# Salinity Tolerance Screening for Biodiesel Cultivars

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## Introduction

The anticipated increase in demand for biodiesel oil feedstock will encourage agricultural producers to expand the area seeded to canola-grade oilseed crops. Biodiesel oil production facilities operating and announced alone will require over a million tonnes of canola oil feedstock per year. Plus, a federal requirement for 2% biodiesel in diesel by 2012 will likely demand another 500 million litres of the biofuel per year. The Canola Council has set an annual production target of 15 million tonnes of canola by 2015 to meet this demand. Biodiesel experts suggest that the two major constraints to the development of the Canadian Biodiesel industry are: (1) assured supply of oilseed feedstocks, and (2) maintenance of the quality of the oil produced. This progress report is generated from a study which addresses the first constraint. The study aims to review the salinity tolerances of potentially high-oil-yielding biodiesel and food-grade feedstock under development and currently available to oilseed growers.

Selected canola cultivars tolerate root-zone salinity equally to that of Harrington barley (Steppuhn & Raney 2005). With this knowledge, producers could expand canola crop area within the 7 million hectares of cultivated lands affected by slight to moderate salinity across the Canadian Prairies (Wiebe et al. 2007; Steppuhn 1996), taking advantage of production options which counter fuel-versus-food concerns. This work will also position Crucifer breeding activity for continued development of high oil-yielding cultivars which better tolerate root-zone salinity.

The researchers contributing to the Agricultural Handbook No. 60 (U.S. Salinity Laboratory Staff 1954) list “rape” as less tolerant of salinity than sugar beet and barley, but more tolerant than most other field crops. In searching for breeding material with which to improve the salinity tolerance of canola, Ashraf and McNeilly (1990) rated *Brassica napus* and *B. carinata* with more salinity tolerance than *B. rapa* and *B. juncea*. Francois (1994) compared ’Westar’ Argentine to ‘Tobin’ Polish canola, confirming that Westar (*B. napus*) was more salinity tolerant than Tobin (*B. rapa*). Francois also found that seed oil contents were not significantly different over the salinity range tested. Conversely, in evaluating six canola cultivars for differences in seed oil content at 27 sites over 3 yr, Shafii et al. (1992) found a relationship with the genotype-environment interaction favouring cooler climates. Ashraf and McNeilly (2004) reported that the NaCl tolerance of *B. juncea*, *B. carinata* and *B. napus* significantly exceeded those of *B. campestris (B. rapa)*, *B. nigra* and *B. oleracea*. Working with *B. napus* cv. Hyola 401 and InVigor 2573, Steppuhn and Raney (2005) determined that salinity did not adversely affect oil concentration in either cultivar until the sulphate test solution exceeded 12 dS/m in electrical conductivity, but then declined as salinity increased.

Salinity generally slows the rate of crop growth, resulting in plants with smaller leaves, shorter stature, and reduced economic yield (Shannon et al. 1994). The inherent ability of crop plants to withstand the effects of elevated solute concentrations of Na, Ca, Mg, $SO_4$, Cl and other ions in root-zone solutions and still produce agricultural products defines salinity tolerance. A wide range of canola-grade oil feedstock cultivars are being evaluated for salinity tolerance in Canada's Salinity Testing Facility at Swift Current (Steppuhn and Wall 1999). The Wheatland Conservation Area (a producer-run, applied research organization) will confirm the test results under field conditions.

## Screening Methods and Tests

At present, the testing facility at Swift Current can accommodate ten cultivars per screening test. Except in the first test, the cultivar 'Westar' will serve as a common cultivar in each test. Screening is based on the salt-sensitivity of plant tissue as measured by seedling emergence, height growth, harvested shoot biomass, and salt accumulation in shoot tissue. The purpose of the screening test is to identify the inherent salt-tolerance for the seedlings tested and to select cultivars for further testing using crop yield functions in response to increasing levels of root-zone salinity. The oilseed crops from which the test cultivars will be selected for screening include:

1) Argentine canola open-pollinated (*Brassica napus*)
2) Argentine canola "Roundup-Ready" hybrid (*Brassica napus*)
3) Argentine canola "Liberty-Link" hybrid (*Brassica napus*)
4) Argentine canola "Clear-Field" hybrid (*Brassica napus*)
5) Polish canola (*Brassica rapa*)
6) Camelina (*Camelina sativa*)
7) Ethiopian mustard (*Brassica carinata*)
8) Mustard (*Sinapis alba*)
9) Canola-quality Juncea (*Brassica juncea*)

In addition, the top oil-producing cultivars of *B. napus*, *B. juncea* and other oilseed crop species offered by co-operating seed companies will also be included.

