University of Birjand

تنشهای محیطی در علوم زراعی
Environmental Stresses In Crop Sciences
Env. Stresses Crop Sci.
Vol. 15, No. 2, pp. 375-392 (Summer 2022)

http://dx.doi.org/10.22077/escs.2021.3740.1908

*Original article*

# Survey of Azotobacter inoculation and cessation of irrigation on yield and some physiological characteristics of rapeseed cultivars

**M.Gholami[1], S. A. Siadat[2*], A. Koochakzadeh[3], M. R. Moraditelavat[3], M. Rafiei[4]**

*1. Ph.D. Student, Department of Plant Genetic and Production Engineering, Faculty of Agriculture, Khuzestan Agricultural and Natural Resources Sciences University, Ahvaz, Iran*
*2. Professor, Department of Plant Genetic and Production Engineering, Faculty of Agriculture, Khuzestan Agricultural and Natural Resources Sciences University, Ahvaz, Iran*
*3. Associate Professor, Department of Plant Genetic and Production Engineering, Faculty of Agriculture, Khuzestan Agricultural and Natural Resources Sciences University, Ahvaz, Iran*
*4. Research Assistant Professor, Lorestan Agricultural and Natural Resources Research and Education Center, Khorramabad, Iran, Iran*

Received 28 September 2020; Accepted 9 October 2021

## *Extended abstract*

### Introduction

Canola is one of the main oilseed crops. The effect of *Azotobacter chroococcum* as one of the biological fertilizer on the quantity and quality of rapeseed can be investigated. The presence of azotobacter in soils has positive effects on plants. Adequate moisture can promote vegetative growth, improve root growth, increase leaf area and durability, prolong flowering period, shoots, number of flowers and grain per pod, seed weight and yield. Moisture stress reduces the quantity and quality of these traits in rapeseed. The aim of this study was to find the right strain, suitable cultivar and appropriate irrigation regime for end of canola season water stress to save water in Lorestan province.

### Material and methods

In order to evaluate the amount of protein, proline, soluble sugars and photosynthetic antioxidant enzymes, chlorophyll and carotenoids of rapeseed cultivars under the influence of inoculation of *Azotobacter chrooccocum* in cut off irrigation conditions, an experiment was conducted in the year 2016-2017 at Sarab Chengai Agricultural Research Station, Khorramabad as a factorial split plot with randomized complete block design with 4 replications. Experimental factors included discontinuation of irrigation at 30% flowering and 30% pod forming stages and optimum irrigation (control), *Azotobacter chrooccocum* included 63, 70 strains and non-inoculated (control) and three rapeseed genotypes including Neptune, Octane and Okapi (control).

### Results and discussion

Results showed that the effect of irrigation interruption on grain yield, proline content, soluble sugars, proteins, antioxidant enzymes and photosynthetic pigments of green tissue was significant. The effect of azotobacter chroococcum strains on all of these traits except grain yield and proline was significant. Rapeseed cultivars differed significantly in terms of seed yield, soluble sugars, enzyme catalase and chlorophyll b, and total chlorophyll a + b. in terms of the accumulation of proline, protein, peroxidase enzyme, carotenoid, chlorophyll a And the chlorophyll a/b ratio was not significantly Different between cultivars. The highest levels of proline, soluble sugars, antioxidant enzymes were obtained in the irrigation cessation treatment from the 30% flowering stage and the highest amount of protein and

*Correspondent author: Seyed Ataollah Siadat; E-Mail: seyedatasiadat@yahoo.com

photosynthetic pigments were obtained from the normal irrigation treatment. Inoculation of rapeseed with *Azotobacter chrooccocum* strains increased the protein, total chlorophyll and carotenoid of the compared to the non-inoculated treatment. Octane and Neptune hybrids outperformed the total chlorophyll a+b of aerial organisms in terms of the enzyme catalase. However, the Okapi (control) cultivar was superior to octane and Neptune hybrids in terms of soluble sugars. The highest grain yield (4559 kg.ha$^{-1}$) was observed in the optimal irrigation (control). In the irrigation cut-off 30% of silique and 30% flowering stages, decreased grain yield (5.99% and 23.65%, respectively) compared to optimal irrigation. Seed yield of Octane and Neptune cultivars were 4584 and 4290 kg ha$^{-1}$, respectively, which were 24.7% and 19.6% more than Okapi (control) cultivars, respectively.

