

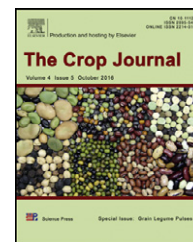
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Achievements and prospects of grass pea (*Lathyrus sativus* L.) improvement for sustainable food production



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ABSTRACT

Grass pea offers an attractive choice for sustainable food production, owing to its intrinsic properties including limited water requirement and drought tolerance. However, low productivity and the presence of a neurotoxin (ODAP) have posed major obstacles to its genetic improvement. Also, biotechnological investments remain limited and the genome is complex and not well understood. Strategies that allow identification of genotypes with reduced ODAP content, coupling of low ODAP content with enhanced yield, and effective seed detoxification methods merit immediate attention. Breeder-friendly genomic tools are being increasingly made available to improve the efficiency of breeding protocols. To this end, the application of next-generation sequencing has provided a means of leveraging the repertoire of genomic resources for this somewhat neglected crop. In this review, we describe progress achieved in *Lathyrus* genetic improvement. We also explore potential opportunities in *Lathyrus* research and identify urgent research needs.

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1. Introduction

Grass pea (*Lathyrus sativus* L.) is a crop of immense economic significance, especially in developing nations including India, Bangladesh, Pakistan, Nepal, and Ethiopia [1–3]. It is often broadcast-seeded into standing rice crops one or two weeks before the rice harvest. This allows grass pea to effectively exploit the residual moisture left after the rice harvest [4,5]. It is also cultivated in China and in many countries of Europe, the Middle East, and Northern Africa. It serves a variety of purposes including food, feed and fodder, owing in part to its nutritive qualities [4,6–9]. Archeological evidence suggests that the domestication of *Lathyrus* dates to the late Neolithic, and precisely to the Bronze Age [2,9]. Prior

to domestication, the crop was presumably present as a weed among other pulse crops. However, evidence based on historical records renders the subject of its origin more contentious [10].

In South Asian countries, grass pea is commonly grown for both grain and fodder purposes. However, the crop has gained more importance for use as animal feed than for use as human food. Animal feed from *Lathyrus* is usually composed of ground or split grain or flour, and is used primarily to feed lactating cattle or other draft animals [11,12]. Human diets include *Lathyrus* as grains that are boiled and then either consumed whole or processed for split dal [13,14].

Grass pea, inherently capable of withstanding temperature extremes, is grown across diverse regions that receive an

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average annual precipitation ranging from 300 to 1500 mm [15,16]. In addition to remarkable tolerance to drought [17], *Lathyrus* has tolerance to excess precipitation and flooding [7,18–20]. It has a hardy and penetrating root system suited to a wide range of soil types including very poor soil and heavy clays [20–22]. Its notable robustness along with its intrinsic ability to biologically fix atmospheric nitrogen makes grass pea an attractive crop for adverse agricultural conditions [4,18,23].

Nutrient-dense food crops with reduced water demands such as *Lathyrus* are likely to play a key role in alleviating global malnutrition. However, to date, very limited research efforts have been devoted to improving *Lathyrus*. The major reason underlying this lack of research effort is the presence of a neurotoxin [β -N-oxalyl-L- α , β -diaminopropionic acid (ODAP)], prolonged consumption of which leads to the neurological disorder lathyrism in humans and domestic animals [24–26]. The disease is more pronounced when grass pea forms the dominant component of the diet and accounts for at least 30% of caloric intake for a period of at least three to four months [18,27]. Influences of a variety of factors on ODAP accumulation in *Lathyrus*, including plant growth stage, nutrients, abiotic stresses (drought, salinity, water, and heavy metal) have been comprehensively reviewed by Jiao et al. [28].

2. Implications for human health

As indicated in the previous section, the major health concern associated with grass pea consumption is the neurotoxin ODAP, which is also known as β -N-oxalyl-amino-L-alanine (BOAA) [29,30]. Irreparable loss of motor function may result from prolonged consumption of *Lathyrus* grains [26]. Instances of lathyrism have been reported from various parts of India [15,29,31]. It was observed that lathyrism could affect anyone consuming a diet consisting of more than 25% grass pea for 3–4 months [15,32]. In view of these findings, the sale or storage of *Lathyrus* has been banned in all states in India except for Chhattisgarh, Maharashtra, and West Bengal under rule 44-A of the Prevention of Food Adulteration Act, 1954 [33].

