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Effects of Biological and Mineral Fertilization on Yield, Chemical Composition and Physical Characteristics of Chickpea (*Cicer arietinum* L.) Seeds

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Abstract: A field experiment was conducted at Ummdagarsi area in Gezira state, central Sudan to study the effects of inoculation with *Rhizobium* and *Bacillus megatherium* var. *Phosphaticum* (BMP), phosphorus and nitrogen fertilization on yield and seed quality of chickpea plants variety Bergag. *Rhizobium* inoculation significantly ($p \le 0.05$) increased yield, ash, fiber, tannin and protein content of chickpea seeds. BMP inoculation significantly ($p \le 0.05$) increased ash, fiber, protein content and cookability. *Rhizobium* and BMP co-inoculation significantly ($p \le 0.05$) increased yield, 100 seed weight, ash, fat, protein content and cookability. Application of chemical fertilizers significantly ($p \le 0.05$) increased yield, 100 seed weight, ash, fat and protein content.

Key words: Rhizobium, Bacillus megatherium var. Phosphaticum, inoculation, chickpea

INTRODUCTION

Legumes are major direct source of food for man and forage for livestock and therefore represent a critical contribution to world food production (Hubbell and Gerald, 1998). They are very important not only as food crops but possess high propensity to grow in depleted soils thereby serving as a medium of fertilizing succeeding crops through their unique symbiotic capability with nitrogen-fixing bacteria which are inhabited in root nodules of the legumes and the nitrogen balance in the soil is thereby preserved (Okaka et al., 2002). The acreage allotted to legumes in Sudan is presently not more than 10% of the total cultivated area, but it is increasing gradually. This increase occurs in the large extension of the areas devoted to crops such as chickpea, faba beans, groundnuts, soybeans and guar.

Chickpea (*Cicer arietinum* L.) Family: Leguminosae, is an important legume crop cultivated in the Northern and the River Nile states in Sudan. In the period of 2006-2008 the average area planted with chickpea was 10000ha and average yield was 1.92 t/ha (FAO, 2008). Chickpea is an important food for millions of people and perhaps the source of protein for the middle and low income groups. It is also an important cash crop for farmers in central and northern Sudan.

Crop productivity can be increased by the application of chemical, organic and biological fertilizers. Fertilization, especially with N and P can generally increase dry matter production up to two-to three folds (Elliott and Abbott, 2003), that is why farmers are applying high amounts of fertilizers which is very costly and hazardous to environment. Crop scientists all over the world are facing the alarming situation induced by the intensive

uses of chemical fertilizers and they are trying to overcome this condition by exploring alternative sources which are cost effective and safe to the environment (Baset Mia and Shamsuddin, 2010). Microbiological fertilizers are an important part of environment-friendly sustainable agricultural practices (Bloemberg et al., 2000). Biofertilization, as compared to the use of chemical fertilizers, is steadily receiving increased attention and recognition from scientists because the microbial inoculants (including Rhizobium and mycorrhizal inoculants) introduced into soil or plant culture will directly or indirectly enhance plant productivity. Biofertilizers must receive more attention in countries like Sudan, with a predominantly low-input agricultural system of production where chemical fertilizers, if available, may not be economically affordable.

MATERIALS AND METHODS

Two field experiments were conducted at Ummdagarsi area in Gezira state, central Sudan (Latitude 14° 55′ N and longitude 33° 11′ E) for two consecutive seasons 2007/2008 and 2008/2009 in a factorial design with four replicates.

In these experiments, chickpea was fertilized with either nitrogen or phosphorus or with both of them. Plants were either inoculated with the introduced *Rhizobium* strain (USDA 3100) or with *Bacillus Megatherium* var. *Phosphaticum* strain (BMP) which was locally isolated, in addition to combination of each (USDA 3100+BMP). Control plants were kept for comparison. The land was prepared by deep ploughing, harrowing then leveling and ridging. The land was then divided into plots 4 x 7 m each. In treatments with nitrogen fertilizer, 43kg N/ha as

urea was broadcasted immediately after sowing. Phosphorus fertilizer was applied at a rate of 43 kg P/ha as triple super phosphate broadcasted before sowing. Certified seeds of chickpea (Cicer arietinum L.) cultivar Bergag were obtained from Hudeiba Research Station, Agricultural Research Corporation, Sudan. Two to three seeds were placed in a hole on the top of the ridge with 20 cm spacing (between holes) and 70 cm between ridges. Plots were immediately irrigated after sowing and then subsequently irrigated at 15 days intervals. Harvest was done at 16 weeks after sowing. Each plot was harvested separately by cutting the plants just above soil level. Plants were then threshed on a large mat, then collected and weighed to determine yield of each plot separately. 100 seeds from the collected samples from each plot were counted in 4 replicates randomly then weighed.

