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Chlorella vulgaris improves growth of *Abrometiella scapigera, Abrometiella brevifolia* and *Abrometiella chloranta* and reduces root attack by nematodes

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Abstract

Research objective: The aim of this study is to investigate the effects of *Chlorella* extract as a biofertiliser on the growth of *Abrometiella* spp. seedlings and to assess whether it has a repellent effect against nematodes

Materials and Methods: The experiments, which began in November 2024, were conducted in the CREA-OF greenhouses in Pescia (PT), Tuscany, Italy (43°54′N 10°41′E) on *Abrometiella scapigera*, *Abrometiella brevifolia* and *Abrometiella chloranta*. The plants were placed in ø12 cm pots; 30 plants per thesis, divided into 3 replicates of 10 plants each. The experimental groups were: i) group without algae, irrigated with water and previously fertilised substrate; ii) group with *Chlorella vulgaris*, previously fertilised substrate. On 2 April 2024, plant height, leaves number, vegetative weight, root weight, root lenght, number of new plantlets, chlorophyll content, leaf area, control of the nematodes *Meloidogyne javanica* and *Meloidogyne incognita* were measured.

Results and Discussion: The experiment showed that the use of *Chlorella vulgaris* extracts can improve the vegetative and root growth of *Abrometiella scapigera*, *Abrometiella brevifolia* and *Abrometiella chloranta* plants grown in pots. The treatment also led to an increase in plant height, number of leaves, length of root hairs, leaf area and number of new seedlings. The trial also showed that the use of *Chlorella* in plant growing media can also increase the photosynthesis rate (SPAD index) and significantly reduce the presence of nematodes, in particular *Meloidogyne javanica* and *Meloidogyne incognita*. According to some researchers, the influence of *Chlorella* extract on cell metabolisms is mainly due to the physiological effect of major and minor nutrients, amino acids, vitamins, and plant growth regulators on cellular metabolisms in treated plants, which increase crop yields and growth. *Chlorella* plants have very high photosynthesis rates, dozens of times higher than other plants. The main reason why *Chlorella* can grow rapidly is because it contains *Chlorella* Growth Factor (CGF), which is quite rich in nucleoproteins, nucleic acids, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), vitamins, amino acids, polysaccharides, complex protein bodies, enzymes, glycoproteins, plant hormones, etc. Its ability to promote plant growth may be due to this factor.

Conclusions: *Chlorella* extract significantly increased *Abrometiella* seedling height, leaf number and leaf area. In particular, *Abrometiella* seedlings treated with chlorella extract developed better root system, chlorophyll a increased significantly and the root length and number of new seedlings also improved significantly. Based on the above results that *Chlorella* extracts contribute to the growth and development of *Abrometiella* plants, we can conclude that with the treatment of *Chlorella* extracts, the application of chemical fertilisers in the production of succulents such as *Abrometiella* can be reduced.

Keywords: Macroalgae; Sustainable applications; Microorganisms; Succulent plants; Nematodes

