Pak. j. life soc. Sci. (2021), 19(1): 15-22 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

Assessing Maize Growth and Physiological Indices under Combined and Individual Influence of *Bacillus* sp. MN-54 and Organic Amendments

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ARTICLE INFO
Received: Feb 10, 2021
Accepted: May 21, 2021

Keywords
Maize
Bacillus sp. MN-54
Biochar
Cattle manure

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ABSTRACT
Use of organic amendments along with biofertilizers are becoming popular day by day for agricultural sustainability and gives hope for a gradual shift from synthetic fertilizers, pesticides**,** and other chemical amendments towards organic and sustainable practices. This experiment was carried for the investigation of biochar, cattle manure**,** and *Bacillus* sp. MN-54 application on the chemical composition, physiology**,** and growth parameters of maize plants individually and coupled with each other. Treatment results were considered significant at $P \leq 0.05$. Cattle manure having low pH combined with *Bacillus* sp. MN-54 treatment significantly improved the transpiration rate, water use efficiency, Relative water content, chlorophyll content, photosynthetic rate, plant height and stomatal conductance ($P \leq 0.05$). The electrolyte leakage was decreased by 58.13% whereas the root growth increased by 116.65% over non-treated plants, when low pH cattle manure was supplemented with *Bacillus* sp. MN-54. Overall, the *Bacillus* sp. MN-54 showed compatibility with the maize variety Gauher-19 and showed significantly positive results when applied with low pH cattle manure, proving its future application in the sustainability of agriculture.

INTRODUCTION

Maize (*Zea mays L.*) is one of the key economical cereal and staple food crop being used in the different temperate and arid regions of the world. Despite the use of genetically high potential maize varieties around the globe (Jockovic et al., 2010), the yield of maize is gradually reducing (Badu-Apraku and Yallou, 2009) due to exhaustion of soil nutrients coupled with harmful impacts of dwindling land fertility. Apart from genetic superiority, higher yields also need agronomic improvements and techniques especially fertilizers; as Phosphorous (P) and Nitrogen (N) are the major macronutrients which adversely affect the growth, development and yield of maize, if deficient (Wu et al., 2005). The microorganisms found in the rhizosphere called plant growth-promoting bacteria (PGPB) and organic amendments such as biochar and cattle manure are found to sustainably improve the agricultural productivity, maintain the soil fertility, and reduce the synthetic fertilizers usage (Mohan et al., 2007; Verheijen et al., 2010; Mrkovacki and Bjelic, 2011; Uzoma et al., 2011; Dhawi et al., 2015; Mohanty et al., 2017).

Rhizosphere possess a great array of microorganisms having both adverse and beneficial influence on physiology and productivity of plants including fungi, bacteria and cyanobacteria etc. (Berg, 2009; Souza et al., 2015; Kim et al., 2016). Rhizospheric bacterial community comprises a variety of strains including *Pseudomonas*, *Azospirillum*, *Bacillus* and *Paenibacillus*. They improve plant growth properties by two pronounced mechanisms i.e. by stimulating the production of growth-promoting phytohormones (Arzanlou et al., 2016; Asari et al., 2017) and imparting heavy metal tolerance (Abubakar et al., 2020) in addition to various other pathways (Glick, 1995; Das et al., 2003; Kejela et al., 2016). Rhizobacteria that helps in the promotion of plant growth and development are very important; as they provide nutrition by promoting nitrogen and phosphorous uptake by plants, improve crop yield and impart agricultural sustainability in different areas i.e. bioremediation, biofertilizers, probiotics and biopesticides (Cakmakci et al., 2006; Nezarat and Gholami, 2009; Abubakar et al., 2020).

Biochar is a carbonaceous natural product produced as a result of organic material (Woods, leaves, FYM) pyrolysis under anaerobic conditions. A variety of products can be used for biochar production such as crop residues, twigs, different types of manure and many other types of organic wastes (Enders et al., 2012). Biochar has increased the yield, water holding capacity, nutrient use efficiency and soil health (Verheijen et al., 2010), cumulative yield of sorghum and rice by 75% with compound fertilizer (Steiner et al., 2007) and 140% rise in radish (*Raphannus sativus*) yield after 4 years of biochar application (Chan et al., 2008). Biochar is also known as soil enhancer due to improved nutrient availability, physicochemical properties of plants (Jones et al., 2012), source of nutrients (Atkinson et al., 2010; Sohi et al., 2010), improving water holding capacity (Glaser et al., 2002), checking nutrient losses (Sohi et al., 2009) and improving microbial population along with their activity In the rhizosphere (Lehmann et al., 2011).

