

Objectives and modern techniques in pea (*Pisum sativum* L.) breeding in Argentina: A review

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Espósito, M.A.^{1,2,3}; García, A.N.³; Guindón, F.²; Cazzola, F.¹; Bermejo, C.^{1,2}; Gatti, I.^{1,4}

ABSTRACT

Pea is considered an essential component of sustainable farming systems. The main objective of pea researchers in Argentina and worldwide is to improve yield components and composition of the seed to enhance nutritional characteristics. Breeding programs that use conventional and nonconventional techniques are a very important tool to develop new varieties that can be used more efficiently in production systems but also adapt to changing market needs.

Keywords: pea breeding, molecular markers, induced mutations, genetic engineering.

RESUMEN

El cultivo de arveja o guisante se considera un componente esencial de los sistemas agrícolas sostenibles. El principal objetivo de los investigadores de arveja en Argentina y en todo el mundo es mejorar los componentes del rendimiento y la composición de la semilla para mejorar las características nutricionales. Los programas de mejoramiento genético que utilizan técnicas convencionales y no convencionales son una herramienta muy importante para desarrollar nuevas variedades que puedan ser utilizadas más eficientemente en los sistemas de producción pero que también se adapten a las necesidades cambiantes del mercado.

Palabras clave: mejora genética de arveja, marcadores moleculares, mutaciones inducidas, ingeniería genética.

¹Universidad Nacional de Rosario (UNR), Facultad de Ciencias Agrarias, Cátedra de Mejoramiento Vegetal y Producción de Semillas, Campo Experimental J.F. Villarino. CC 14 (S2125ZAA) Zavalla, Santa Fe.

²Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR)-CONICET. Campo Experimental J.F. Villarino. CC 14 (S2125ZAA) Zavalla, Santa Fe.

³Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Oliveros, Ruta Nacional 11, 353 km (2206) Oliveros, Santa Fe.

⁴Consejo de Investigaciones Universidad Nacional de Rosario (CIUNR), Maipú 1065, 2.º piso, oficina 209, (2000), Rosario, Santa Fe.

⁵Instituto Nacional de Tecnología Agropecuaria (INTA), Centro de Investigaciones Agrarias (CNIA), Centro de Investigación en Ciencias Veterinarias y Agronómicas (CICVyA), Instituto de Genética "Ing. Agr. Ewald A. Favret" (IGEAF), Dr. Nicolás Repetto y De los Reseros s/n (1686), Hurlingham, Buenos Aires, Argentina.

Correo electrónico: esposito.maria@inta.gob.ar

INTRODUCTION

The increase in world population will lead to a food shortage, which raises the question whether current agriculture will be able to satisfy the food demand of the more than nine billion people that will inhabit the planet in 2050 (Gerland *et al.*, 2014). Food security is the main challenge for scientists and the farming community. A partial substitution of animal proteins for plant proteins could meet this growing demand within the framework of the sustainable development of agricultural and food systems (Dorin *et al.*, 2011).

Pea is an annual grain legume originated in Southwest Asia. It is adapted to temperate climates and reduces the negative impact of wheat monoculture. (Nemecek *et al.*, 2008; Duc *et al.*, 2010; Jensen *et al.*, 2012). Peas are a readily available source of protein, complex carbohydrates, vitamin C and minerals (Dahl *et al.*, 2012). The protein content in legume seeds is up to 30%, unlike that of cereals, which is up to 13% and equal to the protein content found in meat (18-25%) (Pandey *et al.*, 2016). Compared to cereals, legumes are rich in lysine and poor in methionine and cysteine (Kotlarz *et al.* 2011). There is an increase in riboflavin and niacin content after germination (Swaminathan, 1988). They fix atmospheric nitrogen in symbiosis with soil bacteria (Pandey *et al.*, 2016; Blackshaw *et al.*, 2005), so nitrous oxide is reduced. (Jeuffroy *et al.*, 2013). They also reduce wind and water erosion (Battany and Grismer, 2000), reducing soil impedances (Villamil *et al.*, 2006; Alvarez *et al.*, 2017), soil runoff and nitrate leaching (Omae and Nagumo, 2016). They increase carbon capture (Blanco-Canqui, 2012) and nutrient absorption (Möller *et al.*, 2008) Legumes have a fundamental role as diversification crops, breaking the cycles of pests and diseases (Nemecek and Kägi, 2007; Hayer *et al.*, 2010; Macwilliam *et al.*, 2014), developing populations of beneficial insects for crop defense (Pullaro *et al.*, 2006).

