

Allelopathic effects of Hulless barley (*Hordeum vulgare* L.) on rape (*Brassica campestris* L.)

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ABSTRACT

Hulless barley (*Hordeum vulgare* L.) is alpine crop grown only in the Qinghai-Tibetan Plateau. The allelopathic effects of its 66-varieties were evaluated on rape by the relay-seeding co-culture in agar and in Pot culture. Two varieties (Qing 0039 and Qing 0415) significantly inhibited the root length (66.67% and 71.81%, respectively) in *Brassica campestris* L. The results of Response index (RI) values were similar to the relay-seeding co-culture in agar test. The leachates of its 5-varities (Tibet 1295, Qing 0079, Qing 0373, Tibet 0921 and Qing 0230) inhibited the rape seedlings growth. Their inhibition was drastic (100% inhibition) in root length and shoot length but was less (< 80% inhibition) on dry weight. In Cluster analysis, varieties in Group 1 (Qing 0415 and Qing 0039) were inhibitory to root length and dry weight of rape. In the relay-seeding co-culture in agar and leachate experiments the variety Qing 0039 proved most allelopathic. Its roots significantly inhibited the rape seedlings growth up to 5-7 cm distance. The extracts from its whole plants contained 9 compounds (tricin 4'-O-(threo- β -guaiacylglyceryl) ether (**I**), triclin 4'-O-(erythro- β -guaiacylglyceryl) ether (**II**), salcolin C (**III**), methyl ferulate (**IV**), dibutylphthalate (**V**), (+)-syringaresinol (**VI**), (+)-(7R, 7'R, 7"S, 7"SS, 8S, 8'S, 8"SS, 8"SS)-4", 4"-dihydroxy-3, 3', 3'', 3'''-, 5, 5'-hexamethoxy-7, 9'; 7', 9'-diepoxy-4, 8"; 4', 8"-bisoxo-8, 8'-dineolignan-7", 7"', 9", 9"'-tetraol (**VII**), triclin (**VIII**) and chlorogenic acid (**IX**)), in which compounds **I**, **II** and **V** were most allelopathic.

Keywords: Allelopathy, allelopathic varieties, *Brassica campestris*, *Hordeum vulgare*, Hulless barley, growth, inhibition, seedlings growth, varieties

INTRODUCTION

Hulless barley (*Hordeum vulgare* var. *nudum* Hook. f.), called naked barley, is an ancient crop cultivated in Chinese Qinghai-Tibetan Plateau, North African Plateau, Andean highlands of South America and Russian Caucasian mountains (32). The current research on Hulless barley is for making wine, health food and in producing the renewable energy sources (15). Wheat (*Triticum aestivum* L.) is closely-related to Hulless barley (*Hordeum vulgare* var. *nudum* Hook. f.) (42). The weeds infestation in Hulless barley is very serious problem and controlled with herbicides. Allelopathy is one of the alternatives to herbicides that can be used for weed control (9,23,31). Plants may chemically affect the performance of neighbouring plants through allelopathy (27). Wheat and barley are allelopathic to seed germination and seedling growth of weeds (9). Kimber and Guenzi

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(4,5) reported the variability in the allelopathic activity of wheat germplasm. Root exudates of several wheat and barley varieties showed significant inhibitory effects on weeds and other plants. Barley is traditionally weeds smothering crop. The 289 barley varieties from China, Japan, Mexico and Australia have variable allelopathic activity (9). This study aimed to evaluate the allelopathic activity of Hulless barley varieties native from the Qinghai-Tibetan Plateau on seeds germination and seedlings growth of rape (*Brassica campestris*).

MATERIALS AND METHODS

Sixty-six varieties of Hulless barley (*Hordeum vulgare*) (Table 1) bred in Qinghai-Tibetan Highland were provided by Seed Complex Library in Xining, Qinghai Province, China. The seeds of rape (*Brassica campestris*) were purchased from the local markets. Uniform rape seeds were selected for the tests (undersized and damaged seeds were discarded). The seeds were sterilized by soaking in 5% NaClO solution for 10 mins and then washed thrice with sterile water. The germination of rape seeds was 70% to 100%.

The experimental treatments consisted of 3 factors: A. Rape (*Brassica campestris* var. Haoyou 11, B. 66 Hulless barley varieties [(i). seeds, (ii) leachates] C. barley var. 0039 (i). water extract, (ii). seeds, (iii) Compounds isolated from barley var. Qing 0039]. The treatments were replicated thrice in Complete randomised design. Hulless barley was sown in April and harvested in September in 2013.

Bioassays

(i). Relay seeding and co-culture: The allelopathic effects of 66 varieties of Hulless barley (*Hordeum vulgare*) were evaluated by relay-seeding co-culture in agar (40). We divided 66 varieties of Hulless barley (*Hordeum vulgare*) in to 6 groups for ease of testing their bioactivities in Bioassays. Briefly, a glass beaker (500 ml) containing 30 ml of 0.3% water agar (no nutrients) was autoclaved. After cooling the agar medium was added to petri dishes (9 cm dia). Before the bioassay, all the petri dishes and solutions were sterilized at 121 °C for 20 min in the high-pressure steam sterilization Autoclave to prevent the fungi or bacterial growth during the germination and seedling growth. Fourteen surface-sterilized and pre-germinated hulless barley seeds of each of the 66 varieties were sown aseptically (by sterilizing the petri plates, filter, papers, seeds and extracts), in 4 rows with 2 cm distance (between the seeds and between the rows in petri dishes). The petri dishes were placed in growth cabinet ($356 \pm 0.16 \times 10^3$ lux, Light 13 h, 11 h Dark, 25 °C/ 13 °C D/N). On third day, 9 pre-germinated seeds of rape (*Brassica campestris* var. Haoyou 11) were sown aseptically in 3 rows alternating with rows of Hulless barley with 1 cm distance between the rows of barley and rape (Fig. 1).

The control was only rape seeds sown in petri dishes with distilled water. All treatments were replicated thrice in Complete Randomised design. After sowing the rape seeds, the petri dishes were kept in growth cabinet for 7 days. Thereafter, the Petri dishes were taken out from the growth cabinet to record seed germination and seedling growth (root length, shoot length, dry weight) and their inhibition (%) was calculated as under:

Germination rate (%) = $(1 - \text{seed numbers of Treatment} / \text{seed numbers of Control}) \times 100\%$

Inhibition rate (%) = $(1 - \text{values of Treatment} / \text{values of Control}) \times 100\%$

The values were root lengths, bud lengths, fresh weights and dry weights.

Where, T (Treatment): Root length, shoot length and fresh weight under aqueous extracts of samples and C (Control) : Distilled water.

Response Index (RI) was also calculated as under (26):

If $T > C$, $RI = 1 - C/T$.

If $T < C$, $RI = T/C - 1$.

RI values ranged from +1 to -1, positive values indicate stimulation and negative values indicate inhibition. The absolute value of RI varied directly with the strength of the effect.

In Relay seeding study, the response index (RI) was calculated as per the formula (26) given above.



Figure 1. Position of rape and hulless barley seeds sowed in the petri dish.

(ii). Leachates of Hulless barley varieties

Leachate Preparation: The 66-varieties of Hulless barley were grown in the field in 2013 in Huangzhong County, Qinghai Province, China. There were total of 4 plots with 50 m x 50 m for every plot. The row spacing was 15 cm and the seed rate was 200 kg/ ha. During the crop growth, total rainfall was 450 mm and no artificial irrigation was given. We collected the underground roots and Rhizosphere soil (upto 10 cm depth) after sieving through 0.01 μm sieve of 66 varieties of Hulless barley at maturity (End September). These plant samples were dried in shade for about 7 days at room temperature. These dried samples were cut into 3 cm pieces and then 10 g material was soaked in 100 ml distilled water in 150 ml Erlenmeyer flask. These flasks were sealed with parafilm and placed in controlled environment growth cabinet (28 $^{\circ}\text{C}$) for 3 days, thereafter, the liquid leachate was filtered through 0.22 μm Millipore filter and was stored at 4 $^{\circ}\text{C}$ for tests.

Leachate of variety Qing 0039

We collected plant samples of variety Qing 0039 plants, from the farmers fields in Huangzhong County, Qinghai Province, China, at 3-stages: (i). Seedling (Early June, about

30 days after sowing), (ii). Flowering (Early July, about 60 days after sowing and (iii). Maturity (Late in September, about 150 days after sowing), respectively. After collection the plant samples were partitioned in 3- parts (root, leaf and stem) dried in shade for 7 days at room temperature. Thereafter these were cut into 3 cm small pieces. Then 15 g material was soaked into 150 ml and 300 ml distilled water separately for 48 h at room temperature. The liquid leachate was then filtered through 0.22 μm filter membrane and stored at 4 $^{\circ}\text{C}$ for tests (17). The distilled water was added to leachate to prepare its 5% and 10% concentrations.

The bioassay was performed in 9 cm dia petri plates lined with 2 filter papers and irrigated with 6 ml leachate or water as per treatment. In each petri plate, 20 seeds of rape were sown on top of filter paper. The Petri dishes were placed in the incubator (8 h light, 16 h dark, 24 $^{\circ}\text{C}$). The root length, shoot length and dry weight were determined after 7 days (1).

Pot Culture: Sand was sieved through 0.1 mm sieve, autoclaved and dried. Three seeds of barley variety Qing 0039 and 3-seeds of rape were sown per pot (15 cm dia, 20 cm depth). The distance between Qing 0039 and rape rows was 2, 3, 4, 5, 6 and 7 cms respectively. Control was sown only with seeds of rape and irrigated with distilled water. When the rape grew to 2-leaves stage, the allelopathic effects of barley variety Qing 0039 (sown at different distances from the rape rows), were evaluated on seedlings growth (root length, shoot length and dry weight) of rape by RI values (39).

Physiological parameters: The allelopathic effects of barley varieties were determined on rape in pot experiment. The physiological parameters determined were : chlorophyll content, nitrogen content, root activity, SOD content, CAT content, MDA content and conductivity (18).

(iii). Isolation and identification of chemical compounds in Hulless barley

^1H , ^{13}C , DEPT, ^1H - ^1H -COSY, HSQC and HMBC NMR spectra were obtained on a Bruker AM-400 and a DRX-500 spectrometers. FAB-MS spectra were recorded on a JEOL JMS-HX 110 instrument. Chromatographic stationary phases used were silica gel (200-300 mesh), Sephadex LH-20 (25-100 μm , Pharmacia) and MCI-gel CHP20P (75-150 mm, Mitsubishi Chemical). Compounds in thin-layer chromatography were detected by spraying with 5% H_2SO_4 followed by heating.

