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Crop wild relatives of grasspea (*Lathyrus sativus* L.) for designing future climate resilient cultivars

Gitanjali Sahay¹, Surendra Ghrtilahre*¹, Nidhi Kumari¹, Alok kumar¹, Dibendu Datta¹ and Girish Prasad Dixit²

¹ICAR-Indian Institute of Pulses Research, Regional Station Bhopal, India

²ICAR-Indian Institute of Pulses Research, Kanpur, India



ABSTRACT

*Grasspea (*Lathyrus sativus*) is a hardy crop often referred as an insurance against the vagaries of weather fluctuations amid the ongoing scenario of global climate change. To confront the challenges of climate change and attain the food and nutritional security, there is a need to introduce and promote climate-resilient crops within our current cropping systems. Grasspea is an ideal candidate that represents a promising leguminous crop for adaptive farming. There is substantial diversity in crop wild relatives (CWRs) of grasspea, yet crop domestication tends to reduce genetic diversity. Genetic diversity along with trait identification and characterization of the genetic resources is a prerequisite for crop breeding programmes and it is known that the degree and quantity of genetic variability in a specific crop determine the success of its genetic gain. Therefore, in this current review, we seek to explain the potential of grasspea as a valuable asset in addressing the issues emanating from climate change. As grasspea serves as a model crop for sustainable agriculture, we have sought to elucidate the importance of crop wild relatives in breeding programs for the incorporation of specific traits related to yield parameters and biotic and abiotic stress tolerance. In the realm of *Lathyrus* genetic enhancement, we have concentrated on the advancements achieved to date and emphasize possible research avenues such as enhancing yield, creating superior varieties with reduced β -ODAP levels, and developing cultivars resistant to biotic and abiotic stresses.*

Keywords: Grasspea, climate change, crop wild relatives, utilization.

1. Introduction

The global human population has surpassed 8.0 billion and is expected to rise to 9.7 billion by the 2050s. The proportion of malnourished population has also increased substantially over the decades and which is why nutritional security assumes importance apart from addressing the issues of food security. Moreover, the world is presently facing climate change and its harmful impact on agriculture, which represent another risk to food security [29]. Climate change is causing our agriculture to consistently deal with unpredictable and intense rainfall, drought-like conditions, extreme temperature fluctuations, unexpected insect infestations, and minor disease outbreaks, all of which ultimately adversely influence the geographical spread and productivity of various crops, thereby lead to a reduction in crop yields and output [72]. To reduce the effects of climate change and attain food and nutritional security, we must incorporate and promote climate-resilient crops in our agricultural practices.

Grasspea (*Lathyrus sativus* L.), the underutilized crop, is widely considered as a resilient crop which can thrive well under marginal soils and low input conditions apart from

withstanding harsh weather including temperature extremities and soil moisture stress [52] and, hence, adapted to diverse geographical regions. Grasspea exhibits tolerance to multiple abiotic stresses like drought, waterlogging, and salinity besides possessing resistance to a range of pests and diseases. It contains significant protein levels (18-34%) along with high yield potential and nitrogen-fixing abilities compared to other legumes [100]. All these traits make *grasspea* an outstanding crop for ensuring nutritional security, especially in the context of climate change in general and drought in particular. A few global research and development organizations like Global Crop Diversity Trust and Millennium Seed Bank took initiative to conserve and promote grasspea as a crop of choice for supporting the nutritional security. Grasspea contain a neurotoxin, β -N-oxalyl-L- α , β -diaminopropionic acid (β -ODAP) in seeds and seedlings, which is known to induce neuro-lathyrism if grasspea seeds constitute over one-third of a person's calorie consumption for a continuous period of three months or more [21]. Such situations are rare but for various reasons the potential of grasspea has been ignored and its cultivation diminished fast over a short period of time and could not gain prominence in the recent past because of the aspersions of neuro-lathyrism among other reasons.

Characterization, evaluation and documentation of crop genetic resources are important for conservation of genetic diversity and their utilization for crop improvement for successfully harnessing the potential of economic traits trapped in undocumented germplasm.

*Corresponding Author: **Surendra Ghrtilahre**

DOI: <https://doi.org/10.21276/AATCCReview.2026.14.02.41>

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The domestication of crops reduces genetic variability, yet there is significant genetic diversity among the crop wild relatives (CWRs) [96]. The CWRs possess characteristics that enhance resilience to biotic and abiotic threats, as well as improved nutritional quality and yield. The *Lathyrus* genus, also known as grasspea, includes around 160 species, with *L. sativus*, *L. hirsutus*, and *L. cicera* being significant for food and feed purposes, whereas *L. latifolius* and *L. odoratus* are grown as ornamental plants in Europe. Of all the crops, *L. sativus* is the most significant and extensively grown for human consumption [66]. Compared to other crops, limited initiatives have been taken for the genetic enhancement of *Lathyrus* spp. regarding yield, adaptability, and nutritional quality (low ODAP). The CWRs can significantly contribute to the genetic enhancement of cultivated *Lathyrus* spp. since they represent a valuable and underutilized source of beneficial traits [82]. Nonetheless, a significant number of CWRs still lack evaluation and characterization. Substantial initiatives are being pursued globally to collect and conserve *Lathyrus* germplasm. The International Centre for Agricultural Research in the Dry Areas (ICARDA) holds 4,000 accessions, followed by the Conservatoire Botanique National des Pyrénées et de Midi-Pyrénées in France (approximately 4,000 accessions) and the ICAR-National Bureau of Plant Genetic Resources in India, which possesses around 2,600 accessions. In this current review, we discussed the progress made in *Lathyrus* genetic improvement and its potential opportunities as an important crop resource for combating climate change. The significance of crop wild relatives in pre-breeding programmes were also discussed with the aim of highlighting the importance incorporating specific traits into the breeding lines to make the varieties more resilient for sustainable agriculture and also to improve the quality traits.

