OBJECTIVES AND MODERN TECHNIQUES IN PEA BREEDING AT N.A.R.D.I. FUNDULEA

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INTODUCERE

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).

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).

OBJECTIVES IN PEA BREEDING PROGRAMS:

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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.

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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).

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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).

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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 and free from deterioration and damage caused by insects, freezing process, 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).

a.   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.

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.

PEA (PISUM SATIVUM L.) BREEDING IN ROMANIA

In 1962, a pea breeding program was initiated at NARDI Fundulea to increase production in quantity and quality. Starting with 80 year, the objective of breeding program was to obtained varieties with tendril formation (af locus), tolerance to shatering (def locus) with good behavoir at harvest and partial resistance to powdery mildew desease. As a result of the breeding works, 13 spring pea (Marina, Alina, Dorica, Mona, Rodil, Aurora, Nicoleta, Evelina F, Ștefania F, Anastasia F, Olivia F, Otilia F, Silvia F) varieties and numerous lines of the afila type were obtained, with high yield and good resistance to lodging and shatering.

In 2010, was initiated at NARDI Fundulea, winter pea breeding program, this is leaded to the creation of winter pea varieties with good winter hardiness, earliness, and good adaptability and stability at the climatic conditions of Ramania. The program started with three varieties winter pea originated by Austria and SUA and spring pea varieties and lines of perspective created at NARDI Fundulea.

Obtaining two generations per year, one in the field and one in controlled champ to the acceleration of genetic progress and the creation of Romanian winter pea varieties faster. At this moment we have a diversified germplasm of winter peas and 7 varieties of winter peas (Lavinia F, Ghittia F, Olguța F, Andrada F, Petra F, Flavia F, Antonia F).

Breeding work at NARDI Fundulea has been both conventional and modern, using molecular markers analysis for identified the lines with frost tolerance and earliness. For this traits was used molecular markers EST1109, AD59 and AD159.

CONCLUSIONS

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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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. 2019), rust (Rai 4 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). Marker-Assisted Selection

Conventional breeding methods for disease resistance arebased 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 etal. 2022), Fusarium root rot resistance (Coyne et al. 2019), pea enation or seed-borne mosaic virus (Swisher Grimm and Porter 2020), rust resistance (Singh et al. 2023), ascochyta blight (Jha et al 2017), and Aphanomyces root rot (Desgroux et al. 2016) are available for MAB.

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 F2 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. In order to achieve broad and persistent resistance 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 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.

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. (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.

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.