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UNIVERSITÀ DEGLI STUDI DI UDINE
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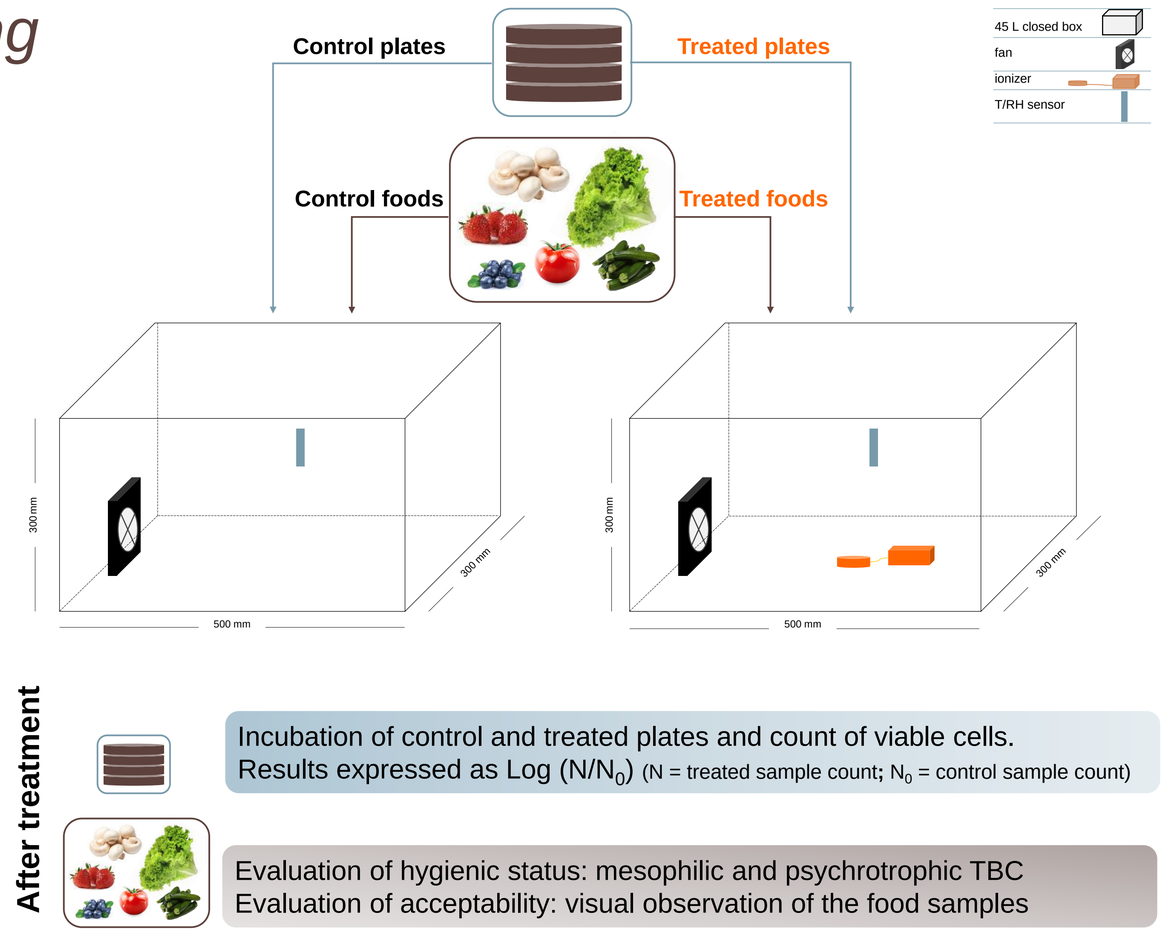
USE OF IONIZATION TECHNOLOGY TO CONTROL MICROBIAL CONTAMINATION IN FOOD STORAGE EQUIPMENT

Application of air ionizers inside domestic refrigerator for improving preservation of fruits and vegetables

AIMS

Food waste and food quality represent two worldwide challenges, especially in the context of domestic consumption.

The aims of this project are to improve food freshness and to reduce waste by keeping control of microbial spoilage and quality loss through the application of air ionization technology inside domestic refrigerators.
To achieve this purposes, the evaluation of the hygienic status and the assessment of the physico-chemical properties of foods after the exposition to ionization are evaluated.



APPLICATIONS

Air ionizers can be applied inside a domestic refrigerator to improve the storage of fruits and vegetables increasing the period of shelf-life. By enhancing the quality and the freshness of the foods, the consumers would be encouraged to consume them reducing the wastes.

The effect of two different ionizers have been evaluated independently on representative microbial species (*Listeria monocytogenes* 284 and *Pseudomonas fluorescens* L22) at room (RT*) and refrigeration (RefT**) temperatures and on foods (lettuce, mushroom, tomato, zucchini, strawberry and blueberry) at RefT**.

*22.6 °C ± 0.9 °C; 96 %RH ± 3 %RH
**5.3 °C ± 0.6 °C; 97 %RH ± 1 %RH

RESULTS

Both ionizers showed to have an effect in the decrease of microbial load, but the entity of the reduction was depended on the device. Ionizer A caused higher microbial reductions on either microorganisms, due to the levels of ozone generated (Fig. 1). Ionizer B, which effect was done by NAI alone, was effective only on *L. monocytogenes* 284 (Fig. 2). The results were reversed in the case of food treatment (Table 1). The amount of ozone generated by ionizer A was too high, resulting in evident oxidation phenomena, especially in the case of lettuce, even if it was used in an intermittent mode. In addition, TBC grew over time, probably because ozone damaged food tissue causing the release of nutrients. Ionizer B, instead, did not cause important visible oxidations maintaining the TBC stable over 4 days. However, more investigations should be done to understand the effect of the ionization treatment also on the physico-chemical properties of foods.

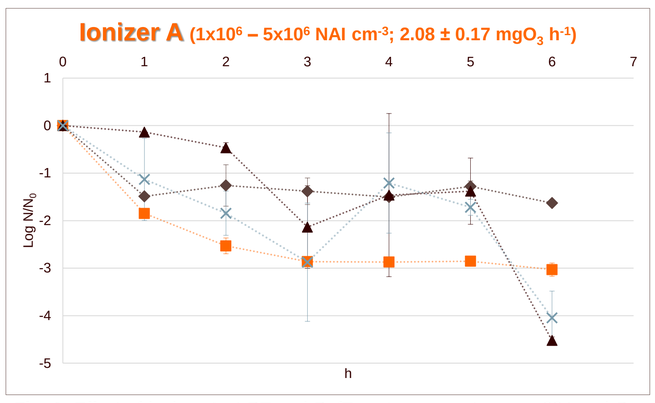


Fig. 1. Effect of ionizer A at RT and RefT on *L. monocytogenes* 284 and *Ps. fluorescens* L22

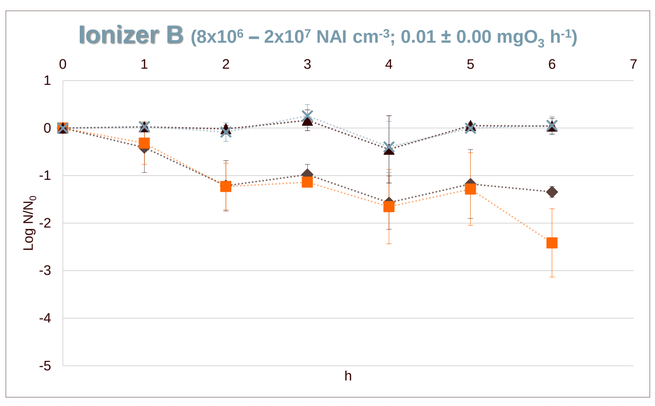


Fig. 2. Effect of ionizer B at RT and RefT on *L. monocytogenes* 284 and *Ps. fluorescens* L22

