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Allelopathic potential of improved rice (*Oryza sativa L.*) varieties against *Echinochloa crus-galli* L.

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ABSTRACT

This study was done to evaluate the allelopathic abilities of 40-Sri Lankan improved rice varieties to control the *Echinochloa crus-galli* L. (Barnyard grass-BYG) using rice/BYG mixed cultures in trays, Double Pot Technique test and a field experiment. In tray experiment, significant inhibition in plant height and dry weight were observed in rice/BYG mixed-cultures than monoculture controls. Varieties which showed highest and lowest inhibition (%) in tray experiment were further tested in Double Pot Technique test and later in field experiment. Among the 40 rice varieties, Ld 365, Ld 368, Ld 408 and Ld 356 proved most inhibitory to BYG growth and 40% inhibition in BYG dry matter accumulation. Hence, these varieties can be used for further allelopathic studies.

Keywords: Allelopathy, *Echinochloa crus-galli L.,* growth inhibition, *Oryza sativa L.,*

INTRODUCTION

Rice (*Oryza sativa L.*) is mainly grown in South and South-east Asia. In Asia, yield losses due to uncontrolled weed growth in direct seeded lowland rice is 45-75%, and 50% in transplanted lowland rice (32). The weed communities in rice crops varies according to climate and the growing environment (4). Regardless of weed composition, weeds compete with crops for resources, especially during establishment and early growth stages (45). Barnyard grass (BYG- *Echinochloa crus-galli L.*) most yield limiting weed in irrigated rice systems of Sri Lanka and is better adapted to grow under dry conditions rather than wet conditions. BYG can affect rice yield at densities as low as 1-3 plants/m^2 (39). Now most paddy farmers practice direct seeding to minimize cost of production and due to water scarcity in paddy fields. These practices are conducive to growth of BYG in paddy fields and this weed may become a serious problem in future (22).

Chemical weed control is becoming increasingly unpopular due to environmental concerns, hence, in recent years, much efforts are being made to develop alternative weed management strategies. Development of natural herbicides, use of allelopathy and other natural suppression mechanisms of weeds as alternatives to herbicides have many benefits. Plant based herbicides are more systemic and easily biodegradable than synthetic ones (37). Synthetic herbicides have only 17 known molecular sites of action (16) whereas, plants produce a complex mix of chemicals whose active ingredients could have multiple sites of action and which could minimize the threat of developing herbicide resistance.

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Thus use of natural herbicides and allelopathy etc. would be favoured than synthetic herbicides. Allelopathy and natural suppression mechanisms in integrated weed management systems may potentially reduce the synthetic herbicide dose for weed control. Allelopathy is the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment (38). Allelopathic capability could provide the competitive edge to crop during the germination and establishment, when weeds competition is maximum (27). During 1988 and 1989 the allelopathic potential in rice was first discovered by Dr. Robert Dilday in Stuttgart, Arkansas in rice accessions. Thereafter numerous papers were published worldwide on allelopathic abilities of rice germplasm and allopathic potential of numerous rice accessions (1,6,7,9,10,11,12,14,17,19,20,24,28,34,35,36). Several allelochemicals were also extracted from different parts of rice plants and these chemicals are assumed to act in an additive or synergistic way rather than in an isolated way (13). Some of the genes responsible for rice allelopathy have also been identified (43,44). Various laboratory screening techniques have been developed to measure allelopathy and separating it from the interference of resource competition. Laboratory bioassays are most convenient, effective and simplest way to estimate the allelopathic potential of rice, as large number of rice cultivars can be examined in a limited time and space all year round (26). Among several bioassays, sandwich method (18), agar medium selection (17,44), plant box method (26), Double Pot Technique (25), hydroponic methods (21) and relay seedling method (33) have been reported and tested for bioassays.

As weeds compete with crops for resources, especially during establishment and early growth stages (45), hence, this study was done to (i). Evaluate the allelopathic abilities of Sri Lankan improved rice varieties at their seedling stage on growth of *Echinochloa crus-galli* L. (Barnyard grass-BYG) using rice/BYG mixed cultures in trays, (ii). Selected varieties were further examined for their allelopathic abilities using a Double Pot Technique and in the field.

