IHJPAS. 37 (1) 2024
Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407

Effect of Mycorrhizal Inoculation and Fertilization with Plant Residues on the Growth of Chard Plant

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Received 1January 2023, Received 28 March 2023, Accepted 2 April 2023, Published in 20 January 2024

doi.org/10.30526/37.1.3168

Abstract

In order to study the effect of inoculation with mycorrhiza and fertilization with plant residues on the growth of plants, we used two factors: the first two levels of mycorrhiza inoculation, *Glumus mossea* (0 and 10 g.pot^{-1}) and the second factor, four levels of plant residues (10 g.pot^{-1}) celery plant residues, 10 g pot^{-1} mint residues, and 10 g pot^{-1} black bean seed residues. Mychorrizal treatment (10 g pot^{-1}) increased the number of mycorrhiza spores and the infection percentage of mycorrhizal by 917.44% and 13088.23%, respectively; celery treatment (10 g.pot^{-1}) increased the chlorophyll index in the leaves and height of the chard plant by 31.34% and 94.04%, respectively; and black seed treatment (10 g.pot^{-1}) increased the percentage of dry matter in the leaves and the percentage of carbohydrates in the leaves by 81.51% and 53.36%, respectively.The results showed the bilateral interactions between the experimental factors that the treatment of mycorrhizal inoculation exceeded (10 g pot^{-1}) and celery (10 g.pot^{-1}) residues in most of the study parameters in each of the Total Chlorophyll index in the leaves (SPAD), plant height, percentage of dry matter in the leaves (%), percentage of carbohydrates in the leaves, number of mycorrhiza spores, and infection percentage of mycorrhizal were (46%, 150.89%, 139.88%, 92.07%, 3283.45%, and 4000%, respectively, compared to the control treatment.

Keywords: Celery, Mint, Black bean, Mycorrhiza, Chard plant.

1. Introduction

Beta vulgar L var. *cilica* (Chard) plant is an important annual that belongs to the Chenopodaceae family; its leaves are simple with an alteration arrangement on the stem; its cultivation is widespread in Iraq; and it is widely used as food and supplements. Chard contains nutrients such as calcium, ferric, and vitamin A, B, and C and is also rich in many substances necessary for growth, such as Saponin, Raphhanin, Asparagine and Betanine [1]. The technology of inoculation with mycorrhiza, known as plant growth-promoting fungi (PGPF), is one of the successful technologies in working to stimulate the plant to grow [2] and increase the content of active compounds in it [3]. Because of the effective role of mycorrhiza in the processing of plant nutrients, plant hormones, organic acids, the formation of chelating compounds, and the improvement of soil texture [4], it raises plant efficiency and promotes growth through symbiotic living between fungus and plants, as well as works to increase plant tolerance to environmental stresses and resistance to pathogens [5].

Apium graveolens L. (Celery) belongs to the Umbelliferae family and is an annual, special-flavored plant that contains volatile oils. The stem is a hollow branch of the internodes, and the leaves are alternate pinnate compounds. The celery contains volatile oils of 1.5–3%, limonene 60–70%, vitamin A, B, and C, glycoside and flavonoid [6].

Mentha viridis L. (Mint) belongs to the Laminaceae family and is an aromatic herbaceous plant. Its leaves are simple opposite, auric, and the stems are four-sided. Mint contains volatile oils Eucalyptol, Limonene, Menthol, Carvone, Methanol, Pinene, Methyl-acetate and Menthone, there are also Terpenene, Flavonoids and Phenolic compounds [7].

Black bean (*Nigella sativa* L.) is a plant of the Ranunculaceae family and is an erect annual herbaceous plant with small ovate-pyramidal seeds and black colors [8]. Seeds contain lactones, glycosides, resins, and tannins, as well as 17 amino acids included in essential amino acids [9], proteins such as Albumin and Globulin saccharides, Ascorbic acid, Carotene, Niacin, Thiamine (B_1), Riboflavin (B_2), Pyroxene (B_6), and Flavonoids [10]. Black bean seeds contain Calcium, Ferric, Copper, Zinc, Phosphorus, Sodium, Potassium, Magnesium, and Manganese [11].

