a fluorescent lamp has more blue radiation than that from a sodium lamp which emits more red light. The physiological effect of these wavelengths is confirmed by the fact that blue and far-red radiations inhibit lettuce seedlings and hypocotyl segments lengthening; this effect is reversed by GA_3 treatments and red light (21, 22).

Growth and morphology were dramatically affected by light quality also in potato plantlets; incandescent lamps induced longer stem length but smaller leaf area, number of leaves, and fresh and dry weights, than those recorded with a light quality as determinated by cool white + Agrolite fluorescents and cool white fluorescent + incandescent combinations (19).

Other light qualities. Research in progress in our Department seems to indicate that other light qualities different from red and blue are also able to influence shoot proliferation (12). As shown in Figure 2, yellow light increased the number of shoots more than the other light qualities. In another trial green light caused highest proliferation. It is still to be verified if it was the cause, but spectra

of both light qualities displayed a well developed peak at 550 nm. Shoot quality also was improved by green and yellow light.

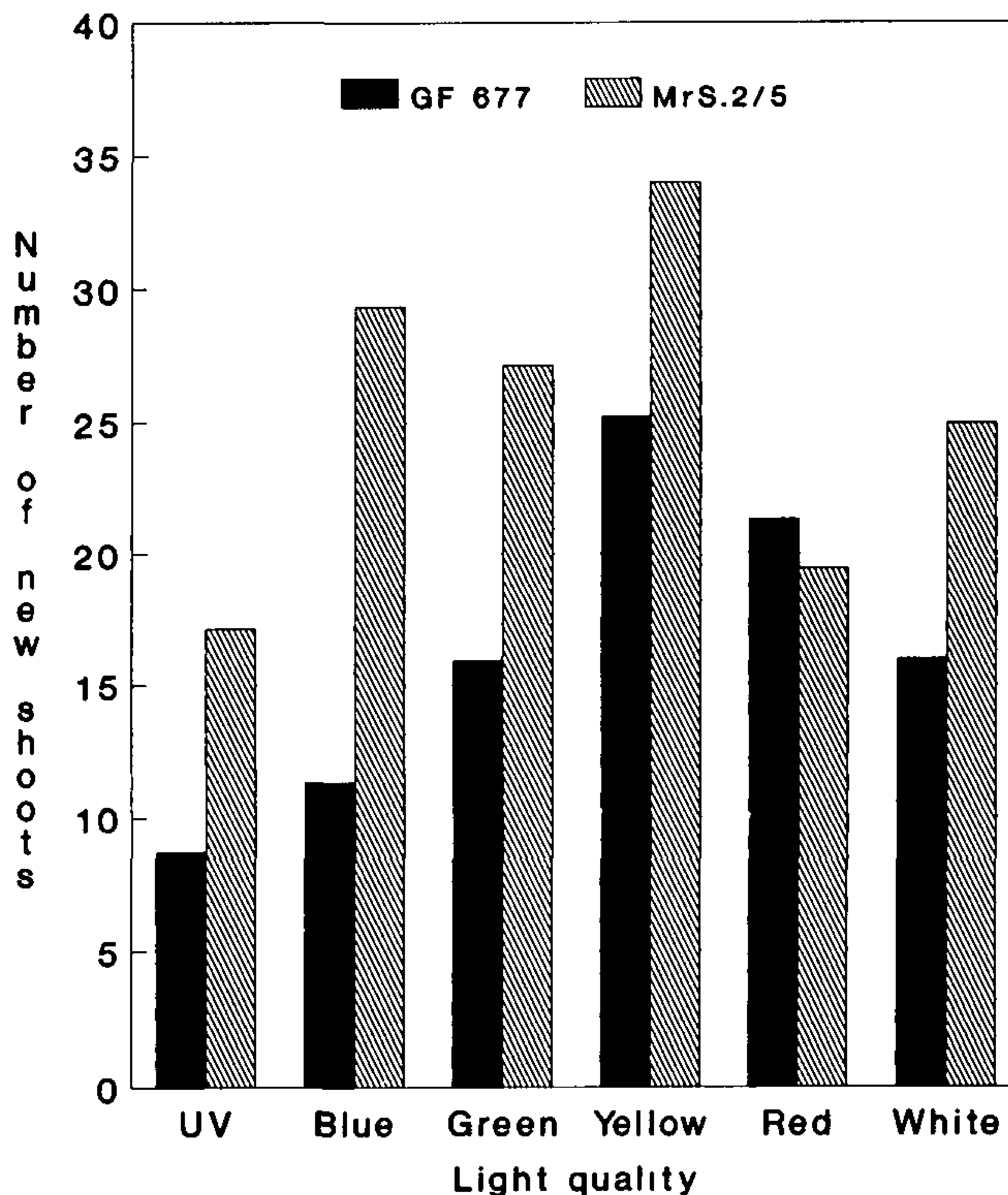


Figure 2. Number of new shoots occurring on primary explants as influenced by different light qualities

