

Effects of Dual Inoculation of Azotobacter and Mycorrhiza with Nitrogen and Phosphorus Fertilizer Rates on Grain Yield and Some of Characteristics of Spring Safflower

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Abstract—In order to evaluate the Effects of dual inoculation of Azotobacter and Mycorrhiza with Nitrogen and Phosphorus levels on yield and yield components of spring safflower, this study was carried out in field of Farahan university in Markazi province in 2007. A factorial in a randomized complete block design with three replications was used inoculation of Azotobacter (with inoculation and without inoculation) and Mycorrhiza (with inoculation and without inoculation) with Nitrogen and Phosphorus levels [F0= N0+ P0 (kg.ha-1), F1= N50+ P25(kg.ha-1), F2= N100+ P50(kg.ha-1) and F3= N150+ P75 (kg.ha-1)] on spring safflower (cultivar IL-111). In this study characteristics such as: Harvest index, Hectolitre weight, Root dry weight, Seed yield, Mycorrhizal Colonization Root, Number of days to maturity were assessed. Results indicated that treatment (A0M1F3) with grain yield (1556 kg.ha-1) and treatment (A0M1F0) with grain yield (918 kg.ha-1) were significantly superior to the other treatments and according to calculated, inoculation seeds in planting date with Azotobacter and Mycorrhiza to cause increase grain yield about 5/38 percentage. we can by inoculation safflower seeds with Azotobacter and Mycorrhiza too easily at the time sowing date. The purpose of this research, study and evaluation the role of biological fixation N and P, to provide for feeds plants.

Keywords—Spring safflower, grain yield, inoculation, Azotobacter and Mycorrhiza.

I. INTRODUCTION

THIS The main oil seed crops of Iran are canola, sunflower, soybean and cotton. They cover largest of the total oil seed production of the country. Farmers in Iran don't produce safflower (*Carthamus tinctorius* L.) in a large scale because it does not have high grain yield and low oil content, almost cultivar to exist have long duration in growth period to compare another oil seed crops were planted in country. Safflower oil is used by farmers locally. However, safflower can be a potential oilseed crops for low-rainfall areas.

Safflower has been grown for centuries, primarily for its colorful petals to use as a food coloring and flavoring agent,

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for vegetable oils and also for preparing textile dye in the Far East, Central and Northern Asia and European Caucasian [1]. It has also received considerable interest recently as forage [2]. Vegetable oil is one of the fundamental components in foods and has important functions regarding human health and its nutritional physiology. The demand for vegetable oils for food purposes has entailed a considerable expansion of oilseed crops all over the world [3]. Particularly, consumers have demanded healthier oils, naturally low in saturated fat such as olive, safflower, canola and sunflower oils. The seeds contain 35-50% oil, 15-20% protein and 35-45% hull fraction [4]. Safflower can also be grown successfully on soil with poor fertility and in areas with relatively low temperatures [5]. Most of the experiments have indicated that vesicular-arbuscular mycorrhiza (VAM) was able to alter water relation of its host plants. effects of VAM on morphology, metabolism and protective adaptation of host plants in the condition of drought stress. Mechanism that VAM can enhance resistance of drought stress in host plant may include many possible aspects: (1) VAM improves the properties of soil in Rhizosphere; (2) VAM enlarges root areas of host plants, and improves its efficiency of water absorption; (3) VAM enhances the absorption of P and other nutritional elements, and then improves nutritional status of host plant; (4) VAM activates defence system of host plant quickly; (5) VAM protects against oxidative damage generated by drought; (6) VAM affects the expression of genetic material [6]. More and more experiments have indicated that VAM was able to alter water relations and played a great role in the growth of host plant in the condition of drought stress. Augé compiled the existing literature on plant water relations, drought and VAM symbiosis [7]. VAM symbiosis improved absorption capacity, and increased the growth of its host plant, which was proved in sugarcane, mung bean, apple, orange, wheat, tomato and wild jujube [8]. There is a great correlation between nutritional status of plant and its drought resistance, while VAM changed the nutritional status of its host plant. P concentrations themselves may affect host water balance, but it is often fixed in soil and not available to plant. Phosphatase produced by VA fungi play an important role in translating fixed or insoluble into soluble P, which can be used by plant freely. At the same time, hyphae are also important ways of P transported in soil. Other elements such as Zn and Cu can also not flow freely in soil [9]. The absorption of Ca, Si, Ni, Co etc. was also reported increased by VAM symbiosis [10]. It is

