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

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ABSTRACT

Guar (*Cyamopsis tetragonoloba* (L.) Taub.), a grain legume grown in India, Pakistan and the United States, draws interest for irrigated environments in the Mediterranean areas. A limitation to widespread guar adaptation in this environment is its low capability to develop N-fixing *Rhizobium* nodulation. A field study was conducted in South-Eastern Sicily, Italy in 2005 and 2006, to examine three levels of *Rhizobium* inoculation (non-inoculated, inoculated at sowing, inoculated at sowing plus at the third true leaf) and two levels of applied phosphorus fertilizer (0 and 40 kg ha^{-1} P). Plant height, pods per plant, seed yield, number of nodules per plant and seed galactomannan content were measured. No significant difference was found due to year of cultivation. Plant height was not significantly affected by *Rhizobium* or P fertilizer. Phosphorus fertilizer promoted the number of pods per plant, especially when bacteria inoculation was applied. Seed yield was significantly increased by inoculation and phosphorus levels. Inoculant caused a significant increase in seed yield (by 34%) from 2.2 t ha^{-1} (I_0) to 3.4 t ha^{-1} (I_2), whereas phosphorus application enhanced yields by 18% when P_0 (2.7 t ha^{-1}) were compared with P_{40} plots (3.3 t ha^{-1}). Without inoculant there was no nodulation, whereas the number of nodules per plant increased to 14 in plots with the highest level of inoculation. Fertilizer P application did not affect *Rhizobium* nodulation. Galactomannan content was not influenced by inoculation and P fertilization, however increased seed yield resulting from inoculation and P fertilizer led to increased galactomannan production per hectare.

Key words: guar, nodulation, Rhizobium inoculation, phosphorus, yield, galactomannans.

1. Introduction

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

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

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

Due to low guar grain yield compared to most legume crops, improved N fixation is an affordable and more sustainable way to increase guar seed yield and improve the ecosystem services role of guar in cropping systems. Some authors report adequate *Rhizobium* nodulation of guar hence fixation of atmospheric nitrogen (Elsheikh and Ibrahim, 1999) with a high positive effect on subsequent crops due to nitrogen fixation (Tucker and Foraker, 1975; Rao et al., 1995; Saxena et al., 1997). A common problem in current guar cultivation in both new and historical production environments, remains the limited capability of the crop to produce root nodules (Erdman, 1948; Kumar and Singh, 2002; Trostle, 2019). Conflicting information is reported on the effects of nodulation on guar grain yield. Khurana et al. (1978) found seed production of 32 different guar genotypes was not correlated with nitrogen fixation. Rao et al. (1995) indicated a significant modification of soil biological properties due to three years of continuous rotation with guar. This is the result of a strong rhizosphere effect promoted by the presence of guar roots. The observation has great importance since efficient biological nitrogen fixation in guar can play an important role in reducing the ‘crop environmental impact’ (Gresta et al., 2014) compared to other crops like cotton or corn, for a profitable crop rotation system.

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

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

2. Materials and methods

2.1 Field experiment

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

TABLE 1

The experiment was laid out in a split-plot design. Inoculant treatments included three levels as main plot (non-inoculated, I_0; inoculated at sowing, I_1; and inoculated at both sowing and at the third true leaf, I_2). Phosphorus treatment levels were the subplot (0 -P_0 and 40 -P_{40} kg ha^{-1} of P_2O_5) applied immediately before sowing. The P source was granular super simple phosphate 20% spread and incorporated 15 cm in the soil. All treatments (six) were replicated three times. No nitrogen fertilization was supplied to avoid any possible suppressive effect on nodulation.

The guar-specific inoculant strain CB3035 (Nodulaid 100, Bio-Care Technology Pty, now BASF, Australia) was obtained in powder form, diluting 10 g of inoculant into 1 litre of distilled water, as directed by the label. Seeds and rhizobial suspension (I_1, I_2) were mixed until the seeds were well coated, and the adhesive contained in Nodulaid helped the seeds stick to the peat.

For soil surface spray inoculation (I_2), 50 g of inoculant was diluted in 5 liters and was applied on the rows with the help of an atomizer at a dose of 100 l ha^{-1}.

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

Weeds were managed by hand. Sprinkler irrigation was adopted to all the treatments with a total water supply of 3100 m^3 ha^{-1} in 2005 and of 3650 m^3 ha^{-1} in 2006, restoring 100% of the guar crop's evaporative demand, estimated by class A pan evaporation, corrected through the use of the lowest crop coefficients (Kc) suggested for soybean by Doorenbos and Kassam (1979). Irrigation was applied 8 and 10 times for 2005 and 2006, respectively, and stopped 45 days before harvest.