The first step upon obtaining the seed will be to test for germination and vigour. The germination testing in an environmentally-controlled growth cabinet with petri dishes, fine sand, plotting paper, and distilled water requires 200 test seeds per cultivar. This test is conducted in 70% relative humidity and under a regime of alternating 12 hour-periods with and without supplementary lighting. A radical length of 12 mm specifies germination.

## Screening Procedures

The oilseed screening conducted in the Salt Tolerance Testing Facility follows common procedures in all cultivar tests. Nine-litre pots filled to 8 L with pure silica (mean particle sizes from 0.1 to 0.3 mm in nominal diameter) provide seedbeds for two cultivars in each pot. The seedbeds are flushed four times daily with a modified, half-strength Hoagland=s nutrient solution consisting of $Ca(NO_3)_2$, $KNO_3$, $KH_2PO_4$, $MgSO_4$, chelated Fe, $NH_4NO_3$, KCl, $H_3BO_4$, plus trace elements including Mn, Zn, Cu, Si, and Mo (Hoagland and Arnon 1950). Randomly selected solutions are salinized by adding $CaCl_2$, $MgSO_4$, and $Na_2SO_4$ to obtain salinized test solutions plus the nutrient-only control and are added to the test solutions prior to seeding. This procedure duplicates the common field situation where seeds must be placed directly into saline seedbeds, typical for dryland prairie conditions and practices. Solution electrical conductivities ($EC_{sol}$) in dS/m relate to equivalent electrical conductivity of saturated soil paste extracts ($EC_e$) in dS/m, as detailed in Ayers and Westcot (1985), by the approximate relationship: $EC_e$.0.5($EC_{sol}$).

Each hydroponic flushing of the root-zone continues for twelve minutes until the sand is completely saturated, after which the solutions drain into 612-litre reservoirs for the next flushing. Water lost by evapotranspiration is replenished weekly or when necessary to maintain the concentrations of salts in solution. The electrical conductivity ($EC_{sol}$) of each solution is checked initially and twice weekly.

The screenings are conducted with an appropriate time course for day/night time sequences (adjusted in 15-minute ephemeral increments) mimicking a May seeding at 51$^o$ north latitude. Supplemental lighting from 475-W sodium lamps positioned 1.5 m above the sand surfaces extend day-lengths. Lamps are strategically positioned overhead in order to obtain measured radiant intensities averaging 7.9 kJ m$^{!2}$ min$^{!1}$ with a uniformity coefficient of 0.9 within the entire test laboratory. Day/night temperatures are reset hourly according to a 24-hour diurnal schedule, and range from 14 to 23EC with ambient temperatures maintained within one or two degrees of the set-points.

Two irrigations with the test solutions precede seeding in order to firm the seedbed. A template guides placement of each seed into a known position within each seedbed. This allows assessment of emergence and survival associated with each seed on a daily basis. Any protrusion of the plant above the sand surface counts it as emerged. Records are kept on paper copies of the seeding template. This practice results in daily counts per pot of the number of newly emerged plants and their survival with time.

Each screening utilizes 60 pots laid out in groups of three forming 20 replicate blocks each containing one pot salinized according to one of the three salt levels and randomized within each block and the 60-pot layout. Depending on the test, four or five seeds of one cultivar are position-placed 6 mm deep in either the east or west half of each test pot following a random choice. Measurements in the screening tests include: plant emergence and survival, the average number of seedlings which survive 35 days or longer after seeding, the average number of days following seeding for the seedlings to reach maximum emergence, plant heights, growth stage development (according to a code by Lancashire et al. 1991), the harvested shoot weight, and salt accumulation in the plant tissue. Selected preliminary results based on these measurements from two screening are reported herein. The first test was conducted in 2000 and the second in 2007.

**First Test** The ten cultivars selected for salt-tolerance screening in this test consisted of one Polish and eight different Argentine types, plus one mustard (Table 1). The percent germination associated with each seed-lot of the ten selected cultivars each measured 89% or higher when measured 14 days following seeding. Of the ten cultivars, only the SW Arrow seed failed to reach 89% germination or greater by the seventh day. Only, four of the ten cultivars registered less than 89% after the first five days following seeding: SW Arrow, Q2, InVigor 2273, and Quest. These seedlings demonstrated the lowest vigour among the ten test cultivars selected.

All treatments were replicated four-fold with four seeds per cultivar per replicate. The sand was flushed four times daily with the modified Hoagland's nutrient solution salinized to one of three sulphate-based salinity levels [with solution electrical conductivity]:
(1) negligible, nutrient-only [1.4 dS $m^{-1}$],
(2) the low end of moderate [9 dS $m^{-1}$], and
(3) the low end of severe [18 dS $m^{-1}$].

