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RESEARCH PROJECT PROPOSALS



As agreed during the Round Table Discussion, each country will develop its own appropriate selective breeding protocols and other related studies. In this connection, project research proposals were submitted and reviewed during the Round Table Discussion taking into consideration resources available and making sure that there will be delineation of efforts.

Thus, the following research activities will be conducted in respective participating countries:

- 1. Genetic improvement of *Macrobrachium rosenbergii* – Indonesia**
- 2. Genetic characterization, domestication and genetic improvement of *Macrobrachium rosenbergii* - Philippines**
- 3. Selective breeding program for genetic improvement of *Macrobrachium rosenbergii* – Thailand**

The Summary Proposals of each research activity are included in the following pages of this Report.





SUMMARY PROPOSAL



ACTIVITY TITLE : Genetic Improvement of *Macrobrachium rosenbergii*

COUNTRY : Indonesia

STATION : Sukabumi - Sukamandi

SOURCE OF EXTERNAL FUNDING {If any}: GOI

DURATION: In Months {indicate total months}: 24 months

DATE STARTED: January 2004

EXPECTED DATE OF COMPLETION: December 2005

PROPOSERS {Name}:	PARTICIPATION {% time}
1. Dr. Estu Nugroho	75
2. Mr. Maskur	75
3. Mrs. Lies Emmawati	100
4. Mr. Agus Sasongko	100
5. Dr. Ketut Sugama	25

RATIONALE

Freshwater prawn population in Indonesia is unique and the geographical distribution of about 19 species covers almost all the islands of Indonesia, thus Indonesia has been recognized as the center of origin of the freshwater prawn. However the potential genetic resource is not yet utilized in the country's freshwater prawn culture industry. Despite the development of the freshwater prawn culture in Indonesia, some problems have been found, e.g. declining growth rate, low survival rate, low resistance to diseases and small edible portion. An alternative means to solve these problems is to produce good quality seeds through the application of selective breeding.

OBJECTIVES

The main objective of this activity is to produce high quality seeds and develop a stock candidate breeders to support the development of a sustainable freshwater prawn culture industry.

BRIEF METHODOLOGY

1. Broodstock collection

Giant freshwater prawns will be collected from South Kalimantan, South Sumatera, South Sulawesi and Bali. Collection from the wild would be taken for at least 100 pairs of prawns from each source.

2. Characterization using molecular marker

Microsatellite DNA and mitochondria DNA will be used to examine the freshwater prawn stocks collected from the various sites.

3. Tagging system

Plate of plastic will be used for tagging to identify the broodstock collected from each site.

4. Breeding system

The breeding system would be developed for full-sibs type for at least five pairs from



each collection site. After which the brood stock will be reared in concrete tanks.

5. Larval rearing

Larval rearing of *Macrobrachium* would make use of the clear water system at a density of 50 – 100 larvae/l. Artificial feed and Artemia will be used in the larval rearing lasting for 35 days.

6. Nursery of the post larvae

Nursery of the post larvae would make use of concrete tanks or earthen ponds and stocking of post larvae will be 25 – 50 individuals/l for 30 days rearing. Feeding rate of about 10 –15 %/day will be adopted at feeding frequency of 3 – 4 times daily.

7. Grow-out culture of fingerlings

Grow-out culture of fingerlings will be done in earthen ponds at a stocking density of 5 – 7 individuals/m² for 5 months culture. Feeding rate will be about 3 – 15 %/day and a feeding frequency of 3 – 4 times daily.

8. Selection

Combination of family and individual selection will be used to improve the growth rate and salinity tolerance of *Macrobrachium rosenbergii*.

9. Multiple location testing

Offspring obtained from the selection and control lines will be tested in different locations of the country to evaluate the culture potentials of the stocks.

