Effect of High Temperature Stress on Yield and Yield Components of Three Brassica Species.

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Introduction:

The changing government policy, the world price situation, the increasing awareness of farmers of sustainability and the availability of technology have encouraged farmers in the semiarid prairie to diversify their cropping system. Canola is an ideal choice for a oilseed crop in the system as the early maturing dwarf sunflowers have failed in the region (Miller, 1998). However, crops in semiarid prairie face many stresses of which water and temperature are often the most important. Canola is a cool season crop and is believed to suffer from high temperature stress. Analysing relationship between seed yield of canola and the growing season temperature has indicated negative relationship even in relatively cooler and moist Melfort and Scott (Nuttal et.al., 1992; Brandt and McGregor, 1997). The mean temperature in semiarid prairie is increasing (Cutforth; personal communication) and global warming is expected to result in marked increase in events of extreme temperatures. Higher wind speed and water deficit in the region are expected to increase heat stress experienced by crops. In such dry conditions plant temperature is often higher than the air temperature (Mahan, et. al., 1995). Hence, there is an urgent need for identifying a *Brassica* species or genotype which can fit in semiarid prairie cropping system.

The most common oilseed *Brassica* species grown in Canada include *B. napus L.*, known as Argentine canola, *B. rapa L.*, known as Polish canola and *B. juncea L.*, commonly known as mustards. Argentine canola has a better yield potential, but is considered more susceptible to abiotic stresses. Nuttal (1992) estimated 0.4 t/ha reduction in Argentine canola yield for every 3° C rise in temperature. Polish canola is also susceptible to heat stress (Brandt and McGregor, 1997). Mustard is reported to be better adapted to semiarid prairie than other two species (Rakow, 1995). However, in spite of resistance to shattering and blackleg disease, poor oil quality of *B. juncea L.* was restricting its market and thereby adoption in the prairie. Recent success in developing new lines of *B. juncea L.* with oil quality similar to canola genotypes, has renewed interest of *B. juncea L.* in semiarid prairie. Limited research on the direct effect of high temperature stress on B. species has been done in the past and no research on the heat stress tolerance of *B. juncea L.* or comparing *B. juncea L.* with other oilseed B. species is reported. Therefore there is need to generate information on heat stress to help determine which B. species is best suited to semiarid prairie.

The seed yield of oilseed Brassica plant is determined by number of pods, seeds per pod and seed weight (McGregor, 1981). Number of pods is determined by the initiation of floral bud and is dependent on the plant biomass and the combined stress level at that time. Seeds per pod depends on photosynthetic supply during fertilization, while seed weight depends on the continued supply of photosynthates after fertilization until maturity. Thus yield adjustment takes place from seeding to maturity. However, reproductive stage has been identified as the most susceptible stage for temperature stress in most of the crops in which temperature response is studied (Hall, 1992; Paulsen, 1994). From a farmers survey Brandt and McGregor (1997) concluded that abiotic stresses are affecting canola grown in Saskatchewan between flowering and pod formation stage.

The overall aim of the current study was to determine the most sensitive crop growth stage for high temperature stress among three oilseed Brassica species, variation in critical temperature and to assess whether canola quality *Brassica juncea L.* is a better crop for semiarid prairie from high temperature tolerance perspective. Further, the B. species were evaluated for their ability to recover from heat stress.

Materials and Methods:

A controlled environment growth chamber experiment was conducted at the Semiarid Prairie Agriculture Research Station, Swift Current, Canada. A canola quality *Brassica juncea L.* genotype, CQ1, was compared with one genotype from each species, namely *Brassica* napus (Quantum), *B. rapa L.* (Parkland) and *B. juncea L.* (Cutlass) for high temperature tolerance. Milk cartoons of 2 L capacity filled with soil (Swinton silt loam) were used for the study. Peat moss was mixed in to the top soil layer to prevent crusting. Several seeds, treated with vitavax, were planted in the milk cartoons and later thinned to one plant per pot at the 2-3 leaf stage. All genotypes used in this trial have different growth and development patterns, therefore, to narrow down the development phase, planting was staggered within a temperature treatment. Plants were watered regularly to avoid water stress. Beginning three weeks after seeding, every alternate week nutrient solution(4.2 g of 20-20-20 dissolved in 1 L water) was used for watering the plants. Aphid infestation was controlled by spraying systamic insecticides. Self incompatiblity and failure of pollination (Morrison, 1993) are problems in such studies. Therefore two plants of each genotype were used in each replication and they were treated as one experimental unit. During flowering, both the plants were shaken together to ensure better pollination. Randomised complete block design with 6 X 5 factorial treatment structure and four replications was used for the study. Plants were rearranged within replication until flowering.

