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Original Research Article

Seed biopriming with *Moringa oleifera* leaf and bark extracts improves seed germination and early seedling vigor of rice

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ABSTRACT

Seed priming is a pre-sowing treatment that promotes rapid and uniform germination, seedling establishment, and early vigor, depending on the priming agent and its concentration. This study evaluated the potentiality of Moringa extract (*Moringa oleifera*) as a biopriming agent for enhancing seed germination and seedling growth of rice. Water extraction method was applied for the extraction of Moringa bark and leaf extracts (MBE and MLE) followed by diluting 10, 15, 20 and 25 times. Nine priming treatments such as- control (no priming), MBE10, MBE15, MBE20, MBE25, MLE10, MLE15, MLE20 and MLE25 were used in the experiment with four replications. A clear advantage of biopriming with Moringa extract over no priming was evident for rice seed germination and seedling growth and vigor and bark extract performed better than leaf extract. In general, higher concentration resulted in better performances for most of the parameters studied. For germination parameters, the maximum germination (93.75%), shortest mean germination time (5.30) and utmost germination index (71.00) were recorded for MBE10. For seedling growth parameters, highest shoot length (15.60 cm), highest root length (11.85 cm), highest shoot dry weight (0.0483 g), highest root dry weight (0.0535 g) and highest root-shoot ratio (0.76) were also recorded from biopriming with MBE10. The findings demonstrate that Moringa bark extract has considerable potential as a seed biopriming agent for enhanced seed germination, seedling growth and vigor of rice. Further studies should characterize the bioactive compounds in Moringa extracts and elucidate the mechanisms responsible for the observed responses.

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INTRODUCTION

Rice is a global staple crop which feeds over 50% of the global population and supplies about one-fifth of dietary energy worldwide (Rahman & Zhang, 2022). Bangladesh ranks third worldwide in rice production, following China and India (Mamun *et al.*, 2021). Bangladesh's high rice production has largely kept pace with, and often outpaced, population increase, but the relationship is tight because rising population continuously raises demand while land, water, and climate constraints make further gains harder (Jamal *et al.*, 2023). The solution to the challenges is to be the increasing agricultural production efficiency and sustainability. Seed priming is an effective invigoration technique that enhances germination rate, uniformity, and seedling emergence under stress conditions (Anwar *et al.*, 2021b). Seed priming activates key germination-related metabolic processes before radicle emergence, facilitating faster and more uniform seedling establishment. During priming, seeds are partially hydrated using solutions with controlled osmotic potential and subsequently re-dried before sowing. Seed priming has been reported to promote faster crop growth, earlier flowering, and improved yield (Harris *et al.*, 1999; Kaur *et al.*, 2002; Islam *et al.*, 2026).

Moringa (*Moringa oleifera)* is an important vegetable of Moringaceae family in many tropical and sub-tropical countries including Bangladesh. Its annual production is comparatively higher than many other secondary vegetables. Most of the parts of moringa have medicinal value. Leaves, flowers and unripe fruits are used as vegetables, and roots and barks are used for medicinal purposes (Anwar *et al.* 2007). Moringa has also attracted considerable attention as a potential source of natural plant growth-promoting substances. Extracts derived from various plant tissues, especially the leaves, have been shown to promote seed germination, stimulate plant development, and improve crop yield (Foidl *et al*., 2001; Phiri & Mbewe, 2010).

Moringa leaf and bark extracts have been stated to promote plant growth when applied either as seed treatments or foliar sprays (Nouman *et al*., 2012; Soares *et al*., 2021). The beneficial effects of MLE are attributed partly to its rich composition of naturally occurring growth-promoting compounds, including zeatin, cytokinins, vitamins, and essential mineral elements (Yasmeen *et al*., 2014). Furthermore, MLE application has been proven to improve plant growth and productivity under drought and salinity stress by modifying physiological and metabolic responses (Yasmeen *et al*., 2013). In rice 3% Moringa leaf extract improved germination timing, germination index, and final germination percentage (Khan *et al*., 2022). Benefits also extend to stressful conditions. In wheat under salinity, Moringa-primed seeds had better germination character and about 10% higher germination than water-primed seeds (Ahmed *et al*., 2021). In tomato, Moringa leaf aqueous extract promoted plumule and radicle growth and increased fresh and dry seedling weight (Abdelhameed *et al*., 2025). Despite extensive research on seed priming for improving germination and early seedling development across different crops, the use of extracts derived from various parts of *Moringa oleifera*, especially bark extract, as a priming agent to enhance rice seed germination and seedling vigor remains largely unexplored in Bangladesh. Keeping the above research gap in mind, a laboratory experiment was conducted to investigate the potentiality of Moringa bark and leaf extracts as a seed biopriming agent and to identify the best Moringa plant part for extraction along with suitable concentration for improving germination, early seedling development, and vigor in rice.

