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Genome-wide association study of heat stress-tolerance traits in spring-type *Brassica napus* L. under controlled conditions☆

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ABSTRACT

High temperatures have a detrimental effect on growth, development, and yield of *Brassica napus*. Even a short period of heat stress can lead to yield losses of 15%–20%. A collection of spring-type accessions available in Germplasm Resources Information Network (GRIN) were used to assess the effect of short periods of high-temperature stress at the early flowering stage of *B. napus*. Two sets of accessions with three replications per set were grown in a greenhouse at 22/18 °C day/night temperatures. Plants from the second set at the 6-day flowering stage were exposed to heat-stress conditions (maximum temperature up to 35 °C) in a plant growth chamber for five days. The heat-stressed plants were then allowed to recover in a greenhouse. Pollen sterility, sterile/aborted pods, and number of pods on main raceme were recorded for both control (set 1) and heat stressed (set 2) plants. Heat susceptibility indices for all three traits were calculated and an association-mapping study was conducted using 37,539 Single Nucleotide Polymorphisms (SNPs) to identify genomic regions controlling the heat stress traits. A total of 5, 8, and 7 quantitative trait loci (QTL) were associated with pollen sterility, sterile/aborted pods, and number of pods on main raceme, respectively. Together they explained respectively 46.3%, 60.5%, and 60.6% of phenotypic variation. Candidate genes in the QTL regions included genes associated with flowering, male sterility, pollen abortion, embryo abortion reducing pollen development, and pod development.

1. Introduction

Canola (*Brassica napus*, AACC, 2*n* = 4*x* = 38) is an amphidiploid species of *Brassica* derived from two diploid species, *Brassica rapa* L. (2*n* = 20, AA) and *Brassica oleracea* L. (2*n* = 18, CC) [1]. It is the second largest oil-producing crop in the world after soybean (Foreign Agricultural Service, USDA, October 2015, https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola/). Canola is cultivated as a major oilseed crop in Canada, Europe, China, Australia, USA, and the Indian subcontinent. North Dakota is the largest canola-producing state, with about 84% of U.S. canola production, and annually

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contributes about U.S. $384 million to the national economy (five-year average from 2011 to 2015; USDA-NASS 2016, https://quickstats.nass.usda.gov/).

It is predicted [2] from various climate models that the global mean temperature will increase by 1–4 °C by the end of the 21st century. The increasing temperature will create an adverse environment that impairs agriculture and crop production [3]. Abiotic stress changes the morphological, physiological, biochemical, and molecular properties of plants. Crop growth at flowering stage is highly sensitive to heat stress [4,5], and causes flower abortion, pollen sterility, and reduced pod development and seed set and reduces the assimilatory capacity and productivity of crops [6,7]. Certain genotypes are more tolerant to heat stress and the tolerance is controlled by multiple genes [8,9].

Canola is strongly affected by heat stress. Temperatures of 15–20 °C are optimal for growth and development of canola. High temperatures (over 27 °C) cause pollen sterility, pod abortion, and yield loss of the crop [10–15]. It has been estimated [15] that every 1 °C temperature increases from the range optimal for growth and development during pod setting of canola causes a 10% yield reduction. Heat stress during the pre-anthesis stage reduces pollen fertility, whereas post-anthesis heat decreases the female fertility of *B. juncea* [16].

Heat-stress tolerance in plants is a complex phenomenon involving numerous biochemical and metabolic activities such as antioxidant activity, membrane lipid unsaturation, gene expression and translation, protein stability, and accumulation of compatible solutes [17]. Heat stress negatively affects the developmental and physiological processes, reproduction, and adaptation of plants [18]. Heat stress tolerance is a polygenic trait that makes it difficult to introgress multiple favorable alleles into cultivars [19,20].

