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Changes in chlorophyll content and fluorescence of leaves of winter rapeseed affected by seedling vigor and cold acclimation duration

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Abstract

Cultivation of winter rapeseed (*Brassica napus* L.) has been increased in the Northwest regions of Iran. Winter frost is one of the major environmental constraint limiting growth and then yield of rapeseed in these regions. Thus, a factorial experiment on the basis of RCB design was conducted under controlled environmental conditions in 2007, to investigate the effects of seed vigor (three vigor levels) and cold acclimation duration (0, 14, 28 and 42 days) on freezing tolerance capability of winter rapeseed. Plant survival at -12°C was significantly decreased with decreasing seed vigor level. Plants produced from the highest vigor seed lot (SV_1, control) had the greatest freezing tolerance (61.1% survival), compared with those from other two poor vigor seed lots. Prolonged acclimation duration improved survival of seedlings from both high and low vigor seed lots. Chlorophyll a, b and a+b concentration of seedlings produced from high vigour seed lot (SV_1, control) was higher than those of the other two seed lots. Concentration of chlorophyll components was significantly decreased in cold acclimated plants during the acclimation period ($P \leq 0.05$). A significant reversible decrease in the maximum quantum yield of the PSII (F_v/F_m) was found in plants of different seed lots during acclimation to low non-freezing temperature. The results clearly suggest that seed vigor can strongly influence freezing tolerance and plant survival in winter oilseed rape, through effects on seedling vigor before frost occurrence.

Key words: Chlorophyll, winter rapeseed, seedling vigor, cold acclimation, freezing tolerance.

Introduction

Rapeseed is an important oil crop worldwide and has a significant potential for cultivation in West and Northwest of Iran. However, seedling establishment and freezing tolerance are the factors affecting production consistency of winter-type rapeseeds in these regions. To achieve high yield capacity and yield stability, crops have to survive and grow during the cold season. A fundamental component of the winter survival capacity is represented by the freezing tolerance which is based on an inducible process, known as hardening or cold acclimation that occurs when plants are exposed to low non-freezing temperatures[3].

In both annual and in most winter herbaceous plants, freezing tolerance is not affected by the photoperiod, but acclimation is mainly triggered by low non-freezing temperatures. Cold acclimation is a rather rapid process in many herbaceous species [13]. During cold acclimation, several physiological changes occur, including alteration of lipid composition in plasma membranes [22], accumulation of protectant compounds, such as carbohydrates, free amino acids or other osmolytes, and induction of new gene activity [6, 14]. The primary function of cold acclimation is to stabilize the membranes against freezing injury [11].

Different factors may influence cold acclimation and frost resistance. Many reports are concentrated on the effects of genotypic differences on freezing tolerance [15, 17]. The potential of seed vigor to influence freezing tolerance of rapeseed seedlings is recently documented (Ghassemi-Golezani *et al.* unpublished), but changes in leaf chlorophyll content and fluorescence of these seedlings are not yet reported.

Chlorophyll fluorescence responds to changes in PSII photochemistry, and therefore represents a convenient and rapid tool to evaluate the capacity of the photosynthetic machinery at low temperature [18]. Determination of the maximum quantum yield of the PSII photochemistry by the ratio of variable to maximal fluorescence in dark-adapted state, Fv/Fm, has been used as a diagnostic probe for measuring chilling stress-induced injury of photosynthesis. Measures of chlorophyll fluorescence have been used to screen genotypes for chilling tolerance in several sensitive species such as maize [5, 20] and rice [2, 21]. Nevertheless, little information is available on the effect of cold acclimation duration on chlorophyll fluorescence in winter rapeseed.