Extraction and isolation of compounds: We collected the leaf and stem of Hulless barley variety Qing 0039 during the seedling stage (Early June) and at 2-4 leaves stage. After collection, the samples were dried in shade at room temperature for 7 days. Thereafter, the dried samples were cut into 3 cm long pieces. Then, these pieces were soaked into 150 ml Erlenmeyer flask with 100 ml MeOH for 7 days. The MeOH extract was concentrated in vacuo to yield a residue, which was dissolved in distilled water and filtered by filter paper. The water-soluble fraction was then desalted and the Dianion- HP20 column was eluted by distilled water. Then, the fraction was tested by HPLC-2030, eluted with MeOH- H_2O (60:40). Afterwards, the fraction was subjected to silica gel (H, 10-40 μm), MCI-gel CHP20P (75-150 mm), Sephadex LH-20 gel (25-100 μm) and HPLC analysis gel to give following nine compounds:

Tricin 4'-O-(threo- β -guaiacylglyceryl) ether (1)

Yellow solid, molecular formula $C_{27}H_{26}O_{11}$, 1H NMR (600 MHz, cd_3od) : δ 7.29 (2H, s, 12,12'- Ar-H), δ 7.09 (1H, d, $J=1.98$ Hz, 2- Ar-H), 6.94 (1H, dd, $J=1.92, 8.16$ Hz, H-6'), 6.81 (1H, d, $J = 8.1$ Hz, 22- Ar-H), 6.76(1H, s, 23- Ar-H), 6.54(1H, d, $J = 2.22$ Hz, 24-Ar-H), 6.27(1H, d, $J = 2.22$ Hz, 6- Ar-H), 5.07(1H, d, $J = 6.48$ Hz, 9- Ar-H), 4.36(1H, td, 16-CH), 4.01 (6H, s, 14, 14' -OCH₃), 3.90(3H, s, 3H, 28-OCH₃), 3.86(2H, m, 18-CH₂), 3.48 (1H, dd, $J=3.66, 12.0$ Hz, 17-CH); ^{13}C NMR(150 MHz, cd_3od) : δ 183.7(C-8), 166.1(C-5), 165.0(C-3), 163.2(C-10), 159.3(C-7), 154.6(C-13,13'), 148.8(C-27), 147.2(C-25), 141.0(C-15), 133.5(C-21), 127.9(C-11), 120.9(C-22), 115.8(C-24), 111.7(C-23), 105.6(C-4), 105.1(C-12,12'), 100.2(C-9), 95.2(C-2), 88.9(C-6), 74.4(C-17), 62.0(C-16), 57.0 (C-28), 56.9(C-14,14'), 56.4(C-18).

Tricin 4'-O-(erythro- β -guaiacylglyceryl) ether (2)

Yellow solid, molecular formula $C_{27}H_{26}O_{11}$, 1H NMR (600 MHz, $dmsO$) δ 7.31 (s, 2H, 12,12'- Ar-H), 7.05 (s, 1H, 2- Ar-H), 6.93 (s, 1H, 23- Ar-H), 6.75 (d, $J = 8.0$ Hz, 1H, , 22-Ar-H), 6.70 (d, 1H, 24-Ar-H), 6.57 (d, 1H, 6- Ar-H), 6.21 (d, 1H, 9- C=CH), 5.16 (d, $J = 4.6$ Hz, 1H, 16-CH), 4.80 (t, 1H, 17-CH), 4.37 – 4.10 (m, 2H,18-CH₂),3.88 (s, 6H, 14, 14' -OCH₃), 3.75 (s, 3H, 28-OCH₃). ^{13}C NMR (150 MHz, $dmsO$) δ 182.3(C-8), 165.3(C-5), 163.6(C-3), 161.9(C-10), 158.0(C-7), 153.5(C-13,13'), 147.5(C-27), 146.0(C-25), 140.0(C-15), 133.8(C-21), 125.8(C-11), 119.9(C-22), 115.2(C-24), 111.5(C-23), 105.3(C-4), 104.8(C-12,12'), 104.2(C-9), 99.6(C-2), 94.9(C-6), 87.0(C-17), 72.7(C-16), 60.7(C-28), 56.9(C-14,14'), 56.1(C-18).

Salcolin C (3)

Yellow amorphous powder, molecular formula $C_{28}H_{28}O_{11}$, 1H NMR (600 MHz, cd_3od) δ 7.13 (s, 2H, 12,12'- Ar-H), 6.89 (d, 1H, 2- Ar-H), 6.78 – 6.68 (m, 2H, 22,23- Ar-H), 6.64 (d, $J = 1.1$ Hz, 1H, 24- Ar-H), 6.44 (t, 1H, 6- Ar-H), 6.18 (t, $J = 1.8$ Hz, 1H, 9-C=CH), 4.45 (q, 1H,16-CH), 3.96 – 3.87 (m, 1H, 17-CH), 3.85 (d, $J = 0.6$ Hz, 6H, 14, 14' -OCH₃), 3.83 – 3.77 (m, 3H, 28-OCH₃), 3.72 (dd, $J = 19.0, 6.3$ Hz, 2H,18-CH₂), 3.27 – 3.21 (m, 3H, 20-OCH₃). ^{13}C NMR (150 MHz, cd_3od) δ 183.8(C-8), 166.2(C-5), 165.3(C-3), 163.2(C-10), 159.4(C-7), 154.7(C-13,13'), 148.8(C-27), 147.4(C-25), 140.6(C-15), 131.1(C-21), 127.6(C-11), 122.1(C-22), 115.6(C-24), 112.2(C-23), 105.8(C-4), 105.5(C-12,12'), 105.1(C-9),100.2(C-2), 95.2(C-6), 86.7(C-17), 83.9(C-16), 61.9(C-20), 57.2(C-28), 56.9(C-14), 56.8(C-14').

Methyl ferulate (4)

White crystal powder, molecular formula $C_{11}H_{12}O_4$, 1H NMR (600 MHz, cd_3od) δ 7.59 (d, $J = 15.9$ Hz, 1H, 5- Ar-H), 7.16 (d, $J = 1.8$ Hz, 1H, 6- Ar-H), 7.05 (d, $J = 1.9$ Hz, 1H, 7-Ar-H), 6.78 (d, $J = 8.2$ Hz, 1H, 9-C=CH), 6.34 (d, $J = 15.9$ Hz, 1H,10-C=CH), 3.86 (s, 3H,12-OCH₃), 3.74 (s, 3H,1-OCH₃). ^{13}C NMR (150 MHz, cd_3od) δ 168.3(C-11), 149.2(C-4), 148. 0(C-3), 145.4(C-9), 126.2(C-8), 122.7(C-7), 115.0(C-5), 113.8(C-10), 110.3(C-6), 55.0(C-12), 50.6(C-1).

Dibutylphthalate (5)

Light yellow oil, molecular formula $C_{16}H_{22}O_4$, 1H NMR (600 MHz, cd_3od) δ 7.68 (dd, $J = 5.7, 3.3$ Hz, 2H, 1,1'- Ar-H), 7.56 (dd, $J = 5.7, 3.3$ Hz, 2H, 2,3'- Ar-H), 4.25 (t, $J = 6.6$ Hz, 4H, 5,5'-CH₂), 1.71 – 1.56 (m, 4H, 6,6'-CH₂), 1.45 – 1.31 (m, 4H, 7,7'-CH₂), 0.93 (t, J

= 7.4 Hz, 6H, 8,8'-CH₃). ¹³C NMR (150 MHz, cd₃od) δ 167.9(C-4, C-4'), 132.3(C-3, C-3'), 131.1(C-2, C-2'), 128.6(C-1, C-1'), 65.4(C-5, C-5'), 30.4(C-6, C-6'), 19.0(C-7, C-7'), 12.9(C-8, C-8').

(+)-syringaresinol (6)

Transparent oil, molecular formula C₂₂H₂₆O₈, ¹H NMR (600 MHz, cd₃od) δ 6.65 (d, *J* = 3.4 Hz, 4H, 3,3',3'',3'''-ArH), 4.72 (s, 2H, 9,9'-Ar-OH), 4.26 (dd, *J* = 7.0, 4.6 Hz, 2H, 6,6'-CH), 3.90 – 3.86 (m, 4H, 8,8'-CH₂), 3.84 (d, *J* = 3.6 Hz, 12H, 5,5',5'',5'''-CH₃), 3.14 (s, 2H, 7,7'-CH). ¹³C NMR (150 MHz, cd₃od) δ 147.92, 134.78(C-2,2',2'',2'''), 131.70(C-1,1'), 103.09(C-4,4'), 86.20(C-6,6'), 71.33(C-8,8'), 55.37(C-5,5',5'',5'''), 54.09(C-7,7').

(+)-(7R, 7'R, 7''S, 7'''S, 8S, 8'S, 8''S, 8'''S)-4'', 4'''-dihydroxy-3, 3', 3'', 3''', 5, 5'-hexamethoxy-7, 9'; 7', 9-diepoxy-4, 8''; 4', 8'''-bisoxo-8, 8'-dineolignan-7'', 7''', 9'', 9'''-tetraol (7)

White amorphous powder, molecular formula C₄₂H₅₀O₁₆, ¹H NMR (600 MHz, cd₃od) : δ 6.97 (2H, dd, *J*=1.86, 4.2 Hz, 7,7'- Ar-H), δ 6.78 (2H, dt, 5,5'- Ar-H), 6.73 (2H, dd, *J*=1.86, 8.1Hz,8,8'- Ar-H), 6.69 (4H, d, *J* = 1.5Hz, 15,15',15'',15'''- Ar-H), 4.90(2H, dd, *J*=3.84, 5.46Hz,17,17'- Ar-H), 4.76(2H, t, *J* = 2.64, 2.34 Hz, 9,9'-CH), 4.28(4H, m, 18,18'-CH₂), 3.94(2H, dt, 11,11'- Ar-H), 3.91(2H, ddd, H-9b, 9'b), 3.84 (12H, s, 14,14',14'',14'''-OCH₃), 3.83(6H, d, *J*=4.98 Hz, 4,4'-OCH₃), 3.60(2H, dt, 19,19'-CH). ¹³C NMR (150 MHz, cd₃od): δ 154.53(C-20,20',20'',20'''), 154.52(C-3, 3'), 148.63(C-2, 2'), 146.84(C-21, 21'), 138.86(C-16, 16'), 136.12(C-6, 6'), 134.77(C-7,7'), 120.70(C-8,8'), 115.63(C-5,5'), 111.40(17,17'), 104.27(C-11,11'), 87.22(C-15,15',15'',15'''), 74.05(C-9,9'), 73.05(C-18,18'), 61.70(C-12,12'), 56.7(C-14, 14', 14'', 14'''), 56.3 (C-4,4'), 55.7(C-19,19').

Tricin (8)

Light yellow needle-shaped crystal, molecular formula C₁₇H₁₄O₇, ¹H NMR (600 MHz, cd₃od) δ 7.19 (s, 2H, 11, 11'- Ar-H), 6.60 (s, 1H, 7- Ar-H), 6.44 (d, *J* = 1.7 Hz, 1H, 2 - Ar-H), 6.19 (d, *J* = 1.9 Hz, 1H, 6- C=CH), 3.95 (s, 6H, 13-OCH₃, 13'- OCH₃). ¹³C NMR (150 MHz, cd₃od) δ 183.1(C-5), 165.4(C-1), 165.3(C-3), 162.6(C-8), 158.7(C-9), 149.0(C-12, c-12'), 140.6(C-14), 122.0(C-10), 104.7(C-11,C-11'), 104.5(C-4), 103.8(C-6), 99.5(C-2), 94.5(C-7), 56.4(C-13, C-13').

