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## **Response of some New Chickpea Genotypes to Rhizobial Inoculation and Foliar Application with Plant Growth Promoting Rhizobacteria (PGPR)**

## Rehab A. M. Abd El-Rahman<sup>1\*</sup> and H. H. Abo Taleb<sup>2</sup>



<sup>1</sup>Department of Legume crop research, Field Crop Research Institute, Agricultural Research Center, Giza, Egypt. <sup>2</sup>Department of Agricultural Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center,

Giza, Egypt.

## ABSTRACT



A field experiment was carried out during 2014/2015 and 2015/2016 seasons at Sids research station, Bani Suef governorate, Agricultural Research Center (ARC), to evaluate the response of eight chickpea genotypes of a wide divergent origin were used; namely, seven introduced from IRCADA G1 (FLP0893C), G2 (S091013), G3 (S090642), G4 (FLP0846C), G5 (FLP0872), G6 (FLP0847C) and G7 (FLIP08-141C) and one local variety Giza195 (G8) cultivated under Egyptian soil conditions to bacterial applications as rhizobial inoculation alone or in combination with foliar application of pink-pigmented facultative methylotrophic bacteria (PPFMs), bacteria as The Plant Growth Promoting Rhizobacteria (PGPR) comparing with un-inoculated control fertilizered with 50kg N/fed. Results showed that, rhizobial inoculation in combination with foliar application with PPFMs bacteria produced the highest average chickpea seed yield per feddan (6.48 and 6.86 ardab) in both seasons, respectively, with no significant difference from rhizobial inoculation alone which produced 6.08 and 6.75 ard. fed.<sup>-1</sup>. Both bacterial treatments recorded the highest values of all studied traits. Concerning studied chickpea genotypes, Giza195 (G8) was the best in all studied characters with no significant difference from G3 (S090642) and G6 (FLP0847C). The highest chickpea seed yields were obtained from Giza195(7.75 and 8.15 ard. fed.<sup>-1</sup>) followed by G3 (7.08and 7.19 ard. fed.<sup>-1</sup>) and G7 (7.51 and 7.70 ard. fed.<sup>-1</sup>) under the combination of rhizobial inoculation and foliar application with PPFMs bacteria in both seasons, respectively. These results demonstrated the necessity of inoculation with specific rhizobial alone or in combination with PGPR bacteria to maximize growth and yield of chickpea genotypes.

nitrogen and

Keywords: chickpea, rhizobial inoculation, PPFMs, PGPR

## INTRODUCTION

Chickpea (Cicer arietinum L.) is one of the earliest cultivated crops and the third widely grown edible legumes in tropical, sub-tropical and temperate regions of the world; it is grown in about 50 countries. Chickpea is an important cash crop for farmers in Middle and Upper Egypt, it can fix up to 140 kg Nitrogen ha<sup>-1</sup> and meet up to 80% of its nitrogen requirement from symbiotic nitrogen fixation (Alhudaiji 2015). Chickpea has the highest nutritional contents. That is rich in fiber and minerals (phosphorus, calcium, magnesium, iron, and zinc). Its lipid fraction is high in unsaturated fatty acids in addition to having high protein content (20-22%), (Singh et al., 2008). Crop legumes, grown in rotation with cereal crops, can improve yields of cereals and contribute to the total nitrogen pool in the soil. Legumes especially chickpea occupies special position regarding nutrition as well as soil fertility and improvement. It has the ability to grow well in poor soils as well as to improve them because of its efficient N fixation system. It can happily grow on marginal, poorly fertile sandy loam land. Soil factor exert greater influence than bacterial inoculation on plant growth, nitrogen fixation and nutrient uptake of plant (Neumann et al., 2011). Legume -Rhizobium symibiosis is undoubtedly the most important N<sub>2</sub>-fixing process and play a subtle role in providing

considerable environmental and agricultural importance, since they are responsible for most of the atmospheric nitrogen fixed on land (Graham and Vance 2003). Sustainable production depends upon the manipulation of all genetic and environmental factors that influence crops by exploiting high yielding varieties and manipulation of its symbiotic system. PGPR induces plant's nutrient acquisition, disease tolerance and plays a vital role in crop yield. Growth and yield of the plant have been improved by repeated inoculation with highly effective rhizobia (Hynes et al., 2008) and/or co-inoculation with PGPR (Tsigie et al., 2011). Improving Biological Nitrogen Fixation (BNF) in food crops may increase plant-based protein for human consumption and increase growth of subsequent crops with lesser chemical inputs. Vessev (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.

maintaining/improving

Symbiosis between legumes and rhizobia are of a

soil

fertility.

These bacteria are collectively known as 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. Implementation of PGPR-based biofertilizer technology presents economic, environmental and agronomic benefits and could be used to a larger degree to partially replace the synthetic fertilizers to improve economic yield under natural conditions, (Adesemoye and Kloepper, 2009). Repeated incorporation of rhizobia with more effective strains coupled with the addition of "helper bacteria" can add to the BNF of the crop. Strain x variety interaction is as important as that of strain or crop variety alone (Abi-Ghanem et al., 2011). Selection of best microbial strains and plant variety/cultivar is important because a strong interaction effect of cultivar x microbe has been reported on BNF in soybean (Israel et al., 1986), lentils and peas. Comparison of N<sub>2</sub> fixation among various rhizobial strains demonstrated 42% variability among strains while 81% variability among different lentil cultivars (Abi-Ghanem et al., 2011). This further supports the fact that each host cultivar has a variable potential for nitrogen fixation and response toward rhizobial inoculation.