According to the results, Interaction Effects showed that rapeseed cultivars produced the highest protein content in the treatment of normal irrigation and inoculation with 63 and 70 *Aztobacter chroococcum* strains. The lowest green organ protein was obtained from treatments of non-inoculated and irrigation discontinuation from 30% flowering and 30% pod forming stages. Irrigation interruptions led to a significant increase in proline concentration in canola. In irrigation cessation treatment, 30% flowering and normal irrigation were observed with the lowest and highest proline concentrations, respectively. Probably related to its role in regulating osmosis to stabilize cellular membranes and proteins, inhibiting free radicals under stress. Irrigation witholding at 30% flowering and 30% pod forming resulted in a significant increase in antioxidant peroxidase and catalase enzymes activities. *Azotobacter chrooccocum* strains significantly reduced the levels of peroxidase and catalase enzymes in the rapeseed aerial parts. It is possible that by inoculating the *Azotobacter chrooccocum*, the plant is less likely to show signs of stress. Irrigation discontinuation significantly reduced chlorophyll a, b, and total levels compared to normal irrigation, possibly due to increased chlorophylase enzyme activity. The interactions between irrigation interruptions, azotobacter inoculation and rapeseed cultivars on the amount of chlorophyll a showed that Neptune and Octane hybrids produced the highest amount of chlorophyll a in the treatment with 70 strain in normal irrigation treatment. The same effects on chlorophyll b levels showed that the highest chlorophyll b levels were observed in octane and Neptune hybrids at 63 and 70 strains in normal irrigation and the lowest chlorophyll b content was observed in octane-free treatment without inoculation with irrigation cessation treatment from 30% flowering stage. Octane hybrid produced the highest total chlorophyll a + b in normal irrigation and bacterial inoculation of the strain 70. The highest accumulation of carotenoids was 0.430 mg g$^{-1}$ FW in octane hybrid in strain 63 and normal irrigation. The lowest accumulation of carotenoids in was obtained from the untreated bacterium. The concentration of soluble sugars in the cultivars varied. The highest soluble sugars in the Okapi cultivar weighed 56.341 mg g$^{-1}$ FW and produced less sugar Neptune with 54.89 and octane with 51.960 mg g$^{-1}$ FW. Therefore, it seems that the accumulation rate of these osmotic regulators is related to higher drought resistance of cultivars.

***Keywords*:** Antioxidant Enzymes, Carotenoid, Chlorophyll, Proline, Soluble sugars

**Table 1. Analysis of variancesof the effect of azotobacter inocullum on yeild, proline, unstructured soluble sugars, protein, catalase and peroxidase antioxidant enzymes photosynthetic pigments content of canola (*Brassica napus* L.) cultivars in terminal cessation of irrigation condition.**

| S.O.V | df | grain yeild | proline | unstructured soluble sugars | protein | peroxidase | catalase |
|---|---|---|---|---|---|---|---|
| **Block** | 3 | 2983472 $^{**}$ | 0.002 $^{ns}$ | 20.68 $^{ns}$ | 0.0015 $^{ns}$ | 0.0006 $^{ns}$ | 0.0267 $^{**}$ |
| **Irrigation** | 2 | 11318330$^{**}$ | 0.05 $^{**}$ | 2118.8 $^{**}$ | 0.105 $^{**}$ | 0.35 $^{**}$ | 0.061 $^{**}$ |
| **Block × Irrigation** | 6 | 878382$^{*}$ | 0.002 $^{ns}$ | 39.27 $^{ns}$ | 0.0027 $^{**}$ | 0.0018 $^{ns}$ | 0.00044$^{ns}$ |
| **Bacteria** | 2 | 725732$^{ns}$ | 0.001 $^{ns}$ | 335.6 $^{**}$ | 0.0627 $^{**}$ | 0.137 $^{**}$ | 0.024 $^{**}$ |
| **Variety** | 2 | 12428648$^{**}$ | 0.0002 $^{ns}$ | 172.75 $^{**}$ | 0.00025 $^{ns}$ | 0.0001 $^{ns}$ | 0.0083 $^{**}$ |
| **Bacteria × Irrigation** | 4 | 187984$^{ns}$ | 0.025 $^{**}$ | 314.9 $^{**}$ | 0.0052 $^{**}$ | 0.031 $^{**}$ | 0.0059 $^{**}$ |
| **Variety × Irrigation** | 4 | 950570$^{*}$ | 0.0003 $^{ns}$ | 85.13 $^{**}$ | 0.00084 $^{ns}$ | 0.0036 $^{ns}$ | 0.0019 $^{**}$ |
| **Bacteria × Variety** | 4 | 109719$^{ns}$ | 0.00072 $^{ns}$ | 12.77 $^{ns}$ | 0.00024 $^{ns}$ | 0.0017 $^{ns}$ | 0.0007 $^{ns}$ |
| **Bacteria × Variety × Irrigation** | 8 | 198607$^{ns}$ | 0.00069 $^{ns}$ | 9.37 $^{ns}$ | 0.00017 $^{ns}$ | 0.0025 $^{ns}$ | 0.00036 $^{ns}$ |
| **Error** | 72 | 368857 | 0.001 | 22.35 | 0.00088 | 0.002 | 0.00029 |
| **CV(%)** | | *14.78* | *9.46* | *8.72* | *7.25* | *7.8* | *3.48* |

