INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND ANALYSIS

ISSN(print): 2643-9840, ISSN(online): 2643-9875

Volume 06 Issue 02 February 2023

DOI: 10.47191/ijmra/v6-i2-37, Impact Factor: 6.261

Page No. 781-799

Grass pea (*Lathyrus sativus*) a Crop of Future for Sustainable Agriculture in The Changing Environmental Conditions

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ABSTRACT: Grass pea (*Lathyrus sativus*) is an ideal pulse crop for sustainable agriculture due to its superior agronomic traits such as deep penetrating root system, resistance to many biotic and abiotic stresses and rich protein content. Even with these superior agronomic traits, the crop is considered as an orphan due to its lower productivity and presence of neurotoxin, b-N-ozalyl-L-a-diamino-propanoic acid (ODAP). By following classical and mutational breeding approaches scientists have developed varieties with lower ODAP (< 0.06 %) and efforts are underway to reduce it to zero. However, till date, there are limited biotechnological research in *Lathyrus sativus* for targeted crop improvement due to unavailability of genome sequence. The recently published draft genome sequence of European accession (LS007) is an important breakthrough and this would help in developing varieties with lower neuro toxin and higher productivity. In this review, the superior agronomic traits, resilience of the crops to various biotic and abiotic stresses, latest genetic research and biotechnological tools developed in *L. sativus*, are discussed.

KEYWORDS: Grass pea • *Lathyrus sativus* • Orphan crops • Neurotoxin • Tissue culture • Molecular and genetic approaches

Abbreviations: ODAP: β - ODAP: β-*N*-oxalyl-L-α, β-diamino propionic acid. BUSCO: Benchmarking Universal Single-Copy Orthologs. RAPD: Random Amplified Polymorphic Deoxy Ribonucleic Acid; RFLP: Restriction Fragment Length Polymorphism; SRAP: Sequence Related Amplified Polymorphism; AFLP: Amplified Fragment Length Polymorphism. ICARDA: International Center for Agricultural Research in the Dry Areas. CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

1. INTRODUCTION

Orphan crops are underutilized or neglected (Dawson and Jaenicke 2006) crops. These crops are characterized either by low yield (finger millet) or poor nutrition (cassava)/ toxic substances (grass pea) (Bermejo and León 1994). In developing countries orphan crops are very important because they provide financial as well as nutritional security to poor farmers of that specific area. However, they have been poorly utilized (in terms of use and cultivation) by the industry and scientific community as compared to other commercial crops such as wheat, corn and rice (Foyer et al. 2016). The negligence of these crops was mainly due to the antinutritional factors, poor market demand and unestablished supply chain for seed material (Cullis and Kunert 2017). Orphan leguminous crops mainly includes Bambara groundnut (*Vigna subterranea*), Cow pea (*Vigna unguiculata*), and Grass pea (*Lathyrus sativus*). These legumes are grown by resource-poor farmers in small, marginal or sub marginal lands using their own multiplied seeds (Diane 2016)

The grass pea has been cultivated across the world, from pre-historic era. The seeds are rich sources of protein, monounsaturated fatty acids and is the only source of rare amino acid L-homoarginine (Jammulamadaka et al. 2011). Humans can consume *Lathyrus* seeds after boiling or cooking, and it can be fed to lactating animals to increase the milk yield (Enneking 2011). *Lathyrus sativus* is known for its tolerance to many abiotic (flood, salinity, drought, and waterlogged conditions) stresses and more productive in water deficit conditions (Jiang et al. 2013; Piwowarczyk et al. 2016 a). It is cultivated mainly in the drought affected areas across the world which receive an annual rain fall of 300-1500 mm (Dixit et al. 2016). In India and Bangladesh, it has been grown as cover crop in waterlogged paddy fields (Campbell et al. 1993). This crop produces substantially reliable yields than other crops in drought prone areas and is popularly known as insurance crop (Vaz Patto and Rubiales 2014). This crop has a deep penetrating root system, suitable for cultivation in various soil types (fertile to heavy clay), responds well to the favorable climatic

conditions and produces higher yields. Under suitable agroclimatic conditions and good management practices the grass pea yield was found as high as ~5 tons/ha (Girma and Korbu 2012).

Besides these good agronomic traits, higher protein and fatty acid contents, the crop is disliked because of the presence of neurotoxin (ODAP) in the seeds (Murti et al. 1964). The toxin is found to cause paralysis (irreversible loss of motor function) known as 'lathyrism when the seeds were eaten alone in substantial quantity (Lambein 2009). Because of this, the crop is unpopular in most of the countries and not cultivated on large area. The development of superior lines with reduced/zero toxin and good agronomic traits (abiotic stress tolerance, low input farming systems) would gain more attention. *Lathyrus sativus* is one of the prime legume crops in Kew's Millennium Seed Bank and the Global Crop Diversity Trust project. The prime objective of this initiative is to preserve the genetic variability and elite germplasm having important traits that could be utilized to improve major food crops for attaining the sustainable food production and food security (Dempewolf et al. 2014).

From the point of food security, sustainability and the present scenario of global climate change, the grass pea is considered as an important germplasm resource (Boukecha et al. 2017) and more focus should be given for developing the improved varieties. The modern breeding approaches such as molecular marker and advanced biotechnological tools such as CRISPR can offer new opportunities for rapid crop improvement (Ibitoye and Akin-Idowu 2010). These techniques could be applied to develop high yielding, robust, high disease resistance lines with low toxin content. In this review various aspects of *Lathyrus* such as origin, cultivars, distribution, nutritional aspects, biotic, abiotic stress tolerance and genetic research, important biotechnological tools applied for crop improvement are discussed.

2. BOTANICAL DESCRIPTION, CENTERS OF ORIGIN, DISTRIBUTION, AND CULTIVATION OF *L. SATIVUS*

The stem of grass pea is erect, winged, soft and succulent in nature. The leaves are alternate, evenly pinnate and have tendrils. Like other pea family this plant also has solitary axillary inflorescence. The flowers are hermaphrodites, pentamerous and have typical papilionaceous corolla. The flowers have two standard petals or keels. The fruits are flat pods, and the seeds are non-endospermic with globular to angled shape and observed in various colors. The *L. sativus* is a diploid and has a total chromosome no of 14 (n=7) (Roy 1936). Initially many authors considered Southwest and central Asia as the center of origin however, now it is traced back to Balkan Peninsula (Townsend 1974). In Iraq many wild species of *L. sativus* were reported but it was unclear about their pedigree. Jackson and Yunus (1984), reported the earliest archaeological evidence (8000 BC) of *Lathyrus* in Jarmo, Iraqi Kurdistan. The remnants of *Lathyrus* were reported in Ali Kosh (9500-7600 BC) and Tepe Sadz (7500-5700 BC) and these were the evidences found for the food crops in those places (Jackson and Yunus 1984). In India the archaeological evidences were reported (2000-1500 BC) for the usage of *Lathyrus* and author indicated that the crop could have been transferred from West Asia (Saraswat 1980).

Vavilov (1951) predicted and allocated Central Asia and Abyssinian regions as the probable centers of origin for *Lathyrus.* Vavilov observed and reported the *Lathyrus* crop diversity is as similar to lentils and broad beans. In Southern and Southwestern regions of Asia only small seeded *Lathyrus* were reported. The *Lathyrus* producing the white flower and large seeds were reported in the Mediterranean region (Jackson and Yunus 1984). The information from archaeo-botanical and phyto-geographical indicated that, this crop could have been originated from Balkan Peninsula and dates to early Neolithic period or beginning of 6th century. As the domestication of agricultural crops is expanded from near East to Europe (6000BC), *Lathyrus* could be the first legume crop which has been domesticated during that period (Kislev, 1989).

