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# Improvement of Faba Bean Yield Using *Rhizobium/Agrobacterium* Inoculant in Low-Fertility Sandy Soil

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**Abstract:** Soil fertility is one of the major limiting factors for crop's productivity in Egypt and the world in general. Biological nitrogen fixation (BNF) has a great importance as a non-polluting and a cost-effective way to improve soil fertility through supplying N to different agricultural systems. Faba bean (*Vicia faba* L.) is one of the most efficient nitrogen-fixing legumes that can meet all of their N needs through BNF. Therefore, understanding the impact of rhizobial inoculation and contrasting soil rhizobia on nodulation and N<sub>2</sub> fixation in faba bean is crucial to optimize the crop yield, particularly under low fertility soil conditions. This study investigated the symbiotic effectiveness of 17 *Rhizobium/Agrobacterium* strains previously isolated from different Egyptian governorates in improving the nodulation and N<sub>2</sub> fixation in faba bean cv. *Giza 843* under controlled greenhouse conditions. Five strains that had a high nitrogen-fixing capacity under greenhouse conditions were subsequently tested in field trials as faba bean inoculants at Ismaillia Governorate in northeast Egypt in comparison with the chemical N-fertilization treatment (96 kg N·ha<sup>-1</sup>). A starter N-dose (48 kg N·ha<sup>-1</sup>) was applied in combination with different *Rhizobium* inoculants. The field experiments were established at sites without a background of inoculation under low fertility sandy soil conditions over two successive winter growing seasons, 2012/2013 and 2013/2014. Under greenhouse conditions, inoculated plants produced significantly higher nodules dry weight, plant biomass, and shoot N-uptake than non-inoculated ones. In the first season (2012/2013), inoculation of field-grown faba bean showed significant improvements in seed yield (3.73–4.36 ton·ha<sup>-1</sup>) and seed N-yield (138–153 Kg N·ha<sup>-1</sup>), which were higher than the uninoculated control (48 kg N·ha<sup>-1</sup>) that produced 2.97 Kg·ha<sup>-1</sup> and 95 kg N·ha<sup>-1</sup>, respectively. Similarly, in the second season (2013/2014), inoculation significantly improved seed yield (3.16–4.68 ton·ha<sup>-1</sup>) and seed N-yield (98–155 Kg N·ha<sup>-1</sup>) relative to the uninoculated control (48 kg N·ha<sup>-1</sup>), which recorded 2.58 Kg·ha<sup>-1</sup> and 80 kg N·ha<sup>-1</sup>, respectively. Interestingly, faba bean inoculated with strain *Rlv* NGB-FR 126 showed significant increments in seed yield (35%–48%) and seed N-yield (34%–49%) compared to the inorganic N fertilizers treatment (96 kg N·ha<sup>-1</sup>) over the two cropping seasons, respectively. These results indicate that inoculation of faba bean with effective rhizobial strains can reduce the need for inorganic N fertilization to achieve higher crop yield under low fertility soil conditions.

**Keywords:** *Rhizobium*; *Agrobacterium*; inoculation; soil fertility; faba bean

## 1. Introduction

Faba bean (*Vicia faba* L., broad bean, horse bean) is a major grain legume widely cultivated in many countries for food and feed purposes [1]. Due to its multiple uses, high nutritional value, and ability to grow over a wide range of climatic and soil conditions, cultivation of faba bean is suitable for sustainable agriculture in many marginal areas [2].

Faba bean is one of the oldest legume crops grown in Egypt [3]. However, production has declined considerably from 523,000 tonnes in 1998 to 158,000 tonnes in 2014 [4]—often a result of susceptibility to foliar diseases, the effects of parasites [5], and/or competition with other crops. Egypt now is the world's largest importer of faba bean; its annual requirement of half million tonnes accounts for over half of global imports [6]. Therefore, increasing faba bean production and improving yield quality is a major target to meet the demand of the increasing Egyptian population, since faba bean constitutes a major part of the diet of Egyptian people [7].

Arid land with low nutrient availability, like most of the Egyptian land available for agriculture expansion [8], covers around 30% of the world's land area [9]. In such poor ecosystems, application of high levels of inorganic fertilizers is a common practice to compensate for nitrogen deficiency which is very costly and is a crucial obstruction toward increasing production of food crops including legumes [10]. In addition, more than 50% of the applied nitrogen fertilizers are somehow lost through different processes which not only represent a cash loss to the farmers, but also a source of pollution for the environment [11]. Consequently, there has been a growing interest in environmental friendly sustainable agricultural practices [12].