Cattle and poultry manure is considered an important organic supplement for improving plant growth, health and enhanced yield of the crops (Lyimo et al., 2012). Cow and goat manure is also equally beneficial for growth and yield of maize, increasing leaf surface area and photosynthetic rate without having any adverse effects on the plant (Hariadi et al., 2016). Cattle manure along with other additives such as biochar and Plant growth promoting rhizobacteria (PGPR) is considered an important factor in today's scenario of increasing the yield of crops under population explosion and also imparting drought and heavy metal tolerance to different crops (Abubakar et al., 2020).

This research is continuation of our previous research (Abubakar et al., 2020) where we investigated the ameliorative role of these treatments i.e., biochar, Cattle manure and *Bacillus* sp. MN-54 on chemical composition, growth and physiological parameters of maize plants under the toxic condition of chromium. Various studies have been undertaken to ascertain the results of PGPR, biochar and cattle manure on a variety of crop species but there is scarcity of research investigating the cumulative effect of the above-mentioned treatments on maize plants under normal growing conditions. Therefore, in this study impact of low and normal pH cattle manure, biochar and *Bacillus* sp. MN-54 were examined for their effects on the physiology, nutritive profile and subsequently on development and yield of maize crop with the normal growing environment.

MATERIALS AND METHODS

Biochar preparation

Anaerobic approach through heat degradation of organic material was used to prepare biochar (Pyrolysis) at the temperature of 350°C, which resulted in the production of biochar, bio-oil, and syngas (Kumar et al., 2013).

Preparation of inoculant

Bacillus sp. MN-54 was stored in a nutrient broth which was modified with 10% glycerol at -75°C and shifted on 10th strength tryptic soy agar i.e., 10 per cent TSA; then strains were selected and prepared in 10 per cent TSA. Bacterial culture was inoculated with the help of full bacterial loop and incubated for 72 h and 28°C at 100 revolutions per minute on **an** orbital shaker incubator (S300S, Firstek Scientific, Taiwan). The optical density (OD) was set at 0.5 and readings were recorded at 600 nm wavelength using spectrophotometer (Evolution-300LC, **Thermo** Electron Corporation, England) to obtain a consistent bacterial population (10^8 - 10^9 cfu.ml^{-1}) in nutrient broth right before its application.

Pot tests in the greenhouse

A pot trial was carried out at Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad (UAF) to examine the composite impact of bacterial strain *Bacillus* sp. MN-54 with organic amendments on the development and growth of maize plants. Bacterial inoculations were applied @ 20ml per pot; while cattle manure and biochar were applied @ 1% (w/w). Six seeds of maize variety Gauher-19 were sown in pot but only two plants per pot were maintained after germination.

Data collection

Data was collected through standard techniques during the whole duration of the experiment.

Agronomic parameters

Shoots fresh and dry weight

Shoot fresh weight was recorded soon after harvesting the crop and dry weight was measured after air-drying the sample, in an oven at 65ºC until constant weight, using an electrical balance (Chyo MJ-3000, YMC Co. Ltd, Japan).

Shoot length

Plant shoot length was measured at harvesting with the help of measuring scale, the shoot length of maize plants **was** recorded from bottom of the plant to the top portion.

Roots fresh and dry weight

Fresh weight of roots **was** recorded by removing the whole roots portion from pots. For three days, roots were dried in air and placed at 65ºC in an oven for one weak. After the complete drying of the sample, dry weight was recorded by using electric balance (Chyo MJ-3000, YMC Co. Ltd, Japan).

Physiological parameters

Measurement of photosynthetic rate, transpiration rate and stomatal conductance of plants

Physiological response of maize plants such as transpiration rate, photosynthetic activity, water use efficiency and stomatal conductance were recorded by using IRGA (LCA-4, Germany). These parameters were measured by selecting the fully matured and prolonged leaves at midday (Ben-Asher et al., 2006).

Measurement of relative water and chlorophyll contents

Total chlorophyll contents of maize plants were recorded by using chlorophyll meter i.e., (SPAD-502, Konica Mintola Sensing, Inc., Japan). While Relative water content (RWC) were measured by weighing a fresh leaf and incubated for 48 hrs. into the water at 4ºC and then again weighing the leaf. RWC was measured by using the formula of Mayak et al. (2004).

$$\text{RWC} = \frac{\text{Turgid weight} - \text{Dry weight}}{\text{Fresh weight} - \text{Dry weight}} \quad (1)$$

Assessment of leaf cell death of maize plant

The cell death was measured according to the method of (Jambunathan, 2010). Dust was removed by washing the leaves, then cut and autoclaved for 20 minutes at 120ºC. Electrical conductivity (EC1) and EC2 were measured before and after autoclaving the leaf sample, respectively using EC meter (Model 4070, Jenway Ltd., England). Electrolyte leakage (EL) of sampled leaves were determined by using the following formula:

$$\text{EL (\%)} = \frac{EC2 - EC1}{EC2} \times 100 \quad (2)$$

Chemical parameters

Plant analysis

Samples of shoots and roots were disintegrated in 2ml solution of sulphuric acid and 1ml solution of hydrogen peroxide. Maize plants were ground and digested by following the method of Wolf (1982).

