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Development of a protocol for frost-tolerance evaluation in rapeseed/canola (*Brassica napus* L.)

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ABSTRACT

Spring frost can severely damage or even kill rapeseed/canola (*Brassica napus* L.) seedlings. A protocol for large scale screening of rapeseed germplasm under frost-simulating conditions has not yet been developed. Accordingly, the present study was conducted to develop a protocol for screening rapeseed germplasm under artificial frost-simulation conditions in a plant growth chamber and in a greenhouse. Nine rapeseed varieties, including three commercial hybrids, three spring types, and three winter types were used. Cold acclimation at 4 °C was applied for 0, 7, or 14 days to two-week old seedlings. The seedlings were treated with four freezing temperatures (−4 °C, −8 °C, −12 °C, and −16 °C). The length of the freezing period was 16 h, including the ramping of temperature down from 4 °C and up from the respective freezing temperature to 4 °C. Plants were allowed to recover at 4 °C for 24 h before they were moved back to the greenhouse. Frost damage was scored on a 0–5 scale, where 0 denotes completely dead and 5 denotes no damage. Seedling survival from the freezing treatment increased from the non-acclimation to the cold acclimation treatment. However, no significant differences ($P < 0.05$) were found between 7 and 14 days of acclimation. Frost treatment at −4 °C resulted in significant differences in seedling damage relative to the other three temperatures, with the −16 °C treatment resulting in the highest overall seedling damage. Significant differences were found between the spring type and the other two types (hybrid and winter). However, no significant differences were found between the hybrid and winter types. The suggested protocol for the assessment of frost tolerance is acclimation of two-week old seedlings for 7 days at 4 °C followed by frost treatment at −4 °C for 16 h.

1. Introduction

Rapeseed/canola (*Brassica napus* L.) is an important crop for the U.S. state of North Dakota (ND), which produces about 84% of the U.S. crop. It is grown primarily in the northeast and north central parts of the state. Canola is considered to be a healthy oil for human consumption compared to other vegetable oils because of favorable combinations of the essential fatty acids in seeds [1].

Frost susceptibility is an abiotic stress that impairs plant growth and crop production [2]. Frost at the seedling stage of rapeseed can be harmful and may destroy the whole crop. The

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frost-free date in North Dakota is generally considered to be May 25, but the date can vary from northern to southern regions of the state and also from year to year. Given that canola is grown in the northern part of the state, the frost-free period tends to start later. The average air temperatures for Langdon, ND in April and May are 4 °C and 11 °C, respectively [3]. However, the minimum temperatures during the same time period are −2 °C and 4 °C, respectively. The severity of frost injury depends on moisture condition, plant growth stage, cold severity, duration of cold temperature, and other factors. Canola seedlings are not affected by a light spring frost that causes leaf wilting but not browning. Frost damage can be seen on leaves and symptoms can include wilting, bleaching, or in extreme cases, plant death. Bleaching occurs owing to phyto-oxidation of pigments in leaves [4]. Wilting is caused by a loss of water from cells. Resistance to chilling by frost is complex and may be difficult to incorporate. Canola growers usually look for blackened cotyledons and/or leaves as an indicator of frost damage necessitating replanting. It is necessary to wait for 5–10 days to confirm whether the plants are recovering by generating green shoots at the growing point of apical meristems in the center of the frozen leaf rosette. Canola is more susceptible to frost at the cotyledon stage than at the three- to four-leaf stage. When early spring-seeded canola is exposed to cold temperature, the defense mechanism allows the plant to withstand cold temperature via gradual hardening of plant tissue. Slow-growing seedlings are harder and less susceptible to cold than rapidly growing seedlings. In spring canola, the process of unhardening the plants to initiate active growth is rapid [5]. Usually, winter type canola is capable of hardening faster, can tolerate cold temperatures for a longer time, and is unhardened slower, reducing frost damage [6]. However, variation in frost hardiness is also available within winter- and spring-type germplasm.

