

Review: A brief history of grasspea and its use in crop improvement

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Introduction

Grasspea (*Lathyrus sativus* L.) is an annual pulse crop belonging to the family Fabaceae⁽⁴⁾ and the tribe Viciae. Grasspea is known by a wide range of common names, include chickling vetch, Indian vetch (UK and USA), khesari or Batura (India) and dhal⁽⁴⁴⁾. Along with *Pisum*, *Lens* and *Vicia*, this genus may be a source of new and useful genetic traits for closely related genera for future plant breeding work improving commercially valuable species.

The *Lathyrus* genus is believed to have originated in southwest and central Asia, with a significant subsequent spread to the east of the Mediterranean basin^(62, 63). All grasspea lines appear to divide into two geographical origins - one group derives from the Indian subcontinent, and another from the Mediterranean/European region, which typically has higher yields and larger seeds⁽²⁴⁾. Morphologically, this crop resembles field pea, but its leaflets are long and grass-shaped rather than rounded, and it has a deep taproot system^(65, 66). This species is now widely distributed throughout Eurasia, North America, temperate South America and East Africa^(44, 62, 63) with a small amount being cultivated in Australia⁽⁵⁸⁾. The most recent studies of grasspea have examined over 140 accessions in detail⁽⁵⁰⁾.

Agronomic and economic importance of grasspea

There are around 187 species and subspecies in the *Lathyrus* genus. Grasspea is the only member of this genus that is widely cultivated as a food crop⁽⁴⁴⁾, while *L. odoratus* (sweet pea) is grown commercially for its flower morphology⁽⁵¹⁾.

Grasspea performs well under adverse agricultural conditions, and its many cultivars possess different attributes including the ability to resist both drought and flooding, high climatic adaptability and the ability to grow in cool climates and at high altitudes^(65, 66). Grasspea cultures also have the ability to adapt to saline, alkaline, clay or otherwise poor soils, and are hardy and easy to cultivate^(3, 61). In addition to nutritional benefits, grasspea has an important role as a legume crop in crop rotations, reportedly adding

around 67 kg ha⁻¹ of nitrogen to the soil in a single season and conferring yield and protein benefits on the subsequent non-legume crop^(44, 68).

Grasspea is grown primarily as a winter pulse crop, and is grown for stockfeed and human consumption in the Middle East, France, Spain, India, Bangladesh, China, Pakistan and Nepal, Asia and Africa^(24, 44, 45, 62), and is known as the 'poor men's diet' in the Central region of India⁽⁶⁰⁾. This crop is cultivated only as a fodder crop in Australia, Europe and North America, and is recommended for low quality soils of southwestern Australia⁽⁵⁹⁾. Grasspea is valued as a nutritious staple food and fodder crop primarily due to its relatively high protein content (18–34% dry weight in seeds, 17% in mature leaves) and its high lysine content^(18, 53, 58).

One of the major drawbacks of grasspea is the fact that the seeds contain a major anti-nutritional compound β -*N*-oxalylamino-L-alanine (BOAA) (also known as β -*N*-oxalyl-L- α , β -diaminopropionic acid or ODAP)^(6, 61, 68, 70). Following prolonged or excessive consumption of grasspea, the neurotoxin causes a drastic paralytic disease known as 'lathyrism' or 'neurolathyrism', manifesting as paralysis of the leg muscles, muscular rigidity and weakness^(43, 66). Recent studies have found that genotype is the main determinant of BOAA concentration, with little if any effect from environment⁽²⁴⁾.

Grasspea genetics and applications

Genetic studies on *Lathyrus* spp. are rare. Detailed chromosome analysis of grasspea shows that grasspea has a chromosome number of $2n = 14$, with two metacentric and five submetacentric chromosomes^(28, 33). Total metaphase chromosome length is estimated at 40.3 μ m⁽⁴⁷⁾.

Meiosis and chromosome pairing have been examined in grasspea, *L. odoratus* and *L. pratensis*^(29, 30). Variation in genome size⁽⁴⁷⁾ or karyotype^(28, 31, 32, 33, 34) in *Lathyrus* spp. has also been studied. Preliminary studies on the phylogenetic distance between seven *Lathyrus* species have been undertaken⁽¹⁾. Studies have taken place on the inheritance of genetic traits

such as BOAA content, flower and seed coat colour^(58, 65, 66) and the genetic diversity in germplasm collections of *Lathyrus* spp.⁽⁵²⁾.

Grasspea has been identified as an important source of novel genes for use in grain legume breeding programs, both for abiotic and biotic stress resistance. Compared to other legumes, grasspea is resistant to many insect pests⁽⁶⁶⁾. *Lathyrus* spp. is a possible source of genes for cold tolerance⁽⁵²⁾ or downy and powdery mildew resistance genes⁽⁴⁴⁾. In Syria, host-plant screening in *Lathyrus* spp. has taken place, uncovering potential sources of resistance to powdery mildew [induced by *Erysiphe pisi* Syn. (syn. *E. Polgoni* D.C.)], botrytis blight (induced by *Botrytis cinerea* Pers. ex Fr) and ascochyta blight (induced by *A. pisi* Lib.). These sources of resistance are gradually being incorporated into pulse breeding programs over time⁽⁵²⁾.

