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The Effect of Iron Fertilization on Nodulation, Yield and Yield Traits of Soybean Genotypes with Different Maturity Groups as Affected by *Brady Rhizobium* Inoculations

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Abstract

Iron (Fe) deficiency is the major constraint for soybean cultivation in calcareous soils. Its availability affects symbiotic association of the host plant and the endosymbiont and eventually the yield of soybean. However, the effectiveness of integrated application of *Bradyrhizobium* and Fe fertilizer on nodulation and yield of soybean genotypes with different maturity group has not been studied. Therefore, the objective of this study was to evaluate the effect of *Bradyrhizobium* inoculated soybean genotypes with different maturity group on FeSO4 and Fe-EDTA nutrient requirement supplied through the foliage and applied directly to the soil. The experiment was conducted under greenhouse and field conditions. Six soybean genotypes, three late maturing (Wogayen, TGx-1336424 and Belsa) and the remaining three medium maturing (GIZA, Afgat and Gishame) were used for greenhouse experiment. Based on the greenhouse result, only two promising genotypes, namely GIZA and TGx-1336424, were selected for further investigation under field experiment. Eight treatments which synthesized by combining three *Bradyrhizobium* inoculations, with and without Fe fertilizer (0 and 4 mg kg^{-1} soil), including N-treated (20 kg N ha^{-1}) and the control, were tested in pot experiment arranged in a completely randomized design (CRD). Four levels of inoculation factorially combined with three levels of Fe-EDTA application were used for field experiment. These treatments were laid out in a spilt plot in randomized complete block design (RCBD) with three replications. The results of the present study indicate significant improvement of most investigated traits of tested medium maturing genotypes, but not for late maturing soybean genotypes under greenhouse conditions. A significant increase of nodule number (NN) and nodule dry weight (NDW) with increasing rates of Fe for selected genotypes were observed in field experiment. Application of Fe, however, showed differential effect on both genotypes on other investigated traits. All investigated traits, except NN, NDW and shoot dry matter (SDW) did not improve significantly by Fe application for TGx-1336424 genotype. In contrast, significant improvement of number of pods per plant (NPP), total biomass yield (TBY), grain yield (GY) and plant total nitrogen concentration (PTC) with increasing rates of Fe were observed for GIZA genotype. The regression analysis indicates different degree of dependence of TBY, NDW, NN and GY of both genotypes with increasing rates of Fe application with different *Bradyrhizobium* sp. inoculation treatments. Hence, it can be concluded that the effect of Fe application is dependent on maturity groups of soybean genotypes and effectiveness of inoculated *Bradyrhizobium* sp.

Introduction

About 10^6 ha (i.e. over 6% of the world's land) of agricultural soils in the world are problematic and unsuitable for crop production due to some kind of salinity (Flowers and Yeo, 1995). Leguminous crop cultivation in the saline soils of semi-arid climates is increasingly advocated as a strategy to amend soil fertility and to reverse or avert soil fertility decline, thus preventing serious nitrogen (N) deficits and sustaining agricultural production in many tropical agro-ecosystems. An essential source of N input into the soil is the biologically-fixed N_2, which is used directly by the plant, and so is less susceptible to volatilization, denitrification, and leaching (Graham, 2008). About 80% of this biologically-fixed N_2 comes from symbiosis involving leguminous plants and various rhizobial species. Of which, soybean (*Glycine max*) plays an important role in the global and agricultural nitrogen (N) cycles by facilitating biological fixation of atmospheric N_2 into plant-available N in the symbiotic process with *Bradyrhizobium* spp. The N_2 fixation potential of soybean varied from 0 to 185 kg N ha^{-1} with an average value of about 84 kg N ha^{-1} (Russelle and Birr, 2004). However, soil stresses, such as soil salinity, can adversely affect N_2 fixation by influencing both the host plant and the symbiotic bacteria (Rai, 1987).

Iron (Fe) deficiency, which results in chlorosis in crops, is a widespread problem in arid and semi-arid regions of the world (Papastylianou, 1990; Rashid and Din, 1992) in soils with a range of pH 7.5 and 8.5 (Guerinot and Yi, 1994; Mengel *et al.*, 2001). This soil pH range often reduces Fe availability below the sufficient threshold level that meets plant needs. Fe is an essential micro-nutrient required by both legumes and root nodulating-bacteria, which is an essential component of nitrogenase, leghaemoglobin and ferredoxins (Evans and Russell, 1971). Deficiency of Fe may affect N_2-fixation by restricting the host plant growth and/or active functioning of *Rhizobium* spp. alone (Andrew, 1962; Rai *et a1.*, 1984). Fe deficiency also primarily affects the structure and functioning of the chloroplast (Tognini *et al.*, 1996; Soldatini *et al.*, 2000; Morales *et al.*, 2001) which in turn reduces photosynthesis and carbohydrate synthesis (Miller *et al.*, 1984; Terry and Abadia, 1986; Bienfait, 1989). Consequently, it may restrict N_2-fixation by limiting the host plant growth and the active functioning of the *Rhizobium* spp. (Andrew, 1962; Rai *et al.*, 1984).

Several reports indicate that different soybean genotypes have the capacity to reduce different degrees of Fe chlorosis (Byron and Lambert, 1983; Halvis *et al.*, 1999). Al-Showk *et al.* (1986) reported the occurrence of different levels of chlorosis in different genotypes of soybean. Hartzook (1984) also found that groundnut genotypes displayed different levels of sensitivity to Fe deficiency. This study also indicated that Fe absorption efficient genotypes had equally yielded to other Fe chelate treated genotypes. Rai *et al*. (1984) suggested that genetic make-up of genotypes might be responsible for variation of Fe absorption and transport capacity among lentil genotypes.