Table 1. Canola/mustard cultivars[z] screened for salinity tolerance by emergence, growth, and development comparisons, Swift Current Salt Tolerance Testing Lab.

| Designation | Crop | Cultivar | Biological name | Type[y] | Supplier |
|---|---|---|---|---|---|
| Quan | Canola | Quantum | *Brassica napus* L. | A | Agricore |
| Q2 | Canola | Q2 | *Brassica napus* L. | A | Sask. Pool |
| H401 | Canola | Hyola 401 | *Brassica napus* L. | A, H | Advanta |
| 46A76 | Canola | UGG 46A76 | *Brassica napus* L. | A, C(SP) | Proven |
| 2273 | Canola | InVigor 2273 | *Brassica napus* L. | A, LL, H | Aventis |
| 2573 | Canola | InVigor 2573 | *Brassica napus* L. | A, LL, H | Aventis |
| Quest | Canola | Quest | *Brassica napus* L. | A, RR | Agricore |
| Arow | Canola | SW Arrow | *Brassica napus* L. | A, RR | Agricore |
| 41P55 | Canola | UGG 41P55 | *Brassica rapa* L. | P | Proven |
| PenM | Mustard | AC Pennant | *Sinapis alba* L. | Yellow | Sask. Pool |

[z] Selected by Southern Applied Research Association
[y] Legend: A=Argentine P=Polish H=hybrid RR=Roundup resistant LL=Liberty link resistant CF=Clearfield SP=Smart Pursuit resistant Sask. Pool = Saskatchewan Wheat Pool

Salinity affects crop emergence as well as crop yield. Consequently, plant emergence was measured in this screening test for comparisons among the ten canola/mustard cultivars. The average number of seedlings which emerged and survived for 36 days formed one of the measures for the comparisons. A second measure was the average number of days following seeding for the seedlings to reach maximum emergence.

Cultivar comparisons were also served by measuring plant heights and growth stage development weekly during the test. The seeds were planted on October 31st with growth measurements taken on Nov 14, 21, 28, Dec 5, 12, 19, and 27. These dates respectively translated to growth measurements obtained 14, 21, 28, 35, 42, 49, and 57 days after seeding. Pod development was assessed on Dec 28th (Day 58), and the plant shoots harvested on Dec 29th (Day 59).

**Second Test** Of the total number of cultivars to be evaluated, another ten were evaluated in this second test (Table 2). Nutrients and salts were prepared and added to the test solutions prior to seeding (five seeds per cultivar per replicate). All treatments were replicated four-fold. The sand was flushed four times daily with a modified Hoagland's nutrient solution salinized to one of three sulphate-based salinity levels [with solution electrical conductivity]:
(1) negligible, nutrient-only [1.4 dS $m^{-1}$],
(2) midway between slight and moderate [8 dS $m^{-1}$], and
(3) midway between moderate and severe [16 dS $m^{-1}$].

Table 2. Second group of cultivars selected for screening.

| Cultivar | Scientific Name | Type |
|---|---|---|
| AC Arid | Brassica juncea (L.) | Canola-quality oil |
| AC Estlin | Brassica juncea (L.) | Canola-quality oil |
| Centennial Brown | Brassica juncea (L.) | Brown mustard |
| AC Base | Sinapis alba (L.) | Yellow mustard |
| AC Vulcan | Brassica juncea (L.) | Oriental mustard |
| AC Sunbeam | Brassica rapa (L.) | Polish canola |
| Carinata | Brassica carinata (L.) | Ethiopian mustard |
| InVigor 5030 | Brassica napus (L.) | Argentine canola (LL hybrid) |
| InVigor 9590 | Brassica napus (L.) | Argentine canola (LL hybrid) |
| Westar | Brassica napus (L.) | Argentine canola (op) |

LL =liberty link; op=open pollinated

**Results, First Test**

All comparisons presented here are preliminary and not necessarily reflective of outcomes resulting from further screening and analyses.

**Seedling Emergence** Except for cultivars 41P55 (Polish-type canola) and AC Pennant (yellow mustard), seedling emergence whether from any salinized or salt-free seedbed equalled 75% or better of the number of seeds planted (data not shown). Only seeds from four cultivars (Quantum, SW Arrow, 41P55, & AC Pennant) registered a slight drop in average emergence and survival (36 days after seeding) when originating from the 9 dS/m environment as when from the salt-free ambient substrate. Severe seedbed salinity (18 dS/m) resulted in seven of the ten cultivar seedlings showing reduced emergence compared to the nutrient-only treatment: Q2, Hyola 401, InVigor 2273, Quest, SW Arrow, 41P55, and AC Pennant. Of these seven, only the latter two measured an emergence/survival below 50%.

