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(*Cyamopsis tetragonoloba* (L.) Taub.)

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1 ***Rhizobium* inoculation and phosphate fertilization effects on productive and qualitative**
2 **traits of guar (*Cyamopsis tetragonoloba* (L.) Taub.)**
3

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18 ABSTRACT

19 Guar (*Cyamopsis tetragonoloba* (L.) Taub.), a grain legume grown in India, Pakistan
20 and the United States, draws interest for irrigated environments in the Mediterranean
21 areas. A limitation to widespread guar adaptation in this environment is its low
22 capability to develop N-fixing *Rhizobium* nodulation. A field study was conducted
23 in South-Eastern Sicily, Italy in 2005 and 2006, to examine three levels of
24 *Rhizobium* inoculation (non-inoculated, inoculated at sowing, inoculated at sowing
25 plus at the third true leaf) and two levels of applied phosphorus fertilizer (0 and 40
26 kg ha⁻¹ P). Plant height, pods per plant, seed yield, number of nodules per plant and
27 seed galactomannan content were measured. No significant difference was found
28 due to year of cultivation. Plant height was not significantly affected by *Rhizobium*
29 or P fertilizer. Phosphorus fertilizer promoted the number of pods per plant,
30 especially when bacteria inoculation was applied. Seed yield was significantly
31 increased by inoculation and phosphorus levels. Inoculant caused a significant
32 increase in seed yield (by 34%) from 2.2 t ha⁻¹ (I₀) to 3.4 t ha⁻¹ (I₂), whereas
33 phosphorus application enhanced yields by 18% when P₀ (2.7 t ha⁻¹) were compared
34 with P₄₀ plots (3.3 t ha⁻¹). Without inoculant there was no nodulation, whereas the
35 number of nodules per plant increased to 14 in plots with the highest level of
36 inoculation. Fertilizer P application did not affect *Rhizobium* nodulation.
37 Galactomannan content was not influenced by inoculation and P fertilization,
38 however increased seed yield resulting from inoculation and P fertilizer led to
39 increased galactomannan production per hectare.

40
41 **Key words:** guar, nodulation, *Rhizobium* inoculation, phosphorus, yield, galactomannans.
42
43

44 1. Introduction

45 Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a spring-summer grain legume crop with outstanding
46 drought resistance and salinity tolerance (Ashraf et al., 2002; Ashraf et al., 2005; Francois et al.,
47 1990). It is mainly cultivated in semi-arid regions of the world, such as India, Pakistan, and to a
48 lesser extent, the USA and South Africa. Guar also shows a high potential for production in other
49 regions of the world with hot climate and limited water availability (Sortino and Gresta, 2007;
50 Gresta et al., 2013). Several studies (Gresta et al., 2016a; Gresta et al., 2018b; Santonoceto et al., in
51 press) demonstrated that guar can be successfully cultivated in Mediterranean areas, where summer
52 high temperature (above 30°C) and often elevated water salt content (3 - 4 dS m⁻¹) do not allow the
53 cultivation of many crops.

54 In 1975 the U.S. National Academy of Science (1975) included guar among the underexploited
55 tropical plants with promising economic value for its drought and salinity tolerance, for its
56 adaptability to a variety of soils and for its versatile gum.

57 Guar seed endosperm is the main source of galactomannans which are used mostly for industrial
58 applications (Whistler and Hymowitz, 1979, Mathur, 2012) although carob, tara, fenugreek, and
59 senna can provide lesser amounts. Galactomannans are long-branched polymers of mannose and
60 galactose with high molecular weight (Mathur, 2012), which have unique properties (5-8 times the
61 thickening power of starch). These polymers are used as a flocculant, thickener and binder in a large
62 number of industrial sectors including petroleum extraction, paper, pharmaceutical, and cosmetics
63 (Mudgil et al., 2014; Das et al., 2011). Compared to other sources of galactomannans, guar gum has
64 an advantage as a cold-water soluble polysaccharide. The guar meal by-product of galactomannan
65 extraction consists of the seed coat and seed embryo. It has a high protein content, around 50%
66 (Chiofalo et al. 2018; Gresta et al., 2017).

