



GENETIC IMPROVEMENT OF HORSE GRAM (*MACROTYLOMA UNIFLORUM* L.): PROSPECTIVE AND CHALLENGES

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ABSTRACT

Horse gram (*Macrotyloma uniflorum* Lam (Verdc.) is an undervalued and under studied legume though is a good source of proteins, carbohydrates and energy. Horse gram (*Macrotyloma uniflorum* L) is a vital yet underutilized pulse crop due to limited genetic research and improvement efforts. This review explores Prospective, challenges and promising avenues like QTL mapping and Marker-Assisted Selection for targeted breeding, along with the exciting possibilities of genomic selection and speed breeding for faster variety development in horse gram Even gene editing with CRISPR-Cas holds promise, but challenges like incomplete genome knowledge need to be addressed. Additionally, bioinformatics and systems biology approaches offer powerful tools to understand the complex interaction of genes and traits in horse gram. While limitations exist, actively pursuing these advancements can unlock horse gram (*Macrotyloma uniflorum* L) full potential, significantly impacting global food security and sustainable agriculture.

KEYWORDS: Horse gram, genomic resources, MAS breeding.

INTRODUCTION

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc. is a hardy pulse crop of semi-arid tropics that has been poorly studied. Despite its current and historical importance to the diet of a large part of the population in India, there are entrenched biases against horse gram, as it is considered a low status food of the poor, particularly in southern India (Kadam *et al.* 1985; Ambasta 1986). The limited scientific knowledge of this crop is reflected in its status in textbooks, even those produced in India, its main country of production. Horse gram has received far less research than pulses of higher status, such as Indian *Vigna* (*V. radiata* (L.) Wilczek, *V. mungo* (L.) Hepper) or pigeonpea (*Cajanus cajan* (L.) Millsp). Indeed, whilst both the Indian *Vigna* spp. and *Cajanus* have received monographic studies of wild relatives (Tomooka *et al.* 2014; Khoury *et al.* 2015; Mallikarjuna *et al.* 2011) and genetic studies of relationships with wild relatives (Aruna *et al.* 2009; Kassa *et al.* 2012; Saxena *et al.* 2014). Only recently has small scale genetic research been conducted on horse gram (Sharma *et al.* 2015). Horse gram earned its common English name as it has been used as fodder to horses and cattle for centuries (Watt 1889–1893), and less often eaten by the British or higher status Indians;

often in India as a supplement to the bulky-straw fodders used (Nezamuddin 1970). Despite such prejudice, horse gram (*Macrotyloma uniflorum*) ranks among the most important pulse crops of India.

Origin, Classification and Genomic relationship

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc., also known as *Dolichos biflorus* and *Dolichos uniflorus*, is a self-fertile cleistogamous species with a diploid chromosome number of $2n=20$. It is an annual short-day (SD) climbing legume with a thermo- and photosensitive phenology. However, some lines show day-neutral characteristics as well, and these mature within 120–180days. Horse gram is a member of the family Fabaceae, and it is largely cultivated for food in countries in temperate and subtropical regions, including in India, China, Philippines, Bhutan, Pakistan, Sri Lanka, and Australia (Krishna, 2010).

It is an excellent source of phosphorous, iron, molybdenum, vitamins (carotene, thiamine, riboflavin, niacin, and vitamin C), and calcium (Sodani *et al.*, 2006). Its seeds contain about 23% protein and are richer in lysine (an essential amino acid) content than pigeon pea and chickpea, making it a good complement to a cereal-

based diet. Its seed and its extracts are widely prescribed in Ayurvedic medicine to treat numerous health disorders, including rheumatism, renal stones, worm conjunctivitis, and piles.

The seeds also contain important anti-nutritional proteins, such as trypsin inhibitors and lectins (Liener, 1970). Its high levels of dietary fiber and molecular tannins, low levels of lipids and sodium, and slowly digestible starch make it suitable for a cardio diet and for diabetic and obese patients (Bazzano *et al.*, 2001). Furthermore, horse gram is particularly rich in the antioxidants such as polyphenols, proteins, and flavonoids. Because of its large amount of digestible protein and small amount of digestive inhibitors, it is widely used as feed for milch animals and horses. Overall, horsegram has the potential to serve as food, as forage, and as a nutraceutical to combat malnourishment (Morris, 2008).

Despite these benefits, a lack of genomic data has impaired the crop improvement of this species. To date, there have been a very limited number of reports on the transcriptomics and identification of micro RNAs and simple sequence repeat (SSR) markers (Bhardwaj *et al.*, 2013; Kaldate *et al.*, 2017) in this species. Genotyping using next-generation sequencing (NGS) technologies has made it feasible for any crop to acquire many genome-wide single nucleotide polymorphism (SNP) markers over a short period, which can be used for genetic estimation of diversity, association mapping, and genetic enhancement through molecular breeding.

Phylogeny

Horse gram is one of the prospective crops for the future. It is a crop with great nutritional value as well as healing properties. Development of elite cultivars with the purpose of improving crop and commercialization appears to be a necessity in the near future due to its beneficial nutritional value, medicinal value, and superior climate resilience. The development of karyotypes for each species of horse gram is necessary for phylogenetic studies.

