EVALUATION OF SOME CHICKPEA GENOTYPES TO BACTERIAL INOCULATION

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ABSTRACT: Field experiment was carried out during the two successive seasons (2015-2016 and 2016-2017) at Sids Research Station, Agricultural Research Center (ARC), Bani Swif Governorate, Egypt, to evaluate the response of some new chickpea genotypes cultivated under Egyptian soil conditions for rhizobial inoculation alone or in combination with foliar inoculation of Pink Pigmented Facultatively Methylo trophic bacteria (PPFMs) as Plant Growth Promoting Rhizobacteria (PGPR). Nodulation status, some vegetative growth and yield parameters were determined. The obtained results cleared that all chickpea genotypes tested were positively responded to the native soil rhizobia and formed root nodules. Rhizobial inoculation alone or in combination with foliar application of PPFMs bacteria (5 L fed -1 ) scored significant increases in nodule numbers, plant dry weight, yield per plant as well as seed yield at the both seasons as compared to untreated treatments. Using rhizobial inoculation and PPFMs bacteria emphasized the superiority and gave of the highest values at all tested parameters. Generally, the second season gave the highest values at all plant tested parameters as compared to uninoculated ones. Chickpea genotypes GT3, GT4 and GT7 emphasized higher response to cultivated under Egyptian soil conditions and gave higher values for nodules number and dry weight, growth parameters i.e. plant dry weight and plant N-content and yield parameters i.e. yield per plant, seed index and seed yield ton. fed -1 as compared to chickpea variety G195.

Key words: Chickpea genotypes, Rhizobial inoculation, Pink- Pigmented Facultatively Methylo trophic bacteria (PPFMs), Foliar application.

INTRODUCTION

Legumes-Rhizobium symbiosis is undoubtedly the most important N2-fixing process and play a subtle role in providing nitrogen and maintaining/improving soil fertility. Symbiosis between legumes and rhizobia are of a considerable environmental and agricultural importance, since they are responsible for most of the atmospheric nitrogen fixed on land (Graham and Vance, 2003).

Chickpea (Cicer arietinum L.) is one of the earliest grain crops cultivated by man and has been found in Middle Eastern archeological sites dated at 7500–6800 B.C., (Williams and Singh, 1987). Chickpea is highly self-pollinating with an out crossing rate of less than 1%. Two main types of chickpea cultivars are grown globally, representing two diverse sub gene pools: Kabuli and Desi. The Kabuli types are generally grown in the Mediterranean region, southern Europe, western Asia, and northern Africa and the Desi types are grown mainly in Ethiopia and the Indian subcontinent. In spite of the above-mentioned constraints, extensive international breeding efforts have led to the development of over 300 improved varieties (Gowda and Gaur, 2004).

Chickpea is grown in about 50 countries, it can fix up to 140 kg nitrogen ha -1 and meet up to 80% of its nitrogen requirement from symbiotic nitrogen fixation (Abo Taleb (1998) and Al-hudaiji (2015)). Chickpea has the highest nutritional compositions and rich in fiber and minerals (phosphorous, calcium, magnesium, iron, and zinc). Its lipid fraction is high in unsaturated fatty acids in addition to having high protein content (20–22%). (Zohary and Hopf, 2000). Singh et al., (2008) illustrated the genetic relationships between the cultivated chickpea and its wild relatives is a prerequisite to track the evolution of cultivated species and also to determine the close relatives which can be exploited for introgression of
useful traits into the culigen in plant breeding programs and many developing countries are substantial research programs to improve its yield, disease resistance and nutritional quality.

*Vessey (2003)* reported that numerous species of soil bacteria, which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by various mechanisms. These bacteria are correctly known as Plant Growth Promoting Rhizobacteria (PGPR). The search for PGPR and investigation of their modes of action are increasing to exploit them commercially as biofertilizer. The mode of action of the biofertilizers includes fixing nitrogen, increasing the availability of nutrients in the rhizosphere, positively influencing both morphology and growth of roots, and promoting other beneficial plant-microbe symbiosis. The combination of these modes of actions in PGPR is also addressed.

*Madhiayan et al. (2005)* reported that The genus *Methylobacterium*- as PGPR - includes a variety of pink pigmented facultative methylotrophic bacteria (PPFMs) that promote plant growth by generating vitamins, phytohormones (IAA, gibberellins and cytokinins) as well as supply nitrogen to plant through diazotrophy and indirectly reduce or prevent the deleterious effects of pathogenic microorganisms, through induced systemic resistance.

*Etesami and Maheshwari (2018)* stated that, combined use of PGPRs in agricultural environments may be a suitable approach to sustainably integrate with chemical fertilizers and lead to plant health improvements that play an important role in reducing the amount of chemicals to achieve sustainable agricultural productivity.

The present work aims to evaluate new chickpea genotypes response to rhizobial inoculation alone or in combination with application of PPFMs bacteria as PGPR bacterial inoculation and its role in enhancing the vegetative growth, seed yield and yield quality of chickpea plants under Egyptian soil conditions.

**MATERIAL AND METHODS**

1. **Soil used**

A field experiment was layout during the two successive seasons (2015-2016 and 2016-2017) at Sids Research Station, Bani Swif Governorate, Agricultural Research Center (ARC). Physicochemical properties of the used soil was carried out according to *Jackson (1973)* at soil analysis Lab., Soils, Water and Environment Research Institute (SWERI), ARC, Giza, and is shown in Table (1).

| Table 1. Some physico-chemical properties of used soil |
|-------------------------------------------------------|
| Property                               | Values     |
|----------------------------------------|------------|
| **Mechanical analysis**                |            |
| Sand                                   | 19.5       |
| Silt                                   | 34.0       |
| Clay                                   | 64.5       |
| Texture grand                          | Clay loam  |
| **Physical analysis**                  |            |
| S. P. %                                | 48.77      |
| PH                                     | 7.72       |
| E.C. dSm                               | 1.04       |
| Organic Carbon %                       | 0.53       |
| Organic Matter %                       | 0.91       |
| Soluble Nitrogen %                     | 62.46      |
| Total Nitrogen %                       | 0.028      |
| **Chemical analysis**                  |            |
| Available _P %                         | 7.62       |
| Available _K %                         | 311.60     |
| **EDTA_extractable**                  |            |
| Fe                                     | 8.60       |
| Mn                                     | 4.31       |
| Zn                                     | 4.10       |
| Cu                                     | 1.81       |
| **Soluble Cations (meq/l)**            |            |
| Ca$$^{++}$$                             | 3.00       |
| Mg$$^{++}$$                            | 1.36       |
| Na$$^{+}$$                             | 5.12       |
| K$$^{+}$$                              | 0.98       |
| **Soluble Anions (meq/l)**             |            |
| CO$$^{-}_3$$                           | 0.00       |
| HCO$$^{-}_3$$                          | 1.51       |
| Cl$$^{-}$$                             | 1.72       |
| SO$$^{-}_4$$                           | 7.23       |