2. Area, production and economic importance, major drawbacks

Grasspea holds significant economic importance, particularly in developing nations such as India, Bangladesh, Pakistan, Nepal, and Ethiopia. Additionally grown in China, numerous European nations, the Middle East, and Northern Africa, it serves multiple functions, significant for food, animal feed, fodder, and nutraceutical uses. Archaeological findings indicate its domestication occurring in the late Neolithic, specifically during the Bronze Age [22]. Grasspea is consumed by humans as boiled grains, eaten whole, or processed into split dal. In India, grasspea farming is predominant in few states like Chhattisgarh, where there have been some instances of human lathyrism linked to consumption when over two-thirds of the diet consisted of it for extended durations (3-6 months) [22]. In contrast, grasspea holds significant promise as a functional food to address health issues related to cardiovascular disease, hypoxia, and hypertension [52].

Grasspea is the third key rabi season pulse crop in India, covering an area of 0.58 million hectares and yielding an annual production of 0.43 million tonnes [22]. It is mainly grown in Bihar, Madhya Pradesh, Maharashtra, West Bengal, and Chhattisgarh. Most of the land (~70%) is distributed between Chhattisgarh and the Vidarbha area of Maharashtra, which is known for rice cultivation where additional irrigation is provided solely for rice, making grasspea the most suitable option for winter crops in rice fallows. Grasspea is actually regarded as more suitable for farming in those regions because of its resilience and low cultivation expenses.

Grasspea is primarily cultivated in rainfed environments on marginal and sub-marginal areas with low soil fertility. Along with several agro-ecological limitations, the crop encounters multiple biotic stresses, such as powdery mildew (*Erysiphe pisi*), rust (*Uromyces fabae*), downy mildew (*Peronospora lathyrilustris*), thrips (*Caliothrips indicus*), and abiotic stresses like drought and waterlogging stress, which reduce the yield potential by 15 - 25% [20]. Additional elements that negatively influence grasspea yields comprise i) reduced productivity of rice fallows, ii) insufficient availability of improved and higher-yielding seed varieties, iii) limited adoption of innovative crop cultivation methods, iv) insufficient or unbalanced fertilizer application, v) delayed planting and low seed quantities, and vi) weed problems and low financial conditions of farmers. A further limitation is the insufficient transfer of relevant technologies [22].

3. Grasspea genetic resources/wild relatives

Grasspea is largely overlooked and underutilized, yet remains a favored option among resource-poor farmers in Asian nations in marginal regions due to its straightforward cultivation that allows it to thrive under adverse agro-climatic conditions with minimal input needs. The effective use of wild relatives of grasspea can aid in their collection, conservation, domestication, and application in crop enhancement programs for food and fodder. Based on taxonomical and morphological characteristics, grasspea species are divided into five categories: *Nissolia*, *Aphaca*, *Cicerula*, *Clymenum*, and *Lathyrus* [43]. Starting from here, the initial four groups are classified as annual species, whereas the fifth species, *Lathyrus*, is categorized as perennial ones [9]. The origin of *L. sativus* remains uncertain; however, several Mediterranean species such as *L. pseudocicera*, *L. cicera*, *L. marmoratus*, and *L. blepharicarpus* are regarded as potential candidates due to their morphological similarities with cultivated varieties [47]. Crop wild relatives are vital resources and have been essential in the genetic enhancement of various cultivated crops, with grasspea anticipated to follow suit. For example, a toxin-free gene identified in *L. tingitanus* could be used to create high-yielding grasspea varieties with low or toxin-free ODAP levels [108]. *L. cicera*, known for its low β -ODAP content, along with its early maturity and cold tolerance traits, could be utilized for the advancement of grasspea. There has been limited attempts of alien gene transfer in grasspea even though successful production of viable seeds has been achieved in inter-specific hybridization involving *L. cicera*, *L. sativus*, and *L. amphicarpus* [2]. The intra-specific hybrids produced chromosome alterations but still produced viable seeds. Hence, alien gene transfer could be a useful tool for grasspea improvement in *L. sativus* by using *L. cicera* and *L. amphicarpus* which are readily crossable among each other [21].

Despite numerous benefits, limited genetic interventions were employed for enhancing the yield potential and addressing the anti-nutrient issues in lathyrus genotypes globally. Given that this crop is well-suited for the changing global climate, both conventional and modern breeding tools for grasspea genetic improvement should be utilized to develop varieties or enhance germplasm/cultivars with reduced β -ODAP levels and increased yield potential. In the recent past, certain grasspea accessions with low β -ODAP (0.04 - 0.1%) have been found globally (Table 1), including India, Nepal, Bangladesh, Australia, Ethiopia, Poland, Canada, and Turkey.

Furthermore, certain wild relatives such as *L. amphicaropus*, *L. cicera*, and *L. ochrus* have been noted to possess negligible or low β -ODAP (0.01%) genes, potentially aiding in the creation of toxin-free grasspea varieties [47]. Lathyrus is primarily a self-pollinated crop, but a significant amount of cross-pollination (2.0 – 27.8%) has been documented, mainly due to honey bees [34]. Moreover, a comparable pattern of cross-pollination rates has been observed in *L. cicera*, indicating its resemblance to *L. sativus* in terms of floral biology [34]. In the past, conventional breeding for grasspea enhancement concentrated on increasing yield by selecting for a higher number of branches per plant. Nevertheless, certain Lathyrus germplasm accessions (L900239 and L920278) possess characteristics such as double flowers or pods at a single node, which are appealing for improving yield.

Table 1: List of improved Grasspea varieties in India

S. No.	Variety	β -ODAP (%)	Average yield (Kg/ha)
1	Pusa 24 (P 24)	0.20	1655
2	Bio R202 (Ratan)	0.05	2530
3	Prateek (LS82046 x A60)	0.08	1560
4	Mahateora (Ratan x JRL 2)	0.07	1550
5	Bio L 208 (Moti)	0.03	2900
6	Nirmal	0.15	1500
7	Local cultivars	0.50– 2.50	455– 640