A previous study on the chard plant, [1] showed that the use of dried mint and celery leaves in the fertilization of the chard plant (*Beta vulgar* var. *cilica*) achieved an increase in height, number of leaves, and dry weight of the plant and reported [12], when the inoculation of the Sweet Leaf (*Stevia rebaudiana* Bertoni) stimulated plant with mycorrhiza significantly exceeded and achieved an increase in the parameters of vegetative and root growth of the plant. [13] reported that fertilizing with the residues of black bean seeds and mustard achieved a significant superiority in increasing the growth parameters of the Cicer arietinum (chickpea) plant. A study on tomato plants [14] proved the superiority of fertilization with garlic extract in terms of vegetative and flowering growth indicators. In a study on chili pepper (Capsicum annuum L.) [15], explained in his experiment that he used a mixture of Organic and Bio-fertilizer, it gave growth indicators through its role in supplying the plant with nutrients and stimulating the photosynthesis process. [16] confirmed that the treatment of inoculation with mycorrhiza gave the best results in the vegetative growth of the Chrysanthemum morifolium plant compared to the comparison treatment, and [17] obtained a significant superiority and increase in the height of the plant, the number of leaves, dry weight, the number of spores, and the percentage of infection of the roots with mycorrhiza when inoculating the seeds of the chamomile plant (*Matricaria chamomilla* L) with mycorrhiza by 5 $g.pot^{-1}$.

In order to have a clean environment, reduce the use of chemical fertilizers, and increase the production of the chard plant, the study aimed to the possibility of using some biostimuli and plant residues to increase plant growth. Determine the best combination of mycorrhiza and celery residues, mint, and black bean in the growth of the chard plant.

2. Materials and Methods

The experiment was conducted in the Botanical Garden belonging to the Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, for the growing season 2020-2021 in order to study the effect of Mycorrhizal inoculation and the dry vegetative total residues of celery, mustard seeds, and black bean on some growth indicators for the chard plant. The chard seeds were obtained from authorized agricultural equipment shops.

Used in agriculture, soil is taken from the deposits on the banks of the Tigris River. 10 random samples were taken from the soil prepared for agriculture and from different areas well mixed for the purpose of homogenization then analyzed in the central laboratory belonging to the College of Agricultural Engineering Sciences, University of Baghdad, to know their chemical and physical properties according to the methods described in [18], as shown in **Table 1**.

Table 1. Some physical and chemical properties before planting

Properties	value	units
pH	7.56	
EC	0.80	dS m^{-1}
N	17.50	mg kg^{-1}
P	4.13	mg kg^{-1}
K	35.20	mg kg^{-1}
Mg^{++}	0.80	m mol L^{-1}
Ca^{++}	1.30	m mol L^{-1}
HCO_3	0.4	mg L^{-1}
CO_3	Nill	
Silt	165.0	
Clay	85.0	g kg^{-1}
Sand	750.0	
Texture		Loamy sand

In the experiment, plastic bags with a capacity of 10 kg of soil were used. The field was divided into three repeaters; each repeater included 8 experimental units; the experimental unit included 5 plants; and the experiment included two levels of mycorrhiza and plant residues: four levels and three repeaters per treatment to make the number of experimental units 24 units, resulting in 2*4*3.

The second factor is plant residues, with four levels: Control treatment without adding a symbol to it C0. Adding 10 grams of celery plant vegetative total residues that had been dried in an electric oven with a vacuum at 70 °C until the weight stayed the same to each bag of soil that weighed 10 kg was called C1. Adding 10 grams of dried mint leaves (which were kept in an electric oven with a vacuum at 70 °C until the weight stayed the same) to each bag of soil that weighed 10 kg was the C2 treatment. Each bag of soil weighing 10 kg was treated by adding 10 g of black bean residues. The residues of black bean seeds were used after cold oil pressure instruments in an oil press machine extracted oil from them.

Chard seeds were cultured on 6/12/2020. The start of seed germination occurred after 5 days from the date of planting, and then the following data took place at the end of the experiment on 2/3/2021.

2.1. Total Chlorophyll index in the leaves (SPAD):

Measured in leaf by Spad, it's calculated with a device called a Chlorophyll meter equipped by a Minolta company by taking the mean of three readings of three plants randomly selected from each treatment.

