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12 **Comparison of Osmotic Adjustment, Leaf Proline Concentration,**
13 **Canopy Temperature and Root Depth for Yield of Juncea Canola**
14 **under Terminal Drought**

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24   **Abstract**

25   Two glasshouse and two field experiments were conducted in 2013 and 2014 to
26   compare the relative importance of four physiological traits: osmotic adjustment
27   (OA), leaf proline concentration, canopy temperature depression (CTD) and root
28   depth on drought performance of canola quality *B. juncea* (juncea canola).
29   Glasshouse experiments were conducted at The University of Melbourne, Parkville
30   and field experiments were conducted at Horsham, Victoria. The experiments used
31   juncea canola hybrids and their parental lines and were laid out in a randomised
32   complete block design with three replications. The glasshouse experiments consisted
33   of two treatments, well-watered and water deficit from first open flower to maturity
34   whereas the field experiments were sown at a site that received 266 mm annual
35   rainfall in 2014. In the glasshouse, canopy temperature depression was the only trait
36   to show a positive and consistent association with drought performance of juncea
37   canola. Cooler canopy temperature was also associated with improved yield in field
38   experiments. Root depth was positively correlated with CTD in 2014 in glasshouse
39   whereas no correlation of root depth with OA and leaf proline was observed. The
40   results indicated that CTD was the only reliable trait among those tested to screen
41   juncea canola for drought tolerance. Root depth of juncea canola hybrids was a
42   constitutive trait and probably was a result of hybrid vigour.

43   **Key words:** juncea canola, osmotic adjustment, canopy temperature, root depth,
44   terminal drought

45   **Introduction**

46   Brassica oilseeds are likely to face drier and hotter growing environments in future
47   with changing climate (Torrey 2007; Watkins 2008). *Brassica juncea* could expand
48   the options for Brassica oilseeds in the low rainfall cereal belt of southern Australia.
49   Canola quality *Brassica juncea* (juncea canola) is being bred for low rainfall
50   environments of southern Australia that are not suitable for cropping *B. napus*. As
51   the current open-pollinated cultivars of juncea canola need yield improvement (Gan,
52   Malhi et al. 2007; Gunasekera, French et al. 2009), hybrid cultivars of juncea canola
53   are being developed and tested in Australia to improve yield potential of the crop.

54 Inconsistent and unpredictable environmental conditions cause difficulty in direct
55 selection for yield in southern Australia.. Selections based on physiological traits can
56 improve efficiency of breeding programmes for yield improvement (Araus 1996;
57 Richards 1996; Slafer and Araus 1998; Reynolds, Hays et al. 2010). Physiological
58 traits have been shown to improve drought performance (as measured by relative
59 biomass, yield, or other traits under drought stress compared with well-watered
60 conditions) of cereals and other crops. For example, increased osmotic adjustment in
61 wheat (Morgan 1991; Morgan 2000), reduced anthesis-silking interval in maize
62 (Bruce, Edmeades et al. 2002; Bänziger, Setimela et al. 2006), stay-green in sorghum
63 (Rosenow, Quisenberry et al. 1983; Borrell, Hammer et al. 2000) and deeper rooting
64 in rice (Courtois, Shen et al. 2003). Deeper rooting has also been found to be one of
65 the most important traits to promote greater biomass production and higher harvest
66 index under water deficit in wheat (Morgan and Condon 1986), sorghum
67 (Santamaria, Ludlow et al. 1990), and sunflower (Chimenti, Pearson et al. 2002).

68 A number of physiological traits have also been shown to be associated with drought
69 performance in Brassica oilseeds. Norouzi, Toorchi et al. (2008) showed that leaf
70 osmotic adjustment had strong positive relationships with shoot dry weight ($r=0.74$)
71 and seed yield ($r=0.57$) of *B. napus* under water deficit. *B. juncea* lines were also
72 observed to be more tolerant of severe water deficit than *B. napus* cv Monty due to
73 higher osmotic adjustment (OA) and more access to soil water under post-flowering
74 drought in Western Australia (Gunasekera, French et al. 2009). Leaf proline
75 accumulation was found to be useful in selection for drought tolerance in Brassica
76 oilseeds in studies by Ma and Turner (2006) and Phutela, Jain et al. (2000). Cooler
77 leaf and (in particular) bud temperatures below ambient were also associated with
78 drought-tolerance (biomass accumulation) in *Brassica rapa* following transient
79 drought stress (Guo, Turner et al. 2015).

