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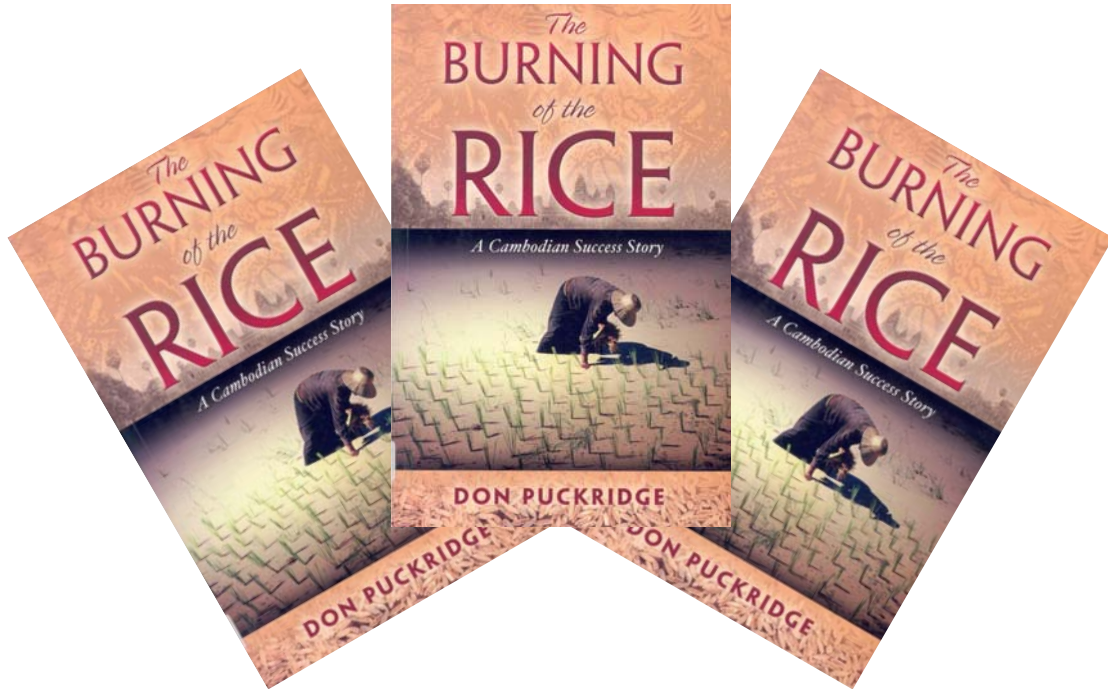
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EDITORIAL

The *Cambodian Journal of Agriculture* is proudly back and, just like before, it will be playing an important role to serve as the forum for all researchers and scientists in Cambodia, and elsewhere, to publish their findings, to share their knowledge and, to communicate more effectively with one another.

In the 21st century, more than ever before, the knowledge of people is expanding rapidly. But yet there is still lack of effective communication sharing that can help us to combat against hunger and to utterly eradicate poverty. It is a worldwide incapability syndrome but it is even more serious in the country like Cambodia where knowledge sharing is still very limited.

For this reason, bringing back the *Cambodian Journal of Agriculture* is especially important for every one to effectively share and disseminate the new knowledge to the development of the country and the social welfare of all. We are fully optimistic that the *Cambodian Journal of Agriculture* will provide significant contributions to promote better linkages, nationally and internationally, in the fields of research and education; and with its contributions we have all the confidences that agricultural productivity in the country will steadily increase. Eventually, hunger will be diminished and poverty will be ultimately eradicated. We therefore urge for your support and contribution.

Contributions of many who responsible to bring back this journal are well acknowledged. Amongst all, the support that the Australian Center for International Agricultural Research (ACIAR) has provided is highly appreciated.

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Editor

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AVAILABILITIES IN CAMBODIA

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អង្គបទសង្ខេប

គ្រោះរាំងស្ងួត គឺជាបញ្ហាដ៏ចម្បងចំពោះផលិតកម្មដំណាំស្រូវក្នុងតំបន់ទំនាបពីទឹកភ្លៀងនៃប្រទេសកម្ពុជា។ ដើម្បីអោយកម្មវិធីបសិដ្ឋកម្មពូជដំណាំប្រកបដោយប្រសិទ្ធភាព កម្មវិធីនោះត្រូវធ្វើការកែសម្រួលច្បាស់លាស់នូវចលករដែលផលិតទៅនឹងគ្រោះរាំងស្ងួតសមស្របជាមួយបរិស្ថានសមស្របដាំដុះ និងបំប្លែងសេណេទិចរបស់វា។ សេរីពិសោធន៍មួយដែលក្នុងនោះបង្កើនបទីកគ្រប់គ្រាន់ និងបង្កើនទឹកជាកូនស្រែមេ ហើយពូជស្រូវប្រពៃណីស្រឡាយកាត់ និងស្រឡាយល្អជាកូនស្រែរង។ សេរីពិសោធន៍នេះត្រូវបានធ្វើជាពីរកន្លែងក្នុងព្រះរាជាណាចក្រកម្ពុជា និងក្នុងរយៈពេលប្រាំឆ្នាំ។ ពូជស្រូវទាំងនេះត្រូវបានវាយតម្លៃសម្រាប់ទិន្នផលគ្រាប់ក្រោមរបបទឹកខុសៗគ្នា និងសម្រាប់ចលករផលិតទៅនឹងភាពរាំងស្ងួតដែលក្នុងនោះមានរយៈពេលលូតលាស់ ការពន្យារពេលចេញផ្កា និងសក្តានុពលទិន្នផលគ្រាប់។

បច្ចេកទេសដែលបានប្រើប្រាស់សម្រាប់បង្កើតនូវភាពរាំងស្ងួតលទ្ធផលនេះគឺ បានទទួលនូវជោគជ័យ ដោយក្នុងនោះអាំងតង់ស៊ីតេរាំងស្ងួតប្រែប្រួលពី ១២ភាគរយ ទៅ ៤៦ភាគរយ ហើយដែលស្រដៀងគ្នាទៅនឹងតំបន់និយមរាំងស្ងួតក្នុងព្រះរាជាណាចក្រកម្ពុជា។ ភាពរាំងស្ងួតដែលបង្កើតឡើងជាទូទៅបានប្រជុំនៅក្នុងគ្រប់ប្រទេស ហើយនោះក៏ដូចគ្នាទៅនឹងតំបន់និយមរាំងស្ងួតដែរ។ អន្តរអំពើរាំងពូជ និងរបបទឹកមានឥទ្ធិពលជាអត្ថន័យ។ សមាសធាតុបំប្លែងនៃអន្តរអំពើរាំងពូជ និងបរិស្ថានសម្រាប់ទិន្នផលគ្រាប់គឺមានទំហំពីរដងកន្លះ ($\sigma^2_{G \times E} / \sigma^2_g = 2.5$) នៃសមាសធាតុពូជតែជាទូទៅទំហំនេះមានកំរិតតូច បើប្រៀបធៀបទៅនឹងលទ្ធផលការសិក្សាក្នុងប្រទេសជិតខាង។ ក្នុងករណីភាពរាំងស្ងួតមានកំរិតមធ្យម (រហូតដល់អាំងតង់ស៊ីតេរាំងស្ងួតដល់ ៣០ភាគរយ នៃការធ្លាក់ចុះទិន្នផល) ភាពឆាប់ចេញផ្កា និងសក្តានុពលទិន្នផលត្រូវបានរកឃើញជាយន្តការដ៏ល្អ។ សក្តានុពល

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ABSTRACT

Drought is a major problem for rice production in the rainfed lowlands of Cambodia. In order to be effective breeding program the appropriate drought tolerance traits for the environments and the genotypic variation for them need to be identified on a routine and reliable basis. A series of experiments with main plots designated as “well-watered” and “managed drought”, and with a range of genotypes including local collections, exotic materials and advanced breeding genotypes were conducted at two sites in Cambodia for five years. The genotypes were evaluated for their yield performance under the different water conditions and for the drought adaptive traits of phenology, flower delay (under drought) and yield potential.

A technique to routinely provide a managed drought environment in Cambodia was developed where the drought intensity ranged from 12- 46 %, which is similar to that in the target populations of environments. The managed drought occurred mainly as a reproductive drought, again consistent with the target environments. There were significant interactions for yield among the genotypes grown under the well-watered and drought conditions. The genotype-by-environment (G x E) interaction component of variance for grain yield was 2.5 times of the genotype component ($\sigma^2_{G \times E} / \sigma^2_g = 2.5$). However, this fraction is relatively small compared to the results obtained from the neighbouring countries.

Early flowering was found to be a good mechanism to escape drought and yield potential contributed to performance under medium drought conditions (up to a drought intensity

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of 30% yield reduction). High yield potential was found mostly in the intermediate flowering genotypes. Three genotypes; IR66327-KKN-8-P1-3R-0, IR66327-KKN-25-P1-3R-0 and IR66327-KKN-54-P1-3R-0 were found to have high yield potential and to be well adapted across all experimental conditions.

It was found that the maximum rate of yield reduction with reducing free water level in the field during flowering time was 1.68 % reduction every centimetre of the declined water level. This information can be employed to estimate potential yield for assistance in selecting tolerant genotypes to drought. Based on these findings, a systemic approach for selection for drought tolerance in rainfed lowland rice in Cambodia is discussed.

KEYWORDS: Rainfed lowland; Yield potential; Pattern analysis; Water availability.

1. INTRODUCTION

A large portion of the world's poor farm in rainfed systems where the water supply is unpredictable and droughts are common. For example, in Thailand about 50 % of all rice land is rainfed and yield losses due to drought are estimated between 11 and 58 % (Jongdee et al., 1997). In contrast to the irrigated rice system, gains from crop improvement of rainfed rice have been modest, in part because there has been little effort to breed and select for drought tolerance in these target environments.

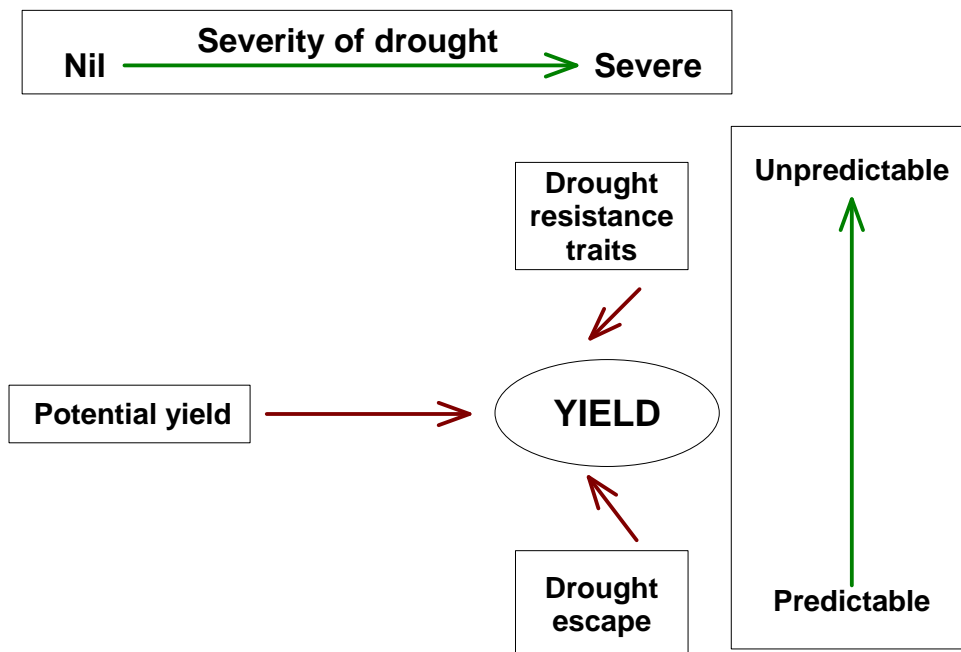


Figure 1. Schematic presentation of medium levels of drought stress in which the potential yield is reduced (from Fukai and Cooper, 2001)

A number of studies have examined the complex processes, mechanisms and traits of rice that provide drought tolerance and better adaptation to these variable rainfall environments. While many putative and specific traits for drought tolerance such as osmotic regulation (Cutler et al., 1980, Yang et al., 1983; Hsiao et al., 1984; Lilley and Ludlow, 1996; Jongdee et al., 1998), root length and root penetration (O'Toole and Chang, 1979; Yoshida and Hesegawa, 1982; Ekanayake et al., 1985; Lilley and Fukai, 1994) have been suggested for selection for drought tolerance, Fukai and Cooper (2001) have summarized this complexity and emphasized three broad mechanisms i.e. yield potential, appropriate phenology and drought tolerance, that influence yield depending on the severity and predictability of the drought. The relationship among these three components in different types of drought is shown in Figure 1. The figure shows that yield potential is an important mechanism for yield when drought stress varies from mild to moderate (where yield is reduced less than 40%), but at more severe stress, a mechanism for drought escape or tolerance is required. Thus, Fukai et al. (1999) indicated that only phenology, high potential yield and ability to maintain high leaf water potential were associated directly with higher GY in their rainfed lowland rice studies in target drought environments in Thailand.

Plant breeders rely on direct selection for grain yield (GY) in the target environments as the main criterion for selection. There is growing evidence in other crops (e.g. pearl millet, Bidinger et al. 1987a,b) that varieties can be developed for improved yield under drought stress yet respond to well watered conditions (i.e. the good years) if there is early selection for yield under both drought and well-watered conditions. However, progress could be enhanced by the judicious choice of parents that provide the genetic materials for the three components of yield under drought viz. yield potential, appropriate phenology and drought tolerance. In practice, rainfed lowland rice breeders often use parents from exotic material for improved yield potential and phenology and local materials to maintain or enhance drought tolerance.

A major factor determining the rainfed lowland environment in relation to drought is rainwater. Water availability in the lowland under rainfed conditions depends on the amount and distribution of rainfall throughout its growing season. Particularly, ample standing water in the field during flowering time is crucial for avoiding yield reduction (Jearakongman et al., 1995). Previous studies on genotype by environment (GxE) interactions for rice yield in Laos and Thailand have

shown genotypic and phenotypic variations in yield response to different water environments (e.g., Inthapanya et al., 2000; Pantuwan et al., 2002). Yield reduction generally increases as delay in flowering time increases due to disappearance of standing water.

There is no information on the variation of grain yield under diverse water conditions among the rice materials used in the breeding program in Cambodia. This study aimed to measure the genotypic variation of rainfed lowland rice genotypes, and their interactions with water conditions at two sites in Cambodia over five years. Moreover, quantifying yield reduction in terms of water availability at flowering time may be helpful to identify drought tolerant genotypes, so relationships between yield reduction and water level in the field during flowering time were investigated.

2. MATERIALS AND METHODS

2.1 Rice genotypes used

Fifteen genotypes (Table 1), which were selected from an

initial trial using eighty genotypes at Prey Veng in 1998, were used to analyse genotypic variance for day to flowering DTF and GY at the Cambodian Agricultural Research and Development Institute (CARDI) and Prey Veng for five years. The 80 genotypes were a mixture of random selections from released varieties, advanced breeding lines (including some from outside Cambodia) and traditional varieties used in the Cambodian breeding program. The set of 15 genotypes represented the range in performance under drought (GY from 1.2 to 1.7 t/ha) and maturity (from 101 to 133 days) among the 80 genotypes tested in 1998 (data not shown). Of the 15 genotypes, seven are Cambodian varieties, one is a Cambodian breeding line and seven are Thai-ACIAR breeding lines and all will be further called as genotype. Base on previous experiment, three of the genotypes are photoperiod insensitive (photoperiod sensitivity index, $PSI \leq 0.3$), four are mildly photoperiod sensitive ($0.3 < PSI \leq 0.7$) and the others are strongly photoperiod sensitive ($PSI > 0.7$). Somaly and Phka Rumchang are aromatic rice varieties.

Table 1. Genotypic information, including the source and photoperiod sensitivity index (PSI), and environment information, including the tested sites and in what year, seeding date (SD), water treatment and site-year-water treatment code.

WW = well-watered treatment, WS = water-stressed treatment.

No.	Genotype	Source	PSI	Site	Year	SD	Water	Code
1	IR46331-PMI-32-2-1-1(P1) (38)	Thai-ACIAR	0.45	Prey Veng	1998	11-Jul	WW	PV98WW
2	IR57514-PMI-5-B-1-2 (40)	Thai-ACIAR	0.24	Prey Veng	1998	11-Jul	WS	PV98WS
3	IR66327-KKN-10-P1-3R-0 (11)	Thai-ACIAR	0.55	Prey Veng	1999	5-Jul	WW	PV99WW
4	IR66327-KKN-25-P1-3R-0 (5)	Thai-ACIAR	0.24	Prey Veng	1999	5-Jul	WS	PV99WS
5	IR66327-KKN-54-P1-3R-0 (16)	Thai-ACIAR	0.53	Prey Veng	2000	18-Jul	WW	PV00WW
6	IR66327-KKN-8-P1-3R-0 (10)	Thai-ACIAR	0.48	Prey Veng	2000	18-Jul	WS	PV00WS
7	IR66368-CPA-84-P1-3R-0 (26)	Thai-ACIAR	0.76	Prey Veng	2001	4-Jun	WW	PV01WW
8	Bang Kuy (acc. 2865) (56)	Cambodia	0.71	Prey Veng	2001	4-Jun	WS	PV01WS
9	CAR4 (77)	Cambodia	0.73	Prey Veng	2002	4-Jun	WW	PV02WW
10	CAR6 (79)	Cambodia	0.73	Prey Veng	2002	4-Jun	WS	PV02WS
11	CIR158-B-B-SB-8-3-2 (62)	Cambodia	0.30	CARDI	1999	2-Jul	WW	CA99WW
12	Khpor Daung (45)	Cambodia	0.81	CARDI	1999	2-Jul	WS	CA99WS
13	Somaly (50)	Cambodia	0.77	CARDI	2000	31-Jul	WW	CA00WW
14	Phka Rumchang (51)	Cambodia	0.77	CARDI	2000	31-Jul	WS	CA00WS
15	CAR3 (76)	Cambodia	0.73	CARDI	2001	4-Jun	WW	CA01WW
				CARDI	2001	4-Jun	WS	CA01WS
				CARDI	2002	13-Aug	WW	CA02WW
				CARDI	2002	13-Aug	WS	CA02WS

Numbers within brackets indicate the genotype numbers used in initial trial for 80 genotypes in 1998. ACIAR = Australian Centre for International Agricultural Research

2.2 Locations

The field experiments were conducted at CARDI (Cambodian Agricultural Research and Development Institute; latitude 11°28'36"N, longitude 104°48'27"E) for four years (1999–2002) and at Prey Veng province (latitude 11°33'40"N, longitude 105°32'42"E) for five years (1998–2002). Soils at both sites are of the Prateah Lang soil type (Plinthustalfs in USDA classification and Acrisols or Luvisols or Planosols in FAO classification) that covers 30% of the total rice growing areas (White et al., 1997).

2.3 Water treatments

The experiment at each site-year combination consisted of two water treatments; flooded, well-watered (WW) and water stressed (WS) (drained fields to develop drought conditions), which were located in adjacent paddies separated by a bund. The WW treatment relied on rainfall, which was in some experiments supplemented with irrigation to provide non-stress conditions. In general, the standing water depth in the WW treatment fluctuated from 0 to 15cm at Prey Veng and 0 to 20cm at CARDI, although in some periods it was not possible to maintain standing water at all times. In the

WS treatment, small canals (10cm in depth) were dug throughout the fields to collect water into a well 50cm deep, dug in the corner of the fields. The water was pumped from the field during the drained period. The field was drained from 1-3 weeks before 50% flowering of the earliest maturity genotype to maturity for 1998-2000 experiments and from 2 weeks after transplanting to maturity for 2001-2002 experiments to simulate a wide range of drought conditions.

