Osmoconditioning as a Means of Counteracting the Ageing of Pepper Seeds during High-temperature Storage

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ABSTRACT

Sweet pepper seeds were osmotically conditioned in 0·4 M mannitol solution for 4 d (at 25 °C, in darkness) before or after storage at 35 °C for up to six months, and their germination and viability was compared with that of untreated seeds stored under the same conditions. Seeds that had been osmoconditioned prior to storage retained a high rate of germination and germinated to a high final percentage (from 80 to 50 per cent) at both 15 and 25 °C throughout the storage period. By contrast, both the rate and total level of germination of untreated pepper seeds declined rapidly at both germination temperatures, and by three months of storage the total level of seed viability was already less than 10 per cent. Seeds that were first stored at 35 °C, and then osmoconditioned just prior to germination, showed a decline in germinability which when tested at 25 °C was the same as for untreated seeds, while tested at 15 °C occurred at a slightly slower rate than for untreated seeds.

It is evident that osmoconditioning prior to storage, in addition to the acceleration of germination, resulted in a dramatic delay of the ageing rate, thus increasing considerably the longevity of seeds. On the other hand, osmoconditioning after storage did not seem to have any significant effect on seed viability, though it enhanced the germination rate.

Key words: *Capsicum annuum*, sweet pepper, seed, germination, osmoconditioning, priming, storage, viability, ageing, longevity.

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is a warm season plant that requires relatively high temperatures for successful seed germination (Sachs, Cantliffe and Watkins, 1980; Perl and Feder 1981; Cantliffe and Watkins, 1983; O'Sullivan and Bouw, 1984). Recommended sowing temperatures for commercial production range from 21 to 28 °C, with germination occurring about one week earlier at 28 °C than at 21 °C (Smith, 1979). At lower temperatures germination is markedly reduced (Sachs *et al.*, 1980) and chilling injury may occur (Harrington and Kihara, 1960).

Several seed treatments have been tested in an attempt to improve the germination of pepper seeds, especially at low temperatures, but the results have proved rather variable. Heydecker, Higgins and Turner (1975) showed an invigoration of pepper seeds by treatment (priming) with polyethylene glycol. This effect was confirmed by Yaklich and Orzolek (1977) in laboratory tests, but could not be demonstrated in the field. Fieldhouse and Sasser (1978) showed pepper seed germination to be improved following priming by 2 per cent KNO_3 and 2 per cent KH_2PO_4 and subsequent drying. Sachs *et al.* (1980) also found KNO_3 to promote germination, but showed the effect to be reduced on drying of the seeds. Fieldhouse and Sasser (1975) reported that the germination of 'Early Calwonder' pepper seeds was promoted by soaking in 1 per cent NaOCl, but this was not confirmed by the experiments of Cantliffe and Watkins (1983).

Relatively little attention has been paid to the storage of osmoconditioned seeds. On several occasions and for rather short periods of storage, osmoconditioned seeds of different species have been reported to either retain their germinability (Heydecker *et al.*, 1975; Atherton and Farooque, 1983) or deteriorate during storage (Nakamura and Enohara, 1980; Ely and Heydecker, 1981). In most works, however, neither storage conditions nor untreated seed controls have been specified so

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that the results are open to criticism. Only in a very recent work by Dearman, Brocklehurst and Drew (1986) has the question of storing primed seeds been properly investigated. It was found that osmoconditioned onion seeds stored at high temperature and moisture and tested at various intervals, showed a slightly increased (or at least similar) viability compared to that of the untreated seeds. Furthermore, both primed and untreated onion seeds, stored at low temperature, retained the initial high viability and their respective germination rates.

As far as it concerns the storage of pepper seeds following priming treatments, O'Sullivan and Bouw (1984) showed that a promotive effect on the germination of seeds of Midway pepper, when primed in 15 per cent salt solution, was retained for 21 d after treatment. Perl and Feder (1981) reported a retention of seed vigour for two months following priming. Aljaro and Wyneken (1985) showed the effects of priming of Yolo Y pepper seeds to be apparent for up to 140 d prior to sowing. However, in other experiments, the promotive effect of priming was reduced rapidly after drying of the seeds (Sachs *et al.*, 1980; O'Sullivan and Bouw, 1984).