Samples were taken from seeds of each plot, ground and used for proximate analysis which were conducted according to the methods of AOAC (1984), except for tannin content which was estimated using the modified vanillin HCl method (Price *et al.*, 1978) and Hydration coefficient and Cookability which were determined using the methods described by (Elsheikh *et al.*, 2009)

Multifactor Analysis of Variance (ANOVA) was used to determine the effect of different treatments on the measured parameters. Least significant difference was used to compare between means (Gomez and Gomez, 1984). Significance was accepted at p≤0.05.

RESULTS AND DISCUSSION

Effects of treatments on chickpea yield: Inoculation of chickpea plants with Rhizobium strain USDA 3100 significantly (p<0.05) increased seed yield in both seasons over the control (Table 1). It is known that Rhizobium inoculant with the compatible strain provides the plant with its requirement of nitrogen. Inoculation might provide up to 80% of nitrogen requirement of groundnut (Adlan and Mukhtar, 2004). This makes the plant more vigorous and enables it to absorb nutrients form the soil, which is finally reflected in increasing its yield. The positive effect of Rhizobium inoculation on yield of some legumes was reported previously by Yadav et al. (2010) and Rugheim and Abdelgani (2011). BMP inoculation insignificantly increased yield in both seasons. Velayutham et al. (2003) found that BMP inoculation significantly increased chickpea seed yield. Co-inoculation with Rhizobium and BMP slightly increased chickpea seed yield in both seasons compared to uninoculated control. The positive effect of Co-inoculation with Rhizobium and BMP is in accord with observation of Rudresh et al. (2004).

Nitrogen fertilization significantly (p≤0.05) increased chickpea seed yield in both seasons over the control. Phosphorous fertilization alone and Nitrogen and Phosphorus interaction significantly (p<0.05) increased

chickpea seed yield in the second season compared to uninoculated control. Osman *et al.* (2010) found that chemical fertilization increased faba bean yield.

100-Seed weight: Rhizobium and BMP inoculation separately significantly (p<0.05) increased 100-seed weight in both seasons (Table 1). Velayutham et al. (2003) reported that seed inoculation with BMP increased the 100-seed weight of chickpea. Coinoculation with Rhizobium and BMP significantly (p≤0.05) increased 100 seed weight in both seasons. The increment in 100-seed weight resulted by coinoculation was reported before by Barea et al. (2005). Phosphorus chemical fertilizer significantly (p<0.05) increased chickpea 100-seed weight in the first season. it was previously reported that application of 43 kg N/ha + 43 kg P₂O₅/ha slightly increased chickpea 100-seed weight in the first season. Application of 40 kg P2O5/ha increased 100-seed weight of chickpea (Velayutham et al., 2003). Nitrogen and Phosphorus interaction was found to increase 100-seed weight of faba bean (Osman et al., 2010).

Effects of treatments on the proximate analysis of chickpea seeds

Moisture content: The moisture content of chickpea seeds was not affected by *Rhizobium* inoculation, BMP inoculation or co-inoculation with *Rhizobium* and BMP (Table 2). Elsheikh *et al.* (2009) reported that there was no effect on moisture content following *Rhizobium* inoculation.

Application of nitrogen fertilizer significantly (p \leq 0.05) increased moisture content of chikpea seeds in the first season and slightly increased it in the second season. Phosphorus chemical fertilizer significantly (p \leq 0.05) increased seed moisture content in the second season and slightly increased it in the first season. The increment in seed moisture content due to application of chemical fertilizers was reported by Rugheim and Abdelgani (2011).

