# PeaMUST (Pea MultiStress Tolerance), a multidisciplinary French project uniting researchers, plant breeders, and the food industry

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NEWS AND VIEWS

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## Abstract

The French government has supported as part of its "Investments for the Future" program a 9-year research project, PeaMUST, devoted to pea and to a lesser extent, faba bean improvement. Focusing on the main causes of yield irregularity that limit pea and faba bean cultivation, an integrated approach, including molecular exploitation of the pea genome sequence, was applied to identify and incorporate favorable alleles and allele combinations in prebreeding material.

**KEYWORDS**
abiotic, biotic, faba bean, genome, legume, pea, *Pisum sativum*, protein, seed, selection, stress, *Vicia faba*

# 1 | INTRODUCTION

Pea (*Pisum sativum* L.) is a key crop for sustainable agriculture in temperate climate zones. Despite its agroecological interest, and value as a rich source of protein in human and livestock diets, the area sown to pea is small in Europe. Pea and other grain legume crops are challenged by leading cereal crops for yield and profitability per unit area, and imported soybean is a cheap and common source of protein for the food and feed industries. To increase the competitiveness of pea, Pea MUltiStress Tolerance (PeaMUST), a 9-year plant biology research project, was conceived, which received 5.5 M€ funding from the French "Investments for the Future" Program. The consortium of 28 research and commercial, mainly breeding, partners has focused on tackling yield irregularity in pea, principally caused by susceptibility to diverse abiotic and biotic stresses. Faba bean was also studied in view of its close phylogenetic similarity to pea and its importance in European cropping systems. The project was organized as shown in Figure 1. Core groups providing new genetic resources enabled four research modules and bioinformatics support. The recently available genomic sequence of pea (Kreplak et al., 2019) has been exploited throughout the project. The commercial partners can benefit from high-throughput genotyping, bioinformatics, upscaling of molecular breeding methods, and collaborate in progeny development and field testing. The project facilitates sharing of expertise of public research on complex trait phenotyping and genomics.

