

# Locating Genes Controlling Allelopathic Effects against Barnyardgrass in Upland Rice

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## ABSTRACT

To understand the genetic control of allelopathy in rice (*Oryza sativa* L.), quantitative trait loci (QTL) mapping was performed using a population of 142 recombinant inbred lines derived from a cross between cultivar IAC 165 (*japonica* upland variety) and cultivar CO 39 (*indica* irrigated variety). The map contained 140 DNA markers. The relay seeding technique, which is a laboratory bioassay measuring the inhibition in weed root growth due to the presence of rice seedlings, was used to evaluate the allelopathic effect of the rice lines. Cultivar IAC 165 showed strong and consistent allelopathic activity against barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], whereas CO 39 was weakly allelopathic. Transgressive segregation for allelopathic activity in both directions was observed in the population. No significant correlation was found between root morphology of the lines and their allelopathic potential, suggesting that allelopathy in rice was under genetic control independent from root morphology. Four main-effect QTLs located on three chromosomes were identified, which collectively explained 35% of the total phenotypic variation of the allelopathic activity in the population. One pair of digenic epistatic loci, not involving any of the main-effect loci, was also detected. Once confirmed, these QTLs may be useful for genetic improvement of allelopathy in rice using marker-assisted selection.

THE increasing population in rice (*Oryza sativa* L.)-consuming countries and changes in farming practices call for new ways to sustainably enhance production. Weeds are one of the most important causes of yield losses in rice. In the upland rice ecosystem, yield loss estimates from weed infestation have ranged from 30 to 100% (Hassan et al., 1994; Pandey, 1996). Moreover, weeds increase production costs and lower rice quality.

In the upland rice ecosystem, hand weeding is still the most common practice to control weeds. Although hand weeding is an effective remedy, it is extremely laborious and time-consuming. The time required for hand weeding varies from 45 to 455 d/ha, corresponding to 40 to 50% of the total crop labor input (Roder et al., 1997). In all rice ecosystems, an increase in wage rates has led to a replacement of manual weeding by chemical control and increased concerns regarding environmental and health effects (Bhuiyan and Castañeda, 1995; Pingali et al., 1995). Consequently, there is an urgent need to identify sustainable weed management systems for rice production that will reduce the use of herbicides and the burden of hand weeding.

Allelopathy is defined as “any direct or indirect harmful or beneficial effect by one plant on another through the production of chemical compounds that escape into

the environment” (Rice, 1984). Rice plants with an allelopathic effect on weeds can mean lower production costs because the need for herbicide application and/or hand weeding is reduced. Thus, incorporating allelopathic genes into rice varieties while maintaining grain yield and quality could benefit farmers and consumers as well as the environment.

Research on allelopathy in rice started in the USA in the late 1980s. During several years, 12 000 accessions from the USDA-ARS rice germplasm were examined in field experiments for their allelopathic potential toward ducksalad [*Heteranthera limosa* (Sw.) Willd.], redstem (*Ammannia coccinea* Rottb.), broadleaf signalgrass [*Brachiaria platyphylla* (Griseb.) Nash], rice flatsedge (*Cyperus iria* L.), sprangletop (*Leptochloa* spp.), and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.]. Two methods were used to record allelopathic activity: the weed-free radial area (cm) from the base of the rice plant, and the percentage reduction in the number of weed plants in a tested plot relative to a nonallelopathic control. These methods have also been used by others (Hassan et al., 1994, 1998; Kim and Shin, 1998). Dilday et al. (1998) identified 412 rice accessions as having evident allelopathic potential against ducksalad, 145 against red stem, and 16 against both weed species. The accessions with allelopathic activity originated from 31 different countries and were genetically very diverse, showing that allelopathic potential is widespread within rice germplasm.

The main problem in conducting allelopathy field experiments is that competition and allelopathy cannot be separated in the field. To overcome this problem, various laboratory screening techniques have been developed to measure allelopathy without the interference of competition (Leather and Einhellig, 1986; Dilday et al., 1991; Fujii, 1992; Kawaguchi et al., 1997). Using a laboratory bioassay called the relay seeding technique, rice varieties suppressing barnyardgrass in the field have significantly reduced the root length of barnyardgrass under laboratory conditions (Olofsdotter and Navarez, 1996). This technique has been routinely used in screening hundreds of rice varieties at the International Rice Research Institute (IRRI) for allelopathy, resulting in the identification of several accessions with strong allelopathic potential.