In India, *Lathyrus* cultivation is concentrated mainly in the Chhattisgarh region, where limited cases of human lathyrism are known. In contrast, a higher incidence of human lathyrism has been reported in Rewa division of Madhya Pradesh state, occurring when *Lathyrus* constituted more than 2/3 of the diet for prolonged periods (3–6 months) [34,35]. A survey of the socioeconomic conditions of *Lathyrus*-growing farmers and their culinary practices was conducted in two villages near Raipur. In these villages, the consumption was related to the size of the farm holdings, with non-farm holding families having greater consumption of *Lathyrus* [36].

Grass pea holds tremendous potential as a functional food to improve health conditions associated with cardiovascular disease, hypoxia, and hypertension [37–39]. Importantly, patents have been granted based on ODAP (as a hemostatic agent) in the USA and China, and an increasing number of therapeutic applications derived from *Lathyrus* may be developed in coming years [38]. Further, as highlighted by Singh and Rao [39], ODAP is increasingly being used for

therapeutic purposes owing to its role in the stabilization of hypoxia inducible factor-1 (HIF-1). In short, the evolving view of grass pea as a functional food is likely to cause a dramatic shift in the ways pulses and lathyrism are perceived.

3. Economic importance in India

Grass pea is the third most important cool-season pulse crop of India, occupying an area of 0.58 million ha with an annual production of 0.43 million tonnes [14]. It is cultivated primarily in Bihar, Madhya Pradesh, Maharashtra, West Bengal, and Chhattisgarh [37]. The majority of this acreage (~70%) is shared by Chhattisgarh and the Vidarbha region of Maharashtra, which is a rice-growing region where supplemental irrigation is available only for rice. Consequently, water is not available for subsequent winter crops, making grass pea the only alternative for a crop following rice [40–42].

Grass pea effectively withstands unfavorable conditions including excessive moisture at sowing, which is often followed by moisture stress at advanced growth stages. In fact, grass pea is preferred for cultivation in such areas owing to its hardy nature coupled with its marginal costs of cultivation. In early 1990s, the socioeconomic impact of grass pea consumption was assessed in a random sample of 100 farmers from Raipur, Bilapur and Bastar. This study revealed that almost 60% of the rice growers included grass pea in their cropping system. Most of the farmers practiced subsistence agriculture with smaller land holdings (of below 5 ha). However, its consumption among non-farmers did not exceed 3% of total food intake. Among pulses, farmers had a preference for chickpea, which accounted for over 35% of total pulse expenses incurred, followed by other pulse crops including pigeonpea (25.3%), blackgram (17.5%), and grass pea (11.2%). The most common use of grass pea was to prepare dal, and nearly 25% of consumers adopted conventional measures to detoxify grass pea grains before consumption. Considerable awareness was found among rural people about the toxic effects of grass pea consumption. Another study conducted in Gondia district in Maharashtra showed that nearly 60% of the population consumed grass pea as a part of their diet; however, the quantity of grass pea consumed per day was reported to be less than 25 g [37].

4. Trends in area and production in India

Given severe legislative control measures imposed by several state governments, acreage under grass pea has declined considerably over the past decades. Although improved varieties containing low amounts of toxin have been developed by the Indian Council of Agricultural Research (ICAR) and associated agricultural institutes, farmers are increasingly shifting towards higher-value crops. Consequently, a continuous reduction has occurred in the area and production of grass pea across India. The national acreage has gradually fallen from 1.67 million ha to 0.58 million ha over the last four decades. A similar trend has been noted in its production, which has declined substantially from 0.84 million to 0.43 million tons over the same time period (<http://agricoop.nic.in>).