Plants were grown in the growth chamber (Model GR96, CENVIRON, Control Environment Ltd., Winnipeg, Canada) at 20/15 °C day night temperature till the high temperature treatments were imposed. The relative humidity (RH) was maintained above 60 % and photosythetic flux at the leaf level was 300 μmol m^{-2} sec^{-1}. Temperature in the canopy was

monitored at two locations within the growth chamber and it was used to adjust the chamber settings. Two high temperature stress treatments, 28/15 and 35/15 °C, were imposed for one week period either during the flowering or the pod formation stage. To optimize the use of high temperature growth cabinets the 28/15 °C plants were seeded 3 weeks before those subjected to 35/15 °C treatment. Further, plants intended to be stressed at flowering stage were planted one week later to have plants transferred into high temperature cabinets (Model PGW36, CENVIRON, Control Environment Ltd., Winnipeg, Canada) in a short period of time. In spite of the staggered planting, there was a small variation in the developmental stage among treatments and/or replications. Therefore, whenever each experimental unit (2 plants in two separate pot) reached required developmental stage, it was moved to high temperature growth cabinets. The RH was maintained above 70 % and regular watering minimized the water stress due to high temperature. Temperature and RH control relied on a 'Viasala sensor' positioned in the lower part of the canopy to sense RH and temperature of growth cabinets. After the stress period, plants were moved back to their respective replications in the growth chamber. At late pod filling stage, plants from 35/15 °C temperature treatment were moved to a green house (growth chamber breakdown necessitated) and maintained at 22 °C till maturity.

Yield and yield components of terminal raceme (shoot) was assessed in all treatment combinations. Total number of viable pods, seeds per pod, thousand seed weight and seed yield per terminal raceme was recorded on at least one plant in each replication. Seed yield per plant was recorded on all plants. The number of flowers that opened in 35/15 °C treatment applied at flowering was recorded. To observe effect of high temperature on floral characters, flowers opened during the first six days of 35/15 °C stress at flowering were marked with color markers and monitored closely. This marking identifies the beginning and end of stress period on the terminal raceme. Outliers from the data were removed by using JMP software (Mahalanobis analysis). All observations were averaged across two plants in each treatment unit and mean values were used for statistical analysis. Analysis of variance was conducted using GLM procedure (SAS Institute Inc., Cary, NC).

Results and Discussion:

The differences noted for the terminal raceme showed a significant effect of one week of high temperature on seed yield and yield forming traits (Table 1). Seed yield of terminal raceme of all genotypes has reduced at 35/15 °C stress at flowering, while it increased with 28/15 °C in *Brassica juncea L.* and B. rapa L. This indicates that the optimum daytime temperature for *B. juncea L.* and *B. rapa L.* cultivar was above 20 °C and is close to 28 °C. In contrast, the decrease in seed yield at 28/15 °C at flowering in *B. napus L.*, though it was statistically non-significant, indicated that the optimum for *B. napus L.* is around 20 °C. All genotypes have a narrow optimum temperature range and either side of that optimum range reduces plant yield (Mahan, et.al., 1995). The terminal raceme yield indicates that Argentine canola may have optimum temperature range cooler than other two species. Our results do not fully agree with the findings

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of Morrison (1993) who reported complete sterile flowers in Argentine canola (Delta and Westar) at 27/17 °C. The differences in the results are probably related to the water stress. In this study plants were watered regularly and humidity was always maintained above 60%. As a results plants retained their stomatal conductance during the temperature stress period to the levels of prestress period. The leaf temperature during the stress period was at least 2 °C lower than air temperature (data not presented). However, our findings closely follow those of Polowick and Sawhney (1988).