# 2 | EVALUATION OF GENOMIC SELECTION FOR ACCELERATING BREEDING CYCLES

In the module "Molecular marker-based prebreeding and breeding approaches," genomic prediction was tested on three types of peas: spring peas (PP), conventional winter peas (Phr), and photoperiod-responsive winter peas (PHr) to evaluate the accuracy of this breeding strategy, as compared with phenotypic selection. Training panels for the three pea types were defined, then phenotyped and genotyped, and equations to predict yield and traits related to response to yield limiting factors, based solely on genotypic data, were developed. The feasibility of genomic prediction in pea using a diversity panel had already been demonstrated (Burstin et al., 2015), and the effect of marker density and training population size had been evaluated (Tayeh et al., 2015). The prediction accuracy of these models was evaluated in PP and Phr breeding panels developed in the project. Predictions were robust for yield but less accurate for the response to limiting factors, probably because these traits were estimated on a limited number of trials. Spring pea lines derived from the first cycle of genomic selection were tested in agronomic conditions and compared with lines derived from phenotypic selection; lines from genomic selection showed a similar performance to lines from phenotypic selection with likely higher yield stability. These encouraging results showed the promising nature of this method for varietal selection in
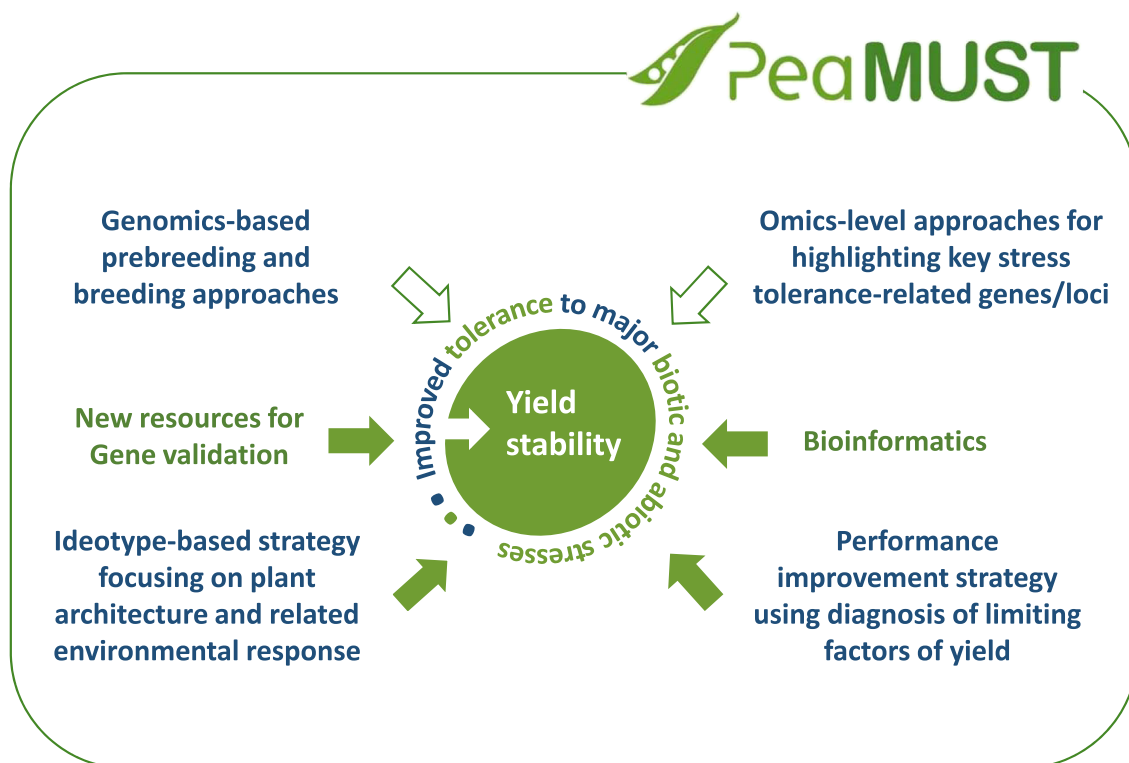


**FIGURE 1** Pea MUltiStress Tolerance (PeaMUST) project organization

pea. By reducing the time required to select interesting lines, breeding cycles can be accelerated.

## 3 | GENETIC AND GENOMIC DETERMINANTS OF RESISTANCE TO MAJOR STRESSES IN PEA AND FABA BEAN

Plant material (near-isogenic lines, NILs and recombinant inbred lines, RILs) for resistance quantitative trait locus (QTL) detection and validation by genome-wide association study (GWAS) were produced in pea and faba bean. In support, genomic resources were generated for these two species. Using exome capture, which combines single nucleotide polymorphism (SNP) discovery and genotyping, 1.9-M SNPs were detected for the pea architecture and multistress (AMS) collection and 1.7 M for the faba bean collection. By sequencing of the faba bean transcriptomes of four parental lines of the RIL populations, 104,000 SNPs were detected, and 2,000 of those have been used for genotyping of segregating populations using genotyping by capture technique (Carrillo-Perdomo et al., 2020).

In pea, Aphanomyces root rot is a major problem to the establishment of the crop, causing severe crop damage and with infected fields being unsuitable for pea cultivation for several years. Major Aphanomyces root rot resistance QTLs previously detected were validated in NILs, and favorable haplotypes were identified from GWAS panels (Beji et al., 2020; Desgroux et al., 2016; Lavaud et al., 2015; Lavaud et al., 2016). Pea NIL evaluations confirmed the significant effect of Aphanomyces resistance QTL combinations with the major QTL on chromosome 7 in decreasing disease severity and limiting yield reduction in highly infested fields. However, isolates were also identified in French pea fields, which were aggressive on these NILs (Quillévéré-Hamard et al., 2018; Quillévéré-Hamard et al., 2020), advocating the pyramiding of multiple resistance alleles for breeding varieties with high and durable levels of resistance (Pilet-Nayel et al., 2017).

