Some studies on maize seed germination and ageing

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Abstract

The effect of immersing dry viable maize seeds (*Zea mays* L. cv. Stowell's Evergreen) in ethanol for 10 min before imbibition on water was investigated. The most striking finding was that the ethanol treatment inhibited maize seed germination, but this was only evident if the treated seeds were placed embryo-side down in contact with the germination paper.

Additional key words: *ethanol,* Zea mays *L.*

Introduction

Seed ageing or the deterioration of seed quality parameters, including loss of viability and reduction of seedling vigour, is a central problem in seed biology (Priestley, 1986). In many seeds, the extent of seed ageing may be detected or quantifiable only after months, or sometimes years, in storage under natural conditions. To aid studies on seed ageing, artificial treatments have been devised by seed physiologists to obtain deteriorated seeds in a shorter period of time, usually within days of treatment. These include placing seeds under extreme conditions (e.g., high humidity and high temperature) to accelerate seed ageing (Priestley, 1986), or exposing seeds to alcohols (Musgrave *et al.*, 1980; Sadjad and Pian, 1980; Mugnisjah and Nakamura, 1986).

The initial objective of the present work was to examine the use of ethanol to obtain deteriorated maize seeds. The most striking finding arising from the initial experiments was that the position of the maize embryo in relation to the germination substrate was critical in the germination of the ethanol-treated seeds but not of the non-treated seeds.

Materials and Methods

Sweet corn seeds (*Zea mays* L. cv. Stowell's Evergreen) were purchased from Ferry Morse Seed Co. (Mountain View, California, U.S.A.) and were used in all the experiments. For germination tests, triplicate lots, each consisting of 40 seeds, were imbibed in darkness at 25°C on one layer of Whatman No. 1 filter paper wetted with 30 ml of distilled water in a loosely-covered plastic tray. The percentage of seeds germinated was scored 4 d from the start of imbibition. In some experiments the germination tests were extended for four more days before a second count of seed germination was taken. Seeds were considered to have germinated when the radicle emerged free of the surrounding seed structures.

In an experiment for treating the maize seeds with ethanol, about 250 seeds were totally immersed in 100 ml of distilled ethanol of the appropriate concentrations for various times in a 250 ml Erlenmeyer flask. The control experiment was to immerse the maize seeds in water in the same manner. The ethanol or water was then decanted and the seeds rinsed three times in all experiments unless stated otherwise, each with 150 ml of distilled water. Subsequently during the germination tests, the maize seeds were placed either with the endosperm part of the seed in direct contact with the wetted filter paper (herein termed embryo-side up) or with the embryo part of the seed in direct contact with the wetted paper (herein termed embryo-side down).

Results

The maize seeds used throughout the experiments with ethanol, had high capacity for germination (Table 1). Soaking the seeds in water did not affect seed germinability. However, pre-sowing soaking in 100% (v/v) ethanol for 10 min adversely affected seed germination, but this was only evident when seeds were placed embryo-side down in contact with the wet filter paper during the germination tests (Table 1). Similarly, a low percentage of seed germination resulted when the maize seeds following the ethanol treatment were wrapped between wet filter paper (data not shown). The seeds of at least two other cultivars of maize exhibited the same response to the ethanol treatment (Leung, unpublished results).

The effect of the brief ethanol treatment just described was investigated further. First, the maize seeds were treated for 10 min with a range of ethanol concentrations before the onset of the germination tests (Table 2). The treatments with ethanol of 25% (v/v) or higher concentrations significantly reduced maize seed germination when embryos were down. For comparison, the effect of imbibition for 4 d on different concentrations of ethanol is shown in Table 3. Up to 2.5% (v/v) ethanol had no or little effect on the germination of maize seeds. However, 5% (v/v) ethanol drastically reduced the percentage of seed germination and 10% (v/v) was found to be completely inhibitory. These adverse effects of the higher concentrations of ethanol were the same, regardless whether the seeds were placed embryo-side up or down.