Table 1. Comparison of the effects of ionizers A and B on *in vitro* and food tests

	Ionizer A	Ionizer B
Device characteristics	Good electrical stability High NAI High ozone	High electrical stability High NAI Very low ozone
Test *in vitro* (RT and RefT)	Good efficacy in the reduction of viability towards different microbial species	Efficacy of the ionization related to the microbial specie
Test on foods (RefT)	Intermittent working mode Strong oxidation phenomena on foods	Continuous working mode No perceivable oxidation phenomena
Example: Lettuce, after 4 days of treatment		

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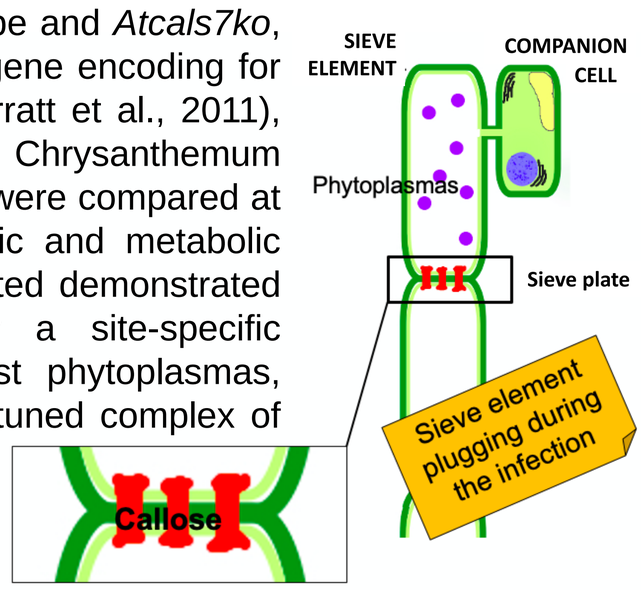
CALLOSE RESPONSE TO PHYTOPLASMA INFECTION: PHLOEM PLUGGING OR SOMETHING MORE?

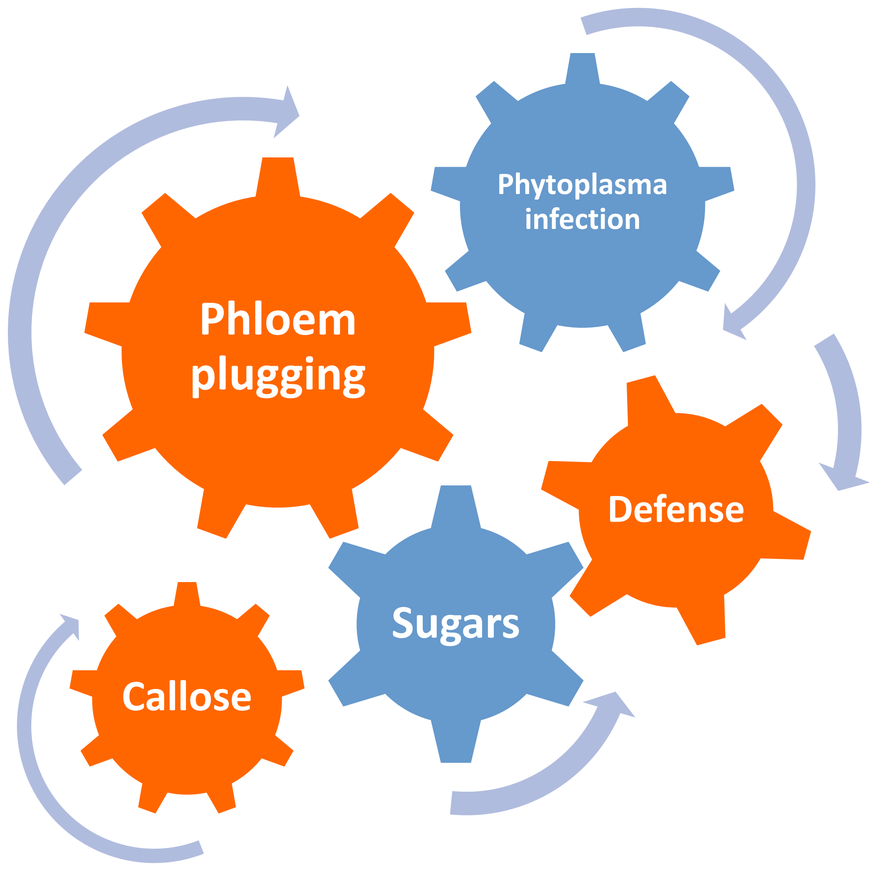
Callose is accumulated in plant tissues as a mechanical response to face pathogens and injuries, but its role is not only confined to a mechanical response.

AIMS

In this work we investigated about the changes in callose metabolism and sugar homeostasis during phytoplasma infection, to highlight how and how much callose and sugars are regulated during infection and involved in plant immune response and signaling.

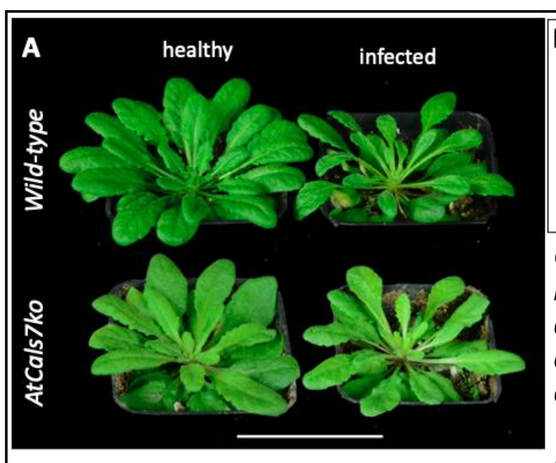
Arabidopsis thaliana wild-type and *Atcals7ko*, a mutant line in which the gene encoding for AtCALS7 was silenced (Barratt et al., 2011), healthy or infected with Chrysanthemum Yellows (CY)-phytoplasma, were compared at morphological, transcriptomic and metabolic levels. Results here presented demonstrated that callose is not only a site-specific mechanical defense against phytoplasmas, but it is also part of a fine-tuned complex of signals to limit the damages related to the infection.



