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Advances in pea breeding and genomics: From traditional techniques to modern approaches

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Abstract

Peas, a highly valued annual legume vegetable with a rich history of domestication, are grown globally as a valuable export-oriented cash crop. Despite an increase in cultivation area and production, there has been only a slight improvement in green pea productivity, from 7.7 to 7.8 t/ha, over the last two decades. The primary focus for genetic improvement in peas is developing resistance to various biotic stressors, including diseases such as powdery mildew, downy mildew, rust, wilt, viral infections, and bacterial blight, as well as pests like leaf miners, aphids, pod borers, and pea stem flies. Traditional breeding approaches have played a significant role in the genetic improvement of peas, resulting in the development of several cultivars in various segments; however, advanced breeding techniques such as marker-assisted selection, genomic selection, and genome editing hold great promise in enhancing genetic improvement by facilitating the identification and selection of desirable traits, such as resistance to biotic and abiotic stressors, improved yield, and increased nutrient content, through the introduction of precise genetic modifications. By targeting specific genomic regions associated with desired traits, these techniques can increase the efficiency and precision of breeding programs, ultimately leading to the development of more resilient and productive pea varieties.

Keywords: *Pisum*, breeding, genomics, marker-assisted selection, biotic, abiotic, stresses.

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Introduction

Peas (*Pisum sativum* L.) is a highly valued annual legume vegetable with a history of domestication that dates back nearly 10,000 years. It belongs to the family Leguminosae, predominately self-pollinated plant species that has played a crucial role in the establishment of modern genetics (Sharma *et al.* 2022a,b). The nuclear genome size of cultivated pea is predicted to be 1C = 4.4 to 4.8 pg DNA, corresponding to the haploid genome size (1C) of 4.45 Gb (Kreplak *et al.* 2019). They are grown over an area of 7.18 and 2.78 million hectares for dry and green seeds, respectively (FAOSTAT 2019). The nutrients present in green peas, such as starch, protein, fiber, minerals, vitamins, and other phytonutrients, make them a popular export-oriented cash crop worldwide, with an export value of more than 1000 million US dollars for green peas in 2021 (https://www.tridge.com). While traditionally a cool season crop, the development of cultivars resilient to certain abiotic stresses has led to an expansion of its cultivation into warmer regions of the world (Bueckert *et al.*, 2015; Dhall and Kaur, 2022; Singh *et al.*, 2023). However, as far as green-pea farming is concerned, it is predominantly carried out in Asian countries, where the

average productivity is high and accounts for more than 87% of the total, in comparison with European countries (Devi *et al.* 2019). In India, it is commercially grown as a winter vegetable in North Indian plains and foothills, besides as a summer crop in the hills. In the hills of North Western Himalayas, agroclimatic conditions favor the cultivation of garden pea as an important off-season vegetable crop (Rana *et al.* 2021). The green pods are available during the summer months, find a ready market in the plains and provide lucrative returns to the growers (Sharma *et al.* 2020). However, despite an increase in cultivation area and production, productivity has only slightly improved from 7.7 to 7.8 t/ha (green peas) over the last two decades (Devi *et al.*, 2023a, 2023b). Meeting the increasing demand for food due to a growing global population while combating various biotic and abiotic stresses has become a significant challenge for crop scientists and producers (Devi *et al.*, 2022).