According to Wang et al. (2015), the genus *Lathyrus* has 187 species worldwide. They are grown from temperate regions of Northern Hemisphere to East Africa and South America as well. Amongst various species of *Lathyrus* cultivated, *L. sativus* is the most widely grown as compared to *L. cicero* and *L. ochrus*. According to Dixit et al. (2016) in India the crop is distributed in various states such as Madhya Pradesh, Bihar, Chhattisgarh, Maharashtra and West Bengal and it's one of the 3rd most important cool season legume in all these states. In these regions, under drought conditions, grass pea is the only option to grow and produce for life stock and livelihood.

In most of the places grass pea is cultivated as rotational crop in the rice field. During 12th five-year plan (2012-2017*)* of India*,* the area under this crop (Lakh hectares) and total production were accounted for 4.93 and 3.88 tons respectively. The Chhattisgarh has the highest area under cultivation (67.26%) and production (59.52%) of *Lathyrus* followed by Bihar. Madhya Pradesh and West Bengal ranked third in area (8.80%) and production (9.56%) respectively. The farmers are still growing various improved varieties which are better and advanced than previous genotypes/cultivars or varieties. The most popular varieties/ cultivars of *Lathyrus* in India are Nirmal, Prateek, Maha Teora, LSD-6, Pusa-24, Ratan, B-1, LS 157-14, RLS-4595, and HD-3. These varieties are most popular due to their low content of ODAP. (https://vikaspedia.in/agriculture/crop-production/package-of-practices/pulses/lathyrus).

3. NUTRITIONAL ASPECTS OF *LATHYRUS SATIVUS*

According to Duke (1981) the seeds of grass pea is a rich source of protein (18.2-34.6%), fat (0.6%), carbohydrate (58.2%) and starch (35%). The seeds were also found to contain sucrose (1.5%), pentosans (6.8%), phytin (3. 6%), lignin (1.5%), albumin (6.69%), prolamin (1.5%), globulin (13.3%), and glutelin (3.8%). It is also a rich source of essential amino acids (in grams per 16 grams of nitrogen) such as valine (4.68g), histidine (2.51g), arginine (7.85g), leucine (6.57g), lysine (6.94g), isoleucine (6.59g), methionine (0.38g), threonine (2.34g), phenylalanine (4.14 g), and tryptophan (0.40 g) (alike other temperate legumes *Lathyrus* also does not contain tryptophan and methionine amino acids). Parida and Ghosh (2016) mentioned the other nutritional aspects of *Lathyrus* such as minerals (calcium- 2.9 mg/100g, phosphorus - 317 mg/100mg and iron- 6.3 mg/100mg), vitamins (niacin- 2.9 mg/100g, carotene-120µg/100g, thiamin- 0.39 mg/100g and riboflavin- 0.17 mg/100g) and can provide 345 Kcals of energy per 100g of seeds. Al-Snafi (2019) also reported that the grass pea seeds are rich sources of minerals like calcium (82.01-118.97 mg/100g), magnesium (98-178 mg/100g), zinc (2.74-4.52 mg/100g), iron (4.64-8.74 mg/100g), manganese (1.16- 1.78 mg/100g) and copper (0.85- 1.23 mg/100g) of *Lathyrus* seeds. Chinnasamy et al. (2005) mentioned that grass pea is a rich source of unsaturated fatty acids (56.3% to 59.98%) than saturated fatty acids (40.01- 3.65 %). The seeds of *Lathyrus* were found to contain various kinds of lipids such as triglycerides (40.01%), diglycerides (68.53%), monoglycerides (52.72%), free fatty acids (43.65%) and phospholipids (41.77%).

4. RELEVANCE OF GRASS PEA CULTIVATION UNDER CHANGING ENVIRONMENTAL CONDITIONS

Climate change is a burning issue. The continuous increase in greenhouse gases has resulted in increased atmospheric temperature and changed the rain fall pattern. The CO_2 is a main greenhouse gas and has a shelf life ~100 years. The global average atmospheric CO_2 concentration is 414.72 ppm (± 0.1 ppm). Due to rise in CO_2 level, the atmospheric temperature is also raising, and Arctic ice is melting at the rate of 12.8% per decade and this has increased the average sea level. In last 100 years, the sea level is increasing at 3.3 mm per year and currently it is at ~178 mm (https: //climate.nasa.gov). Grass pea is one of the neglected crops, but widely grown as forage and food crop in various agroclimatic conditions (Hanbury 2000). The people who live in drought prone areas (Asia and Africa) mostly cultivate this crop, due to its resistance to various biotic and abiotic stresses and higher productivity (Getahun et al. 2002). It can be cultivated without any external fertilizer addition and due to this the cost of cultivation can be greatly reduced (Getahun et al. 2002). *L. sativus* is also important from the point of crop rotation system, its efficient nitrogen fixation improves the soil fertility and indirectly increases the yield of subsequent (mostly cereal) crops. The drought is a part of climate change, and the drought resistant characteristic feature of this crop makes it suitable for cultivation in most of the drought affected areas.

4.1. Studies under important abiotic and biotic stresses

The plant productivity, and quality of the produce is affected by various stresses and these stresses are categorized into abiotic and biotic. The abiotic stresses are believed to be the probable cause for major yield loss. The abiotic stresses include heavy metals, radiation, salinity, floods, drought, and extreme temperatures (Muehlbauer and Tullu 1997). On the other hand, the stress caused by various biological pathogens (bacteria, fungi, virus, nematodes and herbivores) are considered as biotic stresses. Plants are immobile and have developed certain evasive mechanisms to overcome biotic and abiotic stresses. Plant senses the external threats and responds in a defensive way. In a response to the external stimuli, various signal transduction pathways are stimulated to protect the crops and these defensive responses are controlled by a single or group of genes. The signaling pathways are acted from inside cell to outside and are biochemical and physiological in nature.

The various biotic and abiotic stresses and the different tolerance mechanisms observed in grass pea are shown in figure 1.

Plants respond differently to the various abiotic stresses, and they alter the cellular functions to adapt to the changing environment. Chattopadhyay et al. (2011) studied the response of plants after exposing to high salinity and low temperature stress for 36 h. The expressions analyzed using two-dimensional gel electrophoresis clearly indicated the differential expression of genes in the plants which were exposed to different stresses. In this study, stress responsive proteins which were involved in various cellular activities (protein synthesis and degradation, signal transduction, cell metabolism and cell defense) during the stress were identified. The analysis of mRNA from the stress exposed plant provides an accurate information on response of plants and the genes responsible for adoption to the changes. Sometimes the end product analysis will not provide an accurate information when the quantity is too low, and it doesn't correlate with the transcription studies. In that case, the analysis of mRNA would help in identifying the expressed genes qualitatively and qualitatively (Dumas-Gaudot et al. 2004). The reactive oxygen species produced during the stress was known to induce the degradation of enzyme (Rubisco) which fixes CO_2 during photosynthesis in grass pea. Therefore, by following biotechnological approaches, the stress regulated protein could be studied, and strategies can be developed to improve the stress adaption (Chattopadhyay et al. 2011).

