Journal of Experimental Botany, Vol. 33, No. 136. pp. 1068–1075, October 1982

Tomato Seed Germination. Osmotic Pretreatment and Far Red Inhibition

K. GEORGHIOU, C. A. THANOS, T. P. TAFAS AND K. MITRAKOS

Institute of General Botany, University of Athens, Panepistimiopolis, Athens 621, Greece

Received 14 April 1982

ABSTRACT

At 25 °C germination of tomato (*Lycopersicon lycopersicum*) seeds is inhibited by continuous and intermittent far red illumination. It is also inhibited by a single 30 min far red irradiation given 8 h from the start of imbibition. The incubation of seeds in a mannitol solution inhibitory for germination has no effect on the final germination percentage after seeds are subsequently transferred to water. A 30 min far red irradiation at the time of transfer results in partial inhibition of germination. This inhibition can be released by the continuation of osmotic incubation for several days before the transfer to water. At the end of a 7 d dark period of osmotic incubation, inhibition of subsequent germination in water can be realized only by continuous far red illumination. Seeds osmotically pretreated for 7 d and afterwards dried-back show a mean time to 50% germination significantly lower than that of untreated seeds. Moreover, besides single and intermittent, even continuous far red light has no inhibitory effect on the germination of these seeds. It is concluded that, in addition to the already known germination advantages, osmotic presowing treatment also induces the ability of seeds to germinate under unfavourable light conditions.

INTRODUCTION

The control of tomato seed germination by light and phytochrome is well documented (Egles and Rollin, 1968; Mancinelli, 1966; Mancinelli, Borthwick, and Hendricks, 1966; Mancinelli, Yaniv, and Smith, 1967; Yaniv and Mancinelli, 1967; Yaniv, Mancinelli, and Smith, 1967; Yaniv and Mancinelli, 1968). Depending upon the conditions and the cultivar used, tomato seed germination may be prevented by either a single low-energy (Mancinelli *et al.*, 1967) or a prolonged high-energy (Mancinelli *et al.*, 1966) far red (F) illumination. Furthermore, the continuous F requirement for germination inhibition can be successfully fulfilled by an intermittent F irradiation (Yaniv *et al.*, 1967). This substitution has promoted the idea that the prolonged F requirement results from the slow dark transformation of pre-existing phytochrome intermediates to P_{fr}, the active form of phytochrome, during imbibition (Kendrick, 1976; Kendrick and Spruit, 1977).

Osmotic presowing treatment of seeds, a procedure termed 'priming' (Heydecker, Higgins, and Turner, 1975) or 'osmoconditioning' (Khan, Tao, Knypl, Borkowska, and Powell, 1978), has been shown to result in a more rapid and more synchronized radicle emergence (Gray and Steckel, 1977; Guttridge and Bright, 1978; Heydecker, Higgins, and Gulliver, 1973; Knypl, Janas, and Radziwonowska-Jozwiak, 1980; Rennick and Tiernan, 1978; Salter and Darby, 1976. Review papers by: Khan, 1977; Heydecker, 1977; Heydecker and Coolbear, 1977; Hegarty, 1978; Tonkin, 1979; Khan, Peck, and Samimy, 1980/81). In addition, priming may enable seeds to germinate at temperatures lower or higher than that of untreated

seeds (Heydecker *et al.*, 1975; Sachs, 1977). Such an osmotic pretreatment is effective in tomato seeds; at 20 °C and 25 °C, the mean time to 50% germination of primed seeds is reduced to about half that of untreated seeds (Coolbear and Grierson, 1979; Coolbear, Grierson, and Heydecker, 1980; Heydecker *et al.*, 1975). This decrease is even greater at 15 °C (Lightburn, as referred to by Heydecker, 1977), which is explained by the fact that the promotive effect of priming is generally more pronounced at low temperatures (Heydecker, 1977).

In most of the cases studied, the photosensitivity of seeds imbibing under osmotic stress has been reported to change significantly (Thanos and Mitrakos, 1979). Nevertheless, it is only in three instances where an osmotic effect has been investigated and proven to be persistent after the subsequent transfer of seeds to water (with or without re-drying). In the first two cases the osmoticum induces a secondary, light-controlled dormancy in *Chenopodium bonus-henricus* (Khan and Karssen, 1980) and in *Lactuca sativa* L. cv. Grand Rapids (Kahn, 1960; Georghiou, 1981). In the third case, osmotically pretreated *Citrullus lanatus* seeds show a completely different type of photosensitivity during germination (Thanos, 1980).

