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Lathyrus: It's DUS Descriptors, Economic Uses, Nutritional Importance and Problem of Lathyrism

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ABSTRACT

Lathyrus sativus is one of the most neglected crops found in the realm of crops grown by farmers and specially agriculture system in India. Although it is found to be grown in many of the African countries like Somalia, Ethiopia and Bangladesh, Nepal but still it is not very popular among the crops found in India. In West Bengal, it is grown sporadically in the land after the rice harvesting but the local farmers and landraces are going to be wiped out because of fear of lathyrism found in this crop due to certain percentages of BOAA, the chemical which has it's limit beyond 2-3 % has detrimental effect on the central nervous system. As we always know that local landraces always have immense value because of all kind of alleles found in all crops including this crop. The landraces which are grown for 10-20 years are certainly have much more accumulation of different alleles evolved due to genotype and environmental interactions and they have tremendous impact in broadening the genetic base of the crop which will otherwise perish if landraces are not maintained properly by the farmers. The problem of this crop *lathyrus* is that farmers do not usually frequently grow this crop because of fear of lathyrism and economic return of the crop is not very good from the market. So, farmer's fraternity depends on the released varieties of *lathyrus* which are not very much in the market. In this way, farmers varieties are loosing its ground in this crop and genetic base of the crop is gradually becoming narrow. On the other hand, evaluation of this crop according to DUS descriptors should always be done from the conservation point of view. Following the DUS descriptors is the way of defining the genotyopes according to their potentiality of quality and quantitative characters are the most important factors from the plant breeders point of view. But the main challenge for the breeder is to produce the varieties having BOAA less than 0.2% which was set as a safe limit for consumption of this crop. Different breeding methods can be adopted for this crop for enhancing the crop quality and productivity of the crop.

Key words : Lathyrism, BOAA, Landraces, Alleles, Genotype, interactions, DUS descriptors.

Introduction :

Lathyrus sativus, also known as Grass pea (Chromosome number 2n =14), belongs to the family Fabaceae, subfamily Papilionaceae, is a strong legume crop that is considered one of the most resilient to climate changes and can survive during drought conditions. High-quality proteins

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and other advantageous traits can be found in *Lathyrus sativus*. It goes by several names in India, including "khesari," "chickling vetch," "batura," "Indian pea," "Kesare," "karas," "karil," "kasar," "khesari dhal," "lakhodi," "chattramatur," "santal," "teora," "tiuri," and "chickling pea." It goes by the names "Sabberi," "guaya," and "Khesari" in Bangladesh and Ethiopia, respectively. In Italy, it is referred to as "Cicerchiacoltivata," "pisellobretonne," and "pisellocicerchia." In Nepal, it is also known as "Kheshari," while in Pakistan, it is known as "Matri," "mattra," and other names. It has wild relatives in Iraq and is likely a descendant of the closest wild species to it genetically. *Lathyrus* deem (Townsend and Guest 1974; Hopf, 1986). According to Smart (1984), the grass pea is one of the oldest types of pulse crops. It is grown extensively throughout the world, particularly in Bangladesh, India, Nepal, and Ethiopia and to a limited extent in South America, northern Europe, Australia, some parts of Russia, Ukraine, China and also in southwest Asia. It develops as a food source for both green manure and feed purposes as well as for its seeds and vegetative portions. Central, southern, and the Mediterranean regions were the only places where this crop was primarily used.

Origin :

The name *Lathyrus* comes from the Greek word "lathyros", which means "exciting", and refers to the aphrodisiac properties attributed to grass pea (Loudon *et al.,* 1855). *Lathyrus sativus* has such a long agricultural history. Many authors have claimed that *Lathyrus sativus*'s origin was a mystery. Even in Southwest and Central Asia, which is assumed to be the region of origin, it is believed to have been entirely veiled by the natural dispersion cultivation (Smartt, 1984). However, current theories contend that the Balkan peninsula seems to be where the crop originated. There have been reports of wild *Lathyrus sativus* species in Iraq, however it is unclear if they are truly wild species or if they are accessions. Vavilov reported two distinct origin regions for *Lathyrus sativus* in 1951; one of them was the Central Asian Center, which included Afghanistan, the Republics of Tajikistan and Uzbekistan, as well as Western Tian-shan and North West India. Vavilov identified the Abyssinian centre as the second origin centre. According to a report by Jackson and Yunus (1984), 8000 BC-dated ancient archaeological evidence was discovered in the jarmo of Iraqi Kurdistan. Remains of the *Lathyrus* species have been discovered in Iran at Tepe Sadz and Ali Kosh (9500–7500 BC) (7500-5700BC).