Biological nitrogen fixation, especially rhizobia-legumes symbiosis, is one of the alternative solutions and the promising technologies which play an important role in reducing the consumption of chemical N-fertilizers, increasing soil fertility, decreasing the production cost, and eliminating the undesirable pollution impact of chemical fertilizers in the environment [13]. Worldwide, N<sub>2</sub> fixed by nodulated legumes (pulses and oilseeds legumes) is estimated to contribute 21.45 Tg N annually to global agricultural systems [14].

Like other legumes, faba bean contributes to sustainable agriculture by fixing atmospheric nitrogen in symbiosis with soil rhizobia [15]. Faba bean commonly establishes effective nitrogen fixation symbiosis with fast-growing rhizobia of the species *Rhizobium leguminosarum* *sv. viciae* (*Rlv*) [16]. Later, *R. fabae* [17], *R. laguerreanae* [18], *R. etli* [19,20], and *Agrobacterium tumefaciens* [20] were also identified as faba bean-nodulating microsymbionts.

Faba bean is one of the most efficient nitrogen-fixing legumes and faba bean plants can meet all of their N needs through biological nitrogen fixation (BNF) [14,21]. Globally, the amounts of N<sub>2</sub>-fixed by faba bean were estimated in the range from 45 to 300 kg N·ha<sup>-1</sup> [22]. Under different Egyptian field conditions, the amount of N<sub>2</sub>-fixed by this legume ranged between 121 and 171 kg N·ha<sup>-1</sup> [23,24].

Populations of soil rhizobia often vary considerably in their abundance and effectiveness in nodulating and fixing atmospheric nitrogen (N<sub>2</sub>) symbiotically with their legume hosts [25,26]. Low fertile soils, particularly sandy soils, contain insufficient numbers of indigenous rhizobia to form efficient symbiotic relationships with their appropriate legumes. In such cases, the reliance on soil rhizobia as the sole source of inoculants can restrict legume yields [27,28]. Therefore, research on the impact of legume inoculation with efficient rhizobial strains can assist in defining the potential of inoculation to improve legume yields and increase the contribution of legume fixed N to the agriculture system [29].

In a previous study, the taxonomic diversity of 42 rhizobial strains that had been isolated from nodules of faba bean grown under different agro-ecological conditions in Egypt was studied using multilocus sequence analyses (MLSA) [20]. Interestingly, only 17 strains were identified as *Rlv*, while 24 strains were identified as *A. tumefaciens*, and one strain was classified as *R. etli*. All isolated strains formed effective symbioses with faba bean plants in Leonard jar assemblies. The present study is complementary to the previous work, and is intended to investigate the potential of highly efficient strains as faba bean inoculants to enhance the crop yield and productivity under low fertility sandy soils compared to the recommended inorganic N-fertilization (96 kg N·ha<sup>-1</sup>).

## 2. Material and Method

### 2.1. Bacterial Strains

Seventeen Egyptian strains of faba bean nodulating rhizobia including twelve *A. tumefaciens* and five *R. leguminosarum* *sv.* *viciae* [20] were used in this study. All strains were grown in yeast extract-mannitol (YEM) medium [30].

### 2.2. Symbiotic Effectiveness under Greenhouse Conditions

Studies of the symbiotic properties (nodulation and nitrogen uptake) of 17 rhizobial strains were conducted with faba bean cultivar *Giza 843* in a pot experiment. Plastic pots (30 cm diameter) were filled with 10 kg of sandy soil and arranged in a complete randomized block design with three replicates. Low fertility sandy soil samples with no history of inoculation were collected from Agricultural Research Station, Ismaillia Governorate (30°37'00.10" N and 32°14'38.57" E). Soil characteristics used in the pot experiment were determined according to [31], and are shown in Table 1. Four seeds were planted in each pot. Each seed was inoculated with 1 mL of a log phase rhizobial culture ( $10^9$  cells mL<sup>-1</sup>). Growth conditions of faba bean plants were 12–25 °C (night/day), a relative humidity of 50%–60%, and a photoperiod of 10 hr. At flowering stage, 50 days after sowing, plants were uprooted and assayed for dry weight of nodules, shoots and roots dry weight, as well as shoot N-uptake by faba bean plants.