2.2. Plant height (cm plant^{-1}):

Measured from the surface of the soil to the highest point in the main branch of the plant by a ruler.

2.3. Percentage of dry matter in the leaves:

It was calculated to take 5 leaves from each experimental unit and then weighed by a sensitive balance and dried in an electric oven supplied with a vacuum at 70 °C until the stability of the weight took the dry weight. The percentage was calculated according to the following formula: Dry matter percentage = dry weight / fresh weight x 100.

2.4. Percentage of carbohydrates in the leaves:

According to the method of Joslyn (1970) [19] in the laboratories of the Department of Biology, College of Science/University of Baghdad.

2.5. The number of mycorrhiza spores (spore 100g^{-1} dry soil)

The number of mycorrhiza fungi spores was calculated in the laboratories of the Department of Agricultural Research, Center for Integrated Control, Ministry of Science and Technology, Baghdad. According to Gaur and Adholya (1094), it serves as a method of wet sieving for the separation of spores. [20], 50 gm of soil from the area surrounding the root was weighed, and 1000 ml of distilled water was added to it. It was then distributed in test tubes and placed in a centrifuge for five minutes at a speed of 4000 cycles per Minute^{-1} . Then the upper suspended contents were poured out of the tubes, and the sediment at the bottom was taken and distilled water was added to it. The volume was increased to 100 ml with the addition of 50 g of sugar, and the mixture was discarded again by the centrifuge. The filtrate was taken and passed through a group of sieves of varying diameters (180, 125, 63, and 50 µm). Then the contents of the third and fourth sieves were collected in glass containers, and distilled water was used to remove sugar, and the water was Transferred to a volumetric flask, the volume was completed to 25 ml, then 1 ml of the suspension was withdrawn, placed in a petri dish, and examined under a microscope according to the number of spores in 100 g of soil according to the following:

The number of spores in 100 g soil=the number of spores calculated in 1 ml of suspended×25 ×2.

2.5. Percentage of infection with mycorrhizal (%):

The percentage of infection was estimated at the end of the season in the laboratories of the Department of Agricultural Research, Center for Integrated Control, Ministry of Science and Technology, Baghdad. Root samples were taken with the soil to the depth to which the end of the roots reached, and the method mentioned was used by [21]. The percentage of infection was calculated according to the following equation:

% of roots infected with mycorrhiza = (number of affected pieces / total number of pieces) × 100. (**Figure 1, Figure 2).**

A work experiment was designed according to the Randomized Complete Block Design (RCBD). The experiment treatments were randomly distributed with three replicates per treatment. The number of experimental units is 24 experimental units, resulting from (2×4×3). The experimental unit contained 5 plants; 3 homogeneous plants were elected in growth for each experimental unit for the purpose of the study. The data were statistically analyzed using the statistical program Genstat according to the method of variance analysis, and the averages were compared using the lowest significant difference (L.S.D.) at the level of probability 5%.

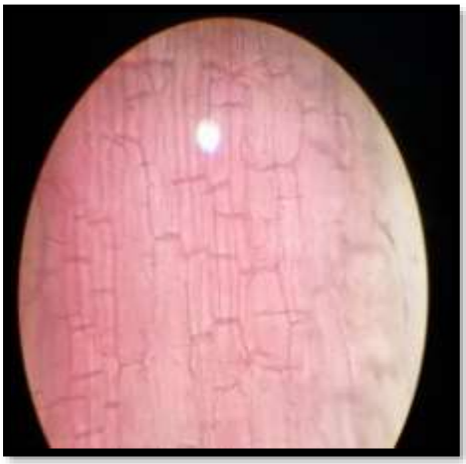
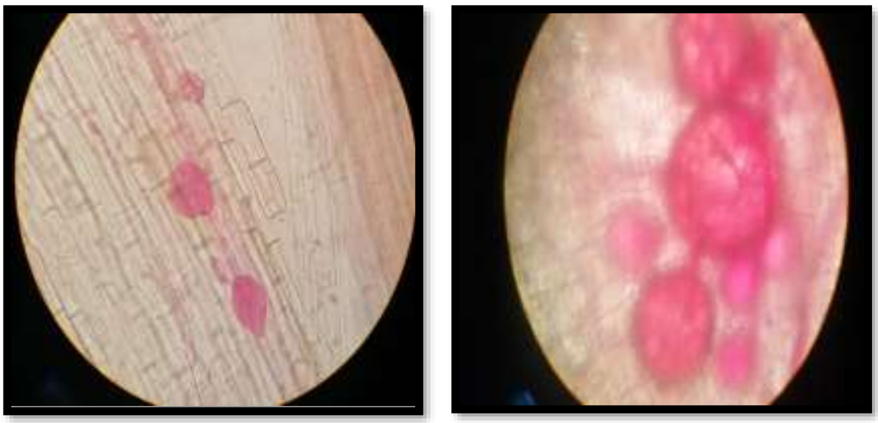


