

## Seed Chlorophyll Influences Vigor in Oilseed Rape (*Brassica napus* L. var *AC Excel*.)

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### Abstract

Seeds of *Brassica napus* L. var *AC Excel* were sorted into four subsamples on the basis of the amount of chlorophyll present. Vigor of the four subsamples was studied and compared using the germination test, accelerated aging test, controlled deterioration test and electrical conductivity test. Laboratory vigor ratings were correlated with field data. Results show that seed chlorophyll had no significant effect on germination. However,

seedling growth and performance were negatively affected by high chlorophyll. High chlorophyll subsamples were more sensitive to increased moisture and temperature than low chlorophyll subsamples. Additionally, seed conductivity study indicates that seed chlorophyll content is directly proportional to seed coat porosity. The usefulness of the four vigor test methods in assessing seed quality among chlorophyll seed lots of *Brassica napus* is also discussed.

**Keywords:** *Brassica napus*, Chlorophyll, Germination test, Seed vigor

## 1. Introduction

*Brassica napus* L. and *B. rapa* L. are commonly known as ‘canola’ or ‘oilseed rape’. *Brassica napus* is the predominant oilseed species in Canada. The canola industry is large and ranks highly among other industries in the Canadian Prairies. The canola variety, *AC Excel*, is one of the early varieties developed at the Saskatoon Research Centre for its high oil content and seed yield. In recent times, intensive breeding programs have resulted in the emergence of several canola varieties which differ in oil profile and agronomic performance. *AC Excel* still remains a reference check for the newly introduced varieties.

Canola seed is grown mainly for edible vegetable oil. The meal is used for the preparation of animal feeds. The presence of chlorophyll in seeds significantly reduces its market value, resulting in lower earnings to farmers and producers. Canola seed crushers downgrade seed lots with numerous green seeds (Ward *et al.*, 1995). When seeds are crushed, the chlorophyll is extracted with oil, producing dark-coloured oil, which is aesthetically unappealing to consumers (Ward *et al.*, 1995). Chlorophyll is a photosensitizer, promoting oxidation of oil in the light, leading to rancidity (Endo *et al.*, 1984a, b; Usuki *et al.*, 1984; Kiritsakis and Dugan 1985). Removal of chlorophyll from oil is by adsorption on bleaching clay, a process which is not only expensive, but may also lead to loss of oil to the bleaching clay (Mag, 1989).

Green seeds are usually formed as a result of high temperatures and drought stress during seed-filling (Prijic *et al.*, 1998). Seed chlorophyll content may be low or absent when plants grow, and produce mature fruits under favorable environmental conditions. Jalink *et al.*, (1998a) reported that the amount of chlorophyll in the seed coat decreases during maturation, a process known as ‘degreening’. Seed-filling appears to be one of the most critical stages during seed development, as any adverse environmental stress during this process may result in seed chlorophyll accumulation and poor seed quality. Sanhewe and Ellis (1996a, 1996b) found that seeds of *Phaseolus vulgaris* L. harvested after the end of the seed-filling phase scored higher in a germination test, resulted in more normal seedlings and possessed better storability than seeds harvested at a less mature stage. In a similar study, Jalink *et al.*, (1998b) reported that seeds of *Brassica oleracea* with the lowest amount of chlorophyll fluorescence had the highest percentage of germination and normal seedlings. Furthermore, they reported that crop management practices such as sowing rate, sowing date and swathing procedures influence the chlorophyll level of harvested seeds. The process of chlorophyll breakdown during seed maturation is not yet properly understood. Ward *et al.*, (1995) reported that chlorophyll is broken down during the late stages of ripening and that the final chlorophyll level in rape seeds can be affected both by the genotype and the environment.

