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Promotion of vigour in cabbage seed by osmotic priming pre-treatment at both vernalisation and non-vernalisation temperatures

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SUMMARY
Osmotic priming of cabbage seeds with polyethylene glycol (PEG 6000), to prevent germination during a vernalising treatment, was investigated as part of a study into potential seed production in the tropics. A preliminary attempt to vernalise cabbage seeds had resulted in weak stemmed seedlings, with fungal infections, as a consequence of the non-primed seeds having germinated while still in the fridge at the low temperatures used for vernalisation (0° – 5°C). Two experiments to determine the effect of PEG 6000 on the viability and vigour of cabbage seed at non-vernalisation and vernalisation temperatures were carried out. In the first experiment, seeds of ten varieties of cabbage were imbibed separately in 302.44 g l^{-1} PEG 6000 in Petri dishes lined with filter papers moistened with PEG solution, or with distilled water as controls. The primed seeds were kept for 2 weeks at 25°C (non-vernalisation temperature) under an 8 h photoperiod, washed in distilled water and sown immediately into 4 cm-square plastic modular trays filled with Levington F2S compost. Priming did not have any significant effect on the proportion of seeds that germinated ($P > 0.05$), but significantly enhanced the coefficient of velocity (CV) at 25°C. In the second experiment, seeds of four varieties of cabbage were primed at 0° – 5°C for 8 weeks prior to sowing in compost. Controls were seeds primed in the same concentration of PEG 6000 and kept at 25°C for 11 d, and seeds sown without priming or vernalisation. Again, priming of cabbage seed with PEG 6000 had no adverse effect on the proportion of seeds that germinated, but promoted the CV (vigour) of seeds kept at vernalisation temperatures (0° – 5°C).

Cabbage (*Brassica oleraceae* var. capitata L.) is one of the most important and popular vegetable crops in the family *Brassicaceae*. Vernalisation (cold treatment) is required to induce cabbage to flower and therefore is the major limitation to cabbage seed production in the tropics. Unlike other brassicas such as *Arabidopsis* and Chinese cabbage, in which plants can sense low temperatures during seed vernalisation (i.e., seed vernalision-responsive types), it is reported that cabbage needs to reach a certain developmental stage (seven-to-nine leaves) or a stem diameter of 6 mm, before it becomes sensitive to low temperatures (i.e., plant vernalisation-responsive type; Ito *et al*., 1966; Friend, 1985; Lin *et al*., 2005). However, in a preliminary study, we found that exposure of cabbage seed to a prolonged period of cold treatment in a refrigerator, in combination with gibberellic acid treatment, appeared to induce flowering, although more work needed to be done to confirm this. To do this, we required a method to apply a vernalisation treatment that did not adversely affect the viability and vigour of the seed. During a preliminary attempt to vernalise cabbage seed for potential seed production in the tropics, germination occurred even at low temperatures (0° – 5°C). This resulted in fungal infections and weak stems, following an initial growth of plants in a refrigerator, which were not present in seeds that had been pre-treated with polyethylene glycol (PEG 6000).

An osmotic priming pre-treatment with PEG 6000 has been reported to allow the initial stages of germination to proceed, but to inhibit the final stage that leads to elongation of the radicle (Karssen *et al*, 1989). Priming is also known to enhance germination and seedling growth in many crops (Ajouri *et al*., 2005; Bradford, 1986; Guedes and Cantliffe, 1980). However, the effect of osmotic priming on cabbage seed quality (i.e., germination and vigour) under vernalisation temperatures (0° – 5°C) for a prolonged period (8 weeks) is not known.

Experiments were carried out to determine the effect of an osmotic priming pre-treatment (PEG 6000) on the viability and vigour of cabbage seed under non-vernalisation and vernalisation temperatures, to address the following questions:

1. How does osmotic priming (PEG 6000) pre-treatment affect the germination and vigour of cabbage seed compared to non-primed seed at 25°C?
2. How does osmotic priming (PEG 6000) pre-treatment affect the germination and vigour of cabbage seeds kept for a prolonged period under vernalisation temperature (8 weeks at 0° – 5°C) compared to non-primed seeds, or to primed seeds kept at a higher (tropical) temperature (25°C)?

