REGIONAL YIELD TRIAL AND NUTRITIONAL VALUES OF NEW MUNGBEAN LINES WITH POWDERY MILDEW AND CERCOSPORA LEAF SPOT RESISTANCE

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การทดสอบผลผลิตในระดับท้องถิ่นและคุณค่าทางโภชนาการของถั่วเขียวสายพันธุ์ใหม่ที่ต้านทานต่อโรคราแป้งและใบจุด

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คำสำคัญ: ปฏิสัมพันธ์ระหว่างพันธุกรรมกับสิ่งแวดล้อม/GGE biplot/ไมโครกรีน/การทดสอบหลายพื้นที่/การวิเคราะห์ปริมาณกลุ่มสาร/ถั่วงอก/*Vigna Radiata (L.)* Wilczek

พืชตระกูลถั่วเป็นแหล่งสารอาหารที่สำคัญและส่งเสริมความมั่นคงทางอาหารของโลก เนื่องจากเป็นแหล่งของโปรตีนและคาร์โบไฮเดรตที่สำคัญนอกเหนือจากโปรตีนจากเนื้อสัตว์ โดยในกลุ่มพืชเหล่านี้ ถั่วเขียว [*Vigna radiata* L. Wilczek] เป็นสปีชีส์ที่มีการปลูกอย่างแพร่หลาย อย่างไรก็ตาม การผลิตถั่วเขียวในปัจจุบันได้รับผลกระทบจากการระบาดของโรคที่สำคัญจากเชื้อรา ได้แก่ โรคราแป้ง (powdery mindew) และโรคใบจุด (Cercospora leaf spot) ที่ส่งผลให้ผลผลิตลดลงอย่างมาก ดังนั้นจึงมีความต้องการพันธุ์ถั่วเขียวที่มีความต้านทานโรคและคุณค่าทางโภชนาการสูงเพิ่มมากขึ้น งานวิจัยนี้มีวัตถุประสงค์เพื่อ 1) ประเมินศักยภาพการให้ผลผลิตของถั่วเขียวสายพันธุ์ใหม่ผ่านการทดสอบผลผลิตในระดับท้องถิ่น 2) ประเมินเสถียรภาพของถั่วเขียวจีโนไทป์ต่าง ๆ ในลักษณะผลผลิต และลักษณะที่เกี่ยวข้องกับผลผลิต และ 3) วิเคราะห์สัณฐานวิทยาและคุณค่าทางโภชนาการของเมล็ด ถั่วงอก และไมโครกรีน ในถั่วเขียวจีโนไทป์ต่าง ๆ ในการทดลองที่ 1 การทดสอบผลผลิตในระดับภูมิภาคและการประเมินเสถียรภาพของถั่วเขียวสายพันธุ์ใหม่ด้วยวิธี GGE biplot โดยปลูกทดสอบถั่วเขียว 8 จีโนไทป์ใน 4 สถานที่ระหว่างฤดูฝนและฤดูแล้ง ซึ่งจากผลการทดลองบ่งชี้ว่า ถั่วเขียวสายพันธุ์ใหม่ทุกสายพันธุ์แสดงความต้านทานโรคใบจุดและราแป้งดีกว่าพันธุ์รับ CN84-1 โดยสายพันธุ์ P22 และ P24 ให้ผลผลิตและเสถียรภาพสูงในหลายสภาพแวดล้อมทั้งสองฤดูเมื่อเทียบกับจีโนไทป์อื่น ๆ สายพันธุ์ P12 ให้ผลผลิตสูงในฤดูแล้งในพื้นที่จังหวัดนครราชสีมา แต่มีข้อจำกัดเรื่องการออกดอกและสุกแก่ล่าช้า ซึ่งการวิเคราะห์ GGE biplot ได้ยืนยันผลลัพธ์ว่าสายพันธุ์ P22 มีทั้งศักยภาพการให้ผลผลิตและเสถียรภาพสูง ขณะที่สายพันธุ์ P08 มีเสถียรภาพสูงสุดในด้านจำนวนฝักต่อต้นและน้ำหนัก 100 เมล็ด นอกจากนี้วิธี GGE biplot ยังเน้นย้ำถึงความสำคัญของปฏิสัมพันธ์ระหว่างพันธุกรรมกับสิ่งแวดล้อม (GEI) โดยพบว่าจังหวัดชัยนาทเป็นสถานที่ทดสอบที่เหมาะสมที่สุดในการแยกความแตกต่างของการให้ผลผลิตในถั่วเขียว โดยรวมสายพันธุ์ P22 และ P24 เป็นสายพันธุ์ที่มีศักยภาพสูงสุดเนื่องจากมีการปรับตัวอย่างกว้างขวางและให้ผลผลิตที่สูงในหลากหลายสภาพแวดล้อม ในขณะที่ P08 และ P12 มีความจำเพาะต่อสภาพแวดล้อม การทดลองที่ 2 เป็นการศึกษาคุณค่าทางโภชนาการ และองค์ประกอบทางโภชนาการผ่านการวิเคราะห์ปริมาณกลุ่มสาร (proximate analysis) ได้แก่ ความชื้น โปรตีน ไขมัน เถ้า ใยอาหาร และคาร์โบไฮเดรต และลักษณะทางสัณฐานวิทยาของเมล็ดและถั่วงอก โดยใช้เมล็ดที่ได้จากการปลูกในสองสภาพแวดล้อมที่ต่างกัน ได้แก่ ฤดูฝนที่จังหวัดพิษณุโลก (PNR) และฤดูแล้งที่จังหวัดชัยนาท (CND) พบว่าพันธุกรรม สิ่งแวดล้อม และ GEI มีผลกระทบอย่างมีนัยสำคัญต่อคุณค่าทางโภชนาการของเมล็ด โดยเมล็ดที่ได้จาก PNR มีค่าความชื้น ไขมัน และคาร์โบไฮเดรตสูงกว่า ขณะที่เมล็ดจาก CND มีปริมาณโปรตีน

มากกว่า โดยเมล็ดถั่วเขียวพันธุ์ CN3 และ CN84-1 มีปริมาณโปรตีนสูง ขณะที่สายพันธุ์ P08 และ P24 มีคาร์โบไฮเดรตสูง นอกจากนี้ยังพบว่า P22 และ P24 มีปริมาณใยอาหารและเถ้าที่สูงตามลำดับ สำหรับถั่วงอกจากถั่วเขียวพันธุ์ CN84-1 เหมาะสมที่สุดในการผลิตถั่วงอกที่มีปริมาณโปรตีนสูง ส่วนสายพันธุ์ P24 และ P08 เหมาะสมกว่าสำหรับปริมาณคาร์โบไฮเดรตสูง ขณะที่สายพันธุ์ P22 ให้ปริมาณใยอาหารสูง ด้านการวิเคราะห์ลักษณะทางสัณฐานวิทยาพบว่าความยาวรากมีความแตกต่างอย่างมีนัยสำคัญระหว่างจีโนไทป์ต่าง ๆ ในการทดลองที่ 3 การประเมินลักษณะทางสัณฐานวิทยาและองค์ประกอบทางโภชนาการของไมโครกรีนถั่วเขียวจีโนไทป์ต่าง ๆ พบว่า ความยาวต้นไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ขณะที่ความกว้างและความยาวใบ และอัตราการให้ผลผลิตมีความแตกต่างทางสถิติระหว่างจีโนไทป์ ซึ่งสายพันธุ์ P08 มีความยาวใบมากที่สุด และสายพันธุ์ SUPER5 มีอัตราการให้ผลผลิตสูงที่สุดซึ่งบ่งชี้ถึงประสิทธิภาพการผลิตไมโครกรีน ในด้านโภชนาการ สายพันธุ์ SUPER5 และ P22 มีปริมาณโปรตีนสูงในขณะที่สายพันธุ์ D5 มีปริมาณคาร์โบไฮเดรตสูงที่สุด ส่วนจีโนไทป์ CN3 และ SUPER5 มีปริมาณคาร์โบไฮเดรตต่ำกว่า ขณะที่พันธุ์ CN84-1 มีปริมาณใยอาหารสูง นอกจากนี้ยังพบความแตกต่างอย่างมีนัยสำคัญของปริมาณไขมัน และเถ้า การศึกษานี้แสดงให้เห็นว่า ถั่วเขียวสายพันธุ์ใหม่มีความต้านทานโรคที่ดีขึ้น มีเสถียรภาพของผลผลิตสูง และคุณสมบัติทางโภชนาการที่เทียบเท่าหรือดีกว่าพันธ์รับและพันธุ์รับรอง โดยสายพันธุ์ P22 และ P24 เป็นตัวเลือกที่ดีที่สุดสำหรับการปลูกในวงกว้าง เนื่องจากมีศักยภาพและความเสถียรสูงในหลายสภาพแวดล้อม การประเมินคุณค่าทางโภชนาการและลักษณะทางสัณฐานวิทยา พบความแตกต่างอย่างมีนัยสำคัญระหว่างจีโนไทป์ทั้งด้านคุณค่าทางโภชนาการและลักษณะทางสัณฐานวิทยา แสดงให้เห็นว่า คุณภาพของเมล็ด ถั่วงอก และไมโครกรีนมีความแตกต่างกันระหว่างจีโนไทป์ต่าง ๆ ซึ่งผลการศึกษานี้ให้ข้อมูลที่สำคัญสำหรับการปรับปรุงพันธุ์ที่มุ่งพัฒนาพันธุ์ถั่วเขียวที่มีความต้านทานโรคให้ผลผลิตสูง และคุณค่าทางโภชนาการที่ดีขึ้น ซึ่งจะช่วยเสริมสร้างความมั่นคงทางอาหารที่ยั่งยืนในอนาคต

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา ปิยังกูร เจือกโว้น
ปีการศึกษา 2567 ลายมือชื่ออาจารย์ที่ปรึกษา

PIYANGKOON JAUKWON : REGIONAL YIELD TRIAL AND NUTRITIONAL VALUES OF NEW MUNGBEAN LINES WITH POWDERY MILDEW AND CERCOSPORA LEAF SPOT RESISTANCE. THESIS ADVISOR : PROF. PIYADA ALISHA TANTASAWAT, Ph.D. 146 PP.

Keyword: Genotype × environment interaction/GGE biplot/Microgreen/
Multi location trial/Proximate analysis/Sprout/*Vigna Radiata* (L.) Wilczek

Legumes are vital sources of nutrition and play a crucial role in global food security due to their high protein and carbohydrate content, serving as an important alternative to animal-based protein. Among these, mungbean [*Vigna radiata* (L.) Wilczek] is a widely cultivated species. However, current mungbean production faces significant challenges from fungal diseases, particularly Cercospora leaf spot (CLS) and powdery mildew (PM), which severely reduce yield. Consequently, there is an increasing demand for mungbean varieties that exhibit improved disease resistance and enhanced nutritional value. This research aimed to: 1) evaluate the yield potential of newly developed mungbean lines through regional yield trials, 2) investigate the stability of different mungbean genotypes based on yield and yield-related components, and 3) analyze the morphological and nutritional characteristics of seeds, sprouts, and microgreens from various mungbean genotypes. Experiment 1 focused on regional yield trials and stability of new mungbean lines utilized the GGE biplot method. The eight mungbean genotypes were tested by planting them at four locations during the rainy and dry seasons. The results indicated that all new mungbean lines showed better resistance traits against CLS and PM compared to the recurrent parent CN84-1. Specifically, lines P22 and P24 demonstrated superior yield and good stability across environments, with consistent performance in both seasons. Line P12 exhibited high yield in Nakhon Ratchasima during the dry season but exhibited delayed flowering and maturity. The GGE biplot analysis confirmed that line P22 combined high yield with stability, whereas P08 showed the highest stability for pods/plant and 100 seed weight. Additionally, the GGE biplot method highlights the significance of genotype environment interaction (GEI). The environmental interaction analysis revealed that Chai Nat was the most suitable testing site for distinguishing yield differences. Overall, lines P22 and P24 were identified as the most promising lines due to their broad adaptation and high yield across various environmental conditions, while P08 and P12 showed environment-specific adaptations. Experiment 2 focused on the nutritional contents through the proximate analysis (moisture, protein, fat, ash, fiber, and carbohydrate) and morphological traits of seeds and sprouts derived from different

mungbean genotypes in two contrasting environments: Phitsanulok during the rainy season (PNR) and Chai Nat during the dry season (CND). Genotypic, environmental, and GEI significantly influenced seed nutritional content. Seeds grown in the PNR had higher moisture, fat, and carbohydrate contents, whereas those seeds from the CND contained more protein. For the mungbean seeds, varieties CN3 and CN84-1 exhibited higher protein content, while P08 and P24 were high in carbohydrates. Additionally, P22 and P24 showed elevated levels of fiber and ash, respectively. For the mungbean sprouts, variety CN84-1 is the most suitable for producing sprouts with high protein content. Lines P24 and P08 are more suitable for higher carbohydrate content, while P22 offers higher fiber content. The morphological analysis revealed significant differences in root length among the genotypes. Experiment 3 focused on the evaluation of morphological characteristics and nutritional profiles of mungbean microgreens from various genotypes, demonstrated that hypocotyl length showed no significant variation, while leaf (length and width), and output ratio differed significantly among genotypes. While line P08 had the longest leaves. Line SUPER5 exhibited the highest output ratio, suggesting superior microgreen production efficiency. Nutritionally, lines SUPER5 and P22 had high protein content, while line D5 had the highest carbohydrate content. By contrast, genotypes CN3 and SUPER5 exhibited lower carbohydrate content. Moreover, significant differences were also observed in fat and ash content. Whereas, the superior fiber content was found in CN84-1. This research demonstrated that newly developed mungbean lines exhibit enhanced disease resistance, high yield stability, and higher or comparable on better nutritional qualities to recurrent parent and certified varieties. Lines P22 and P24 are superior candidates for broad cultivation due to their high performance and stability in various environmental conditions. The nutritional and morphological evaluation revealed significant differences between genotypes in both nutritional characteristics and morphological traits, demonstrating the varying quality of seeds, sprouts, and microgreens across different genotypes. These findings provide critical information for breeding programs aiming to develop mungbean varieties with combined resistance, high yield, and enhanced nutritional values, thereby contributing to sustainable food security in the future.

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Student's Signature Piyangkoon Jaukwon
Advisor's Signature Piyada Theerakulpisut

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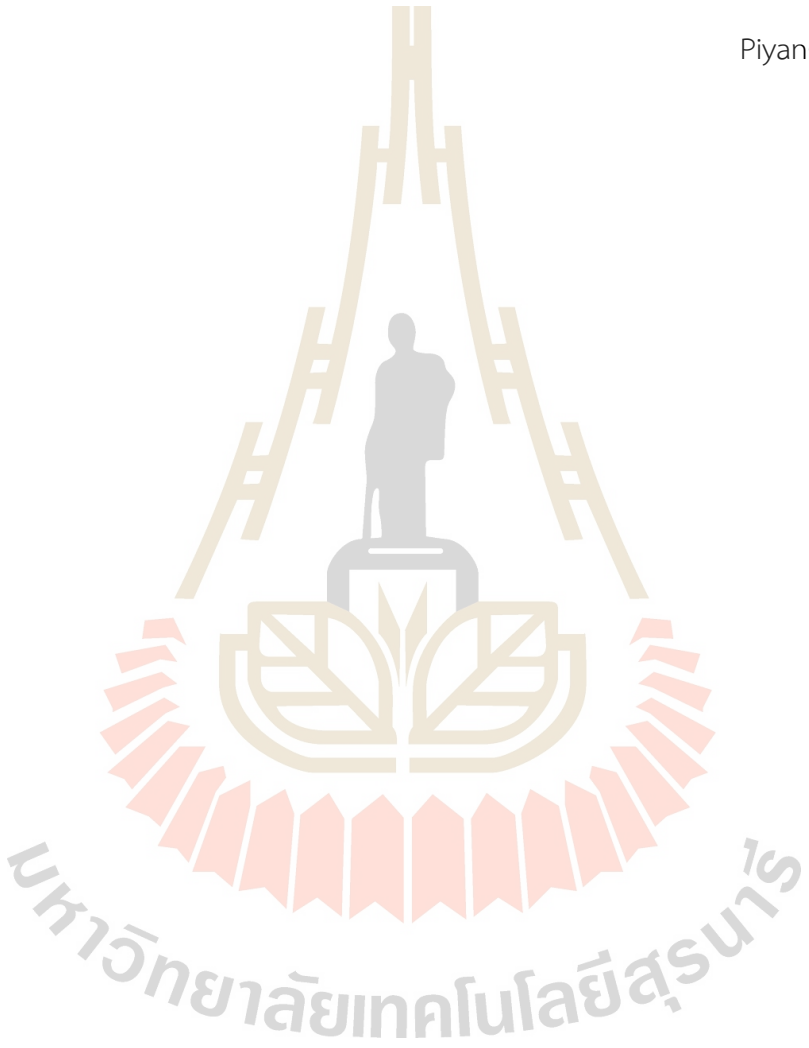

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LIST OF ABBREVIATIONS

A	=	Asynchrony
AEC	=	Average environmental coordination
AMMI	=	Additive main effects and multiplicative interaction
ANOVA	=	Analysis of variance
AOAC	=	Association of Official Analytical Chemists
AUDPC	=	Area under the disease-progress curve
BC	=	Backcross
BNF	=	Biological nitrogen fixation
BP	=	Between paper
CLS	=	Cercospora leaf spot
CN	=	Chai Nat
CND	=	Chai Nat during dry season
CRD	=	Completely randomize design
CV	=	Coefficient of variation
DAP	=	Days after planting
DB	=	Dry basis
DMRT	=	Duncan's new multiple range test
DOA	=	Department of Agriculture
E	=	Environment
FW	=	Fresh weight
G	=	Genotype
GEI	=	Genotype environment interaction
GGE	=	Genotype plus genotype environment interaction
KPS	=	Kamphaeng Saen
MAS	=	Marker assisted selection
MET	=	Multi environment trial
NR	=	Nakhon Ratchasima
PB	=	Phetchabun
PC	=	Principal component
PFD	=	Photon flux density
PN	=	Phitsanulok
PNR	=	Phitsanulok during rainy season

LIST OF ABBREVIATIONS (Continued)

PM	=	Powdery mildew
PPFD	=	Photosynthetic photon flux density
PS	=	Partial synchrony
RCBD	=	Randomized complete block design
S	=	Synchrony
SI	=	Severity index
SPSS	=	Statistical package for the social sciences
SUT	=	Suranaree University of Technology
SVD	=	Singular value decomposition
°C	=	Degree Celsius

CHAPTER I
INTRODUCTION

1.1 Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is a leguminous crop widely cultivated in tropical and subtropical regions, particularly in Asia, including India, Thailand, Vietnam, the Philippines, Indonesia, and China. It is characterized by its short life cycle, typically maturing within 60–90 days after planting (DAP), depending on the variety, agroclimatic conditions, and management practices (Lawn and Ahn, 1985; Kim et al., 2015). Mungbean is relatively easy to cultivate, adaptable to various soil types and climatic conditions, and exhibits drought tolerance. Additionally, it possesses the ability to fix atmospheric nitrogen through symbiotic relationships with rhizobium bacteria in the soil. This nitrogen-fixing capability makes mungbean an ideal candidate for crop rotation systems and as a green manure crop to enhance soil fertility (Nair et al., 2013; Kim et al., 2015). This trait not only improves soil health but also contributes to sustainable agricultural practices by reducing input costs and environmental impacts.

In 2023, Thailand reported a mungbean cultivation area of 663,500 rai, with a total production of 101,825 tons. Compared to the previous year, this represented a 5.48% decrease in cultivation area and a 3.66% decline in production (Office of Agricultural Economics, 2022). Major mungbean-growing regions in Thailand include the provinces of Chai Nat, Phetchabun, and Khon Kaen, where the crop is integrated into diverse cropping systems to improve soil fertility and farmer livelihoods (Udomsak, 2008; Office of Agricultural Economics, 2022). In Thailand, popular mungbean varieties include Kamphaeng Saen (KPS) 2, Chai Nat (CN) 84-1, CN72, CN3, and Suranaree University of Technology (SUT) 1. Mungbean is typically cultivated during the rainy season, late rainy season, and dry season. Mungbean is a nutrient-dense crop, rich in protein, vitamins, and minerals, contributing to food security and nutritional health in resource-limited communities (Ganesan and Xu, 2017).

Mungbean is a crop significant in terms of economics and nutrition, serving as a valuable source of nutrition for food and feed. Its nutritional composition varies considerably depending on genotypes, environmental conditions, and cultivation practices (Skylas et al., 2017; Wang et al., 2021). These variations highlight the need for targeted breeding and cultivation strategies to optimize nutritional quality. The protein and carbohydrate content of mungbean seeds typically range from 20.00-30.00% and 60.00-70.00%, respectively (Somta et al., 2022). Due to its versatile nutritional profile, mungbean able widely to process into a variety of food products, including mungbean flour, desserts, vermicelli, ice cream, and snacks (Bhatty et al., 2000). These products are popular in many Asian cuisines and contribute to the global demand for plant-based protein sources. Additionally, mungbean sprouts are a convenient and highly digestible form of consumption. Sprouts are particularly rich in dietary fiber and phytonutrients, including vitamin C, vitamin A, vitamin B1, vitamin B2, phytochemicals, and gamma-aminobutyric acid (GABA) (Randhir & Shetty, 2005). These nutrients make mungbean sprouts a functional food suitable for individuals of all ages and genders, offering health benefits such as improved digestion, enhanced immune function, and reduced oxidative stress. The nutritional and functional properties of mungbean, combined with its adaptability to diverse agroecological conditions, underscore its potential as a key crop for addressing global food security and nutritional challenges.

Mungbean is a vital agricultural crop, playing a significant role in sustainable food production systems. Ensuring sustainable mungbean production is critical to meet the increasing domestic demand for its consumption. However, domestic production capacity has been insufficient to fulfill this demand, leading to a reliance on imports. According to the Office of Agricultural Economics (2021), Thailand imported 36,385 tons of mungbean in 2021, while domestic consumption reached 109,446 tons during the same period. This import volume accounted for 33.24% of the annual consumption, highlighting the instability in the food supply chain and the need to enhance domestic production. Several factors contribute to the insufficient production of mungbean. These include: low average yield per unit area, often influenced by unpredictable weather conditions; deficiency in the availability of high-quality seeds, and varieties that are well-adapted to local agroclimatic conditions, which limits the potential for robust and consistent crop growth; high labor costs associated with harvesting, exacerbated by non-uniform maturation of the crop; and prevalent plant diseases, particularly powdery mildew (PM; caused by *Sphaerotheca phaseoli*) and Cercospora leaf spot (CLS; caused by *Cercospora canescens*). Tsou et al. (1979) reported that PM can cause yield losses of up to 40% if no disease management

practices are implemented. Similarly, Kelly et al. (2017) emphasized the economic impact of PM on mungbean production. Additionally, CLS can reduce mungbean yield up to 50% if it occurs after the flowering stage (Asian Vegetable Research and Development Center, 1974, 1975). Currently, there are limited mungbean varieties in Thailand that resistant to both diseases.

Effective management strategies for PM include increasing the plant spacing to improve air circulation and reduce humidity; environmental control measures to minimize conditions favorable for disease development; biological control using antagonistic fungi such as *Trichoderma* spp. for seeds and foliar application, which can reduce the accumulation and spread of the pathogen; and chemical control using fungicides such as triadimefon and triazoles (Khunti et al., 2005). However, the reliance on chemical treatments is not sustainable due to their environmental impact, high costs, and development of chemical resistant pathogen strains. An environmentally friendly and sustainable approach to disease management is the development and use of disease-resistant mungbean varieties through plant breeding programs. Resistant varieties can significantly reduce the need for chemical inputs, save labor, and lower cultivation costs while maintaining or improving yield potential.

Improving mungbean varieties for disease resistance can be achieved through various methods, such as conventional breeding and mutagenesis (Reddy et al., 1994; Wani et al., 2014; Javed et al., 2016). However, traditional plant breeding methods are time-consuming and slow to achieve success. Therefore, several molecular markers have been developed to enhance the efficiency of plant breeding programs, including the study of genetic diversity in plants and plant pathogens, as well as the selection of parental lines and hybrids using both qualitative (Fazio et al., 1999) and quantitative traits (Veldboom and Lee, 1994). Specifically, disease resistance genes have been utilized to develop disease-resistant plant varieties, accelerating the progress of plant breeding. The pyramiding of multiple disease resistance genes using marker-assisted gene pyramiding techniques can result in plant varieties with resistance to multiple pathogen races, making it more difficult for resistance to break down.

Mungbean varieties in Thailand, such as KPS2, CN72, CN84-1, and CN3, are known for their high yield potential but remain susceptible to PM and CLS (Chueakhunthod, 2019). Reports from Phruetthithep et al. (2011) and Wongwarat et al. (2023) indicate that Thai-certified varieties, such as SUT1, KPS2, CN72, and CN84-1, are susceptible to PM and CLS. The integration of breeding programs focused on disease resistance, coupled with the adoption of well agronomic practices, is essential to ensure the increased availability of mungbean as a nutritious and sustainable legume

crop (War et al., 2017). To address this disease, there is a pressing need to develop new mungbean varieties with enhanced resistance to PM and CLS, along with high yield and stability. Recently, our laboratory has developed new disease resistant breeding lines with high yield potential, including lines P08, P12, P22, and P24 (Pookhamsak et al., unpublished data). These lines resulted from crosses between the CN84-1 variety and resistant double cross [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)] which were developed through gene pyramiding methods. The resistant V4718, V4758, and V4785 lines were derived from India and obtained from the World Vegetable Center in Taiwan. The PM resistance genes in these lines are controlled by single dominant genes located at different loci on the chromosomes (non-allelic) and independently assorted (Khajudparn et al., 2009). Lines P08, P12, P22, and P24 were developed through backcrossing using the resistant donor parents and CN84-1 as recurrent parent, containing 3-4 resistance genes (2-3 genes for PM and 1 gene for CLS), providing resistance to both diseases and high yield potential resulted from cultivation trials conducted at the preliminary and standard trial levels during 2020–2022, these newly developed mungbean lines derived from backcrossing are eligible for subsequent evaluation through regional trial and farm trials.

This regional trial represents a critical step toward identifying mungbean cultivars that can enhance agricultural productivity and provide sustainable benefits to farmers. Building upon previous research, the present study conducts regional trials across diverse environmental conditions to rigorously evaluate the performance, adaptability, and disease resistance of newly developed lines. Understanding genotype × environment interaction (GEI) is fundamental in breeding programs that aim to develop cultivars with high yield potential and stable performance. GEI arises when different genotypes respond differently across environments, and a particularly important form is crossover interaction, where genotype rankings vary from one environment to another (Smith et al., 2001; Yan and Hunt, 2002). This implies that a genotype superior in one location may not retain its performance elsewhere, underscoring the need for multilocation testing.

To analyze GEI, various statistical methods have been developed, including regression coefficient analysis, summation of squared deviations from regression, additive main effects and multiplicative interaction (AMMI) models, and genotype plus genotype-by-environment interaction (GGE) biplot analysis. These approaches enable the identification of genotypes with either broad or specific adaptability, guiding the selection of stable and high-performing cultivars.

A key focus of this study is disease resistance, a trait highly influenced by environmental conditions. Disease development depends on the interaction of three essential components: a susceptible host plant, a virulent pathogen, and a conducive environment. In the absence or limitation of any one of these factors, disease incidence and severity are significantly reduced (Velásquez et al., 2018; Jiwuba et al., 2020). Therefore, breeding for disease resistance must consider the target environments in which the crop will grow. Regional trials are essential for evaluating the expression and stability of resistance traits, as the effectiveness of resistance genes can vary significantly depending on environmental conditions. The regional yield trials provide crucial data to support the recommendation of cultivars tailored to specific agro-ecological zones, while also identifying broadly adaptable lines. This approach ensures that selected genotypes meet both the productivity and resilience demands of diverse farming systems.

In addition to disease resistance and yield stability, nutritional content is also important, as mungbean is a significant food crop that provides high levels of carbohydrates and protein. Sprouts and microgreens are often more nutrient-dense than ungerminated seeds or mature vegetables, containing higher levels of vitamins, minerals, and bioactive compounds essential for healthy diets (Ebert, 2022). Microgreens are rich sources of vitamins such as vitamin C, minerals including copper and zinc, and phytochemicals (Zhang et al., 2021). Comparative analyses show that germinated seeds tend to have higher macronutrients (lipids, proteins, sugars, amino acids, minerals), while microgreens possess significantly higher concentrations of vitamins and polyphenols (Bhaswant et al., 2024). However, there is limited data on the nutritional composition of new mungbean genotypes. Therefore, the nutritional values of these mungbean genotypes must be rigorously assessed to ensure that breeding efforts address both quantity and quality. Evaluating the nutritional composition of seeds, sprouts, and microgreen is crucial, as it not only supports the development of value-added products but also facilitates the registration and certification of new varieties. By integrating nutritional profiling into breeding programs, researchers can develop varieties that meet both agronomic and dietary needs (Saltzman et al., 2013; Garcia et al., 2016; Bouis and Saltzman, 2017; Salsman et al., 2021), contributing to improved food security and addressing nutritional deficiencies in populations worldwide.