The varieties now identified as allelopathic belong to different isozymic groups and to different ecosystems. There appears to be a higher frequency of allelopathic varieties among tropical japonicas within *Oryza sativa* and among *O. glaberrima* accessions than in other varietal groups (Fujii, 1992; Courtois and Olofsdotter, 1998).

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**Abbreviations:** NRC, no-rice check; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred lines; QTL, quantitative trait loci.

There is strong evidence that allelopathy in plants is due to a complex of chemicals (Rizvi and Rizvi, 1992). Several putative allelochemicals have been identified from soil where allelopathic rice lines have been growing (Mattice et al., 1998) and also from soils containing decomposing rice residues (Chou and Lin, 1976). Although these results infer that allelopathy in rice is a quantitative trait, the only study reported on the genetic basis of allelopathy in rice is that of Dilday et al. (1998) on the  $F_2$  progeny from a cross between allelopathic rice accession PI312777 and rice cultivar Lemont. This study indicated that the allelopathic effect of rice on duckweed was quantitatively inherited.

Manipulation of quantitative traits such as allelopathy based on phenotypic selection is difficult because trials are time-consuming and labor-intensive, and are influenced by environmental factors. In this respect, DNA markers provide us with a powerful tool to dissect and manipulate quantitative trait variation through quantitative trait loci (QTL) mapping experiments and marker-assisted selection. The principle of QTL mapping is to detect association between a particular phenotype and molecular markers, which are segregating in a population. This can be done by using an appropriate experimental design, phenotyping techniques, and statistical analysis. Once the genes of interest have been located and the molecular markers closely linked to these genes have been found, gene manipulation is possible through marker-assisted selection (McCouch and Tanksley, 1991).

In this paper, we report on the first effort to map QTLs associated with allelopathic activity in rice in a recombinant inbred population, using the relay seeding technique for phenotyping.

## MATERIALS AND METHODS

### Plant Material

The mapping population consisted of 250 recombinant inbred lines (RILs) produced by single seed descent from a cross between the *japonica* upland cultivar IAC 165 and the *indica* irrigated cultivar CO 39. Cultivar IAC 165 showed strong and consistent allelopathic activity against barnyardgrass, whereas CO 39 was weakly allelopathic based on our preliminary experiments (data not shown). The population was used previously for mapping genes/QTLs for blast resistance (Mauleon, 1995) and structural root morphology traits (Shen et al., unpublished data, 1999). A subset of 121 RILs with the most complete marker data was used in the phenotyping experiments.

The map used in this study for determining and locating QTLs was built by Mauleon (1995) and upgraded by Shen et al. (unpublished data, 1999). This map contains 140 markers

(microsatellites and RFLP markers) covering all 12 rice chromosomes, with an average distance of 14 cM between markers.

### Phenotyping Experiment

The 121 RILs, the parents, and two checks (Aus 196, a nonallelopathic variety, and a no-rice check) were evaluated for their allelopathic potential using the relay seeding technique (Navarez and Olofsdotter, 1996) with minor modifications. Specifically, 30 sterilized rice seeds (soaked in 2.5% sodium hypochlorite for 10 min and washed three times in distilled water) were sown in two parallel rows (3.0 cm apart, 15 seeds in each row) inside a petri dish, except for the no-rice check (NRC). The seeds were covered with 7.0 g of perlite to restrain arching of the rice roots. The petri dishes were placed inside a germination box and 30 mL of distilled water were added to each germination box outside the petri dishes. The water moved into the petri dishes through a bridge of filter paper, leaving the perlite inside the petri dish aerobic. No nutrient was applied at any time during the experiment. The germination box was covered with a thin transparent plastic sheet held in place by a rubber band. Four days after sowing, another 20 mL of distilled water were added to each germination box. On Day 7, 20 sterilized barnyardgrass seeds (soaked in 2.5% sodium hypochlorite for 10 min and washed three times in distilled water) were sown in one row in between the two rows of rice seedlings on top of the perlite, including the no-rice check. At the same time, 30 mL of distilled water was added to all the germination boxes. On Day 14, two-leaf rice seedlings were trimmed to prevent competition for light between rice and weed seedlings. The setup was placed on wire shelves covered with white styrofoam plates. The light intensities on the shelves ranged from 1300 to 3000 lux. The photoperiod was 12 h. The temperature inside the room was kept between 29 and 33°C. Thus, the relay seeding technique ensured that there was no competition for light, water, or nutrients. Preliminary experiments showed that there was no competition for space and that pH in the water stayed stable in the time course of the experiment (unpublished data, 1999). A reasonable conclusion is therefore that the effect seen is due to allelopathy.