Ash content: Rhizobium inoculation significantly (p \leq 0.05) increased ash content of chickpea seeds in the first season (Table 2). BMP inoculation and coinoculation with Rhizobium and BMP significantly (p \leq 0.05) increased ash content of chickpea seeds in the first season. These results are in accord with the results obtained by Osman et al. (2010) for faba bean. Nitrogen fertilizer significantly (p \leq 0.05) increased ash content of chickpea seeds in the second season. Application of nitrogen and phosphorus chemical fertilizers separately significantly (p \leq 0.05) increased ash content of chickpea seeds. These results are in accord with the observations of Rugheim and Abdelgani (2011) for faba bean in Hudeiba, Sudan.

Table 1: Effects of treatments on chickpea yield (t/ha) and 100 seed weight (g)

	Yield		100 Seed weight		
Treatments	First season	Second season	First season	Second season	
No inoculation					
Control	1.75	1.63	16.17	17.59	
Nitrogen (43 kg N/h)	1.88	1.94	15.33	17.01	
Phosphorous (43 kg P ₂ O ₅ /h)	1.76	1.65	13.77	18.24	
Nitrogen + Phosphorous	1.83	1.94	16.70	14.21	
Mean	1.80	1.79	15.49	16.76	
Inoculation with Rhizobium (USDA 3100)					
Control	1.93	1.90	17.51	17.89	
Nitrogen	1.89	1.95	17.14	17.95	
Phosphorous	1.82	1.90	15.71	17.47	
Nitrogen + Phosphorous	2.06	1.77	18.98	17.54	
Mean	1.93	1.88	17.33	17.71	
Inoculation with Phosphobacterin (BMP)					
Control	1.84	1.83	16.74	17.27	
Nitrogen	1.82	1.85	16.18	18.05	
Phosphorous	1.71	1.80	14.81	17.90	
Nitrogen + Phosphorous	1.86	1.87	16.77	15.66	
Mean	1.81	1.83	16.12	17.22	
Inoculation with Rhizobium + phosphobacterin					
Control	1.90	1.80	17.28	15.64	
Nitrogen	1.77	1.82	16.90	16.54	
Phosphorous	1.94	1.81	16.38	18.22	
Nitrogen + Phosphorous	1.80	1.89	16.30	17.74	
Mean	1.85	1.83	16.96	17.03	
LSD for Rhizobium	0.0692	0.0572	0.4796	0.3953	
LSD for phosphobacterin	0.0692	0.0572	0.4796	0.3953	
LSD for Rhizobium x phosphobacterin	0.0979	0.0810	0.6782	0.5591	
LSD for nitrogen	0.0692	0.0572	0.4796	0.3953	
LSD for phosphorous	0.0692	0.0572	0.4796	0.3953	
LSD for nitrogen x phosphorous	0.0979	0.0810	0.6782	0.5591	

Table 2: Effects of treatments on moisture content (%) and ash content (%) of chickpea seeds

	Moisture		Ash		
Treatments	First season	Second season	First season	Second season	
No inoculation					
Control	5.481	4.858	2.267	2.704	
Nitrogen (43 kg N/h)	6.154	4.922	2.176	3.114	
Phosphorous (43 kg P ₂ O ₅ /h)	5.692	5.106	2.189	2.868	
Nitrogen + Phosphorous	5.066	5.135	2.247	2.866	
Mean	5.667	5.005			
Inoculation with Rhizobium (USDA 3100)					
Control	5.612	5.069	2.864	3.125	
Nitrogen	5.594	5.032	2.893	2.955	
Phosphorous	5.641	5.316	2.395	2.893	
Nitrogen + Phosphorous	6.256	5.254	2.737	2.568	
Mean	5.776	5.167	2.722	2.885	
Inoculation with Phosphobacterin (BMP)					
Control	5.216	4.929	2.990	2.907	
Nitrogen	5.571	5.448	2.447	2.767	
Phosphorous	5.726	4.779	2.545	2.597	
Nitrogen + Phosphorous	5.422	4.827	2.821	2.931	
Mean	5.484	4.996	2.701	2.800	
Inoculation with Rhizobium + phosphobacterin					
Control	5.590	4.921	2.872	2.911	
Nitrogen	5.459	4.857	2.642	2.315	
Phosphorous	5.627	5.369	2.871	2.465	
Nitrogen + Phosphorous	5.435	4.805	2.798	2.665	
Mean	5.528	4.988	2.796	2.589	
LSD for Rhizobium	0.4343	0.2348	0.1002	0.1686	
LSD for phosphobacterin	0.4343	0.2348	0.1002	0.1686	
LSD for Rhizobium x phosphobacterin	0.6142	0.3320	0.1418	0.2384	
LSD for nitrogen	0.4343	0.2348	0.1002	0.1686	
LSD for phosphorous	0.4343	0.2348	0.1002	0.1686	
LSD for nitrogen x phosphorous	0.6142	0.3320	0.1418	0.2384	