It has been found that Fe-EDTA (Ethylenediaminetetraacetic acid) application directly to soils suppressed nodulation in pea plants, mainly due to the effect of the chelate on the formation of lateral roots and the initial processes of nodulation (Lie and Brotonegoro, 1969; Lie and Egerrat, 1988). The hypothesis of the present research was that late maturing genotypes require more nitrogen than medium maturing soybean genotypes. This results in better improvement of nodulation, N_2 fixation and yield of late maturing genotypes by Fe-EDTA application than that of medium maturing genotypes in moderately Fe deficient soils. Therefore, the overall objective of the study was to evaluate the effect of Fe fertilizer application on nodulation and yield of soybean genotypes with different maturity group and effectiveness of *Bradyrhizobium* spp. inoculation in Shinille area of Ethiopia Somali Region.

Materials and Methods

Description of experimental site

The field experiment was conducted in 2012 in the irrigated agricultural field (Shinille Agricultural Demonstration site, Somali Region, Ethiopia), which is semi-arid in nature. The experimental field is located at 09°41′ N latitude and 41°51′ E longitude with an elevation of 1079 m. The soil is dominated by sandy-clay and increases amount of clay further down to lower depth. The site has been used for maize (*Zea mays* L.) and tomato production in the previous years and had no history of inoculation of *Bradyrhizobium* strains for soybean cultivation. There had no any fertilizer application history in this site.

Rhizobial population sizes were estimated with the most probable number (MPN) method (Vincent, 1970) within 2 weeks of sampling, using a base dilution of 10 and the soybean variety Solitaire as the trap host. Then, no rhizobia were detected by a plant infection technique (Brockwell, 1963) at sowing time. The soil physical and chemical properties and the rhizobial population nodulating soybean is indicated in Table 1.

Table 1. Soil samples analysis of the experimental site at Shinile, Somali Region, before sowing

Soil properties	Shinile soil
pH in H_2O	7.74
EC (mS/cm)	4.12
Organic carbon (%)	2.15
Total nitrogen (%)	0.29
Available P (mg kg^{-1})	25.85
Ca (cmol(+) kg^{-1})	31.10
Mg (cmol(+) kg^{-1})	3.22
Na (cmol(+) kg^{-1})	0.14
K (cmol(+) kg^{-1})	2.22
CEC (cmol(+) kg^{-1})	25.90
Zn (mg kg^{-1})	1.19
Fe (mg kg^{-1})	2.2
B (mg kg^{-1})	0.86
NH_4-N (mg kg^{-1})	26.22
NO_3-N (mg kg^{-1})	23
Clay (%)	27
Silt (%)	50
Sand (%)	23
Textural class	Loam
Number of *Bradyrhizobium* nodulating soybean	None

Sources of soybean seeds and inocula

The soybean genotype, which has been tested under field conditions, used in this study was gratuitously provided by the Pawe Agricultural Research Center. Rhizobial strains, namely *Bradyrhizobium japonicum* (TAL-379), *Bradyrhizobium* sp. (UK-Isolate) and *Bradyrhizobium* sp. (Local isolate) which was isolated from Jimma soil, were used as inoculants. These strains were obtained from Holetta Agricultural Research Center (UK-Isolate) and National Soil Research Center (TAL-379 and Local isolate). The local strain had been previously tested for its infectivity under controlled environment in the National Soil Research Center (data not shown).

Sterile fine filter-mud with adjusted pH of 6.7 was used as a carrier. The *Bradyrhizobium* spp. were separately incubated in yeast-extract mannitol (YEM) broth at 30 ^{0}C for 7 days until the number reached 10^9 cells ml^{-1} for inoculant preparation. About 400 ml of *Bradyrhizobium* sp. culture liquid medium was added to 1 kg of carrier (sterilized filter-mud) and mixed thoroughly and packed in plastic bags then incubated at 26–28 ^{0}C for 15 days.

Pot experiment

A plant growth medium containing soil from Shinille agricultural experimental site was prepared based on the absence of indigenous soybean-nodulating *Bradyrhizobium* to facilitate the visual identification of N-deficiency symptoms in plants nodulated by the specific strain. Saline soil, collected (0-20 cm depth) from an area where the field experiment was conducted, was used for the pot experiment. Zero and 4 mg Fe kg^{-1} of soil was used in the form of $FeSO_4$ and applied directly to the soil before planting as described by Moosavi and Ronaghi (2011). Nitrogen (i.e. 20 kg ha^{-1} based) in the form of urea was applied for N treated pots. Six soybean genotypes, three of which were late maturing

(Wogayen, TGx-1336424 and Belsa) and the remaining three (GIZA, Afgat and Gishame) were medium maturing, were used for greenhouse experiment.

The pot experiment was conducted in the semi-controlled greenhouse at Haramaya University, eastern Ethiopia, in 2012. Eight treatments which was synthesized by combining three inoculations with and without Fe fertilizer (0 and 4mg kg^{-1} soil), including N-treated (20 kg N ha^{-1}) and the uninoculated and unfertilized control, were arranged in a completely randomized design (CRD). Seeds were surface-sterilized with ethanol (for 1 min) and sodium hypochlorite (soaked for 5 min) and then washed several times with deionized water and five seeds were sown per pot. The seedlings were thinned to three plants per pot one week after emergence. Pots were regularly watered to 70% water-holding capacity (WHC), avoiding water logging. Rhizobia were cultured to exponential phase in YEM broth, and then 1 ml of culture containing 1×10^8 rhizobial cells per milliliter was injected to 7-day-old seedlings using pipettes. When the seedling reached late flowering and early pod setting stage, the seedling were removed from the pots, the roots were thoroughly rinsed with water, blotted dry on filter paper, and nodules were picked and counted. The total plant and nodule dry weights were recorded after oven drying at 70 ^{0}C for 48 hrs.

