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OSMOCONDITIONING ENHANCES CUCUMBER AND TOMATO SEED GERMINABILITY UNDER ADVERSE LIGHT CONDITIONS

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ABSTRACT

Osmoconditioning of cucumber (*Cucumis sativus* L.) seeds resulted in a considerable promotion of the germination rate and particularly of the final germination percentage at low, suboptimal temperatures. The osmotica applied in this study were mannitol, NaCl, sucrose, and milk (the latter had been used in ancient times for presoaking of cucumber seeds, as mentioned by Theophrastus). It is well known that the germinability of untreated cucumber and tomato (*Lycopersicon esculentum* Mill.) seeds is light-inhibited. However, even under the most adverse light conditions from the photomorphogenetical viewpoint, i.e., under continuous far-red (cFR) irradiation, osmoconditioned seeds were induced to germinate to an extent depending on temperature and on the cultivar used. On the basis of the kinetics of escape from cFR-inhibition, the promotion of germination is attributed to the presence of the active form of phytochrome (P_{fr}) during osmoconditioning. This effect might prove to be of practical importance under various light conditions encountered both in the laboratory and in the field.

The ancient Greek philosopher Theophrastus (372–287 B.C.) is not only the founder of botany, but he can also be considered as the father of seed physiology (Evenari, 1984). Among his numerous observations and critical remarks, there is a reference to some effects of presoaking on seed germination: "Some even presoak the seed of cucumber in milk or water to make its germination faster" (Theophrastus, Enquiry into Plants, Book VII, I:6). Presoaking as an agricultural practice to stimulate germination must have been familiar to Greek farmers long before Theophrastus; as mentioned by Pliny, "Democritus advises soaking all seeds, before they are sown, in the juice of the plant that grows on roof tiles". Pliny himself refers to the presoaking of cucumber seeds in water and honey (cited by Evenari, 1980/81).

The modern era of seed presoaking has only recently been inaugurated by Heydecker and his research team. They developed a technique, simple in concept, but physiologically complex, which enables the radicles to emerge appreciably sooner than usual after sowing (Heydecker et al., 1973). This technique consists of pre-

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imbibing seeds in a biochemically inert osmoticum for a certain period of time before their eventual transfer to water (with or without an intervening redrying). The osmotic treatment of the seeds before sowing, termed "priming" (Heydecker et al., 1975) or "osmoconditioning" (Khan et al., 1978), has been shown to result in (a) a faster and more uniform germination; (b) a wider temperature range of germination; and (c) an increased longevity of the seeds during storage (e.g., Georghiou et al., 1987a).

Normally, tomato and cucumber seeds are dark germinators, although the occurrence of the reversible red–far-red photoreaction has been demonstrated physiologically at low intensity light (Mancinelli et al., 1966; Yaniv et al., 1967; Toole, 1973). It is therefore evident that in both species germination is under phytochrome-mediated light control. Germination of both tomato and cucumber seeds is inhibited by prolonged irradiation with white (incandescent) light. The greatest inhibition is obtained by far-red radiation (710–720 nm), exposure to blue light being less inhibitory (Frankland & Taylorson, 1983).

In previous works (Georghiou et al., 1982, 1987b), we have raised the question of interactions between photosensitivity and osmoconditioning. Some preliminary results obtained with tomato seeds have led to the conclusion that a presowing osmotic treatment in darkness can confer on the seeds the ability to germinate under unfavorable light conditions, e.g., under continuous far-red (cFR) irradiation. It is the purpose of the present work to clarify and expand the current knowledge on the changes caused by osmoconditioning in the photosensitivity of seeds.

MATERIALS AND METHODS

Seeds of cucumber (*Cucumis sativus* L., cvv. 'Ermis' and 'Kalyviotiko') and of tomato (*Lycopersicon esculentum* Mill., cvv. 'Roma' and 'Pakmore') were used in this work. The cucumber cultivars were obtained from a local seed market and the tomato cultivars from Ferry Morse, USA and Castle Seeds, USA, respectively.