2.4 Crop management

The seeding date (Table 1) varied between early-June and mid-August, depending on the beginning of the wet season at the location. The beginning of the wet season is the normal planting time in the target environments. Thirty days old seedlings were transplanted by hand with two to three seedlings per hill with 20 cm spacing between hills and between rows. After 10 days, missing hills were again re-planted. Irrigation was applied at transplanting to maintain an above-ground water level of 3-5 cm in all treatments and was continued until harvest in the WW treatment and until the time of draining water in the WS treatment.

The fields were fertilized with 60-30-30 kg/ha of N-P₂O₅-K₂O. N was applied as urea (46% N) three times (1/3 at transplanting as a basal, at 30 days and at 60 days after transplanting), while the P₂O₅ as triple super phosphate (48% P) and K₂O as potassium chloride (60% K) was applied once as a basal application. Weeds were controlled three and four times by hand in the flooded and drained fields, respectively. The fourth weeding in the drained field was 10 days after draining the water. No pesticide was used.

2.5 Experimental design

The experiments at each location and year were arranged in three randomized complete blocks within the two water treatments. For the analysis of variance, the water-treatments were treated as main-plots, with replicates within main-plots, and genotypes as sub-plots. The sub-plots were 1.2 × 3 m in size (six rows) with no extra row spacing between them. The main-plots (water treatment) were in separate but adjacent fields separated by a bund.

2.6 Measurements

The days to flower (DTF) were measured as the time taken for 50% of the plants in the two center rows of each plot to flower. At maturity plants from an area of 1.04 m² were harvested at ground level from the two center rows leaving one hill at each end of the row as a border. The harvested plants were sun dried for several days and the grain threshed and then weighed. Sub-samples of 150 g were taken and dried in an oven at 70 °C for 2 days. The oven-dried samples were again reweighed to determine the water content to calculate grain yield (GY) on a dry-weight basis. The level of the water table was recorded, weekly, using PVC tubes placed in each corner of the main water treatments plots.

2.7 Photoperiod sensitivity index (PSI) calculation

The PSI was determined, using the procedure described by Immark *et al.* (1997) as follow:

$$PSI = 1 - (F2 - F1) / (SD2 - SD1) \quad [1]$$

Where: F1 = date of 50% flowering of the first seeding, F2 = date of 50% flowering of the second seeding (30 days apart), SD1 = date of the first seeding, and SD2 = date of the second seeding. Genotypes with a PSI = 0 are completely insensitive, whereas those with a PSI = 1 are strongly sensitive.

2.8 Relative water level (WL_{REL})

The relative water level (WL_{REL}) was determined as the difference in paddy water level (WL) between the WW and WS treatments and used as an index of water-deficit to explain the yield reduction (YR) due to drought. The WL_{REL} was calculated based on four water conditions of the WW and WS treatments, as follows:

- (a) When WL of both water treatments was above the soil surface, there was no water deficit, and hence the two water treatments had the same water availability. Thus,

$$WL_{REL} = 0 \quad \text{when } WL_{WW} \geq 0, WL_{WS} \geq 0 \quad [2a]$$

where WL_{WW} and WL_{WS} are free water level for the WW and WS treatments.

- (b) The case of WL_{WW} ≥ 0 with WL_{WS} < 0 occurred frequently after water was drained in the WS field. WL_{REL} for these water conditions was calculated as:

$$WL_{REL} = 0 \quad \text{when } WL_{WW} \geq 0, WL_{WS} \geq 0 \quad [2b]$$

- (c) The case of WL_{WW} < 0 with WL_{WS} ≥ 0 was rare; the occurrence was only a few days during the flowering periods in a few environments. The effect on the determination of WL_{REL} was, therefore, minimal. WL_{REL} for these water conditions was calculated as:

$$WL_{REL} = -WL_{WW} \quad \text{when } WL_{WW} < 0, WL_{WS} \geq 0 \quad [2c]$$

- (d) When WL of both water conditions was below the soil surface, the difference in level between the two water treatments was taken as WL_{REL}. Thus,

$$WL_{REL} = WL_{WS} - WL_{WW} \quad \text{when } WL_{WW} < 0, WL_{WS} < 0 \quad [2d]$$

Since WL was measured weekly, WL between measurement days was estimated by interpolation between the two consecutive measurements. WL_{REL} was averaged over six weeks around flowering (three weeks before and after flowering date) of a genotype or around mean flowering date of an experiment as water deficit during this six week period was found to best correlate with yield reduction due to water stress.

2.9 Data analysis

An analysis of variance (ANOVA) was conducted on the data of DTF and GY using a residual maximum likelihood (REML) program (Robinson *et al.*, 1982). It was assumed that the genotypes represented a random sample of rainfed lowland rice genotypes and that the sites and years were a random sample of those used by the breeding program. The water treatment was a fixed variable. Therefore, treatment means for all random variables were computed as Best Linear Unbiased Predictors (BLUPs). REML software (Robinson *et al.*, 1982) was used to calculate the BLUPs. Two main analyses were performed for DTF and GY. The first was a combined analysis over both water treatments to detect the effects of water, and genotype and their interaction effects at each site. In the second analysis, a combined analysis over sites or years was performed to detect the effects of site or year, water and genotype and their interaction effects.

Also two-way cluster analysis of GY and DTF for the environments and the genotypes was conducted. The cluster analysis for DTF was used for the analysis of the relationships between yield reduction and water level.

3. RESULTS

3.1 The water environments and types of drought

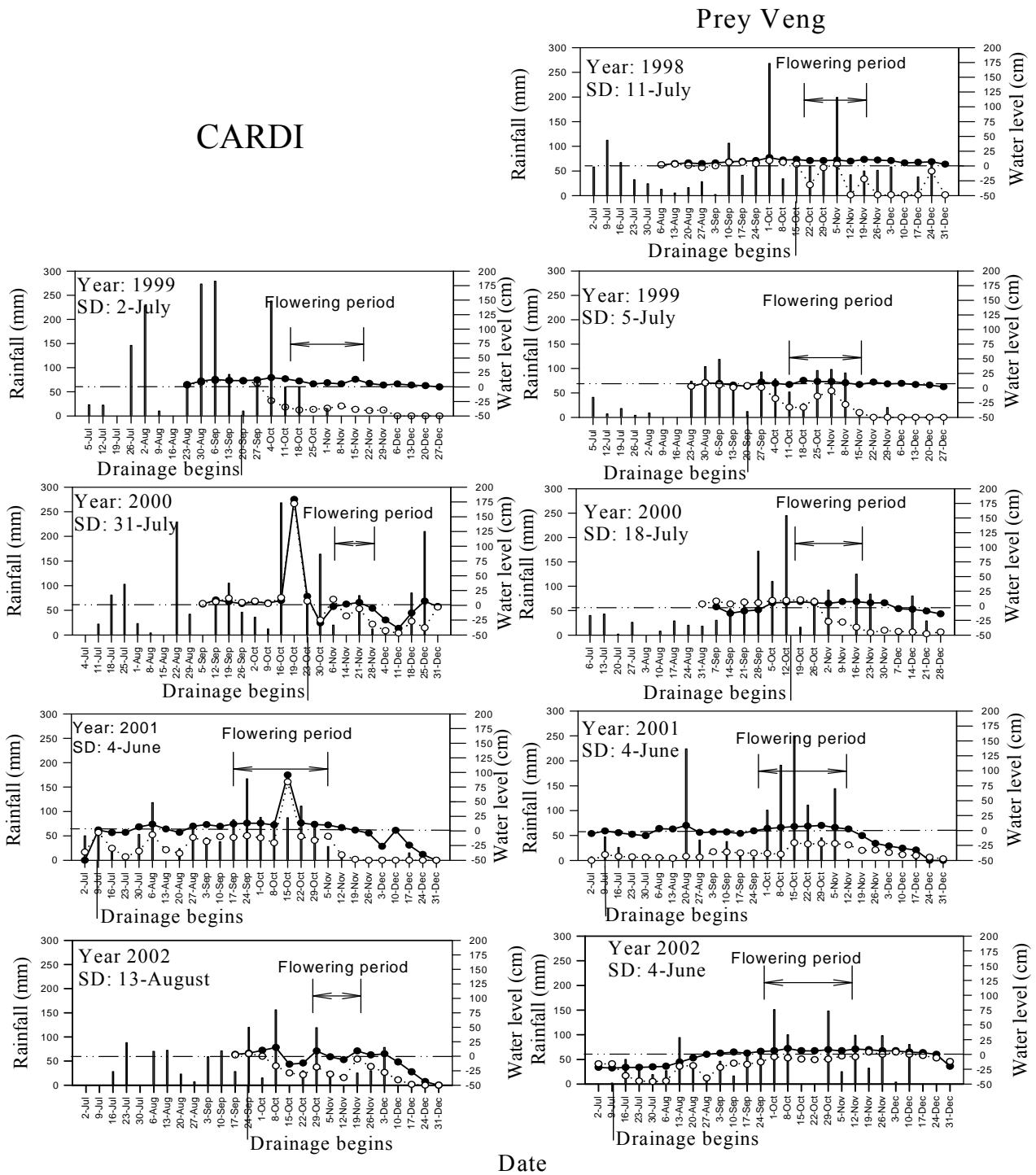


Figure 2. Rainfall (bars) and water levels under the well-watered (solid lines) and the water-stress (dotted lines) treatments at CARDI and Prey Veng from 1998-2002. Dash-dot-dotted lines refer to the ground level within an experiment.

The weekly rainfall and water levels under the WW and the WS treatments in all sites for experiment 1 and 2 are shown in Figure 2. The draining technique was successful in providing drought conditions. The WS treatment reduced yield by as much as 46% in 1999 at the Prey Veng site and by 20% on average over all trials (Table 2). The range of drought conditions can be described as from “severe, pro-

longed drought from booting stage” at Prey Veng in 1999 and “mild, short drought during the grain filling” at CARDI 2000. These droughts are considered to be representative of the target environments of the rainfed lowlands in Cambodia.

Table 2. Mean grain yield (MGY-t/ha) at Prey Veng (PV) and CARDI (CA) under the well-watered (WW) and water-stress (WS) treatments, relative grain yield reduction (GYR), severity of drought (SoD) and period of drought (PoD).

	PV1998		PV1999		PV2000		PV2001		PV2002		CA1999		CA2000		CA2001		CA2002	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
MGY	1.93	1.52	1.98	1.08	1.49	1.17	2.46	1.71	2.21	1.77	2.72	2.07	2.42	2.13	2.63	1.47	2.15	1.47
GYR (%)	21		46		21		30		20		24		12		44		32	
SoD	Mild		Severe		Mild		Moderate		Mild		Mild		Mild		Severe		Moderate	
PoD	Grain filling		From booting stage		From flowering stage		From vegetative stage		at vegetative stage		From booting stage		Short grain filling		From vegetative stage		From vegetative stage	

3.2. Genotypic variation in days-to-flower (DTF) and grain yield (GY)

The combined analysis for 15 genotypes tested at CARDI and Prey Veng in five years shows significant effects of year (Y), water (W), genotype (G) and G × Y, G × site (S) × Y, and G × W × S × Y interactions on DTF (Table 3). The size of the variance component of the G × E interaction ($\sigma^2_{G \times E}$) was about half to that of the genotype component (σ^2_g).

There were significant effects of W, G and G × W × S × Y interaction on GY (Table 3). The G × W component of variance was about half of that for the genotype component but large relative to the other interaction components. The size of the variance component of the G × E interaction ($\sigma^2_{G \times E}$) was 2.5 times to that of the genotype component (σ^2_g).

Heritability (based on genotype means) for grain yield was

estimated for 18 experiments conducted with WW and WS treatments at two sites (Table 4). In the WW treatment, the heritability for GY ranged from moderate 0.42 at Prey Veng 2002 to 0.95 at CARDI 2001. In the WS treatment, the genotype mean heritability also ranged from moderate 0.11 at Prey Veng 1998 to 0.89 at Prey Veng 2000.

All the phenotypic correlations between the mean GY of the 15 genotypes grown under the WS and WW treatments was highly significant (Table 4). The correlation tended to be higher in mild drought conditions (PV98, PV00 and CA99) and lower in moderate (PV01, CA02) and severe (PV99, CA01) drought as would be expected. When drought occurred at very early vegetative stage at Prey Veng in 2002, the early maturing group was affected while the mid and late maturing groups produced reasonably good yields as in WW conditions, resulted in negative correlation.

Table 3. Components of variance ± standard errors ($\sigma^2 \pm SE$), level of significance based on F-test of mean squares from ANOVA and the ratio of genotype-by-environment interaction and genotype component of variance ($\sigma^2_{G \times E} / \sigma^2_g$)¹ for grain yield for 15 genotypes grown under two water treatments at two sites (CARDI and Prey Veng) in five years (1998-2002).

Source of variation	Degree of freedom	Days-to-flower	Grain yield
Year (Y)	4	75.3±75.0**	0.00±0.10ns
Site (S)	1	0.00±85.0ns	0.05±0.13ns
Water (W)	1	2.76±7.86**	0.18±0.27*
Genotype (G)	14	101±40.8**	0.04±0.03*
G × W	14	3.04±1.93ns	0.02±0.01ns
G × S	14	0.00±3.17ns	0.01±0.01ns
G × Y	52	6.23±5.81*	0.01±0.01ns
G × W × S	14	0.64±1.15ns	0.00±0.01ns
G × W × Y	52	1.79±1.72ns	0.00±0.01ns
G × S × Y	44	25.4±6.49**	0.00±0.01ns
G × W × S × Y	44	7.61±2.00**	0.06±0.02**
Error	532	5.39±0.33	0.12±0.01
Heritability (h^2)		0.92	0.65
$\sigma^2_{G \times E} / \sigma^2_g$		0.45	2.5

** = significant at $P < 0.01$, * = significant at $P < 0.05$, ns = not significant.

¹ The G×E interaction component of variance was estimated as the sum of the G×W, G×S, G×Y, G×W×S, G×W×Y, G×S×Y, and G×W×S×Y components for the analysis of two sites in five years.

Table 4. The genotype mean heritability under the water stress (WS) and well-watered (WW) treatments and the phenotypic correlation coefficients between the WS and WW treatments for grain yield of 15 rice genotypes grown at CARDI (CA) for four years and at Prey Veng (PV) for five years.

	CA99	CA00	CA01	CA02	PV98	PV99	PV00	PV01	PV02
Heritability									
Well-watered	0.60	0.62	0.95	0.68	0.82	0.80	0.93	0.71	0.42
Water stress	0.73	0.68	0.73	0.75	0.11	0.84	0.89	0.85	0.32
Phenotypic correlation	0.84	0.62	0.35	0.56	0.85	0.76	0.88	0.65	-0.48

The two-way cluster analysis of the genotypes for GY was truncated at the four-group level of genotypes (GG = genotype group) (Fig. 3) and at the three-group level of environments (EG = environmental group = water-site-year experiment) (Fig. 4) and this retained 76% of the G x E interaction variation for the standardized GY. Genotype Group GG1 was comprised of three genotypes-IR66327-KKN-8, IR66327-KKN-25 and IR66327-KKN-54-with the highest mean GY of 2.23 t/ha. The GG2 comprised of three genotypes and had a mean GY of 1.91 t/ha. The GG3 comprised of four genotypes with a mean GY of 1.88 t/ha. The GG4 comprised of five genotypes and had the lowest mean GY of 1.80 t/ha.

EG1 was comprised of five water-site-year experiments and dominated by WW treatment (four of the five). The EG2 comprised of eight water-site-year experiments and dominated by the WS treatment (five of the eight) mainly from booting stage (four of the five). The EG3 comprised of five water-site-year environments (three were WS treatments and two were WW treatments)

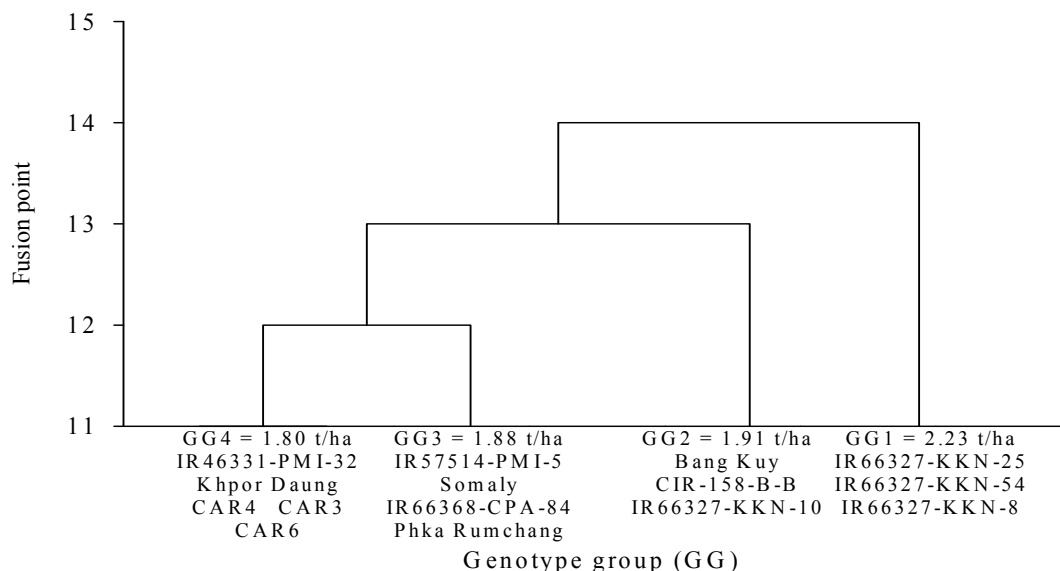


Figure 3. Dendrogram, truncated at the four-group level, for the hierarchical clustering of 15 rice genotypes, based on the matrix of standardized grain yield (GY) best linear unbiased predictors (BLUPs) for 18 water-site-year environments in Cambodia. The mean GY values are shown for each group.

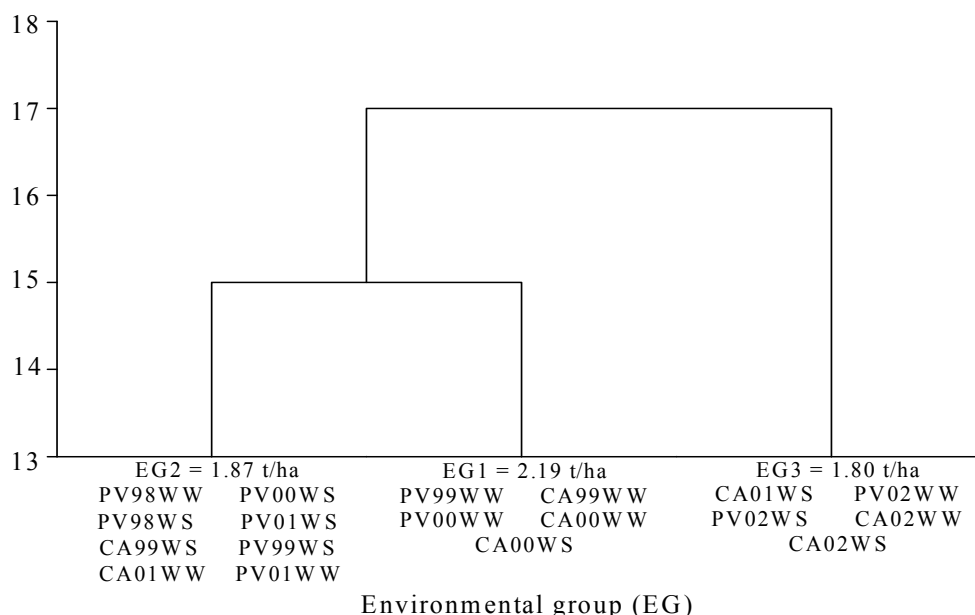


Figure 4. Dendrogram, truncated at the three-group level, for hierarchical clustering of 18 water-site-year environments, based on the matrix of standardised estimated grain yield (GY) best linear unbiased predictors (BLUPs) for the 15 genotypes tested at Prey Veng and at CARDI from 1998 to 2002 in Cambodia.