The method of stressing during flowering or pod development stage adopted in this study revealed significant differences among the genotypes used in this study. Most of the heat stress work on canola or mustard have used continuous stress after particular developmental stage (Morrison, 1993; Morrison et.al, 1989). The method of stressing only 7 days represents the field situation in the semiarid prairie where long term weather data indicates that at least 7 days in a year experiences daily maximum temperature of 32 °C or higher (McCaig, 1996). A short period of extreme temperature at a sensitive stage can be as critical to the crop yield as a mild temperature stress over a longer period (Howarth, 1996). Flowering period in *Brassica* spp. ranges from 2-3 weeks and the plant produces more flowers than its photosynthetic machinery can sustain. As a result most of the late formed flowers are aborted (McGregor, 1981). Therefore this study targeted the critical stage, manifesting the cultivar responses.

The flowering pattern of *Brassica* spp was affected by high temperature stress. Severe stress of 35/15 °C during flowering, progressively reduced the number of flowers opened during the stress (Fig. 1). Thus number of flowers opened between 4th and 6th day of the stress period was only 27 % of that during the first two days of stress in Quantum, lower than Cutlass and CQ1. *B. napus L.* produces fewer pods and branches than *B. rapa L.* (McGregor, 1981) and is less responsive to the favorable conditions that prevail later in the season (Brandt, 1997). In addition, *B. napus L.* was the earliest one to show a stress response among the three species compared.

The recovery from heat stress was different among species and was affected by temperature and growth stage. When the heat stress (35/15 °C) was imposed at pod formation, pod size reduced gradually and we observed unfertilized pods or aborted pods were seen in all genotypes. Later the terminal raceme recovered and produced a few normal pods. The 35/15 °C at flowering was monitored more closely and the flowers opened in high temperature (up to sixth day) were marked. None of the flowers opened in 35/15 °C formed any viable pod in any of the genotypes studied, indicating that 35/15 °C is at or above threshold temperature for all three *Brassica* spp. Photosynthesis measured 3 days into the high temperature stress indicated that the photosynthate was not a limitation for fertilization and pod formation. The effect of water deficit was also minimized in our studies. Therefore in the present study heat stress influenced flowering directly. Similar observation was reported in wheat by Wardlaw et. al. (1980).

In the present study, the flowers opened in the normal temperature after moving back from high temperature stress (35/15 °C) at flowering produced abnormal pods in Quantum, which were plump and short (2-2.5 cm). Similar abnormal pods were noticed by Morrison (1993) and they attributed it to failure of fertilization due to high temperature, leading to parthenocarpy. But, in our study these abnormal pods were invariably formed after stress relief and were more common in Quantum plants stressed at flowering and not in plants stressed at pod formation (which produced many flowers during the stress period). Also it was not common in Maverick and was never seen in *B. juncea L.* genotypes. Further all genotypes in the present study produced seeds after the 35/15 °C stress at flowering. This indicates the abnormal pods might have been produced by some other physiological response. Another possible hypothesis is that the Quantum plant during the stress period continued its photosynthesis and accumulated most of the photosynthates in (in spite of additional loss due to increased respiration) temporary storage organs (stem and leaf). When temperature stress was relieved, most of the stored photosynthate was rushed to pods, the growth of which was still recovering, leading to bulged, short pods. Closer observation of abnormal pods revealed that number of seeds in the abnormal pod were similar to normal pod, but due to lack of space they were squished together. This hypothesis is supported by reduced frequency of such abnormal pods in Quantum plants which were stressed at pod formation. At this stage, the already formed pods acted as sink to absorb the extra flush of photosynthate after stress relief. The senescence of most of the leaves by Quantum after returning to control temperatures might have contributed to the photosynthate flush. In *B. juncea L.* senescence of leaf was slower and so less photosythates were retranslocated to pods as suggested by lack of abnormal pods.