Germplasm resources

The lack of genomic resources and the scarcity of scientific research on this neglected legume prompted us to describe the first whole-genome sequence of the high-yield horsegram variety. The genomic resources of legume crops have opened the door to translational research with greater success in marker-assisted selection, genomic selection, and high-yield varieties. A comparison of the 24,521 gene families of horse gram with five sequenced legumes (adzuki bean, common bean, mung bean, barrel medic, and soybean) showed that 79% (19,456) of the genes are orthologous to those of the other five legumes. These conserved orthologous genes also have conserved gene functions, offering an opportunity for comparative functional genomics studies in horse gram and other species (Varshney *et al.*, 2013).

A minimum of 77.54% of the predicted horsegram genes have a history of duplication. Over the same period of time, 3,044 genes remained SCOs without duplication or loss, indicating the essential role of these genes during the evolution of the legumes.

Genome sequencing

The horsegram variety PHG-9 was chosen for whole genome sequencing, as it has had wide cultivation for its high productivity. The reference genome sequence and re-sequencing of 40 germplasm presented in this manuscript have tremendous scope for the genetic improvement of the horsegram in the near future. The combination of short- and long-read sequencing technologies helped us to assemble up to 83.53% of the total estimated genome size (334Mb). Previous genome sequencing studies of important legumes have reported similar genome coverage (Schmutz *et al.*, 2010, 2014; Varshney *et al.*, 2012, 2013). Further advancements in long-read sequencing technologies and construction of high-density linkage map will facilitate building pseudo molecule-level genome assembly for horsegram in near future. However, the unassembled horse gram genome, which usually consists of repeat related sequences, may contribute to a slight increase in repeat content.

Genetic improvement of horse gram

Horse gram (*Macrotyloma uniflorum* (Lam) Verdec.) is highly proteinaceous important food legume crop belongs to family Fabaceae. Conventional breeding methods are not useful in enhancing production of horse gram because of low genetic variability. Due to limited genetic diversity and unavailability of good varieties, it is susceptible to several diseases and pests. Induced mutations for various characters using physical and chemical mutagens haven been widely used for induction of mutations in horse gram. Mutagens such as gamma radiations, ethyl methane sulfonate (EMS) and sodium azide (SA) were employed to induce genetic variability in different horse gram cultivars. Different kind of mutations viz., chlorophyll mutations, plant growth habit, pod mutations (small pod, early and tall), seed mutations, maturity mutations (early and late) and yield characteristics were observed in M2 and M3 generations.

However, a limited number of efforts have been directed towards understanding quality attributes and resistance to diseases and pests. Mutagenesis if combined with genomic technology may provide powerful tool for the discovery of novel gene alleles underlying several agronomically important traits in this unexploited crop.

The genetic base of horse gram is relatively narrow particularly with respect to traits such as disease resistance. The genetic base of the crop can be broadened by exploiting the wild relative gene pool, molecular breeding and mutation breeding programs. The introgression of traits from the wild relatives can be facilitated by developing closely linked markers for these

traits. Moreover, induced mutations using physical and chemical mutagens provide an opportunity to create genetic variability which can then be utilized in physiological or genetic studies and cultivar development. The available genetic resources in horse gram and mutants induced through mutagenesis if combined with genomics it may prove a powerful tool for genetic improvement of horse gram. Though gamma radiations have been proven a powerful tool for generating genetic variability in horse gram, chemical mutagens may provide a good scope for selection, as a tool for inducing alterations in the genotype to enhance the variability of characters in horse gram.

Hence it is necessary to pay attention to this crop to improve its per hectare yield, disease resistant (YMV-Yello Mosaic Virus), early maturity and grain quality through mutation breeding. Despite enormous potential, the crop has not gained the popularity and is still considered as poor man's food. This review therefore is an attempt to compile the meager information available on mutation research and present status to make the crop competitive and revamp its cultivation.

Identification of QTLs and Marker Assisted Selection (MAS)

Horse gram is a nutritious and stress-resilient legume, referred to as an indicator crop. However, to date, little

scientific work has been devoted to its improvement. Considering that horsegram is important for future sustainable nutrition and food security, it is important to understand its genetic architecture using genomic resources. The first framework linkage map was recently constructed using 211 SSR markers, and the same study mapped QTLs for agronomically important traits (Chahota *et al.*, 2020). This crop has many desirable traits, including drought tolerance, antioxidant activity, antimicrobial properties, and high protein and iron contents. The development and utilization of genomic resources for genetic improvement may be extremely useful.

Availability of molecular markers

The inherent drought tolerance mechanism of horse gram is most likely due to the presence of these TFs in its genome. The identification and cloning of novel TFs in horse gram will assist in understanding signaling and transcriptional regulation for various biotic and abiotic stresses, and the same information can be used to develop varieties with broad-spectrum biotic and abiotic tolerance. Horse gram exhibits an innate defense against pest/pathogens from the genes encoding lectin and lipoxygenase-like functions (Roopashree *et al.*, 2006).

Table 1: List of molecular markers used for construction of linkage map of horse gram.

