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Competitive ability of canola cultivars (*Brassica napus* L.) against their natural weed populations

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Abstract

To determine the competitive ability of canola cultivars against natural weed populations, a field experiment was conducted in northern Iran, Mazandaran province, during 2010–2011 growing season. The experiment was arranged as a factorial randomized complete block design with three replications. Factors were four canola cultivars (Hayola-420, Hayola-308, RGS-003 and PF) and two weed management regimes (weed-free condition and weedy condition). Averaged across weed management regimes, grain yield of RGS-003 was significantly higher than that of other canola cultivars. Weed interference significantly reduced canola grain yield and yield components, regardless of canola cultivar. The reduction in grain yield by weed competition was 15.5% for Hayola-308, 11.1% for RGS-003, 9.8% for PF and 6.6% for Hayola-420. This indicates that canola cultivar had different ability to withstand competition, which Hayola-420 had the highest (43.39) ability to withstand competition, followed by PF (40.28), RGS-003 (38.94) and Hayola-308 (34.54). The rank order of competitive ability of the canola cultivars was RGS-003 = Hayola-420 > PF = Hayola-308. Canola grain yield was positively ($P < 0.01$) correlated with silique number on main stem, grain number in silique of main stem, silique number on side branches, 1000 grain weight, and canola biomass, but negatively ($P < 0.01$) correlated with weed biomass, and not correlated with grain number in silique of side branches, plant height and harvest index. In conclusion, these results confirmed that there was a significant difference among canola cultivars for competitive ability against weeds.

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Introduction

Canola (*Brassica napus* L.) is becoming a significant oil seed crop adapted to the northern Iran, and it is usually cultivated in crop rotation with rice as a winter crop. Canola ranks fifth in production among the world's oilseed crops following soybeans, sunflowers, peanuts and cottonseed (FAOSTAT, 2011). With 7% saturated fats, canola oil contains the least amount of saturated fats among the common edible oils.

Weeds are one of the major limiting factors in agroecosystems and can be controlled by using physical, cultural, biological and chemical methods. Successful weed control is one of the most important practices for economical crop production. Recently, increasing cost of herbicide inputs in intensive crop production systems and incidence of herbicide resistance in weeds have motivated scientists to apply integrated weed management (Zhao, 2006). Using weed-competitive cultivars is an important element in integrated weed management, but often one of the most overlooked tools in weed control. A competitive crop utilizes resources before they are available to the weeds Weed competitiveness (WC) is the ability of a crop to suppress and tolerate weeds (Jannink *et al.*, 2000) which include: 1- weed-suppressive ability (WSA), or the crop's ability to suppress weeds, 2- weed tolerance (WT), or the ability of crop to maintain its yield with weed interference. Variation in competitive ability against weeds was reported not only among crop species, but also among cultivars within species. Although a negative correlation between weed competitiveness and yield have been reported by several researchers (Jennings and Aquino, 1968; Kawano *et al.,* 1974), some researchers which worked on rice (Zhao *et al.*, 2006), safflower (Paolini *et al.*, 1998), Soybean (Jannink *et al.*, 2000), barley (Watson *et al.*, 2006) and corn (Chikoye *et al.*, 2008) have suggested that competitive cultivars could be developed without substantially lowering yields.

There is little information about the extent of genotypic variation in competitiveness of common canola cultivars in Iran. Therefore, the aims of this study were to determine the competitive ability and yield losses in four canola cultivars in the presence of natural weed populations.

Materials and methods

Experimental site and design

A Field experiment was conducted in northern Iran, Mazandaran province, during 2010–2011 growing season. Monthly precipitation and temperature from 1 November of 2010 to 30 May of 2011 are presented in Table 1. Moreover, some soil properties are presented in table 2. The experiment was arranged as a factorial randomized complete block (RCB) design with three replications. Factors included four canola cultivars (Hayola-420, Hayola-308, RGS-003 and PF) and two weed management regimes (weed-free condition, weeded weekly, and weedy condition, not weeded throughout the growing season). Nitrogen fertilizer was applied 150 Kg ha^{-1} as urea (46% N) in three equal splits. One-third amount of N (50 kg) and the entire P (30 kg ha^{-1} of triple superphosphate) and K (50 kg ha^{-1} of potassium sulfate) were incorporated the top 10 Cm of soil a week before sowing. The remaining two-thirds of N were provided in two split doses at budding and flowering stages. Canola cultivars were sown on November 1, 2010 in 3 m by 5 m plots that contain 10 rows spaced 30 Cm apart. Canola emerged about 8 days after seeding to densities of 67 plants m^{-2}.