2. **Seeds used**

Seeds of Chickpea (*Cicer arietinum L.*) variety Giza 195 (G195), and seven genotypes namely: GT1 (FLP0893C), GT2 (S091013), GT3 (S090642), GT4 (FLP0846C), GT5 (FLP0872), GT6 (FLP0847C) and GT7 (FLIP08-141C) were used in field experiment at rate 35 kg.fed$^1$ and kindly supplied by Legume Crop Research Dept. Field Crop Research Institute, (ARC), Giza, Egypt.

3. **Bacterial strains used**

3.1. Two strains of *Mesorhizobium ciceri* namely ICARDA 36 and NIFTAL 1148 specific to Chickpea grown on Yeast extract Mannitol agar (YEM) medium (*Vincent, 1970*) were used as mixture basal peat inoculant at rate 4g inoculant to 100 g seeds at the time of planting as seed coating method according to *Abo Taleb (1998).*

3.2. Two strains of PPFMs bacteria namely *Methylobacterium mesophilicum* and
Methylobacterium radiotolerans grown on Methanol Mineral Salts (MMS) agar medium (Holland and Polacco, 1992) were used as foliar inoculation at rate of 5 L. Fed \(^{-1}\) (Shehata, Sawsan et al., 2006) after 30 days of planting. These strains were kindly obtained from Biofertilizers Production Unit, Agricultural Microbiology Dept., Soils, Water and Environment Research Institute, ARC, Giza, Egypt.

4. Fertilizers used

The recommended doses of P and K fertilizers: 100 Kg superphosphate (15.5 \% P\(_2\)O\(_5\) fed\(^{-1}\)) and 50 Kg potassium sulphate (24 K\(_2\)O fed\(^{-1}\)) were added during field experiment preparation. N-fertilization as ammonium sulphate (20.5 \% N) was applied at 15 and 50 Kg N fed\(^{-1}\) and were added at 15, 21 and 35 days after planting.

5. Treatments

Three treatments with 3 replications were allocated in a completely randomized block design as follows:

1. Un-inoculated plants + 50 Kg N fed\(^{-1}\).
2. Rhizobial inoculation +15 Kg N fed\(^{-1}\).
3. Rhizobial inoculation+ foliar application with PPFMs (5 L. fed\(^{-1}\), at 30 days after planting) + 15 Kg N fed\(^{-1}\).

The plot area was 3x 3.5 m\(^2\).

6. Determinations

6.1. Growth stage: Samples were taken after 75 days of planting to determine: nodulation status (number and dry weight of nodules) according to Vencent (1970) and some vegetative growth parameters (plant dry weight and plant nitrogen content).

6.2. Harvest stage: number of branches, number of pods, seed yield (kg. plot\(^{-1}\) and ton. fed\(^{-1}\)), yield of plant and seed index were determined in samples after 140 days of planting, according to A.O.A.C. (1990).

7. Statistical analysis

Data were subjected to an analysis of variance (ANOVA) and the least significant difference test (LSD) at P <0.05, by using (MSTAT) Program according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

1. Growth stage

1.1. Nodulation status

Data in table (2) show that, the all chickpea genotypes responded to the native rhizobia and formed root nodules bacteria but scored the lowest values of nodules formation in both seasons which ranged from 11 to 22 (nod. no. plant\(^{-1}\)) as compared to other tested treatments. Inoculation with specific rhizobia scored significant increases in both seasons and such increases ranged between 22.7 -254.5% as compared to un- inoculated ones. Inoculation with specific rhizobia in combination with PPFMs as foliar application scored highest value (53 nod. no. plant\(^{-1}\)) and led to gave significant increases in number of nodules ranged from 8.6-34.4% in both seasons as compared to the treatments which received rhizobial inoculation.

1.2. Nodule dry weight (mg plant\(^{-1}\))

Data in Table (3) reveal that, the un-inoculated treatment recorded the lowest value of nodules dry weight (30 mg plant\(^{-1}\)) in both seasons as compared to other treatments. Inoculation with specific Mesorhizobium scored higher values (68-104 mg plant\(^{-1}\)) as compared to un-inoculated treatment in both seasons. Application PPFMs as foliar spraying in the presence of rhizobial inoculation scored highest significant increases and recorded nodules dry weight up to (163 mg plant\(^{-1}\)) as compared to inoculation with specific Mesorhizobium alone. Inoculated chickpea genotypes GT4 having the highest value in nodule dry weight (up to 104 mg plant\(^{-1}\)) among all inoculated chickpea genotypes as compared to uninoculated treatments.

The above mentioned data are in agreement with the observations made by Joshi et al. (2000), Stougaard (2000), Begum et al. (2001) and Ogutcu et al. (2008) who reported that PPFMs plays very important role in root nodule initiation, development and function of many legume plants, i.e. alfalfa, soybean and the number and weight of nodules per plant showed significant response to nitrogen fertilization rates, inoculation with specific rhizobial strains and chickpea varieties. Also, Gopalkrishnan et al. (2018) reported that, rhizobial inoculation as such or in-combination with PGPR enhanced the nodule number, nodule weight and shoot weight over the un-inoculated control of chickpea cultivars.
Table 2. Number of nodules and percentage of increases for various chickpea genotypes as affected by application of different bacterial inoculation at 75 days after planting

