PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXIX cycle, 2023-26)

Project draft

1. Field of interest

AGR07

2. Project title

Study and characterization of bean germplasm

3.Tutor

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

The main cereal grain cultivated throughout the world is maize (*Zea mays* L. ssp. *mays*), maybe because of its versatility (Food and Agriculture Organization of the United Nations, Crops Production, 2009; Zeppa et al. 2012). It can be used as feed for livestock, forage, silage and grain, but also for biofuel and for industrial uses. However human nutrition remains one of the main uses, determining the selection of varieties for producing many locally typical cornmeal such as polenta in Italy, angu in Brazil and mush in the USA. Polenta is a very popular dish in the northern regions of Italy (Zeppa et al. 2012). Different kinds of polenta exist on the basis of the maize variety and of the type of flour milling. Historically Italian polenta maize was obtained from landraces, fitted to the different microclimates peculiar to the several Italian agroclimatic areas (Brandolini and Brandolini 2009). Traditionally the landraces used in Italy for human feeding have a flint or semi-flint kernel texture (Brandolini and Brandolini 2009). Kernels of flint corn mostly have hard, glassy endosperms with smooth, hard seed coats: corn flour composition is well defined, it is mainly composed by starch (80%), proteins (10-15%) and lipids (5%) (Panzeri et al. 2011). However, there have been few specific studies on polenta: it is known that polenta can be a good source of iron and phosphorus, and also of carotenoids, most of which are provitamins A (Venturelli et al. 1990; Brandolini and Brandolini 2009; Rodriguez-Amaya et al. 2008). The bio-fortification of these cultivars (for what concern antioxidant power) could be obtained by introgression of few dominant genes conferring a strong anthocyanin accumulation of kernel tissues, in fact anthocyanins have been studied in several different vegetables, because of their antioxidant power, the putative characteristic responsible for many health benefits (Tsuda 2012). Another important topic to be elucidated regards the effect of the presence of high level of flavonoid pigments on the accumulation of dangerous mycotoxins on kernels. In fact, recent studies indicate that flavonoid compounds could tackle the mycotoxins accumulation (Pilu et al 2011). In maize, fungal infections lead to contamination of grains with mycotoxins, in particular aflatoxins and fumonisins, and to loss of yield. Among the several fungal pathogens, Fusarium verticilloides and F. proliferatum are the most abundant in several wet zones such as northern Italy, causing contamination in the kernels with fumonisin B2, B3 and most frequently B1 (Marasas et al. 2006).

5. Layout of the project (draft)

Among the phytonutrients, anthocyanins have been extensively studied in several different vegetables, because of their antioxidant power, the characteristic supposedly responsible for their capacity for chronic disease prevention. Anthocyanins can also be synthesized in maize even though in Europe the colourless varieties have always been preferred. The maize genotypes displaying the anthocyanin colorations ranging from red to dark blue are grown widely by traditional farmers in Central and South America, even though the majority of maize varieties including those with white or yellow grains have the genetic information for the anthocyanin biosynthetic pathway. Two multigene families are required for the regulation of the anthocyanin pathway: the r1/b1family belonging to the class of bHLH transcription factors and the c1/pl1/p1 belonging to the class of MYB transcription factors. A member of each family must be present and active in the dominant form to activate anthocyanin biosynthesis and according to the combination of alleles of the regulatory genes, the synthesis will be active in different plant tissues (Petroni et al., 2014). The aim of this project is to develop and study new varieties of maize rich in flavonoid pigments, to increase the antioxidant power of this food and study the possible role as antagonist vs the fungi attack. The plants selection will be assisted by molecular markers to speed the breeding activities.

5.1.Materials & Methods:

i) Germplasm selection and breeding activities in experimental field and greenhouse

ii) Spectrophotometric analysis of flavonoids content in the kernels

iii) Marker assisted selection by SSRs and genotyping by sequencing (GBS)

iv) Cloning and sequencing of regulatory genes in the different genetic materials

v) Mycotoxin analysis by HPLC and ELISA test

5.2. Schedule and major steps

main activity	I° year						II° year						III° year					
	O N	D J	F M	A M	J J	A S	O N	D J	F M	A M	J J	A S	O N	D J	F M	A M	J J	A S
Germplasm selection and breeding activities in experimental field and greenhouse			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Spectrophotometric analysis of flavonoids content in the kernels			X									X						X
Marker assisted selection by SSRs		X	X	X	X			X	X	X	X			X	X	X		
Cloning and sequencing of regulatory genes in the different genetic materials						X	X	X	X	X	X	X	X	X	X			
Writing Papers										X	X	X	X	X	X	X	X	X
Formation/Communication and congress activities	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

6. Available funds (source and amount)

- 2022-2024. Bando PSR “Conservazione della biodiversità animale e vegetale”, Regione Lombardia. Progetto RISOLO (“Moltiplicazione, caratterizzazione e conservazione in Lombardia di sementi di qualità di riso destinati alla coltivazione biologica). Durata: 24 mesi; ruolo: Principal Investigator. 150000 Euro
- 2022-2024. Bando PSR “Mais tradizionali lombardi alpini recuperati (MAISAlpi)”, Regione Lombardia. Progetto (Ricerca, caratterizzazione e valorizzazione di cultivar di fagiolo tradizionali lombarde). Durata: 24 mesi; ruolo: Responsabile UO UNIMI. 35000 Euro
- Attualmente coinvolto in AGRITEH nello Spoke 1 (facente parte della “massa critica UNIMI) in diversi task ed in particolare nel 1.3.5. 90000 Euro

7. Literature:

Brandolini, A, and A. Brandolini, 2009: Maize introduction, evolution and diffusion in Italy. Maydica 54**,** 233-242. Food and Agriculture Organization of the United Nations, 2009: Crops Production. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567.

Marasas WFO, Miller JD, Riley RT, Visconti A, 2006. Environmental Health Criteria 219, Fumonisin B1.http://whqlibdoc.who.int/ehc/WHO_EHC_219.pdf

Panzeri, D., V. Cesari, I. Toschi, and R. Pilu, 2011: Seed calorific value in different maize genotypes. Energy Sourc. Part A Recov. Utiliz. Environ. Eff. 33, 1700-1705.

Pilu R., Cassani E., Sirizzotti A., Petroni K., Tonelli C. (2011) Effect of flavonoid pigments on the accumulation of fumonisin B1 in the maize kernel. Journal of Applied Genetics 52(2):145-52.

Rodriguez-Amaya, D. B., M. Kimura, H. T. Godoy, and J. Amaya-Farfan, 2008: Updated Brazilian database on food carotenoids: Factors affecting carotenoid composition. J. Food Compos. Anal. 21**,** 445– 463.

Tsuda, T., 2012: Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. Mol. Nutr. Food Res. 56**,** 159-170.

Venturelli, M. B., B. Purin, and M. Pirola, 1990: Maize for the production of polenta flour. Informatore Agrario 46**,** 70–71, 128–129.

Zeppa, G., M. Bertolino, and L. Rolle, 2012: Quantitative descriptive analysis of Italian polenta produced with different corn cultivars. J. Sci. Food Agr. 92, 412–417.