Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2023.122.129

Research Article
Effects of Rhizobacteria on Agronomic Traits of Two *Gossypium hirsutum* Cotton Varieties

[1]Sardor E. Narmatov, [1]Mukhtor M. Darmanov, [2]Gulnara I. Djumaniyazova, [2]Khurshida S. Narbaeva, [1,3,4]Akmal M. Asrorov, [1]Naim N. Khusenov, [1]Rakhim R. Akhmedov and [1]Zabardast T. Buriev

[1]Center of Genomics and Bioinformatics, Academy of Sciences of Uzbekistan, University Street 2, Tashkent 111215, Uzbekistan
[2]Tashkent State Technical University, University Street 2, Tashkent 100095, Uzbekistan
[3]Institute of Bioorganic Chemistry, Academy of Sciences of Uzbekistan, Tashkent 100047, Uzbekistan
[4]Ajou University in Tashkent, 113A Asalobod Street, Tashkent 100204, Uzbekistan

Abstract

Background and Objective: Stimulation of plant growth by rhizobacteria is a part of agricultural practice. Its enhancement leads to significant improvement in plant biology. In this study, we studied the effect of a mixture of salt-tolerant rhizobacteria containing strains KY041889, KY041694, KY041978 and KY041979 on the growth of two cotton varieties: Porloq-4 and Ravnaq-1. **Materials and Methods:** Experimental plants were grown in three different places in a plot and their agronomic traits, as well as fibre traits, were compared following the seed treatment with bacterial strains. **Results:** Indicators such as plant height, number of sympodia, buds and bolls and seed weight significantly improved after treatment. Bacterial treatment accelerated plant germination and flowering. No negative effects were determined on fibre quality in both varieties. Enhancement was defined in seed weight following the treatment with rhizobacteria but not in the total weight of fibre. **Conclusion:** Isolated rhizobacteria were found to stimulate plant growth which was explained by enhanced levels of agronomic traits such as several buds, flowers, bolls and sympodia.

Key words: Cotton plant, rhizobacteria, Porloq-4, Ravnaq-1, agronomic traits, chlorophyll content, plant growth

Citation: Narmatov, S.E., M.M. Darmanov, G.I. Djumaniyazova, K.S. Narbaeva, A.M. Asrorov *et al*., 2023. Effects of rhizobacteria on agronomic traits of two *G. hirsutum* cotton varieties. Asian J. Plant Sci., 22: 122-129.

Corresponding Author: Akmal M. Asrorov, Center of Genomics and Bioinformatics, Academy of Sciences of Uzbekistan, University Street 2, Tashkent 111215, Uzbekistan

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Beneficial microorganisms are expected to promote plant growth. Many of them deserved naming Plant Growth-Promoting Rhizobacteria (PGPR). Observations explain the potential role of PGPR in agriculture[1]. They are considered beneficial due to their role in plant-water interactions as well as plant nutritional status that can contribute to the development of defensive mechanisms[2]. The PGPRs proliferate in the plants' rhizosphere, support nitrogen fixation and thus cause positive effects on root growth and morphology. This mechanism of action enables them to be used as biofertilizers[3].

The PGPR are also effective against nematodes such as *Meloidogyne incognita*. Xiang *et al.*[4] reported 212 strains of rhizobacteria that were effective against this nematode. Among these strains, *Bacillus* was the major genus that supported the cotton plant growth resulting from the reduced population of *M. incognita*. Rhizobacteria isolate obtained from the roots of the cotton plant was found to reveal detrimental effects to *Helicoverpa armigera* in combination with chitin. The formulation based on *Bacillus subtilis* and chitin caused larval and pupal mortality. Bolls treated with Rhizobacteria showed antifeedant effects on the larvae[5]. But the main plant benefit from rhizobacteria belongs to their development. *Bacillus subtilis* isolates were suggested to represent plant-growth-promoting effects due to their effects on root and shoot dry mass and the crop yield[6].

Rhizobacteria are also expected to respond to a high quantity of metals in soil. Ren *et al.*[7] demonstrated increased availability of Cu under the effects of metal-resistant rhizobacteria. *Pseudomonas thivervalensis* Y1-3-9 and *Burkholderia cepacia* J62 strains increased water-soluble Cu by 10 and 42%, respectively, in Cu-polluted soil. Ascorbic acid and glutathione remarkably increased in rape leaves, grown in soil treated with them and reduced oxidative stress by metals.