Total Nitrogen, Phosphorous, Potassium

Nitrogen content was recorded by using the Kjeldahl apparatus. Phosphorous contents were recorded by treating a digested sample of 5ml with Barton reagents (10 ml) containing 25g of ammonium molybdate and 1.25g of ammonium meta-vanadate. Furthermore, to make volume up to 1 L, HNO_3 was added. Absorbance was recorded at 410nm after 30 minutes by using a spectrophotometer (Evolution-300LC, Thermo Electron Corporation, England) and values were read with the help of a standard curve. Potassium content of sampled leaves were measured by using flame photometer (PFP7, Cole-Palmer Ltd., England) and a standard curve was produced by following Ryan et al. (2001).

Statistical analysis

Statistically, data were analyzed using SPSS version 25 (IBM SPSS Statistics, USA) through analysis of variance (ANOVA). Results were considered significant where P-value ≤ 0.05.

RESULTS

Agronomic parameters

Shoot length

Shoot length is a key morphological indicator of plant growth under different climatic and edaphic conditions. In the present study, shoot length of Maize was significantly increased ($P \leq 0.05$) with the application of different treatments as shown in Table 1. Least shoot length of maize was observed with control whereas with bacterial inoculation, the shoot growth significantly increased to 102cm; but under biochar treatment lesser extent of shoot length i.e., 100cm was observed. Cattle manure having low pH helped the maize seedlings to achieve a relatively higher shoot length i.e., 106cm. Besides individual treatments, combined application of bacterial inoculation and low pH cattle manure significantly increased the shoot length to 118cm as compared to other treatments, where biochar and normal pH cattle manure was applied with bacterial inoculation.

Shoot fresh and dry weight

Analysis of variance presented a significant interaction of dry and fresh weight of maize with different treatments at $P \leq 0.05$, as shown in Table 1. Shoot dry and fresh weight improved with the application of different treatments as that of non-treated plants. Whereas among individual treatments, low pH cattle manure was found as one of the most effective treatment that improved the fresh and dry weight of shoot to 227g and 38g, as compared to 175g and 30g under control conditions, respectively. Bacterial inoculation alone tends to not affect the dry weight of maize shoots**,** but fresh weight was significantly higher. Low pH cattle manure and *Bacillus* sp. MN-54s combined application boosted up the shoot fresh weight to 232g and dry weight to 42g.

Root length

Roots are the first organ of the plant which initially intercepts any changes in growing media and alter their internal mechanisms to grow more deeply under different conditions. Analysis of variance also showed significant interactions among root length and different treatments at a probability level of less than 0.05 as shown in Table 1. Normal root growth was observed under control treatment which increased gradually when treated with bacterial inoculation, normal and low pH cattle manure and biochar i.e., 16cm, 17cm, 16cm and 16cm, respectively. As compared to the individual treatment, root length was higher when bacterial inoculation was applied with biochar and normal pH cattle manure i.e., 23cm. Moreover, a higher rate of root length (24cm) was observed, when low pH cattle manure was supplemented with inoculation of *Bacillus* sp. MN-54.

Table 1: Agronomic parameters of maize under the individual and combined influence of Bacillus sp. MN-54 and organic amendments

Treatment	S.L (cm)	R.L (cm)	S.F.W (g)	S.D.W (g)
Control	96^{d}	11.17^{c}	175.33^{b}	29.81^{d}
Bacillus sp. MN-54	102^{bcd}	16.87^{bc}	218.67^{a}	37.17^{c}
Biochar	100.33^{cd}	17^{abc}	220.67^{a}	37.51^{bc}
Low pH cattle manure	106^{abcd}	16^{c}	227.67^{a}	38.70^{abc}
Normal pH cattle manure	99^{cd}	16.7^{bc}	214.33^{a}	36.44^{c}
Bacillus sp. MN-54 + Biochar	117^{ab}	23.37^{ab}	229.67^{a}	41.68^{ab}
Bacillus sp. MN-54 + Low pH cattle manure	118.33^{a}	24.20^{a}	232.33^{a}	42.14^{a}
Bacillus sp. MN-54 + Normal pH cattle manure	113.33^{abc}	23.33^{ab}	225^{a}	40.20^{abc}

S.L = Shoot length; R.L = Root length; S.F.W = Shoot fresh weight; S.D.W = Shoot dry weight. Means sharing different superscript letters, within the column, differ significantly from each other at $P \leq 0.05$.