Figure 1. The root infection with mycorrhiza in chard plant (treatment M0 C0), 10x.



A. **B.**

Figure 2. The root infection with mycorrhiza in chard plant (treatment M1 C1), A.4x, B. 10x.

3. Results

Table (2) shows a clear and significant effect of the individual study factors on the character of total chlorophyll in the leaf where the inoculation treatment with mycorrhiza exceeded 10 g M1 and recorded the highest rate of 47.25 SPAD compared to the comparison treatment M0, which recorded 43.58 SPAD, and the treatment of 10 g celery residues C1 and recorded 51.00 SPAD compared to the C0 treatment, which gave 38.83 SPAD, and the treatment of duel interaction M1 C1 significantly exceeded and recorded 55.00 SPAD, while the comparison treatment M0 C0 recorded the lowest rate of 37.67 SPAD, a rate of increase (46%).

Table 2. Effect of mycorrhizal inoculation and fertilization with plant residues on the Total Chlorophyll the leaves of the chard (SPAD)

Mycorrhiza	Plant residues				Average M
	C0	C1 (10gm pot $^{-1}$ Celery)	C2 (10gm pot $^{-1}$ Mint)	C3 (10gm pot $^{-1}$ Black bean)	
M0	37.67	47.00	42.67	47.00	43.58
M1 (10gm pot $^{-1}$)	40.00	55.00	44.33	49.67	47.25
Average C	38.83	51.00	43.50	48.33	
L.S.D C		5.519		**L.S.D M**	3.902
L.S.D M*C			7.805		

The results of **Table (3)** show a clear significant effect of the individual study factors in the attribute of elevation as the treatment of inoculation with mycorrhiza exceeded 10 g M1 and recorded the highest value of 38.17 cm.plant $^{-1}$ compared to the comparison treatment M0, which recorded 32.92 cm.plant $^{-1}$, and the treatment of 10 g celery residues C1 and recorded 43.33 cm plant $^{-1}$ compared to the treatment of C0, which gave 22.33 cm plant $^{-1}$, and the treatment of duel interaction M1 C1 outperformed other treatments and recorded 47.67 cm.plant $^{-1}$, while the recorded comparison treatment M0 C0 recorded the lowest value of 19.00 cm.plant $^{-1}$ by increasing its amount (150.89%).

Table 3. Effect of mycorrhizal inoculation and fertilization with plant residues on the height of the chard (cm plant $^{-1}$).

Mycorrhiza	Plant residues				Average M
	C0	C1 (10gm pot $^{-1}$ Celery)	C2 (10gm pot $^{-1}$ Mint)	C3 (10gm pot $^{-1}$ Black bean)	
M0	19.00	39.00	33.00	40.67	32.92
M1 (10gm pot $^{-1}$)	25.67	47.67	36.00	43.33	38.17
Average C	22.33	43.33	34.50	42.00	
L.S.D C		2.910		**L.S.D M**	2.058
L.S.D M*C			4.116		

The study factors achieved a clear and significant effect on the characteristic of the percentage of dry matter in the leaves, as shown in **Table 4.** The treatment of inoculation with mycorrhiza exceeded 10 g M1 and recorded the highest value of 13.17 % compared to the comparison treatment M0, which recorded 11.08 %, and the treatment of 10 g.pot $^{-1}$ black bean residues C3 and recorded 14.83 % compared to the treatment of C0, which gave 8.17 %. The dual interaction treatment (mycorrhiza inoculation 10 g + 10 g.pot^{-1} celery residues) M1 C1 outperformed the other treatments and recorded the highest value of 16.00%, while the comparison treatment M0 C0 recorded the lowest value of 6.67%. a rate of increase (139.88%).

