

Stress Tolerant Orphan Legumes

# MONOGRAPH SERIES



# Horsegram (*Macrotyloma uniflorum* (Lam) Verdc.)





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# **Stress Tolerant Orphan Legumes**

Monograph Series



**The Kirkhouse Trust (KT)** is a UK-registered charity founded by Sir Edwin M. Southern to fund the improvement of legume crops that are important for food and nutrition security in African countries and India and to promote scientific education. The origins of KT are entwined with the development of Sir Ed's molecular biology company, Oxford Gene Technology (OGT). In 1997, Oxford University assigned Sir Ed's microarray patents to OGT in exchange for 10% of the equity. In 2000, OGT's income began to grow, and KT was registered as a charity and endowed with an initial donation from the company.

KT's funding model aims to address its twin objectives of improving legume crops, which are important for smallholder farming systems in target countries and raising national scientific capacity. KT has a hands-on strategy, with a team of international scientific consultants working closely with the Principal Investigators (PIs) and students they mentor, providing technological backup as needed, and hosting PIs and students for study visits in their laboratories.

The STOL consortium was established in 2018 under the Promoting India-Africa Framework for Strategic Cooperation Initiative in partnership with the Indian Council of Agricultural Research (ICAR), Department of Agricultural Research and Education (DARE), Ministry of Agriculture and Farmers Welfare, New Delhi, India. The programme aims to facilitate the introduction and exchange of stress-tolerant orphan legume varieties among partnering Indian and African institutions and assess the relative response of selected species to the higher levels of abiotic stresses expected because of climate change. Crops have been identified as potentially having a crucial role in adapting to climate change in arid parts of Africa and India, and selected species are likely to become the focus of KT breeding programmes in the medium to longer term.

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- **Credit:** Rakesh Kumar Chahota, Professor, Department of Agricultural Biotechnology, CSK HPKV, Palampur, Himachal Pradesh, India

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## FOREWORD

Roughly 2.5 billion people (30% of the world's population) live in semi-arid regions, and approximately a third of these people depend on agriculture for their food security and livelihood. Crop production in these regions has always faced challenges associated with excess heat, drought, a highly variable climate, land degradation, and a loss of biodiversity, which has been exacerbated in recent times by climate change, limited access to technology, poor market linkages, weak institutions, and lack of national and international partnerships. A possible strategy to cope with



climate change is to switch from the cultivation of current crops to ones which are more drought-hardy. These include several minor legume crops, commonly known as orphan legumes, currently being grown to a limited extent in the drier regions of both Africa and Asia to provide food and nutritional security to households. These species have remained relatively neglected by both researchers and industry because of their limited economic importance in the global market.

To promote these orphan legumes, the Kirkhouse Trust initiated a consortium programme on "Stress Tolerant Orphan Legumes (STOL)" in partnership with several African countries and India. The STOL programme aims to facilitate the introduction and exchange of stress-tolerant orphan legume among partnering Indian and African institutions and assess the relative response of selected species and varieties to the higher levels of biotic and abiotic stresses expected because of climate change.

To facilitate the better understanding and cultivation of these new crops among Indian and African partners the STOL project is supporting the publication of a series of monographs for selected orphan legumes and Horsegram (*Macrotyloma uniflorum* (Lam) Verdc.) is one of such crops.

I congratulate the authors of this monograph Dr Rakesh Kumar Chahota, Professor, Department of Agricultural Biotechnology, CSK HPKV, Palampur, Himachal Pradesh, India for compiling and synthesising information to bring out the horsegram monograph, which the Kirkhouse Trust is pleased to publish as part of the STOL monographs series. I am sure this publication will enlighten the policymakers, scientists, extension personnel, entrepreneurs and farmers for the improved production and consumption of horsegram across African countries as well as in India.

Edwin Southan

**Professor Sir Edwin M. Southern** Founder & Trustee of the Kirkhouse Trust



## PREFACE

Horsegram (*Macrotyloma uniflorum* (Lam) Verdc.) is an undervalued crop species which may not command the same widespread attention as do other legumes yet has deep roots in the agricultural and nutritional landscapes of Southeast Asia and Africa, particularly in marginal farming communities. This monograph is dedicated to exploring the multifaceted role of horsegram, from its historical significance to its modern-day potential. I aim to shed light on this resilient, under appreciated legume and emphasize its potential to contribute to global food security, sustainable agriculture and health.

Its cultivation, under very harsh conditions in the North-western Himalayan region has attracted our attention to this crop; this review represents a compilation of over 20 years of work. Despite its limited popularity in mainstream agriculture, the crop's ability to thrive in environments where moisture is deficient makes it invaluable in areas vulnerable to climate change. This monograph seeks to fill gaps in the literature, by providing a comprehensive exploration of horsegram's agronomic traits, its cultural significance and its nutritional importance. The nutritional profile of horsegram features high levels of protein, fibre and essential minerals. It is especially valuable in regions where other crops struggle to grow, making it a crucial component of resilient food systems. The information provided here should serve as an excellent and up-to-date reference for food scientists and food chemists, as well as for researchers involved in human nutrition, dietetics and the chemistry of natural compounds. Beyond its nutritional value, horsegram is also rich in antioxidants and bioactive compounds, which are increasingly being recognized for their potential to combat chronic conditions such as diabetes and heart disease.

Given the increasing pressure on both our agricultural systems and human nutrition, the potential of horsegram has never been more relevant. Through this monograph, we hope to encourage a renewed appreciation for this ancient crop and to stimulate further research into its potential benefits. The author has sought to describe the key factors that influence consumer acceptance of orphan legumes in the diet, as well as the known functional properties of these legumes and legume-based food products. I have made an effort to gather information covering both genetic and non-genetic components, gathered from both public and private sources. While I cannot promise that its coverage is comprehensive, I am confident that it will serve as a helpful tool for researchers working on horsegram improvement. I wish to express my thanks to the Kirkhouse Trust (UK), headed by Prof. Sir Edwin Southern, for providing financial support, and to Drs Prem Mathur and Robert Koebner, acting as consultants to the Kirkhouse Trust, for their critical review and comments. I am also grateful to my colleagues Manisha Gautam, Sunny Choudhary, Jai Chand Rana and Rashmi Yadav for providing necessary information for this monograph.

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December 2024

# **1. INTRODUCTION**

Underutilized crops play an important role in the diet of rural people, especially during periods of drought and hunger (Ramteke et al., 2016). Horsegram (Macrotyloma uniflorum (Lam) Verdc.) is considered as an underutilized crop, largely grown by communities in the Indian sub-continent to preserve their cultural heritage (Chahota et al., 2013b; Bhartiya et al., 2015; Ellis, 2016; Cullis and Kunert, 2017; Fuller and Murphy, 2018). Its centre of origin is in southwestern India (Arora and Chandel, 1972). The name horsegram derives from its centuries-long use as a source of feed and fodder for horses. During the colonial period, it was rarely consumed by either British residents or by more wealthy native Indians. It has a special place in the world of orphan crops for its ability to survive under harsh and challenging environmental conditions. It has also served to improve the nutritional value of cereal-based straw used to feed cattle (Nezamuddin, 1970). In addition to various homoeostatic and therapeutic roles in human nutrition, horsegram has favourable effects on the bowel and colon (Yadahally et al., 2012). It is a fast-growing pulse in dry environments and has a lot of potential for traditional medicinal, nutritional and pharmacological uses.

In India; and to a lesser extent in Myanmar, Sri Lanka, Bhutan, Nepal and Pakistan; it is cultivated as a pulse crop. The crop is mostly grown by poor farmers in tribal communities, particularly in drought-prone parts of India (Krishna, 2010). In India, the crop is cultivated in the states of Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh, Odisha, Bihar, Chhattisgarh, West Bengal and Jharkhand, as well as in the mountain foothill regions of Uttarakhand, Jammu and Kashmir and Himachal Pradesh. Within India, horsegram grain accounts for around 0.33% of the national tonnage of pulses produced.

Low-income groups in several developing countries use it as a food, and millions of rural residents of the Indian sub-continent rely on it as a source of vegetable protein and regard it as a co-staple food (Kadam *et al.*, 1985). The crop is consumed in the form of either whole seed, sprouts, or as wholemeal; its most popular form, especially in the southern Indian States, is as the main ingredient of an appetizer called *Rasam*, while in North Indian States, especially in the hilly regions, the local demand for the grain reflects its unique organoleptic taste. Horsegram is known by a variety of names in different Indian regional or indigenous languages and etymologically such as: *gahot* (Kumaon and Garhwal), *muthira* (Malyalam), *kulattha* (Sanskrit), *kurti-kalai* (Bengali), *ullavallu* (Telegu) and *kollu* (Tamil). The meaning of the word "*gahot*" refers to an ability to "dissolve stones

at the beginning stage of their occurrence" (Pande, 1999; Pati and Bhattacharjee, 2013).

Horsegram features as a component of fodder for cattle and horses in both Australia and Africa. In Australia and Papua New Guinea, the crop is sown in managed grazing land to improve pasture quality. Across Africa, in particular in Ethiopia, The Democratic Republic of Congo (DRC), Sudan, Niger, Somalia and Kenya, its cultivation is mainly for the production of fodder. The species' nondomesticated wild relatives are native to Australia, Papua New Guinea, Africa and India (Blumenthal *et al.*, 1989). Forms of the plant selected for specific desirable characteristics are grown in central, eastern and southern Africa, but not as a pulse crop (Blumenthal and Staples, 1993). Archaeological evidence supports the use of horsegram as a food crop as long ago as 2,000 BCE (Mehra, 2000).

# 2. DESCRIPTION OF THE PLANT

#### 2.1 Morphology

A wide array of phenotypic variation has been documented within germplasm sampled from Andhra Pradesh (Neelam et al., 2014) and from the northwestern Himalayan region (Chahota et al., 2005). This phenotypic plasticity may explain the species' wide adaptability to a variety of edaphic and climatic conditions (Kachroo and Arfi, 1970; Yadav, 2002), which extends to the astringent soil characteristics of eucalyptus forests in Queensland, Australia (Nezamuddin, 1970). Typically, horsegram plants can grow to a height of 90-140 cm. Due to the relative thinness of their stems, the plants typically adopt a twining growth habit, thus requiring a means of support for their growth. The main stem is slightly hairy and bears two to four primary branches and a small number of secondary branches (Fig. 1a). Its trifoliate leaves are composed of oblong or lanceolate leaflets of breadth 2.5-5.0 cm and length 6.0-9.0 cm (Fig. 1b). Each greenish-yellow horsegram flower is 1.3-2.0 cm long and develops from a leaf axil in a cluster of two to five units (Fig. 1c). The form of the flower is papilionate, with a pentamerous ground plan. The calyx consists of five sepals and the corolla of five petals of various size and shape (Fig. 1d). The large petal is referred to as the standard/banner, the two side petals as wings and the two lower ones (which form a closed structure) the keel; because the keel remains closed throughout the growing season, the plant is largely selfpollinating. The stigma is surrounded by ten anthers, so that in the event of an insect visiting the flower, self-pollen tends to be shed directly on to the stigma, hence promoting self-pollination. Insect-borne non-self-pollen is responsible for a very low (<1%) frequency of out-crossing. The flattened pods are scimitar-shaped and grow to a length of around 5 cm (Fig. 1e, f, g). At physiological maturity (Fig. 1h, i, j), the seeds, which are either spherical or slightly flattened in shape, feature a small, curved beak; the seed coat can be brown, grey or black (Fig. 1k, l, m, n) (Nezamuddin, 1970; Smartt, 1985; Neelam et al., 2014).

#### 2.2 Cytology

The genus *Macrotyloma* includes 25 species, harbouring a haploid chromosome number of 10-12 (Allen and Allen, 1981). Inspection of root tip mitoses in the domesticate has confirmed the presence of ten pairs of chromosomes (Halder *et al.*, 2012), comprising five morphologically distinct chromosomes: its karyotype is described by the formula 4Am + 2Bscsm + 2Csm + 2Dsm + 10Em, where type A refers to long and metacentric chromosomes; type



Fig. 1: The horsegram plant: (a) a fully grown plant; (b) trifoliate leaf; (c) flower; (d) various parts of flower; (e) plant bearing green pods; (f) green pods; (g) opened green pods; (h) plant bearing mature pods; (i) mature pods; (j) opened mature pods; (k-n) variation in testa colour.

