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Benefits of inoculation with rhizobacteria on growth and yield of onion (*Allium cepa* L) cv *Violet De Galmi* cultivation

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Abstract

Onion production is tightly dependent on chemical inputs like fertilizers and pesticides which alter environment and crop production in Senegal. To contribute to the improvement of this situation, a biofertilizer based on a mixture of Plant Growth Promoting Rhizobacteria (PGPR) strains is tested in greenhouse on soils sampled from two agroecological areas namely Senegal River valley and Niayes and in field at Sangalkam experimental station in Niayes zone. Even though no significant difference was noted between soils, onion plant length and bulbs weight are enhanced with the PGPR-inoculant in greenhouse experiment in comparison to control. Under field conditions, rhizobacterial inoculation resulted in a significant increase of plant growth parameters such as neck diameter and height compared to the non-inoculated control. Although inoculation treatment was lower than the farmers' practice consisting of conventional mineral fertilizer, it significantly increased average weight and yield of onion bulbs by 75.50% and 69.82% respectively, compared to the control. Rhizobacterial inoculation increased mainly onion plant growth and production in field comparared to the non-inoculated plants.

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Introduction

In sub-Saharan Africa, crop production declining has long been related to soil fertility depletion (Sanchez *et al.,* 1997). In order to address this situation, soil fertility is replenished through diverse agricultural practices. However, conventional mineral fertilizers are not a suitable way in smallholder farms because of their high cost. However, soil microorganisms play a crucial role in soil nutrients availability and their supply to plants for growth stimulation and production enhancement. The well-tested biotechnology of microbial inoculation with rhizobia has been applied worldwide for a long time. Recently, the use of plant growth-promoting rhizobacteria (PGPR) knows an expansion with the aim to improve nutrients availability and biological protection for plants (Verma *et al.,* 2010; Figueiredo *et al.,* 2010). Thus, diverse rhizobacteria occurred in soils are reported to involve in its fertilization through nutrients recycling, plant growth stimulation and yields increasing (Beauchamp 1993; Gupta *et al.,* 2000; Ahemad and Kibret, 2014). According to these authors, PGPR promote plant growth by various direct or indirect mechanisms by acting as biofertilizers, phytostimulators and phytoprotectors.

Onion (*Allium cepa* L.) is one of the most important consumed vegetables in Senegal. This vegetable crop is grown mainly in two agro-ecological areas namely Senegal River Valley and Niayes where it occupies a primordial place in the market gardening for both sown area and production. In these zones, it is impossible to increase land used to grow vegetables due to its specific agro-pedoclimatic conditions. To achieve local market demand, crop production requires application of high amounts of synthetic fertilizers and pesticides. These chemical inputs cause several damages on agricultural soil and affect environment and human health through groundwater pollution and crop contamination. However, plants growth, crop yield and their qualities are reported to be enhanced by positive effects of biofertilizers applied to the soil (Vessey, 2003; Verma *et al.,* 2010). Microbial inoculation practice constitutes an enormous potential for enhancing agricultural and environmental sustainability (Ahemad and Kibret, 2013). PGPR inoculants used on onion cultivation have been reported to increase plant growth, yields and bulb quality (Čolo *et al.,* 2014). According to Ahemad and Kibret (2013), the adaptation of rhizobacteria to soil conditions can increase their efficiency. Due to the probable high specificity of bacteria for growth-promoting ability as reported by Gupta *et al.* (2000), indigenous rhizobacterial strains were isolated from the rhizosphere of field growth onion in Senegal (Ndiaye *et al.,* 2020). This preliminary study allowed the screening of strains with interesting PGP properties to produce high promising biofertilizers for onion growth. The objective of the present study was to investigate the effects of inoculation with native strains of PGPR on onion cultivation in field conditions.

Material and methods

Biological material

Seeds of onion cultivar "Violet de Galmi" used in this study were provided by Senegalese national research Center for Horticultural Development located at Cambérène in Dakar district. Onion seedlings were maintained in nursery for 50 days to set up greenhouse and field experiments. PGPR inoculum consisted of mixture of three indigenous rhizobacterial strains RB51, RB53 and RB54 previously isolated from local onion growth areas and selected for their PGP activities (Ndiaye *et al.,* 2020). These trains presented highest values of auxin production and phosphorus solubilizing in addition to their interesting behavior in vitro regarding some abiotic factors such as pH, temperature and salinity. Strains were cultivated individually in appropriate LB nutrient broth (10^7 - 10^8 cells/ml) and well mixed by incubation for one day on a rotary shaker.

