Effect of Seeding Date, Environment and Storage on Canola Seed Vigour

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Abstract

Seed vigour has been identified as one of the leading factors limiting stand establishment and yield in western Canada. Field studies at Scott, SK demonstrated that seed derived from Fall- and April-sown canola produced higher plant densities, higher biomass at bolting, and higher seed yield than seed derived from May-sown canola. This study established the impact of seeding date on seed quality and vigour, which in turn affected emergence, seedling vigour and yield. Also, seed vigour slowly declines within one year, primarily from seed derived from the May-sown canola. Currently we are in the process of uncovering which genes and proteins are in common with vigour irrespective of seed source. We will combine our analysis with synchrotron technologies for a much more in-depth understanding of what constitutes "seed vigour" to develop a rapid, simple, and inexpensive method that will identify intrinsic characteristics of superior seed lots, as well as seed lots that lose vigour when stored under adverse conditions. In addition, we have initiated a study to compare hormones and metabolites during cold acclimation and freeze-induced injury/recovery to correlate these changes with winter survival. This research will identify traits that can be used in marker-assisted/molecular breeding programs for winter hardiness and possible genetic engineering studies on abiotic stress tolerance of seeds and plants. To further understand the processes involved in stress tolerance, we utilized gene transfer techniques to produce a PNT canola that over-expresses a novel gene which results in higher yields under stressful conditions. These PNT lines were tested in the field over 3 years across Western Canada in non-stressed, moderately stressed, or severely stressed areas. At each location, several lines flowered and matured 1 to 3 weeks earlier. The faster maturating PNT lines (up to 55% more mature at harvest) had increased yields (up to 32% increase) and enhanced seed quality (up to 87% increase in larger and more mature seed) versus the control. These results, both in controlled laboratory tests and in field trials, have been optimistic for genetic engineering of plants for enhanced stress tolerance without losing agronomical important characteristics.

Introduction

Seed germination and seedling emergence result from a sequence of biological events initiated by water imbibition followed by enzymatic metabolism of storage nutrients. All of those events are regulated by the environment and the quality of the seed. Low soil temperature and lack of available soil moisture in the spring delay and reduce seedling emergence, particularly in small

seeded crops such as canola (*Brassica* spp.). The mean spring soil temperature in the canola production area of western Canada ranges from 5 to 13°C at a 5 cm depth. The optimal temperature for canola seed germination and emergence is 15 to 20°C (Kondra et al. 1983). Both suboptimal soil temperature and lack of soil moisture delay and reduce germination percentage and seedling emergence (Kondra et al. 1983; Livingston and de Jong 1990; Blackshaw 1991). Kirkland and Johnson (2000) found that dormant Fall-sown canola flowered and matured up to 36 days earlier than mid-May sown canola. Therefore, developing seeds on plants from the Fall-sown canola were not exposed to the hot and dry condition plants experienced from the mid-May planting date. At the early stages of development, the developing seed is heat sensitive and exposure to temperatures above 28°C inhibits maturation resulting in a yield reduction (Brandt and McGregor 1977; Angadi et al. 1999). Seed derived from Fall-sown canola compared to May-sown canola, had a higher thousand kernel weight suggesting there was less stress during grain filling. The objective of this study was to compare canola seed vigour of seed derived from either Fall-, April-, May-seeding dates. Studies on germination and emergence were conducted in a controlled environment cabinet. In addition, three successive field experiments at Scott, SK sown both in the Fall and early spring (April) were conducted to evaluate plant emergence, biomass at bolting and seed yield.

Materials and Methods

Seed Source

The canola seed source of *Brassica napus* cv. Quest used in this study was harvested from seeding date studies conducted at Scott, Saskatchewan. Seed date treatments were Fall (October 31, 1997; October 28 in 1998), early-spring (April 16, 1998; April 15, 1999) and mid-May (May 18, 1998; May 20, 1999). Methods for plot establishment and maintenance were similar to those outlined in Kirkland and Johnson (2000). Seed source was evaluated for seed size, seed maturity, seed germination and seedling emergence.

Controlled Environment Studies

For the various seed lots proportional seed sizes were calculated using a series of four sieves ranging from 1.7 to 1.0 mm in diameter. The experimental design was completely random. Seed maturity was determined visually using a 10X binocular microscope from four completely random-sub-samples (500 seeds) collected from each seed lot. The following four categories were selected: (1) black-dark purple and smooth - mature; (2) black-dark purple and ridged - mature; (3) orange-brown to yellow-green in colour, ridged and non-uniform - immature; (4) damaged - cracked seed.