**Table 1. Continued**

| S.O.V | df | Photosynthesis pigments content | | | | |
|---|---|---|---|---|---|---|
| | | Carotenoid | Chlorophyll a | Chlorophyll b | Total Chlorophyll | Chlorophyll $\frac{a}{b}$ Ratio |
| **Block** | 3 | 0.0001 $^{ns}$ | 0.0076 $^{*}$ | 0.00021 $^{ns}$ | 0.0091 $^{*}$ | 0.140 $^{ns}$ |
| **Irrigation** | 2 | 0.056 $^{**}$ | 1.43 $^{**}$ | 0.085 $^{**}$ | 2.2 $^{**}$ | 1.93 $^{**}$ |
| **Block × Irrigation** | 6 | 0.00039 $^{ns}$ | 0.0013 $^{ns}$ | 0.001 $^{**}$ | 0.0013 $^{ns}$ | 0.179 $^{ns}$ |
| **Bacteria** | 2 | 0.011 $^{**}$ | 0.316 $^{**}$ | 0.001 $^{*}$ | 0.339 $^{**}$ | 5.62 $^{**}$ |
| **Variety** | 2 | 0.0016 $^{ns}$ | 0.004 $^{ns}$ | 0.0021 $^{**}$ | 0.012 $^{**}$ | 0.138 $^{ns}$ |
| **Bacteria × Irrigation** | 4 | 0.0033 $^{**}$ | 0.243 $^{**}$ | 0.0021 $^{**}$ | 0.279 $^{**}$ | 3.75 $^{**}$ |
| **Variety × Irrigation** | 4 | 0.0027 $^{**}$ | 0.0092 $^{**}$ | 0.0021 $^{**}$ | 0.014 $^{**}$ | 0.44 $^{**}$ |
| **Bacteria × Variety** | 4 | 0.00015 $^{ns}$ | 0.00098 $^{ns}$ | 0.0021 $^{**}$ | 0.0015 $^{ns}$ | 0.50 $^{**}$ |
| **Bacteria × Variety × Irrigation** | 8 | 0.0016 $^{*}$ | 0.014 $^{**}$ | 0.0017 $^{**}$ | 0.024 $^{**}$ | 0.171 $^{ns}$ |
| **Error** | 72 | 0.00036 | 0.0024 | 0.00026 | 0.0025 | 0.096 |
| **CV(%)** | | 5.36 | 7.18 | 6.8 | 5.44 | 10.85 |

ns,*and**:non-significant, significant in 0.05 and 0.01 level, respectively

**Table 2. Meanes comparison of the irrigation and cultivar interaction effects on grain yield, proline, unstructured soluble sugars, protein, catalase and peroxidase antioxidant enzymes photosynthetic pigments content of rapeseed *(Brassica napus* L.)cultivars in terminal cessation of irrigation condition under effect of azotobacter inocullum**

| Treatment | | Grain yeild | Proline | Unstructured soluble sugars | Protein | Peroxidase | Catalase |
|---|---|---|---|---|---|---|---|
| | | kg.ha$^{-1}$ | mg.g$^{-1}$ FW | mg.g$^{-1}$ FW | mg.g$^{-1}$ FW | nmol.min.g$^{-1}$ | nmol.min.g$^{-1}$ |
| **I1** | **v1** | 3430.8 $^{dc}$ | 0.429 $^{a}$ | 65.96 $^{a}$ | 0.381 $^{b}$ | 0.654 $^{a}$ | 0.535 $^{a}$ |
| | **v2** | 3925.5 $^{bc}$ | 0.417 $^{ab}$ | 59.26 $^{ab}$ | 0.364 $^{b}$ | 0.661 $^{a}$ | 0.530 $^{a}$ |
| | **v3** | 3086.3 $^{d}$ | 0.422 $^{ab}$ | 62.43 $^{ab}$ | 0.378 $^{b}$ | 0.643 $^{a}$ | 0.510 $^{ab}$ |
| **I2** | **v1** | 4699.7 $^{a}$ | 0.370 $^{bc}$ | 50.52 $^{cd}$ | 0.388 $^{b}$ | 0.659 $^{a}$ | 0.495 $^{ab}$ |
| | **v2** | 4567.7 $^{ab}$ | 0.372 $^{bc}$ | 50.34 $^{cd}$ | 0.376 $^{b}$ | 0.622 $^{a}$ | 0.534 $^{a}$ |
| | **v3** | 3591.6 $^{dc}$ | 0.383 $^{abc}$ | 56.51 $^{bc}$ | 0.380 b | 0.635 a | 0.495 $^{ab}$ |
| **I3** | **v1** | 4742.2 $^{a}$ | 0.347 $^{c}$ | 46.11 $^{d}$ | 0.464 $^{a}$ | 0.461 $^{b}$ | 0.433 $^{c}$ |
| | **v2** | 5258.3 $^{a}$ | 0.348 $^{c}$ | 46.28 $^{d}$ | 0.477 $^{a}$ | 0.476 $^{b}$ | 0.468 $^{bc}$ |
| | **v3** | 3677.5 $^{dc}$ | 0.349 $^{c}$ | 50.08 $^{cd}$ | 0.472 $^{a}$ | 0.489 $^{b}$ | 0.439 $^{c}$ |