| Parameters | Number of nodules (no. nod. plant⁻¹) |
|------------|-------------------------------------|
| Genotypes | Un inoculated | R. inoculation | % increases | R. + ppfm inoculation | % increase* |
|           | S1 | S2 | x̄ | S1 | S2 | x̄ | S1 | S2 | x̄ | S1 | S2 | x̄ |
| GT1       | 12 | 16 | 14 | 25 | 36 | 31 | 121.4 | 26 | 49 | 38 | 22.6 |
| GT2       | 25 | 19 | 22 | 16 | 38 | 27 | 22.7 | 38 | 53 | 46 | 70.3 |
| GT3       | 14 | 22 | 18 | 19 | 42 | 31 | 72.2 | 22 | 45 | 34 | 9.7  |
| GT4       | 10 | 12 | 11 | 40 | 38 | 39 | 254.5 | 38 | 53 | 46 | 17.9 |
| GT5       | 28 | 16 | 21 | 36 | 44 | 40 | 90.4 | 47 | 51 | 49 | 18.4 |
| GT6       | 18 | 13 | 16 | 26 | 43 | 35 | 118.8 | 27 | 49 | 38 | 8.6  |
| GT7       | 15 | 13 | 14 | 25 | 39 | 32 | 128.6 | 31 | 55 | 43 | 34.4 |
| G195      | 23 | 21 | 22 | 41 | 47 | 44 | 100  | 48 | 57 | 53 | 20.5 |
| L.S.D     | 6.55 | 7.31 | 8.62 | 5.72 | 5.53 | 3.78 |

S: season  
GT: Genotype  
* % of increases as compared to un-inoculated treatment.  
** % of increases as compared to rhizobial inoculation treatment.

Table 3. Dry weight of nodules for various chickpea genotypes as affected by application of different bacterial inoculation at 75 days after planting

| Parameters | Dry weight of nodules (mg plant⁻¹) |
|------------|-------------------------------------|
| Genotypes | Un inoculated | R. inoculation | R. + ppfm inoculation |
|           | S1 | S2 | x̄ | S1 | S2 | x̄ | S1 | S2 | x̄ | S1 | S2 | x̄ |
| GT1       | 31 | 43 | 36 | 65 | 98 | 82 | 70 | 139 | 105 | 139 | 105 |
| GT2       | 86 | 67 | 77 | 40 | 95 | 68 | 106 | 219 | 163 | 219 | 163 |
| GT3       | 32 | 55 | 44 | 50 | 103 | 77 | 52 | 116 | 84 | 116 | 84 |
| GT4       | 24 | 35 | 30 | 110 | 97 | 104 | 110 | 128 | 119 | 128 | 119 |
| GT5       | 77 | 42 | 60 | 91 | 115 | 103 | 142 | 110 | 126 | 110 | 126 |
| GT6       | 61 | 45 | 53 | 71 | 107 | 89 | 72 | 98 | 85 | 98 | 85 |
| GT7       | 35 | 28 | 32 | 59 | 82 | 71 | 88 | 113 | 101 | 88 | 101 |
| G195      | 41 | 39 | 40 | 88 | 103 | 96 | 153 | 167 | 160 | 167 | 160 |
| L.S.D     | 15.70 | 11.40 | 17.8 | 9.31 | 27.11 | 30.75 |

S: season  
GT: Genotype

2. Vegetative growth

2.1. Plant dry weight (g plant⁻¹)

Data in table (4) show that, the high significant differences in plant dry weight were evident among the all tested treatments. Un-inoculated chickpea genotypes recorded the lowest plant dry weight ranged 1.5 to 2.9 g plant⁻¹. Inoculated plants scored higher values ranged from 2.5 to 3.1 g plant⁻¹. Significant increases ranged from 11.5 to 80 % of plant dry weight as compared to uninoculated treatments. Rhizobial inoculation in-combination with foliar PPFMs bacteria gave the highest plant dry weight (2.9 - 3.3 g plant⁻¹) and recorded significant increases up to 11.1% were observed as compared to inoculated treatments as such. Chickpea genotypes GT3, GT4 and GT7 having the highest plant dry weight as compared to other chickpea genotypes tested among the two tested seasons.

These results are in harmony with Peix et al. (2001) and Shukla et al. (2012) who found a positive response of chickpea genotypes to inoculation with rhizobia and /or PPFM bacteria and recorded significant increases in plant dry biomass accumulation compared to uninoculated ones. Generally, the increase in dry matter accumulation due to seed inoculation with rhizobia and PPFM s indicates the favorable response of chickpea genotypes to inoculation (Orf, Heba et al. 2014).
2.2. Plant N-content (mg plant$^{-1}$)

Data in Table (5) reveal that, un-inoculated treatment recorded the lowest values of plant N-content, such values ranged from 41.7 to 74.2 mg plant$^{-1}$ as compared to other treatments among the two tested seasons. Inoculation with specific

| Parameters | Plant dry weight (g plant$^{-1}$) | Genotypes | S1 | S2 | $\bar{x}$ | S1 | S2 | $\bar{x}$ | % increase$^*$ | S1 | S2 | $\bar{x}$ | % increase$^{**}$ |
|------------|----------------------------------|----------|----|----|-----|----|----|-----|----------------|----|----|-----|----------------|
| Un inoculated | GT1 | 1.4 | 1.6 | 1.5 | 2.6 | 2.7 | 2.7 | 80.00 | 2.7 | 2.9 | 3.6 | 3.6 |
| | GT2 | 1.8 | 1.9 | 1.9 | 2.4 | 2.6 | 2.5 | 31.60 | 2.6 | 2.7 | 2.7 | 2.7 |
| | GT3 | 2.6 | 2.8 | 2.7 | 2.5 | 2.9 | 2.7 | 0.00 | 2.8 | 3.1 | 3.0 | 11.1 |
| | GT4 | 2.6 | 3.1 | 2.9 | 2.4 | 3.1 | 2.8 | 0.00 | 3.0 | 3.2 | 3.1 | 10.7 |
| | GT5 | 1.5 | 2.1 | 1.8 | 2.4 | 2.9 | 2.7 | 50.00 | 2.8 | 3.0 | 2.9 | 7.4 |
| | GT6 | 1.9 | 2.7 | 2.3 | 2.7 | 3.2 | 3.0 | 30.43 | 2.8 | 2.9 | 2.9 | 0.0 |
| | GT7 | 2.3 | 2.8 | 2.6 | 2.6 | 3.1 | 2.9 | 11.50 | 3.1 | 3.3 | 3.2 | 10.3 |
| | G195 | 2.4 | 2.6 | 2.5 | 2.9 | 3.2 | 3.1 | 24.00 | 3.2 | 3.4 | 3.3 | 6.5 |
| L.S.D | 0.57 | 0.63 | ---- | 0.11 | ---- | 0.32 | 0.28 | ---- |

$S$: season  
$GT$: Genotype

$^*$% of increase as compared to un-inoculated treatment.
$^{**}$% of increases compared to rhizobial inoculation treatment.

