The Effect of Crop Growth Enhancer Bacteria on Yield and Yield Components of Safflower (Carthamus tinctorius L.)

Shahram Lack¹*, Farshad Ghooshchi² and Hamed Hadi²

¹. Department of Agronomy, Science and Research Branch Islamic Azad University (IAU), Khuzestan, Iran
². Department of Agronomy, Varamin Branch, Islamic Azad University, Iran

Corresponding author: Shahram Lack

ABSTRACT: To examine the effect of crop growth enhancer bacteria on morpho-physiological characteristics and yield of Safflower, an experiment in randomized complete block design (RCBD) was conducted in eight levels with four replications. Bacterial factor consisted of control (absence of bacteria), Azotobacter Chroococcum, Azospirillum Brasilense, Pseudomonas Fluorescens, Azotobacter Chroococcum + Azospirillum Brasilense, Azotobacter Chroococcum + Pseudomonas Fluorescens, Azospirillum Brasilense + Pseudomonas Fluorescens, Azotobacter Chroococcum + Azospirillum Brasilense + Pseudomonas Fluorescens respectively. Therefore there were 32 experimental units or plots. The results showed that the effect of bacteria inoculation on the grain yield, biological yield, yield components and morphological traits such as plant height and number of nodes per plant at 1% probability level was significant. The highest grain yield by 2284 kg ha⁻¹ belonged to treatment in which three crop growth enhancer bacteria were inoculated at the planting time. In contrast, the lowest grain yield (1643.4 kg ha⁻¹) was related to the control treatment (non-inoculated). Based on the obtained results, Safflower seed inoculation with crop growth enhancer bacteria, especially with the combination of bacteria, improved the morphological and physiological characteristics of the crop.

Keywords: Safflower, crop growth enhancer bacteria, yield and yield components

INTRODUCTION

One of the major limitations in realizing potential yield of crops and achieving high yield is to supply adequate nutrients. In conventional and high input agriculture, this problem has been solved by the use of chemical fertilizers. Environmental problems caused by the excessive use of chemical fertilizers, energy and their consumption and production costs and their adverse effects on biological cycles and sustainability of ecological cropping systems are the causes of approaching bio-fertilizers application. Application of bio-fertilizers in feeding crops has been recently considered as a fundamental solution to develop the integrated management system of plant nutrition and to enhance the quality and quantity of food per area unit through the integration of organic and inorganic methods of feeding crops. Accordingly, agricultural development during the transition from conventional agriculture to sustainable agriculture with adequate input as the integration of chemical and organic fertilizer consumption especially bio-fertilizers has been acceptably proposed as an alternative approach to agricultural production and yield maintenance. By definition, bio-fertilizers are composed of one or several kinds of helpful microorganisms in combination with preservatives or their metabolic products that are used to provide nutrient for crops. Different kinds of bio-fertilizers contain symbiotic bacteria, mycorrhizal fungi, and plant growth promoting rhizobacteria (Zahir
et al., 2000). The first Rhizobium inoculums called Nitragin were produced by Hilter and Nobbe in 1985. Industrial production of bio-fertilizers began in Canada in 1905 and in Australia and Sweden in 1914 and the commercial production of crop growth enhancer bacteria began in China since 1979. The research on commercial production of plant growth promoting rhizobacteria (PGPR) in Iran goes back to 1995 (Asadi Rahmani and Falah, 2001). The most important mechanisms of PGPR include bioavailability enhancement of minerals and biological nitrogen fixation and dissolving Phosphorus and Potassium, biological control of pathogens by producing bio-antibiotics, and producing plant growth regulators especially auxins, gibberellins, and cytokinins. Moreover, the influencing mechanisms of such bacteria by producing plant growth regulators include the production of IAA through the use of root exudates and tryptophan amino acid, hydrolysis of ethylene precursor (1-aminocyclopropane-1-carboxylic acid (ACC)) by the enzyme ACC deaminase, and the production of hormones and hormone-like substances through the reaction between nitrite resulted from nitrite respiration of upper plant cells inoculated by Azospirillum and ascorbic acid (Zahir et al., 2004). Pseudomonas, Azotobacter, and Azospirillum bacteria are the most important PGPRs. The positive effect of various plant seeds inoculation with PGPR on different aspects of their growth and development including the effect on the seed germination and seedling vigor has been reviewed and approved, so that (Kloepper et al., 1991) for the first time noticed the seedling green enhancement through the seed inoculation with PGPR and called it plant growth promoting rhizobacteria. Moreover, it has been reported that the maize dry weight increased through the seed inoculation with Azotobacter Chroococcum, the dry and wet weight of leaves and the maize height increased through the seed inoculation with Azospirillum Brasilense (Kapulink et al., 1982) and the maize height, plant wet weight and number of leaves increased through the seed inoculation with Pseudomonas Fluorescens. (Zahir et al., 1998) reported that the 19.8% increase of maize grain yield due to the seed inoculation with Azotobacter and Pseudomonas bacteria. (Zahir et al., 2000) observed the increase of the maize dry weight due to PGPR application and 68.4% increase of dry weight of the maize shoots because of PGPR application. This research aimed to study the effect of application of plant growth promoting rhizobacteria including Azotobacter, Azospirillum, and Pseudomonas on the grain yield and growth characteristics of Safflower and also to determine the best bacterial combination for improving the yield.