Despite this indirect experimental information, current research has not tackled the possibility that photosensitivity changes are caused by osmoconditioning. Therefore, and besides the well known advantages for seed germination, we consider it interesting to question whether osmotic pretreatment would result in an improved germinability under adverse light regimes.

MATERIALS AND METHODS

Tomato (*Lycopersicon lycopersicum* (L.) Karsten ex Farwell, cv. Roma VF) seeds were obtained from ΚΥΔΕΠ, Greece. Germination tests were performed on samples of 50 seeds in 9·0 cm petri dishes, with 5·0 cm^3 of deionized water and two sheets of Whatman No. 1 filter paper. Temperature was kept at 25 °C, throughout the experimental procedure. Germination was counted 4 d after the onset of imbibition. Osmotic presowing treatment was carried out in mannitol solutions, in petri dishes (darkness, 25 °C). Osmoconditioned seeds were washed with deionized water and air-dried for 48 h, to their original moisture content. All manipulations were conducted under a dim green safe light.

The broad band far red light (>700 nm, emission maximum at 750 nm, fluence rate on the level of the seeds 5·0 W m^{-2}) was obtained from ten white incandescent tubes (Phillips philinea 6276X, 60 W) filtered through one red and two blue 3·0 mm plexiglas filters (Röhm 501 and 627, respectively) and a 5·0 cm deep water filter. The green safe light (525–575 nm, emission maximum at 550 nm, fluence rate 10 mW m^{-2}) from a green fluorescent tube (F 15 T8. G.6, 15 W Green-Photo, General Electric) was filtered through a 3·0 mm red orange and a 3·0 mm green plexiglas filter (Röhm 478 and 700, respectively).

Germination values are means of at least eight replications; ± values and vertical lines represent standard errors.

RESULTS

Far red sensitivity of untreated seeds

Over a wide range of temperature (15–30 °C) the germination of tomato seeds is strongly inhibited (<10%) by continuous F. In darkness, germination is optimal (>90%) at 25 °C (Table 2).

Intermittent F irradiation cycles (2 min F–58 min dark) is equally effective in inhibiting germination.

Figure 1 shows the photosensitivity towards F (30 min) during imbibition. It is clear that even a relatively brief illumination can suppress tomato seed germination and maximum inhibition is obtained when F is applied at around 8 h after onset of imbibition.

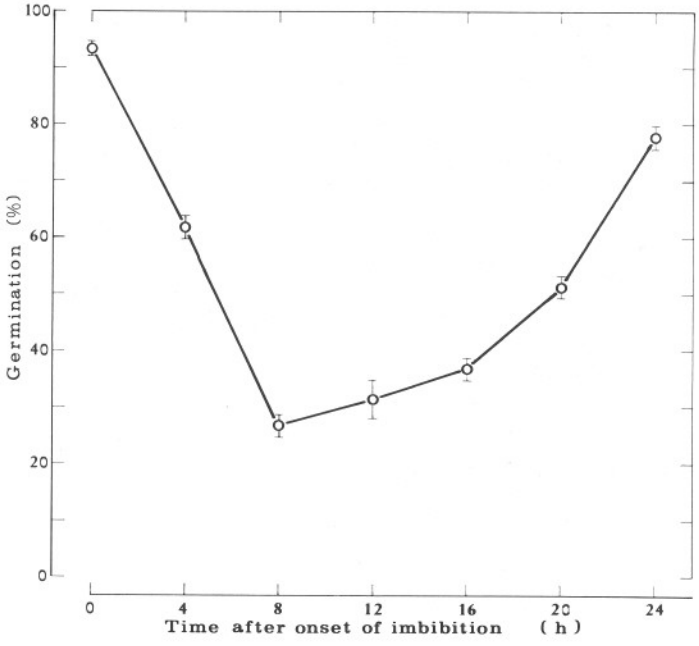

FIG. 1. The effect of brief (30 min) far red light (F) on the germination as a function of time of imbibition at 25 °C. Dark control 94 ± 1%.