Sampling

At the end of the growing period, ten plants from each plot were selected for the measurement of plant height and yield components: silique number in main stem, grain number in silique of main stem, silique number in side branches, grain number in silique of side branches and 1000 grain weight. Plant height was determined by measuring the distance from soil surface to the tip of the plant. To determine the total aboveground biomass and yield per unit area, seeds and straw were collected and dried at 70 ^{o}C for 72 h. Harvested area was 2 m × 3 m in each plot. Grain yield of canola was adjusted to 9%

moisture content. Relative yield loss (RYL) in weedy plots was calculated as the following equation:

Relative yield loss (%) = 100[(weed-free yield – weedy yield) / weed-free yield] [1]

Harvest index (HI) was calculated as the ratio (percentage) of grain dry matter to aboveground dry biomass. Weed biomass was determined by placing 0.5 m × 0.5 m quadrates randomly at 4 places in each weedy plot. Within each quadrate, weeds were harvested by clipping plants at the soil surface, dried to a constant mass at 70 °C for 72 h and weighed.

The following equation was used for evaluating the competitive ability of canola cultivars (Challaiah *et al.*, 1986):

$$CI = (\frac{Ywp}{Ymean})/(\frac{Wi}{Wmean})$$

[2]

Where Y_{wp} is each cultivar yield from the weedy plot, Y_{mean} is the average yield of all canola cultivars from the weedy plot, W_i is weed biomass in each canola cultivar and W_{mean} is average weed biomass in weedy plots.

Also, ability to withstand competition for each canola cultivar was calculated by following equation (Watson *et al.*, 2002):

AWC= 100(Y_{WP} / Y_{WFP})

[3]

Where Y_{WP} is the yield from the weedy plot and Y_{WFP} is the yield from the weed-free plot.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (SAS, 2004). Where the F-ratios were found to be significant, means was compared using fisher's protected LSD at 5% level of probability. Pearson correlation coefficients were calculated using correlation analysis to assess the interrelationships between recorded agronomic traits of canola and weed biomass.

Results and discussion

Canola grain yield, competitive index, ability to withstand competition

The ANOVA indicated that there were significant ($P < 0.01$) effects of cultivar and weed management regime on canola grain yield (Table 3). The interaction effect of cultivar × weed management regime on grain yield was not significant at $P < 0.01$, suggesting that cultivars showed similar responses at both weed free and weedy conditions. Grain yield of RGS-003 was significantly higher than that of other canola cultivars, when averaged across weed management regimes. Weed interference significantly reduced canola grain yield by 22%, regardless of canola cultivar (3007 kg ha^{-1} in weed free compared to 2352 in weedy condition) (Table 4). The decreases of grain yield in the presence of weeds may be attributed to competition between canola and weeds for light, water and nutrient elements. The reduction in grain yield by weed competition was 15.5% for Hayola-308, 11.1% for RGS-003, 9.8% for PF and 6.6% for Hayola-420 (Table 6). This indicates that canola cultivar had different ability to withstand competition, which Hayola-420 had the highest (43.39) ability to withstand competition, followed by PF (40.28), RGS-003 (38.94) and Hayola-308 (34.54). Moreover, significant variations existed for competitive index among canola cultivars. The rank order of competitive ability of the canola cultivars was RGS-003 = Hayola-420 > PF = Hayola-308 (Table 6). These results confirmed that there was a significant difference among canola cultivars for competitive ability against weeds. Similar results were reported by researchers for chick- pea (Paolini, 2006), soybean (Jannink *et al.*, 2000), barley (Watson *et al.*, 2006), and rice (Zhao *et al.*, 2006). On the other hand, these results showed that high yielding ability and high competitive ability can coexist in the same cultivar, and selection for the higher competitive cultivar dose not imply selection for lower yielding ability. Consistent to this result, some researchers (Zhao *et al.*, 2006; Paolini *et al.*, 1998) documented that high yielding ability under weed competition and strong weed suppressive ability are compatible, and can be simultaneously improved in one genotype. In contrast, Kawano *et al.* (1974) suggested a trade-off between high yielding ability under weed competition and strong weed suppressive ability. Correlation analysis (table 7) showed that canola grain yield was positively correlated with silique number in main stem, grain number in silique of main stem, silique number in side branches, 1000 grain weight, canola biomass, plant N concentration and plant N uptake at $P < 0.01$ level, but negatively ($P < 0.01$) correlated with weed biomass, and not correlated with grain number in silique of side branches, plant height and HI. Cultivars with the highest competitive index, RGS-003 and Hayola-420, showed the lowest weed biomass (Table 4 and 6). The biomass of the weeds in mixture with RGS-003 was 74% of that in mixture with Hayola-308. Similarly, significant differences were observed in weed biomass among rice (Zhao *et al.*, 2006), safflower (Paolini *et al.*, 1998), Soybean (Jannink *et al.*, 2000), barley (Watson *et al.*, 2006) and corn (Chikoye *et al.*, 2008) cultivars. It is notable that the dominant weeds in the weedy plots were Persian ryegrass (*Lolium persicum*), wood bluegrass (*poa nemoralis*), londen rocket (*Sisymbrium irrio*) and *vicia sp.*