Frost tolerance is an important trait limiting pea cultivation in Europe, particularly for the development of winter pea with its potential yield bonus. To investigate the genetic determinism of frost tolerance, GWAS was performed using a set of 365 pea accessions. Phenotyping was carried out by scoring frost damage in the field and in controlled conditions (Beji et al., 2020). In a first analysis, 62 SNPs significantly associated with frost tolerance were distributed over six of the seven pea linkage groups. Three previously mapped QTLs were confirmed.

In faba bean, high-density genetic maps were constructed from three RIL populations, and solid blocks of macrosynteny were observed between faba bean and the most closely related sequenced legume species such as pea and *Medicago truncatula* (Carrillo-Perdomo et al., 2020). QTLs were identified on the genetic maps for frost tolerance, as well as bruchid and aphanomyces resistance. Bruchid beetle infestation is particularly problematic in faba bean with increasing restrictions on insecticide use. Syntenic relationships between frost tolerance QTLs in pea and faba bean were revealed. New sources of frost tolerance and bruchid resistance were identified (Carrillo-Perdomo, Klein, et al., 2019; Carrillo-Perdomo, Raffiot, et al., 2019) in a reference faba bean collection. This collection was exome sequenced, and seeds were phenotyped using X-ray 3-D tomography (to detect and quantify bruchid infestation in the bean) for further GWAS analyses. Proteomics, volatile organic compounds (VOCs), and/or RNAseq data were obtained for RIL parental lines and families in order to identify molecular pathways underlying bruchid and Aphanomyces resistance. The knowledge acquired about loci, haplotypes, and genes controlling resistance to major stresses in pea and faba bean will accelerate breeding of durably tolerant varieties.

## 4 | ROLE OF PLANT ARCHITECTURE IN MULTISTRESS TOLERANCE

PeaMUST also focused on the effect of plant shoot and/or root architecture on multistress tolerance. For this, the project exploited naturally existing variability through a collection of genetic resources, including the "Architecture and Multi-Stress" (AMS) collection. In parallel, new material was produced with the introgression of six known mutations affecting shoot and/or root architecture into one spring and two winter pea cultivars. Targeting Induced Local Lesions IN Genomes (TILLING) screens of complementary novel mutations were also performed.

The AMS collection was genotyped and phenotyped for various stresses (drought, Aphanomyces root rot, frost …). Among the significant findings in part of this collection, it was shown that some root architecture traits are correlated to resistance to Aphanomyces root rot (Desgroux et al., 2018).

Studies were also conducted on cell wall composition (pers. comm.), exploring the relationship between cell wall composition and cold sensitivity (Baldwin et al., 2004). The QTLs introgressed into spring and winter cultivars were evaluated for their agronomic performance and responses to a series of stresses. A subsample of 17 lines ("Best of"), mutants, or accessions among the AMS collection was then further tested for drought tolerance on a high-throughput phenotyping platform and for resistance to *Aphanomyces euteiches* on a specific experimental platform. Interesting differences in drought tolerance between cultivars were observed and enhanced tolerance in some mutants as compared with the wild type parent.

## 5 | USING MODELING TO EVALUATE LIMITS TO YIELD

Recently registered pea cultivars were evaluated for yield and adaptation to diverse stresses in a multilocation field network of over 40 environments during 3 years (2013–2015). Agronomic and multi-criteria analyses on this 3-year experimental network demonstrated the better performances of the conventional winter type in most tested areas. A second series of trials was set up in 2018–2019 with more recent cultivars and genotypes derived from the genomic

