**Frost tolerance of faba bean cultivars (*Vicia faba* L.) in
central Alberta**

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Frost tolerance of faba bean cultivars (*Vicia faba* L) in central Alberta

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Faba bean is a crop at high risk for late season frost injury, which results in reduced yield and marketability. After frost exposure, tannin-containing cultivars showed lower proportions of frost damaged seeds when compared to zero-tannin cultivars. Tannin-containing cultivars may mitigate the risk of commercial downgrading due to frost damage.

Key words: Faba (bean), frost, tannin, *Vicia faba* L, pulse

Introduction

Faba bean (*Vicia faba* L) is an annual, cool season pulse crop that is well adapted to the irrigated areas of southern Alberta and regions of the black soil zone (Park et al. 1999). However, in years with early fall frost, faba bean may suffer severe frost damage, resulting in lower yield and poor seed quality (Rowland 1978). During severe and/or prolonged freezing events, blackening or staining can occur to grain that is still filling, negatively affecting

subsequent marketability, especially whole faba grain intended for the human consumption market.

Currently in Alberta, white flowered, zero-tannin faba beans are predominantly used because of higher protein and energy digestibility in monogastric animals (Katell et al 2010). However, these crops have shown poor emergence from cold soil when compared to normal tannin isolines (Kantar 1994 cited in Stoddard et al. 2006). Conversely, all but one winter hardy faba bean cultivar (Gladice) commonly used in Europe have coloured flowers (Link et al, 2010), which contain on average between 47 to 58 times more tannins in the seed coat (Bos and Jetten, 1989). Furthermore, in a cold tolerance screening experiment of faba bean, white flower accessions were found to be the most susceptible, whereas cold tolerant accessions of faba bean and wild relatives had high pigmentation (Inci and Toker, 2011). One Canadian study also reported a zero-tannin cultivar (Snowbird) that presented more visible effects of frost damage compared to normal tannin cultivars, including a late maturity cultivar that had been seeded 13 days later (Braaten 2012).

One possible mechanism explaining the role of tannins in frost protection is their activity as a supercooling promoting agent or anti-ice nucleating agent (anti-INA), which prevents intracellular ice formation and subsequent damage (Koyama et al., 2014).

Although several possible cryoprotectant substances and mechanisms have been previously investigated, the effects of tannin concentration and its correlation to frost damage has not been rigorously studied (Link et al, 2010). In this paper, we discuss observations of a naturally occurring frost event, recorded at three experimental sites that showed a reduction in frost damage among normal tannin compared to zero-tannin cultivars, even after controlling for maturity.

Materials and methods

To determine the extent of late season frost damage to maturing seeds of spring sown faba bean, ten cultivars from two distinct trials were assessed after a frost event that occurred at three test sites on September 10, 2014. Both trials were established at three affected sites in Barrhead (Latitude 54.1181°, Longitude -114.2711°), Morinville (Lat. 53.7979°, Long. -113.4731°) and Vegreville (Lat. 53.4242°, Long. -112.2189°).

In Experiment 1, the adaptability of non-registered European cultivars was being evaluated, while Experiment 2 evaluated the agronomic performance of newly registered cultivars throughout Alberta. The cultivars in each experiment included two types of spring faba bean: normal tannin (T+) so identified by the presence of pigmentation on the flowers and seed coat and zero-tannin (T-), which lacks such pigmentation (Table 1). Both experiments were complete randomized block designs with four replicates at each site.

Plots at all locations were seeded with a custom manufactured Fabro drill, equipped with double shoot atom-jet openers at 20 cm spacing and a seeding rate of 45 plants m⁻². All cultivars received granular rhizobia inoculant and were fertilized with the equivalent to 25 kg P₂O₅ ha⁻¹ at seeding. Standard weed control and other crop maintenance activities were used.

Hourly minimum ambient temperature data of the forty-eight-hour period during which the freezing event occurred was obtained from the nearest meteorological station to each experimental site (data not shown).

Frost damage was assessed by visually determining the number of damaged seeds (blackened) present in a 100 seed sample obtained randomly with a sampling paddle from the harvested grain at each plot. Days to maturity (DTM) were determined prior to desiccation by visually estimating the date when 90% of pods in a plot turned black.

Grain from whole plots was harvested with a plot combine, then dried and weighed to determine yield. Harvested grain samples were sub-sampled to determine the weight of one thousand seeds (TSW).

