Plant Archives Vol. 16 No. 1, 2016 pp. 210-216 *ISSN 0972-5210*

BIODIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) IN BANANA PLANTATIONS IN INDIA

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Abstract

Mycorrhizal fungi are a diverse group of organisms having different symbiotic associations between plants and fungi in a wide range of terrestrial ecosystems. Arbuscular Mycorrhizal Fungi (AMF) provides most of the soil nutrients and dissolved minerals to the plants. In the present study, a survey was conducted to find out the different spore populations of AMF in different banana cultivars in India. Totally, 112 rhizospheric soil samples belongs to approximately 8 different soil types were collected from different banana growing regions of India and analysed for the presence of AMF genera and their spore density. The results revealed that a maximum of 50.8% of AMF spores present in clay type soil and a minimum of 0.2% in silty clay and loamy soil. Finally, AMF spores were isolated from 35 different banana germplasm accessions and observed the presence of 3 different genera *viz*., *Glomus* (7 species), *Acaulospora* (2 species) and *Scutellospora* (2 species). It was also noted that a total of 91.1% of AMF spores isolated were belong to *Glomus* sp. representing that it was the most dominating AMF spores in banana germplasm accessions. Thus, AM fungi was found to be associated with banana in all the locations surveyed and the population of these fungi was observed with high level of spores density.

Key words : AMF spores, *Glomus* sp., *Acaulospora* sp., *Scutellospora* sp., Banana.

Introduction

Mycorrhizal fungi form a symbiotic association in natural environment, which is essential for one or both partners between a fungus (specialized for life in soils and plants) and a root (or other substrate contacting organ) of a plant that is primarily responsible for nutrients transfer (Brundrett, 2002). The fungi play an important central role in many microbiological and ecological processes, influencing soil fertility, decomposition, cycling of minerals and organic matter as well as plant health and nutrition. AMF are important for accessing and recycling nutrients (Haystead *et al*., 1988 and Read, 2008), which can increase plant access to water (Gavito and Varela, 1995), play a role in the formation of soil aggregates (Hamel *et al*., 1997), influence plant biodiversity (Van der Heijden *et al*., 1998) and also help to protect against pests and diseases.

Banana (including plantain) is the world's largest fruit crop with an annual production of 90 million tones and is the fourth most important global food commodity after rice, wheat and maize in terms of gross value production (CGIAR, 1993). It is the staple food for nearly 400 million people worldwide with 200-300 clones, including dessert, cooking types and plantains. Mycorrhizae fungi are probably the most ubiquitous fungi in agricultural soils, accounting for 5–36% of the total biomass in soil and 9–55% of the biomass of soil microorganisms (Olson *et al*., 1999). These fungi are a critical component in agricultural systems because these organisms can increase plant growth (Smith and Read, 1997) and protect against pest and diseases. Banana and plantain cultivars are naturally colonized by arbuscular mycorrhiza fungi. AMF are an important part of sustainable agricultural systems that have low inputs of chemical fertilizers and biocides (Bethlenfalvay and Schüepp, 1994; Jeffries and Barea, 1994; Hooker and Black, 1995). Our present study aimed at isolation and investigates the type of AMF and their spore population present in different banana rhizospheric soil obtained from various banana growing regions of India.

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Materials and Methods

Sample collection

The soil samples were collected from rhizospheric region of different banana cultivars grown in various soil types of major banana growing regions of India *viz*., Tamil Nadu (Trichy, Tanjore, Madurai, Pudukottai, Virudhunagar,

Cuddalore, Karur and Salem districts), Kerala (Iddukki), Maharashtra (Jalgaon) and Tripura (Agartala) during the period of 2010-2012. Soil samples were also collected from rhizosphere region of 18 different Fusarium wilt resistant banana accessions, which were being maintained at National Research Centre for Banana, Trichy (table 1). These soil samples were used for studying the presence of AMF and their spore density.

The soil samples were collected at a depth of the region, where the most root proliferation occurs (usually 30 cm). The samples collected were processed immediately after transporting to the laboratory or air dried for two weeks, stored in sealed plastic bags and maintained at 4°C for up to 6 months until samples could be processed.