67 Due to low guar grain yield compared to most legume crops, improved N fixation is an
68 affordable and more sustainable way to increase guar seed yield and improve the ecosystem services
69 role of guar in cropping systems. Some authors report adequate *Rhizobium* nodulation of guar hence
70 fixation of atmospheric nitrogen (Elsheikh and Ibrahim, 1999) with a high positive effect on
71 subsequent crops due to nitrogen fixation (Tucker and Foraker, 1975; Rao et al., 1995; Saxena et al.,
72 1997). A common problem in current guar cultivation in both new and historical production
73 environments, remains the limited capability of the crop to produce root nodules (Erdman, 1948;
74 Kumar and Singh, 2002; Trostle, 2019). Conflicting information is reported on the effects of
75 nodulation on guar grain yield. Khurana et al. (1978) found seed production of 32 different guar
76 genotypes was not correlated with nitrogen fixation. Rao et al. (1995) indicated a significant

77 modification of soil biological properties due to three years of continuous rotation with guar. This is
78 the result of a strong rhizosphere effect promoted by the presence of guar roots. The observation has
79 great importance since efficient biological nitrogen fixation in guar can play an important role in
80 reducing the ‘crop environmental impact’ (Gresta et al., 2014) compared to other crops like cotton
81 or corn, for a profitable crop rotation system.

82 Biological nitrogen fixation is a renewable source of nitrogen for both the plant being cropped
83 and succeeding crops, representing probably the most important ecosystem service the crop can
84 provide. To promote legume crop nitrogen fixation, commercial enterprises produce bio-inoculant
85 (granular, liquid or powder), containing crop-specific biological nitrogen fixing microorganisms.
86 Among the environmental factors which may affect *Rhizobium* fixation, phosphorus application can
87 improve the efficiency of the *Rhizobium* (Rauti and Ali, 1983; Mand et al., 1991).

88 An experiment was conducted to evaluate the effect of *Rhizobium* inoculation and phosphorus
89 fertilization on grain yield and galactomannan content of guar in a Mediterranean environment.

90

91 **2. Materials and methods**

92 *2.1 Field experiment*

93 A two-year trial was conducted during 2005 and 2006 in Modica, Southern Sicily, Italy
94 (36°49’29’’ N, 14°54’46’’ E, 230 m a.s.l.) on a soil under fallow the previous year, and where guar
95 was never grown before. Texas guar variety ‘Monument’ was manually sowed on June 21, 2005 and
96 May 30, 2006 in plots of 20 m² (5 x 4 m), with rows 0.5 m apart and plants within the row 8 cm.
97 The plant density was equal to 25 plants m⁻² (around 10 kg ha⁻¹). Soil was medium textured with a
98 low P concentration (see characteristics in Table 1).

99

100 **TABLE 1**

101

102 The experiment was laid out in a split-plot design. Inoculant treatments included three levels
103 as main plot (non-inoculated, I₀; inoculated at sowing, I₁; and inoculated at both sowing and at the
104 third true leaf, I₂). Phosphorus treatment levels were the subplot (0 -P₀ and 40 -P₄₀ kg ha⁻¹ of P₂O₅)
105 applied immediately before sowing. The P source was granular super simple phosphate 20% spread
106 and incorporated 15 cm in the soil. All treatments (six) were replicated three times. No nitrogen
107 fertilization was supplied to avoid any possible suppressive effect on nodulation.

108 The guar-specific inoculant strain CB3035 (Nodulaid 100, Bio-Care Technology Pty, now
109 BASF, Australia) was obtained in powder form, diluting 10 g of inoculant into 1 litre of distilled

110 water, as directed by the label. Seeds and rhizobial suspension (I₁, I₂) were mixed until the seeds
111 were well coated, and the adhesive contained in Nodulaid helped the seeds stick to the peat.
112 For soil surface spray inoculation (I₂), 50 g of inoculant was diluted in 5 liters and was applied on
113 the rows with the help of an atomizer at a dose of 100 l ha⁻¹.

114 After treatment, the soil was sprinkler irrigated in order to convey the symbiont into the soil,
115 with about 15 mm of water.

116 Weeds were managed by hand. Sprinkler irrigation was adopted to all the treatments with a
117 total water supply of 3100 m³ ha⁻¹ in 2005 and of 3650 m³ ha⁻¹ in 2006, restoring 100% of the guar
118 crop's evaporative demand, estimated by class A pan evaporation, corrected through the use of the
119 lowest crop coefficients (K_c) suggested for soybean by Doorenbos and Kassam (1979). Irrigation
120 was applied 8 and 10 times for 2005 and 2006, respectively, and stopped 45 days before harvest.