5. Major production constraints

Grass pea is grown predominantly under rainfed conditions and on marginal and submarginal lands that are generally characterized by poor soil health/fertility. Apart from various agroecological constraints, the crop also encounters a range of biotic stresses including powdery mildew (*Erysiphe pisi*), rust (*Uromyces fabae*), downy mildew (*Peronospora lathyri-palustris*), thrips (*Caliothrips indicus*) and abiotic factors including moisture and waterlogging stress, which reduce yield potential by 15%–25% [11]. Other factors that can adversely affect grass pea production include i) lower productivity of rice fallows, ii) inadequate seed supply of improved and higher-yielding varieties, iii) lack of adoption of novel crop production technologies, iv) inadequate or unbalanced use of fertilizer, v) untimely sowing and low seed rates, and vi) weed infestation [43]. Despite results suggesting that the application of micronutrients to deficient soils could be a cost-effective way to increase grass pea production, farmers rarely adopt such practices, owing to their poor economic status [44,45]. Inadequate transfer of applicable technologies remains another constraint.

6. Crop management approaches to improve yields

Grass pea is a winter-season crop adapted to areas with arid or semiarid conditions. In the Indian subcontinent, the crop is generally sown in October or November and harvested in February or March [46]. Sowing date is largely determined by the time when the monsoon rains end, soil type, and soil moisture profile during October. In some rainfed areas of India such as Madhya Pradesh, *Lathyrus* is grown as mixed crop with wheat, chickpea, barley and linseed [43,47]. In general, the seeds are sown about two weeks after plowing at a density of 40–50 kg ha⁻¹.

Among the various nutrients, phosphorus (P) and molybdenum (Mo) influence the productivity of pulses. A study aimed at increasing the productivity of *Lathyrus* under varying levels of P and foliar spraying of Mo was conducted in West Bengal state. Application of P resulted in a marked yield increase. Yield increases were thought to be a consequence of profuse nodulation, with the resulting increase in N fixation influencing the rate of photosynthesis. In addition, a marked increase in the seed yield and overall improvement in yield attributes was obtained by foliar spraying with 0.05% Mo [48]. As a crop grown

under rainfed multiple-cropping systems, *Lathyrus* influenced soil fertility, especially in terms of organic matter, total N, and available P. Higher soil fertility in the rainfed crop systems was evident from higher yields of green gram after *Lathyrus* [49].

In utera cropping systems, *Lathyrus* (cv. Ratan) outperformed chickpea (cv JG-74), lentil (cv JLS-3) and linseed (cv R-552) when grown after medium-duration rice (cv. Mahamaya: 130 days). Utera is a relay cropping system that entails broadcasting seeds of a succeeding crop nearly 15 days prior to the harvest of a rice crop, thereby allowing the succeeding crop to efficiently harness residual moisture. *Lathyrus* yields were higher when seeds were sown under minimal tillage after rice harvest. Application of N:P:K at 80:60:30 kg ha⁻¹ to rice and N of 10 kg ha⁻¹ to *Lathyrus* at planting resulted in the greatest yields for both rice and *Lathyrus*. Leaving 20 cm of rice stubble height resulted in improved growth and highest yields of *Lathyrus*. Rice varieties Mahamaya (130 days) and Bamleshwari (140 days) are preferred for utera cropping (with *Lathyrus*) owing to their greater tolerance to biotic and abiotic stresses (drought/bacterial blight/gall midge) and higher yield potential. Direct drill seeding of *Lathyrus* at rates of 40 and 60 kg ha⁻¹ resulted in similar yields. Seed treatment with *Rhizobium* provided significantly higher seed yield (1101 kg ha⁻¹) than no *Rhizobium* (943 kg ha⁻¹). Application of 20 and 30 kg N ha⁻¹ resulted in significantly higher seed yield than 0 and 10 kg N ha⁻¹ (Personal communication, R.L. Pandey, 2008).

