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Phytochrome activation and osmoconditioning of tomato seeds

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ABSTRACT

Osmotic presowing treatment (OPT) of tomato (*Lycopersicon lycopersicum*) seeds for different periods of time in darkness raises the final germination percentage from about 50% (at both 15 and 25°C) to higher levels, as much as 90% or more. Moreover, it significantly enhances the germination rate (lowering T_{50} to about 40%), compared with the untreated seeds. Intermittent red (iR) light during OPT results in both a reduction (by 1-2 days for 25°C and 3-4 days for 15°C) of the OPT period required for full germination and an advancement of germination rate (by lowering T_{50} in all cases), in comparison with dark-osmoconditioned seeds. Far red irradiations applied immediately after each red one (i(R+F)) during OPT, delay the time course of germination and reduce the final germination percentage compared with untreated seeds, indicating phytochrome involvement in osmotic pretreatment processes. It is concluded that the improvement of germination following a given dark-OPT period is achieved by a shorter period of iR-OPT. Therefore, phytochrome activation during OPT of seeds may be useful not only for the prevention of secondary dormancy induction (observed in some seeds by dark-OPT) but also for (i) the improvement of seed performance induced by dark-OPT and (ii) the reduction of the duration of the osmoconditioning period.

INTRODUCTION

Osmotic presowing treatment (OPT) of seeds, termed "priming" (Heydecker et al., 1975) or "osmoconditioning" (Khan et al., 1978), constitutes a physiological method for the improvement of seed performance. Most attention concerning OPT conditions has been paid to the nature of the osmoticum and its water potential, as well as to the temperature and the duration of the treatment (Akalehiywot & Bewley, 1977; Bodsworth & Bewley, 1981; Coolbear, et al., 1980; Guttridge & Bright, 1978; Heydecker, et al., 1973). On the other hand, only little or no information have been given about light conditions during osmoconditioning. In some cases, where OPT was carried out with either continuous (Heydecker & Gibbins, 1978; Sachs, 1977; Khan, et al., 1983; Blocklehurst & Dearman, 1983) or diurnal, white light irradiation (Guttridge & Bright, 1978; Rennick & Tiernan, 1978), no dark control levels were reported.

Recently, the role of light conditions during OPT has been recognised (Khan et al., 1980/81) and was studied in *Lactuca sativa* (Khan, 1978; Khan et al., 1980/81), *Chenopodium bonus-henricus* (Khan & Karssen, 1980; 81) and *Apium graveolens* (Khan et al., 1980/81). In the first two cases, continuous or intermittent Red (R) light prevented the induction of secondary dormancy, normally caused by prolonged dark osmotic treatments. In the latter case, red or white light during the osmotic treatment not only prevented the establishment of secondary dormancy of celery seeds but also caused a marked advancement of subsequent germination.

The aim of this study, was to investigate the effect of phytochrome activation during osmoconditioning on the subsequent germination of tomato seeds. Thus the objective was to bring together (a) the previously-studied promotive effect of dark-OPT (Coolbear and Grierson, 1979; Goolbear et al., 1980; Georghiou, Thanos, Tafas and Mitrakos, 1982; Heydecker et al., 1975; Lightburn as reffered to by Heydecker, 1977) and (b) the well-documented phytochrome control of tomato seed germination (Mancinelli, Borthwick and Hendricks, 1966; Egles and Rollin, 1968).

MATERIALS AND METHODS

Tomato (*Lycopersicon lycopersicum* (L) Karsten ex Farwell, cv. Roma VF) seeds were obtained from Ferry Morse Seed Company, U.S.A. Osmotic presowing treatments were carried out in petri dishes, each containing 100 seeds immersed in 0.5 M mannitol (Merck) solutions (Ψs=-1.24 MPa), at 25±1°C for 1, 2, 3, 5 and 7 days. This Ψs was chosen because it was the highest to induce total inhibition of germination. Osmoconditioned seeds were washed with deionised water and air-dried at 25°C in darkness, for 48 h, to their original moisture content. Germination tests were performed at 15±1°C and 25±1°C with samples of 50 seeds placed on two sheets of filter paper in glass petri dishes (Ø9 cm) and moistened with 5 ml of deionised water. Germinated seeds were recorded and removed every one (25°C) or two days (15°C).