Secondly, the importance of rinsing the seeds after a 10 min treatment with ethanol was demonstrated (see Table 4). A low percentage of seed germination resulted if the ethanol treated seeds were not rinsed with distilled water. This was evident regardless whether the treated seeds were placed embryo-side up or down. The inhibition of seed germination of ethanol-treated seeds when placed embryo-side up was removed after three or more times of rinsing the treated seeds before sowing. However, germination of those with embryo-side down was still inhibited even after six pre-sowing rinses.

Drying of the maize seeds treated briefly with ethanol before sowing appeared to have nullified the inhibitory effect of ethanol on seed germination (Table 5, cf. Table 1).

The low percentage of seed germination when the ethanol-treated seeds were placed embryo-side down during the germination tests was not a result of ethanol-induced reduction of seed viability. A high percentage of seed germination resulted when treated seeds were turned embryo-side up after four days with embryo-side down (Table 6).

Table 1. Effect on seed germination of soaking dry maize seeds in 100% ethanol before imbibition on water.

Treatment	Position of embryos on subsequent germination test	Seed Germination (%)
1. No immersion	down	97 ± 3[1]
	up	99 ± 1
2. Immersion in H_2O for 10 min	down	88 ± 4
	up	94 ± 3
3. Immersion in ethanol for 10 min	down	13 ± 10
	up	89 ± 6

[1] ± S.E.M.

Table 2. Effect on seed germination of soaking dry maize seeds in different concentrations of ethanol for 10 min before imbibition on water.

% of ethanol (v/v)	Position of embryos in germination test	Seed germination (%)
0 (H_2O)	down	91 ± 5[1]
	up	93 ± 2
6.25	down	75 ± 5
	up	88 ± 2
12.5	down	80 ± 4
	up	93 ± 1
25	down	78 ± 2
	up	90 ± 5
50	down	37 ± 3
	up	90 ± 2
75	down	21 ± 6
	up	91 ± 3
100	down	12 ± 5
	up	87 ± 3

[1] ± S.E.M.

Table 3. Effect on seed germination of imbibing maize seeds continuously for 4 d in different concentrations of ethanol.

% of ethanol (v/v)	Position of embryos in the germination test	Seed germination (%)
0	down	94 ± 2[1]
	up	95 ± 3
1	down	89 ± 6
	up	96 ± 1
2.5	down	77 ± 14
	up	92 ± 4
5	down	41 ± 9
	up	26 ± 6
10	down	0
	up	0

[1] ± S.E.M.

Table 4. Effect on seed germination of the number of rinses of maize seeds following immersion in 100% ethanol for 10 min before imbibition of water.

Number of rinses[1]	Position of embryos in the germination test	Seed germination (%)
0	down	20 ± 8[2]
	up	30 ± 14
3	down	18 ± 9
	up	89 ± 7
6	down	34 ± 17
	up	89 ± 2

[1] Rinsing seeds following immersion in water for up to 6 times had no effect on seed germination.
[2] ± S.E.M.

Table 5. Effect on seed germination of soaking dry maize seeds in H_2O or 100% ethanol for 10 min, followed by drying for 4 d and then rehydration.

Treatment	Position of embryos in the germination test	Seed germination (%)
Drying-Rehydration after:		
Immersion in H_2O for 10 min	down	98 ± 1[1]
	up	98 ± 4
Immersion in ethanol for 10 min	down	89 ± 4
	up	95 ± 3

[1] S.E.M.

Table 6. Germination of maize seeds after soaking in 100% ethanol for 10 min and then placed with the embryo side down for 4 d in contact with the germination substrate. Half the seeds were then placed embryo-side up and germination continued for another 4 d.

Position of embryos on germination substrate		Seed germination (%)		
First 4 d	Second 4 d	After 4 d	Between 4 & 8 d	Total
down	down	22 ± 1[1]	22 ± 5	44
down	up	24 ± 8	67 ± 2	91

[1] S.E.M.