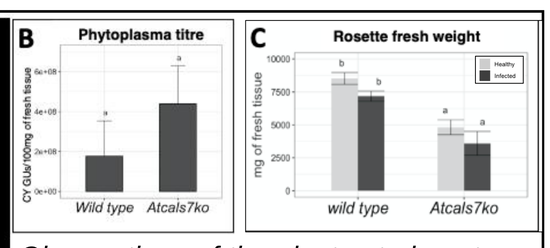


APPLICATIONS

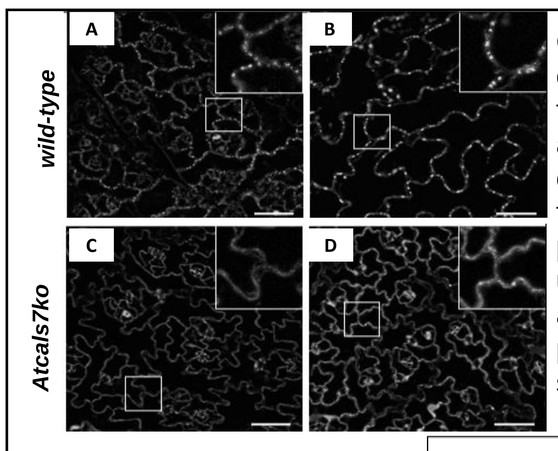
Phytoplasmas are pathogens affecting several economically-relevant crops and fruit trees (as apple or grapevine). The understanding of the mechanisms on which the defense processes are based, could provide important information to improve new strategies to control the infection level in the field. Actually, no effective treatments are able to limit phytoplasma, in exception of the precautionary techniques aimed to limit the spread of the pathogen. The knowledge on the natural bases of the defense will help to set-up environmental-friendly treatments.



Bar corresponds to 5cm



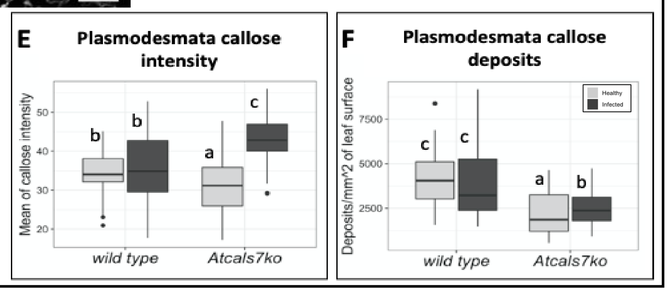
Observations of the plants at phenotype level (A and C) show a smaller dimension in the Atcals7ko line compared to wild type. Both lines show evident symptoms following phytoplasma infection. No differences in phytoplasma titres were found in both Arabidopsis line.



Confocal images indicate drastic changes in symplastic communication in the tissues of the mutant line, as an attempt of the plant to reduce the osmotic shock imposed by the sugar translocation impairment. Following phytoplasma infection, and probably under sugar signalling, communication among cells is further reduced, to block phytoplasma effector spread from the sieve elements to the distal tissues.

Analyses at confocal microscope reveal differences in the density and intensity of callose deposits in wild type plants compared to the mutant line (Fig. E and F) .

Bars=50um



RESULTS

Callose and sugar involvement in the response of plant against pathogens has been object of great interest (Bolton et al., 2009; Leuven et al., 2012; Rojas et al., 2014; Fatima and Senthil-Kumar, 2015; Dodds and Lagudah, 2016; Lee et al., 2016), because they are involved in several signaling processes (Lecourieux et al., 2014) and contribute to plant immune response, functioning as priming molecules for the rapid activation of defense against biotic and abiotic stresses (Bolouri-Moghaddam and van Den Ende, 2012).

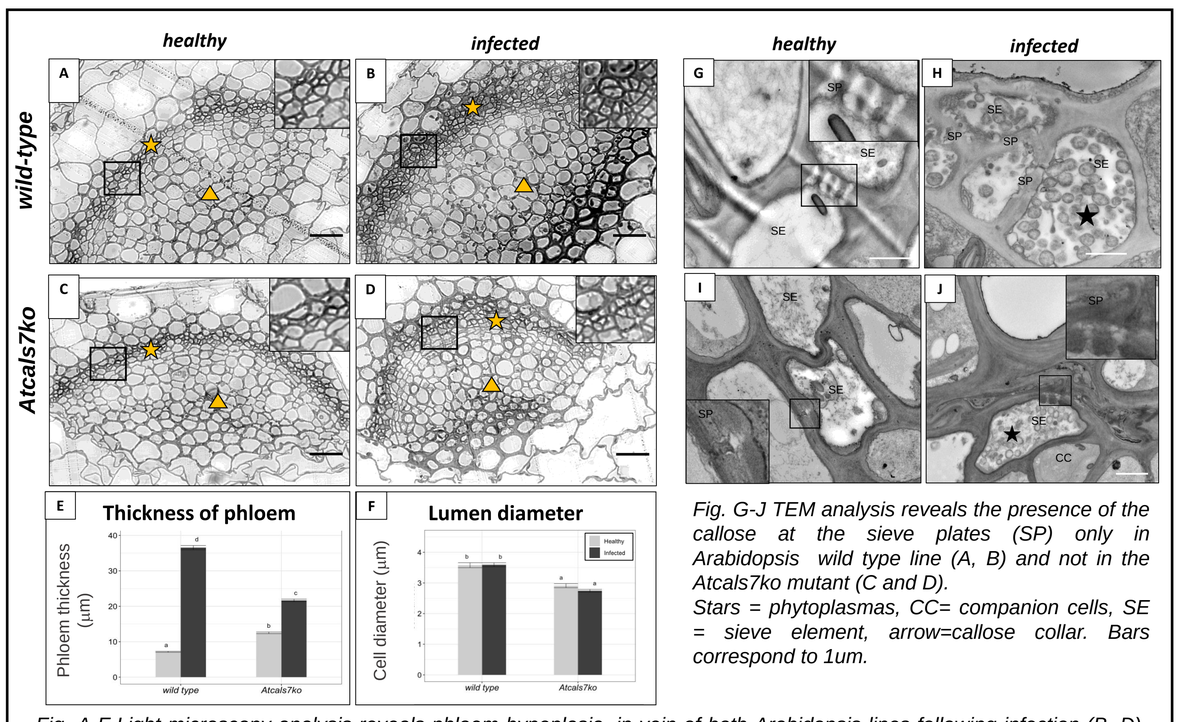
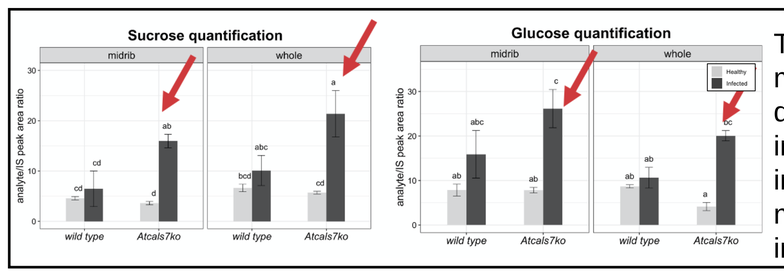


Fig. G-J TEM analysis reveals the presence of the callose at the sieve plates (SP) only in Arabidopsis wild type line (A, B) and not in the Atcals7ko mutant (C and D).
Stars = phytoplasmas, CC= companion cells, SE = sieve element, arrow=callose collar. Bars correspond to 1um.

Fig. A-F Light microscopy analysis reveals phloem hypeplasia, in vein of both Arabidopsis lines following infection (B, D). The lumen diameters in the Atcals7ko line is reduced. Star= phloem, triangle=xylem, bar= 25um

Light (LM, Fig. A-D) and transmission electron microscopy (TEM, Fig. G-J) observations reveal that the loss of callose at the site of phytoplasma infection causes aberrations in the phloem morphology, in particular at the sieve plates (Fig. G-J). The aberrations cause impairment in sugar translocation, as previously reported in *Atcals7ko* line (Barratt et al., 2011).