TABLE I
RESULT OF PHYSICAL AND CHEMICAL CHARACTERISTICS OF SOIL.

| Soil texture | Clay % | Silt % | Sand % | Available K(P.P.M) | Available P (P.P.M) | Total N % | Organic carbon % | T.N.V | PH | EC Mmos.cm ⁻¹ | Depth (cm) |
|--------------|--------|--------|--------|--------------------|---------------------|-----------|------------------|-------|---------|--------------------------|------------|
| loam | 25.0 | 30.0 | 45.0 | 400 | 9 | 0.04 | 0.38 | 10.0 | 7.8 | 1.0 | 30 - 0 |
| loam | | | | 200 -300 | 10 -15 | 0.1> | >1.0 | 10.0< | 6.5-7.5 | 2.0< | Optimum |

still accept by many that VAM enhance resistance of high stress of host plants by improving their nutritional status.

II. MATERIAL AND METHODS

This experiment was conducted in agricultural field of Farahan university (34°30' N, 40°41' E Long., 1779 m sea level) Markazi province in spring of 2007. The soil texture was loamy sandy. The experimental design was used a factorial arrangement in a randomized complete block with three replications. Each plot consisted of 4 rows, 5 m long with 50 cm spaced between rows and 5 cm distance between plants on the rows. Plant density was 40 seed per m². Treatments were include three agent: Azotobacter chroococum (with and without inoculation) with population 10⁸ number per each ml, Mycorrhiza (*Glomus interaradices*), (with and without inoculation) with population 250 - 300 of fungus active organs for each seed planted and used combination of different rate of Nitrogen and Phosphorus in 4 levels: [F₀= N₀+ P₀ (kg.ha⁻¹), F₁= N₅₀+ P₂₅(kg.ha⁻¹), F₂= N₁₀₀+ P₅₀(kg.ha⁻¹) and F₃= N₁₅₀+ P₇₅ (kg.ha⁻¹)] on spring safflower (variety IL-111). Urea (0, 50, 100 and 150 Nitrogen kg.ha⁻¹) was used; It was broadcasted to the plots meanwhile. Treble superphosphate (0, 25, 50 and 75 P₂O₅ kg.ha⁻¹) was banded at seeding time. The plants were thinned after complete emergence in the 6 leaf stage as keeping on rows about 5 cm. In harvest stage, the two middle rows were used for sampling and measured parameters such as: weight hectoliter, mycorrhizal root colonization percent, Length of roots, Harvest index, Root dry weight, Number of days to maturity, oil percent, oil content and grain yield were assessed. Grain yield in each plot measured with 14% humidity. Statistically of the result was done by using MSTAT-C programme. Means were compare using the Duncan's Multiple Range Test (DMRT) at 5% level of probability. Correlation was calculated between grain yield and other plant characters. Assessment of root colonization and spore population

One hundred root segments were examined for each sample. The stained roots were observed under a compound microscope. A root segment was considered as AM positive if it showed any fungal bodies like mycelium, vesicles and arbuscules. Percent of root colonization was calculated as follows:

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

III. RESULTS AND DISCUSSION

Results from the present study are indicate that grain yield have been affected significantly by the inoculation with Azotobacter. In other word, the Azotobacter by increased of activity itself could bring proper amount of nitrogen for feed

plants in Rhizosphere. But mycorrhiza could affect significantly on characters such as; harvest index, hectolitre weight, root dry weight, root mycorrhizal colonization. Maybe, the reasons of no success at inoculation with mycorrhiza to increasing of grain yield, interaction effects and eating between meals with native races of mycorrhiza in soil, soil PH and no to exist enough time for arrived to highest efficiency of mycorrhizal colonization activity. use combination of Nitrogen and Phosphorus levels was significantly on grain yield, oil content, root dry weight, root mycorrhizal colonization and number of days to maturity at 1% level probability and 5% on hectoliter trait.