121 Harvest was conducted in early November in 2005 and late October in 2006. A sampling
122 unit of 2 m² in two inner rows was marked in each plot and plant samples were harvested. The
123 following parameters were measured: plant height, pods per plant and grain yield. Seed yield was
124 adjusted to 13% of moisture. On three plants for each plot, roots were dug with a shovel at 40 cm
125 depth and number of nodules per plant was counted. Galactomannan content was determined
126 following the methodology in Gresta et al. (2013). During the trial, the main climatological
127 parameters were measured and recorded by an in-field data logger (CR21, Campbell Scientific, Inc.,
128 UT, USA). In the first year, from sowing to emergence temperature showed a minimum of 17.6°C
129 and maximum of 29.5°C. After that, temperature ranged from maximum of 30.8°C (average of the
130 first ten days of August) and a minimum of 16.5°C (late October), with an average temperature
131 during the trial period of 27.7°C (Figure 1). Rainfall was 100 mm during the 2005 trial period, 50%
132 of which fell in the month of October, thus not useful for the plants. In 2006, corresponding
133 temperatures were 18.2°C minimum and 23.4°C maximum until emergence, with an average
134 temperature of the whole crop cycle of 29.0°C. Maximum temperatures were over 30°C from
135 midJune to the end of August. During the 2006 trial, rainfall was 185 mm, with 133 mm in October
136 then subsequently dry through harvest.

137

138 **FIGURE 1**

139

140 *2.2 Data analysis*

141 Data collected in this study were processed utilizing a mixed model of analysis of variance
142 (ANOVA), where Inoculation and Phosphorus fertilization were considered as fixed effects, and

143 year as random effect. Galactomannan percentage data were previously transformed using arcsine
144 square root transformation to ensure normality. Tukey's HSD (Honestly Significant Difference) test
145 was adopted to analyze statistical differences between treatments ($P < 0.05$) with DSAASTAT
146 software (Onofri, 2007).

147

148 **3. Results and discussion**

149 The components of the variance and the ANOVA model showed no effect of year of cultivation,
150 whether directly or as an interaction with inoculant or P fertilizer (Table 2). Thus, all data were
151 combined for year of cultivation.

152

153 **TABLE 2**

154

155 Plant height showed no significant variation due to inoculation or phosphorus fertilizer, with a
156 mean value of 84.6 cm (Fig 2a).

157 The interaction of inoculant and P fertilizer resulted in significant increases in pods per plant. Thus
158 phosphorus fertilizer promoted the number of pods per plant, especially when bacteria inoculation
159 was applied (Fig. 2b). The $I_2 \times P_{40}$ treatment resulted in a higher number of pods per plant when
160 compared to non-inoculated without phosphorus, $I_0 \times P_0$ (63.5 vs 36.8 pods per plant). *Rhizobium*
161 bacteria inoculant and phosphorus fertilizer each significantly increased guar seed yield (Fig. 2c).
162 Inoculant led to a significant increase in seed yield (by 34%) from 2.2 t ha⁻¹ (I_0) to 3.4 t ha⁻¹ (I_2),
163 whereas phosphorus application enhanced yields (by 18%) when P_0 (2.7 t ha⁻¹) were compared with
164 P_{40} plots (3.3 t ha⁻¹). These results are in agreement with the findings of Elsheikh and Ibrahim
165 (1999) and Ibrahim et al. (2016) that reported *Bradyrhizobium* strains significantly increased guar
166 yield, protein, crude fiber and mineral content, but had no effect on 100-seed weight. Moreover,
167 this indicates that phosphorus could have an important role in the nitrogen fixation process,
168 confirming the result obtained by other authors (Rauti et Ali, 1983; Mand et al., 1991). In particular,
169 Mand et al., (1991) found soil nitrogenase activity was doubled by applying 40 kg of P₂O₅ ha⁻¹ in
170 guar. Crews (1993) discussed the effect of phosphorus in regulating nitrogen fixation in another
171 legume, alfalfa; soil nitrogenase activity increased in alfalfa where phosphorus was added, and
172 foliar P concentrations and nitrogenase activity were positively correlated. Similar work in guar is
173 merited across different soil types.

174

175 **FIGURE 2**

176

177 Our results report no active presence of any native root nodulating *rhizobia* capable of
178 infecting guar roots (Fig. 2d). In fact, in the absence of inoculant (I_0) we recorded no nodulation.
179 Nodulation was significantly enhanced by inoculant, but not by added P. Furthermore, soil surface
180 spray inoculation (I_2) of *Rhizobium* further increased nodulation, showing the highest number of
181 nodules per plant (13.8).