KEY REFERENCE:

World fish, SEAFDEC, BFAR, INGA, INFIGRAD, IDRC

SCHEDULE OF ACTIVITIES: YEAR 2004

Activity	J	F	M	A	M	J	J	A	S	O	N	D
1. Preparation	█	█										
2. Evaluation			█	█								
3. Selection				█	█	█	█	█	█	█	█	█
4. Hybridization					█	█	█	█	█	█	█	█
5. Multi location testing								█	█	█	█	█
6. Mass Production								█	█	█	█	█



SUMMARY PROPOSAL



ACTIVITY TITLE: Genetic characterization, domestication and genetic improvement of *Macrobrachium rosenbergii*

COUNTRY: Philippines

STATION(S): BFAR-NIFTDC, BFAR-NFFTC, SEAFDEC/AQD

SOURCE OF EXTERNAL FUNDING {If any}: ASEAN-SEAFDEC Special Five-Year Program, Philippine Government

DURATION: In Months {indicate total months}: 24 mos. (Phase I), 36 mos. (Phase II)

DATE STARTED: January 2004

EXPECTED DATE OF COMPLETION: December 2005 (Phase I)

PROPONENTS {Name}:

PARTICIPATION {% time}

Westly R. Rosario	BFAR	30%
Editha C. Roxas	BFAR	50%
Melchor Tayamen	BFAR	30%
Jodecel Danting	BFAR	50%
Maria Rowena R. Eguia	SEAFDEC/AQD	50%
Maria Lourdes C. Aralar	SEAFDEC/AQD	50%
Manuel A. Laron	SEAFDEC/AQD	50%

RATIONALE

The giant freshwater prawn (*Macrobrachium rosenbergii*) is native to tropical countries in South and Southeast Asia, parts of Oceania and the Pacific. It has great potential as a species for rural aquaculture as demonstrated by Thailand and Indonesia. Recent findings show that the Philippine stock of *Macrobrachium rosenbergii*, basically an eastern subspecies (*M. rosenbergii rosenbergii* De Man 1895), is different from the eastern subspecies (*M. rosenbergii dacqueti* Sunier 1925) found in India, Thailand, Malaysia and some parts of Indonesia (New, 2002). Hence, there is an urgent need to develop molecular genetic markers to identify and characterize the different subspecies and/or stocks available in the country. There is also a need to continue ongoing studies on morphometric characterization and domestication of local stocks and the refinement of nursery and grow-out technologies, before any efforts to improve growth and survival in existing culture stocks through genetic modification, can be undertaken.

GENERAL OBJECTIVES

1. To determine the genetic diversity of wild and farmed stocks of freshwater prawns,
2. To develop sound broodstock management and selective breeding methods for the maintenance and/or improvement of the genetic quality of farmed *Macrobrachium rosenbergii* stocks in the Philippines; and
3. To produce quality *M. rosenbergii* seedstock for rural aquaculture.



SPECIFIC OBJECTIVES

1. To identify and differentiate *Macrobrachium rosenbergii* from other indigenous *Macrobrachium* species through morphometric and molecular marker methods;
2. To characterize the different local stocks of *Macrobrachium rosenbergii* using allozyme and advanced DNA-based molecular markers;
3. To refine existing breeding and husbandry techniques for the successful domestication of wild *M. rosenbergii* stocks;
4. To develop viable low-input schemes in the production of quality *M. rosenbergii* seedstock;
5. To evaluate economically important performance traits in the different local *M. rosenbergii* stocks;
6. To formulate and adopt, when necessary, appropriate selective breeding methods for the genetic improvement of local *M. rosenbergii* stocks;
7. To develop local experts in freshwater prawn research and farming through training, information exchange and research collaboration with other international organizations and academic institutions (e.g. Tohoku University in Japan and/or Queensland University of Technology in Australia); and
8. To train local farmers on proper broodstock and culture management of *Macrobrachium rosenbergii*

BRIEF DESCRIPTION OF METHODOLOGY

Research Component

Phase I (2004-2005)

Several known stocks of *Macrobrachium rosenbergii*, from different localities in the Philippines shall be collected and characterized genetically through morphometric and molecular marker analysis (allozyme and microsatellites). Other indigenous *Macrobrachium* species shall likewise be collected and genetically screened to enable the development of an index that will be used to identify and differentiate *M. rosenbergii* from other native *Macrobrachium* species. After determining interspecies and interstock differences based on morphological traits and genetic markers, at least three *Macrobrachium rosenbergii* stocks belonging to genetically diverse populations will be used for breeding and domestication studies.

