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Review Article

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Systematic Review of Mungbean Genetic Improvement: Early Maturity for Efficient Cropping Systems

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ABSTRACT

In recent times, agriculture has become the primary foundation for all food-based products. Agriculture involves the cultivation of various crops, including pulses, which are essential to provide protein-rich food sources and enhance soil health through nitrogen fixation. Pulses come under the legume family. The mungbean (MB) (Vigna radiata L.) is an important pulse crop that significantly contributes to human nutrition and soil health. Moreover, MBs are high in nutrients along with antioxidants, which might provide health benefits. However, the relatively long growth duration of MBs, which delays the subsequent cropping cycle, presents challenges for its cultivation in Rice-Wheat Cropping Systems (RWCS). Mutations in MBs that happen early in maturity can make RWCSs much more effective by letting crops grow in succession more quickly and improving soil health by fixing nitrogen. So, the goal of this review paper is to look at how to genetically improve MB so that it matures quickly, using induced mutations that work for RWCSs. Similarly, this paper delves into the significance of MB for early maturity in rice-wheat (RW) systems, examines various physical and chemical mutagens for the development of early-maturing MB varieties, determines the LD50, and identifies desirable MB mutants that exhibit short duration, high yield, and synchronous maturity.

Keywords: *Rice wheat cropping systems, Agriculture, Nitrogen fixation Mungbean, Early maturity, Physical and chemical mutagens, Genetic improvement*

1. INTRODUCTION

Globally, billions of people get nutrition from pulses. The terms ''legumes'' and ''pulses'' are synonymous, as every pulse is a legume, although not all legumes are considered pulses [1]. Pulses are part of the Leguminosae family, known for its legume or pod fruit. Nearly 11,000 species of legumes are known. Legumes have been grown and utilized as food for ages throughout the world. Within the legume family, MB (Vigna Radiate (VR)) stands out.

Significant crops in Asia exhibit a steadily rising yield [2]. MB is beneficial and a crucial pulse crop with high economic and commercial value. In several Asian countries, like China, India, Bangladesh, and Pakistan, and some Southeast Asian countries, along with dry regions of southern Europe and warmer parts of Canada and the United States, the MB is widely cultivated. The cover cropping of MB (VR) in summer (April–June) is elucidated in Figure 1 [3]. Figure 1 explains the MB's cover cropping (VR) in summer (April–June) [3].

Figure 1: Cover cropping of MB (VR) in summer (April–June)

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This can be attributed to its characteristics as a relatively drought-tolerant, low-input crop, and its short growth cycle of approximately 70 days [4]. In many regions, MB (VR) is a vital legume, which provides high quality due to its nutritional benefits and plays a sustainable role in agriculture. A minor crop, which dry land smallholder farmers could deploy for breaking the downward spiral and augmenting their farms' profitability and sustainability, is termed the MB [5]. MB can be sown as a catch crop between mid-April and the first week of July. Not only will this provide farmers with additional income, but its nitrogen-fixing ability will improve the soil's soil fertility (SF) status, leading to increased yields of both rice and wheat [6]. RWCSs present challenges in terms of crop cycle timing and efficiency. But when early-maturing MB is mixed with RWCSs, the timing and efficiency of crop cycles will be better, which will lead to better resource use and higher overall productivity [7]. Combining early-maturing MB into RWCSs through induced mutation has shown promising results. The short growing season of MB suits well for rice and wheat crops, thus enhancing the overall system productivity and sustainability [8]. Figure 2 illustrates the significance of enhancing the MB genes to promote rapid growth through beneficial mutations for RWCSs.

Figure 2 illustrates the significance of genetic enhancement of MB for early maturity through induced mutations suitable for RWCSs.

Using physical or chemical mutagens to cause mutations is a common way to create new genetic diversity and make MB varieties that mature faster without lowering yield or other important agronomic traits [9, 10].

The identification and growth of early maturing MB strains will not only improve the sustainability of RW cycles but will also help to improve food security and agricultural resilience in areas in which this Cropping System (CS) is common [11]. Also, in Figure 3, the overall review methodology used in the current review paper is explained.