The broad band red light (625-700 nm, emission maximum at 660 nm, total fluence rate 3 $W.m^{-2}$) was obtained from ten red fluorescent tubes (Philips TL 20W/15) filtered through a red plexiglass filter (Röhm 501). White light (fluence rate 9 $W.m^{-2}$) was obtained from eight fluorescent tubes (Philips TL 20W/55). The broad band far red light fluence rate 5 $W.m^{-2}$) and the green safe light (fluence rate 10 $mW.m^{-2}$), under which all manipulations were conducted, were described previously (Georghiou et al., 1982). All fluence rate values refer to the level of the seeds.

Times to 50% germination (T_{50}) were calculated according to the formula:

$$T_{50} = t_i + \frac{(N+1)/2 - n_i}{(n_j - n_i)} \cdot (t_j - t_i)$$

were N is the final number of germinants and n_i, n_j cumulative numbers of seeds germinated by adjacent counts at times t_i and t_j when $n_i < (N+1)/2 < n_j$ (Coolbear et al., 1980). The use of this formula is justified by the sigmoid shape of the germination curves obtained (Figs. 1 and 2), i.e. T_{50} values correspond to turning points (change of slope).

Germination values are means of at least five samples and ± values (as well as vertical lines in figures) represent standard errors.

RESULTS

The effect of 1 day OPT under different light conditions on the subsequent germination at 15 and 25°C is presented in Fig. 1. At 25°C, dark-osmoconditioned seeds germinated faster and to a significantly higher final germination percentage in comparison with untreated seeds. At 15°C, on the other hand, no differences in germination were observed between the above mentioned treatments. When seeds were irradiated with 2 min Red light every 1 h (iR) during OPT, their germination in darkness at both temperatures showed significant differences from dark-osmoconditioned seeds. The rates of germination were increased and the final germination percentages reached almost 100%. When seeds were subjected to 2 min Far Red light irradiations, given immediately after each R one (i(R+F)), they practically lost the capacity for subsequent germination.

Data similar to those shown in Fig. 1 were obtained for 2-, 3-, 5-, and 7-day osmoconditioned seeds. From these data, final germination percentage and time to 50% germination were calculated and are presented in Tables 1 and 2. These tables show clearly the promotive effect of dark-OPT on subsequent germination of seeds at 15°C (Table 1) and 25°C (Table 2), expressed as either an increase in final germination or a decrease of the time taken to reach 50% of total

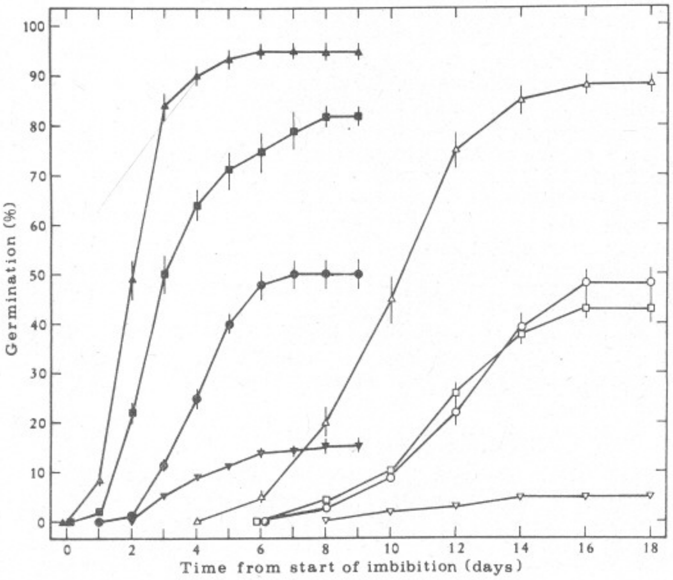

Fig. 1: Time course of dark germination at 15°C (open symbols) and 25°C (closed symbols) of tomato seeds: untreated (O,●), or osmoconditioned for 1 day under darkness (□, ■), iR (Δ,▲), i(R+F) (∇,▼). (The osmoticum was 0.5 M mannitol).