**Table 1.** Physical and chemical properties of different sandy soils used in this study.

Property	Value		
	Greenhouse Experiment	Field Winter Growing Season 2012/2013	Field Winter Growing Season 2013/2014
Texture grade	Sandy	Sandy	Sandy
CaCO <sub>3</sub> (%)	2.85	2.80	1.95
Saturation percent S.P (%)	19.60	27.90	27.60
pH	7.75	8.20	7.94
Electrical conductivity (dS·m <sup>-1</sup> at 25 °C)	0.67	0.90	0.81
Soluble cations (meq/L)			
Ca <sup>+2</sup>	2.12	3.40	3.00
Mg <sup>+2</sup>	1.10	1.30	1.18
Na <sup>+</sup>	2.57	5.30	3.61
K <sup>+</sup>	1.10	0.40	0.35
Soluble anions (meq/L)			
CO <sub>3</sub> <sup>-2</sup>	0.00	0.00	0.00
HCO <sub>3</sub> <sup>-</sup>	1.95	1.20	1.10
Cl <sup>-</sup>	3.00	3.40	3.10
SO <sub>4</sub> <sup>-2</sup>	1.94	5.80	3.94
Total N (%)	0.017	0.024	0.021
Total Soluble-N (mg·Kg <sup>-1</sup> )	8.50	20.00	19.50
Available-P (mg·Kg <sup>-1</sup> )	3.10	4.90	4.45
Available-K (mg·Kg <sup>-1</sup> )	179.00	252.00	238.50
Organic matter (%)	0.31	0.31	0.30
Available micronutrients (mg·Kg <sup>-1</sup> )			
Fe	1.30	4.30	3.92
Mn	0.90	2.10	1.95
Zn	1.00	1.20	1.35
Cu	0.03	0.02	0.05

### 2.3. Field Experiments

Field experiments were carried out at El Wasfeya village, Ismailia Governorate, Egypt (30°34'27.30" N, 32°10'26.21" E) in the two successive winter growing seasons, 2012/2013 and 2013/2014. Soil characteristics from experimental sites were determined according to [31]. The main physical and chemical properties of the soil are shown in Table 1. Faba bean variety *Giza 843* was used due to its tolerance to drought stress conditions. Faba bean seeds were sown in the rate of 100 kg seeds ha<sup>-1</sup> and were cultivated in strips. Each strip (4 m × 12 m) consisted of four plots. Each plot area was 12 m<sup>2</sup> and consisted of four rows, spaced 0.6 m apart. An additional fifth row was placed in each plot and served as a border, and was not involved in calculations. Each strip was spaced apart by 1 m apart to prevent bacterial migrations. Weeds, insects, and fungal pathogens were controlled by chemical spray applications, as required, at rates according to manufacturers' recommendations. At flowering stage, 50 days after sowing, plant samples from each plot were randomly selected from as uniform of an area as possible (in the middle of the second and third lines), in order to avoid heterogeneous conditions or disturbed sites, for estimating nodules dry weight, plant dry matters, and shoot N-content. At harvest, biological yield was determined by the mechanical harvesting of the entire plot using a plot harvester.

### 2.4. Fertilization

All treatments received the recommended dose of phosphate and potassium fertilization in the rate of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 115 kg K<sub>2</sub>O ha<sup>-1</sup>, respectively. All bacterial treatments received 48 kg N·ha<sup>-1</sup> as a starter N-dose. In addition, three un-inoculated controls were involved; (T<sub>0</sub>) the uninoculated non-N fertilized control; (T) the uninoculated with starter N-dose (48 kg N·ha<sup>-1</sup>); and (TN) the uninoculated with full N-fertilizers (96 kg N·ha<sup>-1</sup>).

### 2.5. Inocula Preparation and Seed Inoculation

Vermiculite supplemented with 10% peat was used as a powder carrier [32], packed in polyethylene bags (300 g carrier per bag), sealed and sterilized by gamma irradiation (2.5 × 10<sup>6</sup> rads). Rhizobial strains were grown in YEM medium [30]. Cultures of (1 × 10<sup>9</sup> colony-forming unit mL<sup>-1</sup>) were injected into the carrier to satisfy 60% of water holding capacity. At sowing, faba bean seeds were coated with different rhizobial inoculants at a rate of 10 g of inoculant/1 kg seeds, using Arabic gum solution (16%) as the adhesive agent for seed coating [33].

### 2.6. Statistical Analysis

Data was analyzed for variance using the MSTAT analysis software [34].