MATERIALS AND METHODS

Experimental site, treatments and design

A laboratory-based experiment was conducted at the Agro-Innovation Laboratory, Department of Agronomy, Bangladesh Agricultural University (23°22′ N latitude and 90°33′ E longitude, approximately 18 m above sea level) under subtropical climate. The experiment was single factor, comprising different concentrations of Moringa bark extract (MBE) and Moringa leaf extract (MLE) as follows: i) Control (no priming) ii) MBE10 iii) MBE15 iv) MBE20 v) MBE25 vi) MLE10 vii) MLE15 viii) MLE20 ix) MLE25. The study employed a completely randomized design (CRD) with four replications.

Preparation of moringa extracts

Fully mature *Moringa oleifera* trees growing on the Bangladesh Agricultural University campus were selected for collection of fresh bark and green leaves. The collected plant materials were frozen overnight at −5 °C, mechanically pressed using a locally fabricated extraction machine, and filtered twice through Whatman No. 1 filter paper following the procedure described by Nouman *et al.* (2012). The filtrates were then centrifuged at 8,000 × *g* for 15 min, and the resulting supernatants were collected and diluted with water to prepare the required concentrations for subsequent applications. In case of MBE10 and MLE10 concentration, 100 ml distilled water was added to 10 ml bark and leaf extractions. In case of MBE15 and MLE15 concentration, 150 ml distilled water was added to 10 ml bark and leaf extractions. In case of MBE20 and MLE20 concentration, 200 ml distilled water was added to 10 ml bark and leaf extractions and for MBE25 and MLE25 concentration, 250 ml distilled water was added to 10 ml bark and leaf extractions. The diluted MBE and MLE were further used for seed priming.

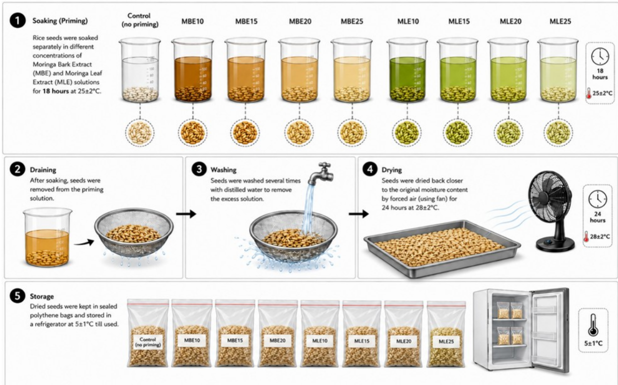

Figure 1. *Flow diagram showing rice seed priming with different concentrations of Moringa bark and leaf extracts.*

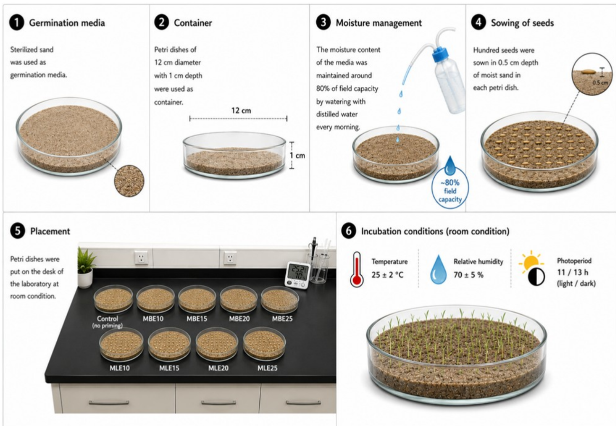

Figure 2. *Flow diagram showing media preparation and sowing of rice seeds (primed and control) for germination test.*

Seed priming

Seeds of rice were soaked separately in different concentrations of bark and leaf extract MBE10, MBE15, MBE20, MBE25, MLE10, MLE15, MLE20, MLE25 solutions respectively, for 18 hours at room temperature (25±2°C). Following priming, the seeds were removed from the treatment solutions and thoroughly washed several times with distilled water to remove residual extract. The seeds were then dried to approximately their original moisture content using forced air for 24 h at 28 ± 2 °C. The dried seeds were placed in sealed polythene bags and stored at 5 ± 1 °C in a refrigerator until further use (Figure 1).