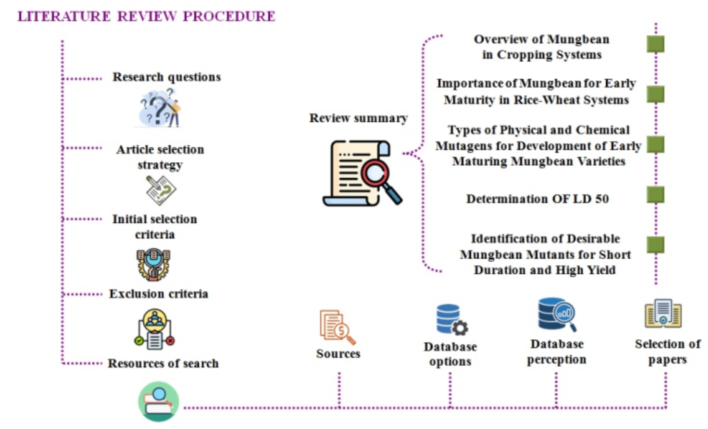

Figure 3: Overall review methodology

2. DEVELOPING RESEARCH QUESTIONS AND SELECTION STRATEGY OF ARTICLE

In operating any investigation, such as a review or thesis, the development of research questions (RQs) is an essential phase. Well-constructed RQs provide clarity and direction, confirming that the study remains focused and relevant. RQs must be specific and formulated in a way that allows for empirical testing. A well-structured selection strategy confirms that the literature reviewed is relevant and high-quality.

2.1. Research questions

Various research questions (RQs) are available with the goal of improving the genetics of MB. The generated research themes and RQs are described in Table 1.

Table 1: Research concepts and Rqs

List	Research concepts	Questions
M_1	*MB in cropping systems.*	*What is the importance of MB in the cropping systems?*
M_2	*MB for early maturity in RWCSs*	*What is the significance of MB for early maturity in RWCSs?*
M_3	*Physical and chemical mutagenesis types*	*How are the physical and chemical mutagens categorized?*
M_4	*LD50 determination*	*Explain the determination of LD50 in the analyzed research articles.*
M_5	*Identification of desirable MB mutants*	*What are studies associated with the identification of desirable MB mutants?*

2.2. Article selection strategy

Selecting eligible articles for review research requires a meticulous approach to ensure a thorough and relevant investigation of the subject. Some of the key methods to follow in the article selection strategy are listed below.

- The article selection process is crucial for locating studies that align with the objectives.
- Searching for related research using keywords is essential to getting answers to the queries.
- Using relevant keywords is vital in the research process, particularly during article selection.

2.2.1. Initial selection criteria

The analytic technique considers significant criteria, such as the paper's language, year, and topic relationship within the desired area. The analysis comprises research publications written only in English. The study focuses on academic publications issued between 2015 and 2023.

2.2.2. Exclusion criteria

The article is removed based on the following criteria:

- The article only related to pulses is omitted.
- Articles published before 2015 are not included.
- Studies focusing just on MB legumes are eliminated

2.2.3. Resources of search

Choosing the right resources and databases and confirming the credibility of the publications are important when conducting a literature review for a research paper. Detailed information regarding the sources, database options, database perception, and paper selection is given as follows:

Sources: To collect data relevant to the research topic, several scholarly search engines, such as Google Scholar, Elsevier, IEEE Xplore, and Springer, are used. The inquiry focused on the "genetic improvement of MB for early maturity through induced mutations suitable for RWCSs" between 2015 and 2024.

Database options: Important databases, such as Scopus, SCIE, and WOS, aid in spotting research publications.

Database perception: The Scopus database stands out for its comprehensive abstract and citation database of peer-reviewed literature found in scientific publications, even though several databases are available. The chosen databases provide distinct advantages in terms of research presentation and substance.

Selection of papers: Lastly, for the systematic review, 60 research articles are chosen. The papers are carefully picked using specific criteria. A graphical representation of the search results for this literature review is depicted in Figure 4.