MATERIALS AND METHODS

To study the effects of Salinity stress on the germination of soybean cultivars, a test was conducted in randomized complete block design with three replications in biotechnology laboratory of Ardabil Islamic Azad University in 2012, that in this experiment, the first factor, were salinity concentrations and the second were cultivars and was used concentrations of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 0.10 Mm. In order to measure germination index, germinated seeds were counted every day. Coefficient of germination rate (CGR), germination rate index (GRI), mean germination time (MGT), final germination percentage (FGP) and germination rate (GR) were calculated by using the formula concerned (table 1):

<table>
<thead>
<tr>
<th>Germination indices</th>
<th>The Formula used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient Velocity Germination (CVG)</td>
<td>$\text{CVG} = 100 \times \frac{\sum N_i}{\sum N_i T_i}$</td>
</tr>
<tr>
<td>germination rate index (GRI)</td>
<td>$\text{GRI} = \frac{G_1}{1 + G_2/2 + \ldots + G_x/x}$</td>
</tr>
<tr>
<td>final germination percent (FGP)</td>
<td>$\text{FGP} = \frac{N_g}{N_t} \times 100$</td>
</tr>
<tr>
<td>mean germination time (MGT)</td>
<td>$\text{MGT} = \frac{\sum N_i T_i}{\sum N_i} = 100 / \text{CVG}$</td>
</tr>
<tr>
<td>germination rate (RS)</td>
<td>$\text{Rs} = \frac{1}{\text{MTG}}$</td>
</tr>
</tbody>
</table>

Analysis of variance and mean comparisons were performed using SAS software and means were compared using Duncan test in the level of 5%.

RESULTS AND DISCUSSION

**Plant height**

According to the results of ANOVA table, the effect of the seed inoculation with bacteria on plant height was significant at 1% probability level (Table 1). Moreover, the comparison of the means (Table 2) showed that the highest height of the plant was related to the treatment in which the Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas at the planting time. Generally, as
the nitrogen available to the plant is increased, the plant height and vegetative growth will increase as well. (Able, 1979) considered the longer rosette stage and longer growing season as the main reasons of the increase of plant height. (Kapulink et al., 1982) reported the increase of the maize height due to the seeds inoculation with (Azospirillum and Zahir et al., 2000) reported that 8.5 % increase of the maize height due to the seeds inoculation with Azotobacter and Pseudomonas. The increase of plant height is probably due to the increase of producing auxins and also the increase of soluble phosphorus by PGPR bacteria.

(Itzigsohn et al., 1993) believe that the increase of morphological growth of maize stem through the use of Azospirillum and Azotobacter is due to the production of phitohormones such as indoleacetic acid, gibberellin and cytokinin. In their studies on (Safflower and Zheng et al., 1993) mentioned that a main characteristic of high-yielding cultivars is their taller height.