80 Measurements of OA, proline and roots require the destruction of the plants and
81 demand a considerable amount of time and resources. In comparison, measurement
82 of canopy temperature is non-destructive and is easily measured with an infrared
83 camera. Measurement of canopy temperature saves time and cost and provides
84 opportunities for repeated and large scale measurements.

There are few reports of comparative studies of physiological traits in relation to their association with drought performance in *Brassica juncea*. This study aimed to assess the relative importance of OA, leaf proline concentration, root depth and canopy temperature to drought performance of juncea canola hybrids and their parents.

2. Materials and Methods

2.1. Plant materials

Three juncea canola hybrids with parental lines and one open-pollinated (OP) juncea canola control cultivar (OasisCL) were included in the glasshouse experiment in 2013 (Table 3). In 2014, eight juncea canola hybrids, three seed parental lines and OasisCL were included in the glasshouse experiment (Table 4). A field trial was undertaken at Horsham (36.6°S, 142.1°E), Victoria in 2014. Trial consisted of two sets of *Brassica* genotypes, set 1 (11 juncea canola hybrids, one juncea canola cultivar and four *B. napus* cultivars) and set 2 (51 juncea canola hybrids, one juncea canola cultivar and two *B. napus* cultivars). The genotypes are listed in Tables 5 and 6.

Table 1

2.2. Experimental details

Glasshouse details

Experiments were conducted in glasshouse (2013, 2014) and field (2014) conditions (Table 1). Glasshouse experiments were conducted at The University of Melbourne, Parkville (37.8°S, 144.9°E). Glasshouse had natural light and the temperature was controlled at 22±4°C.

In the glasshouse experiments, plants were grown in 75 cm long PVC tubes of 10 cm diameter. A bottom layer (3 cm) of the tube was filled with gravel and the bottom cap of the tubes had 4-5 holes to drain water. The tubes were filled with a mixture of 1:1 sand and Red Ferrosols (Isbell 2002) collected from a farm in Creswick (37.43°S, 143.90°E, Victoria) leaving the 5 cm top for irrigation. All the tubes were filled and weighed to ensure that each tube had an equal amount of the soil mixture. The experiment was laid out in a randomised complete block (RCB) design with

three replications. Each genotype was planted in three tubes per replication. In 2013, seeds were sown directly in the tubes in 5-6 holes and thinned at 4-5 leaf stage leaving only one plant in the middle of the tube. In 2014, because of logistical reasons, seeds were sown separately in seedling raising mixture and plants were transplanted to tubes 40 days after sowing. One tube was harvested at first open flower (FOF) and the remaining two were left until physiological maturity. The tubes were rotated within replication every week to avoid spatial effects.

Field details

A field trial was conducted at Horsham (36.6°S, 142.1°E), Victoria in 2014. The field experiments were conducted under rain fed conditions and laid out as an RCB design with three replications. The genotypes were planted in six rows of 10 m long and 25 cm apart.

2.2.1. Water deficit (WD) and well-watered (WW) treatments in the glasshouse

The water treatments were imposed on the two tubes per replicate. One tube received regular watering (WW) until physiological maturity, while the other plant had water deficit (WD) imposed from FOF stage. The plant was irrigated till saturation on the day of FOF and further irrigation was withheld. The tube was put on a layer of paper towels to absorb water from bottom and the top of the tube was covered with plastic to minimise soil evaporation. The tubes were allowed to drain for 24-72 hours after saturation, weighed to estimate the mixture's drained upper limit (field capacity). The weight of the tube when lower leaves started to wilt was taken as the lower extractable limit (wilting point). The amount of available water was calculated for WD treated plants as difference between tube weights at field capacity and at wilting point. Half of the available water was supplied when the lower leaves started to wilt. The drought treatment was given to each WD treated plant until physiological maturity based on tube weights and wilting of individual plants. A disk from the youngest expanded leaf was taken from both WD and WW treated plants to estimate relative water content (RWC). The leaf disks were taken when the WD treated plant wilted for the first time. Fresh weights (FW) of the disks were recorded immediately after sampling and then hydrated by floating on de-ionized water for 3 hours. The disks were wiped dry with tissue papers and turgid weight (TW) was recorded and

oven-dried at 70°C overnight to record dry weight (DW). The RWC was calculated as $RWC\% = \frac{(FW-DW) \times 100}{(TW-DW)}$.