The development and dissemination of disease-resistant, high-yielding, and nutritionally enhanced mungbean varieties will enable farmers to increase production efficiency, reduce reliance on imports, and promote sustainable agricultural practices.

This holistic approach aligns with global efforts to enhance food security, improve nutritional outcomes, and ensure the long-term sustainability of agricultural systems. Leveraging advancements in plant breeding, agronomy, and nutritional science, stakeholders can foster a resilient and productive mungbean sector that meets the demands of a growing population while minimizing environmental impacts.

1.2 Objectives

1.2.1 To assess the potential of novel mungbean lines exhibiting resistance to PM and CLS diseases in regional yield trials.

1.2.2 To determine the yield stability and consistency of new mungbean lines (P08, P12, P22, and P24) based on yield performance and yield-related components compared to recurrent parent and check varieties.

1.2.3 To evaluate the nutritional composition of seeds, sprouts and microgreen from the newly developed mungbean lines recurrent parent and check varieties.

1.2.4 To characterize the morphological traits of sprouts and microgreen from the new mungbean lines compared to recurrent parent and check varieties.

1.3 Research hypotheses

1.3.1 The new mungbean lines resistant to PM and CLS demonstrated yield performance similar or superior to recurrent parent under diverse regional growing conditions at the regional yield trial level.

1.3.2 The new mungbean lines exhibited resistance to PM and CLS diseases in various environments, as confirmed through field condition evaluations.

1.3.3 The morphological and nutritional characteristics of seeds, sprouts, and microgreens from the new mungbean lines are comparable or exceeded those of existing varieties, with notable improvements in key nutritional and morphological traits.

1.3.4 Regional yield trials conducted across multiple locations within the target region provided critical insights into the adaptability, stability, and overall performance of the new mungbean lines, supporting evidence-based decisions for their commercial release and adoption by farmers.

1.4 Scopes of research

1.4.1 This study was divided into three experiments: 1) regional yield trial and stability evaluation thought the GGE biplot analysis 2) nutritional and morphological characterization of seed and sprout and 3) nutritional and morphological characterization of microgreens.

1.4.2 The regional yield trials evaluation of eight Thai mungbean genotypes, consisting of four new mungbean lines (P08, P12, P22, and P24) compared to the recurrent parent (CN84-1) and check varieties (CN3, SUT1, and SUPER5). These genotypes exhibited distinct characteristics, such as large seed size, resistance to PM and CLS, high yield potential, and enhanced nutritional values. All mungbean genotypes were evaluated under the four different environments conditions for regional yield trials evaluation including Nakhon Ratchasima, Chai Nat, Phitsanulok, and Phetchabun, across rainy season and dry season during the years 2023-2024.

1.4.3 The nutritional and morphological characterization of seed and sprout were evaluated under control conditions. Eight mungbean genotypes were included in this experiment, comprising recurrent parent (CN84-1) and check varieties (CN3 and SUPER5) and five new mungbean lines (P08, P12, P22, P24, and D5).

1.4.4 The nutritional and morphological characterization of microgreens were evaluated under control conditions. Nine mungbean genotypes were used, comprising recurrent parent (CN84-1) and check varieties (CN3 and SUPER5) and six new mungbean lines (P08, P12, P22, P24, W5 and D5).

1.4.5 The assessment of nutritional components was performed through proximate analysis, including the determination of carbohydrates, crude protein, crude lipid, crude fiber, and ash content.

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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℃, and nighttime temperatures around 17℃ 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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CHAPTER III

Regional Trial and Stability Evaluation of New Mungbean (*Vigna Radiata* (L.) Wilczek) Lines Resistant to Powdery Mildew and Cercospora Leaf Spot Diseases through GGE Biplot Analysis

3.1 Abstract

This study evaluated the agronomic traits, disease resistance (Cercospora leaf spot; CLS and powdery mildew; PM), and yield stability of eight mungbean genotypes (SUPER5, CN84-1, P08, P12, P22, P24, CN3, and SUT1) across four environments (Nakhon Ratchasima, Chai Nat, Phitsanulok, and Phetchabun) and two seasons (rainy and dry season) during 2023-2024. Results indicated significant differences in yield, disease resistance, and agronomic performance across genotypes and locations. Among new lines, P22 demonstrated the highest yield across all environments with strong adaptability and stability, particularly in areas with high disease outbreak and P24 also exhibited high yield potential and good resistance to both CLS and PM, particularly in Phitsanulok and Phetchabun. The line P12, exhibiting delayed flowering and maturity, showed superior disease resistance and performed well in dry season. Line P08 showed consistent performance across environments, making it a suitable option for areas with variable growing conditions. The study also incorporated a multi-environment stability analysis using genotype plus genotype-by-environment interaction (GGE) biplot model, significant genotype environment interactions (GEI) were observed, P22 showed high yield and stability, particularly for pods/plant and yield. The highest overall stability was found in P08. The analysis revealed that Nakhon Ratchasima and Phetchabun were most representative for 100 seed weight and yield, while Phitsanulok and Chai Nat had more discriminative for yield and pods/plant. This study identified that P22 and P24 showing better adaptation and yield performance which classified them at ideal line.

3.2 Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is a vital legume crop widely cultivated across Asia and other tropical regions, particularly in countries such as India, China, and Thailand. It is valued for its high nutritional content and its ability to improve soil fertility through biological nitrogen fixation (Abbas et al., 2020; Ilyas et al., 2023). This nitrogen-fixing capacity makes mungbean highly suitable for crop rotation systems and as a green manure crop, contributing to enhanced soil health, reduced production costs, and more sustainable agricultural practices (Nair et al., 2013; Kim et al., 2015). In Thailand, major mungbean-growing regions include Chai Nat, Phetchabun, and Khon Kaen, where the crop is integrated into various cropping systems to improve soil fertility and farmer livelihoods (Udomsak, 2008; Office of Agricultural Economic, 2022). Mungbean is cultivated during the rainy, late rainy, and dry seasons. Due to its agronomic benefits and nutritional value, mungbean plays a critical role in sustainable food production systems. However, despite its significance, domestic production is insufficient to meet national demand. In 2021, Thailand imported 36,385 tons of mungbean while domestic consumption reached 109,446 tons, underscoring the fragility of the local supply chain and the need to boost national production capacity (Office of Agricultural Economics, 2021).

One of the key limitations to mungbean productivity in Thailand is the lack of improved varieties that are both high-yielding and adapted to local agroclimatic conditions. Compounding this issue is the prevalence of serious foliar diseases particularly PM and CLS which are among the most devastating diseases affecting mungbean (Pandey et al., 2018; Papan et al., 2021). These diseases cause substantial yield loss, especially under favorable environmental conditions, and hinder the overall growth of the mungbean crop (Barros et al., 2019). Cercospora leaf spot (CLS; caused by *Cercospora canescens*), can lead to yield losses of up to 96% under severe epidemic conditions (Pandey et al., 2018; Abbas et al., 2020; Ilyas et al., 2023). The disease impairs photosynthesis by damaging leaf tissues and chloroplasts, directly affecting plant health and productivity. Similarly, powdery mildew (PM; caused by *Sphaerotheca phaseoli*) and related fungi, can result in yield losses ranging from 50% to 100% at the seedling stage. The complete crop failure depending on environmental conditions and the crop stage (Papan et al., 2021).

While traditional disease management methods including fungicide application and cultural practices are commonly used, they are often costly, inconsistent, and environmentally unsustainable. The use of resistant varieties remains the most effective and environmentally sound approach to controlling PM and CLS in mungbean

production (Pandey et al., 2018; Papan et al., 2021). However, resistance sources in mungbean germplasm remain limited, and their expression can vary depending on the environment. Advances in breeding technologies, including marker-assisted selection (MAS), have enabled the development of improved mungbean lines with dual resistance. Nonetheless, the yield potential, stability, and adaptability of these lines across diverse environments still require comprehensive evaluation (Papan et al., 2021).

Regional yield trials and stability assessments are thus crucial for identifying mungbean genotypes that combine high yield performance with durable resistance to PM and CLS. In Thailand, commercial varieties such as Kamphaeng Saen (KPS) 2, Chai Nat (CN) 72, CN84-1, and CN3 are known for high yield potential but remain susceptible to these diseases (Chueakhunthod, 2019). Therefore, this study aimed to evaluate the agronomic traits, yield performance, and stability of four newly developed mungbean lines P08, P12, P22, and P24 across multiple environments through regional yield trials. The goal is to identify superior genotypes suitable for varietal release that can offer farmers more reliable, productive, and disease-resilient options for mungbean cultivation.

3.3 Materials and methods

3.3.1 Plant materials and breeding procedure

In this study, a total of eight mungbean genotypes were used, and their special features and origins were provided in Table 3.1, including Thai certified varieties (CN3, CN84-1, and SUT1), SUPER5, P08, P12, P22, and P24. The SUPER5 line is resistant to PM and CLS and was developed by Pookhamsak et al. (unpublished data). The P08, P12, P22, and P24 lines were selected from backcross progenies of the recurrent parent CN84-1 and a resistant donor parent, which were developed from backcrossing between CN84-1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)]. The resistant lines V4718, V4758, and V4785 originated from India, as shown in Figure 3.1.

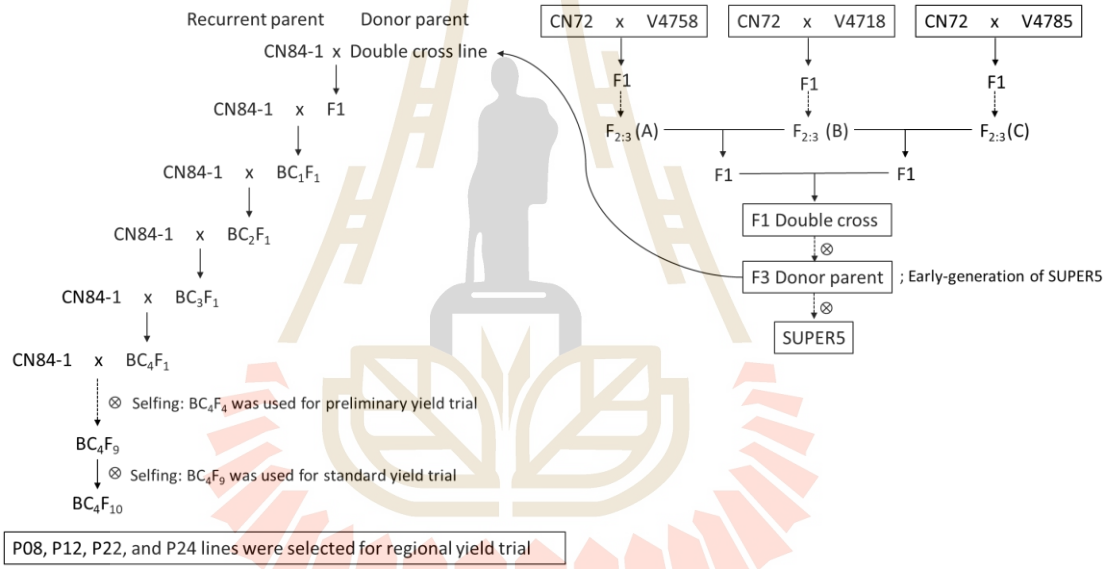

Figure 3.1 Pedigree of backcross progenies from a cross between CN84-1 and resistant double cross line.

Table 3.1 Pedigree and special features of eight mungbean genotypes used in this study.

Genotypes	Pedigree	Special features	Descriptions
SUPER5	Development from double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)]	High resistance to PM[1/] and CLS[2/]	The mungbean resistant line developed by Pookhamsak et al. (unpublished data)
CN3	Selection from mutated CN36	Large seed, high yield, uniform maturity	Thai certified varieties developed at Chai Nat Field Crops Research Center, Thailand
CN84-1	Selection from mutated CN36	Large seed, high yield, high percentage of carbohydrate	
SUT1	UTHONG1 x NP-29	High yield, pods borne above the canopy, moderate resistance to PM	The certified mungbean variety from Suranaree University of Technology (SUT), Thailand
P08	Selected from backcrossing between CN84-1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)]	Large seed, uniform maturity, moderate resistance to PM and CLS	New resistant lines
P12		High yield, rather drought resistance, high resistance to PM, moderate resistance to CLS	
P22		High yield, uniform maturity, abundant pods, moderate resistance to PM and CLS	
P24		Large seed, uniform maturity, moderate resistance to PM and CLS	

[1/] powdery midew, [2/] Cercospora leaf spot

3.3.2 Regional yield trials

The regional trials were conducted to evaluate yield performance, agronomic traits, and resistance ability to PM and CLS under diverse environmental conditions. Four newly developed mungbean lines (P08, P12, P22, and P24) were tested and compared with the recurrent parent CN84-1 and check varieties CN3 and SUT1, as well as the disease-resistant line SUPER5. The experiments were carried out at four locations across Thailand: Chai Nat Field Crops Research Center at Chai Nat Province, Phetchabun Agricultural Research and Development Center at Phetchabun Province, Phitsanulok Seed Research and Development Center at Phitsanulok Province, and the SUT Farm at Nakhon Ratchasima province. The assessment of CLS disease was performed during July to October 2023, while the evaluation of PM disease was conducted from November 2023 to February 2024, across the same four experimental sites. The experimental information and conditions of these sites are presented in Table A.1 and Figure A.6-Figure A.13.

Each experimental field was divided into plots measuring 4 × 6 m per replication with row spacing is 0.5 m and plant spacing is 0.2 cm (total of 8 rows, with 30 plants per row), while the resistant line SUPER5 was planted in a single row for disease comparison. Border rows are planted around the experimental field with 4 rows, using a susceptible variety as a source of disease inoculums. Agronomic data were collected by randomly sampling 10 plants per replication per genotype to calculate the average values. The agronomic practices were implemented across all experimental locations. Prior to sowing, pre-emergence herbicide (alachlor) was applied to control weed growth. Carbofuran (3% G) was incorporated at a rate of 10 g per planting hole as a basal treatment for insect pest management. A compound fertilizer (N-P-K: 12-24-12) was applied at a rate of 30 kg/rai at planting. Ten days after emergence, seedlings were thinned to maintain two plants/hole. To control stem fly larvae, triazophos (40% EC) was sprayed at a concentration of 50 ml per 20 liters of water. At 25–30 days after sowing, an additional application of N-P-K fertilizer (12-24-12) at the same rate (30 kg/rai) was made, followed by hilling up around the base of the plants. Two months after sowing, triazophos (40% EC) was again applied at the same concentration to prevent pod borer infestation (*Maruca vitrata*). Weed control was performed manually as needed when weed pressure was high. Irrigation was provided once a week throughout the growing period.

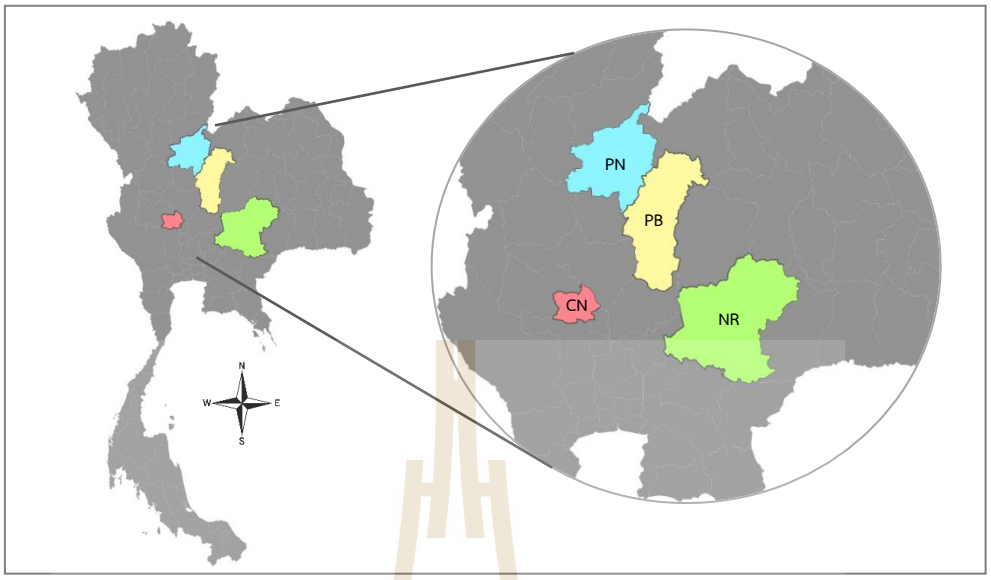

Figure 3.2 Location map of the experimental sites in Thailand, Abbreviation: NR = Nakhon Ratchasima, CN = Chai Nat, and PN = Phitsanulok, and PB = Phetchabun. Map created using pixel map generator (www.pixelmap.amcharts.com).

3.3.2.1 Agronomic traits and diseases scoring

The evaluation of agronomic traits and yield parameters was conducted according to the "Manual for recording mungbean research data" published by the Department of Agriculture (2018) and Chueakhunthod et al. (2020) as follows: Days to flowering: number of days from sowing to 50% of plants in the plot with first pod ripe. Days to maturity: counted from the planting date to the date when 50% of the plants in the plot have the first mature pod. Plant height (cm): measure from soil level to the highest point after the first harvest. Average of ten plants/plot. Lodging score: score the observation plants during the harvesting period based on the angle they lean away from the vertical position over 45°, where the scored indicated 1 = no lodging, 2 = lodging of 1-25% of the plot area, 3 = lodging of 26-50% of the plot area, 4 = lodging of 51-75% of the plot area, and 5 = lodging of more than 75% of the plot area. Branches/plant: number of pod-bearing branches with at least two nodes. Average of ten plants/plot. Clusters/plant: number of clusters having at least one fully grown pod at first harvest including both main stem and branches. Average of ten plants/plot. 100 seed weight (g): weight of 100 randomly selected seeds. Average of ten plants/plot. Pods/plant: number of pods from two harvests. Average of ten plants/plot. Seed weight/plant (g): total seed weight from two harvest times. Average of ten plants/plot. Pod length (cm): maximum length of ten pods (in case of curved pods, the longest straight line from the base to the tip of pod was measured). Average of ten plants/plot.

Seeds/pod: number of seeds/pod of ten pods. Average of ten plants/plot. Yield (kg/rai): calculated by converting the average grain yield in each plot to kg/rai. Each plot had a harvestable area of 24 m², and harvesting was conducted once when more than 80% of the plants reached physiological maturity, approximately 70 DAP.

3.3.2.2 Assessment of PM disease

The assessment of PM resistance during the dry season involves random evaluations conducted at individual plants within each plot. Specifically, 10 plants per plot, aged 55 and 65 DAP, were scored for PM resistance. The observations of resistance levels were divided into four categories (resistance 1.0-3.0, moderate resistance = 3.1-4.7, moderate susceptibility = 4.8-6.4 and susceptibility = 6.5-9.0). The evaluation using a scoring system outlined by Khajudparn (2009), as showed in Figure A.14.

3.3.2.3 Assessment of CLS disease

In the rainy season, evaluations are randomly conducted at the plants within each plot with 10 plants. Assessments occur when mungbean reach 55 and 65 DAP. The scale of CLS severity was divided into three categories (resistance = 1.0-2.5, moderate resistance = 2.6-3.4 and Susceptibility = 3.5-5.0) following the scoring system outlined by Chankaew (2009), as illustrated in Figure A.15.

3.3.2.4 Severity index (SI)

SI is a measurement used to quantify the intensity of a disease in plants. The SI helps to evaluate the effectiveness of disease management practices or the level of resistance in a particular variety. Calculate the severity of disease occurrence using the data of ten plants of disease in each plot. Use the formula developed by Asefa et al. (2016) for calculation.

$$\text{SI}\ (\%) = \frac{\text{Summation of numerical rating}}{\text{No. plants examined} \times \text{Maximum disease score}} x\ 100$$

3.3.2.5 Area under the disease-progress curve (AUDPC)

AUDPC is a quantitative measure used to assess the progression of plant diseases over time. It represents the cumulative amount of disease development throughout the growing season or experiment, providing an overall picture of disease intensity. Calculated based on the SI using the method described by (Campbell & Madden, 1990) as follows:

$$\text{AUDPC} = \sum_{i=1}^{N_i - 1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

When: y_i = SI at the beginning of disease assessment.

y_i+1 = SI at the end of disease assessment.

t_i = time at the beginning of disease assessment.

t_i+1 = time at the end of disease assessment.

3.3.3 Data analysis

Data collected for each trait based on the Randomized Complete Block Design (RCBD) were analyzed. This included data on agronomic and disease resistance traits obtained with triplicate. Mean values for each trait were calculated separately for each location across replications. Subsequently, analysis of variance (ANOVA) was performed using SPSS for Windows Version 14.0 (Levesque, 2007). The combined analysis of variance across locations was conducted based on individual ANOVA from each location, following the method described by (Gomez & Gomez, 1984).

3.3.4 Construction of GGE biplot

The seed yields and yield components (pods/plant and 100 seed weight) collected were analyzed to assess yield stability. GGE biplot model, were applied (Gauch & Hugh, 2006). Yan et al. (2007) expanded the application of the GGE biplot to evaluate genotypes and pinpoint mega-environments. This approach emphasizes the genotype main effect (G) and GEI, while minimizing the influence of non-significant environmental main effects (E). The GGE biplot is generated by plotting the values of genotypes and environments along the first principal component (PC1) versus their corresponding values on the second principal component (PC2), which are derived through singular value decomposition (SVD) of environment-centered data using the given equation.

$$Y_{ij} = \mu + e_j + \sum_{n=1}^{N} \lambda_n \delta_{jn} + \varepsilon_{ij}$$

Where: *Yij* = mean response of i^{th} genotype (i = 1,..., *I*) in the j^{th} environment (j = 1,..., *J*), μ = grand mean, *N* = number of principal components retained in the model, e_j = environment deviations from the grand mean, λ_n = the eigen value of principal component analysis axis, γ = genotype PC score for axis, δ_{jn} = environment PC score for axis, εij = the residual error term.

The optimal genotype was identified based on both mean performance and stability across different environments. Genotype evaluation was carried out and visualized through the "average environmental coordination/axis (AEC/AEA)" perspective

of the GGE biplot, which allows for comparing the mean yield stability across environments within a mega-environment (Naik et al., 2022; Linus et al., 2023; Irfan et al., 2025). The 'symmetrical' GGE biplot analysis was conducted to evaluate the relationships among the test environments and to determine the proportion of variance attributable to GEI. The suitability of experimental sites was assessed using the 'discriminatory power and representativeness' perspective of the GGE biplot. Discriminatory power was reflected in the length of the environmental vector, while representativeness was indicated by the sharp angle between the environmental vector and the AEC (Tamang et al., 2022). Furthermore, the GGE biplot 'Which-won-where' approach was used to evaluate genotype performance under various experimental conditions and categorize test environments into distinct mega-environments. The stability analysis using the GGE biplot method was employed to evaluate stability using R software version 2.13.0 (Jompuk, 2008; R Development Core Team, 2019).

3.4 Results

3.4.1 The agronomic traits and CLS assessment during rainy season at Nakhon Ratchasima

For the agronomic traits and disease resistance of all mungbean genotypes during rainy season at Nakhon Ratchasima (Table 3.2), significant statistical differences were observed among the genotypes for certain traits. Days to flowering, days to pod maturity, seed weight/plant, CLS scoring and AUDPC showed statistically highly significant differences, whereas the other traits did not exhibit significant variation. Most newly developed lines generally flowered between 37.3 to 38.3 days after planting (DAP), which was comparable to the recurrent parent and the check varieties, except for line P12, which flowered later than all other genotypes at 44.3 DAP. For days to pod maturity, most lines were similar to the recurrent parent and the checks, except for P12, which exhibited a longer maturity period and demonstrated asynchronous maturity (<80% of the area reached maturity simultaneously). In terms of seed weight/plant, lines P22 and P24 produced 64.9-97.6% significantly higher weight compared to the recurrent parent and all check varieties. Meanwhile, lines P08 and P12 had seed weight that were comparable to the recurrent parent and check varieties. Under the rainy season conditions in this environment, no lodging issues were observed across all genotypes, and most lines matured uniformly. However, prolonged heavy rainfall during the harvest period (Figure A.6) resulted in reduced yield and high variability with 29.53% and 43.23% C.V. for seed weight/plant and yield, respectively. Nevertheless, under suboptimal environmental conditions, lines P22 and P24 showed

adaptability to produce higher yields than the other genotypes. Agronomic traits assessments clearly indicated that the lines P22 and P24 were superior among genotypes, particularly in pods/plant, seed weight/plant, and yield. Notably, seed weight/plant and yield in P22 and P24 were at least 70% and 44% higher than the recurrent parent, respectively.

The CLS scores at 65 DAP in all new lines tended to be lower than those of the recurrent parent and the check varieties. Line P22 exhibited CLS scores comparable to the resistant line SUPER5. All new lines, except P12, showed resistance levels similar to SUPER5. The development of CLS in all new lines resulted in lower AUDPC scores compared to the recurrent parent and the check varieties.

Table 3.2 Agronomic traits and CLS resistance of new mungbean lines from yield trial in rainy season during July to October 2023 at Nakhon Ratchasima.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	CLS scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.59 c	R	259.38 c
CN84-1	36.9 b[4/]	53.1 bc	S	50.8	1.0	3.4	6.5	8.6	8.1	7.10	3.74 b	146.69	3.34 ab	MR	504.38 a
P08	38.0 b	53.8 bc	S	50.0	1.0	3.2	6.6	8.1	7.7	7.01	3.88 b	126.11	2.59 b	R	392.50 b
P12	44.3 a	57.3 a	A	59.9	1.0	3.8	6.1	8.7	7.8	6.09	2.95 b	104.55	2.68 b	MR	398.33 b
P22	37.3 b	55.0 ab	S	47.5	1.0	4.9	10.5	8.7	8.6	7.06	6.56 a	211.51	2.37 bc	R	381.25 b
P24	38.3 b	53.3 bc	S	50.6	1.0	4.5	11.3	8.6	8.6	6.74	6.40 a	215.25	2.46 b	R	375.00 b
CN3	38.0 b	54.3 abc	S	53.4	1.0	3.9	7.1	8.7	8.3	6.63	3.32 b	117.35	3.41 ab	MR	507.50 a
SUT1	38.3 b	52.5 c	S	58.7	1.0	4.7	7.5	8.5	8.6	6.26	3.52 b	112.61	3.95 a	S	582.13 a
F-test	**	**	N/A[5/]	ns	ns	ns	ns	ns	ns	ns	**	ns	**		**
C.V. (%)	2.7	2.1	N/A	14.0	0.0	22.7	37.0	4.3	7.7	6.06	29.53	43.23	4.17		8.79

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Score the observed plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of CLS severity at 65 DAP, where 1.0-2.5 = resistance, 2.6-3.4 = moderate resistance, and 3.5-5.0 = susceptibility. [4/] Data showing means, different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.2 The agronomic traits and CLS assessment during rainy season at Chai Nat

From Table 3.3 of agronomic traits and disease assessment of new mungbean lines P08, P12, P22, and P24 were compared to the recurrent parent CN84-1, check varieties (CN3 and SUT1) and resistant line (SUPER5) at Chai Nat, significant statistical differences were observed among the genotypes in nearly all traits, except for days to flowering, days to pod maturity, pod length, and AUDPC. The genotypes showed statistically highly significant differences in clusters/plant, pods/plant, and seeds/pod. While plant height, lodging, 100 seed weight, seed weight/plant, yield, and CLS scores exhibited significant difference.

Overall, line P22 exhibited the most favorable agronomic traits, particularly in yield, surpassing both the recurrent parent and the check varieties. Line P12 showed asynchronous maturity, unlike the other new lines which exhibited partial synchrony (80–90% of pods matured simultaneously), consistent with its tendency for longer days to flowering and pod maturity. The plant height of all new lines was not significantly different from the recurrent parent. Lodging was observed in recurrent parent and check varieties. However, the new lines exhibited no lodging. For clusters/plant, line P22 had the highest (9.6 clusters), significantly more than the recurrent parent and CN3 but comparable to the check variety SUT1. Lines P08, P12, and P24 showed comparable clusters/plant to the recurrent parent. In terms of pods/plant, line P22 produced the highest number (27.7 pods), significantly greater than the recurrent parent but comparable to SUT1 and CN3. Lines P12 and P24 also had more pods than the recurrent parent, while line P08 had similar pods number to the recurrent parent. Regarding seeds/pod, no significant differences were observed among the new lines and the recurrent parent, while lines P08 and P22 had significantly more seeds/pod than all check varieties. For 100 seed weight, lines P08, P12, and P24 were comparable to the recurrent parent and CN3. Although P12 had slightly lower seed weight but was not significantly different from the check varieties. In terms of seed weight/plant, line P22 recorded the highest value (15.12 g), 33-41% significantly greater than recurrent parent and check varieties. The other new lines did not differ statistically from recurrent parent and check varieties. For yield, line P22 produced the highest yield (399.60 kg/rai), exceeding the recurrent parent and check varieties by 43–60%. Line

P12 also outperformed the recurrent parent, yielding 321.53 kg/rai, approximately 28% higher. Lines P08 and P24 showed a tendency of 16% and 25% higher yields than the recurrent parent, respectively.

