Pertanika J. Trop. Agric. Sci. 41 (4):1587-1598 (2018)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Evaluation of Different Arbuscular Mycorrhizal Fungi for Selecting the Best for Inoculating Soybean Cultivars MAUS 2 and MAUS 212

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ABSTRACT

A glasshouse experiment was conducted to screen and select the efficient AM fungi for inoculating two drought susceptible soybean cultivars MAUS 2 and MAUS 212. Screening was done using 10 different species of AM fungi. Plant parameters like plant height, stem diameter, biovolume index, total leaf area, dry biomass, P concentration, and mycorrhizal parameters like root colonization, spore number in the root zone soil were recorded according to the standard procedures. Based on the improvement in plant parameters like biovolume index, total leaf area, shoot and root dry biomass, plant P uptake, pod and seed yield, it was concluded that *Ambispora leptoticha* was the best AM fungus for inoculating both the cultivars MAUS 2 and MAUS 212.

Keywords: *Ambispora leptoticha,* AM fungi, plant growth response, soybean

ARTICLE INFO

Article history:
Received: 18 January 2018
Accepted: 21 May 2018
Published: 14 November 2018

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INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is the most important seed legume crop in the world, which contributes to 25 % of the global edible oil, and is the top oilseed crop in India (Agarwal, Billore, Sharma, Dupare, & Srivastava, 2013). In terms of production it has emerged as the most important oilseed crop of India. It stands unique in terms of chemical composition having tocopherol,

ISSN: 1511-3701
e-ISSN 2231-8542

isoflavones, and lecithin besides protein and oil. Soybean protein is called as complete protein due to its amino acids composition and the role of its nutrition value in heart disease and diabetes is well known. In India, soybean is mainly grown as rainfed crop. Its productivity under rainfed conditions is hovering around 1 t/ha despite the yield potential of up to 4 t/ha. The reason for virtually static productivity of soybean is largely due to erratic, uneven and inadequate rainfall and, other abiotic and biotic factors limiting the productivity of soybean.

Application of chemical fertilizers and pesticides to soil is increasing every year to attain maximum yield in crops. In India the use of chemical fertilizers has reached hundred times during the last 5 decades (Food and Agriculture Organization [FAO], 2010). Microbial diversity present in the soil plays a major role in plant growth and conserving the environment. It is well documented that the addition of chemical fertilizers to soil is detrimental to the microbial growth and also deteriorates the soil health and quality. It is therefore essential to reduce the addition of chemical fertilizers by introducing beneficial microbes like mycorrhizal fungi, N fixers, P solubilizers, plant growth promoting rhizomicroorganisms (PGPR) and biocontrol organisms to the soil in order to sustain plant productivity and to maintain soil health (Bollen, 1959). These beneficial microorganisms are applied to crops in order to sustain plant productivity and to maintain soil health.

The role of arbuscular mycorrhizal (AM) fungi on the growth and phosphate nutrition of various plants has been studied extensively (Bagyaraj, Sharma, & Maiti, 2015). All AM fungi are obligate biotrophs and they benefit plants by increasing uptake of diffusion limited nutrients like P, Zn, and Cu, protection from pathogens, tolerance to drought, pathogen protection, beneficial alterations of plant growth regulators and synergistic interactions with beneficial soil microorganisms (Bagyaraj, 2014; Kumar, Ashwin, & Bagyaraj, 2018). Mycorrhizal plants develop extensive root system as compared to non-mycorrhizal plants, which ensures the plant with increased availability of water and nutrient, thereby helping better plant growth and development (Bagyaraj, 2014; Mathimaran, Sharma, Mohan Raju, & Bagyaraj, 2017). Host preference in AM fungi has been reported by earlier workers which enable one to screen and select the best AM fungi for inoculating a particular crop (Chauhan, Bagyaraj, Thilagar, & Ravi, 2012; Srinivasan, Ashwin, & Bagyaraj, 2012).