Table 3: Effects of treatments on fat content (%) and fiber content (%) of chickpea seeds

. ,	Fat		Fiber		
Treatments	First season	Second season	First season	Second season	
No inoculation					
Control	5.848	5.443	3.41	3.28	
Nitrogen (43 kg N/h)	6.486	5.384	3.40	3.93	
Phosphorous (43 kg P ₂ O ₅ /h)	5.999	6.852	3.68	3.41	
Nitrogen + Phosphorous	6.152	5.605	3.69	3.94	
Mean	6.121	5.821	3.55	3.64	
Inoculation with Rhizobium (USDA 3100)					
Control	6.412	6.388	3.86	4.54	
Nitrogen	6.430	5.492	3.76	3.97	
Phosphorous	6.109	5.556	4.08	3.39	
Nitrogen + Phosphorous	6.186	4.570	3.10	4.04	
Mean	6.284	5.502	3.70	3.98	
Inoculation with Phosphobacterin (BMP)					
Control	6.124	5.464	3.61	3.89	
Nitrogen	6.252	6.236	4.01	4.10	
Phosphorous	6.144	5.410	3.51	3.84	
Nitrogen + Phosphorous	6.157	6.591	3.45	3.90	
Mean	6.169	5.924	3.64	3.93	
Inoculation with Rhizobium + phosphobacterin					
Control	6.196	6.877	4.21	3.74	
Nitrogen	6.143	4.416	3.63	4.00	
Phosphorous	6.041	5.434	3.55	3.62	
Nitrogen + Phosphorous	6.112	5.643	4.08	4.24	
Mean	6.123	5.593	3.87	3.90	
LSD for Rhizobium	0.1908	0.2505	0.1591	0.1235	
LSD for phosphobacterin	0.1908	0.2505	0.1591	0.1235	
LSD for Rhizobium x phosphobacterin	0.2698	0.3543	0.2250	0.1746	
LSD for nitrogen	0.1908	0.2505	0.1591	0.1235	
LSD for phosphorous	0.1908	0.2505	0.1591	0.1235	
LSD for nitrogen x phosphorous	0.2698	0.3543	0.2250	0.1746	

Fat content: Co-inoculation with *Rhizobium* and BMP slightly increased fat content of chickpea seeds in the first season (Table 3). Nitrogen fertilizer alone and nitrogen and phosphorus interaction significantly ($p \le 0.05$) increased fat content of chickpea seeds in the first season. Phosphorus fertilizer significantly ($p \le 0.05$) increased fat content of chickpea seeds in the second season. The nitrogen+phosphorus fertilizers gave the highest fat content. The increment in fat content of faba bean due to biological and chemical fertilization was previously reported by Osman *et al.* (2010).

Fiber content: Rhizobium and BMP inoculation separately significantly (p \leq 0.05) increased fiber content of chickpea seeds in the second season. In the first season, inoculation slightly increased chickpea seed fiber content (Table 3). Co-inoculation with Rhizobium and BMP, nitrogen fertilization and nitrogen and phosphorus interaction significantly (p \leq 0.05) increased fiber content of chickpea seeds in both seasons.

Phosphorus fertilizer significantly (p≤0.05) increased fiber content of chickpea seeds in both seasons. The positive effect of *Rhizobium* and BMP inoculation on fiber content was previously reported by Osman *et al.* (2010) and Rugheim and Abdelgani (2011) and contradictory to the findings of Elsheikh *et al.* (2009).

Protein content: *Rhizobium* inoculation significantly (p \leq 0.05) increased protein content in chickpea seeds in both seasons (Table 4). *Rhizobium* inoculation increased nitrogen content and consequently amino acids that bind together forming chains. There are almost 20 amino acids the sequences of which make the different types of the proteins. Proteins are highly essential for living cells since they form antibodies, enzymes and hormones. They provide structure and support for cells and also they carry out transport and storage of atoms and small molecules within cells and throughout the body.

