

Mass rearing of the entomopathogenic nematode *Heterorhabditis* sp. on artificial media

A STUDY was carried out of a Thai isolate of the entomopathogenic nematode, *Heterorhabditis* sp.. This isolate was collected from soil in Roi Et Province, Northeast Thailand. Studies of its biology emphasized its life cycle within the host insect (*Galleria mellonella*), the effect of temperature on its growth and reproduction within the insect, and isolation of its symbiotic bacteria.

Results from studying its life cycle at room temperature ($26 \pm 2^\circ\text{C}$) revealed that the juveniles passed through four stages before becoming an adult (male or female). The adult female could be either hermaphroditic or amphimictic. The first generation of infective juveniles produced from the hermaphroditic female completed its cycle six days (144 hours) within the host insect. The amphimictic female then produced the second generation of infective juveniles, which could complete their life cycle in four days. After ten days, the infective juveniles gradually moved out of the dead body of the host insect.

Low or high temperatures (15°C or below, and 35°C or above) caused the nematodes to stop growing and reproducing. The nematodes developed slowly at

20°C , and produced only one generation of infective juveniles from hermaphroditic females in ten days. The optimum temperature for growth and reproduction was $25 - 30^\circ\text{C}$.

A symbiotic bacterium was isolated from the haemolymph of *G. mellonella* infected with *Heterorhabditis* sp. for 48 hours. The bacterium colony was absorbed with bromthymol blue on NBTA medium. It was circular to irregular in shape, with a clear zone around the colony. It was greenish in color in the center, which was raised, opaque and undulated. The bacterium was classified as *Photorhabdus* sp. *Heterorhabditis* sp. It can be mass produced on a semi-solid media based on KDSM, SBSM or ESM formulae. The yield of infective juveniles was 349 million per liter of KDSM medium, 191 million per liter of SBSM medium, and 293 million juveniles per liter of ESM medium.

News source: **Department of Agriculture, Thailand**

For further information, Nuchanart Tungchitsomkid : Nematology Research Group, Crop Protection Research and Development Office, Department. of Agriculture, Thailand. E-mail: nuchanart@yahoo.com

Genetic analysis of “sink” related grain yield

NEW PLANT TYPE (NPT) rice is being developed with the aim of increasing rice yields. In Vietnam, the Cuu Long Delta Rice Research Institute (CLDRRI) has developed a breeding program to create a NPT rice with a yield potential of 12 - 15 mt/ha. For this, two NPT varieties were crossed with four promising Cuu Long Delta cultivars. A genetic analysis was carried out on the “sink” (panicle length, grain number per panicle, grain weight...) related to rice yield.

Two NPT varieties from IRRI (International Rice Research Institute) with high yielding capacity (IR 70140 and IR 60819) were crossed with nine promising and stable cultivars from the Cuu Long Delta (AS 996, CM 54, CM 16-27, OM 1490, OMCS 98, Nang Thom Cho Dao, IR 20, IR 24 and IR 64).

Field testing was carried out in the Experimental Station of CLRRRI in 1999-2000. Experimental varieties were transplanted with a planting density of 20 x 20 cm. The applied rates of NPK were 90 - 30 - 30 kg/ha in summer-autumn, and 100 - 4 - 30 kg/ha for the spring crop.

Genetic analysis of three cross combinations showed that the parents selected had exerted influence on the genetic relationship of panicle length, grain number per panicle, grain weight, spikelets/primary branch, spikelets/secondary branch and harvest index and grain yield, as evident from path analysis. It is suggested that the selection for grain yield is most efficient if it is based on panicle length, grain number per panicle, grain weight, number of primary branches, number of secondary branches, spikelets/primary branch, spikelets/secondary branch and harvest index, since these characters fulfilled both the requirements of genotype association with yield, and path coefficients analysis.

The results of the genetic analysis were confirmed by multiple regression analysis with maximum weight for these characters in all the crosses.