Osmoconditioning was carried out in darkness at 25°C, using mannitol solutions (Ferak, West Germany). Samples of 25 cucumber or 50 tomato seeds were placed on top of two filter paper sheets moistened with 5 ml of osmotic solution, in 10 cm diameter petri dishes. The molarity of the mannitol solutions was of 0.5 M for tomato and of 0.7 M for cucumber seeds (ψ_s values: –1.239 MPa and –1.734 MPa, respectively). These ψ_s values were chosen since preliminary experiments had shown them to be the highest values inducing total inhibition of germination in tomato and cucumber seeds at 25°C in darkness. For the experiment illustrated in Figure 1, commercially available NaCl, sucrose, and sterilized milk were additionally used. The former two osmotica were adjusted to an osmolarity equal to that of mannitol, while the ψ_s value of milk was somewhat higher, since some germination was eventually permitted. Preliminary experiments showed that the best results can be obtained with a presowing osmotic treatment lasting for 2–3 days (in the cucumber) or 5 days (in the tomato cv. 'Roma'). The osmotically imbibed seeds were subsequently washed with deionized water and air-dried for 48 h to their original weight (and, presumably, moisture content) in a

dark room maintained at 25°C. In the experiment illustrated in Figure 3, one set of osmoconditioned tomato seeds (cv. 'Pakmore') was reimbibed in water without any intervening dehydration.

Germination tests were performed immediately afterwards either in darkness or under cFR light. Both the broad band far-red light (fluence rate: 5 W m^{-2}) and the dim green safelight under which all dark manipulations were conducted (fluence rate: 10 mW m^{-2}) have been described elsewhere (Georghiou et al., 1982). Germination tests were carried out with samples of 25 cucumber and 50 tomato seeds placed on top of two sheets of filter paper soaked with 5 ml of deionized water in 10 cm diameter petri dishes. Visible radicle protrusion was the criterion of germination. Germinated seeds were counted daily and subsequently discarded. Each germination test comprised six samples and was terminated when no further germination occurred. The experimental temperatures were obtained using growth cabinets (Conviron, Canada) maintained at 15 ± 0.5°C, 20 ± 0.5°C, and 25 ± 0.5°C.

RESULTS

Data concerning the promotive effect of osmoconditioning on the germination of cucumber seeds are shown in Figure 1 and Table I. In the cucumber cultivar 'Ermis', the

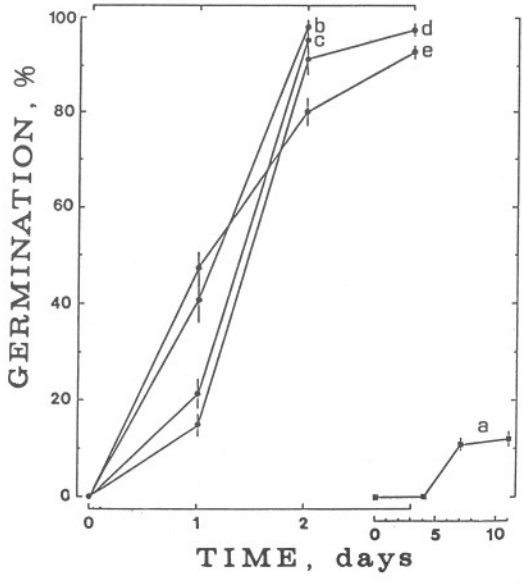

Fig. 1. The time course of germination in 'Ermis' cucumber seeds at 15°C in darkness. a, untreated seeds; b–e, seeds osmoconditioned for 2 days in 0.7 M mannitol (b), NaCl (c), sucrose (d), and milk (e). Vertical bars represent 2SE.

percentage dark germination of untreated seeds at 15°C was extremely low (Fig. 1, curve a), while osmoconditioning resulted in a marked increase of germinability (Fig. 1, curves b–e). More than 90% of the cucumber seeds osmoconditioned for 2 days in one of the four different media used (mannitol, NaCl, sucrose, or milk) germinated within 3 days, whereas none of the untreated seeds had germinated by that time. Obviously, the time course of germination was quite similar in differently pretreated seeds. This was not unexpected, since promotion of seed germination is attributed to the osmotic properties and not to the chemical nature of the presoaking solution.