Lathyrus sativus, however, is thought to have originated in the Bulkan Peninsula during the early Neolithic Period, at the beginning of the 6th millennium BC, according to a combination of phytogeographical and archaeobotanical evidence (Kislev, 1989). As a result of the introduction of agriculture from the Near East, *Lathyrus sativus* is the first crop cultivated in Europe. There is an epidemiological connection between Khesari dal consumption and a motor neural disease known as Lathyrism—the paralysis of the lower limbs in humans.

The toxic principle identified as ß-N-Oxalye-L-, ß diaminopropionic acid (ODAP), also known as ß-oxalyl amino

alanine, is the primary causal agent (BOAA). This toxin can be found in all parts of the plant (Campbell and Tewari, 1997). The paralytic effect occurs due to ODAP toxicity when the seeds of grass pea are consumed as a staple food, that is, 75 percent of the diet intake. It is safe when consumed at 5-30% of total diet intake. Out of the 200 species and subspecies in this genus, only *Lathyrus sativus*, is widely farmed as a food crop (Rahman *et al.*, 1991, Jackson and Yunus , 1984). Mesophytic species of *Lathyrus* are numerous.

History :

Lathyrus sativus has a long agricultural history, but its origin is unknown; however, its primary centres of origin are southwest and Central Asia (Smartt, 1990). According to the ancient Hindu treatise Bhava-prakasa, the triputa pulse causes a man to become lame and cripples and irritates nerves. Even Hippocrates recognised that certain peas were toxic to humans. Neurolathyrism is an ancient disease that has been known since Hippocrates wrote that eating peas on a regular basis could cause 'impotence' in the legs (Cohn & Kislev, 1987).

It was first domesticated between 7,000 and 8,000 years ago in the eastern Mediterranean region, and now it has been grown throughout Asia, North Africa, and southern Europe. This crop was primarily grown in the central and southern parts of Italy. Due to its low seed production (SP), sensitivity to pod breaking, delayed period of maturity, poor adaptability to mechanical harvesting, and presence of the neurotoxic 3-(-N-oxalyl)-L-2, 3-diaminopropionic acid in the seeds, it was almost entirely abandoned after World War II. (Campbell *et al.,* 1993). The *Lathyrus* germplasm collection has shown significant fluctuations in ODAP concentration. (Granati *et al.,* 2003; Siddique *et al.,* 1996; Hanbury *et al.,* 1999) heat seed treatment, conventional breeding, mutagenesis, and in vitro culture have all been used to reduce the quantity of neurotoxins in seeds, as well as genetic selection through these methods. (Tiwari and Campbell, 1996, Padmajaprasad *et al.,* 1997; Rybinski, 2003; Santha and Mehta, 2001).

Few *Lathyrus* species have the propensity to climb utilizing simple or branching tendrils. The grass pea of cultivated *Lathyrus* species has been the most important food grain and fodder since ancient times. Grass pea cultivation was discontinued largely due to presence of high quantity of neurotoxin, ODAP (â-oxalylL-á, â-diamino propionic acid), which, if ingested in significant quantity, caused lathyrism. Grass peas have a high protein content, a high level of adaptation to harsh environments, are disease resistant, and require little input to grow. It does not require irrigation or the application of harmful fertilisers or pesticides. It is a self-protective crop. Grass pea is also known as the most profitable crop because it has the highest productivity of all pulses at about 2.5 tonnes per hectare and requires very little labour and effort. In comparison to all other edible pulses, it is also the cheapest pulse grown in the country. The crop is mostly grown in Madhya Pradesh, Maharashtra, West Bengal, and Bihar in India.

ODAP and Lathyrism :

Grass peas have a reputation for being hazardous because, in some cases,

excessive ingestion can result in the neurotoxic condition neurolathyrism (Lambein and Kuo, 2009). The main nutritional problem with grass pea is that it has neurotoxic non-protein amino acid â-N-oxalyl-L-a,â-diaminopropionic acid (â-ODAP) in its seeds.(Adiga *et al.*, 1962, 1963; Kuo *et al.*, 1998; Murti *et al.*, 1964).

Among other factors, starvation and oxidative stress have been connected to the aetiology of neurolathyrism. By hyper-exciting the neurons, which results in spastic limb movements, the delicate action of â-ODAP induces paralytic neurolathyrism. Children who were given grass pea seeds have slower growth and underdeveloped nervous systems (Grela *et al.*, 2010).

It was decided that the crop should be abandoned as human food due to the incapacitating consequences of extended dependence on grass pea due to its inclusion of the neurotoxic â -N-oxalyl-L-a,b-diaminopropionic acid (ODAP), and seed sales were prohibited in some countries (Enneking, 2011). The cultivation of grass pea was banned in February 1961 due to its association with lathyrism in humans. However, national and international research centres continue to prioritise the enhancement of grass pea due to the growing demand for resilient food grains.