These microorganisms support the assimilation of phosphate ions by improving solubility[8]. The assimilation of phosphorus happens in a form of phosphates. Some PGPR are considered to increase the solubility of phosphates and thus promote their assimilation by plants[9,10] Enhanced levels of nitrogen fixation and indole-3-acetic acid (IAA) are other mechanisms involved in plant-microbe synergy[11,12]. Nitrogen absorbance from the soil by plants is carried as nitrates and ammonium ions, but a nitrate ion is a predominant form that is assimilated by plants[13]. Bacteria belonging to Azotobacter and Azospirillum genera, isolated from the rhizosphere of the cotton plant, were found to promote plant growth that was characterized by nitrogenase activity[14].

The PGPR are known to produce auxins, plant growth-regulating hormones[15,16] and influence root growth[17]. Auxin-producing PGPR was shown to elicit changes in transcripts of hormones, cell wall and defence-related genes[18]. The amount of bacteria in the rhizosphere is 10-1000 times greater than in bulk soil[19]. Phytohormones, plant growth regulators working at low concentrations[20], can be induced in plants by PGPR. A common group of phytohormones plays role in the overproduction of lateral roots and root hairs[21]. Microbes in the soil improve soil organic matter and thus reduce the need for fertilizers[22]. In general, these beneficial microbes, termed PGPR, support the assimilation of organic matter by plants[23].

The treatment of plants with PGPR is expected to lead to several benefits-pH normalization of alkali salinized soils, the balance of soil microbial community in favour of beneficial microorganisms, the balance of nutritional elements in forms that are accessible to plants, a decrease of levels of salinization in saline soils, an increase of fertility of degraded soils. All these features lead to plant growth and improved crop yield.

Plants and bacteria assimilate organic compounds from the soil in a mutualistic manner. Symbiotic soil microorganisms living in the rhizosphere of many plant species have different beneficial effects on plants through various mechanisms such as enhancing chlorophyll content and leaf surface area[24]. The PGPR are involved in different activities and contribute to sustainable crop production[16] by colonizing the plant roots system. In this study, the effects of rhizobacteria were studied that reached the market under the name Rizokom, containing four salt-tolerant strains, on agronomic traits and fibre quality of two cotton varieties, widely grown in Uzbekistan. The chlorophyll content was linked with plant growth parameters.

MATERIALS AND METHODS

Study area: The field experiments were carried out in 2020 and 2021 in the field experimental area of the Center of Genomics and Bioinformatics of the Academy of Sciences of Uzbekistan (41°22'49.2"N and 69°20'26.6"E).

Growing bacterial strains: The active salt-tolerant strains of rhizobacteria, studied in this work, were found to belong to *Bacillus subtilis BS-80* (KY041889), *Bacillus licheniformis BL-83* (KY041694), *Paenibacillus polymyxa PP-113* (KY041978) and *Paenibacillus amylolyticus PA-118* (KY041979). These bacterial strains were produced as bio-preparation Rizokom.

Research objects: Porloq-4 and Ravnaq-1 cotton varieties of *G. hirsutum* were selected as the object of research. Porloq-4 cotton variety was created based on mutual genetic hybridization of Namangan-77 variety with phytochrome A1 gene-attenuated RNAi Coker-312 line using RNA interference[25].

Ravnaq-1 cotton cultivar derived through MAS technology by selecting homozygous samples among the BC5F5 (Andijan-35×L-141) segregation hybrids using the BNL1604 marker that is associated with fibre length and strength[26].

Seed treatment with bacterial preparation: The seeds of Porloq-4 and Ravnaq-1 varieties were sprayed with bacterial preparation (cell titer 7×10^8 CFU mL^{-1}) at room temperature for 8 hrs (stirring every hour). Control seeds were immersed in distilled water, respectively.

Field experiments: The field experiments were carried out for years, in 2020 and 2021. The seeds were sown in three different places in the plot (rows length 10 m, the total number of nests 30). In total 90 plants were studied in each sample. Phenotypic changes were observed. In this study, agronomic characteristics such as seed germination, number of sympodia, flowers and buds in cotton plants, plant height and number of harvested bolls were studied. In addition, raw cotton was collected from each of the research plants. Laboratory analyzes were performed to study the effect of the bacterial preparation on quantitative characteristics such as the cotton fibre length, yield and weight as well as the weight of seeds.

Chlorophyll analysis: The chlorophyll content was determined using the Chlorophyll meter SPAD502 Plus mobile tool. During the early-flowering phase when buds developed, chlorophyll content was studied at the area of 2×3 mm. In early June, a set of 120 samples, 40 plants in three plots, were chosen for each case.

Statistical analysis: Statistical analyses were performed using NSCC-2022 software. Significant differences were calculated using Tukey-Kramer's test.