The obtained data are in agreement to Polacco and Holland (1993) who reported that, application of inoculation with PPFMs resulted increasing of plant dry weight of soybean plants as compared to untreated ones. Holland (1997) reported that the activities of PPFMs could make a biochemically measurable and physiologically meaning full contribution to plant nitrogen accumulation and metabolism. The mentioned data are in harmony with Yates et al. (2007) who reported that PPFM bacteria play very important role in plant nitrogen content, and symbiotically benefit the plant species. Also, Sharma et al. (2016) stated that inoculated peanut seedlings with rhizobia as such or in-combination with PGPR scored significant increase in total nitrogen (N) content (up to 76%) over the non-inoculated control.
3. Harvest stage

3.1. Number of branches (No. Plant⁻¹)

Application of rhizobial inoculation alone or in-combination with PPFMs bacteria led to gave higher values and scored significant increases at number of branches at various tested chickpea genotypes as shown in Table (6). Values of number of branches ranged from 6.0 to 12.6, 8.3 to 13.3 and 10.2 to 13.7 for untreated chickpea genotypes, inoculated plants and rhizobial inoculation in-combination with PPFMs bacteria respectively through the tested seasons. Genotype GT4 recorded the highest value of number of branches per plant and value was up to 13.2 among the two tested seasons at various treatments and followed by GT7 chickpea genotypes (13.1). In this respect, Rudresh et al. (2005) studied the effect of inoculation with Rhizobium on growth attributes and observed that chickpea gave higher plant height (3.3%), number of branches per plant (23.3%) and biomass per plant (144%) as compared to uninoculated control. In similar findings, Elkoca et al. (2008) revealed that rhizobial inoculation increased plant height, shoot dry weight and chlorophyll content in chickpea. These findings are in agreement with that of Giri and Joshi (2010).

Table 6. Number of branches for various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

| Parameters | Genotypes | Un inoculated | R. inoculation | R. + ppfm inoculation |
|------------|-----------|---------------|----------------|----------------------|
|             |           | S1  | S2  | S  | S1  | S2  | S  | S1  | S2  | S  |
| GT1        | 6.0       | 8.3 | 7.2 | 11.6 | 13.3 | 12.5 | 12.7 | 13.3 | 13.0 |
| GT2        | 7.6       | 8.5 | 8.6 | 10.0 | 13.3 | 11.7 | 11.6 | 13.3 | 12.5 |
| GT3        | 8.0       | 11.0 | 9.5 | 10.0 | 12.6 | 11.3 | 10.6 | 12.6 | 11.6 |
| GT4        | 9.6       | 11.6 | 10.6 | 11.6 | 12.6 | 12.1 | 12.8 | 13.5 | 13.2 |
| GT5        | 9.5       | 12.6 | 11.1 | 8.3 | 12.6 | 10.5 | 10.2 | 13.5 | 11.9 |
| GT6        | 11.0      | 11.4 | 11.2 | 11.6 | 11.5 | 11.6 | 11.8 | 12.5 | 12.2 |
| GT7        | 8.3       | 9.6 | 8.9 | 11.6 | 11.3 | 11.5 | 12.5 | 13.7 | 13.1 |
| G195       | 8.3       | 11.0 | 9.7 | 10.3 | 11.6 | 11.0 | 10.8 | 12.5 | 11.7 |
| L.S.D      | 2.55      | 2.11 | --- | 1.63 | 0.81 | --- | 1.17 | 1.08 | --- |

S: season  GT: Genotype

3.2. Number of pods (No. Plant⁻¹)

Data in Table (7) show that, un-inoculated treatment gave the lowest number of pods at all tested chickpea genotypes as compared to other treated treatments in the both two seasons, these values ranged from 21.2 to 39.9 No. plant⁻¹. Inoculation with specific Rhizobium as such or in combination with PPFM bacteria emphasized the superiority in number of pods and recorded values ranged from 33.3 to 69.3 and 35.4 to 72.4 for inoculated plants as such and rhizobial inoculation in-combination with PPFMs bacteria respectively as an average of the two tested seasons.

Table 7. Number of pods of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

| Parameters | Genotypes | Un inoculated | R. inoculation | R. + ppfm inoculation |
|------------|-----------|---------------|----------------|----------------------|
|             |           | S1  | S2  | S  | S1  | S2  | S  | S1  | S2  | S  |
| GT1        | 21.6      | 30.0 | 25.8 | 20.6 | 46.0 | 33.3 | 22.5 | 48.2 | 35.4 |
| GT2        | 22.6      | 28.3 | 25.5 | 59.6 | 69.0 | 64.3 | 58.9 | 72.1 | 65.5 |
| GT3        | 24.6      | 33.3 | 29.0 | 41.6 | 35.6 | 38.3 | 45.3 | 51.5 | 48.4 |
| GT4        | 21.6      | 24.6 | 23.1 | 44.3 | 35.6 | 40.0 | 44.5 | 55.3 | 49.9 |
| GT5        | 20.0      | 22.3 | 21.2 | 33.3 | 35.6 | 34.5 | 47.2 | 57.3 | 52.3 |
| GT6        | 36.6      | 43.3 | 39.9 | 42.3 | 59.0 | 50.7 | 46.7 | 59.2 | 53.0 |
| GT7        | 26.6      | 27.0 | 26.8 | 63.0 | 75.6 | 69.3 | 67.5 | 77.2 | 72.4 |
| G195       | 26.3      | 26.6 | 26.5 | 40.3 | 44.3 | 42.3 | 50.3 | 54.3 | 52.3 |
| L.S.D      | 5.43      | 6.17 | --- | 21.17 | 15.81 | 7.38 | 11.7 | 8.92 | --- |

S: season  GT: Genotype
These data were in harmony with those obtained from Sharar et al. (2000) and Khan et al. (2003) who reported that, Number of pods per plant and number of seeds per plant were reported to be 21.8% and 10.5% higher, respectively in chickpea inoculated with *Rhizobium* over uninoculated control. Similar observations reported in other studies where inoculation of chickpea with rhizobia increased plant growth, ground dry matter, number of pods, seed yield, and nitrogen fixation under various climatic conditions (Fatima et al., 2008).