Current Status of Genetic Resources

To breed the new cultivars, it is essential to focus on enhancing the genetic variability in the base population (Devi *et al.* 2018). In 2013, Smykal *et al.* conducted an inventory of gene banks and identified 98,947 accessions of peas comprising various categories, including landraces (38%), commercial cultivars (34%), mutant or genetic stocks (5%), and breeding lines (13%). Among these accessions, only 1,876 (2%) were wild pea relatives (Smýkal *et al.* 2013; Smýkal *et al.* 2015). Further, there were 706 accessions of *P. fulvum*, 624 accessions of *P. s.* subsp. *elatius*, 1562 accessions of *P. s*. subsp. *sativum* (syn. *P. humile/syriacum*), and 540 accessions of *P. abyssinicum*, although there are some levels of specimen duplication and misidentification that exist (Smýkal *et al.* 2013). The National Institute for Agricultural Research (INRA), France; Australian Grains Gene Bank (AGG); N.I. Vavilov Research Institute of Plant Industry, Russia; US Department of Agriculture (USDA), United States; Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; and International Center for Agricultural Research in the Dry Areas (ICARDA), Lebanon are the six leading active pea germplasm repositories in the world (Table 1). Pea accessions are also conserved in several National Germplasm Repositories across various countries. These repositories contain a substantial number of pea accessions, including 4,558 in Italy, 3,837 in China, 3,609 in India, 3006 in the United Kingdom, 2,896 in Poland, 2,849 in Sweden, 2,311 in Ukraine, and 2,110 in Aberystwyth University in the United Kingdom (Ambrose *et al.* 2023); Parihar *et al.* 2022). Additionally, seven other countries hold over 1,000 accessions of *Pisum* in their national germplasm repositories. These collections are essential in preserving the genetic diversity of pea and ensuring their availability for future research and crop improvement programs. Several web portals, including the European Cooperative Program on Plant Genetic Resources, the Cool Season Food Legume Database, the Genetic Resources Information Network and System-wide Information Network for Genetic Resources, and KnowPulse, have been created internationally to record and disseminate information about pea genetic resources.

Table 1: Leading institutes with peas germplasm collections worldwide [Ambrose *et al.* (2023) and Parihar *et al.* (2022)]

Institute	*Collections*
National Research Institute for Agriculture, Food and Environment (INRAE), France	8,839
Vavilov Institute, Russia	8,203
Australian Grains Genebank (AGG), Australia	7,432
US Department of Agriculture (USDA)	6,827
International Center for Agricultural Research in the Dry Areas (ICARDA)	6,105
Leibniz Institute of Plant Genetics and Crop Plant Research, Germany	5,343
Instituto di Genetica Vegetale, Italy	4,558
Institute of Crop Sciences, China	3,837
National Bureau of Plant Genetic Resources (NBPGR), India	3,609
John Innes Centre, UK	3006

Traditional Breeding Methods and Targeted Traits

In vegetable peas, traditional breeding methods have been used to develop varieties with a range of targeted traits. The most important task of pea breeding is to develop varieties with high and stable production, different maturity types and resistance against biotic and abiotic stresses (Sanwal *et al.* 2021). Besides high yield, specific pod characteristics (proper filing, long, dark green, sweet) and resistance to pests and diseases are the main criteria opted by the breeders for garden pea improvement (Rana *et al.* 2023). One of the most critical attributes in pea plants is earliness, which enables quick maturation and an early crop yield. Longer and visually appealing green pods with more seeds per pod are preferred, as they increase yield and aesthetic value. Sweetness is another important quality, making the peas more palatable to consumers. A high shelling percentage is also desirable, reducing waste during processing. Specific maturity, early or mid, allows for better planning and harvesting. Pea plants suitable for freezing and canning are highly valued for storage and distribution purposes. High protein content and processing quality are desired attributes, while high nutritive value is a must for health-conscious consumers. Resistance to different biotic stressors, such as diseases like powdery mildew (PM), downy mildew (DM), rust, wilt, viral infections, and bacterial blight, and pests like leaf minor, aphids, pod borer, and pea stem fly, are the key focus for genetic improvement. Given the pressing issue of climate change, it is imperative that pea plants possess resilience against abiotic stresses such as

heat, cold, drought, and frost, thereby enabling their survival amidst environmental adversities.

Over the past few decades, various improved varieties have been developed through a traditional breeding approach *viz*., introduction, pure line selection, pedigree method, bulk method, single seed descent, backcross method, and mutation breeding. For example, varieties like Kashi Ageti, Kashi Uday, Kashi Purvi, Kashi Nandini from ICAR-IIVR and at IIHR, Bangalore, several varieties, including Arka Priya, Arka Pramodh, and Arka Apoorva, have been created using pedigree selection. The same technique was used to create the Vivek Matar 3, 6, 11, Kashi Samridhi, Narendra Sabji Matar 6, Matar Ageta 6, and Jawahar Matar 1 and 2. Similarly, demand has also been raised for edible-podded peas (Eshanee *et al*., 2020).