The grain yield performance plot for four groups of genotypes over the three groups of environments indicated that genotype groups showed remarkable changes in relative grain yield (Figure 5). For example, the GG2 was poorest in EG3, but in EG1 it was better than GG4 and GG3. In EG2, GG3 was better than GG4 but its reverse in EG1. Genotype Group GG1 performed consistently better in all environmental groups.

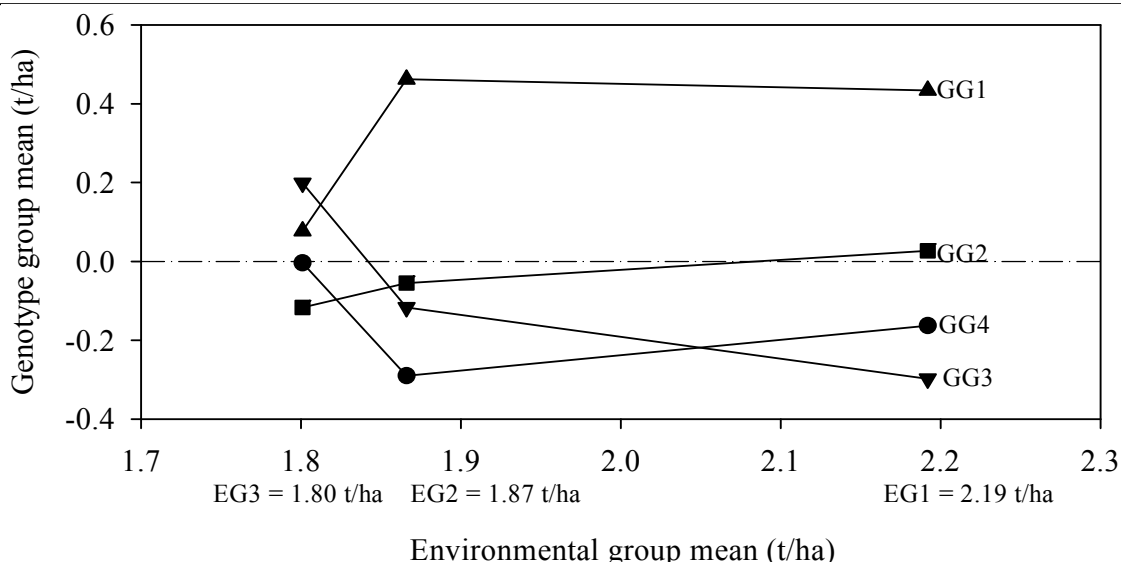


Figure 5. Performance plot for four groups of genotypes and three groups of environments identified by two-way cluster analysis of the matrix of grain yield for 15 rice genotypes tested at 18 water-site-year environments (1998-2002).

3.5 Relationship between grain yield (GY) and days-to-flower (DTF)

There was no significant correlation between the mean DTF across all environments and the mean GY (Figure 6a). However, there was a significant non-linear relationship between DTF and GY in two of the three environmental groups. In the EG1, which was dominated by the WW treatment, there was a significant quadratic relationship (Figure 6b; $R^2 = 0.32^*$) with the intermediate flowering groups (GG1 and

GG2) having the highest GY, followed by the late flowering group (GG4). The early genotypes (GG3) had the lowest GY. There was large variation in GY among the genotypes with intermediate flowering date. In the EG2, where GY was dominated by the WS treatment, the early and intermediate flowering groups had similar GY and the late flowering group had the lowest GY (Figure 6c; $R^2 = 0.28^*$). In the EG3, there was no significant relationship between DTF and GY (Figure 6d).

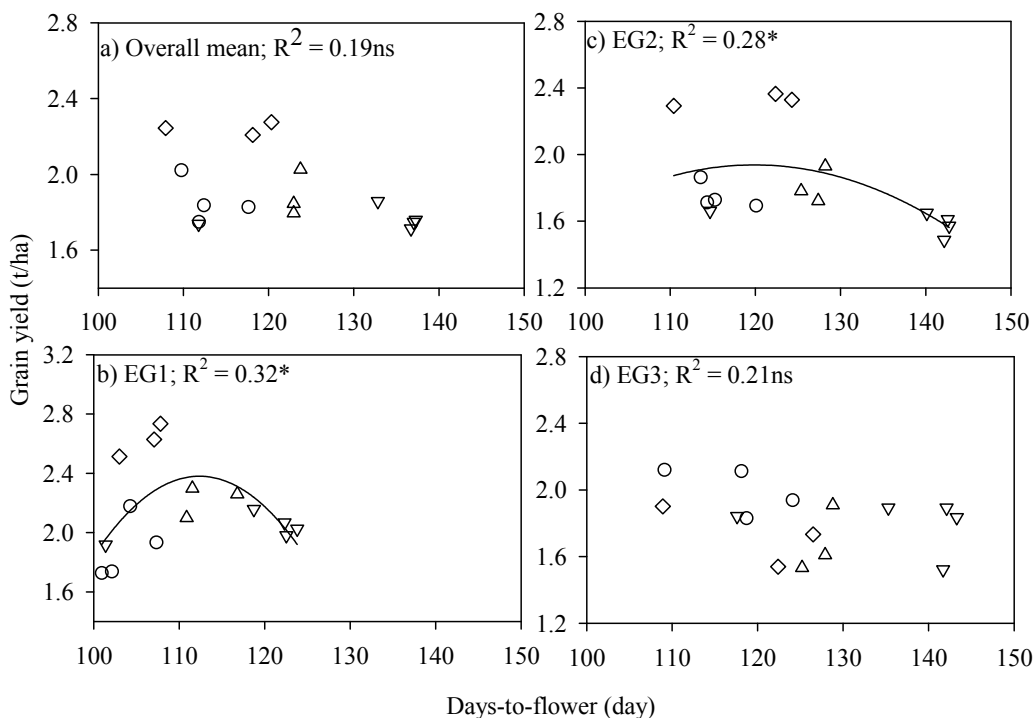


Figure 6. Relationship between days-to-flower and grain yield for 15 rice genotypes for overall mean (a) and three environmental groups (EG1, EG2 and EG3) identified by two-way cluster analysis. \diamond = GG1, Δ = GG2, \circ = GG3 and ∇ = GG4.

3.6 Relationships between yield reduction and relative water level (WL_{REL})

The two-way cluster analysis of the genotype for DTF was truncated at the three-group level of genotypes (early, intermediate and late maturity groups, data not shown). Figure 7 presents plots of yield reduction (%) against WL_{REL} (cm) for the nine drought environments (four years at CARDI and five years at Prey Veng), showing a positive moderate relationship (solid line, $R^2 = 0.28$). Based on the field observations and inferences, 11 data points that had low yield reduction with high WL_{REL} [solid symbols (CARDI 1999 - early, medium and late, CARDI 2001 - late, CARDI 2002 - late, Prey Veng 1998 - late, Prey Veng 2000 - early, intermediate and late, Prey Veng 2001 - early and intermediate)] were eliminated from the other data points (opened symbols) to determine maximum rate of yield reduction to WL_{REL} . These could be explained in connection with the distribution of rainfall during seasons or lateral movement of water from

adjacent field. At CARDI in 2001, the crop was completely submerged by a flash flood from 13-16 October with maximum water depth of 95 cm. This period fell on ± 1 week of mean flowering date for the early maturity group (7 October for the control and 11 October for the WS treatment) so as to affect genotypes that flowered at that time. Thus, the early maturity group had high yield reduction despite low WL_{REL} during flowering time, compared with the other maturity groups. Therefore, this outlying data point was also eliminated in the present analysis. Excluding these 12 data points, one linear relationship for all groups was calculated with zero intercept since there was no effect of maturity on the relationship between yield reduction and water level. By eliminating these extreme data points, thus, the result illustrates that there was a strong and positive relationship between yield reduction and WL_{REL} with every centimetre of declined WL_{REL} increases 1.68 % yield reduction.

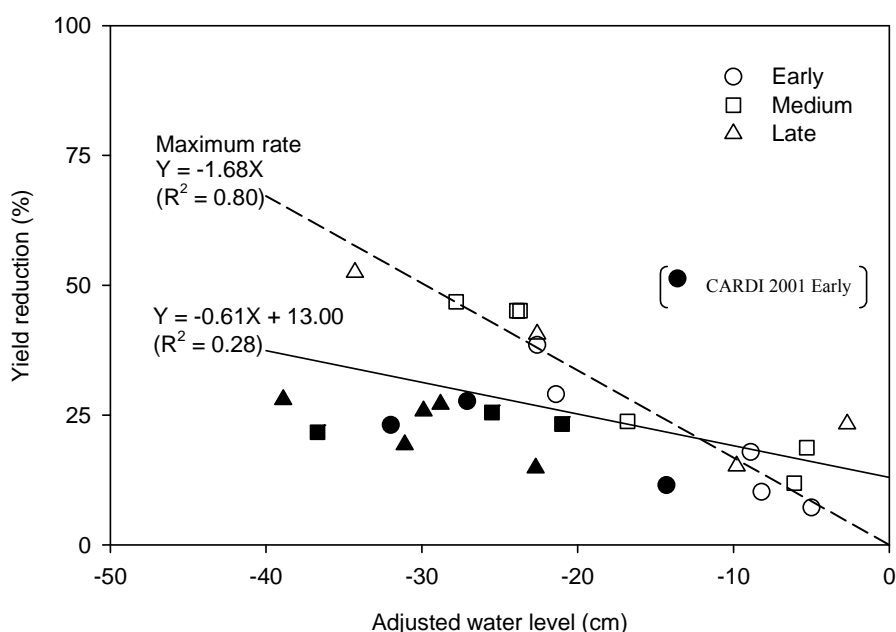


Figure 7. Relationship between grain yield reduction and relative water level around flowering.

4. DISCUSSION

A major issue for rainfed lowland rice plant breeding programs is the identification and manipulation of environments for drought screening that are representative of the target population of environments of the farmers (Fukai and Cooper, 1995). While the historical rainfall pattern may be used to explain yearly variation in GY, the water level in the paddies is a more direct measure of water availability to the current rice crop (Jearakongman et al., 1995). In this study, the establishment of drought environments by draining paddies at different crop growth stages was successful in providing different types of drought environments for screening genotypes. Although the types of drought varied in timing with crop development from prolonged drought for the vegetative stage to a short duration in the grain filling stage, and in intensity from mild to severe drought, the actual stress mainly developed late in the season and as such represents the target environments. The drought effect ranged from a 12 to 46% reduction in GY (only one site was 12%, the others were all greater than 20%). In similar environments in Thai-

land, Wonprasaid et al. (1996) obtained a 40% reduction in GY using the method of draining the paddies about one month before flowering, while Pantuwan et al. (2002) obtained reductions from 19% to 80%. The values obtained from manipulation of the water in our experiments are in keeping with the estimated losses in the farmer fields in Cambodia.

There were marked changes in GYs of genotype groups across environmental groups. In Environmental Group EG1 where there was no shortage of water, the early flowering and photoperiod sensitive GG3 yielded less than the late flowering GG4. This is similar to the finding by Pantuwan et al. (2002) where the early flowering lines tended to have lower yield potential than the late flowering lines. In contrast, in EG2, which was dominated by the WS treatment, late flowering genotypes (GG4) were exposed to the drought for a longer period than the early flowering genotypes (GG3), resulting in a lower GY. Again this finding is similar to that of Jearakongman et al. (1995); Wonprasaid et al. (1996); Cooper and Somrith (1997); Rajatasereekul et al.

(1997); Fukai *et al.* (1999) and Pantuwan *et al.* (2002). These data suggest the disadvantage of late flowering genotypes under prolonged drought, and of early flowering, photoperiod sensitive genotypes, under favourable water conditions. However, one group of genotypes (GG1), made up of three high yielding breeding lines derived from a single cross between IR46331-PMI-32-2-1-1/IR53466-B-118-B-B-20 from the Thai-ACIAR project, had superior GY at most of the sites. Thus, it is possible to select for high yielding genotypes for both well-watered and drought conditions. This finding is similar to that of Rajatasereekul *et al.* (1997).

With respect to the yield reduction-relative water level relationship, water level in the WS treatment fields was designed so soil water conditions were unusual throughout the seasons or after the late vegetative stages, and therefore great yield reduction might be expected. However, in some cases (the 11 excluded data which show less yield reduction despite higher WL_{REL} in Figure 6), rainfall could relieve this severe water stress from the crops because of reduced water stress in the topsoil. In particular, sufficient rainfall during flowering time could result in decreased yield reduction. This indicates that good distribution of rainfall during flowering time is crucial for decreasing yield reduction when standing water in the field disappears. Moreover, the yield reduction-water level relationship established in this study might result from effects of other constraints (e.g., infertility/nutrients stress) on yield reduction with the increased water stress. For example, Inthapanya *et al.* (2000) reported a significant genotype by fertiliser interaction for yield of rainfed lowland rice in Laos. Therefore, to understand the large genotype by environment interaction, the environment factor needs to be broken down into two sub-factors: water availability and soil fertility (Fukai *et al.*, 1999).

5. CONCLUSIONS

The establishment of drought conditions by draining the rainfed lowland paddies can allow plant breeders to select for drought tolerant genotypes directly in the conditions representative of the target environments. There was a $G \times W \times S \times Y$ interaction component of variance for grain yield and this interaction was largely explained by water conditions during the cropping season, rather than by specific year and site factors. Therefore, the selection for drought tolerant genotypes must be conducted under the appropriate target drought conditions taking place these effects into consideration.

The variation of genotype-by-environment interaction for grain yield was also associated with the variation in phenology. Under favourable water conditions, early flowering photoperiod sensitive genotypes were disadvantaged as they had a short vegetative period. Late flowering genotypes, on the other hand, were disadvantaged under prolonged drought as they were exposed to a longer period of drought.

Although genotype-by-environment interaction for grain yield existed, the interaction was not so large as to change the ranking for GY of some genotypes across environments. Consequently, it is possible to select high yielding genotypes (GG1) for both well-watered and drought conditions for both environmental sites and for a range of seasonal conditions.

In terms of plant water availability, it appears that yield reduction can be explained reasonably well by the relative water level during flowering time. The rate of yield reduction to WL_{REL} determined in this study ($-1.68 \% \text{ cm}^{-1}$) defines the maximum level of yield reduction in relation to the

relative water level under rainfed conditions, and this is probably observed at farmer's level. Therefore, potential yield estimated from water level and yield measured under rainfed conditions can assist in identifying drought tolerant genotypes.

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ប្រសិទ្ធភាពនៃត្រកូន និងចកាយទា លើការលូតលាស់របស់ត្រីចំណីបង្ក្រាមស្រះចិញ្ចឹមត្រីចំរុះ
EFFECT OF WATER SPINACH AND DUCKWEED ON FISH GROWTH PERFORMANCE
IN POLY-CULTURE PONDS

San Thy, Khieu Borin^A, Try Vanvuth, Pheng Buntha and Preston T R^B

អត្ថបទសង្ខេប

កូនត្រីសរុបចំនួន ៣៦០ ក្បាល ត្រូវបានយកមកធ្វើពិសោធន៍ក្នុង កូនស្រះចំនួន ១២ ដែលស្រះនីមួយៗមានទំហំ ១០ម^២ ។ សមាសភាពកូនត្រី ពូជដែលបានដាក់ចិញ្ចឹមនៅក្នុងស្រះនោះមាន ៤០ភាគរយ ជាប្រភេទត្រី ទីឡាព្យា (*Oreochromis niloticus*) ៣៥ភាគរយ ជាប្រភេទត្រីកាប- សាមញ្ញ (*Cyprinus carpio*) និង២៥ភាគរយ ជាប្រភេទត្រីកាបឥណ្ឌ (*Cirrhinus mrigal*) ។ ការពិសោធន៍នេះមានចំនួន ៣ប្រការ: ប្រការទី១: ការចិញ្ចឹមតាមបែបធម្មជាតិ ប្រការទី២: ការចិញ្ចឹមតាមបែបធម្មជាតិ ដែលផ្តល់ត្រកូនជាចំណីបន្ថែម និងប្រការទី៣: ការចិញ្ចឹមតាមបែបធម្មជាតិ ដែលផ្តល់ចកាយទាជាចំណីបន្ថែម ។ កាកសំណល់នៃឡូជីវខ្ពស់ត្រូវបាន ប្រើប្រាស់ដោយគុណភាពក្នុងកំរិត ១២០ គ ក្រ អាសូត / ហិកតា។ ត្រកូន និងចកាយទាត្រូវបានផ្តល់ប្រចាំថ្ងៃក្នុងកំរិត ៣-៥ភាគរយ នៃទម្ងន់ខ្លួន ចាប់តាំងពីពេលចិញ្ចឹមរហូតដល់អាយុ ១២០ថ្ងៃ ។

គុណភាពទឹក (pH ភាពជ្រាបទឹក សីតុណ្ហភាពទឹក និងកំរិត អុកស៊ីហ្សែនរលាយក្នុងទឹក) មានលក្ខណៈប្រហាក់ប្រហែលគ្នានៅគ្រប់ ប្រការទាំងអស់ ។ អត្រារស់នៃកូនត្រីគ្រប់ប្រភេទទាំងអស់មានកំរិត ប្រហាក់ប្រហែលគ្នាដែលក្នុងនោះ ត្រីកាបសាមញ្ញ (Comon Carp) រស់បាន ៨៨.៣ភាគរយ ត្រីកាបឥណ្ឌ (*mrigal*) រស់បាន ៩០.៦ភាគរយ និងត្រី ទីឡាព្យា (*Tilapia*) រស់បាន ៩២.២ភាគរយ ក៏ប៉ុន្តែមានត្រីក្នុងស្រះដែល ផ្តល់ចំណីចកាយទា មានអត្រារស់ទាប ។ ការកើនឡើងនៃទម្ងន់ប្រចាំថ្ងៃ របស់ត្រីមានការខុសប្លែកគ្នាទៅតាមប្រភេទត្រី និងការផ្តល់ចំណីបន្ថែម លើកលែងតែស្រះ ដែលផ្តល់ចកាយទា ។ កំណើនទម្ងន់ត្រីសរុបប្រចាំថ្ងៃគឺ ៦.១៧ ១៥.២ និង ២១.២ គក្រ ក្នុង១ហិកតា ក្នុង១ថ្ងៃ (P=0.012, SE±2.74) ដែលធ្វើឱ្យទទួលបានត្រីសរុប ១.៤៥០គក្រ សំរាប់ប្រការទី១ ២.៤៧០ គក្រ សំរាប់ប្រការទី ២ និង ៣.១២០ គក្រ (P=0.007, SE±276) សំរាប់ប្រការទី៣ ក្នុង ១ហិកតា ក្នុងរយៈពេល ៤ខែ ។

យោងលើលទ្ធផលពិសោធន៍ខាងលើយើងអាចសន្និដ្ឋានបានថា

ចកាយទា និងត្រកូន អាចប្រើប្រាស់ជាចំណីបន្ថែមដល់ការចិញ្ចឹមត្រីចំរុះ ទោះបីជាកំណើនទំហំមានលក្ខណៈល្អប្រសើរចំពោះត្រីផ្តល់ចកាយទា ។

ABSTRACT

A total of 360 fingerlings were distributed to twelve ponds of 10 m² each. The composition of fish in a pond was 40%, 35% and 25% of tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*) and mrigal (*Cirrhinus mrigal*) respectively. Treatments were effluent (E), effluent plus water spinach (EWS) and effluent plus duckweed (EDW). The level of effluent applied was 120 kg N ha⁻¹. The water spinach and duckweed were given daily at 3 to 5% DM of fish bodyweight from fingerling stage to 120 days old respectively.

The water quality (pH, water transparency, water temperature and dissolved oxygen) were similar in all treatments. The survival rate was similar for all species (88.3%, 90.6 and 92.4 % of common carp, mrigal and tilapia respectively) however lower survival rate was in ponds fed duckweed. The daily weight gain was significantly difference between species and supplements except the duckweed ponds. The total daily weight gain was 6.17, 15.2 and 21.2 kg ha⁻¹ day⁻¹ (P=0.012, SE±2.74) resulting a total yield of 1,450, 2,470 and 3,120 kg ha⁻¹ per 4 months (P=0.007, SE±276) for E, EWS and EDW respectively.