182 Generally poor to no nodulation is often reported in guar fields across much of the world guar
183 growing region (Abidi et al., 2015; Thapa et al., 2018; Kumar and Singh, 2002). Khurana et al.
184 (1978) in a trial carried out in North India involving 32 genotypes found nodule number per plant
185 ranging from 0.9 to 5.7. Stafford and Lewis (1980), in an inoculation trial reported only 36% of guar
186 plants were effectively nodulated. In contrast, Mal and Yadav (1972) recorded a high number of
187 nodules (46 and 41 on two guar varieties). Mal (1969) reports that guar crop fixes 50-150 kg N ha⁻¹
188 and Wetselaar (1967) estimated from a three-year trial that guar added to the soil plant-system a
189 total of 219 kg N ha⁻¹.

190 Guar researchers and farmers are mistaken to assume measurable contribution of rhizobially-fixed
191 nitrogen without examining the roots for active nodules. As is well known, strains of *Rhizobium* are
192 usually crop-specific as a given rhizobial strain can infect only a particular crop or related crops.
193 Guar can be nodulated by both *Rhizobium* and *Bradyrhizobium* belonging to the cowpea group
194 (Richmond, 1926, Wilson, 1939; Dadarwal et al., 1985). Khandelwal and Sindhu (2012) examined
195 95 *Bradyrhizobium/Rhizobium* strains and reported highly significant differences among different
196 strains in the number and weight of nodules. They report a greater plant productivity related
197 directly to rhizobial nodulation. In contrast, more recently, Thapa et al. (2018), in a greenhouse
198 experiment, found that inoculation with *Rhizobium* strain USDA 3385 on two guar varieties did not
199 increase nodulation versus a control as plants were well nodulated in the control from a regional
200 soil. The lack of nodulation benefit due to the inoculant may be due to lower bacterial count from
201 seedbox powder inoculants.

202 There were no significant responses to both phosphorus and bacteria inoculation application in
203 terms of galactomannan content in seed with an overall mean of 32.4% on dry weight seed basis
204 (Fig. 2e). However, galactomannan yield per hectare was increased due to an increase in seed yield,
205 rising from 0.73 t ha⁻¹ (I_0) to 1.16 t ha⁻¹(I_2),. Galactomannan contents agree with those reported by
206 Gresta et al. (2018a) in a similar environment and slightly higher than those reported by Gresta et al.
207 (2016b) on the same variety. Galactomannan content is not strictly linked to phosphorus application.

208 No references are reported in the literature in this respect so physiological research is needed before
209 developing agronomic hypotheses.

210

211 **4. Conclusions**

212 The present research indicates rhizobial inoculation, in soil lacking of the specific guar *Rhizobium*,
213 is a valuable and sustainable practice to apply in guar cultivation. Phosphorus applications promote
214 seed yield, as well, although it was to a lesser extent. Although some literature reports no increased
215 guar grain yields due to *Rhizobium* inoculation, all guar production systems should be investigated
216 for environmentally and economically favorable nitrogen nutrition of guar via use of a guar-specific
217 *Rhizobium* inoculant as evaluated in this research. No effect of bacteria inoculation and phosphorus
218 application has been observed directly on galactomannan content, however galactomannan yield per
219 ha was increased because of seed yield increase. The results of this trial could be useful for
220 researchers to further develop the nitrogen fixation process in guar, and to farmers to improve
221 agronomic practices of guar, especially in areas where it has never been cultivated before.

222

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226

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Highlights

- Inoculation significantly affected nodule number.
- The interaction of inoculation x phosphate fertilizer significantly affected pods per plant.
- Bacteria inoculation enhanced seed and galactomannan yield per hectare.
- Galactomannan content (%) was not influenced by inoculation and phosphate fertilization.
- The present research proves inoculation as a valuable and sustainable practice in guar cultivation.