AMF spore isolation

The AMF spores were isolated from rhizospheric soil samples by the standard method of wet sieving and decantation as described by Gerdemann and Nicolson (1963). Twenty grams of soil was independently suspended in 250ml of water and placed in a magnetic stirrer for 5 min and placed at rest. After 30 min., the soil filtrate was poured in sieves of different pore sizes ranging from 150µm to 40µm arranged in racks. Then, the filtrate obtained was washed with sterile distilled water and poured onto a Whatman filter paper of 7cm diameter placed in Buchner funnel. The filter paper containing the spores was then transferred in a 9cm Petri-dish for examination under a binocular stereo microscope.

Identification of type of AMF spores

The AMF spores showing different shape, size and color were individually mounted in a slide containing water/ lactophenol/PVLG (Morton, 1988; Morton and Benny, 1990) for identification by viewing under 40x and 100x objective using Olympus stereo zoom binocular microscope. The identification was done based on the morphological characters such as spore colour, size, surface ornamentation and wall structure with reference to the descriptions and pictures provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi and originally published species descriptions.

Quantification of AMF spores

The Arbuscular mycorrhizal fungal spores collected from all the sievings brought from 100g soil were counted under microscope and the total number of spores present was counted by moving the Petri plate slightly and number of spores in each microscopic field was noted. The actual spore density present in rhizospheric soil was calculated by dividing the total number of spores present by the weight of soil taken for analysis.

Results and Discussion

Arbuscular mycorrhizal fungi [AMF] constitute an important component of the soil microbial community and are obligately symbiotic soil fungi, which colonize the roots of the majority of plants. These fungi are so named because they produce characteristic finely branched hyphal structures, termed arbuscules, inside cortical cells of plant roots. AM fungi (AMF) help plants to capture nutrients such as phosphorus and micronutrients from the soil. They improve plant nutrition and promote plant diversity (Van der Heijden *et al*., 1998) and also help to control insect and fungal pathogens (Azcon-Aguilar and Barea, 1996). Hence, our present study aimed to isolate and identify the AMF population in banana plantations which will be useful for the control of banana diseases particularly Fusarium wilt disease and also promote plant growth in the near future.

Totally, one hundred and twelve rhizospheric soil samples were collected from different banana growing regions of India. It was found that the soils collected in this study were belong to eight different soil types constitute different percentage of AMF spore density (table 1).

The result revealed that the AMF density was maximum in clay soil (50.8%), which was followed by sandy loam (16.3%), sand (17.7%), silty loam (5.6%), silty clay-loam (7.1%), clayey loam (2.1) and minimum in loamy and silty clay (0.2%) soils (fig. 1).

Different isolates of AM fungi were collected from different locations and identified by standard manual according to their morphological structures; spore size etc. The soil sample areas show varied environmental conditions. Eleven species of arbuscular mycorrhizal fungi were identified from the collections of different soil types cultivated with different banana cultivars. The arbuscular mycorrhizal fungi identified belong to three different genera *viz.*, *Glomus* (7 species), *Acaulospora* (2 species) and *Scutellospora* (2 species). This result clearly indicated that the banana rhizospheric soil is the habitat of three different AMF fungi such as *Glomus*, *Acaulospora* and *Scutellospora* (table 1).

It was also noted that, a total of 91.1% of AMF spores isolated were belong to *Glomus* spp. representing that it was the most dominating AMF spores in banana plantations (fig. 2). Similarly, Bever *et al*. (1996) identified species of arbuscular mycorrhizal fungi belonged to the genera of *Acaulostis* (9 species) and *Scutellospora* (1 species) and indicated that *Acaulospora* and *Glomus* were the dominant genera and *A. denticulata, A.*

Table 1 : Details of AMF identified in different soil types and banana cultivars.