The quantification of the major sugars involved in the defense signaling was taken in consideration, revealing the increase of their amount in mutant line in case of infection.

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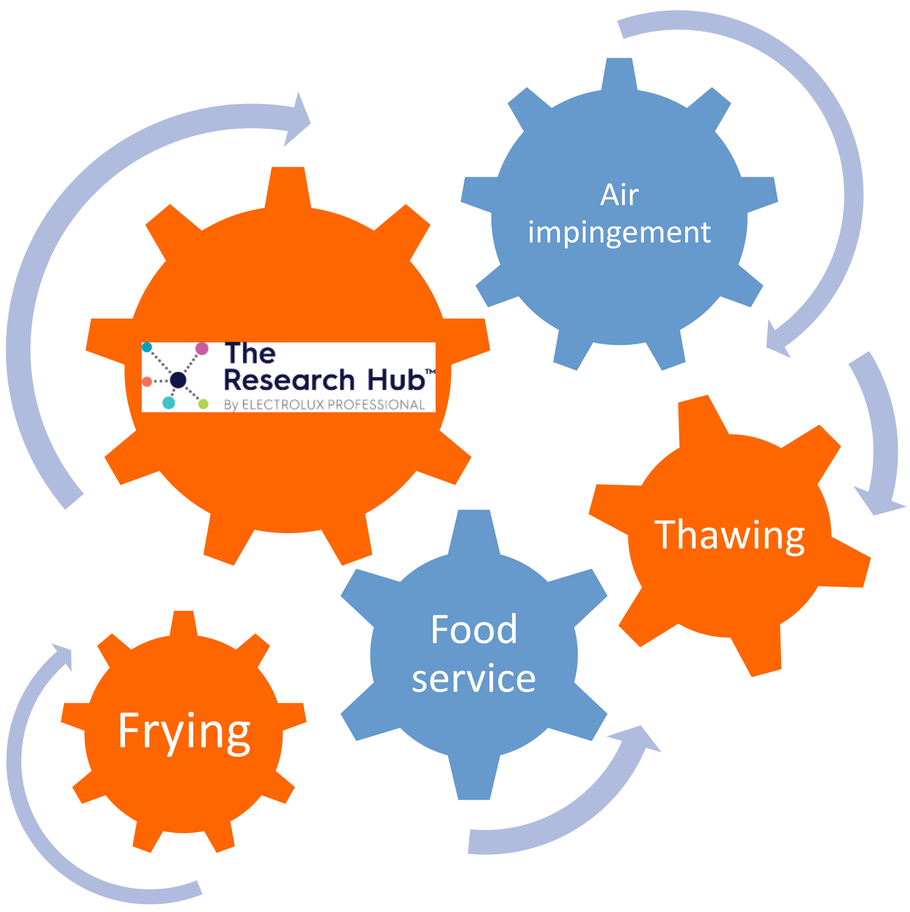
AIR IMPINGEMENT THAWING FOR FOOD SERVICE

Food thawing by air impingement as an alternative to refrigerator thawing before frying in quick service restaurants



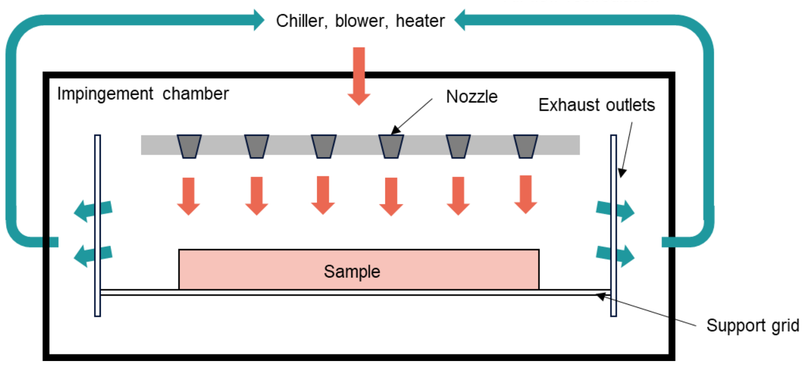
AIMS:

Aim of the project was to develop and validate a process of air impingement (AI) thawing for professional food service. Specific targets were:

- Development of an AI thawing prototype
- Definition of thawing cycles (target 0 °C) by a novel multiobjective optimization technique
- Comparison of the thawing performances of the prototype, with those of conventional refrigerator method (study case of chicken fingers)
- Validation of prototype thawing efficacy on different food products (French fries, squid, cod).

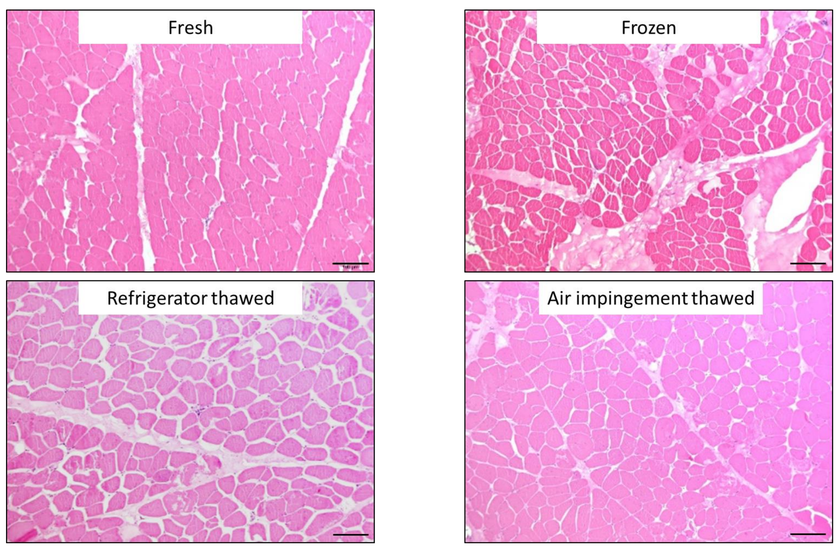
APPLICATIONS:

Quick service restaurants commonly purchase frozen products and thaw them at need before cooking. However, long thawing time associated to refrigerator thawing (even more than 1 day) is space consuming and might lead to large food losses, depending on operators planning skills. There is a need for a faster and easily implementable thawing method, which does not impair food quality after thawing and further frying.



RESULTS:

An efficient AI thawing prototype was developed (fig. left). Additionally, nine AI thawing cycles were selected minimizing thawing time, food temperature and its unevenness. AI allowed reduction of chicken fingers thawing time by 60-85% compared to refrigerator but resulted in similar thawing loss, WHC or firmness. Moreover, an AI cycle, minimizing chicken fingers temperature, showed promising results in preserving fresh tissue structure (fig. below). This cycle was applied before frying of chicken fingers, to verify if structural improvement affected frying performance. Mean values of fat uptake and firmness resulted favourably slightly lower. Thawing by AI and frying of French fries, squid and cod confirmed a significant thawing time reduction as compared to refrigerator while showing comparable thawing loss, cooking loss, firmness and fat uptake.



