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Mutation breeding for improvement of mothbean (*Vigna aconitifolia* (Jacq.) Marechal) adapted to arid conditions: A comprehensive review

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ABSTRACT

The moth bean, belonging to the genus Vigna, is a drought hardy orphan legume that grows well in dry and semi-arid environments. It is particularly common in many parts of Asia, most notably the Indian subcontinent. It differs from other Vigna species and has the potential for strong growth because of its inherent capacity to adapt to a range of biotic and abiotic obstacles to growth. Particularly in rain-fed cropping systems, which make up about 80% of all cultivated land in the world, moth bean can play a critical role in sustaining food grain production, improving nutritional security, and providing a source of income to resource-poor farmers amid rises in global temperatures and frequent drought occurrences. Unlike conventional breeding, mutation breeding through induced mutagenesis presents a powerful tool for creating variability and offers scope for selection and genetic improvement of Mothbean with narrow genetic base. The present paper reviews the improvement of various traits of mothbean through mutation breeding as reported by various scientists.

Keywords: Mothbean, Pulses, Gamma Radiation, EMS, Mutation Breeding

INTRODUCTION

The genus *Vigna* is an important taxon of leguminous plants and comprises about 100 species distributed in Asia, Africa, America, and Australia (Lewis *et al.* 2005). Moth bean [*Vigna aconitifolia* (Jacq.) Marechal], synonym *Phaseolus aconitifolius* (Jacq.) is an orphan legume belongs to the family *Fabaceae*, (*Leguminosae*) and subfamily *Papilionoideae*. However, Marechal *et al.* (1978) proposed changed nomenclature of *P. aconitifolia* (Jacq.) to *V. aconitifolia* (Jacq.) Marechal. It is considered as one of the most primitive species among the *Vigna* genus, with respect to its evolution (Smartt, 1985).The genomes of the Vigna species and particularly those of the Asian Vigna are highly conserved (Kaga *et al.* 2010), and all species are diploid with the same number of chromosomes (2n = 2× = 22). The genome sizes of *Vigna* species are highly variable, ranging from 416 to 1,394 Mb (Parida *et al.* 1990). A comparative genome analysis done by Yundaeng *et. al* (2019) showed high genome synteny between moth bean and mungbean (*Vigna radiata*), adzuki bean (*Vigna angularis*), rice bean (*Vigna umbellata*), and yardlong bean (*Vigna unguiculata*).The karyotype formula, as per Bhatnagar *et al.* (1974), is 1-long (2.7-3.5μm) sub-median centromere + 5 medium (1.96-2.6 μm) sub-median centromere + 1 medium median centromere and four small (<1.95μm) median centromere. Moth bean [*Vigna aconitifolia* (Jacq.) Maréchal], a native crop of the Indian subcontinent is capable of sustaining long dry spells and high temperatures beyond 40°C (Sharma *et al.* 2003). In the past, the wild progenitor of the moth bean was not precisely described, but it reportedly occurred in India (Arora and Nayar 1984). Purseglove (1974) suggested that the moth bean was domesticated in India, Pakistan, Afghanistan, Sri Lanka, and Myanmar. Whyte *et al.* (1989) considered *Vigna trilobata* (L.) Verdc. to be the wild form of moth bean. Moth bean is a short semi-erect hairy annual bushy herb, with a prostrate creeping habit. The seeds of moth bean contain (per 100 g edible portion) water 9.7 g, protein 22.9 g, fat 1.6 g, carbohydrate 61.5 g, Ca 150 mg, 381 mg, P 489 mg, Fe 10.9 mg, Zn 1.9 mg, vitamin A 32 IU, thiamine 0.56 mg, riboflavin 0.09 mg, niacin 2.8 mg, vitamin B6 0.37 mg, folate 649 μg and ascorbic acid 4.0 mg. The essential amino-acid composition per 100 g edible portion is: tryptophan 147 mg, lysine 1248 mg, methionine 220 mg, phenylalanine 1028 mg, valine 734 mg, leucine 1541 mg and isoleucine 1138 mg (Singh and Ansari, 2018; Sharma *et al.,* 2000). Mutation is a change in the genome sequence of an organism which leads in creation of variation