Rizza *et al.* [18] found a significant reversible decrease in F_v/F_m in oat genotypes during acclimation to low, non-freezing temperatures. Rapacz *et al.* [16] also reported the reversible decrease in apparent quantum yield of PSII (F_v/F_m) in spring-type rapeseed. Similar result was also shown by Hurry and Huner [8] in spring and winter wheat. According to Huner *et al.* [7] cold-tolerant winter cereals must maintain the capacity for active photosynthesis during prolonged exposure to low non-freezing temperatures during the cold acclimation period with minimal changes in pigment composition. In this research, we evaluated changes in chlorophyll content and fluorescence parameters during cold acclimation in winter rapeseed plants derived from high and low vigor seeds. The survival of these plants was also tested at -12°C.

Materials and Methods

Seeds of a winter rapeseed (*Brassica napus* L. var. *oleifera* cv. Okapi) were divided to three sub-samples. A sub-sample was kept as control or high vigor seed lot (SV_1). Other sub-samples with about 15% moisture content were artificially deteriorated at 40°C for 5 and 7 days (SV_2 and SV_3, respectively). So, three seed lots were obtained with 98% (SV_1), 85% (SV_2) and 72% (SV_3) seed viability. Seeds were sown in plastic rectangular pots (10 by 27 by 37 cm) containing loam soil: peat (6:1 v:v) and placed on a vegetation yard in three blocks. Such conditions secured natural temperature and light conditions. The daily maximum and minimum temperatures during seedling growth ranged from 24 to 35°C and from 12 to 22°C respectively. After seedling establishment, each pot was thinned to 8 plants.

Four weeks after planting, the seedlings were transferred to a growth chamber adjusted at 4°C with 12 hours (300 µmol m^{-2} s^{-1} PAR) daily photoperiod, for acclimation. At this time, the seedlings were at different growth stages, dependent on seed lot vigor (Fig. 1). After the appropriate acclimation duration (0, 14, 28 and 42 days), pots were placed in three blocks in a low-temperature incubator for the freezing treatment. Initial temperature in the incubator was 1°C. The temperature was subsequently lowered to -1°C over a period of 2 hours, and held at this temperature for 12 hours, to ensure that nucleation occurred evenly. The temperature was then reduced by 1°C per hour down to -12°C. Pots were removed from the incubator and moved back to the growth chamber. Twenty-four hours after the pots were removed from the incubator, all plants were returned to the natural conditions. After 2 weeks, seedling survival in each pot was recorded.

Similar approach was adopted to evaluate freezing tolerance of the seedlings from different seed lots. Therefore, a factorial set of experiment on the basis of RCB design in three replications was arranged to evaluate frost tolerance of seedlings under freezing temperature, affected by three seed vigor levels and four acclimation durations. Chlorophyll concentration (Chl a, Chl b and Chl a+b) was determined according to Arnon [1] using three leaf discs (1.27 cm^2 each) from the youngest fully expanded leaf in each pot. The chlorophyll fluorescence induction parameters were measured in leaves by a chlorophyll fluorometer (OS-30, OPTI-SCIENCES, USA). Fluorescence emission was monitored from the upper surface of the leaves. Dark-adapted leaves (1 h) were initially exposed to the weak modulate measuring beam, followed by exposure to saturated white light to estimate the initial (F_0) and maximum (F_m) fluorescence values, respectively [9]. Variable fluorescence (F_v) was calculated by subtracting F_0 from F_m. The F_v/F_m ratio measures the efficiency of excitation energy capture by open PSII reaction centers, representing the maximum capacity of light-dependent charge separation in PSII [18].

The data obtained from the survival counts, chlorophyll content and fluorescence parameters were analyzed, using general linear models (PROC GLM) of SAS [19] to compare differences among the treatments. Excel software was used to draw the figures.