Existing techniques for breeding, larval rearing and grow-out of these local stocks shall be developed and standardized for use in subsequent stock comparison work. The local stocks and possibly one imported domesticated stock (e.g. from Thailand) will be compared for growth and survival in different culture environments (tank, cage and ponds). Simultaneous studies will also be conducted to assess their reproductive efficiencies. A genetic improvement program on Philippine *Macrobrachium rosenbergii* stocks shall be undertaken if the results of the performance evaluation prove that local stocks are genetically deteriorated and/or inferior compared to the imported Asian farmed stock.

Phase II (2006 onwards)

From the earlier stock comparison runs, a component analysis on the variation in culture traits shall



have been performed and the extent by which genotype, environment and/ or both influence growth (and other traits) shall have been noted. From thence we shall determine which domesticated stock (or stocks) is genetically diverse and has the potential for superior culture performance. Several selective breeding schemes shall be tried to further improve growth, survival and reproductive performance of Philippine *Macrobrachium rosenbergii* stocks especially if a large percentage of the phenotypic variation is due to genetic differences and if the selected trait(s) is highly heritable.

Since the thrust is to promote rural aquaculture development, the selective breeding methods that will be tried should be simple enough for the farmers to adopt. A simple mass selection method and a within-family selection scheme with rotational line crossing will be tried to improve performance traits in the Philippine *Macrobrachium rosenbergii* stock. On the other hand, if the stock comparison studies show that non-genetic factors greatly influence culture performance, then the development of optimum breeding and husbandry methods shall be given more emphasis.

Training Component

Phase I (2004-2005)

The recently completed Aquaculture Biotechnology Research Facility (Biotech Lab) established at the SEAFDEC/AQD premises in Tigbauan, Iloilo, Philippines is for the primary use of the Philippine Department of Agriculture-Bureau of Fisheries and Aquatic Resources. This facility has a Molecular Endocrinology and Genetics (MEG) Laboratory where DNA-based population genetics studies can be conducted. To date however, some equipment and chemicals have yet to be purchased for the facility to become fully operational. Genetic screening of the local *Macrobrachium* stocks can be performed in this laboratory if funds for this project can be allocated for the purchase of the necessary chemicals (primers, chemicals for DNA extraction and electrophoresis etc.) and laboratory equipment (e.g. vertical slab electrophoretic apparatus; refrigerated microcentrifuge, etc).

While waiting for the Biotech Lab-MEG laboratory to become fully operational, it is proposed that at least one staff from BFAR will be trained in a population genetics laboratory in Japan (e.g. Tohoku University) where he/she can start doing some sequence analysis on a Philippine *M. rosenbergii* stock. The preliminary research cum training shall cover the development of microsatellite primers for use in subsequent genetic characterization studies. The skills training will be for a month, preferably during the first quarter of 2004 and the work shall be continued in the Biotech-MEG Laboratory in Iloilo.

Apart from the training in Japan, a week-long training workshop on molecular genetic techniques shall be held in mid-2004 at the MEG Laboratory in Iloilo. BFAR and AQD staff will participate in this training course, which will be conducted by a Japanese visiting scientist from Tohoku University and MRR Eguia of SEAFDEC/AQD. It is important that the visiting scientist will assist the Philippine counterpart in ensuring that the population genetics laboratory of the Biotech Lab-MEG Laboratory is fully functional and that the protocol for laboratory analyses are standardized. From thence, analysis of all the collected samples shall be conducted at the Aquabiotech-MEG laboratory. Subsequent training sessions specifically on statistical analysis using population genetics software shall be conducted as well.

Phase II (2006 onwards)

In preparation for the development of a selective breeding program for the Philippine *Macrobrachium rosenbergii*, a training workshop on the freshwater prawn broodstock management and quantitative genetics will be held and participated in by BFAR and AQD staff.



DETAILED METHODOLOGY

Phase I (2004-2005)

Study I: Survey and collection of stocks of *Macrobrachium rosenbergii* and other indigenous *Macrobrachium* species

Stocks of *M. rosenbergii* and other *Macrobrachium* species shall be collected from sites where these indigenous freshwater prawns are found to be abundant. Based on earlier surveys, the giant freshwater prawn is found in at least seven regions (specifically in 18 provinces) in the Philippines (BFAR et al., 2003). Some members of the project team shall either travel to some of these collection sites or coordinate with BFAR regional offices to be able to obtain at least 100 pc. of freshwater prawn (*Macrobrachium* sp.) from each source. These samples will be brought to BFAR-NIFTC in Dagupan, Pangasinan where all collected Philippine wild stocks of *Macrobrachium* sp. will be kept. BFAR-NIFTC shall start domesticating and breeding some of these stocks for the proposed stock comparison studies. BFAR-NIFTC shall publish an updated record of all the possible sources of wild giant freshwater prawns in the Philippines.