4. Genetic diversity in grasspea

The impact of climate change on farming varies depending on the region and the severity and intensity of extreme alterations in cold, heat, salinity, drought, and flooding conditions. The influence of the environment on crop plants may manifest as extremely low or high temperatures, diminished or surplus water supply, emergence of new pests, saline or acidic soils, and a rise in the occurrence of extreme events. Hence, an appropriate alternative new variety or novel crops is necessary to meet consistent food demand under changing environmental conditions. Noticing the trends of climate change, many agricultural organizations across various nations have adjusted their breeding strategies according to food demand, the growing population rates in their countries, and climate change impacts. Coordinated breeding initiatives and targeted research are essential for enhancing and advancing previously overlooked crops, which are now viewed as promising, and could serve as a significant breeding strategy [8]. Currently, the genus Lathyrus consists of 187 species found across temperate areas of the Northern Hemisphere, tropical East Africa, and South America. Nevertheless, the Mediterranean and Irano-Turanian areas represent the primary hubs of diversity. In the genus *Lathyrus*, *L. sativus* (grasspea) is the sole species extensively grown as a food crop, while other species (*Lathyrus cicera* and *Lathyrus ochrus*) are planted to a lesser extent. Additionally, grasspea exhibits superior agronomic adaptability as a grain and forage legume in vulnerable agro-ecosystems, owing to its ability to withstand challenging climatic factors like drought, flooding, and salinity [103].

Grasspea originated in the Balkan region and it prefers self-fertilization. It has been noted that certain out crossing occurs in the species (ranging from 9.8 – 27.8%) due to honey bees, making it regarded as "a frequently cross-pollinated" crop [45]. The genetic diversity present in the germplasm can be used in the breeding program. Globally, numerous methods of analysis are employed to assess genetic diversity. Grasspea exhibits significant morphological diversity, particularly regarding vegetative attributes like leaf length and tendrils, whereas floral traits display less variation.

Grasspea ecotypes are categorized according to leaf length, flower colour, pod size with markings, seed size and colours, often reflecting their geographical distribution [12]. Moreover, the nutritional characteristics of seeds, their responses to both biotic and abiotic stresses, and yield factors have been assessed to define the existing diversity of accessions for both *L. cicera* and *L. sativus*. Cytological research indicates that the fundamental chromosome number of $x=7$ is prevalent across the genus, and the majority of species are diploid, while polyploidy is uncommon [66]. Nonetheless, it has demonstrated consistency in chromosome count, while significant variations in chromosome size have been crucial in the evolution of *Lathyrus* species. Although it is frequently cross-pollinated, significant variability in *Lathyrus* species has been observed due to uncontrolled pollination occurring during flowering periods. SDS-PAGE analysis of reduced seed globulins revealed intra-specific diversity stemming from variations among the grasspea accessions.

Grasspea accessions from identical geographical locations have exhibited variations in the width, quantity, and intensity of bands observed during SDS-PAGE analysis of albumins and globulins, indicating that the specific seed protein content and its polymorphism remain unaffected by geographical origins [75]. In the context of ODAP, significant variability was noted at both the inter-specific and intra-specific levels [76]

Genetic variation in lathyrus has been evaluated and documented using morphological and biochemical markers, and molecular marker loci that encode storage proteins, isozymes, or DNA-based markers. Induced dwarf mutants of grasspea can be effectively distinguished from each other using allelic variants related to leaf esterase and root peroxidase isozymes [93]. Three isozymes, namely esterase, aspartate aminotransferase, and acid phosphatase have been utilized to assess variability among Ethiopian grasspea genotypes wherein a non-significant relationship with morphological diversity was observed. A wide array of molecular marker systems—including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), inter simple sequence repeats (ISSR), sequence-related amplified polymorphism (SRAP), expressed sequence tag-simple sequence repeats (EST-SSR), and sequence-tagged sites (STS)—have been extensively employed to assess genetic diversity and elucidate phylogenetic relationships among species [32].

5. Breeding for adaptation/ mitigation of climate change

Grasspea is grown with minimal input management due to its resilience against extreme temperatures, drought, waterlogging, salinity, and heat [1]. Simultaneously, it remains unaffected by insect pests, leading to minimal losses in field conditions. Despite numerous favorable features, it hasn't received significant attention due to three primary reasons: firstly, the existence of toxins in the seeds and plants; secondly, the characteristics of the pollination process; and thirdly, the extensive genome size. The global demand for legume protein is rising, prompting researchers to consider other lesser-known pulse crops, such as grasspea, to recognize their potential for growth in marginal areas. To achieve this, it is essential to characterize landraces for their adaptability and utilize them in breeding programs to harness the potential of this crop [30].

Utilizing traditional breeding methods, over 30 enhanced varieties have been successfully created and are cultivated in diverse agro-climatic conditions across different regions of the globe [48]. This focused numerous endeavors on breeding to create genotypes with a reduced level of β -ODAP. However, soil, drought, and climatic factors significantly influence the β -ODAP levels, demonstrating a pronounced genotype \times environment interaction effect [42]. Nonetheless, it is now widely recognized that the toxic effects of β -ODAP can be prevented by incorporating adequate amounts of fruits, vegetables, and cereals into the diet [27].

In general, the genetic enhancement of lathyrus can be divided into three stages. The initial phase (1940-60), lasting nearly two decades, focused primarily on enhancing yield [26], resulting in the development of numerous cultivars suitable for widespread cultivation. The subsequent phase (1974-1990s) focused on creating improved cultivars with reduced β -ODAP levels. Following the assessment of the global lathyrus collection, a notable degree of variation in β -ODAP levels in lathyrus seeds has been noted; spanning from 0.02 to 2.59% [46], yet no β -ODAP-free plants have been found in any wild species or germplasm [24]. Among certain Indian germplasm, the greatest concentration of β -ODAP has been observed, whereas the minimum level (0.02%) has been documented in Australian germplasm from ICARDA. Several significant enhanced lathyrus cultivars, with β -ODAP levels $<$ 0.1%, have been introduced due to numerous national and international breeding efforts, including Gurbuz 1 from Turkey; Ceora and Chalus from Australia; Wasie from Ethiopia; Ali-Bar from Kazakhstan; Bina Khesari 1, Bari Khesari 1, and Bari Khesari 2 from Bangladesh; PUSA 24, Ratan, Prateek, and Mahateora from India [46]. During the third phase (from the 1990s onward), numerous breeding lines and varieties were created that integrated low β -ODAP ($<$ 0.1%) with high-yielding capacity (up to 1.5 tons/ha) and significant resistance to various biotic and abiotic stresses [47].