The two cultivars MAUS 2 and MAUS 212 (drought susceptible) used in the present study were selected based on an earlier field experiment conducted using 25 soybean cultivars obtained from ICAR-Directorate of Soybean Research, Indore and All India Co-ordinated Research Project on Soybean, University of Agricultural Sciences, Bangalore, to investigate their drought adaptive traits. The present investigation was conducted to screen different AM fungi and select the best AM fungi for inoculating two different drought susceptible cultivars

of soybean which yielded more under irrigation compared to other cultivars. The results of the present study will reveal the best AM fungi for inoculating soybean, which will be used to understand the role of the selected AM fungi in enhancing drought tolerance in the two drought susceptible cultivars MAUS 2 and MAUS 212, later.

MATERIAL AND METHODS

The experiment was conducted in a polyhouse at Centre for Natural Biological Resources and Community Development (CNBRCD), Bangalore. The AM fungi cultures used in this experiment were *Funneliformis caledonium, Acaulospora laevis, Rhizophagus fasciculatus, Claroideoglomus etunicatum, Gigaspora margarita, Glomus macrocarpum, G. bagyarajii, F. mosseae, R. intraradices* and *Ambispora leptoticha*. AM fungi used in the study were isolated from various crops by the corresponding author, and some species were procured from various research centres working on AM fungi as detailed in Sreeramulu (1996). All the fungi were maintained in the culture collection of CNBRCD, Bangalore and were selected based on the positive results of earlier studies on other crop plants (Chauhan et al*.,* 2012; Srinivasan et al., 2012, Thilagar & Bagyaraj, 2015). Since AM fungi are obligatory symbionts they were multiplied using traditional "Pot Culture" technique as soil inoculum in pots with Rhodes grass (*Chloris gayana*) as the host using soilrite, perlite and vermiculite in the ratio 1:1:1 (v/v/v basis) under polyhouse condition. After 75 days of growth, shoots of Rhodes grass were cut and the substrate containing spores, hyphae and root bits (cut into about 1 cm pieces) were air dried and used as the inoculum. All the ten AM fungi inocula had infective propagule numbers in the range 1400-1600/ g of substrate (Thilagar, 2015).

Polybags of size of 24 cm x 12 cm with 2.5 kg substrate holding capacity were filled with the sand: soil: compost substrate mixture in 1:1:0.25 (v/v/v). The soil used in this study was collected from an uncultivated field from a depth of 0-15 cm which has been classified as fine, kaolinitic isohypothermic kanhaplustalfs. The substrate had a pH of 6.2 (1:10 soil to water extract ratio), available phosphorus of 5.9 ppm (NH4F + HCl extractable) (Jackson, 1973) and an indigenous AM fungal population of 20 spores/50 g of soil (Jackson, 1973). A planting hole was made in the middle of the polybag up to a depth of 5cm. The polybags were inoculated with 10g of respective AM fungal cultures according to the treatments and were replicated 6 times. Uninoculated control received 10g of soilrite, perlite and vermiculite 1:1:1 (v/v/v basis) with no AM fungi. Two seeds of each cultivar were sown separately per bag in the planting hole and later thinned to leave single plant/ polybag. The polybags were watered whenever necessary.

Sl. No.	Treatments
1	Uninoculated Control
2	Inoculated with *Funneliformis caledonium*
3	Inoculated with *Acaulospora laevis*
4	Inoculated with *Rhizophagus fasciculatus*
5	Inoculated with *Claroideoglomus etunicatum*
6	Inoculated with *Gigaspora margarita*
7	Inoculated with *Glomus macrocarpum*
8	Inoculated with *G. bagyarajii*
9	Inoculated with *F. mosseae*
10	Inoculated with *R. intraradices*
11	Inoculated with *Ambispora leptoticha*

The plants were harvested 90 days after sowing (DAS). At harvest, plant height was recorded from soil surface to the growing tip of the plant using measuring tape and stem diameter was measured 1 cm above the soil surface using digital Vernier Calipers. Biovolume index (BI) (depicts the total volume of a plant) based on its height and stem girth was calculated by the formula given by Hatchell, Berry and Musse (1985). Leaf area per plant was calculated by recording the leaf area in WinDIAS 3 Image Analysis System. Pod and seed weight per plant was calculated by weighing the harvested mature pods from the plant and the separated seeds in a standard weight balance machine.