The importance of osmoconditioning in promoting germination was also demonstrated for the other cucumber cultivar, 'Kalyviotiko' (Table I). The final germination percentage of untreated 'Kalyviotiko' seeds was of ca. 85% at 15°C in darkness. Osmoconditioning for 3 days resulted both in a slight increase in the final germination percentage (up to 91%) and in a considerable enhancement of the germination rate, illustrated by the decrease in the time interval until 50% germination (T_{50}) from 2.9 to 1.6 days (Table I). Likewise, seeds osmoconditioned for 1, 2, 4, and 5 days germinated faster than untreated ones (data not shown). When germination tests were conducted under cFR light, the germination of both untreated and 1-day-osmoconditioned seeds was fully inhibited (final germination percentage: about 5%). On the other hand, the germinability of seeds osmoconditioned for 2–5 days was considerably increased. Seeds presoaked for 3 days presented the highest final germination percentage (ca. 50%, Table I), while either shorter or longer pretreatments with osmotica resulted in lower germination percentages (18%, 38%, and 27% for 2, 4, and 5 days of osmoconditioning, respectively).

Table I also presents the germination characteristics of another lot of 'Ermis' cu-

TABLE I

Final germination percentages and germination rates (T_{50}) of cucumber seeds at different temperatures in darkness (D) or under continuous far-red (cFR) irradiation

Cucumber cultivar	Temperature	Seeds[1]	Final germination (% ± SE)		T_{50} (days)	
			D	cFR	D	cFR
'Kalyviotiko'	15°C	UN	84.7 ± 3.0	5.3 ± 1.3	2.9	3.5
		OC	90.7 ± 1.7	50.7 ± 4.0	1.6	1.8
'Ermis'	20°C	UN	64.0 ± 4.8	4.8 ± 2.3	2.0	1.5
		OC	71.3 ± 3.0	58.7 ± 5.0	0.7	0.6
	25°C	UN	77.0 ± 2.9	37.3 ± 2.9	1.7	1.7
		OC	72.3 ± 4.3	69.3 ± 5.2	0.6	0.6

[1]UN = untreated; OC = osmoconditioned by presoaking for 3 days in 0.7 M mannitol solution at 25°C.

cumber seeds, different from that in Figure 1, tested at 20°C and 25°C. Final germination percentages in the dark were of about 65–75% at both temperatures and they were not affected significantly by osmoconditioning, whose action was restricted to a decrease of T_{50} by about 1 day. In contrast, germination under cFR light was considerably improved by osmoconditioning: the final germination percentage increased from 5% to 60% and from 40% to 70% at 20°C and 25°C, respectively (Table I). It is noteworthy that both the time course of germination (not shown) and the germination rates (as reflected by the T_{50} values) of either osmoconditioned or untreated seeds were not significantly affected by light conditions.

As regards the germination of the two tomato cultivars at 20°C and 25°C, osmoconditioning enhanced the germination rate, decreasing T_{50} by about 1–2 days (Table II). This enhancement occurred even when the germination tests were conducted in darkness, where final germinability was always maximal (90% or higher). On the other hand, cFR light fully inhibited germination of untreated tomato seeds at both temperatures and in both cultivars. The osmoconditioning of 'Roma' tomato seeds resulted in a considerable promotion of germination under cFR light at 25°C (final germination: about 80%, Table II); at 20°C, the germination enhancement was somewhat lower (about 55%, as also illustrated in Figure 2, curve d). In addition, Figure 2 clearly shows a striking similarity in shape of the three germination curves corresponding to untreated seeds in darkness (curve a), to osmoconditioned seeds in darkness (curve c), and to osmoconditioned seeds under cFR light (curve d). An impressive promotion of germination under cFR light was achieved by osmoconditioning in