MATERIALS AND METHODS

Forty commonly cultivated Sri Lankan improved rice varieties (Table 1) were used in this study. The selected rice varieties were collected from the different rice research stations (Ambalanthota, Labuduwa, Batalegoda and Bobuwela). The initial germination of collected seeds was > 80%.

I. Tray experiment

Pre-germinated seeds of rice and BYG were sown (3 x 3 cm spacing) in plastics trays (32 x 14 cm) filled with paddy field soil (approximately 1792cm^3 soil per tray) in alternate rows in glasshouse. In a tray there were 3 rice and 2 BYG rows and in each row there were 9 plants each (Figure 1). Before seeds sowing, the basal dose of plant nutrients (Urea:TSP:$ZnSO_4$, 20, 35 and 5 Kg/ha) was added according to the fertilizer recommendations of Department of Agriculture, Sri Lanka. All other weeds were manually uprooted daily by and water was added daily in tray to maintain similar water level. The control was monoculture of BYG (3 x 3 cm spacing). Randomized complete Block Design (RCBD) with three replications was used for this experiment. Thirty days after sowing, shoot length and dry weight of BYG seedlings were obtained. The inhibition percentage (IP) was used to assess the inhibition of each rice varieties on growth of BYG and IP was calculated as follows:

$$IP = (1 - Treatment/Control) \times 100 \ (12)$$

IP > 0 and IP < 0 indicate inhibitory effects and stimulatory effects respectively.

To evaluate the competition between the selected rice varieties and BYG, the plant height and dry weight of each rice/BYG mixed cultures were used to calculate Relative Competition Intensity (RCI) as under:

$$RCI = (P_{mono} - P_{mix}) / P_{mono} \ (21)$$

Where, P_{mono} : Plant height or dry weight in monoculture (control) and P_{mix} : Plant height or dry weight in mixed-cultures (Treatments). Positive RCI values indicate competitive inhibition and the negative values indicate stimulation.

II. Double Pot Technique

This experiment was done to separate the competition effects and allelopathic effects of selected improved rice varieties on growth and development of BYG. Based on the results of tray experiment, 9- improved rice varieties (Ld 365, Ld 356, Ld 408, Ld 368, Bg 38, Bw 453, Bw 400 and Bw 363) were selected. According to the Double Pot Technique described by Karim *et al.* (25) two types of pots were used, one small and another big. Each small pot had pores at the bottom, but the bigger pot had no pores. After filling up the small and big pots with paddy field soil, the small pots were placed at the top of the bigger pots (Figure 1). The small pots were transplanted with 2-weeks old 3-rice seedlings per pot. BYG weed seedlings of same age were raised in bigger pots around the base of smaller pots. The control treatment was only BYG weed without rice plants. Regular watering and manual weeding was done to ensure normal growth of rice and BYG plants. Treatments were arranged as Randomized Complete Block Design with three replicates and experiment was conducted for 40 days, thereafter, plant heights and dry weight were recorded. The inhibition percentage (IP) was calculated as described earlier.

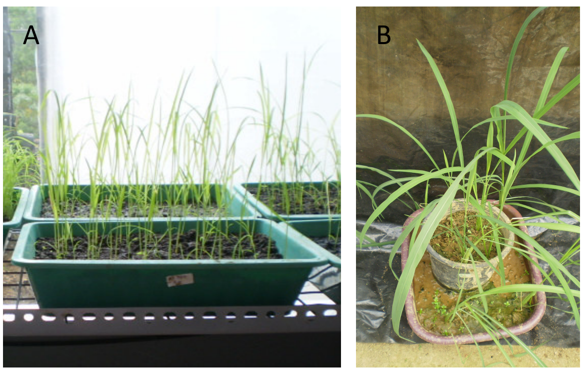

Figure 1. Arrangements of plants in (A) tray experiment and (B) double pot test.