Harvest index: among the all of treatment, highest and lowest harvest index obtained in (A₀M₁F₁) with average 38.07 % and (A₀M₀F₁) with average 33.12% respectively. Therefore, (A₀M₁F₁) was could more successful than another treatment in transport of assimilate from sources to sinks plant and had product highest harvest index. One of benefite Effects of mycorrhiza is on plants photosynthesis, VAM plants often display higher rate of photosynthesis than NM counterparts do, which is consistent with VAM effects on stomatal conductance. Most of the researchers suggested that VAM symbiosis increased the units of photosynthesis, and so as to increase the rates of photosynthetic storage and export at the same time [7]. It has been prove that concentration of chlorophyll and in VAM plants was higher than their control NM plants. Therefore it can product larger grains and enhance economical yield. Harvest index of safflower cultivars under water stress condition ranges from (23.4) to (28.4) % [11]. And another research, harvest index of safflower cultivars reported ranges from (18.5) to (23.5) % [12].

Hectolitre weight: inoculation with mycorrhiza and use chemical fertilizer were significantly effect on hectoliter weight. Therefore, if enough available nutrient existing in around of plants root, plants can absorb higher amount of macro and micro elements and product grain with higher hectoliter weight. Usually grains which have higher 1000-grain weight, they have higher in hectoliter weight compare grains which have lower 1000-grain weight. Treatment (A₀M₀F₀) with average (71.52 Kg.100 litre⁻¹) has higher and (A₀M₁F₃) with average (63.34 Kg.100 litre⁻¹) has lower hectoliter weight between all of treatment. Results were reported by [13] showed which fluctuate 1000-grain weight from (30 to 49 g) and it was correlate with grain yield (r=0.45**), head diameter (r=0.47**) and (r=0.53**)or its components.

Root dry weight: The main effects of inoculation with mycorrhiza was significantly at 5% and use different of nitrogen and phosphorus was significantly at 1% probability level on root dry weight trait. The interaction effect of Azotobacter and use different of nitrogen and phosphorus was

TABLE II
MEAN COMPARISON OF MAIN EFFECTS OF DUAL INOCULATION OF AZOTOBACTER AND MYCORRHIZA WITH NITROGEN AND PHOSPHORUS LEVELS ON SAFFLOWER (VAR. IL-111)

| Treatment | Harvest index (%) | Hectolitre weight (Kg.100Litre ⁻¹) | Root dry weight (g) | Seed yield (kg. ha ⁻¹) | Mycorrhizal Colonization (%) | No. of days to maturity |
|----------------|-------------------|--|---------------------|------------------------------------|------------------------------|-------------------------|
| A ₀ | 34.81 a | 67.07 a | 3.91 a | 1255 b | 7.79 a | 113.3 a |
| A ₁ | 35.85 a | 65.98 a | 4.07 a | 1302 a | 8.04 a | 113.4 a |
| M ₀ | 34.73 b | 67.33 a | 3.86 b | 1268 a | 7.62 b | 113.4 a |
| M ₁ | 35.92 a | 65.72 b | 4.12 a | 1289 a | 8.20 a | 113.3 a |
| F ₀ | 35.60 a | 67.71 a | 3.40 c | 1085 c | 6.41 c | 112.8 b |
| F ₁ | 35.40 a | 67.53 a | 3.71 c | 1202 b | 8.25 b | 113.7 a |
| F ₂ | 34.58 a | 65.82 ab | 4.21 b | 1398 a | 9.25 a | 113.4 a |
| F ₃ | 35.72 a | 65.04 b | 4.65 a | 1429 a | 7.75 b | 113.6 a |

Means which have at least one common letter are not significantly different at the 5% level using (DMRT).

TABLE III
MEAN COMPARISON OF INTERACTION OF DUAL INOCULATION OF AZOTOBACTER AND MYCORRHIZA WITH NITROGEN AND PHOSPHORUS LEVELS ON SAFFLOWER (VAR. IL-111)