Pea is the third most widely grown pulse in the world reaching around 12Mt in 2021 (FAOSTAT 2023) for field pea. Traditionally, the Argentinian core production area is in Buenos Aires and southeastern Santa Fe but it has been extended recently. In the 2021/22 season, the national production reached 225,000 tons, exporting about 136,800 tons in 2022 (SENASA). The main destination is Brazil, followed by India, Senegal, Myanmar, Russia and Italy. The main producers in the world are Canada, followed by Russia, China and India. (FAOSTAT, 2023). Peas are consumed either as human food or animal feed.

Objectives in pea breeding programs

The objective of this work is to review the most relevant aspects of conventional and non-conventional pea breeding worldwide in Argentina. The main traits used as criteria to develop a new variety are yield, tolerance to biotic and abiotic stress, cycle, quality, organoleptic quality parameters, chemical parameters, and nitrogen fixation.

1-Yield

Increasing yield involves maximizing the number of developing seeds per plant. Attempts have been made to increase the number of flowers per node (Gritton, 1986; Devi *et al.*, 2018) instead of increasing the number of grains per pod (Calisaya, 2006). In addition, a higher uniformity in the stage of development of seed or pods has been considered a major objective in breeding programs.

Another important aspect is to improve the efficiency in the harvest of the product, reducing plant height for green and field peas (Singh *et al.*, 2003), increasing the strength of the stem (Banniza *et al.*, 2005) which prevents the lodging of the crop, and using genotypes with the *afila* (*af*) gene (Goldenberg, 1965) that contributes to a significant reduction in harvest losses.

2-Tolerance to biotic and abiotic stresses

Pea cannot tolerate frost at the reproductive stage, so, winter-hardy materials and locating winter-hardy genes for winter-hardy breeding of pea, is an interesting objective too. (Zong *et al.*, 2019). Drought and heat stress during germination, early vegetative stage (Devi *et al.*, 2023) and flowering also affect yield (Yang *et al.*, 2022.) *Ascochyta* blight complex (Kraft *et al.*, 1998) and powdery mildew are very important fungal diseases. Many achievements have been made in terms of resistance to powdery mildew (Pavan *et al.*, 2011), which is based on a single recessive *er-1* gene (Harland, 1948).

3-Cycle

According to Ravasi *et al.* (2020) crop earliness is the most important trait for increasing yield because it allows it to partly escape the unfavorable conditions in the final cycle. During the evolution of legume crops, a reduction in photoperiod sensitivity conferred by mutations in the *ELF3* gene in pea, lentil and chickpea (Ridge *et al.*, 2017), have in each case contributed to the expansion of their ecogeographical range (Williams *et al.*, 2022). In Argentina, having a shorter cycle than winter cereals, is the ideal predecessor of second or late crops of corn, soybeans or sunflower.

4-Quality

Quality traits and post-harvest changes represent an important challenge for breeders (Ambrose, 2008). Good quality peas should be uniformly bright green, of intermediate caliber, fully turgid, sweet taste (Dürschmid *et al.*, 2010) and free from deterioration and damage caused by insects, freezing process (Haiying *et al.*, 2007), mechanical damage, mildew or other diseases for better market price (Anurag *et al.*, 2016). Biofortification is the improvement of the nutritional quality of the edible part of the plant, but unfortunately, there is a limited number of studies related to genetic variation for quality traits in pea, which prevents the full micronutrient enrichment potential of this pulse crop to be reached (Guindon, 2021).

