CHAPTER II
LITERATURE REVIEWS

2.1 Importance of mungbean

Mungbean [*Vigna radiata* (L.) Wilczek], previously known as *Phaseolus aureus* or P. *radiatus* (Döring, 2019), is a leguminous plant that belongs to the family Fabaceae or Leguminosae (McNeill et al., 2012), which comprises approximately 18,000 to 20,000 species (Smýkal et al., 2015; Ranjbar and Zahra, 2016; Silva et al., 2023). Mungbean produces small, green, circular seeds and belongs to the subgenus Ceratotropis. It is a diploid species with 2n = 2x = 22 chromosomes (Mehandi et al., 2019; Swamy, 2023). This legume holds strategic importance in Southeast Asia for both nutritional security and sustainable crop production (Rachie and Roberts, 1974; Konarev et al., 2002). Due to their richness in high-quality protein, essential minerals, and vitamins, mungbean is an integral component of the diet for a vast majority of the Indian population. Mungbean has the ability to fix atmospheric nitrogen through a symbiotic association with *Rhizobium* bacteria (Ali, 1992), which enables it to fulfill its own nitrogen requirements and benefit subsequent crops. It has also been reported to suppress weed flora by approximately 20-45% when intercropped with tall cereal crops, thereby reducing the cost of weed control (Ali, 1988). As a short-duration, year-round crop with tolerance to drought and high temperatures, along with photo-thermal insensitivity, mungbean is considered an ideal crop for intensification and diversification (Mehandi et al., 2019). Mungbean is commonly used in crop rotation with other plants, as its roots can efficiently fix atmospheric nitrogen through symbiosis with *Rhizobium* in the root nodules via biological nitrogen fixation (BNF) (Elahi et al., 2004; Ali and Gupta, 2012; Favero et al., 2021). This process plays a vital role in increasing crop yields on a sustainable basis (Kannaiyan, 1999). Mungbean can fix between 58-109 kg of nitrogen per hectare (Swamy, 2023). Furthermore, the ability of legumes to fix nitrogen from the atmosphere is crucial for agricultural sustainability (Ali and Gupta, 2012). The *Rhizobium* inoculation has been shown to improve nodulation, thereby promoting crop yield (Henzell, 1988). Similarly, Yang et al. (2008) reported an increase in yield due to *Rhizobium* inoculation. Mungbean is a source of high-quality protein, which can be consumed as whole grains, dhal, or in sprouted form, making it an excellent complement to rice for balanced human nutrition. In addition to serving as a prime source of human food and animal feed, fertility by improving soil physical properties and fixing atmospheric nitrogen.

2.2 Mungbeans economic state

There is a growing need to transform global food systems to better align with the objectives of improving human health and environmental sustainability in the future (Godfray et al., 2010; Global Panel, 2016; Springmann, 2016; Willett et al., 2019). By 2050, the consumption of fruits, vegetables, nuts, and legumes will need to double (Sequeros et al., 2021). Legume plants, such as soybean, ground nut, common bean, cowpea, and mungbean, are among the major grain legumes cultivated in Southeast Asia and East Africa. These crops play a crucial role in the transformation of the global food system, as they provide plant-based sources of dietary proteins and essential micronutrients.

Global production of mungbean is approximately 6.0 million tons, derived from a cultivated area of around 43.8 million rai (Gayacharan et al., 2023), with an average yield of 120.17 kg/rai (Nair and Schreinemachers, 2020a). India and Myanmar each account for 30% of the global output, totaling 5.3 million tons. Other significant producers include China, Indonesia, Thailand, Kenya, and Tanzania. As shown in Table 2.1, six countries in Southeast Asia planted mungbean on approximately 2.76 million rai producing about 0.51 million tons of dry grain.

Table 2.1 Mungbean production statistics for the six countries in southeast Asia during 2016-2017.

Countries	Area planted (1000 rai)	Production (1000 t)	Average yield (kg/rai)	References
Cambodia	270	54	196.7	(Ministry of Agriculture Forestry and Fisheries, 2018)
Indonesia	1,242	244	196.7	(Ministry of Agriculture Statistik Pertanian, 2010, 2014, 2018)
Laos	12	3	N/A	(Ministry of Planning and Investment, 2018)
Philippines	252	35	140.0	(Philippine Statistics Authority, 2018)
Thailand	492	86	173.3	(Ministry of Agriculture and Cooperatives, 2018)
Vietnam	498	92	185.0	(Ministry of Agriculture Livestock and Fisheries, 2015)

With the use of high-quality mungbean varieties and appropriate crop management practices, mungbean yields have the potential to reach up to 312.5 kg/rai (Nair and Schreinemachers, 2020a). However, in recent years, many mungbean cultivation areas have been replaced by higher-yielding crops such as sugarcane, feed corn, and cassava. This shift is primarily due to the relatively low yield performance of grain legumes compared to cereals and oilseed crops, as well as the slower rate of yield improvement in legumes (Byerlee and White, 2000; Gowda et al., 2009; Jha et al., 2014).