The need for high quality seed with strong vigor in *B. napus* has generated interest in the study of conditions and factors that influence these characteristics. However, there is limited information on factors which influence seed quality and vigor in canola. Elias and Copeland (1994) reported that storing canola seeds beyond ten months may result in significant drop of seed quality. There have been speculations as to whether seed chlorophyll affects germination and vigor in canola. Ward *et al.*, (1992, 1995) studied the relation between the amount of chlorophyll and seed maturity in oilseed rape (*Brassica napus* L.) and turnip rape (*Brassica rapa* L.), but not much was said about the influence of chlorophyll on germination and vigor. In this study, we have employed the germination test, accelerated aging test, controlled deterioration test and conductivity test to assess the influence of seed chlorophyll on germination and vigor of *B. napus*. Because green seed canola is often not screened during seeding, the aim of our study was to determine whether accumulated chlorophyll has any direct effect on seed germination and vigor. Results of vigor tests will be correlated with field data, such as, number of seedlings per row, seedling fresh weight and biomass to determine whether there is any relationship between seed chlorophyll content and field agronomic performance.

## 2. Materials and methods

### 2.1 Seed Material

Four subsamples of seeds of *Brassica napus* var *AC Excel* with varying amounts of chlorophyll were selected for study. Actual amount of chlorophyll in each subsample was then analyzed spectrophotometrically in the

laboratory.

### 2.2 Spectrophotometric Determination of Seed Chlorophyll

The procedure was a slight modification of American Oil Chemists' Society Official Method AK 2-92 (Mehlenbacher *et al.*, 1992). Seed samples (1.5 gram) were placed in 20 ml polyethylene terephthalate (PET) plastic scintillation vials (Wheaton No. 986741). To each vial was added a stainless steel rod measuring 8 mm by 25 mm, followed by 15 ml of extraction solvent (Heptane:Ethanol; 3:1 [vol : vol]). The vials were then capped, placed in a rack and shaken for 1 hr on an Eberbach reciprocating shaker (280 strokes/minute, 38 mm stroke) in a vertical direction. This thoroughly ground the seeds, and chlorophyll was extracted into the solvent. The vials were then centrifuged at 2500 rpm for 15 min in a IEC Centra GP8 centrifuge equipped with a 216 rotor assembly to clarify the extracted solution. Absorbance of extracts was measured at wavelengths, 630, 665 and 700 using a Hewlett Packard 8453 spectrophotometer equipped with a sipper and a flow through cuvette (1.0 cm path length, 3 mm aperture). The chlorophyll content of seed samples was then calculated as follows:

$$\text{chlorophyll content, mg/kg} = (k \times A_{\text{corr}} \times V) / (m \times l)$$

Where:

$$A_{\text{corr}} \text{ (the baseline corrected absorbance)} = A_{665} - [(A_{700} + A_{630})/2]$$

$A_{665}$  = absorbance at 665 nm

$A_{700}$  = absorbance at 700 nm

$A_{630}$  = absorbance at 630 nm

$k$  = 13 (constant)

$l$  = path length of the cuvette

$m$  = mass of the seed sample, in grams

$V$  = volume of extraction solvent.

### 2.3 Germination Test (GT)

Two hundred seeds were selected at random from each of the four subsamples. Seed moisture was standardized at 10%. Each subsample was pre-incubated for 24 h at 20°C under 100% RH. Fifty seeds from each sub-sample were planted in a petri-dish (2 cm deep, 10 cm diameter) lined with a Whatman # 4 filter paper. Each seed entry was replicated four times and laid out in the growth cabinet maintained at a constant temperature of 20°C, 50% RH, 16 h day light and 8 h darkness. First normal germination count was taken 4 days following incubation, and daily readings continued until day 7. Normal seedlings were harvested after day 7, dried in an oven at 60°C for 6 days, and seedling dry weight was then measured.

### 2.4 Accelerated Aging Test (AAT)

Accelerated aging test was conducted using standard methods (Hamptom and Tekrony, 1995; Onyilagha *et al.*, 2007). 200 seeds were taken randomly from each of the four subsamples and adjusted to 10% moisture level (Hamptom and Tekrony, 1995). Each sub-sample was placed on an accelerated aging tray obtained from Hoffman Manufacturing Inc., Albany, USA. The aging tray was inserted into an inner chamber containing 40 ml of de-ionized water. Seeds were aged at 41°C for 24 h under 100% RH. At the end of the aging period, 50 seeds from each subsample were planted in a petri-dish (2 cm deep, 10 cm diameter) lined with a Whatman # 4 filter paper. Subsequent treatments followed same procedures as in the germination test above.