News source: **Department of Agriculture and Forestry Extension, MARD, Vietnam**

For further information, see *Science & Technology Journal of Agriculture and Rural Development* 6:5. 2002

Application of PCR to diagnose a chicken disease caused by *Mycoplasma gallisepticum*

MYCOPLASMA disease of chickens is a major problem in poultry production. The pathogen has many strains, so that diagnosis of the disease using rapid coagulation on Lamel SPA is not very effective. The aim of this study was to find a more accurate method of diagnosing the disease.

The extraction of nucleic acids from test samples for mycoplasma detection was undertaken, following the CIRAD-EMVT protocol. Primer pairs RP5 and FP2 for polymerase chain reaction were made from gene fragment 16s rARN of *Mycoplasma gallisepticum*. Two hundred and seventy-five diseased samples were used in determining the mycoplasma strains. A Promega kit was used to extract the DNA from the samples.

PCR analysis followed by electrophoresis of PCR products showed that the line of *M. gallisepticum* has 4 bands 401, 143, 199 and 78 base pairs. Meanwhile the line of *M. gallinarum* has 2 bands of 538 and 199 base pairs. This enabled us to distinguish the two strains of mycoplasma by PCR and RFLP. PCR is a highly sensitive method, giving a positive reaction at a concentration of 6.10^{-2} pg DNA or 50 CFU (colony

forming unit). PCR analysis showed that *M. gallisepticum* was detected in 66.0% of samples from diseased chickens, while *M. gallinarum* was detected in 38.5% of samples. However, mycoplasma was detected in only 38.0% of disease samples when SPA was used.

In conclusion, PCR allows us to determine the percentage of infested chickens with mycoplasma more accurately than SPA. This method also allows us to detect pathogens in cattle manure and drinking water, and in chicken embryos that test negative with SPA.

M. gallisepticum and *M. gallinarum* can be distinguished by PCR in combination with restricted enzyme RsaI.

News source: **Department of Agriculture and Forestry Extension, Ministry of Agriculture and Rural Development (MARD), Vietnam**

For further information, contact Le Minh Sat, National Institute of Animal Husbandary, Vietnam: Phan Thanh Phuong, National Institute for Veterinary Research, MARD, Vietnam

E-mail: www.MOSTE@org.vn.

Treatment of litchi trees with “Kiviva”

LITCHI is a high-value fruit crop. In Vietnam, about 27,000 ha are planted in litchi, with a total production of 30,000 mt. Marketing is a problem, because the harvest season is short and the fresh fruit cannot be stored for long. This study attempted to solve the marketing problem by using “Kiviva” to treat litchi trees. “Kiviva” is a commercial product which combines growth stimulator with micronutrients.

The field testing of “Kiviva” was conducted in an experimental orchard of Hanoi Agricultural University in 1999. There were four treatments:

Treatment 1: Untreated (control)

Treatment 2: 12.5 ppm

Treatment 3: 25.0 ppm

Treatment 4: 50.0 ppm

The results showed that the use of “Kiviva” gave a marked improvement in fruit quality and other features. The fruit weight of treated trees was higher than that of the control (22.33 – 22.82 g against 20.20 g), while the rate of fruit drop of treated trees was lower than that of the control (27 - 36% against 47%). This resulted in

higher yields from the treated trees in comparison with the untreated ones (137 - 218 against 130 kg per tree). The strength of the fruit skin, the ratio of fruit flesh to seed, and the rate of fruit set were also better in treated trees than in untreated ones.

One valuable effect of “Kiviva” on litchi was to increase the strength of the fruit skin. This effect gave a marked improvement in the number of undamaged fruit after harvest, transportation and storage. The treatment of litchi with “Kiviva” was also able to delay fruit maturity by 7 – 10 days, thus prolonging the harvest season of litchi, and reducing marketing pressure while making more efficient use of available labor. Fruit treated with “Kiviva” can be stored for 30 days at a temperature of 2°C.

News source: **Department of Agriculture and Forestry Extension, MARD, Vietnam**

For further information, see *Science & Technology Journal of Agriculture and Rural Development* 7: 585-586.