Response of Chickpea to the Rhizobia Inoculation in Different Region of Morocco

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ABSTRACT

The objective of the present study is to assess the effect of rhizobia inoculations on the growth and nodulation of Chickpea. One rhizobia strains were used as control and soils sampled from eleven sites belonging to six different regions of Morocco. The rhizobia inoculation was applied at the time of the seedling, and the nitrogen fertilizer was performed three times the first application at the seedling moment then two and three weeks after the first application. The biomass and nodulation were evaluated under different treatments. The essay was realized at the greenhouse the Faculty of Sciences, Moulay Ismail University. The Inoculation with these root symbionts, increased the biomass accumulation of the chickpea treated compared to the absolute control. However, the best response result was that of the simple rhizobial inoculation which showed a very good growth as well as an important root infection assisted by the number and weight nodule compared to the rhizobial inoculation combined to the nitrogen fertilization and to the control plants. Finally, the symbiosis with rhizobia is an important biological technology to improve the sustainable production of leguminous plant in different agro-ecological regions.

Keywords: *Chickpea, Rhizobia, Inoculation, Growth, Nodulation.*

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1. INTRODUCTION

As in many developing countries, Chickpea present an important legume food crop in Morocco. Chickpea as the other grain legumes is considered as main source of proteins in human and animal nutrition and plays a key role in crop rotations. As so, legumes (like chickpea) are cultivated in rotation with other crops with the aim to improve soil fertility and reduce the incidence of weeds, diseases and pests [1]. In Morocco, during the last decade, the chickpea crop has been progressively extended from semi-arid to arid areas where edapho-climatic conditions such as salinity, pH and temperature may have an adverse effect on the establishment of functional nitrogen-fixing symbiosis [2-4].

The present work aims to assess the response of chickpea of winter ILC 195 in different regions of Morocco. The response was tested for nodulation, dry matter yield in plastic pots carried out in a greenhouse at the Faculty of Science, of Moulay Ismail University.

2. MATERIALS AND METHODS

2.1. The Choice of Soil Sampling Sites

The choice of the sampling sites was based on three criteria. The first type of sapling sites corresponded to the region where the chickpea is usually cultivated, the second type corresponded to the soils having problems according to the chickpea culture and the last one corresponded to the sites where chickpea will be cultivated. For that, the soils were sampled from six regions in Morocco: Sais (Meknes), Chaouia (Settat), Pre-Rif (Tissa), Central Plateau (Maaziz), Middle Atlas (Khénifra) and the Gharb plain (Gharb). The edaphoclimatic characteristics of each region are present in the table 2.

2.2. Soil Sample Collection

In order to preserve the soil in its field natural conditions, the method "Soil core" described by Vincent [5] was used sampling the soil in cylindrical pots (20cm x 16cm) at the rate of 7kg/ha of soil per pot.

Soil samples were collected from 10 sites of 6 different regions in Morocco. From each site 10 kg of soil were randomly sampled. The determination of the different physicochemical characteristics was performed after samples collection.

2.3. Physicochemical Characteristics of Soils

Soil samples taken from representative sites were used for analysis of soil texture and some chemical properties. The analysis were estimated according to the methods described by Van [6].

2.4. Experimentation

2.4.1. Treatments & Experimental Design

The essay was realized according to a design in randomized complete block arranged in split-plot, the chickpea used was the variety ILC 195. A Rhizobia reference strain IAPC1 was used as control to assess the efficiency of the native strains of different soils. Three treatments were executed:

T1: Treatment inoculated with the IAPC1strain;

T2: Treatment without inoculation no nitrogen fertilization;

T3: Treatment fertilized with nitrogen but without inoculation.

Five repetitions per treatment were taken.

2.4.2. Seeds Inoculation

The seeds inoculation of the treatment T1 was done at the sowing moment. The seeds used were coated using a mixture of peat and liquid suspension of Rhizobium strain IAPC1 (5.10^6 viable cells/seed).

2.4.3. Experimentation Conduct

A/ Sowing:

The chickpea seeds were sown at a depth of 1 to 2 cm at a rate of 4 seed per pot. The inoculated seeds were sown at the last time to ovoid contamination of the non inoculated seeds. After two weeks of growth under greenhouse conditions, one plant was removed in order to leave only three plant per pot.