It is concluded that duckweed and water spinach can be used as a supplement to poly-culture fish although fish gained better with the supplement of duckweed.

KEYWORDS: Pond, biodigester effluent, fish, polyculture, water spinach and duckweed *Oreochromis niloticus*, *Cirrhinus mrigal*, *Cyprinus carpio*

INTRODUCTION

Fish constitutes more than 70% of the total animal protein intake of the Cambodians. Fishery contributes from 3.2 to 7.4 percent to the gross domestic product (GDP) although fish caught from natural water such as lakes and ponds has declined from 120 000 - 130 000 tonnes in the 1960s (about 25kg per capita) to 75 000 tonnes in the 1990s (about 10-13 kg per capita) while the population of Cambodia is reaching almost 14 million in 2006 and continues to increase on average at about 2.4 percent. Although it is still slow, the aquaculture has increased from 1610 tonnes in 1984 to 15000 tonnes in 1999 (Nao Thouk, 1999).

The aquaculture systems practiced in Cambodia are: (i) cages culture and (ii) ponds culture. The fish production can be monoculture and poly-culture. However, to optimize the use of natural resources, the integration of animals, crops and fish is an ideal strategy. In recent years, research has been promoted in Cambodia, giving attention

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on the role of biogas as a key component of the farming systems. The recycling of waste gives additional value to both human and animal wastes through gas production, produce good quality of organic materials and the control of pathogens. The effluent from the biogas has been used to produce plankton for fish.

Duckweed has been reported to have good balance of amino acids comparable to milk (Leng *et al.*, 1995). Duckweed is a good quality when it was harvest in the ponds and canals nearby the houses due to the availability of nutrients. Duckweed grown naturally in ponds and canals with poor nutrients grow slowly with long root systems and has poor protein content.

Water spinach is available naturally in the rainy season or it can be planted in the rainy and dry seasons. The two types of water spinach; land and aquatic, are commonly cultivated by farmers. When water is not a constraint factor, aquatic water spinach has the capacity to produce foliage for longer period. Aquatic water spinach produce up to 40 ha⁻¹ cut⁻¹ when applied 200 kg of nitrogen ha⁻¹ year⁻¹ (CelAgrid, unpublished data).

The purpose of the present study is to investigate the supplement of the water spinach and duckweed on the growth performance of the tilapia, common carp and Indian carps kept in poly-culture in the ponds.

MATERIALS AND METHODS

EXPERIMENTAL PERIOD

The experiment was carried out at the Centre for Livestock and Agriculture Development 'CelAgrid' from April 21 to August 20, 2004.

POND CONSTRUCTION AND PREPARATION

A total of 12 ponds were excavated with the capacity of 20 m³ each (4 m length x 2.5 m wide and 1 m deep). The ponds were lined with blue plastic to protect from soil erosion and to avoid filtration of water. After paving the blue plastic, water from pond was pumped into all experimental ponds. Lime (CaO) at 200 g/m² was applied at 20 days before stocking with fish, in order to kill parasites and pathogenic organisms if existed and also to increase the pH (Photo 1).



Photo 1: Ponds arrangement all in a row by paving with blue plastic

EXPERIMENTAL DESIGN

The Completed Randomized Block Design was used. There were 3 treatments: the effluent as control (E), effluent with the supplement of water spinach (EWS), and the effluent

with the supplement of duckweed (EDW) and each treatment replicated 4 times.

Stocking density and fish composition

Each pond was stocked with three fish species at a density of 3 fishes per m². The species were tilapia (*Oreochromis niloticus*), common carp and mrigal (*Cirrhinus mrigal*). The composition was 40 percent of tilapia, 35% of common carp, and 25% for Mrigal which was equal to 12, 10 and 8 heads (30 fish per 10 m²) of tilapia, common carp and Mrigal respectively.

FERTILIZATION AND FEEDING

Biodigester effluent

Ponds were fertilized with effluent from a plastic biogas digester loaded with pig manure with the rate of 120 kg of nitrogen per ha⁻¹ year⁻¹. The chemical composition of the effluent is presented in Table 1.

Table 1: The chemical composition of the effluent

	Mean	Minimum	Maximum
DM, %	3.08	2.69	4.63
OM, %	63.7	50	75
NH ₃ -N mg/litre	400	257	579
N, mg/litre	835	450	1770
NH ₃ -N in proportion of total N, %	55.4	32.7	75.39

Feed supplementation

Feed supplementation (water spinach and duckweed) was given twice daily at 8:00 am and 4:00 pm. The feed supplementation was estimated at 5% DM basis of the fish body weight. Duckweed and water spinach were cultivated at CelAgrid. The chemical composition of the feed supplements is presented in Table 2.

Table 2: The dry matter (%/kg) and crude protein contents (%/kg DM) of the feed supplements

	Duckweed	Water spinach
Dry matter	5.5	9.11
Crude protein	31.1	18.5

MEASUREMENTS AND ANALYSES

The effluent from biogas digester was daily sampling and bulked for the weekly analysis of N, OM, Nitrogen, and Ammonia. The quantity of N was calculated before applying into the fish pond. Fish bodyweight and length were measured at 8:00 am in every 20 days before applying the feed or effluent.

The oxygen and pH of the water in the fish pond was measured in every 4 days and each measurement was taken twice at 6:00 am and at 4:00 pm using the DO₂ meter (Model 9150). The water temperature was measured three times weekly at 6:00 am, at 12:00 am and at 5:00 pm using thermometer placing in each pond for 5 minutes. Water quality was also measured at 12.00 am in every 2 days using Secchi disk.

STATISTICAL ANALYSES

The data were subjected to analysis of variance (ANOVA) by using the General Linear Model (GLM) of the SPSS software (Release 12.0, 2003). Two ways ANOVA is used to determine length and weight of fish, and the descriptive

statistic was used to determine the mean, maximum and minimum of effluent fertilizer, water quality parameters and feed.

RESULTS AND DISCUSSION

WATER QUALITY

There were no differences on water quality parameters (pH, water transparency, water temperature and dissolved oxy-

gen) among the treatments (Table 3). The average of oxygen concentration was close to the minimum to level recommended by Swingle (1969). Values from 0.3 to 1mg/litre over an extended period were considered to be lethal to fish and from 1mg to 5mg/litre the fish survived, but growth was slow. The pH for all treatments was within the range suggested by Swingle (1969).

Table 3: The water quality from different treatments

	Mean value	Minimum	Maximum
	pH value		
E	8.77	8.17	9.42
EWS	8.57	7.92	9.44
EDW	8.48	7.91	9.23
	Water transparency, cm		
E	12.7	9.0	20.3
EWS	13.6	9.0	21.8
EDW	13.8	9.5	21.8
	Water temperature, °C		
E	31.0	28.0	34.7
EWS	31.0	28.1	33.3
EDW	30.7	28.1	34.5
	Dissolved Oxygen, mg/litre		
E	4.57	1.95	8.15
EWS	3.89	1.74	6.23
EDW	4.15	2.10	7.18

E = Effluent, EWS = Effluent plus water spinach, EDW = Effluent plus duckweed

FISH SURVIVAL RATE

The survival rate was similar for all species (88.3%, 90.6 and 92.4 % of common carp, mrigal and tilapia respectively). Ponds fed duckweed had the lowest survival rate compare to the control and water spinach ponds and within those ponds supplemented with duckweed common carp and Mrigal had higher mortality rate compare with Tilapia (Table 4). In contrast to the study by Pich Sophin and Preston (2001) where Tilapia had poorer survival rate either in ponds fertilized with urea-DAP, raw cow manure and effluent from biodigester.

Table 4: Fish survival rate by treatments and species as % of the total number of fish

Species	Control (effluent)	Duckweed	Water spinach	Average
Common carp	92.5	75	97.5	88.3
Mrigal	100	75	96.8	90.6
Tilapia	93.8	89.6	93.8	92.4
Average	95.4	79.8	96.0	

FISH GROWTH IN LENGTH

Fish supplemented with duckweed had largest length disregard fish species (Table 5). The length was 11.9% and 28.3% for Mrigal and 10.5% and 26.8% for Tilapia and 15.6 and 28.3% larger than EDW and E fish respectively. However, the length of Mrigal was the largest even the control group comparing with other species.

Table 5: The effect of duckweed and water spinach on length from individual fish, cm

	Interval harvesting, days						
	0	20	40	60	80	100	120
	Common carp						
E	9.9	11.8	12.2	12.5	13.7	12.5	12.7
EWS	10.2	13.1	14.5	15.7	15.8	16.1	16.3
EDW	10.3	12.0	12.5	13.3	14.0	13.9	14.1
	Mrigal						
E	11.8	13.9	15.5	17.0	17.7	18.2	19.1
DW	11.5	13.3	17.1	20.3	20.4	23.1	24.5
WS	11.7	14.3	16.4	18.0	20.7	21.0	21.9
	Tilapia						
E	10.1	13.3	13.9	14.3	14.7	14.6	14.9
DW	10.7	14.3	16.2	16.9	17.4	18.4	18.9
WS	10.5	13.6	14.5	15.2	15.4	16.4	17.1

FISH INDIVIDUAL WEIGHT

Fish grew faster in the pond supplemented with duckweed followed by those supplemented with water spinach (Table 6). Similar result was also found in Bangladesh where they fed duckweed to Mrigal (William et al., 1991). Duckweed was the appropriate choice to be used as supplement for the small-scale aquaculture as it can be harvested from the natural ponds or canals close to houses in the rainy season. The natural ponds and canals are dried up in the dry season in this case an artificial pond managed by farmers could play

the role to produce good quality duckweed.

Mrigal among the three species had the highest final weight disregard the feed supplement. After 120 days, the weight of Mrigal was 59.2% and 200% in the control pond, 53.9% and 198% in the EWS ponds and 59.4 and 62.1% higher than Tilapia and common carp respectively. The weight of Tilapia in the control pond of the present experiment was similar to the study reported by Pech Sophin and Preston (2001) where they used effluent from biodigester to fertilize Tilapia pond.

Table 6: Weight of fish individual in relation to the species and feed, g/head

Interval harvesting, days	0	20	40	60	80	100	120
Control (fertilizing effluent from biodigester)							
Common Carp	19.5	27.0	28.0	27.5	26.4	26.8	25.5
Tilapia	21.0	38.8	43.8	46.4	44.0	47.6	48.3
Mrigal	19.4	32.7	42.2	51.6	63.3	68.4	76.9
Effluent fertilizer with water spinach supplementation							
Common Carp	17.8	28.0	32.3	37.0	42.0	43.3	43.3
Tilapia	20.8	45.8	53.3	57.1	71.9	74.8	83.8
Mrigal	19.2	36.3	50.9	71.9	104	109	129
Effluent fertilizer with duckweed supplementation							
Common Carp	19.5	36.6	47.5	62.3	60.8	58.3	62.3
Tilapia	22.7	50.5	62.3	75.8	96.0	102	101
Mrigal	20.0	38.0	63.8	110	140	140	161

DAILY WEIGHT GAIN

The daily weight gain was significantly difference between species and supplements except the duckweed ponds (Figure 1). Mrigal grew faster in the all treatments compare with other species and for all species kept in ponds providing only effluent from biodigester at the rate of 1 kg ha⁻¹ day⁻¹ resulted poor growth. Knud-Hansen et. al., (1991) and Lin et. al., (1997) recommended 4 kg ha⁻¹ day⁻¹ as the optimum level for fish kept in the pond and it is four times higher than the rate applied of the present experiment. Pig manure

loaded in an anaerobic biodigester, before applying in the ponds stocked with poly-culture of fish, resulted in a daily growth 0.5 g day⁻¹ for tilapia (Pich Sophin and Preston 2001). However, other study using biodigester effluent with the supplement of commercial feed, the daily weight gain was in a range of 0.15-0.23g day⁻¹ fish⁻¹ (Edwards et al 1988). Thus the daily weight gain of the present study is quite similar the above studies. As feed supplement used in the present study is available in the rural areas, then it is ideally to give the supplement to fish to be able to get better harvest.

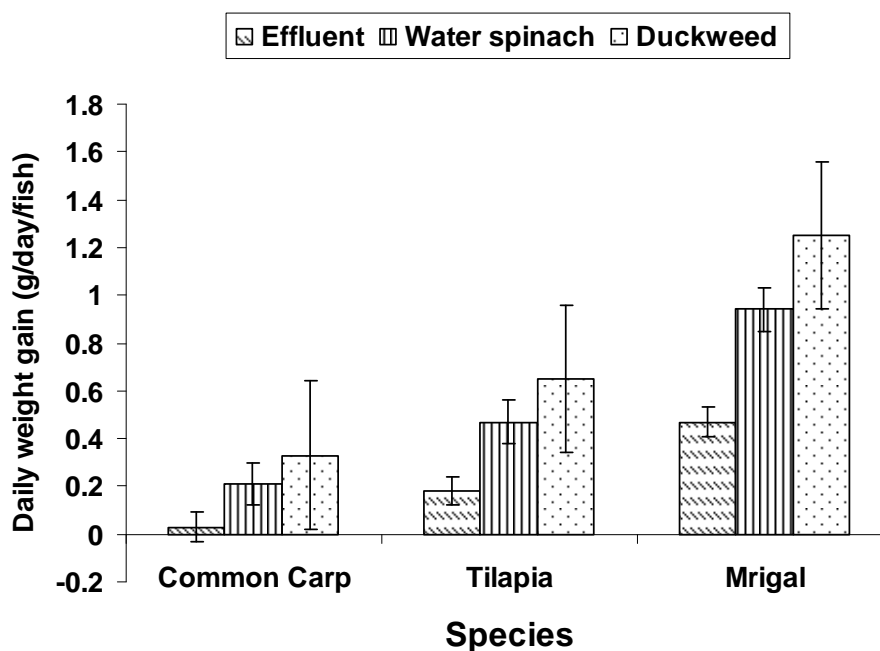


Figure 1: Effect of water spinach and duckweed on the daily weight gain of fish kept in poly-culture ponds.

TOTAL YIELD OF FISH

The total daily weight gain ($P=0.12$), total yield (0.007) and net yield ($P=0.008$) were significantly increased with the supplement of duckweed and water spinach (Table 7). The net yield of fish was 1.2 and 1.9 times for EWS and EDW respectively higher than fish kept in E ponds. The result from our study on fish kept on E ponds is in the agreement with Pich Sophin and Preston (2001) who reported that the fish poly-culture fertilized with effluent from biodigester got an increase of 55% net fish. The explanation that the yield of fish in EDW ponds was better than fish kept in EWS and E

ponds because duckweed protein has a better array of essential amino acids than most vegetable proteins and more closely resembles animal protein (Hillman and Culley 1978). Duckweed is converted efficiently to liveweight by certain fish including carp and tilapia (Robinette *et al* 1980; Hassan and Edwards 1992). The total yield of fish supplemented with duckweed was 3.1 tons ha^{-1} per 4 months which is close to the figure reported by Skillicorn *et al.* (1993) that fish supplemented only with well managed and fertilized duckweed, got an average yield at around 10 tons ha^{-1} year $^{-1}$ annually.

Table 7: Effect of water spinach and duckweed on supplementary unaccompanied feed to fish poly-culture

Treatment	Daily weight gain kg/day/ha	Total yield kg/ha	Net yield kg/ha	The increased net yield, %
Effluent	6.17	1450	848	58.4
Water spinach	15.2	2470	1888	76.4
Duckweed	21.2	3120	2493	79.9
SE/P	2.74/0.012	276/0.007	282/0.008	

CONCLUSIONS

Duckweed and water spinach can be used as a supplement to poly-culture fish although fish gained better with the supplement of duckweed. As a strategy for farmers in rural community, they can use duckweed in the rainy season where it is naturally available but good quality duckweed should be harvested from canals and ponds close to houses. However, land water spinach can be planted and used as supplement to fish in the dry season.

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សំណាយផ្ដោតនៃមហាមន្តោត និងការគ្រប់គ្រង

BROWN PLANTHOPPER OUTBREAKS AND MANAGEMENT

Preap Visarto^A, M. P. Zalucki^B & G.C. Jahn^C

អត្ថបទសង្ខេប

មូលហេតុ និងបញ្ហានៃការផ្ទុះឡើងរបស់ប្រជាករ មហាមន្តោតបានធ្វើការបកស្រាយនៅក្នុងអត្ថបទនេះ។ ការមានពងច្រើន និងភាពរស់រានមានកិរិតខ្ពស់ គឺជាកត្តាដែលអាចធ្វើអោយប្រជាករមហាមន្តោតអាចផ្ទុះបាន។ កត្តាទាំងពីរនេះគេបានដឹងថាបណ្តាលមកពីការប្រើប្រាស់ជីគីមី និងថ្នាំពុលសម្លាប់សត្វល្អិត។ ដូច្នេះហើយបានជាមានការសិក្សាអំពីឥទ្ធិពលនៃថ្នាំសម្លាប់សត្វល្អិត និងជីគីមីទៅលើកំណើតពង និងកិរិតអាចរស់រានរបស់កូនមហាមន្តោតក្នុងលក្ខខណ្ឌស្រែកសិករ។ ម្យ៉ាងវិញទៀតពេលវេលាសំរាប់សាបសំណាបក៏អាចជាកត្តាមួយធ្វើអោយមហាមន្តោតកើតឡើងខ្លាំងដែរ។ យើងបានដឹងថាប្រជាករមហាមន្តោតនៅក្នុងប្រទេសកម្ពុជាបានកើតឡើងជាអន្លើ និងរកេតរកូតខ្លាំងណាស់។ បាតុភូតនេះអាចមានការទាក់ទងគ្នាទៅនិងពេលវេលានៃការសាបសំណាប និងការផ្លាស់ប្តូរទីលំនៅរបស់មហាមន្តោត។ ថ្នាលសំណាប ដែលសាបមុនគេខុសប្រក្រតី អាចផ្តល់ជាទីជំរកដល់មេមហាមន្តោត ហើយពងមហាមន្តោតដែលមាននៅក្នុងសំណាប នឹងញាស់ហើយបង្កជាកូន ពូជជាចៅច្រើនឡើងៗ ហើយដែលអាចឈានទៅរកបន្ទុះប្រជាករដោយដ៏ង។ ដូច្នេះហើយសន្ទូងដែលមានមហាមន្តោតដែលជាដើមហេតុនៃបន្ទុះប្រជាករ ត្រូវបានយកមកធ្វើការសិក្សា។ លើសពីនេះយើងបានដឹងថាបន្ទុះប្រជាករមហាមន្តោតនៅក្នុងតំបន់អាស៊ីត្រូពិច គឺបណ្តាលមកពីការរកជាពូជពូជចៅ ពីពីរទៅ បីជំនាន់ហើយដែលមានការទាក់ទងទៅនឹងទំហំប្រជាករដំបូង គឺជាដើមហេតុនៃបន្ទុះនេះ។ ហើយដោយភាពផ្ទុះនៃប្រជាករ មហាមន្តោតនៅប្រទេសកម្ពុជាមានលក្ខណៈជាបណ្តុំរាយប៉ាយនោះបញ្ហាទាំងនេះ ត្រូវបានធ្វើការសិក្សា និងពិភាក្សានៅក្នុងអត្ថបទនេះដែរ។ លើសពីនេះការសិក្សាអំពីប្រសិទ្ធិភាពនៃសត្វល្អិត មានប្រយោជន៍ក្នុងការកំចាត់មហាមន្តោតក៏ត្រូវបានលើកយកមកពិភាក្សាដែរ។ និយាយជាប្រមូលកត្តាទាំងអម្បាញាណដែលបានរៀបរាប់កន្លងមកនេះ ត្រូវបានពិភាក្សា និងសំយោគដើម្បីឈានទៅរកសេចក្តីសន្និដ្ឋាន និងអនុសាសន៍មួយសំរាប់ការងារអនុវត្តន៍ និងស្រាវជ្រាវជាបន្តទៀត។

ABSTRACT

The causes and problems of BPH outbreaks are discussed in this paper; increased fecundity and survival are factors that can lead to outbreaks of brown planthopper populations. These two factors are believed to be influenced by the use of chemical fertilizers and insecticides. Therefore the effects of chemical fertilizers and insecticides on fecundity and survival of BPH under Cambodian field conditions were studied. The effect of fertilizer on BPH oöcyte (egg) production was examined. Timing of seedbed establishment as a cause of brown planthopper outbreaks is one of the most possible factors. Outbreaks of brown planthopper in Cambodia are very localized and appear to be random. This phenomenon may be related to the time of seedbed establishment and colonization by migrating or dispersing BPH; rice seedbeds that are established early may provide a suitable host for BPH to colonize and subsequently to be transplanted into fields initiating localized outbreaks. Transplanted rice seedlings as a source of initial BPH infestation is examined. Outbreaks of brown planthopper in tropical Asia are generally related to population build up over two or three generations. The size of the initial population in a field may enable BPH populations to reach outbreaks levels. As outbreaks of BPH in Cambodia appear random and localized, it was thought these spot outbreaks might be from seedlings infested with BPH eggs. Cambodian rice fields have abundant natural enemies and these play an important role in reducing BPH population density. Field experiments on the relationship between BPH establishment and their natural enemies are presented. Overall synthesis of my findings, where the different strands of this work are brought together by focusing on the effects of host plant nutrition, cultivar reaction, timing of crop establishment and predation effects on the growth of populations of BPH. This leads to the final conclusions and recommendations for further research.