2.3. Observations and data analysis

In the glasshouse, plant measurements were taken during the period of FOF and maturity (MAT). MAT was recorded when 80% pods changed colour from green to pale yellow. After harvest, pods were hand-threshed and seed yield per plant was recorded. Leaf samples were collected at least four weeks after FOF and stored at -80°C to assess the degree of osmotic adjustment and leaf proline concentration. RWC of the leaf samples were also recorded as mentioned above.

Measurements of osmotic adjustment

The frozen leaf samples were thawed at room temperature (25°C) and leaf sap was extracted by using a benchtop centrifuge at 14,000 rpm for 5 minutes. Osmolarity of leaf sap was measured by using a freezing point osmometer (Osmomat 010, Gonotech GmbH) which was calibrated using distilled water and NaCl solution. The osmolarity was converted to osmotic potential (OP) as following: $OP = Osmolarity \times (-R) \times T$, where OP, osmotic potential (Mpa); R, gas constant (0.008314); T, temperature (298°K). The OP values were multiplied by RWC values to make them comparable between WD and WW treatments and the difference between OP values between WD and WW treatments of a genotype was taken as OA.

Measurements of leaf proline concentration

Leaf proline concentration was determined using the acidic ninhydrin method developed by (Chinard 1952) and modified by (Bates, Waldren et al. 1973). Thawed samples were ground with 3% salicyclic acid and the plant extracts were incubated in the reaction mixture (sulfosalicyclic acid, glacial acetic acid and acidic ninhydrin) at 96°C for an hour. Toluene was added to the mixture to separate the chromophore and its absorbance was measured at 520 nm using toluene as reference. Proline concentration was calculated using standard curve of L-proline.

Measurement of canopy temperature depression

175 In the glasshouse, three infra-red images of plants were taken in seed filling stage
176 using FLIR T450SC camera (FLIR Systems) from noon to 2.00 pm in sunny
177 weather. The images were processed using MATLAB® software to estimate canopy
178 temperatures. While processing thermal photographs, the background pixels were
179 eliminated by setting the highest and the lowest temperatures within the plant pixels
180 and the plant pixels were averaged to get canopy temperature. The air temperature
181 was measured with a digital thermometer (CheckTemp®1, model HI98509,
182 HANNA Instruments Inc.) for each infra-red image. The difference between the
183 canopy and the air temperature (canopy temperature depression, CTD) was used to
184 compare the onset of stress in different genotypes over time.

185 *Root biomass at depth*

186 After harvest, the bottom cap of the tube was removed and the roots from the gravel
187 (3 cm bottom layer) were collected, washed and oven-dried at 70°C overnight to
188 record dry weight. The root biomass at the bottom was used as an indicator of the
189 root depth.

190 *Measurements in field*

191 Seedling emergence of each field plot was recorded as visual scores (1= very poor
192 emergence, 9=complete emergence). The middle 8 m² of each field plot was
193 machine-harvested to estimate seed yield per hectare. Two infra-red images of the
194 plots were taken at mid-day (1.00-2.00 pm) 6 weeks after the plots reached 50%
195 flowering. Canopy temperature was estimated by processing the images with
196 MATLAB® software. The two measurements were averaged and recorded for each
197 plot. The differences between air temperature and canopy temperature were recorded
198 as canopy temperature depression. The air temperature was measured with a digital
199 thermometer (CheckTemp®1, model HI98509, HANNA Instruments Inc.) for each
200 infra-red image.

201 Data were analysed with SAS v9.1.3 (SAS Institute Inc. 2004). Normal ANOVA
202 was used to analyse the effects of genotypes, treatments and their interaction. Seed
203 yield data were first analysed with emergence score as a covariate. As the emergence
204 scores had no significant effect on seed yield, emergence score was dropped out
205 from the model. Data were transformed for the variables that showed heterogeneity

206 of variance. Means were compared using least significant difference (LSD). Effects
207 of various traits on seed yield were also studied using correlation and regression
208 analysis.

209 **3. Results**

210 **3.1. Water deficit in glasshouse**

211 Water deficit treatment significantly reduced RWC of the fully expanded uppermost
212 leaf in both glasshouse experiments. Genotypic differences for RWC were not
213 significant. RWC for water deficit and well watered treatments averaged 67 and 85%
214 in 2013; and 68 and 88% in 2014, respectively.

215 **3.2. Rainfall during growing season**

216 For the field trial, 2014 was a dry year at Horsham having only 266 mm annual
217 rainfall. Horsham had only 159 mm during the growing season (May to November)
218 and the post-flowering period (Sep-Nov) was severely stressed (only 36 mm rainfall)
219 (Figure 1).

