Introduction

The rice gall midge is a major dipteran pest of rice affecting rice growing region in South, Southeast Asia and Africa. The damage to rice production caused by the gall midge is estimated at more than US$ 500 million annually (Mathur and Krisnaiah, 2004). There are two species of gall midge, Asian gall midge (*Orseolia oryzae*, Wood-Mason) and African gall midge (*Orseolia oryzivora*, Harris and Gang). This thesis will deal largely with the Asian gall midge. Damages to rice from the gall midge have been reported in different rice growing areas of Asia such as China (Lai *et al*., 1984), India (Kalode and Bentur, 1989), Lao LDP (Inthavong *et al*., 2004), Combodia (Jahn and Bunnarith, 2004), Thailand (Tayathum *et al*., 2004). In Thailand, gall midge infestation is widespread in the Northern and Northeastern regions, and has been reported in many provinces such as Nan, Chiang Mai, Chiang Rai, and Ubon Ratchatani. The main symptom of gall midge damage is the formation of gall called the silver shoot instead of the growing point, resulting in failure of rice panicles to develop and so yield losses (Hidaka *et al*., 1974). Adding to difficulties for its control, the gall midge is genetically highly diverse within the species (Katiyar *et al*., 2000). So far six biotypes have been identified in India (Lakshmi *et al*., 2006), four biotypes in China (Tan *et al*., 1993) and three biotypes in Thailand (Thongphak *et al*., 1999; Charapok, 2006). The different biotypes of gall midge have differing reactions on different rice varieties (Kalode and Bentur, 1989), with continuing evolution into new biotypes (Harris *et al*., 2003). Thus new improved varieties may be released as resistant to some biotypes, but they may not be resistant to other and new biotypes.

The resistance to gall midge in different rice varieties has designated plant responses to specific gall midge resistance genes. The gall midge resistance genes in rice varieties were released *Gm1* in 'Samaridhi', *Gm2* in 'Surekha' (Chaudhary *et al*., 1986), *gm3* in 'RP2068-18-3-5' (Kumar *et al*., 1999), *Gm4* in 'Abhaya' (Shrivastava *et al*., 1994), *Gm5* in 'ARC5984' (Kumar *et al*., 1999), *Gm6* in 'Duokang1' (Yang *et al*., 1997) , *Gm7* in 'RP2333-156-8' (Kumar *et al*., 2000), *Gm8* in 'Jhipiti' (Kumar *et al*., 2000), *Gm9* in 'Madhuri line 9' (Shrivastava *et al*., 2003) and *Gm10* in 'BG 308-2' (Kumar *et al*., 2005).

In Thailand, improved varieties resistant to the insect have been released in the past: RD4 in 1973 and RD9 in 1975, by the Thai Rice Research Institute. A local variety called Muey Nawng is widely recognized for its resistance to gall midge. Meuy Nawng 62 M was selected and pure-lined in Chiang Mai from seed lots of local Muey Nawng, and indentified in 1959. Recently, it was reported that only Meuy Nawng 62 M was resistant to some gall midge populations while RD 4 and RD 9 were no longer resistant (Tayathum *et al*., 1995).

The local rice varieties have a wide distribution throughout Northern Thailand (Rerkasem, 2005). Local varieties are genetically diverse and can be given same and different name in different locations (Harlan, 1992). The name Muey Nawng is recognized by farmers for its gall midge resistance in areas where the insect pest poses a serious problem, including Chiang Mai, Chiang Rai and Nan in Northern Thailand. However, different accessions of Muey Nawng have been found to be genetically different (Supamongkol, 2006). This study set out to investigate how the variation among accessions of Muey Nawng is related to its resistance to the gall midge populations from different locations in Thailand. Understanding genetic variation will be useful for breeding program aiming to serve areas with gall midge problem and to use specific genotypes of Muey Nawng that are resistant to specific gall midge populations.

The objectives of the study were

1. To assess the extent of gall midge infestation and its impact on rice yield in farmer's field

2. To determine genotypic variation in farmers' accessions of Muey Nawng, a local rice variety generally recognized for gall midge resistance.