| Treatment | Harvest Index (%) | Hectolitre weight (Kg.100Litre ⁻¹) | Root dry weight (g) | Seed yield (kg. ha ⁻¹) | Mycorrhizal Colonization (%) | No. of days to maturity |
|-------------------------------|-------------------|--|---------------------|------------------------------------|------------------------------|-------------------------|
| A ₀ M ₀ | 33.36 b | 68.64 a | 3.68 b | 1245 a | 9.00 b | 113.3 a |
| A ₀ M ₁ | 36.26 a | 65.50 b | 4.14 a | 1266 a | 6.58 c | 113.4 a |
| A ₁ M ₀ | 36.11 a | 66.03 b | 4.05 ab | 1292 a | 6.25 c | 113.6 a |
| A ₁ M ₁ | 35.59 a | 65.93 b | 4.10 a | 1312 a | 9.83 a | 113.2 a |
| A ₀ F ₀ | 35.84 a | 68.96 a | 2.88 e | 956.8 d | 6.66 d | 112.3 b |
| A ₀ F ₁ | 34.63 ab | 68.57 a | 3.69 d | 1194 c | 8.00 c | 113.8 a |
| A ₀ F ₂ | 33.12 b | 65.69 ab | 4.27 bc | 1454 a | 11.50 a | 113.5 a |
| A ₀ F ₃ | 35.65 a | 65.07 b | 4.82 a | 1416 ab | 5.00 e | 113.7 a |
| A ₁ F ₀ | 35.36 a | 66.46 ab | 3.93 cd | 1213 c | 6.16 d | 113.2 a |
| A ₁ F ₁ | 36.17 a | 66.50 ab | 3.72 cd | 1210 c | 8.50 c | 113.5 a |
| A ₁ F ₂ | 36.05 a | 65.95 ab | 4.15 b-d | 1342 b | 7.00 d | 113.3 a |
| A ₁ F ₃ | 35.80 a | 65.01 b | 4.49 ab | 1441 a | 10.50 b | 113.5 a |
| M ₀ F ₀ | 36.02 ab | 69.75 a | 3.48 cd | 1091 d | 7.66 b | 112.7 c |
| M ₀ F ₁ | 33.45 c | 68.17 ab | 3.48 cd | 1168 cd | 6.50 c | 113.5 ab |
| M ₀ F ₂ | 34.68 bc | 65.61 bc | 4.18 b | 1462 a | 8.50 b | 113.7 a |
| M ₀ F ₃ | 34.79 bc | 65.81 bc | 4.31 b | 1351 b | 7.83 b | 113.8 a |
| M ₁ F ₀ | 35.19 a-c | 65.67 bc | 3.33 d | 1079 d | 5.16 d | 112.8 bc |
| M ₁ F ₁ | 37.35 a | 66.90 a-c | 3.93 bc | 1236 c | 10.00 a | 113.8 a |
| M ₁ F ₂ | 34.49 bc | 66.03 bc | 4.24 b | 1335 b | 10.00 a | 113.2 a-c |
| M ₁ F ₃ | 36.66 ab | 64.28 c | 5.00 a | 1506 a | 7.66 b | 113.3 a-c |

Means which have at least one common letter are not significantly different at the 5% level using (DMRT).

significantly at 1% probability level. The highest and lowest root dry weight obtained in (A₀M₁F₃) with average (5.39 g) and (A₀M₁F₀) with average (2.86 g) respectively. Thus, mycorrhiza fungi can causes higher growth in roots and to increasing root dry weight in plants was inoculated with mycorrhiza. The root dry weight was recorded in inoculation with mycorrhiza condition (average:0.49 g) and without mycorrhiza condition (average:0.46 g) in safflower cultivars [14]. Enlargment of root areas and its efficiency of water absorption, The main absorption apparatus of mycorrhiza extension hyphae with a diameter of 2-5mm can penetrate soil pore inaccessible to root hairs (10-20mm) and so absorb water that is not available to non-mycorrhizal plants [10].

Grain yield: The all of main, twofold and threefold interactions effects of treatments were significantly on grain yield, except main effect of mycorrhiza and twofold

interactions effect of inoculation with Azotobacter and mycorrhiza. Results showed that treatment (A₀M₁F₃) with grain yield average (1556 kg.ha⁻¹) and treatment (A₀M₁F₀) with grain yield average (918 kg.ha⁻¹) were significantly superior to the other treatments. In other word, Mycorrhiza could with symbiosis activity itself, cause to increasing P nutrient in around root plants and addition absorb by roots. with use combination of Nitrogen and Phosphorus levels was significantly at 1% level probability, but threefold interaction effect of inoculation Azotobacter and mycorrhiza with use combination of Nitrogen and Phosphorus levels was significantly on at 1% level probability. The study of evaluated parameters varied greatly among the cultivars. Cv. Remzibey was found to be superior than the other two cultivars with its higher seed yield (1648 kg ha⁻¹), oil content (28.0 %) and oil yield (480 kg ha⁻¹). However, head diameter