Table 2: Physiological parameters of maize under the individual and combined influence of Bacillus sp. MN-54 and organic amendments

Treatment	C.C	O.P	P.R (µmol m^{-2}s^{-1})	S.C (mmolm^{-2}s^{-1})	E.L (%)	W.U.E (µmol m^{-2}s^{-1})	T.R (mmol m^{-2}s^{-1})	PARW/ m^{2}	R.W.C (%)
Control	33^{d}	0.54^{e}	14.87^{c}	155.67^{c}	10.27^{a}	4.37^{d}	10.27^{d}	762.67^{c}	54^{c}
Bacillus sp. MN-54	42^{abc}	0.59^{de}	16.87^{bc}	168.33^{bc}	8.2^{b}	5.4^{cd}	15.57^{c}	899.67^{b}	65^{b}
Biochar	40^{bc}	0.59^{cde}	17.3^{bc}	169^{bc}	7.87^{bc}	5.42^{cd}	16.03^{bc}	902.33^{b}	64.67^{bc}
Low pH cattle manure	39^{bc}	0.62^{cde}	17.17^{bc}	170.67^{bc}	6.17^{d}	5.57^{bc}	15.77^{c}	904^{b}	67.33^{ab}
Normal pH cattle manure	37^{cd}	0.57^{de}	16.7^{bc}	173.33^{b}	6.57^{cd}	5.51^{c}	16.57^{bc}	906.67^{b}	67.33^{ab}
Bacillus sp. MN-54 + Biochar	46^{a}	0.66^{ab}	19.27^{ab}	191.67^{a}	4.1^{e}	6.61^{ab}	21.53^{a}	1075.33^{a}	75^{ab}
Bacillus sp. MN-54 + Low pH cattle manure	46.67^{a}	0.68^{a}	20.47^{a}	194^{a}	4.3^{e}	7.36^{a}	22.13^{a}	1091^{a}	76.67^{a}
Bacillus sp. MN-54 + Normal pH cattle manure	44^{ab}	0.65^{abc}	18.90^{ab}	191^{a}	4.07^{e}	6.84^{a}	20.67^{ab}	1081.33^{a}	75.33^{ab}

C.C = Chlorophyll Content; O.P = Osmotic Potential; P.R = Photosynthesis rate; S.C = Stomatal conductance; E.L = Electrolyte leakage; W.U.E = Water Use Efficiency; T.R = Transpiration rate; PAR = Photosynthetically active radiations; R.W.C = Relative water content. Means sharing different superscript letters, within the column, differ significantly from each other at $P \leq 0.05$.

Table 2: Chemical constituents of maize under the individual and combined influence of Bacillus sp. MN-54 and organic amendments

Treatment	Shoot nitrogen contents (ppm)	Shoots phosphorous contents (ppm)	Shoots potassium contents (ppm)
Control	0.16^{e}	0.98^{h}	3.17^{g}
Bacillus sp. MN-54	0.20^{cd}	1.34^{g}	4.14^{f}
Biochar	0.22^{bc}	1.54^{d}	4.69^{d}
Low pH cattle manure	0.24^{bc}	1.37^{f}	4.41^{e}
Normal pH cattle manure	0.21^{cd}	1.41^{e}	4.11^{f}
Bacillus sp. MN-54 + Biochar	0.30^{a}	2.09^{a}	6.49^{a}
Bacillus sp. MN-54 + Low pH cattle manure	0.28^{a}	1.99^{b}	6.37^{b}
Bacillus sp. MN-54 + Normal pH cattle manure	0.26^{ab}	1.85^{c}	6.01^{c}

Means sharing different superscript letters, within the column, differ significantly from each other at $P \leq 0.05$.

Physiological parameters

Photosynthesis rate and Chlorophyll content

Photosynthesis rate and Chlorophyll content are intermingled growth indicators of plants. Higher stability rate of chlorophyll content is responsible for the improved rate of photosynthesis activity. Analysis of variance also showed a significant interaction between chlorophyll content and photosynthesis rate with different treatments at the $P \leq 0.05$ as data is presented in Table 2. In comparison with control treatment, chlorophyll content and photosynthesis rate of maize was increased with individual treatment of bacterial species, biochar, and cattle manure with low and normal pH. Among all individual treatments, a higher rate of chlorophyll content at 42 SPAD-value was observed with bacterial inoculation whereas improved photosynthesis rate was observed with individual biochar treatment i.e., 17.3. Beside individual treatments, composite application of low pH cattle manure and *Bacillus* sp. MN-54 significantly increased ($P \leq 0.05$) the chlorophyll content to a SPAD-value of 47, which subsequently enhanced the photosynthetic rate to 20.47 µmol m^{-2}s^{-1}.