S. no.	Study site areas	State from which soil was collected	Name of the cultivars	Soil Type	AMF Identified	No. of spores /100g soil
1.	Thiruvarur	Tamil Nadu	Poovan	Clay	*Glomus aggregatum*	80
2.	Thiupanthurai	Tamil Nadu	Karpuravalli	Sandy loam	*Glomus* spp.	80
3.	Varagur	Tamil Nadu	Monthan	Clay	*Scutellospora heterogama*	79
4.	Thiukattupalli	Tamil Nadu	Rasthali	Clay	*Glomus* spp.	82
5.	Valapakkudi	Tamil Nadu	Poovan	Sandy loam	*Acaulospora* spp.	73
6.	Nadu Padi	Tamil Nadu	Pachanadan	Sandy loam	—	—
7.	Thiruvaiyur	Tamil Nadu	Poovan	Sand	*Glomus aggregatum*	75
8.	VairavanKovil	Tamil Nadu	Poovan	Clay	*Scutellospora heterogama*	69
9.	Papanasam	Tamil Nadu	Poovan	Clay	*Glomus aggregatum*	74
10.	Thiruvarur	Tamil Nadu	Monthan	Sandy loam	—	—
11.	ICAR-NRCB	Tamil Nadu	Pachanadan	Sandy loam	*Glomus* spp.	72
12.	ICAR-NRCB	Tamil Nadu	Bluggoe	Silty loam	*Glomus* spp.	119
13.	ICAR- NRCB	Tamil Nadu	Pisang rajah	Clay	*Glomus* spp.	80
14.	ICAR- NRCB	Tamil Nadu	Dudhsagar	Silty clay loam	*Glomus* geosporum	69
15.	ICAR- NRCB	Tamil Nadu	Burrow Cemsa	Sand	*Glomus* spp.	50
16.	ICAR- NRCB	Tamil Nadu	Burmanica	Sand	*Glomus* aggregatum	98
17.	ICAR- NRCB	Tamil Nadu	Local Peyan	Clay	*Acaulospora* spp.	60
18.	ICAR- NRCB	Tamil Nadu	Karpuravalli	Clay	*Scutellospora calospora*	65
19.	ICAR- NRCB	Tamil Nadu	EnnaBennian	Sand	*Glomus* spp.	120
20.	ICAR- NRCB	Tamil Nadu	Ney Poovan	Clay	*Glomus* spp.	110
21.	ICAR- NRCB	Tamil Nadu	Matti	Clay	*Glomus* spp.	65
22.	ICAR- NRCB	Tamil Nadu	Monthan	Sand	*Glomus* spp.	78
23.	ICAR- NRCB	Tamil Nadu	Udhayam	Sand	*Glomus* spp.	125
24.	ICAR- NRCB	Tamil Nadu	Cultivar Rose	Clay	*Acaulospora* spp.	105
25.	ICAR- NRCB	Tamil Nadu	Sabri	Clay	*Glomus viscosum*	87
26.	ICAR- NRCB	Tamil Nadu	Pisang Seribu	Sand	*Glomus etunicatum*	102
27.	ICAR- NRCB	Tamil Nadu	Pisang Jari Buaya	Sand	*Glomus* spp.	95
28.	ICAR- NRCB	Tamil Nadu	Nendran	Clay	*Acaulospora foveata*	78
29.	ICAR- NRCB	Tamil Nadu	GCTCV-119	Clay	*Glomus clarum*	54
30.	ICAR- NRCB	Tamil Nadu	Kluteperod	Clay	*Glomus etunicatum*	132
31.	ICAR- NRCB	Tamil Nadu	Pisang Ceylon	Sand	*Glomus claroideum*	96
32.	VeyalanMedu	Tamil Nadu	Karpuravalli	Clay	*Acaulospora capsicula*	79
33.	Kalakuttikadu	Tamil Nadu	Monthan	Clay	*Acaulospora capsicula*	92
34.	Melur	Tamil Nadu	Monthan	Clay	*Glomus* spp.	105
35.	Madurai	Tamil Nadu	Nendran	Clay	*Glomus* spp.	44
36.	Vadugappati	Tamil Nadu	Nendran	Sandy loam	*Glomus* spp.	17
37.	Melur	Tamil Nadu	Neypoovan	Sandy loam	*Glomus* spp.	52

Table 1 continued...

Table 1 continued...