B to long and sub-metacentric chromosomes, bearing a satellite on the short arm; type C to medium length sub-metacentric chromosomes; type D to short sub-metacentric chromosomes; and type E to short metacentric chromosomes. The length of the individual chromosome ranges from 1.26 to 2.41  $\mu$ m. At diakinesis and metaphase I, the meiocytes form a mean of 9.96 bivalents and 0.08 univalents, while at anaphase I, each meiocyte forms ten chromosomes. A possible explanation for the occurrence of multivalents is that the species is a cryptic polyploid, derived from an ancestor having a haploid number of 7 (Halder *et al.*, 2012). Current work is underway to characterize the karyotypes of two closely related species, namely *M. sar-gharwalensis* and *M. axillare* (Mishra *et al.*, 2024), while the application of the fluorescent *in situ* hybridization technique has allowed comparisons to be drawn as to the site of the nucleolar organizing region in *M. uniflorum, sar-gharwalensis* and *axillare* (Fig. 2). This, along with data derived

from other analyses, has suggested *M. axillare* to be the more likely candidate than *M. sar-gharwalensis* as the progenitor of the domesticate (Fig. 3).

*M. sar-gharwalensis* is of particular interest because its seed has a protein content of 39.5%, while *M. axillare* possesses several desirable agronomic traits, such as bearing a large number of pods per plant, a high seed yield per plant and pronounced tolerance to abiotic stresses. The karyotypes of the domesticate and the two non-domesticates vary somewhat, notably with respect to chromosomal length; such differences may contribute to the sexual incompatibility exhibited by wide hybrids, which hinders the transfer of desirable traits into the domesticate.



Fig. 2: Cytological analysis in Macrotyloma spp.: Conventional karyotype of (I) M. sargharwalensis, (II) M. axillare: (a, b) mitotic metaphase chromosomes; (c) karyotype; (d) Ideogram. (III) FISH-based karyotype of M. uniflorum: (a, b) mitotic metaphase chromosomes; (c) karyotype; (d) ideogram. (IV) Crosses between the domesticate and its wild relatives: (a) M. uniflorum (HPKM 249) × M. sar-gharwalensis (b) M. uniflorum (HPKM 249) × M. axillare.



Fig. 3: The phenotypes of the domesticate and its likely progenitors: (a) *M. axillare,* the probable progenitor of cultivated horsegram; (b) The domesticate; (c) *M. sar-gharwalensis.* 

Very few  $F_1$  hybrids could be recovered from 200 *M*. *uniflorum* × *M*. *axillare* crosses, while none at all were obtained from *M*. *uniflorum* × *M*. *sar-gharwalensis* crosses.

#### 2.3 Taxonomy

*M. uniflorum* belongs to the kingdom *Plantae*; sub-kingdom *Tracheobionta* (vascular plants); super division *Spermatophyta* (seed plants); division *Magnoliophyta* (flowering plants); class *Magnoliopsida* (dicotyledons); subclass *Rosidae*; order *Fabales*; family *Fabaceae* (Table 1). There has been a degree of taxonomic confusion over the species, reflected in the botanical, agronomic as well as the recent archaeobotanical literature of India. In the Indian floristics and archaeobotanical literature, the formal name assigned to horsegram was *Dolichos biflorus* (Watt, 1908; Gamble, 1935; Vishnu, 1989; Kajale, 1991; Saraswat, 1992). The species was later renamed *D. uniflorum* Lam. and subsequently, as a result of its re-assignation from *Dolichos to Macrotyloma* (Smartt, 1985), its present classification became *M. uniflorum* (Lam.) Verdc. (Kingwell-Banham and Fuller,

Kingdom	Plantae
Sub-kingdom	Viridiplantae
Infrakingdom	Streptophyta
Super division	Embryophyta
Division	Tracheophyta
Sub-division	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Fabales
Family	Fabaceae
Genus	Macrotyloma (Wight and Arn.) Verdc.
Species	Macrotyloma uniflorum (Lam.) Verdc.
Cultivated	Macrotyloma uniflorum var uniflorum

Table 1: Taxonomical classification of *M. uniflorum*.

2014). The word *Macrotyloma* is derived from the ancient Greek words *makros* (large), *tylos* (knob) and *loma* (margin), reflecting the knobbly structures formed on the pods (Blumenthal and Staples, 1993). *M. uniflorum* is one of three economically significant *Macrotyloma* species, along with *M. axillare* and *M. geocarpum* (Isely, 1983).

Southworth (2005) restored an ancient word for horsegram \*kol- or \*kolut, based on its occurrence between and across most Dravidian languages. Related terms can be found among the Indic languages, for instance *kulattha* (which originated in Sanskrit) and *juang kulto* (Fuller, 2003; Southworth, 2005), which originated in eastern and central India. Today, the species is referred to as horsegram, kulthi bean, *hurali* or Madras gram. The International Legume Database and Information Service (ILDIS) provides a wealth of taxonomic data covering the distribution, common names, life forms and uses of horsegram, as well as literature references, illustrations and distributional maps.

#### 2.4 Wild relatives

The 24 species belonging to the genus *Macrotyloma* are found across tropical Africa, the east coast of South Africa, Saudi Arabia, Madagascar, the Mascarenes

and southeastern Asia. The *Macrotyloma* species were originally considered as belonging to the genus *Dolichos* L. but have since been allocated as a separate genus based on floral characteristics and unique tuberculate pollen grain sculpturing. The worldwide distribution of *Macrotyloma* and some of its related species is detailed in Table 2. *M. uniflorum* typically grows from a hard rootstock; its stems are suberect, sometimes reaching 0.6 m in length. Its 4-5 mm long stipules are lanceolate and striate. Its leaflets, measuring  $1.0-3.5 \times 0.7-2.7$  cm, have oblique laterals that are rounded at the base (Kumar, 2012); the length of the petiole is 0.8-6.8 cm and that of the petiolules 1-2 mm. Its leaves are pinnately trifoliolate. Its bracts and bracteoles are linear and 2 mm long. Its flowers, with two to five petals, can be either solitary or are carried in an axillary cluster. The length of the rachis and peduncle is up to 1.5 cm.

There are reports of the presence of *M. uniflorum* in South Africa, Ethiopia, Kenya, Tanzania, Angola, DRC and India. It grows in wastelands, rocky outcrops, sandy patches, thickets or bushland at an elevation of 600-1,700 masl (Verdcourt, 1970). Four intrageneric varieties have been identified, namely var. uniflorum, var. stenocarpum (Brenan) Verdc., var. verrucosum Verdc. and var. benadirianum (Chiov.) Verdc. Plants classified as var. uniflorum are found in the wild in southern Asia and Namibia; they form 6-8 mm wide pods. This is the variety which is cultivated in the tropics as a cover and forage crop; var. stenocarpum (Brenan) Verdc. plants are found in India, central, eastern and southern Africa at altitudes of up to 1,700 masl in grassland, bushland and thickets, often on sandy soils. Their pods are 4.0-5.5 mm wide with guite smooth margins, and leaflets are pubescent. This form is also widely cultivated in Australia and California (USA). Plants belonging to var. verrucosum Verdc, are found in eastern and southern Africa at altitudes of up to 550 masl in grassland and thickets; the margin of their 4.0-5.5 mm wide pods are slightly or markedly warty, and their leaflets are pubescent. Finally, plants belonging to var. benadirianum (Chiov.) Verdc. are found in eastern Africa (Somalia, Kenya) at sea level on sand dunes or thin soils on coral rag; their 4.0-5.5 mm wide pods have a slightly warty margin, and their leaflets are highly velvety (Kumar, 2012).

*M. axillare* is a perennial, evergreen form (Mackinder *et al.*, 2001), often forming a woody stem up to 3.5 m in length. Its glabrous or golden adpressed or spreading indumentum stems can be either ascending or trailing. Three types have been recognized: var. *glabrum*, var. *axillare* and var. *macranthum*. The former forms 5-15 mm long pods and 1-3 cm long flowers. Their stems are sparsely (but occasionally heavily) pubescent. Plants classified as var. *axillare* bear 3-7 mm long pods, forming stems which are usually densely (but occasionally sparsely)

Species	Origin	Reference	Distribution
Macrotyloma africanum (Wilczek) Verdc.	Tropical Africa	Gillett <i>et al</i> ., 1971	Angola, Burkina Faso, Burundi, Cameroon, DRC, Ethiopia, Kenya, Malawi, Mali, Nigeria, Rwanda, Tanzania, Zambia and Zimbabwe
<ul> <li>Macrotyloma axillare (E. Mey.) Verdc.</li> <li>Accepted Infraspecifics:</li> <ol> <li>Macrotyloma axillare var. axillare</li> <li>Macrotyloma axillare var. glabrum (E. Mey.) Verdc.</li> </ol> <li>Macrotyloma axillare var. macranthum (Brenan) Verdc.</li> </ul>	Tropical and S. Africa, W. Indian Ocean, SW. Arabian Peninsula and Sri Lanka	Mackinder <i>et</i> <i>al.</i> , 2001	Angola, Botswana, Burundi, Cameroon, Cape Provinces, Comoros, DRC, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Kwa Zulu-Natal, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Nigeria, Rwanda, Senegal, Sierra Leone, Somalia, Sri Lanka, Sudan, Swaziland, Tanzania, Togo, Uganda, Yemen, Zambia and Zimbabwe
<i>Macrotyloma bieense</i> (Torre) Verdc.	Angola	Torre <i>et al</i> ., 1966	Angola
<ul> <li>Macrotyloma biflorum (Schum. and Thonn.) Hepper</li> <li>Accepted Infraspecifics:</li> <li>1. Macrotyloma biflorum var. appressepuberulum Verdc.</li> <li>2. Macrotyloma biflorum var. biflorum</li> </ul>	W. Tropical Africa to Ethiopia and Zambia	Robyns (1954)	W. Tropical Africa to Ethiopia and Zambia
Macrotyloma brevicaule (Baker) Verdc.	Ghana to Chad	Hepper, 1958	Cameroon, Chad, Ghana and Nigeria
<i>Macrotyloma ciliatum</i> (Willd.) Verdc.	South East Asia	Kumar and Sane, 2003	India, Sri Lanka
Macrotyloma coddii Verdc.	Africa: Kalahari- Highveld regional transition zone	Germishuizen and Meyer, 2003	KwaZulu-Natal

#### Table 2: Worldwide distribution of horsegram and related species.

Species	Origin	Reference	Distribution
<i>Macrotyloma daltonii</i> (Webb) Verdc.	Cape Verde, Dry Tropical Africa	Mackinder <i>et al.</i> , 2001	Angola, Botswana, Cape Verde, Chad, DRC, Eritrea, Ethiopia, Guinea, Kenya, Malawi, Namibia, Niger, Nigeria, Senegal, South Africa, Sudan, Tanzania, Uganda, Zambia and Zimbabwe
Macrotyloma decipiens Verdc.	South East Africa	Mackinder et <i>al</i> ., 2001	Mozambique
<ul> <li>Macrotyloma densiflorum var. densiflorum (Baker) Verdc.</li> <li>Accepted Infraspecifics: <ol> <li>Macrotyloma densiflorum var. longicalyx Verdc.</li> </ol> </li> <li>Macrotyloma densiflorum var. densiflorum</li> <li>Macrotyloma densiflorum var. longicalyx Verdc.</li> </ul>	Tanzania to S. Tropical Africa	Figueiredo and Smith, 2008	Angola, DRC, Malawi, Tanzania, Zambia and Zimbabwe
<i>Macrotyloma dewildemanianum</i> (Wilczek) Verdc.	Tanzania to Zambia	Lock, 1989	Malawi, DRC, Tanzania and Zambia
<i>Macrotyloma ellipticum</i> (R.E.Fr.) Verdc.	Tanzania to S. Tropical Africa	Figueiredo and Smith, 2008	Angola, DRC, Malawi, Tanzania and Zambia
Macrotyloma fimbriatum (Harms) Verdc.	Tanzania to S. Tropical Africa	Figueiredo and Smith, 2008	Angola, DRC, Malawi, Mozambique, Tanzania and Zambia
Macrotyloma geocarpum (Harms) Accepted Infraspecifics: 1. Macrotyloma geocarpum var. geocarpum 2. Macrotyloma geocarpum var. tisserantii (Pellegr.)	W. Tropical Africa to Chad.	Terrell, 1977	Benin, Burkina, Cameroon, Chad, Ghana, Guinea- Bissau, Ivory Coast, Mali, Nigeria, Senegal and Togo Introduced into India, DRC and Zambia
<i>Macrotyloma hockii</i> (De Wild.) Verdc.	Southern parts of Africa	Lock, 1989	DR Congo to Zambia.
<i>Macrotyloma maranguense</i> (R. Wilczek) Verdc.	E. Central and E. Tropical Africa, S. Africa	Germishuizen and Meyer, 2003	Burundi, DRC, Kenya, Rwanda, South Africa, Swaziland, Tanzania and Uganda