The present work aims to study the response of new chickpea genotypes to rhizobial inoculation alone or in combination with application of Pink-Pigmented, Facultative Methylotrophic bacteria (PPFMs) as PGPR bacterial inoculation and its role in enhancing the vegetative growth, seed yield and yield quality of chickpea under Egyptian soil conditions.

## MATERIAL AND METHODS

A field experiment was carried out during two successive seasons (2014/2015 and 2015/2016) at Sids research station, Bani Suef governorate, Agricultural Research Center (ARC), to evaluate the response of eight chickpea genotypes of a wide divergent origin; namely, seven introduced from IRCADA G1 (FLP0893C), G2 (S091013), G3 (S090642), G4 (FLP0846C), G5 (FLP0872), G6 (FLP0847C) and G7 (FLIP08-141C) and one local variety Giza 195 (G8) cultivated under Egyptian soil conditions to bacterial applications as rhizobial inoculation alone or in combination with foliar application of PPFMs bacteria as PGPR. Physical-chemical 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. For Bacterial inoculation, 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 a mixture basal peat inoculant at a rate of 4g inoculant to 100 g seeds at planting time as seed coating method, and 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 application at rate of 5 L per Feddan 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. The recommended doses of P and K fertilizers: 100 Kg superphosphate (15.5 % P<sub>2</sub>O<sub>5</sub> fed<sup>-1</sup>) and 50 Kg potassium sulphate (24 K<sub>2</sub>O fed<sup>-1</sup>) were added during soil preparation. For N-fertilization, starter dose of 15 kg N fed<sup>-1</sup> was applied with the two rhizobium inoculation treatments in the form of ammonium sulfate (20.5% N) before the first irrigation. While 50 kg N fed<sup>-1</sup> divided in two equal doses were added to the un-inoculated treatment before the first and second irrigation.

Table 1. Physical-chemical properties of used soil at the experimental site:

Property         Values           Mechanical analysis :         Sand (%)         19.5           Silt (%)         34.0         Clay (%)           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 (%)         62.46           Total Nitrogen (%)         62.46         Total Nitrogen (%)         7.62           Exchangeable P (%)         7.62         Exchangeable R (%)         311.60           EDTA_ extractable :         Fe         8.60         Mn         4.31           Zn         4.10         Cu         1.81         Soluble Cations (meql <sup>-1</sup> ):         Ca <sup>++</sup> 3.00           Mg <sup>++</sup> 1.36         Na <sup>+</sup> 5.12         K <sup>+</sup> 0.98           Clay         Soluble Anions (meql <sup>-1</sup> ):         CO3 <sup></sup> 0.00         HCO3 <sup></sup> 1.51           Cl         1.72         SO4 <sup></sup> 7.23         1.51         1.72	experimental site:	
Mechanical analysis :           Sand (%)         19.5           Silt (%)         34.0           Clay (%)         64.5           Texture grand         Clay loam           Physical analysis :         10.4           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:         Exchangeable P (%)           Exchangeable P (%)         7.62           Exchangeable K (%)         311.60           EDTA_ extractable :         Fe           Fe         8.60           Mn         4.31           Zn         4.10           Cu         1.81           Soluble Cations (meql <sup>1-1</sup> ):         3.00           Mg* <sup>++</sup> 1.36           Na <sup>+</sup> 5.12           K <sup>+</sup> 0.98           Soluble Anions (meql <sup>1-1</sup> ):         0.00           CO3 <sup></sup> 0.00           HCO3 <sup></sup> 1.51           Cl-         1.	Property	Values
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	Mechanical analysis :	
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	Sand (%)	19.5
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	Silt (%)	34.0
Texture grand         Clay loam           Physical analysis :         S. P. %           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 (%)         62.46           Total Nitrogen (%)         0.028           Exchangeable P (%)         7.62           Exchangeable K (%)         311.60           EDTA_ extractable :         Fe           Fe         8.60           Mn         4.31           Zn         4.10           Cu         1.81           Soluble Cations (meql <sup>-1</sup> ):         3.00           Mg <sup>++</sup> 1.36           Na <sup>+</sup> 5.12           K <sup>+</sup> 0.98           Soluble Anions (meql <sup>-1</sup> ):         0.00           CO3 <sup></sup> 0.00           HCO3 <sup></sup> 1.51           Cl <sup>-</sup> 1.72           SO4 <sup></sup> 7.23	Clay (%)	64.5
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 (%)         62.46           Total Nitrogen (%)         62.46           Exchangeable P (%)         7.62           Exchangeable K (%)         311.60           EDTA_ extractable :         Fe           Fe         8.60           Mn         4.31           Zn         4.10           Cu         1.81           Soluble Cations (meql <sup>-1</sup> ):         3.00           Mg <sup>++</sup> 1.36           Na <sup>+</sup> 5.12           K <sup>+</sup> 0.98           Soluble Anions (meql <sup>-1</sup> ):         0.00           HCO <sub>3</sub> <sup></sup> 1.51           Cl <sup>-</sup> 1.72           SO <sub>4</sub> <sup></sup> 7.23	Texture grand	Clay loam
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$\begin{array}{llllllllllllllllllllllllllllllllllll$	S. P. %	48.77
E.C. dSm       1.04         Organic Carbon (%)       0.53         Organic Matter (%)       0.91         Soluble Nitrogen (%)       62.46         Total Nitrogen (%)       0.028         Chemical analysis:         Exchangeable P (%)       7.62         Exchangeable K (%)       311.60         EDTA_ extractable :         Fe       8.60         Mn       4.31         Zn       4.10         Cu       1.81         Soluble Cations (meql <sup>-1</sup> ):         Ca <sup>++</sup> 3.00         Mg <sup>++</sup> 1.36         Na <sup>+</sup> 5.12         K <sup>+</sup> 0.98         Soluble Anions (meql <sup>-1</sup> ):         CO <sub>3</sub> <sup></sup> 0.00         HCO <sub>3</sub> <sup></sup> 1.51         Cl <sup>-</sup> 1.72         SO <sub>4</sub> <sup></sup> 7.23	PH	7.72
$\begin{array}{cccc} Organic Carbon (\%) & 0.53 \\ Organic Matter (\%) & 0.91 \\ Soluble Nitrogen (\%) & 62.46 \\ \hline Total Nitrogen (\%) & 0.028 \\ \hline \\ \hline Chemical analysis: \\ \hline \\ Exchangeable P (\%) & 7.62 \\ \hline \\ Exchangeable K (\%) & 311.60 \\ \hline \\ \hline \\ \hline \\ Fe & 8.60 \\ Mn & 4.31 \\ Zn & 4.10 \\ \hline \\ \hline \\ Cu & 1.81 \\ \hline \\ \hline \\ Cu & 1.81 \\ \hline \\ \hline \\ Ca^{++} & 3.00 \\ Mg^{++} & 1.36 \\ Na^{+} & 5.12 \\ K^{+} & 0.98 \\ \hline \\ \hline \\ Soluble Anions (meql^{-1}): \\ \hline \\ CO_{3}^{} & 0.00 \\ HCO_{3}^{} & 1.51 \\ Cl^{-} & 1.72 \\ SO_{4}^{} & 7.23 \\ \hline \end{array}$	E.C. dSm	1.04
$\begin{array}{cccc} Organic Matter (\%) & 0.91 \\ Soluble Nitrogen (\%) & 62.46 \\ \hline Total Nitrogen (\%) & 0.028 \\ \hline Chemical analysis: \\ Exchangeable P (\%) & 7.62 \\ \hline Exchangeable K (\%) & 311.60 \\ \hline EDTA\_ extractable : \\ Fe & 8.60 \\ Mn & 4.31 \\ Zn & 4.10 \\ \hline Cu & 1.81 \\ \hline \\ Soluble Cations (meql^{-1}): \\ \hline Ca^{++} & 1.36 \\ Na^{+} & 5.12 \\ K^{+} & 0.98 \\ \hline \\ Soluble Anions (meql^{-1}): \\ \hline CO_{3}^{} & 0.00 \\ HCO_{3}^{} & 1.51 \\ Cl^{-} & 1.72 \\ SO_{4}^{} & 7.23 \\ \hline \end{array}$	Organic Carbon (%)	0.53
$\begin{array}{c} \mbox{Soluble Nitrogen (\%)} & 62.46 \\ \hline \mbox{Total Nitrogen (\%)} & 0.028 \\ \hline \mbox{Chemical analysis:} \\ \hline \mbox{Exchangeable P (\%)} & 7.62 \\ \hline \mbox{Exchangeable K (\%)} & 311.60 \\ \hline \mbox{EDTA\_ extractable :} \\ \hline \mbox{EDTA\_ extractable :} \\ \hline \mbox{Fe} & 8.60 \\ \hline \mbox{Mn} & 4.31 \\ \hline \mbox{Zn} & 4.10 \\ \hline \mbox{Cu} & 1.81 \\ \hline \mbox{Soluble Cations (meql^{-1}):} \\ \hline \mbox{Ca^{++}} & 1.36 \\ \hline \mbox{Na^{+}} & 5.12 \\ \hline \mbox{K^{+}} & 0.98 \\ \hline \mbox{Soluble Anions (meql^{-1}):} \\ \hline \mbox{CO}_3^- & 0.00 \\ \hline \mbox{HCO}_3^- & 1.51 \\ \hline \mbox{Cl} & 1.72 \\ \hline \mbox{SO4^-} & 7.23 \\ \hline \end{array}$	Organic Matter (%)	0.91
