**Supplementary data**

**Growth enhancement of *Brassica napus* under both deficient and adequate iron supply by intercropping with *Hordeum vulgare*: a hydroponic study**

**Experiment for study of intercropping effects under adequate supply of nutrients**

**Materials and methods**

Twenty five-day-old oilseed rape plants precultured in 50% Hoagland nutrient solution together with one-week-old barley seedlings were cultivated either as monocrop (MC) or intercrop (IC) in 0.8 L plastic pots filled with 100% Hoagland nutrient solution (pH 5.8–6.0). One oilseed rape was cultivated with 4 barley plants in the IC pots and two oilseed rape or 8 barley plants in the MC pots. Each treatment was composed of eight independent pots. Four replicate pots were used for determination of dry weight and elements concentration and the other four replicate pots were used for biochemical analyses. Throughout the experiment the half of the nutrient solution was substituted by a fresh one every three days. After two weeks growth under environmentally-controlled conditions, gas exchange parameters were determined in the young leaves and then plants were harvested (Field et al. 2000). A group of the similar-sized plants was harvested before cultivation in the MC/IC pots for determination of dry weight. Plants dry weight was determined in the oven-dried samples (70 °C for 48 h), and then the concentrations of nitrogen (Koroleff, 1983), phosphorus and potassium (Jaiswal, 2004) were determined after wet-digestion of leaf samples. In another group of plants, fresh leaf samples were used for determination of total protein (Bradford, 1976) and non-structural carbohydrates (Yemm and Willis, 1954) immediately after harvest.

**References**

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Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248–254.

Field CB, Ball JT, Berry JA. 2000. Photosynthesis: principles and field techniques. In: Pearcy RW, Ehleringer JR, Mooney H, Rundel PW, editors. Plant physiological ecology: field methods and instrumentation. Netherlands: Kluwer Academic Publishers; p 209–253.

Yemm EW, Willis AJ. 1954. The estimation of carbohydrates extracts by anthrone. Biochem J. 57: 508-514.

**Table S1.** Dry biomass of oilseed rape and barley before and after two weeks intercrop in hydroponic medium. Data of each row indicated by the same letter are not statistically different (P<0.05).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Oilseed rape (mg plant–1) | | Barley (mg 4 plant–1) | |
| Before starting treatment | Shoot | 43.9±0.7 a | | 41.0±0.2 a | |
| Root | 4.3±0.3 b | | 43.6±0.4 a | |
| Whole plant | 48.2±0.8 b | | 84.6±0.2 a | |
|  |  | MC | IC | MC | IC |
| Two weeks after IC treatment | Shoot | 394±65 b | 630±84 a | 217±20 a | 205±21 a |
| Root | 24.3±5.7 b | 40.3±5.9 a | 82.0±4.0 a | 77.4±6.1 a |
| Whole plant | 417±69 b | 669±88 a | 299±23 a | 282±27 a |

**Table S2**. Photosynthesis and transpiration rates and stomatal conductance, concentrations of soluble proteins, carbohydrates, nitrogen, phosphorus and potassium in the leaves of oilseed rape (*Brassica napus*) and barley (*Hordeum vulgare*) cultivated as monocrop (MC) or intercrop (IC) in the hydroponic medium for two weeks. Significant differences between MC and IC treatments of each species were indicated by different letters (P<0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Oilseed rape | | Barley | |
| MC | IC | MC | IC |
| Photosynthesis rate  (µmol CO2 m–2 s–1) | 2.83±0.31 b | 5.50±1.89 a | 6.39±1.35 a | 7.55±0.54 a |
| Transpiration rate  (mmol H2O m–2 s–1) | 0.75±0.02 b | 2.21±0.29 a | 0.82±0.14 a | 1.03±0.38 a |
| Stomatal conductance  (mol H2O m–2 s–1) | 0.19±0.00 b | 1.09±0.60 a | 0.12±0.03 a | 0.14±0.05 a |
| Soluble proteins  (mg g–1 FW) | 51.3±4.27 b | 61.2±4.07 a | 77.6±5.28 a | 82.5±3.08 a |
| Soluble carbohydrates  (mg g–1 FW) | 43.68±3.41 b | 53.89±1.23 a | 39.99±3.63 b | 49.93±5.21 a |
| Nitrogen  (mg g–1 DW) | 59.24±2.20 b | 72.29±3.86 a | 98.83±5.94 a | 100.0±4.93 a |
| Phosphorus  (mg g–1 DW) | 29.7±1.68 a | 33.3±7.15 a | 57.4±8.37 a | 67.9±5.48 a |
| Potassium  (mg g–1 DW) | 52.5±2.94 a | 60.1±3.07 a | 65.9±10.8 a | 71.3±15.5 a |