7. Genetic enhancement of *Lathyrus*

7.1. Collection and conservation of genetic resources

Increasing genetic erosion presents a challenge to sustaining *Lathyrus* genetic diversity. There is a pressing need to collect and conserve the genetic variation present across diverse geographical regions. A systematic collection of grass pea in India was initiated in 1967 in Madhya Pradesh [50]. In 1969 germplasm collections were made in different states of India including Bihar, Eastern Uttar Pradesh, West Bengal, and Gujarat, and from tribal areas of Bihar in 1975 [40]. More than 1000 accessions were collected from Madhya Pradesh during 1989–1991. A set of 24 diverse and determinate land races designated as LSP-1 to LSP-24 was collected during 2000 from Kangra valley, Himachal Pradesh [51]. At present, 2720 *Lathyrus* accessions are conserved in collections at the National Bureau of Plant Genetic Resources (NBPGR), New

Table 1 – Accessions identified for agronomically/economically important traits.

Character	Promising accessions
Earliness (<100 days)	IC-120438, IC-120446, IC-120447, IC-120448, IC-120596, IC-120507, IC-120420, EC-200325, EC-209076, EC-208952, RLK-10012, RLK-10013, RLK-10031, RLK-10037, RLK-10050, RLK-10048, RLK-266, RLK-287.
Number of pods (>50 per plant)	BioL-212, BioL-239, BioR-234, DL-265, RLK-430, IC-12507, IC-120497, IC-120537, IC-120422, NIC-18768, NIC-18849, NIC-18851, NIC-18890.
Grain yield (>10 g plant ⁻¹)	JRL-47, Pusa 534, RJK-49, RLK-204, RLK-393, RLK-658, RLK-1009, RLK-1081, IC-120479, IC-120512, IC-120530, IC-120531, IC-120535, NIC-18768, NIC-18890, S-270, P-72, P-176
Seed size (>12 g 100 seed ⁻¹)	EC-209017, EC-209026, EC-209044, EC-200322, RLK-143, RLK-148, RLK-158, BioL-208, BioR-227, LS-8246, Sel. 505
ODAP content (<0.2%)	BioL-212, BioR-202, BioR-231, BioL-222, BioL-208, LS 157-12

Delhi (http://www.nbpr.ernet.in/PGR_Databases.aspx) [52]. In addition, active collections are being maintained at different research stations including IGAU, Raipur, Indian Institute of Pulses Research (IIPR), Kanpur and NBPR, Akola. *Lathyrus* accessions displaying important traits are listed in Table 1.

7.2. Estimating genetic variation for ODAP content

Pronounced variation in *Lathyrus* grain ODAP content was detected by evaluating diverse grass pea accessions. The range of ODAP content varied across studies. Results have included ranges of 0.15%–0.95% among 1128 accessions [1]; 0.06%–0.71% among 1963 accessions [52]; 0.1–2.6% among 576 accessions [53]; 0.1%–0.3% among 1500 accessions [54]; 0.10%–0.78% across 643 accessions [55]; 0.2%–2.0% across 100 accessions [56], and 0.128%–0.872% across 1187 accessions [43,57]. Notably, grass pea germplasm from the Indian subcontinent contained higher ODAP (0.7%–2.4%) than local germplasm (0.02%–1.20%) from the Near East [58].

Studies of the inheritance of ODAP content have produced different conclusions. Analysis of F₂ populations suggested that ODAP content was inherited both quantitatively and qualitatively [59–62]. Furthermore, presence of non-additive effects [60] as well as additive/additive × additive gene effects for ODAP content was observed across other studies [22,63–65]. Other reports have suggested ODAP content to be controlled predominantly by additive genetic variance [50,66]. Results based on reciprocal crosses suggested maternal cytoplasmic effects on ODAP concentration [22]. A study conducted in southwestern Australia that included genotypes of both *L. sativus* and *Lathyrus ciceri* detected significant genotype effects on ODAP [67].