$\begin{tabular}{ c c c c c c } \hline Total Nitrogen (\%) & 0.028 \\ \hline Chemical analysis: \\ \hline Exchangeable P (\%) & 7.62 \\ \hline Exchangeable K (\%) & 311.60 \\ \hline EDTA\_ extractable : \\ \hline EDTA\_ extractable : \\ \hline EDTA\_ extractable : \\ \hline Fe & 8.60 \\ Mn & 4.31 \\ Zn & 4.10 \\ \hline Cu & 1.81 \\ \hline Cu & 1.81 \\ \hline \\ Soluble Cations (meql^{-1}): \\ \hline Ca^{++} & 3.00 \\ Mg^{++} & 1.36 \\ Na^{+} & 5.12 \\ K^{+} & 0.98 \\ \hline \\ \hline CO_3^{} & 0.00 \\ HCO_3^{} & 1.51 \\ Cl^{-} & 1.72 \\ SO_4^{} & 7.23 \\ \hline \end{tabular}$	Soluble Nitrogen (%)	62.46
$\begin{tabular}{ c c c c } \hline Chemical analysis: \\ \hline Exchangeable P (%) & 7.62 \\ \hline Exchangeable K (%) & 311.60 \\ \hline EDTA\_extractable : \\ \hline EDTA\_extractable : \\ \hline EDTA\_extractable : \\ \hline Re & 8.60 \\ Mn & 4.31 \\ \hline Zn & 4.10 \\ \hline Cu & 1.81 \\ \hline Cu & 1.81 \\ \hline Cu & 1.81 \\ \hline \\ Soluble Cations (meql-1): \\ \hline \\ Ca^+ & 5.12 \\ K^+ & 0.98 \\ \hline \\ \hline \\ Soluble Anions (meql^{-1}): \\ \hline \\ CO_3^- & 0.00 \\ HCO_3^- & 1.51 \\ \hline \\ Cl^- & 1.72 \\ SO_4^- & 7.23 \\ \hline \end{tabular}$	Total Nitrogen (%)	0.028
Exchangeable P (%)       7.62         Exchangeable K (%)       311.60         EDTA_extractable :         Fe       8.60         Mn       4.31         Zn       4.10         Cu       1.81         Soluble Cations (meql <sup>-1</sup> ):         Ca <sup>++</sup> 3.00         Mg <sup>++</sup> 1.36         Na <sup>+</sup> 5.12         K <sup>+</sup> 0.98         Soluble Anions (meql <sup>-1</sup> ):         CO <sub>3</sub> <sup></sup> 0.00         HCO <sub>3</sub> <sup></sup> 1.51         Cl <sup>-</sup> 1.72         SO <sub>4</sub> <sup></sup> 7.23	Chemical analysis:	
Exchangeable K (%)         311.60           EDTA_ extractable :         EDTA_ extractable :           Fe         8.60           Mn         4.31           Zn         4.10           Cu         1.81           Soluble Cations (meql <sup>-1</sup> ):           Ca <sup>++</sup> 3.00           Mg <sup>++</sup> 1.36           Na <sup>+</sup> 5.12           K <sup>+</sup> 0.98           Soluble Anions (meql <sup>-1</sup> ):           CO <sub>3</sub> <sup></sup> 0.00           HCO <sub>3</sub> <sup></sup> 1.51           Cl <sup>-</sup> 1.72           SO <sub>4</sub> <sup></sup> 7.23	Exchangeable P (%)	7.62
$\begin{tabular}{ c c c c c } \hline EDTA\_extractable: \\ \hline Fe & 8.60 \\ Mn & 4.31 \\ Zn & 4.10 \\ Cu & 1.81 \\ \hline \\ \hline Cu & 1.81 \\ \hline \\ Soluble Cations (meql-1): \\ \hline \\ Ca^{++} & 3.00 \\ Mg^{++} & 1.36 \\ Na^+ & 5.12 \\ K^+ & 0.98 \\ \hline \\ \hline \\ Soluble Anions (meql^{-1}): \\ \hline \\ CO_3^{} & 0.00 \\ HCO_3^{} & 1.51 \\ Cl^- & 1.72 \\ SO_4^{} & 7.23 \\ \hline \end{tabular}$	Exchangeable K (%)	311.60
Fe       8.60         Mn       4.31         Zn       4.10         Cu       1.81         Soluble Cations (meql <sup>-1</sup> ):         Ca <sup>++</sup> Soluble Cations (meql <sup>-1</sup> ):         Ca <sup>++</sup> 3.00         Mg <sup>++</sup> 1.36         Na <sup>+</sup> 5.12         K <sup>+</sup> 0.98         Soluble Anions (meql <sup>-1</sup> ):         CO <sub>3</sub> <sup></sup> 0.00         HCO <sub>3</sub> <sup></sup> 1.51         Cl <sup>-</sup> 1.72         SO <sub>4</sub> <sup></sup> 7.23	EDTA_extractable :	
$\begin{array}{ccccccc} Mn & & & 4.31 \\ Zn & & & 4.10 \\ \underline{Cu} & & & 1.81 \\ \hline & & & & \\ \hline & & & & \\ Ca^{++} & & & & \\ Mg^{++} & & & 1.36 \\ Na^{+} & & & 5.12 \\ K^{+} & & & 0.98 \\ \hline & & & \\ \hline & & & \\ CO_{3}^{} & & & 0.00 \\ HCO_{3}^{} & & & 1.51 \\ Cl^{-} & & & 1.72 \\ SO_{4}^{} & & & 7.23 \\ \hline \end{array}$	Fe	8.60
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Mn	4.31
$\begin{tabular}{ c c c c c } \hline Cu & 1.81 & & & & \\ \hline & Soluble Cations (meql^{-1}): & & & & \\ \hline & & & & & & \\ Mg^{++} & 1.36 & & & \\ Na^+ & 5.12 & & & \\ K^+ & 0.98 & & & \\ \hline & & & & \\ \hline & & & & \\ CO_3^- & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & & \\ \hline & & & & & \\ CO_3^- & & & & \\ \hline & & & & \\ CO_3^- & & & & \\ CO_3^- & & & & \\ \hline & & & & \\ CO_3^- & & & & \\ \hline & & & & \\ CO_3^- & & & & \\ \hline & & & & \\ CO_3^- & & & & \\ \hline & & & \\ CO_3^- & & & & \\ CO_3^- & & & \\ \hline & & & \\ CO_3^- & & & \\ CO_3^- & & & \\ \hline & & & \\ CO_3^- & \\ CO_3^- & \\ CO_3^- $	Zn	4.10
$\begin{tabular}{ c c c c c } \hline Soluble Cations (meql^-l): & & & & & & & & & & & & & & & & & & &$	Cu	1.81
$\begin{array}{ccccccc} Ca^{++} & & 3.00 \\ Mg^{++} & & 1.36 \\ Na^{+} & & 5.12 \\ K^{+} & & 0.98 \\ \hline \\ CO_{3}^{-} & & 0.00 \\ HCO_{3}^{-} & & 1.51 \\ Cl^{-} & & 1.72 \\ SO_{4}^{-} & & 7.23 \\ \hline \end{array}$	Soluble Cations (meql <sup>-1</sup> ):	
$\begin{array}{cccc} Mg^{++} & 1.36 \\ Na^{+} & 5.12 \\ K^{+} & 0.98 \\ \hline & \\ \hline CO_{3}^{-} & 0.00 \\ HCO_{3}^{-} & 1.51 \\ Cl^{-} & 1.72 \\ SO_{4}^{-} & 7.23 \\ \hline \end{array}$	Ca <sup>++</sup>	3.00
$\begin{tabular}{cccc} Na^+ & 5.12 \\ K^+ & 0.98 \\ \hline & \\ \hline & \\ CO_3^- & 0.00 \\ HCO_3^- & 1.51 \\ Cl^- & 1.72 \\ SO_4^- & 7.23 \\ \hline \end{tabular}$	Mg <sup>++</sup>	1.36
K <sup>+</sup> 0.98           Soluble Anions (meql <sup>-1</sup> ):         0.00           HCO <sub>3</sub> <sup></sup> 1.51           Cl <sup>+</sup> 1.72           SO <sub>4</sub> <sup></sup> 7.23	Na <sup>+</sup>	5.12
Soluble Anions (meql <sup>-1</sup> ): $CO_3^-$ 0.00 $HCO_3^-$ 1.51 $Cl^-$ 1.72 $SO_4^-$ 7.23	K <sup>+</sup>	0.98
CO3 <sup></sup> 0.00           HCO3 <sup>-</sup> 1.51           Cl <sup>+</sup> 1.72           SO4 <sup></sup> 7.23	Soluble Anions (meql <sup>-1</sup> ):	
HCO <sub>3</sub> - 1.51 Cl <sup>-</sup> 1.72 SO <sub>4</sub> - 7.23	CO <sub>3</sub> -	0.00
Cl <sup>-</sup> 1.72 SO <sub>4</sub> <sup>-</sup> 7.23	HCO <sub>3</sub> -	1.51
SO4 <sup></sup> 7.23	Cl	1.72
	<u>SO4</u>	7.23