4.1.1 Soil Salinity

High salinity is one of a predominant abiotic stress in the agriculture. By 2050, more than 20 % of the geographical area and ½ of the irrigated land will be affected by increased salinity (Silva and Geros, 2009). Osmotic stress and ion toxicity are the two main effects of salinity which ultimately affect the crop growth and yield. The increased osmotic stress affects the plants water and mineral (K^+ and Ca^{2+}) uptake capacity. Reduced cell size, membrane function, and metabolic rates are some of the primary effects of salinity stress. Piwowarczyk et al. (2016b) correlated the salinity tolerance of grass pea to the higher antioxidant activity in root cells. The increased accumulation of peroxidases and phenolic compounds were observed in the roots of grass pea plants exposed to salinity stress. As a mechanism of tolerance, the grass pea plants exposed to moderate salinity (100 mM) has shown an increased accumulation of proline (Tokarz et al. 2015). The tolerance of grass pea to moderate salinity is well reported (Campbell et al. 1994).

4.1.2 Drought Stress

During drought, the water availability is decreased, and the plant dies due to water deficiency. Therefore, the drought directly affects growth, yield, and productivity of the crop (Boyer 1982). Plant tolerance to drought is a complex phenomenon of cellular, morphological, physio-biochemical, and molecular responses that enable retention of water under water deficit conditions. The tolerance of a crop to the drought is the complex and a collective mechanism of biochemical, cellular, and morphological changes (Rampino et al. 2006). Grass pea has developed some unique morphological drought tolerance traits such as stems with winged margins, narrow leaves, and extensive deep-root system as compared to other legumes. Because of these morphological features grass pea can adopt and grow faster under drought conditions as compared to other legumes. (Schroeder et al. 1993). Talukdar (2013) studied the growth and yield of lentil and grass pea under water deficit conditions. The growth of the plant, overall dry biomass and grain yields were significantly reduced in lentil as compared to grass pea. Chlorophyll (Chl) a, and Chl a/b ratio, the ratio of potassium to sodium and relative leaf water content, stomatal conductance and net photosynthetic rates were declined in both the crops under severe water stress conditions. During drought plants are known to accumulate specific amino acids, the increased proline level was reported in root nodules and leaf's of *Lathyrus* (Talukdar 2013). During water stress, plant reduce their water requirement by lowering metabolic activities (Jiang et al. 2013). The assimilates/metabolites are directed towards roots to increase their water uptake rate. The secondary metabolites which protect the crop from stress are produced for osmotic adjustment (Rampino et al. 2006). The photosynthetic activity and carbon fixation are significantly lowered during water stress. The reactive oxygen species produced due to drought stress affect the crop by oxidative damage (Farooq et al. 2009). The ascorbate peroxidase and catalase enzymes degrade the reactive oxygen species and reduces the oxidative effects caused due to salt and drought stress (Hossain et al. 2004). The proline accumulation and lipogenase inductions were observed in *L. sativus* under drought conditions (Tyagi et al. 1995). These defense mechanism of grass pea towards drought stress make it resistant to varied climatic conditions. Due to these properties, grass pea can be grown in harsh climatic regions of Western India, Northern Maharashtra, and some parts of Madhya Pradesh. Grass pea resists extreme drought levels and is the only productive crop among other legumes (Vaz Patto et al. 2006).

4.1.3. Cold stress

Extreme low temperature triggers the cold stress. This is one of the abiotic stresses that affect the productivity, quality, and post-harvest life cycle (Bourion et al. 2003). The mechanism of cold tolerance and the expression of cold shock proteins is well studied in psychrophilic bacteria (Russell 1990). The membrane fluidity and permeability are determined by lipid composition of the cell. During the cold stress, the degree of fatty acid saturation, percentage of unsaturated fatty acid content, methyl branching and ratio between ante-iso-branching to relative to iso-branching is increased and total fatty acid chain length is decreased (Russell 1984). The exposure of bacteria from 25 to 5ºC was found to alter the fatty acid composition. In bacteria, under cold stress an increased cis-vaccenic acid and decreased palmitic acid were reported (Suutari and Laakso 1994). The studies with legume *Rhizobium leguminosarum* shown that, the cold shock and cold acclimation proteins were expressed under cold stress. The plants exposed to cold conditions, showed increased unsaturated fatty acid content which have possible role in cold tolerance. The molecular chaperons expressed during cold stress were known to protect the plants from extreme cold (Bourion et al. 2003). The synthesis of molecular chaperon's is induced by many signal transduction pathways such as reactive oxygen species, abscisic acid, protein kinase, protein phosphate and Ca^{2+}. These kinds of cellular mechanisms might be present in grass pea, which makes it tolerant to cold stress.

4.1.4. Heat Stress

When plants encounter heat shock or high temperature, the growth parameters such as germination and photosynthetic efficiency are affected (Toker and Shyam. 2018), fertilization and embryogenesis processes are severely affected (Farooq et al. 2009). The higher temperature stimulates the leaf senescence and reduces the photosynthesis which affects the seed filling and seed

development (Sita et al. 2017). During heat stress, the most interesting aspect observed in *Lathyrus sativus* was development of synezetic knot in early stage of prophase-l instead of leptotene and zygotene stages, variations in nucleic acid and cytoplasmic features. The formation of bridges, multivalent, migration of chromosomes and tripolarity are some of the other effects observed in the next generation due to heat stress (Kumar and Tripathi 2009). During heat stress several abnormalities were observed in the meiotic division, the synezetic knot was reported in stage 1 of prophase of first meiotic division in M2 population of grass pea. Due to prolonged heat stress, the chromosomal aberrations, morphological changes, and sterile pollen grains were also observed in grass pea seedlings. These changes sometimes are heritable or novel sources for creating improved lines (Kumar and Tripathi 2009). The grass pea exposed to temperature stress / or grown under 55ºC for 48 hours have shown variegated flower colour as compared to control (violet flower). These changes were observed by many researchers, and it was verified as a response to heat stress (Kumar and Tripathi 2009). It was also observed that, the production of heat resistance secondary proteins and altered levels of sugars/starch, during pod filling phase in response to heat stress, as a means of drought evasion in grass pea (Henry 2003).

4.1.5. Biotic stresses

The fungi, virus, bacteria, nematodes, and insects are main biotic agents which causes damage to the plants. In Syria and India, *Lathyrus* is moderately resistant to fungal diseases such as powdery mildew (Campbell et al. 1994; Asthana and Dixit 1998). Many efforts are made to develop disease resistant varieties in *Lathyrus*. The grass pe lines, RPLK 26 and RL41 developed by Raipur research center were found tolerate powdery mildew disease. Due to favorable climatic conditions, downy mildew is one of the serious diseases of grass pea in India (Campbell 1997). The varieties such as *L. aphaca* and *L. sativus* (Asthana and Dixit 1998) were found resistant to Downey mildew. Rust is more prevalent in North-western Ethiopia (Campbell 1997), *Uromyces pisi* and *U. viciae-fabae* were found to cause rust in *L. sativus* and *L. cicera* (Farr and Rossman 2013).

Bean Yellow Mosaic Virus causes leaf mottle, vein clearing, leaf deformation and stunting in many leguminous crops. *L. sativus* is resistant to Bean Yellow Mosaic Virus. The Alfalfa Mosaic Virus and Pea Seed-Borne Mosaic Virus were found to infect *Lathyrus* and transmit to next generation through seeds. However, generally the grass pea is moderate resistant to virus diseases (Johansew et al. 1994).