3. To evaluate interaction among Muey Nawng accessions and gall midge populations

Chapter 1

Literature Review

1.1 Rice gall midge

1.1.1 Taxonomy and morphological characteristic

Rice gall midge has been classified into two species: Asian rice gall midge (*Orseolia oryzae*, Wood-Mason) and African rice gall midge (*Orseolia oryzae*, Harris and Gang), in the family Cecidomyiidae and Order diptera. The body of adult gall midge is similar to the mosquito but it is orange in color. The body length of adult is about 4.8 mm for female and smaller at 3.5 mm for male. The length of the fore wing is about 3.8 mm for the female and in 3.0 mm for the male. The antenna, pronotum, leg, compound eye and lateral pleuron on the thorax are black. The eggs are ellipse in shape; their length is 0.45 mm and width is 0.25 mm. The larvae have three instars. The first instar larvae length is about 0.75 mm and light whitish color. The length of the second instar larvae is 1.7 mm, clear of eye spots but otherwise similar to the first instar larvae. The third instar larvae length is 3.5 mm and milky white color. The pupa is brown color and its length is 5 mm for the female and 4 mm for male (Hidaka *et al*., 1974). Moreover, Joshi and Venugopal (1985) investigated the variation in morphology of rice gall midge from different location from India. They found distinct variation in the size of the trochanter and the femer, the structure and shape of the hind tarsal segments and body pigmentation among populations from different locations. However, the rice gall midge is generally classified into Biotypes based virulence on the host plants, feeding, survival, and adaptation to specific locations among insects. So far six biotypes have been identified in India (Lakshmi *et al*., 2006), four biotypes in China (Lai *et al*., 1984) and three biotypes in Thailand (Thongphak *et al*., 1999; Charapok, 2006). Different insect species that have been classified into biotypes such as Hessian fly (Ratcliffe *et al*., 2000), brown plant hopper (Den Hollander and Pathak, 1981) and greenbug (Montllor *et al*., 1983).

1.1.2 Geographic distribution of rice gall midge

Asian rice gall midge is a serious pest of rice in South and Southeast Asia such as China (Pasalu *et al*., 2004), India (Kalode and Bentur, 1989), Cambodia (Jahn and Bunnarith, 2004), Lao PDR (Inthavong *et al*., 2004), Sri Lanka (Kudagamage *et al*., 1988), Bangladesh (Cattling *et al*., 1978), Indonesia (Kartohardjono, 1979) and Thailand (Hidaka *et al*., 1974). The African rice gall midge has been reported as serious pest of rice field in African countries such as Nigeria (Ukwungwu and Joshi, 1992) and Siera Leone (Taylor *et al*., 1995). In Thailand, the problem of rice gall midge is widely distributed in the North, Northeast and other parts of the country. Tayathum *et al.* (2004) reported that rice gall midge was a serious pest of rice field in Chiang Mai, Chiang Rai, Phrae, Nan and Tak in the North, Sakon Nakhon, Nong Khai and Ubon Ratchathani in the Norteast and Suphan Buri, Chai Nat and Chachoengsao in the Central region.