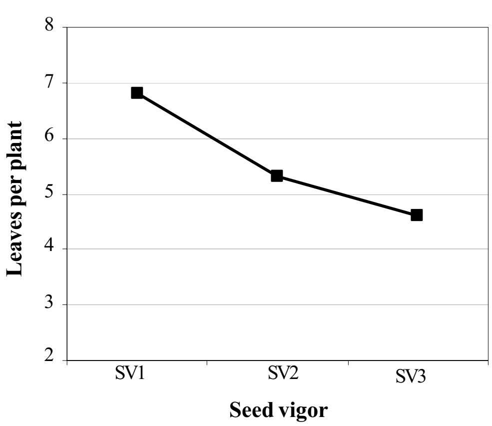

Figure 1. Mean number of leaves for plants produced from different seed lots. SV_1, SV_2 and SV_3: Seed lots with 98, 85 and 72% viability, respectively.

Results

Seedling survival at -12°C was significantly decreased with decreasing the vigor of seed lots (Table 1). None of non-acclimated plants from all seed lots were survived at -12°C. In comparison, seedling survival was considerably improved, due to cold acclimation. Survival of the plants from both high and low vigor seed lots at 28 and 42 days acclimation duration was higher than that at 14 days acclimation (Fig. 2).

Plants from high vigor seed lot (SV_1) had the highest chlorophyll a, b and a+b concentration (43.2, 9.0 and 52.2 mg cm^{-2} respectively). However, the chlorophyll content of the plants from low-vigor seed lots was statistically similar (Table 1). A significant ($P \leq 0.05$) decrease of chlorophyll a, b and a+b concentration was noticed in cold acclimated plants during the acclimation period (Fig. 3). The interaction of seed vigor and acclimation duration for this trait was not significant. Changes in F_v/F_m during 6 weeks of cold acclimation of the seedlings from different seed lots are shown in Fig. 4. F_v/F_m of the seedlings from all seed lots was not significantly different ($P>0.05$). When 4 weeks old plants from different seed lots were acclimated at 4°C, the ratio of variable to maximal fluorescence (F_v/F_m) in dark-adapted state was decreased with increasing acclimation duration up to 14 days. This effect was gradually reversed during longer exposure to low temperature, up to 28 days and thereafter slightly decreased.

Table 1. Frost resistance and chlorophyll a, b and a+b concentration of winter oilseed rape seedlings produced from different vigor seed lots.

Seed vigor level (SV)†	Plant survival (%) at -12℃	Chl a (mg cm^{-2})	Chl b (mg cm^{-2})	Chl a+b (mgcm^{-2})
SV_1	61.1 a‡	43.2 a	9.0 a	52.2 a
SV_2	47.2 b	37.9 b	7.7 b	45.7 b
SV_3	40.3 c	34.1 b	6.8 b	40.9 b

† SV_1, SV_2 and SV_3: Seed lots with 98, 85 and 72% viability, respectively. ‡ Different letters indicate significant difference at $p \leq 0.05$.

Discussion

Cold acclimation facilitated the freezing tolerance of the plants contributed, and as was expected, led to more distinct differentiation of frost hardiness. This positive relationship extended up to 28 days acclimation duration with no significant change up to 42 days acclimation. Prolonged acclimation duration improved survival of seedlings from both high and low vigor seed lots (Fig. 2). Survival of seedlings from high vigor seeds was considerably higher than those from low vigor seeds at freezing temperature (Table 1). This superiority could be resulted from enhancing seedling vigor as indicated by more leaves per plant before acclimation and freezing treatment (Fig. 1) and higher chlorophyll content of leaves during acclimation (Table 1). Therefore, cold acclimation and freezing tolerance of rapeseed can be improved by increasing both the seedling size (Ghassemi-Golezani *et al.* unpublished) and the chlorophyll content of leaves before frost occurrence. These are well-provided, if high vigor seeds were cultivated.

