Effects of arbuscular mycorrhizal fungi and P-solubilizing Pseudomonas fluorescence (ATCC-17400) on morphological traits and mineral content of sesame

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Abstract

Sesame (Sesamum indicum L.) is an important staple crop of the family Pedaliaceae. The commercial production of sesame is still dependent on the applications of chemical fertilizers. Mycorrhiza inoculum resulted in better morphological and biochemical traits in vegetables. Thus, here the outcome of arbuscular mycorrhizal fungi (AMF) and Pseudomonas fluorescence (ATCC-17400) inoculation was studied in the pot culture experiment. Primarily, there seems to be a promising opportunity of AMF in sesame under pot and field trials because of enhanced morphological parameters, especially root weight, and disparities in nutrients and metabolites. The AMF appears to be an option to boost plant growth, mineral content, and sesame yield. The AMF treatment with Pseudomonas fluorescence strain (ATCC-17400) determined the maximum values for the morphological traits and mineral content. Overall, our study highlights mycorrhizal fungi and other microbes efficacy in achieving a successful sesame production.

1. Introduction

One of the most significant challenges of the 21st century is to feed the world competing with the increasing population rate. One of the economically important oil yielding plants is Sesamum indicum (Family: Pedaliaceae), commonly called Sesame. It is considered a vital oilseed source for developing countries as animal protein is costlier (Hassan et al. 2017). Sesame seeds have high oil content with a deliciously nutty aroma, mild taste and flavour, and biodiesel production quality (Habibullah et al. 2015). Sesame seeds contain a substantial amount of carbohydrates, proteins, fibres, essential minerals (Ca, P, Fe, etc.), tryptophan, methionine, lignans, flavonoids, phenolics, saponins and polyunsaturated fatty acids as well as rich in vitamin B and E (Myint et al. 2020).

Moreover, sesame seeds have several health benefits in terms of antioxidant, hypcholesterolemic effects, anticancer and blood pressure reduction activities, emulsifying, and foaming properties (Joshi et al. 2015; Hwang et al. 2017). Seeing the medicinal properties of sesame, some medicinal plants which may have active pharmaceutical compounds and other secondary metabolites are also in demand (Saini et al. 2020a). Thus, people are growing those plants in their home gardens, especially women in this way they are also conserving biodiversity (Saini et al. 2019a). This also increased the consumption of various wild and underutilized fruits with so many therapeutic properties (Cheema et al. 2016). Taking these properties in mind, the sesame plant is selected for the experiment.

Sesame is one of the first crop cultivated for oil production, and during the last 3 decades, the world production of sesame has noticeably increased (FAOSTAT, 2017). Global sesame seed consumption in 2018 was USD 6559.0 million, and it is estimated that sesame seed consumption will reach 7244.9 million by 2024. While in India, consumer preferences for sesame seeds have
increased by 15% in the present time, occupying an area of 1.8 M hectares (Bheda, 2019). To meet the increasing demand of consumers, there should be continuous production of food crops and oilseed crops. Thus, growers use non-renewable inorganic fertilizers in an excessive dose that sometimes creates a problem in the soil and hinders rhizospheric interaction of roots and soil microbes (Saini et al. 2019a). Therefore, a better approach is to choose a biologically active, sustainable, and friendly method. Such a process can be useful in promoting the use of microbes as bio-inoculants, especially Mycorrhizal Fungi (earlier known as Vesicular Arbuscular Fungi) and Phosphate Solubilizing Bacteria (PSB), also known as Plant Growth Promoting Rhizobacteria (PGPR) (Azooz and Ahmad, 2013; Hashem et al. 2019).