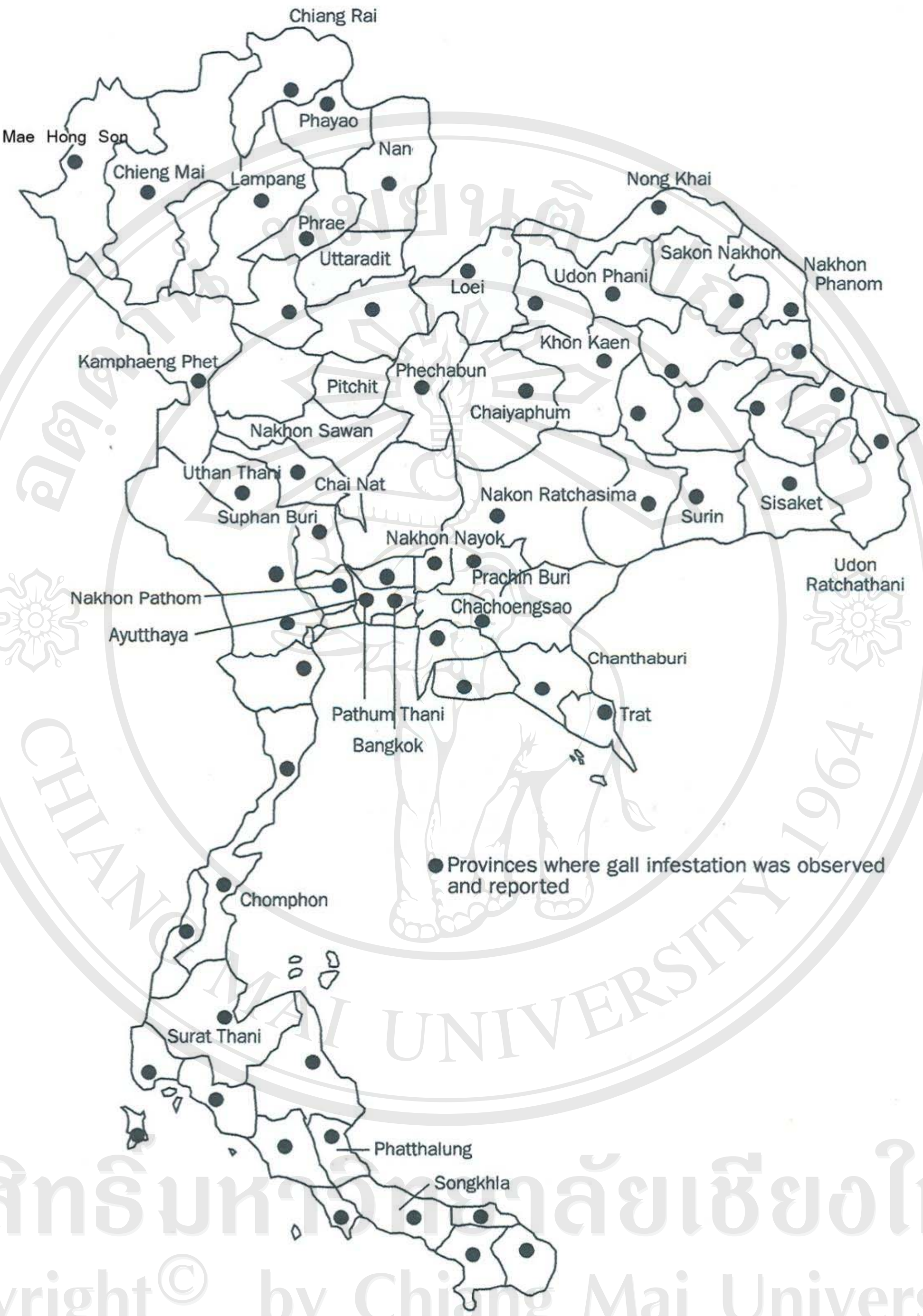

Figure 1.1 Distribution of the rice gall midge in Thailand from 1933-1998 (Tayathum *et al*., 2004).

1.1.3 Mechanism of damage

The life cycle of rice gall midge can be separated into four stages: egg, larvae, pupa and adult. The adult gall midge lays an average of over 200 eggs in groups of two to six on the leaf sheath and leaf blade of rice. The incubation of the eggs varies from one to six day, followed by their hatching into larvae stage. Following hatching, the first instar larvae move down on the leaf surface wet by rain or dew, into the plant's growing point, which fed on by the second and third instars larvae. The larvae stage takes about 14 days. While the larva grows into pupa, the rice plant develops its last leaf into a 'rice gall', which consists of a long ivory-white tube onion leaf or silver shoot , instead of a normal leaf blade. The rice plant produces no more leaf or panicles. The average of pupa stage is about three days. Then, the pupa moves up towards the silver shoot terminal and a hole is made at the upper end of the gall cavity. The skin of pupa then bursts and the gall midge crawls out, dries its wings and flies away. The observed life of the female is two to six days while the male lives for one to two days and the sex ratio is 1:3 (female to male). One life cycle of the gall midge takes about 21-28 day, under favorable condition. Omoloye and Odebiyi (2001) observed that the female African rice gall midge mated once throughout life and its oviposition occurred after mating between 18.00 h to 24.00 h. After the rice harvest, the rice gall midge lives on alternative host plants, where they have migrated to before the rice matures. Up to six overlapping generations of the gall midge may be found in one year. Tayathum *et al.* (1995) reported that rice damage by gall midge can be found from seedling to heading stage of rice plant. In seedling stage, in the seedbeds for transplanted rice, the infested plant is stunted, has unusually high number of tillers, doesn't produce leaf primordia and rolled leaf. Rice plants infested with gall midge in tillering stage have leaves that are short than normal with onion leaf or silver shoot, high number of tillers and stunted. By the time the rice is flowering, the crop can no longer be directly damaged by the gall midge, but it can still develop more silver shoots in the non-productive tillers to increase the number of gall midge adults.