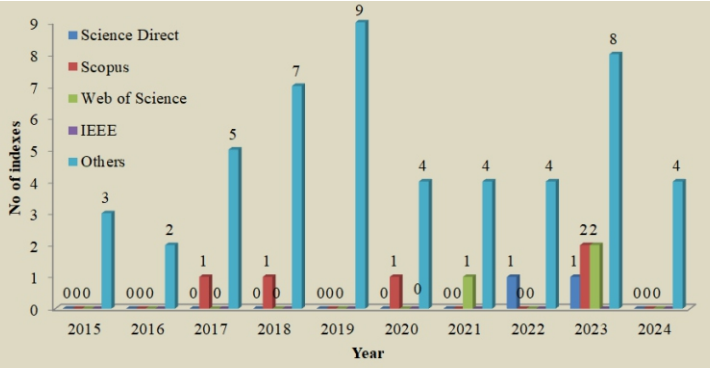

Figure 4: Graphical depiction of the search results for this literature review

Additionally, when compiling a set of 60 research articles for a literature review, calculating and reporting the publication percentage based on various criteria can provide a quantitative overview of the sources. The pie chart of publication percentage analysis is elucidated in Figure 5.

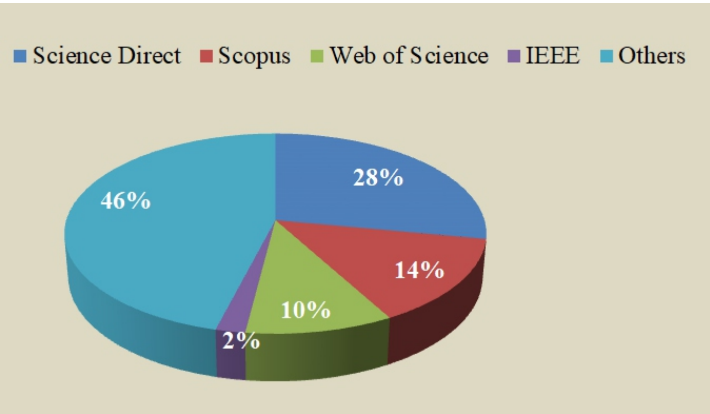

Figure 5: Pie chart of publication percentage analysis

3. LITERATURE REVIEW

There is a purpose to analyze the genetic improvement of MB for early maturity through induced mutation; particularly in the background of RWCSs. MB is rich in protein and other nutrients. Induced mutations can also improve MB cultivars' resistance to pests and illnesses, thus lowering crop losses and the requirement for pesticides.

This paper outlines the fundamentals of MB in CSs, emphasizes the importance of MB early maturity in RW systems, explores various physical and chemical mutagens for the development of early-maturing MB varieties, determines the LD50, and identifies desirable MB mutants for short-duration, high-yield, and synchronous maturity.

3.1. OVERVIEW OF MUNGBEAN IN CROPPING SYSTEMS

Globally, MB, being an excellent source of nutrients, minerals, and vitamins, serves as a highly nutritious food, an important feed, and a fodder crop [12]. MB is a versatile legume, which plays a significant role in CSs, particularly in Asia and Africa [13]. MB is a valuable component of sustainable CSs, offering multiple agronomic, environmental, and economic benefits [14]. Its ability to enhance SF and fit into diverse farming practices makes it an important crop for improving resilience and productivity in agriculture [15].

An evaluation was conducted to examine MB's response to fertility along with lime levels under soil acidity in an alley CS in the Vindhyan area, India. The study aimed to assess the technique for fertility and lime levels in acidic soil, as well as its impact on MB output. The use of 100% RDF resulted in the greatest gain in MB yields, specifically in seed (524 kg/ha), straw (1426 kg/ha), and biological yield (1949 kg/ha) [16].

We studied how adding (i) organic matter, (ii) bio fertilizers, and (iii) crop residues to wheat-MB cropping systems in the Indo-Gangetic plains affected the activity of microbes in the soil. We measured (A) the soil microbial biomass C (C mic), (B) basal respiration, (C) ergo sterol, (D) glomalin, (E) soil enzymes (glucosidases, phosphatases, and dehydrogenases), (F) FDA activity, (G) organic carbon (C org), (H) the ratio of C mic to C org, and (I) the metabolic quotient (qCO). VC, CR, and BF were used in organic methods to improve the soil microbes and C organization in RW systems [17].