7.3. Mutation breeding

The slow progress of grass pea genetic improvement may be due largely to an extremely narrow range of genetic variation resulting from self-pollination and interspecific incompatibility [68]. For these reasons, improvement strategies focusing on mutation breeding have been explored as a way of creating genetic variation. Mutation breeding in grass pea has led to the generation of several viable diploid mutants with marked alterations in plant characters such as growth habit, branching, stem and internodes, leaflets, stipules, flower and pod characters, and seed traits [17]. Examples include dwarf mutants *dwf1*, *dwf2*, and *dwf3* [69,70]. Similarly, branching mutant 1 (*brm1*), *brm2* and profusely branched mutant (PBM) were recovered from varieties “BioR-231” and “Hoogly Local”. A non-winged internode mutant (NWIM) with erect, determinate, and semidwarf habit was developed from the variety BioR-231. Interestingly, the mutant also showed an increased number of primary and secondary branches, along with higher grain yield and low seed ODAP content [71]. Similarly, a fasciated mutant (250 Gy) was detected, characterized by a broad, strap-like flattened stem, clustered canopy leaves, and reduced yield [72]. Mutations affecting leaflet pattern have also been detected by various researchers [68,70,73–75]. Interestingly, mutants (CELM) with erect leaflets displayed radiation use efficiency (RUE) and higher biomass accumulation [73].

Mutant lines derived from varieties “BioR-231”, “BioL-203”, and “Nirmal” showed extensive variation in the flower (corolla) [75]. Mutants with pale-violet (*pvfm*) and white flower (*wfm*) showed high grain-yielding potential [17]. As with flower color, a broader range of seed coat variations was developed by mutagenesis [70,76,77]. Mutants including black-mosaic seed coat (BSCM) and white non-mosaic seed coat (WSCM) have significantly higher grain yields and lower seed ODAP content than wild type plants [17]. The exposed stigma noted in the malformed flower mutation (*mfl*) could substantially increase pollinator activity [68,78]. Similarly, mutants with increased number of pedicels and improved podding capacity were isolated from BioL-203, Nirmal, LSD-3, P-24/3, BioR-231, Hoogly Local, and two local varieties from the eastern Himalayas [17].

Similarly to morphological changes, chromosomal alterations including translocations have been induced in grass pea by mutagenesis [79,80]. Recently, a mutant (*rfl-1*) was isolated from M₂ progeny of EMS-treated BioL-212, exhibiting marked deviations in karyology [81]. Mutants have been developed that show enhanced salt tolerance [82,83], resulting from an increase in the activities of reactive oxygen species (ROS)-scavenging enzymes including superoxide dismutase and ascorbate peroxidase.

Mutagenic treatments have introduced marked biochemical changes in grass pea, a majority of which have been reported to contribute directly or indirectly to plant defense systems. For example, a glutathione (GSH)-deficient mutant (*gshl-1*) was isolated from gamma ray-treated M₂ progeny of the genotype BioL-212. Examination of this mutant under normal and cadmium (Cd)-stressed conditions revealed its greater sensitivity to Cd [84]. By contrast, enhanced tolerance to salt and metal toxicity was obtained through induced mutagenesis [83,85]. One such mutant, *dwf1*, showed a 2.5-fold increase in foliar GSH content and normal growth under Cd stress [69,86].

An EMS-induced mutant, *rfl-1*, was characterized as showing elevated rates of cell division and cell growth. Similarly, mutants including *gshl-1*, an ascorbate (AsA)-deficient mutant (*asfl-1*), and a GSH-overproducing mutant were investigated to assess the role of arsenic (As) on wilt tolerance [85]. In addition to underscoring the effect of As on PAL activity, the study suggested a role for H₂O₂ level and GSH redox during pathogenesis [85].

Gamma-ray mutagenesis produced an *asfl-1* mutant containing only 42% leaf and 20% root ascorbate content relative to the parent genotype BioR-231. The results pointed to the possible occurrence of a rearrangement event involving antioxidant defense machinery in *asfl-1* that effectively mitigated the adverse effect of ascorbate deficiency and permitted survival under salt-stress conditions [87]. Two flavonoid-deficient mutants, *fldL-1* and *fldL-2*, were established as a product of EMS mutagenesis and leaf flavonoid content was found to be reduced by up to 20% in both mutants relative to wild-type genotypes [88].

7.4. Screening against diseases and pests

Thrips are a pest of high economic concern for grass pea cultivation in India. Screening of 56 accessions against thrips at

Table 2 – Low-ODAP varieties and their areas of adaptation.