The plants were harvested 90 days after sowing (DAS). Dry biomass of the shoot and root was determined after drying the plant at 60^0C to a constant weight in a hot air oven. Plant P concentration was estimated colorimetrically following the vanadomolybdate phosphoric acid yellow colour method [9]. AM fungal spore numbers in the root zone soil was estimated by collecting soil samples (50g) from each bag of a treatment and subjecting it to wet sieving and decantation method as outlined by Gerdemann and Nicolson (Gerdemann & Nicolson, 1963). Root bits were stained using trypan blue as outlined by Philips and Hayman (1970) and the per cent mycorrhizal root colonization was estimated by adopting gridline intersect method (Giovannetti & Mosse, 1980). The fungi were ranked for each character and compared pairwise using Duncan's multiple range test at 5% significance level (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Host preference among AM fungi has been reported by earlier workers (Soram, Dutta, & Jha, 2012; Srinivasan et al., 2012; Ulfath Jaiba, Balakrishna, Bagyaraj, & Arpana, 2006), hence selecting efficient symbiotic AM fungi that can be used for inoculating different mycotrophic plants has been stressed (Bagyaraj & Kehri, 2012). In the present study, soybean plants showed varied plant growth responses to different AM fungi. In general, AM fungal inoculation resulted in a significant increase in plant height, stem diameter, plant biomass, total leaf area, phosphorus concentration and yield in both the cultivars of soybean (Tables 1 and 3).

Plant height and stem diameter was significantly more in *G. macrocarpum* inoculated plants in MAUS 2 cultivar, and with *G. bagyarajii* inoculation in MAUS 212 cultivar. This was also true for biovolume index (BI) (Tables 1 and 3). The uninoculated control plants had the least BI (Tables 1 and 3). Studies by Meghvansi, Prasad, Harwani and Mahna (2008) on other soybean sp. with three different AM fungi showed significant improvement over plant growth parameters. Improved plant height, stem diameter and plant biomass because of AM fungal inoculation has been reported in other crops like French bean (Chauhan et al., 2012), chilly (Thilagar & Bagyaraj, 2015) and tomato (Pushpa & Lakshman, 2014).

Total leaf area (TLA) was significantly more in *A. leptoticha* inoculated plants in both cultivars MAUS 2 (Table 1) and MAUS 212 (Table 3). TLA is an important parameter which depicts the photosynthetic activity of the plant which in turn shows the yield capability. Hence in this study the TLA results show that inoculation with most of the AM fungi increases the TLA of the plant and thus the photosynthetic activity which in turn will increase the yield (Mondal, Datta, & Mondal, 2017).

In MAUS 2 in general all the 10 AM fungi increased shoot dry biomass but were statistically on par with control treatment whereas in MAUS 212 cultivar inoculation with *A. leptoticha* showed higher shoot dry biomass compared to all other treatments including uninoculated control. In MAUS 2 cultivar, *A. leptoticha* inoculated plants showed significantly higher root dry biomass compared to other inoculated plants but was on par with *F. mosseae* and *C. etunicatum* inoculated plants (Table 1). *A. leptoticha* inoculation to MAUS 212 cultivar also increased the root dry biomass to the maximum extent but was statistically on par with all other AM fungal inoculated plants except those inoculated with *Gi. margarita* (Table 3). Control plants showed least root dry biomass in both cultivars. Total plant dry biomass was also significantly more in *A. leptoticha* (46.48%) which was on par with *F. mosseae* (28.82%), *G. macrocarpum* (27.44%) and *R. intraradices* (21.10%) inoculated plants compared to control treatment in MAUS 2 cultivar (Table 1). In MAUS 212 cultivar *A. leptoticha* inoculation increased total plant dry biomass significantly by 44.64% compared to uninoculated plants, and was statistically on par with the treatments *F. caledonium* (25.60%) and *R. intraradices* (24.97%). Uninoculated control plants had significantly least total plant dry biomass in both the cultivars (Table 3). Similar observation was reported by Gupta and Janarthanan (1991) where inoculation with *G. aggregatum* in Palmarosa enhanced plant dry biomass. This was further confirmed by reports of Gogoi and Singh (2011) which showed inoculation with *A. delicate* increased plant dry biomass of *Piper longum*.