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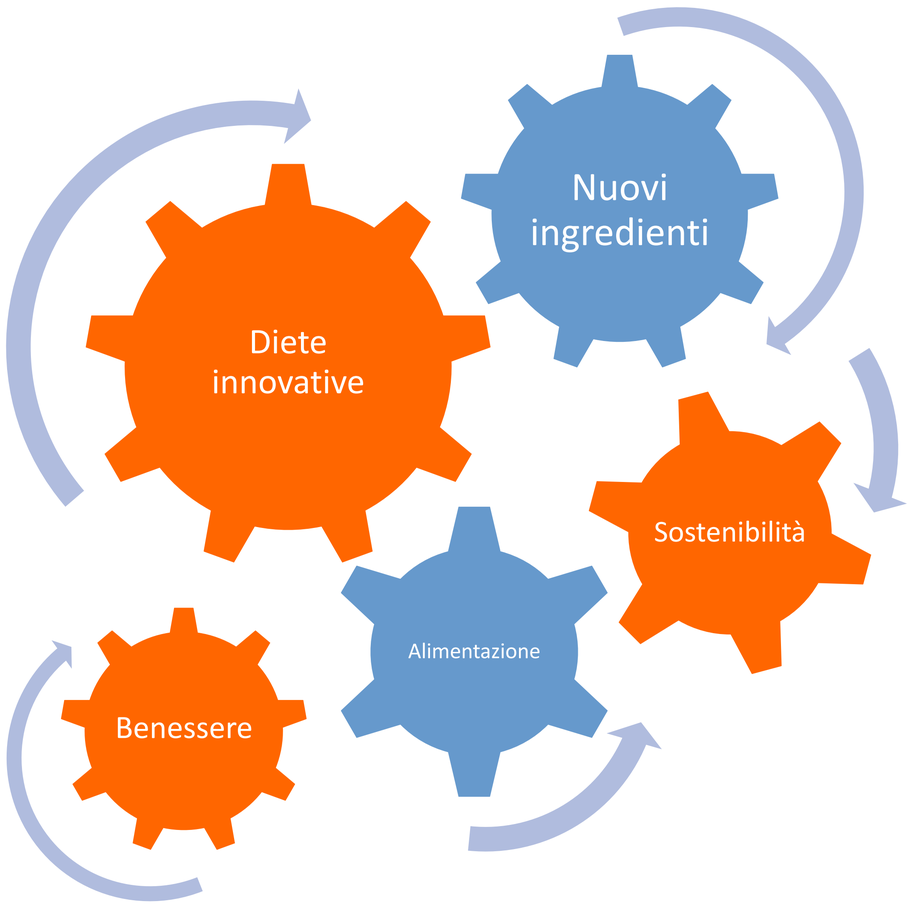
Nuovi ingredienti e sottoprodotti per migliorare sostenibilità, produttività, benessere e qualità delle specie ittiche allevate.

OBIETTIVI:

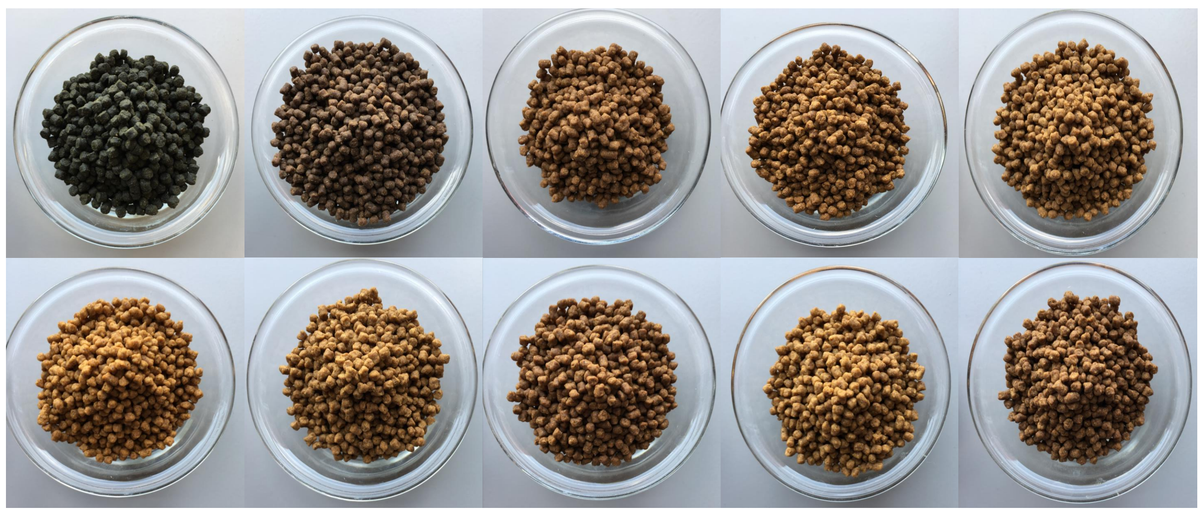
- Caratterizzazione di nuovi ingredienti (insetti, crostacei, microalghe e sottoprodotti della macellazione degli avicoli) per la progettazione di nuove formulazioni mangimistiche

- Valutazione della riposta biologica delle tre specie ittiche (*Dicentrarchus labrax*, *Sparus aurata* e *Oncorhynchus mykiss*) a diete contenenti gli ingredienti innovativi



AMBITO DI APPLICAZIONE:

La ricerca di nuovi fonti proteiche mira di ridurre l'impatto sulla fauna ittica selvatica prelevata per la produzione di farina di pesce e l'utilizzo di materie prime vegetali in competizione con l'alimentazione umana e con altri settori zootecnici. Il progetto ha come scopo l'incremento della sostenibilità dell'acquacoltura intensiva di specie marine e dulciacquicole (spigole, orate e trote) analizzando diversi ingredienti sostenibili da inserire in nuove formulazioni di diete innovative che permettano migliorare, o perlomeno non alterare, le performance di crescita, la qualità e il benessere dei pesci allevati.



RISULTATI:

Il fine del presente studio è stata la caratterizzazione del valore nutrizionale degli ingredienti innovativi presi in esame, determinando la loro composizione centesimale, degli elementi e il profilo degli acidi grassi e degli aminoacidi. Quindi, è stata determinata la digeribilità attraverso il metodo *in vivo* ed *in vitro*, osservando una differenza significativa tra i vari ingredienti. Successivamente sono state formulate e saggiate diete con diverse percentuali di inclusione degli ingredienti test. Le prove zootecniche e i parametri da esse derivanti hanno permesso di stabilire la dieta più performante. Per tutte e tre le specie in esame (*Dicentrarchus labrax, Sparus aurata e Oncorhynchus mykiss*) sono stati osservati i migliori parametri zootecnici nei pesci alimentati con diete inclusive di farine d'insetto e di sottoprodotti della macellazione degli avicoli.