Consequently, mungbean production has not been sufficient to meet rising domestic demand. Despite the expanding needs of the industrial sector, particularly for products such as bean sprouts, vermicelli, and traditional sweets, local production has remained inadequate. According to the Office of Agricultural Economics (2015), domestic mungbean consumption reached 115,317 tons, while national production was only 99,301 tons. This shortfall necessitated the importation of mungbeans from abroad. A significant portion of domestic mungbean use is attributed to the sprouting industry, which utilizes both mungbean and black gram, with demand for sprouting purposes alone estimated at around 70,000 tons annually (Masari et al., 2011). Although mungbean imports are not extensive, they serve to supplement domestic supply, primarily sourced from neighboring countries such as Myanmar and Indonesia. Mungbean exports from Thailand primarily consist of seeds, with key trading partners including Hong Kong, Singapore, Philippines, and Malaysia

Mungbean can be cultivated year-round across all regions of Thailand. However, optimal planting periods are during the late rainy and dry seasons. Several certified mungbean varieties such as CN36, CN84-1, KPS1, and KPS2 are widely adopted by farmers due to their high yield potential and adaptability. Despite its importance, mungbean production in Thailand faces multiple constraints, including limited yields and insufficient output to satisfy domestic needs. Moreover, the crop is vulnerable to a range of diseases that can adversely affect both yield and grain quality.

2.3 Influence of environmental factors on plant growth and development

Plants are continually exposed to dynamic and potentially harmful environmental conditions. As immobile organisms, they have evolved complex and highly specialized defense mechanisms, many of which rely on the production of a wide array of chemical metabolites to help mitigate stress. Mungbean is recognized as a highly adaptable crop with considerable drought tolerance. It can be cultivated in diverse soil types across regions including Southeast Asia, Southeast Africa, Australia, and South America (Parihar et al., 2022). Optimal growth requires evenly distributed rainfall ranging from 400 to 550 mm during the growing season (Bhardwaj et al., 2023). Azimov (2023) reported that mungbean can grow in environments with limited soil moisture and fertility, and it plays a significant role in rain-fed agricultural systems in the dry and intermediate zones of Sri Lanka.

The growth and development of mungbean are influenced by several environmental factors, most notably light, temperature, water availability, and nutrient levels. Understanding how these factors affect plant physiology is essential for optimizing growth strategies and achieving specific cultivation goals, such as enhancing leaf development, promoting flowering, or increasing overall biomass. Furthermore, knowledge of these environmental influences allows for better identification and management of plant stress symptoms, thereby improving crop health and productivity under variable environmental conditions.

2.3.1 Light

Light is a fundamental environmental factor influencing the growth and development of plants. It serves as one of the most critical external cues regulating plant behavior and developmental processes (Whitelam and Halliday, 2007). Plant health and productivity largely depend on the availability and characteristics of light, as it is essential for photosynthesis the process by which plants convert light energy into sugars and starches necessary for growth. When assessing light requirements for tropical crops, including mungbean, three key aspects must be considered: intensity, duration, and quality.

Light Intensity plays a vital role in various physiological processes, impacting plant productivity, stem elongation, leaf pigmentation, and flowering. However, excessive light can be harmful, leading to symptoms such as leaf discoloration, sunburn, browning, and eventual tissue death (Alessandro and Havaux al., 2020). Light Duration, or photoperiod, refers to the length of time a plant is exposed to light within a 24-hour cycle. Photoperiod significantly influences flowering behavior in many species. Aggarwal and Poehlman (1997) observed that mungbean genotypes exhibited variation in flowering responses to photoperiod and temperature. In the equatorial region, an increase in day length was associated with higher mean temperatures and delayed flowering. Bashandi and Poehlman (1974) reported that extending the photoperiod beyond 12 hrs resulted in delayed flowering and increased plant height in mungbean, although the degree of response varied among genotypes. Light quality refers to the wavelength or color of light. Sunlight comprises a full spectrum of wavelengths, ranging from red to violet. Among these, red and blue wavelengths are the most influential in plant development. Blue light primarily supports vegetative growth, especially leaf expansion, while the combination of red and blue light is essential for stimulating flowering. The green coloration of plant foliage is due to the reflection rather than absorption of green wavelengths. A clear understanding of the effects of light intensity, duration, and quality is crucial for managing and optimizing plant growth conditions, especially in controlled environments such as greenhouses or growth chambers.

2.3.2 Temperature

Crop species exhibit varying responses to temperature throughout their life cycles, primarily manifesting as phenological responses at different stages of plant development. Each species has a specified range of maximum and minimum temperatures that delineate the limits of growth and development. Mungbean requires warm-humid climatic conditions, with temperatures ranging between 25°C and 35°C (Bhardwaj et al., 2023). The Reported from Lawn and Ahn, (1985) showed that temperatures of growing season for leguminous plant are > 20°C. The average yield of temperate legumes plant has moderately improved in past half a century, with about a 45-50% increase for most legumes (Araújo et al., 2015). The rate of vegetative development, including the appearance of nodes and leaves, tends to increase as temperatures approach the optimum level for the species. In most plant species, the optimum temperature for vegetative development is typically higher than that for reproductive development. Rising global temperatures associated with global warming and climate change pose a growing challenge to agricultural productivity. These temperature increases lead to morphological, anatomical, physiological, and biochemical alterations in plants, ultimately impacting their growth and development (Macalister et al., 2020) and causing reduced yields in plants.