Both germination and emergence rates were used to determine seed vigour. Germination tests were conducted with primed and non-primed canola seed lots derived from Fall-, April-, and May-sown canola at either a constant 22±C or 8±C in the absence of light in a randomized complete block design. Seedling emergence was evaluated for primed and non-primed seed lots derived from Fall-, April-, and May-sown canola. Twenty seeds of each treatment were seeded at a uniform depth of 2.5 cm in a sandy loam (Entic Haploboroll) soil collected from a field located at the Kernen Crop Research Farm of the University of Saskatchewan, Saskatchewan, Canada.

The study was conducted in a controlled environment chamber maintained at 60% r.h., 14 h photoperiod with fluorescent lights (750 νnmol m^{-2} s^{-1} at pot height) and 8±C light/5±C dark temperature regime. The pots were set up within the chamber in a randomized complete block design with six replications per seed lot.

Field Studies

Three successive field studies were conducted at Scott, SK, Canada in 1999-2000 using the same seed sources as used for the controlled environment studies. Seeding dates for the three experiments were November 16, 1999 (Fall 1999), May 1, 2000 (Spring 2000) and October 31, 2000 (Fall 2000). Studies were conducted on a Dark Brown Chernozemic (Typic Boroll) loam soil containing 31% sand, 42% silt, and 27% clay with 4% organic matter content and a pH of 6.0. The six seed sources were seeded in a randomized complete block design at a rate of 150 seeds m^{-2}. Treatments were replicated eight times for the November 1999, however the May 2000 experiments were replicated four times for the October 2000 experiment due to space limitations. Plot size was 2 x 5 meters. (For a complete description see Gusta et al. (2004) Can J Plant Sci, April issue)

Statistical Analysis

Lab data was analyzed according to a complete random experimental design using the GLM procedure in SAS (2001, SAS Procedures guide, release no. 8.02, Cary, N.C.). Field data was analyzed according to experimental design using the PROC MIXED procedure in SAS (Little et al. 1996).

Results and Discussion

Controlled Environmental Studies

The effect of seeding date on seed size is shown in Figure 1. In both years, over 80% of the seeds from the Fall-sown canola were greater than 1.70 mm in size. A slightly smaller percentage of seed from the April seeding date fell into this category. Only 12.5% of the 1998 seed from the May seeding date were larger than 1.7 mm, whereas 44% of the 1999 seed fell into this category. The majority of the seed from May-sown canola from both years were in the 1.40 to 1.70 mm range for seed size. This result confirms Kirkland and Johnson (2000) findings that the canola seed weight was greater for Fall-sown canola compared to mid-May-sown canola. The distribution of mature and immature seed from the three seeding dates is shown in Figure 2. On average for both years, seed from the Fall- and April-sown canola had the highest percentage of mature seed (59 to 70% of the total seeds) compared to only 16 to 24% of the total seed from the May-sown canola. We have demonstrated that mature smooth black to dark purple seed produce more vigorous seedlings compared to immature orange-brown or yellow-green coloured seeds (Thompson and Gusta, unpublished data).