Table 1. Monthly precipitation and temperature from November to May at Rice Research Institute of Iran.

Month	Year	Precipitation (mm)	Temperature (°C)		
			Maximum	Minimum	Average
November	2010	137	19.7	8.8	14.2
December	2010	0.2	18.7	8.0	13.3
January	2011	210.4	12.1	4.9	8.5
February	2011	218.2	10.0	3.3	6.7
March	2011	164.7	13.3	5.0	9.2
April	2011	94.91	15.4	11.0	13.3
May	2011	16.31	22.3	16.5	19.4

Table 2. Some soil properties (0-30 cm) of experimental field prior to planting.

OC (%)	PH	Sand (%)	Silt (%)	Clay (%)	Texture	CEC (me 100 g^{-1})	Total N (%)	P (mg kg^{-1})	K (mg kg^{-1})
2.7	6.9	20	43	37	Loamy clay	32.1	0.213	8	101

Table 3. Mean squares of ANOVA for canola grain yield (Y), silique number in main stem (SNMS), silique number in side branches (SNSB), grain number in silique of main stem (GNMS), grain number in silique of side branches (GNSSB), 1000 grain weight (TGW), harvest index (HI), canola biomass (CB), weed biomass (WB), and plant height of canola (H) as affected by cultivar and weed management regime.

source	df	Y	SNMS	SNSB	GNMS	GNSSB	TGW	HI	CB	WB	H
R	2	334495 ^{ns}	67 ^{ns}	155 ^{ns}	0.9 ^{ns}	0.3 ^{ns}	0.14^{ns}	3 ^{ns}	1800044^{ns}	2366 ^{ns}	28^{ns}
Cultivar (C)	3	822046 **	94 ^{ns}	402 **	29.8**	9.3 **	0.34**	79**	30730678**	8873**	849**
Weed Regimes (W)	1	2571530**	318 **	2860**	24.6**	13.8 **	0.19**	10 ^{ns}	18568004**	996745**	477**
C×W	3	106456 ^{ns}	33^{ns}	1 ^{ns}	1.8 **	1.0 ^{ns}	0.03^{ns}	14 ^{ns}	295969 ^{ns}	8873**	150^{ns}
Error	14	111087	38	136	0.2	0.5	0.03	8	888475	962	60.9

** Significant at the 0.05 and 0.01probability level, respectively.

ns, not significant.

Table 4. Canola grain yield (Y), silique number in main stem (SNMS), silique number in side branches (SNSB), grain number in silique of main stem (GNMS), grain number in silique of side branches (GNSSB), 1000 grain weight (TGW), harvest index (HI), canola biomass (CB), weed biomass (WB), and plant height of canola (H) as affected by cultivar and weed management regime.

