NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 40, 2023
First Online: March, 2023. DII 2067-5720 RAR 2023-111

EFFECT OF BIOFERTILIZER AND DROUGHT STRESS ON QUANTITATIVE AND QUALITATIVE TRAITS IN SOME WINTER RAPESEED (*Brassica napus* L.) CULTIVARS

Mohammad Reza Safari[1], Mohammad Reza Dadashi[*1,] Abolfazl Faraji[2], Mohammad Armin[3]

[1]Department of Agronomy, Gorgan Branch, Islamic Azad University, Gorgan, Iran

[2]Department of Agriculture and Horticulture, Golestan Province Agricultural and Natural Resources Research and Training Center, Agricultural Research, Education and Extension Organization, Gorgan, Iran

[3]Department of Agronomy and Plant Breeding, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran

[*]Corresponding author. E-mail: morezda@yahoo.com

ABSTRACT

To evaluation the effect of plant growth-promoting rhizobacteria under water deficit condition on quantitative and qualitative traits of winter rapeseed cultivars, an experiment was conducted at Agricultural and Natural Resources Research Center located in North Khorasan Province (2015-2017). Plant growth-promoting *rhizobacteria* (Nitroxin consumption and control) under water deficit condition (after 80, 130, 180 mm evaporation) on quantitative and qualitative traits of winter rapeseed cultivars (Natalie, Okapi, and Neptune) were investigated.

Findings indicated that Nitroxin increased soil K, P, Fe, and Cu concentrations and decreased the soil pH and EC. Indeed, the soil N reduction and P increasing trend were related to the maximum biological yield and harvest index (HI) in the second and first years, respectively. Also, an inverse relationship was observed between No. of pods plant^{-1} (NPP) and No. of seeds pod^{-1} (NSP) among cultivars. Nitroxin was more affected on NSP and seeds weight than NPP under low and mild drought stress. The highest harvest index was observed in the mild stress in three cultivars, but, the Nitroxin consumption was ineffective. Seed yield and its components except for NPP and HI were increased by the Nitroxin consumption. The HI exhibited the inverse and direct relationship between protein and oil content, respectively. High drought stress compared to low drought stress reduced NPP (54.9%), NSP (1.3%), seed yield (52.2%), biological yield (47.5%), seed oil content (5.5%), seed oil yield (54.9%), and seed protein yield (52.7%). The highest NPP (45.5), seed yield (2740 kg ha^{-1}), biological yield (7572 kg ha^{-1}), and oil yield (933 kg ha^{-1}) were obtained from cv. Natalie. In conclusion, Nitroxin biological fertilizer ameliorates the negative effects of drought stress under mild stressful conditions but it had not any considerable effects on reducing the effects of drought stress under severe stress conditions.

Keywords: nitroxin, oil yield, protein content, rapeseed, water stress.

INTRODUCTION

Rapeseed (*Brassica napus* L.) plant possesses remarkable agronomic traits such as tolerance to cold and drought conditions, long growing period, wide range of adaptation to soil texture, dominancy on narrow leaf weeds, spring and winter genotypes, adapted to diverse soil moisture, low production costs and ultimately produces higher oil yield than other cultivated oilseed crops, therefore, it can be recommended for planting in most Provinces of Iran (Khani et al., 2017). Also, rapeseed is one of the most important oil plants in whole world, so that it is the world's third-largest source of oil ranked as after soybean and palm oil (FAO, 2019).

Water scarcity is a major restraint affects the plant growth and yield, especially in the arid and semi-arid regions like Iran. To meet the nutritional demands of the growing population, it is necessary to develop drought-tolerant plants capable of cultivation in a wide range of climates and soil types (Bot and Benites, 2005). Drought stress has been reported to influence the reproductive mechanisms such as flower and pod formation, seed size and the seed filling period depending on the severity and time of stress and growth stage (Birunara et al., 2011). Shabani et al. (2009) reported that

Received 30 November 2022; accepted 13 March 2023.

production potential of rapeseed was decreased by drought stress. Water restriction disturbs the plant nutritional balances. Accordingly, use of bio-fertilizers not only increases the plant's resistance to various environmental stress conditions such as water scarcity and nutrients, but also**,** it improves the soil characteristics and nutrients availability for the plants (Vatan Doost et al., 2019). Soil-based application of growth-promoting bacteria stimulates crop tolerance to abiotic stresses including drought stress and as an attractive strategy has been studied by many researchers (Grover et al., 2011). Growth-promoting bacteria consumption method has been investigated by several researchers and cropping systems (Choudhary et al., 2016; Vurukonda et al., 2016; Van Oosten et al., 2017).

One of the most prominent mechanisms to increase the plant growth by the growth-promoting bacteria is nitrogen fixation. Nitrogen fixation results from the symbiotic relationship between some fungi species and *Rhizobium* bacteria as well as the non-symbiotic cooperation of some micro-organisms and the plant roots in agricultural systems (Vejan et al., 2016). Nitroxin fertilizer contains free-living bacteria such as *Azotobacter* and *Azosperyllium*, capable of fixing the atmospheric nitrogen in the rhizosphere. Furthermore, these biological organisms produce and release some biologically active substances such as pentatonic acid, nicotinic acid, B vitamins and auxins (Wani et al., 2016). In addition, gibberellins production will be stimulated that enhance root growth**;** thereby increase water and nutrients uptake and consequently make better the seed yield and plants productivity (Iqbal et al., 2011).

Majidi (2012) stated that a significant variation in the drought tolerance based on yield-dependent traits among the new rapeseed genotypes will be aided the selection of better promising cultivars. The cultivars have diverse capabilities in the production and maintaining pods, so**,** if the pod formation occurs simultaneously with the suitable environmental conditions**,** the florets number and pod formation are stimulated, so that the cultivars with prolonged reproductive stage and a longer pods will be ultimately higher seed yields mainly due to more NSP (Diepenbrock, 2000).

Considering the water scarcity problem in many areas in the world and the importance of rapeseed as an oilseed, it is necessary to evaluate the rapeseed cultivar responses to degrees of drought stress, and finally to select the promising cultivars for the high seed yield and quality potential. In context of decreases the water availability in many regions and knowing direct effects of drought stress on the metabolic processes of plants and nutrients uptake from the soil (especially nitrogen), the present study aimed to assess the effects of drought stress on some morphological and physiological characteristics of some winter rapeseed cultivars and to evaluate the modulating role of Nitroxin consumption under drought stress.