We looked at how keeping residue and managing nitrogen precisely affected the physical and chemical properties of the soil and crop yield in the maize-wheat-MB system of the Indo-Gangetic Plains. According to the research, the ZT-centric system with maize-centric crop rotations (MWMb) may enhance soil characteristics and system productivity in northern India by retaining crop residue and implementing precision nitrogen management [18].

The influence of varying fertilizer quantities and planting strategies on MB performance in India's semi-arid regions was determined. The experiment consisted of eight treatments and three replications that were laid out in a split-plot design. When MB was cultivated as an intercrop, yield components were considerably greater [19].

The effects of 0-tillage in an MB-wheat cropping system were analyzed, focusing on soil characteristics and crop yield. The evaluation was set up in a randomized block design with three replicates. In both crops, the infiltration rate arose as the frequency of tillage decreased. When compared to CTM-CTW, ZTM-ZTW enhanced organic carbon in the top layer while slightly decreasing pH [20].

MB was described as a protein-rich legume that enhances soil fertility and diversifies CSs. The 15N natural abundance method was used to look at plant growth and N2 fixation in five MB genotypes grown in "2" soil textures. According to the N2 fixation study's findings, selecting an MB genotype might help reduce agricultural systems' nitrogen requirements [21].

3.2. IMPORTANCE OF MUNGBEAN FOR EARLY MATURITY IN RICE-WHEAT SYSTEMS

MB plays a key role in enhancing the productivity as well as sustainability of RWCSs, especially in regions where RW dominates [22]. MB matures quickly—typically within 60–90 days. This allows farmers to plant it in the space between rice harvesting and wheat sowing. Farmers can maximize land use and obtain additional income from a short-duration crop due to MB's rapid growth [23, 24]. Combining MB with RWCSs significantly enhances productivity, improves soil health, and supports sustainable agricultural practices [25]. Its early maturity and various agronomic benefits make it a valuable component for farmers seeking to optimize their cropping strategies [26].

A study was done on wheat-MB-rice crop rotations in a subtropical humid environment to see how tillage and residue retention affected the soil and crop yields. From the first to the third week of June, MB pods were gathered, while, in the second week of November, rice was harvested. Higher residue retention with minimal tillage improved soil properties and crop yields in upland areas, while deeper tillage methods consistently supported rice production in wetland areas [27].

In the wheat-MB T. aman rice cropping system, (i) overall system productivity, (ii) nutrient absorption, and (iii) nutrient equilibrium were defined. Crops were gathered at maturity. For every test crop, data on yields (kg ha-1) were collected. The average yields for wheat, MB, and T. aman ranged from 1415 to 3096 kg ha-1, 1020 to 1463 kg ha-1, and 2999 to 4282 kg ha-1, respectively, indicating that T4 was the most effective treatment [28].

The potential of MB as an additional crop in the RW system of Punjab and Pakistan was discussed. Since most cultivars matured around 100 to 120 days, which was not suited for the cropping schedule, the area under MB cultivation was shrinking. Therefore, better harvest index genotypes might be selected using synchronous maturity in order to maximize seed output [29].

The impact of tillage and crop establishment on weeds and production in an RW-MB rotation was explained. To assess the impact, long-term research was started in 2015 in Patna, Bihar, India. Weed density and variety were impacted by the tillage and crop establishment practices. Rice output declined slightly in the zero-till RW system, while system productivity remained stable due to improved wheat yield [30].

A long-term bed planting strategy was proposed to enhance productivity and fertility in the wheat-MB-rice cropping system. In this study, system productivity, fertility, and nitrogen use efficiency were evaluated. It was suggested that nitrogen fertilizer rates could be reduced if 30% of the straw from rice and wheat, along with 100% residue retention from MB, were maintained. After thirteen years (15 RW-MB crop cycles) with 30% SR, soil organic matter in PRB's surface soil layers increased by 0.82% [31].