Protein content

The total protein content in the pea seed is lower than that in soybean meal and higher than that in cereals (Ludvíková and Griga, 2022). Pea cultivars have shown protein content between 11.38 and 32.60% (Nikolopoulou *et al.*, 2007; Jha *et al.*, 2013, 2015; Guindon *et al.*, 2019; Accoroni *et al.*, 2022). Besides the cultivar effect, the soil and climatic conditions, as well as agrotechnological management, may substantially influence the seed protein content (Ludvíková and Griga, 2022; Coyne *et al.*, 2005 and Guindon *et al.*, 2019) reported that seed type, wrinkled or smooth, affected the composition of pea seeds, with higher protein content in wrinkled seeds, but Jha *et al.*, (2013) didn't observed significant differences between accessions with different seed surface or cotyledon color.

Starch content

The values of starch ranged between 27.6 and 56.3% in different pea accessions (Jha *et al.*, 2013 and 2015). Wrinkled seeds had shown lower starch concentrations than smooth seeds at maturity and field peas with green cotyledons had shown less starch than accessions with yellow ones (Jha *et al.*, 2013).

Micronutrients

The content of micronutrients have been less studied. Cheng *et al.* (2015) evaluated metal concentration in 330 accessions from a core collection of the USDA pea collection, obtaining the following means expressed in $\mu\text{g g}^{-1}$: boron (B): 7.8; Ca: 802.1; copper (Cu): 4.4; Fe: 50.4; magnesium (Mg): 1,685.8; manganese (Mn): 16.0; molybdenum (Mo): 23.2; nickel (Ni): 2.5; phosphorus (P): 5047.5; potassium (K): 12474.3, and Zn: 41.8

Understanding genetic variability among genotypes is important for the selection of lines with a good nutritional profile. Guindon *et al.* (2019) calculated broad sense heritability for quality traits obtaining high values (between 40.43 and 98.29), while (Nemeskéri, 2006) established values of broad sense heritability of 0.78 for xanthophyll content and 0.32 for carotene, and (Ma *et al.*, 2017) found values of broad sense heritability from 83.9 to 98.3 for different minerals (B, Ca, Fe, K, Mg, Mn, Mo, P and Zn).

Total phenolics, tanins, carotens and chlorophyll contents

The quality performance of a genotype is influenced by the cultivation (starch exception) the year and location (Nikolopoulou *et al.*, 2007).

Environmental conditions exhibited a significant effect on starch, fat content, Fe, K, Mg, Mn, P and Zn, alanine, glycine, isoleucine, lysine, threonine content, and trypsin inhibitor activity, Wang and Daun (2004). Amarakoon *et al.* (2012) informed that Zn, Ca, P concentrations were influenced by location, genotype and genotype by environment interaction (G x E). G x E was not significant for phytic acid. Liu *et al.* (2015) showed that variety, environment and their interaction affected P and phytic acid concentration, but for Fe concentration G x E was not significant. For carotenoids and polyphenolics (Marles *et al.*, 2013) demonstrated that only chlorophyll a showed G x E interaction. All other compounds assayed, were reasonably stable across environments, suggesting that breeding for increased levels should be possible.

5-Nitrogen fixation

Pea's ability to fix atmospheric nitrogen in symbiosis with rhizobia (BNF), and its participation in crop rotation is essential for sustainable agricultural systems. (Wysocki and Lozak, 2021; Dowling *et al.*, 2021). Their inclusion also helps to break cycles of pests and pathogens (Macwilliam *et al.*, 2014).