RESULTS AND DISCUSSION

The effects of bio-preparation on the growth and development of cotton plants and agronomic traits were studied in the field. Observations showed that seed germination in samples treated with Rizokom bio-preparation resulted in germination of seeds 2 days earlier compared to control. Following the bacterial treatment, seed germination made 99 and 100% in Porloq-4 and Ravnaq-1 varieties, respectively, whereas, 94-95% germination efficacy was observed in their controls for 12 days (Fig. 1a-b). Two years results revealed significantly enhanced levels between controls and treated samples ($p<0.005$). Rizokom treatments led to no significant differences in both varieties when the 2020 and 2021 year results were compared ($p>0.05$).

Similar effects were observed in the number of flowers in both varieties in 2020 (Fig. 1c-d). But 2021 results in Porloq-4 variety demonstrated sharp increases both in control and treated samples (Fig. 1c). The delay in the number of flowers in the Ravnaq-1 variety in 2021 can be explained by a similar tendency observed in the number of opened bolls (Fig. 1d-e). The effects of bio-preparation on the number of opened bolls led to significant results between controls and the treatments in 2020 and 2021 ($p<0.05$) (Fig. 1e-f). Between the results of 2020 and 2021 of controls and treatments in both varieties, no significant differences were observed ($p>0.05$). Several opened bolls counted on 104, 110 and 116th days of the sowing showed a similar tendency in both varieties in 2020 and 2021 (Fig. 1).

The significant differences were observed in 2021 in both cotton varieties (Fig. 2a). The treatment resulted in 7 and 15 cm differences in the plant height in Porloq-4 and Ravnaq-1 varieties, respectively (Fig. 2a). The treatment with Rizokom bio-preparation significantly increased the number of buds (Fig. 2b) and sympodia (Fig. 2c), in both varieties in 2020. Greater differences were observed in all cases in 2021 in both varieties. The most remarkable difference was found in the number of bolls following the treatment of Porloq-4 samples with rhizobacteria that the level made a 69% greater mean value in 2021 (Fig. 2d). Possibly this phenomenon is attributed to favourable weather in 2021. In 2020, no differences were observed in the plant height following the bacterial treatment.

Cotton samples collected for laboratory analysis were studied for valuable economic characteristics, such as cotton weight in a boll (Fig. 3a), seed weight (Fig. 3b), lint percentage (Fig. 3c) and fibre staple length (Fig. 3d). No significant differences in these parameters were found in all cases. The weight of 1000 seeds was similar in control and treated samples in 2020. More favourable weather led to greater differences in 2021 (Fig. 3b).