3.3. TYPES OF PHYSICAL AND CHEMICAL MUTAGENS FOR DEVELOPMENT OF EARLY-MATURING MUNGBEAN VARIETIES

By using both physical and chemical mutagens, mutation breeding can be used to create MB cultivars that mature early [32]. To produce early-maturing MB cultivars, deploying physical and chemical mutagens is an effective way [33]. Therefore, genetic varieties are increased, allowing breeders to select desired features while supporting sustainable farming practices [34]. Some of the chemical mutagens used in the growth of early-maturing MB varieties include ionizing radiations such as gamma rays and cosmic rays, which are classified as physical mutagens, as well as alkylating agents, acridine derivatives, nitroso compounds, and hydroxylamine [35]. Table 2 shows the research papers that looked at the effects of physical and chemical mutagens on the growth of early maturing MB varieties. It shows what the papers were trying to find, what they found, and what their limitations were.

Table 2 shows research papers that looked at the effects of physical and chemical mutagens on the growth of early maturing MB varieties, including what they were trying to find, what they found, and what their limitations were.

Mutagens	*Findings*	*Limitations*	*Reference no.*
Gamma radiation (physical mutagen)	From the results, it was found that the development of wild MB pools of diverse origins, along with environmental conditions, might aid in conserving the crop's genetic wealth.	Determining the optimal dose of gamma radiation was challenging. Too-low outcomes might not induce sufficient mutations, while too-high outcomes might lead to lethal effects or severe growth impairment.	[36]
Ethyl Methanesulfonate (EMS) [chemical mutagen]	The detected mutation may be better deployed to generate a plant with an enhanced plant type for mechanical harvesting and to increase yield along with yield-related traits.	EMS can occasionally cause random point mutations, potentially leading to harmful traits and complicating the selection process.	[37]
EMS, sodium azide, and gamma radiation are examples of chemical and physical mutagens.	In each mutagenic treatment, higher doses resulted in maximal seedling damage and death as compared to the control.	Defining the optimal concentration of EMS for mutagenesis could be complex. Due to this, too many mutations may be toxic rather than enough.	[38]
Hydroxylamine [Chemical Mutagen]	As per ROS activities and metabolizing antioxidant enzymes, superoxide dismutase was recommended in tolerant genotypes, presenting first-line protection against salt-induced O2.	Mutations induced by hydroxylamine might not be stable, potentially leading to inconsistent expression of salt tolerance traits across generations.	[39]
Gamma rays [physical mutagen]	Through phenotyping, 64 MYMIV-resistant mutants were found in the M3 generation. Marker-assisted genotyping was also used to find the 22 mutants that really were resistant.	Research involving gamma radiation might face regulatory scrutiny, particularly regarding safety and environmental impact.	[40]

A study was done to look at the genetic differences between 17 MB mutants created by electron beams and gamma rays. The study focused on genetic analysis and differences. According to the data, seeds pod^{-1} had the highest heritability (0.92), followed by pod length (0.89), branches plant^{-1} (0.88), and seed weight (0.88) [41].

A total of 204 germplasm MB (VR L.) genotypes were evaluated for their germination ability at 10°C to identify those with cold tolerance mechanisms. The analysis revealed that TTL1, a noble member, significantly contributed to V. radiate's cold tolerance response at the protein level. Research-assisted breeding efforts also contribute to the sustainable growth of MB [42].

3.4. DETERMINATION OF LD 50

LD50 represents the dose of a substance required to kill 50% of the test organisms in the case of MB seeds [43]. Analyzing the LD50 of a substance for MB (VR) in the context of wheat and rice CSs entails determining the concentration of the substance (such as a chemical mutagen or pesticide) that causes a 50% mortality rate in a specific population [44, 45]. Using the right mutagen dose based on LD50 can change the genes of MBs, which is important for making better varieties with traits that people want [46]. In mutation breeding, establishing the LD50 is a crucial step, as it represents the dose of a mutagen that results in 50% mortality for the treated MB population. Researchers can identify the optimal dose that induces genetic mutations without causing excessive plant damage or lethality by determining the LD50. This balance is essential for maximizing mutation efficiency while maintaining plant viability.

