CHAPTER 1
INTRODUCTION

1.1 Significance of this study

Mungbean [Vigna radiata (L.) Wilczek] is known as one of the important legume crops, which is a highly self-pollinated crop with an early maturity. It is an important pulse consumed in many countries, especially in Asian countries including Thailand. The mature seeds contain abundant nutrients and bioactive compounds such as polyphenols, polysaccharides, and polypeptides. In addition, it is rich in essential sources of digestible proteins for humans (about 20.97-32.60%) and vitamins as well as minerals. Mungbean has been associated with health benefits, such as anticancer and immunomodulatory activities (Hou et al., 2019). It is popular among farmers because its life cycle is short (60 to 65 days), and it is drought tolerant. In addition, it can fix atmospheric nitrogen in symbiotic association with Rhizobium providing benefit to other crops (Tomooka et al., 2014). The seeds of mungbean can be processed into various food products such as vermicelli, mungbean flour, cookies, noodles, and sprouts. In Thailand, the demand for consumption is about 100,000 tons/year. In contrast, the productivity of mungbean tends to decrease, resulting in insufficient supply to meet the domestic demand. The productivity of mungbean suffered from several biotic and abiotic stresses including diseases, insect-pests, and weakness to environments. In addition, poor crop management practices and lack of genetic variability are the important factor constraints on yielding potential of mungbean in Thailand (Office of Agricultural Economics, 2020). Among these factors, Cercospora leaf spot (CLS) disease caused by Cercospora canescens Ellis & Martin, which is a hemibiotrophic fungus, inflicts significant yield losses. This disease outbreaks during the rainy season with symptoms of spots on mungbean leaves that appear after the fungus infects and increase in number and size during flowering and pod-filling stages. CLS disease can lead to 23% yield losses if mungbean leaves are damaged by up to 75% (Quebral and Cagampang, 1970). Powdery mildew (PM) is caused by the biotrophic fungus Sphaerotheca phaseoli. It outbreaks in the cool-dry season with the first symptoms of infection being small, circular, white powdery patches on the lower leaves. The disease can develop rapidly and damage at the seedling stage onwards,
which can reduce yield more than 50% and up to 100% at the seedling stage (Quebral and Cagampang, 1970; Khajudparn et al., 2010). In mungbean production, agronomic practices and chemical prevention have been applied to control the disease. However, these are limited by many factors and the chemical is very harmful to health and the environment. Therefore, genetic resistance is a cost-effective and desirable option to reduce yield losses caused by these factors and increase mungbean production and environmental friendliness. The resistance source of CLS is controlled by a single dominant gene, which derived from the V4718 line (Chankeaw et al., 2011; Arsakit et al., 2017). While PM resistance in the V4718 and V4785 is controlled by a single non-allelic dominant gene (Khajudparn et al., 2010, Poolsawat et al., 2017). Nevertheless, the use of resistant varieties having only a single resistance (R) gene may lead to the boom-bust cycle because the resistance function of resistance gene can be lost when the disease mutates to overcome single resistance gene, resulting in the resistant varieties become susceptible (Chukwu et al., 2019). Therefore, the varieties with multiple resistance genes obtained by gene pyramiding can be used to overcome this problem. Pyramiding multiple resistance genes in a single line confer broad-spectrum and durable resistance. Therefore, the varieties with multiple resistance genes obtained by gene pyramiding can be used to overcome this problem. Recently, we successfully developed a resistant line, namely SUPER5 which had CLS, and PM resistance genes derived from the double cross [(CN72 x V4758) x (CN72 x V4718)] x [(CN72 x V4718) x (CN72 x V4785)]. SUPER5 showed higher resistance levels to both CLS and PM diseases than all the resistant parents when evaluated in different environments, indicating that it can be used as an effective resistance source in the mungbean breeding program (Poolsawat et al., 2017; Pookhamsak et al., unpublished data).