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1. Introduction

Chemical fertilizers have increased crop yields in recent decades. However, long-term chemical fertilizer applications have destroyed soil aggregate structure, causing soil compaction and the death of soil beneficial bacteria, and vegetables contain higher nitrate levels than is recommended. The development of bio-fertilizers that are environmentally friendly can be an approach to increasing crop productivity [1]. Plant nutritional constituents have been increased through genetic selection, which has included allele selection, gene and genome duplication, and the creation of new genotypes. These techniques have many advantages, but some of them can pose potential risks to food safety and require special attention to ensure that consumer health is protected. Agricultural practices can benefit from the use of bio-fertilizers as a safe tool for enhancing the nutritional properties of food crops [2]. Bio-fertilizers have been recognized as environmentally friendly compounds with positive effects on plants for many years. Specifically, they reduce the use of chemical fertilizers by increasing the amount of micro- and macronutrients taken up by plants, which positively impacts root growth and root morphology. By interacting with biochemical processes and physiological mechanisms, they also display hormone-like activity. Bio-fertilizers contain active molecules that promote nitrogen assimilation, according to recent studies. It might be explained by the fact that plants treated with bio-fertilizers are induced to synthesize phenylpropanoids in a metabolic pathway associated with these products, which may explain why they can help plants cope with stress [3]. The application of biofertilizer-containing products to pepper plants has been shown to positively affect plant growth in recent studies. A trend in agricultural production has recently been searching for bio-fertilizers. Among the many functional compounds found in *Chlorella*, there are crude protein 50–60%, carbohydrate 15–20%, crude lipid 12–18%, growth hormones, potassium, calcium, magnesium, iron, zinc, vitamin E, B1, B2, C, B6, folic acid, free biotin and chlorophyll, among others. In addition to its high photosynthetic efficiency, *Chlorella* is the only plant on earth that can grow four times in 20 hours, and it is known as the 'canned sun'. Many crops, including *Chinese chives*, spinach, lettuce, wheat, and *Hibiscus esculentus*, have been used as bio-fertilizers with *Chlorella* extract, which has increased their biomass [4,5].

Currently, the application of *Chlorella vulgaris* extract in succulent plants in particular *Abrometiella* spp. has not been observed. To examine whether the extract of *Chlorella* extract can stimulate the growth of *Abrometiella* spp. seedlings as a biofertiliser, further research is needed. The aim of this study is to investigate the effects of *Chlorella* extract as a biofertiliser on the growth of *Abrometiella* spp. seedlings and to assess whether it has a repellent effect against nematodes (Figure 1).

Figure 1 Details of the *Abrometiella* plants used in the trial

2. Materials and methods

The experiments, which began in November 2024, were conducted in the CREA-OF greenhouses in Pescia (PT), Tuscany, Italy (43°54′N 10°41′E) on *Abrometiella scapigera, Abrometiella brevifolia and Abrometiella chloranta*. The plants were placed in ø12 cm pots; 30 plants per thesis, divided into 3 replicates of 10 plants each. All plants were fertilised with a controlled-release fertiliser (1 kg m^{-3} Osmocote Pro®, 6 months with 190 g/kg N, 39 g/kg P, 83 g/kg K)mixed into the growing medium before transplanting.

The experimental groups were:

- Group without algae (CT) (peat 70% + pumice 30%), irrigated with water and previously fertilised substrate;
- Group with *Chlorella vulgaris* (CH) (peat 70% + pumice 30%), previously fertilised substrate

2.1. Chlorella cultivation

According to Kim et al., *Chlorella vulgaris* was separately cultivated in Bold Basal culture medium (BBM). A strain of *Chlorella vulgaris* was placed in a shaking incubator at constant temperature (30 °C) for 6 days at 180 rpm at pH 6.0 in a control room. In a bench scale fermenter with a capacity of 7.5 L, *Chlorella* was harvested when its dry biomass concentration was 180 g/L after seven days.

2.2. Preparation of Chlorella extracts.

For 72 hours, the fresh chlorella was immersed (w/v; 10) in 5000 mL ethanol and stirred constantly, then filtered through whatman 40.8 m filter paper. The extract was then concentrated in vacuo using a rotary evaporator at 50 °C and lyophilized. Following drying at ambient temperature, the condensed ethanol extract was placed in an amber vial and kept at 4 °C in a desiccator. *Chlorella* extracts were diluted with distilled water to a concentration of 0.4 mg/L after first solubilizing with dimethyl sulfoxide (DMSO). A solution of extracts was sprayed on the leaves of *Abrometiella* seedlings, whereas distilled water was sprayed on the untreated control plants. A solution of *Chlorella* extracts was sprayed on each of the 30 plants in each treatment. During spraying, the nozzle was approximately 10 cm away from the *Abrometiella* leaves.