Electrolyte leakage and relative water content

Electrolyte leakage usually **occur** in leaf cells due to the less stability of cellular membrane and this situation results in the more loss of water content from the leaf and shoots cells. A positive relation was observed between electrolyte leakage, relative water content and different treatment at $P \leq 0.05$, data is presented in Table 2. Electrolyte leakage was observed to be increased when treated with *Bacillus* sp. MN-54 inoculation i.e., 8.2%, while lowest was observed with individual treatment of low pH cattle manure i.e., 6.17%. Furthermore, higher relative water content was showed when treated with cattle manure either at the normal or low pH. Composite treatment in the form of normal pH cattle manure with *Bacillus* sp. MN-54 effectively reduced the excessive leakage of electrolyte by 4.07% from the cellular compartments. This reduced level of electrolyte leakage subsequently helped the shoot and leaf cells to maintain a higher level of relative water content when bacterial inoculation was supplemented with cattle manure either at lower pH by 76.67% and high pH by 45.33%, over control plants.

Transpiration rate and Stomatal conductance

Transpiration rate and Stomatal conductance are physiological mechanisms of a plant which prevent the cellular dehydration and wilting. As stomatal conductance increases the rate of transpiration also increases which indicate the ability of plant to efficiently perform their metabolic activities under different treatments. Analysis of variance showed a significant interaction of different treatments with stomatal conductance and transpiration rate at a $P \leq 0.05$, as data shows in Table 2. In all individual treatments, a higher rate of stomatal conductance and transpiration rate was exhibited by cattle manure having normal pH i.e., 173 and 16.57 mmol $m^{-2}s^{-1}$, respectively. Rate of stomatal conductance and transpiration rate increased gradually in and reached at their peak level i.e., 194 and 22.13 mmol $m^{-2}s^{-1}$, respectively when treated with low pH cattle manure and *Bacillus* Sp. MN-54. Contrarily least rate of stomatal conductance and transpiration rate was found for with inoculation of *Bacillus* sp. MN-54 treatment.

Osmotic potential and water use efficiency

Water use efficiency of maize increased with the increased osmotic potential in leaf and shoots. Analysis of variance showed a significant interaction of osmotic potential and water use efficiency with different treatments as compared to their control at $P \leq 0.05$, whereas data is presented in Table 2. Results indicate that as osmotic potential decreased the water use efficiency of maize also decreased therefore linear positive correlation was found between both physiological responses. Lower rate of osmotic potential was observed for cattle manure with normal pH treatment i.e., 0.57 which gradually increased when treated with low pH cattle manure, biochar, and *Bacillus* sp. MN-54 i.e., 0.59, 0.59 and 0.62 respectively. Apart from these, the osmotic potential was noted at their peak level when cattle manure of low pH was supplemented with bacterial inoculation followed by normal pH cattle manure and biochar combination.

Photosynthetic active radiations

Photosynthetically active radiation (PAR) is a physiological process of plants which exhibited the light absorption ability of leave and food preparation capabilities of plants under daylight conditions. PAR activity of plants can be stimulated by different compounds which can take part in growth or scavenging of different oxygen radicals. A significant interaction of PAR activity with different treatments was observed at $P \leq 0.05$, as shown in Table 2. Higher rate of PAR activity (1081 W/m^2) was exhibited by maize when treated with cattle manure having normal pH and *Bacillus* sp. MN-54. Whereas results also revealed that individual treatments did not effectively regulate the PAR activity but treatment in composite form has a clear positive effect on PAR activity of maize plants.

Chemical parameters

Nitrogen, phosphorus, and potassium contents

Nitrogen, phosphorus, and potassium are essential macronutrients required for plant growth. Availability of these nutrients helps the plant to grow more vigorously and at the same time, they directly or indirectly take part in all morphological, physiological, and biochemical responses of plants. Mean values represented here in table 3 showed a significant interaction of these nutrients with different treatments at $P \leq 0.05$, data being presented in Table 3. Uptake of one nutrient had a synergistic effect with other nutrients and their availability increased with the increment of any other nutrient. Least rate of nitrogen uptake was observed in maize i.e., 0.20 ppm when treated with *Bacillus* sp. MN-54 which ultimately caused less uptake of phosphorus (1.34 ppm) and potassium (4.14 ppm). The concentration of nitrogen, phosphorus and potassium was increased with different treatments and reached their highest level when treated with a combination of biochar and *Bacillus* sp. MN-54 i.e., 0.30 ppm, 2.09 ppm and 6.49 ppm, respectively.