Species	Origin	Reference	Distribution
<i>Macrotyloma oliganthum</i> (Brenan) Verdc.	S. Tanzania to S. Tropical Africa	Mackinder et al., 2001	Malawi, Mozambique, Tanzania, Zambia and Zimbabwe
Macrotyloma prostratum Verdc.	Tanzania to Zambia	Lock, 1989	Malawi, Tanzania and Zambia
Macrotyloma rupestre (Baker) Verdc.	DR Congo to S. Tropical Afrixa and Namibia	Figueiredo and Smith, 2008	Angola, DRC, Congo, Malawi, Namibia, Zambia and Zimbabwe
Macrotyloma schweinfurthii Verdc.	Togo to Cameroon, E. Sudan to Ethiopia	Brunel <i>et al.</i> , 1984	Cameroon, Ethiopia, Nigeria, Sudan and Togo
Macrotyloma stenophyllum (Harms) Verdc.	Tropical Africa.	Gillett <i>et al.</i> , 1971	Angola, Benin, Burkina, Burundi, Cameroon, Central African Republic, DRC, Chad, Ethiopia, Guinea-Bissau, Ivory Coast, Mali, Nigeria, Senegal, Sudan, Tanzania, Togo and Uganda
<i>Macrotyloma tenuiflorum</i> (Micheli) Verdc.	W. Tropical Africa to Ethiopia and Angola	Boudet <i>et al</i> ., 1986	Angola, Burundi, Cameroon, Central African Republic, Chad, DRC, Ethiopia, Gabon, Guinea, Mali, Sierra Leone, Togo and Uganda
<ul> <li>Macrotyloma uniflorum (Lam.) Verdc.</li> <li>Accepted Infraspecifics:</li> <li>1. Macrotyloma uniflorum var. benadirianum (Chiov.) Verdc.</li> <li>2. Macrotyloma uniflorum var. stenocarpum (Brenan) Verdc.</li> <li>3. Macrotyloma uniflorum var. uniflorum</li> <li>4. Macrotyloma uniflorum var. verrucosum Verdc.</li> </ul>	Tropical and S. Africa, Indian Subcontinent to Myanmar	Kumar, 2012	Angola, Assam, Bangladesh, Botswana, Cameroon, DRC, Ethiopia, Guinea, India, Kenya, Mozambique, Myanmar, Namibia, Pakistan, Rwanda, Senegal, Somalia, Sri Lanka, Sudan, Tanzania, Uganda, and Zimbabwe Introduced into Java, Nepal, Philippines, Queensland and Taiwan
<i>Macrotyloma stipulosum</i> (Baker) Verdc.	Kenya to S. Tropical Africa	Figueiredo and Smith, 2008	Angola, Burundi, DRC, Kenya, Malawi, Rwanda, Tanzania, Zambia and Zimbabwe

Species	Origin	Reference	Distribution
<i>Macrotyloma kasaiense</i> (R. Wilczek) Verdc.	Congo, DR Congo to S. Tropical Africa and Namibia	Sita and Moutsambote, 2005	Angola, DRC, Malawi, Namibia, Zambia and Zimbabwe

[Source: (IPNI Life Sciences Identifier (LSID) https://www.ipni.org/: names: 505852-1]

pubescent. Plants classified as var. *macranthum* develop 1.2-1.5 cm long flowers. Plants of var. *axillare* have been observed in Sri Lanka, Somalia, Ethiopia, Uganda, Kenya, Tanzania, Angola, Yemen, Senegal, Swaziland, Madagascar, South Africa, Mauritius, La Réunion and Comoro Island. They grow at elevations between 0 and 1,800 masl in areas of open forest, miombo and mixed woodland, as well as on sand dunes in coastal regions or lakeshores and occasionally along rivers (Verdcourt, 1970; Mackinder *et al.*, 2001).

*M. ellipticum* is a woody perennial plant able to grow up to 1 m in height. Its young stems are silky and yellow. Its striate stipules measure 7-9 mm  $\times$  2-3 mm, its petioles are 1.6-3.2 cm long and its rachis length is 2-3 mm. Its leaves are pinnately trifoliolate, and its leaflets are elliptic to oblong-elliptic, obtuse to sub-acute at the apex, apiculate/non-apiculate and circular at the base. An axillary cluster of up to four flowers is borne on 2-3 mm long pedicels. The striate bracts measure 2-3  $\times$  1 mm, and the bracts and the lanceolate bracteoles 3-4 mm. The species is found in Tanzania and DRC at altitudes of 1,350-1,650 masl in wetter miombo, forest, streams and grassland (Mackinder, 1998; Figueiredo and Smith, 2008).

*M. densiflorum* is a perennial plant that grows up to 1.75 m in height. The plant forms several straight branches with ferruginous or silvery hair on the lower stems. Its lanceolate, striate and densely sericeous stipules are 0.8-1.0 cm long. Its leaflets are oblong-elliptic, apiculate, 2.5-9.0 cm  $\times$  1-2.5 cm in length. It forms trifoliolate leaves with a rachis length of 4-5 mm (Mackinder, 1998; Figueiredo and Smith, 2008). The species is indigenous to Tanzania, Angola and DRC, growing in miombo woodland, on the edges of cultivated land and on rock outcrops at an altitude of 1,230-2,000 masl.

*M. africanum* plants are seasonal or sporadic. Its stems often form a tangled mass up to 1.35 m in height and are glabrous or sparsely pubescent, ascending or trailing. The leaves are pinnately trifoliolate with a 1.5-5.0 mm long rachis. The petioles are 1.2-3.0 cm long. The leaves are either glabrous or slightly appressed hairy on both sides, with a rounded base and narrow oval to elliptic-lanceolate

shapes. Petiolules are of length 1 mm. The species occurs in Angola, Ethiopia, Kenya, Tanzania, Burundi, DRC, Nigeria, Cameroon and Mali; it grows from 1,000 to 1,600 masl in grasslands, miombo and mixed deciduous forest, occasionally in wetter locations, on rocky outcrops and in sandy places, as well as in formerly farmed areas and along the roadside (Gillett *et al.*, 1971).

*M. biflorum* is a perennial herb. Its thin, up to 2 m long stem, forms white hairs on all axes. Plants are either prostrate or scandent. The leaves are pinnately trifoliolate, with a petiole length range between 0.8-2.0 cm and a rachis of length 1-2 mm. Its leaflets are elliptic to oblong-elliptic measuring  $1-4 \times 0.5-1.4$  cm, round at the base and apex, and rarely apiculate at the apex. An axillary cluster of a small number of sessile or sub-sessile flowers forms an inflorescence of length 2-3 mm. The species occurs in the Central African Republic, DRC, Guinea, Mali, Sierra Leone, Togo, Upper Volta, Ghana, Nigeria, Cameroon, Chad, Sudan and Angola. It flourishes at altitudes of 1,000-1,500 masl, in grasslands and mixed deciduous forest with sandy soils and rocky outcrops (Robyns, 1954; Verdcourt, 1970).

*M. daltonii* is an annual or perennial herb. Its stems are climbing or trailing, slender and villous, reaching 0.7 m in length. Its stipules are long, ovate and striate, measuring 5-7 mm in length. Its leaves are pinnately tri-foliolate and its petioles are 0.8-5.6 cm long. Its leaflets are elliptic, measuring 2.4-5.8  $\times$  0.8-3.4 cm and its petiolules are 2-3 mm in length. Its flowers are either solitary or occur in axillary clusters of two to four, borne on a 1-2 mm long pedicel. The species is found in the Cape Verde, Nigeria, Sudan and Ethiopia. It is adapted to miombo woodland, along roadsides and on sandy soils, at altitudes of 500-1,500 masl (Verdcourt, 1970; Mackinder, 1998; Mackinder *et al.*, 2001).

*M. oliganthum* is a perennial herb forming stems up to 45 cm long. Its stipules are ovate and striate, measuring 4-8 mm  $\times$  2-3 mm; its leaves are pinnately trifoliolate; its petioles are of length 2.2-6.0 cm; its leaflets are oblong and elliptic; its flowers are either solitary or form an axillary cluster of two or three flowers; its 3-5 mm long bracts are lanceolate and striate. The lengths of its pedicels and bracteoles are, respectively, 3-5 mm and 2 mm. Its calyx tube is sparsely pubescent, of length 2-4 mm; its lobes are triangular and pubescent, measuring 2-4 mm (Mackinder, 1998; Mackinder *et al.*, 2001).

# 3. SPECIES ORIGIN, DISTRIBUTION, CULTIVATION AND CONSUMPTION

#### 3.1 Centre of origin

The species' centre of origin remains obscure. Wild forms of *M. uniflorum* are found in both India and Africa (Verdcourt, 1971), but India is believed to be the site of domestication (Purseglove, 1974; Smartt, 1985). Arora and Chandel (1972) hypothesized that the domesticate originated in southwestern India, but Mehra and Magoon (1974) suggested that it arose in both India and Africa simultaneously. The greatest extent of genetic diversity across the Macrotyloma genus is found in Africa, whereas for the domesticate, the Himalayas and southern India are the regions where the greatest level of diversity exists (Zeven and de Wet, 1982). Southern India is generally considered to be the primary centre of origin of the cultivated form, while the North-western Himalaya is considered as a secondary centre of origin. The domestication of horsegram probably started in India, where its cultivation can be traced back to prehistoric times. It is probable progenitor (*M. axillare*) does not appear to occur in India, although a number of other wild relatives are present. The species *M. sar-garhwalensis*, found in the Gharwal region of Himalaya has only in recent times been recognized as a separate species (Gaur and Dangwal, 1997).

#### **3.2 Species distribution**

In modern times, the crop is mainly grown across the southern Indian states, as well as in the Himalayan foothills (Ramani *et al.*, 2020). Together, the states of Tamil Nadu and Andhra Pradesh account for nearly 90% of the total land area of India used for its cultivation. The crop can be grown with minimal inputs in soils of low to medium fertility (Witcombe *et al.*, 2008) and can tolerate a wide range of temperature (Smartt, 1985). It is, however, intolerant of both frost and water logging. The crop is grown successfully at altitudes as high as 1,500 masl and is typically planted near the edges of fields used to cultivate other crops (Gaur, 1999). Asouti and Fuller (2008) have reported that the species is adapted to dry evergreen open woodlands dominated by *Acacia* and *Albizzia*, a bioclimatic zone similar to those where wild *Macrotyloma* species are found in Africa (Verdcourt, 1971).

The palatability of horsegram for domesticated animals has likely limited the size of the plant population in areas close to human settlement. According to the *Flora of Mizoram*, *M. uniflorum* is a 'common species in open areas' (Singh *et al.*, 2002), implying that it had not at that time been a cultivated as a crop. It is therefore possible that some wild populations do extend into northeastern India and even into neighbouring Myanmar. However, it is also possible that these are feral populations originating from ancient crops. In the eastern Himalaya (Sikkim), horsegram is cultivated at altitudes of up to 1,000 m asl (Atkinson 1882; Watt 1889–1893), while in the northwestern Himalaya, it is grown at altitudes as high as 2,000 m asl. The unique taste of horsegram grown in higher altitudes has stimulated consumer demand, allowing farmers to achieve a premium price for their produce. In recent years, the crop has become a popular pasture species in Australia, Taiwan and The Philippines. Burkill (1966) has noted that the crop was first introduced to South-east Asia as a fodder crop, although archaeological evidence indicates that it was previously grown in Thailand in the period 300 BCE to 100 CE (Castillo *et al.*, 2016).

