PhD School on Agriculture, Environment and Bioenergy

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(XXXV cycle, 2019-21)

Project draft

1. Field of interest

AGR07

2. Project title

Phytic acid, roots development and anthocyanin biosynthesis in *Zea mays* L.

3.Tutor

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4. Relevance of the topic and state of the art

In plants, phytic acid (PA) (myo-inositol-1,2,3,4,5,6-hexakisphosphate; InsP6) represents the major storage form of phosphate (P) in the seeds (from 65 to 85%) [1]. In maize about 80% of phytate is found in the embryo, while 20% in the aleurone layer [2]. During maturation PA is accumulated in inclusions named globoids in the protein storage vacuole [3]. Due to its high negative charge at the physiological pH, PA chelates minerals (such as iron, zinc, potassium, calcium, magnesium), reducing their bioavailability. During germination, phytases degrade PA and P is remobilized to support seedling growth [4]. Only ruminants can degrade PA, due to the presence of phytases in their digestive system. Viceversa, monogastric animals do not have phytase activity: they assimilate only 10% of phytate in feed, while 90% is excreted and the supplementation of feed with phosphorus is necessary (economic problem). Moreover, as excreted, P concentration increases in manure, consequently in soils, contributing to P pollution in groundwater (environmental problem) [5]. Hence, PA is considered an anti-nutrient.

For these reasons, PA reduction or elimination in cereals' seeds has been an important challenge in genetic breeding programs. Among the different strategies used, in the last decades many low phytic acid (*lpa*) mutants have been isolated in all major crops [5]. In maize, *lpa1* mutations are caused by lesions in *ZmMRP4* [6]. These mutations showed a reduction in PA content, accompanied by a proportional increase in P_i, even if the total P remained unchanged. In particular, *lpa1-1* allele showed a 66% reduction in PA [7], while in *lpa1-241* and *lpa1-7* this drop was greater than 80% [8,9]. The seed remained viable in *lpa1-1*, while highest PA reductions (*lpa1-241* and *lpa1-7*) compromised germination capacity and only embryo-rescue allowed us to work with these mutants**.**

lpa mutations are often associated with poor agronomic performance and many negative pleiotropic effects on the seed and plant have been described [7–9]; despite this, little importance has been given to the root system. In recent years it has been noted that under field conditions, *lpa1-1* is more susceptible to drought stress, probably due to an alteration in mature root system [9].

The root system architecture (RSA) plays a key role in the study of drought resistance. In fact, roots have multiple functions: they are fundamental in the uptake of water and nutrients and they provide anchorage of the plant in the soil. Roots are also an site of biosynthesis of hormones and are involved in interactions with the rhizosphere [10].

5. Layout of the project (draft)

The root system architecture (RSA) has been little taken into consideration by breeders in comparison with above-ground parts of the plant. This is due to the difficulty of screening underground habitat, but also due to

the strong influence of the environment on the variability of root structure. Among all the approaches of RSA phenotyping, field-based techniques are laborious and destructive, but are the only ones that consider the interaction of the roots with the surrounding soil. Viceversa, non-soil techniques enable for easy and non-destructive visualization of RSA, as well as precise control of the root growth environment, but may not recapitulate the 3D nature of RSA in the soil [11,12].
Here we use a combination of soil and non-soil techniques with the aim to phenotype the root system architecture in *lpa1-1*. Comparing the mutant root apparatus with the wild phenotype, it will be possible to understand if the different RSA in the two genotypes is the main cause of drought stress sensivity in *lpa1-1*.

A second part of the work will be carried out with the lethal mutations *lpa1-7* and *lpa1-241*. In a previous paper [13] it was observed that *lpa1-241* enhances the accumulation of anthocyanin in the kernel, changing the color of scutellum in the strongest *lpa1-241* mutant from dark red to bluish. This alteration was attributed to a defect in the pigment transport in the vacuole, causing a mislocalized accumulation of these pigments in the cytosol, suggesting that *ZmMRP4* could have a direct or indirect role in anthocyanin transport.
In this project we would like to broaden these observations in order to understand if the same phenomenon is repeated also with the other lethal mutant, *lpa1-7*, not yet studied.

	1st year		2nd year		3rdyear	
Activity	I-VI	VII-XII	I-VI	VII-XII	I-VI	VII-XII
Survey of *lpa1* mutations and sequencing						
Analyse roots development in *lpa1* mutations						
Study a possible interaction between PA accumulation and anthocyanin biosynthesis						

5.1. Materials & Methods

- Breeding activities in experimental field and greenhouse

- Roots image acquisition and analysis

- Cloning and sequencing *lpa1* mutations

- RT-PCR expression analysis

- Embryo-rescue

5.2. Schedule and major steps

6. Available funds (source and amount)

- SOCIAAALP, Fondazione CARIPLO 2019-2022, bando coltivare Valore. 60.000 Euro

- “Pigmented maize cobs waste as an environmental friendly solution to dye natural fibers” PASTEL. CARIPLO-Biotecnologie industriali CARIPLO 2018-2020. 250.000 Euro

- Several other contracts with private companies about 100.000 Euro

6. Literature:

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