DISCUSSION

Investigating the underlying mechanism of PGPRs for improving crop production is an important phenomenon in agricultural sustainability. PGPRs are known for promotion of growth when added to seeds, roots or any other plant parts (Akinrinlola et al., 2018). The focus in optimizing the use of PGPRs should be the characterization of crop species-specific PGPRs, as this study shows the beneficial effects of *Bacillus* sp. MN-54 on the maize plants. Treatments with the combination of low pH cattle manure and *Bacillus* sp.

MN-54 improved the growth, physiological and nutritive parameters of the maize as that for non-treated plants. This advancement of the different parameters shown in this experiment is considered to be the result of an increase in growth-promoting hormones especially gibberellin, which is responsible for the activity of other growth and physiology promoting factors (Gholami et al., 2009). Maize plants inoculated with the *Bacillus* sp. MN-54 were observed taller than non-inoculated control plants. Biochar could improve soil properties such as pH and CEC (Topoliantz et al., 2002). Biochar application also influences soil pH, EC, organic carbon, nitrogen and phosphorous and in return improve the plant growth (Nigussie et al., 2012). The use of biochar with biofertilizers improved the physiology and growth of maize and showed the second-best performance for certain observed parameters. Bacillus sp. improved the root and shoot length of maize significantly either with biochar or cattle manure. Many researchers have reported the increase in various crops root length by bacteria (Vessey, 2003). This root length improvement is advantageous for the host system and root development, improved root penetration, surface area and nutrient absorption (Vasudevan et al., 2002). Higher nutrient availability and usage of those nutrients by plants tend to improve the performance of inoculated plants observed during the pot experiment (Cakmakcı et al., 2001; Ozturk et al., 2003) where the wheat yield was increased because of inoculation. These phenomena can also be the effect of plant growth-promoting hormones being produced by *Bacillus* sp. MN-54 colonizing the root (Hayat et al., 2010). Besides the beneficial role of cattle manure, biochar and *Bacillus* sp. MN-54 in this study, cattle manure and biochar may have provided many more nutrients adding to the nutrient profile of the soil and beneficial to plants as higher macronutrient profile was observed in soils being cured by biochar and cattle manure (Liang et al., 2006; Solomon et al., 2007). Compatibility of our bacterial strain *Bacillus* sp. MN-54 lies in its ability to be a competent colonizer of rhizosphere and growth-promoting hormones production. Enhancement in the nutritive profile can also be ascribed to all three of the treatments as it increases the availability of macronutrients, which is well reported after biochar application (Lehman et al., 2003; Jockovic et al., 2010). Nigussie et al. (2012) observed enhanced nitrogen, phosphorous and potassium uptake in *Lactuca Sativa* after addition of biochar. Linu et al. (2009) and Uzoma et al. (2011) also observed improved nutrient uptake after soil treatment with biochar. There are several studies available investigating the impact of low and normal pH cattle manure, biochar, and *Bacillus* sp. MN-54 but there is no study available at the moment to ascertain the cumulative effect of the above-mentioned treatments apart from our previous study related to the effect of these same treatments in imparting the chromium toxicity tolerance to the maize plants (Abubakar et al., 2020). It was observed that cattle manure, biochar, and *Bacillus* sp. MN-54 possess the ability to increase the overall yield and growth parameters of maize plants, proving to be vital for sustainable agriculture. The treatment of maize plants with low pH cattle manure and *Bacillus* sp. MN-54 performed better than other treatments due to the accumulation of plant growth improving factors and improvement of soil nutrient profile by cattle manure and providing optimum pH for *Bacillus* sp. MN-54 colonization. However, there is a need for identification, isolation, characterization, and commercialization of more rhizobacterial species for sustainable use of biofertilizers in future.

Acknowledgments
This research was supported by Kyungpook National University Development Project Research Fund, 2018.

Authors' contribution

MA, MN, ZA, and SAC conceived, designed, and conceptualized the study. MA and MN performed the main experiment and measured the parameters. ASK, FN, SHY and MA wrote the original manuscript and did the statistical analysis. ASK, FN, SHY, HYP, and CHK helped with manuscript preparation, review, editing, data analysis and manuscript submission. MN, ZA and SAC supervised the work. All authors have read and approved the final manuscript.

REFERENCES

Abubakar M, M Naveed, Z Ahmad, SA Cheema, AS Khan, HY Park and CH Kwon, 2020. Ameliorative Effect of Bacillus sp. MN-54 and Organic Amendments Combination on Maize Plants Growth and Physiology Under Chromium Toxicity. Journal of Agricultural Science, 12: 39-48.

Akinrinlola RJ, GY Yuen, RA Drijber and AO Adesemoye, 2018. Evaluation of Bacillus strains for plant growth promotion and predictability of efficacy by in vitro physiological traits. International Journal of Microbiology, 2018: 101-109.