#### 3.3 Area of production and consumption in India

The total area cultivated under horsegram in India is about 507,000 ha, yielding 262,000 000 kg of seed; thus, the crop's mean seed yield is 516 kg/ha. Horsegram is more popular in the southern Indian states than in the northern ones (Lokeshwar, 1997), due to which 90-95% of its production is concentrated in the states of Andhra Pradesh (16%), Odisha (16%), Tamil Nadu (18%), Maharashtra (18%) and Karnataka (34%) (Kumar, 2007). In terms of production, Karnataka contributes 25.7% of the national output, followed by Odisha (15.5%) and Chhattisgarh (13.3%). Levels of productivity vary greatly, and the three most productive states are Bihar (959 kg/ha), West Bengal (796 kg/ha) and Jharkhand (603 kg/ha) (Anonymous, 2015-15). The crop can be grown in either the Rabi (after the monsoon rains) or the *Kharif* (at the beginning of the first monsoon rains) seasons. Rabi crops are typically sown by subsistence farmers on poorly fertile soils deemed unsuitable for the production of other crops. Although the area sown to horsegram and the volume of its production are higher in Kharif than in Rabi-grown crops, productivity is higher in the latter. Crop yields have been stagnant for some years, and the area under production in India is in decline.

Traditionally, horsegram is inter-cropped with sorghum and various millets, but more recently the application of nitrogenous fertilizer to support the production of maize has greatly reduced the use of this mixed cropping system. Improved varieties of horsegram are available in many parts of the country; dependent on cultivar choice and farming practices, these improved varieties can yield 700-900 kg/ha of grain, in addition to about 1,000 kg/ha of fodder. For a long time, the crop was regarded as underutilized and outside of mainstream agriculture.

The establishment in 1992 of the National Network Project on Arid Legumes, headquartered at the Indian Council of Agricultural Research (ICAR) Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan, focused on strengthening the status of horsegram. From 2015, responsibility for this programme was moved to ICAR-Indian Institute of Pulses Research (IIPR), Kanpur, Uttar Pradesh. Its major emphasis is on the collection, evaluation, characterization and utilization of germplasm, varietal development and on the improvement of production and crop protection technologies.

## 4. PROPERTIES OF DIFFERENT PLANT PARTS

Horsegram has been recognized as a valuable source of dietary protein and other nutrients, such as vitamin C, riboflavin and niacin. Its nutritional value is as high as that of other commonly consumed pulses in all aspects and represents an excellent source of iron, molybdenum and calcium (Bhartiya *et al.*, 2015). In traditional Ayurvedic cuisine, horsegram is considered to have beneficial medicinal properties. In addition to its various homoeostatic and therapeutic roles in human nutrition, the consumption of horsegram has favourable effects on bowel and colon health (Yadahally *et al.*, 2012).

#### 4.1 Leaves

Extracts of horsegram leaf have been shown to exert an antihypercholesterolemia effect: in trials, consumption of leaf extracts over a five-week period resulted in a significant decrease in the subjects' blood content of total cholesterol, triglycerides, low thickness lipoprotein and extremely low thickness lipoprotein, as well as an increase in that of high thickness lipoprotein (Kumar *et al.*, 2013). The various free radical scavenging activities of horsegram's ethanolic leaf concentrate are associated with a high degree of *in vitro* antioxidant action, as shown by superoxide radical seeking tests, ferric reducing antioxidant power assays and reducing power assays (Bharathi and Anand, 2014).

#### 4.2 Seeds

Ayurvedic medicine recommends the use of horsegram seed to treat urinary stones (Yadava and Vyas, 1994; Ravishankar and Vishnupriya, 2012), urinary diseases and piles (Yadava and Vyas, 1994). It can also function as an astringent and a tonic (Brink, 2006), can control irregularity in the menstrual cycle (Neelam, 2007), and combat corpulence, hiccups and certain other ailments. The liquor remaining after cooking horsegram seed has been used to treat common colds, throat infections and fevers (Perumal and Sellamuthu, 2007). Seed extracts have been shown to control the growth of various pathogenic microbes, including *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Gupta *et al.*, 2005). The seed contains antioxidant polyphenols and free radical scavengers, as well as molybdenum (a constituent of haemoglobin), which controls the intake of calcium and iron, thereby aiding in the transport of oxygen into cells (Ramesh *et al.*, 2011; Murthy *et al.*, 2012).

Horsegram seed is high in protein, dietary fibre, micronutrients and several beneficial phytochemicals. The seed is low in fat and contains more dietary fibre than that of the common bean (*Phaseolus vulgaris*) (Kawale *et al.*, 2005). The seed also contains several anti-nutritional compounds which interfere with protein digestibility and limit the bioavailability of certain minerals (Sandberg, 2002). A variety of factors, such as genotype, soil, fertilizer regime, cultural techniques, climatic conditions, post-harvest management and storage conditions all have an impact on the crop's nutritional quality, either directly or indirectly (Hornick, 1992).

#### 4.2.1 Carbohydrates

Horsegram seeds contain a range of carbohydrates, including sugar, fibre and starch (Prasad and Singh, 2015; Gautam and Chahota, 2022). The sugars present are monosaccharides, disaccharides (sucrose and maltose) and certain oligosaccharides. Starch (Table 3) accounts for 43.4% of the seeds' carbohydrate content (Bravo *et al.*, 1999). Both soluble and insoluble fibre are present, the latter being present in higher amounts than in the seed of kidney bean (Kawale *et al.*, 2005). The crude fibre content of the seed coat is higher than in the embryonic axis or the cotyledon, which is relevant since pulse seed coats which are high in fibre and low in protein can be useful in formulating food products designed to improve gastrointestinal health (Sreerama *et al.*, 2010a, b).

#### 4.2.2 Protein

The protein content of horsegram seed can vary from 13% to 32% (Savithramma and Shambulingappa, 1996; Gautam and Chahota, 2022) (Table 3), which is at least as high as that of other commonly consumed pulses such as common bean (19.9%), pigeonpea (Cajanus cajan) (20.3%), black gram (Vigna mungo) (22.0%), chickpea (Cicer arietinum) (18.9%), mung bean (V. radiata) (22.5%) and pea (Pisum sativum) (20.4%) (Longvah et al., 2017). Between 75.3% and 78.8% of horsegram seed protein is classified as either albumin or globulin, 9.9–17.5% as glutelin and 7.0–11.3% as residual protein (Yadav et al., 2004). In contrast, seed of the related species M. sar-garhwalensis contains 38.4% crude protein, which translates into a seed protein content about double that of typical horsegram (Yadav et al., 2004). Horsegram seed protein has a higher lysine content (0.52 g/g nitrogen) than does the seed of either black gram (0.40 g/g nitrogen) or pigeon pea (0.48 g/g nitrogen) (Gopalan et al., 1989), making it a good complement to a cereal-based diet (Venkatesha, 1999; Prasad et al., 2010). The major limiting amino acids present in the seed are methionine, threonine and tryptophan (Khader and Venkat Rao, 1986; Thirumaran and Kanchana, 2000).

#### 4.2.3 Vitamins and fat

Horsegram seed provides a source of the vitamins thiamine B1 (0.4 mg/100 g), riboflavin B2 (0.2 mg/100 g) and niacin B3 (1.5 mg/100 g) (Bhartiya *et al.*, 2015; Gautam *et al.*, 2020). *In planta*, these compounds are believed to participate in metabolic reactions associated with the response to abiotic stress (Asensi-Fabado *et al.*, 2010). The fat content of horsegram seed ranges from 0.5% to 4.0% (Table 3), but this level is increased by dehulling (Sudha *et al.*, 1995). Horsegram

	Nutritional composition	Citation
e	51.90 – 60.90%	Bravo <i>et al.</i> , 1999
	57.20%	Gopalan <i>et al</i> .,1999
	51.90% - 60.90% (Whole)	Sudha <i>et al</i> ., 1995
iydra	56.80% - 66.40% (Dehulled)	Sudha <i>et al.</i> , 1995
arboh	66.60 + 2.10%	Sreerama et al., 2012
ö	37.15%	Ranasinghe and Ediriweera, 2017
	53.27%	Kawale et al., 2020
	46.91 ± 1.90 mg/ml	Gautam <i>et al.</i> , 2020
	17.90 - 25.30%	Bravo <i>et al</i> ., 1999
	22.00%	Gopalan <i>et al</i> .,1999
	23.00%	Venkatesha, 1999
c	17.90% - 25.30% (Whole)	Sudha <i>et al</i> ., 1995
rotei	18.40% - 25.50% (Dehulled)	Sudha <i>et al</i> ., 1995
ц.	22.50 + 1.00%	Sreerama et al., 2012
	24.24%	Ranasinghe and Ediriweera, 2017
	36.84%	Kawale et al., 2020
	21.80 ± 1.10%	Gautam <i>et al.</i> , 2020
Fat	0.58 - 2.06%	Bravo <i>et al.</i> , 1999
	0.50%	Gopalan <i>et al.</i> ,1999
	0.70 - 2.06% (Whole)	Sudha et al., 1995

Table 3: Nutritional composition of horsegram.

	Nutritional composition	Citation
	0.81 - 2.11% (Dehulled)	Sudha et al., 1995
	0.60 - 2.60%	Sreerama et al., 2012
	1.10%	Ranasinghe and Ediriweera, 2017
	3.97%	Kawale et al., 2020
	0.12 ± 0.01%	Gautam et al., 2020
Saturated fatty acids	[Palmitic (21.97%), Arachidic (2.85%), Stearic (2.32%) and Myristic (0.36%)] 27.51%	Mishra and Pathan, 2011
Unsaturated fatty acid	72.49% [Linoleic (42.78%), Oleic (16.15%) and Linolenic acid (13.56%)], 72.49% [Linoleic (40.30-45.60%), Oleic (8.90-16.80%) and Linolenic acid (11.60-14.30%)]	Ranasinghe and Ediriweera, 2017 Mishra and Pathan, 2011
Moisture	11.39%	Gopalan <i>et al</i> ., 1999
	11.55% (Whole)	Sudha <i>et al.</i> , 1995
	9.73% (Dehulled)	Sudha <i>et al.</i> , 1995
	6.80 + 2.00%	Sreerama et al., 2012
	8.90%	Ranasinghe and Ediriweera, 2017
	3.96%	Kawale et al., 2020
	9.72 ± 0.34%	Gautam <i>et al.</i> , 2020
Ash	3.00% - 3.80% (Whole)	Sudha et al., 1995
	2.70 - 3.40% (Dehulled)	Sudha <i>et al</i> ., 1995
	2.70 + 0.0%	Sreerama et al., 2012
	3.34%	Ranasinghe and Ediriweera, 2017
	1.96%	Kawale et al., 2020
	3.41 ± 0.25%	Gautam et al., 2020
Macro- nutrients	Ca - [238 mg / 100 g (Whole), 223 mg / 100 g (Dehulled)]	Sudha <i>et al.</i> , 1995
	Ca - (244–312 mg/100 g)	Sudha et al., 1995
	Ca - (287 mg/100 g)	Khatun <i>et al.</i> , 2013

	Nutritional composition	Citation
	Ca - (0.34%), P (0.27%)	Gopalan <i>et al</i> .,1999
	Ca - (0.287%)	Ranasinghe and Ediriweera, 2017
	P - (311 mg / 100 g)	Kawale et al., 2020
	Fe - (5.89-7.44 mg / 100 g)	Gopalan <i>et al</i> .,1999
	Fe - (6.77 mg / 100 g)	Khatun <i>et al.</i> , 2013
	Fe (0.01%)	Gopalan <i>et al</i> ., 1999
	Fe (68.25 - 92.95 µg/g), K - (13.06 - 14.61 mg/g), Ca - (1.20 - 3.13 mg/g)	Kawale <i>et al.</i> , 2020
	P - (3.83 - 4.43 mg/g), Mg - (1.64 - 1.73 mg/g)	Morris <i>et al</i> ., 2011
	S - (1.85 - 2.46 mg/g)	Gautam et al., 2020
	Fe (0.144 mg), K - (72 mg), Ca - (0.011 mg), P - (0.96 mg), Na - (0.35 mg)	
Micro-nutrients	Cu (10.28 - 13.16 µg/g), Mn (31.26 - 59.85 µg/g), Ni (1.04 - 1.33 µg/g), Zn (29.24 - 38.13 µg/g)	Morris <i>et al</i> ., 2011
	Cu (19.00%), Mg (0.17%), Mn (37.00%), Zn (0.28%)	Ranasinghe and Ediriweera, 2017
	Cu (0.01 mg), Cr (0.001 mg), Mn (0.03 mg), Zn (0.05 mg)	Gautam <i>et al</i> ., 2020
Vitamins	Vitamin A (2.10%), Vitamin C (1.40%), Ascorbic acid (0.70%), Thiamine (0.42%), Riboflavin (0.01%) and Niacin (1.50%)	Ranasinghe and Ediriweera, 2017
	Thiamine (0.40 mg / 100 g), Riboflavin (0.20 mg / 100 g) and Niacin (1.50 mg / 100 g)	Bolbhat <i>et al.</i> , 2012
Fibers	5.63%	Ranasinghe and Ediriweera, 2017
	15.60%	Kawale et al., 2020
	6.45 ± 0.21%	Gautam <i>et al.</i> , 2020

seed contains several fatty acids, of which 27.5% are saturated and the remainder unsaturated. Among the former types is linoleic acid, a compound useful for the treatment of diabetes and cardiovascular disease (Mishra and Pathan, 2011). According to Morris *et al.* (2013), a potential benefit of increasing the dietary intake of horsegram, and thereby balancing the ingestion of linoleic acid and

α-linolenic acid, could be to delay the onset of Parkinson's and Alzheimer's diseases. Horsegram lipids have been documented to be suppress ulcers thanks to the presence of phytosterol esters (Berger *et al.*, 2004) which protect against and heal the acute gastric ulceration induced by alcohol (Jayraj *et al.*, 2000).