S. No	Marker	Markers screened	Polymorphic markers	Percent polymorphism %	Markers mapped	Source
1	HUGMS	63	36	57.14	15	EST SSRs (Sharma <i>et al.</i> , 2015)
2	MUMS	200	55	27.50	45	Genic SSRs (Sharma <i>et al.</i> , 2015)
3	MUMST	100	37	37.0	22	Genic trirepeats (Sharma <i>et al.</i> , 2015)
4	MUMSD	103	44	42.72	20	Genic Direpeats (Sharma <i>et al.</i> , 2015)
5	MUGSSR	99	42	42.42	31	Genomic SSRs (Chahota <i>et al.</i> , 2017)
6	MUSSR	50	24	48.0	16	Genomic SSRs (Chahota <i>et al.</i> , 2017)
7	MUGR	94	30	31.91	20	Genomic SSRs (Chahota <i>et al.</i> , 2017)
8	MUD	96	28	29.17	13	Genomic SSRs (Kaldate <i>et al.</i> , 2017)
9	MUGSR	48	8	16.67	7	Genomic SSRs (Chahota <i>et al.</i> , 2017)
10	RAPD	450	55	12.22	22	Operon Tech, United States of America and Fred Muehlbauer, United States of America
11	Drought specific primers	24	5	20.83	4	Charu and Manoj 2011
12	RcSSRs	196	88	44.90	56	Sato <i>et al.</i> , 2005
13	MtSSRs	104	33	31.73	17	Eujayl <i>et al.</i> , 2004
14	COS	384	8	2.08	7	Douglas R. Cook, UC, Davis, United States of America
—	Total	2011	—	493	24.52	29

Genome editing

Horse gram is a relatively unexplored legume with a plethora of nutritional benefits, a high protein content, inherent resilience to biotic and abiotic stress, and adaptability to climate change. This legume, with its myriad advantages, has yet to gain importance nationally and internationally in terms of its genetic improvement and popularization. Genome-wide association studies (GWAS) have gained popularity as a valuable method for studying complex traits. This was fuelled in large part by advancements in genomic technology, which enabled us to look at genome-wide genetic variations through a variety of genetic materials. As opposed to naive approaches, the implementation of the mixed model system in GWAS significantly reduced the number of misleading false associations. Many techniques have now been introduced to increase processing efficiency or improve statistical capacity in this based on current framework. Genomic variations linked to typical agronomic phenotypes and molecular and biochemical phenotypes have been discovered using these approaches. As an outcome of these linkages, gene cloning and rapid crop breeding employing marker-assisted selection or genetic engineering are now achievable. Mining of high effect rare variants, simulated correlations, refining in options availability of GWAS models and using its findings to achieve deep insights in molecular processes are all current research topics. Further advancements in GWAS techniques and technologies can be supported by ongoing studies in these fields. Keeping in view the depleting water resources and increasing temperature, it is important to identify water use efficient genotypes in different crops to cope with future challenges.

Functional genomics, Bioinformatics and Systems biology approach

Functional genomics is a powerful approach to understanding the roles of genes in complex biological systems and can be highly beneficial for improving crops such as horse gram (Sun X. *et al.*, (2018)). Another critical tool in functional genomics is insertional mutagenesis, such as transfer DNA (T-DNA) tagging, and adapting T-DNA tagging to horse gram would enable the systematic characterization of genes, potentially leading to the discovery of traits beneficial for crop improvement (Varshney *et al.*, (2012)). Functional genomics databases creation for horse gram could provide comprehensive resources for storing, querying, and analyzing large-scale data sets and these databases integrate various types of data, including metabolite profiles and small RNA (sRNA) data, and offer tools for identifying co-expressed genes and significant biological processes (Varshney *et al.*, (2013)).

Moreover, the integration of bioinformatics and systems biology in horse gram research could advance the understanding of its genetic and molecular underpinnings, facilitating the development of improved crop varieties (Xue *et al.*, (2013)). Bioinformatics tools

could be used to analyze the horse gram genome, identify genes, and uncover genetic variations, while systems biology approaches map out complex interactions between genes, proteins, and metabolites (Yang Z. *et al.*, (2007)). This integration allows for comprehensive analysis of gene expression, regulatory networks, and metabolic pathways (Yang *et al.*, (2015)). By combining these approaches, researchers can better understand the biological processes underlying key traits, such as stress tolerance and nutrient utilization, and apply this knowledge to optimize breeding strategies and develop resilient, high performing varieties (Yano *et al.*, (2000)).

CONCLUSIONS

Horse gram (*Macrotyloma uniflorum* (Lam), a nutritious and climate-adaptable crop is undeniable, but genetic improvement hinges on advancements in breeding techniques. QTL identification and Marker-Assisted Selection offer promising avenues to target desirable traits like yield and disease resistance. Additionally, CRISPR-Cas gene editing holds immense promise for precise improvement, although challenges like incomplete genome knowledge and transformation protocols need to be addressed. By overcoming these limitations and actively pursuing these genetic improvement strategies, researchers can unlock Horse gram (*Macrotyloma uniflorum* (Lam)) full potential, significantly contributing to global food security and sustainable agriculture.

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