6. Breeding strategies for harnessing CWR traits:

Compared with other pulse crops, *Lathyrus sativus* (grasspea) has received relatively limited attention in genetic research, despite its cultivation for more than 8000 years [70]. This relative neglect is largely attributed to the presence of the neurotoxin β -ODAP (β -N-oxalyl-L- α , β -diaminopropionic acid) and its historical use primarily as a fodder crop. However, the development of improved plant types with compact growth habit, enhanced seed size, and reduced β -ODAP content could transform grasspea into a high-value pulse crop suitable for semi-arid regions of developing countries [15]. In recent years, substantial progress has been made in grasspea improvement programmes targeting multiple traits simultaneously. These include reduction of β -ODAP levels, enhanced resistance to biotic and abiotic stresses, improvement of nutritional quality, increased nitrogen fixation capacity, and broader utilization as food, fodder, and forage. Such advancements contribute to food security under adverse environmental conditions, support low-input livestock systems, and promote soil conservation when used as a cover crop [37]. A range of traditional and modern breeding approaches have been employed for the genetic improvement of grasspea over time (Fig. 1 & 2). Conventional methods, including introduction, selection, hybridization, and mutation breeding, have played a significant role in crop enhancement [22].



Fig 1&2: Grasspea International trials (ICARDA) conducted at ICAR-IIPR, RS, Bhopal

(i) Traditional breeding:

Slow progress in the field of grasspea research initiated for the development of the low β -ODAP varieties by direct selection from landraces and lines [102]. Tradition breeding which are basically progressed by crossing of the selected genotypes followed by screening, selection and evaluation of the advance generations for the traits of interest such as breeding objective with low β -ODAP content, hybridization of low β -ODAP accessions with high-yielding varieties which are having acceptable agronomic traits [15]. The high yielding traits have always been a selection base for every crop improvement programmes. In crops like grasspea, genotypes possess double podding, higher seed weight per pod or better 100 seed weight have to be selected for developing high yielding varieties. *Lathyrus* is also a source of animal feed both in green as well as in dry forms, hence, the biological yield is also of most importance and need breeding strategies for developing varieties for animal feed [102].

From the past research, being focused on the development of super high yielding genotypes with minimum β -ODAP content, an Indian landmark variety of grasspea "Pusa 24" was selected from the field in Bihar in 1966 [18]. It was reported as the cultivar having low β -ODAP content in their seed (0.2%), and it served as base material for the research and development of low β -ODAP *lathyrus* varieties in India and abroad. After this, research efforts guided for the development of the *Lathyrus* genotypes suitable for upland (LSD1 and LSD2) and for rice-fallow area (LSD 6, LSD 3, LSD 6, Pusa 305 and Selection 1276) which are reported to have low β -ODAP (up to 0.2%) content [26]. By selection from locally heterogeneous population, a cultivar "Quila-blanco" was released in 1983 in Chile and specific features of this variety were white bold grain (100 seed weight 28.7 grams), 24.0% protein content with same time maturity [16].

Attempts have been initiated to establish an association between some morphological marker traits of grasspea with low β -ODAP which would be helpful for ease in selection; however, it has remained still unclear because of their polygenic traits which are highly influenced by genotype x environment interactions [34]. Few varieties and advanced breeding lines have been evolved by combining low β -ODAP content (<0.1%) with high yielding genotypes which are having resistance or are tolerant to biotic and abiotic stresses [82].

(ii) Pre-breeding:

To widen the genetic base of the crops, the introgression of useful genes/alleles from primary gene pool are urgently needed by the use of pre-breeding and wide hybridization. Though inter-generic hybridization is boring, it has also been delivered successful instance of the development of inter-specific and wide crosses with grasspea [22]. The successful inter-specific crosses among grasspea and other *Lathyrus* spp. especially *L. pseudocicera*. Embryo rescue techniques have also been employed to increase the range of species in successful inter-specific crosses [2]. The result of grasspea inter-specific hybridisation explains that the identification, utilisation and transfer of useful traits from exotic or wild germplasm have offered opportunities for development of the low β -ODAP genotypes which are mainly from crossable species like *L. cicera* and *L. amphicarpus* [39]. Hybridizations were also attempted with other crop wild relatives such as *L. chrysanthus*, *L. gorgoni*, *L. marmoratus* and *L. pseudocicera* [36], nevertheless only ovules were developed. Identification of the toxin-free in *L. tingitanus* may be used for developing varieties with low levels of toxin [108]. Further, wild species like *L. clymenum* and *L. ochrus* (81) and *L. cicera* [25] have shown resistant to broomrape, which is not available in the cultivated gene pool.

Introgression of the desirable genes, alleles or QTLs from outside the primary gene pool via pre-breeding and wide hybridization are needed essentially to widen the genetic base of the base parent. Thus, inter-specific crosses have been carried out between grasspea and other *Lathyrus* species like *L. pseudocicera*. Embryo rescue technique is also being used for achieving successful inter-specific crosses [2]. Yunus and Jackson, 1991 [106] reported that the introgression of desirable gene or QTLs of desirable traits and their identification from exotic and wild relatives would offer enough opportunities for grasspea improvement, that too specifically between crossable species like *L. amphicarpus* and *L. cicera*. Other species like *L. marmoratus*, *L. gorgoni*, *L. pseudocicera* and *L. chrysanthus* have also been used for crossing [36]; but, only ovules were developed here in that trial. In wild grasspea species have also clearly shown in many literature that the lowest β -ODAP are obtained in *L. cicera*, followed by that in *L. sativus* and *L. ochrus* [34].

(iii) Mutation Breeding

Grasspea is a climate resilience crop; hence the demand and importance have been increased in recent times, especially in areas of rainfed rice growing belts where it can be cultivated as utera crop without giving additional input management. The improvement in grasspea is hampered because of narrow genetic base which resulted due to self-pollination and inter-specific incompatibility. In-vitro regeneration of grasspea from epicotyl explants or callus derived from epicotyls explants or from root induction of callus-derived shoot can be used, and these explants has to be acclimatised through hardening process [88].