MATERIAL AND METHODS

The experiment was conducted at Agricultural and Natural Resources Research Center of North Khorasan Province (Kohaneh Kand); northwest of Bojnourd Township, Iran during 2015-2016 and 2016-2017 growing seasons. Experiment site elevation was 1050 m.a.s.l with minimum and maximum absolute temperatures, and average temperature with -29, 42 and 12.8°C, respectively. The average annual rainfall was 259 mm. Rainfall mainly occurs in late autumn and early spring. Also, the effective precipitation is about 158.9 mm during the growing season.

Soil sample was collected from 0-30 soil depth prior to the land preparation and the end of the experiment to determine the soil physical and chemical properties (Table 1). The experiment was performed as a split plot factorial based on a randomized complete block design with four replications. Main factors includes irrigation regime (after 80, 130, 180 mm evaporation from class A evaporation pan denoted as low, medium and high drought stress) and the application of Nitroxin (Nitroxin (one L ha^{-1} as a liquid bio-fertilizer with 10^8 CFU per mL) and Non-use of Nitroxin as control) and three rapeseed cultivars (Natalie, Okapi and Neptune) as factorial arrangement in the sub plots. The cultivars were selected based on the region's climate, high yield potential, 1000-seed weight, and high resistance to disease.

Common soil preparations including plowing and disking were followed. Then, according to the soil analysis, the required basal fertilizers were applied. Fertilizers containing primary macronutrients based on soil test (Table 1) including nitrogen (150 kg ha^{-1}), triple superphosphate (150 kg ha^{-1}), and potassium sulfate (100 kg ha^{-1}) were uniformly mixed with the soil surface before planting. The furrows were created at a distance of 50 cm from each other. Seeds were planted on 6 separate lines**,** 4 meter long with a row spacing of 25 cm and 5 cm within line spacing. To perform the experiment, the plots were enclosed and, depending on their area, irrigation interval and the amount of water assigned to each plot were calculated. Drought stress treatments were began from 3-4 foliage growing stage. Nitroxin digestive fertilizer containing nitrogen-fixing bacteria, *Azotobacter* and *Azospirillum* was treated at 10^8 live cells per ml per 1 L ha^{-1} of rapeseed seeds[1]. The seeds inoculation was carried out in the lab and far from direct sunlight. The seeds planted immediately after shade drying at 2 cm sowing depth. Weed control performed as common and with special attention. Also, thinning was done in 4 to 6 leaves stage to obtain the optimum plant density (80 plants m^{-2}). Diazinon and Chlorpyrifos pesticides used against aphids as recommended during April.

[1]*according to the factory's instructions, one litre of biological liquid fertilizer could be used for eight kilograms of rapeseed seeds*

Table 1. The physical and chemical soil parameters in the first and the end of the experiment

Year	Soil depth	pH (1:5)	EC	Sand	Silt	Clay	Soil texture	N	K	P	Fe	Cu	Zn	Mn
Unit	cm	-	dS m^{-1}	%	%	%	-	%	%	%	ppm	ppm	ppm	ppm
2015-16	0-30	7.8	2.7	30	36	34	Clay loam	0.036	200	4	2.42	0.2	0.35	7
2016-17	0-30	7.3	2.1	-	-	-	-	0.029	265	7.5	2.86	0.28	0.24	6

At the end of the growing season (the physiological maturity stage), after excluding the marginal effect, the seed yield and the related yield components were evaluated. From the middle of each plot**,** five plants were randomly selected as representative of each experimental unit, in which the number of branches, NPP and NSP, as well as plant height, height to first branch, height to first pod, and pod length were measured. From the pods counted per plant, 20 pods were randomly selected and 1000-seeds weight and seed yield (based on 14% seed moisture content) were calculated by a precision of 0.001 g. Biological yield (total dry weight) including leaves, stems, pods and seed were weighed. Seed oil content was calculated after Soxhlet extraction (temperature 45°C and dry diethyl ether solvent) and seed oil yield was obtained by multiplying the seed yield at oil percentage (Parhizkar-Khajani et al., 2012). Protein content was measured by kjeldahl method (Lowary et al., 1951) and the seed protein yield was obtained from the corresponding dry weight percentage of seeds.

The data were analyzed by SAS (Statistical Analysis System, 2009), and LSD test was used to compare the mean data.

RESULTS AND DISCUSSION

Soil characteristics: the soil microbial activity increasing by bio-fertilizer consumption reduced the soil pH and EC about 0.5 and 0.6 dS m^{-1} in the second growing season compared to the first growing season, respectively (Table 1). The symbiosis between plants and rhizobacteria increased the plant efficiency in the low nutrient soils. Indeed, bio-fertilizer inoculation of seeds led to increased several indices such as root growth rate, leaf area surface, control of pathogenic factors, chlorophyll content, drought resistance, higher root and shoot weight and increment microbial activity in the soil (Lucy et al., 2004) and finally, increased facility access to nutrients in plants (Dobbelaere et al., 2003).

Nitrogen, zinc, and manganese in soil also decreased at the end of the experiment (Table 1). The decrease in soil nitrogen can be attributed to the increase in nitrogen uptake from the soil by plants due to the consumption of nitrogen fertilizer before cultivation and Nitroxin during the experiment. The increase in the plant protein yield at the Nitroxin biological fertilization confirmed this phenomenon (Table 3). Khalilian Ekrami (2006) suggested that application N_2-fixing bacteria (*Azotobacter* sp. and *Azospirillum* sp.) due to fixing nitrogen and so higher nitrogen absorbance in the soil led to increase of photosynthesis and therefore increment of seed protein yield in the plant. Potassium and phosphorous increased about 65 and 3.5 ppm which due to the chemical fertilizer usage (potassium sulfate and triple superphosphate) at the beginning of the experiment, was not unexpected (Table 1). The increasing the iron and copper concentration occurred duration the experiment (Table 1). Mulder's interaction nutrient chart represented that potassium had a synergistic effect on the iron concentration and as the concentration of potassium in the soil increases, so does the concentration of iron (Mulder, 1953). Copper had an antagonistic effect with nitrogen (Mulder, 1953) so the nitrogen reduction trend in the soil during the experiment increased copper concentration at the end about 0.08 ppm (Table 1). Zinc and manganese decreased at the end of the experiment (Table 1). An increase in soil phosphorus concentration, possibly caused by the use of chemical fertilizers, reduced the zinc concentration in the soil. Zinc is one of the most important elements for many enzymes, and strengthens cell membranes and proteins (Amiri, 2012). The role of zinc in plant defense mechanisms under abiotic stresses indicates that this element is able to reduce the effects of drought and salinity stresses in plants (Amiri, 2012). The plant's uptake of zinc and manganese increases under abiotic stress conditions such as drought. The use of biofertilizers indirectly increased the micronutrients such as Cu, Zn, Mn and Fe (Parvatha Reddy, 2014).

