CHAPTER 2
LITERATURE REVIEWS

2.1 Mungbean importance

Mungbean (Vigna radiata (L.) Wilczek) belongs to the family Fabaceae, subgenus Ceratotropis in the genus Vigna. It is a diploid with a somatic chromosome number of 2n = 22 and a highly self-pollinated crop with an early maturity of about 60-65 days. Mungbean is one of the important legume crops in South and Southeast Asia, including Thailand. It was originated, grown, and domesticated in India as early as 1500 BC. 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 of about 20.97-32.60% and vitamins as well as minerals. Moreover, mungbean has been associated with health benefits, such as anticancer and immunomodulatory activities (Hou et al., 2019; Nair et al., 2019). Currently, the global production is more than 3 million tons with a cultivated area of more than 6 million hectares worldwide (Nair et al., 2013). India is the largest producer of mungbean with the production of 1.9 million tons, followed by China (0.98 million tons), Myanmar (0.40 million tons), Indonesia (0.30 million tons), and Thailand (0.21 million tons). Mungbean is processed into various food products and used as an ingredient in both savory and sweet dishes such as bread, pan cake, mungbean flour, cookies, noodles, sprouts, and vermicelli (Mohan et al., 2020). During 2018-2020 in Thailand, there has been a steady increase in domestic consumption from about 94,000 to 102,000 and 110,000 tons, respectively. However, the productivity of mungbean tends to be decreased due to the low quality of seed, leading to a low germination rate of seedlings. In addition, lack of genetic variability and susceptibility to abiotic and biotic stress are constraints that affect yielding potential of mungbean in Thailand (Office of Agricultural Economics, 2020).

2.2 The major diseases of mungbean

2.2.1 Mungbean yellow mosaic virus (MYMV)

MYMV, serious viral disease, is caused by begomoviruses which are spread to the mungbean crop through whitefly Bemisia tabaci (Gennadius) as an insect vector.
The symptoms appear within two days after the virus infection when this virus enters into the phloem cells of the plant host. The mungbean leaves appear as scattered yellow-color spots and turn into a yellow mosaic pattern, and lastly, resulting in complete yellowing, drying, and withering of leaves. Therefore, the photosynthetic efficiency of leaves is decreased. In addition, the mungbean pods are also infected by the virus and then become smaller in size, leading to 10 to 100% yield loss, depending on mungbean genotypes and stages of crop infection (Mishra et al., 2020).

2.2.2 Cercospora leaf spot (CLS)

*Cercospora canescens* Ellis & Martin, a hemibiotrophic fungus, is one of the major biotic constraints in mungbean crops. It is a cause of CLS in mungbean which is widespread in the warm-wet growing season. The growth and germination of *C. canescens* depend on relative humidity (RH) and temperature. Kumar et al. (2011) demonstrated that the number of conidial germinations was increased at high RH (90-100%) and optimal temperature (25-30°C). The disease reduces size of pods and grains and spreads rapidly in susceptible varieties after planting for 30-40 days. The CLS symptoms initiated on the lower side of the old leaves with the spots on mungbean leaves and spread all over the plant during flowering until the pod-filling stage in the warm-wet growing season (Chankaew et al., 2011; Chand et al., 2012). This disease can reduce mungbean seed yield by 50% to 68% in susceptible cultivars due to the reduction of pods and seeds (AVRDC, 1984; Chinsawangwattanakul et al., 1984).

2.2.3 Powdery mildew (PM)

PM disease in mungbean is caused by the biotrophic fungus *Sphaerotheca phaseoli* that can reduce yield more than 50% in the cool-dry growing season and up to 100% at the seedling stage. In suitable conditions, PM can infect all growing stages of mungbean, especially in susceptible cultivars. The fungus infects all over the plant with white powdery growth which starts on the lower leaves and spreads up to the upper ones, thereby adversely affecting the photosynthetic efficiency of the plant (Khajudparn et al., 2010).