Factor	Y (Kg ha^{-1})	SNMS (No. plant^{-1})	SNSB (No. plant^{-1})	GNMS (No. silique^{-1})	GNSSB (No. silique^{-1})	TGW (g)	HI (%)	CB (Kg ha^{-1})	WB g m^{-2}	H (Cm)
Cultivars										
RGS-003	3223	47	71	21	17	3.88	25.3	12886	184	126
PF	2393	38	52	16	19	3.29	30.3	7904	223	133
Hayola308	2572	39	60	18	16	3.60	32.0	8001	246	109
Hayola420	2531	43	67	18	18	3.63	24.6	10283	160	136
LSD (0.05)	412	7	14	0.6	0.9	0.24	3.5	1196	38	9
Weed management regimes										
free	3007	45	73	19	18	3.69	28.7	10598	0	130
Weedy	2352	38	51	17	17	3.51	27.4	8839	407	121
LSD (0.05)	291	5	10	0.4	0.6	0.17	2.5	825	27	6

Yield components

Yield components of canola are silique number on main stem (SNMS), silique number on side branches (SNMS), grain number in silique of main stem (GNSMS), grain number in silique of side branches (GNSSB), thousand grain weight (TGW). Main effects of cultivar and weed management regime were significant for all yield components except cultivar for Silique number on main stem (SNMS). Also, interaction between cultivar and weed management regime was not significant ($P < 0.01$) for all yield components except for Grain number in silique of main stem (GNSMS). Silique number on main stem was significantly lower in weedy plots (38 No. plant^{-1}) compared to weed free plots (45 No. plant^{-1}), when averaged across canola cultivars. Irrespective weed management regime, the maximum silique number on side branches (71 No. plant^{-1}) was recorded for RGS-003, while the minimum (52 No. plant^{-1}) was for PF. Weeds presence significantly reduced Silique number on side branches by 31% (Table 4). Although under weed free conditions, there was no significant difference between Hayola-308 and Hayola-420 for grain number in silique of main stem, but it was significantly higher for-Hayola-420 than for Hayola-308 in weedy conditions (Table 5). Both weedy and weed free conditions, RGS-003 and PF had the highest and lowest grain number in silique of main stem, respectively. The highest grain number in silique of side branches (averaged across weed management regime) was recorded for PF, followed by Hayola-420, RGS-003 and Hayola-308, (Table 4). Similarly to results for all yield components, grain number in silique of side branches was significantly reduced in the presence of weeds when averaged across canola cultivars. Irrespective weed management regime, significant differences were observed for thousand grain weight among canola cultivars. The highest and lowest thousand grain weight were recorded for RGS-003 (3.88 g) and PF (3.29 g), respectively (Table 4). Averaged across cultivars, thousand grain weights were significantly decreased by weed competition (3.69g in weed free treatment compared to 3.51g in weedy treatment). Among yield components, the maximum reduction by weeds competition was recorded for Silique number on side branches (31%) followed by silique number on main stem (16%), grain number in silique of main stem (11%), grain number in silique of side branches (6%) and thousand grain weight (5%). All yield components except 1000 grain weight were negatively correlated with weed biomass. On the other hand, all yield components except grain number in silique of side branches were positively correlated with canola grain yield (Table 7).

Table 5. Interaction between cultivar and weed management regime for grain number in main silique

	grain number in main silique	
Cultivar	Weed-free condition	Weedy condition
RGS-003	23.36 ± 0.37	20.3 ± 0.28
PF	17.9 ± 0.06	15.1 ± 0.20
Hayola 308	18.8 ± 0.52	17.4 ± 0.38
Hayola 420	19 ± 0.35	18.2 ± 0.31

Table 6. Mean comparison for relative yield loss (RYL), competitive index (CI), ability to withstand competition (AWC).