Shoot Rooting. Little research information is found on the influence of light quality in *in vitro* rooting. Trials on grape (4) showed higher shoot rooting percentages with red light; moreover this radiation increased the number of roots per shoot and gave a greater total root length per shoot compared to blue light. A similar trend was seen by the same author under cool white lamps instead of daylight lamps. This was perhaps because of the higher proportion of red irradiance in cool white lamps. Red light

stimulation of root development was also observed on undifferentiated callus tissue such as with *Helianthus tuberosus* (10).

The effect of NAA and red radiation on root induction appeared independent for *Prunus* GF 655/2 (1). Under red light the addition of NAA to culture medium had no effect on rooting which was 100%. By contrast, under far-red light without NAA, rooting fell to about 10% but rose to 100% with the addition of auxin to the medium. Under white and blue light and in the dark, rooting was conditioned by the presence or absence of auxin in the medium, which would seem to indicate that phytochrome affects metabolic processes concerning root induction.

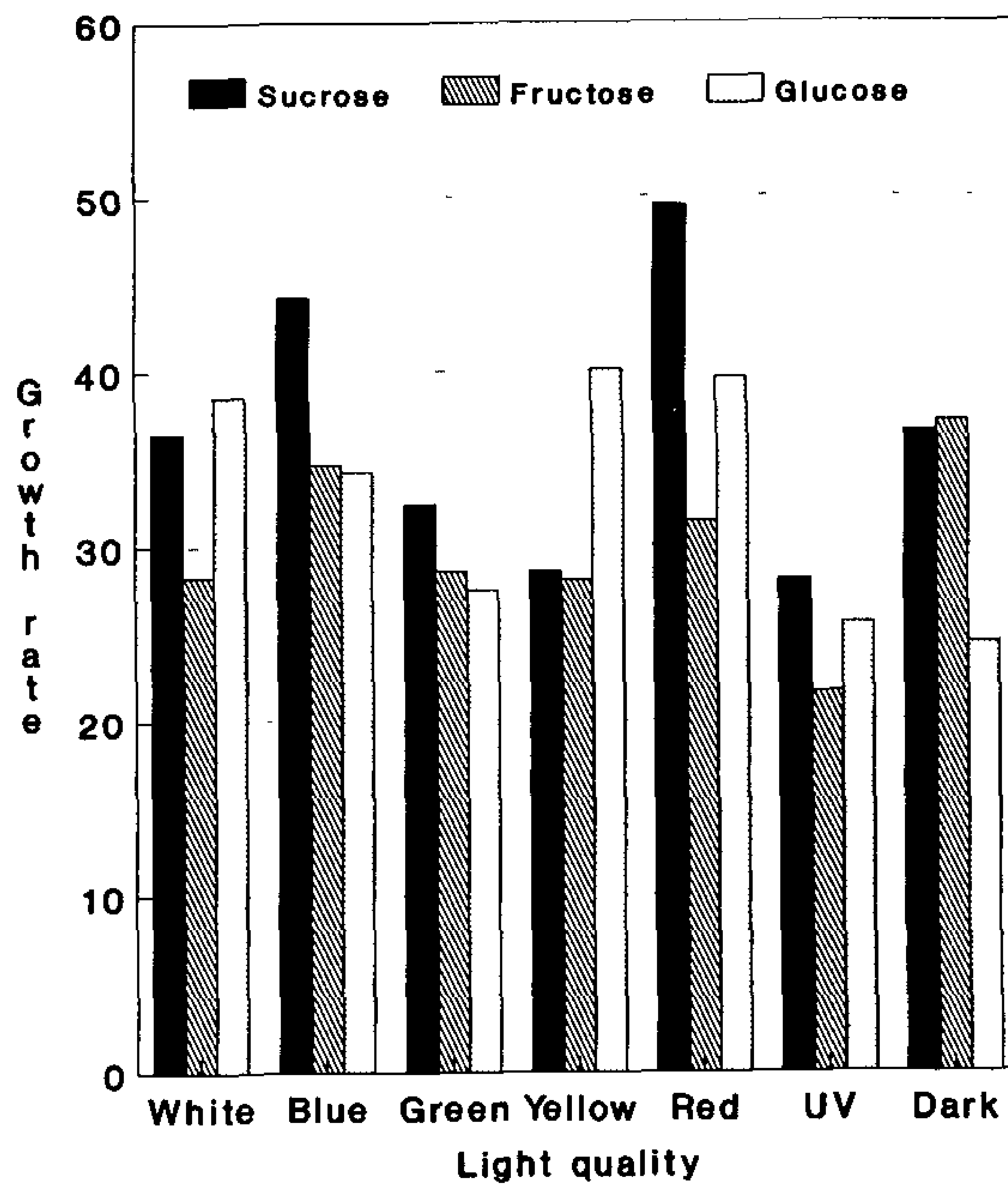
Enhancement of rooting by red light in comparison to white and blue was also observed for MrS 2/5 with 55, 37, and 35% rooting, respectively (12).