BMP inoculation significantly (p \leq 0.05) increased protein content in chickpea seeds in the first season. Coinoculation with *Rhizobium* and BMP significantly (p \leq 0.05) increased protein content in chickpea seeds in the second season. This finding proves the results of Lucas-Garcia *et al.* (2004) for *Lupinus albus* L. Application of nitrogen separately significantly (p \leq 0.05) increased protein cotent in chickpea seeds in the second season as was previously found by Rugheim and Abdelgani (2011) for faba bean. Phosphorus fertilizer significantly (p \leq 0.05) increased protein content of chickpea seeds in the second season. Application of chemical fertilizers significantly (p \leq 0.05) increased protein content of chickpea seeds in the first season.

Table 4: Effects of treatments on protein content (%) and carbohydrates content (%) of chickpea seeds

Table 4. Effects of treatments of protein content (39) an	Protein	, ,	Carbohydrates		
Treatments	First season	Second season	First season	Second season	
No inoculation					
Control	17.83	17.86	65.150	65.847	
Nitrogen (43 kg N/h)	18.08	18.71	63.689	63.928	
Phosphorous (43 kg P ₂ O ₅ /h)	16.54	20.05	65.955	61.706	
Nitrogen + Phosphorous	19.83	16.76	63.007	65.689	
Mean	18.07	17.91	64.449	64.292	
Inoculation with Rhizobium (USDA 3100)					
Control	19.54	18.92	61.700	61.950	
Nitrogen	19.54	19.24	61.772	63.306	
Phosphorous	18.96	20.00	62.807	62.834	
Nitrogen + Phosphorous	17.25	19.61	64.467	63.950	
Mean	18.82	19.44	62.686	63.010	
Inoculation with Phosphobacterin (BMP)					
Control	17.83	18.34	64.218	64.468	
Nitrogen	19.25	16.61	62.462	64.384	
Phosphorous	18.37	18.34	63.690	65.023	
Nitrogen + Phosphorous	18.37	18.09	63.772	63.652	
Mean	18.46	17.84	63.536	64.494	
Inoculation with Rhizobium + phosphobacterin					
Control	17.79	19.56	63.334	61.990	
Nitrogen	17.87	18.65	64.248	64.363	
Phosphorous	17.79	18.90	64.112	64.200	
Nitrogen + Phosphorous	18.37	19.35	63.190	63.985	
Mean	17.69	19.29	63.721	63.635	
LSD for Rhizobium	0.2614	0.4395	0.6615	0.5384	
LSD for phosphobacterin	0.2614	0.4395	0.6615	0.5384	
LSD for Rhizobium x phosphobacterin	0.5549	0.6215	0.9355	0.7614	
LSD for nitrogen	0.2614	0.4395	0.6615	0.5384	
LSD for phosphorous	0.2614	0.4395	0.6615	0.5384	
LSD for nitrogen x phosphorous	0.5549	0.6215	0.9355	0.7614	

These results are in accord with the observations of Babiker *et al.* (1995); Elsheikh *et al.* (2009) and Osman *et al.* (2010).

Carbohydrates content: Rhizobium inoculation alone and co-inoculation with Rhizobium and BMP significantly (p≤0.05) decreased the carbohydrates content of chickpea seeds in both seasons (Table 4). BMP inoculation didn't affect the carbohydrates content chickpea seeds in the first season. Nitrogen fertilizers and nitrogen and phosphorus interaction didn't affect the carbohydrates content of chickpea seeds in both seasons compared to control. Phosphorus fertilizer significantly (p<0.05) increased the carbohydrates content of chickpea seeds in the first season, but not in the second season. This result is in accord with the observations of Osman et al. (2010). Generally, the carbohydrates content in the seeds of leguminous crops was found to decrease with Rhizobium inoculation (Elsheikh, 2001). This may be due to that Rhizobium inoculation increased protein content.

Tannin content: Rhizobium and BMP inoculation separately significantly (p≤0.05) increased tannin content in chickpea seeds (Table 5). This result is in accord with the observations of Elsheikh *et al.* (2009).

Co-inoculation with *Rhizobium* and BMP significantly (p<0.05) increased tannin content in chickpea seeds in the second season. In first season, it slightly increased tannin content in chickpea seeds over the control.