### 2.5 Controlled Deterioration Test (CDT)

200 seeds per subsample were adjusted to 20% moisture level (Hamptom and Tekrony, 1995), and immediately placed into an aluminum foil bag (laminated package composed of 12/20/50 micron polyester/aluminum foil/polythene). The bags were flattened with the edge of hand to remove air, and then heat sealed with a high power electric sealer (Hamptom and Tekrony, 1995; Onyilagha *et al.*, 2007). The aluminum bags with seed were placed side down in a cabinet maintained at 10°C for 24 h for seed moisture equilibration. At the end of the equilibration period, entries were deteriorated in a chamber maintained at 45°C and 98±2% RH for 24 h. Seed samples were planted within one hour after deterioration (Hamptom and Tekrony, 1995) in a thermal-gradient plate. Fifty seeds from each subsample were planted in a petri-dish (2 cm deep, 10 cm diameter) lined with a Whatman # 4 filter paper. Each seed entry was replicated four times. The plates were maintained at a constant temperature of 20°C and 98±2% RH, with 16 h day light and 8 h darkness. First normal germination count was taken 4 days following incubation, and daily readings continued until day 7. Normal seedlings were harvested

after day 7, dried in an oven at 60°C for 6 days, and seedling dry weight was then measured.

### 2.6 Conductivity Test (CT)

For CT, 200 seeds from each of the four subsamples were standardized at 10% moisture, followed by incubation for 24 h at 20°C under 100% RH. At the end of incubation period, seeds were immersed completely in 60 ml de-ionized water. Conductivity was measured after 4 h, 8 h and 24 h periods. The instrument was a VWR 5005 model conductivity meter, equipped with a carbon conductivity cell,  $K = 1.0$ .

## 3. Results

Results of seed chlorophyll analysis, weight of 1000 seeds, germination test, accelerated aging test and controlled deterioration test are listed in Tables 1 - 3. Result of statistical test on influence of aging temperatures and seed chlorophyll on germination and vigor is shown in Table 4. Seed performance was uniform among the subsamples in germination test (GT), irrespective of their varying amounts of chlorophyll. All subsamples achieved over 90% germination 7 days after planting. In the accelerated aging test (AAT) and controlled deterioration test (CDT), the subsample with least chlorophyll (6 ppm) gave higher germination than those with higher amounts of chlorophyll. Also, the subsample with the lowest chlorophyll level consistently achieved over 90% germination 7 days after planting in all tests. In the subsamples with high chlorophyll content, there was no observed pattern with respect to amount of seed chlorophyll and percent germination in the AAT and CDT. Table 4 shows that a combination of aging temperature and chlorophyll influenced germination of seeds in the AAT and CDT, while seed chlorophyll content had significant influence on conductivity. Result of seed conductivity test (CT) is shown in Table 2, and unlike in the AAT and CDT, there is a clearer pattern in the CT with respect to seed chlorophyll content and conductivity. Seed subsample with highest amount of chlorophyll (60 ppm) consistently had highest conductivity, while subsample with least amount of chlorophyll (6 ppm) had least conductivity.

Table 3 shows the correlation between our laboratory vigor results and data obtained from field agronomic studies. Although many high correlation values were obtained, only few were statistically significant ( $r \geq 0.95$  at 5% probability level). The correlation pattern is such that germination counts in laboratory vigor tests taken 4 days after planting apparently had higher correlation with field data in the GT and AAT than counts taken 7 days after. Conversely, germination counts taken 7 days after planting in the CDT had higher correlation with field data than counts taken 4 days after. In the CT, conductivity increased with time (Table 2), however, correlation coefficients were inversely related with increased conductivity (Table 3).