3. Yield parameters

3.1. Yield per plant (g plant⁻¹)

| Parameters | Yield per plant (g plant⁻¹) | Un inoculated | R. inoculation | R. + ppfm inoculation |
|------------|----------------------------|--------------|----------------|----------------------|
| Genotypes | Genotypes | S1 | S2 | x̄ | S1 | S2 | x̄ | % increase* | S1 | S2 | x̄ | % increase** |
| GT1 | 8.3 | 11.6 | 9.9 | 24.4 | 28.3 | 26.4 | 166.7 | 27.5 | 28.6 | 28.1 | 6.4 |
| GT2 | 10.0 | 16.6 | 13.3 | 27.1 | 42.6 | 34.9 | 162.4 | 29.7 | 52.3 | 41.0 | 17.5 |
| GT3 | 13.3 | 15.0 | 14.2 | 12.9 | 23.3 | 18.1 | 27.5 | 31.0 | 29.1 | 27.4 | 51.4 |
| GT4 | 11.6 | 13.3 | 12.5 | 15.3 | 23.3 | 19.3 | 54.4 | 28.2 | 30.2 | 29.2 | 51.3 |
| GT5 | 10.0 | 11.6 | 10.8 | 16.9 | 18.1 | 17.5 | 62.0 | 26.9 | 28.9 | 27.9 | 59.4 |
| GT6 | 8.3 | 15.0 | 11.7 | 18.3 | 25.4 | 21.9 | 87.2 | 30.5 | 33.6 | 32.1 | 46.6 |
| GT7 | 8.3 | 14.0 | 11.2 | 25.6 | 36.0 | 30.8 | 175.0 | 32.7 | 39.2 | 36.0 | 16.9 |
| G195 | 9.6 | 16.6 | 13.1 | 23.3 | 27.8 | 25.6 | 95.4 | 29.3 | 32.9 | 31.3 | 22.3 |
| L.S.D | 2.17 | 1.21 | ----- | 6.33 | 9.81 | ----- | 2.75 | 39.7 | ----- |

S: season  
GT: Genotype
% of increases *as compared to un-inoculated treatment.
% of increase **as compared to rhizobial inoculation treatment.

These data are in agreement with those of Suresh Reddy et al. (2002) who worked on the effect of combined inoculation of PPFMs and *Rhizobium* on groundnut cultivar Co (Gn) 4 and observed that, there was significant increase in plant growth, biomass production and yield parameters of groundnut. Radha et al. (2009) also reported that, inoculation of *Methyllobacterium* isolates in combination with *Bradyrhizobium japonicum* strain SB120 had significant influence on different plant growth parameters, nutrient uptake and yield of soybean plants.

3.2. Seed index (g 100 seed⁻¹)

Data in Table (9) cleared that, both rhizobial inoculations as such or in-combination with PPFMs bacteria gave higher values for seed index at all tested chickpea genotypes as compared to un treated treatments. GT4 and GT7 chickpea genotypes as well as chickpea variety G195 recorded the highest values of seed index and these values were 36.9, 33.6 and 34.6 (g 100seed⁻¹) for GT4, GT7 and G195 respectively among the two tested seasons. The above mentioned data are in harmony with those obtained by Sharar et al. (2000) who reported that number of seeds per plant scored 10.5% higher in chickpea inoculated with *Rhizobium* over uninoculated control. Further, Khan et al. (2003) and Ali et al. (2014) revealed that 1000-seed weight was significantly better with inoculation in chickpea. These findings are in agreement with that of Elkoca et al. (2008), Akhtar and Siddiqui (2009) and Meena et al. (2013) who reported that the performance of the chickpea plants was better in inoculation treatments in comparison to control.

Untreated chickpea genotypes recorded lower values for yield per plants and these values ranged from 9.9 to 14.2 g plant⁻¹ as shown in Table (8). Application of rhizobial inoculation as such did support plant yield and led to gave higher values and scored significant increases ranged from 27.5 to 175 %, as compared to un-inoculated treatments. On the other hand, rhizobial inoculation in-combination with PPFMs bacteria having the highest plant yield values among the all tested treatments in the both seasons and gave percentage increases ranged from 6.4 to 59.4 % as compared to inoculated chickpea genotypes as such.

Table 8. Yield of plant of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

| Parameters | Yield per plant (g plant⁻¹) | Un inoculated | R. inoculation | R. + ppfm inoculation |
|------------|----------------------------|--------------|----------------|----------------------|
| Genotypes | Genotypes | S1 | S2 | x̄ | S1 | S2 | x̄ | % increase* | S1 | S2 | x̄ | % increase** |
| GT1 | 8.3 | 11.6 | 9.9 | 24.4 | 28.3 | 26.4 | 166.7 | 27.5 | 28.6 | 28.1 | 6.4 |
| GT2 | 10.0 | 16.6 | 13.3 | 27.1 | 42.6 | 34.9 | 162.4 | 29.7 | 52.3 | 41.0 | 17.5 |
| GT3 | 13.3 | 15.0 | 14.2 | 12.9 | 23.3 | 18.1 | 27.5 | 31.0 | 29.1 | 27.4 | 51.4 |
| GT4 | 11.6 | 13.3 | 12.5 | 15.3 | 23.3 | 19.3 | 54.4 | 28.2 | 30.2 | 29.2 | 51.3 |
| GT5 | 10.0 | 11.6 | 10.8 | 16.9 | 18.1 | 17.5 | 62.0 | 26.9 | 28.9 | 27.9 | 59.4 |
| GT6 | 8.3 | 15.0 | 11.7 | 18.3 | 25.4 | 21.9 | 87.2 | 30.5 | 33.6 | 32.1 | 46.6 |
| GT7 | 8.3 | 14.0 | 11.2 | 25.6 | 36.0 | 30.8 | 175.0 | 32.7 | 39.2 | 36.0 | 16.9 |
| G195 | 9.6 | 16.6 | 13.1 | 23.3 | 27.8 | 25.6 | 95.4 | 29.3 | 32.9 | 31.3 | 22.3 |
| L.S.D | 2.17 | 1.21 | ----- | 6.33 | 9.81 | ----- | 2.75 | 39.7 | ----- |
Ivanova et al. (1995) and Orf, Heba et al. (2006) reported that, the production of the plant growth regulators like auxins, particularly indole-3-acetic acid (IAA) and indole-3-pyruvic acid, zeatin, zeatin riboside and reacted cytokinins by Methylotrophs and IAA production and nitrogen fixation by Rhizobium has been reported as the factors that enhances plant growth of legumes. The increase in the vegetative growth of the plant is attributed to the increase in the yield of a crop.