III. Field Experiment

Field experiment was conducted at the farmer field in village Ginigala, Sevanagala, Sri Lanka in Maha season (Rice seedlings were transplanted in May and harvested in September, 2014). Based on the results of double pot test, 5-rice varieties (Ld 365, Ld 356, Ld 368 and Ld 408) selected were grown in seedling trays for 21 days, and then plants were transplanted manually at 15 x 20 cm spacing in 1m^2 plots (Figure 2), which were separated individually by small bunds. Randomized Complete Block Design with three replicates was used for this experiment. BYG were grown in seedling trays for 21 days, planted across the rice rows (Figure 2) 2-weeks after rice seedlings were transplanted (2). Herbicides were not used for weed control but all other weeds were removed manually. Other cultural practices (fertilizer application and pest control) were done according to the standard methods of rice cultivation in Sri Lanka (Department of Agriculture, Sri Lanka recommendations). The control was a plot of BYG monoculture. Data was collected, when 50% of BYG plants in the control plots were mature. Three BYG plants from each plot were harvested (leaving the boarder rows) and the plant height, plant dry weight, 1000 gains weight and number of tillers were measured. The inhibition percentage (IP) was calculated as described earlier.

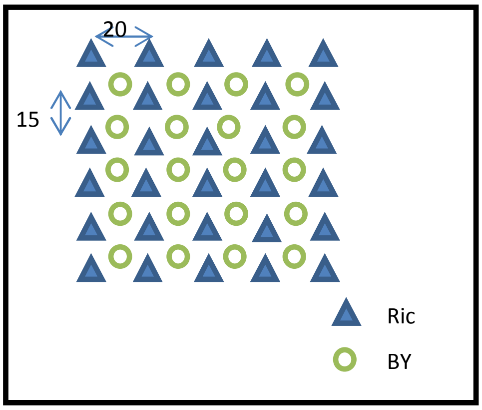

Figure 2. Sowing pattern of rice and BYG in a plot of field experiment. Spacing of rice within and in between the rows was 15 × 20 cm. BYG seedlings were planted at the middle of two rice rows.

RESULTS AND DISCUSSION

Tray experiment

Significant differences in plant height and dry weight were observed in rice/BYG mixed-cultures relative to monoculture controls (Table 1). Among the 40 rice varieties, the highest reduction in plant height of BYG was caused by Ld 365 (58.2%), followed by Ld 356 (49.9%) (Table 1). There were significant differences in inhibition of plant height of BYG between these two rice varieties. Similarly, maximum dry weight reduction in BYG occurred with Ld 365 (93.5%), followed by Ld 368 (83.5%) (Table 1). On the other hand, some varieties (Bw 367, At 353) also stimulated the shoot growth and dry weight of BYG *etc.*

Table 1. Inhibitory effects of rice varieties on barnyard grass (BYG) plant height, dry weight and Relative Competitive Intensity (RCI) of BYG in mixed culture with rice in tray experiment

Rice Variety	Pericarp colour	BYG Inhibition (%)		RCI of BYG mixed with rice	
		Plant height	Dry weight	Plant height	Dry weight
At 303	B	27.9	29.4	0.222	0.272
At 306	W	21.4	16.6	0.245	0.158
At 307	W	31.6	49.0	0.202	0.490
At 308	W	18.4	14.3	0.231	0.143
At 353	W	-8.9	-54.0	0.001	-0.540
At 354	W	-5.4	26.6	0.032	0.247
At 362	B	16.5	-32.2	0.247	-0.322
At 401	B	19.9	18.3	0.236	0.176
At 405	W	15.6	17.2	-0.012	0.165
At 402	B	5.3	18.4	0.031	0.184
Bg 3-5	W	14.7	27.6	0.062	0.254
Bg 38	W	11.2	74.6	0.214	0.739
Bg 304	W	36.0	48.3	0.234	0.466
Bg 305	W	39.7	60.7	0.239	0.595
Bg 358	W	25.7	15.8	0.207	0.151
Bg 359	W	15.0	29.4	0.077	0.288
Bg 360	W	17.2	65.1	0.168	0.502
Bg 403	W	6.6	38.5	0.169	0.366
Bg 406	B	11.5	45.4	0.197	0.454
Bg 407	W	31.8	57.3	0.229	0.573
Bg 407 -H	W	21.1	67.5	0.207	0.665
Bg 454	W	23.1	40.8	0.227	0.390
Bw 267-3	W	22.1	64.5	0.288	0.645
Bw 272-6b	W	8.9	56.9	0.096	0.661
Bw 302	W	7.1	20.5	0.190	0.205
Bw 351	W	12.5	47.4	0.116	0.461
Bw 361	B	28.3	67.2	0.286	0.672
Bw 363	W	31.2	72.9	0.251	0.729
Bw 364	B	28.6	30.4	0.343	0.304
Bw 367	W	-4.9	58.8	-0.160	0.588
Bw 400	B	41.8	76.0	0.305	0.760
Bw 451	W	43.7	58.1	0.362	0.581
Bw 452	B	16.2	36.8	0.255	0.368
Bw 453	W	39.3	72.1	0.398	0.714
H-4	W	37.4	54.1	0.226	0.541
Ld 355	W	10.2	28.7	0.016	0.269
Ld 356	B	49.9	76.4	0.580	0.758
Ld 365	B	58.2	93.5	0.580	0.933
Ld 368	B	46.7	83.5	0.337	0.831
Ld 408	B	37.1	86.2	0.259	0.858
LSD (0.05)		6.9	9.1	-	-