## 4. Discussion

Result from the germination test suggests that under favorable environmental conditions, seed chlorophyll content may have no significant effect on germination of *B. napus* var *AC Excel* (Table 1). However, in the event of any environmental stress, such as high temperature, as in CDT, and/ or moisture, as in AAT, germination of seeds of *B. napus* with high amounts of chlorophyll may be impaired (Tables 1 and 4). This is in agreement with Jalink *et al.*, (1998b) who found in a controlled deterioration test, that seed subsamples of *Brassica oleracea* with lowest chlorophyll fluorescence signal had slightly lower germination and normal seedling percentages than the control seeds, whereas the seeds with highest chlorophyll fluorescence signals had much lower germination and lower normal seedling percentages. In another vigor study using the cold test,

Prijic *et al.*, (1998) found that wrinkled soybean seeds gave better germination than chlorophyll seeds. They suggested that green seeds have non-functional tissues, and may be susceptible to fungal invasion.

Although chlorophyll had no significant effect on germination of subsamples in the germination test, however, its influence on dry weight of seedlings is highly significant (Table 4). Also, seedling dry weight appears to be related to seed weight, such that seeds with highest 1000-seed weight had highest seedling dry weight (Table 1). The result suggests that seed plants raised from large seeds accumulate higher biomass than those raised from small seeds. Similarly, seed plants raised from high chlorophyll seed lots may accumulate less biomass. In nature, accumulation of low biomass by seed plants may translate into low field performance. It is important to note that relation between our 1000-seed weight and chlorophyll content is apparently inverse, a finding which is in agreement with Jalink *et al.*, (1998a), who also suggested that seed quality was inverse to the amount of chlorophyll in the seed coat.

In seed conductivity experiments, the amount of internal metabolites leached through the seed-coat is measured. Thus, it is also a measure of seed-coat integrity, whereby intact seed-coat resists excessive leaching of seed internal metabolites. The integrity of seed-coat is often related to seed vigor, and seed lots with porous seed-coat have high conductivity and low vigor.

Results of our conductivity test (Table 2) show that increase in seed conductivity is directly proportional to seed chlorophyll, and inversely proportional to germination performance of seed subsamples (Table 1). Our results suggest that seeds with high chlorophyll content have higher porous seed-coat than the low chlorophyll subsamples. It is not certain whether high seed-coat porosity is related to seed maturity level. However, Ward *et al.*, (1992, 1995) found a significant correlation between seed chlorophyll levels in harvested seed and time of maturity in eleven *B. napus* cultivars.

Correlation results (Table 3) show that germination test and conductivity test may be most suited for seed vigor evaluation among the chlorophyll samples. Data from the two tests had high correlation with field agronomic data, and significant correlation occurred with field data collected 21 days after planting. The inverse relation between high conductivity (Table 2) and correlation values (Table 3) supports our previous assertion that high conductivity is proportional to reduced field performance. Although germination results from AAT and CDT showed no significant correlation with field agronomic parameters, it is necessary to note that these two tests were stringent enough to predict the adverse effect of high seed chlorophyll on vigor, a characteristic which could not be discerned from the germination test (Table 4). The high correlation between seedling dry weight after seven days incubation and field agronomic data suggests that seedling dry weight may be a useful parameter for assessing vigor in chlorophyll seed lots.

## 5. Summary

Our findings may be summarized as follows:

- a) Amount of seed chlorophyll (up to 60 ppm) may not have any significant effect on germination of *B. napus* seed lots, especially if they are planted during very low environmental stress conditions.
- b) Seeds with high amount of chlorophyll are more prone to deteriorate in the presence of adverse environmental conditions than those with low chlorophyll.
- c) High seed chlorophyll content negatively affects seedling vigor and overall field performance.
- d) Seed-coat porosity is directly proportional to high seed chlorophyll.
- e) High seed weight is proportional to biomass accumulation.