B/ Fertilization:

After the determination of the field capacity of soils, the macro and micro fertilizers were applied in aqueous solution (table 1) before sowing the seeds.

Table-1. Quantities of macro and microelements used for soil fertilization [7]

Element	Dose (mg/kg of dry soil)	Input form
P	100	KH_2PO_4
Na	150	NaH_2PO_4
Mg	1200	$MgSO_4,H_2O$
Zn	220	$ZnSO_4,7H_2O$
Mo	110	Na_2MoO_4
Mn	2080	$MnSO_4,H_2O$
Fe	5	Fe Chelate
Cu	80	$CuSO_45H_2O$
$CaCl_2$	100	CaCl2
B	2860	H_3BO_3

The plant of the treatment T3 have received 200mg of nitrogen as urea form per kilogram of dry soil. The nitrogen input was applied three times at the rate of 66.6 mg (N)/kg of dry soil. The first application was at the sowing moment, then after two and three weeks after sowing respectively.

2.4.4. Assessment of the Response of Chickpea to the Rhizobia Inoculation

Six weeks after sowing, the plants were harvested. The response was assessed determining the nodulation by measuring the number and the dry weight of the nodules, the dry matter field of aerial parts of different plants, the nitrogen percentage and the yield nitrogen in plant for each treatment.

3. RESULTS

3.1. Characteristics of the Sampling Sites

The edaphoclimatic characteristics of the six regions sampled in Morocco are represented in the following table.

Table-2. Characteristics of the Soil sampling Sites [8]

Region	Geology	Dominant soil	Climatology
Central Plateau (Maaziz, Merchouch, Sidi Bettach)	quartzite sandstones schists at the basis and superimposed with important Permo-Triassic deposits and transgressive covring of Miocene and Pliocene.	- hydromorphic soils - Vertisols - ferrallitic	Semi-arid climate, with cold winter and hot summer. Annual rainfall is between 450 and 600 mm. the average annual temperatures are between 17-18°C
Chaouia (Settat)	It belongs to the Atlas domain .the Hercynian basement is the common substratum for the region, flush in several places, and disappears under secondary and tertiary formations. All geological formalities formations are limestones.	- calcimagnesic - Vertisols - fersiallitic	Arid to semi -aride climat with temperate winter. The mean annual rainfall is 408 mm. The mean of the highest temperature 30-36 ° C The mean of the minimal temperature 4 ° C
Gharb (Mokrane)	The soils of the Gharb plain are formed in conditions of little accused relief on fine silt general, the plain have known clayey Quaternary filling.	- Vertisols - little evolved Soils - Subtropical soils isohumic	- Climat Semi-arid inside and sub-humid on the coast - Rainfall exceeds 500mm / year - The cool and wet season lasts seven months from October to April. - The thermal regime is characterized by an average of the monthly maximal temperature that fluctuates between 18.4 and 31 ° C
Middle Atlas (Khenifra)	The Causse Middle Atlas is based on anti-Jurassic land constituting the substratum of the Causse. the Permo Triassic recovers its primary land in discordance and is found over the entire border and on the board thanks to tectonic occidents or erosion that has scraped the overlying Lias.	- Raw Materials series (1-9) - Little evaluated (series 10-15) - Vertisols (25-29 series) - Isohumic (series 36) - Calcimagnesic (series 30-35) - A sesquioxide (series 37-40)	The climate is dissymmetrical: - sub-humid with cold winter (causses areas) - Semi-arid with cool winter (khénifra). The rainfall regime is seasonal Mediterranean with an annual average of 1158 mm in the western border and only 706 mm in Ouiouane and El Hammam. The average of the minimal temperatures are between -1 and 3. The maximal temperatures are between 20 and 40 ° c
Pre-Rif (Tissa)	Field of a hill relief with variable slope depending on the region, various platforms	Vertisols or calcimagnesic soils. little evolved erosion soil	The rainfall varies between 500 mm for the west and 700mm for east
Sais (Fes, Meknès)	Calcareous sediments: * Hard limestone * Flimsy chalky limestone "tuff" Lacustrine information based on the Upper Miocene. Tertiary appear only locally	3 groups: • character steppe soils (isohumic) • Soils calcimagnesic • Soil has sesquioxydes represented by the Mediterranean red soils	According to Emberger and sauvage the climate is considered semi-arid with temperate winter. Aridity and continentality are more pronounced in Fes. - Emberger's Pluviothermic Quotient: 66 ° - Moisture thornthaite index: 12.7 - Mean temperature of the coldest month :4.4 - Mean annual rainfall 530mm

3.2. Physico-Chemical Characteristics of Soils

The results of the analysis of soil sampled mainly the texture and some chemical properties were depicted in the following table.