Chlorogenic acid (9)

White needle-shaped crystal, molecular formula C₁₆H₁₈O₉, ¹H NMR (600 MHz, cd₃od) δ 7.55 (d, *J* = 15.8 Hz, 1H, 9-C=CH), 7.04 (s, 1H, 12- Ar-H), 6.95 (d, *J* = 8.1 Hz, 1H, 11- Ar-H), 6.77 (d, *J* = 7.1 Hz, 1H, 13- Ar-H), 6.26 (d, *J* = 15.9 Hz, 1H, 8-C=CH), 5.33 (s, 1H, 20- Ar-OH), 4.16 (s, 1H, 19-Ar-OH), 3.72 (d, *J* = 8.3 Hz, 1H, 3-CH), 3.30 (d, *J* = 1.2 Hz, 1H, 2-CH), 2.20 (dd, *J* = 29.6, 13.4 Hz, 2H, 1-CH₂), 2.06 (dd, *J* = 25.7, 13.7 Hz, 2H, 5-CH₂). ¹³C NMR (150 MHz, cd₃od) δ 175.7(C-16), 167.3(C-7), 148.3(C-15), 145.8(C-14), 145.5(C-9), 126.5(C-10), 121.7(C-11), 115.2(C-13), 114.0(C-8), 113.9(C-12), 74.8(C-4), 72.18(C-6), 70.7(C-3), 70.0(C-2), 37.5(C-1), 36.9(C-5).

(iv). Statistical Analysis: The Root lengths, shoot lengths and dry weights and RI values were subjected to one-way ANOVA analysis of variance followed by Duncan's multiple-range test to determine significant differences among mean values at the probability level

of 0.05(10). All bioassays were performed three times under identical conditions. Data are presented as means \pm SE.

RESULTS AND DISCUSSION

(i). RELAY SEEDING AND CO-CULTURE

Seed Germination: Most varieties of Hulless barley inhibited the seed germination in rape (Table 1). The varieties Tibet 0100, Qing 0074, Qing 0039, Qing 0112 and Qing 0149, significantly inhibited the seeds germination by 47.92%, 45.83%, 40.42%, 47.92% and 43.75%, respectively.

Table 1. Effects of 66 varieties of Hulless barley (*Hordeum vulgare*) bred in Qinghai-Tibetan Plateau on seed germination of rape.

No.	Library No.	Variety	Shoots number	Seed germination (%)	No.	Library No.	Variety	Shoots number	Seed germination (%)
1	ZDM1597	Qing 0106	12.0 \pm 2.5	75.00	34	ZDM1588	Qing 0097	14.3 \pm 2.0	89.58
2	ZDM1538	Qing 0047	12.3 \pm 1.8	77.08	35	ZDM5694	Tibet 1295	12.7 \pm 2.8	79.17
3	ZDM1533	Qing 0042	11.7 \pm 1.6	72.92	36	ZDM6277	Tibet 1854	14.7 \pm 1.8	91.67
4	ZDM1529	Qing 0038	14.3 \pm 3.1	89.58	37	ZDM1536	Qing 0045	12.3 \pm 1.6	77.08
5	ZDM1561	Qing 0070	12.7 \pm 2.0	79.17	38	ZDM1535	Qing 0044	11.7 \pm 2.1	72.92
6	ZDM1550	Qing 0059	14.0 \pm 2.1	87.50	39	ZDM1585	Qing 0094	10.3 \pm 1.9	64.58
7	ZDM4343	Tibet 0187	12.3 \pm 1.3	77.08	40	ZDM1525	Qing 0034	11.7 \pm 2.6	72.92
8	ZDM5077	Tibet 0921	14.3 \pm 1.3	89.58	41	ZDM6734	Tibet 2311	9.7 \pm 1.0	60.42
9	ZDM1562	Qing 0071	11.3 \pm 2.8	70.83	42	ZDM1569	Qing 0078	8.7 \pm 1.2	54.17
10	ZDM8097	Qing 0300	11.7 \pm 2.5	72.92	43	ZDM1498	Qing 0007	10.3 \pm 2.5	64.58
11	ZDM1546	Qing 0055	14.7 \pm 1.8	91.67	44	ZDM7288	Tibet 2865	9.3 \pm 1.2	58.33
12	ZDM6734	Tibet 2311	8.7 \pm 1.5	54.17	45	ZDM5630	Tibet 1231	12.0 \pm 1.8	75.00
13	ZDM4204	Tibet 0048	12.7 \pm 2.3	79.17	46	ZDM8202	Qing 0414	12.3 \pm 2.0	77.08
14	ZDM1585	Qing 0094	11.0 \pm 1.2	68.75	47	ZDM1523	Qing 0032	11.0 \pm 1.3	68.75
15	ZDM1543	Qing 0052	10.0 \pm 1.2	62.50	48	ZDM1514	Qing 0023	8.7 \pm 0.3	54.17
16	ZDM4436	Tibet 0280	10.3 \pm 1.0	64.58	49	ZDM1502	Qing 0011	9.3 \pm 1.2	58.33
17	ZDM8203	Qing 0415	11.0 \pm 2.5	68.75	50	ZDM1516	Qing 0025	12.0 \pm 2.3	75.00
18	ZDM04256	Tibet 0100	7.7 \pm 0.5	47.92	51	ZDM1518	Qing 0027	11.3 \pm 2.1	70.83
19	ZDM1565	Qing 0074	7.3 \pm 0.4	45.83	52	ZDM1568	Qing 0077	15.3 \pm 1.9	95.83
20	ZDM1572	Qing 0081	10.0 \pm 2.4	62.50	53	ZDM1570	Qing 0079	8.7 \pm 1.0	54.17
21	ZDM1553	Qing 0062	11.3 \pm 1.9	70.83	54	ZDM1580	Qing 0089	10.3 \pm 2.2	64.58
22	ZDM6333	Tibet 1910	14.7 \pm 2.5	91.67	55	ZDM1583	Qing 0091	8.3 \pm 0.3	52.08
23	ZDM6262	Tibet 1839	8.7 \pm 1.7	54.17	56	ZDM1592	Qing 0101	9.7 \pm 0.5	60.42
24	ZDM1559	Qing 0068	10.3 \pm 1.3	64.58	57	ZDM1601	Qing 0110	12.3 \pm 1.4	77.08
25	ZDM1563	Qing 0072	10.0 \pm 1.0	62.50	58	ZDM1602	Qing 0111	14.7 \pm 2.8	91.67
26	ZDM7564	Tibet-ri1669	9.7 \pm 1.3	60.42	59	ZDM1640	Qing 0149	7.0 \pm 1.0	43.75
27	ZDM6739	Tibet 2316	10.3 \pm 2.0	64.58	60	ZDM1721	Qing 0230	11.3 \pm 2.3	70.83
28	ZDM1530	Qing 0039	9.7 \pm 1.8	40.42	61	ZDM8108	Qing 0309	11.7 \pm 2.2	72.92
29	ZDM1603	Qing 0112	7.7 \pm 0.7	47.92	62	ZDM8140	Qing 0334	9.0 \pm 1.4	56.25
30	ZDM7068	Tibet 2645	12.0 \pm 1.6	75.00	63	ZDM8144	Qing 0345	10.3 \pm 1.7	64.58
31	ZDM1534	Qing 0043	10.7 \pm 1.4	66.67	64	ZDM8172	Qing 0373	8.7 \pm 1.9	54.17
32	ZDM1528	Qing 0037	11.3 \pm 2.3	70.83	65	ZDM8173	Qing 0374	12.3 \pm 2.8	77.08
33	ZDM1544	Qing 0053	12.0 \pm 1.5	75.00	66	ZDM8192	Qing 0394	13.7 \pm 2.2	85.42
	CK		15.7 \pm 2.0	97.92					

Data are expressed as means \pm SD from experiments with three replicates.

Seedling growth: The root length is important parameter to evaluate the allelopathic effects. All 66-varieties of Hulless barley inhibited the root length (1.8-71.81% inhibition) (Table 2). Only two varieties Qing 0039 and Qing 0415 were most inhibitory to root length (66.67% and 71.81% inhibition), respectively. Similar inhibition has also been reported in many studies on rice and wheat plants (12,21,43). However, all varieties of Hulless barley were less inhibitory to shoot length (3.35% to 36.60% inhibition). All varieties of Hulless barley also had variable inhibitory effects on dry weight (0.30% to 55.67% inhibition). As per the results of the relay-seeding co-culture in agar and leachates, Variety Qing 0039 proved the most allelopathic inhibitory variety.

Table 2. Allelopathic effects of relay-seeding and co-culture on 66 varieties of Hulless barley (*Hordeum vulgare*) on growth of rape seedlings.

No	Variety ^a	Root length (cm)	Shoot length (cm)	Dry weight(g)
Group I				
	CK1	8.56±0.91	4.46±0.52	0.8332±0.09
1	Q 0106	5.44±0.66	4.15±0.65	0.6087±0.05
2	Q 0047	3.98±0.27	3.62±0.17	0.5145±0.06
3	Q 0042	5.18±0.67	4.00±0.21	0.6075±0.08
4	Q 0038	4.00±0.18	3.62±0.44	0.5167±0.09
5	Q 0070	4.76±0.23	3.51±0.42	0.5198±0.08
6	Q 0059	5.10±0.42	4.20±0.38	0.5213±0.10
7	T 0187	4.69±0.65	3.84±0.22	0.5187±0.04
8	T 0921	3.80±0.25	3.16±0.38	0.5521±0.06
9	Q 0071	4.86±0.46	3.88±0.41	0.5211±0.06
10	Q 0300	4.01±0.33	3.36±0.33	0.4735±0.02
Group II				
	CK2	7.84±0.98	3.75±0.65	0.8125±0.11
11	Q 0055	4.85±0.39	3.53±0.23	0.5208±0.06
12	T 2311	4.47±0.33	3.45±0.34	0.5123±0.06
13	T 0048	6.37±0.75	3.62±0.41	0.6069±0.08
14	Q 0094	4.50±0.69	3.24±0.27	0.5119±0.06
15	Q 0052	5.04±0.78	3.99±0.38	0.5989±0.07
16	T 0280	4.07±0.49	3.08±0.43	0.5135±0.06
17	Q 0415	2.21±0.29	2.86±0.22	0.4458±0.04
18	T 0100	3.32±0.45	3.77±0.25	0.4659±0.05
19	Q 0074	5.62±0.79	4.39±0.56	0.6010±0.06
20	Q 0081	5.21±0.67	3.80±0.42	0.5986±0.06
Group III				
	CK3	8.48±0.90	3.77±0.33	0.5784±0.56
21	Q 0062	3.64±0.44	3.25±0.33	0.2724±0.22
22	T 1910	4.77±0.39	4.06±0.50	0.4711±0.40
23	T 1839	5.11±0.50	3.21±0.17	0.4576±0.38
24	Q 0068	5.31±0.41	3.44±0.22	0.4661±0.33
25	Q 0072	4.64±0.55	3.64±0.39	0.2759±0.17
26	Tr 1669	7.52±0.34	3.46±0.40	0.4581±0.51
27	T 2316	6.62±0.76	3.54±0.41	0.5142±0.47
28	Q 0039	2.83±0.19	2.39±0.29	0.2564±0.19
Group IV				
	CK4	9.18±0.92	5.07±0.61	0.7311±0.09
29	Q 0112	4.65±0.44	4.32±0.19	0.5206±0.66

