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Differences between wheat genotypes in damage from freezing temperatures during reproductive growth

David P. Livingston III[a,b,*], Tan D. Tuong[a], Thomas G. Isleib[b], J. Paul Murphy[b]

[a] US Department of Agriculture—Agriculture Research Service, 840 Method Rd., Unit 3, Raleigh, NC , USA
[b] North Carolina State University, 840 Method Rd., Unit 3, Raleigh, NC, USA

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ABSTRACT

Cereal crops in the reproductive stage of growth are considerably more susceptible to injury from freezing temperatures than during their vegetative growth stage in the fall. While damage resulting from spring-freeze events has been documented, information on genotypic differences in tolerance to spring-freezes is scarce. Ninety wheat genotypes were subjected to a simulated spring-freeze at the mid-boot growth stage under controlled conditions. Spring-freeze tolerance was evaluated as the number of seeds per head at maturity after plants were frozen at −6 °C. Plants that froze, as confirmed by infrared (IR) thermography, died shortly after thawing and consequently the heads did not mature. Only in plants that had no visible freezing (super-cooled) were heads able to reach maturity and produce seeds. In plants that super-cooled four genotypes had significantly higher seed counts after being exposed to freezing than three with the lowest. In addition, significant differences between genotypes were found in whole plant survival among those that had frozen. Genotypes with high whole-plant freezing survival were not necessarily the same as the super-cooled plants with the highest seed counts. Spring-freeze tolerance was not correlated with maturity suggesting that improvement in freezing tolerance could be selected for without affecting heading date. Spring-freeze tolerance was not correlated with freezing tolerance of genotypes of plants in a vegetative state, either under non-acclimated or cold-acclimated conditions indicating that vegetative freezing tolerance is not a good predictor of spring-freeze tolerance.

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1. Introduction

Fall-sown genotypes of cereal crops such as rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) are generally preferred by growers over their spring planted counterparts. A fall-planted crop usually has a higher yield and allows the opportunity to plant a second crop in areas where it can be harvested sufficiently early in the season. After the crop germinates in the fall, low, above-freezing temperatures induce cold-acclimation which makes fall-sown genotypes better able to withstand freezing temperatures during winter.

In addition to cold-acclimation, low temperatures also stimulate vernalization. This ensures that when temperature and day-length requirements are met in the spring (Zadoks growth stage 30; Zadoks et al., 1974), the plant will enter a reproductive phase and flower. Once the plant enters a reproductive phase, the mechanisms whereby cold-acclimation is induced are suspended (Limin and Fowler, 2006; Mahfoozi et al., 2001) and the plant reverts to approximately the freezing tolerance of a non-acclimated plant. Because the plant has lost most of its freezing tolerance and can no longer cold-acclimate, an unexpected freeze can cause considerable damage to the plant, particularly during Zadoks growth stages 35–47 when the developing head is in the boot.

Information on the extent of damage due to spring-freezes is somewhat anecdotal and varies widely depending on weather conditions and stage of reproductive development. Losses from 30% to as high as 90% have been reported (Al-Issawi et al., 2012; Frederiks et al., 2015; Fuller et al., 2007; Thakur et al., 2010). In contrast, yields in Kansas (Paulson and Heyne, 1983) and Oklahoma (Chatters and Schlehuber, 1953), were reportedly higher in years when late spring freezes occurred. However, it is not clear how yields were impacted in specific areas of fields where freeze damage was originally observed.

With the exception of Reinheimer et al. (2004) who made comparisons in field observations between barley plants at the same growth stage, most differences were observed between cultivars at different growth stages. Because early maturing cultivars consistently suffered more damage than those maturing later, differences

* Corresponding author. Fax: +1 9195152305.
E-mail address: dpl@ncsu.edu (D.P. Livingston III).

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Table 1
Seed number per spike and whole plant survival in ten wheat genotypes exposed to freezing temperatures while in mid-boot growth stage (Zadoks 45–47).

	Reproductive Seed/spike		Whole plant[3] Survival		Vegetative % Survival	
Genotype	Super-cooled[1]	not frozen[2]	(%)	Maturity[4]	NA[6]	CA[7]
Progeny 870	21 a[5]	28	100a[5]	late	8.3bc	84a
Progeny 185	20 a	26	100a	late	21abc	75ab
AG South 2056	19 ab	27	77.5b	late	21abc	58abc
Oakes	16 abc	23	90.0ab	med	0.0c	56abcd
AgriMaxx415	12 bcd	26	90.0ab	late	41ab	49abcd
USG 3251	11 cd	21	77.5b	late	46a	73ab
DynaGro Yorktown	10 cd	25	92.5ab	med	28abc	19d
Merl	6.6 d	23	72.5b	late	54a	66abc
SY Harrison	6.6 d	21	87.5ab	late	38ab	38bcd
AG South 2038	4.4 d	25	100a	late	22abc	30cd
LSD ($p = 0.05$)	7.9	NS	Chi square 29.3		37	39