A daily count of the number of plants emerging from the seeds placed in each sand pot provided a measure of the average number of days necessary to reach maximum emergence (Table 3). Of the ten cultivars, only five showed an increase in the number of days with the first (9 dS/m) level of increased salinity. The number of cultivars with an increase in days to maximum emergence for the moderate salinity compared to severe equalled six. A comparison of the number of days to reach the maximum emergence among the three salinity levels for the nine canola cultivars revealed 29, 26, and 19 pots out of 36 wherein the number of days to reach maximum emergence equalled six days or less for the 1.4, 9, and 18 dS/m salinity levels, respectively. Overall, the time for the seeds of the test cultivars to reach their maximum number of emerged plants averaged no more than nine days irrespective of substrate salinity. Perhaps, the most useful conclusion is that salinity seems to have a limited effect on the speed of canola/mustard germination and emergence, especially for the mustard. Possibly, these rather uniform results are tied to seed size and the time required for seed water imbibition.

Table 3. Average[z] number of days following seeding to reach maximum emergence arrayed by cultivar and seedbed solution salinity.

| Cultivar | Seedbed solution salinity | | |
|---|---|---|---|
| | 1.4 dS/m | 9 dS/m | 18 dS/m |
| | - - - - - - - - - - - - - | days | - - - - - - - - - - - - - |
| Quantum | 7.5 | 6.25 | 6.25 |
| Q2 | 7 | 6.5 | 7.5 |
| Hyola 401 | 7.5 | 8.75 | 6 |
| UGG 46A76 | 5 | 5.5 | 6.25 |
| InVigor 2273 | 5.5 | 6 | 6.5 |
| InVigor 2573 | 5.5 | 8.75 | 6 |
| Quest | 6 | 6 | 7.75 |
| SW Arrow | 6 | 6.25 | 6.75 |
| UGG 41P55 | 8 | 6.25 | 7 |
| AC Pennant | 5 | 4.5 | 4.5 |

[z] Average among four replications with four planted seeds per cultivar per pot.

**Seedling Growth** The negative effect of root-zone salinity on canola/mustard plant height was evident as early as 14 days after seeding (data not shown). This effect intensified in all cultivars for plant height comparisons 21, 28, and 35 days following seeding. By the 35th day, root-zone salinity had negatively affected the plant heights of the Polish type canola and the yellow mustard representatives more than any of the eight Argentine type cultivars. By the 42nd day, 41P55 and AC Pennant plants growing without excess salt in the rooting media were Abolting@ (very rapid shoot growth) ahead of the others. Under severe salinity, average plant height of the Polish canola and the yellow mustard were very noticeably curtailed. Also, InVigor 2573 plants ranked taller than any of the other seven Argentine cultivars. On Day 49, 41P55 and AC Pennant plants growing in the 9 dS/m medium showed marked increases in height from the previous two weeks, suggesting that they were bolting as were all the salt-free canola plants. Two days before the shoot harvest (Day 57) all plants of all ten cultivars growing in the 1.4 and 9 dS/m regimes displayed plant heights well into or beyond the bolting stage (Figure 1). Quest in the 9 dS/m environment and InVigor 2573 in the 18 dS/m root zone were taller than the plants of all other canola cultivars.