Field experiment

The field experiment was conducted at the experimental farm of Shinille Agricultural Demonstration site in 2012 using well-structured drip irrigation system. The land was prepared by deep plowing, harrowing and leveling. Then the area was ridged and divided into 3 m x 3 m plot. The main plot treatments consisted of four levels of inoculants: UK Strain, TAL-379, Local Strain and un-inoculated plot combined with three levels of iron fertilizer (Fe-EDTA) (0, 1, and 2% of Fe). The rate of Fe application was adapted from Moosavi and Ronaghi (2011) and supplied through foliar application after three weeks of sowing. Fe-EDTA (1% and 2% Fe) was applied at a rate of 2.3 L ha^{-1}, giving a total of 0.023 kg Fe $ha^{-1}$and 0.046 kg Fe ha−1 as outlined by Franzen *et al.* (2003). The subplot treatments included two soybean genotypes (Giza and TGx-1336424). The treatments were laid out as split plot in a randomized complete block design (RCBD) with three replications. Each soybean cultivar was planted in spacing of 75 cm between rows, 10 cm between plants, 2 m between main plots and 1.5 m between subplots.

Before planting, 20 g of the different *Bradyrhizobium* inoculants were added to different polyethylene bags containing 200 g of soybean seeds. Sugar solution (10%) was added to each bag to enhance proper mixing and adhesion of the *Bradyrhizobium* carrier material on seed coat. Two seeds were sown per hill and then thinned to one seedling per hill. Plots were immediately irrigated after sowing to ensure uniform germination. Subsequently, plots were irrigated by a drip irrigation system at a 7-day interval. Weeds were controlled by hand hoeing over the growth period. A set of five plants from the central three were randomly uprooted at late flowering and early pod setting stage for estimating the nodulation potential (number of nodules per plant and dry weight of nodules) and shoot characteristics (shoot height and shoot dry weight). Dried shoot parts were grounded and analyzed for total N using the Kjeldahl digestion method.

At physiological maturity stage, plants were harvested from a central 3 m x 2.25 m net plot (three rows) leaving two boarder rows. The plant tops (stalks plus pods) were weighed to determine total dry matter yield before threshing and winnowing to separate the grain and determine the yield. Leaves were not included in the total dry matter yield determinations, as they had already fallen to the ground. Moisture was corrected to 11% when determining grain yield.

Statistical data analysis

Each sample was analyzed in triplicate and data were then averaged. Analysis of variance (ANOVA) was conducted using the SAS computer software package. The least significant difference (LSD) was used to separate treatment means at $P < 0.05$ probability level.

Results and Discussion

Greenhouse experiment

The average nodule number (NN) and nodule dry weight (NDW) were significantly ($P < 0.05$) affected by treatments (T), genotype (G) and T x G interaction (Tables 2 and 3). Iron applied in the form of $FeSO_4$ in soil had a significant effect on NN, NDW and shoot dry weight (SDW) for both soybean genotypes. Fe application significantly improved the NN as compared to unfertilized treatment for GIZA and Gishame genotypes. These genotypes revealed significant response in NN for Fe application with both *Bradyrhizobium* inoculations. Iron application integrated with UK-isolate inoculation resulted in significantly higher in NN with Wogayen genotype than UK-isolate inoculated alone. However, the result revealed the non-significant improvement of the average NN due to Fe application as compared to unfertilized plants.

The maximum NN (67.33) was obtained from TGx-1336424 genotype inoculated with local isolate alone. The same genotype also gave the lowest NN (6.67) when inoculated TAL-379 isolate alone. Beside this, a remarkable improvement in NN was obtained in all tested medium maturing soybean genotypes Of the tested late maturing genotypes, only Wogayen genotype revealed a significant improvement in NN by Fe application integrated with *Bradyrhizobium* inoculation compared to the separate respective inoculation treatments.

Table 2. Nodule number of soybean genotypes inoculated with exotic and native *Bradyrhizobium* isolates integrated with iron application

Treatment	Nodule number						
	GIZA	Afgat	Wogayen	TGx-1336424	Gishame	Belsa	Average
Negative control	0.00e	0.00b	0.00e	0.00c	0.00c	0.00c	0.00c
Positive control	0.00e	0.00b	0.00e	0.00c	0.00c	0.00c	0.00c
UK-Isolate	36.33cd	35.00a	48.67b	63.33ab	41.00b	53.33a	46.28a
UK-Isolate + Fe	54.67ab	45.00a	59.67a	44.67ab	43.67b	38.33ab	47.67a
TAL-379	20.00d	10.00b	9.67de	6.67c	9.33c	9.00c	10.78b
TAL-379 + Fe	26.00cd	10.67b	11.67d	7.33c	10.67c	7.33c	12.28b
Local Isolate	38.67bc	40.67a	36.67c	67.33a	39.00b	29.33b	41.94a
Local Isolate + Fe	56.67a	33.33a	32.67c	39.67b	64.67a	39.00ab	44.33a
LSD (0.05)	17.72	12.04	10.86	24.56	15.72	16.40	6.14
CV (%)	21.58	19.50	15.44	30.35	21.35	26.32	23.41
F- value:							
Treatment(T)	36.47***	57.11***	105.17***	32.11***	56.36***	37.47***	235.53***
Genotype(G)							6.56***
T x G							6.31***

***very highly significant at 0.001; Fe- (4mg kg^{-1} soil) in the form of FeSO$_4$; Negative control- neither inoculated nor fertilized; Positive control- Urea treated

Notes: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

Table 3. Nodule dry weight of soybean genotypes inoculated with exotic and native *Bradyrhizobium* isolates and integrated with iron application