Number of pods per plant: the findings indicated that the NPP decreased in all rapeseed cultivars with intensifying drought stress. The highest NPP was observed in low stress condition in cv. Natalie. The minimum NPP was belonged to cv. Neptune under severe stressful conditions (Table 2). In rapeseed, NPP is determined by the flowering time-lapse and so, water stress caused a delay in flowering time-lapse and reduced pods number. Therefore, photosynthetic assimilates are reduced, and the plants under these stressful conditions guarantee their survival at the expense of lower flowering and the reduced pods number (Jamshidi et al., 2015). Furthermore, the shift in conditions from normal irrigation to moisture stress affects the growing traits of plants and make them to show different genetic potentials due to changing conditions (Sabzi et al., 2017). It seems that early flowering and the longer flowering period in cv. Natalie led to a higher NPP than other two cultivars under drought stress. Other researchers also reported a decrease the NPP in canola plants under drought stress (Jamshidi et al., 2015). Khani et al. (2017) stated that drought stress had the most effect on the flowers fertilization and noted that the stress led to quite less photosynthetic assimilates. They also noted that flowering and early development of pods were essential for canola productivity with normal irrigation.

Nitroxin application was able to modify the effect of drought stress on NPP, so that NPP will be increased compared to Nitroxin non-application. Accordingly, increasing the NPP with Nitroxin application was significant under low stress conditions and mild drought stress (Table 3). Also, more florets will become pods in suitable environmental conditions. Plants gain the advantages of the available temperature and radiation for a longer time and produce more assimilates by longer pods formation period. In hence, plants are able to maintain more florets and produce more NPP and ultimately increased seed yield (Abuzeid and Wilcockson, 1989).

Table 2. Interaction effect of drought stress and cultivar on NPP, seed yield and oil yield

Cultivar	Drought stress	NPP	Seed yield (kg ha^{-1})	Harvest Index (%)	Oil yield (kg ha^{-1})
Natali	Low	45.5 a*	2740 a	27.3 b	1273 a
	Medium	36.2 b	2095 b	29.9 a	934 b
	High	24.9 c	1350 c	25.1 b	591 c
Okapi	Low	43.2 a	1821 a	19.3 b	778 a
	Medium	38.8 b	1499 b	22.6 a	627 b
	High	22.5 c	764 c	14.5 c	316 c
Neptone	Low	35.5 a	2548 a	25.7 a	1186 a
	Medium	25.9 b	1741 b	27.2 a	791 b
	High	20.5 c	1283 c	27.7 a	554 c

Values followed by the same letter within the same columns do not differ significantly at p=5% based on FLSD.
* mean comparisons are based on interaction slicing cultivar at three drought stress levels.

Table 3. Interaction effect of drought stress and cultivar on number of pods per plant, seed yield and oil yield

Drought stress	Nitroxin consumption	NPP	1000-seed weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Oil yield (%)	Protein yield (kg ha^{-1})
Low	Control	38.5 b	3.15 b	2121 a	9239 b	988 b	339 b
	Nitroxin	45.54 a	3.27 a	2518 a	10741 a	1170 a	448a
Medium	Control	30.58 b	3.01 b	1507 b	6173 b	722 b	230 b
	Nitroxin	36.1 a	3.15 a	2050 a	7213 a	846 a	400 a
High	Control	21.28 a	2.88 a	1126 a	5023 a	491 a	150 b
	Nitroxin	23 a	2.8 a	1139 a	5472 a	483 a	225 a

Values followed by the same letter within the same columns do not differ significantly at p=5% based on FLSD.
* mean comparisons are based on interaction slicing drought stress at nitroxin levels.

The number of seeds in the pod: there were significant differences in NSP between different rapeseed cultivars. The highest NSP (24.8) belonged to cv. Neptune. cv. Natalie and Okapi had 21.9 and 19.8, respectively (Table 4). NSP is affected by the genetic (Mirzaei et al., 2010). Also, NSP is one of the determinants of yield. The higher NSP, the larger the reservoir for photosynthetic assimilates produced by the plant, which ultimately increases the seed yield (Mirzaei et al., 2010). In present study, although cv. Natalie and Okapi had more NPP than cv. Neptune, but the three were more seeds in pods of cv. Neptune than other cultivars. Eskandari Torbaghan and Eskandari Torbaghan (2016) stated that there is often an inverse relationship between the NPP and NSP, and these traits are more related to crop size.

Table 4. The effects of drought stress and Nitroxin consumption on some quality attributes of three rapeseed cultivars

Treatment		No. of seed pod^{-1}	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest Index (%)	Oil percentage (%)	Oil yield (kg ha^{-1})	Protein percentage (%)	Protein yield (kg ha^{-1})
Drought stress	Low	22.3 a	2370 a	9990 a	24.2 a	45.3 a	1080 a	16.6b	393.42 a
	Medium	22.2 a	1779 b	6693 b	26.6 a	44.0 b	785 b	17.8a	316.66 b
	High	22.0 a	1133 c	5248 c	21.8 b	42.8 c	487 c	16.4 b	185.81 c
Cultivar	Natali	21.9 b	2062 a	7572 a	27.5 a	45.1 a	933 a	15.6 b	321.67 a
	Okapi	19.8 c	1362 c	7202 a	18.9 b	42.1 b	574 b	18.0 a	245.16 b
	Neptone	24.8 a	1858 b	7158 a	26.3 a	42.5a	844 a	17.3 a	321.43 a
Nitroxin application	Control	22.2 a	1618 b	6812 b	24.0 a	42.5 b	734 b	14.9 b	241. 08 b
	Nitroxin	22.2 a	1903 a	7809 a	24.4 a	44.8 a	834 a	19.0a	361.57 a

Values followed by the same letter within the same columns do not differ significantly at p=5% based on FLSD.