Preparation of germination media and seed placement

Sterilized sand was used as the germination medium, and Petri dishes (12 cm diameter × 1.0 cm depth) were used as germination containers. The moisture content of the sand was maintained at approximately 80% of field capacity by adding distilled water every morning. One hundred seeds were sown at a depth of 0.5 cm in moist sand in each Petri dish. The Petri dishes were placed on laboratory benches under ambient conditions of 25 ± 2 °C temperature, $70 \pm 5\%$ relative humidity, and an 11/13 h light/dark photoperiod throughout the germination period (Figure 2).

Data collection

Measurements were taken for final germination percentage (%), mean germination time (MGT), germination index (GI), seedling vigor index (SVI), shoot length (cm), root length (cm), root-to-shoot ratio, seedling fresh weight, shoot dry weight (g), and root dry weight (g). Seed germination was monitored and recorded daily throughout the experimental period, and the final germination percentage and related germination parameters were determined 15 days after sowing (DAS). For assessment of seedling growth and biomass accumulation, ten seedlings were chosen at random to represent the sample and carefully uprooted from each Petri dish at 15 DAS. The seedlings were subsequently used for measurement of shoot and root growth and determination of fresh and dry biomass.

The percentage of emergence was determined at 15 days after sowing (DAS) using the formula below:

Germination percentage (%) = No. of germination seeds at final count / No. of seeds sown x 100

Mean germination time (MGT) was determined using the formula provided by Ellis & Roberts (1981):

$$\text{Mean Germination Time (MGT)} = \frac{\sum Dn}{\sum n} \quad (1)$$

Where "n" represents the number of seeds that germinated on day "D," and "D" is the count of days from the start of germination.

Germination Index (GI) estimates the time (in days) required to reach a specific germination percentage. It was calculated following the formula provided by the Association of Official Seed Analysis (AOSA, 1983)

$$\text{Germination Index (GI)} = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \cdots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}} \quad (2)$$

Seedling vigor index (SVI): The vigor of each treated seed lot was determined based on AOSA guidelines by multiplying the percentage of normal emergence by the seedling shoot height and then dividing the result by 100 (AOSA, 1983; Adebisi, 2004).

Seedling vigor index (SVI) = Seedling length (cm) x Germination percentage / 100

Where, Seedling length = Root length + Shoot length

Shoot length was recorded as the distance from the seedling base to the tip of its longest leaf (cm), while root length was measured from the seedling base to the tip of the longest root (cm). For dry weight determination, ten seedlings were dried in an oven at 60°C for 72 hours. Following drying, the dry weights of roots, shoots, and entire seedlings were measured. The root-to-shoot ratio was obtained by dividing the root dry weight by the shoot dry weight.

Statistical analysis

The recorded data were compiled, organized, and tabulated prior to statistical analysis. Analysis of variance (ANOVA) was performed using the MSTAT statistical software package. Treatment means were analyzed using Duncan's Multiple Range Test (DMRT) at a 5% significance.