220 Figure 1

221 Table 2

222 **3.3. Effects of water deficit on seed yield and physiological traits in glasshouse**

223 In glasshouse experiments, genotypes differed significantly ($p < 0.05$) for all the traits
224 measured: seed yield, OA, proline concentration, canopy temperature depression and
225 root biomass at bottom in 2013 and 2014 (Tables 2-4). In overall, water deficit
226 significantly reduced seed yield, CTD and relative water content whereas water
227 deficit significantly increased leaf proline concentration in both the experiments.
228 Root biomass at bottom was significantly increased under water deficit in 2013
229 whereas it was significantly decreased under water deficit in 2014.

230 In 2013, one hybrid (HJM1Z-2013) under WW and two hybrids (HJM1Z-0027 and
231 HJM1Z-2013) under WD conditions out-yielded OasisCL, respectively (Table 3).
232 Only two parental lines – JO5Z-09677 and JMOZ-910395 had significantly higher
233 OA than OasisCL. All the hybrids and another parental line (JMOZ-907526) had
234 similar or lower OA than OasisCL. Under WW, HJM1Z-2034 and two parental lines

(JMOZ-907526 and JMOZ-910395) had higher proline concentration than other genotypes whereas all the hybrids and parental lines had higher leaf proline concentration than OasisCL under WD. Two hybrids (HJM1Z-0027 and HJM1Z-2013) and a parental line (JO5Z-09677) generally had higher CTD than other genotypes under both treatments. All the genotypes except HJM1Z-1034 had higher CTD than OasisCL under WW. However, only JO5Z-09677 had higher CTD than OasisCL under WD. Only HJM1Z-0027 produced higher root biomass at bottom than OasisCL under WW treatment whereas all the hybrids and parental lines (except JMOZ-910395 and JMOZ-907504) had higher root biomass at bottom under WD treatment.

Table 3

Table 4

In 2014, all the hybrids except HJM1Z-2021 and two parental lines (J07Z-07140 and J07Z-04065) had higher seed yield than OasisCL under WW condition (Table 4). All the genotypes except HJM1Z-2136 had significantly higher OA than OasisCL. Two hybrids (HJM1Z-0039 and HJM1Z-2137) and two parental lines (J07Z-07140 and J07Z-04065) had higher leaf proline concentration than other genotypes under WD treatment. J07Z-07140 had significantly higher proline concentration than other genotypes under WW treatment as well. All the hybrids and parental lines except JO5Z-09677 had lower CTD than OasisCL under WD. However, two hybrids (HJM1Z-0027 and HJM1Z-2133) and parental line (J07Z-04065) had similar CTD to OasisCL under WW treatment. HJM1Z-2021 had significantly highest root biomass at bottom under both treatments.

3.4. Seed yield and canopy temperature depression in field

Genotypes had significant differences for seed yield and canopy temperature in both trials (Tables 5 and 6). In field trial (set 1), five juncea canola hybrids produced significantly higher seed yield than OasisCL and two of the hybrids produced higher yield than 44Y84, the best yielding *B. napus* hybrid (Table 5). Most of the hybrids had higher CTD than juncea canola and *B. napus* control cultivars. OasisCL had higher seed yield than *B. napus* control cultivars in field trial (set 2) (Table 6). Six

265 juncea canola hybrids produced higher yield than OasisCL and two hybrids had
266 higher CTD than OasisCL.

267 Table 5

268 Table 6

269 **3.5. Relationships between seed yield and physiological traits**

270 In 2014, a hybrid (HJM1Z-2021) and a parental line (JO5Z-09677) and OasisCL
271 were excluded from regression and correlation analysis. HJM1Z-2021 had low seed
272 yield because of poor pod and seed set (Table 4). JO5Z-09677 and OasisCL, much
273 earlier than other genotypes, were affected by delayed transplanting (40 days after
274 sowing) and yielded unexpectedly low. Each data point is a mean value.

275 Seed yield was positively related to CTD in the WD treatment in both glasshouse
276 experiments (Fig 2). CTD had strong positive relationships with seed yield under
277 WD in 2013 ($R^2=0.50$) and 2014 ($R^2=0.62$), respectively. Root biomass at bottom
278 also had a strong positive relationship with seed yield under WD in 2014 ($R^2=0.54$).

279 Figure 3 details the relationships between yield and CTD for the field trials. Seed
280 yield had only a weak positive relationship with CTD in both trials with R^2 values of
281 0.41 (set 1) and 0.31 (set 2).