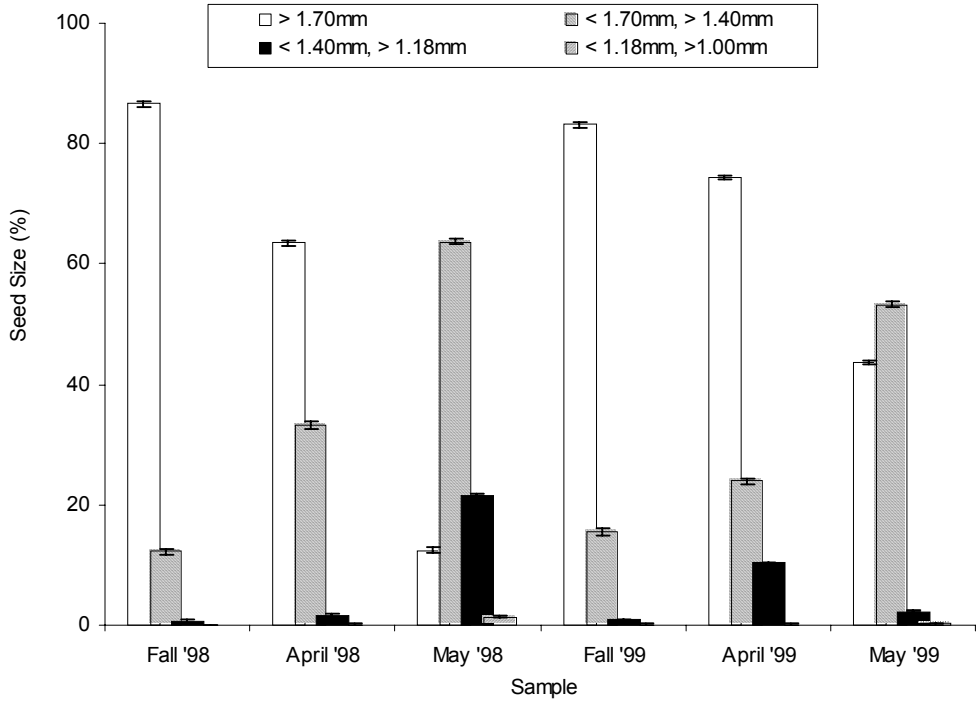

Figure 1. Comparative size (%) of canola seed from Fall, April and May seeding dates harvested in 1998 and 1999. SE = 0.51, LSD = 0.67

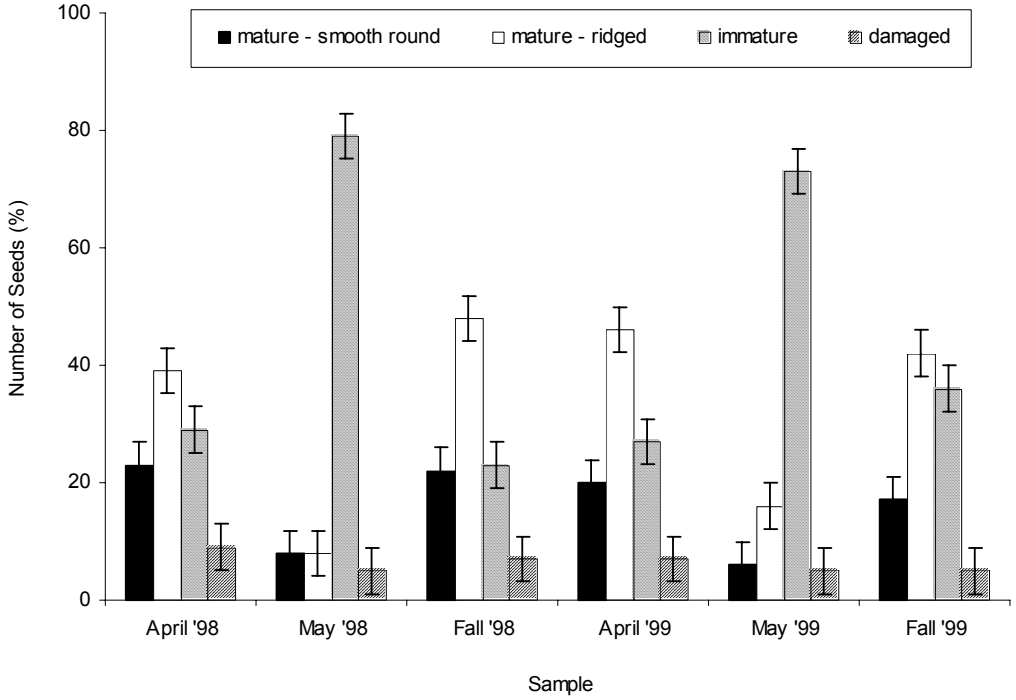

Figure 2. Percentage (%) of mature, immature and damaged seed from Fall, April and May seeding dates harvested in 1998 and 1999. SE = 3.9, LSD = 13.4