Arbuscular Mycorrhizal Fungi (AMF) are obligate symbionts that is thought to be largely promiscuously, colonizing more than 95% of vascular plants (Graham et al. 2017). AM fungi have a mutual relationship between plants receiving carbon source (lipid + carbo hydrate) from the plants and contribute an adequate amount of water and minerals to the plants (Chen et al. 2018). During colonization, AMF produces lipo-chito-oligosaccharides perceived by the plant roots through their signalling molecules (strigolactones and cutin monomers) that activate a typical symbiosis signalling cascade (Bonfante and Genre, 2015; Konieczny and Kowalska, 2017). AMF interaction improves nutritional status, manages the soil microenvironment, restores degraded land, and reduces nutrient leaching (Cavagnaro et al. 2015). PSB (Pseudomonas fluorescens), on the hand, help AMF and vice versa. However, agriculture soil receives a large number of fertilizers, mainly NPK and lacks an excellent nutrient retention system, but AMF/PSB inoculation can manage soil nutrient recycling. (Cavagnaro et al. 2015). Application of AMF and PSB benefit crop development by enhancing plant health and yield (Rouphael et al. 2015). AMF and PSB’s main role is absorbing and translocating mineral nutrients (particularly P) beyond the depletion zone and induce nutraceutical values as well as interfere with the plant growth hormones, thereby influencing better growth along with inducing tolerance to various environmental stresses (Rouphael et al. 2015). PSB and AMF activate the plant’s defense mechanism against pathogen attack (called systemic acquired resistance), which is very well documented (Lanfranco et al. 2018; Saini et al. 2020c).

P. fluorescens is gram-negative, rod-shaped PGPR that colonize plant roots by secreting greenish fluorescent pigment (hence named) particularly under iron scarcity (Kalita and Ram, 2019). Collectively, P. fluorescens helps in dissolving the complex organic phosphate in the soil and mycorrhizal fungi absorb them and transfer to plants (Anokhina et al., 2018).

While other researchers found AMF to be beneficial for the plants and found, how they are interacted with other soil microbes via large scale sequencing or metagenomics studies (Spatafora et al. 2016; Kaushik et al. 2020). Researchers are utilizing biotechnological approaches in the quest for biofortified crops. But, soil amendments using AMF are more ecofriendly and useful (Mahi et al. 2021). Owing to the importance of Sesame with easy compatibility of Pseudomonas fluorescens (PSB), AM fungi – Glomus mosseae (=Funneliformis mosseae), and Acacia lusoria laevis, the present investigation was planned for enhancing the yield of sesame, under pot conditions.

2. Materials and methods

2.1. Study site, soil characterization and pot preparation

Experiment was designed under a controlled temperature of 22 ± 2 °C, 16-h photoperiod light of 8000 lx (in addition to natural sunlight) and 65–70% humid condition in polyhouse of Botany Department, Kurukshetra University, Kurukshetra, during 2018–2019. Soil, taken from the Botanical garden of Kurukshetra University, was first air-dried, sieved through 2 mm sieve, autoclaved to eliminate previous microbial strains, and then mixed with sand in 1:3. Soil pH was 7.5 measured by pH meter (PHS-3C, Shanghai Lida Instrument Factory). Other characters of soil are as follows–sand: 71.05%, clay: 5.24%, silt 36.5%, Ca: 0.76%, K: 4.97: Mg: 0.81%, P: 0.57%, Fe: 12.95%, Zn: 8.77%(Estefan et al., 2013a; Estefan et al., 2013b). The seeds of Sesame variety RT46 were procured from Oil Seed Section of CCS Haryana Agricultural University, Hisar, Haryana 125,004 (India). This variety was chosen because of its cultivation at an extensive scale and its nutritional profile and disease resistance. Later were cleaned with 0.5% (v/v) sodium hypochlorite for 10 min, after washing with water were sown in each pot.

The experiment was carried out in earthenware pots (24.5 × 24), using a randomized complete block design (RCBD) with five replicates of each treatment. To each pot sterilized sand: soil mixture inoculum was added. 1 kg full in control pot and 100 g less in other treatment as AMF soil inocula were applied.