Table-3. Physicochemical characteristics of soil samples

Sites	**Soil texture**					**MO%**	**%C**	**%N**	**C/N**			**N(ppm)**	**P(ppm)**	**K(ppm)**
	SG%	%SF	%S	%L	%A					EAU	Kcl			
Maaziz	8.25	39.93	48.18	8.94	42.87	1.40	0.82	0.14	5.85	6.85	5.79	12.04	9.43	105.6
Merchouch	15.44	22.14	37.56	25.29	37.12	1.39	0.8	0.18	4.44	6.42	5.53	18.24	35.61	274.5
Sidi. Bettach	21.59	19.45	41.04	38.01	20.95	0.44	0.26	0.11	2.36	6.07	5.18	14.06	-	55.55
Khenifra	29.01	9.26	38.27	41.5	20.22	1.18	0.69	0.13	5.30	6.48	5.57	20.6	13.35	70.2
Meknes2	15.58	18.11	33.69	46	20.32	2.33	1.35	0.25	5.4	738	7.39	59.58	21.42	70.2
Meknes1	19.83	27.42	47.25	19.77	32.97	1.74	1.01	0.16	6.31	8.08	7.21	25.31	10.66	252.5
Fes	4	18.37	22.37	53.51	24.17	1.43	0.82	0.36	2.27	8.17	7.28	32.00	32.06	394.4
Gharb	2.39	2.99	5.38	53.27	41.35	1.01	0.59	0.24	2.45	8.56	7.25	22.94	3.32	201.9
Tissa	4.87	9.62	14.49	34.81	50.70	1.51	1.27	0.13	9.76	7.74	7.28	20.6	8.195	480.5
Settat	1.20	10	11.2	36.21	52.62	2.72	1.58	0.3	5.26	8.38	7.08	36.76	16.29	95.5