The pea moth feeds on many plants belonging to Leguminosae family. The moth particularly feeds on pods and seeds there by reduces overall yield of *Lathyrus sp*. (Capinera 2001). Aphid's attack *Lathyrus* sp. and sucks the juice from various parts of the plant. The characteristic sticky honeydew like substances was reported during heavy aphid infestation. The *L. sativus* is generally resistant to thrips, many Indian accessions such as JRL6 and JLR41 have shown greater resistance towards thrips (Asthana 1995). However, the *L. aphaca* was found susceptible (Pandey et al. 1998) to thrips. Nematodes are also reported as major pests of *Lathyrus* sp., particularly cyst (*Heterodera ciceri*) and root knot (*Meloidogyne artiella*) nematodes were found to infect grass pea (Cocks et al. 2000). These abiotic and biotic stress tolerance feature of grass pea mark it as a hardiest legume crop and due to this the area under cultivation of this crop is increasing day by day.

5. BIO SYNTHETIC PATHWAY FOR B-ODAP TOXIN PRODUCTION IN *L. SATIVUS*.

In recent days, β-ODAP production path way was studied using metabolomic tools. The synthesis of β-ODAP is associated with the central carbon metabolism (Liu et al. 2017). The decrease in cysteine and serine amino acid concentration during accumulation of β-ODAP is linked to nitrogen and sulfur metabolism (Liu et al. 2017). The β-ODAP synthesis pathway is complex and partially described in the literature. The initial steps include, the conversion of amino acid serine, into O-acetyl serine and isoxazolin-5-one, and finally to β-(isoxazolin-5-on-2-yl) alanine by β -cyano alanine synthase (Ikegami et al. 1993). The oxalyl-coenzyme A converts, β-(isoxazolin-5-on-2-yl) alanine into 2,3, -L-diamino propanoic acid and subsequently to β-ODAP (Malathi et al. 1970). The enzyme β -cyano alanine synthase is pyridoxal phosphate-dependent enzyme belongs to β-substituted alanine synthase and is the critical enzyme in biosynthesis of β-ODAP. The biotechnological approaches could be applied to regulate the β -cyano alanine synthase enzyme and develop variety with lower β-ODAP. Many scientific reports suggest that mitochondria and chloroplasts are the main sites for β-ODAP synthesis (Ikegami et al. 1993). The content of β-ODAP in *L. sativus* is influenced by both environmental factors and genetic makeup of the plant (Lambein et al. 2010). Drought and heavy metal stress (Zn, Cd and Fe) conditions were known to induce the production of β-ODAP. The biological role of β-ODAP in plant is not defined. However, it is hypothesized that β-ODAP perform various functions such as, zinc ion transportation, protection of photosynthesis under highlight conditions (Zhang et al. 2003) and acts as a hydroxyl ion scavenger (Zhou et al. 2001). Few reports have mentioned its role in inducing resistance against drought and oxidative stress (Jiang et al. 2013).

5.1. Approaches for reduction of β-ODAP in L. sativus

The grass pea could be exploited from the point of food security and to achieve the future demand of food and feed. Grass pea is a leguminous crop, has potential to fix nitrogen from the atmosphere and can be used as a green manuring crop to improve soil fertility. Even though there are many varieties available with low toxin content, due to the stigma of toxicity the seed sale of grass pea is completely banned in some countries and this has affected the funding for research and varietal development. The unavailability of genomic resources is also hampering the exploitation of genetic tools in improving the crop varieties of *L. sativus* (Hao et al. 2017).

5.1.1. Classical breeding approaches to reduce β-ODAP

The prime goal of breeding is to develop commercial varieties with reduced or zero β-ODAP content. The β-ODAP content in grass pea germplasm is in the range of 0.02%–2.59% (Kumar et al. 2011). So far, β-ODAP-free plants have not been observed either in wild species or in germplasm of grass pea (Abd-El-Moneim et al. 2001;). After screening 1082 grass pea accessions, scientists have identified 4 lines of grass pea with reduced β-ODAP (0.007%–0.02% of seed weight) content (Kumar et al. 2011). The grass pea lines with reduced ODAP content have shown poor agronomic characters, which clearly indicates the importance of ODAP in plant growth (Pandey et al. 1998). The low ODAP content (0.08% on seed weight basis) *L. sativus* variety from International Centre for Agricultural Research in the Dry Areas reported a record yield of 1.67 ton·ha^{-1} (Kumar et al. 2011). The content of β-ODAP in grass pea was found to be influenced by various abiotic factors and heavy metal stress and environmental conditions (Tripathy et al. 2015). The current limitation in developing low content β-ODAP varieties is the absence of precise information on genes and enzymes responsible for β- ODAP biosynthesis. Till date genetics-based research on β-ODAP content is very limited, breeding studies with varieties having different β-ODAP levels shown the involvement of two or more genes and loci (Tripathy et al. 2015). However, it's very important to precisely identify the genes involved in toxin production.

5.1.2. Mutational breeding approaches for reducing β-ODAP

The improvement brought through mutation breeding is considered as valuable additions to the conventional breeding. Mutation generates inheritable genetic changes in an organism. Mutations prior to breeding creates the genetic variability and thus increases the probability of isolation of novel genes (Girma and Korbu 2012). Many improved plant characters such as increased number branches (primary and secondary), low seed β-ODAP, higher yield, semi-dwarf, erect, determinate type of varieties (Talukdar and Biswas 2006) and viable diploids with various phenotypes were developed through mutational breeding in *Lathyrus* (Girma and Korbu 2012). The *Lathyrus* mutants are useful to locate morphological markers on genetic material (Talukdar 2009). By induced mutagenesis, various lines comprised of trisomic, tetrasomic, double trisomic, autotetraploids and lines with reciprocal translocations (Talukdar 2012) have been developed. With the help of mutational breeding various mutant varieties resistant to arsenic and salt with low β-ODAP were developed in *Lathyrus*.

6. GENETIC RESEARCH IN *LATHYRUS SATIVUS*

The slow rate of genetic progress in *Lathyrus* is mainly due to narrow genetic variation observed due to self-pollination and incompatibility due to interspecific cross pollination (Nerker 1976). To create variability in the genetic material for phenotypic characteristics alternative approaches such as mutation breeding was followed (Talukdar 2009). The genetic diversity of *Lathyrus* is getting affected due to continuous genetic erosion. For future sustainable program, the diverse genotype exists across various geographical locations needs to be collected and conserved. In India, the collection of *Lathyrus* germplasm was started in the year 1967 and accessions were collected from various states (Bihar, Eastern Uttar Pradesh, West Bengal, and Gujarat) across India (Asthana and Dixit 1998). Between 1989-1991, more than 1000 accessions from Madhya Pradesh (Mehra et al. 1995) and 24, determinate land races named LSP1-LSP24 were collected from Himachal Pradesh, Kangra Valley during 2000, (Kumari 2000). Currently the National Bureau of Plant Genetic Resources (NBPGR), New Delhi maintains ~2720 accessions (Pandey et al. 2008). The important accessions identified for some of the superior agronomic traits are listed in the table 1.