Terminal raceme yield variation due to high temperature was closely followed by variations in number of viable pods (PN_{main}), thousand kernel weight (TKW_{main}) and seeds per pod (SPP_{main}) on the terminal raceme (Table 1). However, the major contribution was from PN_{main}. The role of pods in establishing yield potential in canola has been reported (McGregor, 1981; Brandt and McGregor, 1997; Morrison, 1993). PN_{main} increased with 28/15 °C temperature at flowering in all genotypes. However, there was no response when the same 28/15 °C temperature was imposed at pod development stage. The response with more severe stress (35/15 °C) was quite different from milder stress (28/15 °C), wherein the stress at flowering (most reproductive parts were flowering or younger) reduced PN_{main} drastically, while the same stress at pod formation (wider range of reproductive parts from pod formation to bud stage) accounted for the same or higher PN_{main} than control. The reduction in PN_{main} at flowering is in accordance to Morrison (1993), who proposed that the sensitive stage for heat stress is between late bud to early seed development. But higher or same PN_{main} in heat stress at pod formation cannot be explained from the above. All genotypes in 35/15 °C had fertile pods at the tip portion of terminal raceme (abnormal pods in Quantum were counted as viable pods), which otherwise would have been sterile. This observation defends the theory that canola uses the later formed extra pods for yield formation in the event of failure of earlier formed pods due to stress (McGregor, 1981). Severe heat stress at flowering reduced SPP_{main}, but was not consistent in all genotypes. The possibility of more seeds in the pods produced after stress was relieved might have confounded this

observation. The reduction in TKW$_{main}$ due to severe heat stress of 35/15 °C was observed in the present study, which was also observed in Argentine canola by Morrison (1993).

Seed yields of all Brassica genotypes investigated in this study responded to temperature stress, while significant temperature X genotype interaction (p=0.01) indicated the variation among species. Seed yield per plant followed a trend similar to that of terminal raceme (Table 2). *B. rapa L.* (Parkland) was the most sensitive species in which yield reduction due to heat stress was up to 93% of the optimum temperature. In *B. napus L.* (Quantum) yield reduction due to heat stress was 34%, which was similar to *B. juncea L.* (Cutlass). Canola quality *B. juncea L.* (CQ1), which responded similar to Cutlass at most temperatures, was more sensitive to severe heat stress at flowering. This genotype had different response to severe heat stress including increased plant height (data not shown) and continued flowering during the stress (Fig. 1) when others have reduced flowering. Therefore, probably a higher proportion of flowers in CQ1 suffered from heat stress than other genotypes.

Conclusions:

Cultivars of all three Brassica species were susceptible to midday temperature of above 35/15 °C. The damage to the yield forming traits was more severe when the stress occurred earlier during flowering. Heat stress imposed at pod formation indicated that many of the floral structures had passed sensitive stage and had matured in to productive pods. However, we could not ascertain from our observations in the present study what developmental stages of floral structure are susceptible to heat stress. Among the species, *B. rapa L.* was most susceptible while *B. napus L.* and *B. juncea L.* very close to each other in heat stress response. Canola quality *B. juncea L.* did not establish any superiority over either Cutlass or Quantum. More work in the field and in controlled environment is required to identify the critical temperature, reproductive stage and genotypic variation.

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Table 1. **Effect of high temperature stress on the seed yield and yield forming traits of main shoot (raceme) of different *Brassica* species.**