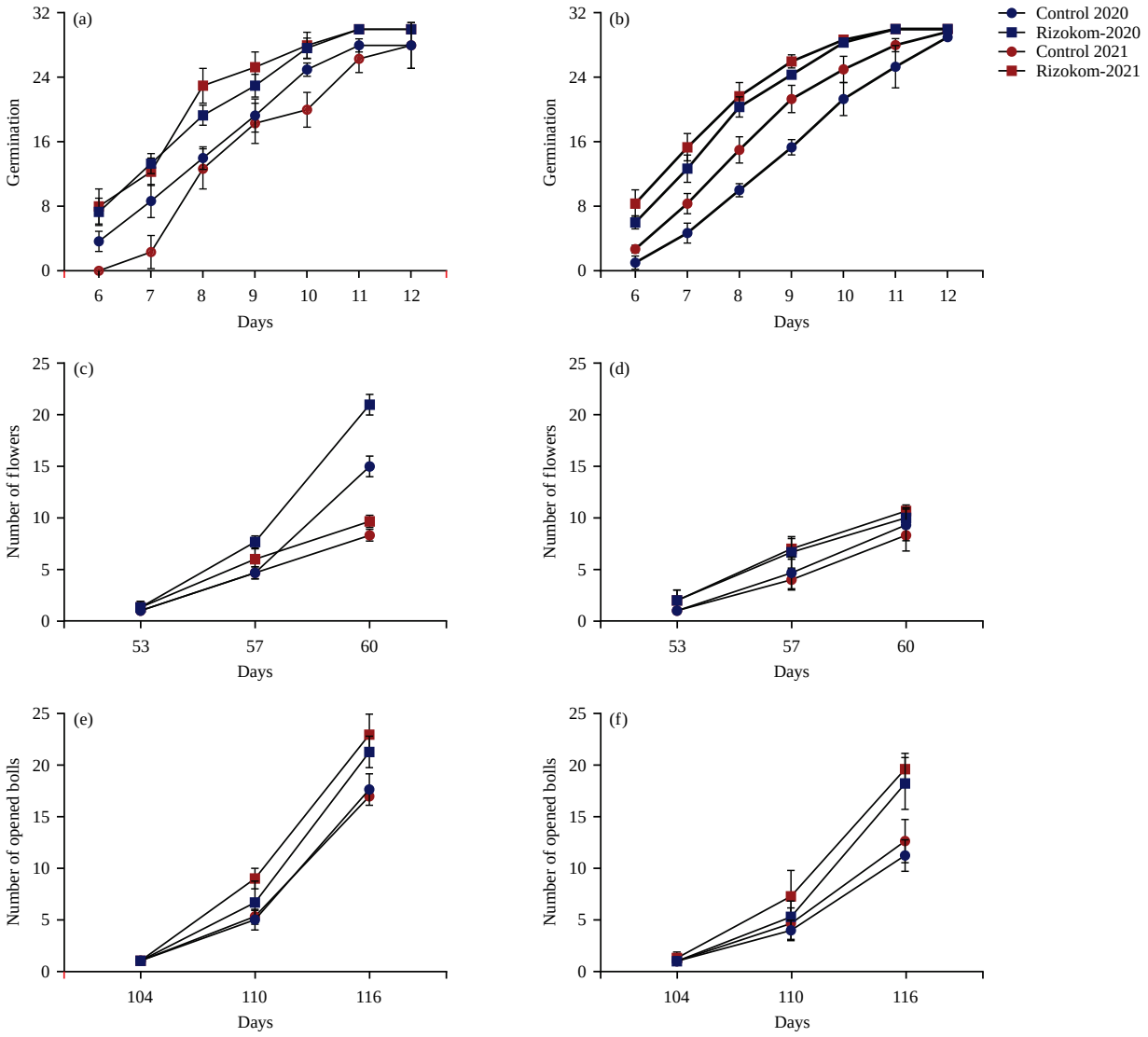

Fig. 1(a-f): Effects of rhizobacteria on agronomic traits of (a, c, e) Porloq-4 and (b, d, f) Ravnaq-1 varieties
Days were calculated after sowing in both year studies

Obtained data in this study were in correspondence with other results. Rhizobacteria were reported to increase plant height, boll weight and seed cotton yield. These effects were even greater when PGPR was applied in combination with diammonium phosphate[27]. Similar results were reported with nitrogen. The combination of PGPR with nitrogen led to significant increases in the microbial population, plant growth and cotton yield[28,29]. These results explain that benefits from PGPR could be enhanced in soil rich in organic residues.

Chlorophyll content, studied in 120 plant samples in each case, revealed their enhanced levels in treated plants (Fig. 4a-b). Though the enhancement level was insignificantly different from the controls by comparison based on Tukey's criteria (Fig. 4c), these results are in correspondence with other results. In the Ravnaq-1 variety, the effects of the rhizobacteria treatment were greater than in Porloq-4, that the mean value made 45.32 (Ravnaq-1) and 44.06 (Porloq-4) while mean values in controls were ~42, respectively (Fig. 4c). Similar results in the effects of Rizokom were observed in the number of sympodia, buds and plant height of Ravnaq-1 and Porloq-4 varieties.

Changes in the chlorophyll content in this study were in correspondence with those observed by Diaz *et al*.[6] *Bacillus* species *B. subtilis*, *B. velezensis* and *B. amyloliquefaciens* did not cause significant differences in chlorophyll content in cotton leaves 21 days after sowing grown in greenhouse