Table-4. Response of Chickpea to inoculation in soils from different sites

Site	Treatment	Nodules Number / Pot	Nodules weight (g/pot)	Dry Matter (g/pot)	%N	Total Nitrogen (g/pot)
Fes	Inoculated	810.80a	1.87 a	21.50a	2.43 b	0.52 b
	Non-Inoculated	88 b	0.18b	33b	3.88a	1.27 a
	Control	511.80a	1.40 a	10.00c	1.85 b	0.26 b
	Signification	**	**	***	***	***
	CV%	52.6	55	14.94	19.1	27.3
Gharb	Inoculated	543a	1.41 a	16.96a	2.55 b	0.44 b
	Non-Inoculated	24b	0.03 b	31b	4.19 a	1.04 a
	Control	396a	1.46 a	16.52a	2.62 b	0.45 b
	Signification	***	***	**	*	***
	CV%	32.4	31.7	18.5	17.7	28.8
Khenifra	Inoculated	670 a	1.68 a	3.31	3.31	0.50 b
	Non-Inoculated	3.60 b	0	3.83	3.83	0.83 a
	Control	164.80 b	0.55 b	2.48	2.48	0.24 c
	Signification	***	***	***	NS	**
	CV%	61.4	53.9	20.2	25.9	32.2
Maaziz	Inoculated	768 a	1.27 a	15.76a	2.751 a	0.42 b
	Non-Inoculated	0b	0b	25.30b	3.24 a	0.85 a
	Control	92.2b	0.25 b	9.02 c	1.76 b	0.13 c
	Signification	***	***	***	NS	**
	CV%	49.8	38.9	26.3	21.8	46.1
Meknes 1	Inoculated	243.8a	0.52 ab	12.62b	2.75	0.42 b
	Non-Inoculated	0.20b	0.02 b	23.50a	4.19	1.85 a
	Control	344 a	0.85 a	9.18 b	2.62	0.13 b
	Signification	*	*	*	NS	*
	CV%	59.9	82.4	44.4	20.9	57.1
Meknes 2	Inoculated	807.6a	1.99 a	22.8a	2.20 a	0.49b
	Non-Inoculated	0b	0b	37.66b	233b	1.05a
	Control	358.4 c	1.12c	16.16c	2.43b	0.38b
	Signification	***	***	***	*	**
	CV%	29.6	46.9	17.3	17.1	22.1
Merchouche	Inoculated	663.60a	1.39 a	15.04a	2.80 b	0.42 b
	Non-Inoculated	7b	0.01 b	24.46a	3.80 a	0.93 a
	Control	490.20a	1.30 a	13.98a	2.69 b	0.41 b
	Signification	***	***	**	**	***
	CV%	38.4	47.8	33.1	26.7	44.8
Settat	Inoculated	953.33a	2.14 a	21.92b	3.63	0.78
	Non-Inoculated	108.8b	0.12 b	32.56a	2.88	0.93
	Control	775.60a	1.46 a	19.42b	2.97	0.63
	Signification	***	***	**	NS	NS
	CV%	21.5	20.6	17.7	45.6	41
Sidi Bettach	Inoculated	484.4 a	1.14 a	16.26a	2.85a	0.48a
	Non-Inoculated	0b	0b	17.84a	4.30b	0.77b
	Control	68.20 b	0.22b	10.00b	1.59c	0.16 c
	Signification	***	***	***	NS	**
	CV%	38.6	37.4	27.1	23.8	33.1
Tissa	Inoculated	62.80 a	1.89 a	21.84b	6.62 b	0.57 b
	Non-Inoculated	8.40 b	0.18 b	31b	4.19 a	1.28 a
	Control	432.8c	1.58 a	16.24b	2.59 b	0.45 b
	Signification	***	***	**	**	***
	CV%	41	45.9	18.1	21.1	26.1

NS : Not significant at probability threshold $p < 0.05$
* : significant at probability threshold $p < 0.05$
** : significant at probability threshold $p < 0.01$
*** : significant at probability threshold $p < 0.001$
The averages of the same column followed by the same letter did not differ significantly according to the Newman and Keuls test (5%).

3.3. Nodulation

Nodulation is a qualitative assessment of the symbiotic fixation of the nitrogen. Based on the results of the present study, we can divide the sites into three groups. The first group concern the sites, which have shown an increase on the nodules number as well as their weight. The second group belong to the site that have demonstrate an increase on the nodules number without affecting their weight. Finally, the last group that depicted a negative response to inoculation (no effect on the nodules number and weight).

For the first group, the inoculation of chickpea with reference strain IAPC1 have significantly increased the nodules number and their weight in the soils of four sites "Maaziz, Sidi Bettach, Khenifra and Meknes 2". The nodules number for these sites was respectively 727, 610, 306 and 125. In addition, the nodules dry weight was respectively 688, 518, 305 and 177mg (Table 4).

For the site, "Tissa" the inoculation have increased the nodules number however, their weight have not increased. The last group belongs to the sites Meknes 1 and Settat. For this group the inoculation have shown no effect either on the nodules number or on their weight. The nodules number was 344 for Meknes 1 and 775.6 for Settat. The nodules weight of Meknes 1 was 850mg and 2.13mg for Settat (Table 4).

The nitrogen input have affected significantly the nodulation even that this inhibition was partial, the nodules were white, very small and their weight was negligible (Table 5).

Table-5. Inhibition of the nodulation by the nitrogen fertilization in soils sampled from different sites

SITES	Treatment without inoculation		Treatment with nitrogen fertilization and without inoculation		% Inhibition of Nodules Number
	Nodules Number	Nodules Dry Weight (g)	Nodules Number	Nodules Dry Weight (g)	
Maaziz	92.20	0.25	0	0	100
Marchouche	490.20	1.30	7	0.01	98.57
Sidi Bettache	68.20	022	0	0	100
Khenifra	164.80	0.55	3.60	0	97.81
Meknes 1	358.4	1.12	0	0	100
Meknes 2	344	0.85	30.20	0.02	91.22
Fes	511.80	1.40	88	0.18	82.80
Gharb	396	1.46	24	0.03	93.9
Tissa	432.8	1.58	8.40	0.01	98.05
Settat	775.60	2.13	108.80	0.12	85.97