Values were calculated from data collected 30 days after transplanting of rice and BYG. Second column represent pericarp colour where, B=Brown rice and W=white rice. RCI: Relative Competitive Intensity, LSD: Least significant difference

Thus the Sri Lankan improved rice varieties during their early growth stages significantly affected the growth and development of BYGt. Allelochemicals released from plants effects the biological and biochemical processes of neighbouring plants and thereby inhibiting or stimulating their growth and development. Allelopathic compounds can influence the plants physiological processes [cellular expansion, cell wall construction, phytohormonal balance, activity of specific enzymes, mineral uptake, photosynthesis, respiration, stomatal movements, protein synthesis *etc*. (3,38,41)]. Allelochemicals also modify soil properties such as soil pH (30) and thereby causing indirect effects on plant growth and development.

The plant height reduction of BYG observed (Table 1) in the present study may also be due to the action of allelochemicals release from rice plants. Probably these allelopathic compounds may affect the nutrients uptake of BYG plants, which ultimately interfered with the development of plant height. However, a few rice varieties showed stimulations of plant height. The stimulations could be due to many reasons such as low concentrations of allelochemicals may cause stimulations to plant growth (38), and due to competition for light with neighbouring plants. In the tray experiment, dry matter accumulation of BYG was reduced in all varieties except in At 353 and At 362. Seedling weight is an important expression of seedlings size and vigour and is important for success in competition for growth resources (8). In fields and in mixed culture experiments, the growth reduction of BYG was affected by both allelopathic and competition effects.

Relative competition intensity values of plant height were positive for BYG except in Bw 367 and At 405 mixed cultures (Table 1). Whereas, RCI values of plant dry weight were positive for BYG except in At 353 and At 362 mixed cultures (Table 1). Ld 365 variety showed the highest competition against BYG. Relative competition intensity (RCI) shows competition between rice varieties and BYG (21). The RCI values for BYG were positive for most of the rice varieties indicating competition in rice/BYG mixed-cultures. Moreover, RCI values for BYG showed that BYG have different responses to stress caused by different rice varieties. The RCI values for BYG in BYG/Ld 365 variety mixed-culture were much higher than other varieties, indicating that Ld 365 was more competitive against BYG than other varieties.

The 40 rice varieties used in this experiment were from 2- groups (as per their seed pericarp colour) i.e, white rice and brown rice. There is a belief that varieties with coloured pericarp have high nutritional properties than white rice. Therefore, it could also be assumed that rice varieties with coloured pericarp may have higher allelopathic properties. Although not for coloured pericarped rice, Jung *et al*. (23) showed that rice residues of varieties with coloured hull have higher allelopathic potential on *E. crusgalli* than varieties with colourless hull. Hence in this experiment inhibition (%) of BYG growth by white and brown rice were analysed (Table 1) and we found that varieties with brown coloured pericarp (Table 1) are more inhibitory to all measured parameters (Table 2).

Table 2. Inhibitory effects of pericarp colour of rice varieties on Barnyard grass in tray experiment

Pericarp colour	BYG Inhibition (%)	
	Plant height	Dry weight
Brown rice	23.3a	47.7a
White Rice	21.6b	41.5b
LSD (0.05)	1.2	1.7

Double Pot Technique

Based on the dry weight reduction in tray experiment, 8 most allelopathic rice varieties (Ld 365, Ld 356, Ld 408, Ld 368, Bg 38, Bw 453, Bw 400 and Bw 363) and one poorly allelopathic (At 353) rice variety were selected for this experiment. Significant differences were observed among the varieties in terms of BYG plant height and dry weight inhibition (Figure 3). Varieties Ld 365, Ld 356 and Ld 408 caused more inhibition in plant height and dry weight, similar to tray experiment. Contrarily, the rice variety At 353 stimulated the BYG plant height and dry weight (Figure 3).