Study II: Development of standard morphometric and morphologic indices for the identification of indigenous *Macrobrachium* sp.

BFAR-NIFTC shall serve as a repository of all collected live *Macrobrachium* sp. samples. They shall document samples from each stock by recording specific morphological and meristic parameters at the different growth stages (egg, larva, post-larvae and adult) that can be used to identify stocks or species. The morphometric data that will be obtained in adult *Macrobrachium* samples will be similar to those measured in *Penaeid* sp. (Goswami et al 1986; Lester and Pante, 1992) but with slight modifications.

Study III: Development of molecular genetic markers for Philippine wild stocks of *Macrobrachium* sp.

A. Allozyme variation study

Tissues (eye, muscle, hepatopancreas, pleopods etc.) samples shall be taken from some of the *Macrobrachium* sp. individuals for preliminary (enzyme-tissue specificity) analysis of allozyme markers. Once specific tissues are identified to exhibit good resolution for polymorphic enzyme loci, these shall be subsequently used in the biochemical screening of at least 30 samples from each stock. Allozyme variation in individuals belonging to the different stocks and species shall be analyzed to determine the genetic structure of existing wild populations of *Macrobrachium* sp. and to identify markers that can be used to distinguish the different species and stocks of *Macrobrachium* sp.

B. Microsatellite marker analysis

Preliminary DNA sequencing shall be done (in collaboration with a Japanese/ Australian university) on some *Macrobrachium* samples to identify and characterize microsatellite loci in wild *Macrobrachium* sp. collected from the Philippines. Primers shall be designed and optimum PCR conditions shall be determined for use in amplifying DNA for subsequent microsatellite variation work. Pleopods from at least 30 individuals per stock of wild *Macrobrachium* sp. shall be collected and stored in ethanol for microsatellite marker analysis. About 5-8 microsatellite loci identified to be polymorphic shall be used to characterize stocks and species collected from the different localities.

As in the allozyme study, the genetic structure and genetic variability of the various stocks and species shall be determined and microsatellite markers unique to each species/ stocks shall be identified. Molecular markers, particularly highly variable microsatellites,



are used in monitoring genetic changes in wild and farmed stocks and are important in the conservation and sustainable management of any aquaculture resource (O Connell and Wright, 1997).

Study IV: Development and refinement of procedures for domestication (breeding, larval rearing and grow-out) of wild *M. rosenbergii* stocks

A. Refinement of breeding and larval rearing techniques for *Macrobrachium rosenbergii* - Evaluation of different live food organisms as starter food for freshwater prawn larvae

This study aims to evaluate the potential of different live food organisms as substitute for *Artemia* in rearing *M. rosenbergii* larvae. In experiment 1, five different live foods (*Moina*, *Brachionus sp.*, *Panagrellus redivivus*, *Ceriodaphnia* and *Artemia*) will be evaluated. In experiment 2, the two most promising substitute for *Artemia* will be fed to *M. rosenbergii* larvae at three feeding levels and frequencies. This is to optimize the use of live food. The larval rearing period will end after 34 - 36 days when 80% of the population attains the postlarval stage (Ang and Cheah, 1986). The proximate composition and fatty acid content of the feed samples will be determined before the feeding experiment. Other studies that will develop and promote the use of low-input seed production and larval rearing schemes will also be pursued.

B. Farming of *Macrobrachium rosenbergii* in modular cages in Laguna de Bay

Since the methods for FW prawn culture in tanks and ponds (Rosario, 2001) have been established, this study aims to adopt with appropriate modifications, the existing technology for the culture of *M. rosenbergii* in ponds to a freshwater lake. Specifically this study, which shall be conducted at SEAFDEC/AQD-BFS aims to determine the growth, survival of this prawn as affected by different stocking densities, varying surface area of artificial substrate, seasonal variation in natural food availability and in polyculture with other freshwater fishes (Nile tilapia, bighead carp). The effect of these parameters on the population structure of different morphotypes as well as the degree of heterogenous individual growth (HIG) in male FW prawns will be assessed (Ranjeet and Kurup, 2002). Finally, the viability of a commercial lake-based culture system for *M. rosenbergii* will be evaluated.