When germination tests were conducted at 22±C, there was not much significant difference in either germination rate or final percent germination for seed derived from 1998 and 1999 (data not shown). There were significant differences when the same germination tests were conducted at 8±C (data not shown). Seed derived from the 1998 May seeding date germinated at a slower rate such that the time for 50% germination (T_{50}) was 4.3 days versus only 3.25 days for the Fall and April seeding dates harvested in 1998. In addition, the final germination count for seed derived from 1998 May-sown canola was only 85% compared to 97% for the other two seed lots (data not shown). Similar comparisons were found for the 1999 seed lots at 8°C. To evaluate seed vigour, we compared seedling emergence at 8±C for both our primed and non-primed seed lots (Fig. 3). While there was no significant difference in either the rate or final number of seedlings for the Fall- and April-sown canola seed lots, there was a significant difference for both of these parameters for seedlings derived from May-sown canola for both the 1998 and 1999 seed lots (Fig. 3A). Seed size was expected to account for the difference in seedling vigour however, differences in rate and final emergence percentages disappeared when the seed lots were primed with ABA (Fig 3B). Seed priming improves germination in many plant species (Bradford et al. 1986), however its mechanism remains unknown.

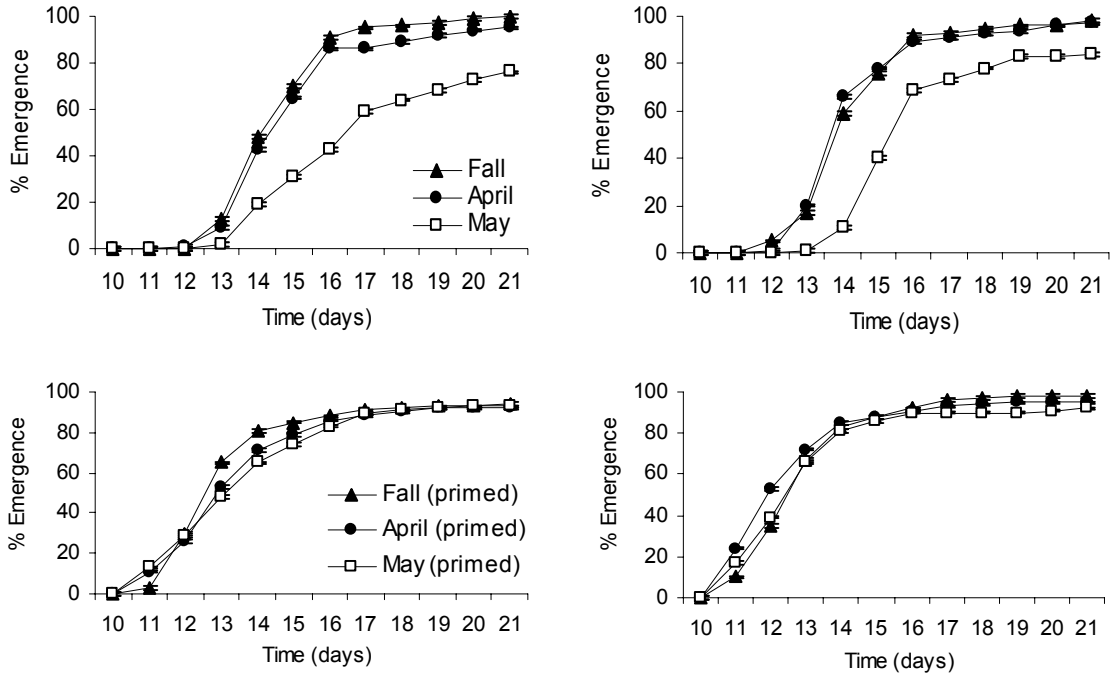

Figure 3. Comparative canola seedling emergence (%) at 8±C of non-primed seed (A) and primed seed (B) from Fall, April and May seeding dates harvested in 1998 and 1999. SE = 0.75, LSD = 2.1

Field Studies

Environmental conditions for the field studies were varied (Table 1 and 2). Results from the three site-years of field studies revealed seed lots derived from Fall-sown canola had the highest seedling emergence compared to seed lots derived from April- and May-sown canola when seeded in the Fall of 1999 and 2000 (Table 3). Field emergence patterns were similar to the results found in the controlled environment emergence study at 8°C, particularly seed derived from Fall- and May-seeded canola (Fig. 3A). Therefore, the quality of the seed has a definite effect on either over-wintering and/or spring emergence. There was also a year effect; seed derived from the 1998 Fall harvest had higher emergence than seed derived from the 1997 Fall harvest for all site-years. Seed derived from 1998 April-sown seed had lower plant emergence than April-sown seed from the 1999-growing season. The growing season in 1998 was hotter and drier than the growing season of 1999, thus the developing seeds may have experienced more stress in 1998 that may have resulted in less seed vigour and accelerated aging (Ken Kirkland, personal communication). The seed source had less of an effect on plant emergence for the experiment seeded in the spring of 2000. Therefore, differences in seed vigour may be more detectable under stressful environmental conditions such as those encountered from fall seeding.