RESULTS AND DISCUSSION

Germination and vigor index

The seed priming method had a significant impact on the germination percentage (Figure 3), mean germination time (Figure 4), germination index and Seedling vigor index (Table 1). All the priming methods except MLE20 and MLE25 priming led to a greater germination percentage compared to untreated seeds. The highest (93.75%) final germination percentage was found when seeds were primed with MBE10, which showed no significant difference compared to the results obtained from several other priming techniques like MBE15 (92.5%), MBE20 (89.50%), MBE25 (88.25%), MLE10 (57.75%), and control (88.5%). Priming with MLE15 resulted in the lowest germination percentage (48.25%). Surprisingly, no germination occurred when seeds were treated with MLE20 and MLE25 (Figure 3). Germination was noticeably delayed in seeds treated with MLE15, which recorded the longest MGT (8.80 days). In contrast, the fastest germination occurred in seeds primed with bark MBE10, where MGT was reduced to only 5.30 days. This result was statistically similar to MBE15 (5.80 days). Intermediate responses were observed in MBE20 (6.40 days) and MBE25 (6.88 days), while MLE10 showed a relatively high MGT of 8.23 days (Figure 4). Overall, bark extract particularly at higher concentrations helped seeds germinate more quickly than leaf extract. The highest GI (71.00) was observed in seeds primed with MBE10, which was statistically similar to MBE15. Treatments with bark extract MBE20 and MBE25 also maintained comparatively high GI values, indicating good germination performance. In contrast, leaf extract at 1:15 concentration (MBE15) resulted in a much lower GI (31.50). No germination occurred in seeds treated with MLE20 and MLE25. Overall, bark extract consistently supported better germination than leaf extract (Table 1). The highest SVI (25.73) was recorded in seeds primed with MBE10, although it was statistically similar to bark extract at 1:15 concentration (MBE15). Moderate vigor was observed in MBE20 and MBE25. In contrast, leaf extract treatments generally reduced seedling vigor. The lowest SVI (8.14) was found in leaf extract at MLE15, followed by MLE10. No seedling development occurred in leaf extract at 1:20 (MLE20) and 1:25 (MLE25) concentrations, resulting in an SVI of zero (Table 1).

Seed priming is a simple, economical, and widely adopted technique that enhances germination uniformity, accelerates seedling emergence, and improves seedling vigor, ultimately contributing to higher crop productivity under stressful environmental conditions (Anwar *et al.*, 2021a; 2024). By initiating key pre-germinative physiological and biochemical processes, seed priming enhances seed performance and promotes vigorous early plant growth (Roy *et al.*, 2013; Paparella *et al.*, 2015; Parvin *et al.*, 2024; Sarmin *et al.*, 2025). Moringa leaf extract (MLE) has attracted considerable research interest because of its rich composition of phytohormones, antioxidants, and essential nutrients, making it a promising natural bio-stimulant for improving plant growth and stress tolerance (Al Khazan, 2020; Haider *et al.*, 2024). Among the treatments, bark extract MBE10 resulted in the highest germination percentage, germination index (GI), seedling vigor index (SVI), shoot length, root length, dry biomass, and root-to-shoot ratio, while recording the lowest mean germination time (MGT). The accelerated germination and improved final germination percentage under MBE10 may be attributed to the activation of key biochemical processes during seed priming, including hydrolysis of stored reserves, activation of germination-related enzymes, and alleviation of seed dormancy (Farooq *et al.*, 2019). These processes facilitate the rapid mobilization of stored nutrients and promote embryo growth, resulting in faster and more uniform seedling emergence. Similarly, Karmore & Tomar (2015) reported that 24 h seed priming significantly reduced the time required for 50% seedling emergence and shortened mean germination time compared with unprimed seeds. Although performance gradually declined with increasing dilution (MBE15, MBE20, and MBE25), these treatments generally remained superior to or statistically comparable with the unprimed control.

Shoot and root length

Shoot and root lengths were significantly influenced by seed-priming treatments (Figure 5). The longest shoots (15.59 cm) and roots (11.85 cm) were recorded in seeds primed with MBE10, which were statistically comparable to those obtained with MBE15 (15.44 and 10.82 cm), MBE20 (14.83 and 10.45 cm), MBE25 (14.52 and 10.35 cm), and control (14.10 and 10.28 cm), respectively. In contrast, MLE15 produced the shortest shoots (10.25 cm) and roots (6.64 cm), followed by MLE10, which resulted in shoot and root lengths of 11.62 and 7.72 cm, respectively. At lower concentrations, leaf extract completely inhibited seedling development. Overall, bark extract was more effective than leaf extract in supporting both shoot and root growth of seedlings. Bark extract is a rich source of minerals, sterols, and growth-promoting compounds, including cytokinins, auxins, and other naturally occurring biostimulants that enhance cell division and improve osmotic regulation during seed imbibition (Arif *et al*., 2023; Kamanga *et al*., 2025). The superior performance of the MBE10 may be attributed to its ability to supply these beneficial compounds at an optimal concentration, thereby enhancing germination and subsequent seedling growth without inducing phytotoxic effects.