SUSHIN
SUSTAINABLE FISH FEEDS
INNOVATIVE INGREDIENTS

PROGETTO Ager
AGROALIMENTARE E RICERCA

FONDAZIONI IN RETE
PER LA RICERCA
AGROALIMENTARE

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Approccio multimodale per la gestione dei bambini trattati per tumore della fossa cranica posteriore

Il ruolo della valutazione neuropsicologica estesa e delle metodiche di neuroimaging post-processing per lo studio dell'outcome cognitivo a lungo termine nei bambini con tumore della fossa cranica posteriore

OBIETTIVI:

1) Studiare le funzioni neuropsicologiche e linguistiche in bambini trattati nella Clinica Pediatrica di Udine per tumore della fossa cranica posteriore (PFT) attraverso specifiche batterie multimodali età-dipendenti
2) Correlare la performance neurocognitiva con il danno anatomico macro e microstruttutrale cerebrale valutato in risonanza magnetica (RM) rispettivamente con lo studio volumetrico e la trattografia

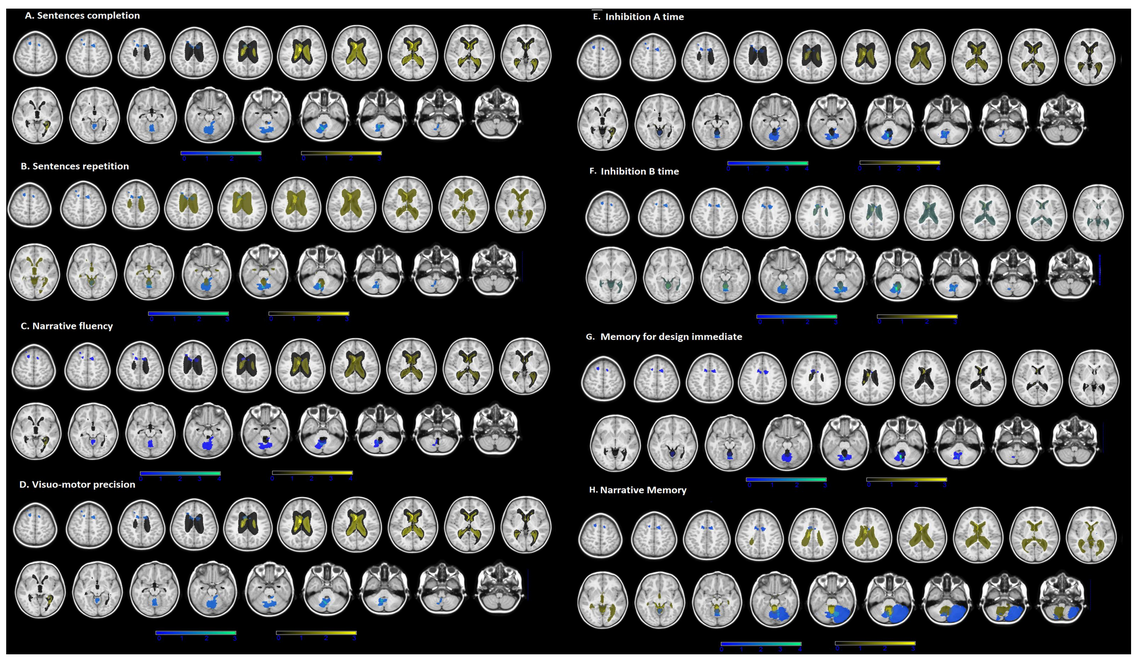

Figura 2

AMBITO DI APPLICAZIONE: L'utilizzo di scale multimodali come la NEPSY-II, la BVL 4-12 e la WISC-IV consente di esplorare in modo approfondito rispettivamente le funzioni neuropsicologiche, il linguaggio e le abilità cognitive nei bambini con tumore del sistema nervoso centrale. Correlare precocemente il danno anatomico cerebrale valutato in RM con gli specifici deficit consente di comprendere meglio le basi fisiopatologiche del danno neurocognitivo, di attivare strategie per ridurre la tossicità trattamento-dipendente migliorando l'adattamento psico-sociale e qualità di vita dei bambini.

RISULTATI: Nei 7 bambini con PFT arruolati abbiamo individuato un deficit della memoria immediata e ritardata, delle funzioni esecutive e del linguaggio. Nello studio post-processing di RM la dilatazione patologica dei ventricoli laterali e la lesione del verme cerebellare sono risultate associate alla compromissione neuropsicologica (figura 2). La ricostruzione dei fasci cerebellari ha mostrato come la ridotta integrità del fascio parieto-ponto-cerebellare correli con un minor quoziente intellettivo e peggiori prestazioni della memoria. Un ridotto volume del fascio spino-cerebellare correla invece negativamente con l'esecuzione di compiti manuali.

In conclusione l'estensione del danno cerebrale macrostrutturale e la perdita della sostanza bianca sono associate ad una performance psicologica inferiore in bambini con PFT (figura 1 e 3). Tali dati preliminari sono importanti per continuare a migliorare i protocolli terapeutici e di follow-up dei piccoli pazienti e ridurne le sequele mediche e neuropsicologiche a lungo termine.

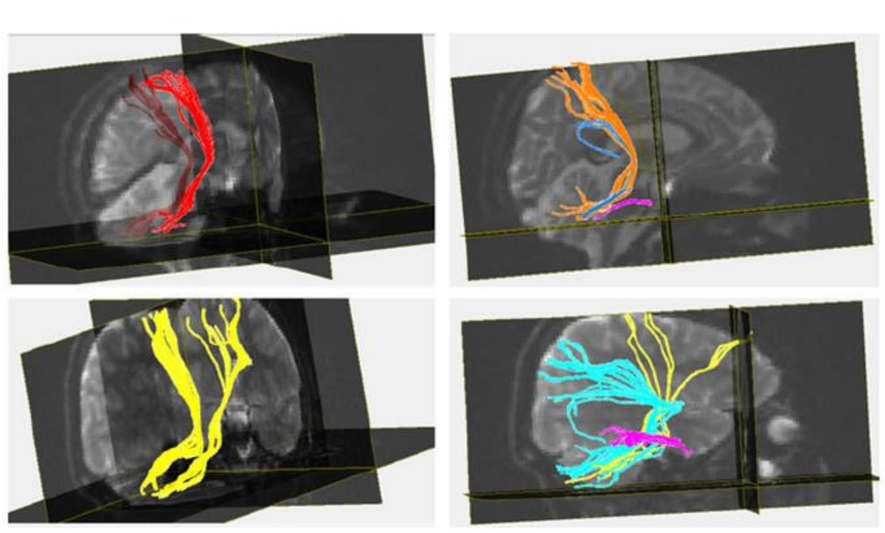

Figura 1

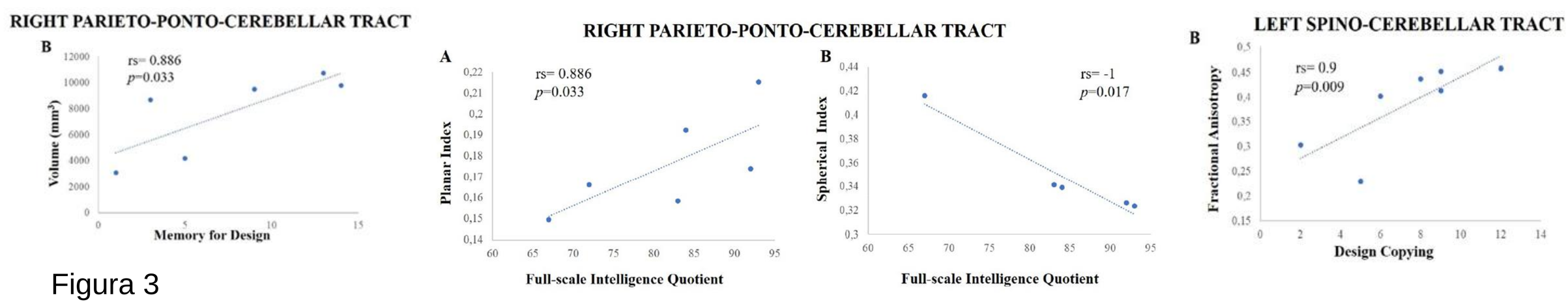

Figura 3

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NOVEL CATIONIC RUTHENIUM(II) COMPLEXES: SYNTHESIS AND CYTOTOXIC ACTIVITY TOWARD ANAPLASTIC THYROID CANCER CELLS