BNF can be increased by improving efficacy and rhizobia infection by repeating inoculation of rhizobia (Vessey, 2004), using more efficient strains (Hynes *et al.*, 1995), or by co-inoculation with "helper organisms" (Mishra *et al.*, 2009; Dileep Kumar *et al.*, 2001). Crop varieties and strain-by-variety specific interactions (Andrews and Andrews, 2017) are also important factors. (Abi-Ghanem *et al.*, 2011).

Several studies have found a strong influence of the variety in FBN. Abi-Ghanem *et al.*, (2011). Mutations in pea associated with root nodulation and N fixation offer an opportunity to develop pea cultivars with greater N fixation potential (Dhillon *et al.*, 2022). Supernodulating and hypernodulating mutants developed in pea are important genetic sources for breeding high BNF varieties.

Further improvement of rhizobia inoculants is necessary (Brockwell and Bottomley, 1995). Strain selection may be possible to improve the ability to infect a wide range of varieties, stimulate the production of large numbers of nodules, and fix N efficiently with minimal consumption of host plant resources (Chalk *et al.*, 2010). Pea has great nutritional and export value (Dhillon *et al.*, 2022). Improving N fixation in pea will also benefit the rapidly growing plant-based protein market for which pea is a preferred ingredient (Boukid *et al.*, 2021).

Pea breeding in the world

Significant achievements have been made through conventional breeding. Cultivars adapted for winter sowing have been developed in Europe and the northwestern US (Hanocq *et al.*, 2009). The introgression of the Hr allele that delays the onset of flowering by avoiding winter frosts (Lejeune-Hénaut *et al.*, 2008) allowed increasing winter resistance (Tayeh *et al.*, 2015).

Breeding for disease and pest resistance in garden pea started two decades ago in Jabalpur, India (Asima *et al.*, 2010). Wild pea species/forms represent valuable donors of resistances to biotic and abiotic factors (Coyne *et al.*, 2020). Since then, cultivars with resistance to some important diseases such as downy mildew, *Fusarium*, and rust were developed, along with desirable characteristics, such as maturity, green pod yield, pod length, and seed quality (Asima *et al.*, 2010). In pea, where variability in germplasm is low due to its autogamous nature, the use of induced mutagenesis for plant breeding, is highly recommended (Burstin *et al.*, 2018; Masry *et al.*, 2019; Pandey *et al.*, 2022). Thirty *Pisum sativum* L. mutant varieties officially were released and recorded in (FAO/IAEA) Mutant Varieties Database. Some of them were developed by hybridization with a mutant variety, others by direct induced mutations techniques. Among the improved traits, lodging resistance, plant structure, early maturity and high seed yield have been reported.

Pea breeding in Argentina

In 2005, a pea-breeding program was initiated at the Faculty of Agrarian Sciences of the Rosario's National University (FCA-UNR) to increase production in quantity and quality. There is an active collection of germplasm from around the world and genetic variability is analyzed through morpho-agronomic and molecular traits (Espósito *et al.*, 2007; Gatti *et al.*, 2011). In 2014, the National Institute of Agricultural Technology (INTA) and the FCA-UNR, joined inter-institutional efforts to promote the local development of pea genotypes adapted to the region. This program, which uses conventional methodologies, has so far obtained two new commercial lines of green and yellow field peas, leafletless and with high adaptation to local agroecological conditions and high yield potential. In addition, it shows good behavior at harvest and partial resistance to powdery mildew disease, *Erisiphe pisi* DC (Syn. E. polygona DC). This disease can reduce the yield

up to 59% and affect the industrial quality of the grain (Warkentin *et al.*, 1996; Carrillo *et al.*, 2012).

The development of new pea varieties needs a decade or more of the use of traditional methodologies. The process time is a limiting factor to obtain improved varieties and recombinant inbred lines (Ril's). Pea breeders tried to formulate and realize a pea ideotype, which would join the maximum desired characteristics both from an agronomic and markets point of view. Another widely used method to improve a pea variety is backcrossing to introduce a single desired trait, such as disease resistance or quality variables, from less adapted materials to elite ones.