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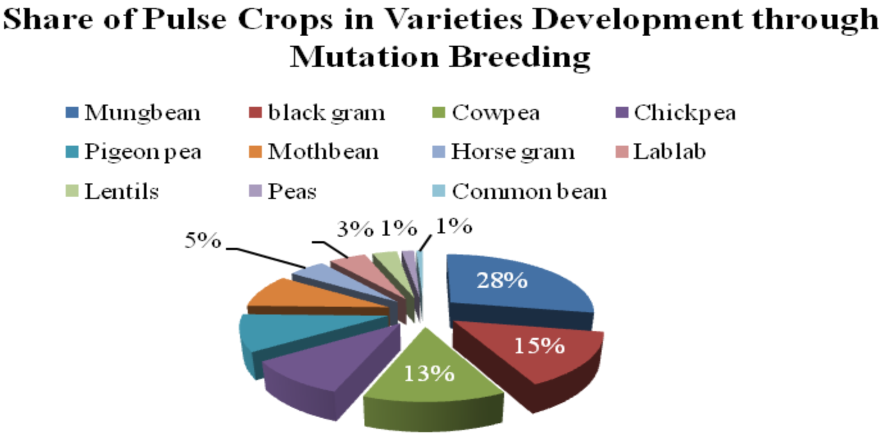
(Ripley, 2013). Purposeful utilization of the induced mutation for crop improvement is known as mutation breeding (Pathirana, 2011). Induced mutation or mutagenesis is the sudden heritable changes in the genome of an organism not caused by genetic recombination or segregation but induced by physical, chemical, or biological agents (Roy chowdhury & Tah, 2013).

Need of mutation breeding in mothbean

In recent years mutation breeding has been gaining ground for inducing genetic resources (Datta *et al.* 1993). Induced mutations contributed significantly to plant improvement programs, even though most of the induced mutations are recessive and deleterious from the breeding point of view (Maluszynski *et al.* 1995). In general, hybridization is difficult in pulses due to cleistogamous flower (Agrawal *et al* 2001). The success of hybridization in moth bean is very low because of certain reasons viz; low cross ability per cent between cultivated and wild species , small flower size, flower shocking, physiological changes during emasculation and pollination, failure of pollen tube to reach to stigma as reported by (Ahn and Hartmann, 1977; Chen *et al.,* 1983; Satija and Ravi, 1997). Genetic improvement through conventional hybridization is cumbersome in mothbean probably due to tiny anthers and poor pod setting (Sharma and Majumdar, 1982). A high proportion of pod settings during artificial hybridization are a prerequisite for successful employment in legume genetic improvement programs. Mahla and Sharma (2022) reported that Pollination without emasculation in mothbean produced about 2-5% hybrid seeds successfully. They achieved about 8% success in the hybrid pod setting. However, Chavan *et al.,* 1966 attempted cross between *Vigna aconitifolia* X *V. tribolata* and F_1 were partially semi seed fertile. Further, Henry (1985) made crosses and found better performance of hybrids over parental variety but subsequent were not tested. In 2006, the first variety, CAZRI Moth-2 (CZM-45), developed through hybridization using Jadia and RMO-40 as parents, was released. The release of early maturing variety RMO-40 in 1994 developed interest in mutagenesis and subsequently series of a short duration varieties was developed through mutagenesis.

Induced mutation in mothbean

Globally, 331 pulse crop varieties have been released through mutation breeding, with India contributing 122 of these. In India, mung bean leads with 34 varieties, followed by black gram (18), cowpea (16), chickpea (12), pigeon pea (12), moth bean (11), horse gram (6), Lablab (6), lentils (4), peas (2), and common bean (1). The dominance of gamma ray-induced mutant varieties highlights the effectiveness of physical mutagens in developing new crop varieties (Jegadeesan & Punniyamoorthy, 2023).



Source: IAEA-MVD (2022)

The commonly held belief is that there's a restricted amount of genetic diversity in mothbean, and expanding this diversity could improve breeding initiatives. As a result, research has been focused on identifying effective mutagenic agents for mothbean, aiming to generate new mutated versions that can be incorporated into breeding programme.