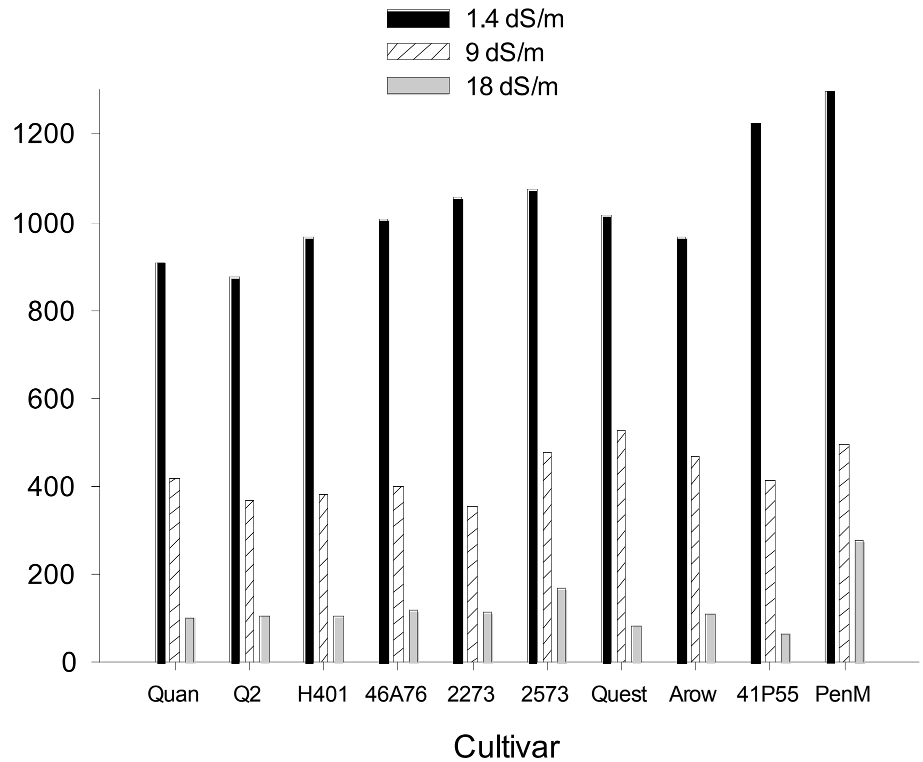


Figure 1. Average plant height (mm), of canola seedlings for cultivars measured 57 days after seeding, subjected to seedbed and root-zone conductivities of 1.4, 9, and 18 dS/m ($EC_{sol}$), First Test.

**Growth Stage** A very slight but consistent retardation of growth stage in all plants of all cultivars growing in the moderate and severe salinity treatments occurred from emergence until the 28$^{th}$ day after seeding (data not shown). By the 28$^{th}$ day, mustard plant development among the 1.4 and 9 dS/m treatments began to advance rapidly. The Polish type canola plants growing without excess salt seemed to be just entering rapid stage advancement. At that time, the Argentine type canola plants appeared to be about one week behind. By the 42$^{nd}$ day after seeding, the AC Pennant and the 41P55 salt-free plants had reached the mid-flowering or anthesis stage, while the 9 dS/m salinity plants were showing inflorescences. This was also about the same growth stage as the nutrient-only, Argentine type canola plants. The delay of growth caused by the root-zone salinity for all plants of all cultivars, especially the canolas, became very evident on Day 57. By harvest, 1.4 and 9 dS/m plants of all cultivars were either well into or approaching inflorescence. This also included the 18 dS/m plants of the mustard. The rest of the plants in the severe salinity environment were still elongating and bolting.

**Pod Development** An assessment of pod development associated with each plant was conducted on Day 58 after seeding (data not shown). The Polish canola (41P55) and the yellow mustard (AC Pennant) representatives in the absence of root-zone salinity were the most advanced in pod development. This advance was also evident in the 9 and 18 dS/m substrates.

In all cultivars, root-zone salinity delayed pod development.

**Shoot Biomass** A visual assessment of all the test plants was made just prior to harvesting the plant shoots. The influence of seedbed and root-zone salinity on the production of shoot (above-ground) biomass harvested 59 days after seeding is consistently evident in all test cultivars (Figure 2). The salinity-free, yellow mustard produced more shoot biomass than any canola cultivar. Among the canola cultivars, Hyola 401 plants without salinity amassed the greatest biomass yield, and the salt-free Q2 the least. With moderate salinity, the InVigor 2573 plants accumulated the most above-ground biomass. Under a severely saline environment, InVigor 2573 was again the most productive. Grown in saline root zones and seedbeds, the yellow mustard and the Polish canola were the least productive.