Resistance Sources for Biotic and Abiotic Stresses

Germplasm for resistance to both biotic and abiotic stresses is an important area of research for peas improvement. From time to time, the crop breeder has devoted efforts to screen the available genetic resources for various biotic and abiotic stresses (Table 2). Although wild species of peas contribute only 2% of the worldwide collection (Smýkal *et al*., 2015), they had played a significant role as the donor for various economic traits (Table 3). The genetic diversity of wild peas can be valuable for breeding for resistance to pests and pathogens, abiotic stress such as extreme temperatures, improved nutritional and fodder value, agro technical advantages like branching and hibernation, and peculiarities of symbiotic nitrogen fixation (Kosterin 2016). Rana *et al*. (2023) identified 10 lines viz., SP7, SN-1, SN-6-1, SN-7-1, SN-2, SN-5-2, SN-6-2, SN-10, SN-21 and SP-28-1 along with Palam Sumool as resistant to powdery mildew disease. Sharma *et al*. (2013) revealed that the conventional breeding approaches of hybridization followed by selection involving commercial susceptible variety and resistant donor parent has resulted in the development of powdery mildew-resistant varieties with light, yellowish green and medium-sized pods and find hindrance in the replacement of existing susceptible variety(ies).

Revolutionizing Pea Crop: Modern Breeding Approaches for Improved Traits

The development of cultivars with improved resistance to biotic and abiotic stresses is a primary goal of crop breeding programs throughout the world. Because of their narrow variability and polygenic inheritance pattern, traditional gene mapping could not be widely employed to map the genes/quantitative trait loci (QTLs) regulating disease resistance (Parihar *et al*. 2022). Furthermore, because quantitatively inherited traits are substantially influenced by environmental factors, DNA-based markers are commonly used to map genes/QTLs regulating quantitatively inherited phenotypes in peas. Several gene/QTLS has now been discovered/mapped in peas of related to various economic traits *viz*., Fusarium root rot (Coyne *et al*. 2015; 2019), rust (Rai

Table 2: Resistant germplasm reported for biotic and abiotic stresses in peas

S. No.	*Trait*	*Source*
Biotic stresses		
1.	Powdery mildew	Jawahar Pea 83, JP4 (JM 6), PRS4, FC 1, EC 326, T 10, P 185, P 288, PC 6578, B 4048,P 6587, P 6588, BHU 159, IC 4604, JP 501, VP 7906, HFP4, EC598878, EC598538, EC598757, EC598704, EC598729, EC598535, EC598655, EC598816, EC381866, IC278261, IC267142, IC218988, IC208378, IC208366, LE 25, ATC 823, KPMR-10, T-10, P-185,6533, 6587,6588, JI 210, DMR 4, DMR 7, DMR 20, HFP 9907 B, Pant Pea -42, VL Matar 42, IPFD 99-13, IPFD 1-10, IPF 99-25, Pusa Prabhat, Ambika, Kashi Samridhi, Kashi Samrath, DPPMR09-1 (INGR21221)
2.	*Fusarium* Wilt	Kalanagini, JP 179, Pusa Vipasha, Early Perfection, Bonneviella, PL 43, Glacier, PI215766, PI244121
3.	Rust	PJ 222117, EC 109188, EC 42959, IC 4604, PJ 207508, JP Batri Brown 3, JP Batri Brown 4, IPF-2014-16, KPMR-936 and IPF-2014-13, PJ 207508, C 12, Wisconsin, DMR 3, Pant P 5, Pant P, 8, Pant 9, HFP 8711 and HUDP 15, IPFD 1-10 JP-4, FC-1, Pant P 11, HUDP 16, JPBB-3, HUP 14
4.	Pea mosaic	America Wonder, Perfection Canner's Gem, Dwarf White Sugar, Little Marvel
5.	Leaf miner	EC 16704, 21711, 25173, P-4107
6.	Pea stem fly	Dwarf Grey Sugar, T 10, T 163
7.	Ascochyta Blight	Kinnauri
Abiotic stresses		
1.	Salinity	New Line Perfection, Market Prize
2.	Cold	VL Mater-6
3.	Moisture	VL Mater-6
4.	High temperature	IIHR 544, Matter Ageta 6, Arka