Treatment	Nodule dry weight						
	GIZA	Afgat	Wogayen	TGx-1336424	Gishame	Belsa	Average
Negative control	0.0000c	0.0000d	0.0000d	0.0000b	0.0000d	0.0000c	0.0000d
Positive control	0.0000c	0.0000d	0.0000d	0.0000b	0.0000d	0.0000c	0.0000d
UK-Isolate	0.2600b	0.2743b	0.3470b	0.4523a	0.4523b	0.3613a	0.3274b
UK-Isolate + Fe	0.6367a	0.3897a	0.4277a	0.3840a	0.3840a	0.3637a	0.4357a
TAL-379	0.4390ab	0.1187c	0.0833c	0.0567b	0.0567c	0.0927c	0.1551c
TAL-379 + Fe	0.3450b	0.0970c	0.1393c	0.0563b	0.0563c	0.0797c	0.1406c
Local Isolate	0.2923b	0.3123ab	0.2833b	0.4467a	0.4467a	0.2260b	0.3207b
Local Isolate + Fe	0.4167ab	0.2433b	0.3130b	0.3883a	0.3883ab	0.2923ab	0.3321b
LSD (0.05)	0.2248	0.0872	0.0796	0.0923	0.0880	0.1019	0.0450
CV (%)	26.62	17.19	14.13	14.64	15.10	20.37	20.38
F- value:							
Treatment(T)	22.36***	67.54***	102.97***	124.83***	81.59***	54.00***	254.06***
Genotype(G)							25.51***
T x G							9.14***

***very highly significant at 0.001; Fe- (4mg kg^{-1} soil) in the form of FeSO$_4$; Negative control- neither inoculated nor fertilized; Positive control- Urea treated

Notes: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

There was also a significant improvement in NDW, including the average NDW, in medium maturing soybean genotypes and Wogayen genotype from the tested late maturing genotypes due to Fe application integrated with UK-isolate inoculation as compared to the inoculation alone (Table 6). The highest (0.6367 g) NDW was obtained from GIZA genotype due to UK-isolate inoculation integrated with Fe application. Dual application of TAL-379 and Fe in TGx-1336424 and Gishame soybean genotypes produced the lowest (0.0563 g) NDW. The data also indicated that NDW reduced due to TAL-379 inoculated with Fe application as compared to the TAL-379 inoculation alone in all genotypes except Wogayen genotype.

A significant variation in shoot dry weight (SDW) due to the treatments for all investigated soybean genotypes, except GIZA genotype, was observed at P<0.05 (Table 4). Iron applied with *Bradyrhizobium* inoculation did not significantly improve the SDW as compared to inoculation alone. Generally, the result revealed that UK-isolate and local isolate inoculation resulted in significantly higher SDW in all genotypes, except GIZA genotype, than the control treatment. The highest (9.933 g) SDW was obtained from Wogayen genotype when inoculated UK-isolate, followed by 9.800 g in the same genotype fertilized inorganic N.

Table 4. Shoot dry weight of soybean genotypes inoculated with exotic and native *Bradyrhizobium* isolates integrated with iron application

Treatment	Shoot dry weight						
	GIZA	Afgat	Wogayen	TGx-1336424	Gishame	Belsa	Average
Negative control	6.367a	4.633b	7.100cd	6.467abc	5.900c	5.367b	5.972d
Positive control	8.600a	8.201a	9.800a	7.900abc	6.467abc	8.367a	8.222ab
UK-Isolate	7.833a	8.233a	9.933a	8.500abc	9.133a	7.667a	8.550a
UK-Isolate + Fe	9.300a	9.033a	8.767abc	7.033bc	8.767ab	8.033a	8.489a
TAL-379	8.767a	7.533a	6.500d	6.033c	6.833abc	7.167ab	7.139c
TAL-379 + Fe	9.300a	6.867ab	8.000bcd	5.533a	6.733abc	7.500a	7.322c
Local Isolate	8.367a	7.533a	9.600ab	9.300ab	6.300bc	8.100a	8.233ab
Local Isolate + Fe	9.533a	7.967a	8.733abc	8.800	8.133abc	8.400a	8.594a
LSD (0.05)	3.9102	2.469	1.728	3.200	2.735	1.963	1.011
CV (%)	16.26	11.61	7.15	15.20	13.29	9.17	12.52
F- value:							
Treatment(T)	1.67^{ns}	6.89**	12.92***	4.47**	4.76**	6.09**	16.20***
Genotype(G)							7.95**
T x G							2.27**

*NS- non significant; **highly significant at 0.01; ***very highly significant at 0.001; Fe- (4mg kg^{-1} soil) in the form of FeSO$_4$; Negative control- neither inoculated nor fertilized; Positive control- Urea treated. Notes: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test*

Field experiment

The effects of iron (Fe) and inoculation (I) significantly (P <0.05) affected the NN of both investigated genotypes (Table 5). However, the data indicated non-significant interaction effect of I x Fe on NN of both genotypes. Iron applied at 2% significantly increased NN including its average value for both genotypes. The highest NN (38.67 and 36.83) were scored by TGx-1336424 and GIZA genotypes, respectively, when inoculated local isolate. Whereas the unfertilized control produced the lowest NN (25.33 and 29.25) for GIZA and TGx-1336424 genotypes, respectively. Inoculation of TAL-379 performed poorly with regards to improvement of number of nodules per plant, including the average value, in both genotypes.

It was noted that NN of both genotypes had a significant quadratic association trend with increasing rates of Fe (graph not indicated). The coefficient of determination was higher for TGx-1336424 genotype ($Y=1.222x^2 + 5.222x + 33.77$, $R^2=0.278$) than GIZA genotype ($Y=1.166x^2 + 3.944x + 39$, $R^2=0.188$). However, the NN produced in each inoculation treatments had a significant linear association with increasing rates of Fe (Figure 1a and b). The coefficients of determination for GIZA genotype inoculated TAL-379 ($R^2=0.611$) and UK-Isolate ($R^2=0.715$) were higher than that for TGx-1336424 inoculated those respective isolate. The UK-isolate inoculation in TGx-1336424 genotype indicated higher coefficient of determination ($R^2=0.675$) than the coefficient (0.491) of GIZA genotype inoculated the same isolate. The highest coefficient of determination in GIZA genotype was $R^2=0.715$, which was obtained by inoculation of local isolate, while NN of TGx-1336424 genotype showed the highest coefficient of determination ($R^2=0.675$) when inoculated UK-isolate.