Hence, mutation breeding is considered an important genetic improvement approach, since it is regarded as an endmost way of generating new variability in crop plants. It can be a better supplement to traditional breeding for creating additional genetic variations which may be used by the plant breeder for the development of the desired genotypes for specific purpose [2]. In grasspea, the chemical mutagens like EMS (ethyl methane sulphonate) and NMU (N-nitroso-N-methyl urea) are more effectively used than radiation mutagen for developing chlorophyll mutant [64]. However, various genotypic response has been observed in exposure to gamma radiation (64). Through mutation breeding, a lot of phenotypic variation have been observed which affects maturity, growth habit of the plants, stem shape, branching patterns, flower colour, hairiness, stipule shape, leaf shape, pod size and colour, differences in the colour of seed coat, streak pattern on the flower [14]. Along with phenotypic changes, chromosomal level changes like translocations were induced through mutagens [90].

Through mutation breeding, salt tolerant lines have been developed which has increased level of anti-oxidant properties such as superoxide dismutase and ascorbate peroxidase [91] and it is also used in the development of the low β -ODAP varieties [90]. Biochemical level changes have also been reported by mutagen treatment and it is being used in plant defense. For example, a glutathione (GSH)-deficient mutant (gsh1-1) was derived by gamma-rays treatment in M2 generation of a genotype, BioL-212 and this mutant reported to have better sensitivity to cadmium [92]. By the use of mutation breeding using EMS (0.01%) and gamma rays (250 Gy), two popular varieties like "Bina Khesari 1" in Bangladesh and "Poltavskaya" in Russia were developed [47]. Met (Methionine) amount production capacity of grasspea was enhanced from 25% in the parent to almost 50% in the altered putative mutant genotype [10]. Induced mutation and somaclonal variation can create new variability which could generate better opportunities for the plant breeders for selecting suitable genotypes with higher yield. A somaclone NGOG 5 derived with increased yield and lower β -ODAP content may be used suitable donor in the lathyrus breeding plans.

(iv) Molecular breeding approach

Molecular breeding provides number of tools and techniques to address the changes of increasing yield, enhancing quality and stabilisation in the production in the scenario of unpredictable climate change [44]. DNA markers and its utilization has helped to facilitate marker-aided selection (MAS) for many crop improvements. Recent advancement in plant genomics research and the development of functional DNA marker at large number gives extra resources to understand crop diversity at species and gene levels which would finally help in speeding up the genetic improvement programme [63]. Grasspea is not as advanced in the field of genomics and molecular research as other pulses crop like chickpea. Till now, three linkage map have been constructed using molecular markers [78]. One of it was constructed by using 11 RAPD markers, one isozyme marker and flower colour [18]. The another map were developed by using 47 RAPDs, 7 cross-amplified pea microsatellite simple sequence repeats (SSR) markers and 13 cleaved amplified polymorphic sequence (CAPS) markers [89]. These maps were utilized for analysis of the quantitative trait loci (QTL) to evaluate a backcross population for its resistance to *Ascochyta* blight.

To better the marker assisted selection (MAS), the basic requirement is the availability of tightly linked molecular markers with the character of interest. In addition to knowing the level of genetic diversity in phylogenetic research, the DNA makers can be used in plant breeding programme for MAS (101). These Markers are utilized to know the actual number of genes, its positions and effects of individual genes/QTLs governing traits of interest by linkage mapping and QTL analysis [16]. The use of molecular markers in plant breeding fasten the generation of new varieties by assisting plant breeders in early stage selection of desirable genotypes with trait of interest based on genetic make up rather than phenotypic appearance [94]. Through the EST-SSR marker system higher amount of conservation is displayed that can be transferred among the species. The numbers of ESTs for *L. sativus* (178) and *L. cicera* (126) are highly confined when compared with those available for *L. odoratus* (8702) [52]. At present, high-throughput and dense genotyping systems have aided association mapping that has an edge over the bi-parental population by generation of a many number of recombinants in a short period of time [19]. Therefore, the development of a complete genetic map for grasspea, and identification of the valuable genes and QTLs for MAS and with possible alignment with other species is crucial. MAS, linkage maps and gene cloning will fasten the introgression of unique genes for low β -ODAP and higher Met contents leading to improvement of the quality of locally popular cultivars.

(v) Tissue culture and Transgenic approach:

Grasspea appears to be promising as a good candidate for both TILLING and Eco-TILLING as the genetic resources limited and it also shows recalcitrant in nature while genetic transformation. Transgenics in grasspea by *Agrobacterium*-mediated transformation and particle bombardment hold high potential following regeneration of whole plants by tissue culture [46]. Also, *Agrobacterium rhizogenes*-mediated transformation followed by somatic embryogenesis was also reported in *L. maritimus* [41]. Till now, very few research were made towards grasspea transformation. Hence, urgently concerted efforts are required for the optimisation of genetic transformation protocol in grasspea and successful generation of transgenic plants with reduced β -ODAP.

By using somaclonal variation, tissue culture has potential to improve the biometrical traits, generation advancement, screening for drought and salt tolerant genotypes, and regeneration of true plant type in grasspea is possible. Grasspea having recalcitrant seed is tough for regeneration under in-vitro conditions. However, the first viable plant regeneration protocol development and optimisation from meristematic tissues in grasspea was reported by Zambre *et al.*, 2002 [107] and following it, different explants from disease-free seedlings (including leaf, internode, hypocotyl, epicotyl and cotyledon,) were used for regenerating plants. Amongst the different explants of grasspea, the epicotyl showed highest shoot proliferation frequency and was reported to be the most responsive. In *L. sativus*, very few somaclones development is reported, but in India, a low ODAP variety (Ratan) have been developed from somaclones derived lines [61]. Somaclonal variations at cytological, morphological and biochemical levels have been reported in four grasspea genotypes (Nirmal, P 24, Nayagarh local, and Dhenkanal local) [98]. A notable promising (high yield) potential somaclone (cv. NDOG 5) with low ODAP content was generated that may be utilized in further grasspea

breeding programmes. A method for reducing the generation cycles and speeding the breeding procedure by using in-vitro system providing up to 4-5 cycles per year of grasspea was reported by Barpete *et al.*, 2020 [13]. However, it is feasible to obtain only few seed per plant through this biotechnological approach, but it can surely contribute towards speeding up the breeding programme of ODAP-free grasspea varieties.