Conventional improvement techniques in the world and in Argentina

The use of DNA markers, along with conventional breeding, can accelerate the selection of desirable traits in cultivar development (Collard and Mackill, 2008) and increase efficacy (Ragot *et al.*, 2007; Collard and Mackill, 2008).

Since the first genetic map, already published in 1948 (Lamprecht 1948), knowledge of the pea genome has expanded consistently along with the development of molecular markers including RAPDs, SSRs, SRAPs and SNPs (Pandey *et al.*, 2021). SNP markers are abundant and amenable to high-throughput genotyping (Tayeh *et al.*, 2015; Jha *et al.*, 2017; Gali *et al.*, 2018; Aznar-Fernández *et al.*, 2020, Tran *et al.*, 2023).

The use of new technologies is necessary to accelerate this process, improving phenotyping and genotyping methods and increasing available genetic diversity. The selection of superior genotypes has some limitations, especially with quantitative traits, with continuous variation and high influence from the environment. The genetic complexity of these traits arises from segregation and interaction of different alleles at multiple loci. The regions within genomes that contain genes associated with them are known as quantitative trait loci (QTLs) and can be efficiently analyzed using molecular markers (Atienza *et al.*, 2016).

Molecular markers that reveal polymorphism at the DNA level have proven to be a very powerful tool for genotype characterization and estimation of genetic diversity (Wang *et al.*, 2015; Teshome *et al.*, 2015; Singh *et al.*, 2021; Sharma *et al.*, 2022), gene mapping and the classification of genetic resources forming groups with similar characteristics (Espósito *et al.*, 2007; Bermejo *et al.*, 2010); providing essential information for the design of strategies for use in breeding programs.

Several reviews that dealt with available genomic resources in pea have been published (Vignesh *et al.*, 2011; Tayeh *et al.*, 2015). Some gel-based markers works have been developed (Wu *et al.*, 2017, Sharma *et al.*, 2020), but advances in next-generation technologies have reduced the costs of DNA sequencing so several works have used high throughput marker genotyping platforms that can develop a relatively large number of polymorphic markers.

Genotyping by sequencing (GBS) is now feasible for high diversity, large genome species. It has been used in different pea studies, like germplasm characterization, genomic selection (Gali *et al.*, 2019) days to maturity, plant height, seed yield and seed weight, linkage map construction and QTL analyses (Ma *et al.*, 2017, Huang *et al.*, 2017; Gali *et al.*, 2018, Guindon *et al.*, 2019; Gawłowska *et al.*, 2021; Sari *et al.*, 2023) such as

protein, mineral nutrients, carbohydrates and several vitamins, pea (*Pisum sativum* L.).

There are also important QTL studies on pea seed quality. QTLs were determined in Va and Vb of the linkage map for seed protein content (Krajewski *et al.*, 2012). In addition to important minerals such as Ca, Fe, K, Mg, Mn, Mo, and P (Ma *et al.*, 2017), QTLs of starch, fiber, and phytate contents (Gali *et al.*, 2019) in pea were also defined. The QTL studies using plant populations derived from interspecific crosses in pea are very limited (Jha *et al.*, 2016).

Kulaeva *et al.* (2017) integrate information about pea markers and provide an easy-to-use online tool, the Pea Marker Database, combining information about known pea gene-based markers.

An international consortium has recently been formed to produce a reference genome sequence for pea. It provides insights into legume genome evolution, with resequencing data for 42 wild, landrace and cultivar *Pisum* genotypes. This is a valuable tool that facilitates the characterization of mutants, enhances pea improvement and allows a more efficient use of the wide genetic diversity present in the genus, enabling genomic assisted crop improvement (Kreplak *et al.*, 2019).