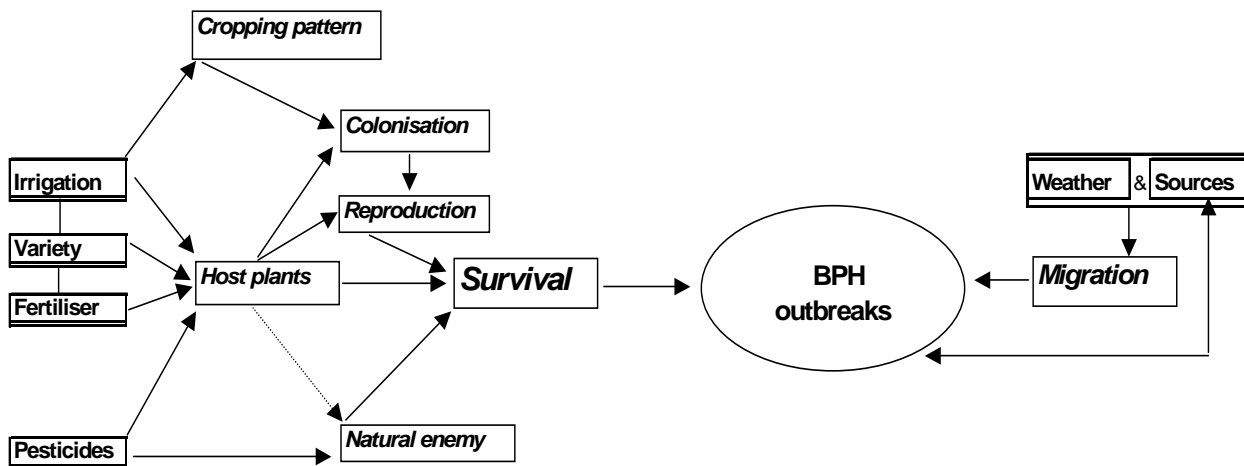
KEYWORDS:

Brown planthopper, outbreak, fertilizer, cultivar, fecundity, egg production, seed bed, & insecticide.

1. INTRODUCTION

Outbreaks occur when the population of a species increases in abundance rapidly over a relatively short period of time (Berryman 1987). As Berryman (1987) points out populations increase when the “environment” becomes more favourable for the reproduction and/or survival of the species and individuals aggregate into an area. In tropical Asia outbreaks of BPH have been wide spread since the introduction of new high yielding cultivars, chemical fertilizer and pesticides. Since BPH can devastate rice fields during an outbreak, this delphacid has the potential to cause tremendous economic losses. The Cambodian government began documenting pest outbreaks in 1991. There has been a BPH outbreak every year in Cambodia since then. Outbreaks of BPH in the Cambodian rice agro-ecosystem appear to be localized and patchy within a field (Preap et al. 2001a).

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Fig.1 Summary of factors related to BPH population outbreaks (Denno 1994, Gallagher et al. 1994, Preap et al. 2001a & b, 2002a &

Most BPH outbreaks in Cambodia have been localized, and on a small scale. These localized outbreaks are thought to be related to several factors, that alone or together influence adult colonization, reproduction and survival of nymphs and adults. These factors are related to host plant quality and natural enemies and will depend on farming practices such as: pesticide application, fertilizer use, host plant cultivar, irrigation, and cropping pattern (Fig 1).

Compared to other countries in the region, for example Vietnam (Pham 2001, 2002) and Thailand (Setboonsang 1993, Vongsilabutr 2001), Cambodia suffers relatively little crop loss to BPH outbreaks. Compared to Vietnam, high yielding cultivars are not widely grown in Cambodia (only 13% of the total rice land), but highly BPH-susceptible traditional cultivars, such as *Eth Chhmoush*, are planted by local farmers. Some Cambodian farmers use a high rate of fertilizer, but extensive outbreaks are very rare. What are the key factors that keep BPH outbreaks in Cambodia to a minimum? In this work I will discuss the factors that contribute to the BPH population changes in the rice agro ecosystem in Cambodia.

2. MATERIALS AND METHODS

The effects of chemical fertilizers and insecticides on fecundity and survival of BPH under Cambodian field conditions experiments were conducted in farmer fields in Samrong district, Takeo province and in CARDI's fields, Phnom Penh in wet season 1999. The effect of fertilizer on BPH oöcyte (egg) production was examined under glass house condition in 1999; I used two rice varieties (IR72 & *Eth Chhmoush*) and two fertilizer rates (low & high) to test the egg production. Egg productions from each female were counted under binocular and compound microscopes. In wet season 2000, I conducted an experiment to examine the effect of timing of seedbed establishment on brown planthopper outbreaks in farmer fields in Samrong district and CARDI's fields. Seed beds were established in four times: very early, early, normal, and late, compared to the normal time of local farmer practices.

To check the influence of initial number of BPH on transplanted rice seedlings as a source BPH population build up and outbreak, I conducted an experiment at the same time and at the same fields of Samrong and CARDI.

Further more, Cambodian rice fields have abundant natural

enemies and these play an important role in reducing BPH population density. Thus, field experiments on the relationship between BPH establishment and their natural enemies are presented.

3. EFFECT OF PLANT NUTRITION AND INSECTICIDE TREATMENTS ON BPH PRODUCTION AND SURVIVAL

Host plant nutrition is thought to influence host plant selection, subsequent insect performance (development, survival, growth) and the consequent population growth and dynamics (Waloff 1980, Denno & Roderick 1990). If brown planthoppers develop on nitrogen-rich host plants more nymphs survive to become larger and more fecund adults (Denno & Roderick 1990, Preap *et al.* 2001a).

Host plant quality influences BPH development. BPH that develop on susceptible plants produced more eggs and nymph survival was much higher than on a resistant cultivar (Table 1). Susceptible plants if treated with a high rate of fertilizer achieve higher yields but also a higher density of BPH, and some times these may reach outbreaks level. In general, what is good for the plant is good for the pest (Jahn *et al.* 2001). In irrigated rice fields, herbivores, predators, and parasitoids increase in abundance with increasing levels of nitrogen fertilizer use (De Kraker *et al.* 2000, Preap *et al.* 2001a).

Many insect species exhibit higher growth rates and decreased development times when their host plant is fertilized with high nitrogen levels (Tabashnik 1982, Fisher & Fiedler 2000, fig.2). Many pests are associated with heavy fertilizer application (Chelliah & Subramanian 1972, Reissig *et al.* 1986). The use of a resistant cultivar can alleviate this pest problem. In Southeast Asia outbreaks of BPH have declined in frequency and scale, as farmers started to plant resistant cultivars such as IR26 and IR36 (Way & Heong 1994). My results indicated that BPH developing on a resistant cultivar had a smaller body, were less fecund and had poorer survival than on a susceptible cultivar (Table 1). Denno & Roderick (1990) suggested that a low level of BPH population occurred on resistant cultivars because of chemical compounds. Yoshihara *et al.* (1979, 1980) and Sogawa (1982) indicated that a decarboxylated derivative of aromatic amino acids and oxalic acid are inhibitors BPH feeding. Minor plant nutrients, such as silicon can increase the resistance of rice plants to planthoppers (Chang *et al.* 2001).

Fig.2 Brown planthopper colony, nymphs & adults



The repercussions can be quite serious when we make drastic changes in the agro-ecosystem without understanding the consequences. In the mid-1980s BPH outbreaks in Indonesia

were induced through insecticide applications (Kenmore *et al.* 1985, Kenmore 1996).

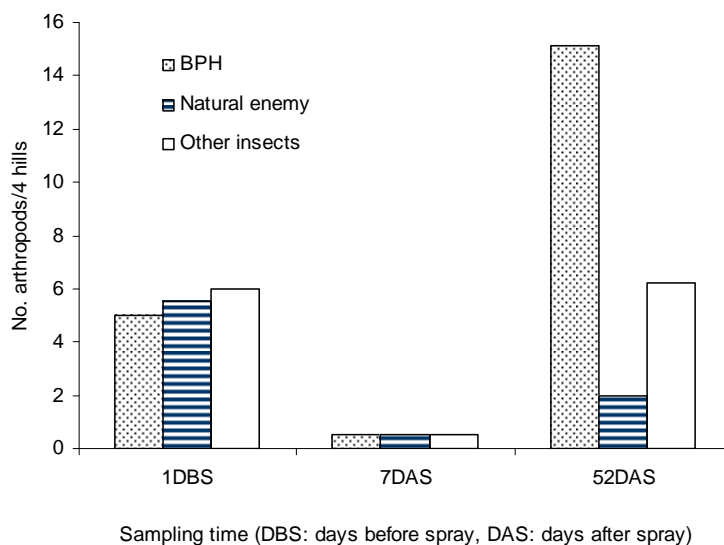
Natural enemies were killed faster than the target pest and BPH population became resurgent and reached outbreak level. Gallagher *et al.* (1994), Way & Heong (1994) and Settle *et al.* (1996) consider that the action of natural enemy is fundamental to sustainable brown planthopper management. The recovery of natural enemies after pesticide application was very poor compared to other insect, particularly BPH. A week after being sprayed almost all the population of natural enemies, other insects and BPH were killed. But 52 days after being sprayed the population of other insect and BPH had recovered better than natural enemies (Fig. 3).

My results in field cage experiments indicated that each *A. inustus* or *P. pseudoannulata*; which are very abundant in Cambodian rice field ecosystem (Preap *et al.* 2001a & b); can kill up to 97% of BPH in 15 days after spiders were introduced into the cage with a ratio of spider to BPH of 1:24 to 1:57 (Preap *et al.* 2001b).

Table1. Body length, fecundity, eggs laid and nymph survival of brown planthopper (+/- se) on resistant and susceptible cultivars treated with low and high fertilizer rates.

Host plant variety	Resistant (IR 72)		Susceptible (Eth Chhmoush)	
	Low	High	Low	High
Body length (mm)	3.74±0.02	3.86±0.01	3.85±0.01	4.34±0.02
Oocytes production (no./female)	101±7	158±8	123±7	338±6
Eggs laid (no. per female)	46±2.3	66±2.5	55±2	72±2
Survival of nymphs (%)	19±1	35±3	28±3	53±4

Fig.3. The population dynamics of natural enemies, BPH and other insects in plots treated with insecticide.



4. COLONIZATION AND INITIAL POPULATION SIZE: ARE THESE THE KEY FACTORS FOR LOCALIZED POPULATION OUTBREAKS

The combined effects of high levels of colonization and improved performance on nitrogen-rich host plants often result in rapid population growth and larger population size.

After apparently randomly landing into fields BPH macrop-terous tend to choose high fertilized-plants (Preap *et al.* 2001a). They tend to move from plant to plant and selected more nutritious plants (susceptible cultivar, high-fertilized) on which to feed and, oviposit. Such plants accumulate locally higher densities. Subsequent populations will be higher; nymphs have a higher survival rate and adults are more fecund (Sogawa 1970, Denno 1985).

The BPH population build up depends on the number of insects initially infesting the crop and the crop stage infested. When the crop was planted with seedlings that had been exposed to BPH at a seedling to adult BPH ratio of 27:1 to 108:1, BPH built up to high numbers and the crop was killed. The higher the numbers of infesting insects the sooner plants were killed, and high-fertilized plants were killed faster than low fertilizer plants (Table 2). Even crops planted with seedlings exposed at the lowest BPH density tested, 216 seedlings to 1 BPH, were seriously affected when the BPH population increased to a high level at 72 days after trans-planting. When colonization started late with a few insects (one per hill 22 days after transplanting) on low fertilized plants BPH did not increase to a high level and subsequently did not affect plant yield. However high fertilizer application is favorable for the BPH population increase and these plants

were seriously affected (Table 2).

Initial population density on seedlings may be a key factor leading to subsequent population increase and local out-breaks and these outbreaks are closely related to the plant quality, crop stage of infestation and natural enemies. The density of the initial population depends on BPH sources, weather (particularly wind systems that influences BPH migration from longer distance) and the target host plants (crop stage and time of crop establishment).

Some areas (Takeo province) are "outbreak prone" because BPH sources are relatively abundant (thousands of hectares of rice crop are grown in the dry season and generally de-velop to the maturing stage by the beginning of the wet sea-son crop). When seedbeds were established very early, which coincides with when BPH migrates from the source crops, seedbeds trapped relatively high numbers of macrop-ters and these formed a high initial colony and subsequently increased to outbreak level, especially on high-fertilised crops (Preap *et al.* 2002a).

In some areas BPH sources are very limited (e.g. CARDI). Even seedbeds established very early had very low initial colonization and no subsequent population increase. With very low incidence at the early stages of a rice crop BPH populations are very stable at a low density during season for both traditional and modern cultivars. For crops that started with a relatively high colonization the population of BPH increased to a high level at the 3rd or 4th generation when the crop reached the reproductive stage (modern high yielding cultivar) and maximum tillering for a traditional cultivar.

Table 2. BPH infestation at different crop stages and the impact of population increase. The crop damage score of 9 indicates very high BPH density and all plants were killed, score of 7 is BPH in high density and almost all plants were killed, and score of 3 is some BPH were observed and plants were slightly affected

Low fertiliser rate

No. adults infested per hill	Crop stage of infestation	At highest BPH density	
		Crop damage score	DAT when achieved
8	Seedling	9	32 DAT
4	Seedling	9	32 DAT
2	Seedling	9	43 DAT
1	Seedling	3	72 DAT
1	22 days after transplanting	3	72 DAT

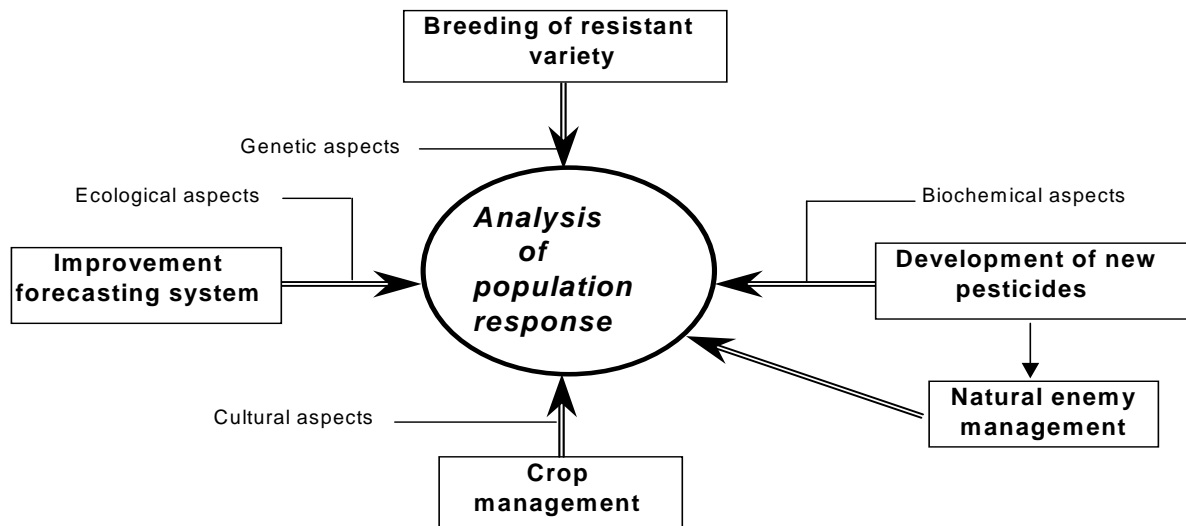
High fertiliser rate

No. adults infested per hill	Crop stage at infestation	At highest BPH status	
		Crop score	Crop stage
8	Seedling	9	7 DAT
4	Seedling	9	22 DAT
2	Seedling	9	43 DAT
1	Seedling	7	72 DAT
1	22 days after transplanting	7	72 DAT

5. APPROACHES AND RECOMMENDATION FOR BPH MANAGEMENT

A long-term strategy for BPH management for both temperate and tropical Asia will rely on: crop management (cultural aspects), breeding of new resistant cultivars (genetic aspects), development of new pesticides (biochemical aspects), natural enemy management (thought proper use of insecticide and improved forecasting systems to better time management intervention (ecological aspects) (Fig. 3).

Fig. 3. Long term approaches for BPH management



Understanding the impact of these factors on BPH population dynamics will enable us to improve BPH management in the long term.

5.1 CROP MANAGEMENT

Using resistant cultivars, fertilizer, seedbed management and other cultural practices all contribute to pest control and crop harvest.

Georghiou (1972) suggested that cultural control methods could reduce insect populations. Cultural control methods are the modification of certain farm operations to make the environment unfavorable for the development and multiplication of the insect pest but favorable for crop production. Cultural control of rice pests incorporates crop production methods. Farmers use cultural control consciously or unconsciously to improve yield by reducing pest number (Reissig *et al.* 1986). Proper land preparation can provide a good crop and minimise use of herbicides. Herbicide application, such as butachlor, quinchlorac or betazone, increased the feeding rate of BPH and may also lead to high population (Wu *et al.* 2002).

The current recommendations for nutrient and pest management are woefully inadequate for predicting edaphic effects on multiple pests and different cultivars (Jahn *et al.* 2001). For example, applying N to promote recovery following stem borer and defoliator damage (Peng 1993, Litsinger 1994) and to increase grain yield may lead to higher planthopper incidence (Cook & Denno 1994, Preap *et al.* 2001a). To date, the application of the science of pest management-nutrient interactions in rice is a simple recommendation to split nitrogen applications, plow straw into the soil to increase silicon uptake or apply K during planthopper outbreaks (Jahn *et al.* 2001).

To avoid a high density of macropters at the seedling stage in “outbreak prone areas” the timing of seedbed establish-

ment needs to be considered. If seedlings are infested with a relatively high number of BPH (see above discussions) then localised outbreaks may occur. For example, at Takeo, Cambodia (“an outbreak prone area”) seedbeds must not be established very early because these seedbeds act like a trap for catching BPH macropterous adults migrating from older dry season crops. These BPH are then transplanted into the crop and this population may subsequently increase to a high level. The need for seedbed field monitoring with yellow pan traps would help growers to make a management decision. Control by using insecticides at the seedling stage should be used if seedbeds are infested with a relatively high number of macropters (yellow pan trap catch more than 2 adults per trap per day). This decision can deal with the right target with minimum risk to the environment. Small scale use of insecticides at the seedling stage in seedbeds will not lead to a huge reduction of natural enemies, as would occur after transplanting, is economical and most farmers can afford it.