Variety	Year of release	Yield (kg ha ⁻¹)	ODAP (%)	Area of adaptation
Ratan (Bio L 212)	1997	1530	0.06	Madhya Pradesh, Maharashtra, Bihar, Chhattisgarh, Orissa, West Bengal
Mahateora	2008	1600	0.08	Madhya Pradesh, Maharashtra, Chhattisgarh
Prateek	2006	1450	0.08	Madhya Pradesh, Maharashtra, Chhattisgarh

Raipur revealed two genotypes, RLK-273-1 and RLK-273-3, with moderate resistance [57]. Similarly, genotypes RLK-1, RLK-281, RLK-617, and RPL-26 were reported to be thrips-tolerant by Asthana and Dixit [40]. In addition to pest problems, powdery mildew and downy mildew represent two major biotic factors severely effecting grass pea [11]. A three-year study performed under condition of natural infection detected promising lines with no visible symptoms of downy mildew [89]. Lines showing moderate resistance to powdery mildew included RPLK-26 and RL-21 [90]. On the basis of field-based screening, IPLy2-10, RPLK-26, JRL-41, and RLS-2 were found to be tolerant to powdery mildew [25]. Screening 96 lines with *Cercospora pisi sativae* f. sp. *Lathyrus misha* revealed eight lines with exceptional resistance or immunity [91]. Germplasm with resistance to downy mildew has also been reported [40,57]. Vaz Patto and Rubiales [92] screened a set of 50 *Lathyrus* accessions for rust disease and established the presence of high resistance in all accessions against *Uromyces viciae-fabae* and *Uromyces ciceris arietini*, and a partial resistance to *Uromyces pisi*. Detailed histological evidence was provided to explain the resistance observed against the rust disease. Similarly, a survey of 151 *L. ciceri* accessions for their response to broomrape (*Orobanche crenata*) led the authors to advocate to harnessing the joint benefits of both genetic resistance and escape mechanisms to develop genotypes with stable resistance against broomrape [93].

7.5. Varietal improvement

The first phase of *Lathyrus* crop improvement in India lasted for almost 20 years (1940–1960) and focused on isolating single plant progenies with substantial yield superiority [25]. As a result, several cultivars were developed and recommended for cultivation. This set of improved cultivars included BR13, LC76 (Bihar); T12 (Gujarat) No. 91/No. 11 (Madhya Pradesh) and B1/B19 (West Bengal).

The second phase (1974–1990) involved development of improved varieties that were relatively free of ODAP. In this phase research was focused on estimating the varying levels of ODAP, and effective breeding protocols were adopted to eliminate or reduce the content of this toxin.

The extensive testing and evaluation led to the release of a low-ODAP (0.2%) variety, Pusa 24, that was particularly adapted to upland cultivation [90,94]. Subsequent research efforts led to the development of six additional varieties (LSD1, LSD2, LSD3, LSD6, Pusa-305, and Selection 1276) with low ODAP content (up to 0.2%). Of these, two varieties (LSD1 and LSD2) were recommended for upland cultivation, while the remaining four (LSD3, LSD6, Pusa 305, and Sel. 1276) were found to be suitable for rice fallows [25,95].

In the third phase (1990s), several varieties and lines were developed that combined low ODAP with high yield potential and resistance to a variety of biotic and abiotic stresses. For

example, BioR-202, BioL-203, BioL-212, BioR-231, and BioL-208 had enhanced yield and high harvest index. As shown in Table 2, BioL-212 was identified and released as “Ratan” in 1997 for cultivation in the North East Plain Zone (NEPZ) and Central Zone (CZ) in India [25,57,96]. Later, two varieties were developed, Prateek (LS 157-14) and Mahateora (RLS 4595), that had very low ODAP content (< 0.1%) and yield up to 1.5 t ha⁻¹ [2].

7.6. Prebreeding and distant hybridization

Genetic improvement of crops having a narrow genetic base may require the use of desirable alleles from outside the primary gene pool. ICARDA has collected and conserved nearly 1555 accessions representing 45 wild *Lathyrus* species from more than 40 countries [2,97]. Screening of wild grass pea for ODAP content has shown that the zero level is virtually absent in the gene pool [98–100]. The lowest ODAP content has been recorded in *L. cicera*, followed by *L. sativus* and *Lathyrus ochrus* [98–100]. Similarly, ODAP content was found to vary between 0.07 and 0.51% across 142 accessions of *L. cicera* [2].