selection work of the project. Lines coming from these first genomic selection trials gave yields equivalent to those of the best current varieties. This was encouraging because the application of genomic selection undoubtedly has a margin for improvement in the future. Some pea crop models were developed and/or implemented throughout the years: DiagVar-Pea for quantifying limiting factors and estimating potential yield, based on DiagVar (Lecomte et al., 2010); Azodyn-Pea for pea crop simulations under various pedoclimatic conditions, with interesting links with the DiagVar diagnosis (Jeuffroy et al., 2012). The FlorSys crop-weed competition model (Colbach et al., 2014) was adapted for peas to study their ability to control weeds, relevant to pea crop production and weed management. Economic analyses of the barriers and levers to the uptake of new pea varieties were also performed in comparison with the wheat varietal innovation, showing the lower actor number, market size, and systemic effects that limit development of the pea crop, despite its agronomic and environmental advantages. Additionally, biochemical analyses of seeds were conducted to characterize and compare seed technological properties of various genotypes (pers. comm.).

## 6 | NEW RESOURCES FOR GENE VALIDATION

To confirm the value of gene candidates identified by some of the PeaMUST results, a gene validation platform was set up in the project. As genetic transformation of pea is slow and difficult, complementary alternative approaches were investigated: TILLING and Virus Induced Gene Silencing (VIGS). Studies conducted on VIGS in pea using bean pod mottle virus (Meziadi et al., 2016, 2017) proved the susceptibility to inactivation by VIGS of genes expressed in both pea aerial and root compartments and showed this technique could be used to study genes expressed in either compartment. VIGS silencing persisted 15 weeks after infection (Pflieger, Geffroy pers. comm.), which confirmed the interest of this technique for validating candidate genes for disease resistance. Unfortunately, (as frost tolerance was one of the traits of interest), VIGS proved inefficient in cold conditions (Pflieger, Geffroy pers. comm.), possibly due to lack of viral replication. More than 48 simple loss-of-function mutants have been obtained from three TILLING collections that were constructed in the spring varieties "Caméor" (Dalmais et al., 2008), in the branched rms3 and rms4-Terese background and in the PHr winter pea genotype 336/11, the latter produced during the project. Both spring and winter varieties can now be screened.

## 7 | BIOINFORMATICS AND DATA MANAGEMENT SUPPORT

The project was supported by data management and computing services. A data repository was created specifically for the project, which made all data available to partners directly after their production and which has facilitated data integration in public and private

repositories. The latest genotyping and sequencing technologies were used for producing numerous genomic resources for pea and faba bean (exome capture SNP detection and genotyping databases on large pea and faba bean panels, transcriptome unigene resources in faba bean). To assure access after project end, results will be integrated and maintained for the Pea community on the GnpIS database at URGI. Some data are already published and can be accessed through the PeaMUST-dedicated web page: https://urgi.versailles.inra.fr/Projects/PeaMUST.

## 8 | CONCLUSION

Funding of the project over 9 years enabled researchers to use approaches requiring a long time frame, needed for prebreeding, to come to fruition. Access to the pea genomic sequence was revolutionary and opens up many prospects for accelerating targeted trait selection in the future. By considering exploitable genetic variation in breeding material, PeaMUST has complemented existing GWAS studies (Gali et al., 2019). Dissemination of the project results continues through the diffusion of brochures, newsletters, booklets, scientific papers, organization of training sessions, website presence, https://www.peamust-project.fr/, and a twitter account https://twitter.com/PeaMUST.

### CONFLICT OF INTEREST
The authors have no competing commercial interests to declare.

### ETHICS APPROVAL STATEMENT
The study did not involve the use of human or animal subjects.

### DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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### REFERENCES
Baldwin, L., Domon, J.-M., Klimek, J. F., Francoise Fournet, F., Sellier, H., Gillet, F., Pelloux, J., Lejeune-Henaut, I., Carpita, N., & Rayon, C. (2004). Structural alteration of cell wall pectins accompanies pea development in response to cold. *Phytochemistry*, *104*, 37–47. https://doi.org/10.1016/j.phytochem.2014.04.011
Beji, S., Fontaine, V., Devaux, R., Thomas, M., Negro, S. S., Bahrman, N., Siol, M., Aubert, G., Burstin, J., Hilbert, J.-L., Delbreil, B., &