Table 9. Seed index of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage.

| Parameters | Seed index (g 100 seed⁻¹) |
|------------|---------------------------|
| Genotypes  |  Un inoculated  |  R. inoculation  |  R. ppfm inoculation |
|            | S1  |  S2  |  S  |  S1  |  S2  |  S  |  S1  |  S2  |  S  |
| GT1        | 26.4 | 28.4 | 27.4 | 27.0 | 27.2 | 27.1 | 28.2 | 31.5 | 29.9 |
| GT2        | 28.5 | 30.5 | 29.5 | 29.5 | 31.8 | 30.7 | 32.5 | 32.6 | 32.6 |
| GT3        | 26.5 | 26.8 | 26.7 | 30.2 | 30.9 | 30.6 | 31.7 | 34.8 | 33.3 |
| GT4        | 26.9 | 26.5 | 26.7 | 29.9 | 30.1 | 30.0 | 36.3 | 37.5 | 36.9 |
| GT5        | 26.3 | 25.7 | 26.0 | 30.0 | 32.8 | 31.4 | 33.6 | 37.5 | 36.9 |
| GT6        | 26.6 | 27.4 | 27.0 | 29.6 | 32.7 | 31.2 | 32.6 | 35.1 | 33.9 |
| GT7        | 29.0 | 27.5 | 28.3 | 29.2 | 31.2 | 30.2 | 33.2 | 33.7 | 33.6 |
| G195       | 28.9 | 29.5 | 29.2 | 29.5 | 32.6 | 31.1 | 33.5 | 35.7 | 34.6 |
| L.S.D      | 1.77 | 2.34 | ---- | 1.93 | 2.11 | ---- | 1.82 | 1.73 | ---- |

S: season  GT: Genotype

3.3. Seed yield (kg plot⁻¹)

Application of various bacterial treatments led to enhance seed yield (kg plot⁻¹) and recorded higher values as compared to untreated treatments which recorded the lowest values for seed yield (kg plot⁻¹) as shown in Table (10). GT7, GT3, GT4 chickpea genotypes and variety G195 were responded to rhizobial inoculation in-combination with PPFMs bacteria and scored the highest seed yield (kg plot⁻¹) and these values were 2.13, 1.99, 1.87 and 2.40 for GT7, GT3, GT4 and chickpea genotypes and G195 respectively. In this respect, Rice et al. (1995) and Ivanova et al. (2001) and Orf, Heba et al. (2006) reported that, the production of the plant growth regulators like auxins, particularly indole-3-acetic acid (IAA) and indole-3-pyruvic acid, zeatin, zeatin riboside and reacted cytokinins by Methylotrophs and IAA production and nitrogen fixation by Rhizobium has been reported as the factors that enhances plant growth of legumes. The increase in the vegetative growth of the plant is attributed to the increase in the yield of a crop.

Table 10. Seed yield (kg plot⁻¹) of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage.

| Parameters | Seed yield (kg plot⁻¹) |
|------------|-----------------------|
| Genotypes  |  Un inoculated  |  R. inoculation  |  R. ppfm inoculation |
|            |  S1  |  S2  |  S  |  S1  |  S2  |  S  |  S1  |  S2  |  S  |
| GT1        | 0.63 | 0.73 | 0.68 | 1.16 | 1.20 | 1.18 | 1.25 | 1.37 | 1.31 |
| GT2        | 1.40 | 1.61 | 1.51 | 1.66 | 1.85 | 1.76 | 1.72 | 1.90 | 1.81 |
| GT3        | 1.70 | 1.85 | 1.78 | 1.95 | 2.10 | 2.03 | 1.97 | 2.01 | 1.99 |
| GT4        | 1.72 | 1.20 | 1.46 | 1.75 | 1.84 | 1.89 | 1.85 | 1.90 | 1.87 |
| GT5        | 1.26 | 1.30 | 1.28 | 1.64 | 1.96 | 1.80 | 1.75 | 1.97 | 1.86 |
| GT6        | 1.22 | 1.19 | 1.21 | 1.56 | 1.65 | 1.61 | 1.65 | 1.72 | 1.69 |
| GT7        | 1.51 | 1.35 | 2.43 | 1.98 | 1.83 | 1.89 | 2.10 | 2.15 | 2.13 |
| G195       | 1.76 | 1.65 | 1.71 | 2.25 | 2.50 | 2.38 | 2.30 | 2.50 | 2.40 |
| L.S.D      | 0.33 | 0.19 | ---- | 0.17 | 0.65 | ---- | 0.27 | 0.61 | ---- |

S: season  GT: Genotype

3.4. Seed yield (ton fed.⁻¹)

Data in Table (11) show that un-inoculated treatment recorded the lowest value of seed yield (0.252 ton fed.⁻¹) in both seasons as compared to other treatments. Inoculation with specific Mesorhizobium scored higher value (0.950 ton. fed⁻¹) as compared to un-inoculated treatment in both seasons. Application with PPFMs as foliar spraying in the presence of rhizobial inoculation scored highest value (0.960 ton fed.⁻¹) as compared to rhizobial inoculation ones. Application of both rhizobial inoculations had a positive effect on seed yield (ton fed.⁻¹) for all tested chickpea genotypes as shown in table (11). Untreated treatments recorded the lowest seed yield values as compared to inoculated treatments as such or in-combination with PPFMs bacteria. The values were 0.810 ,0.718,
0.765 and 0.950 seed yield (ton fed⁻¹) for inoculated chickpea genotypes GT3, GT4, GT7 and G195 respectively and corresponding values at rhizobial inoculation in combination with PPFMs bacteria were 0.796, 0.753, 0.850 and 0.960 (ton fed⁻¹) in the same order. These data were in agreement with those obtained by (Kantar et al., 2003; Ozturk et al., 2003) and Orf, Heba et al. (2006) who reported that significant increases in seed protein content due to bacterial inoculation supported the hypothesis that biological nitrogen fixation by the Rhizobium and PGPR-root associations could be responsible for the observed higher N uptake of inoculated plants.