Using cultural practices such as plant spacing, weeding, cropping pattern (synchronous planting or crop rotation), fertilizer and irrigation management, in pest control may prevent buildup of BPH populations. Farmers can reduce the pest population by flooding the field or plowing under the stubble after harvest. Using these methods, farmers are able to reduce the number of larvae and pupae of rice stem borer for instance (Reissig *et al.* 1986). Cultural control techniques should be compatible with other control methods and with the needs of the crop. Cultural methods must be cost effective although their results may not be immediate or as spectacular as insecticide use (Oka 1979).

Clark *et al.* (1974) pointed out that cultural methods are the priority in controlling pest attack; they are dependable, economical, ecologically sound, and non-polluting. Some cultural methods are already used to combat brown planthopper. Rice is the only suitable host for BPH. On alternate hosts, this insect can sur-

vive but does not multiply well (Mochida & Dyck 1977). Consequently, during a fallow period, the insect population will be much reduced. The population phenology of the pest will be disrupted when the rice crop is rotated with other crops or there is a fallow between two rice seasons (Oka 1979).

Cultural control methods are not broadly satisfying because the same practice may be good for decreasing one pest but not for another (Reissig *et al.* 1986). For example farmers drain the field to control case worm, *Nymphula depunctalis* Guenée, but the yield will decrease if there is insufficient water supply. Oil applied onto the water surface and shaking the plants (Kenmore *et al.* 1984) can kill BPH but has negative effects on the plants-physiology. Rotation crop should be non-hosts for the insects otherwise rotation may increase pest population.

Synchronous planting and creation of a rice crop free period can greatly reduce pest abundance. Pests disperse from field to field and they can maintain high population levels and cause great yield losses in a farm community where planting times of neighboring fields are staggered beyond an interval of 3 to 4 weeks (Reissig *et al.* 1986). Preap *et al.* (2002a & 2002b) found that rice seedbeds established three weeks earlier than normal can lead to a serious yield reduction due to the high level of BPH landing into the seedbeds, especially in beds treated with high rates of nitrogen fertilizer.

5.2 BREEDING OF RESISTANT CULTIVARS

Controlling BPH with resistant cultivars and insecticides is often not adequate (Oka 1979). IR26 and other rice cultivars with the same gene for resistance to BPH were reported to be hopper burned in two widely separated small areas in the Philippines (IRRI 1975). Soon after introduction of the resistant cultivars insecticides were used to control rice pests and caused severe outbreaks of brown planthopper.

Brown planthopper-cultivars are one of the major products of rice pest control research and can go far towards reducing the farmer problems (Matteson *et al.* 1994). A resistant cultivar can slow down the population increase through lowered fecundity, and poorer survival (Preap *et al.* 2001a, 2002b). Tolerance to damage is also possible.

Cultivars containing the *Bph1* or *Bph2* gene were initially highly successful in controlling BPH (Cohen *et al.* 1997). In Southeast Asia, BPH outbreaks declined in frequency and scale after the planting of these cultivars (Way & Heong 1994). Releasing resistant cultivars by the International Rice Research Institute (IRRI), began with IR26 in 1973, and provided effective but often only short-term control of BPH. After overuse of insecticides, massive outbreaks occurred in some areas planted with resistant cultivars (Reissig *et al.* 1982a & b). For example, the cultivar *Cisadane* containing *Bph2* was very popular in central Java, but became susceptible to *N. lugens* after the fields were intensively treated with insecticides (Kamandulu &

Bahagiawati 1990). Planting resistant cultivars would be one method to help keep rice production safe from BPH attacks, but would not help in the long-term if insecticides are widely used and the resistance gene overcome (Gallagher *et al.* 1994, Setboonsarng 1993, Vongsilabutr 2001).

The 'breakdown' of resistance genes was accelerated by over use of insecticides and the planting of large areas to near monocultures of some cultivars, such as IR26 (containing *Bph1*) and IR36 (containing *Bph2*) (Gallagher *et al.* 1994, Cohen *et al.* 1997). Diversifying the use of rice cultivars in rice production would slow down the break down of resistance genes and outbreaks could be minimised. For example, in Cambodia there are more than 3,450 local rice cultivars (Sahai *et al.* 1992, Javier *et al.* 1999) and outbreaks of brown planthopper are very rare and on a small scale (even in susceptible cultivars).

5.3 NEW PESTICIDES

The negative impact of pesticides on rice production increased when outbreaks of brown planthopper occurred in many countries in Southeast Asia (Kenmore *et al.* 1985). The outbreaks of this insect pest were caused by indiscriminate overuse of insecticides (Kenmore *et al.* 1985, Cohen *et al.* 1997). Development of resistance to various insecticides has been reported (IRRI 1970, 1975, Heinrichs 1994, Nagata 1982, 1999, Nagata *et al.* 2001). Insecticides do not kill BPH eggs, but do kill natural enemies (Reissig *et al.* 1982b) and BPH recovered faster than the natural enemy population (Settle *et al.* 1996, Preap *et al.* 2001a, Fig. 2).

However there are pesticides to control the egg stage of this pest. Suzuki *et al.* (1996) and Yamasaki *et al.* (1999) discovered that *S. furcifera* eggs laid in leaf sheaths and mid ribs of leaf blades suffer considerable mortality. The egg mortality reached 77% when a watery lesion is formed (Suzuki *et al.* 1996). Benzyl benzoate, which was extracted from the watery oviposition lesion, exhibited ovicidal activity against *S. furcifera* at concentrations of 6.4 ppm or more (Suzuki 2002). Thus this new compound would be an effective replacement for those products that cannot kill the egg stage. However this compound should widely be tested on the other planthopper species such as BPH.

5.4 SUSTAINABLE MANAGEMENT AND IMPROVEMENT OF FORECASTING SYSTEMS

BPH is one of the most serious insect pests in rice growing countries in Asia both in the sub-tropics and temperate regions. This phenomenon may be due to inappropriate management strategies and the lack of a reliable forecasting system in some countries of Asia. The latter in part reflects a lack of information and understanding of BPH population dynamics. One cannot solve the BPH problem alone without a meaningful exchange of information among neighboring countries and researchers. This is essentially due to long distance migration (eg Watanabe *et al.* 1994, Watanabe 2002). To solve the problem created by this potential pest in every country in the region (e. g. China,

Korea, Japan, Thailand, Cambodia, Laos and Vietnam) will require sharing information and experience among neighboring countries. A network for exchange of information and databases for BPH is being set up for the region. Food and Agriculture Organization (FAO) and Rural Development Administration (RDA) are funding a project called "Development of Information System for Brown planthopper in Asia".

However in Southeast Asia outbreaks are localised and the detection of initial population at the time of crop establishment would be very helpful. The use of yellow pan traps to detect macropters immigrating into the seedbeds or later crop stages is a very simple tool that Cambodian farmers could use. If the number of macropters caught by yellow pan trap is less than 2 per trap per day seedlings will be safe for transplanting. With 2 or more per trap seedlings are highly infested and need to be treated before transplanting or they should be discarded. To increase confidence in this warning indicator a study over a wider range of locations and sampling over a wider range of densities is needed.

Cambodian rice ecosystems have an abundance of natural enemies (Preap *et al.* 2001a, 2001b), which play an important role in keeping the rice pest situation relatively stable compared to some other counties in the region. However the role of each natural enemy in the population dynamics of BPH is not yet understood. Research into maintaining high levels of natural enemies to manage BPH in Cambodian rice fields will be indispensable.

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បំរែបំរួលភាពនៃពន្ធរបស់ត្រីប្រាខ្មៅ នៅក្នុងអាណន្តមេគង្គកម្ពុជា

GENETIC DIVERSITY OF THE MIGRATORY CATFISH *PANGASIU* *BOCOURTI* IN THE CAMBODIA'S MEKONG RIVER

So Nam^{A,B} VOLCKAERT Filip A.M.^B and Srun Limsong^A

អង្គបទសង្ខេប

ការប្រើប្រាស់បំរែបំរួលពន្ធ នៅក្នុងគោលបំណងនៃការគ្រប់គ្រង និងអភិរក្សធនធានជលផលមានសារៈសំខាន់ជាខ្លាំង ។ ការយកមិត្តកុងឌ្រីម៉ូរហ្វីស្វិស (Restriction Fragment Polymorphism of mitochondrial DNA) មកប្រើប្រាស់ដើម្បីធ្វើអង្កេតលើ បំរែបំរួលភាពនៃពន្ធ (genetic diversity) និងរចនាសម្ព័ន្ធ (genetic structure) នៅក្នុងតំបន់ហ្វែរូន ដែលមានឈ្មោះថា សាយតូក្រូមប៊ី-ឌីលួប (Cytochrome b - D-loop region) ដែលមានប្រវែង ២,១គីឡូបេស (2.1 kb) របស់ត្រីប្រាខ្មៅ (*Pangasius bocourti*) ។ ក្នុងត្រីប្រាខ្មៅចំនួន ៩០ក្បាល ដែលត្រូវបាន ចាប់ពីទន្លេមេគង្គកម្ពុជា នៅឆ្នាំ២០០១ ដើម្បីយកមកសិក្សាលក្ខណៈ ពន្ធសាស្ត្រនោះ យើងបានស្រាវជ្រាវឃើញថាត្រីប្រាខ្មៅទាំង៩០ក្បាលនេះ មានមិត្តកុងឌ្រី ឯកប្រភេទ (mitDNA haplotype) ចំនួន ០៧ ។ ជា មធ្យមបំរែបំរួលភាពនៃពន្ធរបស់ត្រីប្រាខ្មៅប្រែប្រួលពី ០,៤៤ ដល់ ០,៦៨ ។ ផ្អែកលើការវិភាគពន្ធសាស្ត្រលើកងប្រូបរបស់យើងទៅលើអត្រាសរុបនៃការ ចាប់យក (Total fixation Index) ដែរវ៉ាយ (Pair-wise F_{ST}) និងទ្រីស្តិ អំពីចម្ងាយពន្ធរបស់ Slatkin (Slatkin's genetic distance) បានបង្ហាញ អោយឃើញថា ត្រីប្រាខ្មៅមានចំនួនមួយពូជតែប៉ុណ្ណោះ នៅក្នុងទន្លេ មេគង្គកម្ពុជា ។

ការបំផ្លិចបំផ្លាញកន្លែងពងកូនរបស់ប្រភេទត្រីប្រាខ្មៅនេះ នឹងធ្វើ អោយមានការជះឥទ្ធិពលអវិជ្ជមាន មិនត្រឹមតែដល់ទន្លេមេគង្គកម្ពុជា ប៉ុណ្ណោះទេ ថែមទាំងដល់អាងទន្លេមេគង្គទាំងមូលទៀតផង ។ ដោយសារ តែត្រីប្រាខ្មៅមានតែចំនួនមួយពូជ ហើយពូជនេះត្រូវបានចែកចាយទៅក្នុង អាងទន្លេនៃប្រទេសជិតខាងទាំងបួន (កម្ពុជា ឡាវ ថៃ និងវៀតណាម) ដូច្នេះប្រទេសទាំងបួននេះត្រូវ មានយុទ្ធសាស្ត្ររួមមួយដើម្បីគ្រប់គ្រងធន ធាននេះ ហើយត្រូវអនុវត្តយុទ្ធសាស្ត្រនេះរួមគ្នាសាពេលអនាគត ។

ABSTRACT

The application of genetic variation is of practical significance in both fisheries management and conservation objectives. Restriction fragment polymorphism of mitochondrial DNA (mtDNA-RFLP) was employed to investigate the genetic diversity and genetic structure in the mtDNA cytochrome b - D-loop region (2.1 kb) of the migratory catfish *Pangasius bocourti* collected in the Cambodia's Mekong River in 2001. We observed seven mtDNA haplotypes among the 90 individuals assayed. On average, intraspecific genetic diversity (i.e. genetic variation) ranged from 0.439 to 0.684. Based on our initial genetic analyses of the total fixation index (G_{ST}) among all samples, pair-wise F_{ST} values and Slatkin's genetic distances, *P. bocourti* seems to represent a single stock in the Cambodia's Mekong River.

The destruction of the spawning ground of this species might have negative impacts on the Cambodia's Mekong River and probably the whole Mekong River basin. Since the stock is shared among the riparian countries of the Mekong, holistic and basin-wide resource management strategies have to be developed and implemented.

KEYWORDS

conservation, fisheries, management, mtDNA, Pangasiidae, restriction fragment length polymorphism (RFLP).

INTRODUCTION

Among the world largest and biologically richest rivers, including the Yangtze and Xijiang (East Asia), Amazon (South America), Mississippi (North America), Congo (Africa) and Danube (Europe), the Mekong River basin is no exception in its faunal characteristics (Banareescu 1992; Myers et al., 2000). With a mean annual discharge of 475 x 10⁹ m³ y⁻¹ the Mekong has its source in High Asia, its middle course flows through the province of Yunnan (P.R. China), the lower course and its tributaries drain Laos, Cambodia, southern Vietnam and Thailand. Its aquatic fauna is very rich with at least 1,200 fish species (Rainboth, 1996). Furthermore, annual floods inundate larger areas, establishing temporary connections between various water-bodies that otherwise remain isolated during the dry season. These flooded areas provide abundant food, spawning areas and fry nurseries for fish. Many fish species migrate laterally between the floodplain and the deeper lake or tributaries, or carry out longitudinal migrations to and within the main Mekong stream. Typically many species spawn within the main Mekong River and its tributaries. Eggs and larvae are swept downstream by the river and into the floodplain where they grow. Other species may spawn directly on the floodplain and in the swollen lakes and reservoirs. The long-range migrations undertaken by some species appear to be spawning migrations, when the fish home to particular spawning areas (Singhanouvong et al., 1996). Environmental deterioration from human activities has disrupted migrations and decreased fish populations.

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Running title: Genetic diversity of *Pangasius bocourti*

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The Asian catfish family of the Pangasiidae includes riverine fishes generally occurring in freshwater, with exception of *Pangasius pangasius*, *P. polyuranodon*, *P. krempfi*, and *P. kunyit*, which may enter in saline waters. They occur from the Indian subcontinent and Burma to continental Southeast Asia (Indochina/Mekong) and Insular Southeast Asia (Indo-Malay Archipelago) (Roberts and Vidthayanon, 1991; Vidthayanon, 1993; Pouyaud et al., 1999). Pangasiidae are moderately to very large catfishes, with at least 21 living species (Vidthayanon, 1993; Rainboth, 1996). They are an important fisheries resource, some of them being cultured widely and some well known as aquarium fishes.

Today, unfortunately, human activities along the Mekong River watershed are increasing rapidly and threaten this heritage of mankind. Fish yield appears to be related to variations in the extent of the yearly flood regime (Ahmed et al., 1998). Since the 1950's nearly 4,000 large and small dams and associated reservoirs and irrigation schemes have been built in the Mekong watershed. This has led to large reductions in the coverage of aquatic habitats, the blocking of accesses of migratory fish species to spawning and fry nursery areas, the altering of the level and quality of water, and the ending of the seasonal ebb and flow that is vital to the cycle of mating and reproduction (Robert, 1993a; Moreau and Ernsberger, 2001). This has resulted in decreasing Mekong populations, including *Pangasius bocourti* (Roberts, 1993b). Hence assessment of population diversity and structure of *Pangasius bocourti* represents imperative scientific information.

Pangasius bocourti (Trey Pra Kchao in Cambodian) (Pangasiidae, Teleostei) plays a major role in the Mekong River ecosystem due to their migratory behavior, feeding ecology, development of aquaculture and commercial values as popular food fish. It is an omnivore feeding on fruit and plant material (Vidthayanon, 1993; Singhanouvong et al., 1996; FishBase, 2002) and molluscs, crustaceans and small fish in nature (Nam So, 2001: pers. obs.). The total estimated production of *P. bocourti* and *Pangasianodon hypophthalmus* was 20,000-40,000 tons for pond culture and 40,000-50,000 tons for cage culture in the An Giang and Dong Thap provinces of the Mekong delta in 2000 (Tung et al., 2001; Tong et al., 2002).

Pangasius bocourti might spawn in the Mekong River between the north end of Koh Rongiev (Kratie), through Koh Kha Nhae and Koh Ou Kande next to the border at Stung Treng province and below the Khone Falls. Fish may concentrate in the Stung Treng province from Koh Baychor (Siembok district) near the Kratie border to Koh Kei (Thalaboriwath district) during April-July (Touch, 2000: pers. comm.; Nam So, 2001: pers. obs.). Poulsen and Valbo-Jorgensen (2001) reported that spawning of *P. bocourti* may take place between Kratie and Stung Treng provinces. The upstream migration of adult fish towards the spawning grounds occurs from November to March and the downstream migration from April to October, followed by the fry and fingerling (Poulsen and Valbo-Jorgensen, 2001).

Within species diversity might be partitioned into variation within and among populations (Wright, 1968). It is necessary to maintain both types of variation to minimize the frequency of extirpation of local populations and to sustain species stability since genetic diversity is a requisite for evolutionary adaptation to a changing environment (Avise, 2000; Frankham, 2002). So far, genetic diversity and genetic differentiation at the population levels has proven to be the

best method to manage the conservation of species (Templeton et al., 1995; Crandall et al., 2000), including fisheries (Ryman & Utter, 1987; Waples, 1991; Pullin et al., 1999). However, their application, particularly in tropical regions, is still in its infancy.

The specific objectives of this study are: (1) to optimize the mitochondrial DNA RFLP markers of *P. bocourti*; (2) to examine genetic diversity, levels of genetic differentiation and population structure; and (3) to assess the implications for fishery management of this species in the Cambodian Mekong River.

MATERIALS AND METHODS

Biological material

Pangasius bocourti samples were collected from five major watersheds in the Cambodia's Mekong River (Fig. 1). Samples were typically taken at three life stages: adult, sub-adult and fingerling. There were difficulties to sample adult fish, therefore fingerlings include about 60% of the fish genotyped. A total of 90 specimens were collected from five sampling localities (Stung Treng, Kratie, Prey Veng, Bassac: Kandal, and Kampong Chhnang), with the sample sizes varying from 12 to 20 individuals per location (Table 1). Tissue samples consisted of fin clips, although muscle tissue was occasionally used. Most of the samples were collected from cage culture operations along the Tonle Sap and Mekong River; some samples were collected from fishers and retailers. Attention was paid to make sure that fish specimens sampled from each location originated from that locality in order to avoid mixing of populations. Fin clips were stored in a salt-saturated DMSO solution (20% DMSO; 250 mM Ethylenediaminetetracetic acid and 5 M NaCl) and kept at room temperature for transport to the laboratory for further DNA analysis. They were transferred to pure ethanol and stored at room temperature upon arrival.

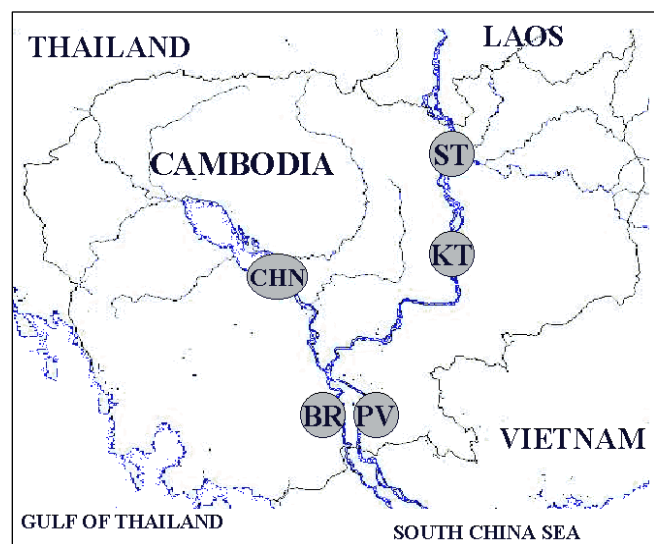


Figure 1. Map of Cambodia showing the geographical locations (named in Table 1) of *Pangasius bocourti* sampled from the Cambodia's Mekong River basin.