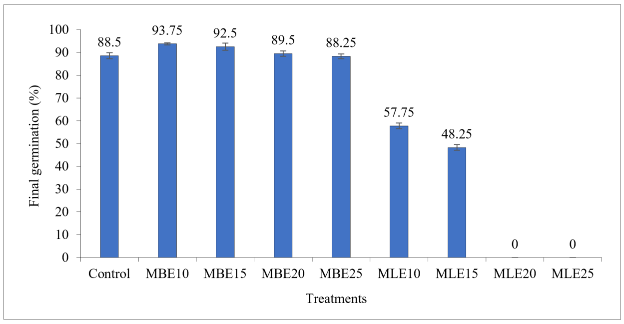

Figure 3. *Effect of Moringa bark and leaf extract on final germination percent of rice.*

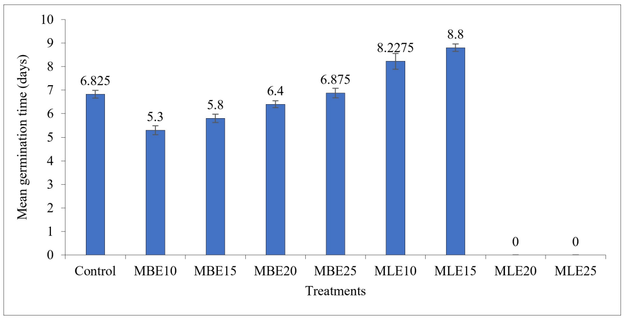

Figure 4. *Effect of Moringa bark and leaf extract on mean germination time of rice.*

Table 1. Effect of Moringa bark and leaf extract on germination and seedling growth of rice.

Treatme-nts	Root-shoot ratio	Root dry weight (mg)	Shoot dry weight (mg)	Seedling dry weight (mg)	Germination index	Seedling vigor in-dex
Control	0.73ab	42.3d	37.3c	79.5d	55.25c	21.56c
MBE10	0.76a	53.5a	48.3a	101.8a	71.00a	25.73a
MBE15	0.70a-c	52.0ab	42.5b	94.5b	65.50b	24.30b
MBE20	0.70a-c	49.3bc	39.8bc	89.0c	59.50c	22.63c
MBE25	0.72a-c	47.5c	39.0c	86.5c	57.50c	21.96c
MLE10	0.67bc	33.3e	24.3d	57.5e	36.75c	11.18d
MLE15	0.65c	27.5f	22.8d	50.3f	31.50e	8.14e
MLE20	0.00d	0.00g	0.00e	0.00g	0.00f	0.00f
MLE25	0.00d	0.00g	0.00e	0.00g	0.00f	0.00f
Level of sig.	**	**	**	**	**	**
CV (%)	9.76	7.25	7.50	5.59	7.46	5.83

Means with the same letters within the same column do not differ significantly. ** = Significant at 1% level of probability.

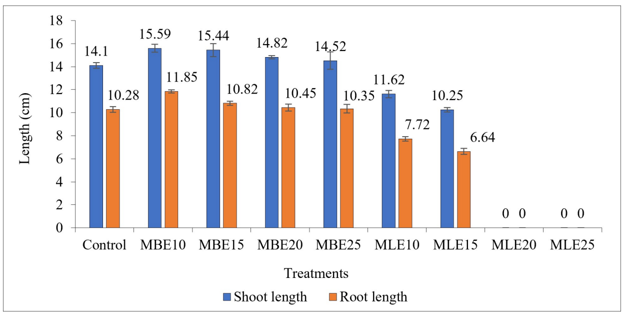

Figure 5. *Effect of Moringa bark and leaf extract on shoot length and root length of rice seedling.*