MATERIALS AND METHODS

Effect of PEG 6000 on the viability and vigour of cabbage seed at non-vernalisation temperatures

Seeds of ten varieties of cabbage (obtained from Warwick-HRI, Wellesbourne, Warwickshire, UK) were imbibed in 5 cm-Petri dishes lined with three filter

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papers. For each variety, three replicate dishes, each containing 20 seeds were treated by moistening the filter paper with 2 ml 302.44 g l^{-1} PEG 6000, providing an osmotic pressure of 1,200 kPa. Three replicate control dishes were moistened with 2 ml distilled water. The dishes were kept in a controlled environment room for 2 weeks at 25°C with an 8 h photoperiod at 400 μmoles m^{-2} s^{-1} photosynthetic photon flux density. The seeds were then washed in distilled water and sown at a depth of 0.5 cm in 46 g Levington F2S compost in each 4 cm-square module, in plastic modular trays. The time of seedling emergence was recorded daily. The coefficient of velocity (CV; Scott *et al*., 1984), a measure of vigour, was calculated as follows:

$$CV = \frac{\sum_i N_i}{\sum_i N_i D_i} \times 100 \quad (1)$$

Where N_i = number emerging on day i; and D_i = days from sowing.

The CV gives an indication of the speed and uniformity of seedling growth (i.e., a higher CV means higher vigour).

The final proportion of seeds germinating was recorded on day-10, in accordance with the International Seed Testing Association Regulations (ISTA, 1993).

Effect of PEG 6000 on the viability and vigour of cabbage seed at vernalisation temperatures

Seeds of each of four varieties of cabbage (including 'K. K. Cross', a variety commonly grown in Ghana, West Africa) were heat-treated to protect against pathogens by placing them in a 50°C water bath for 30 min, after which they were quickly cooled and imbibed in 302.44 g l^{-1} PEG 6000. Fifty seeds of each variety were then placed in 9 cm-Petri dishes (i.e., 50 seeds per dish) lined with three filter papers, moistened with 4 ml PEG solution. This was replicated three times.

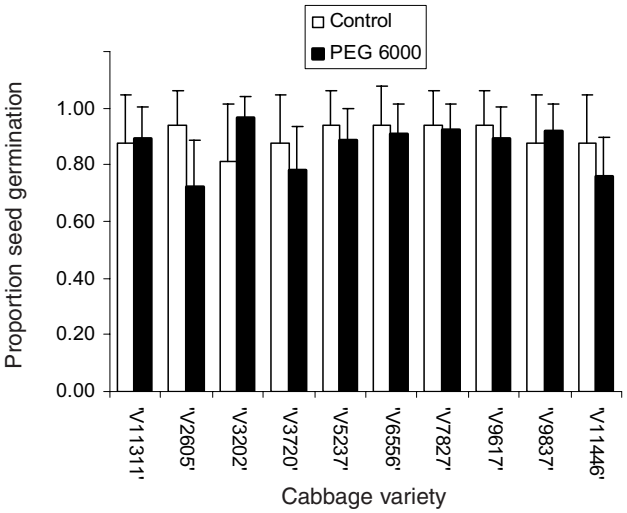

FIG. 1
Lack of effect of priming on the proportion of seed germination in ten cabbage varieties tested. Seeds were primed in PEG 6000, or kept in distilled water (control), at 25°C with an 8 h photoperiod for 2 weeks. Bars show standard error of the mean proportion of seed germination predicted from the regression model. (n = 3).

The seeds were then exposed to vernalisation temperatures (0° – 5°C) for 8 weeks in a refrigerator (referred to as 'Prim + Ver'). After the period of vernalisation, the seeds were washed in sterile water and sown immediately in 4 cm-square plastic modular trays filled with Levington F2S compost. There were two controls. In the first control, seeds (referred to as 'Prim only') were primed as above and kept at 25°C for 11 d prior to sowing of the treated ('Prim + Ver') seeds. This was to ensure that both batches of seeds had the same thermal time and were of similar physiological age. For the second control, (referred to as 'No Prim + No Vern'), seeds were sown without priming or vernalisation. All seeds were planted in similar containers at a depth of 0.5 cm. CV values and the final proportion of seeds germinating were determined as described above.