282 Figure 2

283 Figure 3

284 The physiological traits (OA, leaf proline concentration, CTD and root biomass at
285 bottom) were not related to each other under WW treatment. Under WD, only CTD
286 and root biomass at bottom were positively related ($r=0.789^*$, $n=9$) in 2014.

287 **4. Discussion**

288 The results in the study showed that CT was the most reliable physiological trait to
289 screen germplasm for drought performance. Under water deficit, depression in CT
290 was positively related to seed yield in both glasshouse experiments whereas root
291 biomass at bottom had positive relationship with seed yield only in 2014 (Figure 2).
292 Positive relationship of yield with CT depression in glasshouse was also supported

293 by results from field trials (Figure 3). The other two physiological traits, OA and leaf
294 proline concentration failed to show any relationship with seed yield.

295 Several studies have shown positive relationships between cooler CT and enhanced
296 yields under drought in several crops including Brassica oilseeds. Singh, Singh et al.
297 (1985) used an infrared thermometer to measure CT of Brassica oilseeds and
298 concluded that cooler CT was associated with improved seed yield under drought.
299 Kumar and Singh (1998) also recommended CT as a suitable physiological trait for
300 screening of germplasm for drought tolerance in Brassica oilseeds. Cooler CT under
301 drought are a result of improved stomatal conductance (Manavalan and Nguyen
302 2012). Cooler CT was also associated with greater leaf OA and stomatal
303 conductance in the study by Singh, Singh et al. (1985). The results from this study
304 are in accordance with the study by Olivares-Villegas, Reynolds et al. (2007) in
305 wheat. They compared chlorophyll content, CT, osmotic potential of the leaves, leaf
306 rolling and spectral response of light reflected from the canopy for contribution to
307 drought performance. Canopy temperature was the only trait that consistently and
308 strongly related to yield performance under water deficit.

309 Osmotic adjustment (OA) and proline accumulation have been shown to be useful
310 traits for drought tolerance in Brassica oilseeds (Kumar, Singh et al. 1984;
311 Gunasekera, French et al. 2009; Phutela, Jain et al. 2009; Din, Khan et al. 2011).
312 However, OA and proline concentration were not associated with yield of juncea
313 canola under water deficit in this study. Richards and Thurling (1979) also found no
314 or weak relationship between drought performance and physiological traits (OA,
315 proline, chlorophyll stability) in Brassica oilseeds in Western Australia. The parental
316 line JO5Z-0977 had superior OA than other genotypes in both glasshouse
317 experiments; however, its hybrids had inferior OA to the parent (Tables 3 and 4). All
318 three hybrids had relatively inferior OA but relatively superior proline concentration
319 under WD in 2013. In 2014, three highest yielding hybrids in WD had relatively
320 inferior OA and highly variable leaf proline concentration under WD. It appeared
321 that deeper rooting of hybrids was independent of osmotic adjustment properties and
322 was a constitutive trait. It is likely that deeper rooting behaviour of the hybrids was
323 related to their inherent higher biomass production. Even drought imposed plants
324 were well watered till FOF; it is highly probable that the hybrids, on account of their
325 vigour, were able to produce deeper roots by this stage.

326 Deeper rooting was not associated with drought yield or other physiological traits in
327 2013 when three hybrids and five open-pollinated lines were used in the study (Table
328 1). However, deeper rooting was associated with drought yield (Figure 2) and
329 positively correlated with CTD ($r=0.789$) in 2014 when there were eight hybrids and
330 four parental lines were used. The results indicated that positive relationship of
331 deeper rooting with drought yield and CTD was associated more with hybrids than
332 with parental lines.

333 This study used two different sets of juncea canola hybrids and parental lines in
334 glasshouse experiments in 2013 and 2014. Most of the genotypes were parental lines
335 in 2013 whereas most of them were hybrids in 2014. The two glasshouse
336 experiments showed similar trends in effects of water deficit for seed yield, leaf
337 proline concentration, CTD and relative water content. The hybrids and parental
338 lines had reduced seed yield, CTD and relative water content; and increased leaf
339 proline concentration under water deficit (Tables 3 and 4). However, HJM1Z-0027
340 (a common hybrid in both experiments) had considerably higher yield and yield
341 difference between WD and WW treatments in 2014 than in 2013. HJM1Z-0027 also
342 had much higher leaf proline concentration in WD treatment in 2014 than in 2013.
343 The two experiments showed different trends for root biomass at bottom in 2013 and
344 2014. In 2013, most of the genotypes had increased root biomass at bottom under
345 water deficit. In contrast, most of them reduced root biomass at bottom under water
346 deficit in 2014. It appeared that the different sets of juncea canola hybrids and
347 parental lines showed different trends for root biomass in these experiments. As this
348 study was mainly based on pot experiments, further research is recommended to
349 verify the results.