38.	Melur	Tamil Nadu	Rasthali	Clay	*Glomus* spp.	32
39.	Ilayur	Tamil Nadu	Rasthali	Clay	*Glomus* spp.	18
40.	Ilayur	Tamil Nadu	Monthan	Clay	*Glomus* spp.	53
41.	Kalupatti	Tamil Nadu	Poovan	Clay	*Glomus* spp.	58
42.	Kallupatti	Tamil Nadu	Rasthali	Clay	*Glomus* spp.	18
43.	Virudhunagar	Tamil Nadu	Monthan	Sand	*Glomus* spp.	42
44.	Parampatti	Tamil Nadu	Monthan	Clay	*Glomus* spp.	61
45.	Parampatti	Tamil Nadu	Monthan	Clay	*Glomus* spp.	22
46.	Ilayur	Tamil Nadu	Grand Naine	Sand	*Glomus* spp.	28
47.	Thiruvayur	Tamil Nadu	Poovan	Clay	*Glomus* spp.	11
48.	Thiruvaiyur	Tamil Nadu	Neypoovan	Clay	*Glomus* spp.	2
49.	Cuddalore	Tamil Nadu	Neypoovan	Clay	*Glomus* spp.	65
50.	Cuddalore	Tamil Nadu	Neypoovan	Clay	*Glomus* spp.	5
51.	Pudukottai	Tamil Nadu	Poovan	Clay	*Glomus* spp.	21
52.	Pudukottai	Tamil Nadu	Poovan	Clay	*Glomus* spp.	10
53.	Cuddalore	Tamil Nadu	Poovan	Clay	*Glomus* spp.	35
54.	Cuddalore	Tamil Nadu	Neypoovan	Clay	*Glomus* spp.	57
55.	Pudukottai	Tamil Nadu	Poovan	Clay	*Glomus* spp.	17
56.	Cuddalore	Tamil Nadu	Rasthali	Clay	*Glomus geosporum*	104
57.	Thanjavur	Tamil Nadu	Rasthali	Clay	*Glomus* spp.	22
58.	Thanjavur	Tamil Nadu	Poovan	Clay	*Glomus* spp.	52
59.	Kundampatti	Tamil Nadu	Nendran	Sand	*Glomus* spp.	11
60.	Kundampatti	Tamil Nadu	Neypoovan	Sand	*Glomus* spp.	3
61.	Neithalur	Tamil Nadu	Neypoovan	Clay	*Glomus* spp.	18
62.	Puliyur	Tamil Nadu	Neypoovan	Sand	*Glomus* spp.	5
63.	Salem	Tamil Nadu	Karpuravalli	Clay	*Glomus* spp.	17
64.	Salem	Tamil Nadu	Poovan	Clay	*Glomus* spp.	32
65.	Salem	Tamil Nadu	Dwarf Cavendish	Clay	*Glomus* spp.	45
66.	Salem	Tamil Nadu	Rasthali	Clay	*Glomus etunicatum*	45
67.	Salem	Tamil Nadu	Robusta	Clay	*Glomus* sp.	2
68.	Salem	Tamil Nadu	Karpuravalli	Clay	*Glomus* sp.	2
69.	Salem	Tamil Nadu	Robusta	Clay	*Glomus* sp.	1
70.	Salem	Tamil Nadu	Karpuravalli	Clay	*Glomus* sp.	2
71.	Karur	Tamil Nadu	Neypoovan	Clay	*Glomus* sp.	22
72.	Sirugamam	Tamil Nadu	Neypoovan	Loamy	*Glomus* sp.	7
73.	Sukampatti	Tamil Nadu	Neypoovan	Sandy loam	*Glomus* sp.	12
74.	Sukampatti	Tamil Nadu	Neypoovan	Sandy loam	*Glomus* sp.	16
75.	Sukampatti	Tamil Nadu	Nendran	Sandy loam	*Glomus* sp.	13
76.	Sukampatti	Tamil Nadu	Poovan	Sandy loam	*Glomus* sp.	20
77.	Karur	Tamil Nadu	Nendran	Clayey loam	*Glomus* sp.	32
78.	Devasthanam	Tamil Nadu	Poovan	Clayey loam	*Glomus* sp.	2

Table 1 continued...

Table 1 continued...