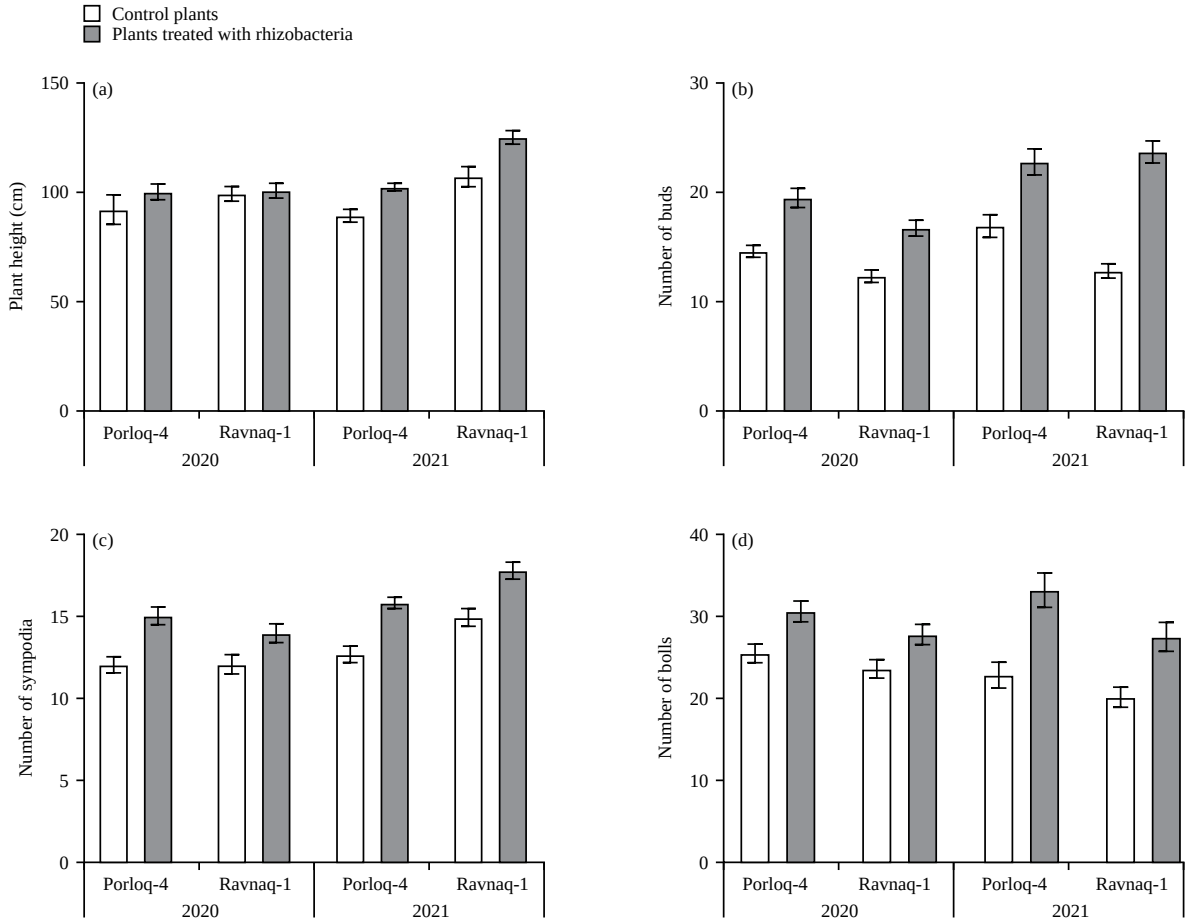

Fig. 2(a-d): Agronomic traits in cotton varieties following the treatment with rhizobacteria, (a) Number of buds were calculated for 18 days after sowing, (b) Plant height was studied at the beginning of August once and (c-d) Number of sympodia and bolls were calculated once in the middle of August

condition. Significant differences in chlorophyll content resulting from *B. megaterium* and isolate of *Enterobacter* sp., were detected in okra plants grown under salt stress[30]. Similar results under salt stress were observed in rice by 1-aminocyclopropane-1-carboxylic acid deaminase-producing PGPR[31]. The effects of rhizobacteria on the chlorophyll content are likely more remarkable under stress conditions.

The bio preparation Rizokom includes four active salt-tolerant strains of cotton rhizobacteria. The active strains of rhizobacteria (KY041889, KY041694, KY041978, KY041979) have polyfunctional properties such as tolerance to high concentrations of chloride and sulphate salts, ability to dissolve hardly accessible minerals and organic compounds, growth-stimulating and root-forming activity. They were also able to reveal antagonistic activity towards cotton phytopathogens: *V. dahliae*, *A. alternata*, *R. solani*, *F. oxysporum*, *F. oxysporum* f.sp. *vasinfectum R-7, F. oxysporum* f.sp. *vasinfectum 316* Ba *F. solani 319*. In the earliest investigations, rhizobacteria, colonized in cotton seedlings, were found to possess antifungal activity. Bacterial isolates showed high activity against *Pythium ultimum* and *Rhizoctonia solani in vitro*. Actinomycetes and Pseudomonas revealed the highest antifungal activity against these pathogens among gram-positive and gram-negative bacterial isolates[32]. *Brevibacillus brevis* SVC(II)14 was demonstrated as another bacterium that results in a beneficial impact on the cotton plant. Its effects were linked with phosphate solubilization, production of IAA, reduction of acetylene and antifungal activity[33].

In the future, we aim to study the effects of bio preparation on early discussed biochemical parameters to link with improved agrochemical traits in cotton plants.