Analysis of variance for frost damage was conducted assuming a Binomial distribution and a logit link function. Such analysis was performed using the GLIMMIX procedure in SAS 9.3. Least square means were obtained through the ilink option. Location, cultivar and location*cultivar were considered fixed effects and block nested within location was considered random. Cultivar*block (location) was also included in the random statement as suggested for generalized binomial linear predictors (Stroup 2015).

DTM was analyzed with the same procedures using a Gamma distribution with a log link function as suggested in Stroup et al. (2015).

Yield and TSW analyses were performed with the same procedures using a Normal distribution. All pertinent comparisons were performed with linear contrast using the estimate option and Scheffe adjustment for multiplicity.

Results

Frost damage and location

All cultivars were subjected to sub-freezing temperatures and exhibited varying proportions of seed damage (Table 1). Mean frost damage across all tests sites ranged from 7.7% to 48.2% in Experiment 1 and from 8.6% to 54% in Experiment 2. The effect of location was significant only in Experiment 2. No significant interactions between cultivar and location were found (Table 1).

Frost damage and cultivar

Cultivars in both experiments showed significant differences in frost damage (Table 1). Contrast analysis showed that tannin-containing cultivars had a significantly lower proportion of frost damage. The group comparison between zero-tannin and normal tannin cultivars showed a difference in frost damage proportions that favoured the tannin-containing cultivars in both experiments (Table 1). Zero-tannin cultivars had 2.2 times more frost damaged seeds than normal tannin cultivars in Experiment 1 and 4.5 times more in Experiment 2.

The association between the presence of tannins and frost damage was statistically significant even after accounting for crop maturity. Overall, cultivars with a relatively late maturity showed a higher percentage of frost damaged seeds; however, normal tannin cultivars compared with zero-tannin cultivars with the same maturity presented a statistically significant difference in frost damage (Table 1). For instance, frost damage for Imposa (T-) more than tripled the pigmented Obelisk (T+) (48.2% vs 15.0% respectively) despite having the same maturity (Table 1). This trend was also reported for early maturing cultivars Snowbird (T-) and Rodeo (T+), where the frost damage for zero-tannin Snowbird more than doubled the tannin-containing Rodeo (19.6% vs 7.7% respectively). A similar trend also occurred in Exp. 2 where Snowbird (T-) and Tabasco (T-) more than doubled the frost damage of Malik (T+) (30.6% and 44.6% vs 7.8%).

Although the inverse relationship between frost damage and tannin content held true when adjusted for maturity, there was still a significant difference in overall frost damage between early and late maturing cultivars among both tannin related groups. Early and intermediate maturing cultivars resulted with less damage seeds than late maturing cultivars of the same tannin profile (Table 1).

The exception to low frost damage and early maturity was Snowbird (T-), which despite being the earliest maturing cultivar had more frost damage than other early and intermediate cultivars in both experiments, as well as the late maturing Obelisk (T+) in Experiment 1 (Table 1).

Frost damage and yield

The yields affected by frost were comparable to mean yields of the same cultivars grown at the same sites during years with no frost events (Table 1). However, there was a negative correlation between frost damage and the yield of affected cultivars (Table 2) and this relationship was much stronger among the zero-tannin cultivars ($r = -0.94$, $P = 0.0165$). Tannin-containing cultivars outperformed zero-tannin cultivars in terms of seed yield in Experiment 1 (Table 1), but had comparable yields in Experiment 2 ($P = 0.056$).

Frost damage and thousand seed weight

Thousand seed weights (TSW) in both experiments were significantly different for location and cultivar, and their interaction was significant only in Experiment 1. In the year of the frost event, there was a significant drop in TSW compared to non-frost years (Table 1). A paired weight comparison (paired T test) of 100 undamaged and 100 frost damaged seeds showed a significant difference in weight ($P < 0.0001$). The reduction in weight for damaged seeds averaged 11.8 g per hundred seeds, or the equivalent of a 21.4% reduction in TSW.

Discussion

Frost damage

The effect of location on frost damage was significant only in Experiment 2 ($P < 0.0001$), but the difference was consistent across sites for both experiments (data not shown). Damage

was most severe at Morinville, followed by Vegreville and Barrhead. Mean frost damage for location appears to be in step with accumulated precipitation (Experiment 1: $r=0.924$, $P=0.094$; Experiment 2: $r=0.998$, $P=0.001$), conceivably because of its influence on development, maturity and drying rate.