#### 4.2.4 Anti-nutritional factors

Horsegram seed contains a diverse range of bioactive compounds, in particular phytic acid and phenolic acid, widely considered to be active as antioxidants. Phytic acid, which can be present either as the free acid, as phytate or as phytin (Oatway et al., 2001), is ubiquitous in legume seeds, accounting for about 78% of the phosphorus content of the seed (Chitra et al., 1995). The concentration of phytic acid in the horsegram embryonic axis fraction is 3.8 mg/g, whereas in the cotyledon fraction the concentration is 8.4 mg/g. Phytic acid is considered to be an anti-nutritional compound, because it inhibits protein digestibility, promotes the 'hard-to-cook' property of the seed (Stanley and Aquilera, 1985) and reduces the bioavailability of calcium, zinc, iron and magnesium (Sandberg, 2002). On the other hand, it acts as an antioxidant (Graf and Eaton, 1990), possesses anticarcinogenic activity (Shamsuddin et al., 1997; Turner et al., 2002), and reduces the rate of cell proliferation, thereby augmenting both the immune response (Reddy, 1999) and the level of hypoglycemic and hypolipidemic activity (Rickard and Thompson, 1997). Sundaram et al. (2013) have reported that tannins, a class of oligomeric, high molecular weight polyphenols, accumulate to a level of 0.1 g/100 g. The seed coat has a particularly high content of phenolic compounds (485 mg/g gallic acid equiv.) (Sreerama et al., 2010a). These compounds have been implicated as protectants against a range of diseases, including heart disease, cancer and inflammation (Tapiero et al., 2002). The principal phenolics present in the seed of horsegram are gallic acid, caffeic acid, chlorogenic acid, vanillic acid, ρ-hydroxybenzoic acid, ferulic acid and protocatechuic acid, along with the flavonoids rutin, apigenin glycoside, kaempferol glycoside, myricetin and guercetin (Sreerama et al., 2010a, Gautam et al., 2022; Gautam and Chahota, 2022).

# 5. USAGE

Horsegram's key agricultural value is as a source of both feed/fodder and green manure (Nezamuddin, 1970; Zaman and Mallick, 1991). Its importance as a therapeutic dietary agent has also long been recognized by practitioners of folk medicine. The various uses are described below:

#### 5.1 Diuretic and anti-calcifying activity

It is estimated that about 12% of the world's population suffers from kidney stones. Horsegram has been found to be effective in the treatment of this condition (Ghani, 2003), and hence enjoys wide popularity across India as a curative and prophylactive (Singla and Kumar, 1985). The consumption of horsegram is believed to increase the flow of urine, thereby creating pressure on a stone and promoting its passage. Horsegram seeds have been found to be at least as effective a treatment as potassium citrate, a conclusion supported by the finding of several authors that including horsegram in the diet promotes the decalcification process needed to break down kidney stones (Gautam *et al.*, 2020).

#### 5.2 Anti hyperglycemic activity

Horsegram seed has been documented to possess antihyperglycemic properties, as shown by an analysis of raw/unprocessed horsegram conducted by Tiwari *et al.* (2013). Its inclusion in the diet can reduce the absorption of carbohydrates, while at the same time providing a significant amount of soluble fibre. It has also been found to be useful as a hypoglycaemic agent and, as it represents a rich source of dietary antioxidants (Siddhuraju and Manian, 2007), it also acts as an antidiabetic (Gupta *et al.*, 2011). Extracts of horsegram seed have been found to exert a hypolipidaemic and hypoglycaemic action (Senthil, 2009), while the consumption of raw horsegram seed reduces insulin resistance (Kaundal *et al.*, 2019).

#### 5.3 Treatment of jaundice and other diseases

Horsegram seed has the potential to treat jaundice. The liquor remaining after cooking horsegram is used to treat jaundice in many southern Indian states (Purushottam *et al.*, 2017; Kashid and Talekar, 2021). Its consumption has been found to reduce the incidence of conditions aggravated by the presence of non-nutritive compounds such as phytic acid, phenolic acid, fibre and enzymatic
inhibitors. Furthermore, its use can reduce blood cholesterol levels and has also been found to be beneficial against gastritis, constipation, sun-burn, certain diseases affecting women (leucorrhoea, menstrual troubles, bleeding during pregnancy, postpartum excessive discharges), colic caused by wind, piles, rheumatism, haemorrhagic disease and intestinal worms (Pati and Bhattacharjee, 2013). Horsegram seed has also recently been shown to prevent atherosclerosis in animals (Shobana *et al.*, 2012) and may be a potential functional food for the control of high cholesterol and heart disease (Kashid and Talekar, 2021).

## **6. GENETIC RESOURCES**

Outside of India, very few initiatives to date have been undertaken to conserve horsegram germplasm. A systematic collection has been assembled by the ICAR-National Bureau of Plant Genetic Resources (NBPGR) New Delhi. In the 1970s, ICAR and the United States Department of Agriculture (USDA) initiated a programme of collecting and conserving horsegram germplasm, and since then germplasm has been collected from almost all horsegram growing areas in India.

### 6.1 Germplasm conservation

At present about 2,588 accessions are curated by various gene banks. The USDA's Germplasm Resources Information Network (GRIN) reports just 29 horsegram accessions; PROTAbase, a database of around 7,000 useful plants of tropical Africa, curates 21 accessions; and the Australian Tropical Crops and Forages Genetic Resources lists in its collection 38 accessions (Brink, 2006). In contrast, the ICAR-NBPGR curates 2,403 accessions, of which 2,392 are indigenous and 11 are exotic (Table 4). Internationally based holdings, as listed in Genesys (https://www.genesys-pgr.org), are summarized in Table 4. Table 5 reports the genetic stocks registered at ICAR-NBPGR.

Centre	Number of accessions
Horsegram germplasm collections from India maintained at ICAR-NBPGR geneban	an States k
Andhra Pradesh	85
Bihar	50
Chhattisgarh	175
Himachal Pradesh	125
Jharkhand	198
Karnataka	87
Kerala	133
Madhya Pradesh	131
Maharashtra	185
Odisha	119

Table 4: Indian Indigenous collection of horsegram germplasm at ICAR-NBPGR genebank (New Delhi) and other countries holding horsegram accessions in their respective gene banks.

Centre	Number of accessions		
Rajasthan	107		
Tamil Nadu	176		
Uttarakhand	188		
Others (Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Delhi, Goa, Gujarat, J and K, Manipur, Punjab, Sikkim, Uttar Pradesh, West Bengal)	633		
Total indigenous collections	2392		
Exotic collection	11		
Total collections	2403		
Horsegram germplasm collections maintained by other countries genebank			
USDA	35		
Australia	38		
Belgium	1		
Costa Rica	1		
Mali	1		
Myanmar	2		
Namibia	3		
Nepal	65		
Pakistan	2		
Panama	1		
Tanzania	11		
South Africa	2		
Zimbabwe	2		
Kenya	21		
Total collection in other countries	185		

### Table 5: List of genetic stocks registered at ICAR-NBPGR.

Accession	Donor identity	INGR No	Pedigree	Novel unique features
IC212722	IC212722/GN-1882	INGR02007	Natural Wild	High Protein content (>35%)
IC0587786	CRHG-6	INGR11017	K-42	Tolerance to Anthracnose
IC0587788	CRHG-8	INGR11018	K-42	Higher fodder yield
IC642009	Mutant of HPKC2	-	HPKC2	Early maturity and erect plant type

#### 6.2 Germplasm characterization

Attempts to characterize the genetic diversity present in Indian germplasm were initiated by Chahota et al. (2005), who evaluated 63 accessions, obtained from ICAR-NBPGR Shimla, with respect to 12 morpho-agronomic characters. Since then, follow-up studies have widened the extent of sampling, including those reported by Durga et al. (2012), Uma et al. (2013), Latha et al. (2013), Alle et al. (2015), Vijay Kumar et al. (2016) and Gomashe et al. (2018). Chahota et al. (2018) further evaluated 360 accessions not only at the morphological level, but also at the genotypic level. A core set of 105 entries was developed for a detailed analysis of the genetic basis of key phenotypic traits (Choudhary et al., 2022). Rana (2010) observed variation with respect to both qualitative (growth habit, leaf and stem hairiness, stem colour, pod colour) and quantitative (plant height, pod length, test weight) traits, while Gomashe et al. (2018), in a comparison involving 66 accessions, remarked that the most variable traits were yield, pod length and the number of pods per plant. A high degree of variation in the number of pods per plant was also reported in a set of accessions collected from Bastar (Chhattisgarh) (Singh et al., 2019). According to Priyanka et al. (2021), among a set of 252 accessions, the highest yielder produced 65.6 g seed per plant. Latha (2006) set out to identify correlations between a number of agro-morphological traits, and in particular, concluded that a higher yield potential was associated with a longer crop life cycle.

Most horsegram accessions are not widely used for commercial cultivation, as they suffer from one or more of an excessively long-life cycle, asynchrony of flowering and maturity, photosensitivity and indeterminacy (Uma *et al.*, 2013). The wild relative *M. sar-gharwalensis* produces seed with a very high protein content, whereas *M. axillare* plants are rather tolerant to low temperature and produce large numbers of pods per plant. Transferring such desirable traits into cultivated types is considered as a potentially effective strategy to generate resilient horsegram crops with higher yield.

#### 6.2.1 Biotic stress tolerance/resistance

Horsegram crops face several biotic constraints throughout the growing season, of which the most serious are caused by insects, microbes and viruses. The efforts made to identify sources of resistance against several biotic stress agents are summarized in Table 6.

#### 6.2.2 Abiotic stress tolerance

Among pulses, horsegram is recognized as being resistance to severe drought, high salinity and the presence of heavy metals (Reddy *et al.*, 2008). Sources of resistance/ tolerance with the potential to be exploited in breeding are detailed in Table 7.

