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Impact of the azotobacter and different nitrogen rates on yield and protein of forage canola cultivars

S.Ebrahimi[1], S.A.M.Modarres Sanavy*[,2], A.H.Shirani Rad[1], H.Iran Nejad[1]

[1]Agronomy Department, Faculty of Agriculture, Aboureyhan Campus, Tehran University, Tehran, (IRAN)

[2]Agronomy Department, Faculty of Agriculture, Tarbiat Modares University, Tehran, (IRAN)

E-mail : Modaresa@modares.ac.ir

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ABSTRACT

In order to study the effect of various nitrogen rates and azotobacter application on qualitative yield and protein percentage of three forage canola cultivars (RGS 003, Hyolla 401and Hyolla 330), an experiment was conducted in Seed and Plant Improvement Institute Research Farm, Karaj (35°48'N, 51°10'E and 1321m H) during crop season 2005. The factors were arranged as factorial split plot in a randomized complete block design with three replications. Two levels of Aztobacter (application and non application) and three nitrogen levels (0, 75,150 $Kg.ha^{-1}$) were randomized to the main plots and cultivars were located in sub-plots. Results showed that Azotobacter application significantly affected on quantitative traits. High nitrogen increased all quantities traits, protein percent and glucosinolate rate in plant forage. There were differences among cultivars for dry and wet forage weight, stem length and sub-branch number. According to this research, 150 $kg.ha^{-1}$ nitrogen produced the most forage yield. But in regards to reduction necessity of chemical fertilizer application in direction of sustainable agriculture, of 75 $kg.ha^{-1}$ nitrogen with Azotobacter application was considered as the best treatment and RGS003 was introduced as the best cultivars.

KEYWORDS

Azotobacter;
Canola;
Nitrogen;
Quality and quantity;
Forage.

INTRODUCTION

Providing necessity animal protein, due to its effect on growth, mental and physical health of society, is interpreted as obvious criterions of growth and development of a country. Regarding to the annual protein consumption and capacity of production of country, is clear that for control of export exorbitant sums foreign exchange for import of these items, it should be hopeful to increase internal production. Whereas, country pastures are in exposure of sever damage and erosion because of irregular grazing of domestics and various droughts, suitable forage plants can be considered as one way for nutrition of available domestics and control of import of forage to state and maintaining of pastures.

All of the researchers that worked on forage plants in the world including Goihl and, Mcelliney[21] Nelson in Australia[20], Amin, *et al*. in Egypt[12] have emphasized on canola for forage production due to its compatibility and favor plant. They suggested the production of the forage from the canola and placing it in the cropping pattern and the crop rotation of the region would be useful.

Clay Pool, *et al*.,[11] found when Helshtain 45-day calves were nourished with Canola feed during 7 weeks before weaning and 8 weeks after weaning, their weight increased to 900 grams. Banelos, *et al*.,[16] with emphasis on enormous volume of producing branch and leaf from forage canola and high quality of forage (low fiber and much protein) as a suitable constituent for forage plants in dry areas.

Many researches were conducted in Iran on canola meal as chicken broiler nutrition (Jalali Haji Abadi, *et al*., 2004) nutrition of shrimp and fish (Anami, 2001) and nutrition of sheep (Shourang, 2004).

Daneshgar (2002), has stated that canola plays an important role in providing feed of domestics with production of enormous amount of forage specially in shortage forage time.

Gallic[34] showed that canola straw especially in varieties which have low acid uresic and glucosinolate, plays effective role for nutrition of domestic animal.

Petkov and Lukaszewski[27], explain that there is significant and positive correlation between increasing of weight of rabbit and canola with soybean meal.

So, comparison between varieties for forage production rate is very important. One of the effective inputs on quantity and quality of forage plants is nitrogen. Generally, nitrogen is very important as a fertilizer for forage plants in order to achieve the maximum forage yield that was suitable in point of quantitative traits such as protein. Optimum determination of nitrogen is very important for avoidance nitrate toxicity (Agha Alikhani., 1993). Canola responses to 200 $kg.ha^{-1}$ N and its dry weight increases with high nitrogen[26]. Bilsborrow, et al.[33] observed that high nitrogen application significantly increased plant height, sub-branches number and plant dry weight. But using chemical fertilizer especially nitrogen fertilizers in order to increase agricultural products in unit area not only increase production costs but also has harmful effects on environmental ecology. For example, the irregular using of nitrogen fertilizers causes pollution of flowing and ground waters and finally poisoning of human, domestic and aquatic[18]. So, in spite of these destructive effects and many other problems, it is necessary to find alternative methods for production enhancement more than before.

Providing required condition and being necessary the more use of natural process such as biological nitrogen fixation is one of the safe production approaches and more important than that is the safety of an environment; that is taken for granted in developed countries.