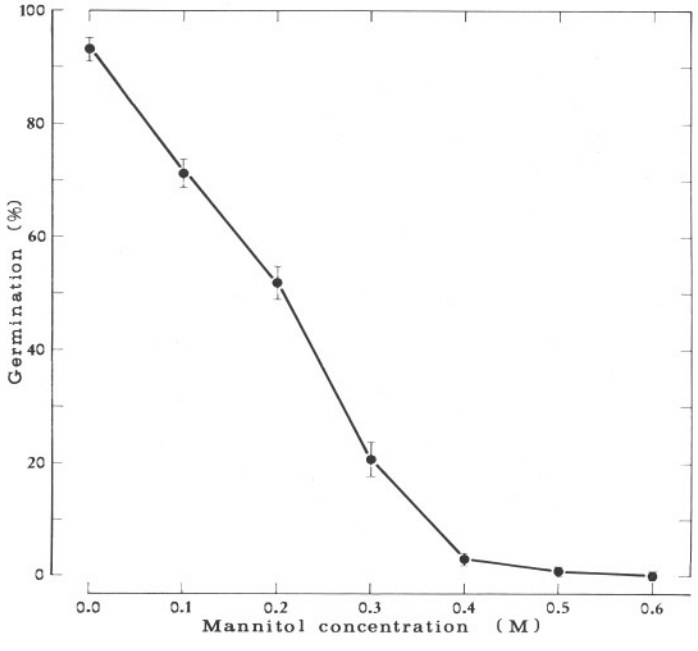

FIG. 2. Final dark germination in osmoticum as a function of concentration.

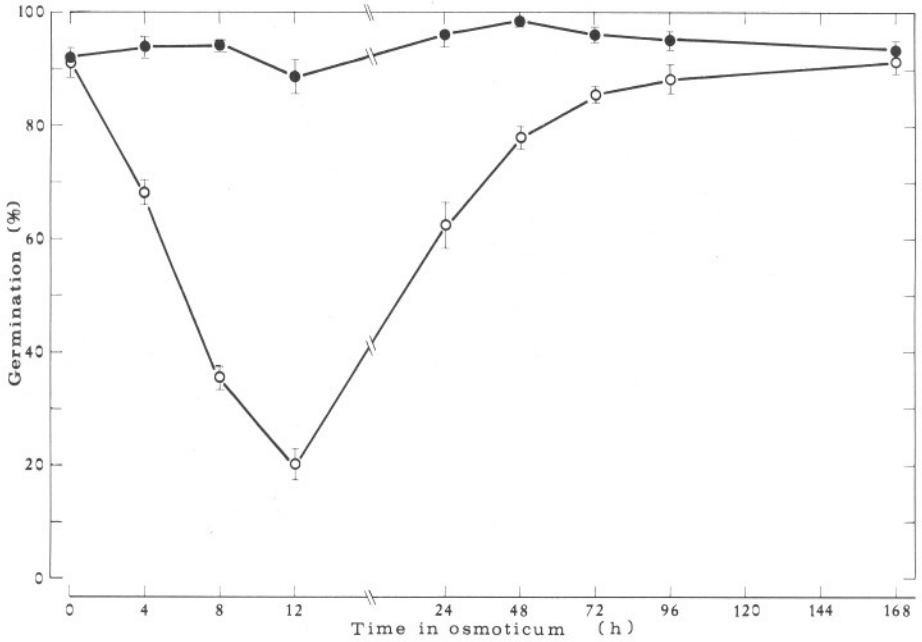

FIG. 3. The effect of brief (30 min) far red light (F) on germination as a function of the duration of osmotic pretreatment at 25 °C. (Seeds are illuminated immediately after transfer to water for germination; the osmoticum used is a 0·6 M mannitol solution; ○: F-treated, ●: dark controls).

Interaction of far red and osmomanipulation

Figure 2 presents the effect of osmotic solutions on dark germination. From the data shown it can be seen that a 0·6 M mannitol solution is totally inhibitory to germination and it is therefore used in subsequent experiments.

Tomato seeds imbibed in the osmoticum for up to 168 h in darkness, germinate fully when they are transferred to water (Fig. 3). A brief (30 min) F irradiation applied immediately after transfer to water results in a significant inhibition during the first 24 h. The kinetics of the inhibition in mannitol is markedly similar to the one obtained in water (Fig. 1) though minimum germination is observed 12 h and 8 h respectively after onset of imbibition. Furthermore, it is interesting to note that the action of F is progressively reduced and is nullified at the 7th day of osmotic incubation (Fig. 3). On the contrary, exposure of these seeds to continuous F results in a suppression of final germination to 15 ± 1%.