Table 4. Effect of mycorrhizal inoculation and fertilization with plant residues on the Percentage of dry matter in the leaves of the chard (%)

Mycorrhiza	Plant residues				Average M
	C0	C1 (10gm pot^{-1} Celery)	C2 (10gm pot^{-1} Mint)	C3 (10gm pot^{-1} Black bean)	
M0	6.67	12.67	10.67	14.33	11.08
M1 (10gm pot^{-1})	9.67	16.00	11.67	15.33	13.17
Average C	8.17	14.33	11.17	14.83	
L.S.D C		2.315		**L.S.D M**	1.637
L.S.D M*C			3.273		

Table 5 shows a clear and significant effect of the individual study factors on the percentage of carbohydrates in the leaves, as the treatment of inoculation with mycorrhiza exceeded 10 g pot^{-1} M1 and recorded the highest value of 13.67% compared to the comparison treatment M0, which recorded 11.33%, and the treatment of 10 g pot^{-1} black bean residues C3, which recorded 14.83% compared to the C0 treatment, which gave 9.67 %. The duel interaction treatment (mycorrhiza inoculation 10 g.pot^{-1} + 10 g.pot^{-1} celery residues) M1 C1 outperformed other treatments and recorded the highest value of 16.00%, while the comparison treatment M0 C0 recorded the lowest value of 8.333% by increasing its amount (92.07%).

Table 5. Effect of mycorrhizal inoculation and fertilization with plant residues on the Percentage of carbohydrates in the leaves of the chard (%).

Mycorrhiza	Plant residues				Average M
	C0	C1 (10gm pot^{-1} Celery)	C2 (10gm pot^{-1} Mint)	C3 (10gm pot^{-1} Black bean)	
M0	8.33	13.00	10.00	14.00	11.33
M1 (10gm pot^{-1})	11.00	16.00	12.00	15.67	13.67
Average C	9.67	14.50	11.00	14.83	
L.S.D C		1.538		**L.S.D M**	1.087
L.S.D M*C			2.174		

Table (6) shows a clear and significant effect of the individual study factors in the characteristic of the number of spores of mycorrhiza inoculation, where the treatment of inoculation with mycorrhiza exceeded 10 g.pot^{-1} M1 and recorded the highest value of 26.25 spores 100 g^{-1} dry soil compared to the comparison treatment M0, which recorded 2.58 spores 100 g^{-1} dry soil, and surpassed the treatment of 10 g pot^{-1} celery residues C1 and recorded 24.43 spores 100 g^{-1} dry soil compared to the treatment of C0, which gave 11.00 spores 100 g^{-1} dry soil. The M1-C1 dual interaction treatment outperformed the other treatments and recorded the highest value of 45.00 spores per 100 g^{-1} dry soil, while the M0-C0 comparison treatment recorded the lowest value of 1.33 spores per 100 g^{-1} dry soil. a rate of increase (3283.45%).

Table 6. Effect of mycorrhizal inoculation and fertilization with plant residues on the number of mycorrhiza spores (spore 100 g^{-1} dry soil).

Mycorrhiza	Plant residues				Average M
	C0	C1 (10gm pot^{-1} Celery)	C2 (10gm pot^{-1} Mint)	C3 (10gm pot^{-1} Black bean)	
M0	1.33	4.67	2.00	2.33	2.58
M1 (10gm pot^{-1})	20.67	45.00	14.33	25.00	26.25
Average C	11.00	24.83	8.17	13.67	
L.S.D C	4.293			**L.S.D M**	3.035
L.S.D M*C			6.071		

Table (7) clearly shows that each study factor had a significant impact on the root infection rate with mycorrhiza fungus. The treatment of inoculation with mycorrhiza exceeded 10 g.pot-1 M1 and had the highest percentage of 22.42% compared to the comparison treatment M0, which had a percentage of 0.17%. The treatment of 10 g.pot^{-1} celery residues C1 had a percentage of 20.17% compared to the treatment of C3, which had a percentage of 6.17%

Table 7. Effect of mycorrhizal inoculation and fertilization with plant residues on Percentage of the root infection with mycorrhiza in chard (%).