Table 1. Mean monthly air temperatures for the 1998-2001 growing season and long-term average (1911-1990) at Scott, Saskatchewan

	April	May	June	July	August	Mean
	Temperature (±C)					
1998	6.8	12.9	14.1	18.2	19.3	14.3
1999	5.1	9.4	13.1	15.0	16.6	11.8
2000	2.1	9.4	13.5	17.4	15.6	11.9
2001	3.9	11.5	13.8	17.9	18.9	13.2
Long-Term Average 1911-1990	3.0	10.0	14.0	18.0	16.0	12.2

Table 2. Mean monthly precipitation for the 1998-2001 growing season and long-term average (1911-1990) at Scott, Saskatchewan

	April	May	June	July	August	Mean
	Precipitation (mm)					
1998	12.1	5.4	63.5	11.0	14.0	106.0
1999	51.7	66.4	42.8	81.0	48.0	289.9
2000	39.1	23.6	38.6	76.4	60.2	237.9
2001	16.5	36.2	48.8	39.7	2.8	144.0
Long-Term Average 1911-1990	23.0	36.0	61.0	62.0	46.0	228.0

Table 3. The effect of seed source on canola responses at Scott, Saskatchewan, 1999-00

	Plant emergence (plants m^{-2})				Biomass at bolting (kg ha^{-1})				Seed Yield (kg ha^{-1})			
	Site year[z]				Site year				Site year			
Seed Source[y]	*Fall*[x] *99*	*Spring*[w] *00*	*Fall*[v] *00*	*Mean*	*Fall 99*	*Spring 00*	*Fall 00*	*Mean*	*Fall 99*	*Spring 00*	*Fall 00*	*Mean*
Fall 97	57	40	14	37	59.1	258.4	--	158.8	3068	2325	1333	2242
April 98	40	35	9	28	55.4	242.0	--	148.7	2983	2386	1043	2137
May 98	12	30	4	15	13.6	59.6	--	36.6	2075	1378	328	1260
Fall 98	80	58	18	52	61.4	268.3	--	164.9	3085	2772	1358	2405
April 99	72	45	13	43	60.5	264.4	--	162.5	3078	2782	1143	2334
May 99	58	51	12	40	45.8	200.4	--	123.1	3096	2193	893	2061
$LSD_{0.05}$	13.8	26.1	6.0	12.7	14.1	61.8		31.2	219.3	477.0	311.7	227.4
Contrasts	*Fall 99*	*Spring 00*	*Fall 00*	*All*	*Fall 99*	*Spring 00*	*Fall 00*	*All*	*Fall 99*	*Spring 00*	*Fall 00*	*All*
Fall vs April	0.01	0.32	0.02	0.05	0.64	0.64	--	0.58	0.55	0.83	0.03	0.28
Fall vs May	<0.01	0.34	<0.01	<0.01	<0.01	<0.01	--	<0.01	<0.01	<0.01	<0.01	<0.01
April vs May	<0.01	0.96	0.15	0.09	<0.01	<0.01	--	<0.01	<0.01	<0.01	<0.01	<0.01
1998 vs 1999 seed				<0.01				<0.01				<0.01

[z] Year and time of seeding.
[y] Year seed lot was sown.
[x] Experiment sown in the Fall of 1999 and harvested in 2000.
[w] Experiment sown in the Spring of 2000 and harvested in 2000.
[v] Experiment sown in the Fall of 2000 and harvested in 2001.

In the two site-years where biomass data could be obtained, seed derived from Fall- and April-sown canola produced the greatest biomass of all the seed lots (Table 3). Although seed source did not have a significant effect on seedling density in the spring-seeded experiment, there was a detectable difference in biomass, indicating differences in seed vigour. Again, the effect of ageing on seed quality was also evident; seed derived from the 1998 seed lot produced less biomass than seed from the 1999 seed lot. Aging of seed cannot totally account for the differences in biomass from the various seed lots. There was no difference in biomass between

the seed lots derived from Fall- and April-seeded canola in both 1998 and 1999. Consequently, it appears aging has a larger effect on seed derived from the May-sown canola. There was a significant difference in biomass in the spring-seeded experiment in 2000 between the seedlings derived from 1998 April- and May-sown canola (242 vs. 60 kg ha^{-1}). This difference could not be accounted for by the difference in plant counts as they were quite similar. This large of a difference in biomass was again observed in the 1999 Fall-sown trial, although biomass accumulation was much less. Differences in environmental constraints during embryogenesis could be a significant factor not previously considered. The effects of seed size and percentage of mature seed (Fig. 1 and 2) likely contribute to the biomass response in the field experiments.