#### Inoculation treatments were as follows:

- 1. Un-inoculated plants + 50 Kg N/fed in two equal doses were added before the first and second irrigation.
- 2. Rhizobial inoculation +15 Kg N fed.<sup>-1</sup> as a starter dose.
- 3. Rhizobial inoculation+15 Kg N fed.<sup>-1</sup> as a starter dose + foliar application with PPFMs (5 L fed<sup>-1</sup>., at 30 days after planting)

The field experiment was laid out in a split-plot in both seasons, where inoculation treatments were arraigned to main plots with three replications and chickpea genotypes in the sub plots. Each sub-plot area was 7.2 m<sup>2</sup>, consisting of four ridges (3.0 m long and 60 cm in width). Planting took place during November (10 and 21 in both seasons respectively). Chickpea seeds were sown in hills distributed on one side of each ridge at 20 cm hill spacing. Before the first irrigation hills were thinned to two plants per hill. **Data recorded:** 

A sample of three guarded hill contain six plants from each sub-plot was taken after 75 days from planting to determine the nodulation status (number and dry weight of nodules per plant) according to Vincent (1970). Also, plant dry weight (g) and plant nitrogen content (g) were determined.

At maturity the two middle ridges of each plot were harvested to determine the seed yield kg plot<sup>1</sup> and converted to ardab (150 kg) per feddan (0.42 ha). Also, five guarded plants were taken from each sub-plot to measure some yield components, such as number of branches and pods per plant, seed yield per plant and seed index.

## Statistical analysis:

Data were subjected to an analysis of variance (ANOVA) and means were compared by the least significant difference (LSD) at 0.05 using MSTAT program according to Snedecor and Cochram (1980).

## **RESULTS AND DISCUSSION**

It is clear from Table 2 that treatment 3 {Rhizobial inoculation+ 15 Kg N fed<sup>-1</sup>+ foliar application with PPFMs (5 L. fed<sup>-1</sup>, at 30 days after planting)} is the best one for all characters in both seasons, data recorded that there were significant differences among inoculation treatments and un-inoculated one which recorded the lowest values of nodules number per plant, nodules dry weight, plant dry weight and N-content. Application of rhizobial inoculation along or in combination with PPFMs application scored high values as compared to un-inoculated treatment.

The highest values for the tested characters were recorded on the plants which received both bacterial treatments (Rhizobial and PPFMs), and these values were 51.50, 136.25, 3.06 and 96.56 for nodules number per plant, nodules dry weight, plant dry weight and N-content in the second season respectively. As shown in Table 2 chickpea genotype Giza 195 followed by chickpea genotypes G5, G7 and G6 recorded higher values for nodules number per plant, nodules dry weight, plant dry weight and plant N-content compared to the other genotypes .These data are in agreement with {Meena et al., (2013) and Orf, Heba et al., (2014)}, who reported that, there are positive response of chickpea genotypes to inoculation with rhizobial alone or in combination with PPFMs, bacteria and recorded significant increases of nodulation situation as well as plant biomass and plant minerals up take. All chickpea genotypes responded to the native rhizobial and formed root bacterial nodules but scored the lowest values of nodules formation in both seasons, as shown in the un-inoculated treatment. Inoculation with specific rhizobia in combination with PPFMs as foliar application scored the highest values, and led to significant increases in number of nodules in both seasons as compared to the treatment received rhizobial inoculation alone. However, rhizobial inoculation alone improved nodulation situation of chickpea plants and significantly increased plant dry weight and N-content per plant compared to the un-inoculated treatment in both seasons.