A toxin-free gene identified in *Lathyrus tingitanus* can be deployed in breeding to develop varieties with low levels of toxin [101]. Wild species including *L. ochrus*, *Lathyrus clymenum* [102], and *L. cicera* [100,103–105] are resistant to broomrape, a trait that is not present in the cultivated gene pool. In addition to its reduced ODAP content, *L. cicera* may be a promising source of other important agronomic traits including earliness and cold tolerance [105].

There have been several successful instances of the development of interspecific and wide crosses with *Lathyrus*. Viable seeds were recovered from wide crosses involving *L. sativus* and two wild species, *L. cicera* and *Lathyrus amphicaropus* [106,107]. Yunus [108] attempted interspecific hybridization using 11 wild species and *L. sativus*, but only a *L. cicera* × *L. amphicaropus* cross yielded viable seeds. Successful interspecific hybrids were also obtained from the crosses *Lathyrus annuus* × *Lathyrus hierosolymitanus* and *Lathyrus odoratus* × *Lathyrus belinensis* [109–111]; *Lathyrus hirsutus* × *L. odoratus* [107,109,112]; *Lathyrus articulatus* × *Lathyrus clymenus* and *L. articulatus* × *L. ochrus* [112,113]; *L. cicera* × *Lathyrus blepharicarpus*, *L. cicera* × *Lathyrus gorgoni*, *L. cicera* × *Lathyrus marmoratus*, *L. cicera* × *L. pseudocicera*, *L. gorgoni* × *L. pseudocicera*, and *L. marmoratus* × *L. blepharicarpus* [97,109,114]; and *Lathyrus rotundifolius* × *Lathyrus tuberosus* and *Lathyrus sylvestris* × *Lathyrus latifolius* [97,106,115]. The results of interspecific hybridization in grass pea suggest that the identification and transfer of desirable traits from exotic and wild germplasm offer many opportunities for *Lathyrus* improvement, especially for readily crossable species including *L. cicera* and *L. amphicaropus*. Biotechnological tools including tissue culture techniques may also be employed to overcome strong reproductive barriers among different species [116].

8. Genomic research in *Lathyrus*

Recent reports have documented genomics tools in grass pea for initiating genomics-based crop improvement [117]. Genetic diversity in *Lathyrus* has been detected using diverse molecular markers including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) [118–121].

An analysis of 53 *Lathyrus* species, three *Vicia* species, and a single variety of *Pisum sativum* using internal transcribed spacer (ITS), nuclear ribosomal and chloroplast (cp) sequence-specific DNA markers supported an existing taxonomic classification based on morphological traits [122]. A limited number (178) of expressed sequenced tags (ESTs) are available at the National Center for Biotechnology Information (NCBI) GenBank for *L. sativus*. However, many more ESTs (8702) are available from *L. odoratus* (http://www.ncbi.nlm.nih.gov/genbank/dbest/dbest_summary/). An initial set of simple sequence repeat (SSR) markers comprising 20 SSRs was developed using an *in silico* survey [123]. The transferability of seven *Medicago truncatula* EST-SSRs was evaluated across 19 accessions belonging to 11 different genera [124]. Seven SSRs were validated across four diverse accessions of *Lathyrus* (*L. sativus*, *L. cicera*, *L. ochrus*, *L. tingitanus*) and *P. sativum* [125]. More recently, genotyping of 176 accessions with EST-SSRs revealed a total of 51 alleles with an average gene diversity of 0.43, and the two subpopulations were recovered using a model-based population structure analysis with authors predicting gene flow among the accessions across the geographical regions in India [126].