Discussion

The present work was initiated to examine in detail the potential use of ethanol stress for maize seed vigour testing and was based on the following published findings. A variety of plant tissues, including seeds, are known to accumulate and metabolise ethanol (e.g., Cossins and Turner, 1959; Jackson *et al.*, 1982). Higher internal levels of ethanol have been found in artificially aged seeds than in the non-deteriorated ones (Crawford, 1977; Woodstock and Taylorson, 1981a; Ellerton and Perry, 1983). Externally applied ethanol has adverse effects on the germination of previously non-deteriorated seeds (Reynolds, 1977; Ellerton and Perry, 1983; Mugnisjah and Nakamura, 1986). Ethanol stress has been proposed to be useful for seed vigour evaluation in maize (Sadjad and Pain, 1980) and in soybean (Musgrave *et al.*, 1980; Mugnisjah and Nakamura, 1986).

Initially the experimental parameters taken into consideration for exposing maize seeds to ethanol include the form of ethanol to be used, that is, whether solution or vapour, and the duration of exposure. The ethanol treatment finally chosen consisted of immersing dry maize seeds for 10 minutes in a solution of ethanol (Table 1). This represented the most convenient and reproducible pre-sowing treatment of maize seeds before a germination test. Soaking maize seeds in 100% ethanol for as little as one minute (data not shown), or 25% or more ethanol for 10 minutes (Table 2) was found to have an adverse effect on seed germination as well as early seedling growth (Leung, unpublished observations). The results here are consistent with those from other work (Musgrave *et al.*, 1980; Mugnisjah and Nakamura, 1986; Alpi *et al.*, 1985).

A surprising finding is that whether the adverse effect of ethanol on maize seed germination was evident or not at the end of a germination test could be related to how the seeds were placed on the germination substrate (e.g., see Table 1). Apparently improved germination resulted when the ethanol-treated seeds were placed embryo-side up. It is possible that even after rinsing the ethanol-treated seeds, there could still be a small amount of residual ethanol left on the seeds. Then if the treated seeds were placed embryo-side down, it might be possible that the embryo was incubated in a solution of residual ethanol, the concentration of which was presumably high enough to have adverse effect on seed germination. By contrast, if seeds were placed embryo-side up, the residual ethanol would evaporate off quickly as the surface of this side of the seeds was exposed to air. This is consistent with the following observations.

Firstly, when the ethanol-treated seeds were rolled between wet germination paper, that is, both the embryo side and endosperm side were hydrated simultaneously and kept moist continuously, the adverse effect of ethanol on seed germination was again evident (Leung, unpublished data). Also, the germination of the maize seeds was inhibited regardless whether the seeds were placed embryo-side up or down in relation to the germination substrate when: (1) 5% or more ethanol was present all the time in the germination substrate (Table 3), and (2) the ethanol-treated seeds were not rinsed before the germination test (Table 4). Finally, drying the ethanol-treated seeds appeared to have completely removed the adverse effect of the ethanol treatment on seed germination (Table 5). Although the treated seeds were dried for 4 d in this experiment, it was subsequently found that it was not necessary to dry the treated seeds completely before rehydration to remove the adverse effect of ethanol. In fact, a brief period of air-drying for 1 or 2 h following a 10 min ethanol soak and rinsing with water was enough (Leung, unpublished observations).

It is noteworthy that soaking for 10 min in ethanol did not reduce maize seed viability (Table 6). The ethanol treatment seemed to simply inhibit or delay maize seed germination when the treated embryos were placed embryo-side down. After 4 d the ungerminated seeds still retained the capacity to germinate (Table 6).

Besides residual ethanol being a possible factor, oxygen availability could be limiting in situations other than embryo-side up. Alternatively, the impact of the initial water uptake, which has been found to be important in legume seed germination (Powell and Mathews, 1978; Woodstock and Taylorson, 1981b; Tilden and West, 1985) might be avoided when the ethanol-treated maize seeds were placed embryo-side up.

It is not clear from the results of the present work whether pre-sowing seed deterioration may be repaired or reversed, as suggested in other work (e.g., Woodstock and Taylorson, 1981b; Tilden and West, 1985). Instead, because of the structure and the size of the maize seed, the evidence presented in this report suggest an additional possibility that some effects of pre-sowing seed deterioration could be avoided during the germination test.

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