Arzanlou M, S Mousavi, M Bakhshi, R Khakvar and A Bandehagh, 2016. Inhibitory effects of antagonistic bacteria inhabiting the rhizosphere of the sugarbeet plants, on Cercospora beticola Sacc., the causal agent of Cercospora leaf spot disease on sugarbeet. Journal of Plant Protection Research, 56: 6-14.

Asari S, D Tarkowska, J Rolcik, O Novak, DV Palmero, S Bejai and J Meijer, 2017. Analysis of plant growth-promoting properties of *Bacillus amyloliquefaciens* UCMB5113 using

Arabidopsis thaliana as host plant. Planta, 245: 15-30.

Atkinson CJ, JD Fitzgerald and NA Hipps, 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant and Soil, 337: 1-18.

Badu-Apraku B and C Yallou, 2009. Registration of Striga-resistant and drought-tolerant tropical early maize populations TZE-W Pop DT STR C4 and TZE-Y Pop DT STR C4. Journal of Plant Registrations, 3: 86-90.

Ben-Asher J, I Tsuyuki, B-A Bravdo and M Sagih, 2006. Irrigation of grapevines with saline water: I. Leaf area index, stomatal conductance, transpiration and photosynthesis. Agricultural Water Management, 83: 13-21.

Berg G, 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology, 84: 11-18.

Cakmakci R, F Dönmez, A Aydın and F Şahin, 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biology and Biochemistry, 38(6):1482-1487.

Cakmakcı R, F Kantar and F Sahin, 2001. Effect of N_2-fixing bacterial inoculations on yield of sugar beet and barley. Journal of Plant Nutrition and Soil Science, 164: 527-531.

Chan KY, L Van Zwieten, I Meszaros, A Downie and S Joseph, 2008. Agronomic values of greenwaste biochar as a soil amendment. Soil Research, 45: 629-634.

Das K, V Katiyar and R Goel, 2003. ‘P’solubilization potential of plant growth promoting Pseudomonas mutants at low temperature. Microbiological Research, 158: 359-362.

Dhawi F, R Datta and W Ramakrishna, 2015. Mycorrhiza and PGPB modulate maize biomass, nutrient uptake and metabolic pathways in maize grown in mining-impacted soil. Plant Physiology and Biochemistry, 97: 390-399.

Enders A, K Hanley, T Whitman, S Joseph and J Lehmann, 2012. Characterization of biochars to evaluate recalcitrance and agronomic performance. Bioresource Technology, 114: 644-653.

Gholami A, S Shahsavani and S Nezarat, 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Academy of Science, Engineering and Technology, 49: 19-24.

Glaser B, J Lehmann and W Zech, 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal–a review. Biology and Fertility of Soils, 35: 219-230.

Glick BR, 1995. The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology, 41: 109-117.

Hariadi YC, AY Nurhayati and P Hariyani, 2016. Biophysical monitoring on the effect on different composition of goat and cow manure on the growth response of maize to support sustainability. Agriculture and Agricultural Science Procedia, 9: 118-127.

Hayat R, S Ali, U Amara, R Khalid and I Ahmed, 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Annals of Microbiology, 60: 579-598.

Jambunathan N, 2010. Determination and detection of reactive oxygen species (ROS), lipid peroxidation and electrolyte leakage in plants, Plant stress tolerance. Springer. pp: 291-297.

Jockovic D, M Stojakovic, M Ivanovic, G Bekavac, R Popov and I Dalovic, 2010. NS maize hybrids: Today and tomorrow. Ratarstvo i Povrtarstvo, 47: 325-333.

Jones D, J Rousk, G Edwards-Jones, T DeLuca and D Murphy, 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biology and Biochemistry, 45: 113-124.

Kejela T, VR Thakkar and P Thakor, 2016. *Bacillus* species (BT42) isolated from *Coffea arabica* L. rhizosphere antagonizes *Colletotrichum gloeosporioides* and *Fusarium oxysporum* and also exhibits multiple plant growth promoting activity. BMC Microbiology, 16: 1-13.

Kim S-I, JS Kwak, JT Song and HS Seo, 2016. Long-term effect of niclosamide on inhibition of bacterial leaf blight in rice. Journal of Plant Protection Research, 56: 323-327.

Kumar S, RE Masto, LC Ram, P Sarkar, J George and VA Selvi, 2013. Biochar preparation from Parthenium hysterophorus and its potential use in soil application. Ecological Engineering, 55: 67-72.

Lehman J, J Pereira da Silva, C Steiner, T Nehls, W Zech and B Glaser, 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil, 249: 343-357.