Table 2. Effect of bacterial treatments on nodulation, Plant dry weight and Plant N- content per plant of some chickpea genotypes after 75 days from planting in two seasons.

No. of Nodulos day Diant day Diant day Diant N. co.														
Character	nodule	s plant <sup>-1</sup>	weight pl	ant <sup>-1</sup> (mg)	weight (g) a	fter 75 davs	plant <sup>-1</sup> (g)							
Seasons	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16						
	Inoculation treatment													
T1	17.88	16.50	48.54	44.25	2.08	2.46	54.09	61.76						
T2	28.50	40.87	73.41	99.91	2.56	2.95	74.21	96.10						
T3	43.38	51.50	98.67	136.25	2.88	3.06	83.00	96.56						
LSD 0.05	4.13	7.28	5.00	4.03	0.226	0.47	2.26	6.37						
	Chickpea genotypes													
Gl	25.00	33.66	55.22	93.33	2.27	2.40	59.73	68.73						
G2	29.00	36.66	77.33	127.00	2.28	2.40	63.87	71.77						
G3	22.33	36.33	45.11	91.33	2.63	2.97	63.97	80.36						
G4	32.00	34.33	81.33	86.66	2.67	3.13	67.40	83.36						
G5	37.67	37.00	103.33	89.00	2.23	2.63	75.00	84.12						
G6	27.33	35.00	68.00	83.33	2.47	2.93	72.47	95.13						
G7	27.67	35.66	64.00	74.33	2.67	3.06	73.27	91.06						
G8 (Giza195)	38.33	41.66	94.00	102.78	2.83	3.07	87.77	103.90						
LSD 0.05	3.26	4.27	9.51	7.82	0.218	0.26	4.28	4.08						
Interaction Sig.	*	*	*	*	*	*	*	*						

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 compared to un-inoculated 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 bacterial inoculation (Orf, Heba *et al.*, 2014).

The obtained data are in agreement with those of Polacco and Holland (1994), who reported that, inoculation with PPFMs increased the dry weight of soybean plants as compared to untreated ones. Holland (1997), reported that the activities of PPFMs could make a biochemical and physiological measurable 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 rhizobial alone or in-combination with PGPR scored significant increase in total nitrogen (N) content (upto76%) over the non-inoculated control.

The effect of bacterial treatments on; No. of branches, No. of pods, 100 seed weight (g) per plant and Seed yield ardab per feddan of some chickpea genotypes in the two seasons are presented in table 3.

Data showed that the un-inoculated treatment gave the lowest values for number of branches (8.55 and 10.50), number of pods (24.70 and 29.15), 100 seed weight (27.47 and 27.66 g/plant) and seed yield ard. fed.<sup>-1</sup> (5.02 and 4.83) in the two seasons respectively. While rhizobial inoculation in combination with PPFMs bacterial foliar application recorded the highest values for the yield components of chickpea genotypes (11.52 and 13.06), (47.55 and 59.38), 32.71 and 34.59) and (6.48 and 6.86) for number of branches, number of pods, seed weight per plant and seed yield per Fadden in both seasons respectively. The second season gave the highest values for the tested traits as compared to season one. These data are in agreement with Rudresh *et al.*, (2005), Giri and Joshi (2010) and Al-hudaiji, M.A.A. (2015), who found that application of bacterial inoculation (rhizobia inoculation alone or in combination with others plant growth promoting rhizobacteria *i.e.* PPFMs) improved the performance of chickpea plants compared to the un-inoculated treatment.

Table 3. Effect of bacterial treatments on; No. of branches, No. of pods, 100 seed weight (g) per plant and Seed yield ardab per feddan of some chickpea genotypes in the two seasons.

Characters	No. of bran	ches plant <sup>-1</sup>	No. of po	ds plant <sup>-1</sup>	100 seed	weight (g)	Seed yield (ard. fed. <sup>-1</sup> )						
Seasons	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16					
	Inoculation treatment												
T1	8.55	10.50	24.70	29.15	27.47	27.66	5.02	4.83					
T2	10.65	12.38	42.21	50.08	29.45	31.28	6.08	6.75					
T3	11.52	13.06	47.55	59.38	32.71	34.59	6.48	6.86					
LSD 0.05	1.01	0.93	2.49	3.40	2.96	2.72	1.11	0.60					
Chickpea genotypes													
G1	10.10	11.63	21.58	40.62	27.48	29.2	3.64	3.95					
G2	9.93	11.69	45.81	56.46	30.17	31.54	5.68	6.48					
G3	9.44	12.06	36.83	40.13	29.47	30.65	6.73	7.21					
G4	11.16	12.55	37.36	38.50	31.03	31.72	6.13	6.06					
G5	9.33	12.90	32.70	38.40	30.19	31.75	5.57	6.22					
G6	11.58	11.80	41.59	53.83	29.60	31.42	5.30	5.53					
G7	10.69	11.53	50.37	59.96	30.47	30.89	6.71	6.44					
G8 (Giza195)	9.69	11.70	37.97	41.73	30.57	32.25	7.11	7.27					
LSD 0.05	0.71	0.62	3.25	4.04	1.82	1.87	0.56	0.52					
Interaction Sig.	*	*	*	*	*	*	*	*					

They added that inoculation treatments had significant influence on different plant vegetative growth traits, nutrient uptake as well as seed yield and gave higher values than the un-inoculated treatments. The above mentioned data are in agreement with the observations made by 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.* alfa alfa and soybean and the number and weight of nodules per plant showed significant response of chickpea varieties to nitrogen fertilization rates and inoculation with specific rhizobial strains.