It would be highly advantageous if the promotive effects of priming on the germination of seeds were retained after drying and storage. It would also be very important to construct viability nomographs for osmoconditioned seeds so that the storage conditions resulting to the predicted viability levels could be chosen beforehand. Hence, we have initiated an investigation into the interactions between osmoconditioning and storage of pepper seeds. Osmoconditioning was applied prior and/or subsequent to storage while various storage periods and temperatures have been tested. The present paper concerns storage at high temperature.

MATERIALS AND METHODS

Sweet pepper (*Capsicum annuum* L.) seeds (lot E 84066) were offered by Enza Zaden, Holland (1985), and the experiments started immediately afterwards. Osmoconditioning was carried out in darkness at 25 °C, using mannitol (Ferak, Berlin) solutions on samples of 100 seeds (in Petri dishes 10 cm in diameter, with 5·5 ml mannitol solution and two sheets of filter paper). Osmoconditioned seeds were washed with deionized water and air-dried for 48 h to their original moisture content (9·8 per cent on wet basis), in a dark room maintained at 25 °C. Untreated and osmoconditioned seeds were stored in light and moisture-proof plastic boxes for up to six months at 35 °C. Germination tests were performed in darkness at 15 and 25 °C on samples of 50 seeds, in Petri dishes. Each dish (10 cm in diameter) was lined with two sheets of filter paper and contained 5 ml of deionized water. Visible radicle protrusion was the criterion of germination. Germinated seeds were recorded and discarded every 2 d, under a dim green safelight. Each germination test was considered finished when no more germination occurred. The temperatures used in the experiments were obtained using growth chambers (W. C. Heraeus GmbH, W. Germany, model BK 5060 EL) set at 15, 25 and 35 °C maintained to within 0·1 °C. Germination values are means of six replicates and ± values as well as vertical lines in figures represent standard errors. T_{50}, the time needed for 50 per cent of final germination, was estimated by the two median values of the germination time courses.

RESULTS

Preliminary experiments prior to storage established that the optimum conditions for osmo-

TABLE 1. *Final germination (per cent ± s.e.) and T_{50} values (d) of pepper seeds at 15 °C, after various osmoconditioning programmes at 25 °C*

Ψ_π of osmoticum (MPa)	Duration of osmoconditioning (d)		
	2	4	6
−0·496	75·7 ± 2·6 21·7	73·0 ± 2·5 16·7	67·3 ± 2·2 14·5
−0·991	71·3 ± 2·2 21·5	74·9 ± 1·7 16·2	65·0 ± 1·3 16·4
−1·487	72·3 ± 0·6 22·3	73·7 ± 3·0 16·1	65·3 ± 3·1 15·6

conditioning of the present pepper seed lot were a treatment of 4 d with 0·4 M mannitol (Ψ_π of osmoticum −0·991 MPa), as shown in Table 1. Osmoconditioning was carried out at 25 °C, but germination was tested at 15 °C since it is at suboptimal temperatures that the effects of priming are most apparent. Using this treatment, seeds germinated to a final percentage of 74·9 ± 1·7, and the time to 50 per cent germination (T_{50}) was 16·2 d. Treatments for a longer time (6 d) resulted in a reduction in the total percentage of seeds germinating, whereas treatments of 2 d resulted in a slower germination rate, as reflected by the higher T_{50} values. When osmoconditioning was