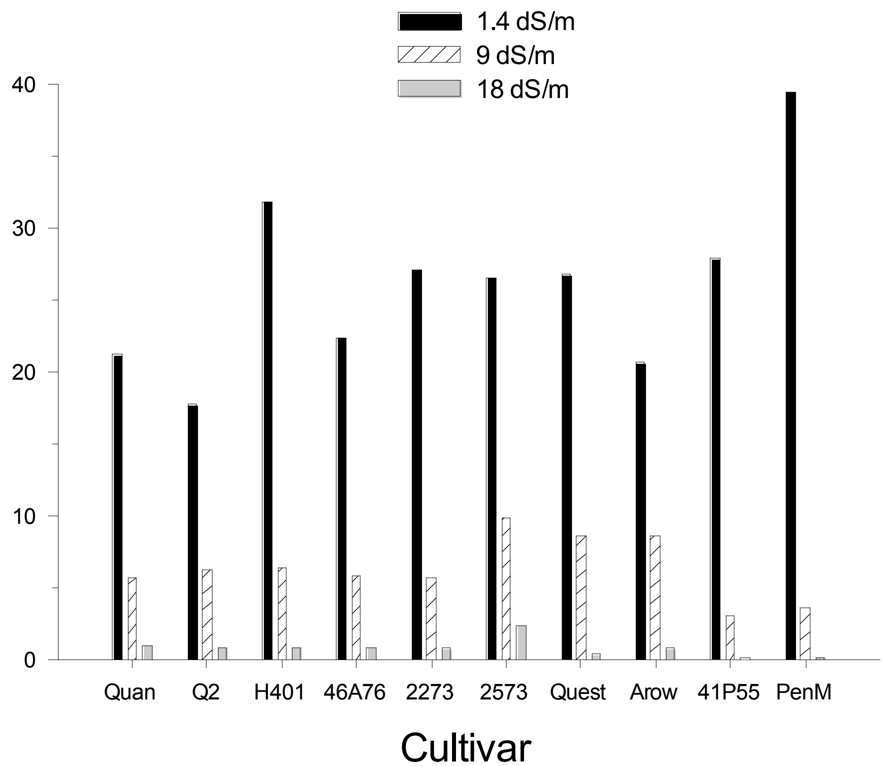


Figure 2. Average above-ground biomass of canola seedlings for cultivars harvested 59 days after planting, subjected to seedbed and root-zone conductivities of 14, 9, and 18 dS/m ($EC_{sol}$), First Test.

A comparison of the relative shoot (above-ground biomass) biomass harvested 59 days subsequent to seeding favoured InVigor 2573 within the 18 dS/m environment (Figure 3). However, SW Arrow plants rated first in relative yield over those of the second-place InVigor 2573 plants within the 18 dS/m rooting environment. This relative ranking resulted because of the low shoot biomass produced by the SW Arrow plants growing in salt-free root zones.

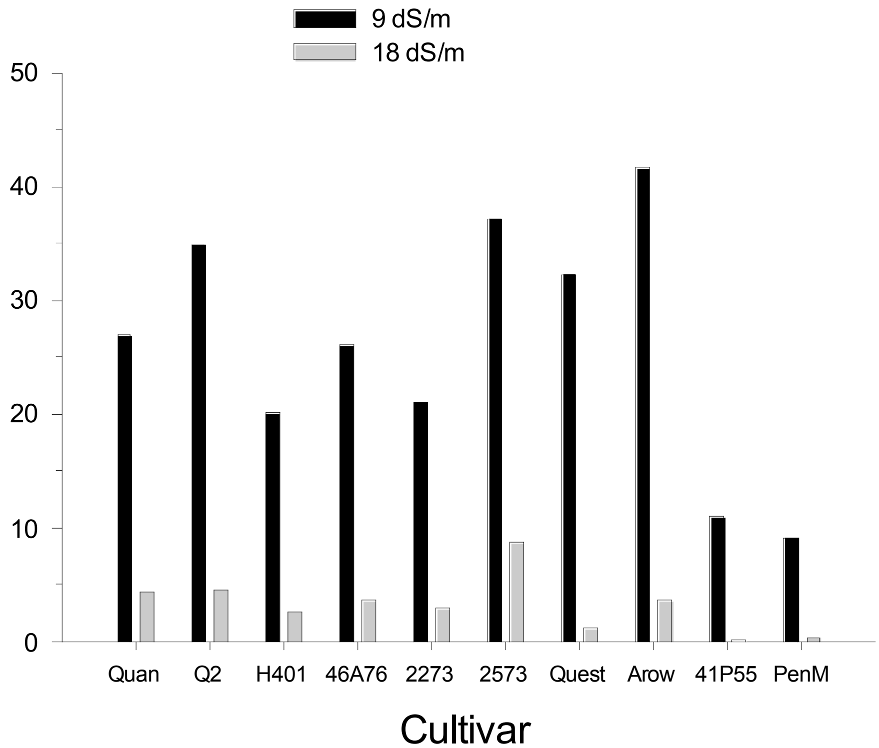


Figure 3. Average relative above-ground biomass expressed as a percentage of the control treatment (1.4 dS/m), of canola seedlings for cultivars harvested 59 days after planting, subjected to seedbed and root-zone conductivities of 9, and 18 dS/m ($EC_{sol}$), First Test.

**Results, Second Test**

All comparisons presented here are preliminary and not necessarily reflective of outcomes resulting from further screening and analyses.

**Seedling Emergence** At 8 dS $m^{-1}$, plants representing each of the ten test cultivars emerged and survived in numbers within 85% of that achieved in salt-free seedbeds (Figure 4). At 16 dS $m^{-1}$, three cultivars (Centennial Brown, InVigor 5030 & 9590) maintained emergence and survival equal to the 1.4 and 8 dS $m^{-1}$ numbers. Westar, Arid, and Base ranked next with 60% survival. AC Vulcan fared as the lowest with only 20% of the survival attained in the 1.4 and 8 dS $m^{-1}$ salinity treatments.

**Plant Height** Salinity decreased the plant heights in all test crop cultivars (Figure 5). Thirty-seven days following seeding, height growth was decreased by 50% or more for all cultivars grown at 16 dS $m^{-1}$.