Study V: *Stock Evaluation*

A preliminary study will be conducted by W. Rosario and his staff at BFAR-NIFTDC to evaluate the reproductive performance of at least three genetically diverse stocks of Philippine *M. rosenbergii* and an imported stock from Thailand. Larval growth and survival shall likewise be assessed using the same stocks in experiments to be conducted by MA Laron of SEAFDEC/AQD and W. Rosario. The quality of the larvae used for these stock comparison studies will be evaluated based on the condition indices developed by Tayamen and Brown (1999). Growth and survival from juvenile to marketable size will be compared simultaneously in ponds and cages using the same *M. rosenbergii* stocks. Pond-based studies will be implemented in BFAR-NFFTC under the supervision of M. Tayamen while cage-based studies shall be conducted at the Binangonan Freshwater Station of SEAFDEC/AQD by MLC Aralar.

Phase II (2006 onwards)

The breeding goals and methodologies for the second phase (selective breeding component) can be described more clearly and in detail once baseline data from Phase I (esp. on the stock comparison work) become



available. Since the program will end in December 2006, preliminary selective breeding work that has been started in 2006 shall be continued for at least until one generation of selection has been achieved.

EXPECTED OUTPUT

Research Component

From the three-year project, baseline data on the genetic structure of wild and farmed *M. rosenbergii* stocks and other indigenous *Macrobrachium* species shall be generated. This information will serve not only as a guide for fishfarmers in identifying the various commercially important giant freshwater prawn stocks and species, it will also become an important reference for the efficient and sustainable management of this valuable aquaculture resource. Philippine-based technologies on *Macrobrachium* culture is not as advanced as those of Thailand and Indonesia, hence through this project, broodstock and culture management methods in ponds and lake-based cages shall have been refined and optimized to suit local conditions using indigenous freshwater prawn stocks which have the potential for superior growth performance. Although genetic improvement and selection work would still have to be continued beyond the three-year program, the fishfarmers will be able to start producing quality seeds by adopting sound breeding and culture practices developed from the results of the domestication and stock performance evaluation studies.

Training and Extension Component

Through the ASEAN-SEAFDEC IRAP program, more local researchers would have been trained on freshwater prawn farming and genetics research. Technology demonstration workshops will be conducted and extension manuals will be written for the fishfarmers as part of the plan to disseminate the technologies developed from the project.

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SUMMARY PROPOSAL



ACTIVITY TITLE: Selective breeding program for genetic improvement of *Macrobrachium rosenbergii*

COUNTRY: Thailand

STATION(S): Aquatic Animal Genetics Research
and Development Institute
(AAGRDI)

SOURCE OF EXTERNAL FUNDING {If any}: ASEAN-SEAFDEC Special Five-Year Program, Government of Thailand

DURATION: In Months {indicate total months}: At least 24 months

DATE STARTED: February 2004

EXPECTED DATE OF COMPLETION: December 2005

PROPONENT {Name}: 1. Mr. Somchart Sukawong (Director of AAGRDI)	PARTICIPATION {% time} 10
2. Dr. Supattra Uraivan and staff	30
3. Dr. Panom K. Sodsuk and staff	30
4. Dr. Srirat Sodsuk and staff	30
5. Mr. Tanan Sangkorntanakit and staff	25
6. Mr. Somsak Roongtongbaisuri and staff	25
7. Mr. Saravuth Jasoh and staff	25
8. Mr. Wisanuporn Ratanatrivong and staff	25

COORDINATOR: Dr. Panom K. Sodsuk
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OBJECTIVES

1. To evaluate the economic traits performance and genetic variation of 9 crosses from 3 stocks of *Macrobrachium rosenbergii*.
2. To improve the economic traits of the best cross by suitable selection procedure.
3. To do PCR conditional optimization and primer test of microsatellite markers that have been developed for *Macrobrachium rosenbergii* by the AAGRDI.
4. To apply polymorphism system of molecular markers (allozymes and/or microsatellites) in the genetic variation evaluation.
(Allozyme markers will be basically and initially used, and microsatellites may be additionally applied later for further selective breeding program.)