However, it is evident that thermo tolerance and biochemical parameters can be efficiently altered and improved through mutagenesis (Harsh *et al.,* 2016). The induction of mutation has already been recognised as a potential technique for crop improvement since the discovery of mutation effects of X-rays (Stadler, 1928). By exposing plants to radiation or other mutagenic agents, scientists can induce random changes in the DNA sequence, including alterations in genes responsible for various traits such as yield, disease resistance, or nutritional content. These induced mutations can sometimes produce beneficial changes that may not occur naturally or through traditional breeding methods. Induced mutations can help regenerate and restore diversity lost during evolution due to adaptation to various stresses (Haq and Shakoor 1980). Therefore, mutation breeding or induced mutation holds great promise for enhancing traditional agricultural arid legumes like Mothbean.

Chlorophyll Mutants

Chlorophyll mutations are generally not useful for plant breeding purpose because of not having any economic value due to their lethal nature, their study could be useful in identifying the suitable mutagen and threshold dose of mutagen that would increase the genetic variability and number of economically useful mutations in the segre-gating generations (Wani and Anis 2004). The reliability of Chl (chlorophyll) mutations' occurrence rate is regarded as a trustworthy measure for assessing the efficiency and potency of mutagenic agents, along with their optimal concentrations. This evaluation helps in determining their suitability for application in mutation breeding (Gustafson, 1951 and Monti, 1968). So chlorophyll mutations are considered as indicators of mutability. Jain *et al.* (2013) produced two types of chlorophyll mutant i.e., *albino* and *xantha* in four varieties of moth bean, namely, RMO-40, RMO-257, Jwala and CZM-1, which seed were treated by four chemical mutagens viz. EMS, MMS, SA and HA. They recorded less frequency of chlorophyll mutants in which *albino* type was less frequent. Khadke and kothekar (2011) recorded four types of chlorophyll mutants such as *xantha, chlorine, albino* and *viridis* by treating seeds of mothbean with EMS and SA, where in case of EMS they observed that frequency of chlorophyll mutants was 2.29% at 0.05%, 3.36% at 0.10% and 3.39% at 0.15%, while it was 1.39% at 0.01, 2.76% at 0.02% and 3.38% at 0.03% in case of SA treatments.

Early Maturing Mutants

Early maturity with high grain yield is prerequisite traits for crops particularly in arid region. Use of induced mutations for obtaining early maturing cultivars has been a frequent breeding objective (Micke 1979). Intermittent drought significantly decreases plant water potential, relative water content, net photosynthesis rate, chlorophyll content, starch, soluble protein, and nitrate reductase activities ((El-Kramany *et al.* 2003; Garg *et al.* 2004a). Conversely, it increases reducing sugars and free amino acids (Garg *et al.* 2004b). These physiological changes result in a substantial reduction in yield. Kumar (2000) reported a semi-erect mutant, CAZRI Moth-l, induced from dew bean cv. Jadia at 30 kR dose of ^{60}Co gamma-rays matured 10 days earlier than the parental variety (85 days). The varieties RMO-257, RMO-225, RMO-435, RMO-423, and RMO-2251 (Table 1) developed through mutation breeding at Rajasthan Agricultural University (RAU), Bikaner, are noted for their early maturity with a maturity range of 60–67 days, exhibited markedly altered plant architecture, were semi-erect to erect in growth habit, and displayed synchronous maturation. The shortened growth period enabled these cultivars to evade terminal drought stress and avoid severe incidences of yellow mosaic disease (YMD) and *Cercospora* leaf spot. Such traits render them particularly suitable for cultivation in arid regions receiving low annual rainfall (200–250 mm) and having a short growing season (Sharma, 2023; Chandora and Rana, 2023).