## 3. Results

### 3.1. Symbiotic Effectiveness under Greenhouse Conditions

The symbiotic efficiency of 17 rhizobial strains related to *Rlv* and *A. tumefaciens* was assessed with faba bean cv. *Giza 843* in a pot experiment under greenhouse conditions. The effect of inoculation on dry weight of nodules, plant dry weight accumulation, and shoot N-content is shown in Table 2. All strains successfully nodulated faba bean and showed different nodulation patterns which ranged from 82 to 366 mg nodules/plant (Table 2). *Rlv* strains NGB-FR 126 and NGB-FR 128 resulted in the highest dry weight of nodules with 366 and 295 mg nodules/plant, respectively. In case of *A. tumefaciens* strains, the maximum dry weight of nodules (230 mg nodules/plant) was produced by strain NGB-FR 39. Nevertheless, the uninoculated controls T<sub>0</sub>, T, and TN resulted in 48, 63 and 52 mg nodules/plant, respectively. Shoot dry weight of faba bean plants increased significantly in response to effective inoculation, however, no significant variations were observed for root dry weight. Out of the tested strains, eight strains (NGB-FR 39, 62, 65, 70, 107, 126, 128, and 142), resulted in significant

increment in shoot dry matter, which ranged from 4.01 to 4.27 g/plant relative to the uninoculated control (T) with 48 kg N·ha<sup>-1</sup>. In the same trend, shoot N-content was clearly affected according to the type of inoculated strains (Table 2). All inoculated strains, except for NGB-FR 26 and 51, produced shoot N content significantly higher than that obtained with non-inoculated control (T). Faba bean plants inoculated by *Rlv* strain NGB-FR 126 showed the highest shoot N-content (152 mg N/plant) which was significant greater than all other treatments, including the full N-fertilized treatment (TN), which recorded 142 mg N/plant (Table 2). On the other hand, all inoculated strains except for strain NGB-FR 26 significantly increased shoot dry weight and accumulated higher N in plants than the uninoculated non-fertilized control (T<sub>0</sub>).

**Table 2.** List of faba bean nodulating rhizobia used in this study, their identity \*, and symbiotic properties under greenhouse condition.

Treatment	Identity *	Dry Weight of Nodules (mg)		Dry wt. (g/Plant)				Shoot N Content (mg N/Plant)	
				Root		Shoot			
NGB-FR 10	<i>A. tumefaciens</i>	103	g	1.68	a	3.62	cdefg	122	hi
NGB-FR 25	<i>A. tumefaciens</i>	82	hi	1.57	a	3.61	defg	123	h
NGB-FR 26	<i>A. tumefaciens</i>	82	hi	1.49	a	3.40	fgh	109	l
NGB-FR 27	<i>A. tumefaciens</i>	96	gh	1.54	a	3.83	abcdefg	130	g
NGB-FR 39	<i>A. tumefaciens</i>	230	d	1.80	a	4.18	abcde	142	de
NGB-FR 51	<i>A. tumefaciens</i>	94	gh	1.47	a	3.62	cdefg	114	k
NGB-FR 62	<i>A. tumefaciens</i>	130	f	1.68	a	4.27	ab	145	bc
NGB-FR 65	<i>R. leguminosarum sv. viciae</i>	172	e	1.78	a	4.16	abcde	141	de
NGB-FR 70	<i>R. leguminosarum sv. viciae</i>	191	e	1.79	a	4.22	abcd	139	ef
NGB-FR 99	<i>A. tumefaciens</i>	104	g	1.49	a	3.67	bcdefg	118	j
NGB-FR 107	<i>A. tumefaciens</i>	100	gh	1.78	a	4.01	abcdef	137	f
NGB-FR 122	<i>A. tumefaciens</i>	182	e	1.62	a	3.82	abcdefg	130	g
NGB-FR 126	<i>R. leguminosarum sv. viciae</i>	366	a	1.94	a	4.26	ab	152	a
NGB-FR 128	<i>R. leguminosarum sv. viciae</i>	295	b	1.89	a	4.24	abc	144	cd
NGB-FR 132	<i>A. tumefaciens</i>	98	gh	1.60	a	3.60	efg	119	ij
NGB-FR 140	<i>R. leguminosarum sv. viciae</i>	146	f	1.87	a	3.82	abcdefg	122	hi
NGB-FR 142	<i>A. tumefaciens</i>	130	f	1.60	a	4.13	abcde	136	f
T <sub>0</sub>		48	j	1.32	a	2.87	h	89	m
T		63	ij	1.46	a	3.34	gh	114	k
TN		52	j	1.91	a	4.32	a	142	cde

\* Bacterial identification based on 16S rRNA sequencing and multilocus sequence typing [20]. Data per plant are means of four replicates (three plants per replicate). Values followed by the same letter within each column are not significantly different at  $p < 0.05$ . T<sub>0</sub>: uninoculated seeds and non-chemical N-fertilizers. T: uninoculated seeds plus starter N-fertilizer (48 kg N·ha<sup>-1</sup>). TN: uninoculated seeds and full N-fertilizer (96 kg N·ha<sup>-1</sup>).