1000-seed weight: the response of different rapeseed cultivars to Nitroxin consumption was different in terms of 1000-seeds weight. There were no significant differences between cv. Okapi and Natalie under Nitroxin consumption and Nitroxin non-consumption. However, Nitroxin consumption in cv. Neptune resulted in higher 1000-seeds weight compared to Nitroxin non-consumption (Figure 1). Given the genetic value of 1000-seeds weight, it seems that if properly feed; fertilizers due to increased photosynthesis potential and more assimilates allocation toward the seeds, produce heavier seeds (Rabiee and Jilani, 2012). So, Nitroxin consumption in cv. Neptune, positively influenced 1000-seeds weight compared to other cultivars.

The weight of 1000-seeds increased significantly under low and mild drought stress after Nitroxin consumption. However, Nitroxin consumption non-significantly decreased 1000-seeds weight under high drought stress (Table 3). It is believed that prolonged seed filling duration increases 1000-seeds weight. Drought stress declines seed filling duration and finally the seed weight (Jalilian et al., 2016). Nitroxin consumption increased 1000-seeds weight under low and mild stress conditions due to enhancing the shoot weight and photosynthetic potential by improving nutrient uptake. In other words, the drought stress that caused leaf loss and reduced the photosynthetic capacity was compensated by the use of bio-fertilizers (Nourzad et al., 2014).

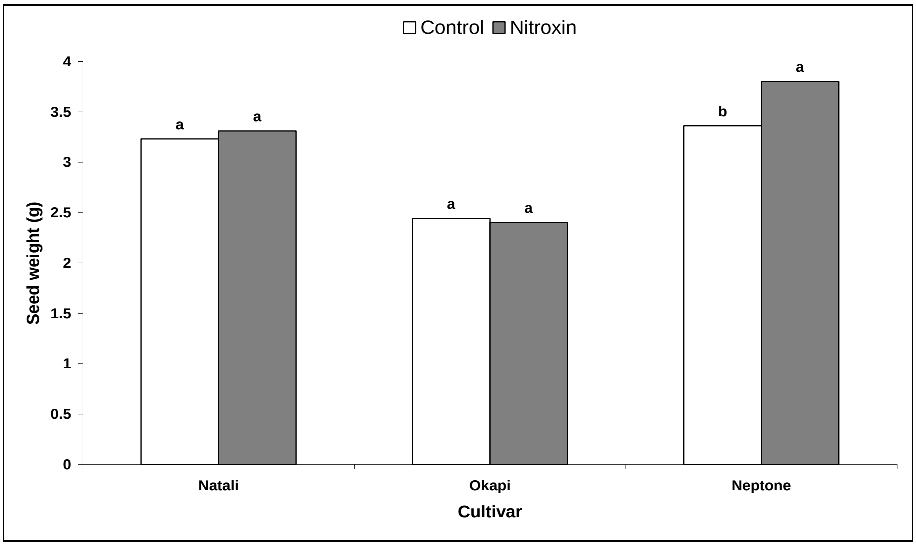

Figure 1. Interaction effect of cultivar and Nitroxin on the 1000-seed weight

Seed yield: seed yield decreased by drought stress in all cultivars (Table 2). The highest seed yield was obtained from low drought stress in cv. Natalie. The least seed yield was belonged to cv. Okapi cultivar under extreme drought stress (Table 2). The significant differences in interactions between drought stress and cultivar is due to genetic differences among cultivars in terms of late season drought tolerance (Praba et al., 2009). The differences in seed yield and its components, in addition to the genetic composition of cultivars have also been attributed to the environmental conditions (Chapman et al., 2000). In fact, the accepted idea for the differences in seed yield is mainly due to the dry matter allocation under drought stress conditions. The suitable available water encourages the plant growth and foliage production. Plants try to reach the maximum vegetative growth to combat the harsh condition under drought stress. Therefore, providing less photosynthetic assimilates for pods and so indirectly impact the seed yield (Gusta, 2012).

Natalie produced higher seed yield under mild and high drought stress than Neptune and Okapi cultivars (Table 2). Nitroxin consumption increased the seed yield under low and mild drought stress (Table 3). No signification differences in seed yield were observed under high drought stress (Table 3). Drought stress reduces cell growth and development, especially in stem and leaves as well as goes to chlorophyll depletion. Before mentioned deteriorations reduce the photosynthesis, vegetative growth**,** yield components and subsequently decrease seed yield (Barnabás et al., 2008). Nitroxin consumption failed to increase the seed yield under high drought stress. Drought stress decreased dramatically seed yield both in Nitroxin consumption and non-consumption (Table 3). However, seed yield reduction was less in Nitroxin consumption. It was reported that Nitroxin fertilizer increased photosynthesis and improved dry matter production by improving soil microbial activity and the availability of various growth hormones and stimulants as well as mineral nutrients availability. This has ultimately improved seed yield (Panwar et al., 2014).

Biological yield: more biological yield was obtained in the second year (7888 kg ha^{-1}) compared to the first year (6733 kg ha^{-1}). Biological yield includes the dry weight of all aerial parts of the plants that are affected by genotype and growing environment conditions (Rokhafrooz et al., 2016). On the one hand, it seems that the longer the flowering period of rapeseed in the second year of the experiment increases the chance of flowering and the longer the growth period, followed by an increase in dry matter and biological yield. On the other hand, biologically yield was more in the second year due to the less sunny hours, more rainfall, and favorable weather conditions, especially at the beginning of seedling growth, which led to uniform germination and proper seedling establishment (Rabiee and Jilani, 2012).