2.3. Determination of chlorophyll content

SPAD value of chlorophyll a and chlorophyll b were measured by a chlorophyll analyzer (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Inc.); each sample was measured three times and their average value was calculated

On 2 April 2024, plant height, leaves number, vegetative weight, root weight, root lenght, number of new plantlets, chlorophyll content, leaf area, control of the nematodes *Meloidogyne javanica* and *Meloidogyne incognita* were measured.

2.4. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results and Discussion

The experiment showed that the use of Chlorella vulgaris extracts can improve the vegetative and root growth of Abrometiella scapigera, Abrometiella brevifolia and Abrometiella chloranta plants grown in pots (Figure 4 and Figure 5). The treatment also led to an increase in plant height, number of leaves, length of root hairs, leaf area and number of new seedlings (Table 1, Table 2 and Table 3). The trial also showed that the use of Chlorella in plant growing media can also increase the photosynthesis rate (SPAD index) (Figure 2) and significantly reduce the presence of nematodes, in particular Meloidogyne javanica and Meloidogyne incognita (Figure 3). In many crops, including Chinese chives, spinach, lettuce, wheat and Hibiscus esculentus, Chlorella extract can be used as biofertilizers, increasing the biomass of these plants [6]. The average height of Chinese chives treated with Chlorella was 3.7 cm shorter than that of those untreated. Chlorella treated Chinese chives had 0.5 mm wider leaves and 30.3 g heavier fresh leaves than those of untreated ones. A Chlorella treatment increased the commercialization and yield of Chinese chives by 11.9% and 18.3%, respectively, while a chlorella treatment increased the thickness and number of spinach leaves by 27.9% and 41.8%, respectively [7,8,9]. A combined seed and soil inoculation of Chlorella vulgaris accelerated Hibiscus esculentus growth and increased pod yield by 63.6% and 31.5% [10]. According to a recent study on wheat, Chlorella extract is beneficial for promoting plant growth. The Chlorella extracts were found to increase the plant height by 30% compared to control plants, and to increase the total biomass of the above- and below-ground parts by 22% and 51%, respectively [11]. Approximately 20% of the world's sugar production comes from sugar beets (Beta vulgaris subsp. vulgaris), which are commercially important biennial roots crops [12]. By increasing the efficiency and regularity of this critical process for

B. vulgaris seeds, Chlorella extracts improved sugar beet germination. It has also been found that Chlorella extracts contain cytokinin, which could be the reason for the increase in photosynthetic biomass [13]. A morpho-biometric study was conducted on lettuce seedlings by monitoring their chlorophyll, carotenoid, and total protein levels after application of chlorella extracts, foliar sprays, and root drenches [14,15]. According to the results, Chlorella extract significantly increased the dry matter, chlorophyll, carotenoid, and protein contents in lettuce seedlings [14]. According to some researchers, the influence of Chlorella extract on cell metabolisms is mainly due to the physiological effect of major and minor nutrients, amino acids, vitamins, and plant growth regulators on cellular metabolisms in treated plants, which increase crop yields and growth [15,16]. Chlorella plants have very high photosynthesis rates, dozens of times higher than other plants. The main reason why Chlorella can grow rapidly is because it contains Chlorella Growth Factor (CGF), which is quite rich in nucleoproteins, nucleic acids, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), vitamins, amino acids, polysaccharides, complex protein bodies, enzymes, glycoproteins, plant hormones, etc. Its ability to promote plant growth may be due to this factor [15,17–21].

Table 1 Evaluation of *Chlorella vulgaris* on agronomic characters on plants of *Abrometiella scapigera*

Abrometiella scapigera	**PH (cm)**	**LN (n°)**	**VW (g)**	**RW (g)**	**RL (cm)**	**NP (n°)**	**LA (cm²)**
CT	11.29 b	24.42 b	38.43 b	22.48 b	5.85 b	2.64 b	22.38 b
CH	15.34 a	32.40 a	42.73 a	23.92 a	6.31 a	6.21 a	24.64 a
ANOVA	***	***	***	***	**	***	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05); Parameters: PH = plant height (cm); LN = leaf number (n°); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); NP= new plantlets; LA: leaf area (cm2). Treatments: CT=control; CH=chlorella vulgaris