Crop species exhibit distinct responses to temperature variations throughout their life cycles, often observed as changes in phenological development across different growth stages. Each crop has defined minimum and maximum temperature thresholds that determine its capacity for growth and productivity. According to Lawn and Ahn (1985), leguminous crops generally require growing season temperatures above 20°C for optimal development.

Over the past five decades, the average yields of temperate legume crops have shown moderate improvements, with increases of approximately 45-50% for many species (Araújo et al., 2015). The rate of vegetative development such as node and leaf formation typically accelerates as environmental temperatures approach the species’ optimal range. Notably, the temperature threshold for vegetative growth is often higher than that for reproductive development in many plant species. However, global climate change and the associated rise in average temperatures present increasing challenges for agricultural productivity. Elevated temperatures can induce a wide range of morphological, anatomical, physiological, and biochemical changes in plants, which may negatively affect growth and development. These stress responses can ultimately result in significant yield reductions (Macalister et al., 2020).

Extreme temperatures, whether excessively low or high, can negatively impact plant development, leading to growth inhibition, morphological abnormalities such as spindly growth, leaf damage, or premature leaf drop. In general, cooler nighttime temperatures are more conducive to plant growth compared to elevated night temperatures. For instance, the optimal upper temperature limit for growth in cool-season crops like broccoli (*Brassica oleracea* L.) is approximately 25°C, whereas warm-season crops such as maize (*Zea mays* L.) can tolerate temperatures up to 38°C (Hatfield and Prueger, 2015). Fluctuations in temperature can also disrupt the phenological development of crops such as soybeans. Exposure to high temperatures during critical reproductive stages can impair pollen viability, hinder fertilization, and negatively affect grain or fruit formation (Hatfield and Prueger, 2015). Even moderate heat stress defined as temperatures 1 to 4°C above the species-specific optimum has been shown to reduce yields (Wagstaffe and Battey, 2004; Timlin et al., 2006; Tesfaendrias et al., 2010). In contrast, cold stress can affect both the vegetative and reproductive phases of plant development, with the reproductive stage being particularly vulnerable to damage (Nishiyama, 1995).

2.3.3 Water

Water is a fundamental component of plant life, comprising approximately 70-95% of plant tissue (Lambers and Oliveira, 2019). It is a critical limiting factor in plant growth and development. When the rate of transpiration exceeds the rate at which roots absorb water, plants experience a water deficit, resulting in stunted growth, a condition commonly referred to as drought stress. Water plays multiple essential roles in plants: it serves as a medium for transporting nutrients and minerals from the soil through the roots into the plant's vascular system and is a key participant in photosynthesis. During this process, water molecules are split to release oxygen and generate glucose, which sustains the plant’s energy requirements. Additionally, water is vital for maintaining turgor pressure, which supports cell rigidity and overall plant structure. It also helps regulate plant temperature. Due to water's high specific heat capacity, plant tissues can absorb or release substantial amounts of heat with minimal temperature change, offering protection against thermal extremes.

Despite mungbean relatively low water requirement compared to other legume species and its adaptability to rainfed conditions, drought stress remains a major abiotic factor limiting its productivity (Chaiyapan et al., 2023). In response to drought conditions, plants activate a range of morphological, physiological, and biochemical mechanisms to mitigate stress. These include increasing relative water content, enhancing antioxidant enzyme activity, reducing excised leaf water loss, and modifying root length all of which contribute to improved stress tolerance (Kalaji et al., 2018; Lontom et al., 2020).

2.3.4 Nutrition

Plants require a diverse range of nutrients to support their growth, development, and metabolic processes. These essential nutrients are primarily absorbed from the soil and can be broadly categorized into two groups: macronutrients and micronutrients. According to Barker and Pilbeam (2015), plants require approximately 14 essential mineral elements for optimal growth and development. Macronutrients are needed in larger quantities and play crucial roles in the formation of plant tissues, proteins, and enzymes. In contrast, micronutrients, although required in smaller amounts, are equally vital. They are involved in numerous physiological and biochemical processes, including primary and secondary metabolism, cellular defense mechanisms, signal transduction, gene expression, energy transfer, and hormone reception (Vatansever et al., 2017). Water also plays a critical role in nutrient transport and the regulation of photosynthesis, which is driven by sunlight. Mungbean is commonly cultivated in marginal soils with minimal inputs, making it highly susceptible to various abiotic stresses that significantly reduce seed yield (Singh and Singh, 2011). Among these stresses, soil salinity poses a major challenge, particularly in coastal regions where mungbean is frequently grown as a rice fallow crop (Kumar et al., 2012). Exposure to salinity stress throughout the crop’s life cycle can lead to substantial yield losses in mungbean.