30	T 2645	4.30±0.51	3.66±0.21	0.4688±0.04
31	Q 0043	5.54±0.71	4.90±0.59	0.5579±0.08
32	Q 0037	5.19±0.39	4.38±0.43	0.5010±0.07
33	Q 0053	5.62±0.41	4.49±0.19	0.5611±0.08
34	Q 0097	5.36±0.37	4.61±0.55	0.6980±0.04
35	T 1295	5.15±0.60	4.71±0.21	0.5232±0.09
36	T 1854	5.05±0.22	4.52±0.31	0.5589±0.06
37	Q 0045	5.46±0.71	4.74±0.51	0.6450±0.07
38	Q 0044	5.79±0.61	4.78±0.39	0.6110±0.06
Group V				
	CK5	8.65±0.91	3.90±0.45	0.8214±0.81
39	Q 0094	6.45±0.59	3.25±0.39	0.4423±0.41
40	Q 0034	6.48±0.53	3.97±0.27	0.6170±0.71
41	T 2311	7.04±0.69	3.52±0.33	0.5935±0.44
42	Q 0078	6.18±0.71	3.92±0.47	0.6889±0.53
43	Q 0007	6.55±0.27	4.18±0.18	0.5139±0.29
44	T 2865	4.85±0.59	3.30±0.40	0.4421±0.34
45	T 1231	6.57±0.67	2.99±0.28	0.4701±0.48
46	Q 0414	5.08±0.49	3.24±0.32	0.5120±0.55
47	Q 0032	6.51±0.52	4.07±0.41	0.5190±0.44
48	Q 0023	5.18±0.66	3.44±0.38	0.5096±0.52
Group VI				
	CK6	7.79±0.81	4.19±0.33	0.7001±0.61
49	Q 0011	5.41±0.42	4.22±0.49	0.6550±0.51
50	Q 0025	5.15±0.44	3.05±0.37	0.4895±0.38
51	Q 0027	6.68±0.61	3.93±0.21	0.5231±0.44
52	Q 0077	5.24±0.52	3.52±0.41	0.6860±0.53
53	Q 0079	5.88±0.55	3.96±0.19	0.6010±0.71
54	Q 0089	6.56±0.70	4.38±0.44	0.4721±0.31
55	Q 0091	4.85±0.39	3.12±0.31	0.4421±0.42
56	Q 0101	6.27±0.66	3.99±0.40	0.6980±0.71
57	Q 0110	5.28±0.54	3.54±0.19	0.6980±0.80
58	Q 0111	6.31±0.72	4.17±0.28	0.4721±0.65
59	Q 0149	5.18±0.67	3.42±0.37	0.4932±0.55
60	Q 0230	7.65±0.77	4.16±0.41	0.7213±0.81
61	Q 0309	5.19±0.49	4.38±0.39	0.4932±0.55
62	Q 0334	5.62±0.53	4.49±0.32	0.6310±0.71
63	Q 0345	5.36±0.48	4.61±0.39	0.7050±0.89
64	Q 0373	5.15±0.58	4.71±0.41	0.4912±0.42
65	Q 0374	4.55±0.43	3.52±0.37	0.4221±0.42
66	Q 0394	5.36±0.51	4.61±0.41	0.6770±0.56

^aQ: barley cv. Qing, T : barley cv. Tibet and Tr : barley cv. Tibet-ri. Data are expressed as means ± SD from experiments with three replicates. Values which were greater than ck values were not counted.

RI values

RI values < zero means inhibitory effects and RI values > zero means promotive effect (Fig. 2). Only two barley varieties 'Qing 0415' and 'Qing 0039' were most inhibitory to root length of rape. However most varieties had weak inhibitory effects on shoot length (Fig. 2b). Most varieties were moderately inhibitory to dry weight (Fig. 2c and 2d).

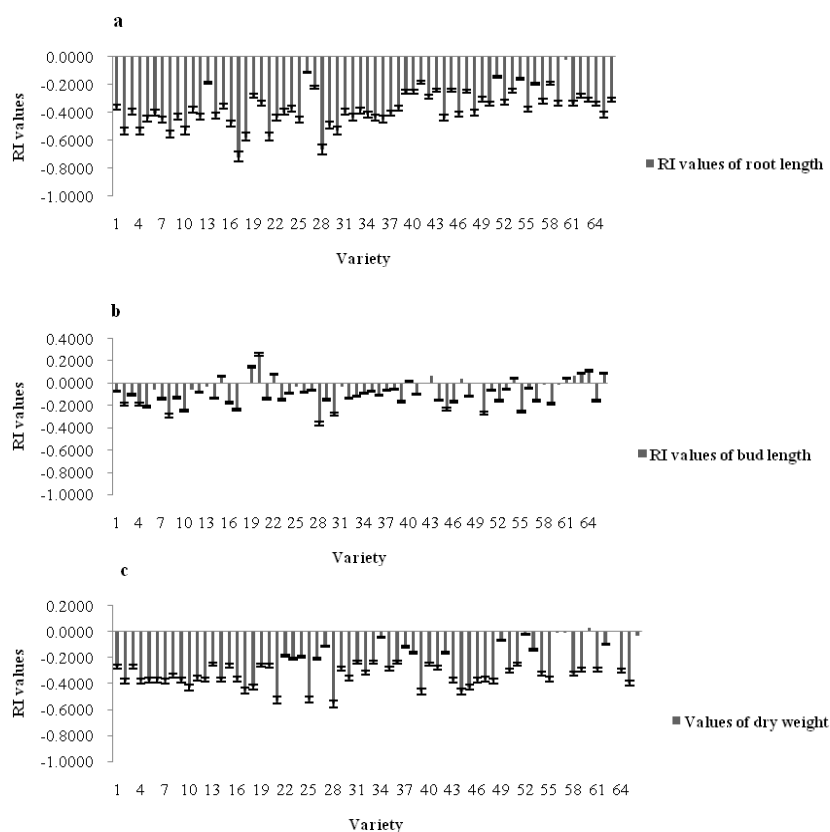


Figure 2. RI values of 66 varieties of Hulless barley on root length (a), shoot length (b), and dry weight (c) of rape.

(ii). LEACHATES

Root length: The inhibitory effects of leachates of 66-varieties of hulless barley on root length, shoot length and dry weight of rape are given in Table 3. Generally, leachates of 66-varieties of hulless barley were inhibitory to root length (inhibition: 1.52% to 100%), while only 7-varieties (Qing 0415, Qing 0039, Tibet 1295, Qing 0079, Qing 0373, Tibet 0921 and Qing 0230) were more inhibitory > 70% inhibition. Five varieties (Tibet 1295, Qing 0079, Qing 0373, Tibet 0921 and Qing 0230) were most inhibitory (inhibition upto 100%) (Table 3).

Shoot length: The leachates of 66-varieties of Hulless barley were also inhibitory to shoot length, with inhibition rates of 4.17% to 100%. Nine varieties (Tibet 1839, Qing 0039, Qing 0397, Qing 0071, Qing 0079, Qing 0373, Tibet 0921, Tibet 1295 and Qing 0230) had appreciable inhibitory effects > 60%. Moreover, five varieties (Tibet 1295, Qing 0079, Qing 0373, Tibet 0921 and Qing 0230) were most inhibitory (inhibition upto 100%) (Table 3).

Dry weight: The leachates of 66-varieties of Hulless barley were inhibitory to dry weight (inhibition 0.62% to 99.70%). There were 21 inhibitory varieties (Q0079, T2645, Q0373, T0921, T1839, Q0039, Q0052, Q0110, T1295, T2311, Q0044, Q0394, Q0230, Q0068, Q0091, Q0037, T2316, Q0038, Q0415, Q0414, and Q0034) with inhibition rates > 90% (Table 3).

Table 3. Allelopathic effects of leachates of 66 varieties of Hulless barley on growth of rape seedlings.

No	Variety ^a	Root length (cm)	IR ^b (%)	Bud length (cm)	IR ^c (%)	Dry weight(g)	IR ^d (%)
Group I							
1	Tr1669	0.22±0.03	39.52	1.11±0.13	-2.45	0.0872±0.01	-31.13
2	Q0397	0.23±0.04	37.87	0.33±0.04	69.54	0.0121±0.01	81.80
3	Q0059	0.16±0.06	56.38	0.87±0.11	19.58	0.1043±0.01	-56.84
4	Q0071	0.38±0.04	-3.29	0.18±0.04	83.31	0.0110±0.01	83.46
5	T0100	0.22±0.05	39.17	0.76±0.06	29.36	0.0494±0.01	25.71
6	Q0032	0.23±0.04	36.18	0.73±0.09	32.68	0.0445±0.01	33.08
7	Q0027	0.19±0.01	48.00	0.43±0.03	59.69	0.0357±0.01	46.32
8	Q0079	0.00	100.00	0.00	100.00	0.0032±0.01	95.18
9	Q0062	0.28±0.04	25.25	0.73±0.08	32.78	0.0089±0.01	86.62
10	T0187	0.22±0.01	39.60	0.59±0.07	45.40	0.0582±0.01	12.48
11	Q0094	0.42±0.03	-14.84	1.56±0.11	-44.40	0.0758±0.01	-13.98
	CK1	0.37±0.04		1.08±0.18		0.0665±0.01	
Group II							
12	T2645	0.25±0.02	38.82	0.42±0.03	55.81	0.0046±0.01	94.61
13	Q0011	0.33±0.04	19.59	0.74±0.09	21.97	0.0604±0.01	29.27
14	T1231	0.47±0.03	-15.97	1.51±0.19	-60.09	0.0965±0.01	-13.00
15	T1854	0.28±0.03	31.47	0.50±0.07	46.97	0.0120±0.01	85.95
16	Q0077	0.48±0.06	-17.82	1.45±0.11	-53.41	0.0876±0.01	-2.58
17	Q0373	0.00	100.00	0.00	100.00	0.0025±0.01	97.07
18	Q0309	0.30±0.02	26.58	0.54±0.06	42.73	0.0132±0.01	84.54
19	T0921	0.00	100.00	0.00	100.00	0.0018±0.01	99.70
20	Q0101	0.31±0.04	24.35	0.69±0.06	26.73	0.0469±0.01	45.08
21	T1839	0.30±0.04	26.58	0.33±0.04	65.53	0.0068±0.01	92.04
22	Q0042	0.29±0.05	29.64	0.84±0.06	11.18	0.0839±0.01	1.76
23	Q0025	0.35±0.03	13.40	0.82±0.09	12.98	0.6120±0.08	-16.63
24	T1910	0.33±0.04	19.59	0.46±0.06	51.52	0.0231±0.01	72.95
25	Q0081	0.33±0.04	19.24	1.08±0.11	-14.19	0.0673±0.01	21.19
	CK2	0.41±0.05		0.94±0.10		0.0854±0.01	
Group III							
26	Q0023	0.33±0.04	20.60	1.06±0.13	13.47	0.0425±0.01	36.38
27	Q0045	0.33±0.02	21.80	0.68±0.08	44.90	0.0210±0.01	68.56
28	Q0070	0.30±0.02	27.82	0.63±0.07	48.98	0.0089±0.01	86.68
29	Q0149	0.25±0.04	39.85	0.60±0.08	51.02	0.0081±0.01	87.87
30	Q0094	0.33±0.03	21.43	1.03±0.18	16.26	0.0603±0.02	14.29
31	Q0300	0.37±0.01	11.35	1.38±0.15	-13.00	0.0517±0.02	22.60
32	Q0039	0.08±0.01	82.09	0.05±0.01	95.93	0.0019±0.01	98.20