Senthikumar et al. (2002) and Shehata, Sawsan (2006) established The increase in the yield due to compatible nature of Methylobacterium & Rhizobium and they found that, combined influence on phyllosphere by methylotrophs, which are plant growth promoting phyllosphere (PGPP) bacteria, and on rhizosphere by Rhizobium, which is nitrogen fixing bacterium, might have resulted in increased plant growth and yield parameters.

Table 11. Seed yield (ton fed⁻¹) of various chickpea genotypes as affected by application of different bacterial inoculation at harvest stage

| Parameters         | Seed yield (ton fed⁻¹) | R. inoculation | R. + ppfm inoculation |
|--------------------|------------------------|----------------|-----------------------|
|                    | Un inoculated          | S1  | S2  | S  | S1  | S2  | S  | S1  | S2  | S  |
| Genotypes          |                        |     |     | x̄ |     |     | x̄ |     |     | x̄ |
| GT1                | 0.252                  | 0.292| 0.772| 0.464| 0.504| 0.484| 0.500| 0.548| 0.524|
| GT2                | 0.560                  | 0.644| 0.602| 0.664| 0.740| 0.702| 0.686| 0.760| 0.724|
| GT3                | 0.680                  | 0.740| 0.710| 0.780| 0.840| 0.810| 0.788| 0.804| 0.796|
| GT4                | 0.688                  | 0.480| 0.584| 0.700| 0.736| 0.718| 0.740| 0.760| 0.753|
| GT5                | 0.504                  | 0.520| 0.512| 0.656| 0.784| 0.720| 0.700| 0.788| 0.744|
| GT6                | 0.488                  | 0.476| 0.482| 0.624| 0.660| 0.642| 0.660| 0.688| 0.674|
| GT7                | 0.604                  | 0.540| 0.572| 0.780| 0.732| 0.756| 0.840| 0.860| 0.850|
| G195               | 0.704                  | 0.660| 0.682| 0.900| 1.00 | 0.950| 0.920| 1.00 | 0.960|
| L.S.D              | 0.168                  | 0.153| -----| 0.128| 0.191| -----| 0.182| 0.175| -----|

S: season GT: Genotype

CONCLUSION

In the present study it could be concluded that: under Egyptian soil conditions, necessity exists for inoculation with specific rhizobia alone or in combination with PGPR bacteria to maximizing the development and yield production of chickpea plants. All tested genotypes of chickpea emphasized the superiority of response to inoculation with specific rhizobia and foliar application with PPFM bacteria. PPFMs did support nodule form, plant growth and yield and reduce using chemical fertilizers specially nitrogen fertilizers. GT3, GT4 and GT7 chickpea genotypes gave a positive results and higher values for the all tested chickpea parameters in comparison to chickpea G195 variety under Egyptian soil conditions.

REFERENCES

Abo Taleb, H.H. (1998). Intercropping of legumes and non-legumes as an approach to maximize input of biological N₂- fixation in plant - soil system. Ph.D Thesis, Fac. of Agric., Cairo Univ. Egypt, pp: 12-14.

Akhtar, M.A. and Siddiqui, Z.A. (2009). Effects of phosphate solubilizing microorganisms and Rhizobium sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. African J. Biotech., 8: 346-350.

Ali, S.; Charles, T.C. and Glick, B.R (2014). Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol. Biochem., 80, 160–167.

A.O.A.C. (1990). Association of Official Analytical chemists. 15th ed. Vol.1 United States of America, 40-64 pp.

Begum, A. A.; Leibovitch, S.; Migner, P. and Zhang, F. (2001). Inoculation of pea (Pisum sativum L.) by Rhizobium leguminosarum bv. viciae preincubated with naringenin and hesperetin or application of naringenin and hesperetin directly into soil increased pea nodule under short season conditions. Plant and Soil, 237: 71–80.

Elkoca, E.; Kantar, F. and Sahin, F. (2008). Influence of nitrogen fixing and phosphorus
solubilizing bacteria on the noduleation, plant growth, and yield of chickpea. J. Plant Nutrition, 31: 157–171.

Etesami, H. and Maheshwari, D.K. (2018). Ecotoxicology and Environmental Safety, (156): 225–246.

Fatima, Z.; Bano, A.; Sial, R. and Aslam, M. (2008). Response of chickpea to plant growth regulators on nitrogen fixation and yield. Pakistan Journal of Botany, 40(5): 2005-2013.

Giri, N. and Joshi, N.C. (2010). Growth and yield response of chick pea (Cicer arietinum) to seed inoculation with Rhizobium sp. Nature Science, 8: 232-236.

Gopalakrishnan, S.; Srinivas, V.; Venula, A.; Samineni, S. and Rathore, A. (2018). Influence of diazotrophic bacteria on nodulation, nitrogen fixation, growth promotion and yield traits in five cultivars of chickpea. International Crops Res. Institi. for the Semi-Arid Tropics (ICRISAT), Patancheru., (502): 324.

Gowda, C.L. and Gaur, P.M (2004). Global scenario of chickpea research present status and future thrusts. In: Ali, M, Singh, BB, Kumar, S; Dhar, V (eds) Pulses in New Perspective, Indian Society of Pulses Research and Development, IIPR, Kanpur, India, pp. 1–22.

Graham, P.H. and Vance, C.P. (2003). Legumes: Importance and Constraints to Greater Use. Plant Physiol., 131: 872-877.

Holland, M.A. (1997). Methylobacterium and plants. Recent Res. Develop. Plant Physiol., 1:207-213.

Holland, M. A. and Polacco, J. C. (1992). Urease null and hydrogenase – null phenotypes of a phytoaggregate bacterium reveal altered nickel metabolism in two soybean mutants. Plant Physiol., 98: 942-948.

Ivanova, E. G.; Doronina, N. V. and Trotsenko, Y. A. (2001). Aerobic methylobacteria are capable of synthesizing auxins. Microbiology, 70: 392-397.

Jackson, M. I. (1973). Soil Chemical Analysis. Constable and Co., Ltd. London.

Joshi, J; Mahmoud, S. A.; Holland, M. A.; Minsmje, E. M.; Dadson, R.B.; Omer, M.A.; Hashem, F.M. and Abdel-wahab, S.M. (2000). PPFMs;Are these the future biofertilizers? Proc.12th Annual Agronomy Society meeting, Dt-lous, Mo. USA.