It is reported that due to the non-responsive nature for organogenesis or somatic embryogenesis, the genetic transformation rate is low in legume crops. The epicotyl explant of Indian grasspea accession and was co-cultured with two disarmed *Agrobacterium tumefaciens* strains (EHA 105 and LBA 4404) in the systematised genetic transformation protocol [11]. Following several efforts by researchers from Ethiopia, a productive protocol of grasspea of regeneration for transient genetic transformation of two Ethiopian-origin grasspea varieties is systematised. The seed protein quality of grasspea was tried to be improved by Ethiopian scientists and similarly through gene coding the genetic transformation was planned for improving the Met content [28]. Very recently, grasspea *Agrobacterium*-mediated genetic transformation was found to enhance the tolerance to fungal pathogens and nutritional quality without any ill- effect on the seed protein quality, while, lower level of β -ODAP (up to 73%) was furthermore recorded by Kumar *et al.* [49]. In addition, anti nutritional metabolite, oxalic acid (OA) is a recognised precursor of β -ODAP. The lower level of OA in transgenic seeds of grasspea (about 75%) was linked to an increase in seed micronutrients *ie.* calcium, iron, zinc, manganese, and magnesium [49]. Thus, there is immense possibility of genetic transformation for better nutritious utilization of grasspea.

7. Breeding for biotic stress tolerance

Disease and Insect Resistance in CWRs

Grasspea is comparatively resistant to various insect pests and diseases [67]. However, few pathogens and insect pests cause damage to the crop. The diseases *viz.*, Powdery mildew (*Erysiphe* sp.), Downy mildew (*Peronospora lathyri-palustris*), Grey mould (*Botrytis* sp.), Rust (*Uromyces* sp.), *Ascochyta* blight (*Ascochyta pisi*, *A. rabiei* and *Mychosphaerella pinodes*), and leaf spot (*Cercospora pisi sativae*) are of significant importance in terms of damage they cause to the crop [29]. Likewise, Thrips (*Caliothrips indicus*), Aphids (e.g. *Aphis craccivora*), Pod borer (*Etiala jhinkinella*), and Bruchid beetles are major insect-pests of grasspea [67]. Apart from diseases and insects, the root parasitic weed (an obligate root parasite), broomrape (*Orobanche crenata* Forsk. and *O. foetida* Poir), is another major constraint in grasspea cultivation especially in Mediterranean and sub-Saharan African regions [74]. In case of heavy *Orobanche* infection severity, grasspea fail to produce good grain yield.

The *Lathyrus* spp. contains resistance against *Erysiphe* spp., *Ascochyta* spp., *Uromyces* spp., *P. lathyri-palustris*, and *C. pisi sativae*. Additionally wild *Lathyrus* spp. Like *L. articulatus* and *L. aphaca* and *L. ochrus* have complete and moderate resistance against *Orobanche* spp. [1]. Resistance has been reported against powdery mildew and rust pathogens in *Lathyrus* spp. Patto and their collaborators have been at the forefront of research on *Lathyrus* resistance, specifically focusing on rust as well as powdery mildew [67; 65; 66]. They have conducted extensive assessments and provided detailed descriptions of resistance against rusts and powdery mildew across various

Lathyrus species. A collection consisting of 150 accessions of *L. cicera* and 50 accessions of *L. sativus* were evaluated for resistance against *Uromyces* spp. and *E. pisi*. A wide range of disease reactions was observed against the test pathogens in this collection. In another study from Bangladesh, 75 accession lines were screened for rust and powdery mildew resistance, out of which 27 were found resistant to *Uromyces* spp. and 38 lines were resistance to *E. pisi* [83]. *L. belinensis* is completely resistant to powdery mildew pathogen and though with difficulty it can be crossed with *L. odoratus* which is highly susceptible and therefore the powdery mildew resistance can be introgressed from *L. belinensis* to *L. odoratus* [68]. In *L. belinensis*, a single dominant gene governs the powdery mildew resistance.

In another comprehensive study conducted by Martins *et al.*, [59] a global collection of 189 *L. sativus* accessions for resistance to *E. pisi* and *E. trifolii*, were examined revealing a broad spectrum of responses to pathogens. The researchers found that while partial resistance to *E. trifolii*, which had not been previously characterized, was relatively less common, it was more prevalent in the case of *E. pisi*. Additionally, Martins *et al.* [59] identified seven and twelve distinct single nucleotide polymorphic molecular markers (SNPs) associated with responses to *E. trifolii* and *E. pisi* based on genome-wide association study (GWAS). Almeida *et al.* [5] studied the resistance mechanism in *L. sativus* against rust by RNA sequencing of un-inoculated *L. sativus* leaves and *U. pisi* inoculated leaves of partially rust-resistant (BGE015746) and susceptible (BGE024709) grasspea genotype, and several pathogenesis related genes were up-regulated in the resistant genotype while these were down-regulated in susceptible one. A mildew resistance locus O (MLO)-like gene, and MLO-related transcripts were considered as potential resistance genes.

Ascochyta blight infection on grasspea has commonly been linked, associated with *A. rabiei* and *Mycosphaerella pinodes* (telomorph *Dydimella pinodes*) [12], and only recently also to *A. lentis* var. *lathyri* has also been reported [38]. *Lathyrus* spp., including *L. sativus*, *L. cicera*, *L. ochrus*, and *L. clymenum*, exhibit considerably higher resistance to *A. rabiei* compared to field pea cultivars [33]. *A. lentis* var. *lathyri* demonstrates a distinct preference for infecting grasspea exclusively, while *A. lentis* isolates obtained from lentils are capable of infecting grasspea but with limited efficacy. Moreover, cross-inoculation studies have revealed that various grasspea accessions exhibit significant susceptibility to *M. pinodes*, while displaying immunity or high resistance to isolates of *A. rabiei*, *A. lentil*, and *A. fabae* [12]. This resistance holds significant importance due to its potential transferability to the field pea (*Pisum sativum*) crop, which is closely related in terms of phylogeny. *Ascochyta* previous researches suggest that two independently segregating genes, operating in a complementary epistatic manner may control the quantitative resistance of *L. sativus* to ascochyta blight [89]. In a study conducted by Almieda *et al.* [4], the early response of the leaf gene expression profile in a resistant genotype of *L. sativus* to infection by *A. lathyri* was compared to un-inoculated control sample from the same genotype utilizing deep Super SAGE for analysis. The results indicated up-regulation of defense-related genes associated in and/or regulated by the ethylene pathway, over-expression of cellulose synthase and lignin biosynthesis genes was observed during the plant pathogen interaction. This was the first genome-wide overview of the gene expression profile of the *L. sativus* response to *Ascochyta* infection [4].