Regarding CLS scores at 65 DAP, lines P12, P22, and P24 had comparable scores to the resistant line SUPER5, with P12 showing the lowest score among the new lines. As CLS incidence was generally low under these environmental conditions, all genotypes exhibited low disease scores and were classified as resistant. The AUDPC values were consistent with the CLS scores at 65 DAP, with the new lines tending to have lower AUDPC values than the recurrent parent and SUT1, however, they were not significantly different.

Table 3.3 Agronomic traits and CLS resistance of new mungbean lines from yield trial in rainy season during July to October 2023 at Chai Nat.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	CLS scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.03 c	R	202.78
CN84-1	33.8[4/]	53.2	S	61.4 bc	2.3 a	6.5 c	15.7 d	9.3	11.0 a	6.68 a	10.75 b	249.45 c	1.88 a	R	288.33
P08	33.7	53.3	PS	56.1 c	1.0 b	6.6 bc	17.2 cd	8.9	10.9 a	6.45 ab	11.39 b	290.19 bc	1.73 ab	R	273.33
P12	35.3	59.7	A	68.5 ab	1.0 b	7.5 bc	20.9 bc	9.1	11.2 a	5.95 bc	12.67 ab	321.53 b	1.27 bc	R	226.67
P22	36.7	58.0	PS	56.1 c	1.0 b	9.6 a	27.7 a	8.7	10.6 ab	6.09 abc	15.12 a	399.60 a	1.53 abc	R	253.33
P24	33.3	54.0	PS	58.6 c	1.0 b	7.9 bc	20.8 bc	9.1	10.7 ab	6.39 ab	12.37 ab	312.78 bc	1.47 abc	R	246.67
CN3	34.3	55.3	S	59.9 bc	1.5 ab	8.1 b	19.1 bcd	8.6	9.9 c	6.27 ab	11.00 b	275.06 bc	1.68 ab	R	268.33
SUT1	34.7	57.8	PS	69.3 a	2.1 a	9.0 a	23.6 ab	9.2	10.2 bc	5.66 c	11.37 b	278.79 bc	1.81 ab	R	280.83
F-test	ns[5/]	ns	N/A	*	*	**	**	ns	**	*	*	*	*		ns
C.V. (%)	4.6	4.8	N/A	7.7	12.3	9.3	11.0	2.7	2.7	4.73	11.67	9.96	7.92		12.31

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Scores the observed plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of CLS severity at 65 DAP, where 1.0-2.5 = resistance, 2.6-3.4 = moderate resistance, and 3.5-5.0 = susceptibility. [4/] Data showing means different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.3 The agronomic traits and CLS assessment during rainy season at Phitsanulok

The results of the experimental cultivation of new mungbean lines P08, P12, P22 and P24 compared to the recurrent parent CN84-1, check varieties (CN3 and SUT1) and resistant line (SUPER5) at Phitsanulok were shown in Table 3.4. Most traits did not show clear differences among the genotypes, except for days to flowering, lodging and 100 seed weight, which showed statistically significant differences, while CLS scores and AUDPC showed highly significant differences.

Line P12 exhibited the longest day to flowering (44.3 days), while the other new lines had shorter days to flowering (36.0-38.0 days), which comparable to the recurrent parent and the check varieties. The maturity of P12 was classified as asynchronous (<80% of the area reached maturity simultaneously), whereas lines P22 and P24 exhibited synchrony maturity (>90% of the area reached maturity simultaneously), with greater uniformity than the recurrent parent and comparable to the check varieties. SUT1 showed the greatest plant height (98.6 cm), which resulted in a significantly higher lodging score than P24 and CN3. The new lines exhibited lodging scores not significant from the recurrent parent. For clusters/plant, P22 tended to be higher with 12.2 clusters than the recurrent parent CN84-1 (7.2 clusters) and the check varieties CN3 and SUT1 (7.1–10.1 clusters). Pods/plant for line P22 tend to have higher than other genotypes with 34.8 pods. The 100 seed weight of all new lines was similar to the recurrent parent but significantly higher than SUT1. No statistically significant differences were observed in seed weight/plant. However, lines P22 and P24 (23.61 and 18.13 g, respectively) tended to have 20-79% higher than the recurrent parent and check varieties. Consequently, yield of P22 and P24 (420.84 and 415.90 kg/rai, respectively) also tended to be higher. The highest yield was observed in P22, which exceeded the recurrent parent and check varieties by 18–33%.

For the CLS scores at 65 DAP, all new lines (1.67–2.33) and the recurrent parent CN84-1 (1.92) were classified as resistant. Line P12 had the lowest CLS score (1.67) among the new lines, lower than the recurrent parent (1.92) and significantly lower than all check varieties (2.67-2.83). Lines P08, P22, and P24 also tended to have lower CLS scores compared to the check varieties CN3 and SUT1, both classified as moderate resistant. For AUDPC, line P12 (265.00) recorded the lowest score among the new lines, comparable to the resistant line SUPER5 (216.81), and significantly lower than the recurrent parent CN84-1 (328.75) and all check varieties. The AUDPC values of P08, P22, and P24 (289.17–327.59) did not significantly differ from the recurrent parent but were significantly lower than the check varieties CN3 and SUT1 (381.67 and 387.50, respectively).

Table 3.4 Agronomic traits and CLS resistance of new mungbean lines from yield trial in rainy season during July to October 2023 at Phitsanulok.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	CLS scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.08 d	R	216.81 d
CN84-1	35.2 bc[4/]	58.0	PS	83.3	2.3 ab	7.2	20.8	10.6	12.0	7.0 a	15.10	346.71	1.92 bc	R	328.75 b
P08	36.0 bc	66.3	PS	74.6	1.8 ab	7.8	19.4	9.6	11.7	7.0 a	13.80	338.45	2.33 abc	R	327.59 b
P12	44.3 a	66.7	A	87.6	3.1 ab	7.6	18.7	9.5	11.5	6.68 a	12.66	330.22	1.67 c	R	265.00 cd
P22	38.0 b	59.7	S	71.9	2.7 ab	12.2	34.8	9.9	11.9	7.08 a	23.61	420.84	2.17 abc	R	314.26 bc
P24	36.0 bc	58.3	S	70.1	1.5 b	9.9	27.3	9.9	12.0	6.83 a	18.13	415.90	2.17 abc	R	289.17 bc
CN3	35.0 c	56.7	S	76.5	1.5 b	7.1	19.6	9.8	11.5	7.0 a	13.16	315.34	2.83 a	MR	381.67 a
SUT1	38.0 b	59.3	S	98.6	3.2 a	10.1	26.8	10.3	11.5	6.11 b	14.96	356.56	2.67 ab	MR	387.50 a
F-test	*	ns[5/]	N/A	ns	*	ns	ns	ns	ns	*	ns	ns	**		**
C.V. (%)	4.0	10.9	N/A	9.2	11.4	14.2	12.6	2.0	3.6	4.03	14.32	11.19	6.55		8.79

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Scores the observed plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of CLS severity at 65 DAP, where 1.0-2.5 = resistance, 2.6-3.4 = moderate resistance, and 3.5-5.0 = susceptibility. [4/] Data showing means, different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.4 The agronomic traits and CLS assessment during rainy season at Phetchabun

The results of the experimental cultivation of new mungbean lines at Phetchabun were shown in Table 3.5. Most traits did not show statistically significant differences among the genotypes, except for plant height and pod length, which exhibited significant differences. In contrast, lodging, CLS scores at 65 DAP and AUDPC showed highly significant differences.

The maturity of almost all genotypes was classified as synchrony (>90% of pods matured simultaneously), except for P24 and SUT1, which were classified as partial synchronous (80–90%). Most new lines had plant height comparable to the recurrent parent and check varieties, except for P08 (61.7 cm), which was shorter than both the recurrent parent CN84-1 (75.5 cm) and SUT1 (78.1 cm). Line P12 (81.6 cm) and variety SUT1 exhibited greater height and exhibited more lodging than other genotypes. Although clusters/plant and pods/plant did not show statistically significant differences, P22 recorded the highest values in both traits (7.4 clusters and 20.7 pods, respectively). For pod length, all new lines had similar average length (9.2 - 9.3 cm), which was shorter than CN84-1 (10.0 cm) but not significantly different from check varieties CN3 and SUT1 (9.2 and 9.6 cm, respectively). Lines P22 and P24 had the highest seed weight (11.60 and 11.61 g, respectively), showing a tendency to outperform other lines and varieties. Similar trends were observed in yield, with P22 and P24 (388.79 and 368.67 kg/rai, respectively) tending to produce 9-32% higher yields compared to the recurrent parent CN84-1 (339.54 kg/rai) and check varieties CN3 and SUT1 (293.92 and 295.83 kg/rai, respectively).

Most new lines had CLS scores at 65 DAP not significantly different from the recurrent parent, except for P24, which had lower scores (2.10), significantly lower than the recurrent parent (2.83) and all check varieties (3.45-3.59). Lines P12 and P24 were classified as resistant, similar to the resistant line SUPER5. While other new lines were classified as moderate resistance (P08 and P22). The CLS scores corresponded with AUDPC values other than SUPER5, line P24 recorded the lowest AUDPC (310.00), significantly lower than other new lines (350.00–388.33), the recurrent parent CN84-1 (382.50), and the check varieties CN3 and SUT1 (445.00 and 458.75, respectively). While other new lines had significantly lower AUDPC than the check varieties.

Table 3.5 Agronomic traits and CLS resistance of new mungbean lines from yield trial in rainy season during July to October 2023 at Phetchabun.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	CLS scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.05 e	R	204.58 e
CN84-1	32.7[4/]	45.7	S	75.5 ab	1.8 b	6.3	18.3	10.0 a	11.5	6.47	9.89	339.54	2.83 bc	MR	382.50 bc
P08	32.0	46.0	S	61.7 c	2.3 b	6.1	15.4	9.2 b	11.5	6.39	9.70	321.71	2.88 b	MR	388.33 b
P12	37.0	50.0	S	81.6 a	4.7 a	7.1	18.1	9.3 b	11.6	5.90	9.63	318.69	2.50 c	R	350.00 c
P22	36.7	52.3	S	67.6 bc	2.0 b	7.4	20.7	9.2 b	11.1	6.35	11.60	388.79	2.60 bc	MR	360.00 bc
P24	34.7	51.0	PS	71.3 abc	1.7 b	7.1	20.0	9.2 b	10.9	6.31	11.61	368.67	2.10 d	R	310.00 d
CN3	33.0	45.7	S	70.2 abc	1.7 b	6.6	15.7	9.2 b	11.2	6.56	9.09	293.92	3.45 a	MR	445.00 a
SUT1	34.8	48.5	PS	78.1 ab	3.8 a	6.8	17.1	9.6 ab	11.2	5.83	9.71	295.83	3.59 a	S	458.75 a
F-test	ns[5/]	ns	N/A	*	**	ns	ns	*	ns	ns	ns	ns	**		**
C.V. (%)	6.2	7.7	N/A	8.5	8.5	8.8	13.5	2.5	4.3	5.22	17.18	10.97	2.20		5.13

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Scores the observed plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of CLS severity at 65 DAP, where 1.0-2.5 = resistance, 2.6-3.4 = moderate resistance, and 3.5-5.0 = susceptibility. [4/] Data showing means, different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.5 The agronomic traits and CLS assessment during dry season at Nakhon Ratchasima

From the data presented in Table 3.6, significant differences were observed among mungbean genotypes in clusters/plant, seeds/pod, and yield, while highly significant differences were found in lodging, 100 seed weight, PM scores, and AUDPC.

The new mungbean lines were compared with the recurrent parent CN84-1 and the check varieties CN3 and SUT1. In terms of maturity, line P12 was classified as having partial synchrony (80–90% of pods matured simultaneously), similar to SUT1, whereas the other genotypes were classified as synchrony (>90%). Plant height did not differ significantly among genotypes; however, line P12 tended to be taller than the other new lines. Lodging was not observed in most genotypes, except in SUT1, which showed lodging issue. Line P12 produced significantly more clusters/plant (6.3 clusters) than CN84-1 (4.6 clusters) and was comparable to SUT1. Other new lines exhibited 5.0–5.3 clusters, which tend to be higher than CN84-1. Lines P12 and P22 showed a tendency for higher pods/plant (13.5 and 13.7 pods, respectively) than CN84-1 (10.7 pods). For seeds/pod, all new lines were comparable to the recurrent parent, but line P12 (10.7 seeds) had a significantly higher value than CN3 and SUT1 (9.1 and 8.9 seeds, respectively). For 100 seed weight, the four new lines were similar to both the recurrent parent and check varieties, except for line P08, P22 and P24, which had significantly higher 100 seed weight (6.45 - 6.68 g) than variety SUT1. In seed weight/plant, lines P12 and P22 tended to have higher values (8.08 and 7.03 g, respectively) than the other genotypes. In terms of yield, line P12 produced a significantly higher yield than CN84-1, with an increase of 28.9%, and exceeded the check varieties (CN3 and SUT1) by 20.8 - 29.7%. Lines P08, P22, and P24 exhibited yields not significantly different from both the recurrent parent and SUT1, while P22 resulted significant higher yields than variety CN3.

The PM scores at 65 DAP indicated that all four new lines and SUT1 exhibited moderate resistance. The recurrent parent CN84-1 and CN3 showed higher disease severity and was classified as moderately susceptible. The AUDPC followed a similar trend, showing no statistically significant differences among the new lines, the recurrent parent, and the check varieties. However, the resistant line SUPER5 exhibited the lowest AUDPC and was classified as resistant.

Table 3.6 Agronomic traits and PM resistance of new mungbean lines from yield trial in dry season during December 2023 to March 2024 at Nakhon Ratchasima.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	PM scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.23 c	R	123.69 c
CN84-1	41.0[4/]	57.3	S	47.0	1.3 bc	4.6 b	10.7	9.2	10.5 ab	6.53 ab	6.23	194.64 bc	5.24 ab	MS	479.63 ab
P08	39.7	56.7	S	49.7	1.0 c	5.0 ab	12.7	8.9	9.4 bc	6.68 a	6.61	209.94 bc	4.43 ab	MR	532.41 ab
P12	40.7	57.7	PS	53.3	1.5 bc	6.3 a	13.5	8.9	10.7 a	6.18 bc	8.08	250.84 a	4.52 ab	MR	539.81 ab
P22	40.0	57.0	S	49.4	1.3 bc	5.3 ab	13.7	8.6	9.7 abc	6.65 ab	7.30	234.00 ab	4.65 ab	MR	543.52 ab
P24	40.0	56.7	S	46.2	1.3 bc	5.0 ab	10.9	8.4	9.7 abc	6.45 ab	6.72	209.15 bc	3.14 b	MR	460.19 ab
CN3	39.3	55.7	S	49.7	2.0 b	4.5 b	10.8	8.5	9.1 c	6.87 a	6.05	193.44 c	5.76 a	MS	587.96 a
SUT1	37.0	55.0	PS	54.5	2.8 a	6.3 a	14.5	8.9	8.9 c	5.81 c	6.48	207.68 bc	4.77 ab	MR	395.37 b
F-test	ns[5/]	ns	N/A	ns	**	*	ns	ns	*	**	ns	*	**		**
C.V. (%)	8.5	7.3	N/A	11.9	8.6	13.9	27.7	3.5	6.7	3.84	10.95	9.53	7.16		18.29

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Scores the observed plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of PM severity at 65 DAP, where 1.0-3.0 = resistance, 3.1-4.7 = moderate resistance, and 4.8-6.4 = moderate susceptibility, 6.5-9.0 = susceptibility. [4/] Data showing means, different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.6 The agronomic traits and CLS assessment during dry season at Chai Nat

According to the data presented in Table 3.7 from the experimental cultivation at Chai Nat, most agronomic traits showed non-significant differences among mungbean genotypes. However, 100 seed weight, PM scores, and AUDPC exhibited highly significant differences.

When compared with the recurrent parent CN84-1 and the check varieties CN3 and SUT1. All genotypes except P12 classified as synchrony (>90%). Line P12 exhibited partial synchrony (80–90% of pods matured simultaneously), which corresponded with it delay days to flowering (63 days). Lodging issue was not observed in this trial; most genotypes showed less than 25% lodging within the cultivation area. For 100 seed weight, most new mungbean lines had weights similar to the recurrent parent CN84-1 and the check variety CN3, except for P12, which showed a lower weight than all other genotypes. In other agronomic traits, the new lines showed favorable tendencies compared to the recurrent parent and check varieties. For the clusters/plant, line P12 tends to produce higher clusters/plant among all genotypes with 9.3 clusters followed by P24 with 8.6 clusters. Similarly, lines P12, P22, and P24 showed a tendency of higher values for pods/plant and seed weight/plant (20.24-21.3 pods and 11.93-12.98 g, respectively) compared to the recurrent parent and check varieties. Consequently, these three lines exhibited promising yield levels, with yields of 327.34, 329.29, and 305.87 kg/rai, respectively.

The PM scores at 65 DAP indicated that PM was violent under the environmental conditions of Chai Nat, resulting in susceptibility across new lines, recurrent parent and check variety CN3. Nevertheless, the new lines tended to have lower PM scores than the recurrent parent and check variety CN3, ranging from 7.03 to 7.81. Notably, line P08 had a significantly lower PM score than the recurrent parent. Similarly, AUDPC in lines P08 and P12 were significantly lower than those of the recurrent parent and tended to be lower than CN3.

Table 3.7 Agronomic traits and PM resistance of new mungbean lines from yield trial in dry season during December 2023 to March 2024 at Chai Nat.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	PM scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3.24 d	MR	506.33 d
CN84-1	40.2[4/]	59.3	S	71.6	1.3	7.2	17.1	9.6	11.6	6.79 a	11.57	261.78	8.06 a	S	1393.21 a
P08	40.7	59.7	S	70.3	1.0	6.8	17.3	9.5	12.2	6.53 a	11.29	294.45	7.03 bc	S	1236.21 bc
P12	41.7	63.0	PS	69.8	1.7	9.3	21.3	9.2	11.8	5.72 c	12.77	327.34	7.50 ab	S	1231.48 bc
P22	41.0	58.7	S	64.4	1.0	8.0	20.9	9.4	12.1	6.46 a	12.98	329.29	7.81 a	S	1322.74 ab
P24	40.3	57.7	S	63.4	1.0	8.6	20.4	9.2	11.6	6.63 a	11.93	305.87	7.41 ab	S	1289.51 ab
CN3	39.7	59.3	S	65.1	1.2	6.9	17.6	9.4	11.7	6.74 a	11.83	293.33	7.73 ab	S	1316.70 ab
SUT1	39.5	58.3	S	72.7	1.8	8.0	19.7	9.8	11.5	6.09 b	11.89	298.44	6.68 c	S	1140.53 c
F-test	ns[5/]	ns	N/A	ns	ns	ns	ns	ns	ns	**	ns	ns	**		**
C.V. (%)	4.6	3.6	N/A	21.9	14.0	17.0	15.4	5.3	8.3	2.75	12.26	10.51	1.03		4.77

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Scores the observation plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of PM severity at 65 DAP, where 1.0-3.0 = resistance, 3.1-4.7 = moderate resistance, and 4.8-6.4 = moderate susceptibility, 6.5-9.0 = susceptibility. [4/] Data showing means different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.7 The agronomic traits and CLS assessment during dry season at Phitsanulok

The results of the experimental cultivation of new mungbean lines P08, P12, P22, and P24 during dry season at Phitsanulok have been presented in Table 3.8. Most agronomic traits showed non-statistically significant differences among genotypes, except for 100 seed weight, which differed highly significantly.

When compared with the recurrent parent CN84-1 and the check varieties CN3 and SUT1, most genotypes exhibited synchrony maturity (>90% of the area reached maturity simultaneously), except for line P12, which showed partial synchrony (80–90%), P12 also tended to produce more clusters/plant and pods/plant than the recurrent parent. In terms of 100 seed weight, line P08 exhibited 7.13 g, which was comparable to the recurrent parent CN84-1 (7.26 g) and significantly higher than the check variety SUT1 (5.66 g). For yield, lines P08 and P24 tended to produce higher yields than both the recurrent parent and the check varieties, with the yields of 320.59 and 313.02 kg/rai, respectively. All new mungbean lines possessed distinct superior agronomic traits. However, line P08 tends to have higher seed weight/plant and yield than the recurrent parent CN84-1 (8.89 g and 299.52 kg/rai, respectively), with an average 9.64 g and 320.59 kg/rai, which was the highest among all genotypes.

For PM scores at 65 DAP, the environmental conditions at Phitsanulok were not conducive to PM development and less symptoms were observed. All genotypes were classified as resistant. The PM scores of new mungbean lines did not differ significantly among genotypes. However, line P24 showed the lowest PM score among the new lines (1.15), while the resistant control line SUPER5 showed no disease symptoms. The AUDPC followed a similar trend with PM scores, all four new lines exhibited lower AUDPC than the recurrent parent CN84-1.

Table 3.8 Agronomic traits and PM resistance of new mungbean lines from yield trial in dry season during December 2023 to March 2024 at Phitsanulok.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	PM scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.00	R	222.00
CN84-1	40.5[4/]	61.0	S	57.8	1.0	4.4	14.1	9.6	10.9	7.26 a	8.89	299.52	1.85	R	316.67
P08	40.3	60.0	S	49.4	1.0	4.9	13.5	9.5	10.8	7.13 ab	9.64	320.59	1.78	R	309.00
P12	41.3	60.7	PS	57.3	1.0	5.6	15.4	9.2	11.0	6.43 d	8.85	282.84	1.30	R	255.56
P22	39.3	60.7	S	50.2	1.0	4.6	13.6	9.2	10.9	6.71 cd	9.12	298.68	1.55	R	283.80
P24	39.0	60.0	S	50.3	1.0	4.3	12.5	9.3	10.8	6.81 bcd	9.44	313.02	1.15	R	238.89
CN3	38.3	59.7	S	54.5	1.0	5.5	14.6	9.2	10.6	7.07 abc	9.39	298.56	2.09	R	343.62
SUT1	38.5	58.7	S	57.3	1.0	5.3	16.7	9.2	10.6	5.66 e	9.64	293.13	1.11	R	234.72
F-test	ns[5/]	ns	N/A	ns	ns	ns	ns	ns	ns	**	ns	ns	ns		ns
C.V. (%)	3.0	1.8	N/A	8.6	0.0	16.9	15.1	3.1	4.7	3.10	16.34	17.01	11.98		20.11

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Scores the observed plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of PM severity at 65 DAP, where 1.0-3.0 = resistance, 3.1-4.7 = moderate resistance, and 4.8-6.4 = moderate susceptibility, 6.5-9.0 = susceptibility. [4/] Data showing means, different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, ns = not significant, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.8 The agronomic traits and CLS assessment during dry season at Phetchabun

The results of the experimental cultivation of new mungbean lines P08, P12, P22, and P24 during the dry season at Phetchabun are presented in Table 3.9. Most agronomic traits showed non-statistically significant differences among genotypes. However, days to flowering and lodging showed significant differences, while 100 seed weight, PM scores, and AUDPC showed highly significant differences.

When compared to new mungbean lines with the recurrent parent CN84-1 and the check varieties CN3 and SUT1, line P12 exhibited the longest duration to flowering at 48.7 days, which also led to a longer duration to maturity (64.3 days). The remaining new lines had days to flowering and days to maturity ranging 43.0-44.3 days and 61.7 to 63.0 days, respectively. In terms of maturity classification, line P12 exhibited partial synchrony (80–90%), similar to CN3, while the other genotypes displayed synchronous maturity (>90% of the area reached maturity simultaneously). Line P12 produced the highest clusters/plant (7.9 clusters) and pods/plant (19.7 pods), showing a tendency to outperform other genotypes. For 100 seed weight, lines P08, P22, and P24 were comparable to the recurrent parent CN84-1 and check variety CN3. Regarding seed weight/plant and yield, all new mungbean lines tended to produce higher values than both the recurrent parent and the check varieties, with yields ranging from 312.95 to 337.88 kg/rai.

For the PM scores at 65 DAP, line P12 exhibited the lowest PM score among the new lines, with a lower score than both the recurrent parent and the check variety CN3. The other lines also showed scores lower than the recurrent parent, but they are not significantly different. Lines P12, P22, P24, and the variety SUT1 were classified as moderately resistant, whereas CN84-1 and CN3 were categorized as moderately susceptible. For AUDPC values, lines P12, P22, and P24 exhibited lower values than both the recurrent parent CN84-1 and check variety CN3. The AUDPC value of line P08 was lower but not significantly different from that of the recurrent parent. These results clearly indicated that the new mungbean lines demonstrated better resistance to PM compared to the recurrent parent.

Table 3.9 Agronomic traits and PM resistance of new mungbean lines from yield trial in dry season during December 2023 to March 2024 at Phetchabun.

Genotypes	Days to flowering	Days to maturity	Maturity[1/]	Plant height (cm)	Lodging[2/]	Clusters/plant	Pods/plant	Pod length (cm)	Seeds/pod	100 seed weight (g)	Seed weight/plant (g)	Yield (kg/rai)	PM scores[3/]		AUDPC
SUPER5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.31 d	R	256.17 e
CN84-1	43.3 b[4/]	61.5	S	66.6	1.3 b	7.4	17.7	9.1	10.2	7.30 a	11.03	271.34	5.72 a	MS	824.85 a
P08	44.0 b	63.0	S	68.5	1.3 b	7.0	18.6	9.3	11.2	7.51 a	13.21	337.88	5.20 a	MS	731.48 ab
P12	48.7 a	64.3	PS	68.4	3.0 a	7.9	19.7	8.8	10.3	6.81 b	12.08	315.78	4.08 bc	MR	575.93 cd
P22	43.0 b	61.7	S	62.5	1.0 b	6.4	18.6	9.0	10.5	7.36 a	12.11	312.95	4.67 ab	MR	670.37 bc
P24	44.3 b	63.0	S	65.6	1.7 b	7.2	18.9	8.9	10.4	7.23 a	11.92	325.72	4.71 ab	MR	688.62 bc
CN3	41.7 b	60.3	PS	66.9	1.8 ab	6.4	18.3	8.8	10.3	7.27 a	11.62	298.63	5.16 a	MS	739.71 ab
SUT1	44.7 b	61.2	S	61.1	1.8 ab	7.3	18.7	9.4	10.8	6.73 b	11.03	282.77	3.46 c	MR	498.77 d
F-test	*	ns[5/]	N/A	ns	*	ns	ns	ns	ns	**	ns	ns	**		**
C.V. (%)	4.5	2.4	N/A	7.0	12.3	11.2	11.0	3.1	4.0	2.95	11.57	12.50	2.96		10.32

[1/] Maturity stages including synchrony (S): more than 90% of pods mature simultaneously at the first harvest, partial synchrony (PS): 80–90% of pods mature simultaneously, and asynchrony (A): less than 80% of pods mature simultaneously. [2/] Scores the observed plants based on the angle leaning away over 45°, where 1 = no lodging, 2 = lodging of 1-25% of area, 3 = lodging of 26-50% of area, 4 = lodging of 51-75% of area, and 5 = lodging more than 75% of area. [3/] The scores of PM severity at 65 DAP, where 1.0-3.0 = resistance, 3.1-4.7 = moderate resistance, and 4.8-6.4 = moderate susceptibility, 6.5-9.0 = susceptibility. [4/] Data showing means, different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [5/] N/A = not available, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$

3.4.9 Combined variance analysis for yield of mungbean genotypes

The combined variance analysis of seven mungbean genotypes across four experimental locations and two seasons is presented in Table 3.10, including the experimental fields in Nakhon Ratchasima (dry season), Chai Nat (rainy and dry seasons), Phitsanulok (rainy season), and Phetchabun (rainy and dry seasons). The experimental fields in Nakhon Ratchasima (rainy season) and Phitsanulok (dry season) were excluded from the combined analysis due to excessive variation. The results demonstrated that plant yield in the six fields was markedly affected by genotype (G) and location (L) with, although season (S) and their interactions (G × L, G × S, and G × L × S) exhibited non-significant effects.