Major efforts in grass pea breeding over the past 50 years have been focused on lowering the ODAP concentration, resulting in the production of various varieties, including low ODAP types. (Kumar *et al.*, 2011). Today, it is also agreed that ODAP content in and of itself does not appear to be a problem because grass pea is safe to consume by both humans and animals if consumed as part of a balanced diet (Lambein and Kuo, 2009) and because seeds can undergo various processing techniques, such as fermentation, pre-soaking in alkaline solutions, cooking, and so on, that can partially detoxify them (Kuo *et al.*, 2000; Kumar *et al.*, 2011).

Less than 0.2% of ODAP concentration is considered safe for human ingestion (Abdel Moneim *et al.*, 1999). It is used for food, feed, and fodder. Generally, the seeds are hulled and parched before being used as food. It is frequently consumed as a soup, sauce, pancake-like unleavened bread, dal, flour, paste balls, fried and roasted seeds, immature pods, and young vegetative parts as a green vegetable, and other similar domestic cooked preparations. The toxin can be removed from the seeds by simply soaking them in hot water and discarding, or by consuming it after the coat has been removed. Thus, the toxin is detoxified up to 60% by the aforementioned methods, and other processes such as fermentation can achieve up to 90% detoxification.

L. sativus contains a toxic principle, its cultivation has been prohibited since 1954 under the Prevention of Food Adulteration Act (PFA, 1954) until it is declared that it can be consumed within the limit of < 0.2% ODAP content (Abdel Moneim *et al.*, 1999). The 1152 variety of Grass pea is the most common in India. So, the percentage composition of major nutrients in 1152 varieties of seeds is as follows: protein-18.2- 34.6 percent, fat 0.6 percent, and carbohydrate-58.2 percent, with starch accounting for 35 percent (Duke, 1981).

Grass pea : A blessing or a curse?

Grass peas can be used for a number of things, including as human food as well as animal feed and fodder. Albumins (14%), globulins (66%), glutelins (15%), and prolamins (5%), constitute the protein found in the seeds of *L. sativus* (Chandna and Matta, 1994). Human diets include *Lathyrus* as grains that are boiled and then either consumed whole or processed for split dal, as opposed to animal diets from grass pea, which often consist of ground or split grain or four and are generally used to feed lactation cattle or other animals (Enneking, 2011).

In addition, the fact that 58% of the fatty acids in grass pea are polyunsaturated makes it an excellent food for humans (Grela *et al.*, 2010). The grass pea gives many low-income communities food and nutrition security since it is a nutrient-dense food/feed crop with excellent drought tolerance and little input requirements for its cultivation. Like other orphan legumes, grass pea is still untapped in terms of its potential as a source of chemicals that can improve human health. For example, l-homoarginine is only found in this source.

Consequently, grass pea is a superb example of a prospective "functional food" from the perspective of nutraceuticals (Llorent-Martnez *et al.*, 2017). L-homoarginine is an amino acid that has benefits for treating cardiovascular disease (Rao, 2011; Singh and Rao, 2013) and treating the effects of hypoxia, or insufficient oxygen supply at the tissue level, which is linked to the development of cancer tumours (Ke and Costa, 2006; Jammulamadaka *et al.*, 2011). Therefore, a regular dietary intake of l-homoarginine from tiny amounts of grass pea needs additional research because it may be beneficial for human health (Rao, 2011).

Furthermore, grass pea, like most grain legumes, is deficient in the sulfur containing amino acids methionine and cysteine, but is high in lysine, which is deficient in cereals (Ravindran and Blair, 1992 Mahler-Slasky and Kislev, 2010). L. sativus' amino acid profiles are comparable to several grain legumes (Hanbury *et al.*, 2000). A lack of necessary sulfur-containing amino acids, such as methionine, which is essential in the central nervous neurological system (Amara *et al.*, 1995), which can be overcome cereals in a well-balanced diet (Lambein and Kuo, 2004).

However, grass pea has a reputation for being toxic because, under certain conditions, it has been linked to neurolathyrism, a neurological disorder in humans and domestic animals (Lambein and Kuo, 2009), due to its high concentration of the neuro excitatory -N-oxalyl-l,diaminopropionic acid (ODAP) (Vaz Patto and Rubiales, 2014). This paralyzing, but not lethal, condition is more severe when grass pea is the primary component of the diet, accounting for at least 30% of calorie intake for at least 3-4 months (Kumar, 1998).