2.4 The crucial diseases of mungbean

2.4.1 Mungbean yellow mosaic virus (MYMV)

One of the most devastating diseases affecting mungbean production across Asia is MYMV (Sudha et al., 2013). The first report of an MYMV outbreak in Thailand was recorded in Kamphaeng Phet Province, located in the northern region of the country (Thongmeearkom et al., 1981). The disease is highly destructive and has been reported to cause near-total yield loss in infected mungbean fields (Honda, 1986). In India, Usharani et al. (2004) documented significant yield losses due to MYMV in farmer-managed fields in Tamil Nadu.

The causal agent of the disease is transmitted by the tobacco whitefly (*Bemisia tabaci* Genn.) (Thongmeearkom et al., 1981). MYMV continues to spread rapidly into new regions, causing substantial economic losses up to 85% in mungbean under severe infection (Karthikeyan et al., 2013), and even complete (100%) yield loss when infection occurs at early growth stages (Kitsanachandee et al., 2013).

2.4.2 Powdery mildew (PM)

The first report of PM in cowpea, caused by *Sphaerotheca phaseoli*, was documented during the summer of 2003 in Turkey (Soylu et al., 2004). The damage caused by this pathogen can range from 20-40% under cold and dry conditions during the reproductive stage and can reach up to 100% during the seedling stage. White circular patches appear on the lower leaves, eventually spreading to the upper leaves. The development of PM is favored by cool temperatures (20-25°C) and moderately humid conditions, though excessive wetness does not promote disease progression. Symptoms include small white powdery spots that can spread to cover the entire leaf surface. The use of PM-resistant cultivars is considered one of the most effective strategies for managing this disease. Several mungbean genotypes, such as V4189, V4207, V4668, V4574, V4718, V4758, and V4785, have been reported to exhibit resistance to PM (Nair et al., 2020b). Among these, the genotypes V4718, V4758, and V4785 demonstrate particularly high levels of resistance to PM (Chueakhunthod et al., 2020).

2.4.3 Cercospora leaf spot (CLS)

CLS is a significant disease affecting mungbean, caused by the fungus *Cercospora canescens*. The first documented report of this disease was made in 1960 by Munjai et al. (1960) in India. CLS severely impacts both the quality and yield of mungbean production, with yield reductions ranging from 50-80%, depending on the severity of the infestation. In extreme cases, yield losses may reach up to 93% if the outbreak is not effectively managed (Lal et al., 2001; Kaur, 2004; Chand et al., 2012). The fungus is dispersed through both air and soil, and *C. canescens* can infect both the upper and lower surfaces of plant leaves. Its hyphae are approximately 2-4 microns wide, septate, and exhibit a brownish-green color. The conidiophores, which are stalk-like structures that bear conidia (spores), may be either straight or curved and range in color from light brown to dark brown, often with few branches. The conidia are clear, colorless, needle-shaped with sharp tips, and measure approximately 2.5-6.0 microns in width (Phengsintham, 2013).

The disease cycle of *C. canescens* begins when spores fall onto plant leaves and germinate under favorable weather conditions, particularly in warm and humid environments. The spores germinate, and the fungus grows and reproduces continuously until the end of the growing season. After the planting season ends, the spores can persist in plant debris for an extended period, ranging from 1 to 4 months, awaiting the next growing season to infect plants again (Pool and McKay, 1916). The disease typically spreads when plants are around 30 to 40 days old, leading to leaf shedding and affecting the size of pods and seeds (Grewal et al., 1980). Skaracis et al. (2010) reported that humidity exceeding 90%, daytime temperatures between 27-30°C, and nighttime temperatures around 17°C create optimal conditions for CLS disease in sugar beet crops. The host range of *Cercospora* spp. is diverse, primarily affecting plants within the Fabaceae family, particularly genera such as *Phaseolus* spp. and *Vigna* spp.

The fungus *C. canescens* initiates leaf damage, resulting in the formation of necrotic spots, typically ranging from 3-15 millimeters (mm) in diameter at the center. In the early stages, these spots appear brown, gradually turning gray with a red-brown border. Symptoms can be observed on both the upper and lower surfaces of the leaves (Munjal et al., 1960). Vakili (1977) reported that lesions caused by *C. canescens* and *C. cruenta* on mungbean typically manifest as round lesions measuring 8-15 mm, with orange to light brown colors. The lesions turn gray as the fungus produces spores. Additionally, the disease may affect leaf stems, flower stems, and pods. Uddin et al. (2013) reported that lesions caused by *C. canescens* on mungbean initially appear as water-soaked spots on the leaves. As the lesions age, individual spots may merge, resulting in larger lesions. Severe infestations can lead to leaf distortion and deformation (Daub and Ehrenshaft, 2000).