Table 1. Soil characteristics of the experimental field in Modica, Southern Sicily, Italy

Traits	unit	value
pH	-	7.2
Sand	%	39
Silt	%	24
Clay	%	37
Total calcium carbonate equivalent	%	18,6
Total nitrogen (Kjeldahl)	‰	2,1
Phosphorus (Olsen)	ppm	49,2
Potassium (Ammonium acetate)	ppm	355,2

Table 2Analysis of variance for *Rhizobium* inoculation and applied P fertilizer, Sicily, Italy (2005-2006)

Source of Variation	DF	Plant height	Pods per plant	Seed Yield	Nodules plant	galactomannan content
Year (Y)	1	2550.25 ^{ns}	603.73 ^{ns}	0.49 ^{ns}	36.00 ^{ns}	0.98 ^{ns}
Inoculation (I)	2	7.58 ^{ns}	794.49 ^{ns}	5.76 ^{**}	688.86 [*]	6.22 ^{ns}
Y x I	2	60.25 ^{ns}	301.13 ^{ns}	0.009 ^{ns}	27.75 ^{ns}	1.23 ^{ns}
Fertilizer (F)	1	491.36 ^{ns}	1322.00 ^{ns}	3.54 [*]	44.4 ^{ns}	0.17 ^{ns}
Y x F	1	110.25 ^{ns}	17.11 ^{ns}	0.005 ^{ns}	1.77 ^{ns}	1.99 ^{ns}
I x F	2	284.69 ^{ns}	23.12 [*]	0.25 ^{ns}	17.86 ^{ns}	2.07 ^{ns}
Y x F x I	2	7.58 ^{ns}	28.01 ^{ns}	0.15 ^{ns}	19.19 ^{ns}	1.80 ^{ns}
Residual	12	38.28	56.64	0.15	52.56	3.17

df degree freedom; ns not significant; ** significant at $P \leq 0.01$; * significant at $P \leq 0.05$.