Despite this negative connotation, -ODAP in grass pea has been regarded as a multifunctional plant metabolite. Under extended water stress, an increase in ODAP content has been seen (Hanbury *et al.*, 1999). Under polyethylene glycol (PEG)-induced circumstances of water stress, polyamines in the seedlings have

demonstrated a close association with ODAP level (Xing *et al.*, 2000a). The amount of ODAP in grass pea seeds is similarly impacted by the accumulation of abscisic acid (ABA) and H_2O_2 in response to water stress (Xing *et al.*, 2000b). Under conditions of water stress, it is hypothesized that ODAP neutralises the hydroxyl radical to safeguard glycolate oxidase function. (Zhang *et al.*, 2003; Zhou *et al.*, 2001).

Pharmacological Effects :

Antioxidant effects :

Lathyrus sativus antioxidant activity was assessed using DPPH scavenging activity, reducing power, - carotene bleaching inhibition, and TBARS formation inhibition. *Lathyrus sativus* exhibited high flavonoid concentrations and antioxidant activity [DPPH scavenging activity: 18.95 0.64 soaked and 15.23 0.48 mg/ml cooked; Reducing power: 3.94 0.06 soaked and 3.26 0.11 mg/ml cooked; -carotene bleaching inhibition: 0.92 0.16 soaked and 0.82 0.08 mg/ml cooked, and TBARS inhibition: 3.47 0.25 soaked and 1.62 0.09.

Nervous effects :

The open field and hole-cross methods were used to assess the CNS depressant activity of methanolic extracts of *Lathyrus sativus* seeds (200 and 300 mg/kg bw). At both tested doses (200 and 300 mg/kg), the methanolic extract significantly (p 0.001) decreased the locomotor. The open field and hole-cross methods were used to assess the CNS depressant activity of methanolic extracts of *Lathyrus sativus* seeds (200 and 300 mg/kg bw). At both tested doses (200 and 300 mg/kg), the methanolic extract significantly (p 0.001) decreased the locomotor activity of mice in open field and hole-cross methods, which were comparable to the standard drug, diazepam (1 mg/kg).

Antidiabetic effect :

The methanolic extract of non-boiled and boiled *Lathyrus sativus* seeds extract significantly and dose-dependently reduced blood glucose concentrations in glucoseloaded mice. Blood glucose levels were reduced by 37.7, 44.8, and 48.8 percent at extract doses of 100, 200, and 400 mg/kg of non-boiled seeds, respectively. At the same doses, the methanolic extract of boiled seeds reduced blood glucose levels by 31.0, 45.6, and 47.3 percent, respectively.

1.9 Structural formula of ODAP

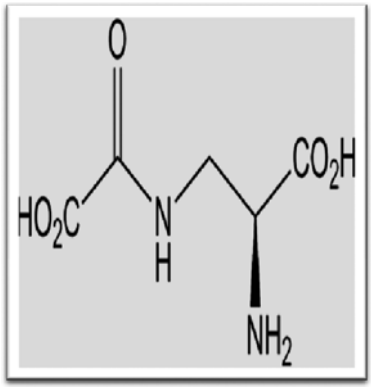

Figure 1.7. Structural formula of ODAP

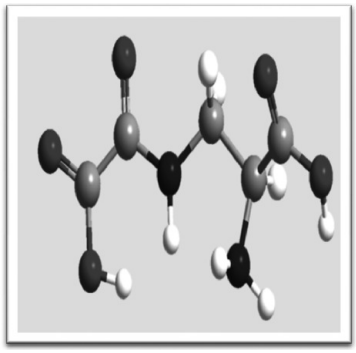

Figure 1.8. 3D form of ODAP

Taxonomy :

Kingdom : Plantae Subkingdom: Tracheobionta Class : Magnoliophyta Subclass : Rosidae Superdivision : Spermatophyta Division: Magnoliophyta Order : Fabales Family : Fabaceae Subfamily : Papilionoideae, Tribe : Vicieae Genus : *Lathyrus* L. Species : *Lathyrus sativus* L.

Forage and animal feed :

In Bangladesh, young plants are used as grazing or as cattle fodder. Before allowing the crop to rejuvenate and be harvested for seed, fields are typically permitted to be grazed by cattle. *Lathyrus* has a great deal of potential as a fodder crop. Gowda and Kaul (1982) claim that by intercropping maize with other crops, it was possible to produce 7–10 t/ha of fodder without harming the maize's granular yield. The most crucial element in South East Asian crop yield is frequently the stems and chaff left over after harvest. Usually, the plants are removed when they are still green but after the pods have ripened. As a result, biomass is able to retain its optimum nutritious content while also yielding high-quality seeds.

In surveys of Indian farmers, it is frequently discovered that the value of animal feed is more crucial in determining the output of this crop than that of human food. As per studies, in Pakistan's Sind province, 40% of the crop is used for human food, 60% of the crop is used as forage, animal feed or fodder, and 10% is left over for seed.