BRIEF METHODOLOGY

1. Microsatellite markers

- 1.1 PCR condition of microsatellite primers will be optimized taking into consideration the annealing temperature, amount of DNA template, MgCl₂, primer and enzyme concentration, etc.
- 1.2 The primers will be tested through a number of trials for the screening of the *Macrobrachium rosenbergii* samples from different stocks using the optimized PCR condition.

2. Selective breeding program

- 2.1 Reciprocal crosses of 3 stocks of *Macrobrachium rosenbergii* (1 wild and 2 domesticated stocks) producing 9 crosses will be carried out at the two AAGRDI hatcheries (Pathumthani and Phetchaburi).
- 2.2 Performance will be evaluated between the 9 crosses under 4 environmental conditions in four different provincial areas, Pathumthani, Uttaradit, Chumphon and Burirum. In each environment, all crosses will be reared together in 3 ponds. The crosses are identified by different colors being injected into the prawn muscle.
- 2.3 Genetic variation will be evaluated between the 9 crosses based on allozyme markers.
- 2.4 In each environment, the cross with the best performance will be chosen for the selective breeding program (4 environments may not be the same cross)
- 2.5 The within-family selection procedure will be used to improve the economic traits of the chosen cross.
- 2.6 Performance and genetic variation will be evaluated in each selected generation. (Later, microsatellite markers may be used to the allozymes)
- 2.7 After 3 generations of selection, the selected lines will be evaluated under farm conditions.

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The Southeast Asian Fisheries Development Center (SEAFDEC), a regional treaty organization based in Bangkok, Thailand was established in December 1967 to promote fisheries development in the region. Its member countries are Japan, Malaysia, the Philippines, Singapore, Thailand, Brunei Darussalam, the Socialist Republic of Vietnam, Union of Myanmar, Indonesia and Cambodia. The Council of Directors who represents SEAFDEC Member Countries is the policy-making body of the organization.

SEAFDEC does research on appropriate fisheries technologies, trains fisheries and aquaculture technicians, and disseminates fisheries and aquaculture technologies. Four departments were established to pursue these objectives

The Training Department (TD) in Samut Prakan, Thailand (1967) for marine capture fisheries training

The Marine Fisheries Research Department (MFRD) in Singapore (1967) for fishery post-harvest technology

The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development

The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of marine fishery resources in the exclusive economic zones (EEZs) of SEAFDEC Member Countries

SEAFDEC/AQD is mandated to

Promote and undertake aquaculture research that is relevant and appropriate for the region

Develop human resources for the region

Disseminate and exchange information on aquaculture

The Aquaculture Department in the Philippines maintains four stations: in Iloilo Province, the Tigbauan Main Station and the Dumangas Brackishwater Station; in Guimaras, the Igang Marine Substation; and in Rizal, the Binangonan Freshwater Substation.



Tigbauan Main Station Dumangas Brackishwater Station



Igang Marine Substation



Binangonan Freshwater Station

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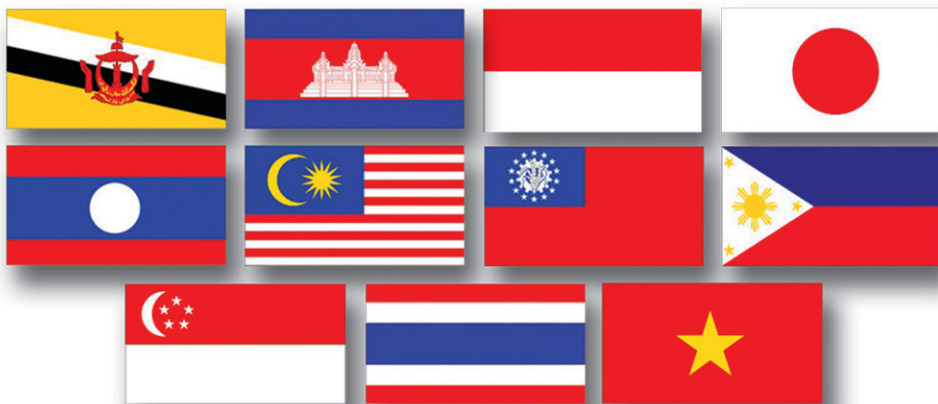
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