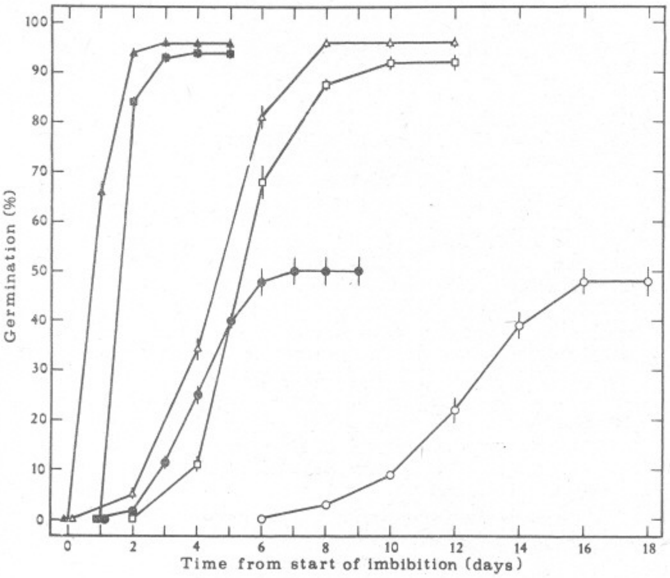

Fig. 2: Time course of dark germination at 15°C (open symbols) and 25°C (closed symbols) of tomato seeds: untreated (O,●), or osmoconditioned for 5 or 7 days under darkness (□,■) and iR (Δ, ▲) respectively. (The osmoticum was 0.5 M mannitol).

TABLE 1. Maximum germination percentage (N) and time to 50% of final germination (T_{50}) of tomato seeds osmoconditioned under different light conditions (Darkness, D; 2 min R/1 h, iR; [2 min R+2 min F]/1 h, i[R+F]) for different periods of time (1,2,3,5 and 7 days). Germination tests were carried out in deionised water and darkness at 15 °C

Length of osmoconditioning period (d)	D		iR		i[R+F]
	N (%)	T_{50} (h)	N (%)	T_{50} (h)	N (%)
1	43±3	270	88±2	240	5±1
2	61±2	218	91±2	190	8±1
3	80±2	206	94±1	173	10±2
5	92±2	124	96±2	106	8±3
7	90±2	132	94±1	115	9±1

Untreated seeds: N = 48±3% , T_{50}= 295 h

TABLE 2. Maximum germination percentage (N) and time to 50% of final germination (T_{50}) of tomato seed osmoconditioned under different light conditions (Darkness, D; 2 min R/1 h, iR; [2 min R+2 min F]/1 h, i[R+F], for different periods of time (1,2,3,5 and 7 days). Germination tests were carried out in deionised water and darkness at 25 °C

Length of osmoconditioning period (d)	D		R		i[R+F]
	N (%)	T_{50} (h)	N (%)	T_{50} (h)	N (%)
1	82±2	64.7	95±2	47.4	15±2
2	88±1	40.8	95±1	38.4	19±3
3	90±2	38.4	95±1	36.0	22±4
5	99±1	36.0	99±0	26.4	20±2
7	94±1	36.0	95±1	16.8	26±2

Untreated seeds: N = 50±3% , T_{50}= 96.0 h

TABLE 3. Reduction (%), of time to 50% final germination (T_{50}) caused by iR (compared with D)

Length of osmoconditioning period (d)	Reduction of T_{50} (%)	
	15 °C	25 °C
1	11.1	26.7
2	12.8	5.9
3	16.0	6.2
5	14.5	26.7
7	12.9	53.3

germination percentage. Intermittent R light irradiation during OPT resulted in virtually full induction of germination even by 1 day of osmoconditioning. As a consequence, further effects of increasing the length of the osmoconditioning period were restricted to the decrease of the time to 50% of final germination. When i(R+F) was applied during osmoconditioning, germination at both 15 and 25°C was always lower than that of the untreated seeds. For this reason, T_{50} values for i(R+F)- treated seeds were not calculated.

Table 3 shows the effect of iR during osmoconditioning on the reduction of the time to 50% germination. This reduction is expressed as a percentage, on the basis of the corresponding dark-OPT T_{50}. It is clear that while the iR effect remains relatively constant at 15°C (11-16%) it is extremely variable at 25°C (6-53%) according to the length of the osmoconditioning period.