### 3.2. Evaluation of Faba Bean Inoculation under Field Trials

Two field experiments were conducted over two successive growing seasons (2012/2013 and 2013/2014), in low fertility sandy soils at Ismaillia Governorate in order to investigate the symbiotic properties of the rhizobial strains that had a high nitrogen-fixing capacity under controlled conditions in the greenhouse. The results showed that all selected strains were able to nodulate faba bean cv. Giza 843 under field-grown conditions (Tables 3–6).

In the first season (2012/2013), at flowering stage, the highest nodules dry mass per plant was achieved by *Rlv* NGB-FR 128 (367 mg/plant) followed by *Rlv* NGB-FR 126 (322 mg/plant), which was significantly higher than that obtained by other tested strains (Table 3). On the other hand, the uninoculated controls (T<sub>0</sub>, T, and TN) showed nodulation status ranged from 11–83 mg nodules/plant. Highly significant differences were observed in the dry matter of faba bean plants according to different rhizobial inoculations (Table 3). Faba bean plants inoculated by *Rlv* NGB-FR 126 and *A. tumefaciens* NGB-FR 62 showed the highest shoot dry weights (6.2 and 6.1 g/plant, respectively), which were significantly higher relative to the full N-uninoculated control (TN) that resulted in 5.4 g/plant. Similarly, *Rlv* NGB-FR 126 resulted in the maximum shoot N-content (281 mg N/plant), which was

significantly greater as compared to the uninoculated controls where the accumulated N in shoots ranged from 128–258 mg N/plant.

**Table 3.** Effect of different rhizobial strains on nodulation, growth parameters, and shoot N content of faba bean plants after 50 days of sowing under field conditions (winter growing season 2012/2013).

Treatment	Dry wt. of Nodules (mg/Plant)		Dry wt. (g/Plant)				Shoot N Content (mg N/Plant)	
			Root		Shoot			
NGB-FR-39	85	e	0.84	d	4.3	e	172	d
NGB-FR-62	287	c	1.21	a	6.1	ab	256	b
NGB-FR-70	143	d	1.08	b	4.7	e	181	d
NGB-FR-126	322	b	1.20	a	6.2	a	281	a
NGB-FR-128	367	a	1.14	ab	5.5	bc	251	b
T <sub>0</sub>	28	f	0.69	e	3.1	f	128	e
T	83	e	0.96	c	4.8	de	211	c
TN	11	f	1.19	a	5.4	cd	258	b

Data per plant are means of four replicates (three plants per replicate). Values followed by the same letter within each column are not significantly different at  $p < 0.05$ . T<sub>0</sub>: uninoculated seeds and non-chemical N-fertilizers. T: uninoculated seeds plus starter N-fertilizer (48 kg N·ha<sup>-1</sup>). TN: uninoculated seeds and full N-fertilizer (96 kg N·ha<sup>-1</sup>).

**Table 4.** Effect of different rhizobial strains on different growth parameters and yield of faba bean plants under field conditions (winter growing season 2012/2013).

Treatment	Plant Height (cm)	No of Branches/Plant	No of Pods/Plant	No of Seeds/Plant	Seed Index	Yield (ton/ha)				Seed N Yield kgN/ha						
						Straw	Seed	Straw	Seed							
NGB-FR-39	89	d	3.0	a	18	cd	48	de	75.7	c	3.6	bcd	2.83	d	85	e
NGB-FR-62	127	b	3.3	a	22	ab	65	ab	79.2	a	4.6	ab	4.29	a	150	a
NGB-FR-70	90	d	3.3	a	15	de	43	e	72.3	d	2.9	cd	2.78	d	83	e
NGB-FR-126	134	a	3.7	a	24	a	72	a	79.0	a	5.9	a	4.36	a	153	a
NGB-FR-128	128	b	3.3	a	20	bc	61	bc	78.3	ab	4.4	b	3.73	b	138	b
T <sub>0</sub>	72	e	3.0	a	13	e	31	f	69.2	e	2.4	d	1.76	e	53	f
T	92	d	3.3	a	19	bc	53	cd	76.1	bc	3.8	bc	2.97	d	95	d
TN	101	c	3.7	a	22	ab	62	bc	78.1	abc	4.5	ab	3.24	c	114	c

Values followed by the same letter within each column are not significantly different at  $p < 0.05$ . T<sub>0</sub>: uninoculated seeds and non-chemical N-fertilizers. T: uninoculated seeds plus starter N-fertilizer (48 kg N·ha<sup>-1</sup>). TN: uninoculated seeds and full N-fertilizer (96 kg N·ha<sup>-1</sup>).

**Table 5.** Effect of different rhizobial strains on nodulation, growth parameters, and shoot N content of faba bean plants after 50 days of sowing under field conditions (winter growing season 2013/2014).