The highest biological yield was observed in optimal irrigation conditions. Biological yield declined significantly by increasing the drought stress (Table 4). Zabet and Hoseinzadeh (2011) illustrated that reducing plant height and the number of nodes in the stem was a reason why drought stress reduced cell divisions and vegetative growth, thus reducing plant biological yield. In drought stress, photosynthesis decrease due to leaf area reduction by reducing cell division and turgor potential and consequently, the whole plant growth and development, plant height and leaf fall, as well as reducing stomata conduction to prevent water loss and thus less absorption of carbon dioxide and chlorophyll content. Accordingly, the biological yield is affected as a reservoir determines the seed yield (Shokouhfar and Abofatilehnezhad, 2013). The increase in dry matter and eventually in biological yield under low drought stress due to more development and longer green period of the leaves, leads to the produce the larger assimilate sources (Rabbani and Emam, 2011).

The biological yield increased from 6812 to 7809 kg ha^{-1} (12.8%) with Nitroxin consumption compared to non- consumption (Table 4). *Azotobacter* in the Nitroxin can increase the plant vegetative growth and shoot weight by producing the growth-promoting metabolites such as auxin, cytokinin, and gibberellin. It seems that the production of such metabolites has increased vegetative growth and dry matter yield in the plant (Yousefpoor and Yadavi, 2014).

Harvest index: the harvest index was significantly higher in the first year (26.2) than in the second year (22.2). The harvest index indicates the percentage of photosynthetic assimilates transfer from source to the sink (Rabiee and Jilani, 2012). In fact, it seems that none effect of the year on the seed yield (data was not shown) and higher biological yield in the second year has been the most important factor in the significant increase in harvest index in the first year compared to the second year.

The highest harvest index was observed in mild stress (26.6), which it had not a significant difference to the low irrigation conditions (24.2), but after increasing the intensity of drought stress, the harvest index decreased significantly and reached to 21.8 (Table 4). Shokouhfar and Abofatilehnezhad (2013) reported that the reason for the decrease in total dry matter due to significant differences between different irrigation regimes was the positive and high correlation between dry matter with photosynthesis and leaf area index in the vegetative stage.

They also stated that severe drought stress in the reproductive stage has a greater effect on seed yield than total dry matter, thus reducing the harvest index (Shokouhfar and Abofatilehnezhad, 2013). Also, the sharp decrease in the NPP, which has an important role in the yield production, is one of the important reasons for the decrease in the harvest index in the high drought stress (Shokouhfar and Abofatilehnezhad, 2013).

The increase in the harvest index under mild stress in the present study indicated that although mild stress has reduced seed and biological yield, but its reducing effect on biological yield had been higher (Rokhafrooz et al., 2016). This is in accordance with the result of Emam and Niknejad's research (2011).

Natalie (27.5) and Neptune (26.3) cultivars excelled compared to the Okapi (18.9) (Table 4). Harvest index is one of the criteria used in estimating the efficiency of assimilates distribution to seed or economic part of plants, and it is considered as one of the important indicators in increasing yield due to less impact on the environment conditions (Yadollahi et al., 2016). The higher harvest index means that the share of seeds in the total dry matter produced by the plant has increased. In other words, a large amount of photosynthetic assimilates is transferred to the seeds. However, in drought stress, the transfer of assimilates is slow due to reduced transpiration. Cultivars with higher harvest index in drought stress had performed better in assimilates transfer (Richard, 2004).

Oil content: oil content was correspondingly decreased by drought stress. The oil content under low drought stress was 45.3% and under mild and high stress conditions decreased by 1.3% and 2.5%, and reached to 44% and 42.8%, respectively (Table 4). There are conflicting reports on the effect of drought stress on oil content. Basically**,** the oil content is controlled by several genes (Hobbs et al., 2004) and the damage to a large number of control genes due to drought is unlikely (Xiao et al., 2009). So, the decrease in oil content due to drought stress is not prevail (Aslam et al., 2009). Besides**,** Ghodrati (2013) stated that the oil content is controlled by the genetic characteristics of the cultivar. However, since the source of oil is from the photosynthetic products (sugars) and this process is greatly reduced under drought stress especially under critical growth stages.

Nitroxin consumption reduced the seed oil content in different rapeseed cultivars (Figure 2). However, the decrease was not significant in cv. Okapi and cv. Neptune, which is contrary to Yasari and Patwardhan (2007). However**,** it is consistent with the results of Zarei et al. (2014). One of the most important qualitative characteristics of rapeseed is oil content. Seed oil content in rapeseed cultivars apart from genetics, depends on the environmental factors such as temperature, nutritional conditions and water availability. It seems that high vegetative growth as a result of Nitroxin consumption usually results seed oil content reduction.

In fact, with more nitrogen content, more substrates are provided for protein synthesis, and thus, more photosynthetic assimilates dedicated to protein synthesis reduce the potential for carbohydrates and lipids biosynthesis and accumulation, which in turn reduce the seed oil content (Hasanzadeh Ghorttapeh and Javadi, 2016).

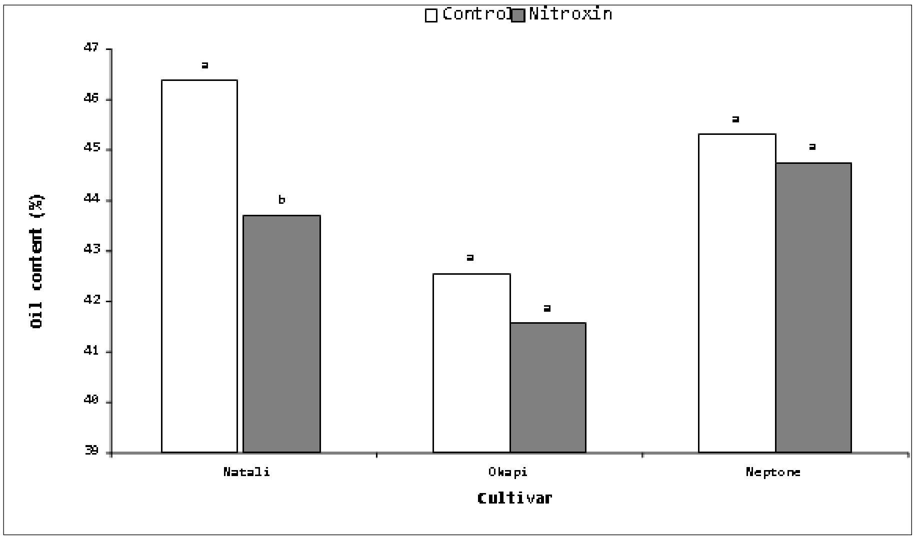

Figure 2. Interaction effect of cultivar and Nitroxin on oil content

Oil yield: nitroxin consumption significantly increased seed yield under low and mild stress conductions. But, this increase was not significant under high drought stress (Table 3). Given the high correlation between seed yield and oil yield, it can be stated that the optimum oil yield is related to the seed yield. Nitroxin consumption under stressful conditions increased the seed and oil yield in rapeseed by providing the adequate nutrients and stimulants. Even under adverse nutritional conditions, these bacteria can increase the plant growth and yield (Raei et al., 2016). Oil yield is a function of oil content and seed yield; however, under stressful conditions, the oil content increases and oil yield may decline as a result of the severe decrease in seed yield due to water deficit stress (Shubhra et al., 2004).