Figure 2 shows the optimal germination curves obtained after 7 (test at 25°C) and 5 days (test at 15°C) of osmoconditioning, under darkness or iR. It is evident that dark-OPT can dramatically improve both the rate and the final percentage of germination. The application of iR during osmoconditioning (Tables 1 and 2) decreases both the OPT-period required for promotion of full germination (by 1-2 d at 25°C and by 3-4 d at 15°C) and the subsequent germination time (Table 3). However, for the optimal osmoconditioning periods presented in Fig. 2, the promotive effect of iR is minimal compared with darkness since it is restricted only to a T_{50} decrease of about 20 h in both cases.

Finally, the promotive action of light given as a single brief red irradiation, as well as the ability of intermittent white (fluorescent) light (iW) to substitute for iR, are now being investigated. These light treatments have been tested during one day of OPT, for reasons of convenience. The preliminary results have shown that either a brief (10 min) red light irradiation (given after 8 or 16 h of imbibition in osmoticum) or iw light can substitute for iR irradiation.

DISCUSSION

Even though dark germination of untreated seeds of the tomato seed lot used is unusually low (about 50% at 25°C compared with > 80% in previously published photophysiological or osmoconditioning studies), the germination behaviour of dark-osmoconditioned seeds is quite similar to those reported earlier for other tomato cultivars (Coolbear et al., 1980; Heydecker et al., 1975; Heydecker, 1977; Rumpel & Szudyga, 1978), and for the same cultivar obtained from another source (Georghiou et al., 1982). Consistent with the above mentioned studies, final germination percentages of nearly 100% were obtained here after dark-osmoconditioning, and mean times to 50% germination of dark-osmotreated seeds were less than half of untreated ones. This decrease is strikingly similar (about 40% of the untreated control) for both temperatures, though it is achieved after 2 and 5 days of dark-OPT, when germination is tested at 25 or 15°C respectively (Tables 1 and 2).

Attempts to improve seed performance led to the combination of osmoconditioning with the infusion of growth regulators (Khan, 1977; Cantliffe & Watkins, 1983) or with phytochrome activation by appropriate irradiation. To our knowledge, the second combination proved to be: (a) indispensable for the priming of lettuce (Khan, 1978) and chilled *Chenopodium bonus-henricus* seeds (Khan & Karssen, 1980), (b) unnecessary for soyabean and sweet corn seeds (Khan et al., 1979), and (c) useful for further improvement of the beneficial results of dark-OPT in celery seeds (Khan et al., 1981/81). For the first case, light action might be attributed to the prevention of the appearance of a deeper dormancy state resulting from dark imbibition.

In the case of tomato seeds studied here, the presence of an increased fraction of the active form of phytochrome (P_{fr}) during OPT, significantly enhances the beneficial results of dark-osmoconditioning. Therefore, the rate of germination is increased in every case, though at 25°C the decrease of T_{50} is variable (Table 3). The reasons for such a variability are unknown, but if absolute values are considered there is a markedly similar reduction of T_{50} by about 20 h at both temperatures (at optimal OPT periods, Fig. 2). Moreover, with respect to light action during OPT, what is more important in our opinion is the reduction of the OPT time required for full germination (Tables 1 and 2). The shortening of the imbibition period in the osmoticum is very important from an application point of view, since seeds might not need protection against microbial infestation, as is the case during prolonged imbibition.

Although the mechanisms underlying the promotion of germination by osmoconditioning are still poorly understood, it seems likely that metabolic changes such as RNA and protein synthesis, reported by Coolbear and Grierson (1979) and Khan et al. (1980/81) might be accelerated by increased P_{fr} levels. The increase of P_{fr} level by red light in tomato seeds during OPT is considered indisputable, since the fraction of phytochrome present as P_{fr} in dark-germinating tomato seeds was found to be only about 40% (Mancinelli et al., 1967). Moreover, when i(R+F) is applied during OPT, P_{fr} levels are significantly decreased and germination is practically inhibited (Tables 1 and 2). Thus, the active suppression of P_{fr} level by far red irradiations, not only prohibits osmoconditioning but induces a deeper state of seed dormancy as well.

It is considered very premature to suggest an interaction (if any) between phytochrome and osmoconditioning effects on seed germination. The only conclusion is that for the best results of osmoconditioning in seeds with low or moderate P_{fr} levels (such as tomato or celery), the continuous or intermittent application of red or white fluorescent light during OPT is profitable.

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