Recently, 19 primer-pairs were designed by surveying an EST database of *L. sativus* [127]. Three hundred EST-SSR markers were screened across 24 grass pea accessions to identify 44 polymorphic loci [128]. In addition to *L. sativus*-specific DNA markers, 24 EST-SSRs were chosen to analyze transferability of *Medicago truncatula*-specific marker in three legume species [129]. Ponnaiah et al. [130] also reported the development of seven *Lathyrus*-specific EST-SSR markers. In addition to SSRs, 156 intron-targeted amplified polymorphic (ITAP) markers from *M. truncatula* and field pea and DNA markers specific to defense-associated genes in field pea were also shown to have utility for *Lathyrus*. Cleaved amplified polymorphic sequence (CAPS) and derived-CAPS (dCAPS) were also developed for *Lathyrus* [131]. Based on a linkage analysis of 75 markers including RAPD, morphological and isozyme markers, map locations could be determined for 69 markers on 14 linkage groups (LGs) covering 898 cM of the genome [132]. Similarly, mapping of 64 markers including RAPD and sequence tagged site (STS)/CAPS led to the establishment of nine LGs that collectively spanned 803 cM of the genome [133] (reviewed in [117]).

Recently, next-generation sequencing (NGS) platforms were used to develop 50,000 SSR markers for grass pea [134]. Later, 30 of these high-throughput SSRs were employed to analyze 266 *Lathyrus* accessions and 17 relatives from Africa, Europe, Asia and ICARDA [135]. The possibility of gene flow between the European and African accessions, indicated by the population structure analysis, was further supported by unweighted pair group method with arithmetic mean (UPGMA)-based cluster analysis and principal component analysis (PCA). Recently, RNAseq analysis of rust-responsive grass pea genotypes enabled

construction of a reference transcriptome assembly with 134,914 contigs [136]. Similarly, coupling Illumina technology with serial analysis of gene expression (SAGE) analysis yielded a set of 738 UniTags expressed differentially in the leaves of *L. sativus* as a response to *Ascochyta lathyr* inoculation [137]. Functional categorization associated these genes with processes involved in “biotic/abiotic stress”, “cell metabolism”, and “hormone signaling”. Given the observation that only peroxidase showed differential expression, the authors proposed that a ROS burst might make only a minor contribution to resistance against *A. lathyr*. These modern molecular tools represent powerful public resources for genome mapping and molecular breeding in grass pea.

9. Future opportunities and research needs

Substantial regions of rice fallows are scattered across India. The location, extent (11.65 million ha), and gross environmental conditions of these areas have been determined using publicly available databases and a geographic information system (GIS) approach. These rice fallows permit cultivation of an additional crop that can efficiently use moisture retained in the soil. Smaller landholdings constitute an enormous, albeit underused, resource for poor farmers who remain confined to subsistence farming, and have restricted opportunities for enterprise and income diversification. Growing a crop, especially a legume crop such as *Lathyrus*, after rice would have beneficial effects on soil fertility and soil health.

Similarly, in the Eastern Indo-Gangetic plains a sizeable area is monocropped using medium- and long-duration varieties of rice. The lack of irrigation water and delay in vacating the rice field does not normally permit double cropping. The top soil layer generally dries out at the time of rice harvesting and planting a post-rainy season crop is not feasible. Under such conditions, relay cropping of *Lathyrus*, especially in medium-deep soils, could transform these monocropped areas into double-cropping areas, increasing the productivity and sustainability of the agricultural system. However, such expansions require the development and promotion of low-toxin varieties that are suited to relay cropping and mechanized farming.

Below are listed areas of investigation that deserve immediate attention:

- Large-scale collection, conservation, and evaluation of germplasm from unexplored regions.
- Accelerated development of low- or near-zero-level ODAP genotypes.
- Breeding for increased forage and fodder production.
- Improved understanding of drought-tolerance mechanisms.
- Identification of sources of resistance against biotic stresses.
- Identification and genetic manipulation of enzymes responsible for ODAP production.
- Increasing accuracy and throughput of ODAP detoxification method.
- Refinement in production technologies used for utera cultivation.
- Generation of saturated linkage maps and other cost-effective genomic tools.

- Implementing newly developed genomic resources in existing breeding protocols to accelerate efficient development of new varieties.

We consider that meeting the above-mentioned research priorities will help not only to improve grass pea yield but also to introduce this crop to areas where it is not currently produced.

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