The effect of light on root elongation *in vitro* was also studied in *Dracaena fragrans*. Root elongation in blue and red light was promoted almost as well as in corresponding white light intensity, while in far-red light root elongation was inhibited as well as in darkness (24).

Light Quality Effect on Callus Growth and Organogenesis. Light in the near-ultraviolet (371 nm) region of the spectrum inhibited callus growth as observed in embryo cultures of *Pseudotsuga menziesii* (9), and in tobacco callus cultures at 16 h/day irradiance above 150 $\mu W\ cm^{-2}$, whereas irradiance of 24 $\mu W\ cm^{-2}$ was promotive (20). UV again inhibited growth at irradiance of 40 $\mu mol\ m^{-2}\ sec^{-1}$ in *Actinidia deliciosa* callus cultures (Figure 3), when sucrose, fructose, and glucose were used as energy sources in the medium (13). Similarly, near-ultraviolet light showed a negative effect on shoot initiation in tobacco callus cultures (20).

Growth and shoot formation in tobacco callus were stimulated by treatment of blue light (467 nm) at irradiance from 100 to 500 $\mu W\ cm^{-2}$ for 16 h day (20); shoots were also produced when cultures were exposed continuously for 5 weeks to blue light at a high irradiance of 1550 $\mu W\ cm^{-2}$ (26).

Compared to green and red light or darkness, continuous blue light (450 nm) for 3 weeks at 1500 $\mu W\ cm^{-2}$ increased the fresh weight of the pith callus of *Pelargonium zonale* (25). In carrot callus (17), on the other hand, blue light depressed growth and red and polychromatic light enhanced it as revealed in the higher mitotic division rate of callus cells. Red light applied to callus produced by pine embryos also induced formation of adventitious shoots (7); callus from kiwi fruit leaves (11) gave the best performance in terms of callus growth rate (Fig. 3 above) and shoot organogenesis (Fig. 3 below).