The mungbean cultivated in Phitsanulok during the raining season demonstrated the highest yield, averaging 360.57 kg/rai, which was considerably superior to yields from other locations, exceeding them by 18-68%. This may have resulted from the lack of substantial rainfall during the harvesting time (Figure A.12), in contrast to other areas cultivated in the same season. Alternatively, it may be ascribed to more advantageous environmental conditions for mungbean cultivation relative to other locales and seasons. The average yield of all mungbean genotypes was 303.95 kg/rai. The yields of the new mungbean lines P12, P22, and P24 were significantly better than the recurrent parent CN84-1, whereas P08 exhibited non significantly different from the recurrent parent. Line P22 achieved the highest average yield of 347.58 kg/rai across six environments, surpassing the recurrent parent CN84-1 by 24% (279.95 kg/rai), while line P24 obtained 323.02 kg/rai, which is 15% larger than the recurrent parent. Nakhon Ratchasima (dry season) and Chai Nat (rainy season) showed significant differences in yield among genotypes, with average yields of 214.24 kg/rai and 306.64 kg/rai, respectively. Line P12 had the highest yield at Nakhon Ratchasima (250.84 kg/rai), while P22 had the highest yield at Chai Nat (399.60 kg/rai). Other new lines also exhibited higher or comparable yields relative to the recurrent parent and all check varieties. Although, yields in Chai Nat (dry season), Phitsanulok (rainy season), and Phetchabun (rainy and dry seasons) were not significantly different among genotypes, the new lines tended to produce higher yields than both the recurrent parent and all check varieties.

Table 3.10 Combined analysis of yield (kg/rai) of seven mungbean genotypes across four environments and two seasons.

Genotypes	Nakhon Ratchasima	Chai Nat		Phitsanulok	Phetchabun		Overall mean
	Dry season	Rainy season	Dry season	Rainy season	Rainy season	Dry season	
CN84-1	194.64 bc[1/]	249.45 c	261.78	346.71	339.54	271.34	279.95 d
P08	209.94 bc	290.19 bc	294.45	338.45	321.71	337.88	298.77 bcd
P12	250.84 a	321.53 b	327.34	330.22	318.69	315.78	310.73 bc
P22	234.00 ab	399.60 a	329.29	420.84	388.79	312.95	347.58 a
P24	209.15 bc	312.78 bc	305.87	415.90	368.67	325.72	323.02 ab
CN3	193.44 c	275.06 bc	293.33	315.34	293.92	298.63	278.29 d
SUT1	207.68 bc	278.79 bc	298.44	356.56	295.83	282.77	286.68 cd
Mean	214.24	306.64	303.48	360.57	332.45	306.44	303.95
F-test	*[2/]	*	ns	ns	ns	ns	**
Genotypes (G)			**				
Locations (L)			**				
Seasons (S)			ns				
G x L			ns				
G x S			ns				
G x L x S			ns				
C.V. (%)			13.01				

[1/] Data showing means, different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [2/] ns = not significant, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

3.4.10 Evaluation of GEI using symmetrical GGE biplot analysis

The yield potential and stability of all mungbean genotypes, including CN84-1, P08, P12, P22, P24, CN3, and SUT1, were evaluated across four locations: Nakhon Ratchasima, Chai Nat, Phitsanulok, and Phetchabun. Agronomic traits, including pods/plant, 100 seed weight, and yield, were assessed and displayed in Patterns A, B, and C, respectively.

A symmetrical GGE biplot analysis was conducted to evaluate the relationships among the test environments and to determine the proportion of variance attributable to the GEI, as explained by principal components (PC) 1 and PC2. The results revealed that the cumulative variances of PC1 and PC2 accounted for 96.25% of the GEI for pods/plant, 96.7% for 100 seed weight, and 95.05% for yield. Based on the symmetrical GGE biplot, the relationship between test environments was assessed using environmental vectors, which are lines drawn away from the biplot origin at x,y = 0.0,0.0 to each environment as shown with green lines in Figure 3.3. Environments forming acute angles between their vectors were positively correlated, whereas obtuse angles indicated negative correlations. Right angles suggested no correlation between environments. In addition, the distance between two environments reflected their ability to discriminate among the mungbean genotypes. For pods/plant (Figure 3.3, Pattern A), Chai Nat and Phetchabun exhibited a positive correlation, providing the most consistent genotype data across both locations. For 100 seed weight and yield (Figure 3.3, Pattern B and C), Nakhon Ratchasima and Phetchabun were positively correlated, indicating the most consistent genotype responses in these environments for both traits.

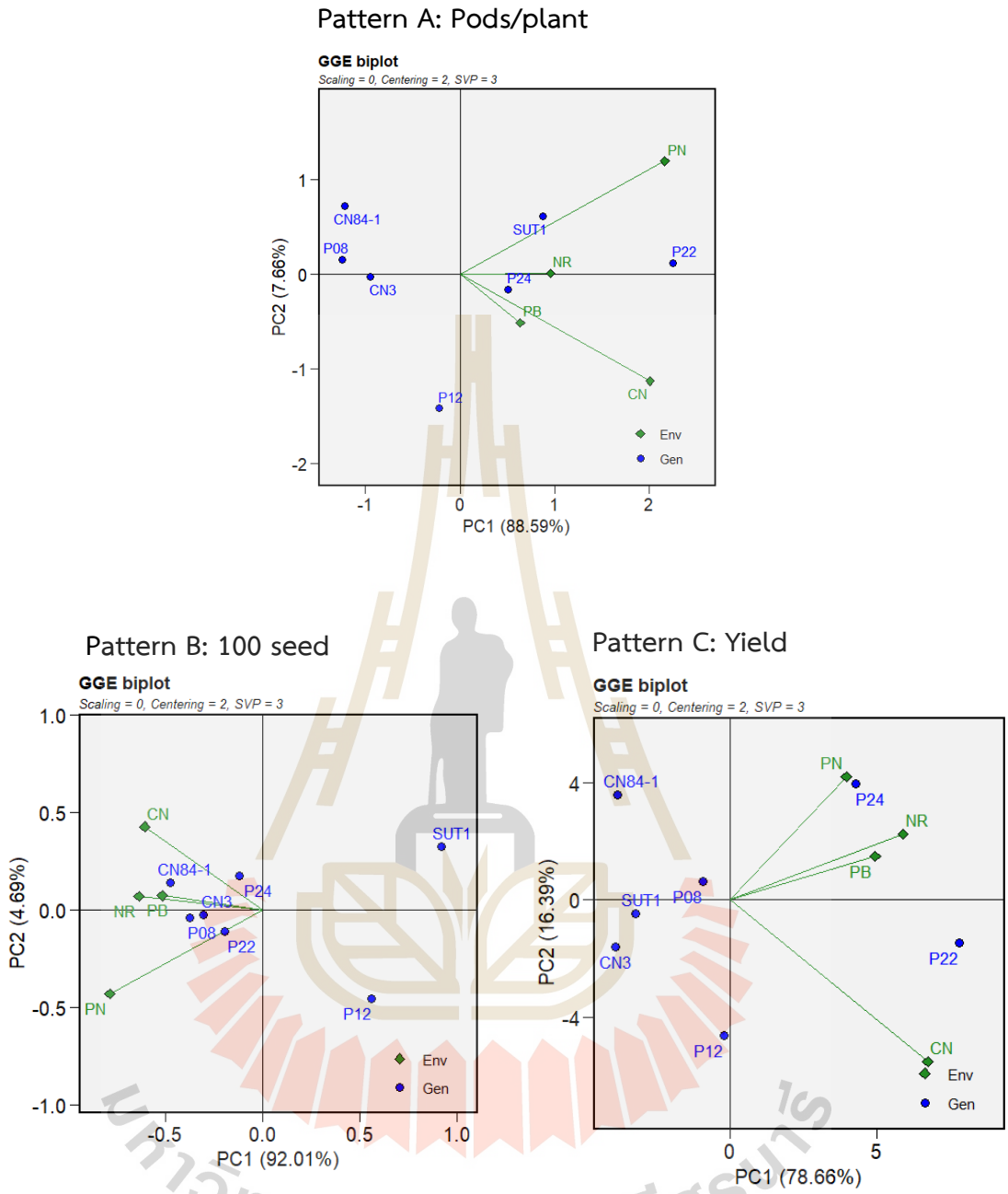

Figure 3.3 The GGE biplot 'Symmetrical' pattern illustrating the effects of the first two principal components (PC1 and PC2) of seven mungbean genotypes evaluated across four locations and two seasons. Abbreviation: NR = Nakhon Ratchasima, CN = Chai Nat, PN = Phitsanulok, and PB = Phetchabun.

3.4.11 Evaluation of genotypes based on mean performance and stability

A stability analysis of mungbean genotypes across four test locations was performed utilizing the GGE biplot approach under the ‘Mean vs. Stability’ perspective (Figure 3.4). The biplot displays the ranking of genotypes according to their overall performance across various locations, utilizing the AEC to visually represent both mean performance and stability. This examination demonstrates the performance and stability of each genotype. The stability of the genotype and GEI are represented by a green line. The vertical axis is called the 'AEC ordinate,' while the horizontal axis is the 'AEC abscissa.' The length of the genotype's projection along this line indicates its stability longer projections signify lower stability.

For pods/plant (Figure 3.4, Pattern A), line P22 exhibited the highest performance compared to other genotypes, followed by SUT1, P24, and P12, respectively. In contrast, line P08 and CN84-1 had the lowest pods/plant. However, line P08 was closest to the AEC abscissa, indicating the highest stability among all genotypes. Since the AEC ordinate reflects the degree of instability, the low projection of P08 onto this axis further confirmed its stable performance across environments. Additionally, P22 and P24 showed relatively high pods/plant with good stability as well. In the analysis of 100 seed weight (Figure 3.4, Pattern B), the AEC abscissa extended in the negative direction to the left side, indicating that the recurrent parent CN84-1 had the highest 100 seed weight, followed by lines P08, CN3, P22, P24, and P12, respectively. Although line P08 had slightly lower seed weight than CN84-1, but it exhibited greater stability, as reflected by its proximity to the AEC abscissa and minimal projection onto the AEC ordinate. Both P08 and CN3 were classified as genotypes with large seeds and high stability. For the performance and stability of yield as shown in (Figure 3.4, Pattern C), line P22 achieved the highest average yield, followed by P24, P12, and P08. Variety CN3 had the lowest yield among all genotypes. Notably, line P22 combined high yield and stability. Although line P08 exhibited moderate yield performance, it exhibited the highest level of stability among the new mungbean lines, as indicated by its close alignment with the AEC abscissa and low value on the AEC ordinate.

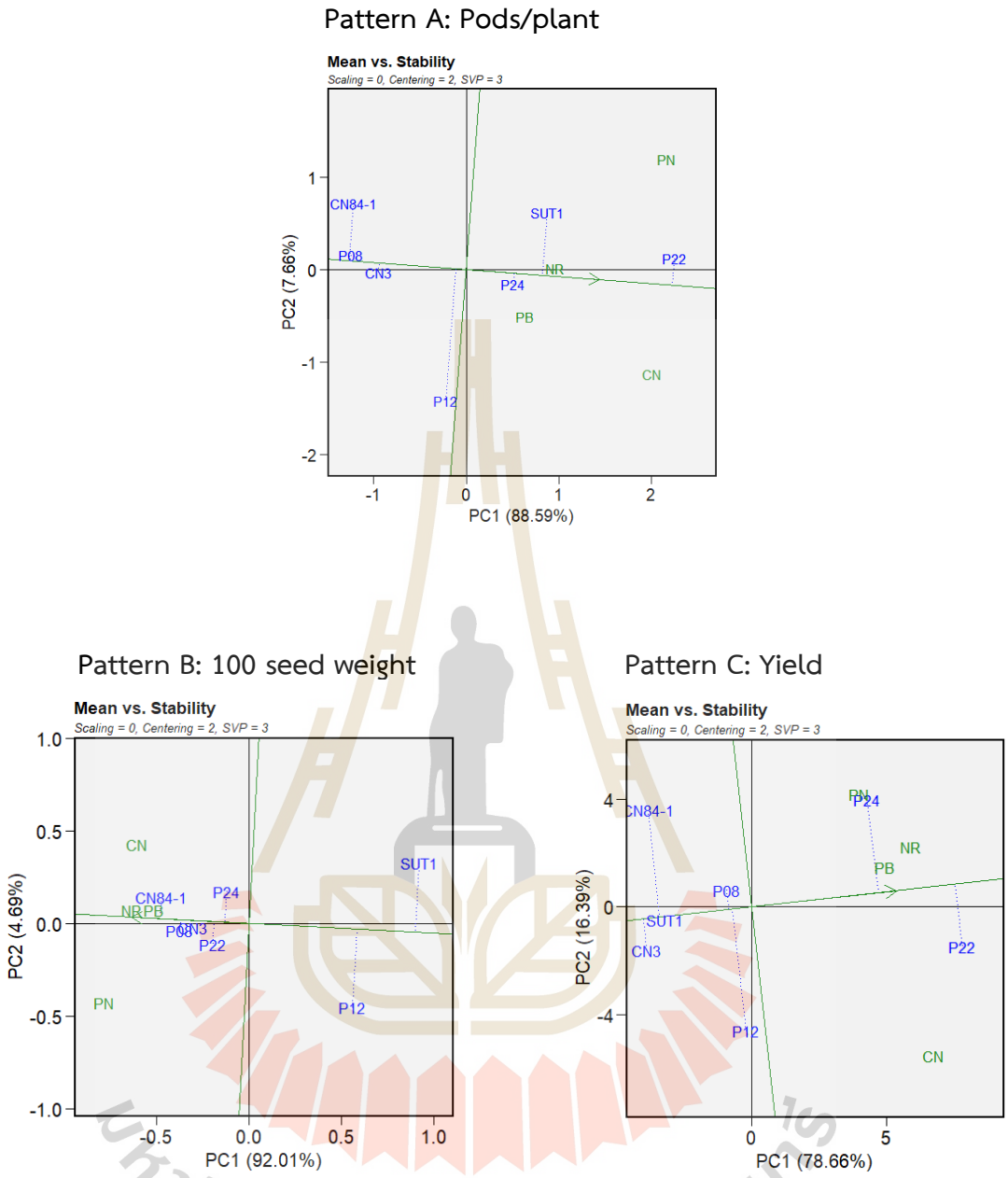

Figure 3.4 The GGE biplot 'Mean vs. stability' pattern illustrating interaction effect of seven mungbean genotypes evaluated across four locations and two seasons. Abbreviation: NR = Nakhon Ratchasima, CN = Chai Nat, PN = Phitsanulok, and PB = Phetchabun.

3.4.12 Identification of mega environments and 'Which-won-where'

The suitability of mungbean genotypes across environments was assessed using the GGE biplot represented as a polygon showing 'Which-won-where' pattern. Vertical lines, the so-called "equality lines," are then subsequently drawn from the origin (0.0,0.0) to different sides of polygon to divide it into sectors called "mega environment". This graph helps to pinpoint major environments and genotypes with specialized resilience. The G+G×E variation accounted for 96.25%, 96.70%, and 95.05% for pods/plant, 100 seed weight, and yield, respectively (Figure 3.5).

For pods/plant, the polygon was divided into only a single sector, as all test locations were grouped within the same boundary. Line P22 demonstrated the highest pods/plant performance across all locations, followed by variety SUT1 (Figure 3.5, Pattern A). Regarding 100 seed weight, only one sector was also identified. The recurrent parent CN84-1 exhibited the highest 100 seed weight performance across locations, followed by line P08 (Figure 3.5, Pattern B). In contrast, for yield (Figure 3.4, Pattern C), the test environments were grouped into two distinct sectors representing 2 mega environments. The first group included Phitsanulok, while the second group comprised Nakhon Ratchasima, Phetchabun, and Chai Nat. The result found that line P22 showed the highest yield and was the best adapted to the second group of locations, followed by P24 and P24 was best suited for cultivation in the first group.

Considering genotype-specific adaptation to environments, observation based on the proximity of close genotype and environment points on the biplot graph. The result indicated that pods/plant of P22 was particularly well-suited adaptation to Phitsanulok and Chai Nat, while variety SUT1 was best to Nakhon Ratchasima (Figure 3.5, Pattern A). For 100- seeds weight, CN84-1 was the most suitable adaptation for Phetchabun, followed by Nakhon Ratchasima and Chai Nat, respectively, whereas line P08 was particularly adapted to Phetchabun and Nakhon Ratchasima as shown in (Figure 3.5, Pattern B). Regarding yield, the high yield production and suitable adaptation of line P24 was best at Phitsanulok followed by Nakhon Ratchasima and Phetchabun, respectively. Whereas line P22 showed better adaptation to Nakhon Ratchasima, Phetchabun, and Chai Nat (Figure 3.5, Pattern C).

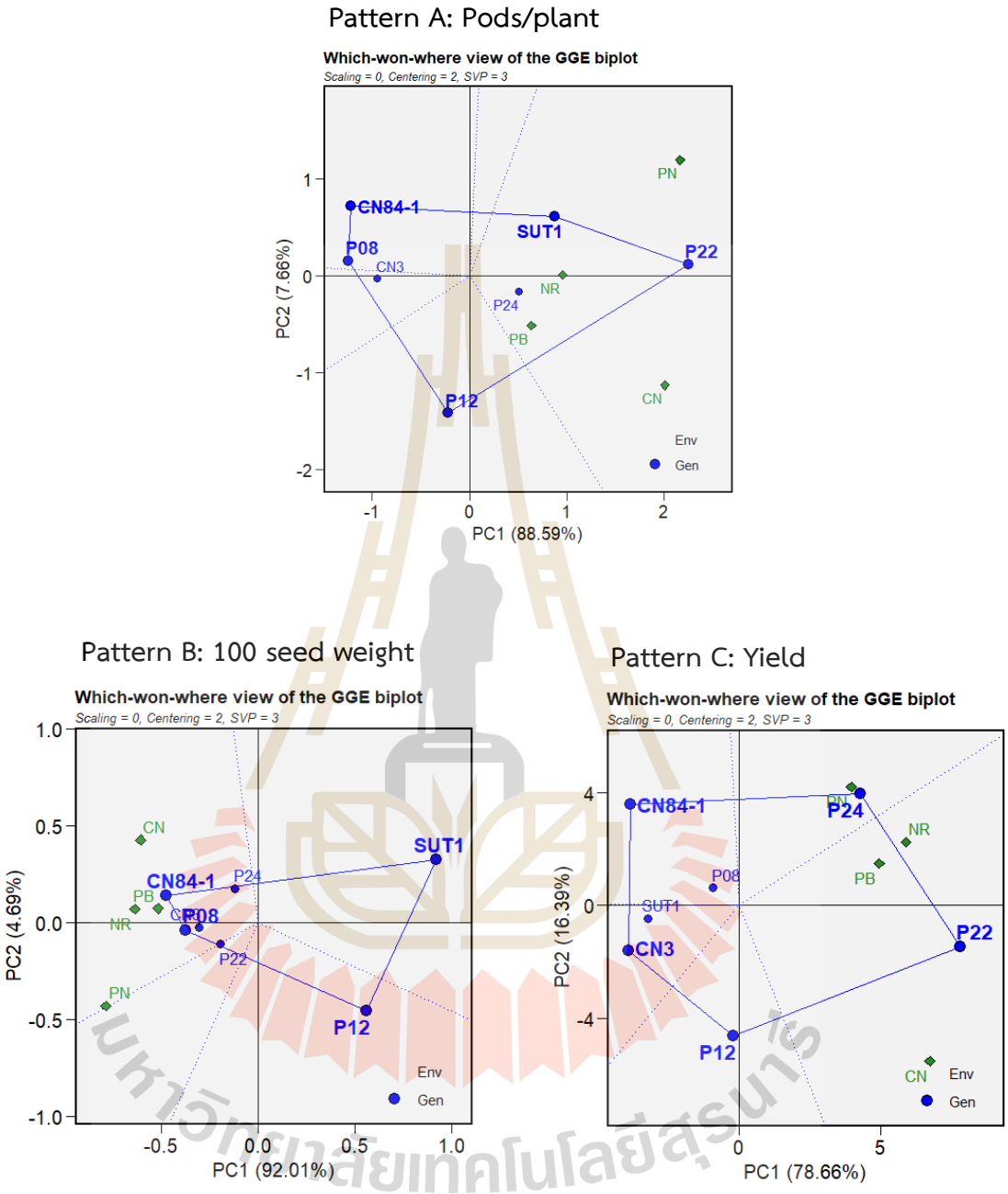

Figure 3.5 The polygon GGE biplot 'Which-won-where' view displaying the genotype main effect plus G×E interaction effect of seven mungbean genotypes evaluated across four locations and two seasons. Abbreviation: NR = Nakhon Ratchasima, CN = Chai Nat, PN = Phitsanulok, and PB = Phetchabun.

3.4.13 Assessment of testing locations: discriminative vs. representativeness and desirability index

The evaluation potential of each environment for distinguishing the mungbean genotypes, GGE biplot analysis under the Discrimitiveness (the ability of an environment to distinguish genotype) vs representativeness (the ability of an environment to represent all other evaluated environments) pattern was performed (Figure 3.6). The analysis used the AEC, illustrated by a green arrow originating from the biplot center point (0.0,0.0), which represents the average variance of PC1 and PC2 across all tested environments. In the GGE biplot approach, three variables are crucial for evaluating test locations: discrimination power, representativeness, and desirability index. The "discriminating ability" specified by extent of the environmental vector that corresponds to standard deviation within experimental location. Environments vectors with longer vectors are more effective at discriminating against the different genotypes classified as "discriminating locations" due to their ability to differentiate between genotypes. The representativeness of experimental sites is driven by acute angles formed by environment vectors and the AEC abscissa. The acute angles with the AEC abscissa, identifying as "most representative" experimental locations. The desirability index of experimental sites combines both its discriminative ability and representativeness.

For the discriminative environment indicated that Phitsanulok was the most discriminative environment for pods/plant and 100 seed weight, while Chai Nat was the most discriminative environment for yield (Figure 3.6, Pattern A, B, and C). Regarding the representativeness of each location, which can be assessed by the angle relative to AEC abscissa, it was found that for pods/plant and 100 seed weight, the Nakhon Ratchasima location served as the most representative environment. In contrast, for yield, the Phetchabun location was identified as the most representative and provided a highly stable testing environment for yield evaluation (Figure 3.6, Pattern C). The desirability index indicated that Nakhon Ratchasima had the highest desirability for 100 seed weight and yield, while Chai Nat showed the highest desirability for pod/plant. These locations exhibited long environmental vectors and formed acute angles with the AEC abscissa.

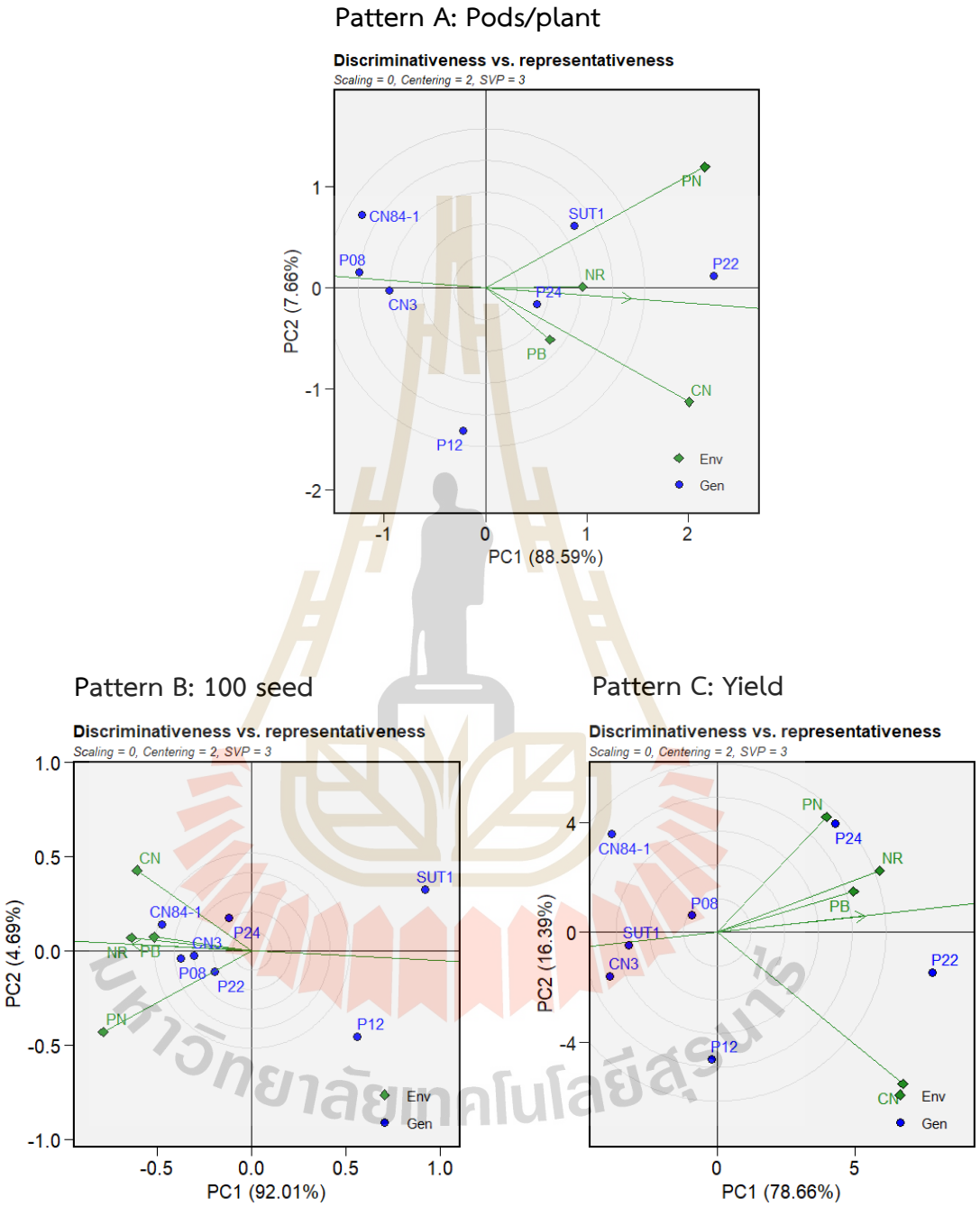

Figure 3.6 The GGE biplot 'Discriminativeness vs. Representativeness' pattern for genotype comparison with ideal genotype showing G+G×E interaction effects of seven mungbean genotypes evaluated across four locations and two seasons. Abbreviation: NR = Nakhon Ratchasima, CN = Chai Nat, PN = Phitsanulok, and PB = Phetchabun.

3.5 Discussion

The regional yield trial of eight mungbean genotypes CN84-1 (recurrent parent), SUPER5 (resistant line), two check varieties (CN3 and SUT1), and four new mungbean lines (P08, P12, P22, and P24) was conducted. The four new lines were derived from BC_4F_{10} generation plants developed through a backcrossing method. The donor parent was obtained from an F_3 generation of a double cross between genotypes CN72, V4718, V4758, and V4785, subsequently backcrossed to the recurrent parent CN84-1, which possessed high yield but was susceptible to diseases, for breeding new varieties introducing resistance to both CLS and PM. The double cross was derived from [(CN72 × V4758) × (CN72 × V4718)] × [(CN72 × V4718) × (CN72 × V4785)] (Pookhamsak et al., unpublished data). The donor parent lines V4718, V4758, and V4785 were resistant lines sourced from India (Nair & Schreinemachers, 2020). All three donor lines have been reported to carry resistance to PM (Chueakhunthod et al., 2020). Additionally, V4718 has a single dominant gene conferring resistance to CLS (Chankaew et al., 2011; Tantasawat et al., 2020). CLS and PM are the major diseases affecting mungbean production. CLS, caused by *Cercospora canescens*, can reduce mungbean yield by up to 50% if infection occurs after the flowering stage (Asian Vegetable Research and Development Center, 1974, 1975). PM, caused by *Sphaerotheca phaseoli*, can result in yield losses of up to 40% if disease management practices are not implemented (Tsou et al., 1979). The Indian donor lines V4718, V4758, and V4785 possess resistance genes that effectively counter these diseases.

Following the development and preliminary evaluation of these resistant lines, previously the superior lines P08, P12, P22, and P24 were selected through standard trials and subsequently included in this regional trial (multi-environment trials). This study involved the evaluation of key agronomic traits, disease resistance (CLS and PM), stability and performance of the new mungbean lines. Regional yield trials were conducted at four locations (Nakhon Ratchasima, Chai Nat, Phitsanulok, and Phetchabun) across two seasons (rainy and dry seasons) during the years 2023-2024.

3.5.1 Performance of new mungbean lines across environments

3.5.1.1 Line P08

During the rainy season, P08 exhibited agronomic traits that were mostly lower than or comparable to the recurrent parent CN84-1 across all environments. The yield had tended to be lower among new mungbean lines, except for P12. Line P08 demonstrated moderate resistance to PM and CLS in all environments except at Phetchabun. In the dry season trials at Nakhon Ratchasima and Chai Nat, P08 tended to produce lower yields than P12, P22, and P24 but outperformed the recurrent parent

at both sites. Its level of PM resistance varied according to disease outbreak, ranging from susceptible (Chai Nat) to resistant (Phitsanulok), and was comparable to the recurrent parent and check varieties. Agronomic traits of P08 during the dry season were generally better than during the rainy season across all locations.