Treatment	Dry wt. of Nodules (mg/Plant)		Dry wt. (g/plant)				Shoot N Content (mgN/Plant)	
			Root		Shoot			
NGB-FR-39	309	e	1.86	c	10.4	c	312	c
NGB-FR-62	421	d	1.86	c	10.2	c	307	c
NGB-FR-70	711	b	2.32	b	12.9	b	402	b
NGB-FR-126	814	a	2.53	a	14.9	a	483	a
NGB-FR-128	581	c	1.73	c	9.4	d	283	d
T <sub>0</sub>	19	f	1.10	e	5.8	f	151	f
T	59	f	1.51	d	8.3	e	233	e
TN	14	f	1.80	c	9.5	d	285	d

Data per plant are means of four replicates (three plants per replicate). Values followed by the same letter within each column are not significantly different at  $p < 0.05$ . T<sub>0</sub>: uninoculated seeds and non-chemical N-fertilizers. T: uninoculated seeds plus starter N-fertilizer (48 kg N·ha<sup>-1</sup>). TN: uninoculated seeds and full N-fertilizer (96 kg N·ha<sup>-1</sup>).

**Table 6.** Effect of different rhizobial strains on different growth parameters and yield of faba bean plants under field conditions (winter growing season 2013/2014).

Treatment	Plant Height (cm)		No of Branches/Plant		No of Pods/Plant		No of Seeds/Plant		Seed Index		Yield (ton/ha)			Seed N Yield kgN/ha		
											Straw	Seed				
NGB-FR-39	125	c	4.5	a	18	b	52	b	90.5	bcd	6.6	c	3.51	b	109	b
NGB-FR-62	123	cd	4.3	ab	17	bc	45	bc	92.3	abc	6.5	c	3.43	bc	107	b
NGB-FR-70	150	b	4.5	a	20	a	61	a	93.0	ab	8.4	b	4.35	a	148	a
NGB-FR-126	173	a	4.5	a	21	a	64	a	95.3	a	8.9	a	4.68	a	155	a
NGB-FR-128	116	d	4.0	ab	14	d	40	cd	89.0	cd	6.1	d	3.16	c	98	b
T <sub>0</sub>	80	f	3.0	c	8	e	25	e	81.3	f	3.4	f	1.58	e	47	d
T	102	e	3.5	bc	13	d	32	de	84.8	e	5.3	e	2.58	d	80	c
TN	117	cd	4.0	ab	15	cd	38	cd	88.5	d	6.0	d	3.17	c	104	b

Values followed by the same letter within each column are not significantly different at  $p < 0.05$ . T<sub>0</sub>: uninoculated seeds and non-chemical N-fertilizers. T: uninoculated seeds plus starter N-fertilizer (48 kg N·ha<sup>-1</sup>). TN: uninoculated seeds and full N-fertilizer (96 kg N·ha<sup>-1</sup>).

At harvest, in the first season (2012/2013), rhizobial inoculation induced significant increases in plant height, number of pods/plant, number of seeds/plant, and seed index of faba beans according to the type of inoculated strain (Table 4). Inoculated plants with strains NGB-FR 62, 126, and 128 enhanced plant height (127–134 cm), which was significantly higher than the uninoculated control plants (T). Likewise, inoculation with strain NGB-FR 126 produced the highest number of pods/plant (24 pods), which was significantly greater than the uninoculated control (T), that produced 19 pods/plant. Similarly, strains NGB-FR 62 and 126 gave the maximum number of seeds/plant and seed index, which were significantly higher than the uninoculated control (T). On the other hand, number of branches had no significant variations among tested strains and the uninoculated controls (T<sub>0</sub>, T, and TN). Faba bean inoculated with *Rlv* NGB-FR 126 and *A. tumefaciens* NGB-FR 62 produced the maximum seed yield (4.36 and 4.29 ton·ha<sup>-1</sup>, respectively) and the maximum seed N-yield (153 and 150 kg N·ha<sup>-1</sup>, respectively), which were significantly higher compared to the full-N fertilized uninoculated control (TN).

In the second season 2013/2014, at flowering stage, the significant effect of rhizobial inoculations on nodulation and plant growth parameters was obvious (Table 5). Strain *Rlv* NGB-FR 126 produced the highest dry weight of nodules (814 mg nodules/plant), while the uninoculated controls (T<sub>0</sub>, T, and TN) gave nodulations with a range of 14–59 mg nodules/plant. Faba bean plants inoculated with *Rlv* NGB-FR 126 recorded the maximum root dry weight (2.53 g/plant), shoot dry weight (14.9 g/plant), and shoot N-content (483 mg N/plant) with significant increases higher than the full N-fertilizer uninoculated control (TN), which resulted in 1.8 g/plant, 9.5 g/plant, and 285 mg N/plant, respectively (Table 5).