2.3 Sources of CLS and PM resistance

An important further determining success in mungbean cultivar development is genetical resistance sources. The sources of CLS and PM resistance genes have been reported in several countries such as India, Taiwan, Pakistan, including Thailand. Several genotypes showed resistance to CLS and PM i.e., Pusa105, VC3960-88, BARI
Mung-2, C2/94-4-42, CO-3, M5-22, M5-25, HUM-1, ML-1194, V4718, V4758, and V4785 etc. (Mishra et al., 1988; Hartman et al., 1993; Haque et al., 1997; Wongpiyasatid et al., 1999; Iqbal et al., 2004; Chankaew et al., 2011; Singh et al., 2017) and SUPER5 (Pookhamsak et al., unpublished data). Inheritance of CLS and PM resistance, and the genetic basis of CLS resistance in mungbean has been characterized as differently determined by a single dominant or recessive gene, depending on the genotype. Mishra et al. (1988) studied the inheritance of CLS resistance in 20 crosses of mungbean. They found that the segregation ratio of CLS resistance in all 14 F2 crosses was 3:1 for susceptible and resistant progenies. Using crossed between Pusa 105, PDM 15, PDM 2, PDM 113 and PDM 115 (resistant to CLS) and ML 267, Hyb 12-4 and PDM 62 (susceptible to CLS) indicates that the inheritance of CLS resistance is controlled by a single recessive gene. Inconsistent with Singh et al. (2017), who evaluated the CLS inheritance in 18 mungbean crosses using 6 CLS susceptible varieties and 3 CLS resistant genotypes of mungbean (HUM-1, ML-1194, and ML-1229) under field conditions. The F1 generation of all the crosses showed resistance to CLS, indicating that the inheritance of CLS resistance was dominant over susceptibility. Moreover, the segregation ratio in the F2 and F3 populations was observed to be 3:1 (resistant: susceptible). This denotes the inheritance of resistance to CLS with the control of a single dominant gene. In addition, the inheritance of PM has also been studied. Chaitieng et al. (2002) revealed that the resistant lines to PM are controlled by a single dominant gene, according to progenies derived from crossing between two donor parents (SUT4 and VC1210A) and a recurrent parent (CN36). Furthermore, in our laboratory, Khajudpan et al. (2007) studied the inheritance of PM resistance using progenies from 3 resistant lines V4718, V4758, and V4785. They found that the resistance in the F1 × CN72 and F2 population segregated in a ratio of 3:1 and 15:1 for resistance and susceptibility, respectively in the progenies. These confirmed that PM resistance in these resistant lines is controlled by a single dominant gene.

2.4 Breeding for disease resistance

2.4.1 Conventional breeding methods

Plant breeding methods can be divided into conventional and biotechnological methods. Conventional plant breeding is the development or improvement of cultivars for desirable traits using procedures that are based on the phenotypic selection of hybrids from segregating progenies. The interesting traits may be controlled by one or a few genes (qualitative traits) or by numerous genes
Several methods have been used for self-pollinated species to develop new cultivars such as mass selection, pure line selection, pedigree selection, bulk selection, single seed descent, and backcrossing, etc. The most widely used methods are as follows:

2.4.1.1 Pedigree selection

Pedigree selection is a frequently used method to select superior genotypes from segregating populations while recording the ancestry of selected plants. Segregating populations obtained from a cross between two parents with different genetic backgrounds are highlighted. Pedigree selection starts at the early generations based on visual evaluations and depends on the degree of genetic variability within individuals. Selection of segregating populations is generally to obtain the progenies with desirable characters from both parents and is also based on the record of pedigree, which determines the genetic relationships among individuals selected. Therefore, breeders can trace back to an individual F₂ plant from any subsequent generation. This method is efficient for improving qualitative traits, such as disease resistance, or easily classifiable traits, such as height, maturity, and flowering date. However, this method requires selection of progenies in every generation. It is difficult to handle many crosses simultaneously and time-consuming. Moreover, desirable genotypes may be in early segregating generations (Breseghello and Coelho, 2013; Acquaah, 2015). Sadiq et al. (1998) developed mungbean for large seed size, high yield, and resistance to MYMV through the pedigree breeding method. About 2,500 F₂ plants were generated from F₁ between NM 20-21 and 1482E and selected for desirable progenies until the F₅ generation. One breeding line showed the highest grain yield and resistance to MYMV, which was later developed into a new mungbean variety (NM 89). However, it took them over 8 years for the breeding process.