Drought stress decreased the seed oil yield in all three cultivars studied. However, cv. Neptune and cv. Natalie were more tolerant than cv. Okapi. In all studied cultivars, the highest seed oil yield was obtained under low drought stress. Also, the lowest seed oil yield belonged to cv. Okapi under high drought stress (Table 2). It has been reported that severe drought stress reduced the oil produced in oily plants by reducing the amount of unsaturated fatty acids and increasing the ratio of saturated fatty acids, which eventually increases the oil saturation degree (Karimi-Kakhaki and Sepehri, 2010). However, according to the results, the difference in cultivars under drought stress conditions was more than that of non-stress conditions. So it seems that genetic differences between cultivars are the functional reason to devote the tolerance or susceptibility in cultivars.

Seed protein yield: drought stress affected the seed protein yield. The highest seed protein yield (393.42 kg m^{-2}) was obtained in optimal irrigation conditions and showed a significant difference with other treatments. Seed protein yield was decreased by 19.51%

and 52.77%, following drought stress from mild to severe levels, respectively (Table 4). Protein yield is a function of both seed yield and protein content has a direct relationship with both factors (Vahdi et al., 2015). Drought stress seems to affect seed quantity and quality in two ways. First by closing the stomata and reducing photosynthesis potential and so less assimilates transmission to the seeds and the reduced seed yield, and later by affecting the nitrogen fixation. Hence, the plant faces nitrogen deficiency and subsequently the seed yield and oil as well as protein yield declines (Hirel et al., 2007). The highest seed protein yield belonged to cv. Natalie (321.67 kg m^{-2}) and cv. Neptune (321.43 m^{-2}), and cv. Okapi had the lowest seed protein yield (245.16 kg m^{-2}) (Table 4). The differences in seed protein yield in rapeseed cultivars may be due to their diverse genotypes efficiency for the nutrient uptake and differences in the conversion of absorbed elements for dry matter and proteins biosynthesis (Chamorro et al., 2002). Ghorbanzadeh Neghab et al. (2013) in an experiment on nine soybean cultivars showed that genotype has effect significant on the protein yield, so that, Zan, Sanjari and Columbus cultivars were reported as high protein containing cultivars.

Nitroxin consumption with all drought stress (low, mild and high stress) conditions increased the seed protein yield compared to non-consumption (no fertilizer application) (Table 3).

CONCLUSIONS

Drought stress reduced the No. of seeds pod^{-1} and consequently the seed yield but Nitroxin biological fertilizer ameliorated the negative effects of drought, more obvious in conditions of moderate drought. The results revealed that there were significant differences among cultivars in terms of No. of pods $plant^{-1}$, No. of seeds pod^{-1}, 1000-seeds weight, and seed yield. The cv. Natalie had more qualitative and quantitative yield under stressful and normal conditions than other cultivars due to higher 1000-seeds weight, despite of having fewer pods. The minimum and maximum seed oil content and protein content was obtained from cv. Okapi. Drought stress also had a significant effect on oil content, oil yield, and protein yield.

A negative relationship was also observed between the harvest index and protein content, and a positive one between harvest index and seed yield. Protein content decreased in drought stress condition because of the nitrogen uptake would be transformed to the osmolytes like proline.

REFERENCES

Abuzeid, A., and Wilcockson, S., 1989. *Effects of sowing date, plant density and year on growth and yield of Brussels sprouts (Brassica oleracea var. bullata subvar. gemmifera).* Journal of Agricultural Science, 112: 359-375. DOI: https://doi.org/10.1017/S0021859600085816

Amiri, A., 2012. *The effect of zinc nutrition on the vegetative growth characteristics of almond rootstocks (Prunus amygdalus L.) in saline conditions.* Ms.c Thesis in Horticultural Sciences, Faculty of Agriculture and Natural Resources, Isfahan University of Technology.

Aslam, M., Nelson, M., Kailis, S., Bayliss, K., Speijers, J., Cowling, W., 2009. *Canola oil increases in polyunsaturated fatty acids and decreases in oleic acid in drought stressed Mediterranean type environments*. Plant Breeding, 128: 348-355. https://doi.org/10.1111/j.1439-0523.2008.01577.x

Barnabás, B., Jäger, K., Fehér, A., 2008. *The effect of drought and heat stress on reproductive processes in cereals*. Plant, Cell & Environment, 31: 11-38. DOI: 10.1111/j.1365-3040.2007.01727.x

Birunara, A., Shekari, F., Hassanpouraghdam, M., Khorshidi, M., Esfandyari, E., 2011. *Effects of water deficit stress on yield, yield components and phenology of canola (Brassica napus L.) at different growth stages*. Journal of Food, Agriculture and Environment, 9: 506-509.

Bot, A., and Benites, J., 2005. *The importance of soil organic matter: key to drought-resistant soil and sustained food production*. Food & Agriculture Org., Rome, Italy.