TABLE 1. *The effect of post-illumination osmotic treatment on the far red (F) inhibitory action at 25 °C. (Germination is counted after an additional 4-d dark period in water)*

Treatment	Germination (%)
12 h in osmoticum + 30 min F	20·40 ± 2·79
12 h in osmoticum + 30 min F + 3 d in osmoticum	43·60 ± 2·31
12 h in osmoticum + 30 min F + 7 d in osmoticum	82·40 ± 2·48

Table 1 shows that the effect of 30 min F can be reversed by an additional period of osmotic treatment, intervening between illumination and transfer to water. In addition, the longer this period the larger the reversion.

Photosensitivity of osmoconditioned seeds

Figure 4 shows the germination time course of tomato seeds osmotreated for 7 d and subsequently air-dried. It is clear that there is a considerable acceleration of germination compared to untreated seeds. So, the time for 50% germination is reduced from about 50 h to 30 h.

Moreover in experiments not presented here, it is observed that final germination of osmoconditioned seeds is fully manifested (91 ± 2%) at 15 °C while it is considerably lower in untreated seeds (40 ± 2%).

Table 2 compares the photosensitivity of osmoconditioned seeds with untreated and osmotreated (not dried) seeds. Germination after osmoconditioning cannot be suppressed by either brief (30 min) nor continuous F illumination. On the other hand germination of untreated seeds is inhibited by both brief and continuous (as well as intermittent) F, while

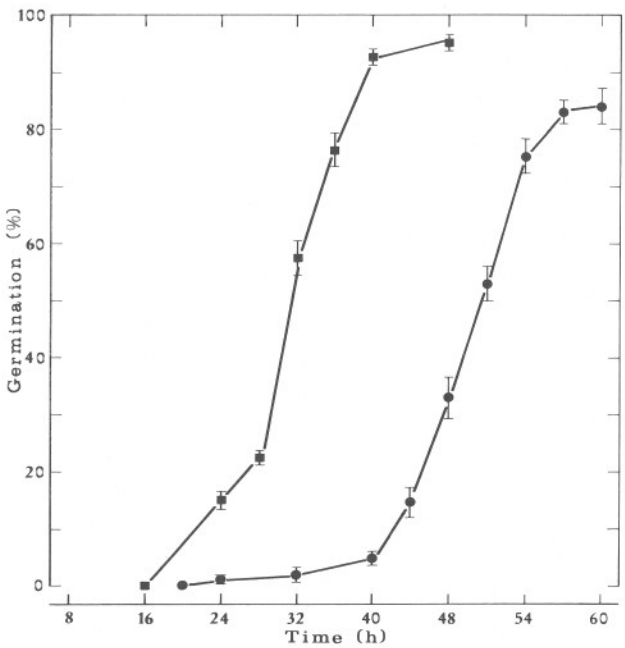

FIG. 4. Time course of dark germination at 25 °C (●: untreated and ■: osmoconditioned seeds).

TABLE 2. *The far red light (F) effect on the germination (%) of untreated, osmotreated and osmoconditioned seeds at 25 °C*

	Untreated	Osmotreated (not dried)	Osmoconditioned (dried)
Darkness	92·00 ± 2·00	93·00 ± 1·50	93·60 ± 2·21
30 min F[a]	16·40 ± 2·56	91·00 ± 1·00	91·60 ± 0·75
Intermittent F[b]	12·00 ± 1·00	—	88·35 ± 2·00
Continuous F	7·20 ± 0·83	15·00 ± 1·25	93·00 ± 1·29

[a] Given 8 h after onset of imbibition in untreated and osmoconditioned seeds and at the time of transfer in osmotreated ones.

[b] 2 min F h^{-1}.

osmotreated (not dried) seeds do germinate after brief but not under continuous F illumination.

DISCUSSION

The germination behaviour of the tomato cultivar Roma VF is studied here for the first time. The results obtained are quite similar to those reported for the cultivars Ace, Porte, Glamour and St. Pierre (Mancinelli *et al.*, 1967; Egles and Rollin, 1968). Our results are consistent with the above mentioned ones since maximum inhibition by 30 min F is observed 8 h after onset of imbibition (Fig. 1). It is worth noting that though continuous F illumination totally inhibits germination of Roma VF seeds (Table 2) a single brief F irradiation inhibits only a proportion of the seed population (Fig. 1, Table 2). A similar response has been reported for Ace, Porte and Glamour cultivars (Mancinelli *et al.*, 1966) as well as for St. Pierre seeds (Egles and Rollin, 1968). The inhibition of seed germination by a brief F irradiation is rather unusual and has only been observed in certain lots of partially light-requiring Grand Rapids lettuce achenes (Vidaver and Hsiao, 1972). In these cases, the action of a single F illumination is attributed to the presence in the seeds of an amount of the active phytochrome form (P_{fr}) sufficient to promote a proportion of the population to germinate in darkness.