On Day 17, the perlite was washed off the roots of barnyardgrass and the root length of 10 weed seedlings from each germination box was measured. Assuming that allelopathic chemicals released from the rice plants will inhibit the growth of weed roots, the average root length of the 10 weed seedlings was used as an indicator of the allelopathic potential of the rice plants in question. The screenings were carried out as a completely randomized design with three sets of independent experiments, each replicated twice, staggered in time because of space constraints.

### Statistical Analysis

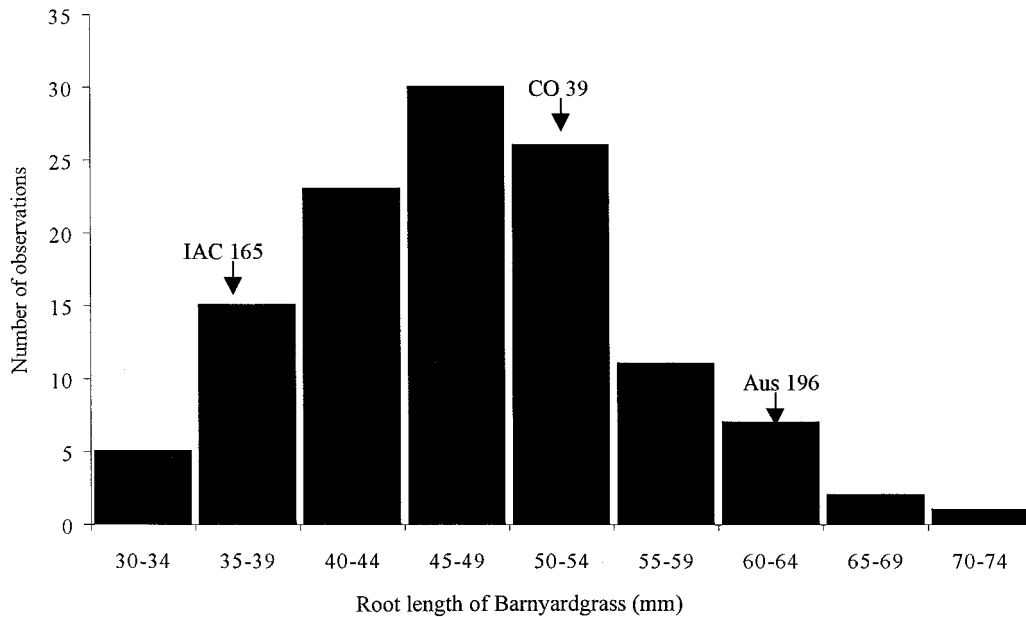
Analysis of variance was performed to partition the different sources of variation for weed root length using SAS PROC GLM (SAS Inst., 1990). The broad-sense heritability for the

**Table 1. Mean and standard deviation for allelopathy (expressed as barnyardgrass root length) of the controls (NRC and Aus 196), parents of the population (IAC 165 and CO 39), and recombinant inbred lines (RILs).**

	NRC†	Aus 196	IAC 165	CO 39	RILs		$h^2$ ‡
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Range	
Barnyardgrass root length, mm	95.2 ± 5.4	59.4 ± 12.5	38.0 ± 11.9	53.3 ± 13.5	47.7 ± 8.2	26.1–71.9	0.68

† NRC, no-rice check.

‡  $h^2$ , broad-sense heritability across experiments.