Seed yield Mutants

In 2013, Jain and colleagues subjected four moth bean varieties (RMO-40, RMO-257, Jwala, and CZM-1) to four distinct chemical mutagens, namely, EMS, MMS, SA, and HA. In the third generation (M3), they observed that 15 progenies displayed significantly improved seed

Table 1: Mothbean mutant cultivars have been released in India and have been certified for cultivation

Mutant Variety	*Year of Release*	*Type of Mutant development*	*Character improvement*
CZMO 18-5 (CAZRI Moth 5)	2022	Mutant of GMO-2 using Gamma rays – 200 Gy	Light green foliar till maturity, high yield potential yield, better disease resistance
CZMO 18-4 (CAZRI Moth 4)	2022	Mutant of CAZRI Moth-2 using Gamma rays – 200 Gy	High potential with better disease resistance
RMO-2251 (Marudhar)	2016	Induced mutant from moth bean variety RMO-225	Erect stem with 3-5 branches, fodder remain green upto maturity, average incidence of YMV
Rajasthan Moth-257(RMO-257)	2005	A mutant derived from jadia moth through 30 kR + 0.6% EMS.	tolerant to yellow mosaic virus, tolerant to thrips and white flies
CAZRI Moth -3 (CZM-99)	2005	Induced mutant from moth bean variety RMO-40	Erect upright growth habit characterized with synchronous maturity, foliage remains green even when whole pods mature, showing escaping against ymv, highly stable in productivity with high degree of drought tolerance potential.
RMB-25	2004	Induced mutant from moth bean variety RMO-40 using Gamma Radiation, a dose of 300 Gy was employed	Early maturing, Multi clustered, semi-spreading type, moderately tolerant to YMV and bacterial blight
RMO-423	2004	A mutant derived from moth bean variety, RMO-40	Late appearing young leaves are deeply penta-lobbed while, other leaves are broad and palm, shaped.; stem pigmentation absent
MARU BAHAR (RMO-435)	2002	A mutant derived from RMO-40 through 60 kR gamma radiations.	Erect, medium nearly, good yield potential, leaves green coloured, mutant from RMO-40
CAZRI MOTH-1 (CZM-79)	1999	A mutant from jadia cultivar at 30 kR dose of ^{60}Co gamma-rays	Semi-erect, Bolder grain (l000-grain weight 32g), more pods/plant (67), higher grain protein (25%), more resistance potential towards Yellow Mosaic Virus disease
MARU VARDHAN (RMO-225)	1999	A mutant derived from jadia through 40kg.+0.6% EMS	Primary branches /plants:6-7;cluster/leaf axis: 2-3;cluster/plant : 14 ; pods/cluster: 7.6;pods/plant: 116;seed/pod : 5-6;peduncle length: 2.5;pod length: 5;leaf type : uniform, shallow lobbed medium leaves; test weight:28; grain colour: light brown.
FMM-96	1997	A mutant selected from variety Jadia after its irradiation with 30 kR. dose of gamma rays.	Erect plant habit 6-7 pods/cluster, leaflets light green and less dissected.
RAJASTHAN MOTH-40	1994	A mutant derived by treating seeds of Jwala variety with 400 Gy gamma rays + 0.1% EMS.	First early variety, erect growth, suited to low rainfall, widely grown in low rainfall zones, short stature, less biomass. Its average seed yield ranged from 600 to 900 kg/ha (compared to 400-500 kg/ha of parent) in All India Coordinated Varietal trials.
MARU MOTH-1	1988	Variety evolved through chemical mutation.	A semi-spreading growth habit and a peduncle length ranging from 0.5 to 3.0 cm, Resistant to yellow mosaic virus
JMM-259 (Maru-Moth-1)	-	A mutant from Jadia variety.	Plant height 30 cm, with prominent main axis, semi-spreading with dark green leaves. Pods in clusters on main as well as primary and secondary branches. Suitable for early and late sown conditions. This variety is more suitable than jadia during drought years by virtue of earliness in maturity.

Source: https://seednet.gov.in/SeedVarieties/CentralVariety.aspx & https://nucleus.iaea.org /sites/mvd/SitePages/ Variety.aspx yield per plant, exhibiting yield-contributing traits of greater magnitude compared to the best check variety, RMO-225. Henry and Daulay (1983) treated seeds of Jadia variety of mothbean with aqueous solutions of EMS with 0-3% concentrations and isolated 25 mutants which showing a higher number of pods than the parents in the M_2 generation. They observed that mutants JMM-211, JMM-259 and JMM-60 consistently maintained superiority in yield and pod number per plant over then parent 'Jadia' under varying condition of rainfall. Kumar (2000) recorded mean grain yield potential of mutant CAZRI Moth-I induced from dew bean cv. Jadia at 30 kR dose of ^{60}Co gamma-rays, was realized almost 10.5% higher over the national and 22.0% higher over the regional checks, respectively.