One of the biological approaches for increasing agricultural productions is potential application of useful soil living beings that are able to fix biological nitrogen (NBF) or to produce motivated chemical for plant growth. One of these beings is soil living bacteria of azotobacter species. Right now some of the countries use these bacteria as biological fertilizer to produce many kinds of crops such as cereal, forage, vegetables, and fruits[18].

Ravikumar and Co-Work's[36] studied the effect of Azobacter on the plant species in the coastal regions and saw that the inoculation of Azotobacter caused to increase the growth of the plant and quantity of the chlorophyll in the leaf. There are many reports about both azotobacter and nitrogen fertilizer on crops like wheat. For instance Adris[30] reported that appropriate combination of animal fertilizer, azotobacter, and mineral nitrogen caused wheat yield improvement, and decreased nitrogen application to 50 percent. Kadar et al.[1] reported that using of azotobacter could decrease utilization of urea to 20 percent.

According to the findings of Arshad Khalil, et al.[2] canola seed inoculation with plant growth promoting bacteria, significantly increased grain yield. Similar job was done by Bertrand, et al.[7]. They reported a special increase in the weight of dry root of canola, about 11 to 52 percent by using Plant Growth Promoting Rhizobacteria (PGPR). Deferitaz, et al. reported that PGPR caused an increase in the plant height, number, bud weight and yield of canola that cultivated in pot.

At present, PGPR are applied to produce agriculture crops, for instance, cereal, forage plants, summer crops in some countries[1].

Along with this and due to the importance of forage rape cultivars and the role of mineral nitrogen on yield increment, the current research in order to study the quantitative and qualitative of different rape forage cultivars under the influence of different levels of nitrogen fertilizer with and without azotobacter have been designed.

MATERIAL AND METHODS

To investigate the different levels of nitrogen and using of Azotobacter on yield, percent of protein and glucosinolate content, of the forage cultivars in Mahdasht (Placed in Karaj) a field study was conducted in the Research Field in Karaj Seed and Plant Improvement Institute. The experiment was performed in complete block design arrangement in factorial split with tree replications. Azotobacter in two levels (with and without azotobacter), and three levels of nitrogen (0, 75 and 150 $Kg.ha^{-1}$ of pure nitrogen) were randomized in main plot units. Sub-plots were the various levels of cultivars included Hoyolla 401, Hyolla 330 and RGS003.

In this experiment each sub-plot had 6 rows, length of each row was 3 meters, and the space between plots was considered 1 meter. In the end of June, the operation of the preparation of the land was done with disking to break the clods, leveling the land by leveler and creating the stream and the mound by furrower. Weed was controlled by herbicide (Trifloralin) in the quantity of 2-2.5 $liter.ha^{-1}$ pre-cultivation and blended with the soil.

The dry seeds were sown. Before seed sowing, the weighted seeds in the separate vessels were inoculated with the Azotobacter in 20/June/2004 (1 kg per hectare). The seeds of control treatments were sown without any inoculation.

Different levels of nitrogen fertilizer in two times (cultivation and bolting stages) and in equal amounts were spread on soil by hand. First irrigation was performed after cultivation as that the furrower became full of water. All treatments were irrigated every 7 days after first irrigation until harvest time. Biomass and dry matter of each treatment was measured on plants at 50% flowering and 2.4 square meters of four middle rows in each plot after eliminating the margin effect (0.5 meter from tip and end of each row). Before harvesting 10 plants were selected randomly from each plot, and the number of sub-branches and the plants height were measured. The percentage of protein was determined for each sample by Kejldahl method. The rate of glucosinolate was also detected by estimating the amount of glucose in the sample by spectrophotometer (Glucose in the samples is changed to laconic acid and hydrogen peroxide by presence of peroxides and then 4-amino-Anti peril and phenol alter the color of the solution to red color. The intensity of color was measured by spectrophotometer in the wave length of 490-550 that is spectrum of Glucose.

Variance analysis for all the traits was calculated by SAS software (SAS Institute Inc. 1997). Mean comparison for main and interaction effects were performed with Duncan's multiple range test was applied to compare the means at $P\leq0.05$ (Steel and Torrie, 1980).

RESULT AND DISCUSSION

In evaluating the agricultural designs in forage plant the most current trait that is taken for granted through examination factors, is forage yield. It should be noticed that forage coming from canola cultivars, has this capability to be consumed as fresh forage, silage forage or even hay, so a report considering the weight of wet forage is also matter of concern. Looking at variance analysis table (TABLE 1), statistically, the effect of azotobacter, nitrogen, and cultivar was significant on biomass ($p\leq0.01$). Plants treated with azotobacter produced more biomass with average production of 45124 $kg.ha^{-1}$ than plants without azotobacter (TABLE 2).