2.4.1.2 Backcross breeding

Backcross breeding is the method used to transfer one or a few target genes from a donor parent (a resistant variety; R) to the recurrent parent (a susceptible high yielding variety; S). The progeny that contains 50% genetics of each parent is called F₁. The progeny is repeatedly backcrossed (BC) to the recurrent parent to recover all the desirable genes in subsequent generations, until nearly 99% genetic background of the recurrent parent is achieved in BC₆ generation. Backcross breeding has been used to develop new varieties for CLS and PM resistance in mungbean. Chaitieng et
al. (2002) improved new mungbean varieties from three different crosses including a cross between VC3689A (resistant line) as donor parent and KPS1, KPS2, and PSU1 (susceptible varieties) as recurrent parents, and then backcrossed to their recurrent parents. The SUT2, SUT3, and SUT4 varieties resistance to CLS disease were obtained from this program. Meanwhile, PM resistance was obtained from two crosses between 2 donor parents (SUT4 and VC1210A) and recurrent parent (CN36). The F1 and BC1 to BC3 progenies were found to be resistant to PM when evaluated under greenhouse and field conditions. In addition, the resistant lines had higher resistance levels than their parents in the BC3F3 generation (Chaitieng et al., 2002). This supports that backcrossing method can successfully transfer the resistance genes to susceptible varieties.

2.4.1.3 Gene pyramiding

Gene pyramiding is defined as the method aimed to combine multiple desirable genes/QTLs from multiple parents into a single genotype. A pyramid could be constructed with major genes, minor genes, or any other types of gene that conferred disease resistance. The varieties carrying multiple genes can be responsible for broad-spectrum resistance to diseases and against different isolates or races of pathogens. The gene pyramiding scheme can be divided into two parts. The first part is the crossing scheme, whose strategy is to cumulate resistance genes that have been identified in multiple parents into a single genotype. This is called the root genotype. The second step is the fixation scheme, which purposes to fix all resistance genes into homozygosity by selfing for avoiding the segregation of resistance genes (Figure 2.1). However, conventional breeding is still limited due to genotype-environment interactions. The selection for disease resistant genotypes can be performed only once per year. For instance, CLS and PM diseases occur only in the rainy and cool-dry seasons, respectively, so the breeding program is making slow progress. Therefore, the procedures for developing a new variety may take 8 to 10 years, resulting in high production costs. Moreover, conventional breeding makes it difficult to pyramid numerous resistance genes into a single genotype because it cannot distinguish resistant plants with varying numbers of resistance genes.
2.4.2 Biotechnological methods

Recently, plant breeders use new technologies to solve the problems of conventional breeding approach i.e., time-consuming, high labor cost and uncontrollable environments. To speed up the breeding process or make it more efficient, biotechnological methods can overcome these problems with the advancement of molecular genetic knowledge.