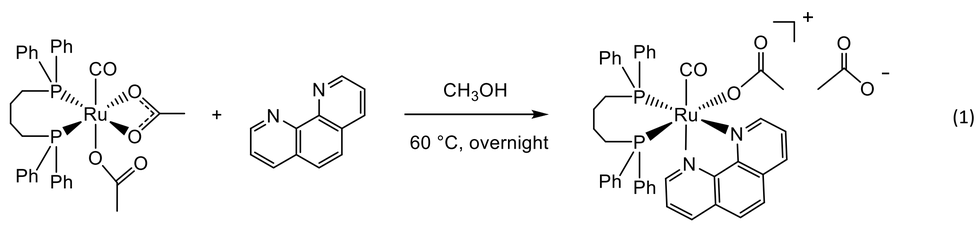
Denise Lovison, Lorenzo Allegri, Federica Baldan, Giuseppe Damante, Walter Baratta.

INTRODUCTION

Anaplastic thyroid cancer is a rare histotype of thyroid tumor characterized by a dramatic poor prognosis. The use of Doxorubicin and Cisplatin, showed no relevant effects in term of patients survival. For these reasons, the development of new compounds showing anticancer activity is still challenging. Transition metal complexes have been extensively investigated in the last decades as promising anticancer drugs, on account of their ability to interact with biomolecules through the metal or by intercalation with the ligands. Alternatives to the platinum derivatives are ruthenium complexes, which have been investigated as efficient anticancer drugs due to their low *in vivo* toxicity and inhibition of the cancer cell growth.

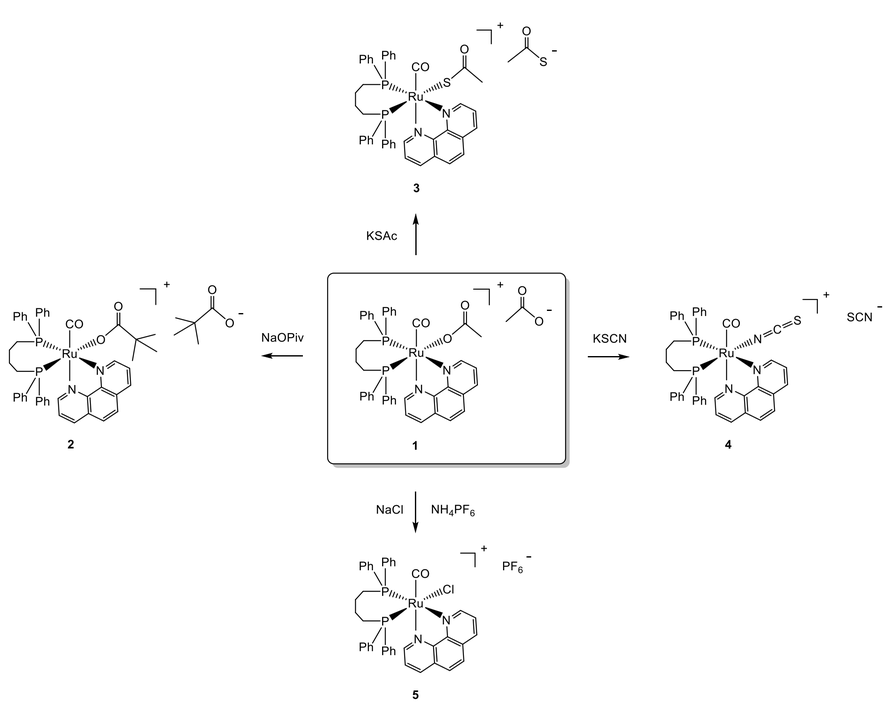
DISCUSSION

Herein we report the synthesis of new cationic carboxylate, thioacetate thiocyanate and chloride complexes [RuX(CO)(dppb)(phen)]X (X = anionic ligand) from the acetate precursor [Ru(η^1-OAc)(η^2-OAc)(CO)(dppb)] (Eq.1).



(1)

Due to the presence of a *trans* phosphine, a facile substitution of the OAc ligand is permitted. As a matter of fact, **1** promptly reacts with alkali carboxylate, thiocarboxylate, isothiocyanate and chloride salts, affording the derivatives [RuX(CO)(dppb)(phen)]Y, by displacement of OAc (**Scheme 1**).



Scheme 1. Syntheses of complexes **2-5** from 1 by displacement of the coordinated acetate in MeOH at 60 °C.

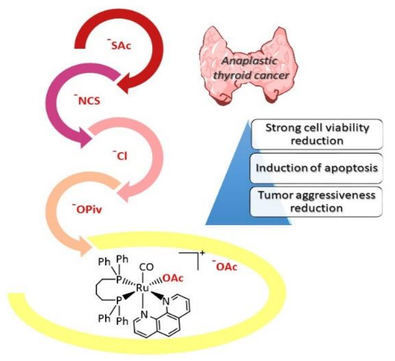


Figure 1. High cytotoxicity and antimetastatic properties toward anaplastic thyroid cancer is displayed by the cationic ruthenium(II) complexes [RuX(CO)(dppb)(phen)]Y (X = Y = OAc, OPiv, SAc, NCS; X = Cl, Y = PF6) easily obtained from [Ru(η1-OAc)(η2-OAc)(CO)(dppb)] with phen and substitution of the acetate ligand.

RESULTS

In order to test the effects of these complexes on cell viability in ATC cell lines, we performed an MTT assay after different doses administration of the compounds for 24, 48 and 72 h treatments. All tested compounds exhibited a strong cell viability decrease at different doses after 48 or 72 h treatment, showing a good effectiveness even compared with Cisplatin administration. Based on these data, we calculated EC_{50} at 72 h and the values are reported in **Table 1**. Interestingly, the pivalate **2** and thioacetate **3** reduce the cell viability at significant lower concentrations as low as 0.21-0.09 μM, more than twenty-times lower than Cisplatin.

Complex	EC_{50} of SW1736 cells [μM][a]		EC_{50} of 8505C cells [μM][a]	
	48 h	72 h	48 h	72 h
1	2.10 ± 0.41	1.24 ± 0.16	3.02 ± 0.21	2.40 ± 0.54
2	0.50 ± 0.08	0.19 ± 0.04	1.35 ± 0.47	0.10 ± 0.02
3	0.33 ± 0.03	0.21 ± 0.06	0.50 ± 0.07	0.09 ± 0.03
4	3.10 ± 0.68	2.77 ± 0.25	2.20 ± 0.62	2.80 ± 0.28
5	2.83 ± 0.25	2.80 ± 0.45	2.85 ± 0.31	2.84 ± 0.15
Cisplatin	16.20 ± 2.75	6.40 ± 1.54	12.00 ± 3.48	5.20 ± 1.82

Table 1. EC_{50} (μM ± SD) of complexes **1-5** and Cisplatin in ATC cells.