The theory behind Double Pot Technique was that the allelochemicals secreted from rice plants will drain from the pores of smaller pot and collected in the bigger pot. Therefore, allelopathic effect and competition effect can be separated using this technique. Although growth conditions and data collection times were different in Double Pot Technique than tray experiment, but results were comparable. Rice varieties with highest and lowest inhibition percentages on BYG growth in tray experiment and Double Pot Technique are similar. (Table 1 and Figure 3). As an example Ld 365 and At 353 showed highest and lowest performances respectively both in tray and double pot test (Figure 3).

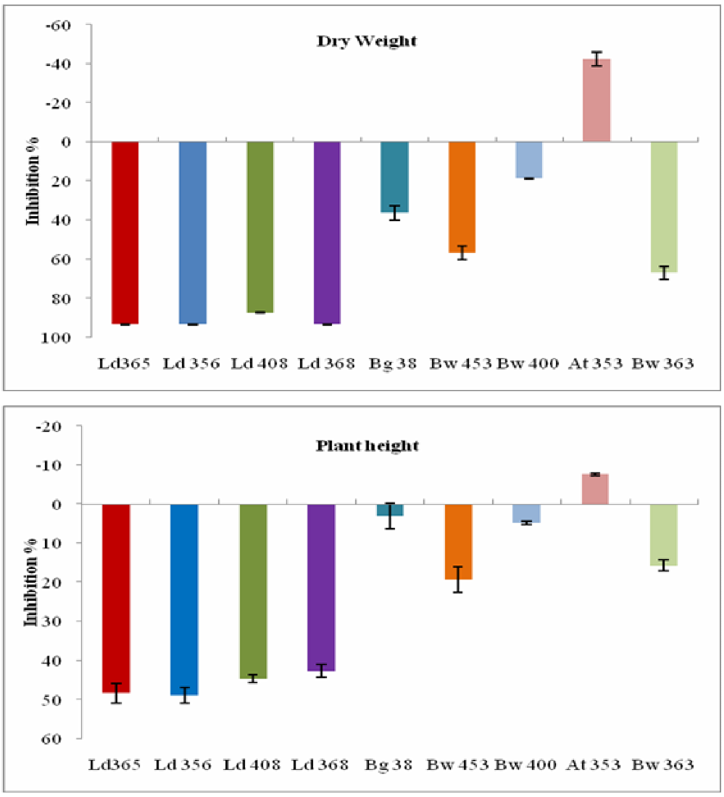

Figure 3. Inhibitory effects of rice varieties on barnyard grass (BYG) plant height and dry weight in Double Pot Technique. Values were calculated 42 days after establishment of rice and BYG in pots.

Field Experiment

The four strongly allelopathic rice varieties (Ld 365, Ld 356, Ld 368 and Ld 408) and one poorly allelopathic variety (At 353) identified in Double Pot Technique were used. These varieties significantly inhibited the BYG growth and reduced the dry weight (38.4 to 42.6 %), while the dry weight reduction by the poorly allelopathic variety was 20.3 %. In this study all Ld varieties caused similar dry weight reduction, but 1000-grain weight and number of tillers showed significant differences. Significant differences were not observed in plant height inhibition of all Ld varieties and in At 353. Lowest reductions in 1000-grain weight and number of tillers were observed in At 353 (Figure 4).