79.	Devasthanam	Tamil Nadu	Poovan	Clayey loam	*Glomus* sp.	2
80.	Karur	Tamil Nadu	Neypoovan	Clay	*Glomus* sp.	15
81.	Karur	Tamil Nadu	Neypoovan	Silty clay	*Glomus* spp.	11
82.	Karur	Tamil Nadu	Poovan	Clayey loam	*Glomus* spp.	2
83.	Kerala- Estate	Kerala	Monthan	Sandy loam	*Glomus* spp.	72
84.	Korandakad	Kerala	Poovan	Silty loam	*Glomus* spp.	64
85.	Maraiyoor	Kerala	Karpuravalli	Silty loam	*Glomus* spp.	63
86.	Maraiyoor	Kerala	Neypoovan	Sandy loam	*Glomus* spp.	35
87.	Maraiyoor	Kerala	Karpuravalli	Silty loam	*Glomus* spp.	63
88.	Amaravathi Dam	Tamil Nadu	Poovan	Sandy loam	*Glomus* spp.	53
89.	Kumuli Munar	Kerala	Grand Naine	Sandy loam	*Glomus* spp.	58
90.	Devikulam	Kerala	Karpuravalli	Silty clay	*Glomus* spp.	82
91.	Muttadi	Kerala	Vellai Poovan	Sandy loam	*Glomus* spp.	2
92.	Vallarakumpara	Kerala	Palayam Kondan	Clayey loam	*Glomus* spp.	49
93.	Kattapananear	Kerala	Hill Banana	Silty clay loam	*Glomus* spp.	73
94.	Tata Estate	Kerala	Poovan	Silty loam	*Glomus* spp.	52
95.	Muttadi	Kerala	Pachai singam	Silty loam	*Glomus* spp.	59
96.	Santhampara	Kerala	Poovan	Clay	*Glomus* spp.	72
97.	Madupatti Dam	Kerala	Pachanadan	Silty clay	*Glomus* spp.	45
98.	Palayankondan	Kerala	Neypoovan	Sandy loam	*Glomus* spp.	35
99.	Kumuli	Kerala	Hill Banana	Clay	*Glomus* spp.	28
100.	Munnar	Kerala	Robusta	Clayey loam	*Glomus* spp.	20
101.	Thekkadi	Kerala	Red banana	Silty clay	*Glomus* spp.	22
102.	Kumuli	Kerala	Nendran	Silty loam	*Glomus* spp.	31
103.	Jalgoan	Maharashtra	Grand Naine	Clay	*Glomus* spp.	10
104.	Jalgoan	Maharashtra	Grand Naine	Clay	*Glomus* spp.	24
105.	Jalgoan	Maharashtra	Grand Naine	Clay	*Glomus* spp.	18
106.	Jalgoan	Maharashtra	Grand Naine	Clay	*Glomus* spp.	15
107.	Jalgoan	Maharashtra	Grand Naine	Clay	*Glomus* spp.	29
108.	Nagichera	Tripura	Grand Naine	Sandy loam	*Glomus* spp.	17
109.	Krishnapur	Tripura	Grand Naine	Sandy loam	*Glomus* spp	12
110.	Kanchanmalla	Tripura	Grand Naine	Sandy loam	*Glomus mossae*	38
111.	Belonia	Tripura	Grand Naine	Sandy loam	*Glomus* spp.	27
112.	Kurti	Tripura	Grand Naine	Sandy loam	*Glomus* spp.	11

foveata, *A. spinosa*, *A. tuberculata*, *G. claroidenum*, *G. clarum*, *G. constricum* and *G. monosporum* were the dominant species in the tropical rainforest of Xishuanogbanna in China. Victor *et al*. (2014) reported that 23 AMF morphospecies belonging to four genera were registered and 11 corresponded to *Glomus*, 10 *Acaulospora*, one to *Gigaspora* and one to *Ambispora*.

The variation in the occurrence of different genera and species of AM fungi with banana cultivars might be due to variations in soil chemical characteristics. Root colonization and plant mycorrhizal dependency may be genotype and environment dependent traits (Smith and Read, 1997). Earlier report showed significant differences in AM Fungal population density between different soil types (Gaidashova *et al*., 2010). Occurrence of *Glomus*