Table 3: Wild genetic resources for genetic improvement of peas

Donor Species	*Pathogen/Insect*	*Reference*
P. fulvum	Bruchid resistance	Aznar-Fernandez and Rubiales (2019)
	Powdery mildew resistance	Fondevilla *et al.* (2010)
	Virus resistance	Konecná *et al.* (2014)
P. fulvum	Pea weevil, rust, powdery mildew and ascochyta blight	Kosterin (2016)
P. sativum subsp. *elatius*	Nematode	Vito and Perrino (1978)
	Broomrape	Valderrama *et al.* (2004)
	Fusarium wilt	McPhee *et al.* (1999)
	Ascochyta blight	Fondevilla *et al.* (2005); Carrillo *et al.* (2014)
	White mold	Porter *et al.* (2009)
P. sativum subsp. *elatius* (Accession JI2055)	low temperatures up to and -20°C	Ali *et al.* (1994)
P. sativum ssp. *syriacum* (P665)	Bruchid resistance	Aznar-Fernández and Rubiales (2019)

et al. 2016; Barilli *et al.* 2018); powdery mildew (Pavan *et al.* 2013; Ma *et al.* 2017; Sun *et al.* 2019); and Common root rot (Desgroux *et al.* 2016), etc.

Marker-Assisted Selection

Conventional breeding methods for disease resistance are based primarily on the principles of Mendelian genetics. Classical breeding is limited by the length of screening procedures and reliance on environmental factors. Hence, the deployment of molecular markers linked to resistance genes could be an alternative. This is the most reliable screening procedure to increase the efficiency of isolating disease resistance lines using marker-assisted selection (MAS). A close association of markers with a trait of interest is the prerequisite of MAS, which identifies the target traits without assessing their phenotype in the early generation (Tayeh *et al.* 2015a). Both bi-parental and association mapping approaches have been utilized in the identification of closely associated markers with genes controlling disease resistance in pea. Such gene-linked markers control resistance to Powdery Mildew (Devi *et al.* 2022; Table 4), *Fusarium* root rot resistance (Coyne *et al.* 2019; Table 5), pea enation or seed-borne mosaic virus (Swisher Grimm and Porter 2020), rust resistance (Singh *et al.* 2023; Table 6), ascochyta blight (Jha *et al.* 2017), and Aphanomyces root rot (Desgroux *et al.* 2016) are available for MAB. Accessibility of the reference genome will pave the way toward finding the genes of interest and understanding the genetic background of individuals at the genome level by deploying molecular markers responsive to high-throughput genotyping.

Marker-Assisted Backcrossing (MAB)

Backcrossing is the most commonly used method of incorporating one or more gene into elite varieties in breeding. In this method of breeding, the parent used to have large number of desirable attributes but is deficient in only a few characteristics (Allard 1999). The method was first described in 1922 and was widely used between the 1930s and 1960s. MAB procedure includes three steps of selection foreground, recombinant and background selection (Holland 2004). The first step (foreground selection) involves use of a molecular marker to identify/screen the desired gene or QTL (Hospital & Charcosset 1997). The second step (recombinant selection) involves the selection of back cross progeny with target gene and recombinants between the target locus and tightly linked flanking markers and the last and final step (background selection) involves the selection of recurrent parent (RP) by utilizing unlinked markers to the target locus. The marker-assisted backcrossing (MABC) has been successfully used for the introgression of QTLs for *Aphanomyces* root rot (ARR) resistance into several recipient genotypes (Lavaud *et al.* 2015).