3.5.1.2 Line P12

During the rainy season, line P12 exhibited characteristics that generally tended to be lower than those of the recurrent parent CN84-1, particularly in seed weight/plant and yield across all environments, except at Chai Nat. Line P12 also exhibited longer days to flowering, days to maturity, and greater plant height, which contributed to an increased risk of lodging during the rainy season. However, results of the dry season showed non-significant differences in plant height among genotypes, although P12 still exhibited higher lodging rates than other genotypes. Additionally, P12 matured later than the other genotypes. Line P12 showed moderate resistance to PM and high resistance to CLS across all environments. In the dry season, the agronomic traits of P12 tended to perform better than the recurrent parent, except at Phitsanulok, where it recorded the lowest values among all tested genotypes, however, the differences were not statistically significant. Conversely, at Nakhon Ratchasima, P12 exhibited the highest yield, outperforming all check varieties and recurrent parent, followed by its performance at Chai Nat. Resistance to PM was consistent with that of P08, with P12 demonstrating better resistance than the recurrent parent. Overall, P12 showed better adaptation and stronger disease resistance during the dry season compared to the rainy season across all environments, with particularly outstanding performance observed at Nakhon Ratchasima during the dry season.

3.5.1.3 Line P22

During the rainy season, P22 consistently exhibited agronomic traits superior to those of the recurrent parent CN84-1 and outperformed P08, P12, and P24 across all environments. P22 demonstrated moderate resistance to PM and moderate to high resistance to CLS. In the dry season, P22 showed higher yields than all check varieties and recurrent parent across all environments, with the highest yield recorded at Chai Nat, followed by Nakhon Ratchasima and Phitsanulok. Although yield tended to be lower in the dry season compared to the rainy season, P22 maintained better PM resistance than the recurrent parent, especially at Chai Nat, where it was high in disease outbreak. Despite the severe PM outbreak at Chai Nat, P22 still achieved the highest yield among all tested genotypes. This highlights P22 strong adaptability and

disease resilience, contributing to its superior yield stability, particularly at Chai Nat across both seasons.

3.5.1.4 Line P24

During the rainy season, P24 exhibited agronomic traits superior to those of the recurrent parent CN84-1 across almost all traits and environments, with particularly outstanding performance at Nakhon Ratchasima. However, its yield was generally lower than or comparable to P22. P24 demonstrated moderate resistance to PM and good resistance to CLS across all environments. In the dry season, P24 consistently outperformed both the recurrent parent and check varieties in all environments, achieving the highest yields particularly at Phitsanulok and Phetchabun. Disease evaluations revealed that P24 generally had the lowest disease scores among the new mungbean lines. Overall, line P24 demonstrated superior performance compared to the recurrent parent and check varieties across both seasons and showed excellent adaptability, maintaining high yield performance in several environments.

3.5.2 The combined variance analysis of yield

The combined variance analysis of yield was conducted to determine whether environmental factors or seasonal variation significantly influenced mungbean yield. The analysis highlighted significant effects of G and L on yield performance, whereas the effects of S, G × L, G × S, and G × L × S interactions were not significant. These findings suggest that genotypic differences and specific location factors were the primary determinants of yield variation, consistent with patterns previously reported in legume crops, including mungbean (Kang, 1997; Yan et al., 2007). Among the agronomic traits evaluated in this study, yield was identified as the most critical trait of interest to farmers. Based on the combined variance analysis across the environments where data combination was possible, line P22 consistently exhibited superior yield performance, followed by line P24. In contrast, the recurrent parent CN84-1 consistently recorded the lowest average yield among all tested genotypes, with the differences being statistically significant.

3.5.3 Stability analysis from GGE biplots approaches

Multi-environment stability analysis using GGE biplots provided additional insights into the performance consistency of the mungbean genotypes across diverse environments. Assessing various genotypes in different locations presents a considerable challenge. Therefore, selecting appropriate test locations is crucial for conducting efficient and cost-effective multi-environment trials (Parihar et al., 2017; Munaro et al., 2020). The GGE biplot approach provides an effective solution to these challenges by enabling the evaluation of genotypes and identifying the best

experimental locations, which can be categorized into distinct mega-environments, irrespective of their agroecological zones (Naık et al., 2022). The significant presence of Genotypes and Locations in combined analysis of yield association underscores the necessity of multilocation evaluation of genotypes (Irfan et al., 2025).

The ‘symmetrical’ GGE biplot was conducted to examine the relationship between genotypes and environments based on pods/plant, 100 seed weight, and yield. For pods/plant, Chai Nat and Phetchabun exhibited a positive correlation, providing the most consistent genotype data across both locations. For 100 seed weight and yield, Nakhon Ratchasima and Phetchabun were positively correlated, indicating the most consistent genotype responses in these environments for both traits.

In GGE biplot view ‘Mean vs. Stability’ the AEC abscissa denotes elevated average performance, while the AEC ordinate indicates genotype stability and depicts the contribution of genotype to GEI (Yan et al., 2007; Pour-Aboughadareh et al., 2023; Basnet, 2024; Kunwar et al., 2024; Irfan et al., 2025). The result revealed that line P22 combined high performance and stable yield across environments, while P08, despite moderate yield, demonstrated the highest stability. P24 showed relatively high pods/plant with well stability. Whereas CN84-1 had the highest 100 seed weight, followed by lines P08 and CN3 respectively. Both P08 and CN3 were classified as genotypes with large seeds and high stability. Line P22 achieved the highest average yield and stability, followed by P24, P12, and P08. Variety CN3 had the lowest yield among all genotypes. The combination of performance and stability is essential for the recommendation of new cultivars for wide-scale cultivation (Yan & Tinker, 2006)

The GGE biplot 'Which-won-where' analysis demonstrated environment-specific advantages of genotypes. According to the GGE biplot analysis, an ideal experimental location should be identified based on its ability to designate genotypes, represent the mega-environment, and have a high desirability index (Yan et al., 2007; Badu-Apraku et al., 2020; Mohammadi et al., 2023; Basnet, 2024). For pods/plant, Line P22 demonstrated the highest pods/plant performance across all locations, followed by variety SUT1, while for 100 seed weight, CN84-1 retained superiority. Line P22 performed best for yield in environments such as Nakhon Ratchasima, Chai Nat, and Phetchabun, whereas P24 showed superior adaptation to Phitsanulok. These observations emphasize the importance of multi-trait selection across environments to maximize genetic gains in mungbean breeding (Yan et al., 2007).

Furthermore, the ‘Discriminativeness vs. Representativeness’ visualization enabling the elimination of unnecessary testing locations while preserving trial

heritability and genetic gain, thus enhancing the selection process in a cost-effective way (Yan et al., 2007; Badu-Apraku et al., 2020). The GGE biplot analysis showed that Phitsanulok was the most discriminative environment for pods/plant and 100 seed weight, whereas Chai Nat was the best for discriminating against yield differences. For representative environment, Nakhon Ratchasima was ideal for pods/plant and 100 seed weight evaluations, while Phetchabun was identified as the most representative environment for yield testing. The desirability index indicated that the maximum desirability index for pods/plant, 100 seed weight, and yield were observed at Chai Nat, Nakhon Ratchasima, and Nakhon Ratchasima, respectively. Resulting from long environmental vectors and formed acute angles with the AEC abscissa. Such analyses highlight the critical role of selecting optimal testing environments to ensure effective genotype differentiation and reliable selection decisions (Yan et al., 2007).

3.6 Conclusion

The evaluation of new mungbean lines across multiple environments and seasons. Among the tested lines, P22 and P24 consistently exhibited superior yield performance across various environments. Line P22 achieved the highest overall yield, with strong adaptability and stability, particularly in environments with high disease outbreak. P24 also demonstrated excellent yield potential, especially at Phitsanulok and Phetchabun, indicating its broad adaptability across both favorable and moderately stressed conditions. Both lines exhibited synchronous pod maturity. Line P12, showing resistance to CLS and moderate resistance to PM, presented delayed flowering, maturity, and high lodging under rainy season. However, in the dry season, P12 was achieving the highest yield at Nakhon Ratchasima. These results suggest that P12 is better suited for dry season cultivation. Line P08 exhibited agronomic performance comparable or lower than recurrent parent. Although its yield was lower than that of P22 and P24. Line P08 maintained better performance than CN84-1 under dry season and exhibited high yield stability across environments. The ‘symmetrical’ GGE biplots resulted that Chai Nat and Phetchabun were positively correlated for pods/plant, while Nakhon Ratchasima and Phetchabun responses for 100 seed weight and yield. Line P22 demonstrated high yield and moderate stability, while P08 showed the highest stability in yield. The GGE biplot ‘Mean vs. Stability’ analysis revealed that line P22 exhibited the highest performance and stability in pods/plant and yield. P08 demonstrated the highest stability for pods/plant and 100 seed weight. Line P24 exhibited well performance with high stability, while CN3 showed the lowest yield.

The ‘Which-won-where’ analysis revealed significant GEI, with line P22 showing the best performance for pods/plant and yield, particularly in Phitsanulok and Chai Nat. CN84-1 and P08 were the top performers for 100 seed weight. Line P24 was best at Phitsanulok whereas line P22 showed better adaptation to Phetchabun and Chai Nat. The GGE biplot analysis ‘Discriminative vs. Representativeness’, resulting that Chai Nat was the most discriminative for yield. Phetchabun was the most representative for yield. The desirability index indicated that Nakhon Ratchasima had the highest desirability for 100 seed weight and yield, while Chai Nat showed the highest desirability for pods/plant.

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CHAPTER IV
Nutritional and Morphological Characterization of New Mungbean (*Vigna radiata* l.) Lines: Implications for Sprout Quality and Environmental Variation

4.1 Abstract

This study aimed to characterize the nutritional composition of seeds and sprouts derived from newly developed mungbean lines, and to assess their morphological characteristics as well as the influence of environmental variation on nutritional quality. The proximate nutritional composition of mungbean (*Vigna radiata* (L.) R. Wilczek) seeds and sprouts, including moisture, crude protein, crude fat, total ash, crude fiber, and carbohydrates, along with the morphological traits of sprouts (hypocotyl diameter, hypocotyl length, root length and output ratio) were evaluated across seven genotypes comprising two check varieties (CN3 and CN84-1) and five newly developed lines (P08, P12, P22, P24, and D5). Plants were cultivated under two contrasting environments: Phitsanulok during the rainy season (PNR) and Chai Nat during the dry season (CND). Significant effects of genotype (G), environment (E), and genotype-by-environment interaction (GEI) were detected for most nutritional traits, highlighting the complexity of nutritional variation. Seeds and sprouts of plants grown under PNR conditions exhibited higher fat and carbohydrate contents, whereas those from CND had elevated protein and ash levels. The check varieties CN3 and CN84-1 consistently showed high protein content, while lines P08 and P24 were superior in carbohydrate accumulation in both seeds and sprouts. Line P12 demonstrated high fiber content in seeds, whereas P22 was notable for fiber enrichment in sprouts. Moreover, P24 exhibited elevated ash content in sprouts. Crude fat levels in both seeds and sprouts showed only minor variation across genotypes and environments. Morphologically, root length was the only trait that discriminates among genotypes. Line D5 exhibited desirable sprout characteristics, including short roots and high output ratio, beneficial for commercial production. These findings confirm the potential of the new mungbean lines, with nutritional and morphological performance. Furthermore, environmental conditions significantly influenced nutrient accumulation, suggesting their importance in breeding programs targeting seed quality improvement.

4.2 Introduction

Mungbean (*Vigna radiata* (L.) R. Wilczek) is a legume crop of global importance, especially in tropical regions, where it is widely cultivated due to its nitrogen-fixing ability by symbiosis with *Rhizobium* sp. (Chaiyapan et al., 2023). This characteristic makes it a valuable soil-enriching crop, commonly used in crop rotations or as green manure to improve soil fertility. The crop is economically and nutritionally significant, serving as an essential source of food and feed. Mungbean seeds are also a rich source of macronutrients, comprising up to 67.10% carbohydrates and 32.60% protein content (Dahiya et al., 2015). Their high protein content not only contributes to their nutritional value but also makes mungbean an excellent candidate for hydrolysate production (Karami and Duangmal, 2024). Moreover, their compositional profile supports their versatility as raw material for a wide range of processed food products (Bhatty et al., 2000). Mungbean sprouts are highly regarded for their enhanced digestibility and enriched profile of phytonutrients, including vitamins C, A, B1, B2, and gamma-aminobutyric acid (GABA), which contribute to their functional food value (Randhir and Shetty, 2005). Both mungbean and black gram (*Vigna mungo*) are widely utilized in sprout productions due to their rapid germination and favorable nutritional profiles (Masari et al., 2016). Beyond their nutrient composition, mungbean consumption has been linked to a range of health-promoting effects, including antioxidant, anticancer, and anti-inflammatory activities (Hou et al., 2019). Furthermore, mungbean-derived products such as vermicelli, characterized by a low glycemic index (GI), have shown potential in supporting glycemic control and cardiovascular health, particularly in individuals with diabetes and related metabolic disorders (Yeap et al., 2012; Hou et al., 2019).

The nutrient composition of plants is governed by a complex interplay of genetic factors, environmental conditions, and their interactions (genotype-by-environment interactions; GEI), all of which critically influence both the quantity and quality of nutrients accumulated in seeds (Pregitzer et al., 2013; Cong et al., 2015; Asaro et al., 2016; Napier et al., 2023). Understanding these relationships is essential for advancing breeding strategies and agronomic practices aimed at improving the nutritional value of legumes. Nutrient accumulation is a dynamic process, particularly intensified during the reproductive stage, and individual nutritional traits often respond differently to genetic and environmental influences. For example, in maize, crude protein content is predominantly affected by environmental conditions, while lipid accumulation is more strongly determined by genetic factors (Cong et al., 2015). GEI is also a key contributor to phenotypic variation in agronomic and nutritional traits

(Napier et al., 2023). In legumes, traits such as protein concentration and anti-nutritional factors like phytate are significantly influenced by genotype, environment, and their interactions (Gore et al., 2021). Environmental variables, including light parameters, also play an important role in legume growth and nutrient accumulation (Vaidya and Stinchcombe, 2020). Additionally, factors such as soil properties and moisture levels impact the symbiotic relationship between legumes and *Rhizobium* spp., which is vital for nitrogen fixation and thus seed nutrient content (Yeremko et al., 2025).

Recently, several new mungbean lines with enhanced resistance to major fungal diseases powdery mildew (PM) caused by *Sphaerotheca phaseoli* and Cercospora leaf spot (CLS) caused by *Cercospora canescens* and high yield potential have been developed, including line D5 (Papan et al., 2024) and lines P08, P12, P22, and P24 (Pookhamsak et al., unpublished data). While these lines represent promising genetic resources for sustainable mungbean production, their nutritional profiles, particularly concerning seasonal or environmental variation, have not yet been investigated. In contrast, the nutritional composition of existing commercial mungbean varieties has been relatively well documented. For instance, variety CN3 contains approximately 58.37% carbohydrate, 24.05% protein, 1.03% fat, 4.50% fiber, and 4.12% ash, while CN36 and CN72 have reported carbohydrate contents of 56.17% and 56.35%, respectively (Jomsangawong et al., 2022). Moreover, Department of Agriculture (2018) also reported that the variety CN84-1 contained 54.85% carbohydrate. Overall, mungbean seeds typically contain 50.00–70.00% carbohydrates and 20.00-30.00% protein, although these values can vary depending on genotypes and environmental conditions (Somta et al., 2022). Despite this existing knowledge, it remains unclear whether the improved disease-resistant lines also possess favorable nutritional characteristics comparable to or exceeding those of established varieties.

The present study aimed to characterize the nutritional compositions of seeds and sprouts derived from these newly developed mungbean lines and to assess the influence of environmental variation on their nutritional quality. The results may offer valuable insights for the food industry in selecting mungbean lines with superior nutritional attributes and provide a foundation for breeding programs targeting enhanced nutritional value in mungbean.

4.3 Materials and Methods

4.3.1 Plant materials

Seeds of seven mungbean genotypes (CN3, CN84-1, P08, P12, P22, P24, and D5) were collected from the regional yield trials conducted at two locations in Thailand during the 2023–2024 growing seasons: the Phitsanulok Seed Research and Development Center (Phitsanulok province) during the rainy season (PNR), and the Chai Nat Field Crops Research Center (Chai Nat province) during the dry season (CND). Standard agronomic practices were followed during seed sowing and crop management to ensure uniform growth and development across genotypes. Plants were grown to physiological maturity, defined as 70 days after planting (DAP), at which point more than 80% of the pods had reached maturity. The experimental design consisted of plots measuring 4 × 6 m per replication, arranged in a randomized complete block design (RCBD) with three replications. Each plot contained 8 rows with a row spacing of 0.5 m and intra-row plant spacing of 0.2 m, totaling 30 plants per row. Supplemental irrigation was provided weekly via sprinkler systems to maintain optimal soil moisture. The soil characteristics and environmental conditions of these sites were presented in Table A.1 and Figure A.8 and Figure A.11, respectively. Additionally, details of the mungbean genotypes along with their specific characteristics were provided in Table 4.1.

Table 4.1 The information and characteristics of seven mungbean genotypes.

Genotypes	Pedigree	Special features	Descriptions
CN3	Selection from mutated CN36	Large seed, high yield, uniform maturity	Thai certified varieties developed at Chai Nat Field Crops Research Center, Thailand
CN84-1	Selection from mutated CN36	Large seed, high yield, high percentage of carbohydrate	
P08	Selected from backcrossing between CN84-1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)] (V4718, V4758, and V4785 were originated from India) (Pookhamsak et al., unpublished data)	Large seed, uniform maturity, moderate resistance to PM[1/] and CLS[2/]	New resistant lines
P12		High yield, rather drought resistance, high resistance to PM, moderate resistance to CLS	
P22		High yield, uniform maturity, abundant pods, moderate resistance to PM and CLS	
P24		Large seed, uniform maturity, moderate resistance to PM and CLS	
D5	Selected from backcrossing between SUT1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)] (SUT1 Variety developed at SUT, Thailand) (Papan et al., 2024)	Uniform maturity, moderate resistance to PM, abundant pods, pods borne above the canopy, and trichomeless	

[1/] powdery mildew, [2/] Cercospora leaf spot

4.3.2 Samples preparation

Seed samples were prepared by removing impurities, drying them at 45°C for 24 hrs, and then grinding them into a fine powder. The powdered samples were subsequently stored at -20°C to preserve their integrity for nutritional analysis. Sprout samples were prepared using a modified method based on Wang et al. (2021). Mungbean seeds were washed with sterile distilled water and soaked in warm water at 40°C for 30 min. After soaking, the seeds were kept in dark conditions at room temperature for 8 hrs. Following the soaking period, the seeds were rinsed with sterile distilled water and germinated using the between paper (BP) method. The germination process was conducted in a dark, temperature - controlled chamber at 25°C, with the seeds watered twice daily using sterile distilled water for 72 hrs. After germination, the sprouts were dried at 45°C for 24 hrs and ground into a fine powder. The powdered samples were then stored at -20°C for subsequent nutritional analysis.

4.3.3 Proximate analysis

The contents of crude protein, crude fat, crude fiber, and total ash were analyzed following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2019). The total carbohydrate content was calculated by deducting the sum of these proximate components from the total. The parameter was measured in triplicates.

4.3.3.1 Moisture content

The measurement of moisture content, petri dish was placed in a hot air oven at 105°C for 2 hrs. Then, it was placed in a desiccator for 30 min until the temperature of the petri dishes equaled room temperature. Next, the petri dish was weighed using an analytical balance to four decimal places, 2-3 g of sample was placed into the petri dish with the cover slightly ajar and returned to the hot air oven at 105°C for 2 hrs or until dried. After drying, the petri dish containing the sample was allowed to cool in the desiccator for 30 min until the temperature of the petri dishes equaled room temperature. Then, the petri dish with the dried sample was weighed using the analytical balance to four decimal places. The drying process at 105°C was repeated for 1 hr or until the weight difference between two consecutive weighing was no different than 3 mg. The moisture content was calculated using the following equation:

$$\text{Moisture Content (\%FW)} = \frac{(W2 - W3)}{(W2 - W1)} \text{x } 100$$

When: W1 = Weight of the empty petri dish (g)

W2 = Weight of the petri dish with sample before drying (g)

W3 = Weight of the petri dish with the dried sample (g)

4.3.3.2 Crude protein content

The total protein content was determined using the Kjeldahl method. Approximately 0.5-1.0 g of sample was placed into a digestion tube, followed by the addition of 3 g of accelerating agent (a mixture of copper sulfate and potassium sulfate in a 1:10 ratio) and 20 mL of concentrated H_2SO_4. A blank sample was prepared by omitting the sample. Digestion was carried out in a Digestion System at 380 °C for 100 min until a clear blue solution was obtained. After digestion, the tubes were removed and cooled for about 20 min before proceeding to protein distillation using a UDK 149 Automatic Kjeldahl Distillation Unit (VELP Scientifica, Italy). For distillation, 20 mL of 4% boric acid (H_3BO_3) solution mixed with 2–3 drops of mixed indicator (methyl red and bromocresol green at a 1:1 ratio) was prepared in an Erlenmeyer flask for each sample. The distillation was performed with the following program: 45 mL H_2O, 60 mL of 40% NaOH, and a distillation time of 4 min. During distillation, ammonia gas (NH_3) released from the sample reacted with NaOH and condensed into the boric acid solution, causing a color change from pink to green. The resulting solution was then titrated with 0.1 N HCl until the endpoint, indicated by a color change back to pink, was reached. The volume of HCl used was recorded.

$$\text{Crude protein content (\%DB)} = \frac{(\text{A} - \text{B}) \text{ x N x } 1.4007 \text{ x F}}{\text{W}_\text{t}}$$

When: A = Volume of HCl used for sample titration (ml).

B = Volume of HCl used for blank titration (ml).

W_t = Weight of the sample (g).

N = Concentration of the HCl (N).

F = Factor (specific to mungbean, which is 6.25).

4.3.3.3 Crude fiber content

Crude fiber analysis was performed using the Fibertec 2010 automatic analyzer (Foss Tecator, Denmark). Approximately 0.5-1.0 g of sample was placed into a filtered crucible. Then, 150 mL of 1.25% hot H_2SO_4 was added to each tube, along with 3 drops of n-octanol antifoaming agent to minimize foaming. The sample was boiled for 45 min. After boiling, the mixture was filtered until dry by activating air

suction, followed by releasing the acid from the sample through valve opening. The samples were washed three times with hot H_2O and filtered to dryness. Next, 150 mL of 1.25% NaOH solution was added, again with 3 drops of n-octanol antifoam, and the sample was boiled for 45 min. After boiling, the sample was filtered and washed three times with hot H_2O. Subsequently, the sample was rinsed with acetone (C_3H_6O) 3 times, each with 25 mL. The filtered crucible containing the residue was removed from the extractor and dried in an oven at 105 ℃ for 2 hrs, then cooled in a desiccator for 30 min. Following drying, the crucible was incinerated in a muffle furnace at 500 ℃ for 2 hrs. The furnace was allowed to cool until the temperature fell below 250 ℃ before opening; the furnace must remain closed for at least 3 hrs prior to sample removal or until the temperature is below 200 ℃. Finally, the crucible was cooled again in a desiccator for 30 min before further analysis.

$$\text{Crude fiber content (\%DB)} = \frac{W_2 - W_3}{W_1} \text{ x } 100$$

When: W_1 = Weight of sample (g).

W_2 = Weight of sample + crucible after oven dry (g).

W_3 = Weight of sample + crucible after burn in furnace (g).

4.3.3.4 Ash content

Analysis of ash content started with weighing the constant weight in hot air oven at 105℃ for 2 hrs, 2-3 g of the dried sample put into a crucible. The samples were incinerated on a hot plate until smokeless to form a lump. Subsequently, incinerate in a furnace at 500℃ for 3 hrs or until a light gray or uniform white ash is obtained. After removal from the furnace, it is allowed to cool to room temperature in a desiccator for 30 min. The weight was recorded, and the ash content calculated according to the following formula:

$$\text{Ash content (\%DB)} = \frac{W_2 - W_1}{S} \text{ x } 100$$

When: W_1 = Weight of the crucible.

W_2 = Weight of the crucible and sample after incineration.

S = Weight of the sample.

4.3.3.5 Fat content

The analysis of fat content was conducted following the Soxhlet extraction method using the Soxtec™ 2050 Auto Fat Extraction System. Initially, the extraction beaker was weighed and dried at 105°C for 1 hr to achieve a constant weight, then cooled in a desiccator. Approximately 1–1.5 g of the sample was weighed and put into the filter paper, then folded and inserted into a cellulose thimble. The cellulose thimble was positioned for extraction and insertion of the extract. Subsequently, 80 mL of petroleum was added to the extraction beaker and put it into the positioned for extraction. The heating program was set with the following parameters: extraction temperature at 180°C, extraction phase: 60 min, rinsing phase of 90 min, and drying phase for 15 min. Upon completion of the program, the extraction beaker containing the extracted fat was removed and dried in a hot air oven at 105°C for 2 hrs. After drying, the beaker was cooled to room temperature in a desiccator for 30 min. Finally, the extraction beaker with the extracted fat was weighed to determine the fat content.

$$\text{Crude fat content (\%DB)} = \frac{\text{B} - \text{A}}{\text{W}} \text{ x } 100$$

When: W = Weight of the sample.

A = Constant weight of extraction beaker.

B = Weight of extraction beaker and extracted fat.

4.3.3.6 Carbohydrate Content

To analyze the carbohydrate content, using the following method outlined by Hailu (2018). Carbohydrate content calculated by subtracting the percentages of moisture, protein, fat, fiber, and ash according to the following formula:

$$\text{Carbohydrate content (\%DB)} = 100 - \text{Moisture} + \text{Protein} + \text{Fiber} + \text{Fat} + \text{Ash}$$

4.3.4 Morphological characteristics of mungbean sprouts

Data collection for hypocotyl diameter, hypocotyl length, and root length: Collected data from 20 sprouts/genotype/replication, with 6 replicates, and measured the results using a ruler and vernier caliper. Hypocotyl diameter (mm): Measure at the midpoint of the hypocotyl once per plant using a vernier caliper. Hypocotyl length (cm): Measure from the base of the hypocotyl to the cotyledon node using a ruler. Root length (cm): Measure from the base of the hypocotyl to the longest tip of the

root. Output ratio: Calculated based on data collected from 6 replicates for each genotype follow by modified method from (Wang et al., 2021), computed as output ratio = fresh weight of sprout (g) / weight of mungbean seeds (g)

A completely randomized design (CRD) was employed for the experimental setup. Data were analysed using the analysis of variance (ANOVA). A mean comparison was conducted using Duncan's New Multiple Range Test (DMRT) to assess the nutrient composition across mungbean genotypes and the morphological characteristics of mungbean sprouts. Additionally, a combined analysis was performed to investigate the effects of genotype, environment and interactions between genotype and the environment. Statistical analyses were conducted using SPSS version 16.0 (Levesque, 2007).

4.4 Results

The nutritional composition, including moisture, crude protein, crude fat, total ash, crude fiber, and carbohydrate contents, was evaluated in seeds and sprouts of seven mungbean genotypes. These included two certified check varieties (CN3 and CN84-1) and five newly developed lines (P08, P12, P22, P24, and D5). The genotypes were cultivated under two contrasting environmental conditions: Phitsanulok rainy season (PNR) and Chai Nat dry season (CND). Combined analysis of variance revealed that genotype (G), environment (E), and genotype-by-environment interaction (GEI) had significant effects ($P \leq 0.01$ or $P \leq 0.05$) on nearly all measured nutritional traits in both seeds and sprouts, though the magnitude and patterns of these effects varied among traits (Table 4.2 and Table 4.3). The significant GEI underscores the differential response of mungbean genotypes to varying environmental conditions. Overall, the nutritional composition patterns were consistent between seeds and sprouts, with CND-grown samples exhibiting significantly higher protein and ash contents, while those from PNR showed elevated fat and carbohydrate levels.

4.4.1 Proximate nutritional composition of mungbean seeds; genotypic, environmental, and genotype-by-environment interaction effects

The nutritional composition of mungbean seeds is summarized in Table 4.2. Moisture content was strongly influenced by G, E, and GEI. Seeds produced under the PNR environment exhibited significantly higher moisture content (11.19%) than those from the CND environment (8.63%), reflecting environmental differences in post-harvest humidity. The significant GEI effect was evident in the differential response of genotypes across environments. Under PNR, all new lines and CN84-1 (11.09-11.44%) had higher moisture content than CN3 (10.51%). Among them, P12 and P22 were comparable to CN84-1, while other new lines showed 1.01- to 1.03-fold lower values. In contrast, under CND, genotypic differences were more distinct: P12 exhibited the lowest moisture content (8.06%) and D5 the highest (9.36%). Notably, P12 and P22 showed significantly lower (1.03- to 1.08-fold) moisture content than both check varieties in CND. When averaged across environments, CN3 maintained the lowest overall moisture level (9.61%), while D5 exhibited the highest (10.28%), indicating combined G and GEI effects.