Role of β-ODAP in plants

In general, it is thought that â-ODAP in plants acts as a hydroxyl ion scavenger,

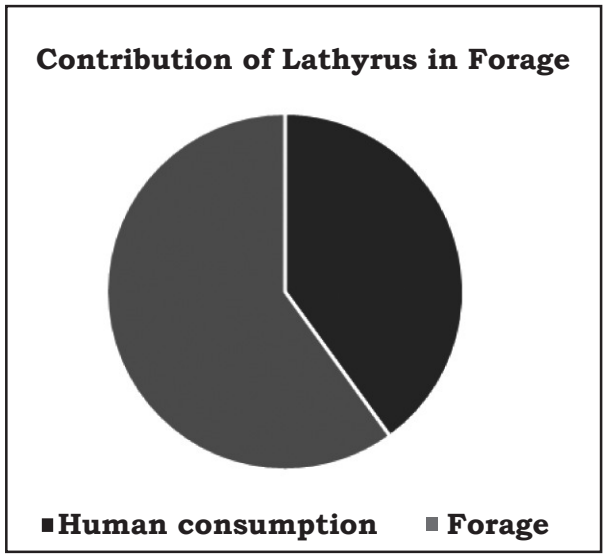

Figure 1.2. Contribution of *Lathyrus* in forage

a carrier molecule for zinc ions, and a safeguard for photosynthesis under intense light conditions. According to several studies, â -ODAP is essential in avoiding oxidative stress and coping with drought.

The biological function of this significant metabolite is still not fully understood. The total eradication of â -ODAP in grass pea should be treated gradually until we have a better knowledge of its function in plants.

As noted in the earlier section, the neurotoxic ODAP, also known as -N-oxalyl-amino-L-alanine, is the main health risk associated to eating grass peas (BOAA). Prolonged ingestion of *Lathyrus* grains may cause irreversible loss of motor function. Lathyrism has been documented in many regions of India.

Variety amelioration research :

With appropriate breeding tactics, grass pea offers enormous potential as a

source of various stress-tolerant genes for general crop development (Hao *et al.,* 2017). Donor germplasm with desirable phenotypes, for example, can be used to create new breeding materials, and available genomic technologies can be employed to accelerate the breeding process. However, when compared to other legume crops, grass pea genomic data are still rare due to its large complex 8.2-Gb genome (Bennett and Leitch, 2012). Furthermore, biotechnology investments continue to be minimal. As a result, virtually little research effort has been committed to crop genetic enhancement to yet.

Molecular toolbox for variety amelioration :

The genetic potential of grass pea could be used to increase food and nutrition security, feed livestock, and boost soil fertility as green manure in difficult drought-prone regions. Regardless of the availability of low toxin lines, the designation of grass pea as a poisonous plant and the prohibition of seed sales in some countries have significantly reduced financing for genetic improvement of grass pea using modern breeding techniques as well as genetics and omics tools. As a result, there are few genomic resources for this orphan legume today, limiting its potential use in crop breeding (Hao *et al.,* 2017).

Cultivation details :

a) Soil type :

Teesta alluvial plain group was the source of the soil used in the experiment. Sandy loam is the major soil type in the field, and it has a limited water-holding capacity. Extremely fertile soil can be found there. This plant can be grown in a different types of soil and it can withstand any type of soil condition because of it's hardy nature.

b) Meteorological parameters :

Weather conditions :

This plant can be grown in wide range of environmental conditions starting from sub-Himalayan terai agro-climate upto coastal saline zone. Tit also has the ability to grow in wide range of temperature conditions starting from cold climatic condition upto higher temperatures.

***Rainfall* :**

After the seeds were sown, it would be good to have rainfall and generally crop is sown in the middle to last of November and if there was rain in the month of December and January, the germination is found good and satisfactory. In the month of February and March there was rain. At the time of harvesting, if there is rain, it would seriously hamper the yield of the crop .

Humidity :

It can withstand a wide range of variation of humidity and it can grow between the maximum percentage of 100% upto and the minimum percentage was 25%.

c) Preparatory tillage :

The topsoil and subsoil of the experimental field were divided twice during cultivation using a cultivator and rotavator. To keep the space tidy, the weeds should be eliminated.

d) Fertilizer application :

For improved yields, a dose of N: P_2O_5: K_2O @ 20:50:20 (Kg/ha) applied in a

common plot at the time of seed sowing is sufficient for maximum yield of the crop.

e) Planting material :

For planting, all natural seeds of every kind should be used. The plot size should be 4m. m x 1.5mt and each allotment has five rows of seeds which should be planted. Row to row and plant to plant spacing should be maintained at 30 cm and 10 cm, respectively. The duration of the crop is found to be from the fourth week of November until the first week of April, last week of April, the crop will reach in the harvesting stage.

f) Preparation of layout :