2.5 Breeding strategies of mungbean

2.5.1 Conventional breeding methods

Plant breeding strategies aim to enhance crop traits such as yield, disease resistance, stress tolerance, and nutritional quality (Breseghello and Coelho, 2013). Traditional or conventional breeding methods in plants improve traits by selecting and crossing plants with desirable characteristics. These methods rely on natural genetic variation and do not involve genetically modified organisms (GMOs) or molecular breeding techniques (Akhtar et al., 2023). Hybridization is employed to obtain the desired traits from closely related individuals and incorporate them into new cultivars. Parents are selected based on their superior performance in predetermined traits. Traditional breeding typically takes about 10 years to release a new cultivar (Bharti and Chimata, 2019). However, conventional plant breeding is not only time-consuming but also expensive. Additionally, newly developed cultivars may not meet practical needs due to the emergence of new diseases or changes in environmental conditions.

The development of mungbean varieties in Thailand has advanced significantly through conventional breeding methods. In 1976, the Department of Agriculture (DOA) released the U-Thong 1 variety, which originated from the Philippines. This variety was selected for its uniform maturity, high yield potential, and larger seed size compared to local cultivars. A decade later, in 1986, Kasetsart University introduced two improved varieties, KPS1 and KPS2, which exhibited high productivity and enhanced resistance to CLS and PM. These cultivars were developed through mass selection from breeding lines VC1973A and VC2778A, respectively, both originally sourced from Taiwan (Srinives, 1994). In 1997, Suranaree University of technology released the SUT1 variety, derived from a cross between U-Thong 1 and NP-29 using the single seed descent method. This variety showed moderate resistance to CLS and PM (Laosuwan, 1999). Mungbean variety CN36 was developed from a cross between Pagasa 1 and PHLV 18 at the Asian Vegetable Research and Development Center (AVRDC), Taiwan. Various traits were evaluated including disease resistance and yield performance across multiple growing locations. The selection resulted in line VC1628A-7, which demonstrated superior agronomic characteristics and adaptability (Chai Nat Field Crops Research Center, 2021).

2.5.2 Marker assisted selection (MAS)

Conventional breeding faces several limitations, including the polygenic nature of many traits, lengthy breeding cycles, and the influence of environmental factors on phenotypic selection. Consequently, the application of MAS offers a promising approach to accelerate the plant breeding process and enhance selection efficiency. The advent of molecular tools has revolutionized plant breeding through MAS, which enables breeders to select traits based on linked genetic markers rather than solely on observable traits. This approach accelerates the breeding cycle and enhances selection accuracy. MAS is particularly valuable for complex or late-expressing traits, such as disease resistance and stress tolerance. Deoxyribonucleic acid (DNA) markers have been widely utilized in MAS to explore structural genomics in crop plants (Muthamilarasan and Prasad, 2015). These markers can detect allelic variation in genes, offering a more precise method of selection compared to traditional breeding (Collard and Mackill, 2008). The study by Wu et al., (2022) using the marker-assisted backcross breeding to transfer the VrPGIP2 gene conferring bruchid resistance into the mungbean cultivar KPS1. The advanced line R67-22 showed high resistance to bruchids and good agronomic traits, making it a promising candidate for cultivar release. Poolsawat et al. (2017) have developed and used markers linked to the PM resistance gene of a cross between CN72 and V4718 using inter-simple sequence repeats (ISSR) I85420 and ISSR-anchored resistance gene analog (ISSR-RGA) I42PL229 markers and closest to the PM resistance gene. Chankaew et al. (2009) investigated the inheritance of CLS resistance in a cross between the mungbean resistant line V4718 and the susceptible variety KPS1. Their findings indicated that resistance in V4718 is governed by a single dominant gene, which can be effectively used to distinguish between susceptible and resistant genotypes.

2.5.3 Mutation breeding

Mutation breeding is a plant breeding method in which new crop varieties with desirable characteristics are generated through induced mutations (Mir et al., 2020). This approach involves using physical agents like gamma rays or chemical mutagens, such as ethyl methane sulfonate (EMS), to induce genetic mutations. These mutations create novel genetic variability that may not naturally occur in a crop species (Yali and Mitiku, 2022). Mutagenic substances cause the desired genetic alterations (Oladosu et al., 2016). Mutant varieties often exhibit beneficial traits, including improved quality or enhanced stress resistance, and can either be directly released or used as parental lines in breeding programs. Genetic changes in plants, including those induced by mutation, contribute significantly to the improvement of crop species (San Martín, 2021). Worldwide, approximately 2,252 mutant variants have been developed across various plant species (Yali and Mitiku, 2022). For example, the mungbean variety CN84-1 is a mutant line of CN36, irradiated with 500 Gy of gamma rays (Ngampongsai et al., n.d.), while the CN3 variety was derived from CN36 through exposure to 400 Gray of gamma irradiation, with selection and evaluation conducted between 2005 and 2018 (Jomsangawong et al., 2022). Other successful mutant-derived varieties include CN72 from Thailand, PsJ-B-II-17-6 and PsJ-S-31 from Indonesia, NM98 from Pakistan, I-176 from China, and PAEC 3 from the Philippines (Watanasit et al., 2001; Ngampongsai et al., 2004, 2008). The integration of these strategies combining conventional breeding with MAS and mutation breeding represents a modern, efficient, and science-based approach to crop improvement, ensuring food security and sustainability in agriculture.