**Table 2. Continued**

| Treatments | | Photosynthesis pigments content | | | | |
|---|---|---|---|---|---|---|
| | | Carotenoid | Chlorophyll a | Chlorophyll b | Total chlorophyll | Chlorophyll $\frac{a}{b}$ ratio |
| | | ---------- mg.g$^{-1}$ FW ---------- | | | | |
| I1 | v1 | 0.295 $^{d}$ | 0.562 $^{b}$ | 0.206 $^{cd}$ | 0.770 $^{b}$ | 2.801 $^{ab}$ |
| | v2 | 0.331 $^{c}$ | 0.534 $^{b}$ | 0.215 $^{cd}$ | 0.794 $^{b}$ | 2.445 $^{b}$ |
| | v3 | 0.324 $^{cd}$ | 0.565 $^{b}$ | 0.196 $^{d}$ | 0.762 $^{b}$ | 2.832 $^{ab}$ |
| I2 | v1 | 0.355 $^{bc}$ | 0.575 $^{b}$ | 0.204 $^{cd}$ | 0.775 $^{b}$ | 2.880 $^{ab}$ |
| | v2 | 0.355 $^{bc}$ | 0.598 $^{b}$ | 0.215 $^{cd}$ | 0.809 $^{b}$ | 2.865 $^{ab}$ |
| | v3 | 0.405 $^{a}$ | 0.575 $^{b}$ | 0.226 $^{c}$ | 0.798 $^{b}$ | 2.583 $^{ab}$ |
| I3 | v1 | 0.405 $^{a}$ | 0.899 $^{a}$ | 0.295 $^{ab}$ | 1.192 $^{a}$ | 3.060 $^{ab}$ |
| | v2 | 0.405 $^{a}$ | 0.955 $^{a}$ | 0.311 $^{a}$ | 1.265 $^{a}$ | 3.079 $^{ab}$ |
| | v3 | 0.379 $^{ab}$ | 0.883 $^{a}$ | 0.275 $^{b}$ | 1.156 $^{a}$ | 3.246 $^{a}$ |

Means followed by the same letters in each column are not significantly different by duncan's test
(I1: Irrigation withholding since 30% flowering stage, I2: Irrigation withholding since 30% pod forming stage, I3: Normal Irrigation v1: Neptun, v2: Octane and v3: Okapi)

**Table 3. Meanes comparison of the irrigation and bacteria effects on grain yield, proline, unstructured soluble sugars, protein, catalase and peroxidase antioxidant enzymes photosynthetic pigments content of rapeseed *(Brassica napus* L.) cultivars in terminal cessation of irrigation condition under effect of azotobacter inocullum**

| Treatments | | Grain yeild | Proline | Unstructured soluble sugars | Protein | Peroxidase | Catalase |
|---|---|---|---|---|---|---|---|
| | | kg.ha$^{-1}$ | mg.g$^{-1}$ FW | mg.g$^{-1}$ FW | mg.g$^{-1}$ FW | nmol.min.g$^{-1}$ | nmol.min.g$^{-1}$ |
| I1 | b1 | 3532.2 $^{bc}$ | 0.447 $^{a}$ | 64.16 $^{a}$ | 0.400 $^{c}$ | 0.657 $^{bc}$ | 0.517 $^{bc}$ |
| | b2 | 3599.1 $^{bc}$ | 0.445 $^{a}$ | 61.39 $^{a}$ | 0.395 $^{c}$ | 0.617 $^{cd}$ | 0.519 $^{bc}$ |
| | b3 | 3311.3 $^{c}$ | 0.376 $^{b}$ | 62.09 $^{a}$ | 0.328 $^{d}$ | 0.684 $^{b}$ | 0.540 $^{ab}$ |
| I2 | b1 | 4505 $^{ab}$ | 0.326 $^{c}$ | 43.75 $^{c}$ | 0.437 $^{b}$ | 0.551 $^{ef}$ | 0.465 $^{de}$ |
| | b2 | 4153.2 $^{abc}$ | 0.367 $^{bc}$ | 52.4 $^{b}$ | 0.395 $^{c}$ | 0.597 $^{de}$ | 0.495 $^{cd}$ |
| | b3 | 4200.8 $^{abc}$ | 0.431 $^{a}$ | 61.23 $^{a}$ | 0.313 $^{d}$ | 0.769 $^{a}$ | 0.565 $^{a}$ |
| I3 | b1 | 4662.8 $^{a}$ | 0.351 $^{bc}$ | 46.14 $^{c}$ | 0.487 $^{a}$ | 0.465 $^{g}$ | 0.440 $^{e}$ |
| | b2 | 4664.6 $^{a}$ | 0.348 $^{bc}$ | 47.43 $^{bc}$ | 0.483 $^{a}$ | 0.432 $^{g}$ | 0.436 $^{e}$ |
| | b3 | 4350.5 $^{ab}$ | 0.346 $^{bc}$ | 48.90 $^{bc}$ | 0.444 $^{b}$ | 0.528 $^{f}$ | 0.464 $^{de}$ |