Cultivar	RYL (%)	CI	AWC
RGS-003	11.1	4.81	38.94
PF	9.8	2.95	40.28
Hayola 308	15.5	2.73	34.54
Hayola 420	6.6	4.70	43.39
LSD (0.05)	4.19	1.62	4.13

Table 7. Correlation coefficients for measurements of canola cultivars as influenced by weed management regime.

parameter	Y	SNMS	SNSB	GNMS	GNSSB	GW	HI	CB	WB
SNMS	0.64^{**}								
SNSB	0.53^{**}	0.54^{**}							
GNMS	0.76^{**}	0.50^{**}	0.65^{**}						
GNSSB	0.04^{ns}	0.11^{ns}	0.20^{ns}	-0.14^{ns}					
GW	0.40^{*}	0.39^{*}	0.72^{**}	0.72^{**}	-0.18^{ns}				
HI	0.12^{ns}	0.19^{ns}	0.20^{ns}	-0.02^{ns}	-0.32^{ns}	0.06^{ns}			
CB	0.77^{**}	0.70^{**}	0.58^{**}	0.83^{**}	-0.04^{ns}	0.55^{**}	-0.51^{**}		
WB	-0.63^{**}	-0.54^{**}	-0.70^{**}	-0.49^{**}	-0.49^{**}	-0.32^{ns}	-0.06^{ns}	-0.49^{**}	
H	0.08^{ns}	0.20^{ns}	0.24^{ns}	0.10^{ns}	0.53^{**}	-0.01^{ns}	-0.16^{ns}	0.22^{ns}	-0.39^{*}

*, ** significant at the 0.05 and 0.01probability level, respectively.
ns, not significant.

Canola biomass

There were significant ($P < 0.01$) effects of cultivar and weed management regime on canola biomass (Table 3). Averaged across weed management regimes, RGS-003 had the highest biomass (12886 kg), followed by Hayola-420 (10283 kg), Hayola-308 (8001 kg) and PF (7904 kg). Irrespective canola cultivar, canola biomass was significantly reduced by weed competition (10589 kg in weed free condition vs. 8839 kg in the presence of weeds) (table 4). Similarly to this result, the reduction in crop biomass in the presence of weeds was reported for rice (Zhao *et al*., 2006), wheat (Eslami *et al*., 2006) and cowpea (Wang *et al*., 2006). The 2-way interaction of cultivar by weed management regimes was not significant ($P < 0.01$), suggesting that the cultivars which produced the largest biomass under weed free conditions also had the largest biomass under weedy conditions (Table 3). Canola biomass was positively correlated with plant N concentration ($P < 0.01$), canola grain yield and yield components except grain number in silique of side branches at $P < 0.01$ level, but negatively correlated with weed biomass and HI at $P < 0.01$ level, and not correlated with plant N uptake and plant height (Table 7).

Harvest Index

Harvest index was not influenced ($P < 0.01$) by the presence of weeds (Table 3), indicating percentage reductions of canola grain yield and of biomass by weeds were similar. A similar result was reported by Paolini *et al.* (1998) for safflower. On the other hand, harvest index was significantly ($P < 0.01$) affected by cultivar (Table 3). Averaged across weed management regimes, Hayola 308 (32%) and PF (30.3%) had a significantly higher harvest index than RGS003 (25.3%) and Hayola 420 (24.6%). Consistent to the results of this study, paolini *et al.* (1998) reported that harvest index differed among safflower genotypes and wan not significantly influenced by the presence of weeds. In contrast, Paolini *et al.* (2006) reported that harvest index of chick-pea genotypes significantly reduced in the presence of weeds.

Plant height of canola

The cultivar and weed management regime effects were significant ($P < 0.01$) for plant height. The 2-way interaction was not significant (Table 3). Averaged over weed management regimes, Hayola 308 was significantly shorter than the other cultivars. Plant height, when averaged over cultivars, was significantly greater in weed free conditions compared to weed conditions (130 vs. 121) (Table 4). There was a significant ($P < 0.01$) negative correlation between plant height and weed biomass. This result is consistent with published data for other crops such as wheat (Coleman *et al.*, 2001) and Soybean (Jannink *et al.*, 2000), Field Pea (McDonald, 2003). In contrast, Gibson *et al.* (2003) reported that there was no significant correlation between rice plant height and weed biomass.