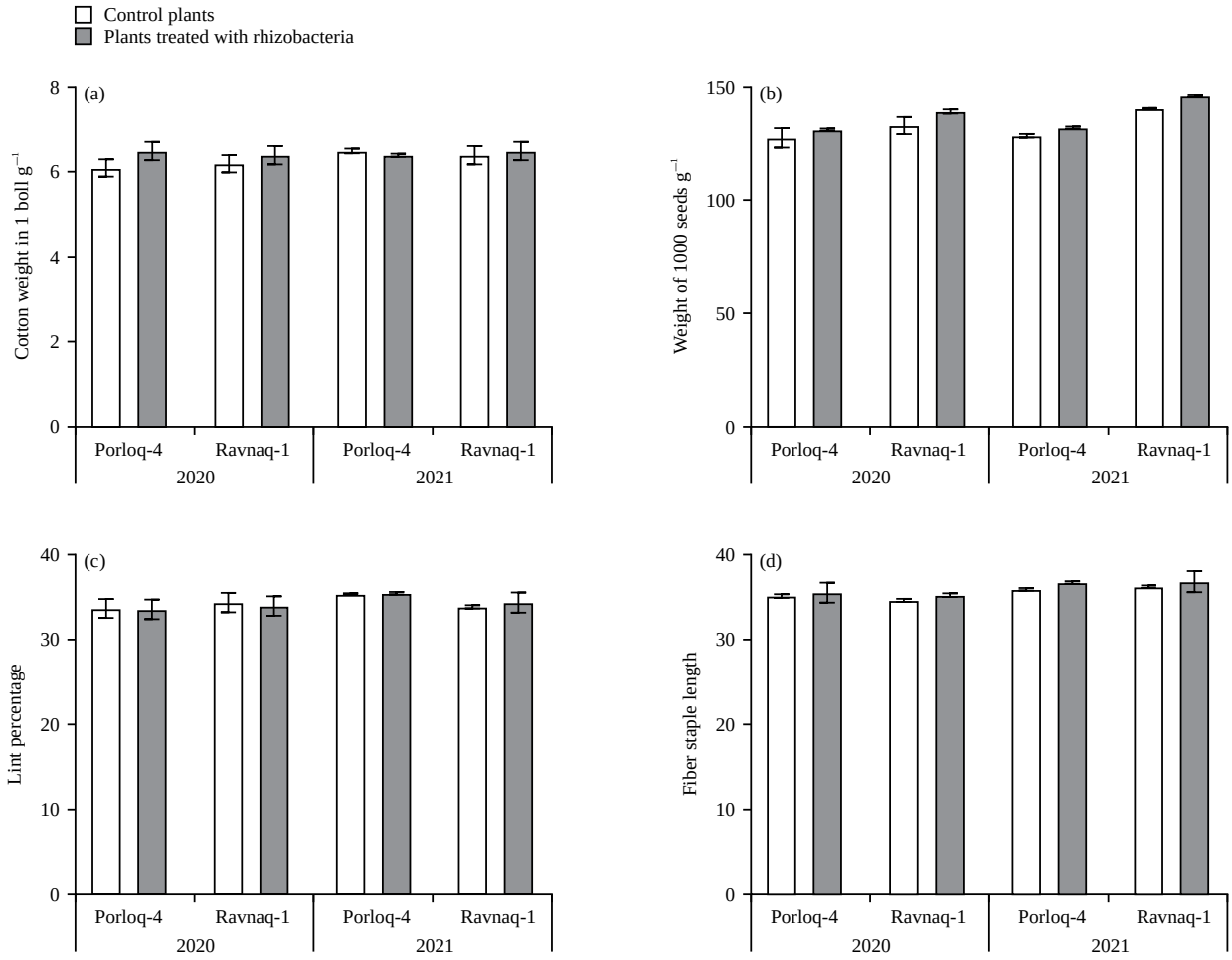

Fig. 3(a-d): Effects of rhizobacteria on fibre parameters in Porloq-4 and Ravnaq-1 varieties

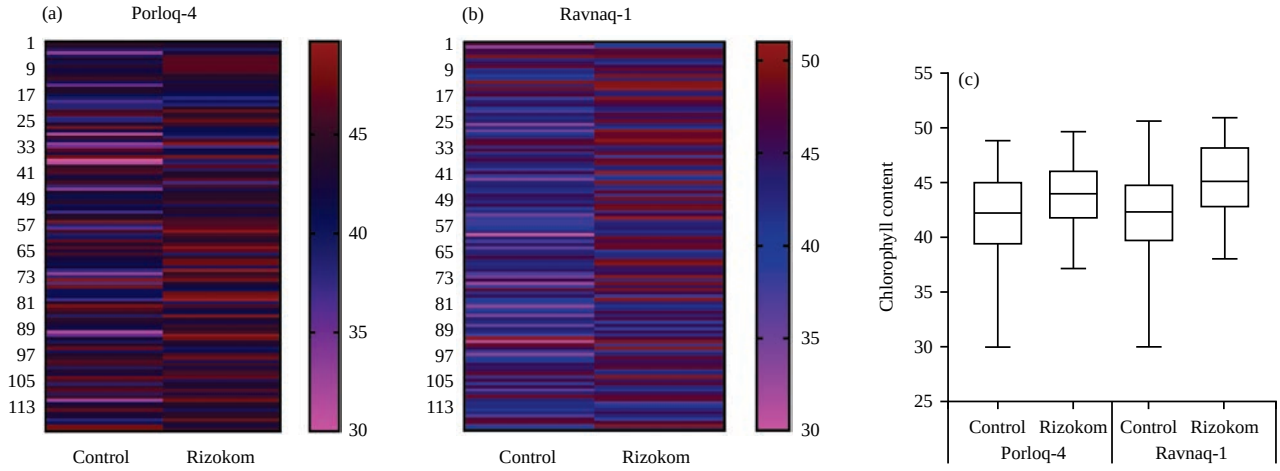

Fig. 4(a-c): Changes in chlorophyll content in cotton leaves after treatment with Rizokom preparation