TABLE II

Final germination percentages and germination rates (T_{50}) of tomato seeds at different temperatures in darkness (D) or under continuous far-red (cFR) irradiation

Tomato cultivar	Temperature	Seeds[1]	Final germination (% ± SE)		T_{50} (days)	
			D	cFR	D	cFR
'Roma'	20°C	UN	94.3 ± 0.6	1.2 ± 0.5	4.3	3.5
		OC	97.7 ± 1.2	56.3 ± 3.0	2.0	1.6
	25°C	UN	89.7 ± 1.3	2.0 ± 0.5	3.2	3.5
		OC	97.0 ± 1.1	80.7 ± 3.1	1.5	2.5
'Pakmore'	20°C	UN	90.0 ± 1.6	2.8 ± 1.2	2.7	1.9
		OC	89.7 ± 1.7	77.0 ± 2.4	0.9	0.8
	25°C	UN	90.0 ± 2.0	5.0 ± 1.2	1.9	2.0
		OC	89.7 ± 1.2	80.0 ± 1.9	0.6	0.6

[1]UN = untreated; OC = osmoconditioned by presoaking for 5 days in 0.5 M mannitol solution at 25°C.

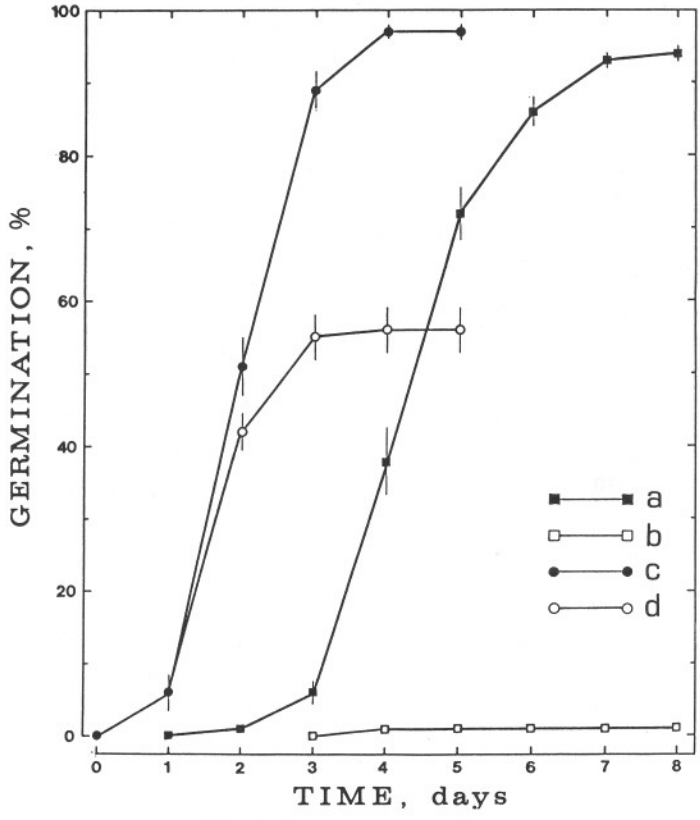

Fig. 2. The time course of germination in 'Roma' tomato seeds at 20°C in darkness (a, c) or under cFR light (b, d). a, b, untreated seeds; c, d, seeds osmoconditioned for 5 days in a 0.5 M mannitol solution. Vertical bars represent 2SE.

'Pakmore' tomato seeds; the corresponding final germination percentages were not affected by temperature (Table II).