The ODAP content in grass pea was found to vary with the accessions. Kumar et al. (2011) analysed 1128 accessions and found ODAP content in the range of 0.15 % to 0.95%. Nagarajan et al. (1968) observed, 0.1 %-0.78 % among 643 accessions (Somayajulu et al. 1975), 0.2-2.0% among 100 accessions (Leakey 1979) and 0.128-0.872 % across 1187 accessions (Pandey et al. 1998). The accessions from subcontinent found to contain higher ODAP content (0.7-2.4 %) than the germplasm collected from the Near East (0.02%-1.2%). The studies with F2 population confirms that, the ODAP content in the plant is governed by both genetic and environmental factors (Tiwari and Campbell 1996). Also, it was observed that, the ODAP content was influenced by gene, gene interactions (non-additive and or additive effects) (Mehra et al. 1993; Pandey et al. 2000; Abd-El-Moneim 2001) and genetic variance. The reciprocal cross study confirms that, the variation in the content is mainly influenced by maternal cytoplasm (Abd-

El-Moneim et al. 2001). The study conducted with *L. sativus* and *Lathyrus cicero* genotypes in Southwestern Australia shown the presence of significant effect of genotype (Hanbury et al. 1999) on ODAP content.

Barpete et al. (2012) have studied chromosome morphology and structural variations in grass pea. The Ratan, Pratik, Pusa 24 Pratik, IC120455, 120500, 120505, 345392, 345401 and 345403 accessions of grass pea shown the total haploid chromosome length of 5.11 µm to 7.30 µm. Lucia and Incoronata (2013), developed a library of single sequence repeat markers for grass pea by sequencing 400 clones. They have identified 7 primers with markable differences in DNA banding. The polymorphism test was conducted with 4 different grass pea accessions using identified primers. The results showed that, the single sequence repeat markers were highly correlated in 3 accessions (*Lathyrus cicera*, *Lathyrus ochrus* and *Lathyrus tingitanus*) of grass pea. Hao et al. (2017) used ribonucleic acid (RNA) sequence analysis for identification of single sequence repeat from two different accessions of grass pea. In the denovo assembly, 1,42,053 transcripts, 27, 431 unique genes and 19,70,104 contigs, 5, 916 SSR markers were identified. Authors designed primer pairs and validated the markers. In the validation study, 30.6 % and 31.06 % markers were confirmed as polymorphs and monomorphs respectively and rest were found complex to identify.

Grass pea total RNA sequencing was performed recently by Xu et al. (2018). RNA was extracted from seedlings of grass pea on 2, 6 and 25 days after sowing and complementary deoxyribonucleic acid libraries were generated and sequenced using the Illumina-Hi Seq 3000 platform. In the study, 2,13,258 unique genes, 39,548 coding sequences were identified. Annotation of the coding sequences with gene ontologies were done by comparing sequences from other legume specie sequenced genomes. This study concluded that, β-ODAP synthesis is primarily regulated by central carbon metabolism and was co regulated by sulfur and nitrogen assimilation. The grass pea genome matches with the close relative *Pisum sativum* (Kreplak et al. 2019). Due to the lack of genetic or molecular maps, the assembling of *Lathyrus* genome is more difficult. The genome sequence for *Lathyrus sativus* (LS007) has been recently published by Emmrich et al. (2020). They have used *Pisum sativum* as a reference genome (6.3Gbp). Whereas, in the kew database (Leitch et al. 2019) the *Pisum sativum* genome size is mentioned as 3.49-5.42 Gpb. The etiolated seedlings were used to extract DNA and to prepare libraries.

Sarwar et al. (1995) selected LS007 (high ODAP European line), LSWT11 (Indian line with high ODAP), and one Indian variety with low ODAP (Mahateora) for RNA sequencing and gene annotation. AUGUSTUS tool was used to predict the protein coding genes (Stanke et al. 2006). The whole genome of LS007 was divided into two assemblies named EIv1 and Rbp. The EIv1 assembly was 8.12 Gbp long and found to contain 1.9 billion Ns and a contig N50 of 597 Kbp scaffold. In the annotation 33,819 high confidence genes were identified. Whereas the Rbp assembly was 6.2 Gbp in length without any Ns and a contig N50 of 155.7 Kbp scaffold. The gene space assessment study conducted using 27 EK -BUSCO database shown 82.8 % and 89.8 %, completeness scores for EIv1 and Rbp respectively.

The genome size of *Lathyrus* reported in literature is highly varying, Emmrich et al. (2020) reported 6.3Gbp and in Kew Plant C-value database it is 8.2 Gbp. Nandini et al. (1997); Ghasem et al. (2011); Ochatt et al. (2013); Macas et al. (2015) reported the genome size of 6.75Gbp, 7.63 Gbp, 7.82-8.90 Gbp, 6.85 Gbp, and 6.52Gbp respectively. The genomes of viciae family and *Lathyrus* genus were found variable in size, and this could be due to the copy number of repeated elements (Leitch et al. 2019; Vondrak et al. 2020). The variation in the *Lathyrus sativus* genome size was attributed to the experimental error or varied genome size of the individual genotype.

The long terminal repeats are class1 transposable elements. Ogre are one of the long terminal repeat retrotransposons or Ty3/gypsy type long terminal repeat retro transposons and are recently discovered in legumes which constitute up to 40% of the genome in some species. (Neumann et al. 2003; 2006). These Ogre are exceptionally big in size and may go up to 25Kbp and have an extra open reading frame 1 and are located upstream of gag gene (Macas and Neumann 2007). The sequence data by Emmrich et al. (2020) showed that the Orge -content in LS007 nuclear genome was 37%. Whereas these results were lower than the values (45.5%) reported by Macas et al. (2015) for a commercial line. The differences in the Orge content could be due to the variation in genotypes between Ls007 and commercial lines.

The availability of various genetic tools helped breeders to establish efficient methods in *Lathyrus sativus* (Bohra et al. 2014). Like any other external phenotypes, molecular markers help in detecting the diversity and variations within and among the species at DNA level. These molecular based identifications are independent of environmental factors. The molecular markers can be used to identify the positions, number, effect of genes or quantitative trait loci that controls pest, disease, protein, β-ODAP concentration, and other traits (Campbell et al. 1993). For this purpose, RAPD, RFLP, SRAP, AFLP (Marghali et al. 2016) and the microsatellite or simple sequence repeat markers are appropriate (Soren et al. 2015). The expressed sequence tags available in public domain databases are very limited for *Lathyrus*. Only, 178 and 126 expressed sequence tags are available for *L. sativus and L. cicero* respectively in NCBI database. Whereas ~8702 expressed sequence tags are reported for *L. odoratus* and these acts as potential source of molecular markers. The simple sequence repeat markers are highly conserved and are transferrable between

species. The cross-species amplification helps in comparative genomic mapping and new markers development for various orphan crops (Gutierrez et al. 2005).

The satellites are important tools for measuring the genetic diversity or species specificity in plants. It has been claimed that in *Lathyrus sativus,* satellites are believed to be originated from LTRs of Orge (Vondrak et al. 2020). In satellites certain DNA motifs are highly repetitive or tandemly duplicated, because of their high level of repetitiveness it's more difficult for assembling the grass pea genome. FabTR53_LAS_A, is a satellite element observed in grass pea and is estimated to be around 289.8 Mbp or 4, 40,000 copies across the genome of LS007 (Emmrich et al. 2020). The Fluorescence in-situ hybridization study confirms the presence of FabTR53_LAS_A in sub-telomeric regions of the chromosomes (Vondrak et al. 2020). There are 20 simple sequence repeat markers have been identified and developed in *Lathyrus* by insilco survey. The 7, expressed sequence tags and simple sequence repeat markers from *Medicago trancatula* were evaluated for their transferability across 19 accessions of 11 different genera of *Lathyrus*. The genotyping of 176 accessions of *Lathyrus* using expressed sequence tags and simple sequence repeat markers indicated the presence of 51 alleles with gene diversity of 0.43. The model-based population structure analysis revealed the presence of two subpopulations and authors predicted the gene flow among the accessions (Soren et al. 2015).