**Table 3. Continued**

| Treatments | | Photosynthesis pigments content | | | | |
|---|---|---|---|---|---|---|
| | | Carotenoid | Chlorophyll a | Chlorophyll b | Total Chlorophyll | Chlorophyll $\frac{a}{b}$ ratio |
| | | ---------- mg.g$^{-1}$ FW ---------- | | | | |
| I1 | b1 | 0.3483 $^{bc}$ | 0.751 $^{c}$ | 0.219 $^{b}$ | 0.973 $^{d}$ | 3.471 $^{a}$ |
| | b2 | 0.3266 $^{c}$ | 0.601 $^{e}$ | 0.214 $^{b}$ | 0.814 $^{ef}$ | 2.848 $^{bc}$ |
| | b3 | 0.2758 $^{d}$ | 0.309 $^{g}$ | 0.18 $^{c}$ | 0.494 $^{h}$ | 1.756 $^{e}$ |
| I2 | b1 | 0.361 $^{b}$ | 0.562 $^{ef}$ | 0.220 $^{b}$ | 0.777 $^{fg}$ | 2.626 $^{cd}$ |
| | b2 | 0.361 $^{b}$ | 0.674 $^{d}$ | 0.205 $^{bc}$ | 0.874 $^{e}$ | 3.342 $^{a}$ |
| | b3 | 0.343 $^{bc}$ | 0.512 $^{f}$ | 0.221 $^{b}$ | 0.732 $^{g}$ | 2.361 $^{d}$ |
| I3 | b1 | 0.404 $^{a}$ | 0.814 $^{c}$ | 0.292 $^{a}$ | 1.103 $^{c}$ | 2.825 $^{bc}$ |
| | b2 | 0.396 $^{a}$ | 1.008 $^{a}$ | 0.299 $^{a}$ | 1.304 $^{a}$ | 3.396 $^{a}$ |
| | b3 | 0.388 $^{a}$ | 0.916 $^{b}$ | 0.292 $^{a}$ | 1.207 $^{b}$ | 3.166 $^{ab}$ |

Means followed by the same letters in each column are not significantly different by duncan's test
(I1: Irrigation withholding since 30% flowering stage, I2: Irrigation withholding since 30% pod forming stage, I3: Normal Irrigation, b1: Azotobacter chroococcum 63, b2: Azotobacter chroococcum 70,b3: No Azotobacter)

**Table 4. Meanes comparison of the bacteria and cultivars effects on grain yield, proline, unstructured soluble sugars, protein, catalase and peroxidase antioxidant enzymes photosynthetic pigments content of rapeseed *(Brassica napus* L.) cultivars in terminal cut off irrigation condition under effect of azotobacter inocullum**

| Treatments | | Grain yeild | Proline | Unstructured soluble sugars | Protein | Peroxidase | Catalase |
|---|---|---|---|---|---|---|---|
| | | $Kg.ha^{-1}$ | $Mg.g^{-1}$ FW | $Mg.g^{-1}$ FW | $Mg.g^{-1}$ FW | $nmol.min.g^{-1}$ | $nmol.min.g^{-1}$ |
| B1 | V1 | 4341.2 ab | 0.447 a | 64.16 a | 0.400 c | 0.657 bc | 0.517 bc |
| | V2 | 4805.3 a | 0.445 a | 61.39 a | 0.395 c | 0.617 cd | 0.519 bc |
| | V3 | 3553.5 bc | 0.376 b | 62.09 a | 0.328 d | 0.684 b | 0.540 ab |
| B2 | V1 | 4421.7 ab | 0.326 c | 43.75 c | 0.437 b | 0.551 ef | 0.465 de |
| | V2 | 4551.8 a | 0.367 bc | 52.4 b | 0.395 c | 0.597 de | 0.495 cd |
| | V3 | 3443.4 bc | 0.431 a | 61.23 a | 0.313 d | 0.769 a | 0.565 a |
| B3 | V1 | 4109.8 abc | 0.351 bc | 46.14 c | 0.487 a | 0.465 g | 0.440 e |
| | V2 | 4394.4 ab | 0.348 bc | 47.43 bc | 0.483 a | 0.432 g | 0.436 e |
| | V3 | 3358.4 c | 0.346 bc | 48.90 bc | 0.444 b | 0.528 f | 0.464 de |