**Fig. 1.** Frequency distribution of the root length of barnyardgrass grown together with different recombinant inbred lines. IAC 165 = strongly allelopathic parent; CO 39 = weakly allelopathic parent; Aus 196 = nonallelopathic check. Standard deviation for the parents and population is shown in Table 1.

trait was estimated from the pooled data from the three experiments using the formula  $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_R^2/n)$ , where  $\sigma_G^2$  and  $\sigma_R^2$  were the estimates of genetic and residual variances derived from the expectations of the mean squares of ANOVA, and  $n$  was the number of replications.

### Quantitative Trait Loci Analysis

The mean values of weed root length were used to map main-effect QTLs associated with allelopathy using the mixed linear model approach in QTL MAPPER version 1.0 (Wang et al., 1999). Selected main-effect and epistatic markers were chosen by the software to control the genetic background variation. The probability thresholds were set at  $P = 0.005$  for both main and epistatic effects. The additive effects, epistatic effects, and percentage of variation explained by individual QTLs were estimated by the software.

## RESULTS

Table 1 shows the mean values of weed root length influenced by the parents, RILs, and controls. The root length of barnyardgrass seedlings grown together with IAC 165 was significantly shorter than for those grown together with CO 39 and Aus 196. This indicated that IAC 165 was more allelopathic than both CO 39 and Aus 196. However, the root length of barnyardgrass seedlings grown together with CO 39 and Aus 196 was significantly shorter than the NRC, suggesting that the two rice varieties were not strictly nonallelopathic. The

weed root length in the presence of the RILs showed normal distribution (Fig. 1), with a mean value of 47.8 mm equal to the midparental value and a range from 29.7 to 70.7 mm (Table 1). The presence of transgressive segregation in both directions for weed root length in the RILs suggested that both parents might carry alleles for increased allelopathic activity.

The analysis of variance indicated that there were highly significant differences among the RILs for weed root length. The estimate of the broad-sense heritability for allelopathic activity was reasonably high with a value of 0.68.

Because the RILs were known to segregate for structural root morphology (Shen et al., unpublished data, 1999), the question arose whether plants having a larger root mass were able to exude more chemicals, and were thereby more allelopathic. Thus, we computed the phenotypic correlations between the root morphology data previously obtained for the population in a greenhouse pot experiment (Shen et al., unpublished data, 1999) and the allelopathy data obtained from the relay seeding technique. The results indicated that rice root morphology traits were not significantly correlated with allelopathic activity in this RIL population (Table 2).

Four main-effect QTLs associated with allelopathic potential were identified and mapped to Chromosomes 2, 3, and 8 (Table 3 and Fig. 2). Individually, the QTLs explained from 6.9 to 12.0% of the total phenotypic

**Table 2.** Correlation between root morphology characteristics and allelopathy in the IAC 165 × CO 39 recombinant inbred line population.†

	MRL†	THK	RW0030	RW3060	RW6090	RWB90	TRW	DRW
Allelopathy	-0.0660	-0.1123	-0.1416	-0.0699	-0.1041	-0.0282	-0.1384	-0.0801

† MRL, maximum root length. THK, root thickness. RW0030, root dry weight in a 0 to 30-cm layer (mg). RW3060, root dry weight in a 30 to 60-cm layer (mg). RW6090, root dry weight in a 60 to 90-cm layer (mg). RWB90, root dry weight below 90 cm (mg). TRW, total root weight. DRW, deep root weight.

**Table 3. Main-effect QTLs involved in allelopathy against barnyardgrass in the IAC 165 × CO 39 recombinant inbred line population. Main-effect and epistatic markers were used for background genetic variation control.**

Chromosome	Flanking markers	LOD	Additive effect	Prob†	R <sup>2</sup> ‡
2	RG25-RZ318	2.43	-2.31	0.0009	6.9
3	RZ681B-RZ536B	4.20	-3.05	0.0000	12.0
3	RM16-RM227	1.81	-2.36	0.0039	7.2
8	RG978-RM42	3.40	2.57	0.0001	8.5

† Prob, two-tail probability of Student *t* value for testing the additive effects.

‡ R<sup>2</sup>, relative contribution from the additive effects of the putative QTLs.

variation. The overall contribution of these four QTLs was approximately one-third of the total phenotypic variation. The alleles at three of the main-effect QTLs (Chromosomes 2 and 3) suppressing root growth of barnyardgrass (for increased allelopathic potential) were from IAC 165, whereas the allele on Chromosome 8 was derived from CO 39.