1.1.4 Factors influencing damage

The levels of rice damage by gall midge have been reported to depend on the environmental conditions and management of rice crop. In wet season, heavy cloud cover favors oviposition, prolonged adult life and increased percentage egg hatch of gall midge (Wongsiri *et al*., 1971). In addition, the high humidity is important for the larva moving to the rice growing point and regulation of the egg stage incubation to the first intar larvae of rice gall midge (Patnaik and Satapathy, 1985). In dry season, abundance of rice gall midge host plants after rice crop harvesting is significant for the increasing or rice gall midge damage in the next crop (Srivastava, 1986). Hidaka *et al*. (1974) found the five host plants around rice field in Thailand: *Leersia hexandra* Sw. (swampy rice grass), *Echinochloa colona* (L.) Link (jungle rice), *Paspalum scrobiculatum* Linn. (rice grass paspalum), *Ischaemum rugosum* salisb. (wrinkle duck-beak) and *Oryza rufipogon* Griff. (common wild rice). Moreover, the rice gall midge also finds ideal habitat in the ratoons and volunteers of rice plants. The management during growing rice is important for controlling gall midge damage. Rajamani *et al*. (2004) reported that choices of susceptible rice varieties and high application rate of nitrogenous fertilizers increased gall midge damage in the field.

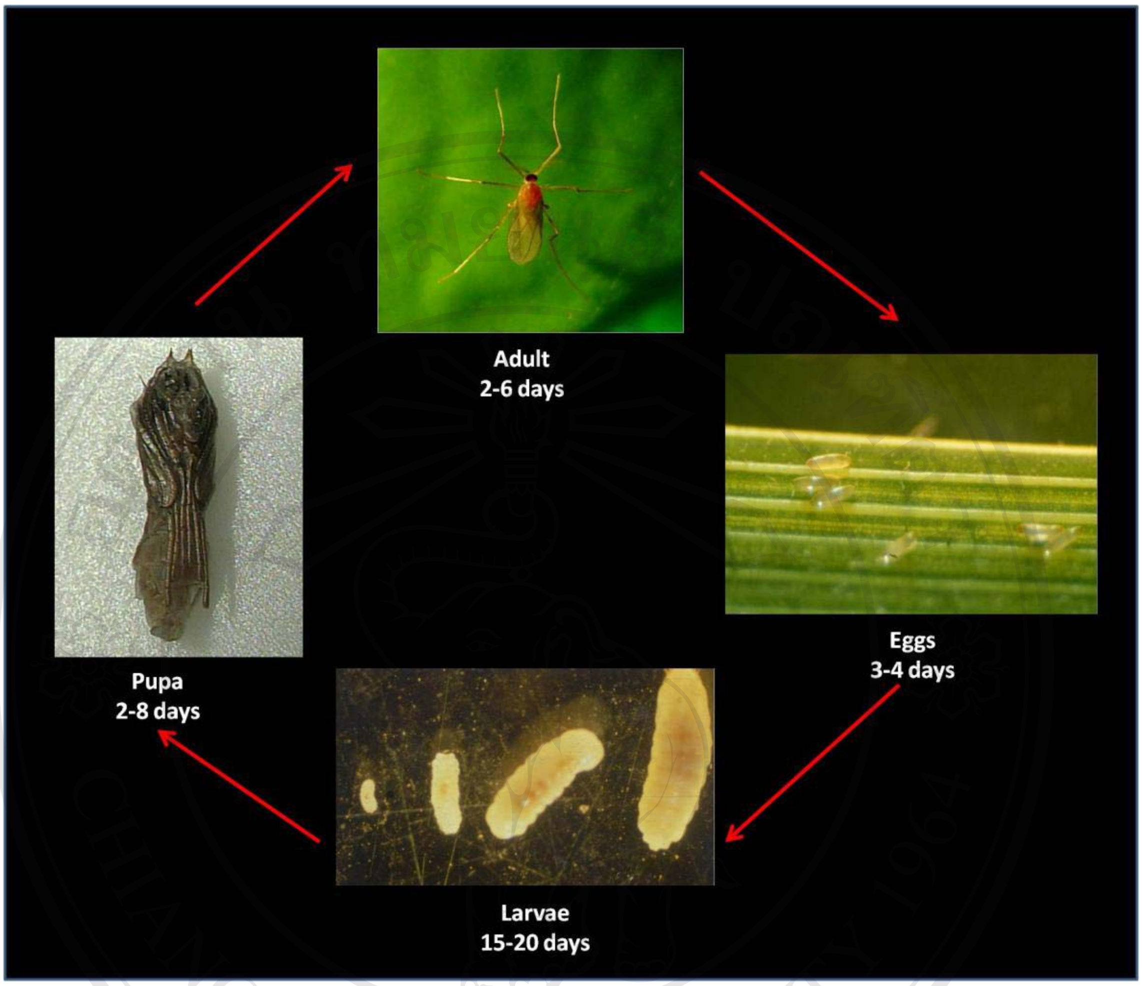

Figure 1.2 The stages life cycle of the rice gall midge.

Figure 1.3 The silver shoots or onion leafs after the rice gall midge damage.

Figure 1.4 The damage to rice production by the rice gall midge.

1.2. Management and cultural control of gall midge

Similar to the management of other pests in crop production, measures for controlling gall midge includes cultural practices, biological control, Chemical control and use of resistant varieties.

1.2.1 Mechanical and Cultural control

Mechanical control is direct or indirect measures taken to kill the insect such as light traps, short duration light, responses to infrared, light reflection and laser phenomena. In addition, cultural control of insect pests includes any modification in the way the crop is grown that results in the lower pest populations or damage such as tillage, water management, crop rotation, crop planting date, trap cropping, vegetational