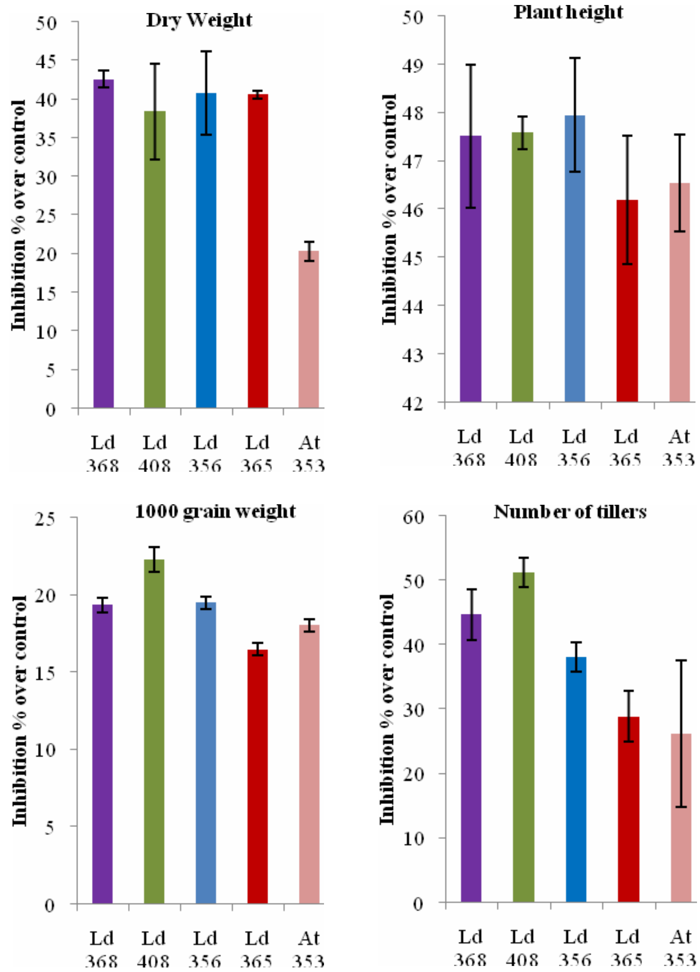

Figure 4. Inhibitory effects of rice varieties on barnyard grass (BYG) shoot dry weight, plant height, 1000- grains weight and number of tillers in field experiment. Values calculated from data collected at the stage when 50% of BYG plants in mature control plots.

In field experiment, corresponding results were observed but instead of stimulations caused by At 353 in the tray and double pot experiments, suppression of BYG growth was noticed (Figure 4). This might be the result of both allelopathic and competition effects in field condition. The inhibitory effects of all other varieties were also less in field than in tray and double pot experiments. This may be the result of inactivation of allelochemicals by soil microbes or dilution of concentration at field conditions. Although, the mechanisms of allelopathic and competition effects are distinct, these two effects are closely related at the field level. Further environmental and management conditions also highly affect the plants in field conditions. However, the consistency of results suggests that the combination of experiments used in this study is very potent way to study allelopathy in rice, particularly when large number of cultivars are to be studied.

Based on the results of these 3- experiments conducted in this study, Ld 368, Ld 408, Ld 356 and Ld 365 rice varieties were found with higher allelopathic potential. These rice varieties can also be used as a source of genes in rice breeding to develop high yielding and weed-suppressive rice varieties. The allelopathic studies conducted by Wathugala *et al*. (40) in field, green house and laboratory bioassays also noted the greatest inhibition of BYG by Ld 365. However, future work is needed to specify and verify the allelochemicals produced by these varieties.

Numerous researches have been done to evaluate the allelopathic potential of rice germplasm in laboratory, green house and field screenings. Allelochemicals released into the environment are highly variable, with plant growth and development stage and as well as the plant origin. Therefore, further experiments under different climatic and management conditions and different developmental stages are important to understand the allelopathic potential of rice varieties. Finally extraction and identification of particular chemicals would help to conclude the allelopathic potential of various rice varieties. Although not for these rice cultivars, several allelopathic chemicals such as p-hydroxybenzoic, ferulic, p-coumaricsyringic and salicylic acids from leaves and straws extracts, decomposing straw and in rice soil have been identified in other countries (5,12,15,29,31).

CONCLUSIONS

We found significant differences among the rice cultivars in terms of inhibition of BYG. The varieties Ld365, Ld 408, Ld 356 and Ld 368 had the highest allelopathic potential than other tested rice cultivars. The consistent ranking of the relative rice allelopathic potential identified in these experiments suggested that the tray experiment is very important method to study the allelopathic effects of rice, if a large number of cultivars are to be screened.

ACKNOWLEDGEMENTS

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