Greenhouse experimental design

A greenhouse pot experiment was carried out at the Faculty of Sciences and Technics of University Cheikh Anta Diop Dakar. Soils collected from Senegal River valley and Niayes zones were separately air dried. Each soil sample was thoroughly homogenized by sieving (2mm) and weighed at 1.5kg into pots. Main soil properties are presented in Table 1.

Table 1. Characteristics of soils sampled from Niayes and Senegal River Valley agro-ecological zones.

Characteristics	Sites	
	Niayes zone	Senegal river zone
Sand (%)	87.0	89.9
Loam (%)	7.5	7.0
Clay (%)	5.5	3.1
pH (H_2O)	6.7	5.6
pH (KCl)	5.8	4.9
C (‰)	4.4	2.4
N (‰)	0.5	0.12
Available P (ppm)	82.0	0.11

Four days after transplantation of two seedlings of onion into pots, they were thinned down to one per pot. Plants allowed to inoculation received 2ml of PGPR inoculant. For each soil type, treatments with five replicates consisted of PGPR inoculation. The control plants received no treatment. Plants were disposed in a completely randomized factorial design. Onion plant culture was maintained by watering to field capacity.

Field experiment for onion inoculation

A field experiment was carried out at Sangalkam agricultural station (14° 46′ 52” N, 17° 13′ 40” W) located in the agroecological zone of Niayes. Seedlings from 50 days grown nursery were transplanted in a randomized complete factorial design with three replicates. Onion plants were cultivated in each plot (1 x 2 m), with 0.10 m and 0.15 m within and between rows, respectively. The three treatments consisted of onion plants inoculated with mixture of PGPR strains, no inoculated plants and local conventional practices using mineral fertilizer (250kg N/ha). Rhizobacterial inoculum was added at the rate of 2ml per plant. At the vegetative maturity, four months after planting, plants were harvested from the microplot (0.45 x 0.50m) delimitated within each plot. Onion bulbs were pulled out from soil. Bulb diameter (size), weight and yield were recorded.

Statistical analysis

Data were statistically analyzed using R software (version 3.6.3). Mean values of different parameters were compared according to the Student-Newman-Keuls test. Analysis of variance (ANOVA) was performed to indicate significant difference between factors at $p \leq 0.05$.

Results and discussion

Greenhouse experiment

The results of rhizobacterial inoculation of onion in the greenhouse on soils of the two onion growing areas are given in Table 2. There were no significant differences between the soils. However, a main effect of rhizobacterial inoculation was observed on plant height and bulbs weight. Thereby, inoculation treatment increase bulbs weight for 45.66% in comparison to non-inoculated control when no significant interaction soil-inoculation was recorded. Table 2 showed that soil-inoculation interaction was significant for onion plants neck diameter and height ($p \leq 0.05$). Regarding vegetative development parameters, PGPR-inoculant did not improve the neck diameter and number of leaves of onion plants compared to the non-inoculated control. These findings are not in consistent with those of Bekaş and Küsek (2021) who reported a significant increase of onions' number of leaves in pot experiment. Furthermore, the significant improvement in onion plant height and bulbs weight obtained of these authors are in perfect agreement with our results. Meanwhile, the findings of Novello *et al.* (2021) also reported a promotion on onion plant growth by rhizobial inoculation.

Table 2. Plant growth and bulb production of onion cultivated in greenhouse

Treatment	Neck_diameter (mm)	Number of leaves	Plant height (cm)	Bulbs_weight (g)
Soil				
Niayes zone	6.11 ± 0.45	5.60 ± 0.52	34.9 ± 11.6	27.9 ± 5.73
Senegal river valley zone	5.85 ± 0.70	5.70 ± 0.48	36.5 ± 2.56	25.9 ± 5.70
Inoculation				
PGPR inoculant	6.10 ± 0.48	5.80 ± 0.42	38.8 ± 2.07 ^{a}	31.9 ± 2.23 ^{a}
Control	5.86 ± 0.69	5.50 ± 0.53	32.5 ± 2.95 ^{b}	21.9 ± 2.45 ^{b}
p-value				
Soil	0.291 ^{ns}	0.660 ^{ns}	0.061 ^{ns}	0.056 ^{ns}
Inoculation	0.329 ^{ns}	0.198 ^{ns}	4.962e-07 ***	2.041e-08 ***
Soil * Inoculation	0.03777 *	0.660 ^{ns}	0.0007264 ***	0.88120

Signif. Codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

This greenhouse trial on both soils and inoculated with a mixture of indigenous strains was conducted with a focus on setting up a field trial for better control and mitigation of instability of environmental factors. Indeed, Jarak *et al.* (2012) reported that positive results in controlled conditions can be

affected by environmental factors that make it not reproductible in field conditions. Moreover, previous studies on onion inoculation with a mixture of PGPR strains have been reported to be more efficient in increasing onion growth and yield parameters compared to single strain application (Čolo *et al.,* 2014; Tinna *et al.,* 2020).