TABLE 1 : Analysis of variance on traits of canola cultivars under different nitrogen rates and azotobacter

S.O.V	df	Biomass	Dry matter	Plant height	Branch number	Protein content	Glucosinolate
Replication	2	*	ns	ns	ns	ns	ns
azotobacter	1	**	**	**	**	ns	ns
nitrogen	2	**	**	**	**	**	**
A*N	2	ns	ns	ns	ns	ns	ns
E(a)	8						
Variety	2	**	**	**	**	ns	ns
A*V	2	ns	ns	ns	ns	ns	ns
N*V	4	**	*	ns	ns	ns	ns
A*N*V	4	ns	ns	ns	ns	ns	ns
E(b)	24						
CV		6.3	3.8	11.7	11.2	8.2	9.4

***And** Significant at 5% and 1% probability Levels respectively and ns No significant**

This difference was resulted from suitable environmental condition, extension of root and better absorption of minerals. In this case, Kennedy, et al[18], Ravikumar, et al[36] and Rodelas[6] reported that the increase of the agricultural products by the azotobacter application. Edris[30] reported that using a compound of Azotobacter, nitrogen and manure caused an accretion in yield.

Mean comparison showed that application of 150 kg.ha^{-1} nitrogen produced the most biomass in plants (TABLE 2). Findings of Jankowski, et al.[26] and Jang[39] also clarifies that an increase in nitrogen enhances the yield of forage rape cultivars significantly. RGS003 had more yield than other cultivars (TABLE 2). This subject is related to genetic potential of mentioned variety. Some researchers like MacGregor[10]. Morison, et al.[19] also attributed the difference between the varieties to the difference of the genotypes.

TABLE 2 : Means comparison for yield and quality in different nitrogen rats, variety and use of azotobacter

Treatment	Biomass ton.ha^{-1}	Dry matter ton.ha^{-1}	Plant height cm	Branch number	Protein content %	Glucosinolate %
Nitrogen fertilizer						
N1	39.9c	6.8c	61.3b	3.7c	15.9c	3.1b
N2	45.5b	8.1b	66.2a	4.7b	17.8b	3.0ab
N3	50.2a	9.2a	67.9a	5.4a	19.7a	3.7a
variety						
V1	44.7b	7.8b	71.4a	5.5a	16.9a	3.4a
V2	40.5c	7.4b	69.0a	5.3a	17.2a	3.2a
V3	53.1a	9.0a	60.4b	3.9b	17.9a	2.9a
Azotobacter						
A1	40.5b	7.2b	63.2b	3.5b	16.1a	3.1a
A2	45.1a	8.1a	69.2a	5.4a	17.3a	3.5a

Means fallowed by the same letter in each column are not significant (Duncan's multiple rang test).
N1 = 0, N2 = 75 N3 = 150 kg.ha^{-1} nitrogen
(V1 = Hyola 401, V2=Hyola 330, V3=RGS 003)
A1 = Non application of azotobacter and A2 = azotobacter application

In this experiment the interaction of nitrogen * variety was only significant (p<0.01) (TABLE 1). In statistical point, RGS003 variety produced the same yield with application of 150 and 75 kg.ha^{-1} nitrogen.

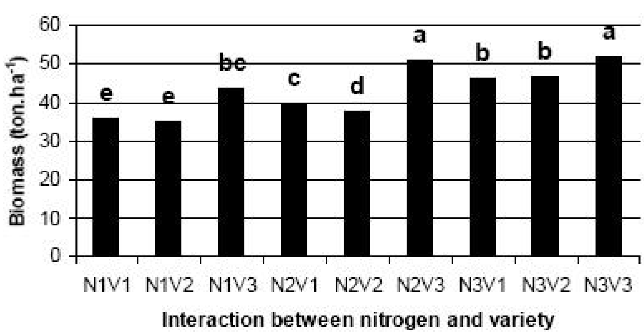

Figure 1 : Interaction between nitrogen and variety on biomass

Effect of Azotobacter, nitrogen and variety on dried weight was also significant and according to the variance analysis table (TABLE 1), the interaction of nitrogen * variety was significant. Using Azotobacter caused to produce 8.1 ton.ha^{-1} of dry forage that was more than non application of it (TABLE 2).

Researchers like Narula and Kumar[32] JagiBoland, et al., (2003) Ahmad, et al.[14], Ravikumar, et al.[36] reported the positive effects of the inoculation of Azotobacter on the dry weight of the plant and it was related to the stimulating growth hormones that azotobacter produced.