Marker-Assisted Gene Pyramiding (MAGP)

Combining several genes into a single genotype is called pyramiding. Pyramiding of genes is one of the key techniques used in conventional breeding. The deployment of DNA markers to plant breeding for gene pyramiding is termed as marker-assisted gene pyramiding (MAGP). Due to the limitations of gene pyramiding, such as the difficulty in identifying plants with many genes, it is difficult to assess plants from F_2 populations with traits that have destructive bioassays since one has to evaluate all traits tested. These limitations are overcome by the use of DNA markers, which are generally non-destructive. MAGP is most frequently employed to combine multiple disease-resistance genes to generate stable disease or insect resistance at the same time, as pathogens often overcome single-gene host resistance over time due to the establishment of new plant pathogen races (Kloppers & Pretorius 1997; Shanti *et al.* 2001; Singh *et al.* 2001). In order to achieve broad and persistent resistance

Table 4: The DNA markers linked to powdery mildew resistant genes

Primer/Locus	*Distance (cM)*	*Marker*	*Gene*	*MP*	*Approach*	*References*
p236	9.8	RFLP	*Er*	F_2	-	Dirlewanger *et al.* (1994)
pI49	18.0	RFLP	*er1*	RIL_S	BSA	Timmerman *et al.* (1994)
pID18	8.7	RFLP	*er1*	RIL_S	BSA	
PD 10	2.1	RAPD	*er1*	RIL_S	BSA	
$ScOPD10_{650}$[a]	2.1	SCAR	*er1*	RIL_S	BSA	
OPL-6	2.0	RAPD	*er1*	F_3	BSA	Tiwari *et al.* (1998)
OPE-16	4.0	RAPD	*er1*	F_3	BSA	
Sc-OPE-16$_{1600}$[b]	4.0	SCAR	*er1*	F_3	BSA	
[@]*Sc-OPO-18*$_{1200}$	-	SCAR	*er1*	F_3	BSA	
OPO-02	4.5	RAPD	*er1*	NILs	-	Janila and Sharma (2004)
OPU-17	10.3	RAPD	*er1*	NILs	-	
ScOPD 10_{650}[a]	3.4	SCAR	*er1*	NILs	-	
A5[c]	20.9	SSR	*er1*	F_2	NA	Loridon *et al.* (2005)
PSMPSAD60[d]	10.4	SSR	*er1*	F_2	BSA	Ek *et al.* (2005)
PSMPSAA374e	11.6	SSR	*er1*	F_2	BSA	
PSMPA5[c]	14.9	SSR	*er1*	F_2	BSA	
PSMPSAA369	24.1	SSR	*er1*	F_2	BSA	
PSMPSAD51	25.8	SSR	*er1*	F_2	BSA	
OPWO4_637	-	RAPD	*Er3*	F_2	BSA	Fondevilla *et al.* (2004)
OPAB01_874	2.8	RAPD	*Er3*	F_2	BSA	
SCAB1 $_{874}$	2.8	SCAR	*Er3*	F_2	BSA	
$ScW4_{637}$	-	SCAR	*Er3*	F_2	BSA	
ScX17$_{1400}$	2.6	SCAR	*er2*	F_2	BSA	Katoch *et al.* (2010)
$ScOPO06_{1100}$y	0.5	SCAR	*er1*	NILs	BSA	Pereira *et al.* (2010)
$ScOPT16_{480}$	3.3	SCAR	*er1*	NILs	BSA	
$ScAGG/CAA_{125}$	5.5	SCAR	*er1*	NILs	BSA	
ScOPE16[b]	9.2	SCAR	*er1*	NILs	BSA	
A5[c]	23.0	SSR	*er1*	NILs	BSA	
BC210	8.2	RAPD/SCAR	*er1*	-	-	Tonguc and Weeden (2010)
$ScOPX04_{880}$	0.6	SCAR	*er1*	NILs	BSA	Srivastava *et al.* (2012)
ScOPD 10_{650}[a]	2.2	SCAR	*er1*	NILs	BSA	
AD60 [d]	9.9[*], 8.7[**]	SSR	*er1*	F_2	BSA	Sun *et al.* (2015)
c5DNAmet	15.4*, 8.1[**]	SSR	*er1*	F_2	BSA	
AD61	0.39	SSR	*Er3*	F_2	BSA	Cobos *et al.* (2018)

Where a, b, c, d denotes the same primer used by different researchers; @ This fragment was only present in susceptible progenies; [*]- *in mapping population*' Xucai 1 × Bawan 6'; [**]- in mapping population' Qizhen 76 × Xucai 1'; Information for the marker BC210 is not available.

against the powdery mildew, it is possible to pyramid the *er1, er2* and *er3* genes into an elite background in the pea (Devi *et al.* 2022).