33	Q0052	0.27±0.03	36.24	0.60±0.04	51.02	0.0056±0.01	91.62
34	Q0110	0.30±0.04	27.82	0.50±0.04	59.18	0.0021±0.01	96.86
35	Q0007	0.29±0.03	29.53	0.86±0.09	29.45	0.0438±0.01	34.43
36	Q0055	0.30±0.05	27.82	0.80±0.08	34.69	0.0261±0.01	60.93
37	Q0111	0.35±0.04	16.67	0.94±0.12	23.58	0.0621±0.02	7.04
38	T1295	0.00	100.00	0.00	100.00	0.0028±0.01	95.81
39	Q0074	0.47±0.06	-12.84	1.98±0.20	-61.58	0.0699±0.01	-4.64
40	T0280	0.29±0.05	31.25	0.76±0.08	38.19	0.0199±0.01	70.21
41	Q0043	0.38±0.04	9.77	0.98±0.19	20.41	0.0098±0.01	85.33
42	Q0106	0.44±0.05	-4.91	1.58±0.12	-28.65	0.0472±0.01	29.34
43	Q0053	0.32±0.03	23.00	1.13±0.11	7.48	0.0433±0.01	35.18
44	T2311	0.25±0.04	39.85	0.60±0.07	51.02	0.0023±0.01	96.56
	CK3	0.42±0.05		1.23±0.13		0.0668±0.03	
Group IV							
45	Q0044	0.25±0.04	34.35	0.60±0.07	37.60	0.0012±0.01	97.51
46	Q0394	0.53±0.06	-40.06	0.97±0.11	-0.54	0.0017±0.01	96.47
47	Q0230	0.00	100.00	0.00	100.00	0.0009±0.01	98.13
48	T2865	0.30±0.04	21.22	0.70±0.09	27.20	0.0051±0.01	89.40
49	Q0089	0.43±0.06	-12.55	1.26±0.16	-30.75	0.0516±0.01	-7.28
50	Q0047	0.30±0.02	21.22	0.72±0.09	25.46	0.0468±0.01	2.70
51	Q0068	0.25±0.03	34.35	0.60±0.07	37.60	0.0011±0.01	97.71
52	Q0091	0.27±0.04	29.97	0.57±0.06	41.06	0.0016±0.01	96.67
53	Q0037	0.38±0.05	1.52	0.83±0.09	14.20	0.0018±0.01	96.26
54	T2316	0.35±0.04	8.09	0.53±0.07	45.40	0.0011±0.01	97.71
55	Q0374	0.28±0.03	26.47	0.48±0.06	50.08	0.0123±0.01	74.43
56	Q0038	0.40±0.03	-5.04	0.80±0.11	16.80	0.0021±0.01	95.63
57	Q0415	0.08±0.01	79.97	0.43±0.06	54.93	0.0015±0.01	96.88
58	Q0414	0.45±0.05	-18.17	0.60±0.05	37.60	0.0021±0.01	95.63
59	Q0034	0.30±0.04	21.22	0.65±0.07	32.40	0.0017±0.01	96.47
60	Q0112	0.31±0.03	17.94	0.78±0.08	18.75	0.0398±0.01	17.26
61	Q0078	0.29±0.04	23.68	0.80±0.09	16.67	0.0376±0.01	21.83
62	T2311	0.33±0.04	13.16	0.92±0.12	4.17	0.0356±0.01	25.99
63	T0048	0.25±0.03	34.21	0.67±0.09	30.21	0.0429±0.01	10.81
64	Q0334	0.36±0.03	5.26	0.73±0.09	23.96	0.0465±0.01	3.33
65	Q0345	0.35±0.04	7.89	0.63±0.07	34.38	0.0399±0.01	17.05
66	Q0072	0.20±0.01	47.37	0.91±0.11	5.21	0.0478±0.01	0.62
	CK4	0.38±0.04		0.96±0.19		0.0481±0.01	

^aQ: barley cv. Qing, T: barley cv. Tibet and Tr: barley cv. Tibet-ri. ^bThe IR was inhibition rate of Hulless barley on root length. ^cThe IR was inhibition rate of Hulless barley on bud length. ^dThe IR was inhibition rate of Hulless barley on dry weight. Data are expressed as means ± SD from experiments with three replicates. Values which were greater than ck values were not counted.

From these results, we found that varieties having appreciable inhibitory effects on rape were all local varieties (Table 4). Also, the inhibitory effects of leachates in most varieties were much higher than in relay-seeding co-culture in agar experiment. The inhibitory effects of wheat leachates were much higher than the aqueous extracts on seed

germination and seedling growth. The inhibition depends on the types and quantities of allelochemicals (34,41).

Table 4. Inhibitory effects of relay-seeding & co-culture and Leachates of 66 varieties of Hulless barley on seedling growth of rape

Relay seeding and co-culture		Leachates	
Inhibition (%)	Barley varieties	Inhibition (%)	Barley varieties
Root length			
General inhibition (1.8-71.8%)	All 66 Varieties	General inhibition (1.5-100%)	All 66 Varieties
Inhibition >60%	Qing 0039 and Qing 0415	Inhibition >70%	Qing 0415, Qing 0039, Tibet 1295, Qing 0079, Qing 0373, Tibet 0921 and Qing 0230
-	-	Inhibition Up to 100%	Tibet 1295, Qing 0079, Qing 0373, Tibet 0921 and Qing 0230
Shoot length			
General inhibition (3.35-36.60%)	All 66 Varieties	General inhibition (4.17-100%)	All 66 Varieties
-	-	Inhibition >60%	Tibet 1839, Qing 0039, Qing 0397, Qing 0071, Qing 0079, Qing 0373, Tibet 0921, Tibet 1295 and Qing 0230
-	-	Inhibition Up to 100%	Tibet 1295, Qing 0079, Qing 0373, Tibet 0921 and Qing 0230
Dry weight			
General inhibition (0.30-55.67%)	All 66 Varieties	General inhibition (0.62-99.7%)	All 66 Varieties
-	-	Inhibition >90%	Q0079, T2645, Q0373, T0921, T1839, Q0039, Q0052, Q0110, T1295, T2311, Q0044, Q0394, Q0230, Q0068, Q0091, Q0037, T2316, Q0038, Q0415, Q0414, and Q0034

Cluster analysis

Our analysis of inhibitory effects of 66-varieties of Hulless barley showed that most varieties were inhibitory to root length and dry weight of rape. Thus we conducted the cluster analysis of all varieties based on these data. The samples were divided into 3-groups according to their inhibitory effects on rape (Fig. 3). The varieties in Group 1 (Qing 0415 and Qing 0039) had significant inhibitory effects. The varieties in Group 2 had moderate inhibitory effects. The varieties in Group 3 had weak or no inhibitory effects. These results were consistent with those of the bioassays.

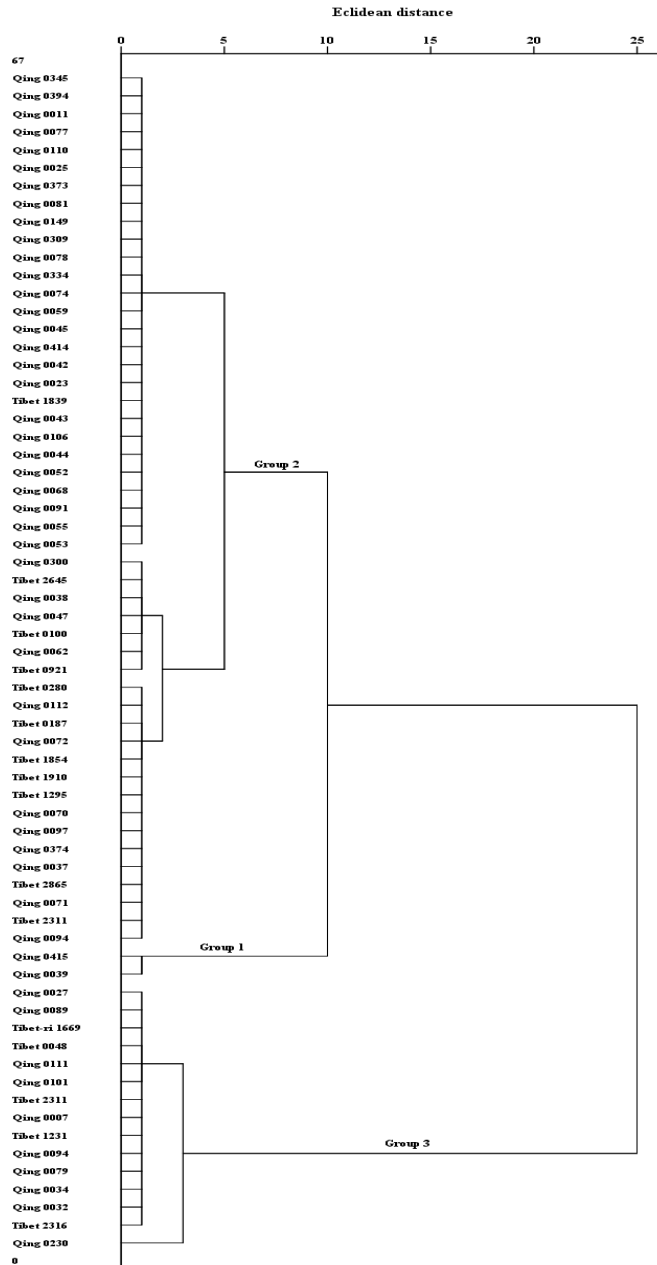


Figure 3. Diagram of Cluster analysis for RI values of 66 varieties of Hulless barley.

(iii). LEACHATES OF BARLEY VARIETY QING 0039

Based on the results of leachates and cluster analysis of 66-varieties, the variety Qing 0039 was identified as allelopathic variety. The allelopathic effects of leachates from its different parts on rape were evaluated by RI values of root length, shoot length, and dry weight in three growth periods (Table 5).

Table 5. Effects of leachates of leaf, shoot and roots of barley variety Qing 0039 on response index of rape.

Period	Part	Leachate concentrations (10 %)			Leachate concentrations (5 %)		
		RI root length	RI shoot length	RI dry weight	RI root length	RI shoot length	RI dry weight
Seedling	Leaf	-0.3303±0.03	-0.4224±0.04	-0.9713±0.06	-0.6279±0.07	-0.0580±0.01	-0.9750±0.09
	Shoot	0.2613±0.03	-0.0157±0.01	-0.9689±0.08	0.0098±0.01	-0.0408±0.01	-0.9571±0.09
	Root	-0.3973±0.03	-0.8762±0.06	-0.9974±0.08	0.0098±0.01	-0.1646±0.07	-0.9569±0.07
Flowering	Leaf	-0.1920±0.01	0.0752±0.01	0.3231±0.03	0.0991±0.01	0.0195±0.01	0.3868±0.03
	Shoot	0.1208±0.01	0.0640±0.02	0.2553±0.04	0.0942±0.01	-0.0094±0.01	-0.3996±0.04
	Root	-0.0770±0.01	0.1746±0.02	0.4310±0.04	-0.0635±0.01	0.0419±0.01	0.3639±0.05
Maturity	Leaf	-0.1363±0.01	-0.4665±0.05	-0.9301±0.09	-0.0908±0.01	0.0107±0.01	0.7198±0.06
	Shoot	0.0566±0.01	0.0907±0.01	-0.0433±0.01	-0.1850±0.02	-0.1109±0.01	-0.0022±0.01
	Root	0.1402±0.01	0.1331±0.01	-0.0588±0.01	-0.1225±0.01	0.2111±0.01	-0.0155±0.01

“L”, “S” and “R” mean different parts of Qing 0039, which are part of leaf and shoot, soil and root. The RI was Response Index of Hulless barley. Data are expressed as means ± SD from experiments with three replicates.