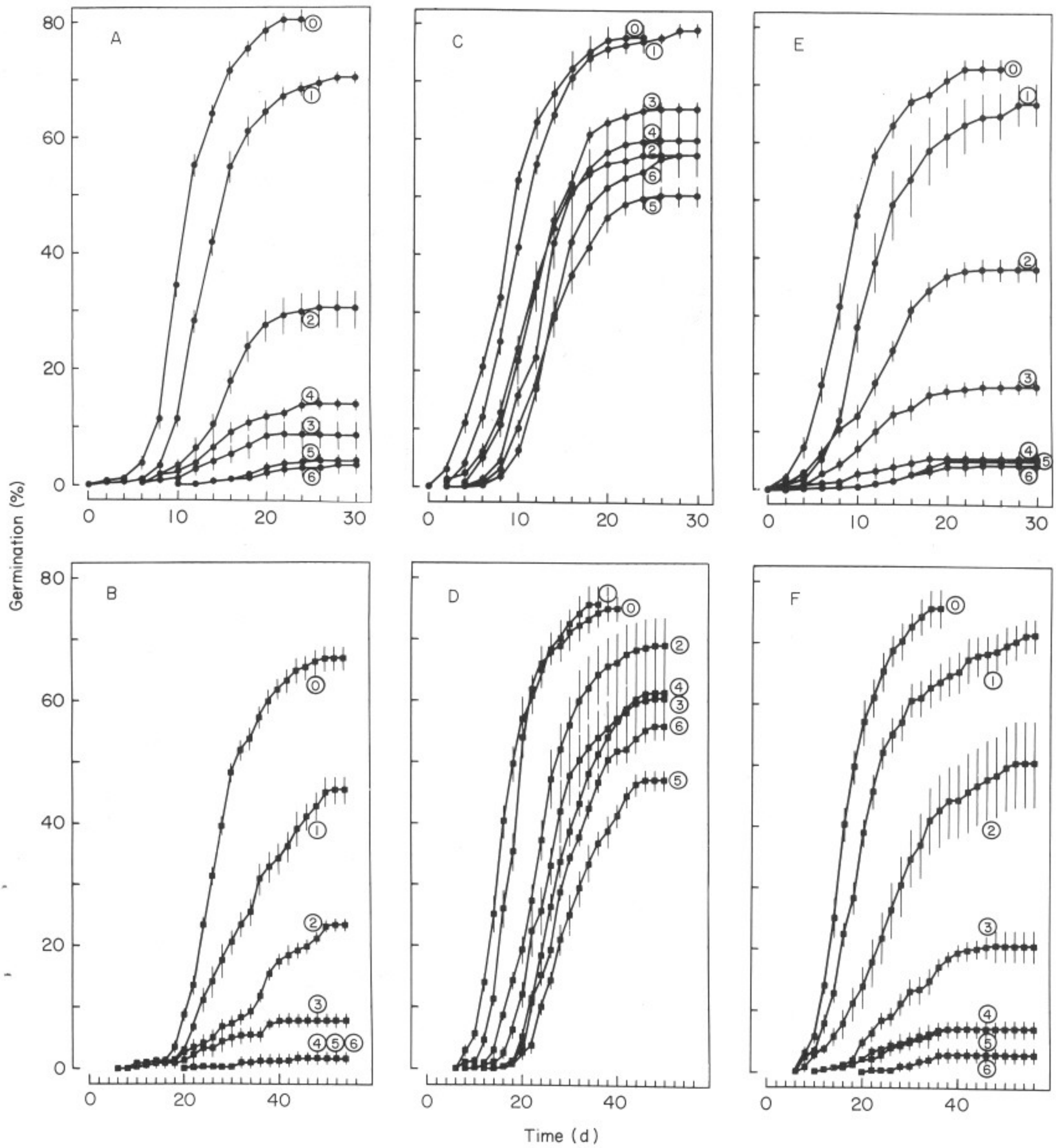

FIG. 1. Germination time courses at 25 °C (A, C, E) and 15 °C (B, D, F) of pepper seeds stored at 35 °C for 0 to 6 months (curves 0 to 6 respectively). A, B, Untreated seeds; C, D, osmoconditioned prior to storage; E, F, osmoconditioned after storage. Vertical bars = 2 s.e.

carried out for 4 d, different osmotica did not show any significant differences, thus the intermediate value ($\Psi_\pi = -0{\cdot}991$) has been adopted.

The germination of pepper seeds at 15 and 25 °C during storage for up to six months at 35 °C is shown in Fig. 1. Germination was tested at the end of each month of storage.

Unprimed seeds prior to storage germinated to a final percentage of about 80 per cent within 22 d at 25 °C (Fig. 1A) and to 70 per cent after 52 d at 15 °C (Fig. 1B). Primed seeds prior to storage germinated to about 80 and 75 per cent in 20 and 34 d at 25 and 15 °C respectively (Fig. 1C, D). Priming thus increased the rate of germination of seeds, although at both temperatures the final percentage of seeds germinating was similar for primed and untreated samples.