2.2. Procurement and mass multiplication of AMF and Pseudomonas fluorescens

Inoculum of Glomus mosseae containing 75–78% colonization (root pieces) plus 670–690 AM spores (w/w) and Acaulospora laevis containing 68–71% colonization (root pieces) plus 550–570 AM spores (w/w) were procured from Forest Pathology Discipline, Forest Protection Division, FRI, Dehradun. Both inoculums were then mass multiplied using Barley (host) for almost 3 months (as AMF generally take 80–90 days for infection and full colonization) to develop the starter inocula for the experiment (Saini et al. 2020c). After mass production, starter inocula was ready containing G. mosseae with 72–74% colonization/infection (barley root pieces) plus 620–640 spores (w/w) and A. laevis with 64–67% colonization/infection (barley root pieces) plus 540–560 (w/w) quantified by gridline intersect method by Adholeya and Gaur (1994); estimation of root colonization percentage was done by Philips and Hayman (1970). Pseudomonas fluorescens (ATCC-17400) was obtained from the CSIR-Institute of Microbial Technology (CSIR-IMTech), Chandigarh, India. It was then multiplied in a nutrient broth medium containing beef extract: 3 g/L; peptone: 5 g/L, and NaCl: 5 g/L, respectively to develop the bacterial colonies. The medium was then incubated at 32 °C for 48 h for proper bacteria growth.

2.3. Noculation of AM fungi and Pseudomonas fluorescens

For single AMF treatment, 100 g of each inoculum was supplemented per pot and 50 g of each inoculum for dual and consortium treatments (Saini et al. 2020b). For P. fluorescens treatment, all the seeds were dipped in the nutrient broth medium for 10 min.

The experiment had seven treatments, as follows:

1. Control (without any bioinoculant)
2. Glomus mosseae (G)*
3. Acaulospora laevis (A)
4. Pseudomonas fluorescens (P)
5. G. mosseae + A. laevis (G + A)
6. G. mosseae + P. fluorescens (A + P)
7. A. laevis + P. fluorescens (A + P)
8. G. mosseae + A. laevis + P. fluorescens (G + A + P)
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2.4. Plant harvest and analysis

Plants were harvested after 120 days of inoculation (DOI), and the effect of bioinoculants on various growth parameters were reported.

2.5. Change in plant height and root length

Plant height (cm) was measured after 120 DOI by 1-meter scale, and roots were also measured after uprooting.

2.6. Root and shoot biomass

After plants were harvested by carefully uprooting and gently washed under running water to remove the adhering soil. Root and shoot dry weight were recorded separately by keeping them in an oven overnight at 70 °C.

2.7. Oil extraction

The sesame seed oil estimation was done by petroleum ether using the Soxhlet's procedure (AOCS, 1997) with a boiling range between 400 and 600°C. 300 ml of normal Hexane was taken, and 10 g of the test was positioned in the thimble and placed in the extractor’s centre. The Soxhlet was warmed up to 60°C. If the solvent was boiling, the vapour increases from the vertical tube to the pinnacle’s condenser. The extract seeps through the thimble’s skin pores and fills the siphon tube, wherever it flows back down into the round outsole flask. This was permitted to carry on for thirty minutes. It was then taken from the hose, dried out in the oven, cooled in the desiccators, and weighed once again to identify the quantity of oil extracted. After the removal, the ensu-ption spectrophotometry (Model Z-2300, Hitachi Co., Tokyo, Japan) that measured at 420 nm (Estefan et al., 2013a,b). Other nutrients' number being highest in mycorrhizospheric plants treated with G. mosseae + A. laevis + P. fluorescens (13.03 ± 0.27) and least in control (6.11 ± 0.27). It is evident from Fig 1 that dry weight of all the inoculated Sesame plants were found to be significant in terms of shoot and root. It was found that supreme increment in shoot dry weight was recorded in the combination of G. mosseae + A. laevis + P. fluorescens (1.58 ± 0.63) followed by G. mosseae + P. fluorescens (1.25 ± 0.53) (Fig. 1). Similarly, the increase in root dry weight was also observed to be maximum with G. mosseae + A. laevis + P. fluorescens (0.47 ± 0.34) followed by G. mosseae + P. fluorescens (0.51 ± 0.43) (Fig. 1).

Based on this investigation, it seems that mycorrhiza's application with other bioinoculants (P. fluorescens) did have much constructive effect on AM spore number and mycorrhizal root colonization. Maximum increment in percent mycorrhizal root colonization was observed in the consortium treatment, i.e., G. mosseae, A. laevis and P. fluorescens (98.41 ± 1.14) followed by the combination G. mosseae + P. fluorescens (74.51 ± 3.83). AM spores' number being highest in mycorrhizospheric plants treated with G. mosseae + P. fluorescens (174.38 ± 3.16) followed by a mixture of G. mosseae + P. fluorescens (157.42 ± 2.92). AM root colonization and AM spores were not observed in P. fluorescens and control treatments.