Crude protein content was significantly affected by G, E, and GEI. Seeds grown in the CND environment accumulated higher protein levels (25.64%) than those in PNR (24.01%). Across genotypes, CN3 and CN84-1 had the highest average protein contents (25.87% and 25.83%, respectively). All newly developed lines exhibited 1.03- to 1.09-fold lower protein contents than these checks. GEI effects were

apparent in shifts in genotype ranking between environments. For example, line D5 achieved 26.79% protein under CND, comparable to the checks, but displayed 1.07- to 1.08-fold lower content under PNR. Among new lines, P12 ranked highest in PNR (24.14%), while D5 performed best in CND, highlighting the genotype-specific responsiveness to environmental conditions.

As with crude protein content, crude fat content was significantly affected by all three sources of variation. But in contrast to protein, seeds grown under PNR conditions accumulated more fat (0.88%) than those grown under CND (0.54%). Significant GEI was evident, particularly in the divergent performance of genotypes across environments. Under PNR, P08 had the lowest fat content (0.49%), comparable to both check varieties. In CND, D5 recorded the lowest value (0.42%), and most new lines, except P24, exhibited 1.11- to 1.50-fold lower fat content than CN3 (0.63%). However, when averaged across both environments, P12 and P22 showed significantly higher crude fat contents (up to 1.53-fold) than the check varieties, while P08 remained comparable to CN3 and CN84-1, revealing a strong G effect modulated by E.

Total ash content was significantly influenced by E and GEI, though genotypic effects were not significant when averaged across environments. Under CND (overall means 4.67%), genotypic differences became evident. CN3 recorded the highest ash content (5.07%), while P24 had the lowest (4.26%). Nevertheless, all new lines, including P24, were statistically similar to CN84-1, indicating limited genotypic variation. Under PNR, differences among genotypes were less pronounced. These findings suggest that ash accumulation is more responsive to environmental variation and specific genotype-environment combinations than to genotype alone.

In contrast to most other nutritional traits, crude fiber content was not significantly influenced by E. However, both G and GEI had highly significant effects. Mean fiber content was similar between PNR (4.20%) and CND (4.40%). Line P12 demonstrated superior performance, with the highest average fiber content across environments (5.47%), significantly exceeding all other genotypes by 1.17- to 1.48-fold. Conversely, P08 had the lowest average fiber level (3.70%). When compared to the check varieties, lines P08 and D5 had similar crude fiber contents to CN3, whereas P22 and P24 were comparable to CN84-1. Fiber accumulation patterns varied across environments: under PNR, P12 again led (6.24%), while in CND, P24 had the highest value (4.84%). Notably, under CND, all new lines except P08 exhibited significantly higher fiber contents (4.19-4.84%) than the check varieties, with increases ranging from 1.01- to 1.19-fold, further illustrating GEI-driven variation.

Carbohydrate levels were significantly affected by G, E, and GEI. Seeds from the PNR environment had higher carbohydrate content (67.03%) than those from CND (64.74%). Lines P08 and P24 consistently exhibited the highest average carbohydrate contents across environments (67.46% and 67.09%, respectively), surpassing the check varieties CN3 and CN84-1 by 1.03- to 1.04-fold. Whereas the remaining new lines exhibited either higher or comparable values relative to the checks. Under PNR, P08 and P24 reached 1.02- to 1.06-fold higher carbohydrate levels than the check varieties, while lines D5 and P12 showed values comparable to CN84-1. In CND, P08 again led (65.97%), followed by P12, P22, and P24, all of which significantly exceeded the checks by 1.01- to 1.04-fold. These findings reflect both stable high-performing genotypes and environment-specific responses.

4.4.2 Proximate nutritional composition of mungbean sprouts; genotypic, environmental, and genotype-by-environment interaction effects

All proximate nutritional composition of mungbean sprouts was significantly affected by G, E, and GEI, as confirmed by a combined analysis of variance (Table 4). Each nutritional trait exhibited distinct patterns of variation influenced by these three factors. Environmental influence was particularly pronounced in moisture content. Sprouts cultivated under the PNR environment exhibited markedly lower moisture levels (81.58%) compared to those from CND (87.30%). Minimal genotypic variation was observed under CND. However, under PNR, genotypic differences became evident; check variety CN84-1 exhibited the highest moisture content (84.06%), significantly surpassing all new lines, which exhibited 1.03- to 1.05-fold lower moisture content. Across environments, CN84-1 and CN3 maintained the highest average moisture levels (85.37% and 85.07%, respectively), whereas P08 consistently showed lower moisture content, highlighting both G and GEI effects.

Crude protein levels were also significantly shaped by G, E, and GEI. Sprouts from CND-grown seeds contained higher protein content (31.51%) than those from PNR (27.97%), underscoring a strong environmental impact. CN84-1 had the highest average protein content across environments (31.00%), significantly outperforming all new lines by 1.02- to 1.11-fold. However, most of them showed comparable protein levels to CN3 except P24. Notably, GEI was evident: under PNR, CN84-1 again had the highest protein level (29.65%), significantly surpassing all other genotypes, followed by D5 and CN3. Whereas, in CND, protein contents among genotypes were more uniform, ranging from 29.83% to 32.35%. Lines P08, P12, and D5 were statistically similar to the check varieties, while P24 consistently exhibited the lowest protein content across both environments.

Crude fat content demonstrated strong G, E, and GEI effects. Under PNR, significant genotypic differences emerged, with lines P08, P24, and D5 showing substantially lower fat levels than CN3 (by 1.27- to 1.40-fold). In contrast, non-significant differences were detected under CND, where fat content ranged narrowly (0.86–1.02%). However, when averaged across environments, P12 and P22 stood out with significantly higher fat contents (1.38% and 1.42%, respectively), while CN3, CN84-1, P08, P24, and D5 had lower values, illustrating combined G and GEI effects.

Total ash content in mungbean sprouts was significantly influenced by all three factors. Under PNR, line P24 exhibited the highest ash content (5.25%), exceeding other genotypes by 1.16- to 1.47-fold. Most other new lines, excluding P08, were statistically similar to CN3 and CN84-1. Under CND, new lines (except P24) had higher ash contents (1.05- to 1.12-fold) than CN84-1, while P24 (4.34%) aligned with both checks. Across environments, P24 maintained the highest ash content (4.80%), outperforming the other genotypes by 1.07- to 1.15-fold, demonstrating strong genotypic superiority as well as GEI.

In contrast to the crude fiber in seeds, the fiber content in sprouts was significantly influenced by E, with sprouts under PNR showing significantly higher levels (3.99%) than those under CND (2.74%). Within the PNR environment, line P22 showed the greatest fiber accumulation, surpassing other genotypes by 1.37- to 4.59-fold, followed by P12. Under CND, fiber levels were more uniform, with P12, P22, P24, and D5 remaining comparable to CN3 and CN84-1. When averaged across both environments, P22 had the highest fiber content (4.65%), outperforming other genotypes by 1.21- to 2.45-fold, reflecting its genotypic potential.

Carbohydrate contents varied significantly with G, E, and GEI. The PNR environment promoted higher carbohydrate accumulation (62.44%) compared to CND (60.39%). Across environments, lines P08 and P24 recorded the highest carbohydrate levels (63.55% and 63.17%, respectively), outperforming others by 1.04- to 1.06-fold. Strong GEI effects were evident: under PNR, P08 achieved the highest content (67.09%), followed by P24, both exceeding other genotypes by 1.03- to 1.14-fold. Lines D5 and P12 remained comparable to the check varieties. In contrast, under CND, P24 led with 62.24%, significantly higher (1.02- to 1.04-fold) than most genotypes except P22, indicating its stable performance across environments. The remaining new mungbean lines had carbohydrate contents comparable to the check varieties CN3 and CN84-1.

Table 4.2 Proximate composition of seeds from seven mungbean genotypes (%DB[1/]).

Genotypes	Moisture			Crude protein			Crude fat			Total ash			Crude fiber			Carbohydrates		
	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average
CN3	10.51 e[2/]	8.72 b	9.61 e	25.05 a	26.70 a	25.87 a	0.59 bc	0.63 a	0.61 bc	3.65	5.07 a	4.36	3.30 c	4.17 b	3.74 c	67.42 b	63.42 d	65.42 bc
CN84-1	11.44 a	8.51 c	9.97 b	25.22 a	26.44 a	25.83 a	0.66 bc	0.48 de	0.57 bc	4.27	4.68 abc	4.47	4.67 b	4.08 b	4.38 b	65.19 cd	64.32 c	64.75 c
P08	11.31 b	8.69 bc	10.00 b	23.72 bc	24.63 c	24.18 d	0.49 c	0.52 cd	0.50 c	3.58	4.72 ab	4.15	3.25 c	4.16 b	3.70 c	68.96 a	65.97 a	67.46 a
P12	11.40 a	8.06 e	9.73 d	24.14 b	24.76 bc	24.45 cd	1.13 a	0.57 bc	0.85 a	4.00	4.80 ab	4.40	6.24 a	4.69 a	5.47 a	64.50 d	65.18 b	64.84 c
P22	11.41 a	8.29 d	9.85 c	24.10 b	25.04 bc	24.57 c	1.19 a	0.55 bc	0.87 a	4.04	4.42 bc	4.22	4.69 b	4.68 a	4.68 b	65.98 c	65.31 b	65.65 b
P24	11.09 d	8.80 b	9.94 bc	22.40 d	25.14 b	23.77 e	0.86 ab	0.59 ab	0.73 ab	3.80	4.26 c	4.02	3.93 bc	4.84 a	4.39 b	69.01 a	65.17 b	67.09 a
D5	11.20 c	9.36 a	10.28 a	23.41 c	26.79 a	25.1 b	1.22 a	0.42 e	0.82 a	3.90	4.75 ab	4.32	3.32 c	4.19 a	3.76 c	68.15 ab	63.84 cd	65.99 b
Mean	11.19	8.63	9.91	24.01	25.64	24.82	0.88	0.54	0.71	3.89	4.67	4.28	4.20	4.40	4.30	67.03	64.74	65.89
G[3/]	**	**	**	**	**	**	**	**	**	ns	*	ns	**	**	**	**	**	**
E			**			**			**			**			ns			**
G x E			**			**			**			*			**			**
C.V. (%)			0.90			1.19			18.98			6.27			9.70			0.88

[1/] DB = dry basis, [2/] Means in the same column with different letters are significantly different based on Duncan's New Multiple Range Test (DMRT), and [3/] * = Significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$. Abbreviation: PNR= Phitsanulok (rainy season), CND = Chai Nat (dry season), G= Genotype, and E = Environment.

Table 4.3 Proximate composition of sprouts from seven mungbean genotypes (%DB[1/]).

Genotypes	Moisture			Crude protein			Crude fat			Total ash			Crude fiber			Carbohydrates		
	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average	PNR	CND	Average
CN3	82.20 ab[2/]	87.93	85.07 a	28.48 b	31.70 ab	30.09 bc	1.43 b	0.86	1.15 b	4.12 bc	4.53 bcd	4.33 b	4.37 c	2.98 a	3.68 bc	61.59 cd	59.92 b	60.76 bc
CN84-1	84.06 a	86.68	85.37 a	29.65 a	32.35 a	31.00 a	1.07 c	0.97	1.02 c	4.50 b	4.26 e	4.38 b	3.05 e	2.94 a	3.00 d	61.73 cd	59.48 b	60.60 bc
P08	79.83 c	86.76	83.29 b	26.86 d	32.05 a	29.45 c	1.02 c	0.86	0.94 c	3.57 c	4.76 a	4.17 b	1.46 f	2.33 b	1.90 e	67.09 a	60.00 b	63.55 a
P12	80.82 bc	88.39	84.60 ab	27.70 c	31.23 ab	29.46 c	1.84 a	0.91	1.38 a	4.17 bc	4.74 ab	4.45 b	4.98 b	2.72 ab	3.85 b	61.31 d	60.40 b	60.86 bc
P22	81.27 bc	87.86	84.57 ab	28.42 b	30.75 bc	29.59 c	1.82 a	1.01	1.42 a	4.09 bc	4.60 abc	4.35 b	6.71 a	2.60 ab	4.65 a	58.96 e	61.04 ab	60.00 c
P24	81.53 bc	86.57	84.05 ab	26.13 e	29.83 c	27.98 d	1.03 c	1.02	1.02 c	5.25 a	4.34 de	4.80 a	3.51 de	2.57 ab	3.04 d	64.09 b	62.24 a	63.17 a
D5	81.34 bc	86.92	84.13 ab	28.57 b	31.94 ab	30.25 b	1.13 c	0.87	1.00 c	4.12 bc	4.49 cd	4.31 b	3.87 d	3.02 a	3.44 c	62.31 c	59.69 b	61.00 b
Mean	81.58	87.30	84.43	27.97	31.41	29.68	1.33	0.93	1.31	4.26	4.53	4.39	3.99	2.74	3.36	62.44	60.39	61.41
G[3/]	*	ns	*	**	**	**	**	ns	**	**	**	**	**	**	**	**	*	**
E			**			**			**			**			**			**
G x E			**			**			**			**			**			**
C.V. (%)			1.23			1.76			8.39			5.48			7.75			1.16

[1/] DB = dry basis, [2/] Means in the same column with different letters are significantly different based on Duncan's New Multiple Range Test (DMRT), and [3/] * = Significant at P ≤ 0.05; ** = highly significant at P ≤ 0.01, and ns=non-significant at P > 0.05. Abbreviation: PNR= Phitsanulok (rainy season), CND = Chai Nat (dry season), G= Genotype, and E = Environment.

4.4.3 Morphological traits of mungbean sprouts

The experimental results on the morphological characteristics of seven mungbean genotypes are summarized in Table 4.4. A significant difference was observed in root length, while hypocotyl diameter, hypocotyl length, and output ratio of mungbean sprouts showed non-statistically significant differences. The average hypocotyl diameter was 2.23 mm, with similar values observed across genotypes. Line P22 exhibited the largest diameter (2.31 mm), while D5 had the smallest (2.16 mm). In terms of hypocotyl length, CN84-1 recorded the longest hypocotyl (5.41 cm), followed by P24 and D5 (4.80 cm). The shortest hypocotyl was observed in line P12 (3.88 cm). Root length showed significant variation among genotypes. Genotypes CN84-1 and P24 produced longer roots (6.64 and 6.60 cm, respectively), 1.24- to 1.27-fold and 1.23- to 1.26-fold significantly higher than CN3 and D5, respectively. Lines P08, P12, and P22 had similar root lengths, with non-significant differences compared to each other and other genotypes. Non-statistically significant difference observed in the output ratio among the genotypes. The average output ratio was 4.27, which CN84-1 and D5 tending to produce higher ratios (4.58 and 4.52, respectively), while variety CN3 (3.77) tended to have a lower output ratio than the other genotypes.

Table 4.4 Morphological characteristics of seven mungbean genotypes sprouts.

Genotypes	Hypocotyl diameter (mm)	Hypocotyl length (cm)	Root length (cm)	Output ratio
CN3	2.24 ± 0.05	4.06 ± 0.36	5.23 ± 0.39 b	3.77 ± 0.10
CN84-1	2.24 ± 0.05	5.41 ± 0.47	6.64 ± 0.35 a	4.58 ± 0.29
P08	2.27 ± 0.06	4.64 ± 0.41	6.20 ± 0.20 ab	4.36 ± 0.48
P12	2.23 ± 0.09	3.88 ± 0.26	5.75 ± 0.32 ab	4.09 ± 0.18
P22	2.31 ± 0.06	4.33 ± 0.32	5.95 ± 0.44 ab	4.25 ± 0.26
P24	2.21 ± 0.04	4.80 ± 0.45	6.60 ± 0.24 a	4.34 ± 0.27
D5	2.16 ± 0.06	4.80 ± 0.48	5.37 ± 0.12 b	4.52 ± 0.30
Mean	2.23	4.56	5.90	4.27
F-Test	ns	ns	*	ns
C.V.%	5.96	19.34	11.69	16.69

* = Significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

4.5 Discussion

4.5.1 Genotypic, environmental, and genotype-by-environment interaction effects on nutritional composition

The proximate nutritional composition of mungbean seeds and sprouts was significantly influenced by G, E, and GEI, as confirmed by combined analysis of variance (Tables 4.2 and 4.3). Most measured traits, moisture, crude protein, crude fat, total ash, crude fiber, and carbohydrate, exhibited considerable variability, emphasizing the combined role of genetic background and environmental conditions in shaping nutritional outcomes.

Moisture content in seeds and sprouts displayed strong environmental sensitivity. Seeds harvested from the rainy-season site (PNR) exhibited significantly higher moisture content than those from the dry-season site (CND), likely due to higher relative humidity and prolonged field exposure before harvest. Seed moisture content ranged from 8.06-11.44%, within the recommended 8.00-12.00% range for prolonged storage and viability (Irfan et al., 2022). In contrast, mungbean sprouts showed higher moisture levels under CND conditions. Elevated moisture in sprouts may reduce shelf life by promoting microbial proliferation, increased respiration, or tissue degradation (Chávez-García et al., 2023). Therefore, PNR-grown sprouts, which had relatively lower moisture content, may offer better postharvest stability.

Crude protein content was largely determined by G but strongly modulated by E. In seeds, check varieties CN3 and CN84-1 consistently showed higher protein levels, highlighting their superior adaptability and nitrogen assimilation capacity. Among the new lines, D5 (with SUT1 as the recurrent parent) exhibited the highest protein content under CND, suggesting a favorable response to nitrogen-enriched soils. Protein content was significantly higher in CND than in PNR, likely reflecting the more fertile clay loam soil in CND, with higher organic matter and nitrogen levels (Table 1). These findings align with Malik et al. (2003), who demonstrated that nitrogen enrichment enhances mungbean seed protein. Adequate nitrogen promotes amino acid synthesis and protein accumulation, while deficiencies limit protein deposition (Uchida, 2000; Azadi et al., 2013; Ge et al., 2024). In sprouts, the new lines, P08, P12, P22, and P24, showed lower protein levels than CN84-1, mirroring seed trends. The reduced protein content in resistant lines may be due to reallocation of metabolic resources toward defense-related proteins such as β-1,3-glucanase and peroxidase, particularly in lines bred for CLS and PM resistance (Inthaisong et al.,

2025). Nevertheless, seed and sprout protein contents observed (22.40–32.35%) were slightly higher than many other common legumes (Li et al., 2010; Gunathilake et al., 2016; Yi-Shen et al., 2018; Idris et al., 2025) and comparable to kidney beans (20.00–30.00%) (Shevkani et al., 2015), cowpea (22.80-25.20%) (Gunathilake et al., 2016), chickpea (19.30–21.00%) (Dahiya et al., 2013), affirming mungbean's role as a protein-dense legume (Anwar et al., 2007).

Crude fat content in seeds and sprouts showed genotypic variation, although the absolute differences were small. Lines P12 and P22 consistently exhibited higher fat levels than other genotypes across both environments. Fat content in seeds ranged from 0.42–1.22%, and in sprouts from 0.86–1.84%. Despite statistical significance, these narrow ranges suggest limited genetic divergence for this trait. P08 consistently exhibited the lowest crude fat in both seeds and sprouts, aligning with consumer preference for low-fat plant-based foods.

Total ash content, representing the mineral composition of plant tissues, varied modestly among genotypes. In seeds, no consistent differences were found between new lines and check varieties across environments. However, in sprouts, line P24 exhibited significantly higher average ash content across both environments than other genotypes, suggesting greater mineral deposition efficiency. Ash consists primarily of essential minerals like calcium, potassium, and sodium (Shokunbi et al., 2023), and high values are nutritionally advantageous. However, overly high ash levels may indicate contamination from soil particles or processing agents (Marshall, 2010), warranting further quality control.

Crude fiber content was primarily governed by G, with notable interactions with the environment. In seeds, P12 displayed the highest average fiber levels, while P22 and P24 had values comparable to CN84-1. P08 and D5 exhibited lower fiber levels, similar to CN3. In sprouts, line P22 showed the highest average fiber content across both environments. High fiber content enhances food texture and contributes to satiety. Cellulose and hemicellulose, key components of crude fiber, may improve crispness and moisture balance in food products (Zdunek et al., 2014; Trandel-Hayse, 2023), offering functional food advantages for high fiber mungbean lines.

Carbohydrate content was strongly influenced by G and E. In both seeds and sprouts, lines P08 and P24 consistently exhibited the highest average carbohydrate levels across environments. Seeds from PNR showed higher carbohydrate content than those from CND, possibly due to altered starch accumulation under high

humidity during pod filling. Carbohydrate accumulation is known to be sensitive to environmental stress, carbohydrate metabolism and accumulation are highly dynamic and respond to various abiotic stresses. These stresses can increase or decrease the concentration of soluble sugars and starch in different plant organs, depending on the type of stress and plant genotype (Rosa et al., 2009; Sehgal et al., 2018), and these findings affirm the role of E and GEI in shaping this trait. Mungbean seeds contained 50.00–60.00% carbohydrates, higher than soybeans (~35%) (Hou et al., 2019), highlighting their potential in starch-based processing. However, carbohydrate content in sprouts was generally lower than in seeds, likely due to metabolic conversion during sprouting. This reduction benefits individuals managing blood sugar levels, while higher carbohydrate sprouts may support energy-intensive needs, such as athletes. Sprouting also enhances antioxidant concentrations and nutrient bioavailability (Tang et al., 2014). Carbohydrate levels are also influenced by the composition of other macronutrients, often increasing when protein levels are low, as seen in the newly developed lines. Specifically, the new lines P08 and P24 demonstrated high carbohydrate content, but conversely, exhibited relatively low protein levels. On the other hand, varieties CN3 and CN84-1, showed a distinct profile with higher protein content but lower carbohydrate levels. This pattern aligns with previous research indicating that traditional varieties tend to have higher protein concentrations, which may be more suitable for nutritional applications where protein is the main focus.

4.5.2 Integrated nutritional response and breeding implications

The combined analysis revealed that GEI significantly influenced all evaluated nutritional traits in both mungbean seeds and sprouts. While genotypes emerged as the predominant factor determining the compositional quality of most traits. Environmental factors, including rainfall distribution, temperature regimes, soil fertility, and nutrient availability, acted as critical modulators of nutrient expression. For instance, the CND environment, characterized by higher nitrogen availability, elevated levels of organic matter and potassium, and relatively stable growing conditions, promoted enhanced accumulation of protein and ash. In contrast, the PNR environment, which was more humid, favored higher moisture and carbohydrate contents, likely due to increased water retention and environmental induced carbohydrate synthesis. The presence of significant GEI effects also indicated that genotypic performance varied across environments, as evidenced by the differing

rank orders of nutritional traits. This variability underscores the importance of GEI in guiding breeding efforts and cultivation practices to optimize specific nutritional traits.

Notably, certain genotypes exhibited distinct nutritional advantages: P08 and P24 consistently accumulated higher carbohydrate levels; P12 and P22 were superior in seed and sprout fiber content, respectively; and D5 demonstrated enhanced protein content under favorable environmental conditions. These genotype-specific strengths reflect their potential utility in targeted nutritional improvement and industrial applications. Moreover, the adaptability of these lines across divergent environments highlights their promise for the development of climate-resilient, nutrient-rich mungbean varieties.

4.5.3 Root length and drought adaptation

The morphological traits of seven mungbean genotypes revealed significant variation in root length, while no significant differences were observed in hypocotyl diameter, hypocotyl length, and output ratio. Genotypes CN84-1 and P24 exhibited greater root length. Root architecture is a critical determinant of early legume seedling performance, particularly under drought stress (Wang et al., 2024; Afonso et al., 2025). Drought-tolerant legume genotypes typically possess increased total root length, higher root density, and deeper root penetration, facilitating more efficient water uptake during soil moisture deficit periods (Khatun et al., 2021; Wang et al., 2024). Especially during the early germination stage, mungbean genotypes with longer roots tend to exhibit better drought tolerance. Several studies have confirmed that mungbean varieties with more developed root systems demonstrate greater drought tolerance and improved physiological traits under stress, including higher relative water content, enhanced membrane stability, and superior yield performance (Bangar et al., 2019; Khan et al., 2025). Nonetheless, additional research is necessary to validate these results.

4.5.4 Hypocotyl dynamics and seedling vigor

Although differences in hypocotyl length were not statistically significant, a trend toward longer hypocotyls was observed, which may correlate with more robust seedling establishment. This observation aligns with the findings of Yu and Huang (2017), who reported that hypocotyl length changes markedly during early plant growth, particularly between seed germination and seedling establishment, highlighting the importance of this trait for successful emergence. While such differences may not always reach statistical significance, longer hypocotyls have been associated with

improved establishment in various plant species and are likely relevant in legumes as well. This trait may contribute to enhanced light competition and mechanical stability during early developmental stages. However, further studies are required to confirm this finding.

4.5.5 Morphological traits and commercial efficiency in mungbean sprout production

The efficiency and marketability of mungbean sprouts are strongly influenced by morphological traits such as hypocotyl diameter, hypocotyl length, root length, and output ratio. Commercially desirable sprouts were typically thick, crisp, and uniform, with a hypocotyl length of 3.0–7.0 cm, as these attributes enhanced texture, appearance, and consumer appeal (Shanmugasundaram, 2007; Gatbonton et al., 2022). High-quality mungbean sprouts were characterized by thick hypocotyls and short roots. The experimental results indicated that these traits varied among genotypes, although all genotypes showed relatively similar characteristics suitable for sprout production and only minor differences, particularly in root length. Notably, line D5 exhibited short roots and tended to have a high output ratio. Short roots were preferred because they simplified cleaning and packaging, while adequate root development supported vigorous sprout growth. However, root length could be shortened through the application of growth regulators. For instance, (Chen et al., 1987) reported that the application of ethephon at concentrations of 10–20 ppm, either as a single or double application, significantly improved sprout quality by reducing root length and increasing hypocotyl thickness. The output ratio was crucial for producer profitability and was associated with vigorous seedling growth and high germination rates. Producers selected mungbean varieties and optimized environmental conditions to increase these traits, thereby ensuring efficient production and meeting market standards. In summary, selecting genotypes with optimal hypocotyl and root traits and high output ratios was essential for producing high-quality, market-preferred mungbean sprouts.

Overall, in terms of nutritional values based on the proximate compositions, CN84-1 emerges as the most suitable mungbean variety for producing sprouts with a high protein content, catering to consumers seeking protein-enriched diets. In contrast, P24 and P08 are preferable choices for sprout production when a higher carbohydrate content and a more desirable taste, often associated with sweetness and appealing mouthfeel, are desired. However, higher carbohydrates may lead to a shorter shelf life compared to genotypes with lower levels, as carbohydrates provide a readily available

substrate for microbial growth, potentially accelerating spoilage. For applications where an elevated dietary fiber content is the priority, P22 would be the optimal choice, offering increased nutritional benefits related to digestive health. These findings underscore that the selection of mungbean variety can be strategically tailored according to the targeted nutritional profile, desired sensory characteristics, and specific end-use applications of the sprouts, balancing nutritional benefits with practical considerations such as shelf life.

4.6 Conclusions

This study comprehensively evaluated the proximate nutritional composition of seven mungbean genotypes, including five newly developed lines, and two check varieties, grown under two contrasting environments (PNR and CND). Significant effects of G, E, and GEI were observed for most nutritional traits. Environmental factors such as humidity, temperature, and soil nutrient content influenced nutrient accumulation. PNR favored higher moisture, fat, and carbohydrate contents, while CND enhanced protein and ash levels. GEI effects indicated environment-dependent genotypic performance, supporting the need for targeted breeding and cultivation strategies. Notably, P08 and P24 exhibited high carbohydrate content, P12 and P22 were rich in seed and sprout fiber, and D5 showed superior protein accumulation under favorable conditions. Although the check varieties CN3 and CN84-1 maintained high protein levels, some new lines outperformed them in specific traits. Among morphological characteristics, root length emerged as a discriminating trait among genotypes. These findings highlight the complex interaction of G, E, and GEI in shaping mungbean nutritional quality. The strong performance of several new lines, some exceeding check varieties in key nutrients, underscores their potential to enhance the nutritional, industrial, and agronomic value of mungbean in sustainable agricultural systems.