Nitrogen fertilization significantly (p \leq 0.05) increased tannin content of chickpea seeds in the second season. This result is in accord with the observations of Babiker *et al.* (1995). Phosphorus fertilization significantly (p \leq 0.05) increased tannin content of chickpea seeds in the second season. Chemical fertilization had no effect on the tannin content of chickpea seeds. This result is in accord with the observations of Osman *et al.* (2010).

Hydration coefficient: *Rhizobium* inoculation significantly (p \leq 0.05) increased the hydration coefficient of chickpea seeds (Table 5). Co-inoculation with *Rhizobium* and BMP significantly (p \leq 0.05) increased hydration coefficient of chickpea seeds in the first season.

Nitrogen fertilization and nitrogen plus phosphorus chemical fertilization significantly (p≤0.05) increased hydration coefficient of chickpea seeds in both seasons. Phosphorus fertilization slightly increased hydration coefficient of chickpea seeds in both seasons. Generally, the hydration coefficient is affected by locality, harvesting time and genotype (Salih and Khairi, 1990).

Table 5: Effects of treatments on tannin content (%), hydration coefficient (%) and cookabiliy (%) of chickpea seeds

	Tannin		Hydration coefficient		Coockabilty	
	First	Second	First	Second	First	Second
Treatments	season	season	season	season	season	season
No inoculation						
Control	0.69	0.73	203.29	205.69	5.85	5.61
Nitrogen (43 kg N/h)	0.68	0.83	210.44	209.28	6.64	6.50
Phosphorous (43 kg P ₂ O ₅ /h)	0.83	0.77	205.21	206.24	7.10	8.35
Nitrogen + Phosphorous	0.54	0.68	207.60	211.07	4.02	7.41
Mean	0.68	0.75	206.64	208.07	5.90	6.97
Inoculation with Rhizobium (USDA 3100)						
Control	0.71	0.91	210.74	209.39	7.58	8.01
Nitrogen	0.85	0.69	210.26	212.30	6.41	8.48
Phosphorous	0.74	0.93	209.79	210.90	7.66	7.39
Nitrogen + Phosphorous	0.73	0.82	216.51	216.04	6.75	7.24
Mean	0.76	0.84	211.83	212.16	7.10	7.78
Inoculation with Phosphobacterin (BMP)						
Control	0.76	0.78	207.24	210.97	6.11	7.21
Nitrogen	0.86	0.79	202.68	204.32	7.17	6.84
Phosphorous	0.77	0.71	210.02	207.26	7.14	6.68
Nitrogen + Phosphorous	0.65	0.94	205.76	213.19	6.64	8.18
Mean	0.76	0.80	206.42	208.93	6.76	7.23
Inoculation with Rhizobium + phosphobacterin						
Control	0.70	0.77	209.64	210.54	7.01	7.50
Nitrogen	0.74	0.83	215.83	209.12	8.06	6.18
Phosphorous	0.76	0.92	209.51	210.10	8.18	6.19
Nitrogen + Phosphorous	0.59	0.84	208.99	208.22	7.40	6.22
Mean	0.70	0.84	210.99	209.49	7.66	6.52
LSD for Rhizobium	0.0456	0.0372	2.2005	2.8129	0.2717	0.2653
LSD for phosphobacterin	0.0456	0.0372	2.2005	2.8129	0.2717	0.2653
LSD for Rhizobium x phosphobacterin	0.0645	0.0526	3.1120	3.9781	0.3843	0.3752
LSD for nitrogen	0.0456	0.0372	2.2005	2.8129	0.2717	0.2653
LSD for phosphorous	0.0456	0.0372	2.2005	2.8129	0.2717	0.2653

Cookability: *Rhizobium* and BMP inoculation separately significantly ($p \le 0.05$) increased cookability of chickpea seeds in both seasons (Table 5). Co-inoculation with *Rhizobium* and BMP significantly ($p \le 0.05$) increased cookability of chickpea seeds in the first season.

Nitrogen and phosphorus, separately and nitrogen and phosphorus interaction significantly (p≤0.05) increased cookability of chickpea seeds. Elsheikh *et al.* (2009) reported an improvement of soybean cookability by *Rhizobium* inoculation.

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