**Table 4. Continued**

| Treatments | | Photosynthesis pigments content | | | | |
|---|---|---|---|---|---|---|
| | | carotenoid | Chlorophyll a | Chlorophyll b | Total Chlorophyll | Chlorophyll $\frac{a}{b}$ Ratio |
| | | ---------- $mg.g^{-1}$ FW ---------- | | | | |
| B1 | V1 | 0.3483 bc | 0.751 c | 0.219 b | 0.973 d | 3.471 a |
| | V2 | 0.3266 c | 0.601 e | 0.214 b | 0.814 ef | 2.848 bc |
| | V3 | 0.2758 d | 0.309 g | 0.18 c | 0.494 h | 1.756 e |
| B2 | V1 | 0.361 b | 0.562 ef | 0.220 b | 0.777 fg | 2.626 cd |
| | V2 | 0.361 b | 0.674 d | 0.205 bc | 0.874 e | 3.342 a |
| | V3 | 0.343 bc | 0.512 f | 0.221 b | 0.732 g | 2.361 d |
| B3 | V1 | 0.404 a | 0.814 c | 0.292 a | 1.103 c | 2.825 bc |
| | V2 | 0.396 a | 1.008 a | 0.299 a | 1.304 a | 3.396 a |
| | V3 | 0.388 a | 0.916 b | 0.292 a | 1.207 b | 3.166 ab |

Means followed by the same letters in each column are not significantly different by duncan's test
(b1: Azotobacter chroococcum 63, b2: Azotobacter chroococcum 70,b3: No Azotobacter, v1: Neptun, v2: Octane and v3: Okapi)

**Table 5. Meanes comparison of the irrigation withholding, bacteria and cultivars effects on grain yield, proline, unstructured soluble sugars, protein, catalase and peroxidase antioxidant enzymes photosynthetic pigments content of rapeseed *(Brassica napus* L.*)* cultivares in terminal cessation of irrigation condition under effect of azotobacter inocullum**