The entire filed plot should be divided into homogeneous plots of 4 m 1.5 m size, and a 1-meter-wide channel separated should be between the two plots, and a 1-meter-wide furrow divided each plot for irrigation and to prevent maximum chances of cross-pollination.

g) Time of sowing :

The crop should be sown in the second half of the November after which it will be found late sown in north Bengal hampering the germination of the crop production. Regular standard operating procedures were used to sow in the fourth week of November, with a 30 cm × 10 cm spacing and a thin coating of earth on top.

h) Crop management :

To make sure the field was weed-free, three rounds of weeding should be carried out, followed by three rounds of hoeing. During the crop's life cycle, other cultural practices, such irrigation, were carried out as necessary as and when necessary depending on the type of soil and area of cultivation.

i) Harvesting season :

The first and second weeks of April saw the completion of the harvest of all grass pea genotypes.

j) Harvesting :

It's time to harvest when the pods start to turn brown and the grains start to resemble dough. Fruit that has been harvested is sun-dried for a week. After 3–4 days of sun drying, harvested materials are bundled in bundles and sent to threshing floors. The process of threshing involves crushing the grain with sticks. A clean seed needs to be sun dried for three to four days in order to reduce moisture content.

Details of observation or methods :

Morphological/agronomical characters :

Use of morphological data specially the DUS descriptors should always beused for general evaluation and maintenance of the crop genotypes. A plant breeder must use the DUS descriptors in order to preserve the material in the filed gene bank and thereafter for specific categorization of the genotypes. It is of UTMOST importance for maintaining any type of crop genotypes and thereby creating the broad genetic base of the crop as a whole. If it is not done properly, the genotypes or some special genotypes will loose its value and the total loss of creating broad genetic base will be lost. The "Descriptors for *Lathyrus* spp." International Plant Genetic Resources Institute, Rome, Italy (IPGRI. 2000) method for DUS Morphological/Agronomical Characters states in this crop are :

Plant height (cm) :

From the ground to the tip of the longest branch, this distance should be measured. The height of the plant was

measured both at vegetative stage and at physiological maturity.

Leaf length (cm) :

Three leaflets should be selected at random from the centre of the main branch and their lengths were averaged.

Leaf width (cm) :

Three leaflets should be selected at random from the centre of the main branch, and their widths were averaged.

Pod length (cm) :

Average length of three pods that should be taken at random from the main branch's base. The information was gathered at physiological maturity.

Pod width (cm) :

It is the average width of three pods that should be randomly selected from the main branch's centre. The information was gathered at physiological maturity.

Seeds per plant :

Each plant's total quantity of seeds should be counted.

Root length (cm) :

When the plant was fully grown, the length of its roots from upper ground level to their tips should be measured in centimetres.

Days to 50% flowering :

The days to 50% flowering were computed from the day of seeding. The data should be recorded when 50% of plants in each plot showed signs of blossoming.

Branches per plant :

Ten randomly chosen plants should be used to count the number of branches on each plant. To determine the number of branches per plant, the total number of primary and secondary branches is to be tallied.

Pods per plant :

The total number of fully grown pods on 10 different plants should be recorded for each plot.

Seeds per pod :

Three randomly chosen pods should be counted, and the average number of seeds will be calculated.

Seed index (100 – Seed weight) (gm) :

100 seeds from each replication should be weighed in grammes on an electronic single pan balance.

Seed yield per plant (gm) :

The complete weight of each plant's seeds should be measured in grammes using a single pan electronic balance.

Days to maturity :

The time period should be measured after seeding when 80 percent of the plants produce ripe pods.

Biological yield/ Plant (gm) :

Data should be collected on the yield of mature, physiologically dried plants after harvest.

Biological yield/ Plot (kg) :

The yield of mature, physiologically dried plants should be measured after harvest.

Some other plant descriptors where data will be recorded maximum at vegetative growth stage (at 50% flowering), unless otherwise specified.

Characterization :

Seedling vigour

Recorded 20 days after germination of seeds

Code	States
3	Poor
5	Intermediate
7	Vigorous

Plant growth rate – stage I

Recorded during emergence to flowering initiation

Code	States
3	Low
5	Medium
7	High

Plant growth rate – stage II

Recorded after flowering initiation

Code	States
3	Low
5	Medium
7	High

Plant growth habit

Recorded at the starting of flowering period.

Code	States
1	Prostrate
2	Spreading
3	Semi-erect
4	Erect

Plant type

By observing the growth of the plants.

Code	States
1	Indeterminate
2	Determinate

Stem colour

Recorded at 50% flowering

Code	States
1	Light green
2	Green
3	Purple – green
4	Purple

Branch arrangements

Branch arrangements

Code	States
1	Evenly distributed throughout the whole plant
2	Mainly on lower part of the plant
3	Mainly in middle part of the plant

Leaf type

By visual observation of plants, it is recorded.