Canola biomass produced in the spring seeded experiment (spring 2000) was nearly four times as great as the 1999 Fall-seeded experiment. This has been observed in previous experiments and may be partially explained by data suggesting that Fall-sown canola requires about 88 fewer growing degree-days to bolt after emergence compared to May-sown canola (Johnson, unpublished data). Therefore, it is bolting much earlier in its physiological development. Even though biomass at bolting was much lower for the 1999 Fall-seeded experiment than the 2000 spring-seeded experiment, yields were not sacrificed. These results suggest there may be a residual vernalization requirement that is met by the Fall-seeding of spring *Brassica napus*.

In all field studies, seed derived from 1998 May-sown canola resulted in the lowest seed yields. This may be due in part to the lower stand density obtained from the seed from May-sown canola however the stand density was not significantly lower in the spring 2000 trial. In the Fall 1999 planting trial, seed derived from 1999 May-sown canola produced a similar yield as the seed derived from Fall-and April-sown canola in both 1998 and 1999. In the other planting trials, results were contrary to the Fall 1999 planting trial. Seed derived from 1999 May-sown canola produced 21 to 34% lower yields than seed from 1998 Fall-sown canola (Table 3) indicating that seed derived from May-sown canola may deteriorate faster than seed derived from Fall-sown canola. In the spring 2000 trial, seed derived from the 1999 May-sown canola yielded 22% lower than seed derived from the 1999 April-sown canola and a similar trend occurred in the fall 2000 planting trial ($p=0.10$). This further substantiates that seed derived from May-sown canola was inferior to seed from Fall- or April-sown canola, particularly as the seed aged. In the Fall 2000 trial, seed derived from Fall-sown canola yielded significantly higher than seed from April-sown canola ($p = 0.03$), which may indicate some deterioration in seed derived from April-sown canola due to aging. The overall mean of the field trials indicated that seed derived from either Fall- or April-sown canola out-yielded seed obtained from May-sown canola. Collectively these results also suggest that seed derived from Fall- or April-sown canola does not age as quickly as seed derived from a May seeding date. This study demonstrates the impact of environment during embryogenesis on seed size and seed maturation that affected seed vigour and seed storage life when these seed sources were field tested.

In summary, this study demonstrates the importance of seeding date on the quality of seeds produced. Germination tests in Petri dishes conducted at 22±C did not reveal differences in seed quality. However, significant differences in seed quality were detected when the test was conducted at 8±C. The largest differences in seed quality are detected in the field, especially under stressful conditions. There are several distinct advantages to the producer to use seed derived from Fall- or April-sown canola in comparison to seed derived from May-sown canola.

First, early seeding resulted in plants that grow and reproduce under cooler and lower evapotranspiration rates compared to May planting dates. The reproductive period for Fall and April seeding was considerably longer compared to the later spring planting date. As a result, the seeds were larger, denser, and more mature than seeds derived from the later planting date. There is some evidence that these seeds do not age as fast as the seeds from May planting dates. Differences in seed vigour were not solely due to seed size but due to other unknown intrinsic differences. This is borne out by the fact that ABA primed seed derived from May-sown canola produced the same seed vigour as seed derived from the April-sown canola (Zheng et al. 1996; 1998). Identification of these intrinsic differences would establish what seed vigour is and allow for better tests for prediction of which seed lots perform best.