Variation in F_v/F_m (Fig. 4) can be interpreted as a reversible photoinhibitory effect having a protective, regulatory function [4, 7, 16]. Nevertheless, during long exposure to cold acclimation other protective mechanisms such as increased capacity of carbon assimilation, increased activities of the scavenger systems for active oxygen species, energy dependent fluorescence quenching and xanthophyll cycle activity are developed [18]. At this stage photosynthesis is well adapted to the new environmental conditions and, as a consequence, F_v/F_m returns to the value found in plants grown at natural conditions. The F_v/F_m ratio represents the PSII functional status, which is not always correlated with leaf chlorophyll content [9, 10, 12]. This ratio is an indicator of stress; leaf values below 0.83 are generally considered indicative of stressed plants. A decline in F_v/F_m might be due to an increase in protective non-radiative energy dissipation or to photoinhibitory damage to the PSII reaction centre [12].

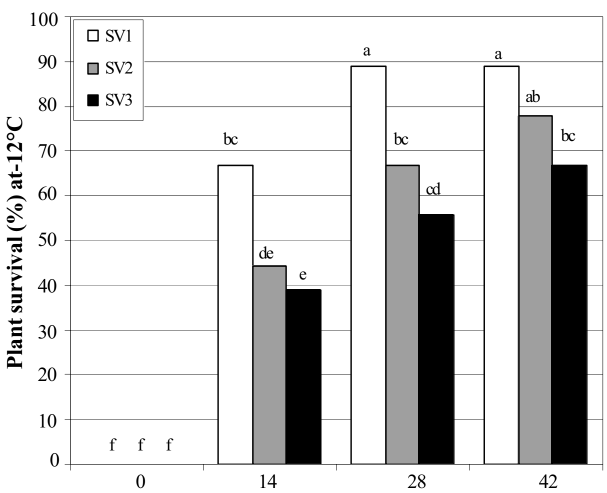

Figure 2. Frost resistance of winter rape seedlings affected by seed vigor level and cold acclimation duration. Different letters indicating significant difference at p≤ 0.05. SV_1, SV_2 and SV_3: Seed lots as described in Fig. 1.

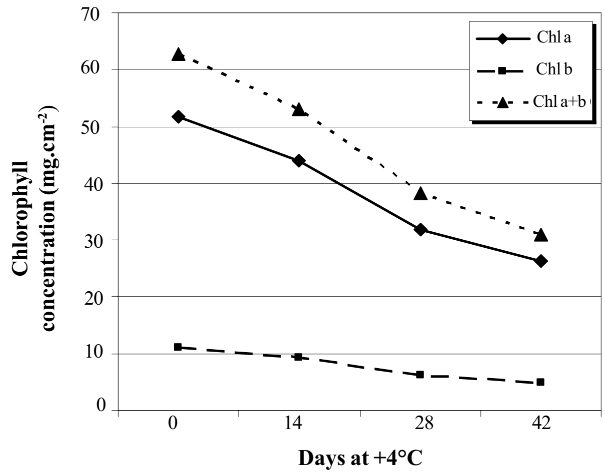

Figure 3. Chlorophyll a, b and a+b content of winter rapeseed leaves during cold acclimation.

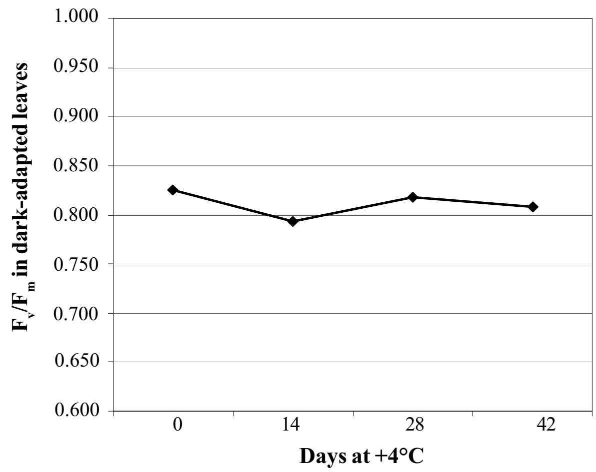

Figure 4. Changes in chlorophyll fluorescence parameters in dark-adapted leaves during cold acclimation observed in winter oilseed rape cv. Okapi.

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