Cultivars showed significant differences (Table 1) in frost damage in both experiments and behaved similarly between locations (Table 1). Differences in frost damage exposed a strong association with the presence of tannins even after accounting for crop maturity. The presence of tannins may explain the large discrepancy in frost damage differences when maturities were comparable. This indicates that early maturity alone may not be a sufficient factor in escaping the deleterious effects of late season frost.

The damage-tannin association agrees with previous findings where white flower genotypes have shown weakness to low temperatures, while tannin-containing genotypes and wild relatives are more tolerant (Inci and Toker, 2011). Many tannin related polyphenols found in faba bean (Baginsky et al, 2013) have also been found to have anti-ice nucleating (supercooling promoting) activity in freezing droplet assays of solutions containing ice nucleators (Kuwabara et al, 2013). As faba bean cultivars with different tannin content are affected differently by frost exposure, the cultivar choice becomes an important tool to manage frost damage avoidance.

Frost damage and yield

Average yield from frost affected cultivars in Experiment 1 were comparable to those from years with no frost. This might suggest that yield would not be reduced by the late season frost, which agrees with previous findings (Rowland et al. 1978) that yields would stabilize when 30% of the plants had the lowest pods turned black. However, the yields from frost damaged

cultivars had a strong, negative correlation with the level of frost damage ($r=-0.85$, $P=0.0005$), especially among those devoid of tannins ($r=-0.93$, $P=0.015$).

Frost damage and thousand seed weight

The decreases in weight of frost damaged seeds when compared to undamaged seeds are in agreement with a three-year study with six faba bean cultivars (Rowland et al. 1978) where it was found that a killing frost led to a reduction in TSW. In addition to this, seed weights from Experiment 1 were significantly decreased compared to previous years when no frost had occurred. Seed weight differences may be attributed to ice formation and injury that causes cell dehydration and turgor losses after thawing (Link et al, 2010). Weight losses in damaged seeds combined with the proportion of frost damage conceivably account for the correlation between frost damage and lower yields.

Whether other morphologic and/or physiologic characteristics play a role in imparting faba bean grain with frost tolerance should be the subject of more study. Further examination of tannin concentration would supplement our understanding of the mechanisms that confer the cultivars with variant degrees of frost tolerance and/or susceptibility.

Conclusions

On average, tannin-containing faba bean cultivars appear to be significantly more tolerant to frost than zero-tannin cultivars, resulting in higher yields and improved seed quality. This relationship appears to hold true even when controlling for maturity. These results have significant implications for further research into the possible cryoprotectant role of tannins and their association with other physiological and/or genetic components in faba bean genotypes. The selection of tannin-containing cultivars that are early maturing would greatly assist in

reducing the risk of frost damage and commercial downgrading, and improve the subsequent marketability into both the human and livestock markets.

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References

- Baginsky, C. Peña-Neira, A. Cáceres, A. Hernández, T. Estrella, I. Morales, H. Petuzé, R. 2013, Phenolic compound composition in immature seeds of faba bean (*Vicia faba* L.) varieties cultivated in Chile. *Journal of Food Composition and Analysis* 31(1):1-6.
- Bos, K. D. Jetten, J. Recent advances of research in antinutritional factors in legume seeds. Proceedings of the First International Workshop on "Antinutritional Factors (ANF) in Legume Seeds", November 23-25, 1988, Wageningen, Netherlands; Wageningen Centre for Agricultural Publishing and Documentation (PUDOC), 1989, 168-171 (Conference paper)
- Braaten, C. 2012. Demonstration of faba beans. Agricultural demonstration of practices and Technologies. Available at:

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<http://www.conservationlearningcentre.com/Portals/0/Projects/2012/Faba%20Bean%20demo%2020110376%20sb.pdf> (accessed on 11.09.16).