Conclusion

From this study, general conclusions can be derived to further our understanding of seed quality and what can be done to enhance seed quality. Environment during embryogenesis and seed maturation has a significant effect on seed quality which affects seed vigour and ultimately yield. Unlike optimal conditions, stressful environmental conditions in the field are able to identify differences in seed quality. Seed held from one year to the next ages faster than previously considered, particularly seed that develops under conditions of environmental stress such as drought, frost or high temperatures. The results may have implications for cultivar evaluation trials such as the Co-op trials. Perhaps seed for the entries should be derived from trials grown at the same location, so that true genotypic differences are measured rather than differences in seed quality. This study was conducted with seed produced from a small geographic area using six seed lots from one genotype. Further study is required to establish whether seed grown under different geographic locations in Western Canada have similar responses to seeding date. Since the environment has such a significant effect on seed vigour, there may be certain geographical locations in western Canada that could produce consistently high quality seed. Also, it is not known if all genotypes respond in a similar fashion as the genotype used in this study. Research should be conducted to identify genotypic variability for seedling vigour as well as to identify genes and intrinsic factors associated with seed stability and vigour. This study also raises some very important questions: How does a seed producer manage his crop to produce consistently high quality seed? Could high yielding open pollinated cultivars match the performance of new hybrid canola cultivars varieties by simply paying close attention to seed quality?

Future Directions

To further understand the processes involved in stress tolerance, we utilized gene transfer techniques to produce a PNT canola that expresses a gene (*Rob-5*, Provisional U.S. Patent filed 11/14/02*)* which results in higher yields under stressful conditions such as drought, heat and frost. These PNT lines were tested in the field over 3 years across Western Canada in non-stressed, moderately stressed, or severely stressed areas (data not shown). At each location, several lines flowered and matured 1 to 3 weeks earlier. The faster maturating PNT lines (up to 55% more mature at harvest) had increased yields (up to 32% increase) and enhanced seed quality (up to 87% increase in larger and more mature seed) versus the control. Microarray expression profiling has been completed and preliminary analysis has uncovered interesting novel findings helpful to determine why some of our PNT plants have enhanced stress tolerance

(data not shown). These results, both in controlled laboratory tests and in field trials, have been optimistic for genetic engineering of plants for enhanced stress tolerance without losing agronomical important characteristics.

Fall seeding trials have demonstrated the impact of seeding date on seed vigour which in turn affects seedling vigour, seed quality and yield. Intrinsic factors that constitute seed quality are relatively unknown and the true value of seed quality on seedling vigour and yield is only now being recognized. Currently we are in the process of elucidating by both genomic and proteomic systematic approaches which genes and proteins are in common with vigour irrespective of seed source. By conducting such a mass comparison we will identify proteins that deal strictly with seed vigour and storage life, not just proteins that respond to a change in temperature or treatment conditions. From proteomic analysis we can identify proteins either up-regulated or down-regulated in seedlots of high or low quality. In addition, we have just initiated a study to compare hormones and metabolites during cold acclimation and freeze-induced injury and recovery to correlate these changes with winter survival. We plan to combine our proteomic, genomic, and metabolomic analysis in both plants and seeds to better understand how *Brassica napus* alters its physiology, biochemistry, and molecular biology during cold acclimation.

We have available to use on the University of Saskatchewan campus the Canadian Light Source (CLS) synchrotron which may be used to perform tomographic analysis of intact seeds. Tomography uses X-rays to create a three-dimensional image of internal structures. The principle is used in medical CAT scans and can be adapted to non-destructive examination of individual seeds. This initial work will determine if a technique using synchrotron light to analyze the oxidation of molecules or the valence state of metals in seeds can be developed. If so, it will allow comparisons between different seeds i.e. old and new seeds, high and low vigour seeds, seeds of different ecotypes/cultivars. Using this knowledge, we hope to develop a rapid, simple, and inexpensive method that will identify intrinsic characteristics of superior seed lots, as well as seed lots that lose vigour when stored under adverse conditions such as high temperature and high humidity.

Not enough is being done to combat questions raised in this industry such as “what constitutes vigour” and more importantly how can we test for it and predict storage life. These tough but extremely important questions have been a major concern with Canadian-based seed organizations and testing facilities for quite some time. The results obtained from this research project may be used by the plant breeder, seed companies and quality testing labs to select for high vigour cultivars, high quality seed lots and predict seed storage life. This will allow more rapid, less subjective and more accurate seed quality analysis by moving from current manual subjective tests to objective-based testing. To be able to test multiple samples at once, with increased reproducibility and accuracy, would definitely be an advantage to the Canadian seed companies in order to stay competitive in the industry. This is a win-win situation for canola growers in Saskatchewan. It will provide more options to growers throughout the region to more confidently purchase the highest yielding canola varieties and hybrids in the marketplace backed by superior agronomic testing lead to increased value of crop production and improved seed quality.

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