The F sensitivity curve of germination for tomato seeds imbibed in osmotic solution during the pre-illumination period (Fig. 3) follows a similar pattern to that of seeds kept throughout in water (Fig. 1). The minimum germination observed are in both cases around 20%. Furthermore, it must be noted that the latter curve precedes the former by about 4 h and this can be interpreted in terms of different hydration rates in water and osmoticum.

From the results concerned with the interaction of osmotic treatment and F, three points are considered important: (a) The reversion of brief F inhibitory action on germination by an additional period in mannitol solution; (b) The ineffectiveness of brief F (but not continuous F) on seeds pretreated for a long period and immediately transferred to water; (c) The ineffectiveness of both brief and continuous F on germination when osmotreated seeds are dried prior to rehydration in water. There are several possible interpretations. The first point could be explained either by the slow transformation of phytochrome intermediate forms to P_{fr} (Kendrick and Spruit, 1977) or by the action of a small amount of P_{fr} during the long period of osmotic suppression of germination. The second result could be due to the escape of germination control from the low-energy reaction of phytochrome, according to the postulation proposed by Kendrick (1976). The interpretation for the effect of dehydration in the third case is completely obscure though it seems that germination has proceeded further than the second point of light control (i.e. the high-irradiance response) proposed by Kendrick (1976).

Although polyethylene glycol (PEG) and salt solutions are usually adopted for seed osmoconditioning, it is shown here that mannitol is a good osmoconditioning medium. Mannitol pretreatment of tomato seeds results in (i) a more rapid manifestation of germination at 25 °C (Fig. 4, where the 20 h advance in germination is strikingly similar with the one observed by Coolbear and Grierson, 1979, in tomato seeds pretreated in PEG for 12·5 d), and (ii) a considerably higher percentage of final germination at 15 °C (in agreement with data reported by Sachs, 1977, on watermelon seeds pretreated in different salt solutions). Furthermore it is clear that, although the underlying control mechanisms cannot be elucidated at the moment, osmotreated tomato seeds present a considerably increased germinability under F, in comparison with untreated seeds. Therefore, it is concluded that the ability for germination under adverse light conditions should be added to the beneficial results of osmoconditioning.

LITERATURE CITED

COOLBEAR, P., and GRIERSON, D., 1979. Studies on the changes in the major nucleic acid components of tomato seeds (*Lycopersicon esculentum* Mill.) resulting from osmotic presowing treatment. *J. exp. Bot.* **30,** 1153–62.

—— —— and HEYDECKER, W., 1980. Osmotic presowing treatments and nucleic acid accumulation in tomato seeds (*Lycopersicon lycopersicum*). *Seed Sci. Technol.* **8,** 289–303.

EGLES, D., and ROLLIN, P., 1968. La photosensibilité des graines de tomate var. St. Pierre. *C.r. hebd. Séanc. Acad. Sci., Paris*, **266**(D), 1017–20.

GEORGHIOU, K., 1981. Phytochrome control on the germination of non-photoblastic lettuce achenes (*Lactuca sativa* L. cv. Grand Rapids). Ph.D. Thesis. University of Athens.

GRAY, D., and STECKEL, J. R. A., 1977. Effects of pre-sowing treatments of seeds on the germination and establishment of parsnips. *J. hort. Sci.* **52,** 525–34.

GUTTRIDGE, C. G., and BRIGHT, S., 1978. Accelerating and synchronizing germination of strawberry seeds by osmotic pre-treatments. *Euphytica*, **27,** 843–8.

HEGARTY, T. W., 1978. The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. *Pl. Cell and Environ.* **1,** 101–19.

HEYDECKER, W., 1977. Stress and seed germination: an agronomic view. In *The physiology and biochemistry of seed dormancy and germination*. Ed. A. A. Khan. Elsevier/North-Holland Biomedical Press, Amsterdam. Pp. 237–82.

—— and COOLBEAR, P., 1977. Seed treatments for improved performance—survey and attempted prognosis. *Seed Sci. Technol.* **5,** 353–425.