Marker-Assisted Recurrent Selection (MARS)

MARS is one of the MAS techniques that relies on recurrent selection and is mostly used for discovering and identifying

Table 5: The DNA markers linked to Fusarium wilt resistant genes (Race 1)

Marker	*Distance (cM)*	*Market type*	*References*
ACG:CAT_222	1.4	AFLP	McClendon *et al*., (2002)
Y15_1050	4.6	RAPD	McClendon *et al*., (2002)
Y15_999*Fw*	-	SCAR	Okubara *et al*., (2005)
AA5-225	2.7	SSR	Loridon *et al*., (2005)
AD134-213	2.5	SSR	Loridon *et al*., (2005)
Fw_Trap_480,	1.2	TRAP	Kwon *et al*., (2013)
Fw_Trap_340	1.2	TRAP	Kwon *et al*., (2013)
Fw_Trap_220	1.2	TRAP	Kwon *et al*., (2013)
THO	1.9[a] , 0.9[b]	CAPS	Jain *et al*., (2015)
Mt5_56	3.9[b]	CAPS	Jain *et al*., (2015)
AnMtL6	3.5[a]	CAPS	Jain *et al*., (2015)
PRX1TRAP13	2.6[a]	TRAP	Jain *et al*., (2015)

[a]- in mapping population 'Lifter'/'Radley'; [b]- in mapping population 'Shawnee'/'Bohatyr'

numerous genomic regions that express complex traits as well as assembling best genotypes within a single population or among related populations (Eathington *et al*. 2007; Ribaut *et al*. 2010). MARS approach allows for genotypic selection and intercrossing among the selected individuals could be done in the same crop season for one cycle of selection, which improves the effectiveness of recurrent selection and progress of the procedure, particularly by integrating multiple beneficial genes or QTLs (Gazal *et al*. 2015). MARS has been suggested for the "forward breeding" of native genes and pyramiding several QTLs for complex traits like grain yield and biotic and abiotic resistance (Ribaut *et al*. 2010).

Transcriptomics

Transcriptomics has become one of the most developed fields in the post-genomic era. The transcriptome is the complete set of RNA transcripts in a specific cell type or tissue at a specific developmental stage and/or physiological condition, which includes messenger RNA, transfer RNA, ribosomal RNA, and other non-coding RNAs. Transcriptomics focuses on gene expression at the RNA level and provides genome-wide information on gene structure and function in order to reveal the molecular mechanisms involved in specific biological processes (ZhiCheng and Chen 2013). Ramachandran *et al.* 2011 used comparative transcriptomics to investigate general and plant-specific adaptations during rhizosphere colonization. *Rhizobium leguminosarum* biovar *viciae* was grown in the rhizospheres of pea, *alfalfa* (a non-host legume), and sugar beet (non-legume). Gene expression data were compared to metabolic and transportome maps to better understand the rhizosphere adaptation. Iveta *et al.* (2017) analyzed seed coat and pod anatomical structure, identified metabolic compounds associated with water-impermeable seed coat, and identified differentially expressed genes involved in pea seed dormancy and pod dehiscence. Anatomical, metabolomic, and transcriptomic analyses were performed on wild dormant, dehiscent *P. elatius* (JI64, VIR320) and cultivated, non-dormant *P. sativum* (JI92, Cameor) and recombinant inbred lines (RILs). There were significant differences in the texture of the testa surface, the macrosclereids' length, and the seed coat's thickness.

Transgenic Technology

Shade *et al.* (1994) tested the possibility of transgenic pea seeds expressing the α-amylase inhibitor of the common bean are resistant to bruchid beetles by introducing the AI-Pv gene into peas (*Pisum sativum*) via a strong seed-specific promoter. AI protein levels in pea seeds were comparable to bean seeds, and peas were resistant to cowpea and Azuki bean weevils. (Perrin *et al*. 2000) demonstrate that grain legume seeds can produce biologically active recombinant antibodies and that field pea seeds can produce recombinant pharmaceutical macromolecules. With well-established agricultural practices worldwide and seeds that are easily stored and distributed, the field pea (*Pisum sativum L*.) appears well-suited for the production of high-value molecules such as recombinant antibodies.