Storage at 35 °C brought about a rapid decline in both the rate of germination and the final germination percentage of untreated seeds. After two months storage, the number of seeds germinating was only 30 or 25 per cent of the total (tested at 25 and 15 °C respectively) as shown in Figs. 1A and B. After three months storage, the viability at both germination temperatures was less than 10 per cent. By contrast, seeds which had been osmoconditioned prior to storage, retained a much greater capacity to germination (Fig. 1C, D). After six months of storage at 35 °C, the final germination was over 50 per cent (tested at both 25 and 15 °C). Furthermore, in seeds that were primed prior to storage, although the time to the onset of visible germination was increased with lengthening storage time, the rate of germination during the exponential phase was quite similar throughout the storage period (Fig. 1C, D). The mean time of germination (T_{50}) for primed seeds rose from 8 to 14 d (25 °C) and from 16 to 28 d (15 °C) over the six months of storage; the T_{50} for untreated seeds prior to storage was 11 and 27 d at 25 and 15 °C respectively.

When seeds were first stored at 35 °C and then primed just prior to testing for germination (Fig. 1E, F), both the rate and the total percentage of germination declined throughout storage. When tested at 25 °C, the rate of germination and the total germination were similar to that of unprimed seeds (cf. Fig. 1A, E). At 15 °C, it was observed that the reduction in rate and total germination of seeds that were primed after storage for one, two or three months was not as great as for the corresponding untreated seeds (cf. Fig. 1B, F). For storage times of more than three months, the germination was extremely low (less than 10 per cent) in both cases.

The value of priming in reducing the rate of deterioration of pepper seeds during storage is most clearly demonstrated in Fig. 2. Here, the final percentage of seeds germinating throughout the whole six-month storage period is compared

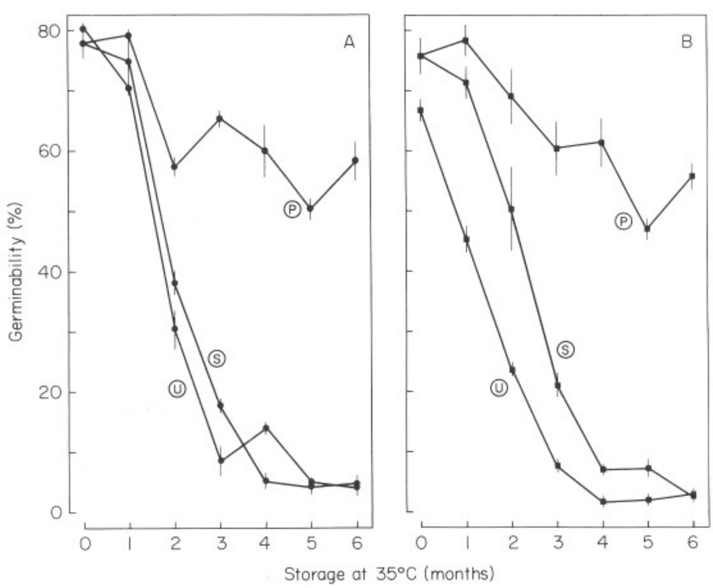

FIG. 2. Final germination of pepper seeds at 25 °C (A) and 15 °C (B) as a function of the duration of storage at 35 °C. U, untreated; P, osmoconditioned prior to storage; S, osmoconditioned subsequent to storage. Vertical bars = 2 s.e.

for all treatments. It is most striking that whereas the final germination percentage of untreated seeds fell to less than 10 per cent within three months (at both germination temperatures), seeds that were osmoconditioned prior to storage retained a very high level of viability throughout the entire storage period. Indeed, if the germination scores in Fig. 2 are linearly extrapolated to obtain a mean viability period, then for seeds primed prior to storage this period is in excess of 10 months. Seeds primed after storage showed a germinability curve of about one month longer than that of untreated seeds (tested at 15 °C), but no differences were observed at 25 °C.

DISCUSSION

Deterioration of seed during storage and the ability of seeds to germinate satisfactorily at low temperatures are of particular relevance to peppers. Seed ageing occurs relatively quickly at high temperatures, especially in warm countries such as Greece where seeds are frequently stored without refrigeration. On the other hand, successful germination at low temperatures is desirable especially for the production of spring crops (Sachs *et al.*, 1980).