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

FIGURE 1

2.2 Data analysis

Data collected in this study were processed utilizing a mixed model of analysis of variance (ANOVA), where Inoculation and Phosphorus fertilization were considered as fixed effects, and year as random effect. Galactomannan percentage data were previously transformed using arcsine square root transformation to ensure normality. Tukey's HSD (Honestly Significant Difference) test was adopted to analyze statistical differences between treatments ($P < 0.05$) with DSAASTAT software (Onofri, 2007).

3. Results and discussion

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

TABLE 2

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

The interaction of inoculant and P fertilizer resulted in significant increases in pods per plant. Thus phosphorus fertilizer promoted the number of pods per plant, especially when bacteria inoculation was applied (Fig. 2b). The I_2 x P_{40} treatment resulted in a higher number of pods per plant when compared to non-inoculated without phosphorus, I_0 x P_0 (63.5 vs 36.8 pods per plant). *Rhizobium* bacteria inoculant and phosphorus fertilizer each significantly increased guar seed yield (Fig. 2c). 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), whereas phosphorus application enhanced yields (by 18%) when P_0 (2.7 t ha^{-1}) were compared with P_{40} plots (3.3 t ha^{-1}). These results are in agreement with the findings of Elsheikh and Ibrahim (1999) and Ibrahim et al. (2016) that reported *Bradyrhizobium* strains significantly increased guar yield, protein, crude fiber and mineral content, but had no effect on 100-seed weight. Moreover, this indicates that phosphorus could have an important role in the nitrogen fixation process, confirming the result obtained by other authors (Rauti et Alì, 1983; Mand et al., 1991). In particular, Mand et al., (1991) found soil nitrogenase activity was doubled by applying 40 kg of P_2O_5 ha^{-1} in guar. Crews (1993) discussed the effect of phosphorus in regulating nitrogen fixation in another legume, alfalfa; soil nitrogenase activity increased in alfalfa where phosphorus was added, and foliar P concentrations and nitrogenase activity were positively correlated. Similar work in guar is merited across different soil types.

FIGURE 2

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

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

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

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

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

4. Conclusions

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

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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 2
Analysis 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≤0.01; * significant at P≤0.05.