It is apparent from Table 1 that percent of seed protein and oil content varied among different treatments. Maximum protein and oil content was recorded in the consortium of G. mosseae + A. laevis + P. fluorescens (Protein: 21.08 ± 0.87; Oil: 46.30 ± 0.64) followed by G. mosseae + P. fluorescens (Protein: 19.67 ± 0.64; Oil: 43.95 ± 0.95) as compared to control (Protein: 17.11 ± 0.68; Oil: 38.58 ± 0.33). Data in Table 2 revealed that P, Mg, K, Ca, Fe, and Zn uptake was significantly affected by AM fungi and P. fluorescens in seeds. The highest value of P was recorded in G. mosseae + A. laevis + P. fluorescens (1.33 ± 0.79), followed by G. mosseae + P. fluorescens (1.19 ± 0.77) over control (0.65 ± 0.56). Likewise, other nutrients were also found maximum in the same consortium treatment (K: 7.91 ± 0.65; Ca: 1.88 ± 0.71; Mg: 1.81 ± 0.54; Fe: 16.51 ± 0.39; Z: 12.11 ± 0.77) as compared to control one (K: 5.88 ± 0.73; Ca: 0.87 ± 0.58; Mg: 0.86 ± 0.55; Fe: 13.02 ± 0.34; Z: 9.39 ± 0.45).
Fig. 1. Variation among the 8 different of sesame for plant height (cm), shoot dry weight (gm), root dry weight (gm) and root length (cm).

Table 1
Influence of AM fungi and *Pseudomonas fluorescence* on growth and yield parameters of Sesame.

| Treatments   | Root colonization (%) | AM spore number/10 g soil | Protein content (%) | Oil content (%) |
|--------------|------------------------|---------------------------|---------------------|-----------------|
| Control      | 0 ± 0                 | 0 ± 0                     | 17.11 ± 0.68        | 38.58 ± 0.33    |
| G           | 61.39 ± 3.29          | 111.22 ± 1.58             | 18.11 ± 1.52        | 41.08 ± 0.75    |
| A           | 48.91 ± 3.21          | 78.33 ± 5.38              | 18.31 ± 1.51        | 39.73 ± 0.66    |
| P           | 0 ± 0                 | 0 ± 0                     | 17.68 ± 0.42        | 39.18 ± 0.71    |
| G + A       | 74.51 ± 3.83          | 132.55 ± 1.58             | 19.34 ± 1.57        | 42.08 ± 0.75    |
| G + P       | 87.53 ± 1.25          | 157.42 ± 2.92             | 19.67 ± 0.64        | 43.95 ± 0.95    |
| A + P       | 63.31 ± 1.67          | 120.74 ± 2.24             | 18.76 ± 1.58        | 41.87 ± 0.74    |
| G + P + A   | 98.41 ± 1.14          | 174.38 ± 3.16             | 21.08 ± 0.87        | 46.30 ± 0.64    |

L.S.D (P ≤ 0.05) 6.622 12.618 3.505 2.331

ANOVA (F 7,16) 386.297 372.955 3.571 72.859

| Treatments   | Calcium g 100 g FW | Potassium g 100 g FW | Magnesium g 100 g FW | Phosphorus g 100 g FW | Iron mg g FW | Zinc mg g FW |
|--------------|-------------------|---------------------|----------------------|-----------------------|--------------|--------------|
| Control      | 0.87 ± 0.58       | 5.88 ± 0.73         | 0.86 ± 0.55          | 0.65 ± 0.56           | 13.02 ± 3.44 | 9.39 ± 0.45   |
| G           | 1.05 ± 0.39       | 6.87 ± 0.92         | 1.11 ± 0.81          | 0.89 ± 0.45           | 15.07 ± 2.22 | 10.75 ± 0.78  |
| A           | 1.08 ± 0.55       | 6.08 ± 0.64         | 1.36 ± 0.53          | 0.75 ± 0.58           | 14.25 ± 0.21 | 10.53 ± 0.78  |
| P           | 0.94 ± 0.72       | 5.35 ± 0.57         | 0.95 ± 0.68          | 0.71 ± 0.68           | 13.87 ± 2.33 | 10.19 ± 0.56  |
| G + A       | 1.69 ± 0.52       | 6.46 ± 0.36         | 1.58 ± 0.47          | 1.08 ± 0.56           | 16.01 ± 0.52 | 11.97 ± 0.65  |
| G + P       | 1.48 ± 0.38       | 7.69 ± 0.73         | 1.67 ± 0.62          | 1.19 ± 0.77           | 15.94 ± 0.63 | 11.75 ± 0.62  |
| A + P       | 1.36 ± 0.39       | 7.08 ± 0.61         | 1.51 ± 0.29          | 0.97 ± 0.53           | 16.64 ± 0.63 | 11.07 ± 0.51  |
| G + P + A   | 1.88 ± 0.71       | 7.91 ± 0.65         | 1.81 ± 0.54          | 1.33 ± 0.79           | 16.51 ± 0.39 | 12.11 ± 0.77  |