Each specimen was identified and classified according to Roberts and Vidthayanon (1991), Vidthayanon (1993) and Rainboth (1996).

Table 1 Sampling locations and sample sizes for *Pangasius bocourti* sampled in the Cambodia's Mekong River. Letters (BR, CHN, KT, PV and ST) correspond to sampling sites as illustrated in Fig. 1.

Sampling site	Sample size
ST Mekong Stung Treng	20
KT Mekong Kratie	12
PV Mekong Prey Veng	19
BR Bassac River Kandal	19
CHN Tonle Sap Kampong Chhnang	20

MtDNA extraction and RFLP genetic analysis

Tissue of 50-100 mg was digested with proteinase K in CTAB (Cetyltrimethyl-ammonium bromide) buffer and incubated at 55°C overnight. DNA was extracted using a standard phenol-chloroform extraction procedure (Sambrook et al., 1989).

The primers used to amplify the cytochrome b (cytb) and D-loop regions were the complimentary ND5/6 and HN20 (Bernatchez and Danzmann, 1993). Polymerase chain reaction (PCR) mixture contained approximately 10-100 ng of DNA, 0.8 µM of forward and reverse primer each, 200 µM of each dNTP, 0.5 U of Globalstar Taq polymerase (Eurogentec, Seraing, Belgium) and the corresponding 1x reaction buffer, 2 mM MgCl₂ and water in a final volume of 25 µl. Amplification was performed in a thermal cycler (Trioblock, Biometra, Goettingen, Germany) programmed as follows: 95°C for 3 min, 35 cycles at 95°C for 30 s, 46°C for 30 s, 72°C for 2 min 30, followed by a final extension at 72°C for 7 min. PCR amplification yielded a fragment of approximately 2100 base pairs, which were visualized by ethidium bromide in 1.2% agarose gel.

The amplified fragments were subsequently screened for polymorphism using five restriction endonucleases: HinfI, MboI, AluI, TasI, and FnuDII. All these enzymes recognize tetranucleotide palindromic sequences. Five or 7 µl of each

PCR product were digested overnight with 10 U of the restriction enzyme in a final volume of 10-12 µl. The restriction fragments were visualized under UV light on 1.2 - 2% agarose gels stained with ethidium bromide. The molecular size of each restriction fragment was measured by densitometry (Gel documentation system, Amersham Biosciences, New Jersey, USA), based on comparison with a comigrating 100 bp molecular weight ladder.

The RFLP pattern produced by each endonuclease was assigned a letter so that each composite mtDNA haplotype was defined by a five-letter code (Table 2).

Data analysis

Endonuclease fragment patterns could be interpreted easily in terms of gain or loss of restriction fragments (Table 2), which were used as data to analyze genetic relationships within *P. bocourti*.

Data analysis, including mtDNA gene diversity (within population) (Nei, 1987), total fixation index (G_{ST}) among populations (Weir and Cockerham, 1984; Long 1986), pairwise F_{ST} values between populations (Reynolds, Weir and Cockerham, 1983; Slatkin, 1995) and genetic distances (Slatkin 1995) was done with the software package ARLEQUIN version 2.0 (Schneider et al., 2000).

Results*Distribution of mtDNA haplotypes*

Of the five restriction enzymes used, three (HinfI, MboI and AluI) were polymorphic in *Pangasius bocourti* (Table 2).

We observed seven mtDNA haplotypes among the 90 fish tested in the five samples (Table 2). The number of haplotypes within a single sampling locality varied from three to six: four were detected at Stung Treng, three at Kratie, five at Prey Veng, three at Bassac river and six at Kampong Chhnang. Haplotypes Pb1, Pb2 and Pb3 added up to 79 (87%) out of the 90 fish analyzed. The dominant haplotype (Pb1) was found in 55 (61%) *P. bocourti*. At Kratie, 75% of fish tested had the common haplotype (Pb1), followed by 70% at Stung Treng. Three singletons (unique haplotypes) were detected: two (Pb6 and Pb7) at Kampong Chhnang and one (Pb5) at Prey Veng.

Table 2. Absolute (in brackets) and relative frequencies and distribution of the composite haplotypes and presence/absence of restriction fragments resolved among *Pangasius bocourti*. Restriction enzymes are HinfI, MboI, AluI, TasI, and FnuDII. Capital letters identify fragment patterns. Abbreviation letters refer to sample locations illustrated in Fig. 1. HC denotes Haplotype Code.

HC	Haplotype	Restriction fragments	ST	KT	PV	BR	CHN	Total
			% (n=20)	% (n=12)	% (n=19)	% (n=19)	% (n=20)	% (n=90)
Pb1	AAAAA	1011111010011101111010111111111111	70 (14)	75 (9)	53 (10)	58 (11)	55 (11)	62 (55)
Pb2	AABAA	101111101001110111101100111111111111	10 (2)	8 (1)	16 (3)	11 (2)	15 (3)	14 (13)
Pb3	ABAAA	101111101101010101001011111111111111	10 (2)	-	21 (4)	31 (5)	10 (2)	12 (11)
Pb4	ABBAA	101111101101010101001100111111111111	10 (2)	17 (2)	5 (1)	-	10 (2)	9 (8)
Pb5	ACAAA	101111101001111101011011111111111111	-	-	5 (1)	-	-	1 (1)
Pb6	ADBAA	101111101110010001101100111111111111	-	-	-	-	5 (1)	1 (1)
Pb7	BBBAA	011111110101010101001100111111111111	-	-	-	-	5 (1)	1 (1)

mtDNA haplotype diversity

The restriction fragments for each endonuclease in *Pangasius bocourti* (Table 2) were used to estimate genetic variation. Gene diversity was estimated to measure population fitness. We detected an average gene diversity of 0.505 at Stung Treng, 0.439 at Kratie, 0.684 at Prey Veng, 0.614 at Basac, 0.684 at Kampong Chhnang (Table 3).

Table 3. Sampling sites, number of individuals used for RCR-RFLP analysis, number of mtDNA haplotypes and gene diversity for *Pangasius bocourti* sampled in the Cambodia's Mekong River.

Sampling site	Number of individuals	Number of haplotypes	Gene diversity
ST	20	4	0.505 ± 0.125
KT	12	3	0.439 ± 0.158
PV	19	5	0.684 ± 0.092
BR	19	4	0.614 ± 0.095
CHN	20	6	0.684 ± 0.103

Table 4. Pairwise F_{ST} values (above diagonal) and corrected Slatkin's genetic distances (below diagonal) of *Pangasius bocourti*. No value is significantly different at $P < 0.05$.

	BR	CHN	KT	PV	ST
BR	-	0.0000	0.0000	0.0000	0.0000
CHN	-0.0108	-	0.0000	0.0000	0.0000
KT	-0.024	-0.0356	-	0.0000	0.0000
PV	-0.048	-0.0262	-0.0435	-	0.0000
ST	-0.029	-0.0123	-0.0657	-0.0374	-

DISCUSSION

Based on the mtDNA PCR-RFLP analysis, it is obvious that: (1) The catfish *Pangasius bocourti* has a moderate level of genetic diversity in the Cambodia's Mekong River; (2) The populations of *P. bocourti* are not significantly differentiated; and (3) There is no obvious evidence for genetic structure in *P. bocourti* in the Cambodia's Mekong River.

P. bocourti samples had a similar distribution of mtDNA haplotypes in the Cambodia's Mekong River. Two haplotypes were ubiquitous and none were frequent and unique to a particular river. At Kampong Chhnang in Tonle Sap the highest number of haplotypes was found, which might suggest that we are either dealing with a mixed group or a more ancestral population. The location is known to be of recent geological origin and as a feeding ground, with an abundance of food and habitats for fish species adapted to periodic water-level change (Lim et al., 1999). Hence we opt for the mixed group hypothesis.

The overall genetic diversity in *P. bocourti* was higher than other fish such as whitefish *Coregonus clupeaformis* in North America, which was reported to range between 0.000-0.351 (Bernatchez and Dodson, 1991) and 0.067-0.700 (Lu et al., 2001); Atlantic salmon *Salmo salar*, 0.00-0.682 (King et al., 2000); brown trout *Salmo trutta* in a Danish river system, 0.000-0.860 (Hansen et al., 1995), in Spain, 0.000-0.712 (Machordom et al., 2000) and in tributaries of the Austrian Danube, 0.181-0.772 (Weiss et al., 2001). However, the values for brown trout (0.511-0.931) in Atlantic, Adriatic, Danubian, Mediterranean and marmoratus areas (Bernatchez, 2001) were higher than *P. bocourti*. Furthermore, the genetic diversity of *P. bocourti* in the Cambodia's Mekong River was higher than the Pimelodid catfishes *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum* in the Bolivian Amazon basin (0.054-0.667) (Coronel et al., 2004).

The reduced genetic diversity at Kratie and Stung Treng might be attributed to four causes: (1) incomplete sampling

(i.e. n = 12, Kratie), (2) stochastic consequences, including genetic drift and inbreeding (Hedrick 1992; Frankham, 2002), (3) historic bottlenecks (Wilson and Bernatchez, 1998), or (4) human-associated effects, including fishing pressure (Ryman and Utter, 1987; Frankham 2002). These genetic consequences might be caused by the small population size assumed to be present at Kratie and Stung Treng. The fisheries in the areas are poorly documented for various reasons and hence no population dynamic data are available.

Based on our analyses, the overall G_{ST} value among all samples suggests very weak or no genetic structure and insignificant differentiation in *P. bocourti*. Additional analyses, including pair-wise F_{ST} values and Slatkin's genetic distances also reflect no genetic structure and differentiation at all along the stretches of the Cambodia's Mekong River.

The Mekong River has, so far, been poorly studied, which complicates a comparative approach. However, one possible comparison could be made with several carp species, which showed minimal phylogeographical differentiation along a 500-km stretch of the Yangtze River in China (Lu et al., 1997). Also, the migratory pirarucu (*Arapaima gigas*) shows a lack of genetic structuring, a high level of gene flow and low effective population sizes in the Amazon basin (Hrbek et al., 2001).

There are no obvious natural or artificial barriers among all sampling sites (Stung Treng, Kratie, Prey Veng, Bassac River and Kampong Chhnang) covering the four major branches of the Cambodia's Mekong River. Hence, it is possible that *P. bocourti* might constitute a single stock, probably due to strong migration or high gene flow among all sampling locations in the Cambodia's Mekong River. This scenario parallels a hypothesis based on ecological surveys along the Mekong River using indigenous fishers' knowledge that there are two distinct populations of *P. bocourti* in the Mekong River. One population occurs from the Mekong delta through Cambodia to the Mukdahan-Sovannaket area

in southern Laos; another population occurs from around Boulikhamxay-Nong Kai provinces to around Chiang Rai-Bokeo provinces (Lao-Thai border) in the north (Poulsen and Valbo-Jorgensen, 2001).

In conclusion, the migratory catfish *P. bocourti* should initially be considered as a single stock in the Cambodia's Mekong River basin. The destruction of the spawning ground of this species might have negative impacts on the Cambodia's Mekong River and probably the whole Mekong River basin. Since the stock is shared among the riparian countries of the Mekong, holistic and basin-wide resource management strategies have to be developed and implemented.

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សេចក្តីជូនដំណឹង

វិទ្យាស្ថានស្រាវជ្រាវ និងអភិវឌ្ឍន៍កសិកម្មកម្ពុជា មានកិត្តិយសសូមជម្រាបជូនដំណឹងដល់ លោក លោកស្រី អ្នកនាងកញ្ញា ជាអ្នកស្រាវជ្រាវទាំងអស់ អោយបានជ្រាបថា ដោយយល់ឃើញពីគុណសម្បត្តិ និងសារប្រយោជន៍នៃទស្សនាវដ្តីកសិកម្មកម្ពុជា ក្នុងការផ្តល់លទ្ធភាពជូនអ្នកស្រាវជ្រាវខ្មែរ ទាំងឡាយឱ្យមាន ឱកាសបង្កើនសមត្ថភាពស្រាវជ្រាវរបស់ខ្លួនតាមរយៈការសរសេរ ការបកស្រាយ និងចូលរួមពិភាក្សានូវរាល់គំហើញវិទ្យាសាស្ត្រផ្សេងៗ ដែលជាកត្តាចាំបាច់មិនអាច ខ្វះបានសម្រាប់អ្នកស្រាវជ្រាវ ដើម្បីជាការពង្រឹងវិស័យស្រាវជ្រាវជាតិ ហើយក៏ដើម្បីជាកិត្តិយសដ៏ខ្ពង់ខ្ពស់សម្រាប់ប្រទេសជាតិយើងដែរនោះ វិទ្យាស្ថានស្រាវជ្រាវ និងអភិវឌ្ឍន៍កសិកម្មកម្ពុជា បានខិតខំព្យាយាមជំរុញអោយមានការបង្កើតឡើងនូវទស្សនាវដ្តីកសិកម្មកម្ពុជានេះ និងធ្វើយ៉ាងណាឱ្យទស្សនាវដ្តី នេះបានរស់រានជីវិត ឡើងវិញក្រោយពីត្រូវអាក់ខានមួយរយៈ ។

នាពេលបច្ចុប្បន្ន ក្រោយពីមានការបង្កើតឡើងជាថ្មីនូវក្រុមប្រឹក្សាពិនិត្យ (Editorial Board) របស់ទស្សនាវដ្តី ដែលមានការចូលរួមពីអង្គការពាក់ព័ន្ធ ជាច្រើន វិទ្យាស្ថានបាននិងកំពុងរៀបចំដំណើរការបោះពុម្ពទស្សនាវដ្តីកសិកម្មកម្ពុជា (Cambodian Journal of Agriculture) នេះឱ្យមានជាប្រក្រតីភាពឡើងវិញ ដូចដែលវិទ្យាស្ថានធ្លាប់បានធ្វើការរៀបចំ និងបោះពុម្ពផ្សាយជាហូរហែរកន្លងមកដើម្បីជាការផ្សព្វផ្សាយទាំងក្នុង និងក្រៅប្រទេស ។

អាស្រ័យហេតុនេះដើម្បីអោយទស្សនាវដ្តីនេះអាចមានសកម្មភាព និងដំណើរការទៅមុខបាន វិទ្យាស្ថានស្រាវជ្រាវ និងអភិវឌ្ឍន៍កសិកម្មកម្ពុជា ក៏ដូចជា ក្រុមប្រឹក្សាពិនិត្យនៃទស្សនាវដ្តីកសិកម្មកម្ពុជា មានក្តីសង្ឃឹមយ៉ាងមុតមាំ និង ជឿជាក់ចំពោះការចូលរួមគាំទ្រពីសំណាក់ លោក លោកស្រី អ្នកនាង កញ្ញា ទាំងឡាយ ដែលមានបំណងចង់បង្ហាញពី ការរកឃើញវិទ្យាសាស្ត្រផ្សេងៗ ក៏ដូចជាបទពិសោធន៍ល្អៗជូនដល់អ្នកស្រាវជ្រាវដទៃទៀត និងក៏ដូចជាចង់ជួយពង្រឹង វិស័យស្រាវជ្រាវជាតិយើងផងដែរក្នុងការផ្តល់នូវអត្ថបទស្រាវជ្រាវផ្សេងៗសម្រាប់ជាការបោះពុម្ពក្នុងទស្សនាវដ្តី ។

សូមអរគុណ

ព័ត៌មានបន្ថែមសូមទំនាក់ទំនង:

វិទ្យាស្ថានស្រាវជ្រាវ និងអភិវឌ្ឍន៍កសិកម្មកម្ពុជា
ផ្លូវជាតិលេខ ៣ សង្កាត់ប្រទេសឡាង ខណ្ឌដង្កោ រាជធានីភ្នំពេញ ប្រអប់សំបុត្រលេខ ០១ ភ្នំពេញ
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ការណែនាំសម្រាប់អ្នកនិពន្ធ

តម្រូវការទូទៅ

ការបោះពុម្ពផ្សាយនៅក្នុងទស្សនាវដ្តីកសិកម្មកម្ពុជា (CJA) អាចជារបាយការណ៍ដើមនៃលទ្ធផលស្រាវជ្រាវ (អត្ថបទ ឬ កំណត់ត្រាខ្លីៗ) អាចជាលិខិតដែលបញ្ជូនទៅអ្នកត្រួតពិនិត្យ ជាការផ្សព្វផ្សាយពាណិជ្ជកម្ម ឬ ការប្រកាសនូវដំណឹងនានា។ កំណត់ត្រាស្រាវជ្រាវ មិនត្រូវសរសេរលើសពី ២១០០ ពាក្យ ឬ ២១០០ ពាក្យ មិនត្រូវអោយលើសពីកន្លះទំព័រដែរ។

តួអក្សរ និង កំណត់ត្រា

អត្ថបទដែលបានរៀបចំត្រូវធ្វើមកអ្នកនិពន្ធដោយប្រព័ន្ធអេឡិចត្រូនិក (តាមរយៈទូរអក្សរ ឬ តាមរយៈថាស) ក្នុងនោះត្រូវមាន តារាង និងក្រាហ្វិក ឯកសារយោង ចំណងជើងតារាង និងចំណងជើងក្រាហ្វិក។ រូបភាពត្រូវតែជារូបដើម ឬ ថតចម្លង (Scan) អោយច្បាស់ដើម្បីធានានូវគុណភាពរបស់រូបសម្រាប់ទស្សនាវដ្តី។ អត្ថបទដែលធ្វើមកកាន់ទស្សនាវដ្តីកសិកម្មកម្ពុជា (CJA) អាចជាភាសាអង់គ្លេស ឬភាសាខ្មែរ។ ក្នុងករណីដែលអត្ថបទជាភាសាអង់គ្លេសត្រូវប្រើប្រភេទអក្សរ Time New Roman ដោយមានការបកប្រែជាភាសាខ្មែរនូវចំណងជើង និងសង្ខេបដោយប្រើប្រភេទអក្សរ Limon ។ ចំពោះអត្ថបទជាភាសាខ្មែរត្រូវប្រើប្រភេទអក្សរ Limon ដោយមានការបកប្រែជាភាសាអង់គ្លេសនូវចំណងជើង និងសេចក្តីសង្ខេបដោយប្រើប្រភេទអក្សរ Times New Roman ។

រចនាសម្ព័ន្ធ

- ចំណងជើង :** ត្រូវនៅទំព័រទី១ នៃអត្ថបទ ឬ កំណត់ត្រា។ ចំណងជើងត្រូវសរសេរអោយបានខ្លី ប៉ុន្តែច្បាស់លាស់ និងឆ្លើយតបទៅនឹងអត្ថបទ។
- អ្នកនិពន្ធ :** នៅខាងក្រោមចំណងជើង ត្រូវដាក់ឈ្មោះអ្នកនិពន្ធទាំងអស់ដែលពាក់ព័ន្ធក្នុងការស្រាវជ្រាវ។ ដកឃ្លាពីឈ្មោះអ្នកនិពន្ធមួយទៅឈ្មោះ អ្នកនិពន្ធមួយដោយប្រើសញ្ញា Comma (,) ហើយឈ្មោះអ្នកនិពន្ធចុងក្រោយគេត្រូវដាក់ឈ្មោះ "និង" " and " នៅពីមុខ។ ឈ្មោះអ្នកនិពន្ធ/អត្ថបទនានា គួរតែមាននៅក្នុង Footnote នៃទំព័រទី១។ គួររៀបចំជាបញ្ជីនូវឈ្មោះអ្នកនិពន្ធ និងបញ្ជាក់ពីអាសយដ្ឋាន និងឯកសារពាក់ព័ន្ធផ្សេងៗនៅក្នុងឃ្លាទី១នៃ Footnote ហើយក្នុងឃ្លាទី២ គួរដាក់បញ្ចូលនូវ ប្រភពមូលនិធិ ប្រសិនបើទាន់បានបង្ហាញនៅក្នុងសេចក្តីផ្តើមអំណរគុណ។
- សេចក្តីសង្ខេប :** អត្ថបទនីមួយៗត្រូវមានសេចក្តីសង្ខេបជាពីរភាសា គឺភាសាខ្មែរ និងភាសាអង់គ្លេស។ សេចក្តីសង្ខេបត្រូវអោយខ្លីតែច្បាស់លាស់ហើយត្រូវសរសេរមិនលើសពី ២៥០ ពាក្យ សំរាប់អត្ថបទ និង ១៥០ ពាក្យ សំរាប់កំណត់ត្រា។ ត្រូវរៀបរាប់អំពីសនិទានភាព ទិសដៅ វិធីសាស្ត្រ លទ្ធផលគន្លឹះ និង សារៈសំខាន់របស់វា ពិសេសសំរាប់កសិកម្មកម្ពុជា។ បន្ទាប់ពីរៀបសេចក្តីសង្ខេបត្រូវរៀបចំតាមលំដាប់ដោយ សេចក្តីផ្តើម ដែលរួមបញ្ចូលនូវការវិភាគ ទៅលើបណ្តាលយសាស្ត្រពាក់ព័ន្ធហើយបន្តដោយខ្លីៗ សម្ភារៈ វិធីសាស្ត្រ លទ្ធផល ការពិភាក្សា សេចក្តីសន្និដ្ឋាន (តាមចិត្ត) សេចក្តីផ្តើមអំណរគុណ (តាមចិត្ត) និងឯកសារយោង។ លទ្ធផល និងការពិភាក្សាអាចបញ្ចូលគ្នា ហើយសេចក្តីសន្និដ្ឋានអាចមាននៅក្នុងផ្នែកពិភាក្សា។