Leachate concentration 5%: In seedling period, leaf and shoot leachates at 5% concentration significantly inhibited the root length and dry weight. Soil extract also significantly inhibited the dry weight. The root leachates at 5% concentration significantly reduced the dry weight. The leachates of all varieties were inhibitory to rape. In seedling period, the RI values of root length, shoot length and dry weight of leaf and shoot part at 5% concentration were : -0.6279, -0.0580 and -0.9750, the RI values of soil part were 0.0098, -0.0408 and -0.9571, the RI values of root part were : 0.0098, -0.1646 and -0.9569, respectively. In flowering period, the RI values of root length, shoot length and dry weight of leaf and shoot part at 5% concentration were : 0.0991, 0.0195 and 0.3868, the RI values of soil part were : 0.0942, -0.0094 and -0.3996, the IR values of root part were : -0.0635, 0.0419 and 0.3639, respectively. At maturity stage the RI values of root length, shoot length and dry weight of leaf and shoot part at 5% concentration were : -0.0908, 0.0107 and 0.7198, the RI values of soil part were : -0.1850, -0.1109 and -0.0022, the RI values of root part were : -0.1225, 0.2111 and -0.0155, respectively.

Leachate concentration 10%: During the seedling periods, leaf and shoot leachates at 10% concentration significantly inhibited the dry weight of rape (Table 5). The leachates significantly inhibited the dry weight but root part significantly inhibited the shoot length

and dry weight. Leachates of all varieties were inhibitory to rape. At maturity stage only leaf and shoot leachates significantly inhibited the dry weight.

In seedling period, the RI values of root length, shoot length and dry weight with leaf and shoot leachate of 10% concentration % were : -0.3303, -0.4224 and -0.9713, the RI values of soil part were : 0.2613, -0.0157 and -0.9689, the RI values of root part were : -0.3973, -0.8762 and -0.9974. In flowering period, the RI values of root length, shoot length and dry weight of leaf and shoot part at 10% concentration were : -0.1920, 0.0752, and 0.3231, the RI values of soil part were 0.1208, 0.0640 and 0.2553, the RI values of root part were : -0.0770, 0.1746 and 0.4310, respectively. At maturity stage, the RI values of root length, shoot length, and dry weight of leaf and shoot part at 10% concentration were : -0.1363, -0.4665 and -0.9301, the RI values of soil part were : 0.0566, 0.0907 and -0.0433, the RI values of root part were : 0.1402, 0.1331 and -0.0588, respectively.

The roots of Qing 0039 variety had significant inhibitory effects on rape seedlings. Some crops (rice, wheat and beans) and other plants, exerts significant inhibitory effects on weeds in the seedling period (17,28,36,37), indicating that the seedling period is most sensitive to allelopathy. Furthermore, root secretions also had allelopathic effects. There are different kinds of allelochemicals in root exudates e.g. terpenoids etc. The terpenoids had allelopathic effects on the seed germination and growth (16,30).

(iv). POT CULTURE

The allelopathic effects of Qing 0039 sown at different distances with Rape were studied in pot experiment and evaluated by RI values of root length and dry weight of rape (Table 6). When the distance between the Qing 0039 and rape rows was increased, the inhibitory effects on root length, shoot length and dry weight were enhanced upto 6 cm distance. The inhibitory effects were highest at distance of 5 cms and 7 cms. These results indicated that the effective distance upto which allelopathic effects occurred was 5-7 cms.

Table 6. Effects of row spacing of barley variety Qing 0039 on seedling growth (root, shoot and dry weight) of rape in pot culture.

Distance (cm)	RI root length	RI shoot length	RI dry weight
2	-0.0232±0.01	-0.1743±0.06	0.0257±0.01
3	-0.1032±0.04	-0.1105±0.05	-0.0265±0.01
4	-0.0545±0.02	-0.1481±0.04	-0.0320±0.01
5	-0.1589±0.06	-0.2523±0.08	-0.1846±0.04
6	0.2164±0.09	0.0655±0.02	0.0385±0.01
7	-0.1299±0.05	-0.1893±0.08	-0.1680±0.06

The RI was Response Index of Hulless barley. Data are expressed as means ± SD from experiments with three replicates.

Physiological Indexes of rape

Photosynthesis: The differences in physiological indexes of rape sown at various distances from Qing 0039 variety are shown in Fig. 4 and Table 7. The chlorophyll content of rape at 5 cm distance began decreasing (Fig. 4a). The chlorophyll content was lowest at 5 and 7 cm. Allelochemicals decreases the chlorophyll content and thereby photosynthesis rate (8,13,20).

Nitrogen content: When the distance between the variety Qing 0039 and rape increased, the nitrogen content first decreased and then increased at distance of 5 cm (Fig. 4b). Generally, the nitrogen contents were high at 5-7 cm distance.

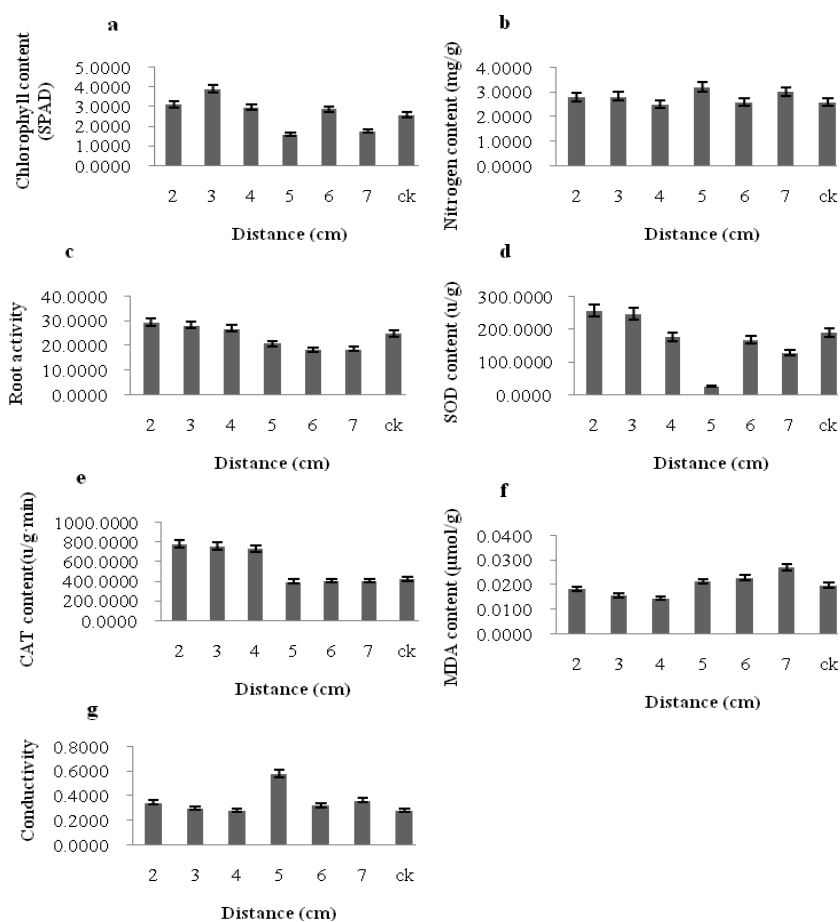


Figure 4. Physiological indexes of Rape in pot experiment.

Physiological indexes of rape (a). chlorophyll content, (b). nitrogen content, (c). root activity, (d). SOD content, (e). CAT content, (f). MDA content, (g). conductivity.

Root activity: When the distance between the variety Qing 0039 and rape increased, the root activity decreased (Fig. 4c). The root activity was lowest at the 5-7 cm distance. It indicated that the effects of Qing 0039 on root activity were highest at 5-7 cm distance. Owing to the inhibitory affects of crop on receptor plants, the root activity of plants also decreased significantly, hence, it becomes difficult for the plant to grow normally (36).

Table 7. Physiological Indexes of rape at different row distances in pot experiment.

Distance (cm)	Chlorophyll content (SPAD)	Nitrogen content (mg/g)	Root activity	SOD content (u/g)	CAT content (u/g·min)	MDA content (μmol/g)	Conductivity
2	3.1227±0.64	2.8000±0.56	18.6667±6.46	256.5224±11.31	783.7500±20.25	0.0183±0.01	0.3450±0.08
3	3.9246±0.35	2.8333±0.46	18.2821±5.46	247.3971±10.46	756.2500±28.16	0.0156±0.01	0.3020±0.10
4	2.9845±0.13	2.5000±0.31	20.8974±3.16	177.2337±14.65	731.2500±31.23	0.0145±0.01	0.2800±0.05
5	1.6208±0.29	3.2000±0.87	26.9231±2.85	26.7676±3.46	400.2500±24.65	0.0214±0.01	0.5820±0.12
6	2.8865±0.51	2.6000±0.13	28.3333±1.64	169.5278±15.31	409.3750±32.10	0.0231±0.01	0.3240±0.13
7	1.7732±0.37	3.0333±0.31	29.5897±3.12	128.5654±9.76	406.8750±12.11	0.0273±0.01	0.3630±0.06
ck	2.5838±0.38	2.6000±0.22	15.0513±4.19	189.6035±8.86	426.3000±19.64	0.0199±0.01	0.2800±0.04

The RI was Response Index of Hulless barley. Data are expressed as means ± SD from experiments with three replicates.

Enzymes

SOD: When the distance between the variety Qing 0039 and rape increased, the SOD content decreased (Fig. 4d). At distance of 5 cm, the SOD content decreased significantly and was lowest. However at 2, 3, 4, 6, and 7 cm the SOD content increased.

CAT: When the distance between the variety Qing 0039 and rape increased, the CAT content decreased significantly (Fig. 4e). The CAT contents were equal at the 5-7 cm distance. It is reported that the allelochemicals inhibited the activity and synthesis of enzymes in plants. For example, tannins inhibit the activity of SOD, CAT, amylase and other enzymes. The physiological and biochemical mechanisms of plant root exudates inhibits the key enzymes of plant during the seed germination period (3,28,35).

MDA content: When the distance between the variety Qing 0039 and rape increased, the MDA content first decreased and then increased at distance of 5 cm (Fig. 4f). The MDA contents got higher and higher at 5-7 cm distance. Since allelochemicals had inhibitory effects on the activity and synthesis of enzymes in plants, the activity of enzymes like SOD and CAT was decreased. The content of MDA and free radical increased when the plant was in stressed environment (19).

Conductivity: When the distance between Qing 0039 and rape increased, the conductivity first decreased and then increased at 5 cm (Fig. 4g). The allelochemicals could harm the cell membrane of recipient plant and increases the cell membrane permeability, thereby increases the conductivity (6,14,29).