Table 2 Evaluation of *Chlorella vulgaris* on agronomic characters on plants of *Abrometiella brevifolia*

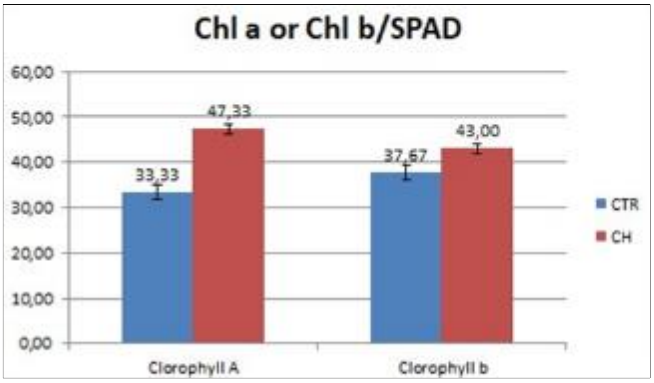
Abrometiella brevifolia	**PH (cm)**	**LN (n°)**	**VW (g)**	**RW (g)**	**RL (cm)**	**NP (n°)**	**LA (cm²)**
CT	12.63 b	20.11 b	37.19 b	21.86 b	4.90 b	3.84 b	23.71 a
CH	16.16 a	29.00 a	41.44 a	24.36 a	6.06 a	6.81 a	25.04 a
ANOVA	***	***	***	***	*	***	ns

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05); Parameters: PH = plant height (cm); LN = leaf number (n°); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); NP= new plantlets; LA: leaf area (cm²). Treatments: CT=control; CH=*chlorella vulgaris*

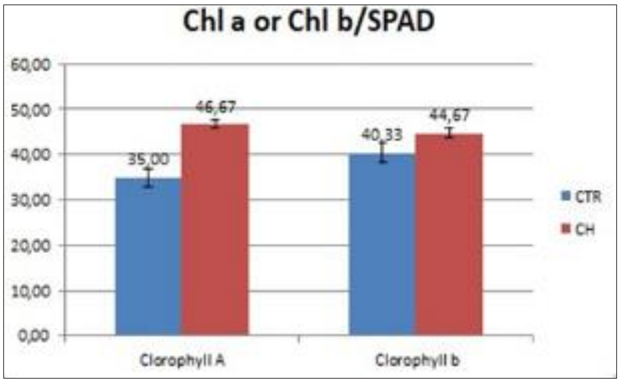
Table 3 Evaluation of *Chlorella vulgaris* on agronomic characters on plants of *Abrometiella chloranta*

Abrometiella chloranta	PH (cm)	LN (n°)	VW (g)	RW (g)	RL (cm)	NP (n°)	LA (cm²)
CT	11.99 b	18.84 b	34.91 b	23.79 a	4.92 b	2.22 b	21.65 b
CH	15.15 a	28.22 a	41.53 a	25.73 a	6.59 a	5.11 a	25.12 a
ANOVA	***	**	**	ns	**	***	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05); Parameters: PH = plant height (cm); LN = leaf number (n°); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); NP= new plantlets; LA: leaf area (cm²). Treatments: CT=control; CH=*chlorella vulgaris*