2.5.4 Genetic engineering

Genetic engineering techniques enable the precise excision and transfer of specific DNA sequences, known as candidate genes, from a wide range of sources including animals, viruses, bacteria, fungi, or even synthetic sequences designed in the laboratory. These genes can then be introduced into target plants using methods such as *Agrobacterium*-mediated transformation or the biolistic (gene gun) approach. Compared to traditional breeding techniques, genetic engineering significantly shortens the time required to develop new plant varieties with desired traits. Candidate genes related to disease resistance often play crucial roles in the interactions between plants and microbial pathogens by restricting pathogen virulence. These include genes encoding enzymes that degrade pathogen cell walls, toxins that inhibit nucleic acid synthesis, and other molecules that interfere with pathogen survival. Additionally, such genes can boost the plant's own defense mechanisms by promoting the synthesis of antimicrobial peptides, phytoalexins, and reactive oxygen species (ROS), which collectively enhance the plant’s resistance to infection. For instance, enhanced resistance against MYMV has been achieved in a related legume species, blackgram (*Vigna mungo*), through the expression of the soybean replication initiation protein (Rep) gene. Haq et al. (2010) demonstrated that blackgram plants co-inoculated with infectious constructs of the soybean isolate of MYMV and an antisense Rep gene construct exhibited significant resistance to the virus. Despite these promising results, genetic engineering does not completely replace conventional breeding methods, especially in countries like Thailand, where regulatory, economic, and public acceptance challenges limit the commercial application of genetically modified crops.

2.5.5 Genome editing

The development of genetically engineered crops that do not carry selectable marker genes for antibiotic resistance has advanced significantly with the emergence of genome editing technologies (GETs). These technologies have become valuable tools for plant breeders due to their precision, efficiency, and ability to make targeted modifications in plant genomes much faster than traditional breeding methods. GETs enable specific alterations at precise locations within the DNA sequence, such as small insertions or deletions, allowing for gene silencing, modification of gene function, or the introduction of new, functionally important genes. This precision offers distinct advantages over conventional breeding techniques that rely on random mutagenesis or longer breeding cycles. Genome editing approaches depend on the creation of targeted double-strand breaks in DNA facilitated by programmable nucleases. Among these, the CRISPR/Cas9 system derived from a bacterial adaptive immune mechanism has become widely adopted due to its RNA-guided specificity and relative ease of use. CRISPR/Cas9 allows researchers to direct nucleases to exact genomic sites, thereby enabling precise edits. Successful applications of CRISPR/Cas9-mediated genome editing have been reported in various crop species to enhance disease resistance. Wang et al. (2016) employed CRISPR/Cas9 along with sequence-specific nucleases to target the OsERF922 gene at multiple loci in the rice genome to improve resistance against rice blast disease. Their study demonstrated a significant reduction in blast symptoms across all six edited mutant lines compared to wild-type plants at both seedling and tillering growth stages. Importantly, no notable differences in key agronomic traits were observed between the edited T_2 mutant lines and the wild-type controls, indicating that the genome edit conferred disease resistance without compromising plant growth or yield-related characteristics.

2.6 Regional yield trials and stability

Regional yield trials, also known as multi-environment yield trials (MET), are a crucial component of developing new crop varieties tailored to specific agroecological regions. These trials involve evaluating the performance of different crop varieties across multiple locations within a defined region to assess their adaptability, yield potential, and resistance to pests, diseases, and environmental stresses. Regional yield trials are typically conducted during the final stages of genotype selection in plant breeding programs. They can be carried out by research institutions or farmers themselves (McGuire et al., 2003). By conducting regional yield trials, researchers ensure that crop varieties are well-suited to the unique climatic and soil conditions of specific regions, thereby optimizing productivity and minimizing the risks posed by environmental stressors. These trials play an essential role in evaluating the yield and stability of genotypes and hybrids (Alwala et al., 2010).

Regional yield trials play a critical role in selecting crop varieties that are well-suited to local conditions and climates across different regions. These trials help farmers make informed decisions by identifying varieties that offer optimal performance and profitability for their farms. Regional yield trials are essentially networks of experiments in which a set of cultivars is assessed to provide genotype recommendations (Shaner et al., 1982; Hildebrand and Poey, 1985). Moreover, regional trials allow breeders and agricultural researchers to allocate resources more efficiently, focusing on developing varieties with traits that directly address the needs and challenges of specific regions.

An important factor in stability studies is the yield potential across multiple locations. Plant yield is primarily influenced by the environment (E) rather than genotype (G) and genotype-environment interaction (GEI). In the absence of GEI, testing in a single environment would suffice for cultivar evaluation. Therefore, understanding the GEI observed in regional yield trials is crucial in breeding programs for identifying high-yielding cultivars with either broad or specific adaptability (Smith et al., 2001; Yan and Hunt, 2002). A particular GEI of interest in breeding programs is one that causes a change in the ranking of cultivars across different environments, known as crossover interaction. This suggests that a cultivar performing well in one environment may not maintain its performance in another environment. Several statistical methods have been developed to analyze GEI, such as calculating regression coefficients, summing squared deviations from the regression, and using additive main effects and multiplicative interaction (AMMI).