Temperature Stage °C		Seed Yield g	Pod Number	TKW g	Seeds Pod[-1]
--------------------- Canola Quality *Brassica* juncea 1 --------------------					
20/15 (Control)		0.88	34	3.16	8.3
28/15	Floweringgg	1.87	68	2.92	9.6
28/15	Pod	0.83	36	3.11	7.5
35/15	Floweringg	0.23	12	2.24	6.0
35/15	Pod	0.96	61	2.46	6.2
		**	***	+	
Lsd (0.10)		0.53	16	0.80	ns
----------------------- Cutlass (*Brassica* juncea) -----------------------					
20/15 (Control)		0.52	16	3.57	9.4
28/15	Floweringg	1.73	50	3.34	10.4
28/15	Pod	0.53	23	3.92	5.4
35/15	Floweringg	0.10	13	1.54	4.4
35/15	Pod	1.01	39	2.24	11.8
		***	***	**	*
Lsd (0.10)		0.30	10	0.80	3.42
---------------------- Quantum (*Brassica* napus) --------------------					
20/15 (Control)		1.66	32	4.29	11.42
28/15	Floweringg	1.22	43	3.37	9.79
28/15	Pod	0.85	28	4.45	7.14
35/15	Floweringg	0.09	14	2.10	2.81
35/15	Pod	0.87	32	2.56	8.82
		**	***	***	*
Lsd (0.10)		0.60	7	0.70	3.68
----------------------- Parkland (*Brassica* rapa) ----------------------					
20/15 (Control)		0.31	32	2.97	-
28/15	Floweringg	0.82	46	2.27	-
28/15	Pod	0.05	13	2.83	-
35/15	Floweringg	0.01	3	1.03	-
35/15	Pod	0.21	46	1.16	-
		**	*		
Lsd (0.10)		0.19	25	ns	

+, *, **, *** Represent F-Test significance at 0.10, 0.05, 0.01 and 0.001 levels, respectively.
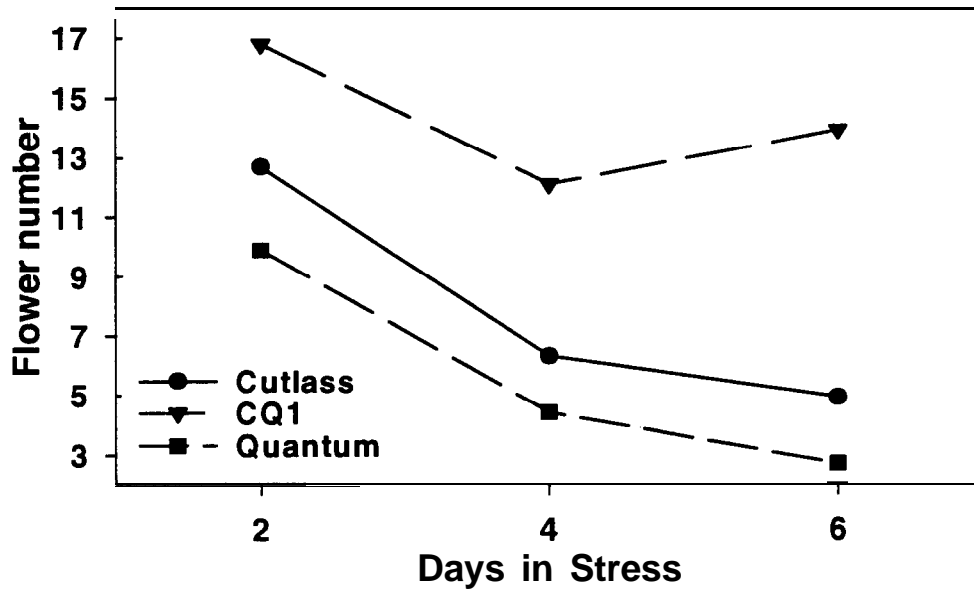
Fig.1.   Effect of 35/15 °C temperature stress at flowering on the rate of flower production on the terminal raceme of Argentine canola and Brassica juncea.

Table 2.   Effect of high temperature stress on the seed yield (plant') of different **Brassica** species.

Temperature Stage °C	CQ1	Cutlass	Quantum	Parkland	Mean
20/15 (Control)	7.63	6.76	9.60	3.89	7.02
28/15 Flowering	8.73	7.94 .	7.67	5.84	7.79
28/15 P o d	8.37	6.93	8.35	1.23	6.22
35/15 Flowering	3.39	5.25	6.30	0.38	4.16
35/15 P o d	6.79	6.08	8.65	1.50	5.74
	*	+	+	**	**
Lsd (0.10)	2.13	1.47	1.90	1.81	1.28

+, *, ** Represent F-Test significance at 0.10, 0.05 and 0.01 levels, respectively.