Several approaches have been used to improve new resistant varieties such as conventional breeding, gene editing, and genetic engineering. However, gene editing and genetic engineering are still limited in Thailand. Moreover, the improvement of new varieties resistant to several diseases through conventional breeding is very difficult because it cannot monitor the number of resistance genes in each plant. In addition, conventional breeding programs are limited by seasonal dependency, time-wasting, and high cost. Recently, marker assisted breeding (MAB) can be used to overcome the limitations of conventional breeding by selecting a trait of interest at the DNA level. The use of molecular markers to assist selection in plant is called
marker-assisted selection (MAS) which can reduce the time of breeding programs, especially for developing the resistant varieties to CLS and PM diseases which occur only in rainy and cool-dry seasons, respectively. Marker-assisted backcross breeding (MABB) and marker-assisted gene pyramiding are applications of MAS for plant breeding. MABB and gene pyramiding aims to improve a specified trait by transferring one or a few genes or QTLs from one genetic source to the elite variety (recurrent parent), and still have similar genetic background to the recurrent parent. Papan et al. (2022) developed a new resistant mungbean line (H3) by using MABB for transferring CLS and PM resistance genes into an elite variety KING. Similarly, Pookamsak et al. (unpublished data) used MABB for screening and selecting a mungbean resistant line (SUPER5), which was highly resistant to CLS and PM diseases. Tantasawat et al. (2021) identified a major quantitative trait locus (QTL) (qCLSC72V18-1) controlling resistance to CLS using I16274 and VrTAF5_indel markers in a cross between CN72 (susceptible variety) and V4718 (resistant line). In addition, Poolsawat et al. (2017) also found a QTL controlling PM resistance which mapped between I42PL229 and I85420 markers in a cross between a susceptible CN72 and resistant V4718 using ISSR and ISSR-RGA markers. In addition, the I27R565 marker was also identified to be linked to the PM resistance gene obtained from CN72 × V4785 crosses (Tantasawat et al., 2021). In addition, background selection through MAS for pyramiding desirable genes is effective for minimizing unlinked regions from the donor segment and selecting within the early backcross (BC) generations (Hasan et al., 2015). Papan et al. (2022) and Pookhamsak et al. (unpublished data) used these markers to develop mungbean lines resistant to CLS and PM (H3 and SUPER5), respectively. These markers would ease and accelerate development of new mungbean varieties resistant to the diseases through MAS.

Disease evaluation under field conditions is important procedure to identify genotypes with varied disease severity. It is an important tool for plant breeding, which is helpful for classifying between resistant and susceptible plants. Additionally, it can confirm homozygous alleles in pyramid lines because crossing over might occur between homologous chromosome. In some cases, weather conditions in the field may not always be favorable for uniform disease spread, which eventually may lead to failure of the overall experiment. The detached leaf assay (DLA) provides rapid identification of probable pathogen infectiousness and plant resistance. It is one of the rapid, low-cost laboratory-based techniques used to evaluate disease resistance levels against several diseases including CLS. Subedi et al. (2019) reported that the DLA
method can be used reliably to differentiate CLS resistant and susceptible genotypes of fenugreek. It required small amount of plant materials, which used less area than field evaluation. Moreover, this technique is easy to control the optimal conditions for disease development. This study attempts to pyramid CLS and PM resistance genes into high-yielding mungbean KING and H3 variety/line using marker-assisted backcross breeding (MABB) and bioassay.

1.2 Research objectives

1.2.1 To assess the genetic background of the recurrent parent and donor line using SSR and EST-SSRs markers distributed throughout the mungbean genome and select the markers showing polymorphisms between both parents to be used in marker-assisted background selection.

1.2.2 To pyramid a CLS resistance gene and 2 PM resistance genes into the high yielding mungbean variety/line (KING and H3) through marker assisted backcross breeding and bioassay.

1.2.3 To compare levels of resistance to CLS and PM diseases between selected backcross progenies, and their recurrent and donor parents.

1.3 Research hypotheses

1.3.1 The polymorphic markers between parents that are not associated with CLS and PM resistance genes can be used for background selection.

1.3.2 The pyramid ed progenies selected by marker-assisted background selection to have high RPG recovery may have similar yield to their recurrent parents.

1.3.3 The pyramid lines selected through marker-assisted foreground selection may show higher levels of disease resistance to CLS and PM or exhibit board-spectrum resistance.

1.4 Research scope

This experiment was divided into 3 parts. The first experiment focused on identification of polymorphic SSR and EST-SSR markers distributed throughout the genome of mungbean and quantify the recurrent parent genome (RPG) recovery, using BC₁F₁ and BC₂F₁ of 2 populations derived from a cross between KING (CLS and PM susceptible variety) × SUPER5 (CLS and PM resistant line) and H3 (CLS resistant and PM moderately resistant line) × SUPER5.

The second experiment focused on pyramiding a CLS resistance gene and 2 PM resistance genes into KING and H3 (recurrent parents) through MABC. The seeds of
F1 generation of each population were used to backcross to their recurrent parents and generate seeds up to BC3F1 generations. Six markers linked to a CLS and 2 PM resistance genes (foreground selection); InDel marker (VrTAF5_Indel) and ISSR (I16274) markers flanked the CLS gene from V4718, ISSR (I85420) and ISSR-RGA (I42PL222) markers flanked the PM 1 gene from V4718, ISSR-RGA (I27R565) and InDel marker (VrMLO12_Indel3) associated with PM 3 gene from V4785 were used for foreground selection. In addition, the evaluation of CLS resistance was performed in F1-BC2F1 generations in laboratory by DLA.

The third experiment focused on PM evaluation under field conditions. The BC2F1 progenies were evaluated at the field level in cool-dry season at Suranaree University of Technology Farm, Nakhon Ratchasima province, Thailand.

1.5 References