In order to investigate further the role of phytochrome in osmoconditioning, curves reflecting the escape from the inhibitory action of cFR light were drawn for 'Pakmore' tomato seeds soaked in water or in 0.5 M mannitol. In addition to the osmoconditioning treatment as defined in this work (i.e., with an intervening desiccation), an osmotic treatment without any subsequent dehydration was also performed. The three escape curves obtained were obviously sigmoid and very close to each other (Fig. 3). However, a delay of about 7 h was observed between the germination of the seeds presoaked in water (T_{50} = 15 h, Fig. 3, curve a) and those presoaked in mannitol solution and subsequently dehydrated (T_{50} = 22 h, Fig. 3, curve b). Curve c, corresponding to seeds presoaked in mannitol and transferred directly to water and cFR light, without

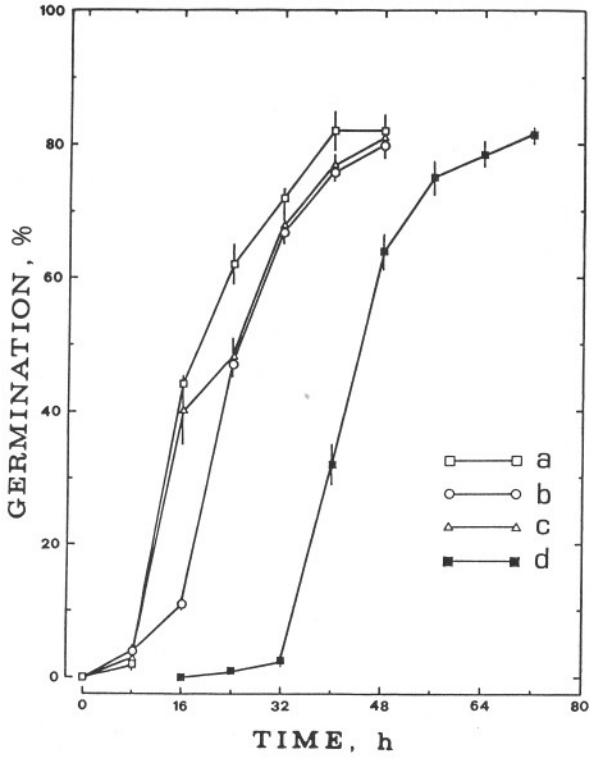

Fig. 3. The kinetics of escape from the inhibitory action of cFR light in 'Pakmore' tomato seeds at 25°C. Time refers to the soaking period (starting from the onset of hydration) in darkness preceding the eventual transfer of the seeds to cFR light, where final germination was scored after an additional period of 3 days. During their maintenance in darkness, seeds were soaked either in water (a) or in 0.5 M mannitol solution (b, c). Mannitol-osmoconditioned seeds were washed and either dehydrated for 2 days (curve b) or not subjected to dehydration and reimbibed in water immediately before their transfer to cFR light (curve c). The time course of seed germination in darkness is illustrated by curve d. Vertical bars represent 2SE.

previous dehydration, fell in between curves a and b. The time course of dark germination was considerably delayed (T_{50} = 42 h, Fig. 3, curve d), although its curve was nearly identical in shape to the other escape curves.

DISCUSSION

Osmoconditioning of cucumber seeds was shown to decrease T_{50} values considerably (by about 1–2 days), thus increasing the germination rate throughout the temperature range tested (15–25°C). This is in agreement with a previous report by Heydecker et al. (1975) who observed that cucumber seed germination at 20°C was faster by 1 day as a result of a 14-day osmoconditioning period in PEG-6000 at 15°C. It is well

known that when cucumber seed germination occurs at low temperatures (equal to or lower than 15°C, depending on the cultivar and/or the physiological age of the seed population), the final germination levels of untreated seeds are extremely low. This fact is illustrated in Figure 1 (curve a). Osmoconditioning of cucumber seeds resulted in an impressively increased germination rate and high germination percentages in darkness (Fig. 1, curves b–e), which confirms the ability of this treatment (already reported for other species) to promote significantly germination at suboptimal temperatures and to broaden at the same time the temperature range of seed germination.