In this aspect, many investigators, reported that rhizobial inoculation alone or in combination with PGPR enhanced nodulation status (number and dry weight of nodules per plant) plant dry weight and N-content per plant along with improving the yield and yield components of chickpea and many legume crops such as alfa alfa, soybean and groundnut compared to the un-inoculated treatment {Gopalakrishnan *et al.*, (2018), Rudresh *et al.*, (2005), Elkoca *et al.*, (2008), Giri and Joshi (2010), and Meena *et al.*, (2013)}.

In the two years of trials under field conditions the interaction of chickpea genotypes and bacterial inoculations significantly affected all traits investigated, compared with control. The interaction was highly significant for all the studied characters in both seasons except of 100 seed weight (g) in 2015/16 season.

Data in Table 4 showed the interaction between the genotypes and bacterial treatments, for number of nodules per plant and nodules dry weight per plant (mg), The variety G 195 gave the highest values of number of nodules/plant (53 and 57) followed by G 2 (46 and 53), G4 (46 and 53) and G 5 (49 and 51) in both seasons, respectively with treatment 3, while for nodules dry weight plant, the highest values were obtained from the variety G 195 in both seasons followed by the genotypes G4 and G5 in the first season and the genotypes G2 and G4 in the second season

number of	nouui	co unu i	iouures	urj ne	Sin per	plant	n une en	0 beabon	<b>J</b> •					
Character		N	o. of nod	ules plar	nt <sup>-1</sup>		Nodules dry weight plant <sup>-1</sup> (mg)							
Season		2014/15			2015/16			2014/15		2015/16				
Treatment / Genotype	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3		
Gl	12.0	25.0	38.0	16.0	36.0	49.0	31.0	65.0	69.7	43.0	98.0	139.0		
G2	25.0	16.0	46.0	19.0	38.0	53.0	86.0	40.0	106.0	67.0	95.0	219.0		
G3	14.0	19.0	34.0	22.0	42.0	45.0	33.3	50.0	52.0	55.0	103.0	116.0		
G4	10.0	40.0	46.0	12.0	38.0	53.0	24.0	110.0	110.0	35.0	97.0	128.0		
G5	28.0	36.0	49.0	16.0	44.0	51.0	77.0	91.0	142.0	42.0	115.0	110.0		
G6	18.0	26.0	38.0	13.0	43.0	49.0	61.0	71.0	72.0	45.0	107.0	98.0		
G7	15.0	25.0	43.0	13.0	39.0	55.0	35.0	72.3	84.7	28.0	82.0	113.0		
G8 (Giza195)	21.0	41.0	53.0	21.0	47.0	57.0	41.0	88.0	153.0	39.0	102.3	167.0		
LSD 0.05		5.64			7.40			16.48			13.55			

Table 4. The interaction effect between different bacterial inoculation treatments and chickpea genotypes on number of nodules and nodules dry weight per plant in the two seasons.

Data in Table 5 show the interaction effect between the genotypes and bacterial treatments, on Plant dry weight (g) after 75 days and N- content  $\text{plant}^{-1}$  (g). The highest values for Plant dry weight were 3.2 and 3.4 g for genotype G8followed by G7 (310 and 330) with treatment 3 in both seasons respectively. The desirable value for N- content plant<sup>-1</sup> (g) were 112.5 and 134.8 for genotype (Giza 195) G8 under treatment no. three in both seasons respectively.

Character		Plant dr	y weight	(g) after	r 75 days	5		Plant N- content plant <sup>-1</sup> (g)				
Season		2014/15			2015/16			2014/15		2015/16		
Treatment / Genotype	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
G1	1.53	2.60	2.70	1.60	2.70	2.90	37.6	84.9	56.7	45.8	93.2	67.2
G2	1.83	2.40	2.60	1.90	2.60	2.70	53.9	74.1	63.6	56.9	82.7	75.7
G3	2.60	2.50	2.80	2.93	2.90	3.10	57.9	61.4	72.6	63.2	96.2	81.7
G4	2.60	2.40	3.00	3.10	3.10	3.20	70.2	61.9	70.1	78.1	82.8	89.2
G5	1.50	2.40	2.80	2.10	2.80	3.00	49.9	78.4	96.7	61.7	94.7	95.9
G6	1.90	2.70	2.80	2.70	3.20	2.90	48.9	74.2	94.3	63.9	115.3	106.2
G7	2.30	2.60	3.10	2.80	3.10	3.30	45.9	76.4	97.5	53.2	98.3	121.7
G8 (Giza195)	2.40	2.90	3.20	2.60	3.20	3.40	68.4	82.4	112.5	71.3	105.6	134.8
LSD 0.05		0 377			0.45			7 4 3			7.07	

Table 5. The interaction effect between bacterial inoculation and chickpea genotypes on; plant dry weight (g) and N- content per plant (g) after 75 days in both seasons.

The results in Table 6 showed that the best genotype (G4) under treatment three in the first season recorded 12.8 for number of branches per plant and G7 under treatment three recorded 13.7 in the second season. The highest number of pods per plant were obtained from G7 chickpea genotypes under bacterial treatment no. three, which recorded 67.5 and 77.2 in both seasons, respectively.