L.S.D (P ≤ 0.05) 1.401 0.185 0.218 1.33 0.218 0.129 1.721

ANOVA (F 7,16) 113.458 172.849 165.556 211.213 122.901 181.231

1G: *Glomus mosseae* (=*Funneliformis mosseae*), A: *Acaulospora laevis*, P: *Pseudomonas fluorescens*, ±: Standard deviation.

2Mean value followed by different alphabet/s within a column do not differ significantly over one other at P ≤ 0.05 (Duncan’s Multiple Range Test).

Table 2
Influence of AM fungi and *Pseudomonas fluorescence* on the mineral content of sesame seed.

| Treatments   | Calcium g 100 g FW | Potassium g 100 g FW | Magnesium g 100 g FW | Phosphorus g 100 g FW | Iron mg g FW | Zinc mg g FW |
|--------------|-------------------|---------------------|----------------------|-----------------------|--------------|--------------|
| Control      | 0.87 ± 0.58       | 5.88 ± 0.73         | 0.86 ± 0.55          | 0.65 ± 0.56           | 13.02 ± 3.44 | 9.39 ± 0.45   |
| G           | 1.05 ± 0.39       | 6.87 ± 0.92         | 1.11 ± 0.81          | 0.89 ± 0.45           | 15.07 ± 2.22 | 10.75 ± 0.78  |
| A           | 1.08 ± 0.55       | 6.08 ± 0.64         | 1.36 ± 0.53          | 0.75 ± 0.58           | 14.25 ± 0.21 | 10.53 ± 0.78  |
| P           | 0.94 ± 0.72       | 5.35 ± 0.57         | 0.95 ± 0.68          | 0.71 ± 0.68           | 13.87 ± 2.33 | 10.19 ± 0.56  |
| G + A       | 1.69 ± 0.52       | 6.46 ± 0.36         | 1.58 ± 0.47          | 1.08 ± 0.56           | 16.01 ± 0.52 | 11.97 ± 0.65  |
| G + P       | 1.48 ± 0.38       | 7.69 ± 0.73         | 1.67 ± 0.62          | 1.19 ± 0.77           | 15.94 ± 0.63 | 11.75 ± 0.62  |
| A + P       | 1.36 ± 0.39       | 7.08 ± 0.61         | 1.51 ± 0.29          | 0.97 ± 0.53           | 16.64 ± 0.63 | 11.07 ± 0.51  |
| G + P + A   | 1.88 ± 0.71       | 7.91 ± 0.65         | 1.81 ± 0.54          | 1.33 ± 0.79           | 16.51 ± 0.39 | 12.11 ± 0.77  |

L.S.D (P ≤ 0.05) 1.401 0.185 0.218 1.33 0.218 0.129 1.721

ANOVA (F 7,16) 113.458 172.849 165.556 211.213 122.901 181.231

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2Mean value followed by different alphabet/s within a column do not differ significantly over one other at P ≤ 0.05 (Duncan’s Multiple Range Test).
5. Discussion