350 **5. Conclusions**

351 Osmotic adjustment and leaf proline concentration have been recommended as
352 useful traits to screen germplasm for drought tolerance in oilseed Brassicas.
353 Recently, non-destructive and easier techniques like measurement of canopy
354 temperature have been successfully used as screening tools for drought tolerance in
355 crops including Brassicas. The study presented a comparison of four physiological
356 traits viz OA, proline, canopy temperature and root depth in relation to their
357 contribution in drought performance of juncea canola. Glasshouse results indicated

that measurement of canopy temperature was the only reliable method to screen drought tolerance of juncea canola. Depression of canopy temperature was positively associated with yield under water deficit in glasshouse and with deeper rooting in 2014. The positive relationships between CTD and drought yield was also supported by results from field trials. Measurement of canopy temperature is non-destructive, easy to use and requires less time and resources than the measurement of OA and proline. Measurement of canopy temperature also provides opportunities for repeated and large scale measurements and could be easily adopted in breeding programs where high throughput is required.

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460 **Table 1** Experiments conducted in glasshouse and field in 2013 and 2014.

Year	Experiment	No. of genotypes	Date sown	Location
2013	Glasshouse	8	10 July	Parkville, Victoria
2014	Glasshouse	12	27 July	Parkville, Victoria
2014	Field trial	16 (set 1), 54 (set 2)	8 June	Horsham, Victoria

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462

463 **Table 2** Mean squares from ANOVA for seed yield and physiological traits in glasshouse
464 experiments.

Source	Seed yield g/plant	Osmotic adjustment Mpa	#Leaf proline concentration mg/kg	Canopy temperature Depression, °C	#Root biomass at bottom mg/plant
2013					
Rep.	0.001 (2)	0.006 (2)	0.003 (2)	13.9 (2)	0.267 (2)
Gen.	6.98** (7)	0.249** (7)	0.0399** (7)	2.3* (7)	0.21* (7)
Trt.	3.1* (1)	-	1.626** (1)	16.9** (1)	0.25* (1)
Gen. × Trt.	0.409 (7)	-	0.121 (7)	0.7 (7)	0.09 (7)
Error	0.75 (30)	0.004 (14)	0.050 (30)	0.8 (30)	0.057 (30)
2014					
	Seed yield g/plant	#Osmotic adjustment Mpa	#Leaf proline concentration mg/kg	Canopy temperature depression, °C	#Root biomass at bottom mg/plant
Rep.	1.72 (2)	0.007 (2)	0.19 (2)	0.15 (2)	0.057 (2)
Gen.	31.9** (11)	0.098** (11)	0.26** (11)	2.7** (11)	24.0** (11)
Trt.	269.41** (1)	-	4.53** (1)	26.7** (1)	63.4** (1)
Gen. x Trt.	6.96** (11)	-	0.057 (11)	0.90 (11)	2.63** (11)
Error	0.97 (46)	0.004 (22)	0.039 (46)	0.83 (46)	0.55 (46)

465 Rep., replication; Gen., genotype; #, Data were analysed on log transformed values because of
466 heterogeneity of variance. * and ** denote significance at 0.05 and 0.01 alpha levels, respectively.
467 Figures in the parentheses are the degrees of freedom.

468 **Table 3** Seed yield and physiological traits of juncea canola in glasshouse 2013.