Table 6: The DNA markers linked to rust resistant genes

Marker	*Distance (cM)*	*Market type*	*Gene/QTL type*	*Resistance against*	*References*
SC10-82$_{360}$	10.8	RAPD	*Ruf*	*Uromyces fabae* (Pers.) de-Bary	Vijayalakshmi *et al.* (2005)
SCRI-71$_{1000}$	24.5	RAPD	*Ruf*	*Uromyces fabae* (Pers.) de-Bary	Vijayalakshmi *et al.* (2005)
OPY111316	6.0	RAPD	*Up1*	*Uromyces pisi* (Pers.) Wint.	Barilli *et al.* (2010)
OPV171078	13.4	RAPD	*Up1*	*Uromyces pisi* (Pers.) Wint.	Barilli *et al.* (2010)
AA505- AA446	10.8	SSR	*Qruf* (Major QTL)	*Uromyces fabae* (Pers.) de-Bary	Rai *et al.* (2011)
AD146-AA416	7.3	SSR	*Qruf 1* (Minor QTL)	*Uromyces fabae* (Pers.) de-Bary	Rai *et al.* (2011)

They transformed peas with a cDNA encoding the single-chain Fv fragment scFvT84.66 to test their suitability for the production of biologically active antibodies. This scFv is derived from the monoclonal antibody T84.66, which recognizes the well-studied carcinoembryonic antigen. The antibody can be used to diagnose human cancers *in-vitro* and *in-vivo*. The seed-specific legumin. A promoter was used to control the expression of scFvT84.66 cDNA. They directed the antibody to the endoplasmic reticulum for improved stability and high accumulation. Transgenic plants produced up to 9 g of functional scFvT84.66 per gram fresh weight in their seeds. The transgene was stably inherited and represented in the progeny, and the antibody continued to exist after two months of storage at room temperature in dried transgenic seeds. Schroeder *et al*. (1993) raised transgenic peas in a glasshouse to produce flowers and viable seeds. The bar and nptII genes were expressed in both the primary transgenic pea plants and their progeny, with a typical 3:1 Mendelian inheritance pattern. Northern blot analyses and assays for neomycin phosphotransferase and phosphinothricin acetyl transferase activity were used to confirm the transformation of regenerated plants. When sprayed at field practice rates, morphed plants were resistant to the herbicide Basta. (Puonti-Kaerlas *et al.* 1990) assessed the production of transgenic pea (*Pisum sativum* L.) plants by *Agrobacterium tumefaciens*-mediated gene transfer. A transformation system was developed that allows the regeneration of transgenic pea plants from calli selected for antibiotic resistance. After several passages on the regeneration medium, shoot organogenesis was reproducibly induced on hygromycin-resistant calli but not on kanamycin-resistant calli. Regenerated shoots could then be rooted and moved into the greenhouse. Furthermore, the effects of various callus-inducing and growth media on organogenesis were studied. DNA analysis confirmed the transformation of the calli and regenerated plants.

Speed Breeding

The expanding human population and dynamic environment have raised serious concerns about global food security, with the current rate of improvement of several important crops insufficient to meet future demand. This slow rate of improvement can be attributed in part to crop plant generation times. We present a method known as speed breeding,' which significantly reduces generation time and accelerates breeding and research programs. Time is an important factor in breeding programs. Shortening plant cycles allows programs to be more efficient (Watson *et al.* 2018). (Federico *et al.* 2021) presented various pulse genetic improvement advances and introduced a speed breeding framework for pea (*Pisum sativum* L.) that includes hybridizations and generation advancement in a growth chamber. To accelerate photosynthesis, flowering, and early seed harvest, the technique employs optimal light quality, light intensity, day length, and temperature control. It is compatible with other breeding technologies, does not involve transgenesis or gene editing, and is marketed as a game changer for increasing program efficiency.