Field experiment

As there were no differences on soils according to the previous results in greenhouse, a field experiment for inoculation with PGPR was carried out in Niayes zone. The data obtained are analyzed in comparison with the farmers' practice and non-inoculated plants. The results recorded in Table 3 showed that the application of chemical fertilizers at conventional fertilization rate produced the best levels of vegetative growth of onion plants. However, Stamenov *et al.* (2018) reported an onion production under conventional growing conditions equivalent to those obtained with inoculation. In this present investigation (Table 3), the application of PGPR-based biofertilizers significantly improved onion plants neck diameter and height growth by 37.22% and 18.08% respectively compared to the non-inoculated control. These results on onion cultivation in field conditions are in agreement with those obtained by many authors that reported high positive effect of applied PGPR inoculants on onion plants growth parameters such as plant height (Čolo *et al.* 2014; Afify *et al.,* 2018). In addition, for these authors, benefits of PGPR inoculation were observed on onion bulb weight and yield which was in accordance with our findings. According to the results mentioned in Table 4, the application of rhizobacteria-based biofertilizers to onion crops significantly improved average bulbs weight (82.50%) and yield (69.82%) compared to the non-inoculated control and without mineral fertilization. Our results are in accordance with those of Afify *et al.* (2018) who obtained positive effect of microbial inoculation of onion plants that increased bulbs weight and total bulbs yield. Similar results were previously reported by Čolo *et al.* (2014) who applied a mixture of PGPR inoculant. In addition, significant difference is noted on the morphological quality parameters of the bulbs such as equatorial and longitudinal diameters. Indeed, Novello *et al.* (2021) revealed that onion bulbs size, evaluated by both minor and major bulb diameters, was higher through rhizobacterial inoculation compared to non-inoculated control. Trognitz *et al.* (2016) identified bacteria that function simultaneously as plant growth promoting and biopesticides. Multifunctional rhizobacterial strains isolated from onion rhizosphere were considered as potential bio-inoculant (Phales, 2018). According to our study, the mixture of PGPR strains inoculated on onion plant cultivation may have potential to provide a successful biofertilizer. Therefore, further study of the functional characteristics and genotypic identification of inoculated strains is necessary to determine their phylogenetic relationship with biofertilizing, phytoprotecting and phytostimulating reference strains.

Table 3. Onion plants growth in field under PGPR biofertilizer at Sangalkam experimental station in Niayes zone.

Treatment	Neck_diameter (mm)	Plant height (cm)	Number of_leaves
Farming practice	9.45 ± 0.47 a	36.4 ± 0.53 a	6.96 ± 0.09 a
Inoculation	8.92 ± 0.84 a	28.2 ± 1.60 b	5.98 ± 0.08 b
Control	5.60 ± 0.38 b	23.1 ± 1.03 c	5.17 ± 0.31 b
Mean ± SD	7.99 ± 1.88	29.2 ± 5.92	6.04 ± 0.80
CV (%)	7.50	3.90	3.18
Pr(>F)	0.000443 ***	2.15e-05 ***	8.39e-05 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4. Onion bulbs production and yield in Niayes zone at Sangalkam experimental station

Treatment	Equatorial diameter (mm)	Longitudinal diameter (mm)	Bulbs mean weight (g)	Bulbs yield (t/ha)
Farming practice	3.04 ± 0.18 a	3.51 ± 0.19 a	129.2 ± 2.15 a	35.8 ± 0.38 a
Inoculation	2.86 ± 0.21 a	3.49 ± 0.21 a	112.5 ± 2.84 b	28.7 ± 1.11 b
Control	1.92 ± 0.13 b	2.84 ± 0.19 b	64.1 ± 0.56 c	16.9 ± 0.09 c
Mean ± SD	2.60 ± 0.54	3.28 ± 0.37	101.9 ± 2.04	27.2 ± 8.30
CV (%)	6.73	5.91	2.04	2.50
Pr(>F)	0.0004 ***	0.00872 **	5.39e-08 ***	1.28e-07 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Conclusions

This study did not showed significant difference of PGPR inoculation in greenhouse experiment on soils sampled from the two main onion cultivation zones in Senegal. However, these biofertilizer-based rhizobacterial mixture enhenced main onion plant growth parameters in field compared to the non-inoculated control. In addition, onion bulbs weight and yield are highly increased by the applied of rhizobacterial inoculant.

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