| Treatments | | | Grain yeild | Proline | Unstructured soluble sugars | Protein | Peroxidase | Catalase |
|---|---|---|---|---|---|---|---|---|
| | | | kg.ha$^{-1}$ | mg.g$^{-1}$ FW | mg.g$^{-1}$ FW | mg.g$^{-1}$ FW | mol.min.g$^{-1}$ | nmol.min.g$^{-1}$ |
| I1 | B1 | V1 | 3277 $^{ef}$ | 0.455 $^{ab}$ | 67.925 $^{a}$ | 0.402 $^{bc}$ | 0.670 $^{bcd}$ | 0.517 $^{bcd}$ |
| | | V2 | 3961.6 $^{b-f}$ | 0.430 $^{abc}$ | 61.973 $^{ab}$ | 0.392 $^{bc}$ | 0.675 $^{bcd}$ | 0.530 $^{a-d}$ |
| | | V3 | 3357.5 $^{def}$ | 0.457 $^{a}$ | 62.59 $^{ab}$ | 0.405 $^{bc}$ | 0.627 $^{c-f}$ | 0.505 $^{b-e}$ |
| | B2 | V1 | 3654.1 $^{c-f}$ | 0.452 $^{ab}$ | 63.738 $^{ab}$ | 0.402 $^{bc}$ | 0.605 $^{c-g}$ | 0.535 $^{abc}$ |
| | | V2 | 3937.5 $^{b-f}$ | 0.430 $^{abc}$ | 57.978 $^{abc}$ | 0.392 $^{bc}$ | 0.637 $^{cde}$ | 0.535 $^{abc}$ |
| | | V3 | 3205.8 $^{ef}$ | 0.452 $^{ab}$ | 62.450 $^{ab}$ | 0.390 $^{bc}$ | 0.610 $^{c-g}$ | 0.495 $^{b-f}$ |
| | B3 | V1 | 3360.7 $^{def}$ | 0.380 $^{a-f}$ | 66.205 $^{a}$ | 0.337 $^{cd}$ | 0.687 $^{bc}$ | 0.597 $^{a}$ |
| | | V2 | 3877.5 $^{b-f}$ | 0.392 $^{a-f}$ | 57.82 $^{abc}$ | 0.307 $^{d}$ | 0.672 $^{f-j}$ | 0.532 $^{a-d}$ |
| | | V3 | 2695.6 $^{f}$ | 0.355 $^{c-f}$ | 62.253 $^{ab}$ | 0.340 $^{cd}$ | 0.692 $^{bc}$ | 0.532 $^{a-d}$ |
| I2 | B1 | V1 | 5025.8 $^{abc}$ | 0.327 $^{f}$ | 42.368 $^{e}$ | 0.450 $^{ab}$ | 0.560 $^{e-i}$ | 0.452 $^{def}$ |
| | | V2 | 4934.2 $^{a-d}$ | 0.315 $^{f}$ | 41.950 $^{e}$ | 0.425 $^{ab}$ | 0.527 $^{g-k}$ | 0.487 $^{b-f}$ |
| | | V3 | 3555 $^{c-f}$ | 0.337 $^{ef}$ | 46.920 $^{de}$ | 0.435 $^{ab}$ | 0.565 $^{e-i}$ | 0.455 $^{def}$ |
| | B2 | V1 | 4601.6 $^{a-e}$ | 0.367 $^{c-f}$ | 51.325 $^{cde}$ | 0.400 $^{bc}$ | 0.597 $^{c-g}$ | 0.482 $^{b-f}$ |
| | | V2 | 4387.2 $^{a-e}$ | 0.372 $^{b-f}$ | 50.700 $^{cde}$ | 0.395 $^{bc}$ | 0.587 $^{d-h}$ | 0.517 $^{bcd}$ |
| | | V3 | 3470.8 $^{c-f}$ | 0.362 $^{c-f}$ | 55.170 $^{bcd}$ | 0.390 $^{bc}$ | 0.607 $^{c-g}$ | 0.485 $^{b-f}$ |
| | BB3 | V1 | 4471.8 $^{a-e}$ | 0.415 $^{a-e}$ | 57.873 $^{abc}$ | 0.315 $^{d}$ | 0.820 $^{a}$ | 0.550 $^{ab}$ |
| | | V2 | 4381.7 $^{a-e}$ | 0.427 $^{a-d}$ | 58.388 $^{abc}$ | 0.310 $^{d}$ | 0.852 $^{ab}$ | 0.597 $^{a}$ |
| | | V3 | 3748.9 $^{c-f}$ | 0.450 $^{ab}$ | 67.443 $^{a}$ | 0.315 $^{d}$ | 0.835 $^{ab}$ | 0.547 $^{ab}$ |
| I3 | B1 | V1 | 4720.4 $^{a-e}$ | 0.347 $^{c-f}$ | 46.605 $^{de}$ | 0.477 $^{a}$ | 0.422 $^{l}$ | 0.427 $^{ef}$ |
| | | V2 | 5520 $^{a}$ | 0.345 $^{c-f}$ | 44.743 $^{de}$ | 0.490 $^{a}$ | 0.475 $^{i-l}$ | 0.465 $^{c-f}$ |
| | | V3 | 3748.1 $^{c-f}$ | 0.360 $^{c-f}$ | 47.0 $^{de}$ | 0.492 $^{a}$ | 0.500 $^{h-l}$ | 0.427 $^{ef}$ |
| | B2 | V1 | 5009.5 $^{abc}$ | 0.347 $^{c-f}$ | 45.295 $^{de}$ | 0.480 $^{a}$ | 0.432 $^{kl}$ | 0.420 $^{f}$ |
| | | V2 | 5330.7 $^{ab}$ | 0.352 $^{c-f}$ | 45.273 $^{de}$ | 0.490 $^{a}$ | 0.422 $^{l}$ | 0.452 $^{def}$ |
| | | V3 | 3653.7 $^{c-f}$ | 0.345 $^{c-f}$ | 51.733 $^{cde}$ | 0.480 $^{a}$ | 0.442 $^{jkl}$ | 0.437 $^{ef}$ |
| | BB3 | V1 | 4496.8 $^{a-e}$ | 0.347 $^{c-f}$ | 46.445 $^{de}$ | 0.435 $^{ab}$ | 0.527 $^{g-k}$ | 0.452 $^{def}$ |
| | | V2 | 4924.1 $^{a-d}$ | 0.347 $^{c-f}$ | 48.820 $^{cde}$ | 0.452 $^{ab}$ | 0.532 $^{f-j}$ | 0.487 $^{b-f}$ |
| | | V3 | 3630.7 $^{c-f}$ | 0.342 $^{def}$ | 51.448 $^{cde}$ | 0.445 $^{ab}$ | 0.525 $^{g-k}$ | 0.452 $^{def}$ |