A. leptoticha inoculation to MAUS 2 and MAUS 212 cultivar resulted in highest pod weight and seed weight compared to uninoculated plants which had the least yield (Tables 2 and 4). Inoculation with *A.*

leptoticha to MAUS 2 cultivar increased pod and seed weight by 78.12% and 40.17% respectively. Similarly in MAUS 212 cultivar *A. leptoticha* inoculation increased pod and seed weight by 42.54% and 23.79% respectively. Increased crop yield due to AM fungal inoculation has been reported by earlier workers in several plants like chilly (Thilagar & Bagyaraj, 2015), tomato (Al-Karaki, 2006) and cucumber (Ortas, 2010). This is because of improved nutrient supply by AM fungi to plants, especially in P deficient soils (Berruti, Lumini, Balestrini, & Bianciotto, 2016).

The phosphorus concentration of the plants also increased significantly due to inoculation with all the AM fungi studied compared to uninoculated plants in both the cultivars. Shoot, root and total plant P concentration (excluding pod & seeds) was significantly more in *A. leptoticha* treatment compared to all other AM fungal treatments and the control in both the cultivars. It is well known that AM fungi improve plant growth mainly through enhanced nutrition of diffusion limited nutrients like P. Variation in the plant P status in relation to fungal species is well documented (Rajan, Bagyaraj, & Arpana, 2005; Soram et al., 2012). In the present study plants raised in the presence of *A. leptoticha* showed an increase of 91.29% and 92.00% in total plant phosphorus concentration in MAUS 2 and MAUS 212 cultivars respectively (Table 2 and 4) compared to plants without inoculation. Such an enhanced plant P concentration because of AM fungal inoculation has been reported in other crops (Wang, Pan, Chen, Yan, & Liao, 2011). The high-affinity phosphate transporter (PT) in AM fungal and the nutritional aspects of AM fungal symbiosis have been studied extensively from both physiological and molecular perspectives. AM fungi are capable of significantly improving plant mineral nutrient acquisition by scavenging larger volume of soil, mainly in low-nutrient conditions, and it has clearly been demonstrated that plants possess a symbiotic Pi uptake pathway (Berruti et al., 2016).

In the present study, mycorrhizal parameters, such as extramatrical spores in the root zone soil and percent mycorrhizal root colonization, were considerably higher in all the inoculated treatments compared to the uninoculated control treatment in both the cultivars; however *A. leptoticha* produced significantly more spores in root zone soil of both the cultivars compared to other AM fungal treatments (Tables 2 and 4). The existence of host preference by AM fungi investigated by earlier researchers brought out that the extent of mycorrhizal root colonization and the spore count in the root zone soil varied with different AM fungi and that the host plant responds best to a particular AM fungal symbiont (Bagyaraj, 2011; Helgason et al., 2002; Vandenkoornhuyse, Ridgway, Watson, Fitter, & Young, 2003). The extent of colonization and the spore count varied with different AM fungi. In the present study it can be concluded that the soybean cultivars MAUS 2 and MAUS 212 responded best to inoculation with *A. leptoticha* (which confers

maximum growth benefits) compared to all other fungi used in this study. The cultivars being drought susceptible it is possible that inoculation with the selected AM fungus can confer drought tolerance, which needs

Table 1

Influence of different AM fungi on height, stem diameter, biovolume index (BI), total leaf area, and shoot, root and total dry biomass of drought susceptible soybean cultivar MAUS 2