Genome Editing

Gene editing is a novel genetic engineering technology that uses engineered nucleases, also known as "molecular scissors," to insert or delete specific genes. Precision genome editing is appealing compared to other breeding approaches because of its speed, flexibility, and lack of transgenes (Gaj *et al*. 2013). For technical and regulatory reasons, neither conventional nor transgenic breeding techniques can meet the increased production demand. CRISPR/Cas9 genome editing technology has recently gained traction in plant biology and crop breeding in response to this challenge. To validate the efficiency of a CRISPR/Cas9 system, (Gaun *et al.* 2023) created a transient transformation system of hairy roots mediated by *Agrobacterium rhizogenes* strain K599. PsU6.3-tRNA-PsPDS3-en35S-PsCas9 was developed as an efficient vector through further optimization. By Agrobacterium-mediated genetic transformation, we used this optimized CRISPR/Cas9 system to edit the pea phytoene desaturase (PsPDS) gene, which causes albinism. This is the first report of successful gene-edited pea plant generation via this method.

CRISPR-Cas9 gene editing techniques have been used in pea to precisely edit genes important for developing resistant lines to various biotic stresses. The development of bioinformatics tools and databases has increased knowledge of genomics, proteomics, and metabolomics in pea for biotic stresses. With the advent of modern tools such as gene editing, conservation of wild type and landraces has raised concerns about regulatory frameworks drafted in various countries. A collaborative effort combining traditional breeding with modern biotech tools, nanotechnology, and speed breeding will assist molecular breeders in designing climate-resilient pea varieties with resistance to biotic stress (Kumar *et al*. 2022). Emerging new breeding approaches such as CRISPR, speed breeding, and genomic selection are beginning to shift the pea breeding paradigm. The rich omics resources and omics-enable breeding approaches will improve genetic gain in pea breeding and accelerate the release of novel pea varieties to meet rising productivity and quality demands (Pandey *et al.* 2021).

Conclusively, the pea researcher's community has made significant progress in enhancing the genetic gain of peas through conventional and molecular breeding techniques. With the help of advanced genomic tools such as comprehensive genetic maps and reliable DNA markers, the introgression of resistance genes from various sources can be accelerated. These achievements pave the way for the development of improved pea varieties with enhanced resistance to pests and diseases, improved yield, and better

nutritional qualities. Researchers are exploring new avenues, such as using machine learning algorithms to predict traits and developing new gene editing tools that are more precise and efficient. These innovations could help us to create even more resilient and productive pea varieties and to unlock new possibilities for their use in food production and other industries.

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सारांश

यह वार्षिक फलीदार एवं विश्व स्तर पर नगदी फसल के रूप में एक मूल्यवान निर्यातोन्मुखी सब्जी फसल है जिसके खेती में सम्मिलित होने का समृद्ध इतिहास है। पिछले दो दशकों में मटर की खेती के क्षेत्रफल एवं उत्पादन में वृद्धि होने के बावजूद हरी मटर की उत्पादकता में बहुत कम वृद्धि (7.7-7.8 टन/हेक्टेयर) हुई है। मटर में आनुवांशिक सुधार के लिए प्राथमिक दृष्टि से चूर्णिल आसिता, मृदुरोमिल आसिता, रस्ट, उकठा, विषाणु संक्रमण, जीवाणु झुलसा एवं जैविक तनावों और साथ ही साथ कीटों जैसे-लीफ माइनर, एफिड्स, फली छेदक और मटर के तने की मक्खियों के प्रति प्रतिरोध विकसित करना है। मटर के आनुवांशिक सुधार में पारम्परिक प्रजनन पद्धतियों ने महत्वपूर्ण भूमिका निभाई है जिसके परिणामस्वरूप विभिन्न पकाव समूहों में किस्मों का विकास हुआ। हालांकि, उन्नत प्रजनन तकनीकों जैसे-मार्कर सहायता प्राप्त चयन, जीनोमिक चयन और जीनोम संपादन प्रमुख है। अनुवांशिक सुधार को बढ़ाने में वांछित गुणों की पहचान और चयन की सुविधा प्रदान करना भी आवश्यक है जैसे- जैविक प्रतिरोध, अजैविक तनाव, बेहतर उपज और पोषक तत्वों की मात्रा में वृद्धि आदि है। मटर की उच्च गुणवत्ता सहित अधिक उत्पादन वाली किस्मों के विकास में आधुनिक प्रजनन तकनीकें कार्यों की दक्षता और निपुणता बढ़ा सकती है।