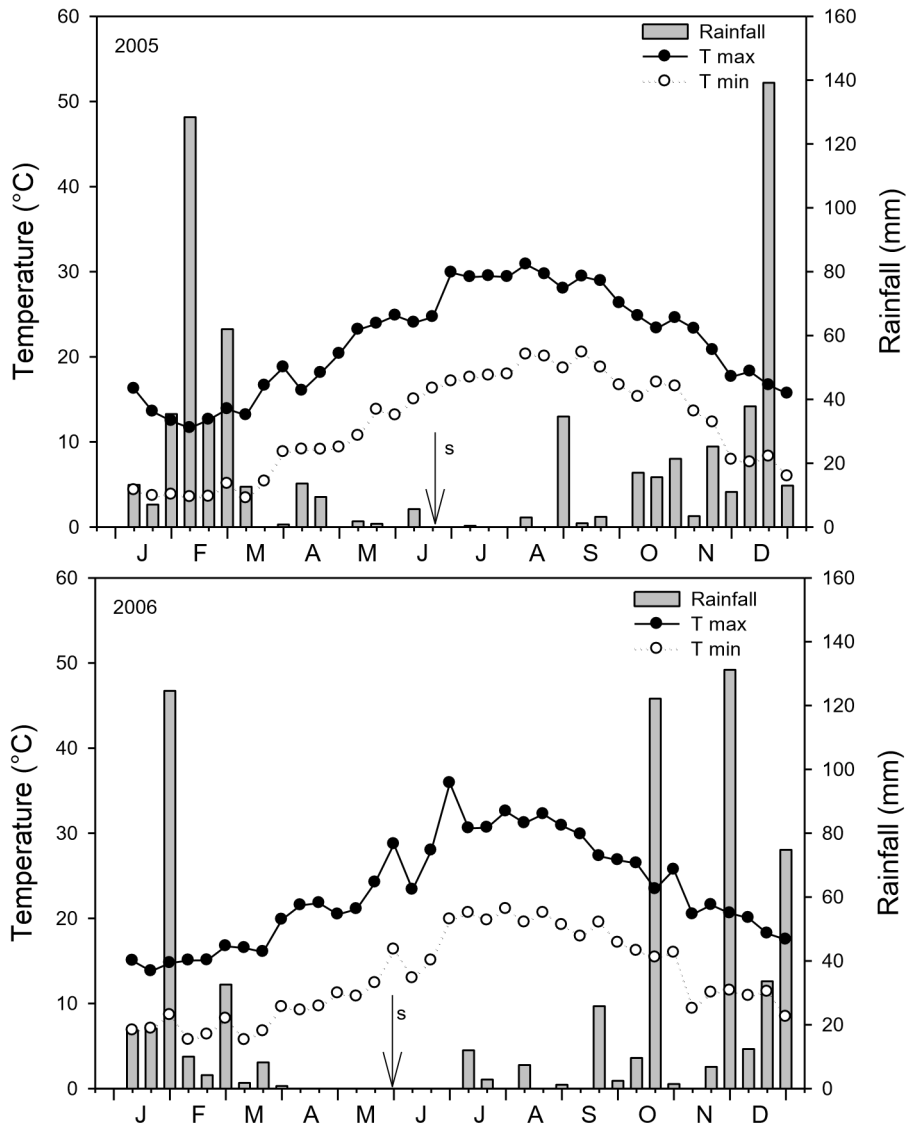
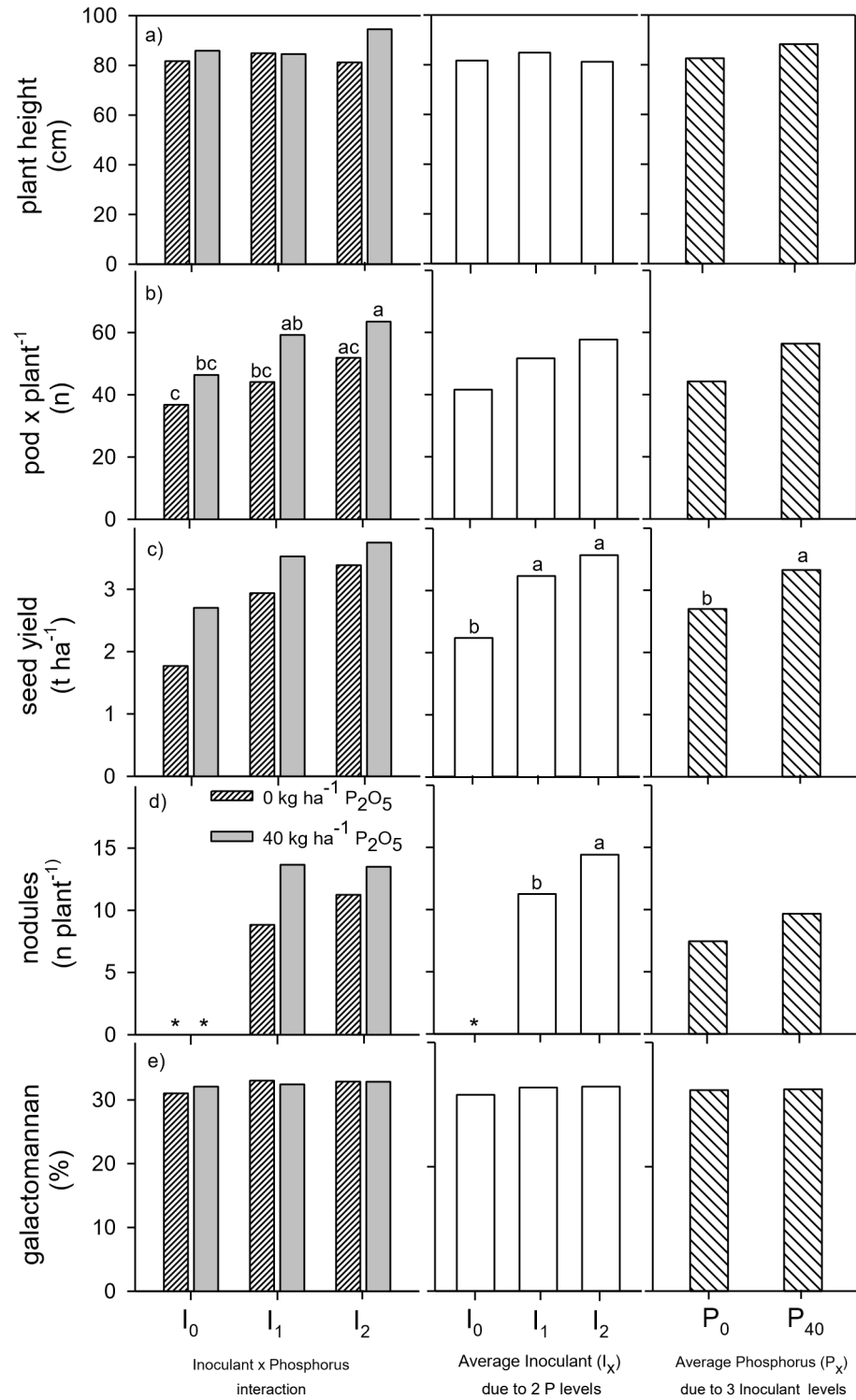


Fig. 1. Temperature and rainfall in the two-year of trial in the experimental field. The arrow with the 's' indicates the sowing time (Modica, Southern Italy).



* Indicates no nodules

Fig. 2. Studied variables in relation to inoculation and phosphorus fertilization. Different letters indicate significant difference between treatments.