Code	States
1	Tendril
2	Phyllody
3	Simple (lamina not bifurcated into leaflets)
4	Bipinnate
5	Multipinnate
99	Other

Number of leaflets per leaf

Code	States
1	One pair
2	Two pairs

Leaf colour

It was recorded by visual observation of selected plants

Code	States
1	Light green
2	Green
3	Dark green
99	Other

Leaf size

Recorded at 50% flowering from the middle area of the main branch.

Code	States
3	Small
5	Medium
7	Large

Leaflet shape

Code	States
1	Linear
2	Lanceolate
3	Ovate – lanceolate
4	Ovate
99	Other

Flower bud size

Just before opening

Code	States
3	Small
5	Medium
7	Large

Flower size

Code	States
3	Small
5	Medium
7	Large

Flower colour

Score on fresh, open flowers for score standard, wing and keel colours separately

Code	States
1	White
2	White – blue
3	Blue
4	Grey
5	Light yellow
6	Yellow
7	Pink
8	Orange
9	Red
10	Violet -blue
11	Violet
99	Other

Flower vein colour

Code	States
1	Blue
2	Grey
3	Violet
4	Yellow
99	Other

Calyx colour

Code	States
1	Light green
2	Green
3	Green – purple
99	Other

Calyx teeth length

Code	States
1	Shorter than tube
2	Equal to tube
3	Longer than tube

Calyx teeth shape

Code	States
1	Sharp
2	Blunt
99	Other

Pod shape

Code	States
1	Oblong – elliptical
2	Medium oblong – elliptical
3	Curved
4	Beaded
5	Board – linear
6	Board – elliptical
99	Other

Pod curvature

Recorded on mature pods.

Code	States
1	Straight
2	Slightly curved
3	Curved

Pod beak shape

Code	States
1	Pointed
2	Blunt
99	Other

Pod beak length Recorded on a fully expanded immature pod from end of last loculus up to the pod tip

Code	States
0	Absent
3	Short
5	Medium
7	Long

Immature pod colour

Code	States
1	Yellow- cream
2	Light -green
3	Green
4	Dark -green
5	Green – purple
6	Light -purple
7	Purple
99	Other

Mature pod colour

Code	States
1	Yellow- green
2	Violet – mottled
3	Grey

Constriction of pods between the seeds

Code	States
0	Absent
3	Slight
5	Medium
7	Pronounced

Pod dehiscence

Scored one week after maturity

Code	States
0	No shattering
3	Low shattering
5	Medium shattering
7	High shattering

Seed shape

Code	States
1	Oblate or flattened
2	Triangular
3	Rhomboid
4	Square
5	Obtraingular
6	Spherical
99	Other

Seed size

Code	States
3	Small
5	Medium
7	Large

Seed coat colour

Code	States
1	Greyed-white
2	Yellow – white
3	Grey
4	Brown
5	Yellow – green
6	Pink
7	Red – purple
8	Black
9	Grey mottled
10	Green mottled
99	Other

Seed coat surface

Code	States
1	Smooth
2	Tubercular

β ODAP analysis :

Spectrophotometric assay for ODAP concentrations in individual seeds :

Individual seeds were assayed to determine the ODAP concentration of seed batches produced in various situations. A rubber-padded peg was used to clamp the seeds, and a benchtop drill was used to drill them (Xenox, Fohren, Germany) to extract seed meal from the storage cotyledons. Drilling into the flattened side of the seed, which is opposite the embryonic axis, was done with care to avoid the embryonic axis. This was done to allow the seeds to germinate later to produce progeny, if necessary. Seed meal was collected in a microcentrifuge tube and freeze dried for 48 hours (BenchTop SLC, Virtis , Gardiner, New York, USA). The weights of dried meal samples were recorded electronically to allow for later normalisation. To measure ODAP concentrations in seed samples, a scaled-down variant of the spectrophotometric method modified by Briggs et al (1983) was used. For extractions and reactions to take place in plate format, volume and sample weights were scaled down. This enabled parallelized sample processing with multichannel pipettes and rapid measurement with a plate spectrophotometer. The seed meal was used to extract free amino acids, 600 µl % v/v ethanol in distilled water were added to every sample, followed by incubation at room temperature in a shaking incubator for 22 hours. For 10 minutes, samples were centrifuged at 16250 g in a benchtop centrifuge. An aliquot of each extracted sample's supernatant was transferred to a 96 well microtitre plate (sterilin, Newport, UK) and 160 µl of 3M KOH solution were added to each well. To prevent leakage, plates were tightly clamped and tightly sealed between aluminium plates. To hydrolyze ODAP to L-DAP, the plates were dipped in a 95° C water bath for 30 minutes. To prevent the ethanol in the solution from boiling off, the plates were submerged in room temperature water before drying and releasing the clamps.