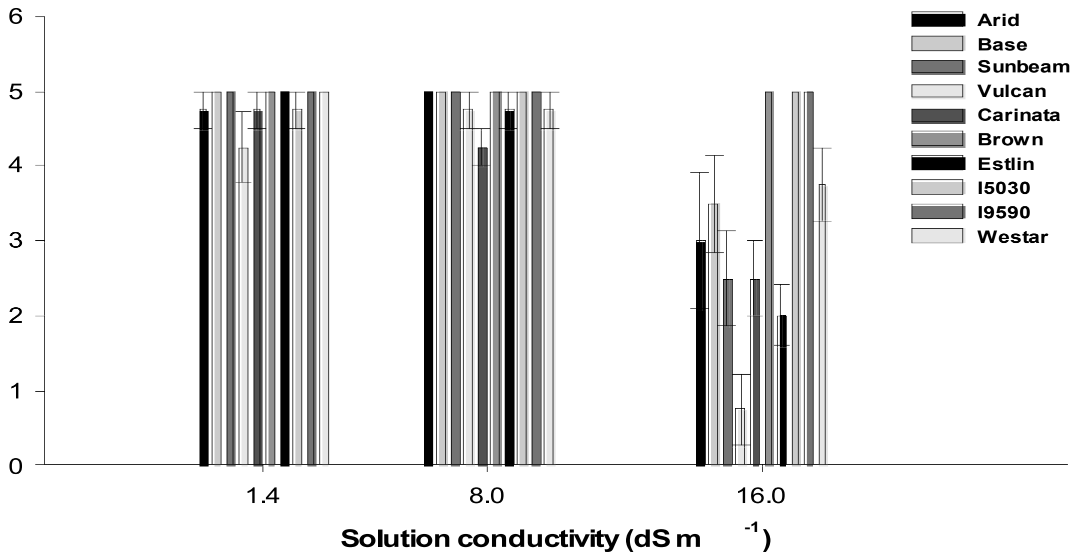


**Figure 4.** Average emergence and survival of selected canola/mustard cultivars (Second Test) associated with three salinity levels; error bars indicate standard error.

**Shoot Biomass** Plants were harvested and measured for shoot biomass per plant at full flowering (between 48 and 69 days following seeding) (data not shown). Generally, the plants growing at the 16 dS $m^{-1}$ showed delayed onset of boot and flowering stages. At 8 dS $m^{-1}$, the average shoot biomass per plant of InVigor 5030 equalled that grown with the nutrient-only solution. AC Arid, AC Sunbeam, AC Vulcan, Carinata, Centennial Brown, and Westar plants produced noticeably less shoot biomass at 8 than at 1.4 dS $m^{-1}$. InVigor 9590 and AC Base biomass yields per plant at 8 dS $m^{-1}$ were significantly greater than these five, but less than their respective shoot averages at 1.4 dS $m^{-1}$. AC Estlin, a *Brassica juncea* cultivar, fared the worst. At 16 dS $m^{-1}$, shoot biomass per plant was reduced in all cultivars; the InVigor cultivars produced the most per plant. These were followed by Westar, Carinata, and AC Base. AC Arid and Estlin, AC Sunbeam, AC Vulcan, and AC Centennial Brown had the lowest plant biomass yields.

Tentative comparisons of the relative biomass produced per plant revealed decreasing salinity tolerances at 8 dS $m^{-1}$ of InVigor 5030 ≥ Arid = InVigor 9590 > Westar = Base = Vulcan = Centennial Brown > Sunbeam = Carinata = Estlin (Figure 6). At 16 dS $m^{-1}$, the decreasing order was InVigor 9590 > InVigor 5030 > Westar ≥ Arid = Base = Vulcan = Sunbeam = Carinata = Centennial Brown = Estlin. [All results are preliminary.]

