Sodium Hypochlorite Concentration, Temperature, and Seed Age Influence Germination of Sweet Pepper

E.M. Khah

Home Products Handling Cooperative Administration, 27 Karolou Street, Athens 104 37, Greece

H.C. Passam

Department of Horticulture, Agricultural University of Athens, 75 Iera Odos, Votanikos, Athens 118 55, Greece

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Abstract. The effects of NaOCl on the germination behavior of seeds of sweet pepper (*Capsicum annuum* L. cv. E-84066) were related to the concentration of NaOCl, treatment duration, solution temperature during treatment, incubation temperature during germination, and the age of the seed. Rate and percentage of germination were slower and lower, respectively, when seeds were exposed to 3% NaOCl solution at 35C for 20 or 40 min. When treatment time and temperature were reduced, or the NaOCl concentration lowered, the adverse effects were less. Fresh seeds were more sensitive than seeds that had been stored for 10 months at ambient temperature, and inhibition of germination was greater when the incubation temperature during germination was lower or higher (15 or 35C) than the optimum (25C).

Treatment of seeds with hypochlorite solutions is known to reduce the losses in germination caused by pathogens. It may also bring about changes in seed metabolism that additionally affect germination (Thomas, 1981).

The published effects of NaOCl on the germination of pepper seed are conflicting. Thus, NaOCl has been reported to either promote (Fieldhouse and Sasser, 1975; Macit, 1981) or inhibit (McCollum and Linn, 1955) germination, or to reduce the rate but not the total germination (Cantliffe and Watkins, 1983). These differences of observation may have resulted from differences in treatment times and concentrations (Dempsey and Walker, 1973, 1978). However, other important factors (e.g., treatment temperature), known to influence the effect of NaOCl on lettuce (*Lactuca sativa* L.) germination (Drew and Brocklehurst, 1984), have not been recorded.

Seeds of 'E-84066' sweet pepper from Enza Zaden, Holland, were isolated from fully mature fruits of plants cultivated in soil in plastic-covered greenhouses. After drying at room temperature (27 ± 5C) to constant weight, seeds were enclosed in envelopes and stored in darkness at 25 ± 3C until required. Sodium hypochlorite solutions were freshly prepared from Klinex (Lever, Athens, Greece), a commercial product containing 14.5% NaOCl.

Treatments were carried out on batches of 300 seeds that were immersed in 250 ml of

deionized water or NaOCl solution (1%, 2%, or 3%) for up to 40 min while being stirred constantly. The solution was thermostatically maintained at 15, 25, or 35C. Following treatment, the seeds were filtered out of solution and, except where otherwise indicated, washed with eight changes of deionized water. Total washing time was 4 min. After blotting off the liquid of the final wash with paper towels, six replicates of 50 seeds each were then placed on three layers of absorbent paper that had been moistened with 10 ml deionized water in 9-cm petri dishes.

The petri dishes were incubated in darkness at 25 ± 1C in constant-temperature incubators (CDR, Athens, Greece). Germination was recorded daily for 18 to 20 days. In one experiment to determine the effect of incubation temperature on germination, the incubators were set at 15 ± 1, 25 ± 1, 30 ± 1, and 35 ± 1C, respectively. The criterion of germination in all experiments was visible protrusion of the radicle with a minimum length of 2 mm.

For each germination test a completely randomized design with six replications was used. For each sample date, means were compared using the method of least significant differences or by determination of SE. The rate of germination was indicated by the T_{50} value derived from graphs, which denotes the time required (days) to reach 50% of the final germination percentage.

When pepper seeds were treated with water or increasing concentrations of NaOCl for 5 or 40 min at 15C within 2 to 3 weeks of seed removal from the pod, the typical time courses of germination were sigmoid (Fig. 1). Treatment with 1%, 2%, or 3% NaOCl for 5 min had no significant effect on the rate or final percentage of germination (Fig. 1A) in comparison with the control (water). However, when treatment was carried out for 40 min, both the rate of germination and the final germination level were increasingly inhibited with increasing concentrations of NaOCl (Fig. 1B). Stimulation of germination by NaOCl treatment was absent, as it was in other experiments using lower concentrations (0.25% and 0.5%) of NaOCl (data not shown). Omission of the standard washing procedure following treatment with 1% or 3% NaOCl for 5 min had no significant effect ($P = 0.05$) on the rate or percentage of germination (Table 1). However, when treated

Table 1. Effect of washing on the rate of germination (T_{50}) and on the final germination percentage of freshly isolated pepper seeds following treatment with NaOCl at 15C.

NaOCl concn (%)	Duration of treatment (min)	Seed			
		Final germination (%)		T_{50} (days)	
		Unwashed	Washed	Unwashed	Washed
0 (H ₂ O)	5	92.0	91.0	6.6	6.7
1	5	90.0	93.7	6.2	6.9
3	5	93.3	90.0	6.6	6.6
0 (H ₂ O)	40	94.0	93.3	7.1	7.5
1	40	92.7	88.0	6.6	6.6
3	40	70.0	73.3	11.1	8.5
LSD ($P = 0.05$)	5	3.35		0.94	
LSD ($P = 0.05$)	40	3.37		0.73	

Table 2. Effect of incubation temperature on the rate of germination (T_{50}) and on the final germination percentage of freshly isolated pepper seeds following treatment with NaOCl at 35C.