Trait	Source	Reference
Yellow mosaic virus resistance	AK-38, HG-GP, DPI-2278, Paiyur-1 and Paiyur-20	Prema and Rangaswamy, 2017
Yellow mosaic virus resistance	HG 46, HG 59, HG 18, HG 72, HG 11 and AK 38	Durga <i>et al</i> ., 2014
Wilt resistance	HG 63, 58, 50 and Palem 2	Durga et al., 2014
Resistance against pest (Callosobruchus chinensis)	Palem-1, Palem-2, AK-21 and NSB-27	Divya et al., 2013
Resistance against pest (Callosobruchus chinensis)	Palem-2	Divya et al., 2012
Yellow mosaic virus resistance	AK-38, HG-14, HG-52, HG-59, HG-63 and HG-75	Parimala <i>et al</i> ., 2011
Powdery mildew	AK-38, HG-14, HG-52, HG-59, HG-63 and HG-75	Parimala <i>et al.</i> , 2011
Pest resistance	Nagamangala Pac 9 strain	Roopashree et al., 2006
Yellow mosaic virus resistant	2-R, 7-R, 8-R and 9R	Sankar <i>et al</i> ., 2002

#### Table 6: Genetic resources identified for various biotic stresses in horsegram.

#### Table 7: List of potential donors of abiotic stress tolerance/resistance.

Trait	Source	Reference
Heavy metal (nickel) stress tolerance	HGR-4	Edulamudia <i>et al</i> ., 2021
Heavy metal (chromium) stress tolerance	Variety Madhu	Dhali et al., 2021
Salinity stress tolerant	Paiyur-2	Kanagaraj and Sathish, 2017
Moisture stress tolerance	D13	Yasin <i>et al</i> ., 2016
Drought tolerant	M-249	Bhardwaj <i>et al</i> ., 2013a
Drought tolerant	M-249	Bhardwaj <i>et al</i> ., 2013b
Drought resistant	HPK4	Bhardwaj <i>et al</i> ., 2013b
Drought tolerant	Cultivar VZM1	Reddy <i>et al.</i> , 2008
Heavy metal (lead) stress tolerance	Cultivar VZM1	Reddy <i>et al.</i> , 2005
High antioxidative activity	Cultivar VZM1	Reddy <i>et al.</i> , 2005
Salinity stress tolerance	Cultivar VZM1	Reddy <i>et al.</i> , 1998

## 7. BREEDING SYSTEMS

Efforts to genetically improve horsegram have mainly concentrated on exploiting the genetic variation represented in the primary gene pool. The approach has not been overly successful, probably because the crop's genetic base is somewhat narrow. The introduction of landrace materials, exotic germplasm, and wild relatives can theoretically serve as a source of novel variation. In comparison to other pulse crops, investment in horsegram breeding and fundamental research has been rather meagre (Sharma et al., 2015a), and the crop remains prone to a variety of diseases and pest damage. The primary breeding objectives are much the same for horsegram as they are for other pulses: the focus is to develop varieties with a higher yield potential, with tolerance/resistance against commonly occurring biotic and abiotic stresses, and to produce seed of good cooking quality. The move towards mechanical harvesting will require breeders to generate plants which mature early and synchronously, are photo-insensitive, produce non-shattering pods, and are determinate and erect. Raising the seeds' protein content is also a priority. Being a self-pollinated crop, mass selection, pure line selection, hybridization, mutation breeding and various biotechnological approaches are all appropriate. Given the crop's predominantly autogamous habit, maintenance of seed purity requires controlled seed multiplication only every 4-5 years.

### 7.1 Selection methods

### 7.1.1 Mass selection

Mass selection is the simplest, most widely used, and oldest technique to enhance crop yield. It involves selecting many plants of similar phenotype and mixing their progeny to produce a new variety. Whether any progeny testing is incorporated or not, the method is easy, effective and highly economical. Cultivating a given variety over a prolonged period does risk the loss of genetic purity as a result of either seed admixture and/or cross fertilization; however, in a self-pollinated crop like horsegram, out-crossing can also be exploited as a means of introducing new genetic variation - the idea is to bulk seed from a group of elite parents and then continue selection on the basis of phenotype alone across a number of successive generations. Instead of bulking the seed from a collection of plants, the procedure can be improved selecting specific plants as the source of seed for the next generation - a strategy which is well-suited to addressing low heritability traits. The horsegram variety HPK-4 (and others) were bred in this way.

#### 7.1.2 Pure line selection

Pure line selection, also known as individual plant selection, is the process of identifying a superior plant(s) and using its offspring to create the next generation. This approach is commonly used to purify elite lines from a mixed population, such as a landrace. Three phases are typically involved in pure line selection: first, a substantial number of superior plants is chosen from the source population; then the offspring of these plants are assessed, frequently over a period of years; and finally, extensive, replicated trials are carried out to validate the lines' performance.

#### 7.1.3 Hybridization

Hybridization is used to develop a crop variety by combining favourable trait(s) present in two or more lines. Hybridization in horsegram is hampered by the small size of its flowers, but a number of varieties have been released using this technique. A national programme has been initiated in India to use intra-specific hybridization as a means of genetically improving horsegram. Given the paucity of genetic variation in horsegram's primary gene pool, wide hybridization offers a potentially powerful means of broadening the crop's genetic base. M. axillare is the probable progenitor of the domesticate, so represents in principle the most appropriate donor of novel genes governing important traits such as the number of seeds produced per plant and the number of seeds set per pod, as well as tolerance to drought and low temperature (Fuller, 2018). However, it has been observed that hybrids created by crossing the domesticate with *M. axillare* are sterile, probably due to the differences in the sizes of the the two species' chromosomes (Mishra et al., 2024). A second potential donor is M. sar-gharwalensis, which produces seed of high protein content, and forms erect, bushy plants; however, to date it has not been possible to generate a hybrid with the domesticate. In future this may be possible to achieve by exploiting embryo rescue technology. The genus Macrotyloma comprises more than 25 species, but whether any of these harbour beneficial traits which can be introgressed to horsegram has yet to be fully assessed. A wide hybridization programme has been undertaken at Chaudhary Sarwan Kumar Himachal Pradesh Agriculture University (CSK HPAU), focusing on the transfer of a number of desirable traits from *M. axillare* and *M. sar-garhwalensis*.

### 7.1.4 Mutation breeding

The scantiness of natural variation for a number of important traits has encouraged efforts to induce *de novo* variation *via* either  $\gamma$  irradiation or chemical mutagenesis based on ethyl methane sulfonate (EMS). The deleterious effect on germination, seedling height and plant survival following exposure of the seed of the variety Rayat-1 to high concentrations of EMS has been documented by Awate and Bolbhat (2015). Irradiating seed of the variety HPKC-2 with 250 Gy of y rays was successful in inducing a number of interesting alterations in phenotype (Chahota et al., 2013a). Examples of traits targeted in this way include maturity time, determinacy and dwarfness. A mutation breeding programme in horsegram was initiated at CSK HPAU in 2009: the programme selected the varieties HPKC-2 and VLG-1 (both well adapted to the local region as targets in which to induce desirable variants. After exposure to three doses of y irradiation (150, 250 and 350 Gy), three types of mutants were generated: the first group, represented by selection HPKM193, combined semi-dwarfism, determinacy, photo-insensitivity and early flowering with early and synchronized maturity; the second group, represented by selection HPKM317, combined dwarfism, determinacy, a bushy habit and photo-insensitivity with synchronous medium maturity; and the third group, represented (HPKM150, HPKM151 and HPKM201) differed from the parental type only with respect to seed colour (Fig. 4). All these materials have been incorporated into the breeding programmes undertaken at various institutes.



Fig. 4: Desirable mutants induced by exposure to γ irradiation of HPKC2: (a) Parent type HPKC2; (b) HPKM317; (c) HPKM193.

HPKM191, a very early flowering and early maturing mutant, has been registered by NBPGR and included in the horsegram National Crossing programme. The mutagenesis-derived varieties CRHG-6 and CRHG-8 were registered by the ICAR Plant Germplasm Registration Committee in 2011 and released for cultivation in southern India: both are less susceptible to shattering and improved with respect to tolerance to yellow mosaic virus, powdery mildew and mite infestation (Salini *et al.*, 2014).

According to Bolbhat *et al.* (2012), treatment with EMS was more effective than  $\gamma$  irradiation in inducing pollen sterility. Bolbhat *et al.* (2012) have reported the use of both  $\gamma$  irradiation (100–600 Gy) and EMS (0.2–0.6%), separately and in combination, to mutagenize the variety Dapoli Kulthi-1. Increasing the severity of exposure resulted in a steady reduction in the seeds' ability to germinate and in the seedlings'ability to elongate both the root and shoot. Mutants were recovered with respect to plant height, primary branch number, the number of pods produced per plant, the number of seeds set per pod, pod length, 1,000 grain weight and yield per plant. Mutagenesis has also been effective in improving the digestibility of horsegram seed (Bolbhat and Kamble, 2015).

#### 7.1.5 Varieties of horsegram released in India

Most of the varieties that have been developed to date for release in India, either at the state or the national level, have been selected from local germplasm. Many of these varieties are not well adapted outside the region in which they were selected, primarily because of their photo-sensitivity (Table 8). The variety Co-I was bred in the Ramanthapura district of Tamil Nadu (southern India). Its yield was 20 to 25% higher than the local variety. In 1976, the varieties Hebbal Hurali-1 and Hebbal Hurali-2 were released for cultivation in Karnataka (southern India). Each of these varieties are earlier maturing (90-100 vs 120 days) than their progenitor (PLKY-32 and EC-1460, respectively). In addition to their being highly productive, both Hebbal varieties are photo-insensitive, thereby permitting their year-round cultivation. Other releases from the All-India National Programme on Arid legumes include VL Gahat 1, VL Gahat 10, CRHG-01, CRHG-02, CRHG-03, KBHG-1, BJPL-1, AK42, AK-21, Indira kulthi 1, VL Gahat 8, VL Gahat, 19, Palem 1 and CRIDA 18 (Anonymous 2013).

Source of release	Recommended	Variety		Growth habit		Year of
	state		Days to maturity	Characteristics	Average yield (kg/ha)	release
Sardar Dantiwada Agricultural University, Krushinagar, Gujarat	Gujarat, Rajasthan, Uttarakhand, Jharkhand, Uttar Pradesh and Maharashtra	Gujarat Dantiwada Horsegarm1 (GHG-5)	89-100	Resistant to root rot, moderately resistant to powdery mildew, Collar rot, <i>Cercospora</i> leaf spot and leaf blight.	500-600	2012
Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu	Tamil Nadu, Karnataka, Andhra Pradesh and Odisha	Paiyur-2	100-106	For Sept- Oct sowing	800-900	2001
University of Agricultural Sciences, Dharwad, Karnataka	Uttar Pradesh	PHG 9	100-105	Semi-spreading thick foliage	200-002	2001
Central Research Institute for Dryland Agriculture, Hyderabad, Telangana	Telangana and Andhra Pradesh	CRIDA LATHA (RHG 4)	72-110	Tolerant to Yellow Mosaic Virus, powdery mildew, leaf blight, root rot and mites	800	2010
All India Coordinated Sorghum Improvement Project, Bijapur, Karnataka	Karnataka	GPM-6, PHG-9	120-130	Resistant to Yellow Mosaic Virus, moderately resistant to Rhizoctonia root rot	800-900	2008
Central Research Institute for Dryland Agriculture, Hyderabad, Telangana	Tamil Nadu, Karnataka, Telangana and Andhra Pradesh	CRIDA-1- 18 R	72-102	Tolerant to Yellow Mosaic Virus, powdery mildew, leaf blight and root rot	800	2009

Table 8: State-wise released varieties of horsegram in India.

Source of release	Recommended	Variety		Growth habit		Year of
	state		Days to maturity	Characteristics	Average yield (kg/ha)	release
	Andhra Pradesh and Telangana	PDM 1 and VZM 1				
	Tamil Nadu	Co-1, 35-5- 122, 35-5-123			Better yield than the native varieties	1970
Maharana Pratap University of Agricultureand Technology, Udaipur, Rajasthan	Gujarat	Pratab Kulthi-1 (AK- 42), GHG-5	83-87	Protein 30% with lush green foliage with wax deposition	1000- 1200	2005
Maharana Pratap University of Agriculture and Tech nology, Udaipur, Rajasthan	Rainfed areas of Northwestern parts of India	Arja Kulthi 21	70-105	Early maturing	800-900	1998
Vivekananda Parvatiya	Uttarakhand	VL- Gahat-8	92-106	Anthracnoseand stem rot resistant	1200	2007
krisni Anusangnan Sansthan, Almora, Uttarakhand		VL Gahat-10	110-115	Resistant to Yellow Mosaic Virus, root rot and leaf spot	1000	N
		VL Gahat, 19	88-94	Multiple disease resistant	500	2010
		VL Gahat 15	95-105	Resistant to Anthracnose and leaf spot	500-600	2009
Rajendra Agricultural University, Pusa (Samastipur), Bihar	Rajasthan	KS-2, Pratap Kulthi (AK-42)	80-85	Early maturing, brown seeds	600-700	1991

Source of release	Recommended	Variety		Growth habit		Year of
	state		Days to maturity	Characteristics	Average yield (kg/ha)	release
Acharya N. G. Ranga Agricultural University, Hyderabad, Telangana	Andhra Pradesh and Telangana	Palem-1, Palem-2, Paiyur-2, PHG-9	80-85 100-105	Early maturing, Semi-spreading	1000- 1200 800-900	1998
Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh	Chhattisgarh	Indira Kulthi-1	92	Up lands under rainfed condition with sowing time of 15 August onwards	700	2010
Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyala, Palampur, Himachal Pradesh	Himachal Pradesh	Baizu	125	Field resistant to anthracnose and very good cooking quality	750	1991

## 8. BIOTECHNOLOGICAL INTERVENTION

Interventions based on DNA technology could have a substantial positive impact on the yield potential and yield stability of horsegram. The National Centre for Biotechnology Information (NCBI) database curates only 1,025 horsegram expressed sequence tags, while an additional set of 1,050 has been acquired from stress-induced plants by Reddy *et al.* (2008). Bhardwaj *et al.* (2013a) have generated a substantial body of transcriptomic sequence data from the shoots and roots of plants subjected to drought stress, which led the recognition of >21,000 unique genes. Microsatellite (SSR) sequences are relatively commonplace in the genome. Although the genome size of horsegram is relatively small (estimated to be 389 Mbp, according to Shirasawa *et al.* (2021)), the extent of the genomic resources available for horsegram remains limited.

