Scientia Horticulturae 228 (2018) 76–80

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Original article

A seed treatment to prevent shoot apical meristem arrest in *Brassica oleracea*

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ARTICLE INFO

Keywords:
Blind plants
Blindness
Seed quality
Seed soaking

ABSTRACT

Brassica oleracea plants can suffer from shoot apical meristem arrest, when sown at cold temperatures, giving rise to so-called blind seedlings that stop development and the formation of new leaves. We developed a seed treatment that strongly reduces the occurrence of this meristem arrest in kohlrabi and cabbage seedlings. The developed treatment involves soaking the seeds in water for one hour at 22 °C before sowing at 0–2 °C. The reduction of susceptibility to induction of shoot apical meristem arrest could be maintained when seeds were dried after the pre-soaking treatment. A strong reduction in susceptibility could also be obtained by incubating the seeds for four hours with a limited amount of water to 20% moisture content. However, when the hydration was performed at seed moisture levels between 30 and 50%, the sensitivity to shoot apical meristem arrest was regained upon drying. The developed treatment to prevent the occurrence of shoot apical meristem arrest can easily be upscaled for implementation at the commercial level.

1. Introduction

Brassica (*Brassica oleracea*) plants can suffer from shoot apical meristem (SAM) arrest, commonly referred to as 'blindness' (de Jonge et al., 2016; Forsyth et al., 1999; Mounsey-Wood, 1957; Wurr et al., 1996). This phenomenon occurs in several *Brassica oleracea* crops, including kohlrabi and broccoli, and leads to high economic losses for plant growers. Blindness can manifest itself by a variety of abnormal phenotypes, including seedlings without apical shoot and leaves or seedlings with one, two, or very few true leaves that are often distorted and severely reduced in size. Although the degree of SAM arrest and consequently, the type of abnormalities, can vary between individual plants, here we refer to all these as SAM arrested. Recently it was shown that the sensitivity for SAM arrest has both a genetic origin and is influenced by seed production or handling conditions, while its expression depends on the environment during germination and or seedling growth (de Jonge et al., 2016).

The physiological basis of SAM arrest is still not well understood. Forsyth et al. (1999) studied calabrese (*B. oleracea* var. italica) plants throughout the winter period and found that blindness was characterized by a cessation of leaf primordium production by the SAM, which loses its meristematic capability but remains alive. Previous studies on SAM arrest in brassica suggested that the exposure to low temperatures during germination and initial plant growth is one of the main causal factors (Mounsey-Wood, 1957; Wurr et al., 1996). Based on the response to cold, a method for inducing SAM arrest in seedlings from sensitive brassica seed lots has been developed (de Jonge et al., 2016). This method allows estimating susceptibility to SAM arrest of brassica seed batches. In this screening procedure dry brassica seeds are imbibed on cold wet filter papers and exposed to 0–2 °C for ten days prior to sowing in soil at around 20 °C. In the same study, it was shown that SAM arrest is significantly and proportionally influenced by the temperature used during this so-called "cold-induction", in which lower temperatures induced a higher number of arrested plants.

Considering the assay, it seems that cold water imbibition may lead to SAM arrest either by causing direct damage to the meristem or by inducing a change in its physiological state. Whatever may be the origin of this syndrome, it remains meaningful to further investigate the effects of temperature during initial seed water uptake as such knowledge may reveal strategies for preventing SAM arrest in brassica. In this study, we discovered that a pre-soaking of sensitive brassica seeds at room temperature, before the cold induction, had a preventive effect on the occurrence of SAM arrest. This protective effect was conserved even after drying the seeds back to their initial moisture content (MC) (ca.

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http://dx.doi.org/10.1016/j.scienta.2017.10.015
Received 13 April 2017; Received in revised form 28 September 2017; Accepted 11 October 2017
Available online 17 October 2017

6–7% on fresh weight basis) and may provide a method to prevent the expression of SAM arrest in susceptible seed-lots from *B. oleracea* varieties. The objective of this study was to test this cure treatment with different sensitive *B. oleracea* seed lots and to determine the optimal parameters as temperature, duration and seed moisture level.

2. Materials and methods

2.1. Seed samples

B. oleracea seeds of kohlrabi varieties Opimes (batch no. 273.580 and 273.586, 2009 harvest), Olivia (batch no. 188.902, 2006 harvest) and the cabbage variety Stanton (batch no. 1645, 2008 harvest) were used in this study. The seeds had been stored at 15 °C (or 20 °C in the case of the Stanton seeds) and 30% relative humidity (RH). The experiments were performed in 2010, 2011 and 2012.

The seed batches were previously identified as highly susceptible to SAM arrest (> 20% arrested) by using the SAM arrest screening test developed by (de Jonge et al., 2016). In germination tests at 20 °C, the standard temperatature for *B. oleracea* (ISTA, 2017), the seeds performed well with 100% germinating seeds.