Genotype (Cross)	Seed yield		Osmotic adjustment Mpa	#Leaf proline concentration mg/kg		Canopy temperature Depression, °C		#Root biomass at bottom mg/plant		Relative water content, %	
	g/plant										
	WW	WD		WW	WD	WW	WD	WW	WD	WW	WD
HJM1Z-0027 (JO5Z-09677 × JMOZ-907526)	3.268	3.255*	-0.059	11.9	45.8*	3.4*	2.4	68.7*	57.5*	85	69
HJM1Z-1034 (JO5Z-09677 × JMOZ-910395)	3.272	2.811	-0.163	67.2*	87.5*	2.5	1.8	17.1	27.0*	86	69
HJM1Z-2013 (JO5Z-09677 × JMOZ-907504)	3.799*	3.204*	-0.015	13.6	117.1*	3.5*	2.6*	29.1	37.5*	85	58
JO5Z-09677(Parental line)	2.910	2.580	-0.342*	17.9	51.8*	3.6*	2.5	21.7	49.1*	85	63
JMOZ-907526 (Parental line)	1.222	1.172	-0.017	53.2*	136.4*	3.0*	1.3	15.6	33.8*	82	69
JMOZ-910395 (Parental line)	1.153	0.534	-0.204*	61.6*	73.0*	3.0*	1.6	2.9	23.8	83	67
JMOZ-907504 (Parental line)	0.877	0.778	-0.142	15.6	34.5	3.2*	1.9	15.7	19.5	86	66
OasisCL (Control cultivar)	2.736	2.257	-0.163	11.5	21.3	2.6	2.2	34.3	21.4	85	73
Treatment mean	2.405	2.074	-0.138	31.6	70.9	3.1	2.0	25.6	33.7	85	67
LSD (0.05)	0.941	0.941	-0.032	-	-	0.3	0.3	-	-	7.9	7.9
CV%	17.1		13.4	12.8		14.5		16.3		6.4	

469 WW, well-watered; WD, water deficit; #, Data were analysed on log transformed values because of heterogeneity of variance and the means are back-transformed
 470 values. * denote that the value is significantly higher than OasisCL by LSD (0.05) test.

471
 472 **Table 4** Seed yield and physiological traits of juncea canola in glasshouse 2014.

Genotype (Cross)	Seed yield	#Osmotic	#Leaf proline	Canopy	#Root biomass	Relative
	g/plant	adjustment	concentration	temperature	at bottom	water
		Mpa	mg/kg	depression, °C	mg/plant	content, %

	WW	WD		WW	WD	WW	WD	WW	WD	WW	WD
HJM1Z-0027 (JO5Z-09677 × JMOZ-907526)	6.895*	3.502*	-0.039*	28.6	185.2	3.76	1.17	62.6*	11.6*	85	67
HJM1Z-0039 (JO5Z-09677 × JMOZ-907555)	8.207*	3.601*	-0.267*	80.8*	221.5*	3.26	1.33	72.0*	39.9*	82	69
HJM1Z-1006 (JO5Z-09677 × JMOZ-909618)	7.53*	3.518*	-0.282*	56.2	151.6	1.46	1.48	32.2*	29.3*	84	71
HJM1Z-2133 (J07Z-07140 × JMOZ-909618)	9.378*	3.755*	-0.196*	25.4	131.0	3.86	1.72	67.9*	40.6*	83	64
HJM1Z-2136 (J07Z-07140 × JMOZ-907526)	7.371*	4.684*	-0.011	34.6	56.3	2.86	2.10	72.2*	50.0*	88	62
HJM1Z-2137 (J07Z-07140 × JMOZ-907555)	9.973*	4.039*	-0.125*	70.1*	260.8*	2.26	1.90	38.3*	30.1*	91	69
HJM1Z-2023 (J07Z04065 × JMOZ-909618)	7.365*	4.14*	-0.063*	38.8	170.0	3.16	1.78	48.9*	38.6*	90	76
HJM1Z-2021 (J07Z04065 × JMOZ-903635)	0.283	0.045	-0.087*	54.9	167.0	2.86	1.15	116.7*	94.5*	88	70
J05Z-09677 (Parental line)	1.891	0.920	-0.447*	21.8	148.1	3.43	2.50	9.3	6.3	89	75
J07Z-07140 (Parental line)	11.607*	4.209*	-0.056*	198.2*	316.7*	3.67	1.48	47.5*	26.7*	93	64
J07Z04065 (Parental line)	8.215*	2.191	-0.185*	67.2*	195.9	3.87	2.15	44.7*	10.2	92	62
OasisCL (Control cultivar)	4.046	1.730	-0.006	39.2	126.4	4.22	2.67	16.1	3.7	95	71
Treatment mean	6.897	3.028	-0.147	59.7	177.5	3.06	1.79	52.4	31.8	88	66
LSD (0.05)	1.144	1.144	-	-	-	1.5	1.5	-	-	10.5	10.5
CV%	19.8		18.1	8.3		14.2		12.4		6.3	

WW, well-watered; WD, water deficit; #, Data were analysed on log transformed values because of heterogeneity of variance and the means are back-transformed values. * denote that the value is significantly higher than OasisCL by LSD (0.05) test.