2.4.2.1 Genetic engineering

Genetic engineering also called genetic modification is the direct manipulation of an organism genes using biotechnology. This method is carried out by cutting a specific sequence of target gene from any organisms and introduced into another organism through *Agrobacterium* transformation or biolistic method. Several genes i.e., genes related with yield, nutritional value, and resistance (R) genes, have been used to transfer into plant species. Haq et al. (2010) transferred soybean replication initiation protein (Rep) from soybean into another *Vigna* species (blackgram). They found that blackgram progeny carrying Rep gene showed relatively high resistance to MYMV. This method can reduce the time it takes to release new cultivars to farmer. However, it cannot be used for commercial in Thailand.
2.4.2.2 Genome editing

Genome editing technologies (GETs) are advanced molecular biology techniques that allow more effective, precise, and rapid engineering of plant genomes. GETs are involved with changing the DNA of an organism at a specific locus in the genome through small deletions and insertions for gene silencing or changing gene function. Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein-9 nuclease (CRISPR/Cas9) are widely used in this method. CRISPR/Cas9 is based on RNA-guided engineered nucleases, extensively applied in various organisms, including plants (Zhang et al., 2018; Wada et al., 2020). Recently, gene editing techniques have been studied in legume family for the improvement of significant agronomic traits and interesting characteristics. Ji et al., (2019) using CRISPR/Cas9 for symbiosis receptor-like kinase gene inactivation in cowpea by means of hairy root transformation mediated by Agrobacterium rhizogenes K599. CRISPR/Cas9-mediated genome editing technology effectively disrupted this essential SNF gene in cowpea.

2.4.2.3 Molecular marker-assisted breeding (MAB)

MAB is one of the biotechnological methods for plant breeding. It is a useful alternative tool for monitoring or selecting the desirable traits at the DNA level by using molecular markers linked to the desirable traits such as disease resistance genes etc. MAB is powerful and reliable when compared with conventional breeding methods. It can help select a trait of interest at the seedling stage and in all environments without suitable conditions, providing year-round selection. Therefore, the selection of desirable traits is progressive. Moreover, it can identify and differentiate the number of target genes/QTLs in the individual plant that is suitable for gene pyramiding (Jiang, 2013). The use of DNA markers to assist in plant breeding is called marker-assisted selection (MAS) which is selected based on the genotype of markers, either directly or indirectly. It divided into 5 approaches consisting of early generation selection, marker-assisted backcrossing (MABC), gene pyramiding, combined marker assisted selection, and marker assisted evaluation of breeding materials (Collard and Mackill, 2008).

2.4.2.3.1 Marker-assisted backcrossing (MABC)

The MABC is the backcrossing technique assisted by molecular markers to
select the desirable traits, such as disease resistance from a donor parent into high-yielding variety (recurrent parent). Conventional backcrossing takes at least 5-6 generations to recover the recurrent parent while using this technique can save at least 2-4 generations of backcross. The MABC is used to select against the donor genome which may accelerate the recovery of the recurrent parent genome through two steps consisting of foreground and background selection.

In the foreground selection, using of markers linked to or flanked the target genes or quantitative trait loci (QTL) (about < 5 cM on either side) to screen putative plants that carried the target genes or QTLs from the donor parent. Molecular studies on disease resistance genes have been identified for MAS in mungbean breeding program. Alam et al. (2014) found two QTLs linked to MYMV resistance gene from a BM1 × BM6 cross. These QTL flanked between CEDG275-CEDG006 and CEDG041-VE5503 SSR markers. Poolsawat et al. (2017) identified two markers linked to the PM resistance which were inter-simple sequence repeat (ISSR) (I85420) and ISSR-anchored resistance gene analog (ISSR-RGA) (I42PL229) markers. These markers were obtained from a cross between CN72 (susceptible variety) and V4718 (resistant line). They were closest to the PM resistance gene with a distance of 9 and 4 cM, respectively, and showing only 0.72% recombination of both markers. Moreover, they found 2 ISSR-RGA (I27R211 and I27R565) markers associated with the PM resistance in another cross of CN72 and V4785. Arsakit et al. (2017) also found two SSR markers (VR393 and CEDG084) linked to 2 major QTLs for CLS and PM resistance in the CN72 × V4718 cross.