Chamorro, A.M., Tamagno, L.N., Bezus, R., Sarandón, S.J., 2002. *Nitrogen accumulation, partition, and nitrogen-use efficiency in canola under different nitrogen availabilities*. Communication in Soil Science and Plant Analysis, 33: 493-504. https://doi.org/10.1081/CSS-120002759

Chapman, S., Cooper, M., Hammer, G., Butler, D., 2000. *Genotype by environment interactions affecting grain sorghum. II. Frequencies of different seasonal patterns of drought stress are related to location effects on hybrid yields*. The

Australian Journal of Agricultural and Resource Economics, 51: 209-222. https://doi.org/10.1071/AR99021

Choudhary, D.K., Varma, A., Tuteja, N., 2016. *Plant microbe interaction: an approach to sustainable agriculture.* Springer Nature Singapore Pte Ltd., DOI: 10.1007/978-981-10-2854-0

Diepenbrock, W., 2000. *Yield analysis of winter oilseed rape (Brassica napus L.): a review*. Field Crops Res 67: 35-49. https://doi.org/10.1016/S0378-4290(00)00082-4

Dobbelaere, S., Vanderleyden, J., Okon, Y., 2003. *Plant growth-promoting effects of diazotrophs in the rhizosphere*. CRC Crit. Rev. Plant Sci., 22: 107-149. https://doi.org/10.1080/713610853

Emam, Y., and Niknejad, M., 2011. *An introduction to the physiology of crop yield (2nd ed.).* Shiraz University Press.

Eskandari Torbaghan, M., and Eskandari Torbaghan, M., 2016. *The effect of seed rate on grain yield and some agronomic traits of spring sown canola under dryland farming conditions*. ESCS 8: 149-158. https://doi.org/10.22077/ESCS.2016.211

FAO., 2019. Available http://www.fao.org/faostat /en/#data/QC/visualize

Ghodrati, G.R., 2013. *Effect of drought tension on grain yield and quantitative characteristics of promising genotypes of spring canola.* Plant Physiology, 5: 67-82.

Ghorbanzadeh Neghab, M., Babaie, H.R., Rassam, G., Dadkhah, A.R., Khoshnood Yazdi, A., 2013. *Response of seed yield components, protein and oil content of soybean cultivars to various sowing date in Shirvan*. Journal of Crop Improvement, 15: 31-41. https://doi.org/10.22059/JCI.2013.36097

Grover, M., Ali, S.Z., Sandhya, V., Rasul, A., Venkateswarlu, B., 2011. *Role of microorganisms in adaptation of agriculture crops to abiotic stresses*. World J. Microb. Biot., 27: 1231-1240. https://doi.org/10.1007/s11274-010-0572-7

Gusta, L., 2012. *Abiotic stresses and agricultural sustainability*. J. Crop Improv., 26: 415-427 https://doi.org/10.1080/15427528.2011.650296

Hasanzadeh Ghorttapeh, A., and Javadi, H., 2016. *Study on the effects of inoculation with biofirtilizers (Azotobacter and Azospirillum) and nitrogen application on oil, yield and yield components of spring canola in West Azerbaijan*. Journal of Crop Production and Processing, 5: 39-50. https://doi.org/10.18869/acadpub.jcpp.5.18.39

Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. *The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches*. Journal of Experimental Botany, 58: 2369-2387. https://doi.org/10.1093/jxb/erm097

Hobbs, D.H., Flintham, J.E., Hills, M.J., 2004. *Genetic control of storage oil synthesis in seeds of Arabidopsis*. Journal of Plant Physiology, 136: 3341-3349. https://doi.org/10.1104/pp.104.049486

Iqbal, N., Nazar, R., Khan, M.I.R., Masood, A., Khan, N.A., 2011. *Role of gibberellins in regulation of source-sink relations under optimal and limiting environmental conditions*. Current Science, 100: 998-1007.

Jalilian, J., Amirnia, R., Gholinezhad, E., Abbaszadeh, S., 2016. *The effect of supplemental irrigation and seed priming on seed yield, yield components and some characteristics of vetch.* Journal of Crop Improvement, 18: 625-637. 20.1001.1.83372008.1395.18.3.7.0

Jamshidi, Z.A., Hasanloo, T., Naji, A., 2015. *Evaluation of physiological and biochemical characteristics of four canola (Brassica napus L.) cultivars in drought condition.* Field Crop Research, 13: 583-597.

Karimi-Kakhaki, M., and Sepehri, A., 2010. *Effect of deficit irrigation at reproductive growth stage on remobilization of dry matter in four sunflower (Helianthus annus L.) cultivars*. Iranian Journal of Field Crop Science, 12: 422-435. 20.1001.1.15625540.1389.12.4.5.8

Khalilian Ekrami, H., 2006. *The effects of sulfur-oxidizing bacteria (Thiobacillus), nitrogen fixation on S.C 704 cultivar of corn yield and yield components*. MSc Thesis, Faculty of Agriculture, Azad University of Tabriz (In Persian with English abstract).

Khani, R., Bakhtvari, A., Eslam, B., Sarabi, V., 2017. *Effects of drought stress on canola (Brassica napus L.) genotypes yield and yield components.* Field Crops Research, 15: 914-924 DOI: 10.22067/gsc.v15i4.58356

Lowary, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. *Protein measurement with the folin phenol reagent*. Journal of Biological Chemistry, 193: 256-275.

Lucy, M., Reed, E., Glick, B.R., 2004. *Applications of free living plant growth promoting rhizobacteria.* Antonie van Leeuwenhoek, 86: 1-25. https://doi.org/10.1023/B:ANTO.0000024903.10757.6e

Majidi, M., 2012. *Identification of canola cultivars for drought tolerance in germination and seedling growth stages using principal component analysis.* Journal of Crop Production and Processing, 2: 41-52. 20.1001.1.22518517.1391.2.4.4.0

Mirzaei, M.R., Dashti, S., Absalan, M., Siadat, A., Fathi, G.E., 2010. *Study the effect of planting dates on the yield, yield components and oil content of canola cultivars (Brassica napus L.) in dehloran rejoin*. Journal of Crop Production, 3: 159-176.

Mulder, D., 1953. *Les elements mineurs en culture fruitiere.* Convegno Nazionale Fruitticoltura, French Montana de Saint Vincent: 118-198.

Nourzad, S., Ahmadian, A., Moghaddam, M., Daneshfar, E., 2014. *Effect of drought stress on yield, yield components and essential oil in coriander (Coriandrum sativum L.) treated with organic and inorganic fertilizers*. Journal of Crop Improvement, 16: 289-302.