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CHAPTER V
Morphological Variation and Nutritional Analysis of New Mungbean (*Vigna radiata* (L.) Wilczek) Lines for Microgreen Production

5.1 Abstract

This study aims to identifying mungbean genotypes suitable for high-efficiency and nutritionally rich microgreen production. The evaluation including morphological and nutritional composition of microgreens, nine mungbean genotypes were examined, including two certified varieties (CN3 and CN84-1) and seven new lines (SUPER5, P08, P12, P22, W5, and D5). Morphological assessment revealed no significant variation in hypocotyl length across genotypes, with an average of 13.46 cm. However, significant genotypic differences were observed in leaf morphology (length and width) and output ratio. Line SUPER5 exhibited the highest output ratio (4.63), indicating superior microgreen production efficiency. Additionally, SUPER5 combined high protein content (46.55%) with high productivity, making it a promising candidate for microgreen cultivation. Line D5 had the highest carbohydrate content (33.51%), while CN3 and SUPER5 displayed lower carbohydrate levels. CN84-1 showed the highest fiber content (13.37%), and CN3 had the highest ash content (11.49%). Fat content exhibited only slight variation, and moisture content remained relatively stable among genotypes. These findings highlight substantial genetic diversity in both morphological and nutritional traits, supporting the potential for targeted mungbean breeding to enhance microgreen yield and nutritional quality.

5.2 Introduction

Microgreens is the young seedlings of vegetables and herbs harvested shortly after germination, have gained considerable attention as functional foods due to their dense nutritional profiles and bioactive compounds (Xiao et al., 2012). Many research studies have indicated that microgreens contain higher nutritional value compared to their mature counterparts (Morris, 2003; Xiao et al., 2012; El-Nakhel et al., 2020; Singh et al., 2023). These nutrient-rich microgreens offer higher concentrations of vitamins, minerals, antioxidants, and proteins compared to their mature counterparts, making them attractive for health-conscious consumers and urban agriculture (Seth et al., 2025). Their short growth cycle and adaptability to controlled environments further enhance their commercial potential.

Among various crops used for microgreen production, legumes such as mungbean [*Vigna radiata* (L.) Wilczek] present promising opportunities due to their inherent high protein content and beneficial phytochemicals (Dhoot et al., 2017). Mungbean is a widely cultivated pulse crop in Asia, valued for its nutritional quality and agronomic adaptability (Habibullah & Shah, 2007). While mungbean sprouts are commonly consumed, the potential of mungbean microgreens is still unexplored, particularly regarding genetic variation in morphological traits and nutritional composition that could influence yield and quality. Previous studies have demonstrated significant genotypic variation in legume microgreens affecting biomass accumulation, leaf morphology, and nutrient density (Zhao et al., 2022; Barlongo & Mercado, 2024). Understanding such variation is essential for breeding programs aimed at improving microgreen yield and nutritional value. Moreover, nutritional analyses indicate that microgreens often surpass mature seeds in protein and micronutrient content due to accelerated metabolic activity during early growth (Xiao et al., 2012; Ebert, 2022).

Despite these insights, there is limited information on the morphological and nutritional diversity among new mungbean lines specifically cultivated for microgreen production. This study aims to evaluate morphological variation and nutritional profiles across different mungbean genotypes to identify superior lines for microgreen cultivation, thereby supporting targeted breeding and commercial production of nutrient-dense mungbean microgreens.

5.3 Materials and methods

5.3.1 Plant materials

In this study, a total of nine mungbean genotypes (CN3, CN84-1, SUPER5, P08, P12, P22, P24, W5, and D5) were used, and their special features and origins were provided in Table 5.1, including Thai certified varieties (CN3 and CN84-1) and newly developed mungbean lines (SUPER5, P08, P12, P22, P24, W5, and D5).

5.3.2 Samples preparation

Seed samples were prepared by removing impurities, drying them at 45°C for 24 hrs, and then grinding them into a fine powder. The powdered samples were subsequently stored at -20°C to preserve their integrity for nutritional analysis. Sprout samples were prepared using a modified method based on (Wang et al., 2021). Mungbean seeds were washed with sterile distilled water and soaked in warm water at 40°C for 30 min. After soaking, the seeds were kept in dark conditions at room temperature for 8 hrs. Following the soaking period, the seeds were rinsed with sterile distilled water and germinated using coconut coir and peat moss mixed in a 1:1 volume ratio. The mixture was then placed into microgreen growing trays and leveled to a depth of approximately 1 inch. Pre-soaked mungbean seeds were evenly spread across the surface of the growing medium and lightly covered with a thin layer of the same medium. The trays were placed in the growth chamber environments controlled maintained at 25°C and kept moist by regular watering. They were stored in darkness for 3 days, followed by exposure to artificial light with a photosynthetic photon flux density (PPFD) of 20.19 μmol/m²·s at wavelengths of 400–700 nm and a photon flux density (PFD) of 21.46 μmol/m²·s at 380–780 nm for 4 days, making a total growing period of 7 days.

Table 5.1 Pedigree and special features of nine mungbean genotypes from used in this study.

Genotypes	Pedigree	Special features	Descriptions
CN3	Selection from mutated CN36	Large seed, high yield, uniform maturity	Thai certified varieties developed at Chai Nat Field Crops Research Center, Thailand
CN84-1	Selection from mutated CN36	Large seed, high yield, high percentage of carbohydrate	
SUPER5	Development from double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)] (V4718, V4758, and V4785 were originated from India)	High resistance to PM[1/] and CLS[2/]	The mungbean resistant lines developed by Pookhamsak et al. (unpublished data)
P08	Selected from backcrossing between CN84-1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)]	Large seed, uniform maturity, moderate resistance to PM and CLS	New resistant lines
P12		High yield, rather drought resistance, high resistance to PM, moderate resistance to CLS	
P22		High yield, uniform maturity, abundant pods, moderate resistance to PM and CLS	
P24		Large seed, uniform maturity, moderate resistance to PM and CLS	
W5	Selected from backcrossing between CN72 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)]	High yield, abundant pods, pods born above the canopy, high resistance to PM and CLS	
D5	Selected from backcrossing between SUT1 and double cross lines [(CN72×V4758) × (CN72×V4718)] × [(CN72×V4718) × (CN72×V4785)]	Uniform maturity, moderate resistance to PM, abundant pods, pods borne above the canopy, and trichomeless	

[1/] powdery midew, [2/] Cercospora leaf spot

5.3.3 Proximate analysis

The contents of crude protein, crude fat, crude fiber, and total ash were analyzed following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2019). The total carbohydrate content was calculated by deducting the sum of these proximate components from the total. The parameter was measured in triplicates.

5.3.3.1 Moisture content

The measurement of moisture content, petri dish was placed in a hot air oven at 105°C for 2 hrs. Then, it was placed in a desiccator for 30 min until the temperature of the petri dishes equaled room temperature. Next, the petri dish was weighed using an analytical balance to four decimal places, 2-3 g of sample was placed into the petri dish with the cover slightly ajar and returned to the hot air oven at 105°C for 2 hrs or until dried. After drying, the petri dish containing the sample was allowed to cool in the desiccator for 30 min until the temperature of the petri dishes equaled room temperature. Then, the petri dish with the dried sample was weighed using the analytical balance to four decimal places. The drying process at 105°C was repeated for 1 hr or until the weight difference between two consecutive weighing was no different than 3 mg. The moisture content was calculated using the following equation:

$$\text{Moisture Content (\%FW)} = \frac{(W2 - W3)}{(W2 - W1)} \text{x } 100$$

When: W1 = Weight of the empty petri dish (g)
W2 = Weight of the petri dish with sample before drying (g)
W3 = Weight of the petri dish with the dried sample (g)

5.3.3.2 Crude protein content

The total protein content was determined using the Kjeldahl method. Approximately 0.5-1.0 g of sample was placed into a digestion tube, followed by the addition of 3 g of accelerating agent (a mixture of copper sulfate and potassium sulfate in a 1:10 ratio) and 20 mL of concentrated H_2SO_4. A blank sample was prepared by omitting the sample. Digestion was carried out in a Digestion System at 380 °C for 100 min until a clear blue solution was obtained. After digestion, the tubes were removed and cooled for about 20 min before proceeding to protein distillation using a UDK 149 Automatic Kjeldahl Distillation Unit (VELP Scientifica, Italy). For distillation, 20 mL of 4% boric acid (H_3BO_3) solution mixed with 2–3 drops of mixed indicator (methyl red

and bromocresol green at a 1:1 ratio) was prepared in an Erlenmeyer flask for each sample. The distillation was performed with the following program: 45 mL H_2O, 60 mL of 40% NaOH, and a distillation time of 4 min. During distillation, ammonia gas (NH_3) released from the sample reacted with NaOH and condensed into the boric acid solution, causing a color change from pink to green. The resulting solution was then titrated with 0.1 N HCl until the endpoint, indicated by a color change back to pink, was reached. The volume of HCl used was recorded.

$$\text{Crude protein content (\%DB)} = \frac{(\text{A} - \text{B}) \text{ x N x } 1.4007 \text{ x F}}{\text{W}_\text{t}}$$

When: A = Volume of HCl used for sample titration (ml).
B = Volume of HCl used for blank titration (ml).
W_t = Weight of the sample (g).
N = Concentration of the HCl (N).
F = Factor (specific to mungbean, which is 6.25).

5.3.3.3 Crude fiber content

Crude fiber analysis was performed using the Fibertec 2010 automatic analyzer (Foss Tecator, Denmark). Approximately 0.5-1.0 g of sample was placed into a filtered crucible. Then, 150 mL of 1.25% hot H_2SO_4 was added to each tube, along with 3 drops of n-octanol antifoaming agent to minimize foaming. The sample was boiled for 45 min. After boiling, the mixture was filtered until dry by activating air suction, followed by releasing the acid from the sample through valve opening. The samples were washed three times with hot H_2O and filtered to dryness. Next, 150 mL of 1.25% NaOH solution was added, again with 3 drops of n-octanol antifoam, and the sample was boiled for 45 min. After boiling, the sample was filtered and washed three times with hot H_2O. Subsequently, the sample was rinsed with acetone (C_3H_6O) 3 times, each with 25 mL. The filtered crucible containing the residue was removed from the extractor and dried in an oven at 105 °C for 2 hrs, then cooled in a desiccator for 30 min. Following drying, the crucible was incinerated in a muffle furnace at 500 °C for 2 hrs. The furnace was allowed to cool until the temperature fell below 250 °C before opening; the furnace must remain closed for at least 3 hrs prior to sample removal or until the temperature is below 200 °C. Finally, the crucible was cooled again in a desiccator for 30 min before further analysis.

$$\text{Crude fiber content (\%DB)} = \frac{W_2 - W_3}{W_1} \text{ x } 100$$

When: W_1 = Weight of sample (g).

W_2 = Weight of sample + crucible after oven dry (g).

W_3 = Weight of sample + crucible after burn in furnace (g).

5.3.3.4 Ash content

Analysis of ash content started with weighing the constant weight in hot air oven at 105°C for 2 hrs, 2-3 g of the dried sample put into a crucible. The samples were incinerated on a hot plate until smokeless to form a lump. Subsequently, incinerate in a furnace at 500°C for 3 hrs or until a light gray or uniform white ash is obtained. After removal from the furnace, it is allowed to cool to room temperature in a desiccator for 30 min. The weight was recorded, and the ash content calculated according to the following formula:

$$\text{Ash content (\%DB)} = \frac{W_2 - W_1}{S} \text{ x } 100$$

When: W_1 = Weight of the crucible.

W_2 = Weight of the crucible and sample after incineration.

S = Weight of the sample.

5.3.3.5 Fat content

The analysis of fat content was conducted following the Soxhlet extraction method using the Soxtec™ 2050 Auto Fat Extraction System. Initially, the extraction beaker was weighed and dried at 105°C for 1 hr to achieve a constant weight, then cooled in a desiccator. Approximately 1-1.5 g of the sample was weighed and put into the filter paper, then folded and inserted into a cellulose thimble. The cellulose thimble was positioned for extraction and insertion of the extract. Subsequently, 80 mL of petroleum was added to the extraction beaker and put it into the positioned for extraction. The heating program was set with the following parameters: extraction temperature at 180°C, extraction phase: 60 min, rinsing phase of 90 min, and drying phase for 15 min. Upon completion of the program, the extraction beaker containing the extracted fat was removed and dried in a hot air oven at 105°C for 2 hrs. After drying, the beaker was cooled to room temperature in a desiccator for 30 min. Finally, the extraction beaker with the extracted fat was weighed to determine the fat content.

$$\text{Crude fat content (\%DB)} = \frac{B - A}{W} \text{ x } 100$$

When: W = Weight of the sample.

A = Constant weight of extraction beaker.

B = Weight of extraction beaker and extracted fat.

5.3.3.6 Carbohydrate Content

To analyze the carbohydrate content, using the following method outlined by Hailu (2018). Carbohydrate content calculated by subtracting the percentages of moisture, protein, fat, fiber, and ash according to the following formula:

$$\text{Carbohydrate content (\%DB)} = 100 - \text{Moisture} + \text{Protein} + \text{Fiber} + \text{Fat} + \text{Ash}$$

5.3.4 Morphological data of microgreens

Data collection for the morphological traits including hypocotyl length, leaf length, and leaf width involved measuring twenty sprouts per genotype per replication, with six replications in total (n = 180). Measurements were taken using both a ruler and vernier caliper. The hypocotyl length of microgreens was measured from the base hypocotyl to the shoot tip. Leaf length was determined from the leaf base to the tip, while leaf width was measured at the widest part of the leaf, typically at the midpoint. Output ratio: calculated based on data collected from six replicates for each genotype follow by modified method from (Wang et al., 2021), computed as output ratio = fresh weight of microgreens (g) / weight of mungbean seeds (g)

5.3.5 Data analysis

A completely randomized design (CRD) was utilized for experimental arrangement. The data were subjected to analysis of variance (ANOVA), followed by mean comparisons using Duncan's New Multiple Range Test (DMRT) to evaluate differences in nutrient composition among mungbean genotypes as well as the morphological traits of mungbean microgreens. All statistical analyses were performed with SPSS software version 16.0 (Levesque, 2007).

5.4 Results

5.4.1 The morphological traits and output ratio of microgreen from various genotypes

The evaluated from nine mungbean genotypes were morphological traits and output ratio of microgreens summarized in Table 5.2. The average hypocotyl length was 13.46 cm, ranging from 12.70 cm in line P08 to 14.07 cm in variety CN84-1, with non-statistically significant differences observed among genotypes. The average leaf length was 3.43 cm and differed significantly, with line P08 exhibiting the longest leaf length (3.66 cm), followed by lines P22 and W5, respectively, while line P24 recorded the shortest (3.27 cm), however, it was not significantly different from all other genotypes except P08. Leaf width also showed highly significant differences, with an average of 1.29 cm. Line SUPER5 had the narrowest leaves (0.89 cm), which were significantly smaller than those of the other genotypes, while variety CN3 had the widest leaves (1.43 cm), although the differences were not statistically significant compared to CN84-1, P08, P22, and P24.

The output ratio, an important indicator of microgreen production efficiency, also varied significantly among genotypes. Line SUPER5 demonstrated the highest output ratio (4.63), significantly outperforming P12, P22, and P24. However, its output ratio was not significantly different from the remaining genotypes. Most of the newly developed lines except P12 had an output ratio compared to the recurrent parent CN84-1.

Table 5.2 Morphological traits and output ratio of microgreen from nine mungbean genotypes.

Genotypes	Hypocotyl length (cm)	Leaf length (cm)	Leaf width (cm)	Output ratio
CN3	14.02 ± 0.44[1/]	3.32 ± 0.07 b	1.43 ± 0.04 a	3.70 ± 0.71 ab
CN84-1	14.07 ± 0.47	3.45 ± 0.06 ab	1.38 ± 0.03 a	3.91 ± 0.30 ab
SUPER5	13.71 ± 0.30	3.32 ± 0.06 b	0.89 ± 0.02 c	4.63 ± 0.38 a
P08	12.70 ± 0.72	3.66 ± 0.13 a	1.39 ± 0.02 a	3.51 ± 0.29 ab
P12	13.63 ± 0.26	3.31 ± 0.07 b	1.27 ± 0.03 b	2.20 ± 0.03 c
P22	12.83 ± 0.79	3.54 ± 0.09 ab	1.42 ± 0.03 a	3.17 ± 0.29 bc
P24	13.84 ± 0.29	3.27 ± 0.08 b	1.39 ± 0.05 a	2.97 ± 0.21 bc
W5	13.24 ± 0.15	3.53 ± 0.11 ab	1.26 ± 0.03 b	3.48 ± 0.20 ab
D5	13.04 ± 0.40	3.51 ± 0.07 ab	1.20 ± 0.01 b	3.54 ± 0.42 ab
Mean	13.46	3.43	1.29	3.46
F-test	ns[2/]	*	**	*
C.V. (%)	8.72	5.97	5.99	3.77

[1/] Data showing means ± standard error (S.E.) different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [2/] ns = not significant, * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$.

5.4.2 Nutritional compositions of mungbean genotypes

The proximate nutritional composition of microgreens from nine mungbean genotypes is presented in Table 5.3. Moisture content showed non-statistically significant differences among genotypes, with an average value of 92.41%. In contrast, crude protein, total ash, crude fiber, and carbohydrate content exhibited highly significant differences, while crude fat showed significant differences. Moisture content ranged from 91.80% in P24 to 92.97% in SUPER5, with similar values observed across genotypes. Crude protein content varied significantly, with line SUPER5 exhibiting the highest value (46.55%), followed closely by line P22 (46.14%). Lines P22 and P24 had significantly higher protein contents than the recurrent parent CN84-1, with increases ranging from 1.08- to 1.03- fold, respectively, while line D5 showed the lowest protein content (41.78 %). Crude fat content also showed significant differences, ranging from 1.16% in SUPER5 to 1.93 % in D5. Line P24 (1.83%) had significantly higher fat content

than the recurrent parent CN84-1 (1.31%). Line D5 had significantly higher fat content compared to CN3, CN84-1, SUPER5, and W5. Total ash content was the highest in CN3 (11.49%), significantly exceeding that of most new mungbean lines, except for P08 and P12. The lowest total ash contents were recorded in lines P22, P24, and W5 (9.11, 9.08, and 9.41%, respectively), which differed significantly from the other genotypes. Crude fiber content ranged from 11.33% in P22 to 13.37% in CN84-1. Variety CN84-1 exhibited the highest crude fiber content, outperforming the lines SUPER5, P12, P22, and W5. The lowest crude fiber content was observed in line P22 (11.33%), which was significantly lower than other genotypes. Regarding carbohydrate content, line D5 showed the highest value (33.51%), though it was not significantly different from lines P12, P24, and W5. The lowest carbohydrate contents were found in CN3 and SUPER5, with values of 29.51 and 28.91%, respectively, showing statistically significant differences. These results indicate that mungbean microgreens exhibit considerable genotypic variation in protein, fat, ash, fiber, and carbohydrate contents, while moisture content remains relatively stable across genotypes.

Table 5.3 Proximate composition of microgreen from nine mungbean genotypes (%DB[1/]).

Genotypes	Moisture	Crude protein	Crude fat	Total ash	Crude fiber	Carbohydrates
CN3	92.67 ± 0.33[2/]	44.51 ± 0.23 b	1.34 ± 0.15 bcd	11.49 ± 0.06 a	13.15 ± 0.16 ab	29.51 ± 0.29 c
CN84-1	92.34 ± 0.39	42.62 ± 0.12 d	1.31 ± 0.11 cd	10.94 ± 0.02 ab	13.37 ± 0.17 a	31.76 ± 0.15 b
SUPER5	92.97 ± 0.33	46.55 ± 0.11 a	1.16 ± 0.19 d	10.96 ± 0.04 ab	12.42 ± 0.10 b	28.91 ± 0.26 c
P08	92.35 ± 0.07	42.65 ± 0.15 d	1.72 ± 0.01 abc	11.02 ± 0.14 ab	12.90 ± 0.07 ab	31.70 ± 0.06 b
P12	92.81 ± 0.88	42.56 ± 0.33 d	1.76 ± 0.03 abc	10.99 ± 0.13 ab	12.43 ± 0.29 b	32.27 ± 0.29 ab
P22	92.93 ± 0.23	46.14 ± 0.26 a	1.64 ± 0.28 abcd	9.11 ± 0.48 c	11.33 ± 0.46 c	31.78 ± 0.57 b
P24	91.80 ± 0.48	43.73 ± 0.16 c	1.83 ± 0.07 ab	9.08 ± 0.43 c	12.87 ± 0.21 ab	32.50 ± 0.26 ab
W5	92.38 ± 0.12	44.50 ± 0.26 b	1.38 ± 0.19 bcd	9.41 ± 0.12 c	12.37 ± 0.28 b	32.33 ± 0.45 ab
D5	92.22 ± 0.41	41.78 ± 0.08 e	1.93 ± 0.08 a	10.21 ± 0.38 b	12.58 ± 0.39 ab	33.51 ± 0.91 a
Mean	92.50	43.89	1.56	10.36	12.60	31.58
F-Test	ns[2/]	**	*	**	**	**
C.V. (%)	0.79	0.81	16.69	4.37	3.66	2.37

[1/] DM = dry basis. [2/] Data showing means ± standard error (SE) different letters in column indicate statistically significant differences at 95% confidence level by comparing means using Duncan's New Multiple Range Test (DMRT). [3/] * = significant at $P \leq 0.05$; ** = highly significant at $P \leq 0.01$, and ns=non-significant at $P > 0.05$, Moisture reported as fresh weight.

Figure 5.1 Morphological appearance of nine mungbean (*Vigna radiata*) genotypes grown as microgreens.

5.5 Discussion

Microgreens are a new type of crop gaining popularity as they are tender young seedlings harvested at the stage when the first true leaves appear (Treadwell et al., 2020). In contrast, sprouts are germinated seeds that are the smallest and youngest form of plants (Dubey et al., 2024). The primary difference among sprouts, microgreens, and baby greens (which represent a later stage of microgreens) lies in their plant size and cultivation duration (Treadwell et al., 2020). Microgreens are collected later than sprouts but earlier than baby greens (Verlinden, 2020). Microgreens can be regarded as superior substitutes for sprouts owing to their higher nutritional content and more pronounced flavor and taste (Puccinelli et al., 2019). Specifically, microgreens are harvested immediately after their newest leaves develop, whereas baby greens are typically harvested when they reach a height of 5 to 10 cm or after 15 to 40 days from seed germination (Dubey et al., 2024). In this study, nine mungbean genotypes were evaluated which revealed significant genotypic variation in both morphological traits and nutritional composition of microgreens, highlighting the potential for targeted breeding programs. The absence of significant differences in hypocotyl length across genotypes suggests this trait is less influenced by genetic factors in microgreen production, consistent with studies emphasizing stability in certain morphological traits under controlled conditions (Kessy et al., 2024). However, leaf morphology (length and

width) and output ratio exhibited marked genotypic differences, with SUPER5 demonstrating superior yield efficiency. These findings align with reports that genetic background strongly influences biomass accumulation and harvest efficiency in legume microgreens (Zhao et al., 2022; Rani et al., 2025).

Nutritional analysis further underscored genotype-specific profiles. Microgreens and sprouts also differ in their chemical composition (Choe et al., 2018). The nutrient content on a dry weight basis, compared between microgreen and mungbean seeds, showed variation. According to a review of over thirty studies on seed nutrition conducted by (Dahiya et al., 2015), the average values for moisture, crude protein, crude lipid, crude fiber, ash, and carbohydrate content in mungbean seeds were 9.80, 23.80, 1.22, 4.57, 3.51, and 61.00%, respectively. Compared to studies on microgreens, most nutrient components show higher quantities in microgreen nutritional except for carbohydrate content, which decreases by up to 30% when seeds are grown into microgreens. Meanwhile, moisture content increases by more than eight times. This increase in moisture influences the practical use of microgreens for consumption. Due to the lower carbohydrate content, microgreens are particularly suitable for health-conscious diets (Zhang et al., 2021). Moreover, microgreens often contain greater concentrations of phytochemicals, minerals, and vitamins compared to their fully matured counterparts (Xiao et al., 2012; Yadav et al., 2019). Additionally, protein content in microgreen lines SUPER5 (46.55%) and P22 (46.14%) exceeds that typically found in mature mungbean seeds (Habibullah & Shah, 2007), highlighting the enhanced nutritional values of microgreens accelerated metabolic activity during early growth stages. Conversely, the lower protein content observed in line D5 (41.78%) suggests a trade-off between carbohydrate and protein accumulation, as evidenced by its highest carbohydrate level (33.51%). Such inverse relationships between macronutrients have been documented in legume microgreens, where genetic variability influences resource allocation (Barlongo & Mercado, 2024).

Moisture content remains relatively stable, averaging 92.5% across genotypes, in contrast to significant variation observed in other traits. Variety CN3 exhibited elevated higher ash levels (11.49%), indicating distinct metabolic priorities among genotypes, potentially associated with environmental adaptation mechanisms (Kessy et al., 2024). Ash primarily consists of mineral salts such as calcium, potassium, and sodium, which reflect the overall mineral composition of the microgreen (Shokunbi et al., 2023). Although high ash content is nutritionally advantageous, excessively elevated ash may indicate contamination from external sources like soil particles, metal residues, cleaning agents, chalk, or sand (Marshall, 2010). These findings align

with broader legume research, where variability in ash content reflects differences in mineral uptake and partitioning among genotypes (Habibullah & Shah, 2007). Crude fiber refers to the portion of plant material composed mainly of cellulose, hemicellulose, and lignin that is indigestible by human enzymes (behrLabor-Technik, 2023; Kuzio & Zibula, 2023). It is traditionally measured by acid and alkaline digestion methods, which capture the insoluble fiber fraction but do not account for all dietary fiber components, especially soluble fibers (Trowell, 1976; Mutter, n.d.). Crude fiber analysis is commonly used in animal nutrition and food quality assessments but is considered a limited measure of total dietary fiber (Trowell, 1976; Muinos, 2022). Crude fiber is a subset of dietary fiber, primarily comprising insoluble fiber components. It functions by increasing fecal bulk through water absorption and swelling within the gastrointestinal tract (Barber et al., 2020; He et al., 2022; Alahmari, 2024), which aids in preventing constipation, reducing intestinal transit time, and lowering the risk of colorectal diseases, including cancer. Microgreens exhibiting high crude fiber content, such as line CN84-1 with 13.37%, are likely to confer superior health benefits compared to other genotypes, potentially enhancing overall digestive health and disease prevention.

The observed diversity in output ratio and nutritional traits aligns with the high genetic variability reported in mungbean agronomic traits (Zhao et al., 2022). For instance, line SUPER5 has a combination of high protein and yield efficiency positions as a prime candidate for microgreen focused breeding, while P12 exhibited a low output ratio and may limit its commercial viability. These insights support the prioritization of traits like synchronized maturity and nutrient density in breeding programs, as emphasized in recent studies on value-added legume products (Barlongo & Mercado, 2024).

Overall, based on the morphological and nutritional characteristics, SUPER5 emerged as the most suitable candidate for microgreen production with high protein content and output ratio prioritized, making it ideal for health-focused and protein-enriched products. For applications focusing fiber, CN84-1 is preferable due to their superior values. Conversely, if the goal is to enhance carbohydrate content and texture, D5 is recommended, as they exhibited the highest carbohydrate level.

5.6 Conclusion

This study highlights the significant genotypic variation observed among mungbean genotypes, both in terms of morphological traits and nutritional composition of microgreens. The results indicate that hypocotyl length remained consistent across the genotypes. In contrast, leaf morphology (length and width) and output ratios showed substantial variation. Among the genotypes, SUPER5 emerged as the most suitable for microgreen production, excelling in protein content and output efficiency along with P22 also exhibited high in protein content. D5 displayed the highest carbohydrate level, whereas CN3 and SUPER5 exhibited lower carbohydrate content. The superior fiber content was higher in CN84-1, while CN3 had the highest ash content. Fat content showed only slight differences across the genotypes. The moisture content remained consistently high across all genotypes. Overall, the considerable diversity in both morphological and nutritional characteristics among mungbean microgreen genotypes provides valuable insights for breeding programs aimed at developing superior microgreen varieties with enhanced agronomic performance and nutritional quality.

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CHAPTER VI
CONCLUSION

Regional yield trials are multi-location field evaluations of a set of newly developed mungbean lines across representative environments, serving as a crucial step in plant breeding programs. They enable breeders to assess genotype performance, adaptability, and yield stability under diverse agro-ecological conditions. Simultaneously, the nutritional study of mungbean is equally important, as legumes are a vital source of plant-based proteins and play a significant role in enhancing global food security. In this thesis, the newly developed mungbean lines with resistance to powdery mildew (PM) and Cercospora leaf spot (CLS) were comprehensively evaluated in terms of agronomic performance, disease resistance, yield stability, nutritional quality, and their potential for sprout and microgreen production.