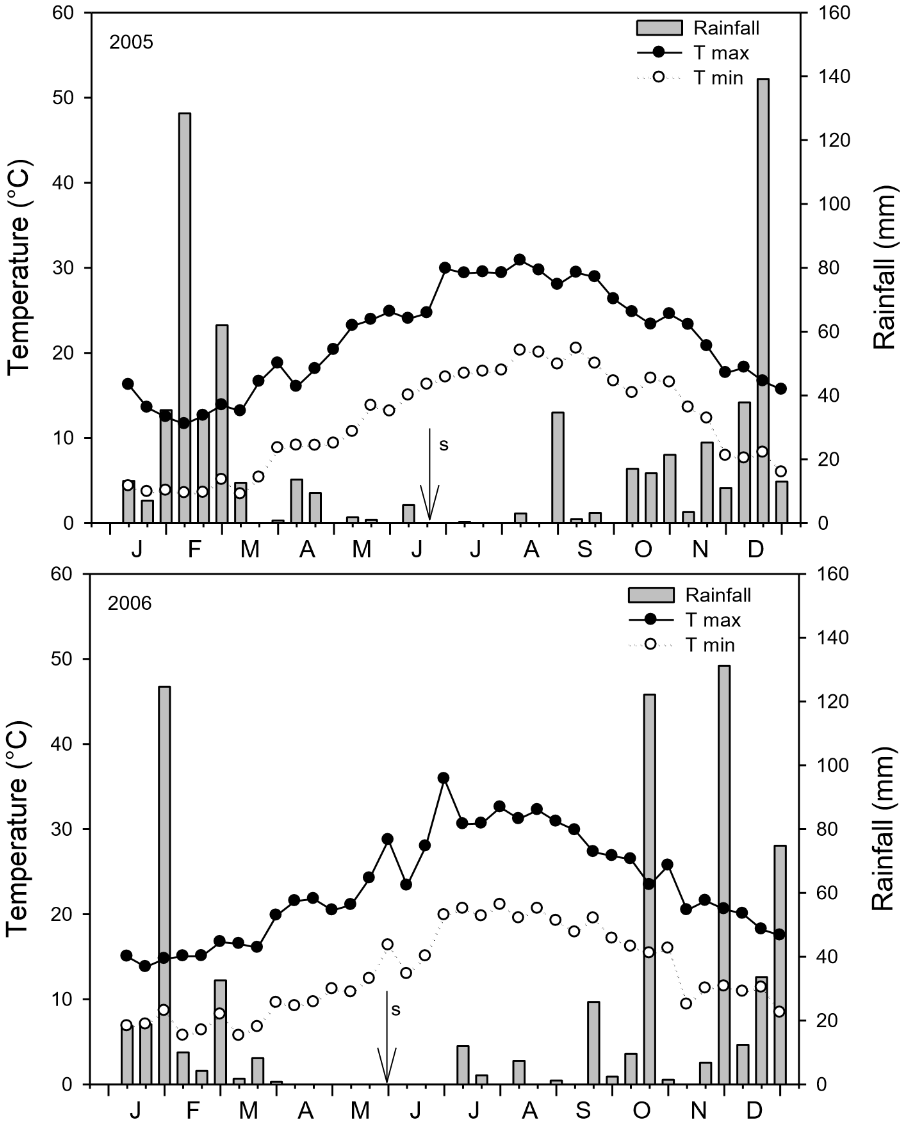
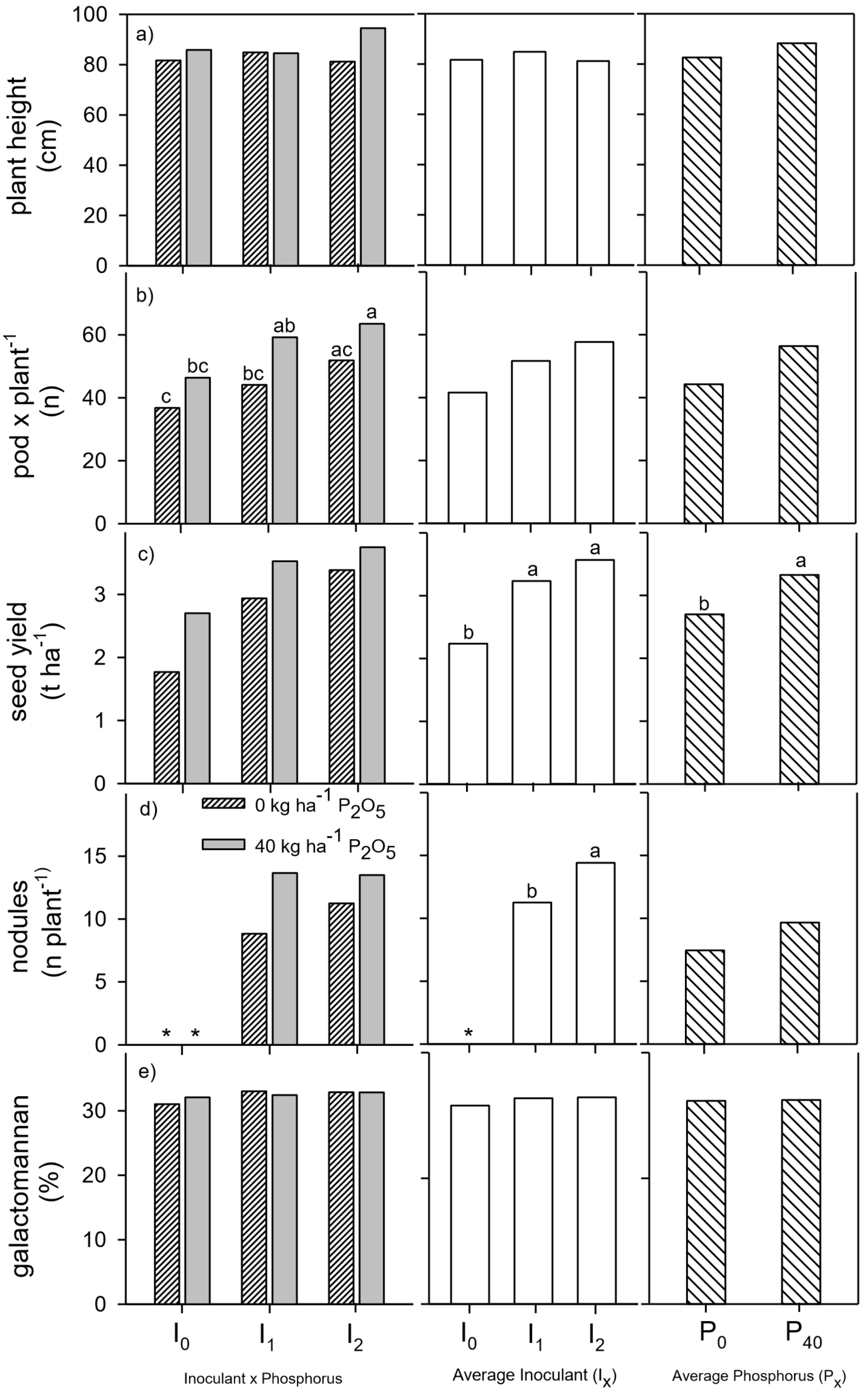


Fig. 1. Temperature and rainfall in the two-year of trial in the experimental field. The arrow with the ‘s’ indicates the sowing time (Modica, Southern Italy).



* Indicates no nodules

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