### 8.1 Marker-assisted selection

DNA-based markers represent a key means of identifying genomic regions harbouring genes determining a given agronomic trait. CSK HPAU curates a collection of >700 horsegram accessions, of which 360 are under phenotypic evaluation and genotypic characterization. Prior marker-based diversity studies have confirmed the presence of plenty of DNA sequence variation within this germplasm (Sharma et al., 2015b). Using a panel of 360 accessions, Chahota et al. (2017) combined data collected with respect to 24 morphological traits with genotyping based on 30 SSRs; the indications were that two separate gene pools exist, one comprising materials sourced from the Himalayan foothills and the other from southern India. A set of 117 SSRs were mined from transcriptome sequence data by Kaldate et al. (2017) and used to document the genetic diversity present in a panel of 48 diverse accessions in horsegram. A set of SSRs present within expressed sequence tag sequences, along with a number of intron length polymorphism markers, has been developed by Liu et al. (2008) and Choudhary et al. (2022) for diversity analysis, linkage mapping and quantitative trait locus (QTL) discovery.

Tables 9 and 10 present the current state of knowledge regarding genes/ QTL identified from several mapping populations. Next generation sequencing technologies (Thudi *et al.*, 2012) are now beginning to be deployed to acquire more extensive genomic data, as exemplified by the work of Chahota *et al.* (2017), who were able to use *de novo* acquired DNA sequence to identify a significant number of SSRs. Sharma *et al.* (2015b) and Chahota *et al.* (2020) used DNA-based markers

Trait	Type of mapping population	Pedigree	Population size	Reference
Morphological and phonological traits	RILs	HPKM249 X HPK4	162	Katoch <i>et al</i> ., 2022
Drought stress tolerance	RILs	HPKM249 X HPK4	162	Katoch <i>et al</i> ., 2022
Drought and yield related	RILs	HPKM249 X HPK4	190	Chahota <i>et</i> <i>al</i> ., 2020

#### Table 9: Trait specific mapping populations exploited for mapping important agronomic traits.

#### Table 10: Genes/QTL underlying variation for key traits as identified by mapping.

Trait	Genes/QTLs*	Reference
Drought stress tolerance	7 QTLs for relative water content, 4 QTLs for root volume, 8 QTLs for root length	Choudhary et al., 2022
Reproductive period	qRP01	Katoch et al., 2022
Early maturity	qMT01, qMT02	
Days to 50% flowering	qFL01	
Plant height	qPH01	-
Primary branches	qPB01, qPB02, qPB03	-
Secondary branches	qSB01, qSB02, qSB03	-
Drought stress related traits	qCHL01, qMDA01, qPRO01, qRD01, qRF01, qRL01, qRL02	Katoch <i>et al</i> ., 2022
Drought stress tolerance	qDFW01, qDTM0, qRL01, qNSPP01,	Chahota <i>et al</i> ., 2020
Yield related	qDFW02	Chahota <i>et al</i> ., 2020
Drought stress tolerance	MuWRKY3	Kiranmai <i>et al</i> ., 2018
Drought stress tolerance	MuNAC	Pandurangaiah et al., 2014
Drought stress tolerance	NAC, MYB, WRKY, bHLH, AP2-EREBP	Bhardwaj et al., 2013b
Drought stress tolerance	Dehydrin	Ramya <i>et al</i> ., 2013
Salt stress tolerance	MuNAC4	Pandurangaiah et al., 2013
Drought stress tolerance	NAC, MYB, WRKY, AP2-EREBP, C3H, PHD and bHLH, GNAT, TIG and G-2, SNF2, bZIP, TRAF FAR1, Calmoldulin binding factor, dehydrin	Bhardwaj et al., 2013b
Drought stress tolerance	Hsp70, GST, CRT, CDPKs, CLB, CIPKs, CaMBPs, MAP Kinase, LTPs	Reddy <i>et al.</i> , 2008

to explore the genetic relationship between the genomes of the domesticate and its two wild relatives: their conclusion was that *M. axillare* is more closely related to the domesticate than *M. sar-ghawalensis*, confirming the conclusions reached from cytological and molecular marker data.

### 8.2 Linkage mapping and QTL identification

A framework linkage map based on the segregation of 211 molecular markers in a population of 190 recombinant inbred lines (RILs) derived from a cross between HPK4 and HPKM249 (Table 9) has been published by Chahota et al. (2020). The map comprises 13 linkage groups (LGs) and spans 1,423.4 cM, with a mean inter-marker interval of 9.6 cM. The map was used to characterize the genetic basis of eight agronomic traits, for which the RILs were evaluated over two years. Five QTL, explaining variation for four traits, of which two were related to the drought response (number of days to temporary wilting and root length), one to yield (number of seeds per plant) and one to phenology (number of days to maturity) were mapped to five different LGs, each associated with a LOD score of >4.0. Katoch et al. (2022) used 162 RILs bred from the same parental combination to identify the genomic regions harbouring genes responsible for early maturity and some yield-related traits. In this case, the map derived from a rather larger set of data (493 markers) comprised ten LGs and spanned 1,541.7 cM (mean intermarker interval of 5.2 cM). In all, 27 QTL (LOD >2.5) associated with 24 traits were detected, using phenotypic data acquired from three environments: 15 of the QTL each explained at least 10% of the variation, and five were stable across the environments (Table 10). The largest single QTL, which explained 53.4% of the variance, was associated with root length (Fig. 5). Choudhary et al. (2022), rather than using a biparental population for QTL discovery, performed a genome-wide association study, based on 20,241 single nucleotide polymorphisms (SNPs) scored on a set of 96 accessions. Nearly half (43.8%) of the SNPs mapped to an intergenic region, while nearly one quarter (21.6%) were located within an intron. Seven QTL were identified as associated with relative water content, four with root volume and eight with root length. Some of the QTL sites coincided with those of genes related to the abiotic stress response.

### 8.3 Whole genome sequencing

Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishwavidyalaya (CSK HPKV) in collaboration with Kazusa DNA Research Institute (Chiba, Japan), have recently acquired the genome sequence of the variety HPK4 (Shirasawa*et al.*, 2021). The overall length of the assembled genome was 294.7 Mb, but the true size of the



Fig. 5: QTL responsible for the variation in several agronomic traits.

genome was estimated to be somewhat larger, lying in the range 388-398 Mbp. The genome assembly reported by Bhardwaj *et al.* (2013b) was based on ~30,000 shoot and root transcript sequences, of which ~3,500 appeared to be unique to horsegram. The HPK4 genome assembly predicted the presence of 36,105 protein-coding genes, of which 14,736 appeared to be horsegram-specific; most of the latter genes mapped to the centromeric region of several chromosomes. As sequence diversity tends to be similarly concentrated in these chromosomal regions, the inference was that many of the genes responsible for morphological diversity belong to the group of horsegram-specific genes. However, exploration of the genetic diversity represented in a set of 89 accessions using a dd-RAD-Seq approach identified only 277 SNPs, which implied that the genetic base of these accessions was rather narrow. A synopsis of the current state of whole genome sequencing in horsegram is given in Table 11.

Estimated genome size	Availability of reference/draft genome (with data base ID)	Sequencing chemistry used	Genome coverage %	No. of predicted genes	Reference
259.20 Mb	PRJDB5374	Illumina HiSeq, 2000	89.00	36,105	Shirasawa et <i>al</i> ., 2021
279.12 Mb	PRJNA721661	Illumina HiSeq 2500	83.53	24,521	Mahesh <i>et al</i> ., 2021

able 11: Whole genome sequen	ce information	ı available in	horsegram.
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## 9. TISSUE CULTURE TECHNIQUES

An efficient means of regenerating plants from *in vitro* cultured cells or callus is required for both *in vitro* mutation breeding (somaclonal variation) and for transgenesis. Tejavathi *et al.* (2010) have established an effective protocol for the regeneration of numerous shoots from callus formed following the *in vitro* culture of shoot tips or cotyledonary nodes. The various steps involved in the procedure are illustrated in Fig. 6. Horsegram plants can also be regenerated from cell suspension cultures (Mohamed *et al.*, 2004), in which embryogenic callus is first induced from leaf segment explants, which are then transferred to a liquid medium to allow the embryos to differentiate and mature (Fig. 7). An effective



Fig. 6: (a) Development of callus from cotyledonary explant (b) Profuse callusing; (c) Shoot development from cotyledonary callus; (d) Proliferation of multiple shoots.



Fig. 7: (a) Microscopic view of the stages of somatic embryogenesis; (b) Regenerating callus (c) young plant regenerated from a somatic embryo; (d) hardened plant.

means of transforming horsegram has also been established, based on the use of an *Agrobacterium tumefaciens* strain containing the binary vector pCAMBIA2301 (Fig. 8). The protocol has been detailed by Amal *et al.* (2020).



Fig. 8: General protocol for *Agrobacterium*-mediated genetic transformation of horsegram.

## **10. AGRONOMY**

Horsegram grows in a wide range of soil types, from granitic sands to heavy clays, provided the soil pH is in the range 6.0 to 7.5; it can even tolerate moderately saline soils. It prefers either a tropical or a sub-tropical climate, where the yearly rainfall lies in the range 500-700 mm, and the air temperature ranges between 20°C and 35°C. Horsegram is intolerant of frost and prolonged periods of low temperature (Purushottam *et al.*, 2017). It also cannot survive water logging.

In India, horsegram is cultivated from sea level up to 2,200 m asl in a range of soil types and across a number of agro-climatic zones. However, 80% of the national production is concentrated in semi-arid dry land environments located in the southern states of Tamil Nadu, Andhra Pradesh and Karnataka. Smaller areas of cultivation are found in Madhya Pradesh and Chhattisgarh. The crop is important in the northwestern Himalayas (Himachal Pradesh and Uttarakhand), where traditional cultivation practices are used to grow the crop under organic and rainfed conditions on soils considered unfit for the cultivation of other crops. Kharif-grown crops raised in low rainfall areas are typically sown in late June, flower from mid-July to mid-August and are harvested in mid to late October. It is also grown as a late catch crop if the season has been too dry to grow other pulses. In Uttarakhand and Himachal Pradesh, horsegram is generally grown mixed with either finger millet and maize. The stems of the main crop are left in the ground after harvest to provide mechanical support for the horsegram plants, which avoids the loss of horsegram biomass through rotting. In the Uttarakhand hills, horsegram is a component of the popular "Barah Anaaja" system of traditional mixed cropping in which seeds of twelve food grains are mixed and grown. In southern India, the crop is sown between September and November, primarily as a mixed crop along with niger (Guizotica abyssinica), various forms of millet or pigeon pea. Its reputation for drought tolerance favours its use in low rainfall locations, where it is typically raised as a rainfed crop.

### 10.1 Crop management

Horsegram plants develop vigorously when supplied with well-rotted farmy ard manure (FYM). If the soil's NPK status is low, an application of 500-600 kg/ha of FYM is recommended, coupled with a basal fertilizer treatment of 20-25 kg/ha N, 25-30 kg/h P and 12.5 kg/ha K. The provision of *Rhizobium* inoculum can improve the plants' ability to absorb nutrients. Treatment of the seed with fungicide helps to protect the plant from various seed-borne diseases (Purushottam *et al.*, 2017).