Shoot, root and seedling dry weight

Seed-priming treatments significantly influenced shoot dry weight, root dry weight, root-shoot ratio, and total seedling dry weight (Table 1). The highest shoot dry weight (48.3 mg), root dry weight (53.5 mg), root-shoot ratio (0.76), and seedling dry weight (101.8 mg) were recorded in seeds primed with MBE10. The shoot dry weight under MBE10 was statistically comparable to that of MBE15, while the root dry weight and seedling dry weight were statistically similar to those recorded with MBE15, MBE20, MBE25, and the control. Bark extract treatments at 1:20 (MBE20) and 1:25 (MBE25) also produced relatively higher shoot dry weights than the corresponding leaf extract treatments. In contrast, MLE15 resulted in the lowest shoot dry weight (22.8 mg), root dry weight (27.5 mg), root-shoot ratio (0.65), and seedling dry weight (50.3

mg), with MLE10 also producing comparatively lower root and seedling dry weights. Notably, no measurable shoot biomass was produced under MLE20 and MLE25. Overall, bark extract treatments were more effective than leaf extract treatments in promoting biomass accumulation and seedling growth, with MBE10 showing the most favorable overall response. The contrasting responses observed between bark and leaf extracts can largely be attributed to their differences in chemical composition. In *M. oleifera*, leaf tissues contain considerably higher concentrations of phenolic and flavonoid compounds than other plant parts. Previous studies have reported that the polyphenol content of leaf extracts is more than twice that of root or seed extracts, while flavonoids are present only in negligible amounts in non-leaf tissues (El-Rokiek *et al*., 2022). These bioactive compounds are known to play a major role in the allelopathic effects of moringa. At appropriate levels they may stimulate seed germination, but at higher concentrations they can disrupt cellular redox homeostasis, impair membrane integrity, and interfere with enzyme activity, ultimately inhibiting germination (Perveen *et al*., 2020; Kamanga *et al*., 2025). The results of this experiment revealed a consistent trend across all ten germination and seedling growth parameters, indicating that seed performance was influenced more by the combined effects of extract source (bark vs. leaf) and dilution level than by the priming treatment alone. In contrast to the generally expected concentration-dependent response, the leaf extract exhibited an unusual pattern in the present study. While the MLE10 produced moderate improvements in several germination parameters, germination was completely inhibited at MLE20 and MLE25. Because these treatments represent progressively more dilute solutions rather than higher extract concentrations, the complete loss of germination in the most dilute treatments, despite partial germination at the more concentrated dilutions (MLE10 and MLE15), represents an unexpected reversal of the anticipated dose–response relationship (Benramadan & Alsabri, 2025). This anomaly warrants careful investigation rather than being interpreted solely based on treatment mean comparisons. The observed response suggests that factors other than extracting concentration may have influenced seed germination and should therefore be considered when interpreting the effects of the leaf extract.

Conclusion

The study demonstrated that *M. oleifera* extract can serve as a promising natural biopriming agent for improving rice seed germination and early seedling vigor. Among the treatments, Moringa bark extract diluted 10 times (MBE10) showed the most consistent and superior performance, producing the highest germination percentage, germination index, seedling vigor index, shoot length, root length, shoot dry weight, root dry weight, and root-to-shoot ratio. Bark extract was markedly more effective than leaf extract in promoting germination, seedling growth, and biomass accumulation. In contrast, leaf extract showed reduced performance, with MLE20 and MLE25 completely inhibiting germination. However, the results are preliminary because the experiment was conducted under laboratory conditions using a single rice cultivar and without chemical characterization of the extracts. Further multi-season field studies are therefore needed to validate the effectiveness of MBE10, standardize extract composition, and determine its practical potential under field conditions.

DECLARATIONS

Author contribution statement: Conceptualization: M.P.A. and S.K.P.; Methodology: M.P.A.; Software and validation: M.P.A., M.Z.H., and N.N.P.; Formal analysis and investigation: M.P.A.; Resources: M.P.A.; Data curation: M.Z.H., N.N.P. and M.N.S.; Writing—original draft preparation: M.Z.H., N.N.P., M.N.S.; Writing—review and editing: M.P.A. and S.K.P.; Visualization: M.P.A.; Supervision: M.P.A. and S.K.P. All authors have read and agreed to the published version of the manuscript.

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Ethics approval: This study was conducted in view of the institutional ethical guidelines and does not harm the human participants.

Consent for publication: All co-authors gave their consent to publish this paper in AAES.

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