**Table S3.** The two-way ANOVA (mean of squares‎) of the effect of culture mode (CM) and Fe deficiency (Fe) and their interactions on the responses of oilseed rape and barley. \*\*\* significant at <0.001, \*\* significant at <0.01, \* significant at <0.05, ns: non-significant

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Oilseed rape | | | | Oilseed rape | | | |
|  | Fe | CM | Fe×CM |  | Fe | CM | Fe×CM |
| Shoot DW | 1.594\*\*\* | 0.258\*\* | 0.00209ns | Root Apo Fe | 3208520.518\*\*\* | 10767.118ns | 0.423ns |
| Root DW | 0.00440\*\*\* | 0.00225\*\* | 0.0000275ns | Root Sym Fe | 684711.918\*\*\* | 13268.477ns | 1334.953ns |
| Chl a+b | 44.679\*\*\* | 2.795\*\*\* | 0.0274ns | Proton release (1st) | 0.000131ns | 0.000636ns | 0.00000869ns |
| Photosynthesis | 42.218\*\*\* | 32.462\*\*\* | 8.165\*\* | Proton release (2nd) | 0.00948\*\*\* | 0.0000482ns | 0.000394ns |
| Transpiration | 0.206ns | 1.504\* | 2.477\*\* | SPAD (resupply) | 697.729\*\*\* | 448.070\*\*\* | 87.138\*\*\* |
| Stomatal cond | 0.245ns | 1.729\*\* | 0.181ns | Leaf Fe (resupply) | 43984.459\*\*\* | 55376.685\*\*\* | 29221.974\*\*\* |
| Fv/Fm | 0.0124\*\* | 0.00119\* | 0.000751ns | Root Fe (resupply) | 1964939.484\*\*\* | 1099.985ns | 106190.552ns |
| F'v/F'm | 0.0363\*\*\* | 0.0178\*\*\* | 0.0078\* | Barley | | | |
| qP | 0.00167ns | 0.000304ns | 0.0078ns |  | Fe | CM | Fe×CM |
| qN | 0.00121ns | 0.0176\*\* | 0.0171\*\* | Shoot DW | 0.000570ns | 0.00000963ns | 0.0000501ns |
| FRO activity (3rd) | 4763.516\*\*\* | 844.287\* | 752.139ns | Root DW | 0.000128ns | 0.0000007ns | 0.00000259ns |
| FRO activity (7th) | 208891.479\*\*\* | 3242.711\* | 1382.566ns | Chl a+b | 3.150\*\*\* | 0.200\*\* | 0.0773\* |
| FRO activity (10th) | 165381.926\*\*\* | 3081.549ns | 2408.141ns | Photosynthesis | 109.922\*\*\* | 2.551ns | 4.022ns |
| FRO activity (14th) | 72562.662\*\*\* | 3522.410ns | 2256.836ns | Transpiration | 0.0149ns | 0.120ns | 0.0317ns |
| PEPC activity | 1023.143\*\*\* | 184.863\* | 110.025ns | Stomatal cond | 0.108ns | 0.0919ns | 0.0673ns |
| PAL activity (1st) | 0.0285ns | 2.319ns | 79.393ns | Fv/Fm | 0.00412\*\*\* | 0.0000834ns | 0.000316ns |
| PAL activity (2nd) | 3.395ns | 17.324ns | 66.398ns | F'v/F'm | 0.00142ns | 0.000383ns | 0.00000406ns |