From the survey of *Lathyrus sativus* expression sequence database, 19 pairs of primers were designed (Shiferaw et al. 2012). After screening 300 expression sequence tags database and simple sequence repeat markers from 24 *L. sativus* accessions, 44 polymorphic loci were identified (Sun et al. 2012). In *M. truncatula* and field pea, along with simple sequence repeats, intron-targeted amplified polymorphic markers (total 159) were also observed. The variant of RFLP, a cleaved amplified polymorphic sequence and its derivatives were also developed for molecular marker analysis in *Lathyrus* (Almeida et al. 2014). The linkage analysis of 75 markers in grass pea resulted in identification of 69 markers location on 14 linkage groups covering 898 cM of genome (Chowdhury and Slinkard 1999). By mapping 64 markers, 9 linkage groups covering 803 cM genome was established in grass pea (Skiba et al. 2004).

Using next generation sequencing tools 50,000 simple sequence repeat markers were developed in *Lathyrus* (Yang et al. 2014), out of these 30 markers were used to evaluate 266 accessions and 17 related materials from ICARDA, Africa, Europe, and Asia (Wang et al. 2015). In the population analysis, gene flow was observed between the Europe and African accessions and results were further supported by unweighted pair group method with arithmetic mean-based cluster and principal component analysis. The RNA sequences analysis of rust infected *Lathyrus sativus* genotype led to a development of reference transcriptome assembly containing 1, 34, 914 contigs (Almeida 2014). Similarly, the analysis of differentially expressed uni tags (total 738) in the leaves of *Lathyrus sativus*, infected by *Ascochyta lathyri* indicated the involvement of various processes such as, biotic and abiotic stresses, cell metabolism and cell signaling (Almeida 2015). And in the study the peroxidases showed differential expression and authors predicted that reactive oxygen species might involve in inducing resistance against rust pathogen (*Alternaria lathyri*). Hence, molecular markers are powerful tools for genome mapping and breeding in grass pea. The various genetic approaches applied in varietal improvement of grass pea is shown in figure. 2.

7. TISSUE CULTURE AND GENETIC TRANSFORMATION TECHNOLOGIES FOR IMPROVEMENT OF *L. SATIVUS*

The plant tissue culture is a technique where a plant cell, tissue or organ can be cultured on a chemically defined medium in an artificial environment to get new plant. By this method disease free clones can be produced and can be transported from one place to another place of the globe. This technique is very useful for embryo rescue and genetic improvement by other biotechnological approaches such as transformation and CRISPR. The presence of neurotoxin ODAP is an undesirable trait in the grass pea plant. It is possible to make this crop neurotoxin free by using the most advanced biotechnological approaches with combination of genomics and plant breeding. In grass pea tissue culture, a range of explants were tested for plant regeneration by somatic embryogenesis, shoot morphogenesis or shoot organogenesis as shown in table 2.

In tissue culture studies, the source of explant is very important to get the plant regeneration. Usually, high plant regeneration probability would be from the meristematic cells of the young explants in comparison to mature tissue. So, in many of the plants the seedling grown explants were commonly used. In perennial and cross- pollinated species, the seedling grown explants are not very recommended due to heterogenous nature of the seeds due to recombination during meiosis. As grass pea is an annual and self- pollinated crop, so in most of the reports the explants were taken either from *in vitro* grown seedling or from green house, nursery or field grown plants. The same trend exists and in majority of these reports the explants were taken from epicotyl, hypocotyl, cotyledonary node, young leaf and stem from *in vitro* grown seedlings (Barik et al. 2004; Barpete et al. 2014 b; Saha et al. 2015). The tissue culture response is highly dependent on the quality and conditions of the donor plants, type, position or age and stage of explant and concentration of growth regulators (Gulati and Jaiwal 1994). The orientation of the explant on the culture medium is also very much important in tissue culture (Figure 3). The orientation of sterilized explant in contact to the medium can highly influence either the shoot regeneration from the cut edges or embryogenic callus formation (Garcia-Luis et al. 2006).

However, the culture conditions like ambient temperature, humidity, dark or light conditions including type of light and photoperiod can also affect the developmental stages of the *in vitro* grown cultures.

The medium and growth regulators also play a very important role in plant regeneration efficiency. The most widely used medium in grass pea plant tissue culture is Murashige & Skoog's medium (Murashige and Skoog's 1962) or sometimes Gamborg' s B-5 basal medium (Gamborg et al. 1968) or modified Murashige & Skoog's medium. Sinha et al. (1983) have used six cultivars of grass pea, L.D.S. 1, L.D. S. 2, L.D.S. 3, L.D.S 4, L.D.S. 5, L.D.S. 6 with various ODAP concentrations in seeds (245, 426, 328, 254, 423 & 295 mg/ 100 g seeds respectively). Stirred suspension bioreactor media (Sinha 1980) was used as basal medium. The best results were shown after supplementation of picloram and 6-Benzylamino purine into stirred suspension bioreactor media for plant regeneration by organogenesis. Genotype dependence is also a major issue in many crop plants if the callus phase prolonged. The genotype dependence was observed in grass pea, out of 6 cultivars of LD series only LDS. 1 showed regeneration of shoots & roots on a medium containing picloram, 6-Benzylamino purine and adenine sulphate. Ochatt et al. (2001) reported the recalcitrance nature of Grass pea in LB, L12 and LIII genotypes, where LIII was found more recalcitrant compared to other two genotypes on various media combinations. They have also used protoplast from hypocotyl but did not observe plant regeneration from any of the three genotypes. Zambare et al. (2002) used LS 8246 and LS 82046 breeding lines for collection of explants (apical meristem and axillary buds) from the plants grown in fields. They used Gamborg' s B-5 basal medium for regeneration and observed the organogenesis. The plant regeneration by direct organogenesis was also reported from seed without intervening callus phase in LS8246 (Malik et al. 1992; 1993). Although the probability of the soma-clonal variation is almost negligible in terms of direct organogenesis, the *Agrobacterium* mediated, or particle gun gene delivery methods are not recommended due to low transformation efficiency in grass pea (Dillen et al.1997).