**Table 5. Continued**

| Treatments | | | Photosynthesis pigments content | | | | |
|---|---|---|---|---|---|---|---|
| | | | Carotenoid | Chlorophyll a | Chlorophyll b | Total Chlorophyll | Chlorophyll $\frac{a}{b}$ Ratio |
| | | | ---------- mg.g$^{-1}$ FW ---------- | | | | |
| I1 | B1 | V1 | 0.352 $^{e-h}$ | 0.735 $^{de}$ | 0.192 $^{fg}$ | 0.930 $^{de}$ | 3.0832 $^{a}$ |
| | | V2 | 0.337 $^{ghi}$ | 0.760 $^{cde}$ | 0.235 $^{cde}$ | 0.997 $^{cd}$ | 3.262 $^{abc}$ |
| | | V3 | 0.355 $^{e-h}$ | 0.760 $^{cde}$ | 0.230 $^{c-f}$ | 0.992 $^{cd}$ | 3.320 $^{abc}$ |
| | B2 | V1 | 0.287 $^{j}$ | 0.587 $^{fgh}$ | 0.212 $^{c-g}$ | 0.797 $^{fg}$ | 2.820 $^{b-e}$ |
| | | V2 | 0.357 $^{d-h}$ | 0.607 $^{fg}$ | 0.220 $^{c-g}$ | 0.825 $^{efg}$ | 2.815 $^{b-e}$ |
| | | V3 | 0.335 $^{ghi}$ | 0.607 $^{fg}$ | 0.210 $^{c-g}$ | 0.820 $^{fg}$ | 2.910 $^{b-e}$ |
| | B3 | V1 | 0.245 $^{k}$ | 0.365 $^{j}$ | 0.212 $^{c-g}$ | 0.582 $^{i}$ | 1.752 $^{gh}$ |
| | | V2 | 0.300 $^{ij}$ | 0.235 $^{k}$ | 0.190 $^{g}$ | 0.425 $^{j}$ | 1.250 $^{h}$ |
| | | V3 | 0.282 $^{j}$ | 0.327 $^{jk}$ | 0.215 $^{c-g}$ | 0.475 $^{j}$ | 2.267 $^{efg}$ |
| I2 | B1 | V1 | 0.350 $^{e-h}$ | 0.580 $^{fgh}$ | 0.217 $^{c-g}$ | 0.792 $^{fg}$ | 2.745 $^{c-f}$ |
| | | V2 | 0.360 $^{d-h}$ | 0.607 $^{fg}$ | 0.207 $^{c-g}$ | 0.810 $^{fg}$ | 3.020 $^{bcd}$ |
| | | V3 | 0.372 $^{b-h}$ | 0.497 $^{hi}$ | 0.235 $^{cde}$ | 0.730 $^{gh}$ | 2.112 $^{fg}$ |
| | B2 | V1 | 0.362 $^{c-h}$ | 0.677 $^{ef}$ | 0.197 $^{efg}$ | 0.872 $^{ef}$ | 3.492 $^{ab}$ |
| | | V2 | 0.360 $^{d-h}$ | 0.672 $^{ef}$ | 0.212 $^{c-g}$ | 0.877 $^{ef}$ | 3.222 $^{abc}$ |
| | | V3 | 0.360 $^{d-h}$ | 0.672 $^{ef}$ | 0.205 $^{d-g}$ | 0.872 $^{ef}$ | 3.312 $^{abc}$ |
| | BB3 | V1 | 0.352 $^{e-h}$ | 0.467 $^{i}$ | 0.197 $^{efg}$ | 0.662 $^{hi}$ | 2.402 $^{d-g}$ |
| | | V2 | 0.345 $^{fgh}$ | 0.515 $^{ghi}$ | 0.225 $^{c-g}$ | 0.740 $^{gh}$ | 2.355 $^{d-g}$ |
| | | V3 | 0.332 $^{hi}$ | 0.555 $^{ghi}$ | 0.240 $^{cd}$ | 0.792 $^{fg}$ | 2.325 $^{d-g}$ |
| I3 | B1 | V1 | 0.407 $^{ab}$ | 0.812 $^{bcd}$ | 0.285 $^{b}$ | 1.092 $^{bc}$ | 2.877 $^{b-e}$ |
| | | V2 | 0.430 $^{a}$ | 0.815 $^{bcd}$ | 0.297 $^{ab}$ | 1.110 $^{b}$ | 2.772 $^{b-f}$ |
| | | V3 | 0.375 $^{b-g}$ | 0.815 $^{bcd}$ | 0.292 $^{b}$ | 1.107 $^{b}$ | 2.825 $^{b-e}$ |
| | B2 | V1 | 0.412 $^{ab}$ | 1.015 $^{a}$ | 0.300 $^{ab}$ | 1.312 $^{a}$ | 2.825 $^{b-e}$ |
| | | V2 | 0.402 $^{abc}$ | 1.017 $^{a}$ | 0.307 $^{ab}$ | 1.322 $^{a}$ | 3.312 $^{abc}$ |
| | | V3 | 0.375 $^{b-g}$ | 0.992 $^{a}$ | 0.290 $^{b}$ | 1.277 $^{a}$ | 3.470 $^{ab}$ |
| | BB3 | V1 | 0.395 $^{a-d}$ | 0.870 $^{b}$ | 0.302$^{ab}$ | 1.172 $^{b}$ | 2.900 $^{b-e}$ |
| | | V2 | 0.382 $^{b-f}$ | 1.035 $^{a}$ | 0.330 $^{a}$ | 1.365 $^{a}$ | 3.152 $^{abc}$ |
| | | V3 | 0.387 $^{b-e}$ | 0.842 $^{bc}$ | 0.245 $^{c}$ | 1.085 $^{bc}$ | 3.445 $^{abc}$ |

Means followed by the same letters in each column are not significantly different by duncan's test
(I1: Irrigation withholding since 30% flowering stage, I2: Irrigation withholding since 30% pod forming stage, I3: Normal Irrigation, b1: *Azotobacter chroococcum* 63, b2: *Azotobacter chroococcum* 70,b3: No Azotobacter, v1: Neptun, v2: Octane and v3: Okapi)