3.4. Dry Matter Yield

The inoculation have increase the dry matter yield that varied from 9.02 and 16.16 g/pot mainly for the five following sites: Maaziz, Sidi Bettach, Khenifra, Meknes 2 and Fes. The percentage of the dry matter yield was 74% for Maaziz, 64.5% for Khenifra, 62.6% for Sidi Bettach, 38.8% for Meknes2 and 20.5% for Fes (Table 4). The inoculation have shown no effect on the yield dry matter especially for the two sites Meknes 1 and Settat. However, the application of nitrogen fertilizer have significantly increase the yield dry matter for all the studied sites.

3.5. Nitrogen Content and Total Nitrogen

The inoculation have increase significantly the nitrogen content in the shoot of the inoculated plants compared to the control that was not inoculated. The best response was observed for two sites Sidi Bettach and Maaziz with the following percentage 79% and 56% respectively (Table 4).

The total nitrogen yield of the shoot plants was also increased due to inoculation especially for the sites Sidi Bettach and Khenifra with the following percentage 108% and 200% respectively (Table 4).

4. DISCUSSION

The degradation of the agricultural land have been aggravated hence the use rehabilitation tools is required to sustain their productivity [9, 10]. This act may be ensured through the use of biological alternative such the rotation [11] in order to improve the physico-chemical properties of the soil [12] in addition to increase its flora and fauna diversity, to increase the yield and the quality of subsequent crops [13-15].

The present study aims to evaluate the response of chickpea to the inoculation using soils sampled from different region in Morocco. The conduct of the essay in the greenhouse provide the possibility to study the behavior of plant in many and different soils. However, the sampling step must be done carefully in order to preserve as possible the natural state of soils and by the way ovoid the nitrogen mineralization [16, 17]. Once the legumes find two sources of nitrogen, they use with preference the nitrogen combined in the soil not the atmospheric nitrogen [18, 19].

These authors have reported that many other factors affect significantly the response of legumes to the inoculation mainly those environmental and nutritional.

Rhizobia is another tool known by its importance in the rehabilitation of poor soils especially on nitrogen. These microorganisms are characterized by their ability to tolerate the main environmental factors [20, 21] and improve the growth and biomass of legumes and therefore increased yields. These results are in great agreement with ours, which can be explained by the fact that the rhizobial inoculation guarantees better absorption of nutrients, protection against pests and pathogens and the induction of the systemic resistance of legumes [22-26]. In addition, the rhizobia secrete auxins that promote root growth which in turn stimulates root infection [27, 28]. This could explain our results that showed a good infection assisted by the number and the weight of nodules.

However, it is recommended to identify combinations with native strains promoting the growth of plants to improve the local productivity of chickpea crops [20]. Anterior study revealed that the symbiotic effectiveness is dependent on the particular combination of the strain and legume species, indicating the selective and specific compatibilities between bacterial and plants [29]. This result is verified by our study through which we noticed a significant improvement in shoot biomass of chickpea inoculated with rhizobia. This is explained by the fact that the growth responses of the inoculated legumes are influenced by the symbionts and the compatibility of the interactions between them (reference strain and the native strains) and those with the host plant [29-31] and also by the application of fertilizers mainly nitrogen ones. Moreover, there was a significant decrease in the number and the fresh weight of the nodule of all the studied sites; this could be explained by the inhibitory effect exerted by the nitrogen fertilizer on the rhizobia that is in perfect agreement with the results proved by Franzini, et al. [32].

Beside the importance of rhizobial inoculation with reference strain, it is better to isolate and identify the most efficient strain for each soil. Therefore their use as biofertilizer in order to have a direct application to improve soil fertility, to reduce chemicals and consequently participate to the protection of the environment supporting a sustainable agricultural system.

5. CONCLUSION

In conclusion, our results have confirmed the important effects of the inoculation with rhizobia on the growth of chickpea in soils sampled from different region of Morocco. These results show an interesting way of rehabilitation of degraded lands that are in current deficient in nutrients. Symbiosis with rhizobia is a biological alternative that ensure the improvement of the sustainable production of legumes in different agro-ecological regions.

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