In order to determine the effect of microbial inoculation, it is very evident to know the feasibility of those microbes, particularly their combine outcome as different microbes have a different degree of colonization (Ambrosini et al. 2016). The present investigation showed that microbial inoculated sesame plants grew well with the different combinations used compared to control plants. It is apparent from the results that consortium treatment having AMF and P. fluorescens was proved to be the best treatment. AM fungi are involved in stimulating strigolactones responsible for improving root and shoot growth, which might be why our study sesame plant height and root-shoot dry weight ratio increased compared to control (Liu et al., 2018). The hyphopodia of AMF enter the plant root cortex to obtain lipid plus carbohydrate from the host plant (Sesame) and help the roots with increase uptake of nutrients, significantly P, which is compulsory for nucleic acids, enhancing the vegetative growth (Bona et al. 2017). This might be the reason for the higher plant height and plant weight of sesame with PSB and AMF inoculation. Treatment/s comprises of A. laevis and G. mosseae in sesame showed diverse results in all parameters studied because the extent of sap (water + minerals) absorption might differ due to different colonization (Wang and Jiang, 2015). Similarly, these AM fungi, when combining with P. fluorescens, possess a different absorption rate (Rashid et al. 2016). AMF produce organo-poly saccharides exudates that are decisive for soil porosity and quality, additionally when AMF inoculants mixed with P. fluorescens activity of these exudates become more commendable (Rashid et al. 2016).

P. fluorescens and AMF secrete some phosphatase enzymes that solubilize orthophosphates, by this plant’s roots get easy access of not only P but other nutrients also like Mg, N, Zn, Cu, Fe, Ca, Se, etc. (Saini et al. 2019b; Johri et al. 2015). That’s why in our experiment nutrient level is increased. AMF and PSB increase the β-glucosidase, hydrolase and urease activities that may lead to good microbial growth in the rhizosphere, consequently maintaining the soil micro-environment and help in growth (Song et al. 2015).

The amount of different minerals absorbed directly affects phytohormones, which also get activated (Park et al. 2017). This increased in plant growth hormones and nutrients enhance the protein and oil content in our sesame plant. Resultantly, AMF and PGPR application enhanced the mineral nutrition absorption leading to enhanced photosynthesis (Arif et al. 2016). As discussed, there is an increase in nutrient accumulation in plants via AMF, one such nutrient, i.e., Nitrogen also absorbed faster, which is very beneficial for plants’ protein formation (Balestrini et al. 2018).

Additionally, it is reported that there is up-regulation of some aquaporin genes, nitrate transporters and plant glutathione-S-transferases by applying AMF and PSB, which facilitate and maintain ionic/water balance in the plants even under drought stress (Jia-Dong et al. 2019). The increment of G. mosseae and A. laevis in sesame plants positively correlated to AM colonization (Ambrosini et al. 2016). This might be the reason for increased AM colonization and AM spore in the experiment. Gholinezhad and Darvishzadeh (2019) also reported an increase in sesame protein and oil content when inoculated by Funneliformis mosseae and G. intraradices. Harikumar (2017) also confirmed an increase in yield and oil content, up to 50% in Sesame when inoculated by AMF. Work done by these researchers and many others corresponds to our findings. Moreover, under stress conditions, the symbiotic fungi like AMF thrive and provide extra nutrients to the roots, producing effects such as development and higher yields. AMP can protect plants from rapidly changing climates. As a result, more studies are required to understand the AMF role in increasing crop quality and crop plants’ productivity (Ahanger et al. 2014; Hameed et al. 2014; Kumar et al. 2015; Latef et al. 2016; Malhi et al. 2021). The present assessment gives a current impression of the numerous nutrient requirements of AMF and their effects on different plant growth stages, including their crucial importance to oilseed crops like sesame, thereby demonstrating the significance of these dependencies on AMF.

6. Conclusion

Pseudomonas spp. and AMF are important microbial bioinoculants that have been shown to promote plant growth, yield and protect plants from pathogens and insects. These inocula help plants to attain healthy growth and high yield, even under stress condition. AMF and PSB can stimulate strigolactones which are iron-chelating agent important for plant growth hormones. It is very well acknowledged that AMF and PSB facilitates nutrient accumulation and is also found in the experiment. Future work will address that by application of bioinoculants, the use of synthetic fertilizers can be minimized with a view to maximize the yield. Overall, the amalgamation of AMF and PSB is the best treatment for attaining better growth and increased water and mineral absorption. Several essential components of the cellular and metabolic processes are maintained by minerals. Sesame biofortification using AMF would be a long-safe way to win over mineral deficiency and fight hidden hunger.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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