The seed should preferably be sown at a depth of 1.5-2.0 cm in a well ploughed field. A within-row spacing of 10 cm and a between-row spacing of 30 cm is recommended. However, many subsistence farmers simply broadcast the seed. When mechanically drilled, the crop can be established on 30 cm wide ridges, separated by 1.2-1.8 m furrows. Current varietal recommendations include BR 5, BR 10 and Madhu in Bihar; HPK-2 and HPK-4 in Himachal Pradesh; PDM 1 and VZM 1 in Andhra Pradesh; K82 and Birsa Kulthi in Jharkhand; S27, S8, S39 and S1264 in Odisha; Co1, 35-5-122 and 35-5-123 in Tamil Nadu; Hebal Hurli 2, PHG 9 and KBH 1 in Karnataka; Maru Kulthi, KS 2, AK 21 and AK 42 in Rajasthan; and VLG 1 in Uttarakhand (Kumar, 2005). Seed production is reduced by water stress, so the crop should be irrigated if possible if the field becomes dry (Purushottam *et al.*, 2017), especially during flowering, pod formation and seed development.

### 10.2 Control of pests and diseases

Within three days of sowing, chemical weeding methods may be applied to manage weeds. Spraying with 2 ml/l aqueous pendimethalin should control the growth of early developing weeds. Manual weeding should be performed 14 days after sowing. Insect pests can be controlled by two applications, separated by ten days, of 2 ml/l aqueous quinalphos. methyldematon, dimethoate and phosphomidan to suppress aphids, leafhoppers and whiteflies, respectively. Root rot wilt is controlled by drenching with a 0.1% solution of bavistin, while leaf spot and powdery mildew are controlled by spraying with 1% bavistin (Purushottam *et al.*, 2017). Horsegram plants are attacked by a number of common pests (aphids, stem fly, leaf hopper and whitefly) and diseases (*Cercospora*, root rot wilt, leaf crinkle, leaf curl and mosaic virus): details of the pests/pathogens and recommended control measures are given in Table 12.

### **10.3 Harvesting methods**

The horsegram crop is harvested when the leaf tissue turns from green to strawcoloured. Harvesting is commonly manually-based, as mechanical harvesting leads to excessive seed loss because of shattering. The crop is typically cut with a sickle and the chopped plants are laid out on a threshing floor for 2-3 days for drying. Threshing is usually accomplished by beating the dry material with a stick. For long-term storage, cleaned seed should be left to dry down to a moisture content of <12%, and then be kept in a cool, dry environment. The remaining biomass, including the pods, is suitable for use as cattle fodder (Purushottam *et al.*, 2017).

#### Table 12: Insect/pest/disease management.

Insect/Pest/Disease	Symptoms	Control measures
- The	As a result of sucking the liquid by adults and nymphs the leaves get brown and crumpled, and the plants appear ill.	Spray with 1 ml/l oxydemeton methyl 25 or 1.7 ml/l dimethoate 30 EC.

Aphids



Adults and nymphs suck the juice from the leaves, causing the leaves to become brown and the surface of the leaves to become uneven. Leaves dry up and fall off.

Spray with 1 ml/l cypermetharin, 2.5 ml/l or sulfoxaflor or chlorpyrifos, 1 ml/l dimethoate

Jassids



This is a polyphagous caterpillar, which bores a hole in pods and occasionally feeds on the seed. Spray with 2.5 ml/l or 2 ml/l quinolphos 25 EC

Pod borer



White fly (vector for yellow mosaic virus) Young leaves first show signs in the form of yellow, dispersed, spherical dots spread over the leaf lamina. The leaves that have been affected become necrotic. Plants that are infected develop more slowly and produce fewer flowers and pods. The pods are stunted and generally immature, although the seeds that do develop are shrivelled. Spray with 2 ml/l oxydemeton methyl 25 EC or 1.7 ml/l dimethoate 30 EC and repeat after 15 days, if necessary

Insect/Pest/Disease	Symptoms	Control measures
Foot rot wilt	Infected seedlings begin to desiccate; if not controlled, the whole plant will die within a few days.	Drench the infected areas with 0.1% bavistin
Cercospora leaf spot	A fungal disease that forms small brown- black spots on the leaves. On Older plant the leaflet infection is on older leaves and may cause serious defoliation.	Spray with 1% bavistin at the appearance of disease.
Powdery mildew	A disease caused by <i>Erysiphe polygoni</i> that produces white powdery patches on both surfaces of the leaves and later on covers the entire leaf. In severe infection foliage become yellow and causing premature defoliation.	Spray with 1.5 ml/l wettable Sulphur or 0.5 ml/l propiconazole or 1% bavistin at the appearance of disease.
Leaf anthracnose	A disease caused by <i>Colletotrichum</i> <i>truncatum</i> . Symptoms are circular, black, sunken spots with dark centre and bright red orange margin on leaves and pods.	Spray with 1% bavistin at the appearance of disease.

### 10.4 Post-harvest management

The most detrimental pests for stored legume seeds in India (and elsewhere) are storage pests, especially bruchids, which cause significant losses in terms of quantity and quality to stored pulses. To minimize such losses, seeds can be kept in mud containers containing up to 100 kg of seed; the container is sealed with a lid after the addition of a mixture of whole grains and clay (1% to 2%). Vegetable oils can also be used to repel pulse beetles. The most suitable such oils are sesame, castor and niger. Whole grains are coated with a 0.5-1.0% oil infusion prior to storage, a treatment which discourages pest insects from laying eggs and/or hatching larvae on grain surfaces. Neem oil (2-5 mL/kg) has also been found to be useful to inhibit insect activity. Storage vessels should ideally be moisture proof and airtight and should be protected from excessive thermal fluctuation (Lal and Verma, 2007). A particularly effective means of controlling bruchids is based on storage in PICS bags, in which up to 80 kg of seed is packed into three 80–100-micron polythene bags; the method works by suffocating any bruchids present in the grain. Experiments have shown the absence of infestation even after nine months of storage (Lal and Verma, 2007).

Chemical control of storage pests is also possible, both initially upon storage and/or during the storage period to prevent reinfestation (Lal and Verma, 2007). Fumigation with either methyl bromide or magnesium (or aluminium) phosphide gas is an effective (albeit hazardous) means of killing storage pests. A safer form of fumigant, as demonstrated by Divya *et al.* (2016), is to increase the concentration of carbon dioxide in the storage vessels. Seed can be stored in a carbon dioxide rich environment for up to six months without any negative effects on germination or seedling vigour. Preventing rodent damage is a challenging task. Rodents can be managed in storage facilities utilising a variety of mechanical (trapping), chemical (poisoning) and physical (sonic and ultrasonic emitters) methods.

## **11. CURRENT RESEARCH PRIORITIES**

Horsegram is an easy to cultivate, nutritious legume, which is relatively resistant to pests and diseases. The US National Academy of Sciences has described it as a possible food source for the future based on its nutritional and medicinal value, as well as on its ability to tolerate drought (National Academy of Sciences, 1978). With some breeding intervention, particularly focused on plant architecture, it has the potential to become a commercially viable crop (Cullis and Kunert, 2017). However, the area cultivated to horsegram has been in marked decline, possibly reflecting societal scorn, changing lifestyles and a lack of research supporting the cultivation of orphan crops. Both the scientific community and the private sector have given it little attention, either because of its unfavourable taste and/ or its poor cooking quality, and as a result, the crop suffers from a narrow genetic base and an inadequately explored genome (Deodhar, 2016; Kamei et al., 2016). Notwithstanding its good nutritional value and medicinal potential, it is only rarely cultivated on prime land. The consequence is that horsegram is grown as a low input crop on marginal land. Despite some investment into genetic improvement, most of the crop is produced using low-yielding landraces, while the availability of improved germplasm is rather limited. Expanding its productivity, and thereby its area of cultivation, will require correction of its indeterminacy and twining growth habit, increasing its plant height, removing its photosensitivity, breeding for earlier flowering, synchronizing crop maturity (Chahota et al., 2013a) and introducing shattering resistance. A substantial research effort will be needed to elucidate the genetic basis underlying variation in these key traits.

In addition, there is a need to educate the public about the value of horsegram, to stimulate demand. Improvements in pulse production and nutrition will only be accomplished if the cultivation of currently orphan pulses such as horsegram can be encouraged by providing a package of appropriate technology. Varietal improvement could be accelerated by taking advantage of genomics-assisted breeding, a concept which combines conventional breeding with genomic resources, and is being applied to secure genetic advances in the major pulse crops (Varshney *et al.*, 2009, 2021). The availability and accessibility of the necessary genomic tools, which includes markers linked to key traits, is a prerequisite for this breeding approach. Overall, the message is that research is urgently needed into this legume (Chel-Guerrero *et al.*, 2002; Arinathan *et al.*, 2003) to promote its use as a nutraceutical forage and food for malnourished populations of the world (Morris, 2008).

# **12. RESEARCH CENTRES**

The research centres currently involved in the horsegram improvement in India are given in Table 13.

Institution	Specialization and research activities	Website
Indian Institute of Pulses Research, Kalyanpur, Kanpur	• To uphold the increase in production of kharif pulses (pigeonpea, mungbean, urdbean, cowpea, horse gram, moth bean, cluster bean, rajmash and rice bean) at national level, through intensification and enhanced genetic gain of cultivars.	https://iipr.icar. gov.in/
	• Plant Genetic Resource management (collection, evaluation, maintenance and utilization) in association with ICAR-IIPR and ICAR-NBPGR.	
	• Development of high yielding varieties including hybrids with multiple stress tolerance and enhanced nutritional quality for different agro-ecological zones.	
University of Agricultural Sciences, Yettineyudda Campus, Krishi Nagar, Dharwad	<ul> <li>To develop location-specific varieties and crop production technologies for pulses.</li> <li>Capacity building of State Agricultural Department officials/NGO's/Farmer's in improved pulse production technologies in southern Indian states in association with UAS, Dharwad.</li> <li>Plant genetic resources management of pulses and their utilization.</li> <li>Genetic enhancement in cowpea and horsegram for yield and multiple stress resistance.</li> </ul>	https://iipr. icar.gov.in/ dharwad/
Central Research Institute for Dryland Agriculture, Santoshnagar, Hyderabad	Crop improvement research in developing drought tolerant varieties by various crop AICRPs.	https://www. icar-crida.res. in/
Indira Gandhi Krishi Vishwavidyalaya, Raipur	<ul> <li>Developing educational and research networks in agriculture and allied field for the state agriculture and allied sectors.</li> <li>Imparting knowledge and education to the people engaged in agriculture and allied fields.</li> </ul>	https://igkv. ac.in/web/igkv. aspx

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Institution	Specialization and research activities	Website
	<ul> <li>Facilitate accelerated dissemination of improved technologies, knowledge and information.</li> <li>Promote effective, efficient and</li> </ul>	
	decentralized governance by introducing best management practices.	
Thakur Chhedilal Barrister College of Agriculture and Research Station, Ratanpur Road, Sarkanda, Bilaspur- Chhattisgarh - 495006	• To advancing sustainable farming practices through innovative research and technology. Our mission is to enhance agricultural productivity, promote environmental stewardship, and improve food security.	https://igkv. ac.in
CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, 176062	• To undertake location specific research in fundamental and applied aspects of crop improvement, natural resource management, plant protection, animal health, animal production and livestock product technology, home sciences and such basic sciences which help in the growth and development of various disciplines of agriculture and animal husbandry.	https:// hillagric.ac.in/
ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora Uttarakhand, 263601	<ul> <li>Basic, strategic and adaptive research for improving productivity and quality of important hill crops with emphasis on conservation and efficient utilization of natural resources</li> <li>Development of post-harvest technologies for value addition.</li> </ul>	https://vpkas. icar.gov.in/
University of Agricultural Sciences (UAS), Gandhi Krishi Vignan Kendra, Bangalore-560065, Karnataka	<ul> <li>Germplasm collection, evaluation, conservation and Utilization.</li> <li>Conventional and markerassisted improvement.</li> </ul>	https://www. uasbangalore. edu.in/
Sardarkrushinagar Dantiwada Agricultural University (SDAU), Palanpur, Gujarat	• To explore state of art agricultural technologies in consonance to the psycho- socio-economic and ecological status of the area for enhancing and sustaining the natural base to make agriculture more sustainable, remunerative and eco-friendly.	https://www. sdau.edu.in/
Regional Research Station of the Maharana Pratap University of Agriculture and Technology, MPUAT, Bhilwara, Rajasthan	• To develop improved varieties/ hybrids, economically viable production and protection technology for crops, farm mechanization, renewable energy sources, post-harvest technology, home science, animal production, etc.	https://www. mpuat.ac.in/

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