Insect resistance too is one of the desirable traits in *Lathyrus* improvement programme though research in this direction is very limited. There are very meagre studies focussing on the evaluation of *Lathyrus* spp. for their resistance to major pests of *Lathyrus* or the mechanism of resistance. In one of very few studies by Annis and O'Keeffe [7] response of two *Lathyrus* spp. (*L. tingitanus* and *L. sativus*) to pea weevil (*Bruchus pisorum*) infestation was studied and it was found that female weevils didn't prefer *Lathyrus* spp. for oviposition when given an option between pea and *Lathyrus* spp. The resistance of *Lathyrus* spp. to pea weevil is due to antibiosis and antixenosis and reported to be located in the pod [7]. High protein content and ODAP in *Lathyrus* seeds attract more bruchid infestation. Tsialtas *et al.*, [99] evaluated eight *L. cicera* accessions for bruchid resistance; significant difference in the resistance level was found. *L. cicera* cv. *Rodos* seeds which has lowest protein and ODAP content but highest iron content in comparison to seven *L. cicera* accessions collected from the mainland and insular Greece was most resistant to bruchid [99].

With the mounting pressure imposed by climate change, there has been a renewed focus on developing resilient varieties capable of withstanding prevalent biotic stresses. Grasspea, in particular, possesses a valuable array of resilient traits that make it a potential donor for genes responsible for adaptive characteristics like disease resistance, drought tolerance, flood tolerance, salinity tolerance, and heat tolerance [29]. Identifying the origin of these adaptive traits is crucial, and pre-breeding attempts play a pivotal role in this endeavor. Additionally, comprehending the genetic control of these traits is essential as it enables the identification of promising genomic targets. This knowledge, in turn, facilitates the development of molecular tools that can aid in precision breeding for stress resistance in grasspea.

8. Breeding for abiotic stress tolerance

Grasspea is a hardy plant that withstands extreme temperatures, drought, flooding, and high salinity. It flourishes in warm environments and poor soils while providing satisfactory yields even under adverse circumstances [29]. Thus, it serves as a beneficial source of characteristics for cultivating climate-resilient crops, including associated legumes like peas [66]

8.1 Drought and waterlogging:

This can be achieved by improving water use efficiency (WUE) and/or acquiring phenological traits that confer drought tolerance. Grasspea seems to be mainly a crop capable of enduring or evading drought situations, characterized by late maturation and senescence in Mediterranean-like climates that have a relatively short growing period and terminal drought. Narrow, winged leaves that can curl their edges inward function as drought avoidance strategies, while early vigor, blooming, and ripening serve as escape strategies. This would be highly important for achieving improved economic yields in short-season environments, like those found in the Mediterranean [95; 40; 85]. The robust and deep root systems of grasspea enhance water use efficiency in flood conditions [79]. Grasspea is often utilized as a "relay crop" or "paira crop" in the rice fallows of South East Asia because it can flourish on leftover soil moisture. It has been assumed that it might serve as the foundation for its reasonable tolerance to both drought and flooding.

Indeed, during flooding, the deeper soil layers stay aerated, allowing a deep-rooted system to enable Grasspea to circumvent flood limitations [79]. Dixit et al. [22] and Girma and Korbu [28] emphasize the submergence tolerance of grasspea, particularly during the germination phase

8.2 Salinity:

Salinity ranks among the primary factors contributing to global soil degradation. It lowers osmotic potential, making it more difficult for plants to extract water, resulting in surface crusting, hindered water infiltration, diminished root zone aeration, and ultimately affecting healthy plant development and crop production. The rise in sea level frequently leads to seawater intrusion and soil salinization in coastal regions of Bangladesh, an area significant for grasspea cultivation. Regions such as arid and semi-arid are likewise vulnerable to desertification and salinization [20; 62]. The strong action of certain antioxidant compounds and the efficient isolation of harmful ions in the rhizome/rootlet, shoots, and tendrils enable grasspea to withstand salinity [97]. These findings provide deeper insights into the tolerance mechanism of grasspea to salinity and enhance its status as a crucial crop for enduring salinity stress

8.3 Heat:

Grasspea is a crop for cold seasons, with a daily maximum temperature exceeding 25°C recognized as the upper limit for heat stress. The severity of heat stress effects primarily relies on the intensity and duration of exposure, along with the level of elevated temperature. Temperatures above 30°C during flowering can lead to reduced pollen viability, increased flower drop, and decreased seed setting and pod filling, ultimately causing a decline in grain yield [79]. Cleistogamous flowering characteristics safeguard the male (anthers) and female (stigmas) structures from heat-induced dehydration during the flowering phase, while initial development is vital to avoid heat stress during pod-filling [3]. Kumar and Tripathi [45] examined the impact of temperature factors on grasspea and highlighted its heat resilience. In the research, F₁ generation seeds of *Lathyrus* were subjected to 55 °C for 48 hours, and subsequent phenological and cytological examinations of F₂ generation plants revealed that seed production was unaffected by the heat stress, and the impact on flower sterility was not significant enough to change the fertility of the grasspea plants.

9. Genome editing

Genome editing, also known as gene editing and genome engineering, is a form of genetic engineering in which DNA is either inserted, deleted, or modified/replaced within the genome of a living organism. Unlike previous genetic engineering methods that inserted genetic materials randomly into a host genome, genome editing focuses on specific insertion or deletion sites at targeted locations. The fundamentals of genetic modifications via programmable nucleases include selecting the target genomic regions and attaching the effector DNA-binding domain (DBD), creating double-strand breaks (DSBs) in the target DNA using restriction endonucleases, and repairing DSBs through homology-directed recombination (HDR) or non-homologous end joining pathways [81]

In the *Lathyrus* genus, there has been a positive increase in the genetic information available; however, this data remains limited compared to other pulse crops.