The result of this current experiment revealed that Fe application and inoculation alone had significant effect on NDW at $P < 0.05$ (Table 5). Highest rate of Fe (2% Fe) resulted in significantly higher NDW than the unfertilized control. However, the NDW induced at 1 and 2% Fe application showed the non-significant difference for both soybean genotypes. The NDW for TGx-1336424 and GIZA genotypes varied from 0.2861 to 0.3250 g and from 0.2596 to 0.3173 g, respectively. Iron applied at 2% Fe increased the NDW by 13.6 and 22.2% over the unfertilized control for TGx-1336424 and GIZA genotypes, respectively. On top of this, TGx-1336424 inoculated local isolate and GIZA genotype inoculated UK-isolate produced significantly higher NDW than any of other treatments, which induced 0.4666 and 0.4300 g, respectively.

Nodule dry weight of GIZA genotype had a significant quadratic association with Fe rates of application. However, this association for TGx-1336424 genotype was non-significant (graph not indicated). Beside this, NDW produced by both soybean genotypes due to different *Bradyrhizobium* sp. inoculation had a significant ($P < 0.05$) and quadratic association with Fe rates of application, except GIZA genotype inoculated TAL-379, whose association was found to be linear (Figure 2a and b). The highest coefficient of determination was recorded in all inoculated treatments for GIZA as compared to TGx-1336424 genotype inoculated the same isolate. The maximum ($R^2=0.623$) coefficient of determination was obtained from GIZA genotype inoculated UK-isolate, followed by the coefficient of determination ($R^2=0.440$) in TGx-1336424 genotype inoculated UK-isolate.

The present finding indicated that *Bradyrhizobium* inoculation (I), Fe rates of application (Fe) and I x Fe interaction had significant ($P < 0.05$) effect on shoot dry weight (SDW) (Table 5). Iron application at 2% significantly increased the SDW in TGx-1336424 genotype as compared to the control. Generally, Fe application in GIZA genotype did not affect significantly the SDW; instead significant reduction of SDW occurred at 2% Fe application as compared to the control. The SDW varied from 48.40 to 56.56 g for GIZA genotype and from 56.74 to 66.81 g for TGx-1336424 genotype. Iron applied at 2% resulted in 17.7% increase in SDW in TGx-1336424 genotype over unfertilized control. While the reduction in SDW for GIZA genotype was estimated at 15.1% as compared to the control. On top of this, the average SDW was significantly increased due to Fe application. Moreover, all *Bradyrhizobium* inoculations significantly enhanced SDW, including the average SDW, in both genotypes. Significantly higher SDW including average SB due to TAL-379 and UK-isolate inoculation over the other treatment in the TGx-1336424 genotype and the was recorded. In GIZA genotype, TAL-379 gave significantly higher SDW than the other treatments.

Iron and I x Fe interaction did not affect significantly ($p \leq 0.05$) the shoot height (SH) (Table 6). However, increasing rates of Fe application increased SH of both tested genotypes of soybean. The lowest SH (65.25 cm) for TGx-1336424 genotype was obtained from unfertilized control, while GIZA genotype scored the lowest SH (61.0 cm) at 1% Fe fertilized plants. TAL-379 and UK-isolates inoculation onto TGx-1336424 genotype significantly increased SH than in the uninoculated treatment. The research finding also showed significantly higher SH of GIZA genotype when inoculated TAL-379 isolate than the inoculated treatment. Generally, *Bradyrhizobium* inoculation increased the SH of TGx-1336424 and GIZA genotypes by 19.2 and 11.5%, respectively, over the uninoculated control.

Iron (Fe), inoculation (I) with *Bradyrhizobium* isolates and I x Fe interaction had significant ($P < 0.05$) effect on number of pods per plant (NPP) of GIZA genotype (Table 6). Only inoculation treatments displayed significant variation in the NPP for TGx-1336424 genotype. Iron application at 2% concentration resulted in significantly higher NPP than that of the unfertilized control in GIZA genotype. Though Fe application did not affect significantly the NPP of TGx-1336424 genotype, the highest NPP was recorded at 2% Fe application. The lowest NPP obtained from TGx-1336424 and GIZA genotypes were 131 and 137, respectively, when treated 1% Fe fertilizer. Significant variation in the NPP was also observed among different inoculation treatments. Inoculations with all *Bradyrhizobium* isolates resulted in significantly higher NPP and its average value for TGx-1336424 than the uninoculated control. However, only TAL-379 inoculation significantly increased the NPP of GIZA genotype.

Table 5. Nodulation and shoot dry matter at late flowering and early pod setting stage of two soybean genotypes (TGx-1336424 and GIZA) across different rates of iron and inoculation of exotic and native *Bradyrhizobium* isolates

	Nodule Number				Nodule dry weight (g)				Shoot biomass (g)		
Treatment	TGx-1336424	GIZA	Average		TGx-1336424	GIZA	Average		TGx-1336424	GIZA	Average
Fe-rate											
F_1	29.25b	25.33b	27.29c		0.2861b	0.2596b	0.2728b		56.74b	56.56a	56.65a
F_2	33.08ab	30.17b	31.63b		0.2951ab	0.2845ab	0.2898b		61.27ab	54.97a	58.12a
F_3	38.67a	36.83a	37.75a		0.3250a	0.3173a	0.3212a		66.81a	48.40b	57.60a
LSD (0.05)	5.74	5.43	3.82		0.0381	0.0447	0.0285		5.90	6.07	4.10
Inoculation											
TAL-379	35.11b	28.67b	31.89c		0.3473b	0.3098b	0.3286b		69.20a	61.42a	65.31a
UK-Isolate	42.22b	46.56a	44.39b		0.3943b	0.4300a	0.4122a		74.41a	50.99b	62.70a
Local isolate	57.33a	47.89a	52.61a		0.4666a	0.4088a	0.4377a		59.79b	48.17b	53.98b
Control	0.00c	0.00c	0.00d		0.0000c	0.0000c	0.0000c		43.02c	52.66b	47.84c
Mean	33.67	30.78	33.22		0.3021	0.2871	0.2946		61.61	53.31	57.46
LSD (0.05)	7.32	6.93	4.86		0.0486	0.0570	0.0362		7.52	7.75	5.21
CV(%)	16.70	17.3	17.0		12.38	15.3	13.83		9.39	11.2	10.22
F-value											
Iron (Fe)	8.50**	14.11***	22.08***		3.56*	5.24*	8.69***		9.12**	6.32**	0.39ns
Inoculation (I)	167.57***	157.98***	320.28***		276.37***	184.37***	441.58***		51.16***	8.29**	33.73***
I x Fe	0.98ns	1.62ns	2.52*		0.49ns	0.84ns	1.20ns		5.88***	3.08*	3.99**