Using of 150 kg.ha^{-1} nitrogen produced the highest forage dry weight (9.2 ton.ha^{-1}) (TABLE 2). Qayyum et al.[35] reported that an enhancement in the level of nitrogen fertilizer upto 120 kg.ha^{-1} increased grain yields, but an increase in higher level of 120 nitrogen has an opposite effect. Aufhamer, et al.[37] and Budzynski[8] also reported that an increase of nitrogen to 330 kg.ha^{-1} would increase plant dry weight.

The effect of variety on dry matter is also meaningful and RGS003 was in a better state, by considering other cultivars (TABLE 2).

TABLE 2, shows the interaction between cultivar and nitrogen. As you see RGS003, receiving 150 Kg fertilizers per hectare (N3) has the highest yield but yield of this cultivar was 75 Kg (N2). Level of fertilize are in a statistical category, therefore, considering the decrease in the consumption of chemical fertilizers in line with sustainable agriculture to introduce N_2V_3 (RGS003 with 75 Kg consumption per hectare) as a logical treatment.

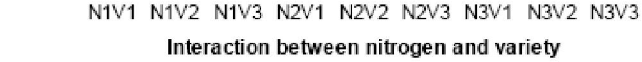

Figure 2 : Interaction between nitrogen and variety on dry matter

The height of plant from the ground is affected by nitrogen rates, using azotobacter, and variety (TABLE 1).

As by using azotobacter the height of plant is 69.2 cm, and without that, 63.2 cm and this was meaningful (TABLE 2). This is for the food that increases the height of plant. Results of Kader et al.[1] and Zahir et al.[2] are similar with our results. Variance in variety mostly has genetic aspect, and Hyolla 401, had more height, but was at the same category with Hyolla 330 (TABLE 2). Variance of other rates of cultivar was declared by PezeshkPur, et al.[32]. With an increase in nitrogen consumption, height of the plant was increased, because internodes were more grown as nitrogen fertilizer caused an increase in plant's length. A finding of Sidlauskus and Gife about an increase in length of stem by nitrogen approves the results. In this experiment nitrogen, azotobacter and variety on number of sub branches in the bushes in surface was one percent meaningful, and also none of the opposite effects on the number of sub branches came to a result (TABLE 1). Using of Azotobacter included more sub branches than not to use it, Beria and Azkon[23] also declared the reason of increase in number of sub-branches in various types of plants conveying growth hormones by azotobacter.

Number of sub-branches in fertilizer's level was 150 Kg nitrogen more than other levels.

Bilsborrow, et al[33] also found that an increase in nitrogen consumption, the number of sub-branches will increase. Qayyam, et al[35] found that nitrogen level increases the number of sub-branches to 120 Kg per hectare. Hyolla 401 variety had the highest number of sub-branches in bush, but was in the same statistical category with Hyolla 330 (TABLE 2), May be it is originated of genetics.

Among the treatments, only the effect of nitrogen on protein came to meaningful (TABLE 1). Effect of azotobacter and variety, on the percent of protein was not statistically of a meaning. 150 $Kg.ha^{-1}$. Nitrogen had an average of 19.7 percent protein more than two other levels between various ranges (TABLE 2).

No distraction can be observed, but considering the comparative average observed, but considering the comparative average chart, RGS003, has better variety than the others varieties (TABLE 2). Results of Wang et al[38] Pouzet et al[3] Sykres et al[24] points that with an increase of nitrogen, protein content also increases, and approves the findings of this research. Zamber, et al.[31] reported an increase of protein by azotobacter in zero levels to 100 Kg nitrogen fertilizer per hectare, however, Zaied, et al[28] reported the effect of azotobacter on the percent of protein in wheat seed didn't significant effect.

According to the results, bacterial treatment effect and variety on the amount of glucosinolate was not meaningful, and it was influenced by the nitrogen rate, moreover none of the interaction was meaningful (TABLE 1). An increase in nitrogen to 150 Kg caused an increase in the amount of glucosinolate. (TABLE 2) approves the results of research by Baily[29] Astra et al[13] Also approves the above results.

Augustinussen, et al (1993) declared the amounts of glucosinolate will not be affected by fertilizer treatment, and these amounts are less than the limit.

CONCLUSION

Results showed that despite the yield of forage in 150 Kg nitrogen with use of azotobacter, with a movement toward the sustainable agriculture and correct and beneficial use of soil sources to cultivate cultivar in 75Kg of nitrogen used of azotobacter. Also, regarding to the higher RGS003 we can use it.

Generally bacteria for plant's growth have some benefits in consideration with chemical fertilizers. A decrease in environmental pollution, and considering less expenses, there availability (need to make and produce them), power to produce and issue, these bacteria, shows the reasons that biological fertilizers are more productive than chemical ones. Idris[30] concluded that compound of azotobacter and mineral Nitrogen saves the consumption of Nitrogen for 50%.

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