Promotive effects of priming on the germination of pepper seeds at low temperatures have previously been described, but in most cases seeds were sown on completion of, or soon after, the priming treatment (Heydecker *et al.*, 1975; Yaklich and Orzolek, 1977; Watkins and Cantliffe, 1983; Mazor, Perl and Negbi, 1984). In studies where pepper seeds were dried prior to germination there was frequently a reduction in the priming effect (Sachs *et al.*, 1980; O'Sullivan and Bouw, 1984). Primed seeds were stored for up to two months by Perl and Feder (1981) and up to 140 d by Aljaro and Wyneken (1985) prior to germination, but apart from this there is little information on storage-priming interactions.

In the present experiments, osmoconditioning prior to storage was found to have two major effects: (a) significant promotion of the rate of germination of pepper seeds, especially at 15 °C, (b) dramatic reduction of the rate of deterioration during storage at 35 °C. As far as it concerns the former effect, results are in agreement with previous works using polyethylene glycol or salt osmotica (Heydecker *et al.*, 1975; Yaklich and Orzolek, 1977; Cantliffe and Watkins, 1983; O'Sullivan and Bouw, 1984; Mazor *et al.*, 1984; Aljaro and Wyneken, 1985). The promotive effects of priming on pepper seed germination are apparently independent of the cultivar, although differences do occur between seed lots (Perl and Feder, 1981). However, it is stressed that in the present experiments seeds were always dried back to their original moisture content prior to germination and probably for this reason the rates of germination were somewhat lower than those reported elsewhere (Yaklich and Orzolek, 1977; Sachs *et al.*, 1980). The seeds used in our experiments showed no signs of ageing prior to the start of this work; for example, osmoconditioning did not increase the final percentage of seed germination prior to storage. In addition, the decline of viability in the untreated seeds stored at 35 °C did not start immediately but was clearly manifested after two months. The non-germinating 20 per cent of the initial seed population should be attributed to either damaged or dormant seeds (Ellis, Hong and Roberts, 1985).

The maintenance of a high percentage of total germination throughout the storage period in seeds osmoconditioned prior to storage, may indicate priming to bring about a delay in the ageing processes characteristic of seeds (Roberts, 1979; 1981). Beneficial effects on viability maintenance in a number of crop seeds were also achieved by a temporary hydration treatment (e.g. Basu and Pal, 1980). Maintenance of seed vigour (Woodstock, 1973; Savino, Haigh and Leo, 1979) is indicated by the consistently higher rates of germination (lower T_{50}) of pre-storage-primed seeds in comparison with untreated seeds.

When seeds were primed after storage, the total germination was higher than for untreated seeds during the first three months, but only when germination was tested at 15 °C (Figs 1B, F and 2B), where germinability declined to its half-value after about 2·5 and 1·5 months of storage, respectively. When tested at 25 °C there was apparently no effect of post-storage priming and half germinability was attained after about two months in both cases. The rate of germination of post-storage primed seeds was also faster, as expected, than that of unprimed seeds over the first three months and especially when tested at 15 °C. Therefore, it seems that post-storage osmoconditioning may have only a marginal effect on the final seed germination level at suboptimal temperatures, in addition to the enhancement of the germination rate.

The viability curve of untreated pepper seeds, stored at 35 °C, shows a rapid decline, thus attaining its half-value (V_{50}) after 2–2·5 months. While post-storage priming was practically of no effect, osmoconditioning of pepper seeds prior to storage resulted in a dramatic increase of longevity (V_{50} longer than six months). This threefold (at least) lengthening of pepper seed viability is not conflicting to previous data for other seeds (Savino *et al.*, 1979; Dearman *et al.*, 1986) and could be attributed to the, generally and rather obscurely, so called invigoration of seeds (Heydecker *et al.*, 1975). It might also be linked to the repair processes reported for other seeds (Sanchez and Miguel, 1983; Ward and Powell, 1983; Burgass and Powell, 1984). Presumably, these biochemical or biophysical processes could lead to either an enduring or rejuvenated seed population. Therefore, it is concluded that the retardation of ageing should be added to the already established beneficial results of osmoconditioning (i, faster and more uniform germination; ii, increase of germinability at sub-optimal temperatures, thus broadening the germination temperature range; and iii, ability of germination under adverse light conditions; Georghiou *et al.*, 1982).

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