Lejeune-Henaut, I. (2020). Genome-wide association study identifies favorable SNP alleles and candidate genes for frost tolerance in pea. *BMC Genomics*, *21*(536). https://doi.org/10.1186/s12864-020-06928-w

Burstin, J., Salloignon, P., Chabert-Martinello, M., Magnin-Robert, J. B., Siol, M., Jacquin, F., Chauveau, A., Pont, C., Aubert, G., Delaitre, C., Truntzer, C., & Duc, G. (2015). Genetic diversity and trait genomic prediction in a pea diversity panel. *BMC Genomics*, *16*, 105. https://doi.org/10.1186/s12864-015-1266-1

Carrillo-Perdomo, E., Klein, A., Kreplak, J., Deulvot, C., Raffiot, B., Desmetz, C., Magnin-Robert, J.-B., Houtin, H., Aimé, D., Leveugle, M., Duborjal, H., Trigui, G., Falque, M., Leppik, E., Frérot, B., Tayeh, N., Gallardo, K., Marget, P., Burstin, J., & Aubert, G. (2019). Towards bruchid resistance in pulses. *Abstract: 9th International Congress on Legume Genetics and Genomics*, pp. 188. 13-17 May 2019.

Carrillo-Perdomo, E., Raffiot, B., Ollivier, D., Deulvot, C., Magnin-Robert, J. B., et al. (2019). Identification of novel sources of resistance to seed weevils (Bruchus spp.) in a faba bean germplasm collection. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01914

Carrillo-Perdomo, E., Vidal, A., Kreplak, J., Duborjal, H., Leveugle, M., et al. (2020). Development of new genetic resources for faba bean (Vicia faba L.) breeding through the discovery of gene-based SNP markers and the construction of a high-density consensus map. *Scientific Reports*, *10*, 6790. https://doi.org/10.1038/s41598-020-63664-7

Colbach, N., Collard, A., Guyot, S. H. M., Meziere, D., & Munier-Jolain, N. M. (2014). Assessing innovative sowing patterns for integrated weed management with a 3D crop: Weed competition model. *European Journal of Agronomy*, *53*, 74–89. https://doi.org/10.1016/j.eja.2013.09.019

Dalmais, M., Schmidt, J., le Signor, C., Moussy, F., Burstin, J., Savois, V., Aubert, G., Brunaud, V., de Oliveira, Y., Guichard, C., Thompson, R., & Bendahmane, A. (2008). UTILLdb, a *Pisum sativum* in silico forward and reverse genetics tool. *Genome Biology*, *9*(2). https://doi.org/10.1186/gb-2008-9-2-r43

Desgroux, A., Baudais, V. N., Aubert, V., le Roy, G., de Larambergue, H., Miteul, H., Aubert, G., Boutet, G., Duc, G., Baranger, A., Burstin, J., Manzanares-Dauleux, M., Pilet-Nayel, M. L., & Bourion, V. (2018). Comparative genome-wide-association mapping identifies common loci controlling root system architecture and resistance to *Aphanomyces euteiches* in pea. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.02195

Desgroux, A., L'Anthoene, V., Roux-Duparque, M., Riviere, J. P., Aubert, G., Tayeh, N., Moussart, A., Mangin, P., Vetel, P., Piriou, C., McGee, R. J., Coyne, C. J., Burstin, J., Baranger, A., Manzanares-Dauleux, M., Bourion, V., & Pilet-Nayel, M. L. (2016). Genome-wide association mapping of partial resistance to Aphanomyces euteiches in pea. *BMC Genomic*, *17*. https://doi.org/10.1186/s12864-016-2429-4

Gali, K. K., Sackville, A., Tafesse, E. G., Lachagari, V. B. R., McPhee, K., Hybl, M., Mikic, A., Smykal, P., McGee, R., Burstin, J., Domoney, C., Ellis, T. H. N., Tar'an, B., & Warkentin, T. D. (2019). Genome-wide association mapping for agronomic and seed quality traits of field pea (*Pisum sativum* L.). *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.01538