TABLE IV
MEAN COMPARISON OF INTERACTION OF DUAL INOCULATION OF AZOTOBACTER AND MYCORRHIZA WITH NITROGEN AND PHOSPHORUS LEVELS ON SAFFLOWER (VAR. IL-111)

| Treatment | Harvest index (%) | Hectolitre weight (Kg.100 litre ⁻¹) | Root dry weight (g) | Seed yield (kg .ha ⁻¹) | Mycorrhizal Colonization(%) | No. of days to maturity |
|--|-------------------|---|---------------------|------------------------------------|-----------------------------|-------------------------|
| A ₀ M ₀ F ₀ | 35.77 ab | 71.52 a | 2.90 e | 995.7 h | 10.00 bc | 112.3 c |
| A ₀ M ₀ F ₁ | 31.18 d | 70.84 ab | 3.23 de | 1159 g | 7.66 d-f | 113 bc |
| A ₀ M ₀ F ₂ | 32.15 cd | 65.39 c | 4.38 bc | 1547 a | 11.67 a | 114 ab |
| A ₀ M ₀ F ₃ | 34.35 b-d | 66.79 a-c | 4.24 bc | 1277 d-g | 6.66 fg | 113.7 ab |
| A ₀ M ₁ F ₀ | 35.92 ab | 66.40 bc | 2.86 e | 918 h | 3.33 h | 112.3 c |
| A ₀ M ₁ F ₁ | 38.07 a | 66.29 bc | 4.16 bc | 1230 e-g | 8.33 de | 114.7 a |
| A ₀ M ₁ F ₂ | 34.08 b-d | 65.99 bc | 4.17 bc | 1360 b-e | 11.33 ab | 113 bc |
| A ₀ M ₁ F ₃ | 36.95 ab | 63.34 c | 5.39 a | 1556 a | 3.33 h | 113.7 ab |
| A ₁ M ₀ F ₀ | 36.26 ab | 67.98 a-c | 4.06 bc | 1186 fg | 5.33 g | 113 bc |
| A ₁ M ₀ F ₁ | 35.71 ab | 65.49 c | 3.73 cd | 1178 fg | 5.33 g | 114 ab |
| A ₁ M ₀ F ₂ | 37.21 ab | 65.84 c | 3.99 b-d | 1376 b-d | 5.33 g | 113.3 bc |
| A ₁ M ₀ F ₃ | 35.24 a-c | 64.82 c | 4.38 bc | 1426 a-c | 9.00 cd | 114 ab |
| A ₁ M ₁ F ₀ | 34.46 b-d | 64.94 c | 3.80 b-d | 1239 d-g | 7.00 ef | 113.3 bc |
| A ₁ M ₁ F ₁ | 36.63 ab | 67.50 a-c | 3.71 cd | 1242 d-g | 11.67 a | 113 bc |
| A ₁ M ₁ F ₂ | 34.89 a-c | 66.07 bc | 4.30 bc | 1309 c-f | 8.66 cd | 113.3 bc |
| A ₁ M ₁ F ₃ | 36.36 ab | 65.21 c | 4.60 b | 1456 ab | 12.00 a | 113 bc |

Means which have at least one common letter are not significantly different at the 5% level using (DMRT)

(2.34 cm), seed per head (33.06), 1000 seed weight (41.8 g), palmitic (11.0 %) and stearic (2.8 %) acid contents of cv. Dinçer and linoleic acid content (75.6 %) of cv. Yenice were higher than those of cv. Remzibey [1]. Previous literature reports cite seed yield of safflower ranging from 1168 to 3325 kg .ha⁻¹ [15]-[20]. Thus, the lowest and highest yields observed in the current study are somewhat similar those found in the preceding works.