diversity, fertilizer use and harvest time (Schellhorn *et al*., 2000). Some cultural practices have been recommended against the gall midge, but there a few reports of their efficacy on-farm. Plowing ratoons and volunteers of previous crop and weeding out of the host of gall midge after harvesting can reduce damage (Nwilene *et al*., 2006). Porchit (2005) suggested that the only mixture between local Muey Nawng (Resistant variety) and SPT1 (Susceptible variety) has a lower silver shoot damage in SPT1 under mixture than SPT1 pure. Moreover, Nacro *et al*. (2006) reported lower destruction by African rice gall midge in earlier transplanted crop under irrigated conditions.

1.2.2 Biological control

Several natural enemies of the gall midge has been identified to distribute widely in paddy fields, and separated in parasite and predator. The hymenopterous parasitoids of the rice gall midge so far identified are *Phatygaster oryzae*, *P. foersteri*, *Neanastatus cinctiventris* and *Obtussiclava oryzae* (Kobayashi and Kudagamage, 1994). The *phatygaster* sp. is found parasitized gall midge larvae stage and gall development on the rice. Hidaka *et al*. (1994) reported that the Thunbug (*Ophionia indica*) was predator of rice gall midge in paddy field in Thailand. Moreover, Nwilene *et al*. (2006) have been found *Cytorhithus viridis* (Hepidoptera: Miridae), *Conocephalus longipennis* (Orthoptera: Tettigoniidae), *Anaxipha longipennis* (Orthoptera: Gryllidae), *Ladybird beetles* (Coleoptera: Coccinellidae)) destruction of

African rice gall midge in field. However, none of these enemies have, however, been developed for use in rice farming.

1.2.3 Chemical control

Chemical pest control is still constituted an important part insect pest management in paddy field, involving chemicals such as carbofuran, carbaryl, diazinon, fenthion, fonofos, malathaion, mrthomyl and phorate, etc., for control of insects (Edwards, 2000). The systemic carbofuran is recommended at the serious area of gall midge (Nwilene *et al*., 2006). Ukwungwu (1990) recommended the granular isazofos at dasage rate 0.75 and 1.0 kg a.i ha^{-1}, for control the African rice gall midge in Nigeria. However, insecticides have adverse impact on the environment or farmers' health. The ne t section will deal with the use of resistant varieties.