The allelopathic effects of Qing 0039 occurred between 5 and 7 cm, i.e. the allelopathic distance of variety Qing 0039 is 5-7 cm. Moreover, the allelopathic effects of Qing 0039 in sand culture were different from those growing in agar in petri dishes. There were some differences in the form and quantity of root exudates between the various cultural methods. When the root exudates are released in the soil, the environment affects them by oxidizing, deoxidizing, hydradating and also influenced by the microbes. Allelopathic effects are the results of combined action of all kinds of allelochemicals and environment (24,25). In future studies, we will isolate and characterize the compounds

syringaresinol (VI) (7), (+)-(7R, 7'R, 7''S, 7'''S, 8S, 8'S, 8''S, 8'''S)-4'', 4'''-dihydroxy-3, 3', 3'', 3''', 5, 5'-hexamethoxy-7, 9'; 7', 9-diepoxy-4, 8''; 4', 8'''-bisoxy-8, 8'-dineolignan-7'', 7''', 9'', 9'''-tetraol (VII) (44), triclin (VIII) (26) and chlorogenic acid (IX) (2), by comparing their spectroscopic data with literature (Fig. 5). The compounds III, IV, V, VI, VII, VIII and X were obtained for the first time from Hulless barley.

Bioactivity of compounds

The allelopathic bioactivity of compounds I-IX isolated from the variety Qing 0039 on rape were evaluated at different concentrations (10, 25, 50, 100, 200 and 500 µg/ml) by RI values of root length and shoot length (Fig. 6 and Table 8).

Table 8. RI values of 9 compounds on root length and shoot length of rape.

Compound	RI values on root length						RI values on shoot length					
	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	500 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	500 µg/mL
1	0.23 ±0.01	0	-0.10 ±0.03	-0.63 ±0.03	-0.79 ±0.09	-1.00 ±0.10	0.14 ±0.01	0.26 ±0.03	0.15 ±0.03	0.14 ±0.08	-0.79 ±0.19	-1.00 ±0.16
2	-0.01 ±0.01	-0.01 ±0.01	-0.12 ±0.04	-0.42 ±0.06	-0.53 ±0.05	-0.62 ±0.10	0.16 ±0.01	0.07 ±0.01	0.01 ±0.01	0 ±0.01	-0.45 ±0.03	-0.76 ±0.03
3	-0.14 ±0.03	-0.08 ±0.02	0.14 ±0.01	0 ±0.01	0.05 ±0.01	-0.03 ±0.01	0.03 ±0.01	0.06 ±0.01	0.07 ±0.04	0.01 ±0.01	-0.02 ±0.01	-0.01 ±0.01
4	0.23 ±0.06	0.21 ±0.01	0.13 ±0.01	0.13 ±0.03	-0.10 ±0.06	-0.58 ±0.10	-0.01 ±0.01	0.02 ±0.01	0.10 ±0.03	-0.49 ±0.03	-0.54 ±0.05	-0.74 ±0.08
5	0.17 ±0.03	0.14 ±0.03	-0.11 ±0.03	-0.56 ±0.08	-0.68 ±0.05	-0.82 ±0.12	0.05 ±0.01	-0.11 ±0.01	-0.29 ±0.01	-0.44 ±0.02	-0.71 ±0.08	-1.0 ±0.12
6	0.08 ±0.02	0.08 ±0.01	0.16 ±0.03	0.65 ±0.08	-0.23 ±0.05	-0.48 ±0.08	-0.06 ±0.01	0.01 ±0.01	0.20 ±0.01	-0.38 ±0.02	-0.44 ±0.01	-0.67 ±0.08
7	0.13 ±0.04	0.01 ±0.01	0.01 ±0.01	0.05 ±0.01	-0.03 ±0.01	0.04 ±0.01	-0.12 ±0.05	0.07 ±0.01	0.20 ±0.01	0.19 ±0.06	-0.03 ±0.01	-0.06 ±0.01
8	0.35 ±0.10	0.43 ±0.04	0.34 ±0.03	0.23 ±0.03	-0.01 ±0.01	-0.12 ±0.03	0.29 ±0.05	0.30 ±0.05	0.15 ±0.04	0.05 ±0.01	0.08 ±0.02	0.01 ±0.01
9	0.26 ±0.09	0.22 ±0.05	0.22 ±0.05	-0.07 ±0.01	-0.25 ±0.05	-0.41 ±0.04	0.04 ±0.01	0.05 ±0.01	0.03 ±0.01	-0.50 ±0.03	-0.64 ±0.05	-0.69 ±0.01

1: triclin 4'-O-(threo-β-guaiacylglyceryl) ether, 2: triclin 4'-O-(erythro-β-guaiacylglyceryl) ether, 3: salcolin C, 4: methyl ferulate, 5: dibutylphthalate, 6: (+)-syringaresinol, 7: (+)-(7R, 7'R, 7''S, 7'''S, 8S, 8'S, 8''S, 8'''S)-4'', 4'''-dihydroxy-3, 3', 3'', 3''', 5, 5'-hexamethoxy-7, 9'; 7', 9-diepoxy-4, 8''; 4', 8'''-bisoxy-8, 8'-dineolignan-7'', 7''', 9'', 9'''-tetraol, 8: triclin and 9: chlorogenic acid. Data are expressed as means ± SD from experiments with three replicates.

Compounds I, II and V: These had significant inhibitory effects on the root length at 100, 200 and 500 µg/ml concentrations (Fig. 6a), While the compounds IV, VI and IX only had moderate inhibitory effects on root length at 500 µg/ml concentration. The inhibitory effects of compounds depend on their concentration. The higher the concentration, the more significant were the inhibitory effects. The compounds I, II and V were the main allelopathic compounds of variety Qing 0039 of Hulless barley, inhibitory to the root length of rape.

Compounds I, V and IX : These had significant inhibitory effect on shoot length at 200 and 500 µg/ml concentrations (Fig. 6b). However, compounds II, IV and VI were significantly inhibitory at concentration of 500 µg/ml. Yet compounds IV, V, VI and IX

had moderate inhibitory effects at 100 $\mu\text{g}/\text{mL}$ concentration. The inhibitory effects of compounds were dependent on their concentration. The compounds I, II, IV, V, VI and IX were characterized as the main allelopathic compounds of variety Qing 0039 of Hulless barley on shoot length of rape.

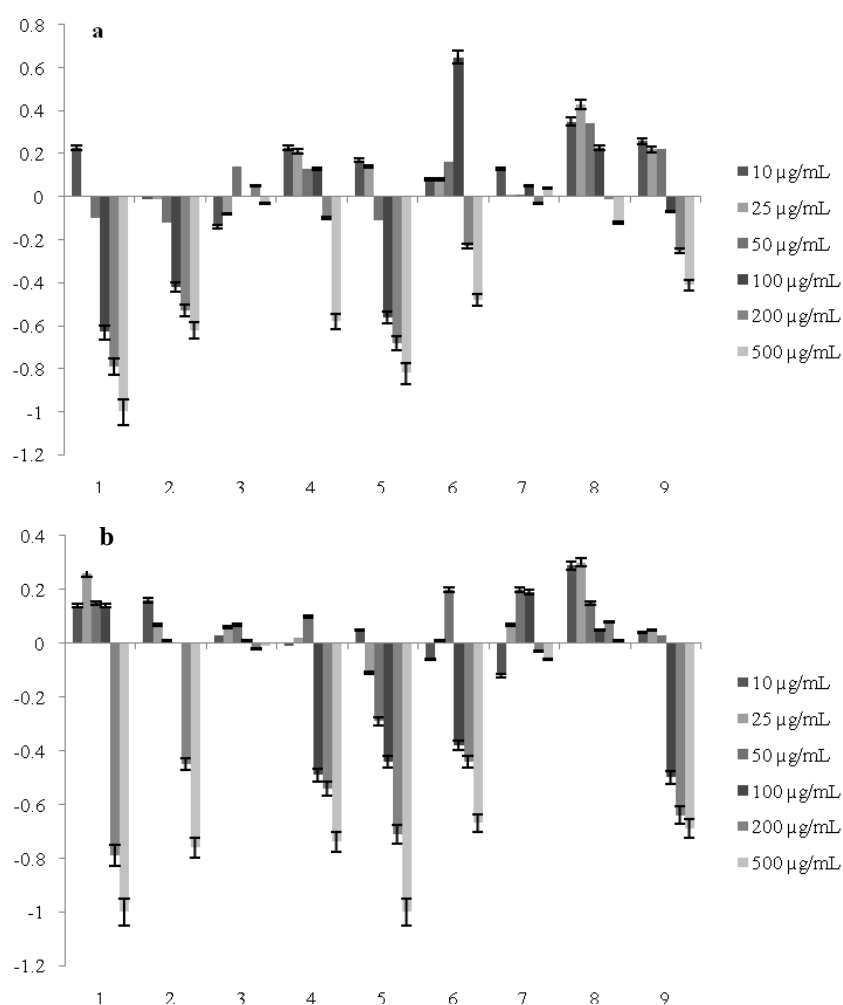


Figure 6. RI values of 9 compounds on root length (a) and shoot length (b) of rape. 1: triclin 4'-*O*-(threo- β -guaiacylglyceryl) ether, 2: triclin 4'-*O*-(erythro- β -guaiacylglyceryl) ether, 3: salcolin C, 4: methyl ferulate, 5: dibutylphthalate, 6: (+)-syringaresinol, 7: (+)-(7*R*, 7'*R*, 7''*S*, 7'''*S*, 8*S*, 8'*S*, 8''*S*, 8'''*S*)-4'', 4'''-dihydroxy-3, 3', 3'', 3''', 5, 5'-hexamethoxy-7, 9'; 7', 9'-diepoxy-4, 8''; 4', 8'''-bisoxy-8, 8'-dioneolignan-7'', 7''', 9'', 9'''-tetraol, 8: triclin and 9: chlorogenic acid.

Taken together, the compounds I, II and V were the main compounds of variety Qing 0039 of Hulless barley allelopathic to rape. The compounds I and II were the same compounds but with different configurations, yet the compound I was more inhibitory to rape than compound II. The compound I had higher antitumor activity than compound II (33).

CONCLUSIONS

Of the 66 hulless barley varieties screened for bioactivity against the seed germination and seedling growth of rape, only two varieties (Qing 0039 and Qing 0415) significantly inhibited the root length (66.67% and 71.81%, respectively) in *Brassica campestris* L. In the relay-seeding co-culture in agar and leachate experiments, the Variety Qing 0039 proved most allelopathic. Its roots significantly inhibited the rape seedlings growth upto 5-7 cm distance. Its leachate from both parts of leaf and stem contained 9 compounds (**I**), triclin 4'-O-(erythro- β -guaiacylglyceryl) ether (**II**), salcolin C (**III**), methyl ferulate (**IV**), dibutylphthalate (**V**), (+)-syringaresinol (**VI**), (+)-(7R, 7'R, 7''S, 7'''S, 8S, 8'S, 8''S, 8'''S)-4'', 4'''-dihydroxy-3, 3', 3'', 3'''-, 5, 5'-hexamethoxy-7, 9'; 7', 9-diepoxy-4, 8''; 4', 8'''-bisoxy-8, 8'-dineolignan-7'', 7''', 9'', 9'''-tetraol (**VII**), triclin (**VIII**) and chlorogenic acid (**IX**), in which compounds **I**, **II** and **V** were most allelopathic.