*NS- non significant; * significant at 0.05; **highly significant at 0.01; ***very highly significant at 0.001;* F_1*- no Fe fertilized;* F_2*- 1% Fe application (0.023 kg Fe* ha^{-1}*);* F_3*- 2% Fe application (0.046 kg Fe* ha^{-1}*); Notes. -Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test*

The research data indicated the significant ($P < 0.05$) effect of Fe and I x Fe interaction on number of seeds per pod (NSP) (Table 3). Slight improvement of NSP was recorded at 1% Fe application in both genotypes. On top of this, significant variation in NSP due to *Bradyrhizobium* inoculation as compared to uninoculated treatment was observed. TAL-379 inoculation significantly increased NSP in both genotypes over the uninoculated control. The data, however, indicated the non-significant difference in the average value of NSP among the inoculation treatments. The NSP varied from 2.1800 to 2.6256 and from 2.3667 to 2.7000 for TGx-1336424 and GIZA genotypes, respectively. Generally, *Bradyrhizobium* inoculation improved the NSP up to 20.4 and 14.1% over uninoculated control, for TGx-1336424 and GIZA genotypes respectively.

I x Fe interaction had a significant ($P < 0.05$) effect on total biomass yield (TBY) for both soybean genotypes (Table 7). However, the effect of Fe rates of application on TBY in TGx-1336424 genotype was non-significant. The TBY of this genotype was decreased when increased the Fe rates from 1 to 2%, though 4% slight increase in TBY due to 1% Fe application as compared to the unfertilized plants. The highest (7816.4 kg ha^{-1}) TBY for TGx-1336424 genotype was obtained at 1% Fe application. However, in GIZA genotypes, increase in Fe rates of application resulted in significant improvement of TBY. The highest TBY (7106.5 kg ha^{-1}) for GIZA genotype was obtained from 2% Fe application, which was improved by 18.4% compared to unfertilized control. The non-significant variation of TBY in GIZA genotype was exhibited due to 1 and 2% Fe applications.

Inoculating local and UK-isolates on TGx-1336424 genotype resulted in a significant increase in TBY over the control. In GIZA genotype, only TAL-379 inoculation produced significantly higher TBY than that of the other inoculation treatments. However, the data revealed the non-significant variation in the average TBY among the inoculation treatments. The highest TBY (9207.8 Kg ha^{-1}) of TGx-1336424 genotype was obtained from UK-isolate inoculation with an average TBY of 7595.2 kg ha^{-1}. Similarly, the highest TBY (8374 kg ha^{-1}) from GIZA genotype was obtained from TAL-379 inoculation with an average TBY of 6687.2 kg ha^{-1}.

The research result revealed that Fe rates application and *Bradyrhizobium* inoculation (I) had a significant effect on grain yield (GY) for GIZA genotype (Table 7). On top of this, the I x Fe interaction had significant effect on the average GY. In this genotype, Fe application at 2% resulted in significantly higher GY production than the unfertilized control. The GY also varied from 1961 to 2196.60 kg ha^{-1}, which produced 12% higher GY at 2% Fe application than that of the unfertilized control. The average GY exhibited significant variation and the highest GY was obtained at 2% Fe application. In contrast, the effect of Fe rates of application was non-significant on GY of TGx-1336424 genotype.

Inoculations of elite isolate of symbiotic N_2-fixer displayed significant effect on GY of GIZA genotype but not observed on TGx-1336424 genotype. The research data showed that *Bradyrhizobium* isolates inoculation resulted in significant variation in GY of both soybean genotypes. Inoculation with UK and local isolates produced significantly higher GY in both genotypes than that of the uninoculated control. The GY of TGx-1336424 varied from 1722 to 2519.18 kg ha^{-1} with average value of 2254.77 kg ha^{-1}, while the maximum GY of GIZA genotype was 2277.33 kg ha^{-1}, which was 72.7% over the uninoculated control. The UK-isolate inoculation produced significantly higher average value of GY than the other inoculation treatments. The lowest GY in both genotypes, including the average value of GY, was obtained from soybean genotypes inoculated TAL-379 isolate.

The research finding revealed that Fe application, I and their interaction had a significant effect on total plant nitrogen concentration (PTC) in GIZA genotype, but not in TGx-1336424 genotype (Table 7). Iron application at 2% Fe resulted in significantly increased the PTC in GIZA genotype as compared to the unfertilized control. The PTC varied from 3.9175 to 4.0792% for TGx-1336424 genotype and from 3.8383 to 4.2050% for GIZA genotype. In TGx-1336424, the PTC decreased with increasing Fe rates of application. Even though there was no significant variation in the average PTN along the Fe rates, the data generally indicated an increase in PTC along increasing rates of Fe application.