1.3 Gall midge resistance in rice

1.3.1 Genotypic variation in gall midge resistance in rice

The difference in response to gall midge damage among rice varieties has been well recognized. Resistance to gall midge in rice varieties is, however, varied with the specific biotype of insect (Bentur *et al*., 1994). For example, Phaguna had resistance to gall midge biotype 1, 2 and 5, while it is susceptible to gall midge biotype 3 and 4 from India. Tayathum *et al.* (1995) reported that three populations of gall midge collected from Nan, Ubon Ratchathani and Chachengsao provinces in

Thailand had variation of percentage damage of gall midge on nine rice varieties. Moreover, the reactions in resistance to gall midge in rice varieties have been classified into highly resistance to highly susceptible (Sankpal and Dumbre, 1980).

Gene-for-gene hypothesis has been used to explain the relationship between disease and resistance in plant (Flor, 1971). The interaction between gall midge and rice plant was shown to correspond to the hypothesis. Most of the resistance genes have been described as dominant allels (R alleles), while the virulence of the gall midge biotypes were recessive genes. For example, the studies of the ten-allelic gall midge resistance genes and six different biotypes of gall midge in India were found corresponding the hypothesis (Sardesai *et al*., 2001). However, the inheritance of an avirulent gall midge biotypes were maternally derive x chromosome, which gall midge biotype was a avirulent biotype when the female is a avirulent biotype (Behura, 2000).

Reaction between rice varieties and gall midge has also been observed to vary with time. In Thailand, rice varieties released as resistant to gall midge in 1959 (Muey Nawng 62M), 1973 (RD4) and 1975 (RD9) by the Thai Rice Research Center Institute (DOA, 2003). Muey Nawng 62 M was selected as a pure ling from farmers' seed of Muey Nawng in Chiang Mai province. The local variety Muey Nawng is recognized by farmers throughout the North as resistant the gall midge. Currently, it was discovered that RD4 and RD9 was susceptible to all gall midge population from Nan province (Tayathum *et al*., 2004). Tayathum *et al*., (1995) reported that the most of the popular Thai rice varieties, such as KDML105 RD6 and RD10 were tested to there population of gall midge from Nan, Ubon Tatchathani and Chachengsao provinces under green house condition. They found all of these popular rice varieties to be susceptible to gall midge. However, many local versions of Meuy Nawng are popular among farmers and are widely distributed in these areas Northern Thailand where gall midge is a serious problem (Supamongkol, 2006). The rice known as Muey Nawng generally shares the main characteristics, such as tall plant type, with bold glutinous grain. However, considerable diversity, including at the molecular level, has been found in the seed of Muey Nawng kept by different farmers. It would therefore be useful to know how these different versions of Muey Nawng respond to the gall midge.

1.3.2 Mechanism of rice resistance

The defense mechanism of plant resistance to insect has been classified in to three types of mechanism, consisting of non-preference, antibiosis and tolerance (Pathak and Saxena *et al*., 1976). Non-preference is defined as some unattractive characteristics for oviposition, feeding and shelter for the insect pest. African rice gall midge was reported to prefer the Asian rice varieties over the African rice varieties for egg laying. When given the choice, the African gall midge laid many more eggs on *Oryza sativa* varieties than they did on any of the *O. glaberrima* entries (Omoloye *et al*., 1999). However, anthocyanin pigmentation of the leaf sheath was not found to have any effect on gall midge preference or non-preference and thus gall midge resistance of rice varieties (Satyanarana *et al*., 1987; Kumar *et al*., 1997). Antibiosis is the resistance mechanism in which that the plant produces some biochemical substrate that is harmful to the insect pest. The adverse effect of the chemical may be growth retardation, resulting in a declination in insect size or weight, reduced rate of some metabolic process and increased restlessness, some nutritional blockage and larval mortality. On the other hand, the plant can grow, develop, reproduce and survive normally (Panda and Khush, 1995). The concentration of o-dihyroxyphenols in the vegetative shoot apices in gall midge resistant varieties IET7009, IET7008 and Siam29 was found to be much higher than in susceptible varieties Java, IR20 and TN1 (Joshi and Venugopal, 1984). However, analysis of the total phenols at the stem base in twenty-nine gall midge resistant and susceptible varieties were not correlated between resistance and susceptible varieties (Amudhan *et al*,. 1999). The hypersensitive response (HR) was found the symptoms on resistance variety Phaguna after avirulent gall midge biotype1 from India damage (Bentur and Kalode, 1996). It showed premature tillering, brown of central leaf and tissue necrosis at the apical meristem. The tolerance represents a plant's ability to not suffer a yield loss and can grow and reproduce itself or to repair injury part of plant after insect damage (Pathak and Saxena, 1976). Omoloye *et al.* (2002) studied the tolerance and responses of four rice cultivars (Cisadane, Bw 348-1, Tox 4093-17 and ITA 306 (local check)) to damage by African rice gall midge. The results indicated that the tolerance in Cisadane was a higher in the percentage of seedling survival, number of fertile tiller, productive panicle and grain yield per hill than local check.