Lehmann J, MC Rillig, J Thies, CA Masiello, WC Hockaday and D Crowley, 2011. Biochar effects on soil biota–a review. Soil Biology and Biochemistry, 43: 1812-1836.

Liang B, J Lehmann, D Solomon, J Kinyangi, J Grossman, B O'Neill, JO Skjemstad, J Thies, FJ Luizão, J Petersen and EG Neves, 2006. Black Carbon Increases Cation Exchange Capacity in Soils. Soil Science Society of America Journal, 70: 1719-1730.

Linu MS, J Stephen and MS Jisha, 2009. Phosphate solubilizing *Gluconacetobacter* sp., *Burkholderia* sp. and their potential interaction with cowpea (*Vigna unguiculata* (L.) Walp.). International Journal of Agricultural Research, 4: 79-87.

Lyimo H, R Pratt and R Mnyuku, 2012. Composted cattle and poultry manures provide excellent fertility and improved management of gray leaf spot in maize. Field Crops Research, 126: 97-103.

Mayak S, T Tirosh and BR Glick, 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiology and Biochemistry, 42: 565-572.

Mohan D, CU Pittman Jr, M Bricka, F Smith, B Yancey, J Mohammad, PH Steele, MF Alexandre-Franco, V Gómez-Serrano and H Gong, 2007. Sorption of arsenic, cadmium, and lead by chars produced from fast pyrolysis of wood and bark during bio-oil production. Journal of Colloid and Interface Science, 310: 57-73.

Mohanty SR, G Dubey and B Kollah, 2017. Endophytes of Jatropha curcas promote growth of maize. Rhizosphere, 3: 20-28.

Mrkovacki N and D Bjelic, 2011. Plant growth promoting rhizobacteria (PGPR) and their effect on maize. Ratarstvo i povrtarstvo, 48: 305-312.

Nezarat S and A Gholami, 2009. Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize. Pakistan Journal of Biological Sciences, 12: 26-32.

Nigussie A, E Kissi, M Misganaw and G Ambaw, 2012. Effect of biochar application on soil properties and nutrient uptake of lettuces (Lactuca sativa) grown in chromium polluted soils. American-Eurasian Journal of Agricultural & Environmental Sciences, 12: 369-376.

Ozturk A, O Caglar and F Sahin, 2003. Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilization. Journal of Plant Nutrition and Soil Science, 166: 262-266.

Ryan J, G Estefan and A Rashid, 2001. Soil and plant analysis laboratory manual. ICARDA, Aleppo, Syria.

Sohi S, E Lopez-Capel, E Krull and R Bol, 2009. Biochar's roles in soil and climate change: A review of research needs. CSIRO Land and Water Science Report, 5: 1-57.

Sohi SP, E Krull, E Lopez-Capel and R Bol, 2010. A review of biochar and its use and function in soil. Advances in Agronomy, 105: 47-82.

Solomon D, J Lehmann, J Thies, T Schäfer, B Liang, J Kinyangi, E Neves, J Petersen, F Luizão and J Skjemstad, 2007. Molecular signature and sources of biochemical recalcitrance of organic C in Amazonian Dark Earths. Geochimica et Cosmochimica Acta, 71: 2285-2298.

Souza RD, A Ambrosini and LM Passaglia, 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and Molecular Biology, 38: 401-419.

Steiner C, WG Teixeira, J Lehmann, T Nehls, JLV de Macêdo, WE Blum and W Zech, 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant and Soil, 291: 275-290.

Topoliantz S, J-F Ponge, D Arrouays, S Ballof and P Lavelle, 2002. Effect of organic manure and the endogeic earthworm Pontoscolex corethrurus (Oligochaeta: Glossoscolecidae) on soil fertility and bean production. Biology and Fertility of Soils, 36: 313-319.

Uzoma K, M Inoue, H Andry, H Fujimaki, A Zahoor, and E Nishihara, 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use and Management, 27: 205-212.

Vasudevan P, MS Reddy, S Kavitha, P Velusamy, RSD Paul Raj, SM Purushothaman, VB Priyadarisini, S Bharathkumar, JW Kloepper, and SS Gnanamanickam, 2002. Role of biological preparations in enhancement of rice seedling growth and grain yield. Current Science, 83: 1140-1143.

Verheijen F, S Jeffery, A Bastos, M Van der Velde and I Diafas, 2010. Biochar application to soils. A critical scientific review of effects on soil properties, processes, and functions. EUR 24099 EN, European Commission, Luxembourg.

Vessey JK, 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 255: 571-586.

Wolf B, 1982. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. Communications in Soil Science and Plant Analysis, 13: 1035-1059.

Wu S, Z Cao, Z Li, K Cheung and MH Wong, 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma, 125: 155-166.