Table 6. Plant height at late flowering and early pod setting stage, number of pods per plant and number of seeds per pod of two soybean genotypes (TGx-1336424 and GIZA) across different rates of iron and inoculation with exotic and native *Bradyrhizobium* isolates

Treatment	Plant height at flowering (cm)				Number of pods per plant				Number of seeds per pod		
	TGx-1336424	GIZA	Average		TGx-1336424	GIZA	Average		TGx-1336424	GIZA	Average
Fe-rate											
F_1	66.50a	61.00a	63.75a		138.8a	138.9b	138.9ab		2.4108a	2.5792a	2.4950a
F_2	65.25a	61.67a	63.46a		131.0a	137.4b	137.4b		2.5225a	2.6292a	2.5758a
F_3	68.25a	67.25a	67.75a		139.1a	147.7a	147.7a		2.4142a	2.6075a	2.5108a
LSD (0.05)	6.24	8.411	5.07		15.2	9.5	9.5		0.2106	0.2428	0.1556
Inoculation											
TAL-379	70.67a	71.33a	71.00a		134.7a	176.1a	155.4a		2.6256a	2.7000a	2.6628a
UK-Isolate	73.00a	61.00ab	67.00ab		145.9a	153.3b	149.6a		2.6244a	2.6622ab	2.6433a
Local isolate	61.78b	56.89b	62.61bc		149.8a	153.0b	151.4a		2.3667ab	2.6922a	2.5294a
Control	61.22b	64.00ab	59.33c		115.0b	102.9c	108.9b		2.1800b	2.3667b	2.2733b
Mean	66.67	63.51	64.99		136.3	146.3	141.3		2.4492	2.6054	2.5272
LSD (0.05)	7.96	10.73	6.44		19.4	15.9	12.1		0.2686	0.3096	0.1977
CV(%)	9.18	13.03	11.17		10.92	8.34	9.634		8.43	9.1394	8.819
F-value											
Iron (Fe)	0.73ns	2.08ns	2.62ns		1.14ns	6.84**	4.02*		1.14ns	0.13ns	0.89ns
Inoculation (I)	8.79***	4.91**	8.85***		9.88***	57.78***	45.89***		9.92***	4.06*	11.63***
I x Fe	2.15ns	0.87ns	1.25ns		0.85ns	4.29**	1.67ns		2.01ns	1.44ns	1.68ns

*NS- non significant; * significant at 0.05; **highly significant at 0.01; ***very highly significant at 0.001; F_1- no Fe fertilized; F2- 1% Fe application ;(0.023 kg Fe ha^{-1}); F3- 2% Fe application (0.046 kg Fe ha^{-1});; Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test*

Table 7. Total biomass and grain yield and total plant tissue nitrogen of two soybean genotypes (TGx-1336424 and GIZA) across different rates of iron and inoculation with exotic and native *Bradyrhizobium* isolates

Treatment	Total biomass yield (Kg/ha)			Grain yield (Kg/ha)			Total plant tissue nitrogen (%)		
	TGx-1336424	GIZA	Average	TGx-1336424	GIZA	Average	TGx-1336424	GIZA	Average
Fe-rate									
F_1	7500.0a	6003.1b	6751.5b	2226.11a	1961.31b	2071.21b	4.0792a	3.8383b	3.9588a
F_2	7816.4a	6952.2a	7384.3a	2290.76a	1973.09b	2131.92ab	4.0442a	3.8525b	3.9483a
F_3	7469.1a	7106.5a	7287.8a	2247.45a	2196.60a	2222.03a	3.9175a	4.2050a	4.0613a
LSD (0.05)	735.4	744.63	506.78	124.01	153.07	95.39	0.1772	0.1568	0.1146
Inoculation									
TAL-379	6604.9b	8374.5a	7489.7a	2259.14b	1503.89c	1881.51c	4.0267a	4.0311b	4.0289a
UK-Isolate	9207.8a	6913.6b	8060.7a	2518.40a	2205.90a	2766.40a	4.0711a	4.2444a	4.1578a
Local isolate	9032.9a	6049.4bc	7541.2a	2519.18a	2277.33a	2398.25b	4.0544a	4.0200b	4.0372a
Control	5535.0c	5411.5c	5473.3b	1722.39c	1319.05b	1520.72d	3.9022a	3.5656c	3.7339b
Mean	7595.2	6687.2	7141.2	2254.77	1826.53	2141.72	4.0136	3.9653	3.9894
LSD (0.05)	938.1	949.8	644.0	158.17	174.32	121.21	0.2261	0.2	0.1456
CV(%)	9.50	10.92	10.61	5.39	7.40	6.38	4.33	3.88	4.11
F-value									
Iron (Fe)	0.85ns	8.03**	5.29**	0.88ns	11.69***	7.40**	2.87ns	21.89***	3.478
Inoculation (I)	57.03***	27.74***	44.51***	85.75***	142.26***	292.27***	1.74ns	31.08***	21.71***
I x Fe	2.68*	2.00ns	2.08ns	2.42ns	1.15ns	2.52*	2.32ns	8.15***	5.41***

NS- non significant; * significant at 0.05; **highly significant at 0.01; ***very highly significant at 0.001; F_1_- no Fe fertilized; F2- 1% Fe application ;(0.023 kg Fe ha_$^{-1}$_); F3- 2% Fe application (0.046 kg Fe ha_$^{-1}$_);; Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test_

Fe chlorosis is commonly observed in groundnut and soybean crops in Shinille areas, Somali region, Ethiopia. The present study was, therefore, launched to explore whether the soybean genotypes having different maturity groups need different rates of Fe applied through foliage with the intention of obtaining highest yields. Papaslyhanou (1993) found better result when Fe chelate was applied at branching and before flowering stage and the phytotoxicity was found to be moderate. The present work indicated significantly low NN and NDW in Fe unfertilized plants regardless of the genotypes. Similarly, O'Hara *et al*. (1988b) had reported that iron deficiency led to decreased nodule number and mass in peanuts. The current investigation is also in conformity with the finding of Hartzook (1984) who reported that iron chelate treated standard genotype performed equally with that the genotype that had inherently efficient iron absorption. Nevertheless, the present result indicated a significant improvement in NN and NDW due to Fe application in the medium maturing genotypes only, where Fe was applied directly through the soils. The amount of Fe applied directly to soil was sensitive for immobilization and could reduce the availability of Fe for significant improvement of NN and NDW in late maturing soybean genotypes. Differential responses of genotypes to iron application could also be related to genetic make-up of the host, which regulates the absorption, translocation, metabolism, and N_2- fixation (Rai *et al*., 1984).