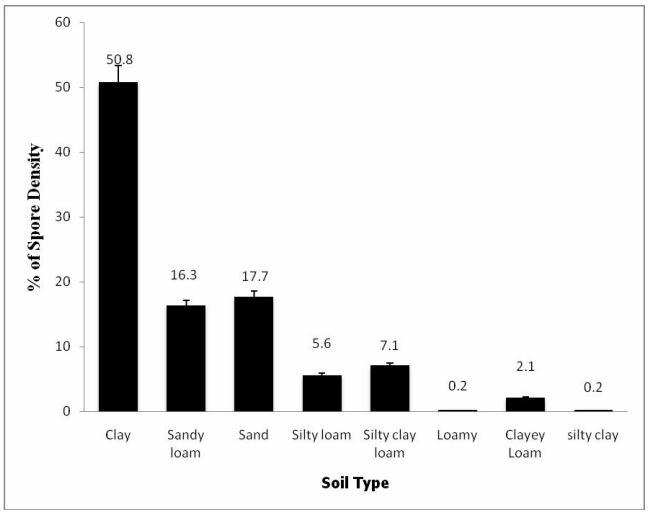

Fig. 1 : Spore density of AMF in different soil types in India.

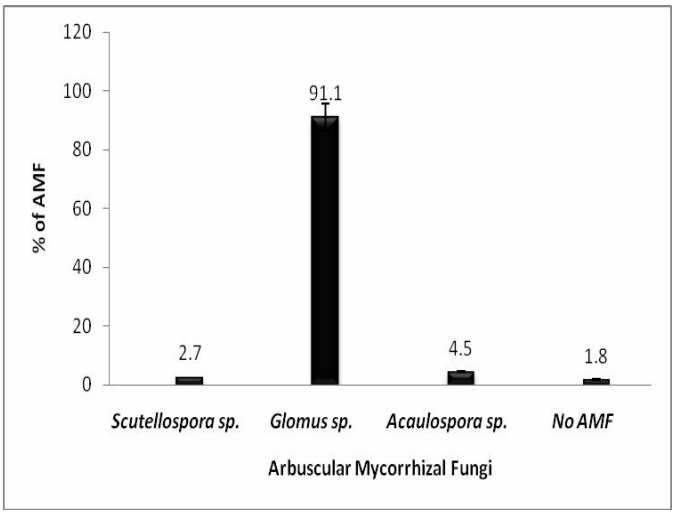

Fig. 2 : Occurrence of AMF in banana plantations.

sp. in food crops also has been observed by earlier workers (Thakur and Lakhanpal, 1990; Bharat and Bharadwaj, 2002). Walker *et al*. (1982) reported that the spore population of AMF in different types of apple orchards in Shimla district of Himachal Pradesh (apple belt) resulted in 525 arbuscular mycorrhizal fungal spores (or sporocarp) and samples were wet sieved from the 118 soil sample, from which 27 species of arbuscular mycorrhizal fungi were identified.

In our present study also, the total number of spores was presented in 100g of soil observed under microscope. The maximum number of spores of about 132 spores of *Glomus etunicatum* was observed from rhizospheric soil sample of cv. Klueteperod from NRC farm in Trichy, Tamil Nadu. The moderate number of spores of about 65 spores of *Glomus* spp. of Matti and Ney Poovan variety was observed from the soil sample of NRC, Trichy and Cuddalore. The minimum of 2 spores of *Glomus* sp. in clay and loamy soil samples were observed from the soil samples of cv. Poovan obtained from Karur district of Tamil Nadu. Surprisingly, no AMF spores were observed in some of the soil samples like sandy loam soil from Nadupadi and Thiruvayur of Tamil Nadu State. This may be due to seasonality, edaphic factors, host-dependence, age of the host plants, the sporulation abilities of arbuscular mycorrhizal fungi, and the dormancy and the distribution patterns of arbuscular mycorrhizal fungal spores in the soils have been reported previously (Walker *et al*., 1982; Sylvia, 1986; Koske, 1987; Gemma and Koske, 1988; Bever *et al*., 1996; Zhao, 1999).

Conclusion

In the present investigation, the AM fungi were invariably found association with banana plantations in most of the locations surveyed and population of these fungi observed were under the three genera *viz*. *Glomus*, *Acaulospora* and *Scutellospora* and the frequency of occurrence of *Glomus* sp. in different cultivars of banana with highly variant types of soil environment like silty clay, silty loam and sandy loam types of soil, respectively. These association benefit the banana plants by increasing growth, nutrient uptake and defense mechanisms by the influence of AM fungi.

Acknowledgements

The authors gratefully thank the ICAR-National Research Centre for Banana for financial support by sponsoring a project.

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