[1] Mean of three replications in each of three years with 10 plants per rep ($n = 90$). These plants were exposed to freezing conditions but according to infra red thermography they did not freeze at −6 °C.
[2] Unfrozen control. Mean of four replications with 10 plants per rep ($n = 40$).
[3] Chi square analysis, $n = 40$ plants. These plants were exposed to freezing conditions and according to infra-red thermography the ones which died had frozen.
[4] Maturity measured separately under field conditions.
[5] Numbers with the same letter within the column are not significantly different from each other according to Tukeys HSD at $p = 0.05$.
[6] Non acclimated, two week old plants, frozen at −8 °C.
[7] Cold acclimated for three weeks, eight week-old plants, frozen at −18 °C.

between cultivars were considered to be due to differences in growth stage (Fredericks et al., 2015; Shroyer et al., 1995) This led to the conclusion that "little or no difference exists in susceptibility of wheat varieties at the same growth stage and therefore little opportunity to increase freezing resistance in improved varieties" (Shroyer et al., 1995).

However, Reinheimer et al. (2004) identified barley genotypes with low floret sterility after a spring freeze and reported QTL associated with spring freeze tolerance. Fuller et al. (2007) reported differences in freeze damage between two wheat cultivars using electrical conductivity measurements. In addition, differences between wheat and barley with regard to the tolerance of heads to freezing temperatures have been reported (Livingston and Swinbank, 1947; Suneson, 1937; Waldron, 1932).

It is generally accepted that differences between genotypes in spring freeze tolerance are a result of differences in maturity with early cultivars being more susceptible than later ones (Fredricks et al., 2015). In fact, some researchers have recommended planting later maturing cultivars as the best means to avoid damage caused by spring freezes (Singles and Marcellos, 1981; Livingston and Swinbank, 1947). However, to our knowledge a test under controlled conditions that would enable freezing a selection of genotypes as they all reach the same growth stage has not been developed.

The purpose of this study was to devise a procedure to evaluate the ability of winter wheat germplasm to withstand a spring freezing event while in the boot stage prior to emergence from the flag leaf. In addition we wanted to determine if spring-freeze tolerance could be predicted by measuring the freezing tolerance of genotypes in the vegetative state as cold-acclimated plants and/or as non-acclimated seedlings.

2. Materials and methods

2.1. Plant materials

Ninety cultivars and germplasm lines submitted to the North Carolina Official Variety Test (NC-OVT) in 2013 (Supplemental Table 1) were subjected to a spring-freeze simulation under controlled conditions. They were planted in Fafard #2 potting mix (Sungro Horticultural Distribution, Agawam, MA) in 2.5 cm × 12 cm cone-tainers (Hummert, Int., Earth City, MO) suspended in racks containing 100 plants in a 10 by 10 grid (Fig. 1A). Seeds were germinated in a growth chamber at 13 °C for 10 days. Under these conditions plants had emerged from the soil and had a single leaf. Racks containing plants were moved to a chamber at 3 °C for 8 weeks to induce vernalization and then transferred to a greenhouse at 20–25 °C under 12-h supplemental light until mid boot stage (Z45–Z47). Plants were watered daily and fertilized weekly with a dilute solution of Miracle Grow fertilizer (Scotts Co., Marysville, OH). Under these conditions each plant produced a single dominant tiller with a well-developed head. Occasional immature secondary and tertiary tillers were kept until after the plant was frozen. Secondary tillers produced after freezing were removed prior to harvesting the head of the primary tiller.

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By growing plants individually in cone-tainers in staggered plantings (one to two weeks apart) it was possible to select individual plants of later maturing genotypes that were the same growth stage as earlier genotypes. All individual plants were therefore frozen at mid-boot, (Z45–47). This is the growth stage when most wheat cultivars in North Carolina are exposed to unexpected spring freezes. (Fig. 1C). This preliminary experiment was repeated three times; each experiment was considered a replication for a total of three replications.

The three hardiest, the three least hardy and four intermediate lines were selected from the preliminary test for a smaller, more detailed experiment, and to confirm results from the larger experiment. This experiment was conducted in three separate years with three replications in each year under the same conditions described above. Each replication contained 10 plants for a total of 90 plants of each genotype.

2.2. Infrared thermography

In year 3 (2015), freezing of two representative cultivars was monitored by infra-red thermography using a FLIR T620 video camera (FLIR Systems, Wilsonville, OR) with a 45° lens. The camera lens was inserted through a hole in the door of the freezer and monitored the freezing of multiple copies of AG South 2056 (hardy) and Merl (non hardy) (see Table 1) from 0 °C to −6 °C and until thawed. The camera was connected to a computer by USB cable and the freeze test was recorded using Research IR software (FLIR Systems,