Number of nodes per plant

The effect of the seed inoculation with bacteria on the number of nodes per plant was significant at 1% probability level (Table 1). The comparison of the means (Table 2) showed that the highest number of nodes per plant was related to the treatment in which Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas during planting time. Although its difference was not statistically significant with other levels in which Safflower seeds were inoculated with Azotobacter and Pseudomonas separately or together and or were inoculated with Pseudomonas, it was statistically significant with other levels at Duncan’s 5% probability level. The number of nodes per stem is very significant because it determines the number of leaves in plant and increases the light intake and consequently increases the plant photosynthesis (Reddy et al., 1997).

Stem diameter

According to results of ANOVA (Table 1), the effect of the seed inoculation with bacteria on stem diameter was significant at 1% probability level of probability. Also the comparison of the means (Table 2) indicated that the highest stem diameter was related to the treatment in which Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas at the planting time. Although its difference was not statistically significant with other levels in which Safflower seeds were inoculated with Azotobacter and Azospirillum and also with Azospirillum and Pseudomonas together, it was statistically significant with other levels at 5% probability level. The use of various levels of Phosphorus and nitrogen fertilizers led to a significant difference by means of making a better balance of nutrients, increasing vegetative growth of plants and consequently increasing the stem diameter for maintaining plant shoots through generating more supporting fabrics by thicker stems. By studying 16 qualitative traits of (Safflower, Ramanchandran and Goud, 1982) reported that there was a positive significant correlation between the grain yield and the stem diameter, plant height and length of lateral branches.

Number of bolls per plant

The effect of the seed inoculation with bacteria on the number of bolls per plant was significant at 1% probability level. The highest number of bolls per plant was related to the treatment in which Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas during planting time. Although its difference was not statistically significant with other levels in which Safflower seeds were inoculated with Azotobacter and Azospirillum separately or together and also with Pseudomonas, it was statistically significant with other levels at 5% probability level (Tables 1, 2).

By the increased availability of nitrogen and phosphorus the number of available sinks will increase. Generally, nitrogen fertilizers increase the number of bolls per plant by affecting the production of raw sap cytokinin of wood vessels and increasing the number of lateral branches (Arsalan et al., 1997; Gilbert and Tucker, 1987) reported
that the number of bolls per Safflower increased from 220 per square meter in the control treatment (lack of nitrogen) to 260 per square meter with the use of 150 kg nitrogen per hectare.

**Number of seeds per boll**

The effect of the seed inoculation with bacteria on the number of seeds per boll was significant. The highest number of seeds per boll was related to the treatment in which Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas at the planting time. Although its difference was not statistically significant with the treatment in which Safflower seeds were inoculated with Azotobacter and Azospirillum together and also with Azotobacter and Pseudomonas and with Azospirillum and Pseudomonas together, it was statistically significant with other levels at 5% probability level.

Lack of nitrogen during the formation of seed in the boll leads to the decrease of the number of formulated reservoirs during the physiological maturity. Consequently, the decrease of reservoir capacity and the shorter growth period which both result from the lack of nitrogen, will lead to the decrease of grain yield. On the other hand, phosphorus is a vital element for the growth of plant and its shortage is considered as one of the growth limiting factors. Since phosphorus plays an important role in plant metabolism by providing the required energy for the plant, the increased availability of this element which depends on the activity of phosphorus dissolving bacteria could lead to the increased inoculation of plant at reproductive phase and the preparation of large reservoir to increase the final grain yield. Growth enhancer bacteria will increase the usable phosphorus for the plant (Kucey et al., 1989; Salimpour et al., 2010) and will increase plant growth enhancer substances such as Indoleacetic acid.

(Gilbert and Tucker, 1987) reported that the number of seeds per boll increased 16% and 25% respectively by the use of 50 and 100 kgNha⁻¹ in comparison to the lack of nitrogen.

**1000-Grain weight**

The effect of the seed inoculation with bacteria on 1000-grain weight was significant at 1% probability level. Also the comparison of means (Table 2) showed that the highest weight of 1000-grain was related to the treatment in which Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas at the planting time. Although its difference was not statistically significant with other levels in which Safflower seeds were inoculated with Azotobacter and Azospirillum and also with Azospirillum and Pseudomonas together, it was statistically significant with other levels at 5% level.