—— HIGGINS, J., and GULLIVER, R. L., 1973. Accelerated germination by osmotic seed treatment. *Nature, Lond.* **246,** 42–4.

—— —— and TURNER, Y. J., 1975. Invigoration of seeds? *Seed Sci. Technol.* **3,** 881–8.

KAHN, A., 1960. An analysis of 'dark-osmotic inhibition' on germination of lettuce seeds. *Pl. Physiol., Lancaster*, **35,** 1–7.

KENDRICK, R. E., 1976. Photocontrol of seed germination. *Sci. Prog., Oxf.* **63,** 347–67.

—— and SPRUIT, C. J. P., 1977. Phototransformations of phytochrome. *Photochem. Photobiol.* **26,** 201–14.

KHAN, A. A., 1977. Preconditioning, germination and performance of seeds. In *The physiology and biochemistry of seed dormancy and germination*. Ed. A. A. Khan. Elsevier/North-Holland Biomedical Press, Amsterdam. Pp. 283–316.

—— and KARSSEN, C. M., 1980. Induction of secondary dormancy in *Chenopodium bonus-henricus* L. seeds by osmotic and high temperature treatments and its prevention by light and growth regulators. *Pl. Physiol., Lancaster*, **66,** 175–81.

—— PECK, N. H., and SAMIMY, C., 1980/81. Seed osmoconditioning: physiological and biochemical changes. *Israel J. Bot.* **29,** 133–44.

—— TAO, K.-L., KNYPL, J. S., BORKOWSKA, B., and POWELL, L. E., 1978. Osmotic conditioning of seeds: Physiological and biochemical changes. *Acta Hort.* **83,** 267–78.

KNYPL, J. S., JANAS, K. M., and RADZIWONOWSKA-JOZWIAK, A., 1980. Is enhanced vigour in soybean (*Glycine max*) dependent on activation of protein turnover during controlled hydration of seeds? *Physiolog. vég.* **18,** 157–61.

MANCINELLI, A. L., 1966. Broad-spectrum light sources, photoconversion of phytochrome and some physiological responses in tomato seed germination. *Annali Bot., Roma*, **28,** 675–86.

—— BORTHWICK, H. A., and HENDRICKS, S. B., 1966. Phytochrome action in tomato seed germination. *Bot. Gaz.* **127,** 1–5.

—— YANIV, Z., and SMITH, P., 1967. Phytochrome and seed germination. I. Temperature dependence and relative P_{FR} levels in the germination of dark-germinating tomato seeds. *Pl. Physiol., Lancaster*, **42,** 333–7.

RENNICK, G. A., and TIERNAN, P. I., 1978. Some effects of osmopriming on germination, growth and yield of celery (*Apium graveolens*). *Seed Sci. Technol.* **6,** 695–700.

SACHS, M., 1977. Priming of watermelon seeds for low temperature germination. *J. Am. Soc. Hort. Sci.* **102,** 175–8.

SALTER, P. J., and DARBY, R. J., 1976. Synchronization of germination of celery seeds. *Ann. appl. Biol.* **84,** 415–24.

THANOS, C. A., 1980. Photophysiological study of watermelon (*Citrullus lanatus* cv. Sugar Baby) seed germination. Ph.D. Thesis. University of Athens.

—— and MITRAKOS, K., 1979. Phytochrome-mediated germination control of maize caryopses. *Planta*, **146,** 415–7.

TONKIN, J. H. B., 1979. Pelleting and other presowing treatments. In *Advances in research and technology of seeds, Part 4*. Ed. J. R. Thomson. Centre for Agricultural Publishing and Documentation, Wageningen. Pp. 84–105.

VIDAVER, W., and HSIAO, A. I.-H., 1972. Phytochrome transformations in lettuce seed irradiated at various temperatures. *Pl. Physiol., Lancaster*, **50,** 249–51.

YANIV, Z., and MANCINELLI, A. L., 1967. Phytochrome and seed germination. II. Changes of P_{FR} requirement for germination in tomato seeds. Ibid. **42,** 1147–8.

—— —— 1968. Phytochrome and seed germination. IV. Action of light sources with different spectral energy distribution on the germination of tomato seeds. Ibid. **43,** 117–20.

—— —— and SMITH, P., 1967. Phytochrome and seed germination. III. Action of prolonged far red irradiation on the germination of tomato and cucumber seeds. Ibid. **42,** 1479–82.