Several studies have demonstrated that many members of the *Lathyrus* genus possess resistance to ascochyta blight caused by *M. pinodes*^(5, 10, 11, 69). Glasshouse trials conducted at the Victoria Institute for Dryland Agriculture in Victoria, Australia, with controlled inoculation conditions found that 59 accessions *Lathyrus* spp., representing ten species including *L. sativus*, exhibited higher levels of resistance to ascochyta blight than did *P. sativum*^(13, 48). Attempts were made to produce field pea–grasspea hybrids by conventional crossing methods followed by embryo culture, but this work was unsuccessful (E. Pang, pers. comm.). Furthermore, preliminary screenings of *L. sativus* accessions indicated a degree of intraspecific variation in ascochyta blight resistance (E. Pang, pers. comm.). The inheritance of resistance to this disease was studied and resistance was found to be controlled by two major recessive genes⁽¹³⁾.

Two potential techniques, more advanced than conventional crossing, offer possibilities for transferring any identified resistance gene(s), such as those from grasspea, across the species barrier. These are cloning of a resistance gene from the donor species, followed by transformation and regeneration of the transgenic host species directly using *Agrobacterium* spp., or through asymmetric somatic hybridisation *via* protoplast fusion^(19, 40). Genetic transformation through techniques such as these provides a means for the genetic improvement of the genome of crop species. Judicious application of biotechnological tools holds great potential for alleviating some of the major constraints to productivity of these crops in the agricultural systems throughout the world⁽⁵⁷⁾.

A transformation/regeneration procedure using *Agrobacterium*-mediated gene delivery has been

developed successfully for one agronomically important crop, field pea^(2, 21, 22, 55). This has since been used to transfer the cDNA encoding an α -amylase inhibitor from common bean into field pea, conferring resistance to pea weevil⁽⁵⁶⁾, and to herbicides^(21, 22). *Agrobacterium*-mediated transformation of field pea has also been shown to be effective across a range of genotypes^(46, 49). However, the use of such a technique to transfer ascochyta blight or other resistance from grasspea into field pea is not yet possible, because the relevant ascochyta blight genes have not been identified cloned. Once this has been determined, a more rapid means of transformation of agronomically important crops such as field pea, using valuable genes from grasspea, should be possible.

Grasspea tissue culture

The use of wild relatives for introducing resistance genes originates from the ongoing search for a broader gene pool for continued crop improvement. Resistance may also be induced by the use of mutagenic agents⁽³⁹⁾ or tissue culture techniques to produce somaclonal variants^(35, 36). However, the disadvantage of induction of resistance is the unpredictability of the resulting combination of other traits in the new plant, or reduced fertility⁽²⁰⁾. While the intentional introduction of new, known traits is desired, interspecific or intergeneric barriers must be overcome. Many techniques are available for this task, including conventional crossing with embryo rescue, somatic hybridisation *via* protoplast fusion, or transformation using *Agrobacterium tumefaciens* or *A. rhizogenes* plasmid vectors. However, each of these techniques requires tissue culture protocols to ensure regeneration of mature hybrids. Plant regeneration from *in vitro* cultures is the major limiting factor in production of transgenic plants, from either protoplast- or *Agrobacterium*-derived sources.

Tissue culture is an effective way to study many aspects of cell development and differentiation⁽¹²⁾, and relies on the ability of cells to undergo sustained division on a solid or liquid growth medium. The basal medium consists of macro- and micronutrients, trace elements and carbon substrates such as sucrose or glucose, with a pH of around 5.6–6.0⁽¹⁵⁾. Additives such as coconut water, a source of nutritious liquid endosperm^(16, 54) have been seen to stimulate cell growth⁽⁶³⁾. The first report of successful isolation and culture of protoplasts in *Lathyrus* spp. was from callus of *L. odoratus*⁽⁵¹⁾. There are only two reports of protoplast isolation in grasspea; an Australian group⁽⁴²⁾ were the first to isolate grasspea protoplasts, from cell suspension cultures, where in the following year a French group⁽¹⁷⁾ isolated grasspea protoplasts from embryonic axis shoots.

Molecular biology using grasspea

As genetic transformation of many pulse species has met with limited success in the last decade, the search continues for new or improved methods of transferring genes across the sexual barrier⁽⁹⁾. The techniques of *Agrobacterium*-mediated gene transfer, electroporation and microprojectile bombardment all depend on having a knowledge of specific genes to be transferred^(7, 8, 14, 25, 26, 27, 38), where protoplast electrofusion allows large segments of the genome to be transferred. Electrofusion is an experimental option for consideration in cases where the gene(s) of interest have not yet been characterised or isolated.

Molecular markers are used in plant genome studies to assess genetic diversity, identify plants at cultivar level, for construction of genetic maps, as specific probes for screening of traits in breeding programs and to tag genetic traits⁽³⁷⁾. For example, RAPD analysis has been used in cluster analysis studies for classification of two *Lathyrus* species, *L. odoratus* and *L. larifolium*⁽²³⁾.

One potential application of molecular markers in *Lathyrus* spp. is the development of SCAR primers tightly linked to the gene(s) for ascochyta blight resistance⁽¹³⁾. These markers could then be used to select somatic hybrids with the resistance marker gene incorporated into the genome. This would provide a rapid and non-destructive method for screening large numbers of putative hybrid plants, to identify those containing the introduced resistance gene.

Several studies have developed protoplast sources for grasspea, through shoot, callus and suspension cell cultures, to develop protoplast isolation and purification protocols^(17, 41, 42). Electrofusion between field pea and grasspea protoplasts has been carried out to introduce useful resistance genes from grasspea into field pea^(17, 41, 42). Analysis of markers linked to the genes for ascochyta blight resistance in grasspea has been performed⁽⁴¹⁾.

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