Plant type Mutants

Kharkwal (2000) successfully induced dwarf, bushy and compact plant mutants in pulse crops such as mungbean, chickpea, cowpea, and others. Jain *et al.* 2013 identify and classify various morphological mutants and categorized these mutants into ten distinct classes, namely: revertant mutants, bushy mutants, bifurcated stem mutants, bolted mutants, sterile mutants, miniature mutants, branched mutants, mutants with increased peduncle length, mutants with a spreading habit, and mutants with increased primary branches. Henry and Daulay (1983) observed transformation from a spreading "viny" growth habit to a more compact and manageable "semicompact" form.

Protein Mutants

According to Patil *et al.* (1998), Gamma radiation treatment induced differed in nutritional composition from their parent PLMO-10. They found increased fat, crude fibre, iron and calcium contents in the grain while some mutants showed increased protein, and tryptophan contents. The mutant MB-M-10 had almost double the Iron content found in majority of grain legumes. A semi-erect mutant CAZRI Moth-I induced from dew bean cv. Jadia at 30 kR dose of ^{60}Co gamma-rays had higher grain protein (25%) than the national check Maru Moth-I (22.7%) (Kumar D., 2000). Khadke and Kothekar (2018) observed that some mutants exhibited a reduction of trypsin inhibitor (TI) content and seed proteins while Seeds of local variety of moth bean were treated with EMS and SA. Trypsin is an enzyme that plays a crucial role in the digestion of proteins. It breaks down proteins into smaller peptides, facilitating their absorption in the digestive system. The presence of trypsin inhibitors can interfere with this process. Khadke and Kothekar (2018) studied the effect of EMS and SA on moth bean trypsin inhibitor content. On electrophoresis, various viable and micro mutants of moth bean were found to have between three and seven iso-inhibitors of trypsin. Some viable mutants had considerable differences in their TI profiles when compared to the control. The TI content of these mutants reduced by 25 to 45 percent, and the seed protein content of micro mutants and viable mutants differed significantly.

YMV Resistant Mutants

Yellow mosaic virus (YMV) is the most destructive viral disease affecting yield potential of mothbean both qualitatively and quantitatively and ability to cause yield loss up to 85% (Nene, 1972; Varma and Malathi, 2003). The radiation-induced mutant cultivar CAZRI Moth-1 of moth bean has been developed with resistance to Mungbean yellow mosaic virus disease (Basandrai *et al.,* 2011)

CONCLUSION

Moth bean (*Vigna aconitifolia*) stands out as a leguminous crop with significant potential due to its resilience in harsh environments and its nutritional value. Despite its importance, traditional breeding approaches face numerous challenges due to the plant's genetic characteristics and reproductive biology. Induced mutagenesis has emerged as a valuable tool for enhancing the genetic diversity and improving various traits of moth bean. This technique has proven effective in addressing key agronomic challenges, including yield enhancement, early maturation, disease resistance, and nutritional quality. Research has demonstrated the effectiveness of different mutagenic agents in generating beneficial mutations in moth bean. These induced mutations have led to the development of several improved cultivars with desirable traits such as increased yield, early maturity, and enhanced resistance to biotic stresses like Yellow Mosaic Virus (YMV). Chlorophyll mutations, while not directly useful for breeding, serve as reliable indicators for the effectiveness of mutagenic treatments. Additionally, significant progress has been made in producing mutants with improved seed yield, protein content, and plant architecture. Mutant varieties like CAZRI Moth-1 and Maru Moth-1 have showcased the potential of mutagenesis in improving moth bean's adaptability and productivity. The successful incorporation of these mutants into breeding programs highlights the viability of this approach in overcoming the limitations of conventional breeding methods. Continued research and breeding efforts will be essential to fully exploit the genetic potential of moth bean, ensuring its contribution to food security and sustainable agriculture in arid and semi-arid regions**.**

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