However, a degree of progress has been observed in the development of molecular markers for *L. sativus*, enabling the assessment of diversity and variation both among and within species [52]. Certain PCR-based molecular markers, such as the Simple Sequence Repeat (SSR) method in grasspea, have significantly enhanced the ability to differentiate among various *L. sativus* accessions and assess the genetic variability within the species [5]. Furthermore, molecular markers can be utilized to identify the quantity, location, and specific impacts of genes/quantitative trait loci (QTLs) that govern beneficial traits, such as resistance to insect pests, β -ODAP and protein levels, along with other traits significant for agriculture through genetic linkage mapping and association mapping [77]. The use of next-generation sequencing (NGS) on *Lathyrus* has provided an opportunity to expand the pool of genomic resources for this species. Grasspea offers significant potential as a reservoir of various stress-resistant genes for enhancing common crops through appropriate breeding techniques [35]. For example, donor germplasm exhibiting the preferred phenotypes can be utilized to create some encouraging breeding resources. The existing genomic tools can also be utilized to accelerate the breeding program.

10. Allele mining

The two main objectives for conserving vital germplasm in global gene banks are: first, to protect crucial germplasm from extinction, and second, to screen, characterize, and document this significant germplasm to identify superior genes/alleles that could potentially lead to the development of enhanced breeding lines. To identify superior genes/alleles, researchers require extensive data on the genome sequences of a specific organism. Extensive sequence data for various crop species can be found in the public domain. This sequence data would aid in identifying and isolating superior and improved alleles of key genes from the crop gene reservoirs. After identifying the gene/alleles of interest, allele mining can be performed to locate appropriate alleles at each locus and determine the genotypes where these alleles are present. These genotypes can serve as parents for hybridization in a breeding program. Consequently, allele mining is a method utilized for analyzing natural allelic variations or desirable alleles of a candidate gene that influence key agronomic traits and may have significance in *lathyrus* genetic enhancement. This aids in understanding the evolution of alleles/genes, discovering new haplotypes, and creating allele-specific molecular markers beneficial for marker-assisted selection (MAS)

Numerous transcriptome studies have demonstrated the generation of a large number of genome-wide SSRs and SNPs markers for effective utilization in mapping-related cloning research and molecular mapping [35]. In 2014, the initial study on global gene expression profiling in *lathyrus*-pathogen interactions related to rust resistance was conducted [4]. In reaction to *Aschochyta* blight infestation, an additional focus was placed on obtaining a molecular understanding of the grasspea crop [4]. Additionally, transcriptome analyses have been conducted to pinpoint genes and regulatory pathways that govern β -ODAP flux during various growth stages of the grasspea cultivar "LZ" [104]. Rathi et al. [71] detailed the time-dependent impacts of water scarcity on the transcriptome and metabolome. In total, 5201 genes from various functional categories, such as structural cell wall proteins, cytoplasmic enzymes, and transcription factors, were identified as responsive to water deficit.

Additionally, metabolome profiling revealed 59 metabolites responsive to dehydration, comprising amino acids and sugar alcohols.

11. Conclusions

A growing population has heightened demands on agriculture to guarantee global food and nutritional safety. This problem is aggravated by climate change. Despite the uncertainty of future climate scenarios, research indicates a decline in agricultural productivity ahead. Climate elements like elevated temperatures and inconsistent rainfall affect pest outbreaks, soil quality, water availability, plant physiology, and metabolic processes in plants [58]. A promising method currently being explored that could significantly aid is the strategic and focused use of crop wild relatives (CWR) in lathyrus enhancement initiatives. Failing to address this challenge could severely impact access to adequate and healthy food, the worldwide economy, and societal stability. The preservation of crop wild relatives (CWRs) is essential and must be tackled methodically on an international, national, or regional level to satisfy future food production needs and adjust to climate change. Missions for collection and conservation initiatives ought to focus on the characteristics desired by users, such as breeders. Targeted efforts can focus on identifying accessions suited to abiotic and biotic stresses like heat, drought, and salinity by analyzing the distribution of these species across environmental gradients that exhibit typical stresses [84]. Lathyrus is a genus comprising approximately 160 species, primarily located in Europe and Asia, with a secondary hub in the Americas. Its diversity is mainly concentrated in the Mediterranean and Irano-Turanian areas [51]. Several species of Lathyrus are cultivated for human food, animal feed, and fodder (*L. sativus*, *L. cicera*, *L. ochrus*, and *L. clymenum*), in addition to being grown for ornamental uses (*L. odoratus*, *L. latifolius*, and *L. sylvestris*). Additionally, they offer advantages by acting as soil nitrifiers and stabilizing dunes [80]. Grasspea is commonly known as an "insurance crop" because of its capacity to flourish in subpar soil and offer economic, social, and nutritional support for underprivileged farmers [55]. It serves as an essential energy resource for more than 100 million individuals in regions prone to drought in Asia and Africa. The plant is adaptable and can endure harsh temperatures and heavy rainfall [52]. Simultaneously, its genetic capacity as "climate resilient" has been ignored, generating a need to enhance its climate-adaptive characteristics to strengthen the economy of underutilized land resources. The comprehensive perspective integrates agronomic quality and variety-improvement approaches to boost resilience against climate change. Climate change may generate new areas conducive to the expansion of grasspea, which has significant resilient characteristics [29]. A successful breeding programme for effectively identifying climate-resilient traits depends on a varietal improvement strategy, which encompasses Genome-Wide Association Studies (GWAS) that focus on the phenotype of complex traits adaptable to climate change and interspecific hybridization. The Agronomic quality-oriented program involves G×E interaction research for yield and quality consistency along with intercropping. CWRs can enhance crop yield and stability by offering advantageous characteristics, such as resistance to biotic and abiotic factors, [60; 31]. One possible impact is that numerous existing crop varieties might require replacement in cultivated regions as a result of climate change [54].

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