Identifying frost tolerance in canola would be beneficial for growers, especially in North Dakota, but also in other places where early planting poses the threat of frost damage. Screens for frost tolerance in canola using artificial growing conditions have not been established. Field testing of frost tolerance relies heavily on weather conditions each year and these cannot be predicted. Thus, screening for frost tolerance under controlled environmental conditions may help to identify frost-tolerant germplasm and can also be performed multiple times in a year, increasing screening capacity over that by field testing.

Canola displays different growth habits. The winter type is grown mainly in western Europe and part of the USA. Vernalization is required for flowering of winter-type rapeseed. The spring type is grown in Canada, USA, Australia, India, eastern Europe, and other countries. China grows mainly a semi-winter type. Owing to its severe winters, North Dakota grows only spring-type canola.

This study aimed to identify a protocol for screening frost tolerance in canola under artificial frost-simulation conditions.

2. Materials and methods

Nine canola varieties chosen from two growth types, were planted in a randomized complete block design (RCBD) with three replicates and eight plants per line per replicate were grown in the greenhouse for two weeks at 20 °C. The photoperiod was 16 h of light and 8 h of dark and the average humidity was 47.3%. The varieties grown included three commercial hybrids (DKL 70–07, Pioneer 45H26, and Sprinter), three spring lines (NDSU151000, Hi-Q, and Kanada), and three winter lines (Fashion, ARC 2180–1, and Galileo). The hybrids are commercial varieties commonly grown in North Dakota and were chosen for this reason. These winter and spring type varieties are commonly used in the North Dakota State University canola breeding program. Because the varieties represented two growth habit types and are commonly used in the breeding program, we chose these winter- and spring-type varieties for this study.

After two weeks of growth, plants were moved to the plant growth chamber for acclimation at 4 °C with a 12-h photoperiod provided by GE Ecolux F32T8 SP35 Eco (32 W T8) style bulbs (General Electric Company). Three acclimation times (0, 7, and 14 days) were used. A total of 216 seedlings (9 varieties × 8 seedlings/variety × 3 acclimation times) per replication per freezing treatment were used. Seedlings were fertilized with 20–20–20 water-soluble fertilizer prior to cold acclimation.

An ESPEC BTU-433 freezing chamber (ESPEC North America, Inc.) was used for frost simulation. Four freezing temperatures were tested: −4 °C, −8 °C, −12 °C, and −16 °C. The total time for frost simulation was 16 h, including the lowering and raising of the temperature from and to 4 °C, along with holding at the minimum temperature. Sixteen h of treatment was chosen, based on overnight freezing temperatures in North Dakota.

In the −4 °C treatment, the temperature started at 4 °C and was lowered at −2 °C h^{-1} over 4 h to reach the treatment temperature. The seedlings were kept at −4 °C for 8 h. The temperature was raised again to 4 °C at a rate of 2 °C h^{-1}, requiring another 4 h. In the −8 °C treatment, the temperature started at 4 °C and was lowered at −2 °C h^{-1} for 6 h to reach the treatment temperature. The seedlings were kept at −6 °C for 4 h. The temperature was raised again to 4 °C at 2 °C h^{-1} over another 6 h. In the −12 °C treatment, the temperature started at 4 °C and was lowered at −3 °C h^{-1} over 5.33 h to reach the treatment temperature. The seedlings were kept at −12 °C for 5.34 h and the temperature was again raised to 4 °C at 3 °C h^{-1} over another 5.33 h. Finally, in the −16 °C treatment, the temperature started at 4 °C and was lowered at −3 °C h^{-1} for 6.66 h to reach the treatment temperature. The seedlings were kept at −16 °C for 2.67 h. The temperature was again raised to 4 °C at 3 °C h^{-1} over another 6.66 h (Table 1).

After frost simulation, seedlings were placed in the growth chamber at 4 °C for 24 h before being moved back to the greenhouse for scoring seedling damage and evaluations. Scoring was performed every three days starting three days after the frost treatment. Each plant was scored individually using a 0 to 5 scale, where 0 denoted dead, 5 denoted no damage, and scores of 1–4 were based on visual estimation of frost damage. Notes on general plant color were also taken. The experiment was replicated three times. A total of 2,592 seedlings (9 varieties × 8 plants/variety × 3 acclimations × 4 frost treatments × 3 replications) were scored in the greenhouse and in the growth chamber.