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Table 1. Percent germination of seeds of *Brassica napus* var *AC Excel* with varying amounts of chlorophyll in a germination test (GT), accelerated aging test (AAT) and controlled deterioration test (CDT). Seed weight and dry weight of normal germinated seedlings are also shown

Variety	Chlorophyll content (ppm)	1000 seed wt. (g)	GT			AAT			CDT		
			4 days	7 days	Seedling dry wt. (mg)	day 4	day 7	Seedling dry wt. (mg)	day 4	day 7	Seedling dry wt. (mg)
AC Excel	6	2.7b	78.0a	97.0a	98.9a	85.5a	98.5a	94.6a	52.0a	93.5a	83.8a
AC Excel	15	3.2a	74.5a	90.5a	99.8a	70.5ab	84.0b	94.5a	25.5b	81.5b	85.6a
AC Excel	29	2.4c	73.0a	95.5a	91.9a	58.0b	90.5ab	82.1ab	6.5c	56.0c	46.6b
AC Excel	60	2.1d	70.0a	92.5a	72.7b	62.5b	82.5b	68.1b	30.5b	71.0b	48.8b

means within columns followed by the same letter are not significantly different (LSD,  $p=0.05$ ).

Table 2. Electrical conductivity measurements of seeds of *B. napus* with varying amounts of chlorophyll

Variety	Chlorophyll content (ppm)	Conductivity ( $\mu$ S) over time (h)		
		4 h	8 h	24 h
AC Excel	6	16.3d	26.7c	47.5c
AC Excel	15	23.6c	36.2b	65.1b
AC Excel	29	28.2b	39.7b	67.7b
AC Excel	60	36.9a	49.2a	86.3a

means within columns followed by the same letter are not significantly different (LSD,  $p=0.05$ )

Table 3. Pearson correlation between results of laboratory vigor tests and field agronomic data of *B. napus* var *AC Excel* with varying amounts of chlorophyll in their seeds

Type of test and time after incubation	Seedlings / row		Fresh weight		Biomass	
	14 DAS	21 DAS	14 DAS	21 DAS	14 DAS	21 DAS
Germination:						
4 days	0.86	0.96	0.28	0.86	0.62	0.95
7 days	0.01	0.29	-0.36	-0.01	-0.25	0.22
Seedling dry wt.	0.91	0.82	0.73	0.92	0.90	0.86
Accelerated aging:						
4 days	0.75	0.93	-0.09	0.74	0.37	0.89
7 days	0.43	0.67	-0.11	0.42	0.14	0.61
Seedling dry wt.	0.97	0.91	0.65	0.98	0.90	0.94
Controlled deterioration:						
4 days	0.44	0.68	-0.46	0.43	-0.00	0.62
7 days	0.73	0.85	-0.09	0.72	0.37	0.83
Seedling dry wt.	0.96	0.92	0.40	0.95	0.77	0.95
Conductivity:						
4 hours	-0.90	-0.96	-0.37	-0.90	-0.69	-0.96
8 hours	-0.86	-0.95	-0.32	-0.86	-0.63	-0.94
24 hours	-0.82	-0.92	-0.31	-0.82	-0.60	-0.90

$r \geq 0.95$  significant at 5% probability level; DAS = days after seeding.

Table 4. Influence of aging temperatures and chlorophyll content on germination of seeds of *Brassica napus* var *AC Excel* with varying amounts of chlorophyll in a germination test (GT), accelerated aging test (AAT) and controlled deterioration test (CDT). Influence of chlorophyll on conductivity (CT) is also shown

Variables	GT			AAT			CDT			CT		
	4 days	7 days	seedling dry wt.	4 days	7 days	seedling dry wt.	4 days	7 days	seedling dry wt.	4 h	8 h	24 h
Temperature	ns	ns	ns	*	ns	ns	**	**	ns	-	-	-
Chlorophyll	ns	ns	***	**	**	***	***	***	***	***	***	***

\* at  $p=0.05$ ; \*\* at  $p=0.01$ ; \*\*\* at  $p=0.001$ ; ns = not significant.