Among the four main-effect QTLs, the two located on Chromosome 3 were not unlinked because the distance between the two intervals was 23.5 cM and they were acting in the same direction. The RM16-RM227 interval had the lowest LOD score. However, these two markers were among the few markers with a nonnegligible number of missing data (approximately one-third) and the resolution for this interval might be increased once these data are completed.

One pair of digenic epistatic intervals was also detected between an interval on Chromosome 1 (RZ801-RG83B) and one on Chromosome 9 (RG553-RZ206) (Table 4). None of these intervals was significant for main effect. Epistasis explained an additional 6.9% of the total phenotypic variation. The epistatic effect was of the same order of magnitude as the smallest individual QTL main effect.

None of the main-effect QTLs for allelopathy was

positioned in the same place as the main-effect QTLs for root morphology (Shen et al., unpublished data, 1999).

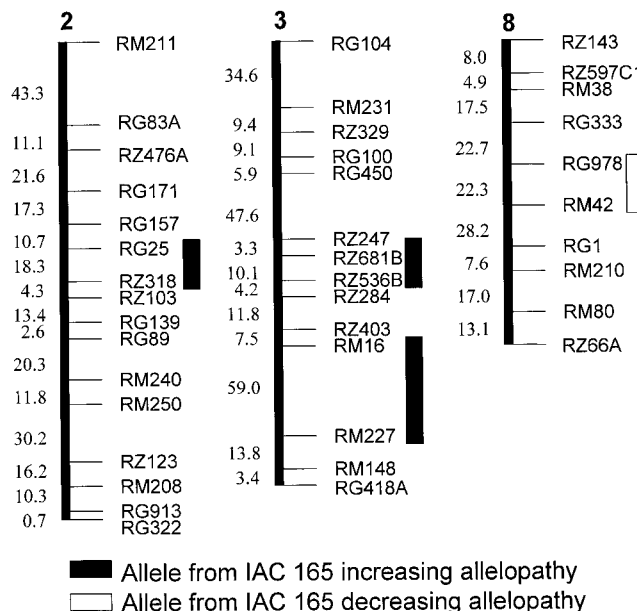
### DISCUSSION

The use of allelopathy has been considered as a potential and environmentally friendly approach for weed control in crop production. However, no intentional breeding efforts have been made to genetically improve allelopathic potential in crops, largely because of our poor knowledge on this phenomenon. In the present study, using the relay seeding technique for phenotyping and DNA markers, we were able, for the first time, to map main-effect and epistatic QTLs associated with the allelopathic activity of rice plants against a common weed species, barnyardgrass. Several important results regarding allelopathy in rice were obtained and merit further discussion.

Our observation that even nonallelopathic varieties such as Aus 196 and CO 39 had certain degrees of allelopathic activity was consistent with the transgressive segregation in the RILs and the QTL allele for increased allelopathy from CO 39. This result indicates that allelopathic potential is widely present in rice germplasm, and is one of the inherent properties of rice plants evolved during the processes of natural and artificial selection. Although the mechanism(s) of the allelopathic activity of rice against barnyardgrass remains largely unknown, the absence of phenotypic correlation between the root mass and allelopathic activity of the lines suggests that the variation in root mass is not the reason for the variation in allelopathic activity.

Our mapping results indicate that allelopathy in rice is a typical quantitative trait involving several loci and possibly some degree of epistasis.

The identified main-effect and epistatic QTLs have relatively large effects, suppressing weed root growth



**Fig. 2. Location of the QTLs controlling allelopathy against barnyardgrass in the IAC 165 × CO 39 recombinant inbred line population (mixed model approach with *P* = 0.005, and main and interaction markers as background genetic variation control).**

**Table 4. Epistatic QTL involved in allelopathy against barnyardgrass in the IAC 165 × CO 39 recombinant inbred line population. Main-effect and epistatic markers were used for background genetic variation control.†**

Chromosome	Interval <i>i</i>	Chromosome	Interval <i>j</i>	LOD	$a_i$	$a_j$	$aa_{ij}$	Prob	$R^2(aa_{ij})$
1	RZ801-RG83B	9	RZ553-RZ206	2.75	-0.57	0.34	2.15	0.0037	6.9