- Crépon, K. Marget, P. Peyronnet, C. Crrouée, B. Arese, P. Duc, G. 2010. Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Research* 115(3): 329-339.
- Inci, N.E. Toker, C. 2011. Screening and selection of faba beans (*Vicia faba* L.) for cold tolerance and comparison to wild relatives. *Genetic Resource and Crop Evolution* 58: 1169-1175.
- Koyama, T. Inada, T. Kuwabara, C. Arakawa, K. Fujikawa, S. 2014. Anti-ice nucleating activity of polyphenol compounds against silver iodide. *Cryobiology* 69(2):223-228.
- Kuwabara, C. Wang, D. Endoh, K. Fukushi, Y. Arakawa, K. Fujikawa, S. 2013. Analysis of supercooling activity of tannin-related polyphenols. *Cryobiology*. Aug; 67(1):40-49.
- Link, W. Balko, C. Stoddard, F.L. 2010. Winter hardiness in faba bean: Physiology and breeding. *Field Crops Research* 115: 287-296.
- Park, B. Lopetinsky, K. Bjorklund, R. Buss, T. Eppich, S. Lafflame, P. Miller, N. Olson, M. Piquette, K. 1999. *Pulse Crops in Alberta*. Alberta Agriculture, Food and Rural Development, Publishing Branch.
- Rowland, G.G. 1978. Effects of planting and swathing dates on yield, quality and other characters of faba beans (*Vicia faba*) in central Saskatchewan. *Canadian Journal of Plant Science* 58: 1-6.
- Stoddard, L.F. Balko, C. Erkin, W. Khan, H.R. Link, W. Sarker, A. 2006. Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes. *Euphytica* 147: 167-186.

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Stroup, W. W. 2015. Rethinking the analysis of non-normal data in plant and soil science.
Agronomy Journal 107:811-827.

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Table 1. Least squares means and P values from analysis of variance for the effects (fixed) of location, cultivar, and cultivar by location on faba bean frost damage, days to maturity (DTM), yield and thousand seed weight (TSW). Replicates within location were considered random effects. Significant effects ($P < 0.05$) are bolded.

Cultivar	Experiment 1				Cultivar	Experiment 2			
	Frost damage ^a	Days to maturity ^b	Yield	TSW		Frost damage	Days to maturity	Yield	TSW
	%		kg ha ⁻¹	g 1000 seeds ⁻¹		%		kg ha ⁻¹	g 1000 seeds ⁻¹
Alexandria (T+)	9.9	123	9772	541		-	-	-	-
Babylon (T+)	9.7	123	9734	478		-	-	-	-
Imposa (T-)	48.2	124	6873	543		-	-	-	-
Obelisk (T+)	15.0	124	9597	488	Malik (T+)	7.8	119	8043	631
Pyramid (T+)	10.6	123	10491	496	Snowbird (T-)	30.6	119	8526	471
Rodeo (T+)	7.7	122	9326	579	Snowdrop (T-)	54.0	123	7126	383
Snowbird (T-)	19.6	121	8809	452	Tabasco (T-)	44.5	119	7080	320
SEM	3.78	0.47	300.7	10.5		4.74	0.69	285.8	13.4
Location	0.6792	<0.0001	0.0032	0.005		<0.0001	<0.0001	0.0002	0.0007
Cultivar	<0.0001	0.0002	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001
Location * Cultivar	0.6534	0.4151	0.0407	0.0348		0.3751	0.007	0.0159	0.1563
T+ vs T-	<0.0001- ^c	0.6662	<0.0001	0.0036		<0.0001-	0.085-	0.056	<0.0001
Late (T+) vs late (T-)	0.0002-	0.8699	<0.0001	<0.0001-		-	-	-	-
Early (T+) vs early (T-)	0.0133-	0.7845	0.0912	<0.0001		0.0010-	0.9403	0.1027	<0.0001
Early vs late	0.0008-	<0.0001-	0.0003	0.9791-		-	-	-	-
Early vs intermediate	0.3863	0.0006-	<0.0001-	0.1220		-	-	-	-
Late vs intermediate	<0.0001	0.0175	<0.0001-	0.1155		-	-	-	-
No frost vs frost exposed ^d			0.0575-	<0.0001+					

^a: Frost damage and days to maturity were assume to follow binomial and gamma distribution respectively, yield and TSW were fit through Normal distributions.

^b: Analysis of variance for days to maturity data pertains to visual rating from eight site-years including all sites in 2014 (frost year).

^c: Contrasts signs indicate direction of contrast estimate. T+ indicated tannin-containing and T- indicates zero-tannin.

^d: Mean site-year contrast of yield and TSW (shaded area) are from a long-term adaptability study (eight site-years) study that includes 2014 and same cultivars.

Table 2. Correlation Coefficients of faba bean cultivar means (n = 11)

	Yield	DTM	TSW
Damage	-0.84918	-0.01143	-0.63016
<i>P-value</i>	0.0009	0.9734	0.0377
Yield		0.30133	0.33363
<i>P-value</i>		0.3679	0.3160
DTM			0.10421
<i>P-value</i>			0.7604

Figure 1 . Distribution of frost damage in Experiment 1 and Experiment 2 in central Alberta, 2014.

Snowbird and Snowbird_r :are same cultivar in both experiments.

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