We found the median lethal dose (LD50) and growth reduction dose (GR50) of gamma irradiation (GI) that was meant to cause mutations in wheat by looking at MB. In laboratory studies, the seed germination, survival, and seedling length of irradiated seeds were assessed in 7 days. Under laboratory settings, DBW 187 and K 1006 had LD50 values of 272.71 and 278.61 and GR50 values of 316.22 and 346.73, respectively [47].

A 60Co radiation source was used to expose 100 seeds of the MB cultivar Kampar to different gamma ray doses (0, 100, 200, 300, 400, 500, 600, and 700 Gray). This was done to find the LD50 value. The LD50, based on the survival of the plants, was found to be 619.875 Gray, indicating that the Kampar cultivar exhibited relatively low radio sensitivity to gamma rays.[48].

The LD50 values and combined effects of EMS (Ethyl Methanesulfonate) and SA (Sodium Azide) on seedling traits were studied in MB genotypes, such as "Pusa 1031" and "Pusa 1431." EMS treatment resulted in LD50 values of 58.81 mM for 'Pusa 1031' and 45.04 mM for 'Pusa 1431.' In contrast, the LD50 for both genotypes treated with sodium azide was determined to be 0.047 mM [49].

The morphological changes in MB induced by gamma radiation were observed in this study. A slightly lower dosage of 550 Gy, in addition to the 600 Gy (LD50 value), was used to irradiate the MB seeds. A dosage of 550 Gy of gamma irradiation resulted in a higher frequency of chlorophyll-deficient and morphologically altered mutants [50].

Researchers looked at how gamma irradiation (GI) changed the shape and yield of two types of green gram, called "CO 6" and "CO 8." To see what effect gamma radiation had on both types of V. radiata, progenies from the M2 generation were looked at. The LD50 for both CO 6 and CO 8 was determined to be 450 Gy, based on germination rates observed on the 15th day after planting. However, an inverse relationship was found between the mutagen doses and seedling survival rates 51.

The study presented the effects of gamma irradiation (GI) on germination and seedling development in green gram, with the goal of inducing variety in the MB variety. The focus was on the GAM 8 genotype during the seedling phase, utilizing gamma (γ) irradiation. A significantly low germination percentage of 22.38% was observed at 700 Gy. However, the ideal LD50, determined through Probit analysis based on germination percentage, was found to be 540.26 Gy [52].

3.5. IDENTIFICATION OF DESIRABLE MUNGBEAN MUTANTS FOR SHORT DURATION, HIGH YIELD, AND SYNCHRONOUS MATURITY

MB (VR) is a vital pulse crop known for its nutritional value and rapid growth [53]. Developing mutants with short duration, high yield, and synchronous maturity can significantly enhance productivity and adaptability, especially in inter-CS with crops like rice and wheat [54]. Finding good MB mutants that have a short life span, high yields, and synchronous maturity is very important for improving crop resilience and productivity in many farming systems [55, 56].

High-yielding, extra short-duration MB lines derived from gamma radiation were selected. In the M5 generation, 20 mutant individuals were identified as promising candidates with early maturity, each producing 25 g of seeds per plant. Furthermore, six mutants were recognized for their exceptionally early maturity, yielding 30 g of seeds per plant [57].

A DNA marker was used to develop thirteen mutant lines of DX208 MB that matured simultaneously. The yield was evaluated in these thirteen mutant MB lines from the M6 generation, alongside the DX208 control variety. When analogized to the control variety (DX208) (2.60 tons/ha) ($p < 0.01$), greater yields (2.99–3.29 tons/ha) were produced by DX8-1-28-8B, DX6-5-1-10, DX4A-3-3-1, and DX2-1-26-5 [58].

The study determined how high non-additive gene activity regulated synchronous maturity in MBs. The genetic material included ten lines, three testers, and thirty F1 hybrids. A randomized complete block design was used, with three replications. The expression of all examined features revealed a poor control of additive genes. It was recommended that selection could be delayed until segregating generations [59].