Mannitol as an agent for the osmoconditioning of cucumber seeds can be successfully substituted by NaCl, sucrose, or milk (Fig. 1, curves c–e). Thus, the presoaking treatments used by ancient farmers should be considered as the first applications of osmoconditioning with a view to improving germination. Of course, this procedure was founded on practical knowledge, and the beneficial results of osmoconditioning were probably attributed to the high nutrient value of milk and honey or to some medicinal effect of the osmotically dense juice from the sarcophyte growing on roof tiles. Nevertheless, these interpretations could not be used for the promotion of germination caused by water pretreatment. This latter effect has been demonstrated for several species, e.g., rice (Basu & Pal, 1980). Anyway, it may be considered that both osmotic and water presoaking treatments bring the seeds to the edge of germination by allowing the accomplishment of the first, preparatory part of seed germination (Heydecker et al., 1975).

In untreated tomato seeds, germination inhibition by cFR light was complete throughout the temperature range investigated (Table II), while in cucumber seeds, cFR inhibition was only partial at higher temperatures (Table I); however, its extent might also depend on the vigor of the seed lot. The difference in the photosensitivity of cucumber and tomato is well known and it is attributed to phytochrome (Frankland & Taylorson, 1983). Osmoconditioning in darkness of tomato and cucumber seeds resulted in a significant level of germination under the otherwise inhibitory cFR light. This apparent escape from the inhibitory action of cFR is very impressive throughout the temperature range tested. Thus, osmoconditioned cucumber and tomato seeds germinated not only faster but also at significantly higher levels as compared with untreated seeds, even under cFR. These results confirm the previously reported increase in germinability in osmoconditioned tomato seeds under brief, intermittent, and continuous far-red irradiation regimes (Georghiou et al., 1982).

Continuous far-red light was chosen since it corresponds to a domain of the visible spectrum which is the most unfavorable from the photomorphogenetic viewpoint (Frankland & Taylorson, 1983). This implies that the beneficial results of osmoconditioning reported in this work may be even significantly higher under various adverse light regimes usually encountered either in the field (e.g., light filtered through a dense leaf canopy) or in the laboratory (e.g., incandescent light). This suggestion relies both on the phytochrome control of seed germination (at least in the so-called light-sensitive seeds) and on the phytochrome transformations and photostationary states occurring under various light conditions.

The mechanisms of osmoconditioning are generally obscure, but it is certain that they interfere with the germination mechanisms themselves and with the biochemical/biophysical modifications involved in germination, especially at its early stages. It is known that the active form of phytochrome (P_{fr}) is present in considerable quantities in both cucumber and tomato seeds in darkness. It is thus reasonable to conclude that the increase in the germinability of osmoconditioned seeds under cFR light is equivalent to an escape from cFR-induced inhibition and this may be a consequence of the action of P_{fr} during osmoconditioning. It is further suggested that the action of P_{fr} might be oriented towards either a change in the seed photosensitivity itself and/or simply towards the accomplishment and overcoming of the photosensitive steps in the seed germination process. This latter interpretation is supported by the time courses of germination in osmoconditioned seeds under cFR light, the curves being quite similar in shape to those recorded in darkness (e.g., in Fig. 2). The seeds which eventually fail to germinate under cFR light (a fact more obvious in tomato) seem to represent the slower-germinating, less vigorous fraction of the seed population. But the decisive evidence lending support to the hypothesis postulated above is provided by the results shown in Figure 3. The almost concurring cFR escape curves for the three treatments tested strongly suggest than an irreversible (cFR-escaping) phytochrome action has taken place almost simultaneously in every treatment. It is obvious that this escape effect can equally well occur during presoaking in mannitol and, which is more important, it can persist during an intervening dehydration. The retardation observed in the escape curve of the osmoconditioned seeds (Fig. 3, curve b), in comparison with that of the water-soaked ones (Fig. 3, curve a) might be attributed to the slower imbibition rate and/or the additional rehydration period required. However, this delay should be regarded as quite short, when the entire time course of germination is taken into consideration.

The major conclusion of this work is the possibility to enhance the germinability of cucumber and tomato seeds under unfavorable light conditions; the enhancement is induced by osmoconditioning through an irreversible phytochrome-mediated event. This result constitutes an additional beneficial effect of osmoconditioning, which might also apply to other plant species, at least to the overtly photosensitive ones.

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