A linear logistic regression analysis (binomial) and analysis of variance were used to analyse the proportion of seeds that germinated and their CV values, respectively, using the Genstat (Release 8.1) statistical package.

RESULTS

Effect of PEG 6000 on the viability and vigour of cabbage seed at non-vernalisation temperatures

Priming did not have any significant effect on the proportion of seeds germinating ($P > 0.05$; Figure 1). For CV, there was no variety × priming interaction, nor a significant main effect of variety; therefore, only the main effect of priming is presented (Table I). Priming with PEG 6000 did enhance the CV for all ten varieties.

Effect of PEG 6000 on the viability and vigour of cabbage seeds at vernalisation temperatures

None of the treatments had any significant effect ($P > 0.05$) on the proportion of cabbage seeds that germinated (Figure 2). Thus, there was no evidence that priming had any adverse effect on the percentage germination of cabbage seed kept at vernalisation temperatures (0° – 5°C) for 8 weeks.

Similar to the results obtained in the first experiment, there was neither a variety × priming treatment interaction, nor a variety main effect on the CV of cabbage seed kept under the two temperatures ($P > 0.5$). Priming treatment had a significant effect ($F_{(2, 24)} = 20.6$; $P < 0.001$); therefore only the main effect of treatment is presented (Table II). The CVs for seeds that had received priming treatments were higher than non-primed seeds ('No prim + No Ver' control). Among the priming treatments, seeds that had been primed and exposed to vernalisation temperatures (0° – 5°C) for

TABLE I
Enhancement of the average coefficient of velocity (vigour) of cabbage seeds vernalised at 0° – 5°C by osmotic priming[†]

Treatment	Coefficient of velocity (d^{-1})
Priming (PEG 6000)	34.4
No priming (Control)	23.0
SED*	4.16
Probability (P)	0.014

[†]Seeds were primed in PEG 6000 or distilled water (control) and kept at 25°C with 8 h light and 16 h night for two weeks.
*SED, standard error of the difference. (n = 3).

TABLE II
Enhancement of the coefficient of velocity (vigour) of cabbage seeds (at 0° – 5°C) by osmotic priming

Treatment	Coefficient of velocity (d^{-1})
Prim only†	42.72
Prim + Var	48.00
No Prim + No Var	36.23
SED*	1.299
Probability (*P*)	< 0.001

†'Prim only' = seeds primed and kept at 25°C for 11 d. 'Prim + Ver' = seeds primed and exposed to vernalisation temperature (0° – 5°C) for 8 weeks. 'No Prim + No Ver' = seeds sown without priming or vernalisation.
*SED, standard error of the difference. (n = 3).

8 weeks ('Prim + Ver') had a significantly higher CV than those that were primed and kept at 25°C for 11 d.

DISCUSSION

The fact that the proportion of germination of primed and non-primed seeds was not statistically different between the ten cabbage varieties in the two experiments (Figure 1; Figure 2) indicated that priming with PEG 6000 had had no adverse effect on the embryo, either at the imbibition or the emergence stage. It has also been suggested that part of the improvement in germination performance that results from priming may arise from repair, during priming, of deterioration sustained previously during maturation or storage (Dearman *et al.*, 1986). Primed seeds actually gave higher CV values in both experiments (Table I; Table II). The results obtained in the present study confirmed the results obtained by Khan *et al.* (1980), where primed cabbage seeds kept at 15°C had accelerated emergence and gave increased plant fresh weight.

The second experiment showed that cabbage seeds can be primed and vernalised at the same time, without any adverse effect on viability. This means that seeds of seed vernalision-responsive plants could be vernalised for a long period of time without growth and loss of viability. When seeds imbibe, their water content reaches a plateau and changes little until emergence of the radicle (Bradford, 1986). Priming up to this point can have a positive effect, while an extended duration of priming will negatively affect germination. This implied that, at 8 weeks, the primed cabbage seeds had still not gone beyond the point at which their viability was adversely affected. Similarly, osmotic priming is also reported to more-than-double germination rate, increase the uniformity of germination, and raise the upper temperature limit for germination of celery seeds (Brocklehurst and Dearman, 1983).