At harvest, plant height was significantly increased upon inoculation by all tested strains, which was greater than the uninoculated control (T). In the same trend, inoculation by all rhizobial strains, except in case of NGB-FR 128, produced significant increments in number of pods/plant (17–21 pods) and number of seeds/plant (45–64 seeds) relative to the uninoculated control (T) which gave 13 pods/plant and 32 seeds/plant, respectively. Crop yield and seed N-yield of the inoculated faba bean in the second season (2013/2014) surpassed those obtained by the un-inoculated controls (Table 6). All over again, the capacity of *Rlv* NGB-FR 126 to produce the uppermost seed yield (4.68 ton·ha<sup>-1</sup>) and seed N-yield (155 kg N·ha<sup>-1</sup>) was confirmed and was significantly greater than the full-N fertilizers treatment (TN), which recorded 3.17 ton·ha<sup>-1</sup> and 104 kg N·ha<sup>-1</sup>, respectively (Table 6).

Over the two experimental seasons, seed inoculation and nitrogen fertilization treatments (48 and 96 kg N·ha<sup>-1</sup>) produced significantly higher seed yield and seed N-yield compared to non-inoculated non-fertilized control (T<sub>0</sub>), indicating that N availability under such low fertility soil is a major constraint for crop productivity.

#### 4. Discussion

Low fertility of soil is one of the major constraints limiting crop productivity [8]. The success of legume grain crops is dependent on their capacity to form effective nitrogen-fixing symbioses with root-nodule bacteria. However, many soils may do not have adequate amounts of native rhizobia in terms of number, quality, or effectiveness to enhance biological nitrogen fixation [29]. *Rhizobium*-legume association can be manipulated, through inoculation under N-limiting field conditions, to improve crop production easily and inexpensively [35]. Where natural N<sub>2</sub> fixation is not optimal, inoculation is essential, ensuring that a high and effective rhizobial population is available in the rhizosphere of the plant [36]. The use of *Rhizobium* inoculants in legumes is the oldest agro-biotechnological application [37]. Several reports demonstrated significant improvement of yield and yield components in faba bean with *Rhizobium* inoculation [29,38–40].

Generally, the common practice of faba bean cultivation in Egypt is planting the seeds without inoculation. Therefore, most farmers depend on application of high levels of chemical fertilizers to supply N to plants, particularly under sandy soil conditions with low fertility nature. Since biological N<sub>2</sub> fixation is not active at early stages of plant growth, especially under low fertility soils, a starter N-dose (48 Kg N·ha<sup>-1</sup>) was applied in this study to enhance plant growth and eventually improve the grain yield production. The application of a starter N-dose with the rate of 48 Kg N·ha<sup>-1</sup> was previously reported to increase nodulation and nitrogen fixation of faba bean under Egyptian soil conditions [41]. In another study, an amount of 40 kg N·ha<sup>-1</sup> was used as a starter N-dose by [42], when they measured the field performance of rhizobial inoculants for some important legumes (lentils, soybeans, faba beans, and peanuts) in Egypt under both clay loam Nile Delta soils and virgin sandy soils. Our results are consistent with previous studies which have reported that the application of an amount of N fertilizer enhances nodulation of different legume crops [33,43,44].

In the present study, we reported the potential use of *Rhizobium*/*Agrobacterium* inoculants as a powerful alternate source of N in low nutrient ecosystems. Under greenhouse conditions (Table 2), all strains nodulated faba bean cultivar *Giza 843*. Out of the tested strains, eight strains (NGB-FR 39, 62, 65, 70, 107, 126, 128, and 140) could establish an effective nitrogen fixation association with this cultivar, producing a dry weight and shoot N content significantly higher than those obtained by the uninoculated control (T) with 48 Kg N·ha<sup>-1</sup>. Previous studies have identified that there are often strong relationships between shoot dry matter and the amount of N<sub>2</sub> fixed [45,46].