A



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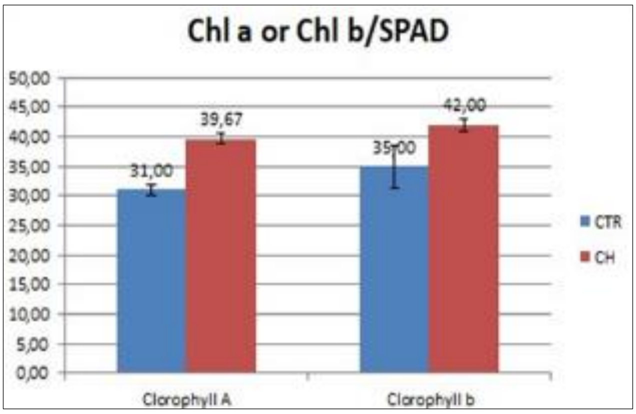
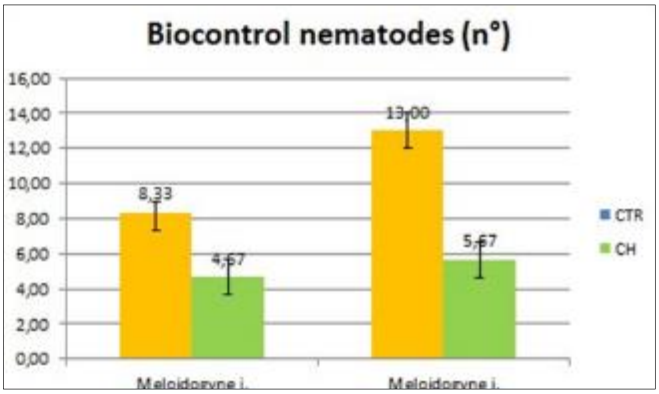


C

Figure 2 Effect of *Chlorella* extracts on the Chlorophyll content of *Abrometiella scapigera* (A), *Abrometiella brevifolia* (B) and *Abrometiella chloranta* (C) plants



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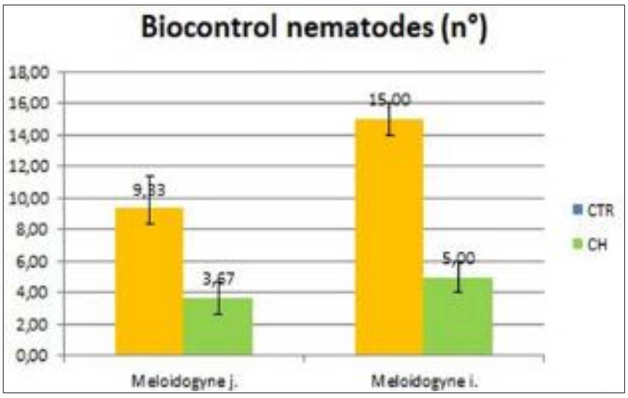

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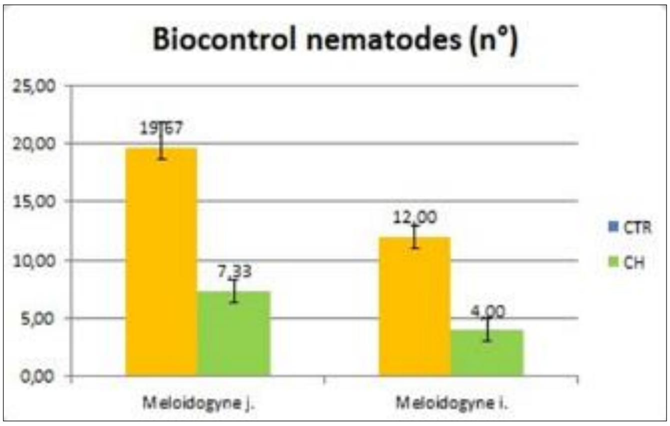

C

Figure 3 Effect of *Chlorella* extracts on the nematodes biocontrol (*Meloidogyne javanica* (j.) and *Meloidogyne incognita* (i.) of *Abrometiella scapigera* (A), *Abrometiella brevifolia* (B) and *Abrometiella chloranta* (C) plants