តារាង : តារាងទាំងអស់ត្រូវដាក់លេខរៀង ហើយត្រូវមានចំណងជើង។ Headnote ដែលមានព័ត៌មានផ្សេងៗពាក់ព័ន្ធទៅនឹងតារាងទាំងមូល គួរចាប់ផ្តើមនៅបន្ទាត់ទីមួយ។ តារាងគួររៀបចំទៅតាមទំហំកូឡោនគំរូរបស់ទស្សនាវដ្តី (ទំហំ ៨ ស.ម ទៅ ២១ ស.ម) ហើយចំនួនកូឡោននៅក្នុងតារាងគួរអោយមានចំនួនតិច។ ការបំបែកចំណងជើងតូចៗ ពីចំណងជើងកូឡោនមេច្រើនពេកគឺមិនល្អទេ ហើយចំណងជើងវែងពេកក៏គួរជៀសវាងដែរ ដោយប្រើការសរសេរពន្យល់ខ្លីៗជំនួសវិញ ដែលការសរសេរទាំងនោះមានលក្ខណៈស៊ីត្តាទៅនឹង Head note ។ តួអក្សរទី១ នៅខាងដើមគួរសរសេរជាអក្សរធំ។

និមិត្តសញ្ញា នៃខ្នាតរង្វាស់ផ្សេងៗ គួរដាក់ក្នុងរង្វង់ក្រចកខាងក្រោមចំណងជើងកូឡោន។ បុព្វបទសំរាប់ឯកតាគួរជ្រើសរើសយ៉ាងណាមិនអោយមានចំនួនលេខច្រើនពេក។ ក្នុងករណីមិនអាចជៀសវាងបានគួរដាក់ចំនួននោះដោយមេគុណ ១០ នូវរាល់តំលៃទាំងឡាយក្នុងតារាង។ កំណត់សំគាល់ខាងក្នុងតារាងគួរតែរក្សាទំហំអក្សរអោយតូច និងត្រូវរក្សាទុកសំរាប់ការបរិយាយជាក់លាក់ផ្សេងៗក្នុងកូឡោន។

បន្ទាត់ផ្នែកអាចដាក់ខាងលើ និងខាងក្រោមចំណងជើងកូឡោន និងនៅខាងក្រោមបង្អស់នៃតារាងតែប៉ុណ្ណោះ។ ចំពោះបន្ទាត់បញ្ជីវិញមិនគួរប្រើទេ។ រាល់តារាងនីមួយៗត្រូវឆ្លើយតបនៅក្នុងអត្ថបទ ហើយចំណុចសំគាល់តូចមួយនៅក្នុងតែមម័រ (Margin) គួរសរសេរបង្ហាញពីទីតាំងពិតប្រាកដរបស់តារាងនៅក្នុងអត្ថបទ។ តារាងខ្លីៗអាចដាក់បញ្ចូលទៅក្នុងអត្ថបទក្នុងលក្ខណៈជាប្រយោគ និងមិនចាំបាច់មានចំណងជើងទេ។ លើកលែងតែក្នុងករណីពិសេសប៉ុណ្ណោះដែលទិន្នន័យអាចត្រូវបានបង្ហាញទាំងក្នុងតារាង និងក្នុងក្រាហ្វិក។ បើពុំនោះទេគួរប្រើក្រាហ្វិកវិញក្នុងករណីចាំបាច់។

ក្រាហ្វិក : ក្រាហ្វិកទាំងឡាយណាដែលមិនល្អ (ឧ. ក្រាហ្វិក ស្ថិតក្នុងទ្រង់ទ្រាយពិបាកអាស/យល់) នឹងត្រូវបញ្ជូនអោយយកទៅពិនិត្យដើម្បីកែសម្រួលឡើងវិញ។ ចំពោះអ្នកនិពន្ធដែលមិនអាចរៀបចំជា ក្រាហ្វិកផ្សេងៗបាន គួរទំនាក់ទំនងជាមួយអ្នកត្រួតពិនិត្យ។ សញ្ញា បូក (+) រឺ គុណ (x) គួររៀបរាប់។ ការពន្យល់ពី និមិត្តសញ្ញាផ្សេងៗគួរតែដាក់នៅក្រោមចំណងជើងនៃក្រាហ្វិក ហើយអក្សរដែលដាក់ក្នុងក្រាហ្វិក គួរមានជាអក្សរវិមា។ អក្សរទាំងពីរនៃក្រាហ្វិក ត្រូវបញ្ជាក់ពី បរិមាណដែលបានវាស់ឡើង ឬរាប់ហើយត្រូវដាក់ឯកតា SI ក្នុងរង្វង់ក្រចក។

រូបថត : រូបថតត្រូវមានគុណភាពច្បាស់ល្អ។ លក្ខណៈសំខាន់ៗនៃរូបថតដែលត្រូវបានបញ្ជាក់គឺច្បាស់លាស់នៅក្នុងអត្ថបទ ត្រូវតែបង្ហាញអោយបានច្បាស់ (ឧ. ដាក់លេខកូដនៅពីលើអក្សរ / ដាក់សញ្ញាព្រួញ)។ រូបថតពណ៌ធម្មជាតិ នឹងត្រូវទទួលយក ប្រសិនបើវាមានសារៈសំខាន់ក្នុងការជួយអោយអោយយល់ពីលទ្ធផល ផ្សេងៗ។

ទារមន្ត្រី : ចំពោះរុក្ខជាតិ, ភ្នាក់ងារចំលងដី និងកត្តាផ្សេងៗ ត្រូវសរសេរជាអក្សរឡាតាំងក្នុងទំរង់ទ្រេត និងអ្នកដែលបានប្រើប្រាស់/បរិយាយមុនគេ (ឧ. rice, *oryza sativa* L.)។

ខ្នាតរង្វាស់ : ប្រព័ន្ធខ្នាតរង្វាស់អន្តរជាតិ (SI) ត្រូវយកមកប្រើប្រាស់ក្នុងរាល់អត្ថបទដែលត្រូវផ្ញើមកទស្សនាវដ្តីកសិកម្មកម្ពុជា។ ខ្នាតរង្វាស់ផ្សេងទៀតអាចបង្ហាញ នៅក្នុងរង្វង់ក្រចកខាងក្រោយខ្នាតរង្វាស់ SI បើសិនជាខ្នាតរង្វាស់ទាំងនេះអាចជួយសម្រួលអោយការសម្រេចតែងតែងយល់អំពីការងារដែលបាន រៀបរាប់ ពីខាងដើម។ ខ្នាតរង្វាស់ដែលត្រូវភ្ជាប់គ្នាពីរដង មិនត្រូវប្រើប្រាស់ទាំងនៅក្នុងទំរង់ដាច់កតាស្តុកស្តាញពេកទេ (ឧ. គួរប្រើ mg/sheep. day, មិនគួរប្រើ mg/sheep/day or mg⁻¹ sheep⁻¹ day⁻¹)។ ទស្សនាវដ្តីកសិកម្មកម្ពុជា ត្រូវប្រើអក្សរកាត់ "L" សំរាប់ឯកតាគិតជា លីត្រ "mL" សំរាប់ ឯកតាគិតជា មីលីលីត្រ។ ខ្នាតរង្វាស់សំរាប់ប្រើប្រាស់ ក្នុងបណ្តុរអ៊ីយ៉ុង (mmol/kg) គួរប្រើចំពោះប្រភេទបណ្តុរ អ៊ីយ៉ុងទោល ឧ. Na⁺, K⁺, CaO.5⁺។ ឯកតាដែលណែនាំអោយប្រើ សំរាប់បណ្តុរអ៊ីយ៉ុង និងសំរាប់សមត្ថភាពបណ្តុរអ៊ីយ៉ុង គឺ cmol(+)/kg [ឬ cmol(-)/kg] កន្លែងដែលមានបញ្ជា (+) រឺ (-) គឺសំដៅលើអាយ៉ុង និងការចុង (បន្តកអគ្គិសនី)។ ឯក- តាដែលណែនាំអោយប្រើសំរាប់ថាមពលកំដៅ អគ្គិសនី គឺ dS/m ឬន្លែខ្នាត mS/cm ត្រូវបានគេទទួលស្គាល់ជាង។

ការវាយតម្លៃលើលទ្ធផល

អត្ថបទស្រាវជ្រាវត្រូវមានការពិពណ៌នាដោយសង្ខេប និងច្បាស់លាស់ ស្តីពីវិធីរៀបចំប្លង់ពិសោធន៍ និងលំអិត ក្នុងករណីដែលការវិភាគវិវិយ័ង ឬការ វិភាគតាម Regression Models ត្រូវបានប្រើក្នុងការវាយតម្លៃដើម្បីអោយអ្នកអានអោយយល់ច្បាស់អំពីវិធីគណនាករិតលំអៀង។ ការវិភាគស្ថិតិ គួរពិពណ៌នា ដោយសង្ខេប ហើយប្រសិនបើចាំបាច់ត្រូវភ្ជាប់ឯកសារយោងជាជំនួយផង។ ចំនួនឯកតាតម្លៃមធ្យម និងរង្វាស់អំពីបំរែបំរួលផ្សេងៗគួរត្រូវបានបង្ហាញ។

ឯកសារយោង

ឯកសារយោង : ឯកសារយោង ត្រូវបានលើកយកមកសម្រាប់អានដោយឈ្មោះអ្នកនិពន្ធ និងមានដាក់កាលបរិច្ឆេទច្បាស់លាស់ (ប្រព័ន្ធរបស់ លោក Harvard) ហើយមិនត្រូវសរសេរជាលេខទេ។ រាល់ឯកសារយោងទាំងអស់នៅក្នុងអត្ថបទ ត្រូវដាក់បញ្ចូលទៅក្នុងបញ្ជីនៅទំព័រចុងក្រោយបំផុតនៃទស្សនាវដ្តី ដោយមាន បញ្ជាក់ពីឈ្មោះអ្នកនិពន្ធ ដែលត្រូវរៀបរាប់តាមអក្សរក្រចក។ រាល់ឯកសារយោងដែលបានបញ្ចូលទៅក្នុងបញ្ជី ត្រូវតែដូចគ្នាទៅនឹងឯកសារយោងនៅក្នុង អត្ថបទ។ នៅក្នុងអត្ថបទ ឈ្មោះរបស់សហអ្នកនិពន្ធពីរនាក់ត្រូវភ្ជាប់ដោយឈ្មោះ "និង" ឬប្រើប្រាស់ពីបីនាក់ឡើងទៅ ដាក់ឈ្មោះអ្នកនិពន្ធទី១ រួចបន្តដោយ 'et al.'។ ចំនួន- ដែលមានឯកសារយោងលើសពីមួយនៅក្នុងអត្ថបទ ឯកសារយោងទាំងនោះត្រូវដាក់តាមកាលប្បវត្តិគ្រឹមត្រូវ។ ចំណងជើងឯកសារនិងលេខទំព័រ ដំបូង និងខាងចុង បំផុតត្រូវបង្ហាញក្នុងរាល់ឯកសារយោងទាំងអស់។ អត្ថបទដែលមិនបានទទួលយកទៅបោះពុម្ពមិនអាចដាក់ បញ្ចូលទៅក្នុងបញ្ជីឯកសារ យោងតែអាចបង្ហាញនៅ ក្នុងអត្ថបទដោយពាក្យថា "ទិន្នន័យមិនបានបោះពុម្ពផ្សាយ" ឬ "ទស្សនៈផ្ទាល់ខ្លួន"។ ប៉ុន្តែការប្រើប្រាស់ឯកសារយោងទាំងនេះគឺមិន ត្រូវបានលើកទឹកចិត្តអោយប្រើ ទេ។ អ្នកនិពន្ធទាំងអស់គួរតែយកលំនាំតាមទស្សនាវដ្តី ដែលទើបនឹងចេញផ្សាយឱ្យបំផុតនូវរបៀបបង្ហាញឯកសារយោងផ្សេងៗ ទាំងក្នុងសៀវភៅ និងក្នុងអក្សរសិល្ប៍ ផ្សេងៗ។ ចំណងជើងពេញនៃសាមញ្ញកម្មត្រូវតែដាក់បង្ហាញមកជាមួយដែរ។

ខាងក្រោមនេះនឹងបង្ហាញពីគំរូខ្លះៗ នៃរបៀបដាក់ឯកសារយោងក្នុងអត្ថបទ :

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ការព្រួយបារម្ភ

ដើម្បីផ្តល់អត្ថបទអោយមកបោះពុម្ពផ្សាយ ត្រូវធានាថាលទ្ធផលដែលបានធ្វើរបាយការណ៍មិនបាន ឬមិនធ្លាប់បោះពុម្ពផ្សាយ ឬក៏ពុំត្រូវបោះពុម្ពផ្សាយនៅកន្លែងណាផ្សេង។ សេក្តីសង្ខេបលទ្ធផលនៃការរកឃើញនៃសន្និសីទ ឬនៅក្នុងអត្ថបទបោះពុម្ពផ្សាយណាមួយមិនត្រូវបានចាត់ទុកជាការបោះពុម្ពផ្សាយជាមុននោះទេ។ ទោះបីជាយ៉ាងណាក៏ដោយ ប្រសិនបើមិនយល់ច្រើនដូចជាតារាង និងក្រាហ្វិក ត្រូវបានបោះពុម្ពផ្សាយមុនហើយនោះ ការបន្ថែមមិននឹងខ្លះៗទៀតមិនអាចចាត់ទុកថាអត្ថបទនោះជាអត្ថបទថ្មីឡើយ។ ចំពោះអត្ថបទដែលមានអ្នកនិពន្ធច្រើនការផ្តល់នូវសំដាប់បោះពុម្ពដោយទស្សនាវដ្តី ត្រូវបានចាត់ទុកថាមានការរកភាពគ្នារវាងអ្នកនិពន្ធទាំងនោះ។ ពេលផ្តល់អត្ថបទដល់ទស្សនាវដ្តីអ្នកនិពន្ធទាំងអស់ត្រូវចុះហត្ថលេខាលើបែបបទ "អាជ្ញាប័ណ្ណបោះពុម្ពផ្សាយ"។

អាសយដ្ឋានទំនាក់ទំនងសម្រាប់ការផ្តល់អត្ថបទ

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រាល់អត្ថបទទាំងអស់ត្រូវត្រួតពិនិត្យដោយអ្នកជំនាញ យ៉ាងតិចណាស់ពី ០២នាក់ ឡើងទៅ។

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Contributions to the *Cambodian Journal of Agriculture* (CJA) may be original reports of research (paper or note), letters to the editor, advertisements, or announcements. Research notes should not be more than two pages in length, while advertisements or announcements should not be more than ½ pages.

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Manuscripts should be submitted electronically, including any tables and figures, the references, table heads and figure captions. Photos must be original or scanned at magazine quality. The manuscript submitted to CJA can be in English (US) or in Khmer. In case the manuscript is in English, the text should be in Times New Roman font with a Khmer translation of the title and abstract in Limon font. For Khmer manuscript, the text should be in Limon font with an English translation of the title and abstract in Times New Roman.

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Style

Tables: Table must be numbered and each must be accompanied by a title. A head note containing material relevant to the whole Table should start on a new line. Table should be arranged with regard to the dimensions of the Journal columns (8 by 21 cm), and the number of column in the Table should be kept to a minimum. Excessive subdivision of column headings is undesirable and long heading should be avoided by the use of explanatory notes that should be incorporated into the head note. The first letter, only, of headings should be capitalized. Use asterisk (*, **, ***) only to indicate statistical significance at 0.05, 0.01, and 0.001 levels of probability, respectively.

The symbol of unit of measurement should be placed in parentheses beneath the column heading. The prefixes for units should be chosen to avoid an excessive number of digits in the body of the Table or scaling factors in the headings. When scaling factors cannot be avoided, the quantity expressed should be preceded by the power of 10 by which the value has been multiplied. Footnotes should be kept to a minimum and be reserved of specific items in the columns.

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Photographs. Photographs must be of the highest quality, with a full range of tones and of good contrast. Important features to which attention has been drawn in the text should be indicated (i.e. by coded upper case letters and/ or arrows). Colour photographs will be accepted if they are essential to understanding the results.

Nomenclature: For plants, pathogens, insects and pests, give the Latin binomial in italics and the authority that first mention in the abstract or text (eg. rice (*Oryza sativa* L.)).

Units of measurement: The International system of units (SI) must be used in all manuscripts submitted to the *Cambodian Journal of Agriculture*. Other units may be indicated in parentheses after the SI units if this helps in understanding the work reported. The double solidus must not be used in complex groupings of units (i.e. use mg/sheep. day, not mg/sheep/ day or mg⁻¹ sheep⁻¹ day⁻¹). The CJA uses the abbreviation 'L' for litre 'mL' for millilitre. The units for exchangeable ions (mmol/kg) should be used for single charged ionic species, eg. N⁺, K⁺, CaO.5⁺. The recommended unit for exchangeable ions and ion exchange capacity is cmol(+)/kg [or cmol(-)/kg], where (+) or (-) refers to a unit charge. This recommended unit is numerically equivalent to the non-SI but still widely used mill equivalents per 100g. The recommended unit for electrical conductivity is dS/m, but mS/cm is acceptable.

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Research paper must contain a clear and concise description of the experimental design used with sufficient detail such that, in the case

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Hubick KT, Farquhar GD, Shorter R (1986) Correlation between water-use efficiency and carbon isotope discriminations in divers peanut (*Archis*) germplasm. *Australian Journal of Plant physiology* 13, 803-816.

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Blackmore DJ (1996) Are rural land practices a threat to the environment? In 'Soil science-raising the profile'. (Ed. N Uren) pp. 22-30. (ASSSI and NZSSS: Melbourne)

Wolanski E, Mazda Y, Ridd P (1992) Mangrove hydrodynamics. In 'Tropical mangrove ecosystem'. (Eds AI Robertson, DM Alongi) pp. 43-62. (American Geophysical Union: Washington DC).

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Silver MW (1970) An experimental approach to the taxonomy of the genus *Enteromorpha* (L.) Link. PhD Thesis, University of Liverpool, UK.

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dale GM, Wolf L (1981) The natural distribution of *Eucalyptus* in Australia. Australian National Parks and Wildlife Service.

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Hayman PT, Collett IJ (1996) Estimating soil water: to kick, to stick, to core or computer? In 'Proceeding of the 8th Australian agronomy conference'. Toowoomba (Ed. M Asghar) p.664 (The Australian Society of Agronomy: Toowoomba, Qld)

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