Kantar, F.; Elkoca, E.; Ogutcu, H. and Algur, O. F. (2003). Chickpea yields in relation to Rhizobium inoculation from wild chickpea at high altitudes. J. Agronomy and Crop Science, 189: 1–7.

Khan, M. A.; Ali, A. and Tanveer, A. (2003). Effect of seed inoculation and different levels of phosphorus on the yield and yield components of chickpea. Pakistan J. Life. Soc. Sci., 1: 106-108.

Madhiayan, M.; Poonguzhali, S.; Lee, H.S.; Hari, K.; Sundaram, S.P. and Sa, T.M. (2005). Pink pigmented facultative methylotrophic bacteria accelerate germination, growth and yield of sugar cane clone Co86032 (Saccharum officinarum L.). Biol. Fertil. Soils, 41(5):350-358.

Meena, M.R.; Dawson, J. and Prasad, M. (2013). Effect of biofertilizers and phosphorus on growth and yield of chickpea (Cicer arietinum L.). Bioinfoilet, 10: 235-37.

Orf, Heba, O. M. (2006). Studies on Some Microorganisms Producing Growth Promoting Substances and Their Relation to Nodulation and Productivity of Legumes, MSc. Thesis Fac. of Agric., Ain Shams Univ. Egypt, 120 p.

Orf, Heba O. M.; Wedad, E. E. Eweda; Sawsan, F. Shehata and Abo Taleb, H. H. (2014). Comparative studies between nitrogen fixing methylotrophic bacteria and rhizobia of some legume plants. Minufiya J. Agric. Res., 39 No 2(2): 775 - 792.

Ogutcu, H.; Algur, O. F.; Elkoca, E. and Kantar, F. (2008). The determination of symbiotic effectiveness of Rhizobium strains isolated from wild chickpeas collected from high altitudes in Erzurum. Turkish J of agricultural and forestry, 32:241-248.

Ozturk, A.; Caglar, O. and Sahin, F. (2003). Yield response of wheat and barley to inoculation plant growth promoting rhizobacteria at various levels of nitrogen fertilization. J. Plant Nutrition and Soil Sci., 166: 1–5.

Peix, A.; Mateos, A.A.; Rodriguez –Barrueco, P.F.; Martoanez –Molina, C. and Velazquez, E. (2001). Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium mediterranenum under growth chamber conditions. Soil Biol. Biochem., 33:103-110.

Polacco, J. C. and Holland, M. A. (1993). Roles of urease in plant cells, Int. Rev. Cytol. 145:65-103.

Radha, T. K.; Savalgi, V. P. and Alagawadi, A. R. (2009). Effect of methylotrophs on growth and yield of soybean (Glycine max (L.) Merrill), Karnataka J. Agric. Sci., 22(1): 118-121.

Rice, W. A.; Olsen, P.E. and Lesset, M.E. (1995). Co-culture of Rhizobium and phosphorus solubilizing bacteria in sterile peat. Soil Biol. Biochem., 27: 110-116.

Rudresh, D.L.; Shivaprakash, M. and Prasad, R.D. (2005). Effect of combined application of
rhizobium, phosphate solubilizing bacterium and Tricoderma spp. on growth, nutrient uptake and yield of chickpea (Cicer aritinum L.). Applied Soil and Ecology, 28:139-146.

Senthil Kumar, M.; Madhaiyan, M.; Sundaram, S.P. and Kannaiyan, M. (2002). Compatible nature of pink-pigmented facultative Methylo trophs with other bioinoculants. India J. Microbiol., 92: 339.

Sharar, M.S.; Ayub, M.; Choudhary, A. and Nadeem, M. (2000). Effect of NP application and inoculation on the growth and yield of gram (Cicer aritinum L.). Pak. J. Agri. Sci., 37: 155-157

Sharma, S.; Kulkarni, J. and Jha, B. (2016). Halotolerant rhizobacteria promote growth and enhance salinity tolerance in peanut. Front. Microbiol., 7.

Shehata, Sawsan F.; Abo Taleb, H. H.; Wedad E. E. Eweda and Heba, O. M. Orf (2006). Growth, yield and yield component of inoculated chickpea and faba bean plants as affected by using methylotrophic bacteria. Arab Univ. J. Agric. Sci. Ain Shams, Univ., Cairo, 14 (2):625-639.

Shukla, P.S.; Agarwal, P.K. and Jha, B. (2012). Improved salinity tolerance of Arachishypogaea L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. J. Plant Growth Regul., 31: 195–206.

Singh, R.; Sharma, P.; Varshney, R. K.; Sharma, S. K. and Singh, N. K. (2008). Chickpea Improvement: Role of Wild Species and Genetic Markers, Biotechnology and Genetic Engineering Reviews, 25 (1): 267-314.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods7th Ed., Iowa State Univ. Press, Amr. USA, pp. 255-269.

Stougaard, J. (2000). Regulators and regulation of legume root nodule development. Plant Physiol.,124: 531-540.

Suresh Reddy, B.V. (2002). Studies on pink pigmented facultative methylotrophs as a new bioinoculant for groundnut (Arachis hypogaea L.). M.Sc. (Agri.) Thesis, Tamil Nadu Agric. Univ., Coimbatore (India).

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 255: 571-586.

Vincent, J. M. (1970). A manual for the practical study of the root nodule bacteria. In: International, Biological Programme. Handbook. No. 15. Blackwell Blackwell Scientific Publications, Oxford and Edinburgh. U. K. pp.75-76.

Williams, P.C. and Singh, U. (1987). The chickpea – nutritional quality and the evaluation of quality in breeding programs. In: Saxena MC, Singh KB (eds) The chickpea. CABI Publishing, Wallingford, UK, pp 329–356

Yates, R. J.; Howieson, J. G.; Reeve, W. G.; Nandasena, K. G.; Law, I. J.; Brau, L.; Ardley, J. K.; Nistelberge, H. M.; Real, D. and O’Hara, G. W. (2007). Lotononis angolensis forms nitrogen fixing, lupinoid nODULES phylogenetically unique, fast-growing, pink-pigmented bacteria, which do not nodulate L. bainesii or L. listii. Soil Biology & Biochemistry, 39 (7):1680-1688.

Zohary, D. and Hopf, M. (2000). Domestication of plants in the old world, 3rd edn. Oxford University Press, New York, USA.