Using multivariate and multi-trait indexing methods, genetic analysis of MB breeding traits was done to find the best genotypes. To detect the best genotypes, 166 diverse MB genotypes were evaluated across two seasons using these indexing techniques. MB varieties may play a significant role in maintaining MB production in a short-duration development environment [60].

4. SUMMARY OF THE STUDY

The CS has a significant impact on the physical and chemical properties of soil, as well as on crop productivity. MB, which is cultivated either as a summer crop or else as a Kharif crop due to its higher degree of heat tolerance (40 C+), is a legume crop. 55–60-day varieties might be cultivated in RW rotation without affecting the current cropping pattern. In the past, when MB cultivars were released for the spring/summer season, their longer duration (70-75 days) made them unsuitable for cultivation in RWCSs. The inclusion of MB legumes in RWCSs has demonstrated potential in increasing the system's productivity and maintaining soil fertility. Thus, the presented MB legumes leave a sizable residual effect, particularly nitrogen, which can be utilized by the following crop.

The RQs are classified as M_1, M_2, M_3, M_4, and M_5 to make the review article more inventive, with the relevant RQs and replies given in Table 3.

Table 3: The related RQs and answers

RQ's	*Answers*
What is the importance of MB in the cropping systems?	*This question aimed to clearly understand the importance of MB in the cropping systems, which was explained in Section 3.1.*
What is the significance of MB for early maturity in RWCSs?	*The question explained the significance of MB for early maturity in RWCSs, which was described in Section 3.2.*
How are the physical and chemical mutagens categorized?	*This question helped explain the categorization of physical and chemical mutagens, which was explained in detail in Table 2 of Section 3.3. Ionizing radiations were physical mutagens, and EMS, hydroxylamine, etc. were chemical mutagens.*
Explain the determination of LD50 in the analyzed research articles.	*The determination of LD50 in the analyzed research articles was explained in detail in section 3.4.*
What are studies associated with the identification of desirable MB mutants?	*The studies associated with the identification of desirable MB mutants were explained in section 3.5.*

According to the study, these mutants were able to reduce the growing period by approximately 15–20 days without causing significant yield loss. These advances could increase the feasibility of MB as a rotational crop, improving SF, reducing fallow periods, and providing additional income to farmers.

5. CONCLUSION

This article clearly explains how induced mutations can be used to improve the genes of MB so that it matures early. The analysis of various research articles revealed that the genetic improvement of MB for early maturity through induced mutation presents the best strategy for enhancing the adaptability of this crop within RWCSs. The early-maturing varieties developed through the approach not only fit within the narrow space between rice and wheat cultivation but also maintain comparable yield and nutritional quality. This made MB an ideal prospect for increasing crop diversity and sustainability in intensive agricultural regions. But, when analyzing the research articles, there was a limitation identified. The performance of early-maturing mutants might vary based on local environmental conditions, such as soil type, water availability, and temperature. To confirm adaptability across diverse agro ecological zones, multi-location trials were necessary. Future researchers should take into account this limitation and conduct additional research on MB using multiple trial locations in their studies. Thus, the successful integration of MB into RW rotations can contribute to enhanced SF, improved resource utilization, and greater economic returns for farmers.

Future Scope

It is incredibly promising for the genetic improvement of mungbean (MB) to achieve early maturity, particularly enhancing productivity and sustainability within Rice-Wheat Cropping Systems (RWCS). Moving forward, it's vital to conduct extensive multi-location trials to ensure these early-maturing MB mutants can adapt to various agro ecological zones. Leveraging advanced molecular breeding techniques like CRISPR/Cas9 will also be crucial in making precise genetic modifications that promote early maturity and other beneficial traits. Additionally, exploring how these early-maturing MB varieties can be integrated into cropping systems beyond RWCS will be essential for improving overall efficiency and soil health. Investigating the development of MB varieties resilient to climate change, including drought and heat tolerance, is a must for future sustainability. Moreover, enhancing the nutritional profile of MB varieties to boost their health benefits for human consumption will be a key area of focus. Finally, understanding how farmers adopt these early-maturing MB varieties and develop the best cultivation practices will maximize their advantages.

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Conflict of Interest

The authors claim no conflicts of interest in publishing this manuscript.

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