The increased weight of 1000-grain in these treatment is possibly due to the increased availability of nitrogen and phosphorus and in general the availability of nutrients, the improvement of photosynthesis and better assimilates distribution towards formulated reservoirs and finally plant growth development which results from the increase of plant growing period. The increased weight of 1000-grain through the application of growth enhancer bacteria (PGPR) has been reported by other researchers as well. The research conducted by (Zahir et al., 1998) indicated that due the inoculation of maize seeds with Azotobacter and Pseudomonas the weight of 1000-grain increased 9.6%. (Marschner, 1995) also reported that nitrogen availability will extend the grain-filling stage and will increase 1000-grain weight at the end of growing period by increasing the plant growing period.

**Grain yield**

According to the results of ANOVA, the effect of the seed inoculation with bacteria on grain yield was significant at 1% probability level (Table 1). Also the comparison of means (Table2) showed that the highest grain yield was related to the treatment in which Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas during planting time. Although its difference was not statistically significant with other levels in which Safflower seeds were just inoculated with Azospirillum and or with Azotobacter and Pseudomonas together, it was statistically significant with other levels at 5% probability level.
It seems like that the increase of nitrogen fixation in this treatment could lead to the increase of leaf area and the production of more assimilates and finally the increase of dry matter production and increase of plant yield. On the other hand, the use of Azospirillum and Azotobacter will lead to the increase of dry matter and plant yield by increasing hormonal activity of plant in rhizosphere.

In an experiment carried out in Australia, (Beech and Norman, 2002) stated that the consumption of 80 kgNha⁻¹ with as much grain yield as 2150 kg ha⁻¹ was the most economical treatment in relation to lack of nitrogen consumption with as much grain yield as 1410 kg ha⁻¹.

In another experiment at different irrigation conditions, (Engel and Bergman, 1997) reported that consumption of 0-150 kgNha⁻¹ increased Safflower grain yield but it remained the same by the consumption of 150-200 kgNha⁻¹ and even it decreased by the consumption of nitrogen at limited irrigation conditions.

(Mirzakhani et al., 2009) studied the effects of dual inoculation of Azotobacter and Mycorrhiza at different levels of nitrogen and phosphorus on yield and yield components of spring Safflower. The results of the experiment showed that the highest grain yield was related to the treatment in which Safflower seeds were inoculated with Azotobacter and Mycorrhiza and also 100 kg nitrogen and 50 kg phosphorus were used during planting.

Results of other researches indicated that inoculation of wheat seed with different kinds of Azospirillum could increase the grain yield 23-63% in comparison to the control treatment (Caballlero-Mellado et al., 1992). Also the results indicated that the inoculation of wheat seed with Azotobacter could increase the grain yield up to 30% (Kloepper et al., 1991). The results of another research showed that the inoculation of sunflower seed with Azotobacter Brasilense and Azospirillum lipoferum had a positive effect on plant growth especially under irrigation conditions (Itzigsohn et al., 1995).

The increased yield of some crops due to the application of phosphate dissolving bacteria has been formerly reported by some researchers. In this regard, the increased yield of wheat has been reported by (De Freitas, 2000) and also the increased yield of barley and sugar beet has been reported by Ferrettin et al., 2004) due to the use of phosphate dissolving microorganisms.

**Biological yield**

With regard to the ANOVA (Table 1), the effect of the seed inoculation with bacteria on biological yield was significant at 1% level. Also the comparison of means (Table 2) showed that the highest biological yield was related to the treatment in which Safflower seeds were inoculated with all three bacterial growth enhancers that is Azotobacter, Azospirillum, and Pseudomonas at the planting time. Although its difference was not statistically significant with the treatment in which Safflower seeds were inoculated with Azotobacter and Azospirillum together, it was statistically significant with other levels at 5% probability level.