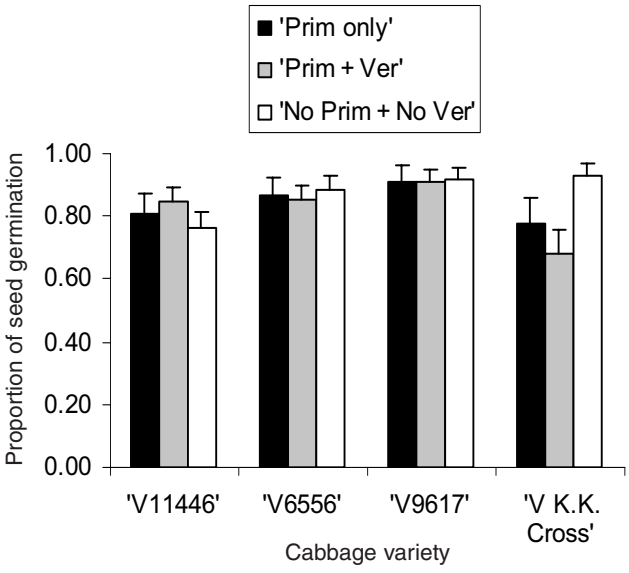

FIG. 2
Lack of effect of priming on the proportion of seeds germinating in four varieties of cabbage kept at two temperatures. 'Prim only' = seeds primed and kept at 25°C for 11 d. 'Prim + Ver' = seeds primed with PEG 6000 and exposed to vernalisation temperatures (0° – 5°C) for 8 weeks. 'No Prim + No Ver' = seeds sown without priming or vernalisation. Bars show standard error of the mean proportion of seed germination predicted from regression model. (n = 3).

Seeds exposed to vernalisation temperatures (0° – 5°C) for 8 weeks ('Prim + Ver') had a higher CV than those primed for 11 d at 25°C (Table II). This may be due to the fact that 8 weeks was enough time for priming to have had an effect on seed coat membrane integrity. Upon wetting, those membrane constituents associated with dormancy, such as C_7, C_8 and C_9 lipid acids, change their position in the membrane and do not revert to their initial position (Bewley and Black, 1982), thus weakening the membrane to allow rapid elongation of the radicle. It has also been reported that, during priming, water imbibition and some major metabolic events prepare the seed for radicle emergence, but radicle elongation is prevented (Karssen *et al.*, 1989). Moreover, slow germinating seeds catch-up with faster ones during priming. This ensures uniform and rapid emergence of seedlings, which may account for the higher CV values after priming in both experiments.

Priming is reported to induce a range of biochemical changes in the seed that are required to start the process of germination (i.e., breaking of dormancy, hydrolysis and the mobilisation of inhibitors, imbibition and enzyme activation; Ajouri *et al.*, 2005) which are a pre-requisite for rapid expansion of cells in the radicle. In primed leek seeds, the significant benefit in germination performance was accompanied by marked increases in protein, DNA and nucleotide biosynthesis. Further, it seems that endosperm weakening, associated with priming, contributed to the higher CV observed in the present studies and as reported for tomato (Haigh, 1988). During priming of tomato seeds, the breakdown of protein bodies was more extensive in endosperm cells at the micropylar region than was observed prior to germination in non-primed seeds (Haigh, 1988). Therefore, there is ample evidence that priming of cabbage seeds, prior to vernalisation, inhibits radicle elongation during vernalisation and improves the rate of germination after sowing.

CONCLUSION

Priming of cabbage seed with PEG 6000 had no adverse effect on the proportion of seeds that germinated, and promoted the CV (vigour) both for seeds kept under vernalisation and non-vernalisation temperatures. In future work, cabbage seeds can therefore be primed and kept at vernalisation temperatures (0° – 5°C) for up to 8 weeks, to promote vigour.

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