Barik et al. (2004) reported the regeneration using cotyledonary nodes of IC-120451, IC-120453, IC-120478, IC-120487 and Nayagarh local, genotypes of *Lathyrus* sp. as an explant. The variable degrees of regeneration were also observed by other workers in these genotypes. The genotype IC-120487 had shown the best response in comparison to other 4 genotypes in Murashige & Skoog's medium supplemented with 6-Benzylamino Purine. However, the good regeneration response was not reported when Murashige & Skoog's media was supplemented with thiadiazuran and the plants have shown stunted growth. In contrast to this Barpete et al. (2014a) reported the efficient regeneration when Murashige & Skoog's medium was fortified with thiadiazuran in combination with indole-3-butyric acid. The direct shoot regeneration was also observed when Murashige & Skoog's basal salt medium was supplemented with Gamborg' s B-5 basal medium vitamins, α-naphthalene acetic acid and 6-Benzyl amino purine or thiadiazuran (Saha et al.2015). 6-Benzyl amino purine is the most widely used cytokinin in legume tissue culture including grass pea (Gulati and Jaiwal 1994; Franklin et al. 1998; Sahoo et al. 2002). There are only very few reports are available on somatic embryogenesis of grass pea (Piwowarczyk and Pindel 2014; Sridhar et al. 2015; Tripathy et al. 2013). Sridhar et al. (2015) reported the higher number of somatic embryos when explants were prepared from internodes of plants in Murashige & Skoog's medium supplemented with 6-benzyl amino purine and 2,4-Dichloro phenoxy acetic acid.

The main purpose of an efficient and reproducible plant regeneration is for mass propagation of clones. The tissue culture technology is also a pre-requisite for genetic transformation and gene editing technologies. An efficient and highly reproducible tissue culture protocol would be needed for genetic transformation studies to change the genetics of any crop. The most desired genetic transformation protocol is an indirect plant regeneration with intervening short-term callus phase. The mode of regeneration could be either by shoot organogenesis or somatic embryogenesis. There are very few reports available on genetic manipulation of grass pea, either β-glucuronidase as reporter gene or neomycin phosphotransferase *II* gene as marker. There is a lots of research focus on genetic transformation studies. Barna and Mehta (1995) have used *Agrobacterium* and Biolistic methods for genetic transformation in grass pea. They have found that, the Biolistic method was more suitable to get the transgenic plants for β-glucuronidase reporter gene via somatic embryogenesis method using young leaflets and nodal segments. The various transformation techniques followed in grass pea are summarized in table 2.

Barik et al. (2005a) have developed a successful *Agrobacterium* mediated transformation protocol in grass pea by using neomycin phosphotransferase II as a marker gene and β-glucuronidase as reporter gene. The transgenic events were confirmed by southern hybridization and T1 progeny was tested for the marker gene integration with 3:1 segregation. They have also mentioned that this protocol would be a model protocol for transformation of targeted genes using *Agrobacterium tumefaciens*. The transfer of various genes for agronomical or nutritive importance could be achieved by following this method. For removal or reduction of ODAP formation, the ODAP biosynthetic pathway must be disturbed to inactivate the function of ODAP regulating gene (s).

Delporte et al. (2012) mentioned that the gene transfer technology could be one of the supplementary and important tools in basic scientific research. These transgenics or gene edited (recently introduced technique) plants would be beneficial in core research tool of biological science. It could also help us to understand the interactions of plants with its environment such as

adaptive behavior and defense strategies. Therefore, genetically improved plants could be a powerful tool for functional analysis of genes.

8. CONCLUSION

Lathyrus sativus is a hardy leguminous crop and mainly grown for fodder and food in various parts of the world. The crop has superior agronomic traits such as higher yield, low water requirement and tolerant to many biotic and abiotic stresses. It can be cultivated as mono crop in rainfed conditions or as a succession crop after paddy harvest to increase the overall annual productivity from unit of land. The crop not only enriches the fertility of the soil but also gives an assured yield to the farmers under extreme drought conditions. The pathway for β-ODAP production is complex and not well understood so far. The transcriptomic study published draft genome sequence of LS007, and next generation sequencing tools have helped in identification of major genes involved in the B-ODAP biosynthesis. Because of these advancements it was possible to do the comparative genome analysis between legume species, to develop molecular markers, and to frame linkage maps for varietal improvement. These biotechnological tools along with modern mutational breeding and tissue culture approaches, can certainly speed up the process of developing *L. sativus* with traits which will aid in societal acceptance. The *L. sativus* with zero toxin and improved agronomic traits could be a crop of the future where it can be recommended to cultivate on marginal lands with promising yields.

9. AUTHOR CONTRIBUTION

All authors have equally contributed for writing this review.

10. CONFLICT OF INTEREST

We declare that all authors have seen, approved the final version submitted, and we don't have any conflict of interest.

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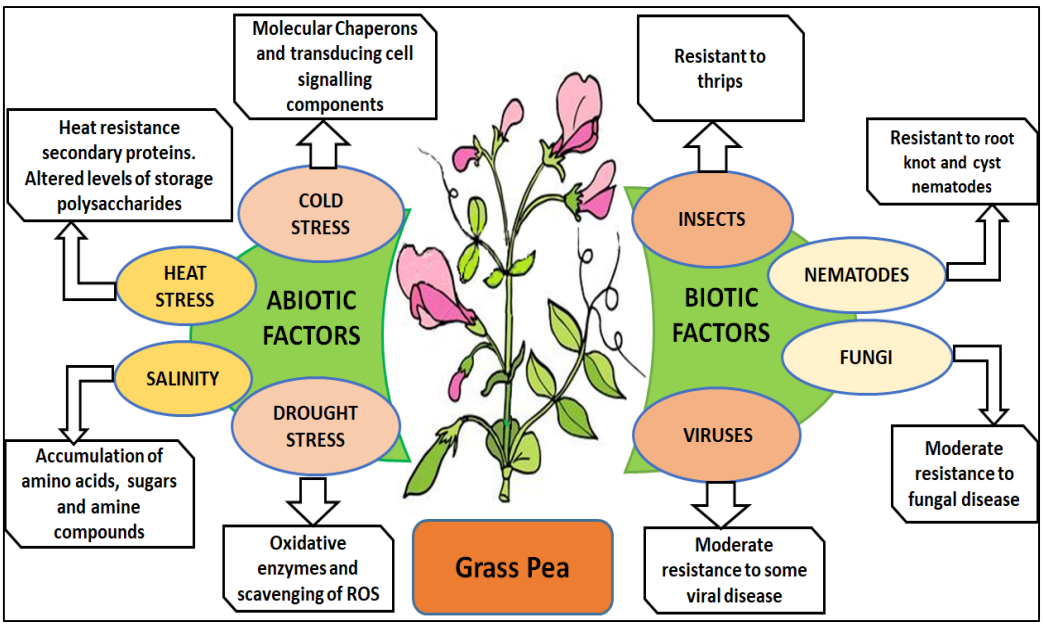

Fig. 1 Relevance of Grass pea in changing environmental conditions and their resistance and adaptability level towards various biotic and abiotic factors