In the first part, eight mungbean genotypes were evaluated in regional yield trials, including three Thai-certified varieties (CN3, CN84-1, and SUT1), the disease-resistant line SUPER5, and four new breeding lines (P08, P12, P22, and P24). Trials were conducted across four multiple locations and two seasons to assess genotype performance under contrasting environmental conditions. Lines P22 and P24 consistently exhibited superior yield and broad adaptability, performing well in both the rainy and dry seasons, particularly under disease outbreaks of CLS and PM. P24 excelled at Phitsanulok and Phetchabun and demonstrating synchronous maturity. P12 showed strong disease resistance and promising dry season performance at Nakhon Ratchasima but was limited by delayed flowering, maturity and rainy season lodging. Although P08 had slightly lower yield than P22 and P24, it displayed the highest yield stability, supporting its suitability for cultivation under variable environments. GGE biplot analyses reinforced these observations, showing P22 to possess both high performance and acceptable stability, P08 to have the highest stability across pods/plant and 100 seed weight, and P24 had balance high yield with moderate stability. The ‘Which-won-where’ and ‘Discriminative vs. Representativeness’ analyses further emphasized the importance of genotype × environment interaction (GEI) and highlighted Nakhon Ratchasima and Chai Nat as ideal testing locations. These result

suggest that P22 and P24 are strong candidates for varietal release, while P12 and P08 offer value in targeted or stress-prone environments.

Building upon these agronomic findings, the second part of the study evaluated nutritional composition and morphological traits of seeds and sprouts from seven genotypes (P08, P12, P22, P24, D5, CN3, and CN84-1) grown in two contrasting environments: rainy season in Phitsanulok (PNR) and dry season in Chai Nat (CND). Significant effects of genotype, environment, and GEI were observed on most nutritional traits, highlighting the critical role of both genetics and growing conditions. Dry season seed samples exhibited stability and higher protein content, while rainy season samples contained elevated levels of moisture, fat, and carbohydrates. For the seeds, varieties CN3 and CN84-1 exhibited higher protein content, while P08 and P24 were rich in carbohydrates. For the sprouts. CN84-1 stands out as the most suitable for producing sprouts with high protein content, ideal for protein-enriched diets. P24 and P08 are preferable when higher carbohydrate content and desirable texture often linked to sweetness and appealing mouthfeel are prioritized. For applications emphasizing fiber, P22 offers additional nutritional benefits to digestive health. Sprout morphologically, root length showed significant variation among the genotypes, with CN3 producing the shortest root length, while CN84-1 and P24 produced the longest roots. However, hypocotyl diameter and length did not show significant differences.

To complement the sprout evaluation and assess further end-use potential, the third experiment focused on the morphological and nutritional characteristics of mungbean microgreens derived from nine genotypes, including a resistant line (SUPER5), the six newly developed lines (P08, P12, P22, P24, W5, and D5) and two varieties (CN3 and CN84-1). While hypocotyl length did not vary significantly among genotypes, substantial differences were observed in leaf (length and width) and output ratio. SUPER5 emerged as the most suitable for microgreen production, excelling in protein content and output efficiency along with P22 also exhibited high in protein content. D5 displayed the highest carbohydrate level, whereas CN3 and SUPER5 exhibited lower carbohydrate content. Fat content showed only slight differences across the genotypes. The superior fiber content was higher in CN84-1. CN3 had the highest ash content, while moisture content remained consistently high across all genotypes.

Taken together, these three interconnected experiments provide a comprehensive assessment of newly developed mungbean lines across multiple dimensions, from field performance to nutritional quality. The regional yield trials confirmed the importance of multi-environment testing in identifying stable, high-

yielding genotypes such as P22 and P24, emphasizing the significance of GEI and the utility of GGE biplot analysis for genotype selection. The nutritional and morphological evaluation of seeds, sprouts, and microgreens highlighted variation in nutrient profiles. Overall, this study demonstrates that regional yield trials are essential in mungbean breeding programs for selecting adaptable and stable genotypes under diverse agro-ecological conditions. Furthermore, the nutritional evaluation of seeds, sprouts, and microgreens reinforces the role of mungbean as a plant-based protein source vital to global food security. The integration of agronomic, morphological and nutritional data in this study supports the selection of elite mungbean lines for varietal release, targeted cultivation, and diversified utilization in plant breeding programs. In terms of nutritional content, the breeding objective influences the selection of genotypes. Genotypes CN3 and CN84-1 have high protein content in seed, while P08 and P24 yield higher carbohydrate content in both seed and sprout production. Similarly, CN84-1 produces sprouts with high protein content, while P08 and P24 produce sprouts with high carbohydrate content. P22 has the highest fiber content in sprouts. Line SUPER5 was the most suitable for microgreen production due to its high protein content and highest output. Meanwhile, CN84-1 exhibited the highest fiber content in microgreens.

The findings from these experiments can serve as a foundation for future breeding strategies aimed at enhancing both yield and nutritional quality in mungbean. The identified elite lines can be further evaluated under farmers' field conditions or integrated into participatory breeding programs to ensure practical applicability and acceptance. Additionally, the nutritional insights gained from seeds, sprouts, and microgreens open new avenues for value-added product development tailored to health-conscious consumers. Further molecular and genomic studies could also validate the observed traits and accelerate the development of superior mungbean variety.

APPENDIX

The characteristics of new mungbean lines derived from the backcrossing method to the recurrent parent CN84-1.

Figure A.1 Appearance characteristics of the new mungbean line P08.

Figure A.2 Appearance characteristics of the new mungbean line P12.

Figure A.3 Appearance characteristics of the new mungbean line P22.

Figure A.4 Appearance characteristics of the new mungbean line P24.

The characteristics of new mungbean lines derived from the backcrossing method to the recurrent parent SUT1.

Figure A.5 Appearance characteristics of the new mungbean line D5.

The weather information of experimental field during the rainy and dry seasons 2023-2024

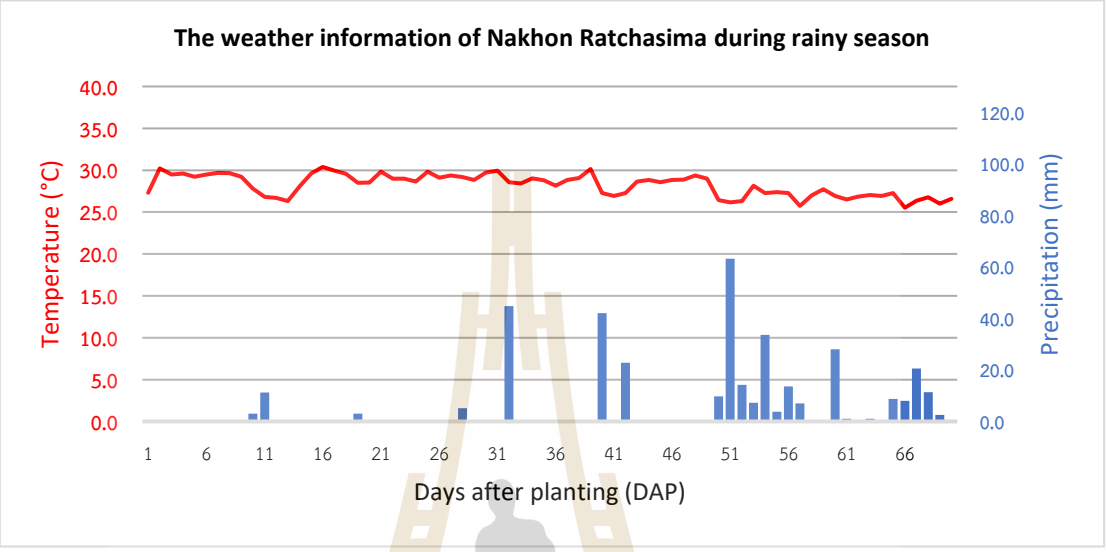

Figure A.6 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Nakhon Ratchasima during rainy season between July 11, 2023 – September 18, 2023, based on the Days after planting of mungbean (x-axis).

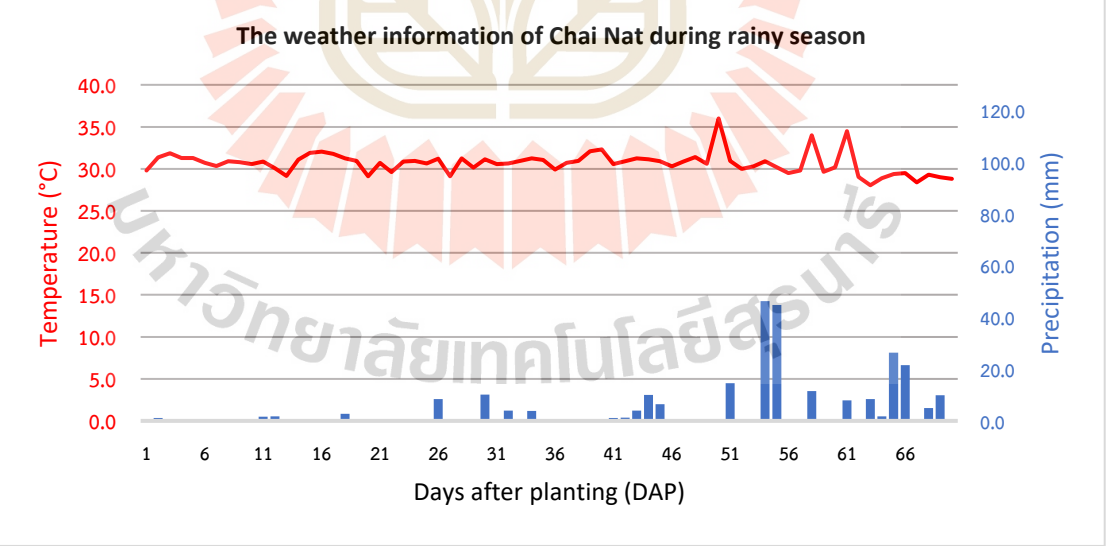

Figure A.7 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Chai Nat during rainy season between July 12, 2023 – September 19, 2023, based on the Days after planting of mungbean (x-axis).

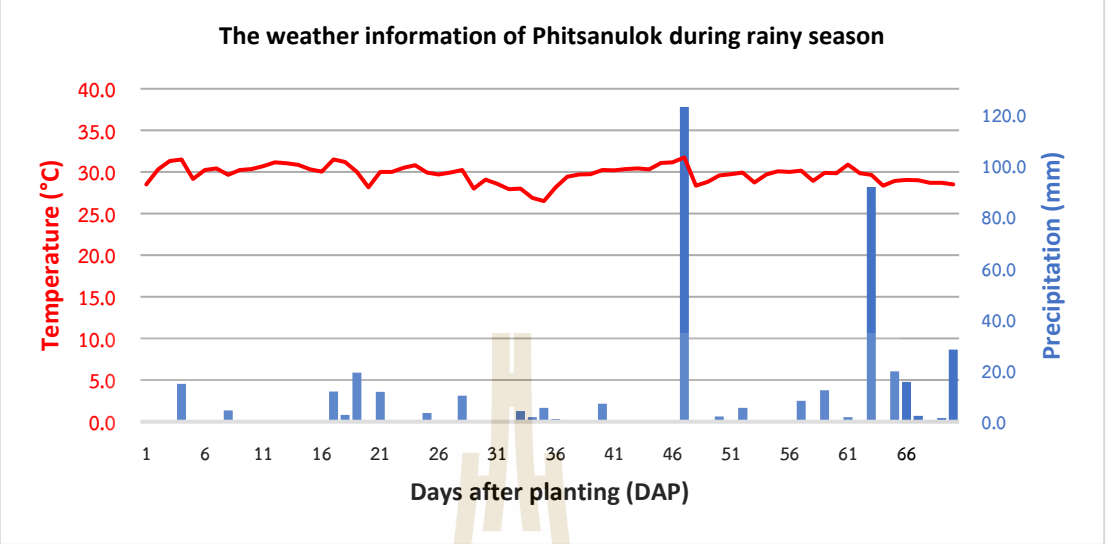

Figure A.8 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Phitsanulok during rainy season between July 7, 2023 – September 14, 2023, based on the Days after planting of mungbean (x-axis).

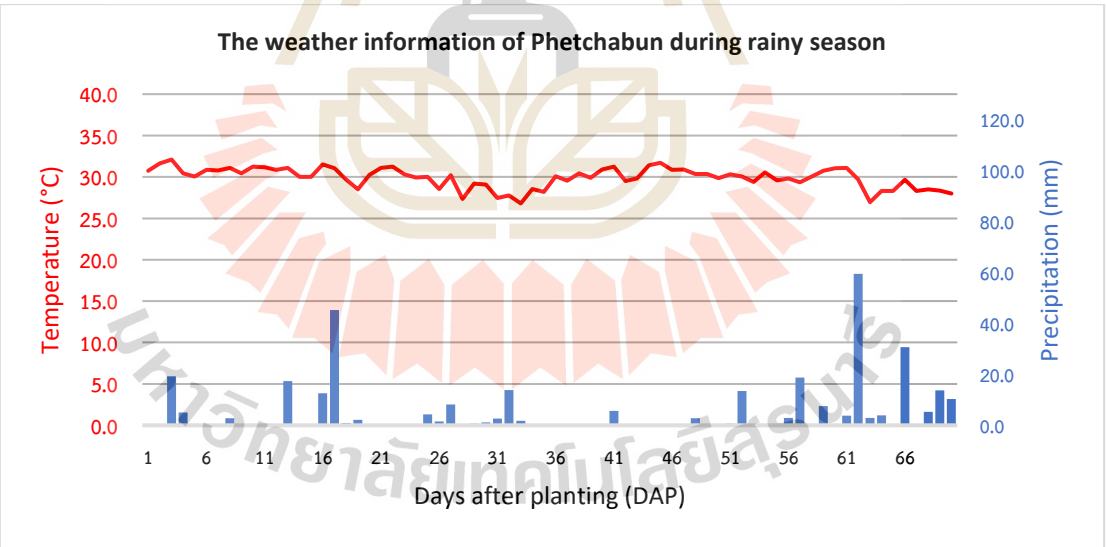

Figure A.9 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Phetchabun during rainy season between July 6, 2023 – September 13, 2023, based on the Days after planting of mungbean (x-axis).

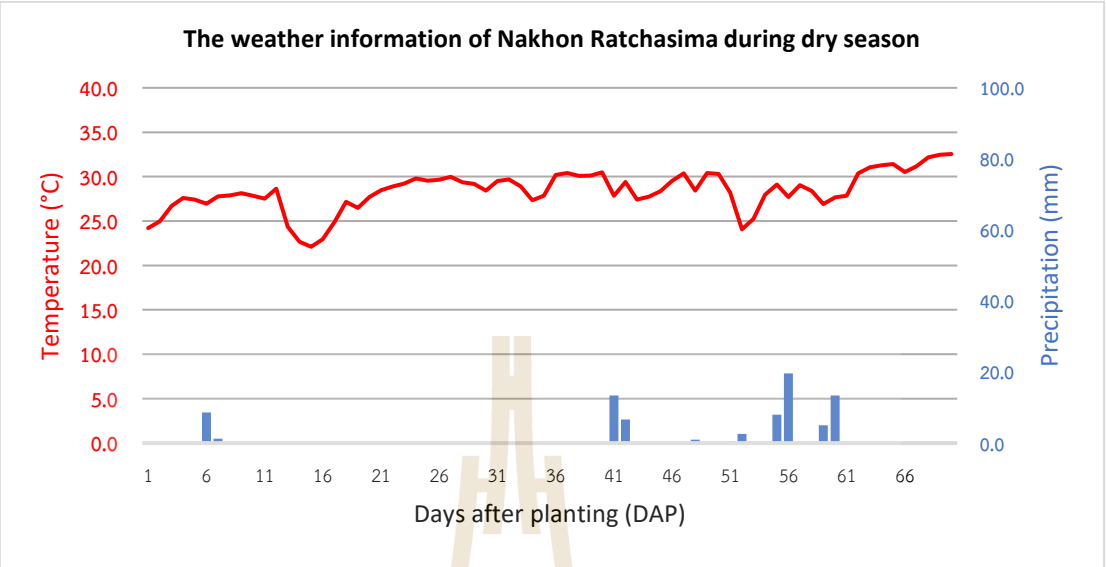

Figure A.10 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Nakhon Ratchasima during dry season between January 29, 2024 – April 7, 2024, based on the Days after planting of mungbean (x-axis).

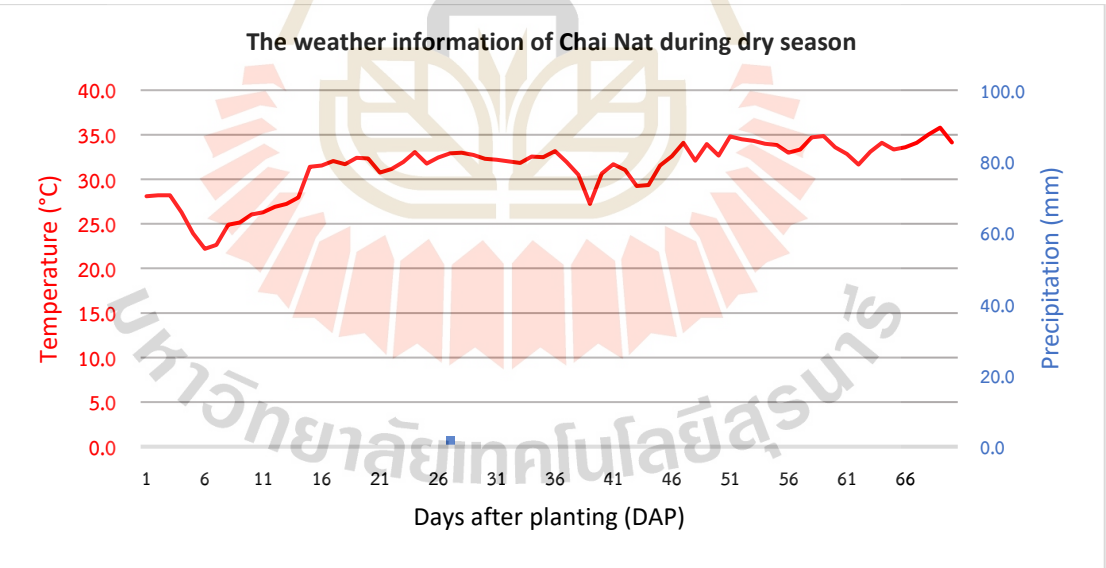

Figure A.11 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Chai Nat during dry season between December 18, 2023 – February 25, 2024, based on the Days after planting of mungbean (x-axis).

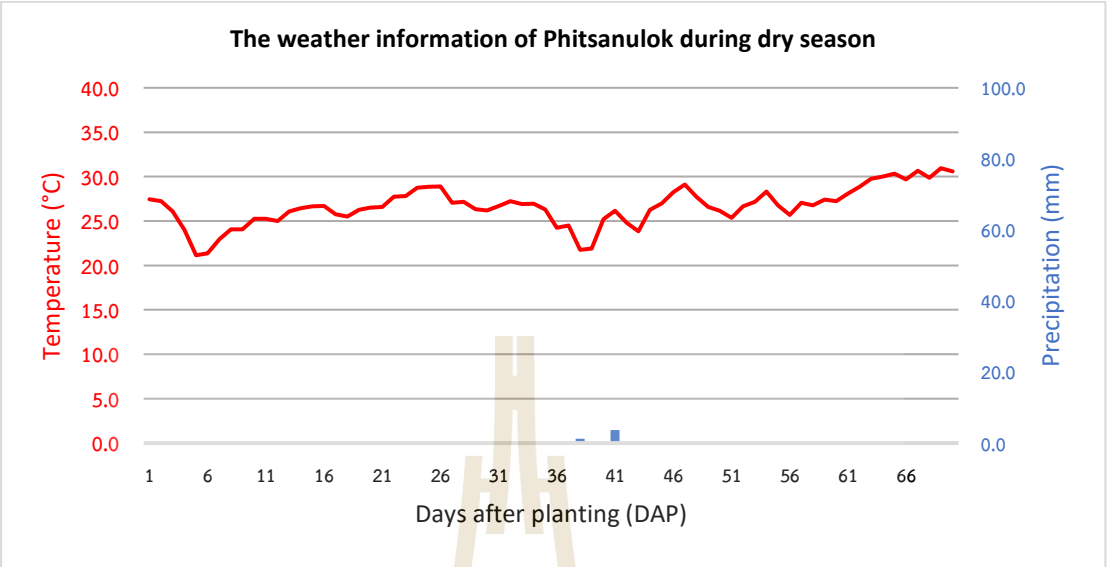

Figure A.12 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Phitsanulok during dry season between December 19, 2023 - February 26, 2024, based on the Days after planting of mungbean (x-axis).

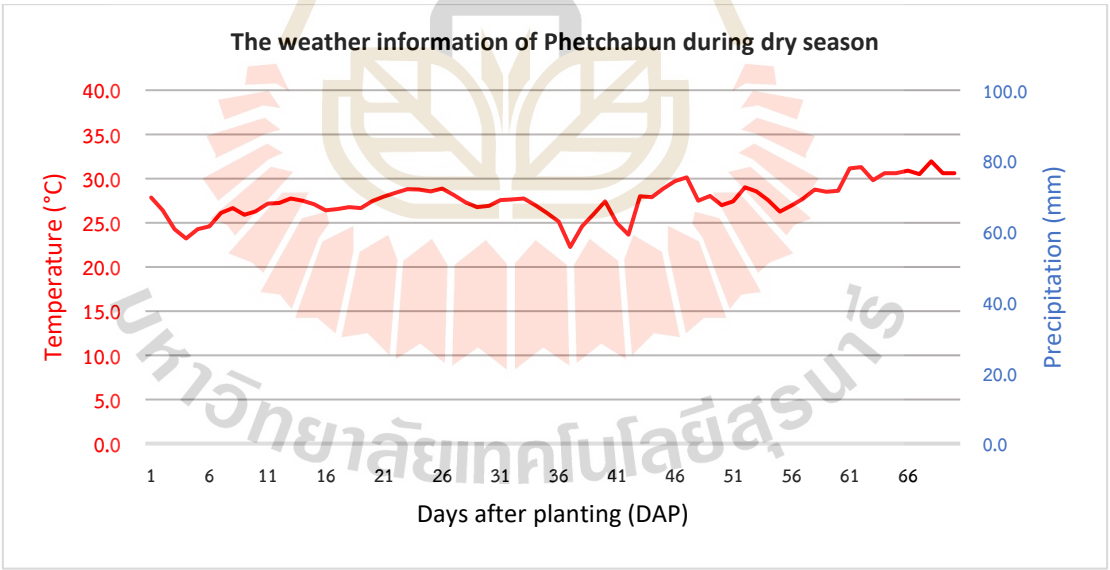

Figure A.13 The Weather information of air temperature (°C; left y-axis) and precipitation rates (mm; right y-axis) at Phetchabun during dry season between December 20, 2023 - February 27, 2024, based on the Days after planting of mungbean (x-axis).

Table A.1 Details of the geographic position and meteorological variables that prevailed at the three locations during the experimental period.

Details	Locations			
Experimental site	**Nakhon Ratchasima**	**Chai Nat**	**Phitsanulok**	**Phetchabun**
Agro-climatic zone	Tropical savanna climate			
Latitude	14°52'37.6"N	15°09'08.3"N	16°50'13.7"N	16°27'21.3"N
Longitude	102°00'15.2"E	100°10'55.1"E	100°22'59.1"E	101°10'00.8"E
Altitude (MSL)	227	17	45	119
pH (soil:water; 1:2)	6.77	6.22	6.29	6.00
EC (uS/cm)	126.03	94.27	58.99	83.76
OM (%)	1.13	1.47	0.70	3.30
N (%)	0.06	0.09	0.04	0.17
P (mg/kg)	26.64	40.33	100.10	27.10
K (mg/kg)	99.73	60.87	27.10	167.26
Soil texture	Sandy Loam	Clay Loam	Sandy Loam	Clay

Assessment of powdery mildew disease

- Score 1: indicates no symptoms of the disease.
- Score 2: indicates the presence of 2-3 lesions on the lower leaves.
- Score 3: indicates the presence of 2-3 lesions on the lower leaves with sporulation.
- Score 4: indicates the presence of numerous lesions on the lower and middle leaves with sporulation.
- Score 5: indicates similar symptoms to score 4, with yellowing or drying of leaves and abundant sporulation.
- Score 6: indicates symptoms like score 5, visible from a distance, with abundant sporulation.
- Score 7: indicates lesions throughout the plant but not exceeding 25% leaf dryness.
- Score 8: indicates symptoms like score 7, with 25-50% leaf dryness.
- Score 9: indicates more than 50% leaf dryness.

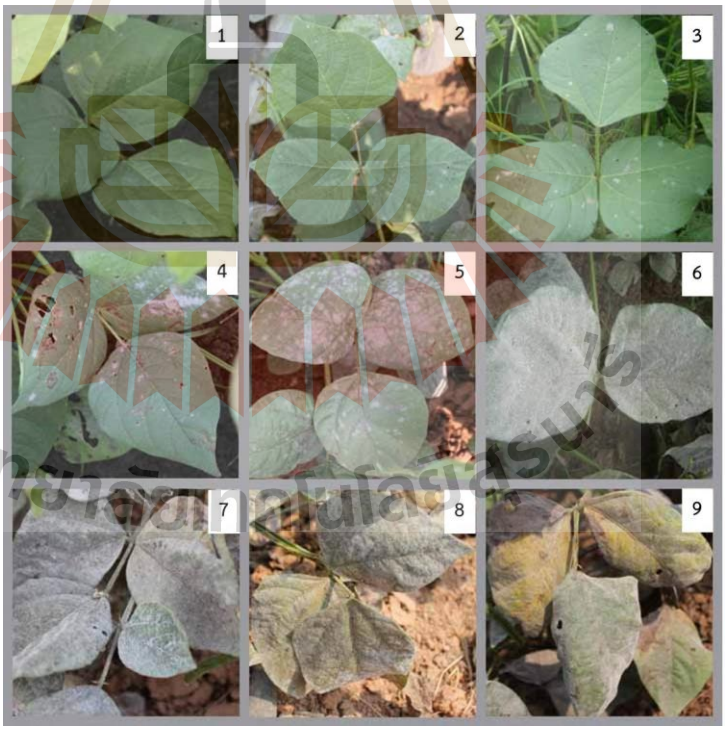

Figure A.14 Scoring criteria for assessing powdery mildew symptoms in mungbean.

Assessment of Cercospora leaf spot disease

- Score 1: Indicates disease incidence at 0% of total leaf area.
- Score 2: Indicates disease incidence at 1-25% of total leaf area.
- Score 3: Indicates disease incidence at 26-50% of total leaf area.
- Score 4: Indicates disease incidence at 51-75% of total leaf area.
- Score 5: Indicates disease incidence at 76-100% of total leaf area.

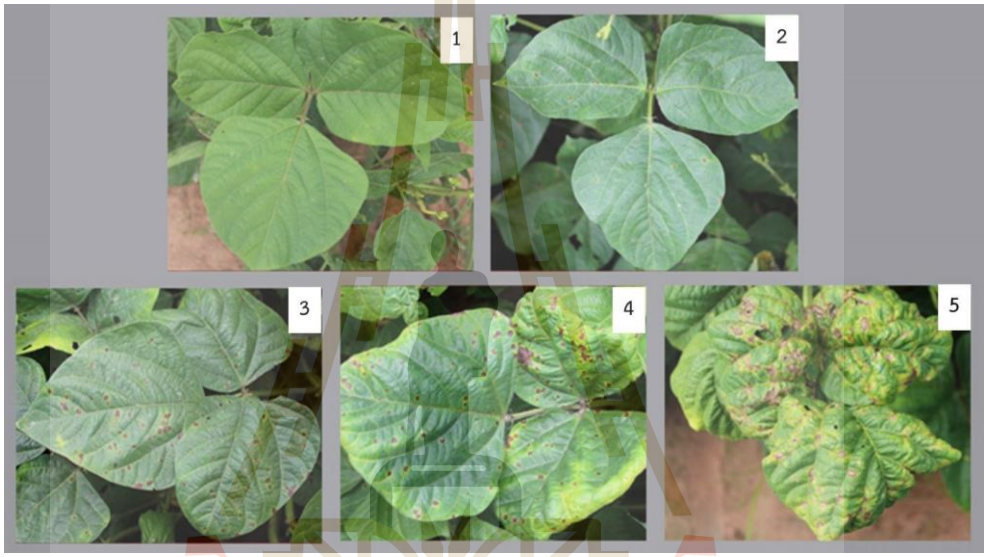

Figure A.15 Scoring criteria for assessing Cercospora leaf spot symptoms in mungbean.

BIOGRAPHY

Mr. Piyangkoon Jaukwon was born on September 17, 1999, at Sisaket, Thailand. He graduated from Princess Chulabhorn Science High School Buriram. He later obtained a Bachelor of Science degree in Crop Production Technology (B.Sc. Crop Production Technology) from Suranaree University of Technology in 2022. During his undergraduate studies, he received the '*Sakayabundit'* (Potential Graduate) Scholarship, which fully covered his tuition fees and provided a monthly stipend. In the same year, he pursued a master's degree in the School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. He received the '*Kittibandit'* Scholarship, which supported his tuition fees and provided a stipend throughout his studies. During his graduate studies, he had the opportunity to present his research at the International Conference "Innovation for Resilience Agriculture," held on February 5-7, 2025, at Chiang Mai University, Thailand. His poster presentation was titled *"Proximate nutritional analysis of newly developed mungbean lines resistant to powdery mildew and Cercospora leaf spot diseases."*