If this positional information available from genome sequencing is combined with linkage, QTL or association mapping, opportunities arise to identify the genes or polymorphisms in genes responsible for variation in a character of interest. Several markers have been associated to traits of interest, such as yield-related traits; aerial and root architecture; plant phenology, morphology, and physiology; abiotic stress resistance, seed nutritional profile and trypsin inhibitors content and lodging resistance. Also, resistance to diseases such as powdery mildew, pea enation and seed borne mosaic virus, fusarium wilt, *Ascochyta blight* and rust (Tayeh *et al.*, 2015).

Unfortunately, there is still little information available on resistance against diseases of economic importance in Pea, being insufficient to achieve effective control. New advances have been made in understanding resistance to *Ascochyta blight* through mapping candidate genes associated with QTL regions (Jha *et al.*, 2017) *Phoma medicaginis* var *pinodella* and, in South Australia, *P. koolunga*. This study aimed to identify candidate genes that map to quantitative trait loci (QTL). Arrays Technology DArTseq platform has been used to identify QTLs Controlling Rust Resistance (Barilli *et al.*, 2018) a wild relative of pea is an important source of allelic diversity to improve the genetic resistance of cultivated species against fungal diseases of economic importance like the pea rust caused by *Uromyces pisi*. To unravel the genetic control underlying resistance to this fungal disease, a recombinant inbred line (RIL, pea weevil (*Bruchus pisorum*) resistance (Aznar-Fernández *et al.*, 2020).

Other recently evaluated characters included QTLs for yield-related traits (Gali *et al.*, 2018, Guindon *et al.*, 2019), for nutritional characters, (Jha and Warkentin, 2020), for metribuzin tolerance (Javid *et al.*, 2017), for frost tolerance (Liu *et al.*, 2017), and leaf shape traits (Zheng *et al.*, 2018).

Marker-assisted selection improves the process of selection and decreases the costs of managing large plant populations. Several genes critical to pea breeding and for which 'perfect' markers or genetically linked markers have been identified, but have established that relatively few pea breeding programs use marker-assisted selection routinely (Warkentin *et al.*, 2015).

INTA and UNR have developed two new commercial lines of green and yellow field peas, leafletless, with high adaptation to local agroecological conditions and high yield potential using conventional breeding. As the time required to increase the genetic gain is a disadvantage, we can also try to use non-conventional methods to increase the efficiency of traditional methods.

Non-conventional improvement methods in the world and in Argentina

The whole breeding process is quite lengthy and laborious – the standard time for releasing a new pea variety is to 10 to 15 years (Brummer *et al.*, 2011) suggested off-season sowing as a methodology to reduce breeding time. However, in pea, two generations per year could be obtained by changing the hemisphere Ochatt and Sangwan (2010).

Another alternative is *in vitro* culture methodology. Different authors (Surma *et al.*, 2013, Ribalta *et al.*, 2017) have developed it to induce *in vitro* flowering, shortening this period using inductive photoperiods and in some cases applications of hormones. *In vitro* plant regeneration is not 100% efficient (Greenway *et al.*, 2012) and is usually genotype dependent (De la Fuente *et al.*, 2013). The rescue of pea embryos can also be carried out *in vivo* along with the flowering acceleration using a hydroponic system with photo and thermo periodic control and reducing the growth of plants by applying antigibberelin (Cazzola *et al.*, 2020). According to Chahal and Gosal (2002), the high cost of materials, labor and the need for specialized staff are limiting factors for the incorporation of *in vitro* acceleration techniques. Therefore, new technologies that improve efficiency and decrease costs are demanded (De la Fuente *et al.*, 2013, Varshney *et al.*, 2019).

In recent years, Speed Breeding was developed in different crops. It includes growth plants using artificial lighting with inductive photoperiods, temperature and humidity control and anticipated grain harvest. It could be initiated at any moment of the year using a large amount of material in a small area (Cobb *et al.*, 2019). In Pea, Cazzola *et al.* (2020) used a hydroponic system, with a 22-hour photoperiod, flurprimidol antigiberelin and early grain harvest, achieving up to 5 generations per year.