Panwar, M., Tewari, R., Nayyar, H., 2014. *Microbial consortium of plant growth-promoting rhizobacteria improves the performance of plants growing in stressed soils: an overview.* Phosphate Solubilizing Microorganisms. Springer: 257-285. DOI: 10.1007/978-3-319-08216-5_11

Parhizkar-Khajani, F., Irannezhad, H., Amiri, R., Oraki, H., Majidian, M., 2012. *Effects of different levels of nitrogen, phosphorus and potassium on quantitative and qualitative characteristics of oil flax*. Journal of Crop Production, 5(1): 37-51.

Parvatha Reddy, P., 2014. *Plant growth promoting rhizobacteria for horticultural crop protection*. Springer Press.

Praba, M.L., Cairns, J., Babu, R., Lafitte, H., 2009. *Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat.* Journal of Agronomy and Crop Science, 195: 30-46. https://doi.org/10.1111/j.1439-037X.2008.00341.x

Rabbani, J., and Emam, Y., 2011. *Yield response of maize hybrids to drought stress at different growth stages.* Journal of Crop Production and Processing, 1(2): 65-78. 20.1001.1.22518517.1390.1.2.5.0

Rabiee, M., and Jilani, M., 2012. *Effect of nitrogen fertilizer levels and planting distances on grain yield and morphological characteristics of second rapeseed cultivation after rice*. International Journal of Agricultural Sustainability, 8(3): 22-33.

Rabiee, M., and Jilani, M., 2012. *Effect of nitrogen fertilizer levels and row spacing on grain yield and morphological characteristics of rapeseed as second cropping after rice*. Agroecology Journal, 8: 22-33.

Raei, Y., Pourmolaie, S., Alami-Milani, M., 2016. *Effect of seed inoculation with nitroxin and different urea chemical fertilizer levels on yield, yield components and oil yield of canola (Brassica napus L.).* Journal on New Biological Reports, 5: 93-98.

Richard, A.R., 2004. *Physiological traits used in the breeding of new cultivars for water- scarce environments*. CSIRO Plant Indstry, Box 1600, Canberra, ACCT, 2601.

Rokhafrooz, K., Emam, Y., Pirasteh-Anosheh, H., 2016. *The effect of chlormequat chloride on yield and yield components of three wheat cultivars under drought stress conditions*. Journal of Crop Production Proccessing, 6(20): 111-123 10.18869/acadpub.jcpp.6.20.111

Sabzi, S., Tahmasebi, Z., Barari, M., 2017. *Study of the yield and some important plant of common bean (Phaseolus vulgaris) genotypes at different moisture levels*. Environmental Stresses in Crop Sciences, 10: 21-30. https://doi.org/10.22077/ESCS.1396.528

Shabani, A., Haghighi, A., Sepaskhah, A., Emam, Y., Honar, T., 2009. *Effect of water stress on physiological parameters of oil seed rape (Brassica napus)*. Journal of Agricultural Science and Technology, 13: 31-43.

Shokouhfar, A., and Abofatilehnezhad, S., 2013. *Effect of drought stress on some physiological traits and biological yield of different cultivars of mung (Vigna radiate L.) in Dezful*. Crop Physiology, 17(5): 49-59

Shubhra, K., Dayal, J., Goswami, C., Munjal, R., 2004. *Effects of water-deficit on oil of Calendula aerial parts.* Biologia Plantarum, 48: 445-448.

Vahdi, N., Gholinezhad, S., Mansourifar, S., Gheyrati Arani, L., Rahimi, M., 2015. *Effect of drought stress on seed yield, oil and protein of soybean (Glycine max) different cultivars*. Journal of Oil Plants Production, 22: 99-113.

Van Oosten, M.J., Pepe, O., De Pascale, S., Silletti, S., Maggio, A., 2017. *The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants*. Chemical and Biological Technologies in Agriculture, 4: 5. https://doi.org/10.1186/s40538-017-0089-5

Vatan Doost, H., Seyed Sharifi, R., Farzaneh, S., Hasanpanah, D., 2019. *The effect of bio-fertilizers on yield and agrophysiological traits of rapeseed under application of nitrogen fertilizer and water restriction conditions*. University of Mohaghegh Ardabili.

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., Nasrulhaq Boyce, A., 2016. *Role of plant growth promoting rhizobacteria in agricultural sustainability - a review*. Molocule, 21: 1-17. https://doi.org/10.3390/molecules21050573

Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M., Skz, A., 2016. *Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria.* Microbiol Research, 184: 13–24. https://doi.org/10.1016/j.micres.2015.12.003

Wani, S.A., Chand, S., Wani, M.A., Ramzan, M., Hakeem, K.R, 2016. *Azotobacter chroococcum - a potential biofertilizer in agriculture: an overview.* Soil Science: Agricultural and Environmental Prospectives: 333-348. https://doi.org/10.1007/978-3-319-34451-5_15

Xiao, B.Z., Chen, X., Xiang, C.B., Tang, N., Zhang, Q.F., Xiong, L.Z., 2009. *Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions*. Mol Plant, 2: 73-83.

Yadollahi, M.T., Tavakoli, M.R., Marjani, A., 2016. *Investigating the compatibility and determining the best planting date for four varieties of canola oilseed in Maneh and Samolghan climatic conditions.* 6th National Symposium on Sustainable Agriculture and Natural Resources, Tehran, Iran.

Yasari, E., and Patwardhan, A., 2007. *Effects of (Azotobacter and Azospirillum) inoculants and chemical fertilizers on growth and productivity of*

canola (Brassica napus L.). Asian Journal of Plant Sci., 6: 77-82.
DOI: 10.3923/ajps.2007.77.82

Yousefpoor, Z., and Yadavi, A., 2014. *Effect of biological and chemical fertilizers of nitrogen and phosphorus on quantitative and qualitative yield of sunflower*. Journal of Agricultural Science and Sustainable Production, 24(1): 95-112.

Zabet, M., and Hoseinzadeh, A.H., 2011. *Determinatin of the most effective traits on yield in mung bean (Vigna radiata L. wilczek) by multivariate analysis in stress and non-stress conditions*. Iranian Journal Pulses Research, 2(1): 87-98.
https://DOI.ORG/10.22067/IJPR.V2I1.12020

Zarei, M., Tadayon, M.R., Tadayyon, A., 2014. *Effect of biofertilizer, under salinity condition on the yield and oil content of three ecotype of hemp*. Journal of Crop Improvement, 16: 517-529.
https://doi.org/10.22059/JCI.2014.53255