Under field conditions, growth and grain yield of faba bean increased significantly in response to inoculation with the most effective rhizobial strains (Tables 3–6). Increases in N<sub>2</sub> fixation translated to greater grain N concentration, and therefore resulted in increased N export from the field at harvest. In the first season (2012/2013), faba bean inoculated with strains *A. tumefaciens* NGB-FR 62 and *Rlv* NGB-FR 126 showed significant increases in seed yield (44%–47%) and seed N-yield (58%–61%), respectively, relative to the uninoculated control (T). While, in the second growing season (2013/2014), inoculation with strains *Rlv* NGB-FR 70 and *Rlv* NGB-FR 126 produced significant increases in seed yield (69%–81%) and seed N-yield (85%–94%), respectively, over the uninoculated control (T). These results are in line with previous report that was published by [29]. They found that in Australia, at sites without soil rhizobia, faba bean grain yield and total grain N increased by 59% and 132%, respectively, due to different inoculation rates.

Unexpectedly, in the first season (2012/2013), faba bean plants inoculated by *A. tumefaciens* strain NGB-FR 39 and *Rlv* strain NGB-FR 70 showed significantly less N uptake compared to the uninoculated control (T) with 48 kg N·ha<sup>-1</sup> (Table 3). This trend was also observed in regards to the final seed N-yield parameter (Table 4). This could be due to the presence of effective indigenous rhizobia or highly competitive but ineffective indigenous strains [47]. Our results are consistent with those published by [48], who reported that N uptake and N<sub>2</sub> fixation response to indigenous soil rhizobia in regards to uninoculated cowpea plants surpassed those of inoculated treatments.

*A. tumefaciens* were previously isolated from the root nodules of several tropical legumes [49]; *Phaseolus vulgaris* [50], *Sesbania* spp. [51], and *Vicia faba* [20,52]. The ability of *A. tumefaciens* to nodulate

legumes roots may be attributed to the possession of a transferred *Sym* plasmid which enabled them to form root nodules and fix nitrogen symbiotically [53]. However, many *Agrobacterium* strains isolated from root nodules failed to re-nodulate their original hosts [50,54], which makes *Agrobacterium* a poor choice for legume inoculation [55]. On the contrary, our results revealed the highly symbiotic stability of tested local *A. tumefaciens* strains to nodulate faba bean roots under both greenhouse and field experiments. The stability of nodulating machinery of *Agrobacterium* strains with soybean was recently reported [33].

Data presented in this study showed that the increase in seed yield in response to rhizobial inoculation was variable depending upon the strain type and climatic conditions of the cropping year. Similar findings were previously reported on soybean by [33,56]. The increments in seed yields in the full N-fertilized plots (TN) and/or inoculated plots, in relation to the uninoculated non-N fertilized plots ( $T_0$ ) controls indicate that, in these soils, nitrogen is a limiting factor, and that crop yields could be strongly improved by means of inoculation or fertilization. However, we found that response to inoculation with the best rhizobial strains was greater than the full N fertilization ( $96 \text{ Kg N}\cdot\text{ha}^{-1}$ ). This study demonstrated the highest potential of rhizobial inoculation as successful alternates of chemical N fertilizers, where effective inoculation with *Rlv* NGB-FR 126 showed significant increments in the final grain yield (35%–48%) and grain N-yield (34%–49%) compared to the inorganic N-fertilized treatments (TN) over the two cropping seasons, respectively. Our results showed that faba bean inoculation could effectively reduce the need of applied inorganic N-fertilizers while achieving higher grain yield. These findings are in line with those published by [36], who reported that, in a field experiment, inoculation of lentil by *Rhizobium* strains Lt29 increased seed yield by 59% while N fertilizer ( $50 \text{ kg urea ha}^{-1}$ ) enhanced yields by 40% over the uninoculated non-fertilized control. Our results are also in agreement with another study [56] which indicated that inoculated soybean under field conditions produced higher or not significantly different seed yields and seed N-yield than the fertilized uninoculated control with  $200 \text{ kg N}\cdot\text{ha}^{-1}$ .

## 5. Conclusions

Field experiments conducted through the two successive growing seasons have demonstrated that nodulation, total N uptake, and faba bean yield and yield components could be significantly improved through the combined use of *Rhizobium/Agrobacterium* inoculations and starter N application ( $48 \text{ kg N}\cdot\text{ha}^{-1}$ ) under low fertility sandy soil conditions. Effective inoculation with strain *Rlv* NGB-FR 126 reduced 50% of the applied chemical N-fertilizers, while maintaining faba bean productivity at levels significantly higher than those that resulted from having added inorganic N inputs ( $96 \text{ kg N}\cdot\text{ha}^{-1}$ ). The results of this study indicate the possibility of using this strain for the development of commercial faba bean inoculants and for achieving better crop yields with reduced usage of N fertilization.

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**Author Contributions:** S.H.Y. designed, conducted the field experiments, analyzed the data and wrote the manuscript. F.H.A. and S.H.Y. prepared the bacterial formulations. S.A.S. conceived the research and revised the manuscript.

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