

**PhD School on Agriculture, Environment and Bioenergy**  
([http://sites.unimi.it/dottorato\\_aab/](http://sites.unimi.it/dottorato_aab/))

(XXXVIII cycle, 2022-25)

**Project draft**

**1. Field of interest**

*Patologia Agraria AGR/12, Genetica Agraria, AGR/07*

**2. Project title**

Genetic and genomic approaches to explore the cuticle-mediated response to biotic and abiotic stress in maize

**3. Tutor** (membro del Collegio dei Docenti) **Paola Casati**

- **Co-tutor** Gabriella Consonni

**4. Relevance of the topic and state of the art:**

Plant epidermal cells are covered by the cuticle, a hydrophobic layer composed of cutin, a polyester made oxygenated fatty acids and waxes, whose main constituents are very-long-chain fatty acids (VLCFAs) and their derivatives, which include alcohols, aldehydes, alkanes, ketones and esters. Waxes can be embedded within the cutin or can accumulate on the outer surface of the epidermis as epicuticular crystals (Bernard and Joubès, 2013). Cuticle provides conservation of internal plant water and protection against damages caused by environmental factors, including UV light, temperature and pathogens, and a (Yeats and Rose, 2013).

Several studies have shown that changes in cuticular composition led to alterations in the cuticle-mediated leaves permeability. In rice and maize, mutants causing a reduction or affecting the organization or transport of waxes (Park et al., 2010; Li et al., 2019) are correlated with increase in transpiration and water loss rates. In addition, positive correlations between increase of wax amount and tolerance to drought stress have been reported in Arabidopsis (Cui et al., 2016) and in wheat (Bi et al., 2016). Vice versa, mutants with reduced wax load exhibit higher sensitivity to drought (Mao et al., 2012; Castorina et al. 2020). Despite growing knowledge about the molecular mechanisms underlying cuticle's biosynthesis and depositions, the genetic and molecular basis of the cuticle-mediated response to environmental stress remain to be investigated. Aims of the project are to obtain a functional characterization of novel genes involved in cuticle deposition and to detect key genes involved in the cuticle-mediated response to environmental stresses in maize

**5. Layout of the project (draft)**

**5.1. Materials & Methods:**

A group of cuticle-related candidate genes has been selected from a previous study (Castorina et al. 2020); they will be further characterized in this project for their role in cuticle biosynthesis and deposition. The involvement in the cuticle-mediated response to stress will be also investigated for selected genes. The project will comprise two main group of activities.

**1. Functional characterization of novel cuticle-related genes.** Loss of function mutant alleles have been obtained from the "BonnMu", a public collection of transposon-insertion lines, located at the Hochholdinger lab (University of Bonn, Germany) and are available for the project.

Task 1.1. Genetic and phenotypic analysis. BonnMu progenies will be propagated and molecularly characterized to confirm the presence of transposon insertions in mutant alleles. Difference between genotypes will be first assessed through visual scoring by looking at the at seedling developmental defects and leaf glossy phenotypes. Cuticle alterations will be assessed with different methodological approaches, such as chlorophyll leaching, water loss and leaf temperature determination.

Task 1.2 Biochemical analysis. On selected genes, the mutant phenotype will be in-depth characterized: the lipid profile will be determined in collaborations with other groups.

## 2. Analysis of the cuticle-mediated response to drought stress and pathogens.

The involvement in the cuticle-mediated response to drought and pathogen infection will be assessed for two interesting genes. The choice will be based on our results and information from the related literature.

Task 2.1. Response to water scarcity. Plants will be grown in controlled conditions, and a mild drought stress will be imposed by withholding irrigation. The response to stress will be compared in mutants and wild types by monitoring appropriate physiological parameters. The expression profile of the candidate genes will be also determined in wild type plants subjected to water stress.