Table 5 Seed yield and canopy temperature of juncea canola hybrids in field trial (set 1) at Horsham in 2014.

Genotype	Seed yield kg/ha	Canopy temperature depression, °C
Juncea canola hybrids		
HJM1Z-3114	1825*	1.4*
HJM1Z-3136	1454	2.2*
HJM1Z-3166	2017*	3.2*
JB0Z-814156	1446	1.6*
JBZ12-03483	1563	1.9*
JBZ12-04229	1592	1.8*
JBZ12-04552	1733*	2.2*
JBZ12-06219	1746*	2.1*
JBZ12-07735	1600	1.6*
JBZ12-08864	1679*	1.2
VT X121 CL	1358	2.1*
<i>B. juncea</i> cultivar		
OasisCL	1404	1.0
<i>B. napus</i> hybrids		
43C80	1292	0.3
43Y85	1279	0.2
44Y84	1546	0.6
Hyola 474CL	1442	1.6
LSD (0.05)	214	0.2
CV%	8.3	3.5

*indicate that the figure is significantly higher than OasisCL by LSD (0.05) test. Figures in bold are significantly higher than the best yielding *B. napus* hybrid (44Y84).

Table 6 Seed yield and canopy temperature of juncea canola hybrids in field trial (set 2) at Horsham in 2014.

Genotype	Seed yield kg/ha	Canopy temperature depression, °C	Genotype	Seed yield kg/ha	Canopy temperature depression, °C
HJM1Z-3142	1363	1.6	HJM1Z-3120	1738*	2.1*
HJM1Z-3143	1219	0.4	HJM1Z-3138	1669*	1.4

HJM1Z-4001	1313	1.8*	HJM1Z-4004	1431	1.2
HJM1Z-4002	1312	0.8	HJM1Z-4005	1507	2.0*
HJM1Z-4003	1225	1.7*	HJM1Z-4008	1420	1.9*
HJM1Z-4006	1081	1.3	HJM1Z-4014	1431	2.0*
HJM1Z-4007	1406	0.8	HJM1Z-4015	1444	1.2
HJM1Z-4009	1312	1.3	HJM1Z-4018	1631	1.7*
HJM1Z-4010	1106	1.4	HJM1Z-4019	1813*	2.8*
HJM1Z-4011	1131	0.6	HJM1Z-4020	1669*	2.2*
HJM1Z-4012	1150	1.2	HJM1Z-4021	1469	1.4
HJM1Z-4013	1094	0.8	HJM1Z-4023	1581	2.8*
HJM1Z-4016	1406	0.7	HJM1Z-4031	1469	1.6
HJM1Z-4017	1225	1.8*	HJM1Z-4132	1431	2.3*
HJM1Z-4022	1269	1.1	HJM1Z-4133	1406	1.1
HJM1Z-4024	1105	0.9	HJM1Z-4135	1469	1.1
HJM1Z-4025	1138	1.1	HJM1Z-4170	1656*	2.1*
HJM1Z-4026	1118	1.0	HJM1Z-4172	1620	1.6
HJM1Z-4027	1288	1.3	HJM1Z-4211	1656*	1.3
HJM1Z-4028	1295	1.6	HJM1Z-4212	1450	1.2
HJM1Z-4029	1287	1.2	HJM1Z-4213	1406	0.6
HJM1Z-4030	1144	0.8	HJM1Z-4215	1481	1.2
HJM1Z-4032	1232	0.6	JB0Z-814156	1550	1.2
HJM1Z-4134	1175	1.4	OasisCL	1438	1.3
HJM1Z-4136	1281	1.1	43Y85	1419	0.3
HJM1Z-4137	1231	1.2	43C80	1393	1.0
HJM1Z-4138	1280	0.5	LSD (0.05)	212	0.3
HJM1Z-4171	1244	0.2	CV%	14.1	3.1

*indicate that the figure is significantly higher than OasisCL by LSD (0.05) test. Figures in bold are significantly higher than the best yielding *B. napus* hybrid (44Y84).

Fig. 1 Climatic conditions in field (Horsham) during the growing season.

Fig. 2 Relationships of yield with canopy temperature depression and root biomass at bottom in glasshouse. WD, water deficit; WW, well-watered; b, regression coefficient; se, standard error of b; R^2 , coefficient of determination. Each data point is a mean value.

Fig. 3 Relationships between seed yield and canopy temperature in field. b, regression coefficient; se, standard error of b; R^2 , coefficient of determination. Each data point is a mean value.

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