In the background selection, the selection of putative plants is based on markers to recover the genome region of the recurrent parent which are not linked to the target gene from the donor parent. However, the progress in recovery of the recurrent parent genome (RPG) depends on the number of markers used in background selection. In mungbean, a total of 53 QTLs related with yield traits or other traits were identified by SSR and EST-SSR markers. Among these, 20 loci are major QTLs (PVE 20%) linked to agronomic traits i.e., 100-seed weight, rate of shattered pods (PDR4W), pod length (PDW), stem internode length (ST11-ST10I), stem length (STL), days to first flower (FLD) (Isemura et al., 2012). These QTLs were distributed on several linkage groups (LGs) which are presented in Table 2.1. Therefore, the use of flanking markers of those QTLs may be associated with a nature of high yielding recurrent parent. Three or four times backcrossing to the recurrent parent in a practical genetic background is sufficient to increase RPG recovery to more than 99%, with selection comparable to BC5.
generation without marker-assisted background selection (Ribaut and Betran, 1999; Hospital, 2003) (Figure 2.2).

Table 2.1 The QTLs related yield traits in BC1F1 populations (Isemura et al., 2012)

<table>
<thead>
<tr>
<th>Traits</th>
<th>LGa</th>
<th>Intervals</th>
<th>LODb</th>
<th>PVEc</th>
<th>Substitution effects</th>
</tr>
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<tbody>
<tr>
<td>100 seed weight</td>
<td>8</td>
<td>VM37-CEDG030</td>
<td>28.1</td>
<td>22.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Pod dehiscence</td>
<td>1</td>
<td>CEDG214-CEDG012</td>
<td>21.4</td>
<td>24.2</td>
<td>-9.93</td>
</tr>
<tr>
<td>Pod size</td>
<td>7</td>
<td>CEDG064-CEDG074</td>
<td>30.1</td>
<td>28.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Internode length</td>
<td>7</td>
<td>CEDG064-CEDG174</td>
<td>22.3</td>
<td>20.5</td>
<td>1.06</td>
</tr>
<tr>
<td>Stem length</td>
<td>9</td>
<td>GMES1216-CEDG166</td>
<td>20.0</td>
<td>20.6</td>
<td>-15.52</td>
</tr>
<tr>
<td>Flowering time</td>
<td>2</td>
<td>GMES0477-CEDG026</td>
<td>46.7</td>
<td>32.9</td>
<td>-11.96</td>
</tr>
<tr>
<td>Internode length</td>
<td>10</td>
<td>CEDG097-CEDG150</td>
<td>19.8</td>
<td>27.1</td>
<td>1.60</td>
</tr>
</tbody>
</table>

a LG = Linkage group.
b LOD= Logarithms of odds.
c PVE= Phenotypic variation value.

Figure 2.2 Expected recovery of RPG comparing MABB and conventional breeding in each BC generation.

2.4.2.3.2 Marker-assisted gene pyramiding (MAGP)

As above-mentioned, Introgression of multiple genes/QTLs may be applied though backcrossing, using multiple parent crossing or complex crossing. However, it is difficult to identify the plants with 1, 2 or more resistance genes by only the phenotypic screening. Therefore, genotypic selection which is used to combine several genes into a single genotype can be helpful identifying the number of genes. The most
frequently used strategy of pyramiding is combining multiple resistance genes which is purposed to enhance broad-spectrum resistance to diseases or pests. Some examples of MAGP are found in several crop species such as wheat, soybean, tomato, and common beans (Carneiro et al., 2010; Hanson et al., 2016; Wang et al., 2017; Yadawad et al., 2017). In addition, Papan et al. (2022) using MAGP in mungbean [Vigna radiata (L.) Wilczek] for improved mungbean varieties with resistance genes and desirable traits. H3 line is an example of mungbean improvement. It showed high yield and was resistant to CLS and PM. These will be useful for the development of new mungbean varieties through MAGP.

### 2.5 References


Jiang, G. L. (2013). Plant breeding from laboratories to fields: Molecular markers and marker-assisted breeding in plants (pp. 45-83). Croatia: InTech.