Y-axis stand for the experimental samples studied in this study (120) in A and B. X-axis: Groups

CONCLUSION

This study has shown that the treatment of seeds of Porloq-4 and Ravnaq-1 cotton varieties with rhizobacteria preparation resulted in enhancement in seed germination, plant height, number of sympodia, flowers and bolls. No significant differences were observed in fibre characteristics such as the fibre length and lint percentage as well as chlorophyll content.

SIGNIFICANCE STATEMENT

The results obtained in this work prove that the treatment of cotton seeds with rhizobacteria, used in this study, enhances the agronomic traits of a cotton plant belonging to *G. hirsutum*. From an economic point of view, the treatment is considered cost-efficient.

ACKNOWLEDGMENT

The work was carried out by The Department of Personalized Agricultural Studies of the Center of Genomics and Bioinformatics of Uzbekistan Academy of Sciences according to Government order.

REFERENCES

1. Xu, J., Y. Feng, Y. Wang and X. Lin, 2018. Effect of rhizobacterium *Rhodopseudomonas palustris* inoculation on *Stevia rebaudiana* plant growth and soil microbial community. Pedosphere, 28: 793-803.
2. Goswami, M. and S. Deka, 2020. Plant growth-promoting rhizobacteria-alleviators of abiotic stresses in soil: A review. Pedosphere, 30: 40-61.
3. Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571-586.
4. Xiang, N., K.S. Lawrence, J.W. Kloepper, P.A. Donald, J.A. McInroy and G.W. Lawrence, 2017. Biological control of *Meloidogyne incognita* by spore-forming plant growth-promoting rhizobacteria on cotton. Plant Dis., 101: 774-784.
5. Vijayasamundeeswari, A., D.L.A. Sankaralingam and R. Samiyappan, 2009. Plant growth promoting rhizobacteria of cotton affecting the developmental stages of *Helicoverpa armigera*. J. Plant Prot. Res., 49: 239-243.
6. Diaz, P.A.E., N.C. Baron and E.C. Rigobelo, 2019. *Bacillus* spp. as plant growth-promoting bacteria in cotton under greenhouse conditions. Aust. J. Crop Sci., 13: 2003-2014.
7. Ren, X.M., S.J. Guo, W. Tian, Y. Chen and H. Han *et al*., 2019. Effects of plant growth-promoting bacteria (PGPB) inoculation on the growth, antioxidant activity, Cu uptake, and bacterial community structure of rape (*Brassica napus* L.) grown in Cu-contaminated agricultural soil. Front. Microbiol., Vol. 10. 10.3389/fmicb.2019.01455.
8. Ahemad, M. and M.S. Khan, 2012. Evaluation of plant-growth-promoting activities of rhizobacterium *Pseudomonas putida* under herbicide stress. Ann. Microbiol., 62: 1531-1540.
9. Paredes, S.H. and S.L. Lebeis, 2016. Giving back to the community: Microbial mechanisms of plant-soil interactions. Funct. Ecol., 30: 1043-1052.
10. Goswami,D., S. Pithwa, P. Dhandhukia and J.N. Thakker, 2014. Delineating *Kocuria turfanensis* 2M4 as a credible PGPR: A novel IAA-producing bacteria isolated from saline desert. J. Plant Interact., 9: 566-576.
11. Glick, B.R., 2012. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica, Vol. 2012. 10.6064/2012/963401.
12. Defez, R., A. Andreozzi, S. Romano, G. Pocsfalvi and I. Fiume *et al*., 2019. Bacterial IAA-delivery into Medicago root nodules triggers a balanced stimulation of C and N metabolism leading to a biomass increase. Microorganisms, Vol. 7. 10.3390/microorganisms7100403.
13. Xu, G., X. Fan and A.J. Miller, 2012. Plant nitrogen assimilation and use efficiency. Annu. Rev. Plant Biol., 63: 153-182.
14. Guzman, A., M. Obando, D. Rivera and R. Bonilla, 2012. Selection and characterization of plant growth promoting rhizobacteria (PGPR's) associated with cotton crop (*Gossypium hirsutum*). Rev. Colomb. Biotecnol., 14: 182-190.
15. Omer, Z.S., R. Tombolini, A. Broberg and B. Gerhardson, 2004. Indole-3-acetic acid production by pink-pigmented facultative methylotrophic bacteria. Plant Growth Regul., 43: 93-96.
16. Gupta, G., S.S. Parihar, N.K. Ahirwar, S.K. Snehi and V. Singh, 2015. Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. J. Microb. Biochem. Technol., 7: 96-102.
17. Jha, C.K. and M. Saraf, 2015. Plant growth promoting rhizobacteria (PGPR): A review. E3 J. Agric. Res. Dev., 5: 108-119.
18. Spaepen, S., S. Bossuyt, K. Engelen, K. Marchal and J. Vanderleyden, 2014. Phenotypical and molecular responses of *Arabidopsis thaliana* roots as a result of inoculation with the auxin producing bacterium *Azospirillum brasilense*. New Phytol., 201: 850-861.
19. Gouda, S., R.G. Kerry, G. Das, S. Paramithiotis, H.S. Shin and J.K. Patra, 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol. Res., 206: 131-140.