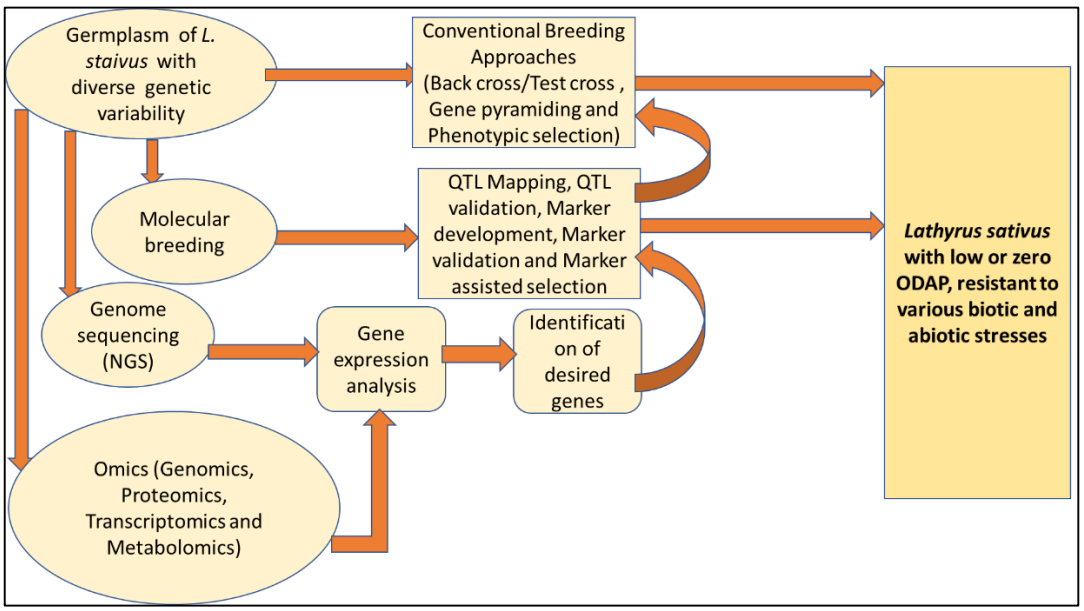

Fig. 2 Genetic approaches for crop improvement in Lathyrus sp.

Fig. 3 Factors which affects the in vitro culture response in Grass pea (*Lathyrus sativus*)

Table 1.

Table 1. Accessions of *Lathyrus sativus* with superior agronomic traits

Agronomic traits	Accession No
ODAP content lower than 0.2%	LS 157-12; BioR-202 and 231; BioL-208, 212 and 222
Seed weight more than 12g 100 seed $^{-1}$	LS-8246; EC-209017; RLK-148, 158 and 143; EC-209044 and 200322; BioL-208 and 227; Sel-505, EC-209026
Grain yield more than 10 g plant^{-1}	JRL-47; IC-120530; IC-120512; RLK-1009, 204, 393, 49, 658, 1081; Pusa-534; IC-120479,120531 and 120535; NIC-18768 and 18890, S-270, P-72 and 176
Number of pods more than 50 plant^{-1}	BioL- 212, 239 and 234; DL-265; IC-12507, 120497, 120537, 120422; NIC-18768, 18851, 18849 and 18890; RLK-430
Early flowering plants (lower than 100 days)	EC-200325, 208952 and 209076; IC-120446, 120447, 120448, 120596, 120420, 120507 and 120438; RLK-266, 287, 10013, 10050, 10012,10031,10037 and 10048

Table 2. Tissue culture studies in grass pea (*Lathyrus* sp.)

S No.	Explant	Regeneration	Medium	Reference	Remarks
1	Stem	Yes	Stirred suspension bioreactor media + Picloram + 6-Benzylamino Purine	Sinha et al. 1982	Shoot Organogenesis
2	Root	yes	Murashige & Skoog's + α-Naphthalene Acetic acid + Kanamycin	Roy et al. 1992	
3	Hypocotyl (protoplast)	Yes	Murashige & Skoog's + Thiadiazuran/ Zeatin	Ochatt et al. 2001	
4	Apical & axillary bud explants		Gamborg' s B-5 basal medium + Thiadiazuran + α-naphthalene acetic	Zambre et al. 2002	

			acid / Indole-3-butyric acid + Coconut water		
5	Cotyledonary node from seedlings		α-Naphthalene Acetic Acid + 6-Benzyl Amino Purine / Thiadiazuran or Murashige & Skoog's +2,4-Dichloro Phenoxy Acetic acid	Barik et al. 2004	
6	Epicotyl segments	Yes	Murashige & Skoog's + α-Naphthalene Acetic acid + 6-Benzylamino purine	Barik et al. 2005a	
7	Epicotyl segments	Yes	Murashige & Skoog's + α-Naphthalene Acetic Acid +6-Benzylamino purine	Barik et al. 2006	Shoot organogenesis
8	Immature zygotic embryo	Yes	Murashige & Skoog's + α-Naphthalene Acetic Acid + 6-Benzyl Amino Purine or Thiadiazuran	Sahin-Demirbag et al. 2008	
9	Internode	Yes	Gamborg' s B-5 basal medium + α-Naphthalene Acetic Acid + 6-Benzyl Amino Purine	Tripathi et al. 2013	Somatic embryogenesis
10	Root segments	Yes	Murashige & Skoog's + 2,4-Dichloro Phenoxy Acetic acid	Piwowarczyk and Pindel 2014	Somatic embryogenesis
11	Stem node	Yes	Murashige & Skoog's + 6-Benzylamino Purine	Barpete et al. 2014a	
12	Embryonic node	Yes	Murashige & Skoog's + Thiadiazuran + Indole-3-Butyric acid	Barpete et al. 2014b	
13	Internode	No (Only Callusing)	Gamborg' s B-5 basal medium + 2,4-Dichloro phenoxy Acetic acid +6-Benzyl Amino Purine or Gamborg' s B-5 basal medium vitamins + α-Naphthalene Acetic Acid + 6-Benzylamino purine	Tripathy et al. 2014	
14	Nodal, Leaf, Internode	Yes	Murashige & Skoog's + Gamborg' s B-5 basal medium and vitamins +6-Benzylamino Purine + Thiadiazuran + α-Naphthalene Acetic acid	Saha et al. 2015	Direct shoot regeneration via shoot morphogenesis
15	Leaf, Internode	Yes	Murashige & Skoog's + 2,4-Dichloro phenoxy Acetic acid + 6-Benzylamino purine	Sridhar et al. 2015	Somatic embryogenesis
16	Root, Petiole, Internode	No	Gamborg' s B-5 basal medium + 6-Benzylamino Purine + α-naphthalene Acetic Acid or Indole-3-acetic acid or 2,4-Dichloro Phenoxy Acetic acid	Piwowarczyk et al. 2016 b	Rhizogenesis
17	Leaf, Root, Stem (1Week old seedlings)	Yes	α-Naphthalene Acetic Acid + 6-Benzylamino purine or Murashige & Skoog's +2,4-Dichloro Phenoxy Acetic acid	Li et al. 2016	
18	Nodal	Yes	6-Benzylamino purine / Thiadiazuran + Murashige & Skoog's +2,4-Dichloro Phenoxy Acetic acid	Catal and Bakoglu 2018	Direct shoot organogenesis

Table 3. Various genetic transformation techniques followed in Grass pea.

S. no.	Explant/ species	Transformation Method	Promoter	Reporter gene/ Selectable marker gene	Gene of interest	Transformation efficiency	Reference
1	Leaf & internodal segments / *Lathyrus sativus*	*Agrobacterium*	35S	β-glucuronidase / neomycin phosphotransferase *II*	-	9-15%	Barna and Mehta 1995
2	Shoot tip and callus/ *Lathyrus sativus*	Bolistic	35S	β--glucuronidase / neomycin phosphotransferase *II*	-	7- 18%	
3	Epicotyl/ *Lathyrus sativus*	*Agrobacterium*	Nopaline, 35S	β-glucuronidase	-	31- 37%	Barik et al. 2005 b
4	Leaves/ *Lathyrus odorata*	Agro infilteration		β-glucuronidase	*phytoene desaturase*	-	Gronlund et al. 2008
5	Embryo/ *Lathyrus sativus*	*Agrobacterium*	GmPM9 promoter	neomycin phosphotransferase *II*	Flammulina velutipes oxalate decarboxylase	-	Kumar et al. 2016