A tetraborate buffer was made. In a 96 well microtitre plate with a clear flat bottom, 30 µl of hydrolysate were mixed with 220 µl of tetraborate buffer for the colour formation reaction (Greiner Bio-one, Alphenaan Den Rijn, Netharlands). Another flat bottom plate was loaded separately with 20 µl of 3M potassium hydroxide solution and 10 µl of non-hydrolyzed supernatant from the overnight extraction, followed by 220 µl of tetraborate buffer.

The reaction mixture in each well was gently tapped sideways on the plate, then incubated at room temperature for 30 minutes. An optical plate reader was used

to measure absorbance at 420 nm. (Peter Martin Ferdinand Emmrich. 2017)

Calculation of ODAP concentration from absorbance readings :

To exclude absorbance caused by the polystyrene plate bottom, the extraction buffer, potassium hydroxide solution, and reagent buffer, as well as other compounds extracted from the seed tissue, the 420nm absorbance readings of non-hydrolyzed samples were subtracted from the readings of hydrolyzed samples. The difference between the two values was used to calculate ODAP concentration. The dry weight of the samples recorded prior to extraction was used to normalise the results.

A set of eleven linear dilutions of L-DAP.HCL was included on each measurement plate as a positive control of the colour forming reaction and to establish a standard curve for calibration of the assay. Aliquots (30µl) of known concentrations of L-DAP. HCL in 2M KOH solution were dispensed into the flat bottom plates. Tetraborate reagent buffer (220 µl) was added to each L-DAP.HCL standard and to one well containing 30µl of 2M KOH solution with no L-DAP.HCL. Concentrations of L-DAP.HCL were chosen to cover the expected range of equivalent seed ODAP concentrations. Standard concentrations used alongside the experiments testing single seeds from different seed batches, and bulk samples from the germplasm collections.

Linear regression of standard curves were calculated using the LINEST function in Microsoft Excel for Windows 2013. The same software was used to calculate seed ODAP concentrations usings the following formula :

$$\text{Conc}=\frac{(A_{hyd}A_{non-hyd})\times V_{ext}}{m_{sample}\times a_{standard}}\times 100\%$$

Conc – Seed ODAP concentration in % w/w

A_{hyd} – absorbance reading of hydrolysed sample after the colour forming reaction

$A_{non-hyd}$ – absorbance reading of non- hydrolysed sample

V_{ext} – Volume of extraction buffer

m_{sample} - Mass of the seed meal sample

$a_{standard}$ - Slope of the standard curve

Figure 1. Field Preparation before sowing

Figure 2. Soaking the seeds for better germination

Figure 3. Sowing of seeds

Figure 4. One month after planting

Figure 5. Mature Plant

Figure 6. Harvested Plants

Figure 7. Wheel hoe for intercultural operation

Figure 8. Irrigation in the field

Conclusion :

Grass pea was earlier known to be an underutilized crop and not known to be consumed by the people of India very much. Earlier, the Govt. imposed ban on this crop for consumption in 1951 to take it solely as a crop or with any other pulses in the food. Later this ban was lifted and many varieties were developed in India which were found to be ODAP content within permissible limit (<0.2%). Within this permissible limit lathyrus is now extensively grown in India and especially UP, Bihar, M.P. and West Bengal. Lathyrus is also known to be cultivated in African countries like Somalia where there are many past instances like famine which devastated the country many times. It can be grown in a wide range of soils and it has the deep root system which can take the soil nutrients deep inside the soil even if it is not surrounded by congenial atmosphere for proper growth. So for the farmers especially the asian countries, it is the boon for them as it can be cultivated without much effort from the farmers.

Lathyrus is a hardy crop and generally disease not frequently found in this crop. But there are some diseases like powdery mildew, downy mildew, rust, ascochyta blight which sometimes cause severe problem in crop production. Breeding for abiotic and biotic stress resistance in lathyrus will yield a lot good varieties in future.

High biomass yield is very important as not only the seed can be taken as pulses but also the herbage is consumed as food. So the vegetative characters are sometimes important for breeders as one of the breeding objectives for crop improvement programme.

In tissue culture laboratory, somaclonal variation can be developed where different variation can be developed in the somaclones of different genotypes. Mutation breeding can be done in the genotypes with mutagenic agents like EMS, MMS or X-ray and gamma ray where the performances of the genotypes can be changed along with ODAP content of the

genotypes within permissible limit. So there are different ways of crop improvement programe by which the genetic base of the crop can be increased and different varieties can be prepared following different breeding methods for crop improvement progrmme.

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