NaOCl concn (%)	Duration of treatment (min)	Incubation temp (°C)							
		Final germination (%)				T_{50} (days)			
		15	25	30	35	15	25	30	35
0 (H ₂ O)	20	93.3	92.0	94.0	84.7	9.5	4.3	4.7	6.5
1	5	90.7	85.3	92.0	85.3	12.4	4.9	4.8	6.2
1	20	96.7	89.3	93.3	88.0	9.5	4.7	5.7	8.2
3	5	96.0	96.7	97.3	88.7	10.3	4.7	4.6	6.3
3	20	38.0	71.3	46.7	27.3	15.9	7.0	9.9	10.9
LSD ($P = 0.05$)		11.7	8.8	6.1	9.1	3.3	1.6	1.0	3.5

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Fig. 1. Germination time courses at 25°C of freshly isolated pepper seeds following treatment for 5 min (A) or 40 min (B) at 15°C with 1) 0% water, 2) 1% NaOCl, 3) 2% NaOCl, or 4) 3% NaOCl. Vertical bars equal ± 1 SE.

with 3% NaOCl for 40 min, the T_{50} of unwashed seed was higher than that of washed seed (indicative of a reduction in germination rate), but the total percentage germination was not significantly different.

When seeds were treated with NaOCl (1% or 3%) for 5 min, the total germination percentage and T_{50} values were similar to those of the control (water), irrespective of treatment temperature (Fig. 2). Only at 35°C did treatment with 3% NaOCl for 5 min cause a significant increase in T_{50} (i.e., reduction in germination rate) in comparison with the control, but the final germination levels were not affected. However, when treatment was extended to 20 or 40 min, NaOCl reduced the rate and final germination percentage of seed in comparison with the control, the degree of reduction depending on the treatment temperature and concentration. Reduction in the rate of germination (i.e., increase of T_{50}) relative to water occurred with 1% and 3% NaOCl, but was greater in the latter. Final germination at 15 or 25°C was significantly lower than in the control only with 3% NaOCl. At 35°C, no seed germinated after treatment with 3% NaOCl for 40 min.

Germination of the fresh (3 days after isolation) seed treated with NaOCl at 35°C was progressively inhibited by increasing the duration of treatment (Fig. 3A). Thus, the T_{50}

Fig. 2. Percentage germination after 20 days incubation at 25°C (A) and the T_{50} values (B) of freshly isolated pepper seeds following treatment with 0% water (white bars), 1% NaOCl (hatched bars), or 3% NaOCl (black bars) for 5, 20, or 40 min at 15, 25, or 35°C. Vertical bars equal ± 1 SE.

increased from 5.0 (control) to 7.8 days (20-min treatment) and 14.5 days (30-min treatment). The total germination decreased from 98% (control) to 54% and 8% (20- and 30-min NaOCl treatments, respectively). By contrast, the germination of seeds that had been stored for 10 months at 25 ± 3 °C was inhibited substantially by 3% NaOCl only when the duration of treatment was > 10 min (Fig. 3B). The degree of inhibition of germination was invariably less than that of the corresponding treatment of the fresh seed.

When the effect of incubation temperature following NaOCl treatment at 35°C was tested, significant reduction of final germination in comparison with the control was observed only after treatment with 3% NaOCl for 20 min, regardless of incubation temperature (Table 2). Inhibition was greatest with incubation at 35°C and least at 25°C. The T_{50} values of seeds treated with 3% NaOCl for 20 min were significantly higher than those of the control, thus indicating a reduction in the rate of germination (Table 2). The T_{50} values were highest for seed incubated at 15 or 35°C. Differences in the T_{50} values for other treatments were mostly nonsignificant ($P = 0.05$).

In conclusion, germination behavior of pepper seed following treatment with NaOCl depended on: 1) the concentration of the NaOCl solution, 2) the duration of treatment, 3) the treatment temperature, 4) the age of the seed, and 5) the incubation temperature during germination.

Inhibition of the rate and percentage of germination by 3% NaOCl is in agreement with previous findings (Dempsey and Walker, 1973; McCollum and Linn, 1955). Stimulation of germination, as reported by Fieldhouse and Sasser (1975) and Macit (1981), was not observed here.

In the previous studies noted, treatment temperatures were not recorded. However, it has been shown that this is an important

Fig. 3. Germination time courses at 25°C of (A) freshly isolated pepper seed and (B) pepper seed that had been stored for 10 months at ambient temperature. Seeds were treated with water for 1) 30 min or 3% NaOCl for 2) 5 min, 3) 10 min, 4) 20 min, or 5) 30 min at 35°C. Vertical bars equal ± 1 SE.

factor that influences the sensitivity of lettuce seed to NaOCl treatment (Drew and Brocklehurst, 1984). Dempsey and Walker (1973) treated pepper seeds immediately after harvest with NaOCl and stored them for 56 days before starting germination tests. In other studies, the age of the seed was not reported (Cantliffe and Watkins, 1983; Fieldhouse and Sasser, 1975). The data presented here show that both treatment temperature and seed age influenced the degree of inhibition caused by NaOCl. Inhibition was also influenced by the incubation temperature during germination. It is important, therefore, that these factors be taken into consideration when NaOCl treatments are undertaken. Since the primary purpose of NaOCl is to provide surface sterilization of seeds (Dempsey and Walker, 1973, 1978), it is essential that sterilization does not inhibit germination. We suggest that, for pepper seeds, treatment with 3% NaOCl at 15 to 25°C for up to 20 min would not inhibit germination.

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