20. Damam, M., K. Kaloori, B. Gaddam and R. Kausar, 2016. Plant growth promoting substances (phytohormones) produced by rhizobacterial strains isolated from the rhizosphere of medicinal plants. Int. J. Pharm. Sci. Rev. Res., 37: 130-136.
21. Sureshbabu, K., N. Amaresan and K. Kumar, 2016. Amazing multiple function properties of plant growth promoting rhizobacteria in the rhizosphere soil. Int. J. Curr. Microbiol. Appl. Sci., 5: 661-683.
22. Bender, S.F., C. Wagg and M.G.A. van der Heijden, 2016. An underground revolution: Biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol. Evol., 31: 440-452.
23. Pii, Y., T. Mimmo, N. Tomasi, R. Terzano, S. Cesco and C. Crecchio, 2015. Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol. Fertil. Soils, 51: 403-415.
24. Raza, W., S. Yousaf and F.U. Rajer, 2016. Plant growth promoting activity of volatile organic compounds produced by biocontrol strains. Sci. Lett., 4: 40-43.
25. Abdurakhmonov, I.Y., M.S. Ayubov, K.A. Ubaydullaeva, Z.T. Buriev and S.E. Shermatov *et al*., 2015. RNA interference for functional genomics and improvement of cotton (*Gossypium* sp.). Frontier Plant Sci., Vol. 7. 10.3389/fpls.2016.00202.
26. Darmanov, M.M., A.K. Makamov, M.S. Ayubov, N.N. Khusenov and Z.T. Buriev *et al*., 2022. Development of superior fibre quality upland cotton cultivar series 'Ravnaq' using marker-assisted selection. Front. Plant Sci., Vol. 13. 10.3389/fpls.2022.906472.
27. Majid, M., M. Ali, K. Shahzad, F. Ahmad and R.M. Ikram *et al*., 2020. Mitigation of osmotic stress in cotton for the improvement in growth and yield through inoculation of rhizobacteria and phosphate solubilizing bacteria coated diammonium phosphate. Sustainability, Vol. 12. 10.3390/su122410456.
28. Anjum, M.A., M.R. Sajjad, N. Akhtar, M.A. Qureshi, A. Iqbal, A.R. Jami and Mahmud-ul-Hasan, 2007. Response of cotton to plant growth promoting rhizobacteria (PGPR) inoculation under different levels of nitrogen. J. Agric. Res. 45: 135-142.
29. Qureshi, M.A., M.A. Ali, F. Mujeeb, M.J. Ahmad, S. Rashid, S. Ullah and M.A. Anjum, 2017. Yield and quality response of cotton to a consortium of PGPR at graded fertilizer levels. Int. J. Biosci., 10: 46-53.
30. Habib, S.H., H. Kausar and H.M. Saud, 2016. Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. BioMed Res. Int., Vol. 2016. 10.1155/2016/6284547.
31. Bal, H.B., L. Nayak, S. Das and T.K. Adhya, 2013. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil, 366: 93-105.
32. Hagedron, C., W.D. Gould and T.R. Bradinelli, 1989. Rhizobacteria of cotton and their repression of seedling disease pathogens. Appl. Environ. Mocrobiol., 55: 2793-2797.
33. Nehra, V., B.S. Saharan and M. Choudhary, 2016. Evaluation of *Brevibacillus brevis* as a potential plant growth promoting rhizobacteria for cotton (*Gossypium hirsutum*) crop. SpringerPlus, Vol. 5. 10.1186/s40064-016-2584-8.