Treatments	Height (cm/ plant)	Stem dia. (mm/ plant)	BI	Total leaf area (cm^2/ plant)	Shoot dry biomass (g/ plant)*	Root dry biomass (g/ plant)	Total plant dry biomass (g/ plant)
Control	90.20d	4.03^{d}	363.51^{bc}	744.82^{e}	5.18	0.71^{c}	5.83^{d}
Funneliformis caledonium	120.50^{ab}	4.28^{bc}	515.62^{ab}	840.11^{cd}	5.72	0.85^{bc}	6.57^{bc}
Acaulospora laevis	101.00^{bc}	4.76^{ab}	479.59^{ab}	770.83^{de}	5.54	0.77^{bc}	6.31^{cd}
Rhizophagus fasciculatus	109.17^{ab}	4.17^{cd}	457.17^{ab}	988.59^{ab}	6.06	0.70^{c}	6.78^{bc}
Claroideoglomus etunicatum	94.17^{cd}	4.77^{ab}	449.19ab	873.49^{bc}	5.64	1.00^{ab}	6.64^{bc}
Gigaspora margarita	119.38^{ab}	4.72^{ab}	563.47^{ab}	897.39^{bc}	5.69	0.76^{bc}	6.45^{bc}
Glomus macrocarpum	123.95^{a}	4.92^{a}	609.834^{a}	1033.00^{ab}	6.50	0.93^{bc}	7.43^{ab}
Glomus bagyarajii	$90.60d^{de}$	4.32^{bc}	391.39^{b}	1112.49^{ab}	6.06	0.84^{bc}	6.90^{bc}
Funneliformis mosseae	99.33^{cd}	4.62^{ab}	460.02^{ab}	1107.26^{ab}	6.39	1.12^{ab}	7.51^{ab}
Rhizophagus intraradices	114.30^{ab}	4.38^{bc}	500.59^{ab}	1005.36^{ab}	6.17	0.90^{bc}	7.06^{ab}
Ambispora leptoticha	106.50^{ab}	4.65^{ab}	492.57^{ab}	1182.46^{a}	7.22	1.32^{a}	8.54^{a}

Note. Means in column with same letters are not significantly different at $P < 0.05$; *Not significant

Table 2

Influence of different AM fungi on pod and seed weight, shoot, root, and total plant P concentration, mycorrhizal spore numbers in root zone soil and mycorrhizal root colonization of drought susceptible soybean cultivar MAUS 2

Treatments	Pod weight (g/ plant)	Seed weight (g/ plant)	Shoot P conc. (%)	Root P conc. (%)	Total plant P conc. (%)	Mycorrhizal spore Nos./ 50 g of dry root zone soil	Mycorrhizal colonization (%)
Control	3.61^{c}	80.09^{c}	0.37^{h}	0.20^{h}	0.57^{h}	105^{f}	66^{d}
Funneliformis caledonium	5.27^{ab}	108.74^{a}	0.61^{c}	0.27^{c}	0.88^{c}	317^{b}	94^{b}
Acaulospora laevis	5.81^{ab}	107.25^{a}	0.50^{f}	0.26^{g}	0.76^{g}	147^{e}	95^{b}
Rhizophagus fasciculatus	5.01^{ab}	101.05^{ab}	0.55^{d}	0.26^{e}	0.81^{e}	138^{ef}	95^{b}
Claroideoglomus etunicatum	4.88^{ab}	108.40^{a}	0.62^{b}	0.26^{c}	0.88^{c}	338^{b}	100^{a}
Gigaspora margarita	4.76^{ab}	98.48^{ab}	0.47^{g}	0.30^{fg}	0.77^{fg}	258^{c}	100^{a}
Glomus macrocarpum	6.07^{ab}	109.25^{a}	0.63^{b}	0.27^{b}	0.90^{b}	270^{c}	100^{a}
Glomus bagyarajii	4.40^{bc}	86.75^{bc}	0.51^{ef}	0.33^{d}	0.84^{d}	185^{d}	95^{b}
Funneliformis mosseae	5.84^{ab}	102.61^{a}	0.52^{e}	0.25^{fg}	0.77^{fg}	130^{ef}	100^{a}
Rhizophagus intraradices	5.55^{ab}	112.17^{a}	0.52^{e}	0.26^{f}	0.78^{f}	197^{d}	100^{a}
Ambispora leptoticha	6.43a	112.26^{a}	0.74^{a}	0.35^{a}	1.09^{a}	371^{a}	100^{a}

Note. Means in column with same letters are not significantly different at $P < 0.05$

further investigation.