It seems like that nitrogen fixation increase in this treatment could lead to the increase of leaf area and more assimilate production and finally increase of dry matter production in the crop. These results indicate that the use of nitrogen has a great effect on the increase of crop biomass. It also seems like that PGPR rhizosphere could be effective in controlling crop diseases by producing various preventive metabolisms such as different kinds of antibiotics and by using antibiosis mechanism and by the relationship between bacteria and pathogens such as parasitic relationships, hunting, competition for occupying suitable places on the root. Moreover through the production of various amino acids and hormones and the increase of absorption and solubility of nutrients such as nitrogen, phosphorus, iron and zinc, the bacteria could enhance the growth and resistance of the plant and will prevent crop diseases as secondary disorder which in turn leads to the increase of plant biomass (Asadi Rahmani and Falah, 2001).
Table 1. Summary of analysis of variance for measured traits

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>Plant height</th>
<th>Number of nodes per plant</th>
<th>Stem diameter</th>
<th>Number of nodes per plant</th>
<th>Number of seed per bolls</th>
<th>1000-Grain weight</th>
<th>Grain yield</th>
<th>Biological yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean square</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>3</td>
<td>2.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.065&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.134&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.588&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2559.099&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treatment</td>
<td>7</td>
<td>614.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.334&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.104&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.796&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16454.371&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>9.66</td>
<td>15.60</td>
<td>0.21</td>
<td>34.729</td>
<td>11.768</td>
<td>1.790</td>
<td>39030.370</td>
</tr>
<tr>
<td>Cv (%)</td>
<td></td>
<td>4.58</td>
<td>16.40</td>
<td>6.83</td>
<td>24.88</td>
<td>11.18</td>
<td>4.55</td>
<td>9.94</td>
</tr>
</tbody>
</table>

Ns.,*,** respectively not significant, significant at 5% level, and significant at 1% level

Table 2. Mean comparison of effects of different treatments of inoculation of seed with growth enhancer measurable traits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Number of nodes per plant</th>
<th>Stem diameter (cm)</th>
<th>Number of nodes per plant</th>
<th>Number of seed per bolls</th>
<th>1000-Grain weight (g)</th>
<th>Grain yield (kg.ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Biological yield (kg.ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.07f</td>
<td>17.23d</td>
<td>5.015e</td>
<td>10.70c</td>
<td>22.14d</td>
<td>26.60e</td>
<td>1643.4d</td>
<td>6682.9e</td>
</tr>
<tr>
<td>Azotobacter chroococcum</td>
<td>64.75d</td>
<td>22.81bcd</td>
<td>6.52cd</td>
<td>20.52ab</td>
<td>29.81bc</td>
<td>28.35cde</td>
<td>1921.7bcd</td>
<td>7883.5c</td>
</tr>
<tr>
<td>Azospirillum brazilians</td>
<td>68.70cd</td>
<td>23.90abc</td>
<td>6.72bcd</td>
<td>22.80ab</td>
<td>30.88bc</td>
<td>29.42bcd</td>
<td>2000.0abc</td>
<td>8271.5b</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>55.67e</td>
<td>20.23cd</td>
<td>6.17d</td>
<td>13.42bc</td>
<td>27.05cd</td>
<td>27.45de</td>
<td>1779.4cd</td>
<td>7401.5d</td>
</tr>
<tr>
<td>Azotobacter+ Azospirillum</td>
<td>77.05b</td>
<td>26.08abc</td>
<td>7.42ab</td>
<td>21.97ab</td>
<td>33.22ab</td>
<td>30.51ab</td>
<td>2137.0ab</td>
<td>9370.9a</td>
</tr>
<tr>
<td>Azotobacter+ Pseudomonas</td>
<td>72.80bc</td>
<td>25.45abc</td>
<td>7.17bc</td>
<td>23.00a</td>
<td>32.24abc</td>
<td>29.63bc</td>
<td>2039.3abc</td>
<td>8403.9b</td>
</tr>
<tr>
<td>Azospirillum+ Pseudomonas</td>
<td>75.00b</td>
<td>26.95ab</td>
<td>7.37ab</td>
<td>20.92ab</td>
<td>32.94ab</td>
<td>30.54ab</td>
<td>2080.7abc</td>
<td>8596.9b</td>
</tr>
<tr>
<td>Azotobacter+ Azospirillum + Pseudomonas</td>
<td>83.27a</td>
<td>30.02a</td>
<td>8.02a</td>
<td>29.85a</td>
<td>37.08a</td>
<td>32.37a</td>
<td>2284.3a</td>
<td>9390.5a</td>
</tr>
</tbody>
</table>

In each column, the difference between two means which have one common letter is not significant at 5% probability level

REFERENCES