Canola germplasm shows comprehensive linkage disequilibrium owing to its limited geographic range and intensive breeding [21]. Genome-wide association study (GWAS) is a powerful tool for characterizing the genetic architecture of traits and identifying multiple candidate genes associated with the traits in many crop species [22–24]. It is based on historical recombination events and genome scanning with high-density DNA markers to locate genetic loci associated with traits of interest at a relatively high level of resolution [25,26]. An association mapping study was performed to identify genomic regions and quantitative trait loci (QTL) controlling three traits affected by heat stress in a collection of spring-type accessions that have practical applications to genotype selection and generation advance.

2. Materials and methods

2.1. Plant materials and phenotyping

Eighty-eight accessions of spring-type *B. napus* from the USDA-ARS Germplasm Resources Information Network (GRIN) (Table S1) were used in this study. These genotypes are a subset of 104 spring genotypes that are available in the diverse GRIN collection. Spring-type canola generally flowers in 40–60 days. The experiment was conducted in a greenhouse and a plant growth chamber at North Dakota State University (NDSU), Fargo, ND, USA during 2014–2015. Two sets of experiments (set 1 and set 2) were conducted using a randomized complete block design (RCBD) with three replications per set. The set 1 experiment (control) was conducted in the greenhouse at 22/18 °C (day/night) temperature until desiccation. Plants in the greenhouse were grown with a 16-h photoperiod provided by natural sunlight supplemented with 400 W HPS PL 2000 lamps (P.L. Light Systems Inc., Beamsville, Ontario, Canada). The plants in set 2 were grown in the same greenhouse and subjected for five days to heat stress in the growth chamber starting at the sixth day after flower initiation. All plants did not flower on the same day. Only the plants at the 6-day flowering stage were transferred into the plant growth chamber. The artificial heat simulation setup in the plant growth chamber was similar to that used in earlier experiments [11–13,27,28] with slight modifications. The heat-stress simulation condition, based on North Dakota weather, included a temperature of 18 °C for 8 h from 22:00 to 6:00 followed by temperature ramping from 18 °C to 35 °C over 6 h from 6:00 to 12:00, maintenance at 35 °C for 4 h from 12:00 to 16:00, and ramping down from 35 °C to 18 °C over 6 h from 16:00 to 22:00. The relative humidity in the growth chamber was maintained at 70% and light was provided for 14 h every day. The plants in the growth chamber were watered twice daily at 300 mL $plant^{-1}$ per application. After the heat treatment, the plants were returned to the original greenhouse room at 22/18 °C day/night temperature and grown until desiccation. Flowering buds were tagged before and after heat stress to characterize pod development during the period of heat stress. Flower buds were collected from the heat-stressed plants on the third day of heat treatment and buds were collected from the control plants at the same time. Pollen sterility, sterile/aborted pods on main raceme, and number of pods on main raceme were recorded for all plants in set 1 (control) and set 2 (heat stress treatment).

2.2. Pollen sterility study

A pollen sterility study was conducted in the laboratory. Flower buds prior to opening were collected from the greenhouse and growth chamber. Ten buds per replication from each accession were collected in an Eppendorf tube containing water and placed on ice during bud collection. The water in the Eppendorf tube was replaced with 30% acetic acid solution (70% ethanol + 30% acetic acid) and the tubes were refrigerated. The preserved buds were opened with forceps on precleaned microslides (75.00 mm × 25.00 mm × 1.00 mm thick) and 2–3 anthers were macerated and pressed with a scalpel on each slide with 1–2 drops of 1% acetocarmine solution. The slide was heated over a spirit lamp for 5–10 s to fix the acetocarmine dye in the pollen grain. Anther debris was removed with a needle and a cover slip was placed on the slide to prevent the entry of air bubbles between the slide and cover slip. Sterile (un- or lightly stained) and viable (stained) pollen were counted under an optical microscope (N-400M, 110–115 V ~60/60 Hz; 0.4 A, halogen lamp 60 V 20 W, AmScope, Irvine, CA, USA). One hundred randomly selected pollen grains per slide were counted and the percentages of fertile and sterile pollen grains were recorded (Fig. 1).

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