| Mal release (1st) | 1972.834\*\*\* | 229.816\*\* | 317.829\*\* | qP | 0.0118\*\*\* | 0.000284ns | 0.0000722ns |
| Mal release (2nd) | 2134.347\*\*\* | 150.338\*\* | 158.754\*\* | qN | 0.0283\*\* | 0.00493ns | 0.00174ns |
| Cit release (1st) | 5390.584\*\*\* | 297.758ns | 472.756\* | PSs release (3rd) | 0.0673\*\* | 0.486\*\*\* | 0.00423ns |
| Cit release (2nd) | 6271.683\*\*\* | 514.604\* | 523.790\* | PSs release (4th) | 0.107ns | 0.913\*\*\* | 0.0917ns |
| Suc release (1st) | 9503.422\*\*\* | 1818.072\*\*\* | 2332.434\*\*\* | PSs release (5th) | 0.0667ns | 0.0959\* | 0.00500ns |
| Suc release (2nd) | 12744.626\*\*\* | 1276.773ns | 1263.407ns | PSs release (7th) | 0.393\*\*\* | 0.0245ns | 0.0921\* |
| Fam release (1st) | 330.950\*\*\* | 23.972\* | 1.730ns | PSs release (14th) | 0.681\*\*\* | 0.146\*\*\* | 0.0545\*\* |
| Fum release (2nd) | 106.726\*\*\* | 9.159ns | 10.201ns | OL Fe | 2327.431ns | 1043.203ns | 482.639ns |
| Phenolics con (1st) | 41942.169\*\*\* | 132.250ns | 319.040ns | YL Fe | 2038.718ns | 1748.491ns | 423.079ns |
| Phenolics con (2nd) | 14082.619\*\* | 4016.054\* | 7460.181\*\* | Root Fe | 687162.010\*\*\* | 40769.259ns | 23489.435ns |
| Phenolics rel (1st) | 534.088\*\*\* | 62.540\* | 85.605\*\* | OL Zn | 50689.496\*\* | 492.103ns | 2555.329ns |
| Phenolics rel (2nd) | 1679.710\*\*\* | 142.290\* | 85.562\* | YL Zn | 7146.685ns | 2152.845ns | 6386.279ns |
| OL Fe | 0.263ns | 1827.876\*\* | 189.405ns | Root Zn | 24174.748\*\*\* | 1295.662ns | 4726.800\* |
| YL Fe | 871.887ns | 1457.797ns | 1048.650ns | Leaf Active Fe | 666255.2209\*\*\* | 22101.166\* | 482.848ns |
| Root Fe | 468110.378\*\*\* | 16786.326ns | 2996.799ns | Root Apo Fe | 6448875.519\*\*\* | 13369.109ns | 3891.552ns |
| OL Zn | 1066.242ns | 37.105ns | 3070.370ns | Root Sym Fe | 177132.041\*\*\* | 55089.647\*\*\* | 34504.632\*\* |
| YL Zn | 233.108ns | 1933.132ns | 3001.085\* | SPAD (resupply) | 337.468\*\*\* | 0.260ns | 63.105\* |
| Root Zn | 45832.172\*\*\* | 37418.099\*\*\* | 9084.543\*\*\* | Leaf Fe (resupply) | 23884.424\*\*\* | 5719.417ns | 1216.703ns |
| Leaf Active Fe | 417987.178\*\*\* | 24687.600\*\*\* | 14913.999\*\* | Root Fe (resupply) | 1217121.407\*\*\* | 32.650ns | 10047.781ns |