However, predicting the overall response of genotypes to environments, and their stability, is not always reliable with these methods (Alwala et al., 2010), due to the multivariate nature of genotype response to the environment. The "GGE Biplot" technique is a graphical method that illustrates the main effects of genotype (G) along with GEI effects. The GGE biplot visually captures genotype-by-environment interaction (GGE) patterns in MET data by plotting principal component scores of both genotypes and environments. The GGE biplot is particularly useful for mega-environment analysis, such as the "which-won-where" pattern, allowing for the recommendation of genotypes specific to mega-environments, genotype assessment (evaluating mean performance and stability), and test environment evaluation. This tool is employed to assess the high-yield potential of different mungbean genotypes across various tested locations to ensure their adaptability. For example, Queme et al. (2010) used the GGE Biplot method to evaluate sugarcane yields in Guatemala, identifying varieties with specific adaptability to certain locations and those with broader adaptability. Similarly, Yan and hunt (2002) utilized the GGE biplot to visually represent the relationship between varieties and environmental conditions in a two-way table, using GE scores as a single value. Varieties with higher GE scores were found to be superior in the studied characteristics and demonstrated efficiency in environmental assessment, aiding in the differentiation of varieties suitable for specific environmental conditions.

Pobkhunthod et al. (2022) conducted a study on multilocation yield trials and yield stability evaluation in *Arachis hypogea* L. (peanuts) involving 12 promising lines tested across 12 different planting locations during both dry and rainy seasons. The study focused on the genotype × environment interaction (GEI) in peanut production. The results indicated that the total variation in seed yield accounted for 64.22%, with principal component 1 (PC1) and principal component 2 (PC2) explaining 45.71% and 18.51% of the variation, respectively. The genotype KUP12BS029-1-1-3 demonstrated high yield potential and stability across multiple locations, followed by KUP12BS030-3-4-1 and KUP12BS030-1-4-3. These promising lines are expected to be released as new peanut varieties in central Thailand.

Wongpiyasatid et al. (2000) conducted regional yield trials in 1998 and 1999 to evaluate twelve newly developed mungbean mutant lines. In the 1998 trial, the lines were grown in 10 experimental plots across 7 locations. Among these, the mutant lines M5-5, M5-1, and M4-2 exhibited superior performance, with average yields of 243, 235, and 229 kg/rai, respectively, outperforming the certified varieties KPS1 and CN36, which yielded 213 and 228 kg/rai. In the 1999 trial, conducted at 5 locations, the lines M5-10, M4-2, M5-5, and M5-22 recorded yields of 240, 240, 236, and 232 kg/rai, respectively, while KPS1 and CN36 yielded 227 and 232 kg/rai. Based on consistently high yields across both years, the mutant lines M5-5, M5-1, and M4-2 were identified as promising candidates for future cultivar development.

Parihar et al. (2022) evaluated 34 mungbean genotypes across 39 locations in five agroecological zones in India to assess genotype × environment interaction. The results showed that environmental factors (54.2%) and genotype × environment interaction (29.7%) had a greater impact on yield than genotype alone (3.0%). While phenological traits varied across locations, they were generally not directly related to yield. Instead, rainfall and relative humidity were found to significantly influence productivity. Heritability-Adjusted GGE Biplot (HA-GGE) analysis identified key sites such as Sagar, New Delhi, and Durgapura as ideal testing environments for selecting widely adaptable genotypes. The study emphasizes the importance of site-specific environmental factors in strategic mungbean breeding.

In a study by Van Giang et al. (2024), GEI was highlighted as crucial for selecting high-yielding, stable mungbean genotypes. The study evaluated eight elite mungbean lines (DTG01-DTG08) and a check variety (DX208) across three locations in Vietnam over three crop seasons. All genotypes outperformed the check, with DTG05 and DTG06 exhibiting the highest yields (110.62 and 118.13 kg/rai, respectively). AMMI analysis revealed that DTG05 performed best in the summer season, particularly at Thanh Hoa and Ha Noi, indicating its potential for commercial cultivation in Northern and North Central Vietnam.

2.7 The proximate analysis of nutrition in mungbean

The proximate analysis of food is a standard laboratory procedure used to determine the approximate composition of food products. Standard Official Methods of Analysis (AOAC) methods were employed for the proximate composition analysis of mungbean seed samples (Kavanagh, 1981). This analysis involves the measurement of various macronutrient components, typically including moisture, protein, fat, ash (mineral content), and carbohydrates. The details of each component are described as follows:

Moisture refers to the water content present in the food, specifically the loss of water and volatile substances during drying (Thangaraj, 2019; Ganogpichayagrai and Suksaard, 2020; Puwastien et al., 2021). Moisture content is determined by weighing the food sample before and after drying it in an oven at a specific temperature until a constant weight is reached. The difference in weight corresponds to the moisture content. Moisture content is a key factor in storage, as it limits the growth of microorganisms, such as fungi and bacteria (Thangaraj, 2019; Ganogpichayagrai and Suksaard, 2020).