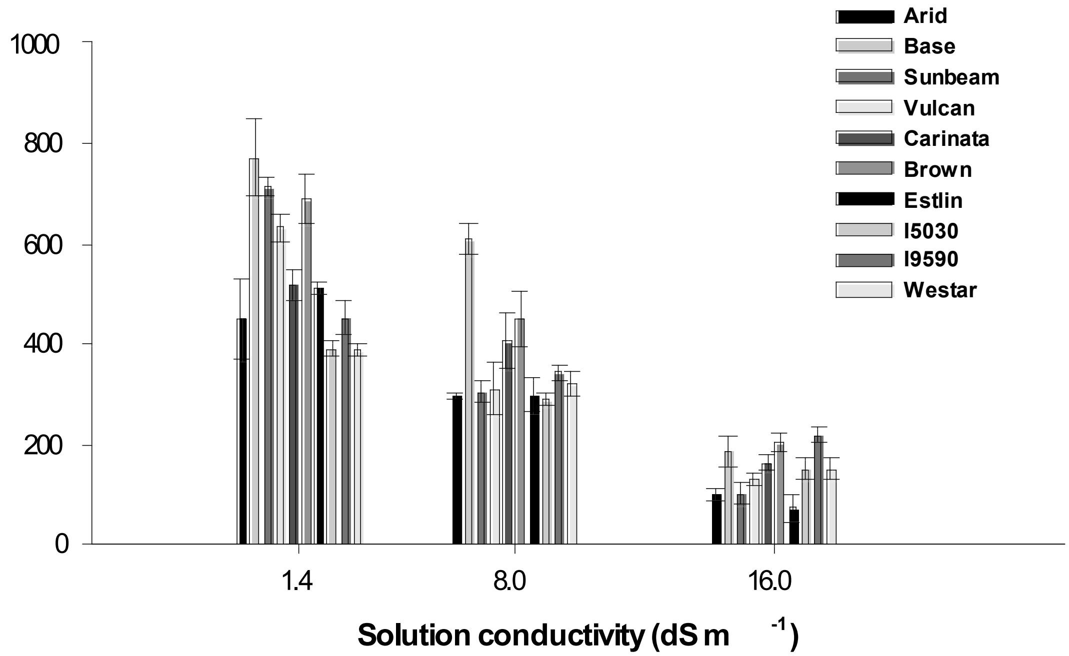


**Figure 5.** Average plant height of selected canola/mustard cultivars (Second Test) associated with three salinity levels; error bars indicate standard error.

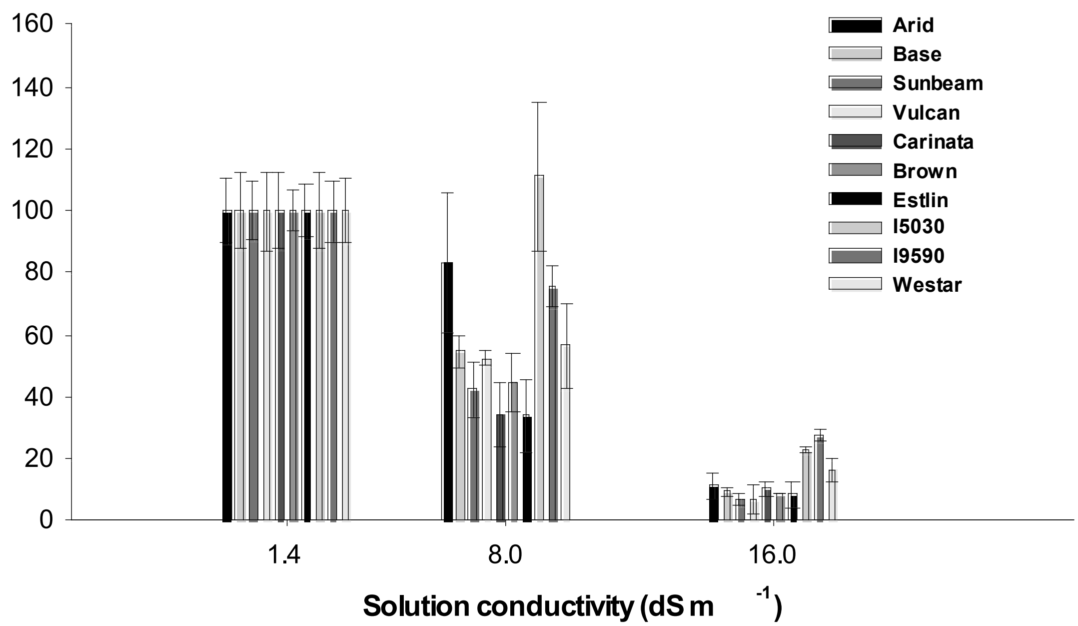


**Figure 6.** Average relative shoot biomass (% of salt-free treatment) of selected plant canola/mustard cultivars associated with three salinity levels; error bars indicate standard error.

## Conclusions and Acknowledgment

Although very preliminary, the result of the comparisons reported here show that the salinity tolerance of three cultivars (AC Arid, InVigor 5030 & 9590) exceeded that of the older cultivar (Westar). On average, the shoot biomass that the three produced per plant while subjected to near-moderate salinity conditions equalled 70% or better for the three grown under the nutrient-only control; the next best equalled 55% for Westar. Another 25 to 30 cultivars are yet to be evaluated and reported. Financial support for this research by the Saskatchewan Canola Development Commission is very gratefully appreciated.

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