Data represented in Table 7 showed that G4 under treatment three had the highest values for 100 seed weight and recorded 36.3 and 37.5 g in both seasons, respectively. The highest chickpea seed yields per feddan (7.75 and 8.34 ardab) were obtained from G8 (Giza 195) under treatment three in the first and second season, respectively.

 Table 6. The interaction effect between bacterial inoculation and chickpea genotypes on number of branches/plant and Number of pods/plant in the two seasons.

Character		No	). of bran	ches plar	nt <sup>-1</sup>	No. of pods plant <sup>-1</sup>							
Season	2014/15				2015/16			2014/15			2015/16		
Treatment / Genotype	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	
G1	6.0	11.6	12.7	8.3	13.3	13.3	21.6	20.6	22.5	30.0	43.6	48.2	
G2	7.6	10.6	11.6	8.5	13.3	13.2	20.9	58.3	58.2	28.3	69.0	72.1	
G3	8.0	9.9	10.4	11.0	12.6	12.6	23.6	41.6	45.3	33.3	35.6	51.5	
G4	9.7	10.9	12.8	11.6	12.9	13.1	21.6	44.3	46.1	24.6	35.6	55.3	
G5	9.5	8.3	10.2	12.6	12.6	13.5	20.3	33.3	44.5	22.3	35.5	57.3	
G6	11.0	11.9	11.8	11.4	11.5	12.5	36.6	42.3	45.8	41.3	61.0	59.2	
G7	8.3	11.6	12.1	9.6	11.3	13.7	26.6	57.0	67.5	26.7	75.9	77.2	
G8 (Giza195)	8.3	10.3	10.4	11.0	11.6	12.5	26.3	40.3	50.3	26.6	44.3	54.3	
LSD 0.05		1.23			1.07			5.63			6.99		

 Table 7. The interaction effect between different bacterial inoculation and chickpea genotypes on 100-seed weight and Seed yield ard. fed.<sup>-1</sup> in the two seasons.

Characters	100 seed weight							Seed yield ard. fed. <sup>-1</sup>						
Season		2014/15		2015/16				2014/15	5		2015/16			
Treatment / Genotype	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3		
G1	26.4	27.8	28.2	27.4	28.7	31.5	2.23	4.19	4.49	2.61	4.32	4.90		
G2	28.5	29.5	32.5	30.5	31.5	32.6	5.01	5.94	6.08	5.68	6.95	6.80		
G3	26.5	30.2	31.7	26.8	30.3	34.8	6.13	6.97	7.08	6.81	7.63	7.19		
G4	26.9	29.9	36.3	26.5	31.1	37.5	6.16	5.55	6.69	4.41	6.81	6.96		
G5	27.0	30.0	33.6	25.7	33.0	36.5	4.51	5.93	6.26	4.53	7.13	6.99		
G6	26.6	29.6	32.6	27.4	31.7	35.1	4.39	5.59	5.93	4.33	6.08	6.16		
G7	29.0	29.2	33.2	27.5	31.4	33.7	5.52	7.09	7.51	4.90	6.73	7.70		
G8 (Giza195)	28.8	29.3	33.5	29.5	32.2	35.0	6.19	7.39	7.75	5.31	8.34	8.15		
LSD 0.05	3.15 3.24				3.24			0.96		0.89				

In this respect, Orf, Heba *et al.*, (2006) reported that, the production of 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 improvement of the vegetative growth of the plant could enhance the crop yield.

These data were in agreement with those obtained by Orf, Heba 2006, who found significant increases in seed protein content due to bacterial inoculation which 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. Senthilkumar *et al.*, (2002) and Shehata, Sawsan, (2006) attributed the increase in the yield to the compatible nature of *Methylobacterium* Rhizobium and they found that, combined influence on phyllosphere by methylotrophs, as a plant growth promoting phyllosphere (PGPP) bacteria, and on rhizosphere by *Rhizobium*, as a nitrogen fixing bacteria, might have important role in increased plant growth and yield parameters.

#### CONCLUSION

Results of the present study proved that: under Egyptian soil conditions, necessity exists for inoculation with specific rhizobial alone or in combination with PGPR bacteria to maximize the development and yield production of chickpea plants. All tested genotypes of chickpea emphasized the superiority of response to specific rhizobial inoculation and foliar application with PPFM bacteria. PPFMs supported nodule formation, plant growth and yield while, reduced the need for inorganic N fertilizer as well as minimized the cost of inputs and environmental pollution. G3, G4 and G7 chickpea genotypes gave higher values for seed yield ard. fed.<sup>-1</sup>. compared to Giza 195 variety under Egyptian soil conditions and could be used in chickpea programs.

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استجابة بعض التراكيب الوراثية الجديدة للحمص للتلقيح البكتيري والرش الورقى بمحفزات النمو رحاب أحمد محمد عبدالرحمن وحاتم أبو طالب ل

العسم بحوث المحاصيل الحقلية معهد بحوث المحاصيل البقولية مركز البحوث الزراعية

تقسم الميكر ويبولوجي - معهد بحوث الأراضي والمياه مركز البحوث الزراعية

الموسمين على التوالي، وهذه النتائج تدل على ضرورة التلقيح البكتيري (الريزوبيا) واستخدام البكتريا المحفزة للنمو (PGPR) مثل (PPFMs) للحصول على أفضل نمو وأعلى إنتاجية للتراكيب الور أثية للحمص