2.2. SAM arrest screening test

To test the sensitivity of seeds for the induction of SAM arrest, we used the screening procedure developed previously de Jonge et al. (2016). For each individual experiment, performed in duplicate or quadruplicate, seeds were placed inside a 9 × 2 cm Petri dish containing two filter paper discs (T 300, AllPaper b.v., Zevenaar, Netherlands) and 9.75 ml of cold (0–2 °C) deionized water. Petri dishes were side-wrapped with Parafilm and placed on ice inside a styrofoam box and immediately covered with ice. After closure the box was placed in a refrigerator set at 2 °C. Alternatively the seeds were first allowed to take up water at room temperature (22 °C ± 2 °C) by incubation on wet filter paper for two h before being transferred to the box with ice. The seeds were maintained at 0 − 2 °C for seven days (or ten days in the case of experiments with variety Stanton seeds). After the cold incubation, the seeds were removed from the Petri dishes and superficially dried using paper towels. Seeds were then directly transferred to coco-peat blocks, (4 × 4 cm and covered by a 1–2 mm layer of sand) or to rock wool plugs (2.4 cm in diameter) in the case of experiments with variety Stanton seeds. The peat blocks or rockwool plugs were moistened with 0.5X Hoagland's solution (Hoagland and Arnon, 1950). The trays were transferred to a growth chamber set at continuous 20 °C and 16-h daylight with a light irradiance of 50 or 75 µmoles/m^2/s. The seedlings were watered three times a week with tap water at 20–22 °C. Three weeks after sowing, when normal seedlings had reached two fully expanded leaves and showed a visible shoot, the frequency of SAM arrested plants was determined by visual scrutiny.

2.3. Pre-soaking and drying

Seeds were placed inside a 150 ml glass beaker containing 100 ml of deionized water at room temperature (21 °C ± 2 °C) for two hours under constant stirring on a magnetic stirrer set at 400 rpm. Afterwards, seeds were separated from the water by vacuum filtration. In the 'pre-soaked' treatment, seeds were directly tested in the screening assay at low temperature (0 − 2 °C), while in the 'pre-soaked, dried' treatment, seeds were dried at 25 °C and 30% RH after soaking until they reached their initial 6–7% MC before being tested in the SAM arrest screening assay at 2 °C.

The method to prevent SAM arrest was optimized by testing different temperatures (16 °C, 22 °C and 28 °C) of soaking water and times of soaking (30 min, 1 h, 2 h and 4 h) at room temperature.

2.4. Limited moisture level treatment

Cabbage seeds of the variety Stanton were pre-soaked to a different MC levels by mixing in a 50 ml tube 0.5 g seeds with a specific amount of water (25 µl till 10 ml) for four hours, at room temperature and subsequently dried back to 6% seed MC. Dry non-soaked seeds exposed to the SAM arrest screening test, served as control (see above).

2.5. Seed moisture content determination

Seed MC of the starting material was determined in triplicate by measuring the seed weight before and after drying for at least 16 h at 105 °C. The seed MC after soaking was determined by measuring the weight gain. For that adhering water was removed first by centrifugation (750 x g, for 2 min at 20 °C).

2.6. Statistical analysis

The standard error of the mean was calculated using Microsoft Excel software.

3. Results

3.1. Effect of water temperature during seed imbibition on SAM arrest

To analyze the effect of water uptake by seeds prior to germination (pre-soaking), we treated seed batches of kohlrabi varieties Opimes and Olivia. Fig. 1A shows the results of the pre-soaking treatment at room temperature as compared to the standard SAM arrest induction assay. With both seed lots the frequency of arrested plants was strongly reduced by the pre-soaking treatment as compared to seeds directly imbibed in ice-cold water. Additionally, we tested the preventive treatment for a sensitive seed batch of a white cabbage variety Stanton, which had been used in our previous studies (de Jonge et al., 2016). The standard assay with direct cold imbibition resulted in 32% arrested

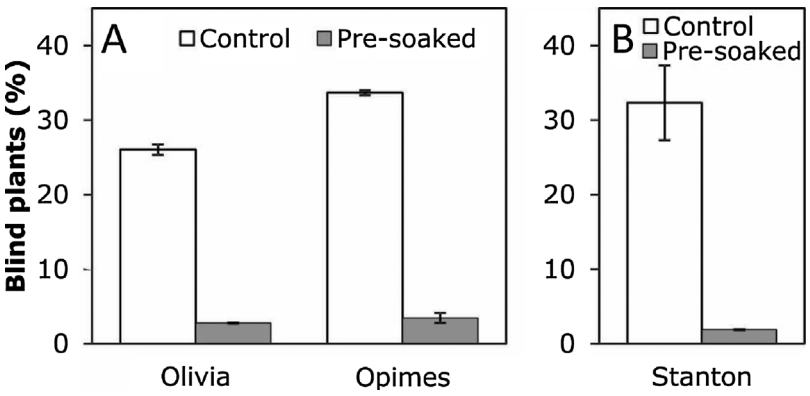

Fig. 1. Frequency of SAM arrested seedlings obtained in the SAM arrest screening test, as influenced by a preventive pre-soaking treatment at 22 °C ± 2 °C. A. Seed lots of kohlrabi cultivars Olivia and Opimes (batch no. 273.580). B. Seed lot of white cabbage cultivar Stanton. Control: untreated seed; Pre-soaked: seed soaked in deionized water at room temperature for 2 h and dried to 6–7% MC. Error bars represent standard error of two replicates with 150 seedlings of Olivia and Opimes and three replicates of 300 seeds of Stanton. Error bars represent standard error of two replicates with 150 seedling.