†  $a_i$  = estimate of the additive effect of locus *i*.  $a_j$  = estimate of the additive effect of locus *j*.  $aa_{ij}$  = estimate of additive by additive epistatic effects. Prob = probability of *t*-tests associated with corresponding epistatic effect.  $R^2(aa_{ij})$  = relative contribution of the epistatic effect to the total phenotypic variance.

by more than 1 cm and individually explaining 7 to 12% of the total phenotypic variation. The two main-effect QTLs on Chromosome 3 can be easily manipulated together as one segment in a marker-assisted selection program because they are linked and are acting in the same direction.

The part of the phenotypic variation explained by epistasis is minor in comparison with the part due to main effect. This feature fits with the predictions of classical quantitative genetic models. For a single seed descent population, additive × additive epistasis should theoretically induce a deviation from normality (Pooni et al., 1977), which is not observed in the distribution of the present population. Moreover, the mean of the population does not deviate from the midparental value, which would also indicate additive × additive epistasis (Snape, 1976; Jinks and Pooni, 1981). The limited importance of epistasis should facilitate the use of the main-effect QTLs, which, once confirmed, can be used in marker-assisted selection for genetic improvement of allelopathy in rice.

The issue of the independence of root system size and allelopathic effect was raised, but our results show that the detected regions can be used to improve allelopathy without an apparent pleiotropic effect on the root system.

Several trials have shown that a rice variety that is allelopathic against one weed species is not necessarily allelopathic toward other species (Dilday et al., 1998; Hassan et al., 1998). A limitation of the relay seeding technique is that it measures the inhibition of root growth of a single weed species. To justify a breeding program for allelopathy, donor varieties should be allelopathic against a wide spectrum of weed species. In parallel experiments, we checked whether the relay seeding technique could be used in evaluating allelopathy against several other weed species [jungle rice, *Echinochloa colona* (L.) Link; itchgrass, *Rottboellia cochinchinensis* (Lour.) W.D. Clayton; saromaccagrass, *Ischaemum rugosum* Salisb.; and horse purslane, *Trianthema portulacastrum* L.]. In most cases, the experiments were not successful because of the impossibility to achieve uniform germination of large amounts of weed seed. The only exception was horse purslane. Unfortunately, all tested rice varieties appeared to similarly reduce the root growth of horse purslane (Jensen, unpublished results, 1999). This lack of measurable rice genetic variability indicated that horse purslane could not be used as a testing species.

The relay seeding technique used in the present study has the advantage of separating allelopathy from competition, which is difficult to achieve in the field. However, laboratory assays must be validated in the field

because what ultimately matters is the expression of the allelopathic effect under field conditions (Inderjit and Dakshini, 1995). In a limited experiment, the allelopathic effect of rice varieties measured using the relay seeding technique was well correlated with their weed-suppressing activity under field conditions (Olofsdotter and Navarez, 1996), which is encouraging. However, flood, rainfall, soil texture, and microorganisms (Tongma et al., 1998) could interfere, and little is known about the conditions that allow allelopathy to take on ecological significance. The test of the population under various field conditions remains to be done.

Finally, while the possibility of developing weed-suppressing crops by regulating their capacity to produce specific allelochemicals has great potential, many questions regarding allelopathy in crops remain largely unanswered. For instance, what are the nature and impact of allelochemicals produced by crop plants on the environment and organisms other than specific weed species? What are their effects on the rice crop itself? The phytotoxic effect of decomposing rice residues in the soil on next years crop is already a problem in some areas of the world (Chou, 1998) and autotoxicity is reported to explain the impossibility of growing two successive upland rice crops in some others (E. Guimaraes, personal communication, 1998). Does the increased expression of allelopathic genes have negative effects on grain yield potential or other desirable traits because of the possible rerouting of metabolites to the roots instead of the grain? The identification of QTLs associated with allelopathy against barnyardgrass achieved in this study marked an initial step in answering many of these questions using the genomics approach. Further efforts to produce near-isogenic lines for the QTLs by marker-assisted selection and to test these QTLs in different genetic backgrounds are under way.

#### ACKNOWLEDGMENTS

The authors thank DANIDA for financial support in the conduct of the study.

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