Jeuffroy, M. H., Vocanson, A., Roger-Estrade, J., & Meynard, J. M. (2012). The use of models at field and farm levels for the ex ante assessment of new pea genotypes. *Eur J Agr*, *42*, 68–78. https://doi.org/10.1016/j.eja.2012.04.005

Kreplak, J., Madoui, M. A., Capal, P., Novak, P., Labadie, K., Aubert, G., Bayer, P. E., Gali, K. K., Syme, R. A., Main, D., Klein, A., Berard, A., Vrbova, I., Fournier, C., d'Agata, L., Belser, C., Berrabah, W., Toegelova, H., Milec, Z., ... Burstin, J. (2019). A reference genome for pea provides insight into legume genome evolution. *Nature Genetics*, *51*(9), 1411. https://doi.org/10.1038/s41588-019-0480-1

Lavaud, C., Baviere, M., le Roy, G., Herve, M. R., Moussart, A., Delourme, R., & Pelet-Nayel, M.-L. (2016). Single and multiple resistance QTL delay symptom appearance and slow down root colonization by *Aphanomyces euteiches* in pea near isogenic lines. *BMC Plant Biology*, *16*. https://doi.org/10.1186/s12870-016-0822-4

Lavaud, C., Lesne, A., Piriou, C., le Roy, G., Boutet, G., Moussart, A., Poncet, C., Delourme, R., Baranger, A., & Pilet-Nayel, M. L. (2015). Validation of QTL for resistance to *Aphanomyces euteiches* in different pea genetic backgrounds using near-isogenic lines. *Theoretical and Applied Genetics*, *128*, 2273–2288. https://doi.org/10.1007/s00122-015-2583-0

Lecomte, C., Prost, L., Cerf, M., & Meynard, J. M. (2010). Basis for designing a tool to evaluate new cultivars. *Agron. Sustain. Dev.*, *30*(3), 667–677. https://doi.org/10.1051/agro/2009042

Meziadi, C., Blanchet, S., Geffroy, V., & Pflieger, S. (2017). Virus-induced gene silencing (VIGS) and foreign gene expression in Pisum sativum L. using the "one-step" bean pod mottle virus (BPMV) viral vector. In M. Kaufmann, C. Klinger, & A. Savelsbergh (Eds.), *Functional Genomics: Methods and Protocols* (3rd ed., Vol. 1654, pp. 311–319). Springer.

Meziadi, C., Blanchet, S., Richard, M. M. S., Pilet-Nayel, M.-L., Geffroy, V., & Pflieger, S. (2016). Bean pod mottle virus: A new powerful tool for functional genomics studies in Pisum sativum. *Plant Biotechnology Journal*, *14*(8), 1777–1787. https://doi.org/10.1111/pbi.12537

Pilet-Nayel, M. L., Moury, B., Caffier, V., Montarry, J., Kerlan, M. C., Fournet, S., Durel, C. E., & Delourme, R. (2017). Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.01838

Quillévéré-Hamard, A., le Roy, G., Lesné, A., le May, C., & Pilet-Nayel, M. L. (2020). Aggressiveness of diverse French *Aphanomyces euteiches* isolates on pea near-isogenic-lines differing in resistance QTL. *Phytopathology in press*. https://doi.org/10.1094/PHYTO-04-20-0147-R

Quillévéré-Hamard, A., le Roy, G., Moussart, A., Baranger, A., Andrivon, D., Pilet-Nayel, M. L., & le May, C. (2018). Genetic and pathogenicity diversity of *Aphanomyces euteiches* populations from pea-growing regions in France. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01673

Tayeh, N., Klein, A., le Paslier, M. C., Jacquin, F., Houtin, H., Rond, C., Chabert-Martinello, M., Magnin-Robert, J. B., Marget, P., Aubert, G., & Burstin, J. (2015). Genomic prediction in pea: effect of marker density and training population size and composition on prediction accuracy. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00941