Mycorrhiza	Plant residues				Average M
	C0	C1 (10gm pot^{-1} Celery)	C2 (10gm pot^{-1} Mint)	C3 (10gm pot^{-1} Black bean)	
M0	0.00	0.33	0.00	0.33	0.17
M1 (10gm pot^{-1})	16.67	40.00	12.33	20.67	22.42
Average C	8.33	20.17	6.17	10.50	
L.S.D C	3.230			**L.S.D M**	2.284
L.S.D M*C	4.568				

4. Discussion

The results in **Tables 2** and **3 show** that the treatments of inoculation with mycorrhiza and celery residues and the dual interaction treatment both increased the plant's height and total Chlorophyll index. This may show how well mycorrhiza and celery work to improve the soil's quality and nutrient availability, since the presence of celery plant residues made the plant respond better to mycorrhiza infection.

It has improved its ability to play a part in making many cytochromes and ferredoxin compounds. Zinc is needed to keep the balance of positive and negative ions, and it helps make tryptophan, which is an amino acid that starts the process of auxin. Manganese [23] is important for starting enzymes in the Krebs cycle and for its part in the electronic transport system in photosynthesis.

Tables 4 and 5 show the effect of the factors of the individual experiment (mycorrhiza, celery, mint, and black bean residues) on increasing the percentage of dry matter in the leaves and the percentage of carbohydrates in the leaves of the plant. Because the residues of black bean seeds contain some essential fatty acids and antioxidants such as glutathione and arginine amino acids

[13]. Encouraged increased growth that coincided with the mycorrhiza inoculation as they worked together to increase nutrients such as iron, copper, manganese, and calcium, as these elements contribute as enzymatic accompaniments and cofactors in cell division and elongation and the opening and closing of stomata [25]. This leads to the delay of the process of chlorophyll demolition, the delay of plant senescence, the continuation of the process of photosynthesis and the processing of the plant with its vital products, and also affects the nutrients and active compounds present in the residues of black seed seeds with the availability of mycorrhiza inoculation and its production of stimulants and growth stimulants such as Cytokinins, Auxins and Gibberellins [12, 26].

This encourages the process of cell division and elongation and the activation of microbiology in the soil and its effective role in the biodegradation of fertilizers to obtain the energy source, which was reflected in an increase in the efficiency of photosynthesis and the formation of protein compounds, which stimulated vegetative growth [17].

It is noted from the results of **Tables 6 and 7** that single study factors and dual interaction play a role in increasing the number of spores and the incidence of mycorrhiza fungus **(Figures 1–2).** The reason may be due to the affinity and efficiency of the symbiotic relationship between the roots of the chard plant and mycorrhiza, and the use of a method and the appropriate amount of inoculation and plant residues encouraged the activity and growth of mycorrhiza.

Infection of the roots with mycorrhiza has made changes in the biochemistry of the cells of the roots and increased the permeability of the cell membranes [27], which was evident in increasing the number of spores and encouraging the colonization of the root by mycorrhiza and increasing the incidence rate, thus increasing the absorption of water and nutrients and the formation of a large root total, which was reflected in vegetative growth [16].

The use of plant residues encouraged the activity of mycorrhiza [28], which is evident in most of the studied traits as the study factors represented by celery residues and their nutrients such as iron, nitrogen, phosphorus, potassium, proteins and mint residues containing essential compounds such as carbohydrates, amino acids, organic acids [3] and secondary compounds such as phenolic acid, Terpenoids, and Flavonoids , and the residues of black bean seeds because of the nutrients and arginine compound, which is the source of nitrogen [29], went in one direction in increasing the same trend in increasing growth indicators represented by Chlorophyll content in the leaves, plant height, Percentage of dry matter in the leaves, Percentage of carbohydrates in the leaves, number of fungus spores and incidence of mycorrhiza [30].

5. Conclusions

The chard plant responds to mycorrhizal inoculation and gives the best results with the use of plant residues represented by celery, mint residues, and black bean seeds in the results of the vegetative growth indicators of the plant.

Acknowledgment

Many thanks to the staff of the botanical garden at the Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad.

Conflict of Interest

There are no conflicts of interest.

Funding

There is no funding for the article.

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