Protein content is determined by measuring the nitrogen content of the food sample using the Kjeldahl or Dumas method. The nitrogen percentage is then multiplied by a factor of 6.25 to estimate the protein content (Nagrale et al., 2018). Different types of foods have varying protein conversion factors (Thangaraj, 2019; Ganogpichayagrai and Suksaard, 2020; Puwastien et al., 2021). Mature legumes, including mungbean, generally contain higher protein levels (Cheng et al., 2019), and the constituent amino acids determine the quality of the protein (Millward et al., 2008; Khan et al., 2019; Khan and Azam, 2021). The nutritional quality of legume proteins can be enhanced through various processing methods, such as in flours and baked goods (Wang et al., 2003; Boye and Pletch, 2010; Fasoyiro and Taiwo, 2012).

Fat content is determined by extracting lipids from the food sample using a solvent, such as ether or petroleum ether. The extracted fat is then weighed to determine its content in the sample. The recommended method for fat extraction is Soxhlet extraction, a technique commonly used to extract lipids from food samples. Petroleum ether is typically employed as the solvent in this method, although other solvents can also be used (Anonymous, 1998; AOAC, 2000).

Ash content refers to the mineral content of the food sample (Ismail, 2024) and represents the inorganic components, such as minerals and salts, remaining after complete combustion at high temperatures (500-600°C) (Nagrale et al., 2018). During this process, water and volatile substances are vaporized, and organic materials are burned in the presence of oxygen to form CO_2 and oxides of nitrogen (Marshall, 2010). Minerals are typically converted into oxides, sulfates, phosphates, chlorides, and silicates. Some elements, such as Fe, Se, Pb, and Hg, may partially volatilize during this procedure, requiring additional methods for specific elemental analysis.

Carbohydrate content is determined by difference, calculated by subtracting the sum of moisture, protein, fat, and ash content from 100%. This method assumes that all remaining components in the food sample are carbohydrates. Different types of carbohydrates are associated with various beneficial physiological effects for human health (Wahlqvist, 2002). However, food composition databases typically report total carbohydrate content measured by difference, without specifying individual carbohydrate components (Zafar et al., 2023).

Mungbean is a rich source of macronutrients, including carbohydrates, proteins, lipids, and dietary fiber. They provide sustained energy due to their complex carbohydrate content and more carbohydrate content (50-60%) than soybeans (Tang et al., 2014), making them a staple in many cuisines. Mungbeans are notable for the good source of protein due to their high protein content (Engel. 1978), making them a valuable plant-based protein source for vegetarians and vegans. Moreover, the dietary fiber present in mungbeans supports digestive health and promotes feelings of fullness, resist obesity, and aiding weight management (Rane et al., 2023).

Zafar et al. (2023) conducted a study on the proximate and chemical composition of 25 mungbean varieties using standard AOAC methods for proximate composition analysis of mungbean seed samples. The results of the proximate assay of the mungbean showed moisture content ranging from 8.31-11.3%, protein content ranging from 20.50-25.40%, fiber content ranging from 3.22-6.76%, and a mean ash content of 3.67%. Dahiya et al. (2015) has been reviewed to assess nutritional properties for mungbean seeds in various genotypes. The results are shown in Table 2.2.

Table 2.2 Proximate nutritional compositions of mungbean seeds.

Macronutrients (%) dry basis	Minimum	Maximum	Average
Moisture	4.10	15.20	9.80
Crude protein	14.60	32.60	23.80
Crude lipid	0.71	1.85	1.22
Crude fiber	3.80	6.15	4.57
Ash	0.17	5.87	3.51
Carbohydrate	53.30	67.10	61.00

Naivikul and Patcharee (1989) studied four mungbean seed samples: U-thong-1, KPS1, Native variety, and U-thong-2. They analyzed the proximate nutritional composition of both seeds and sprouts. The results showed that moisture content increased 9 to 13 times after germination. Protein content slightly decreased from an average of 19.20% in seeds to 17.98% in sprouts. Similarly, fat content was reduced by more than half, ranging from 1.59-1.71% in seeds to 0.63-0.72% in sprouts. Ash content also decreased from 3.44-3.60% in seeds to 2.42-3.08% in sprouts. Fiber content dropped from 3.53-4.30% in seeds to 1.93-2.60% in sprouts. Carbohydrate content in sprouts was 12.00-26.00% lower than in seeds, with averages of 53.94% and 36.52%, respectively.

In the study by Dereje et al. (2023), the proximate composition of microgreens from five *Brassicaceae* species (broccoli, cabbage, kale, mustard, and radish) was evaluated. The analysis revealed that microgreens are rich in essential nutrients. The protein content ranged from 1.81 to 3.41 % fresh weight, while crude fiber content varied between 0.36 and 2.08 %. Fat content was relatively low, ranging from 0.19 to 0.39 %. Ash content, indicative of mineral presence, ranged from 0.59 to 1.17 %. Carbohydrate content was also reported, with values between 2.70 and 3.30 %. These results highlight the nutritional potential of Brassicaceae microgreens, particularly their protein and fiber content, making them valuable for dietary supplementation.

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