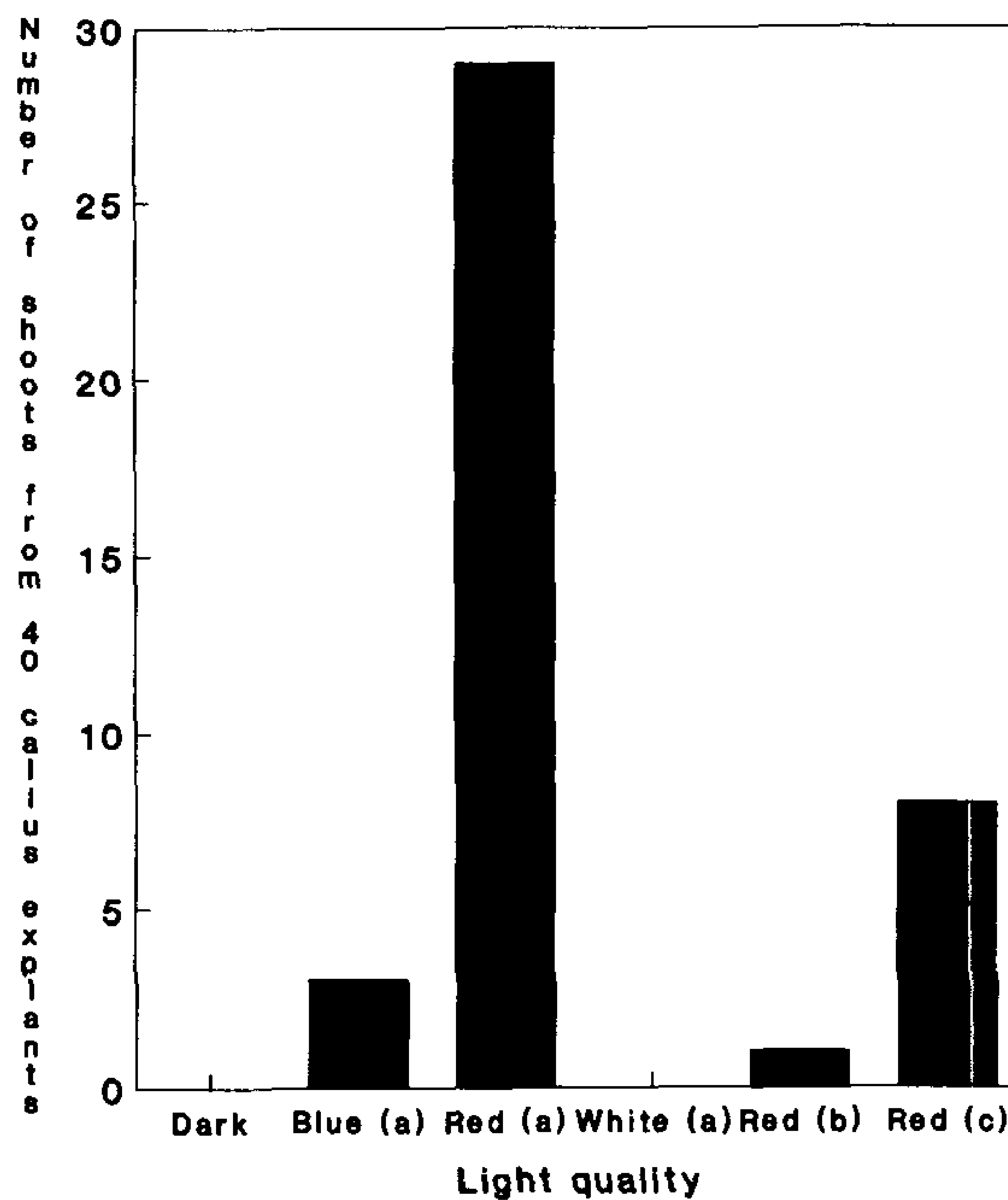


Figure 3. Effect of light quality on growth rate (above) and shoot regeneration (below) in *Actinidia deliciosa* callus. (a) = 40 μmol m^{-2} sec^{-1}; (b) = 15 μmol m^{-2} sec^{-1}; (c) = 5 μmol m^{-2} sec^{-1}.

CONCLUSIONS

Available results so far are not conclusive for practical applications. Variability of response according to species and light quality is a stumbling block on the way to understanding the physiological mechanisms involved in a particular growth performance. It has been shown that no one light quality is effective for all species and all objectives of *in vitro* culture. Much carefully planned research will have to be conducted using standardized experimental methods and characterizing genetic material, which is probably the principal source of non-uniformity in response.

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