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REFERENCES

1. Abdelgaleil, S.A.M. and Hashinaga, F. (2007). Allelopathic potential of two sesquiterpene lactones from *Magnolia grandiflora* L. *Biochemical Systematics and Ecology* **35**: 737-742.
2. Algamdi N., Mullen, W. and Crozier, A. (2011). Tea prepared from *Anastaticahirerchuntica* seeds contains a diversity of antioxidant flavonoids, chlorogenic acids and phenolic compounds. *Phytochemistry* **72**: 248-254.
3. Bansal, G.L. (1997). Allelopathic effects of buttercups on wheat varieties. *Allelopathy Journal* **4**: 139-142.
4. Belz, R. and Hurle, K. (2004). Is there a benzoxazinone mediated potential for weed suppression in *Triticum* L. spp and *Secale 22icolo* L. *Proc. II European Allelopathy Symposium, Allelopathy From Understanding to Allplication*. Institute of soil science and plant cultivation, Pulawy, Poland. Pp. **98**.
5. Bertholdsson, N.O. (2005). Early vigour and allelopathy-two useful traits for enhanced barley and wheat competitiveness with weeds. *Weed Research* **45**: 94-102.
6. Booker, F.L., Blum, U. and Fiscus, E.L. (1992). Short term of ferulic acids onion uptake and water relations on cucumber seedlings. *Journal of Experimental Botany* **43**: 649-655.
7. Cai, X.F., Lee, I.S., Dat, N.T., Shen, G.H., Kang, J.S., Kim, D.H. and Kim, Y.H. (2004). Inhibitory lignans against NFAT transcription factor from *Acanthopanax koreanum*. *Archives of Pharmacology Research* **27**: 738.
8. Chai, Q. and Huang G.B. (2003). Review on action mechanism affecting factors and applied potential of allelopathy. *Acta Botanica Boreali-Occidentalis Sinica* **23**: 509-515. (Chinese).
9. Chen, D.M., Shen, L.H., Chen, X.Y., Lin, R.Y., Sun, H.Y., He, H.Q. and Lin, W.X. (2008). Advanced investigations of triticeae crop allelopathy and molecular ecology. *Chinese Journal of Ecology-Agriculture* **16**: 1053-1059. (Chinese).
10. Cohort Software Inc. (1985). *Costat User's Manual*. Version 3. Cohort Software Inc., Tucson, Arizona, USA.

11. Connick, W.J., Bradow J.M. and Legendre, M.G. (1989). Identification and bioactivity of volatile 23icolour23micals from amaranth residues. *Journal of Agricultural and Food Chemistry* **37**: 792-796.
12. Deng, L.G., Kong, C.H. and Luo, S.M. (1996). Isolation and identification of extract from *Casuarina equiseti folia* branchlet and its allelopathy on seed ling growth. *Chinese Journal of Applied Ecology* **7**: 145-149. (Chinese).
13. Einhelig F.A. (1995). Mechanism of action of allelochemicals in allelopathy. *Allelopathy Journal* **1**: 97-115.
14. Gattas Hallak, A.M., Davide, L.C. and Souza, L.F. (1999). Effects of sorghum (*Sorghub 23icolour L.*) root exudates on the cell cycle of the bean plant (*Phaseolus vulgaris*) root. *Genetics and Molecular Biology* **22**: 95-99.
15. Gou, A.C. and Li, L. (2004). The product development of highland barley has bright prospects. *Barley Science* **4**: 40-44.
16. He, H.B., He, H.Q., Lin, W.X., Chen, X.X., Jia, X.L., Xiong, J., Shen, L.H. and Liang, Y.Y. (2005). Terpenoids in root exudates of different allelopathic rice varieties. *Chinese Journal of Applied Ecology* **16**: 732-736. (Chinese).
17. He, H.B., Wang, H.B., Chen, X.X., Lin, W.X., Jia, X.L., Fang, C.X., Gan, Q.F., Ni, N.A. and Wu, W.X. (2007). Allelopathic effects of aqueous extracts from different parts and root exudates of rice on barnyard grass. *Chinese Journal of Ecology-Agriculture* **15**: 14-17. (Chinese).
18. He, H.B., Wang, H.B., Zeng, C.M., Cai, Z.X., Xiong, J., Qiu, L., Fang, C.X. and Lin, W.X. (2008). Physio-biochemical and molecular response of rice (*Oryza sativa L.*) cultivars with different allelopathic potentials to potassium deficiency. *Chinese Journal of Ecology-Agriculture* **16**: 878-882. (Chinese).
19. He, H.Q. and Lin, W.X. (2001). Studies on allelopathic physio-biochemical characteristics of rice. *Chinese Journal of Ecology-Agriculture* **9**: 56-57. (Chinese).
20. Heji, A.M., Einhelig, F.A. and Rasmussen, J.A. (1993). Effects of juglone on growth, photosynthesis and respiration. *Journal of Chemical Ecology* **19**: 559-568.
21. Hu, F. and Kong, C.H. (1997). Allelopathy of *Ageratum conyzoides* aqueous extract and isolation and identification of its allelochemicals. *Chinese Journal of Applied Ecology* **8**: 304-308. (Chinese).
22. Jeong, R.H., Lee, D.Y., Cho, J.G., Lee, S.M., Kang, H.C., Seo, W.D., Kang, H.W., Kim, J.Y. and Baek, N.I. (2011). A new flavonolignan from the aerial parts of *Oryza sativa L.* inhibits nitric oxide production in RAW 264.7 macrophage cells. *Journal of Korean Society For Applied Biological Chemistry* **54**: 865-870.
23. Khanh, T.D., Chung, I.M., Xuan, T.D. and Tawata, S. (2005). The exploitation of crop allelopathy in sustainable agricultural production. *Journal of Agronomy and Crop Science* **191**: 172-184.
24. Kim K.U., Shin D.H. and Kim H.Y. (1999a) Evaluation of allelopathic potential in rice germplasm. *Korean Journal of Weed Science* **19**: 1-9.
25. Kim, K.U., Shin, D.H. and Lee, I.T. (1999b). Factors affecting the allelopathic potential of rice. *Korean Journal of Weed Science* **19**: 114-120.
26. Kong, C.H., Xu, X.H., Zhou, B., Hu, F., Zhang, C.X. and Zhang, M.X. (2004). Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. *Phytochemistry* **65**: 1123-1128.
27. Li, Y.H., Xia, Z.C. and Kong, C.H. (2016). Allelobiosis in the interference of allelopathic wheat with weeds. *Pest Management Science* **72**: 2146-2153.
28. Li, Y.R. (1993). Allelopathy of plants. *Soil* **5**: 248-251.
29. Lin, R.Y., Rong, H., Zhou, J.J., Yu, C.P., Ye, C.Y., Chen, L.S. and Lin, W.X. (2007). Impact of rice seedling allelopathy on rhizospheric microbial population sand their functional diversities. *Acta Ecology Sinica* **27**: 3644-3654. (Chinese).
30. Macias, F.A. (1995). Allelopathy in search for natural herbicide model. In : *Allelopathy: Organisms, Processes and Application* (Eds., Inderjit, K.M.M. Darkshini and F.A. Einhellig) ACS Symposium Series **582**: 310-329. American Chemical Society, Washington, DC
31. Mao, J., Yang, L.Z., Shi, Y.M., Hu, J., Piao, Z., Mei, L.J. and Yin, S.X. (2006). Crude extract of *Astragalus mongholicus* root inhibits crop seed germination and soil nitrifying activity. *Soil Biology and Biochemistry* **38**: 201-208.
32. Meng, F.L., Qiang, X.L., She, G.J., Tang, Y.W. and Hu, Y.G. (2007). Genetic diversity analysis among Hulless barley varieties form the major agricultural areas of Tibet. *Acta Agriculture Sinica* **33**: 1910-1914. (Chinese).
33. Mohanlal, S., Maney, S.K., Santhoshkumar, T.R. and Jayalekshmy, A. (2013). tricin⁴-O-(erythro- β -guaiacylglyceryl) ether and tricin 4'-O-(threo- β -guaiacylglyceryl) ether isolated from Njavara (*Oryza sativa L.* var. Njavara), induce apoptosis in multiple tumor cells by mitochondrial pathway. *Journal of Natural Medicines* **67**: 528-533.

34. Oueslati, O. (2003). Allelopathy in two durum wheat (*Triticum durum* L.) varieties. *Agricultural Ecosystem Environment* **96**: 161-163.
35. Padhy, B., Patnaik, P.K. and Tripathy, A.K. (2000). Allelopathic potential of Eucalyptus leaf litter leachates on germination and seedling growth of finger millet. *Allelopathy Journal* **7**: 69-78.
36. Qiu, L., Wang, H.B., Xiong, J., Fang, C.X., Wu, W.X., He, H.B. and Lin, W.X. (2008). Regulation effect of exogenous salicylic acid on weed suppression and molecular physiological characteristics of allelopathic rice. *Chinese Journal of Applied Ecology* **19**: 330-336. (Chinese).
37. Qiu, L., Xiong, J., Wang, H.B., Fang, C.X., He, H.B., Li, Z.W. and Lin, W.X. (2008). The nitrogen nutrient efficiency and the expression analysis of the related genes in different allelopathic potential rice (*Oryza sativa* L.) varieties at seedling stage. *Acta Ecology Sinica* **28**: 677-684. (Chinese).
38. Qu, X.Y., Gu, Q.Q., Cui, C.B., Fang, Y.C., Liu, H.B., Zhu T.J. and Zhu W.M. (2004). Structural identification and antitumor activity of secondary metabolites of marine-derived actinomycete 3295. *Chinese Journal of Marine Drugs*, **23**: 1-4. (Chinese).
39. Rizvi, S.J.H., Ketata, H. and Bazazi, D. (2004). Weed suppressing ability of bread wheat genotypes under green house and field conditions. Proc. II European allelopathy symposium, *Allelopathy: From Understanding to Application*. Institute of soil science and plant cultivation, Pulawy, Poland. Pp. 25.
40. Wu, H.W., Haig, T., Pratley, J., Lemerle, D. and An, M. (2000). Distribution and exudation of allelochemicals in wheat (*Triticum aestivum*). *Journal of Chemical Ecology* **26**: 2141-2154.
41. Yu, J.G., Gu, Y., Chang, Z.Z. and Li, R.P. (2013). Allelopathic effects of wheat straw extract and decomposition liquid on rice. *Acta Pedologica Sinica* **50**: 349-356. (Chinese).
42. Zeng, X.Q., Wang, Y.L., Xu, Q.J., Yuan, H.J., Zaxi, L.B. and Ni, M.Z.X. (2013). Assessment of genetic diversity in Tibetan Hulled barley germplasm (*Hordeum vulgare* L. var. *nudum* HK. f.) by SSR primers. *Journal of Triticeae Crop* **33**: 260-267. (Chinese).
43. Zhang, X.K., Liang, W.J., Kong, C.H., Jiang, Y. and Wang, P. (2005). Screening of allelopathic wheat varieties from Chinese germplasm collection. Proceedings, 4th World Congress on Allelopathy. WaggaWagga, Australia **2**: 324-327.
44. Zhu, J.X., Ren, J., Qin, J.J., Cheng, X.R., Zeng, Q., Zhang, F., Yan, S.K., Jin, H.Z. and Zhang, W.D. (2012). Phenylpropanoids and Lignanoids from *Euonymus acanthocarpus*. *Archives of Pharmacology Research* **35**: 1739-1747.