The regression analysis indicated that NN in both TGx-1336424 and GIZA genotypes increased linearly with increase in the rates of Fe application. The highest R^2-values of 0.678 and 0.715 were obtained from TGx-1336424 genotype inoculated with UK-isolate and GIZA genotype inoculated with TAL-379 isolate, respectively, indicating that there was better soybean genotype performance due to Fe application integrated with inoculation with different *Bradyrhizobium* strains. Tang *et al*. (1991) have also showed positive interaction between iron concentrations and *Bradyrhizobium* strains on nodulation of peanuts. It is obvious that *Bradyrhizobium* sp. display differently in performance and in nodule formation, N_2-fixation and form symbiotic association with different genotypes (Okereke and Unaegbu, 1992). O'Hara *et al*. (1988a) found that strains of *Bradyrhizobium* sp. differ profoundly in their ability to obtain Fe from their environment for development of their symbiotic systems in peanut. This ability affected the productivity of soybean genotypes differently due to inoculation with different isolates of *Bradyrhizobum* sp. along with rates of Fe application.

The present study indicated that inoculation with UK-isolate in TGx-1336424 genotype resulted in the highest coefficient of determination in NDW, TBY and GY with increase in the rates of Fe application. This suggests that the effectiveness of the *Bradyrhizobium* isolates is the determinant factor or responsible for remarkable effect of Fe-application on the productivity of any late maturing soybean genotype (TGx-1336424). This also implies that late maturing genotypes could need more N from symbiotic fixation beside the soil N in which the BNF requires Fe for various components of nodule function, i.e. nitrogenase biosynthesis, leghaemoglobin production and bacteroid multiplication. However, the highest coefficient of determination between GY and increasing rates of Fe application in GIZA genotype was obtained in the uninoculated control treatment though higher R^2-value was obtained between NN and NDW with increasing rates of Fe application inoculated with TAL-379 isolate and UK-isolate. This could probably be due to the presence of high soil native N which might have determined the final yield of the medium maturing genotypes, thereby hiding the effect of N derived from symbiotic association with *Bradyrhizobium* sp. In contrast, Tang *et al.* (1991) found that higher Fe concentrations in solution were required for maximum growth of plants reliant on symbiotic nitrogen fixation than that of plants receiving inorganic nitrogen fertilizer.

The findings of the current studies also indicated significant improvement in SDW of TGx-1336424 genotype but reduction in SDW in GIZA genotype with increase in rates of Fe application. It is known that iron application usually increases soybean top dry matter yield (Hodgson *et al.*, 1992; Ghasemi-Fasaei *et al.*, 2002) but higher levels may decrease soybean growth (Roomizadeh and Karimian, 1996). Rai *et al.* (1984) also found that genotypes differed greatly in iron absorption and translocation capacity. High Fe concentration in the plant tissue may reduce the uptake and concentration of Mn in soybean genotypes (Moraghan, 1985), thus lead to poor plant growth (Brand *et al.*, 2000). However, the Fe applied directly to soil enhanced the SDW of medium genotypes, including GIZA genotype, but was not observed in late maturing soybean genotypes. It is obvious that direct soil application of Fe in the form of $FeSO_4$ may not be as effective as application in the form of Fe-chelate as had been previously indicated by Hemantaranjan (1988). Moreover, there might be presence of greater variation in Fe absorption and transport capacity due to difference in genetic make-up among different soybean genotypes (Rai *et al.*, 1984).

The data generated here also indicated that increasing rates of Fe application did not significantly improve the PH, NPP, NSP, TBY, GY and TPC of TGx-1336424 genotypes although Fe application exhibited a significant nodulation enhancement. Beside this, under greenhouse condition, NN, NDW and SDW of late maturing soybean genotypes, including TGx-136424, did not improve with increase in rates of Fe application. A similar finding was reported by Papastylianou (1993) who found that moderately chlorotic peanuts that were fertilized with Fe did not significantly increase in their yield but had bigger nodules than the peanuts that were not fertilized with Fe application. The applied Fe may be also insufficient to get sufficient amount of N from symbiotic N_2-Fixation for significant improvement in the final yield of late maturing peanut genotypes (Robson, 1983; Tang *et al.*, 1991). Tang *et al.* (2006) who also found a similar result in lupine plant reported that the Fe requirement had been very high in lupin, probably due to the fact that it is one of the highest N_2-fixation food legumes, thus requires higher Fe nutrient. Remarkably higher protons production due to need for higher N from symbiotic N_2 fixation may lead to lower rhizosphere pH and, consequently, reduced the availability of some mineral nutrients as well as the effective functioning of rhizobia might be another reason of low response in late maturing genotypes for Fe application (Dakora and Philips, 2002).

The current research data revealed significant improvement in NPP, TBY, GY, and PTC obtained from GIZA genotype with increase in rates of Fe application. This result might be due to the fact that nodulation improvement in GIZA genotype enhances N_2-fixation, which could be sufficient for higher final yield. It is the fact that iron is also a critical micro-nutrient for N_2-fixation due to its essential integral component of nitrogenase, leghemoglobin, and ferrodoxins (Evans and Rossel, 1971). On top of this, it is also important in photosynthesis and carbohydrate synthesis (Miller *et al.*, 1984; Terry and

Abadia, 1986; Bienfait, 1989); it might affect N_2-fixation wherever carbohydrates are involved (Atkins, 1984).

The present study revealed a poor soybean growth in Fe-unfertilized plants irrespective of genotypes. Poor nodulation could be the cause of poor growth as has been indicated in Lupin (Tang and Robson, 1995). Tang *et al*. (1992) also found that iron deficiency generally decreases nodule formation, leghaemoglobin production and nitrogenase activity, leading to low nitrogen concentrations in the shoots in some legumes. In such situations, excess malic and citric acids may accumulate in the cells and normal metabolism and N_2-fixation may be disturbed.

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