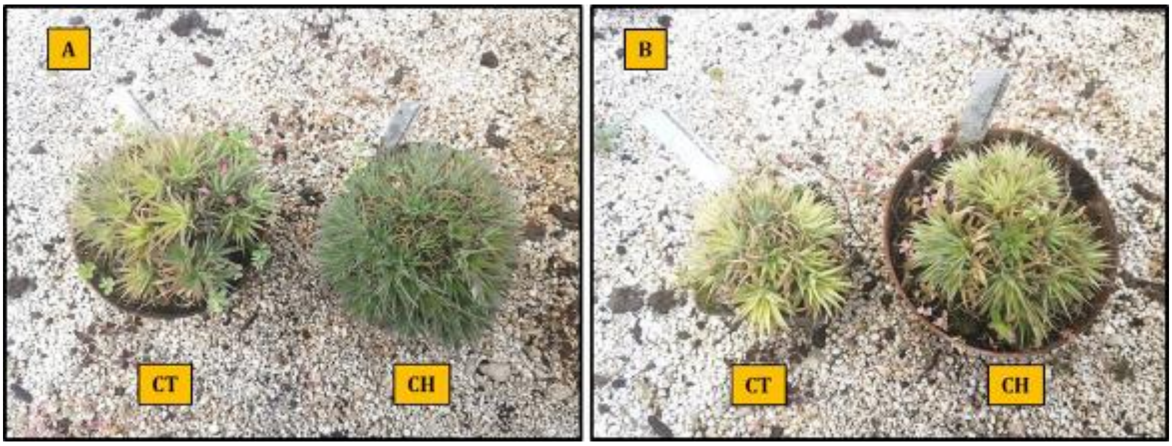

Figure 4 Effect of *Chlorella vulgaris* on vegetative biomass of *Abrometiella chloranta* (A) and *Abrometiella scapigera* (B). Legend: (CT): control; (CH): *Chlorella vulgaris*

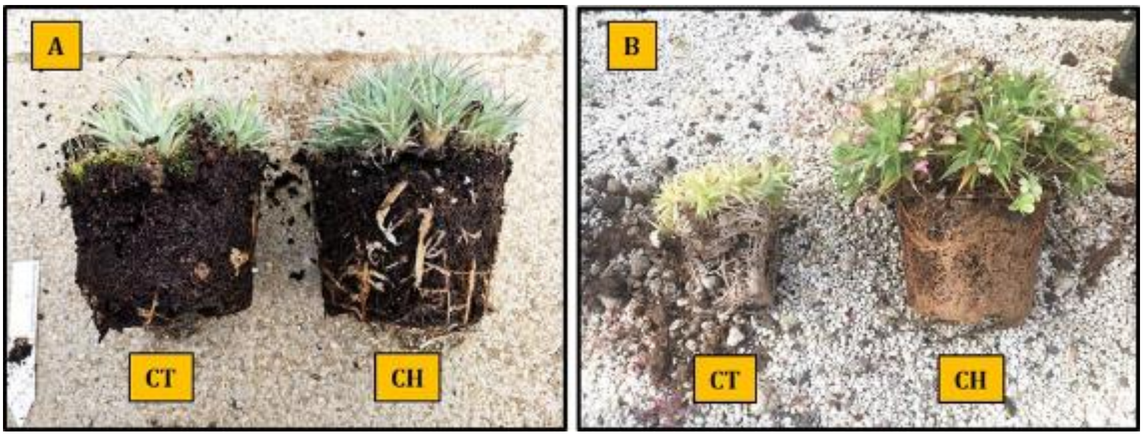

Figure 5 Effect of *Chlorella vulgaris* on root biomass and root hair size of *Abrometiella chloranta* (A) and *Abrometiella scapigera* (B). Legend: (CT) control; (CH): *Chlorella vulgaris*

4. Conclusion

Chlorella extract significantly increased *Abrometiella* seedling height, leaf number and leaf area. In particular, *Abrometiella* seedlings treated with chlorella extract developed better root system, chlorophyll a increased significantly and the root length and number of new seedlings also improved significantly. Based on the above results that *Chlorella* extracts contribute to the growth and development of *Abrometiella* plants, we can conclude that with the treatment of *Chlorella* extracts, the application of chemical fertilisers in the production of succulents such as *Abrometiella* can be reduced.

Compliance with ethical standards

Acknowledgments

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