Task 2.2 Analysis of the response to fungi infection. Maize is susceptible to numerous pathogenic fungi, which can infect the plant in the presence of favorable environmental conditions. Diseases caused by fungi, such as *Fusarium verticillioides*, not only cause significant production losses, but are often associated with the accumulation of toxic metabolites. In this analysis toxinogenic fungi, which play a role in maize both as pathogens and as endophytes will be tested. Conidic suspensions will be inoculated, and colonization will be assessed in the different genotypes by evaluating the symptomatic tissue surface. The presence of fungal structures will also be evaluated by optical microscopy.

### 5.2. Schedule and major steps (3 years):

The schedule of the project is reported in the Gantt chart (Fig.1) in which the duration of each task is indicated. The expected results obtained from the two WPs are listed as deliverable (D) here below and their timing is reported in Fig. 1.

List of deliverables

D1.1 Detection of cuticle-related genes and their effect on cuticle-related seedling phenotypes

D1.2 Characterization of gene functions in the biosynthesis and/or deposition of cuticle components

D2.1 Detection of genes responsive to drought stress

D2.2 Detection of differences between genotypes in the cuticle-mediated response to pathogens

Work package (WP) and Task (T) name	WPs/Tasks	Duration	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Functional characterization of cuticle-related genes	WP1																			
Genetic and phenotypic analysis	Task 1.1	12						D1.1												
Biochemical analysis	Task 1.2	6							D1.2											
Analysis of the cuticle-mediated response to stress	WP2																			
Response to water scarcity	Task 2.1	16																		D.3
Analysis of the response to fungi infection	Task 2.2	12														D.4				

Figure 1 - Timing of the two work packages and their components (Gantt Chart)

### 6. Available funds (source and amount)

“Varietà locali di mais: caratterizzazione per la reintroduzione nel territorio lombardo” (VALOMAYS) Regione Lombardia Programma di Sviluppo Rurale 2014-2020 MISURA 10 – “Pagamenti agro climatico ambientali” SOTTOMISURA 10.2 - 6.000 Euro

### 6. Literature: max 10 citazioni

- Bernard A, Joubès J (2013) Arabidopsis cuticular waxes: Advances in synthesis, export and regulation. *Prog Lipid Res* 52: 110–129
- Bi H, Luang S, Li Y, Bazanova N, Morran S, Song Z, Perera MA, Hrmova M, Borisjuk N, Lopato S (2016) Identification and characterization of wheat drought-responsive MYB transcription factors involved in the regulation of cuticle biosynthesis. *J Exp Bot* 67: 5363–5380
- Castorina G, Domergue F, Chiara M, Zilio M, Persico M, Ricciardi V, et al (2020). Drought-responsive ZmFDL1/MYB94 regulates cuticle biosynthesis and cuticle-dependent leaf permeability. *Plant physiology* 184: 266-282.
- Cui F, Brosché M, Lehtonen MTT, Amiryousefi A, Xu E, Punkkinen M, Valkonen JPPT, Fujii H, Overmyer K (2016) Dissecting abscisic acid signaling pathways involved in cuticle formation. *Mol Plant* 9: 926–938
- Li L, Du Y, He C, Dietrich CR, Li J, Ma X, Wang R, Liu Q, Liu S, Wang G, et al (2019) Maize glossy6 is involved in cuticular wax deposition and drought tolerance. *J Exp Bot* 70: 3089–3099
- Mao B, Cheng Z, Lei C, Xu F, Gao S, Ren Y, et al (2012) *Wax crystal-sparse leaf2*, a rice homologue of WAX2/GL1, is involved in synthesis of leaf cuticular wax. *Planta* 235: 39–52.
- Park JJ, Jin P, Yoon J, Yang JI, Jeong HJ, Ranathunge K, Schreiber L, Franke R, Lee IJ, An G (2010) Mutation in Wilted Dwarf and Lethal 1 (WDL1) causes abnormal cuticle formation and rapid water loss in rice. *Plant Mol Biol* 74: 91–103
- Yeats TH, Rose JKC (2013) The formation and function of plant cuticles. *Plant Physiol* 163: 5–20