Conclusions

In the presence of weeds, canola grain yield and yield components, canola biomass, and plant height were significantly reduced when averaged across cultivars**.** Hayola-420 had the highest (43.39) ability to withstand competition, followed by PF (40.28), RGS-003 (38.94) and Hayola-308 (34.54). The rank order of competitive ability of the canola cultivars was RGS-003 = Hayola-420 > PF = Hayola-308. Cultivars with the highest competitive index, RGS-003 and Hayola-420, showed the lowest weed biomass. In conclusion, significant differences were detected among canola cultivars for competitiveness against weeds.

References

Challaiah O, Burnside C, Wicks GA, Johnson VA. 1986. Competition between winter wheat (*Triticum aestivum*) cultivars and downy brome (*Bromus tectorum*). Weed Science **34**, 689-693.

Coleman RK, Gill GS, Rebetzke GJ. 2001. Identification of quantitative trait loci for traits conferring weed competitiveness in wheat (*Triticum aestivum* L.). Australian Journal of Agricultural Research **52**, 1235–1246, http://dx.doi.org/10.1071/AR01055

Chikoye D, Lum AF, Abaidoo R, Menkir A, Kamara A, Ekeleme F, Sanginga N. 2008. Response of Corn Genotypes to Weed Interference and Nitrogen in Nigeria. Weed Science **56**, 424–433, http://dx.doi.org/10.1614/WS-07-055.1

Eslami SV, Gill GS, Bellotti B, McDonald G. 2006. Wild radish (*Raphanus raphanistrum*) interference in wheat. Weed Science **54**, 749–756, http://dx.doi.org/10.1614/WS-05-180R2.1

Gibson KD, Hill JE, Foin TC, Caton BP, Fischer AJ. 2001. Water-seeded rice cultivars differ in ability to interfere with watergrass. Agronomy Journal **93**, 326-332. DOI: 10.2134/agronj2001.932326x

Jannink JL, Orf JH, Jordan NR, Shaw RG. 2000. Index selection for weed suppressive ability in soybean. Crop Science, **40**, 1087-1094, http://dx.doi.org/10.2135/cropsci2000.4041087x

Jennings PR, Aquino RC. 1968. Studies on competition in rice. III. The mechanism of competition among phenotypes. Evolution **22**, 529-542. DOI: 10.2307/2406878

Kawano K, Gonzalez H, Lucena M. 1974. Intra-specific competition, competition with weeds, and spacing response in rice. Crop Science **14**, 841-845.

McDonald GK. 2003. Competitiveness against grass weeds in field pea genotypes. Weed Research **43**, 48–58, http://dx.doi.org/10.1046/j.1365-3180.2003.00316.x

Paolini R, Faustini F, Saccardo F and Crino P. 2006. Competitive interactions between chick-pea genotypes and weeds. Weed Research **46**, 335–344, http://dx.doi.org/10.1111/j.1365-3180.2006.00513.x

Paolini R, Del Puglia S, Principi M, Barcellona O, Riccardi E. 1998. Competition between safflower and weeds as influencedby crop genotype and sowing time. Weed Research **38**, 247–255, http://dx.doi.org/10.1046/j.1365-3180.1998.00096.x

SAS, version 9.1.3. 2004. SAS Institute. Cary, NC, USA.

Wang G, McGiffen MEJr, Ehlers JD, Marchi ECS. 2006. Competitive ability of cowpea genotypes with different growth habit. Weed Science **54**, 775–782, http://dx.doi.org/10.1614/WS-06-011R.1

Watson PR, Derksen DA, Van Acker RC. 2006. The ability of 29 barley cultivars to compete and withstand competition. Weed Science **54**, 783–792, http://dx.doi.org/10.1614/WS-05-020R3.1

Watson PR, Derksen DA, Van Acker RC, Blrvine MC. 2002. The contribution of seed, seedling, and mature plant traits to barley cultivar competitiveness against weeds. Proceedings of the National Meeting- Canadian Weed Science Society, 49- 57.

Zhao DL, Atlin GN, Bastiaans L, Spiertz JHJ. 2006. Comparing rice germplasm for growth, grain yield, and weed-suppressive ability under aerobic soil conditions. Weed Research **46**, 444-452, http://dx.doi.org/10.1111/j.1365-3180.2006.00529.x