The combination of Speed breeding and Single Seed Decent has the potential to reduce the time required to develop new cultivars and increase the efficiency of breeding programs compared to conventional field systems.

Double haploids (DH) were developed as a quicker method than conventional breeding methods to produce pure lines. DH can be produced by chromosome elimination via wide crosses (Devaux and Kasha, 2009); parthenogenesis and apomixis through gynogenesis or androgenesis from anthers or isolated microspores (Nitsch and Nitsch, 1969; Wedzony *et al.*, 2009). Among these techniques, androgenesis seems to be most promising for induction of haploids in legumes (Gatti *et al.*, 2016) and significant progress has been achieved in dry pea, chickpea, and *Medicago truncatula* Gaertn using it (Ochatt *et al.*, 2009). However, its drawbacks of genotype-dependency and only a single chance of recombination limited its wider application (Yan *et al.*, 2017).

Pea has been described as recalcitrant to doubled-haploids (Bobkov, 2010, 2014; Ribalta *et al.*, 2014) because of its low regeneration frequency of complete haploid plants (Ochatt *et al.*, 2009; Germana *et al.*, 2011).

Recently, Bermejo *et al.* (2020) studied the androgenic response among different taxa of the genus *Pisum* from the pri-

mary and secondary gene pools under the same experimental conditions and found significant differences in the percentage of callus and plant production between the different species and subspecies. They found that *P. fulvum*, *P. sativum* subsp. *elatius* and *P. sativum* var. *arvense* had the highest efficiency in shoot regeneration from another culture (67%, 38% and 40% respectively), becoming potential sources of androgenic competence and androgenesis studies. The commercial varieties tested among *P. sativum* showed significant differences in the callus and plant production, Primogénita FCA-INTA and B101 from the local program giving the best results although with low percentages of plant regeneration (17% and 11%, respectively).

Induced mutagenesis can increase genetic variability at a high mutation frequency. This technique is carried out by subjecting seeds, plants or different plant tissues to mutagenic agents, which can be chemical (ethyl methanesulfonate, sodium azide, etc.) or physical (gamma rays, X-rays, fast-neutrons). Nowadays, scientists continue developing protocols and studying the effects of chemical or physical mutagens over Pea (García *et al.*, 2022; Vinod Bansod *et al.*, 2022; Pandey *et al.*, 2022) and other legume crops (Galili *et al.*, 2021; Tiryaki *et al.*, 2022).

The application of genetic engineering can also play an important role in the non-classical breeding approach to improve agronomic traits. The regeneration potential of grain legumes is lower than that of forage legumes. (Somers *et al.* 2003). Morphogenesis in them is very slow and associated problems like development of albinos, and vitreous tissues and no-response in dedifferentiated calli are very common (Pratap *et al.*, 2010). There are some protocols to obtain stable transgenic plants in beans, soybeans, peas and alfalfa (Zimmermann *et al.*, 2009; Asif *et al.*, 2011). However, the limited success of this technology is associated with a poor regeneration capacity (Atif *et al.*, 2013; Ludvíková and Griga, 2022).

Although some transformation protocols have been developed, the regeneration of transgenic pea plants is not yet routine (Pratap *et al.*, 2018). The efficiency of transformation protocols has been relatively low, 0.1-6.0% (Polowick *et al.*, 2000 and very few close to 10% (Grant and Cooper, 2006). The acceptance of GMOs is also the subject to permanent discussion and their application in breeding programs is subject to legislation and regulations of different countries. Otherwise, it is possible to generate genetic variability using other mentioned techniques.

The climate change that is taking place and the rapid growth of the world population are a reality. Breeding programs are a very important tool to minimize them. They contribute to the creation of the next generation of leguminous crops that not only help agricultural crops to be used more efficiently in production systems but also adapt to changing market needs.

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