1.3.3 Genetic, breeding and selection for gall midge resistance

Studies of inheritance of resistance to rice gall midge in different rice varieties have designated plant responses to specific gall midge resistance genes. Chaudhary *et al.* (1986) studied the inheritance of five resistance varieties (Usha, Samridhi, BD 6-1, Sukekha and IET 6286) to rice gall midge in natural damage. The results suggested that these rice varieties showed the resistance to gall midge with a single dominant gene for resistance. Moreover, the alleles test found the same resistance gene in Usha, Samaridhi and Bd 6-1: this gene was designated *Gm1*. However, Surekha and IET 6286 were shown to have the same resistance gene that is non allelic to Gm1; this gene was designated *Gm2*. Subsequently, other the non-allelic gall midge resistance genes were reported *gm3* in 'RP2068-18-3-5' (Kumar *et al*., 1999), *Gm4* in 'Abhaya' (Shrivastava *et al*., 1994), *Gm5* in 'ARC5984' (Kumar *et al*., 1999), *Gm6* in 'Duokang1' (Yang *et al*., 1997) , *Gm7* in 'RP2333-156-8' (Kumar *et al*., 2000), *Gm8* in 'Jhipiti' (Kumar *et al*., 2000), *Gm9* in 'Madhuri line 9' (Shrivastava *et al*., 2003) and *Gm10* in 'BG 308-2' (Kumar *et al*., 2005). Most of the gall midge resistance genes are dominant resistance gene, except for *gm3*, which is recessive.

Molecular markers link to gall midge resistance gene in rice varieties have been studied. The developments of DNA markers were tightly linked to gene and enable one follow the gene cross between the resistance sources (Mohan *et al*., 1997). Seven gall midge resistant genes were detected by DNA marker on different methods. Birada *et al*. (2004) reported that the progeny-tested of F_2 mapping population derived from cross between W1263 (contained *Gm1* gene) and TN1 (susceptible). They found the SSR markers, RM444, RM316 and RM219 link to resistance *Gm1* gene on the chromosome 9. Subsequence, the other marker resistant genes were reported *Gm2* on chromosome 4 (Rajyshri *et al*., 1998; Himabindu *et al*., 2007), *Gm4* on the chromosome 8 (Nair *et al*., 1996; Mohan *et al*., 1997), *Gm6* on chromosome 4 (Katiyar *et al*., 2001). *Gm7* on chromosome 4 (Sadesai *et al*., 2002) and *Gm8* on the chromosome 8 (Jain *et al*., 2004) Recently, Katiyar *et al* (2001) reported the combination of the two major genes; *Gm2* gene resisted to gall midge biotypes from

India and *Gm6* gene resisted to gall midge biotypes from China by pyramiding of these gene.

This thesis aims to investigate the local Meuy Nawng under the field condition where gall midge is a serious problem in Northern Thailand. Moreover, this study also examines, under field and greenhouse conditions, variation in resistance to gall midge in different accessions of the Meuy Nawng rice variety, collected from different areas in Northern Thailand. The understanding of how different accessions of the local rice variety, Muey Nawng, respond to gall midge should provide useful information for the control of gall midge in rice.