Table 3

Influence of different AM fungi on height, stem diameter, biovolume index (BI), total leaf area, and shoot, root and total dry biomass of drought susceptible soybean cultivar MAUS 212

Treatments	Height (cm/ plant)	Stem dia. (mm/ plant)	BI	Total leaf area (cm^2/ plant)	Shoot dry biomass (g/ plant)*	Root dry biomass (g/ plant)	Total plant dry biomass (g/ plant)
Control	60.00^{e}	4.65^{e}	278.97^{e}	1087.23^{c}	7.24^{bc}	0.69^{e}	7.93^{c}
Funneliformis caledonium	73.67^{e}	4.73^{bc}	348.67^{e}	1427.87^{ab}	8.82^{ab}	1.14^{ab}	9.96^{ab}
Acaulospora laevis	62.83^{e}	4.88^{ab}	306.23^{e}	1158.93^{bc}	7.49^{bc}	1.07^{ab}	8.56^{bc}
Rhizophagus fasciculatus	67.50^{e}	4.90^{ab}	331.20^{e}	1341.83^{ab}	8.22^{bc}	1.13^{ab}	9.41^{bc}
Claroideoglomus etunicatum	61.67^{e}	4.92^{ab}	302.82^{e}	1083.52^{c}	6.61^{d}	1.03^{ab}	7.64^{d}
Gigaspora margarita	64.67^{e}	4.82^{ab}	311.65^{e}	1179.37^{bc}	6.77^{cd}	0.93^{b}	7.70^{d}
Glomus macrocarpum	69.33^{e}	4.90^{ab}	340.80^{e}	1281.93^{ab}	7.18^{bc}	1.13^{ab}	8.31^{bc}
Glomus bagyarajii	111.33^{a}	5.35^{a}	595.62^{a}	1364.79^{ab}	8.50^{bc}	1.07^{ab}	9.57^{b}
Funneliformis mosseae	90.00^{b}	5.27^{ab}	476.03^{b}	1444.70^{ab}	8.65^{ab}	1.01^{ab}	9.66^{bc}
Rhizophagus intraradices	69.17^{c}	4.60^{c}	318.00^{c}	1482.32^{ab}	8.94^{ab}	0.97^{ab}	9.91^{ab}
Ambispora leptoticha	95.00^{b}	5.32^{a}	505.40^{b}	1541.44^{a}	10.30^{a}	1.19^{a}	11.47^{a}

Note. Means in column with same letters are not significantly different at $P < 0.05$

Table 4

Influence of different AM fungi on pod and seed weight, shoot, root, and total plant P concentration, mycorrhizal spore numbers in root zone soil and mycorrhizal root colonization of drought susceptible soybean cultivar MAUS 212

Treatments	Pod weight (g/ plant)	Seed weight (g/ plant)	Shoot P conc. (%)	Root P conc. (%)	Total plant P conc. (%)	Mycorrhizal spore Nos./ 50 g of dry root zone soil	Mycorrhizal colonization (%)
Control	3.55^{d}	66.62^{b}	0.17^{e}	0.08^{f}	0.25^{g}	118^{i}	72^{f}
Funneliformis caledonium	4.44^{ab}	71.72^{ab}	0.24^{c}	0.17^{ab}	0.41^{c}	197^{e}	97^{c}
Acaulospora laevis	4.80^{ab}	77.21^{ab}	0.24^{c}	0.15^{cd}	0.39^{d}	185^{g}	96^{d}
Rhizophagus fasciculatus	4.69^{ab}	77.07^{ab}	0.27^{b}	0.13^{e}	0.40^{cd}	153^{h}	100^{a}
Claroideoglomus etunicatum	3.68^{cd}	74.94^{ab}	0.29^{a}	0.15^{cd}	0.44^{b}	212^{d}	100^{a}
Gigaspora margarita	3.69^{cd}	64.72^{b}	0.21^{d}	0.13e	0.34^{f}	186^{g}	98^{b}
Glomus macrocarpum	4.24^{ab}	65.13^{b}	0.26^{b}	0.17^{ab}	0.43^{b}	153^{h}	100^{a}
Glomus bagyarajii	5.02^{a}	81.84^{a}	0.27^{b}	0.16^{bc}	0.43^{b}	294^{b}	90^{e}
Funneliformis mosseae	4.32^{ab}	76.48^{ab}	0.24^{c}	0.13^{e}	0.37^{e}	227^{c}	100^{a}
Rhizophagus intraradices	4.16^{bc}	65.10^{b}	0.23^{c}	0.14^{de}	0.37^{e}	192^{f}	100^{a}
Ambispora leptoticha	5.06^{a}	82.47^{a}	0.30^{a}	0.18^{a}	0.48^{a}	353^{a}	100^{a}

Note. Means in column with same letters are not significantly different at $P < 0.05$

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