Root Mycorrhizal Colonization: A variety of studies suggest that water extraction by plant roots can be enhanced when they are infected by arbuscular mycorrhiza (AM) fungi. Mycorrhizal colonization fluctuated from 8.7 to 14.4 % and it was not correlate with grain yield or its components [21]. In this study Mycorrhizal colonization fluctuated from 3.3 in treatment (A₀M₁F₃) to 12 % in treatment (A₁M₁F₃) and it was not correlate with grain yield, but it was correlate with 1000-grain weight trait. Too, the all of main, twofold and threefold interactions effects of treatments were significantly on Mycorrhizal colonization, except main effect of Azotobacter. A study was carried out to determine the effectiveness of the arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* on the growth of nine hybrids of grain sorghum and five genotypes of safflower, in soil with deficient N (9 mg kg⁻¹), P (11 mg kg⁻¹) and organic matter (1.4 %), using plants that were and were not inoculated with AMF. Chlorophyll, plant height, dry biomass, root dry weight and mycorrhizal colonization were measured. Mycorrhizal colonized sorghum plants showed a significant (p=0.01) increase in plant height, dry biomass and root dry weight, in comparison with non-colonized plants. Mean mycorrhizal colonization reached 37.5%. Chlorophyll rate showed a differential response between the sorghum hybrids, where only 'Patron' and '83G66' showed no significant increases. In the safflower genotypes, mycorrhizal colonization registered 36.8% on average and significantly increased plant height (p=0.001) and dry biomass (p=0.05), with respect to the non-colonized plants

[21]. Association of arbuscular mycorrhizal (AM) with agricultural crops was assessed at four agro-ecological zones [AEZ-28(Joydebpur), AEZ-9 (Jamalpur), AEZ-11 (Ishurdi) and AEZ-23 (Hathazari)] of Bangladesh during 1999-2000. Mainly cereals, pulses, oilseeds, vegetables and spices crops were selected for assessment. The average AM root colonization in all crops differed among the locations during both years. Average colonization (in two years) was maximum (43.3%) at AEZ-9 (Jamalpur) and minimum (38.8%) at AEZ-28 (Joydebpur). A considerable variation was also observed in average spore population among different AEZs. Higher average spore number (157.4/100 g soil) was recorded at AEZ-23 (Hathazari) and minimum (98.8/100 g soil) at AEZ-28 (Joydebpur). The spore number varied within and between the zones [22].

Number of days to maturity: inoculation with Azotobacter and mycorrhiza could not significant effect on day to maturity. But use levels of nitrogen and phosphorus was significantly on day to maturity at 1% probability. Between all of treatments, (A₀M₁F₁) with average (114.7 days) and (A₀M₁F₀) with average (112.3 days) had highest and lowest

TABLE V
CORRELATION COEFFICIENTS BETWEEN CHARACTERISTICS
1: GRAIN YIELD; 2: HARVEST INDEX; 3: ROOT DRY WEIGHT; 4: ROOT COLONIZATION; 5: HECTOLITRE WEIGHT; 6: DAYS TO MATURITY

| Trait | 1 | 2 | 3 | 4 | 5 | 6 |
|-------|---------------------|---------------------|---------------------|---------------------|---------------------|---|
| GY | 1 | | | | | |
| HI | -0.01 ^{ns} | 1 | | | | |
| RDW | 0.81 ^{**} | 0.15 ^{ns} | 1 | | | |
| RC | 0.25 ^{ns} | -0.24 ^{ns} | 0.04 ^{ns} | 1 | | |
| HW | -0.45 ^{**} | -0.22 ^{ns} | -0.57 ^{**} | 0.15 ^{ns} | 1 | |
| DM | 0.45 ^{**} | 0.07 ^{ns} | 0.43 ^{**} | -0.01 ^{ns} | -0.22 ^{ns} | 1 |

number of days to maturity. Number of days to maturity of safflower cultivars under water stress and non water stress condition reported ranges from (106-114.3 days) to (114-118 days) respectively [12].

IV. CONCLUSION

The success of safflower introduction in new areas will largely depend on the extent of improvement made in yield and oil content [23], [24]. Result from the present study indicated that seed yield, yield components of safflower have been affect significantly by the inoculation with Azotobacter and Mycorrhiza, because this biofertilizers can fix the nitrogen and phosphorus in soil and enhance absorb elements by plant. According to calculated, inoculation seeds in plantig date with Azotobacter and Mycorrhiza to cause increase grain yield about 5/38 percentage.

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