To evaluate whether the cell viability decrease, was due to apoptotic cell death, a Western blot analysis of cleaved-PARP protein levels was performed. We decided to test the carboxylate and thioacetate ruthenium complexes **1-3** which show the lowest EC_{50} values. The cells were treated with complexes **1-3** for 72 hours and then we investigated the cleaved-PARP levels increment, as a well-recognized marker of apoptosis. All three tested complexes were able to induce a strong increment of cleaved-PARP levels in both ATC cell lines. The highest increase was observed with the thioacetate complex **3** administration (**Figure 2**).

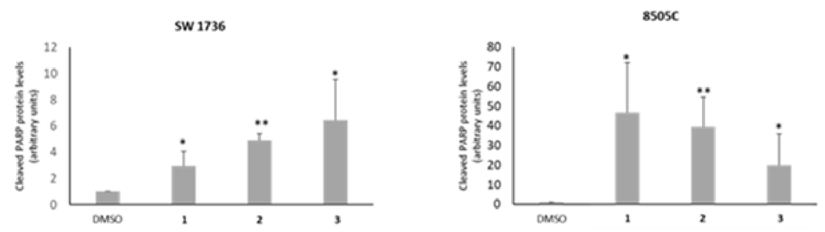


Figure 3. Densitometric analysis of cleaved-PARP fraction levels obtained with Western Blot assay in ATC cells treated with 1-3 at the respective EC_{50} doses or vehicle (DMSO) for 72 h. For each cell line, the results were normalized against Actin and expressed as percentage over control. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by Student's t-test.

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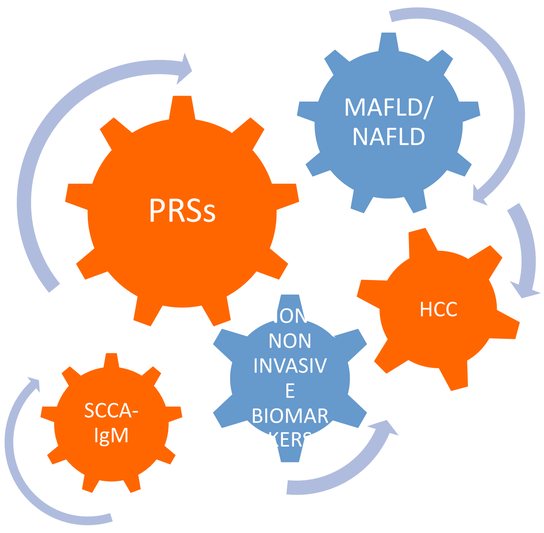
NON-INVASIVE BIOMARKERS FOR THE PREDICTION OF DESEASE PROGRESSION IN METABOLIC ASSOCIATED FATTY LIVER

BACKGROUND:

Metabolic associated fatty liver disease (MAFLD), also known as non-alcoholic fatty liver disease (NAFLD), includes a large spectrum of disease severity, from simple steatosis (NAFL) to steatohepatitis (NASH), with a potential progression to cirrhosis and hepatocellular carcinoma (HCC). During the last decades NAFLD has become one of the leading causes of chronic liver disease worldwide, with a global prevalence of 25-40%.
A complex inter-organ cross-talk involving genetic, hormonal, and environmental factors plays a central role in NAFLD pathogenesis and progression.
The lack of accurate non-invasive biomarkers useful in staging NAFLD and predicting evolutive disease is a major clinical unmet need.
Strong evidence produced in the last years support the role of genetic predisposition in NAFLD and HCC development; variants in gene involved in regulation of hepatic lipid metabolism, such as in PNPLA3, TM6SF2, MBOAT7, and GCKR, are robustly associated with hepatic fat content (HFC) and progression of liver diseases. More recently, Squamous Cell Carcinoma Antigen (SCCA)-IgM has been proposed as marker of cirrhosis and HCC in patients with alcoholic or viral chronic liver disease.



AIMS:

The principal aim of the study was to investigate the association between serum concentration of SCCA-IgM and severity of liver disease in a cohort of patients with NAFLD. Secondly, we examined the impact of a polygenic risk score of hepatic fat content (PRS-HFC), based on well-characterized risk variants, on NAFLD-HCC in a cross-sectional cohort of at risk individuals (NAFLD cross-sectional cohort), and we tried to optimize it by adjustment for a protective variant in HSD17B13 (PRS-5); finally, we identified the best diagnostic threshold.

MATERIAL AND METHODS:

The first cohort included 84 patients with histological diagnosis of NAFLD (39 with NAFL and 45 with NASH). Individuals with comorbidity known to be associated with increase levels of SCCA-IgM were ruled-out. SCCA-IgM levels were measured by the ELISA assay HepaIC®. In the multicentric NAFLD cross-sectional cohort 1,699 patients (of whom 82 from our centre) with NAFLD at different stages of disease and 865 healthy controls were enrolled. Participants were genotyped (in duplicated by TaqMan 5'-nuclease assays) for rs738409 (PNPLA3 I148M variant), rs58542926 (TM6SF2 E167K), rs641738 C>T variant at MBOAT7, rs1260326 (GCKR P446L), and rs72613567 (HSD17B13:TA). Genetic risk variants were combined in PRS-HFC and then adjusted for HSD17B13 (PRS-5). Mendelian randomization analyses were used to assess the causal role of genetic predisposition to NAFLD on HCC development. Diagnostic accuracy of polygenic scores was evaluated by ROC curves, and the best cut-off was identified.

RESULTS:

For the first part of the study 84 patients (39 with NAFLD and 45 with NASH) were enlisted. Patients baseline characteristic are shown in Table 1. SCCA-IgM levels were statistically significantly higher in patients with NASH than in those with simple steatosis (31.0 ± 7.2 ua/mL vs 9.2 ± 1.8 ua/mL respectively; p=0.007, Figure 1) and particularly in those with higher levels of fibrosis at the histological level (p<0.05, figure 2). SCCA-IgM levels at linear correlation analysis correlate with age (p <0.05), BMI (p=0.01), homocysteine (p=0.02), and histological degree of fibrosis (SAF-F score, p < 0.05).

In the multicentric case-control cross sectional study 1,699 subjects with NAFLD at different stage of disease and 865 controls were enrolled. Patients characteristics are shown in Table 2. PRS were influenced by the severity of liver disease, however while PRS-HFC increased progressively, PRS-5 was higher in patients with severe fibrosis than in those with HCC.

Genetic risk variants confer an increase in the risk of HCC that is proportional to the increase in the risk of NAFLD/MAFLD (p=0.02, Figure 4, B). Moreover, we observed a direct relationship between the risk of NAFLD/MAFLD and severe fibrosis, and between severe fibrosis and HCC. Using PRS in Mendelian randomization, we showed that HFC was causally associated with HCC independently of clinical variables (such as age, sex, T2D, or obesity) (p=1*10-5) and partly also of severe fibrosis (p<0.05). The AUROC of PRS-HFC for HCC diagnosis was 0.64. The best cut-off for the score was 0.532, with a 80% specificity and 43% sensitivity. PRS-5 accuracy to detect HCC was similar, and the best cut-off identifier was 0.495 (Figure 5). Finally, we demonstrated that positive PRS-HFC tests improved the detection of HCC in individuals over 40 years (p=1.0*10-2) and in diabetic patients independently of severe fibrosis (p=2.4*10-2) and PRS-5 predicted HCC risk also in non-obese subjects (p=4.2*10-2) (Figure 6).

	NAFL (n=39)	NASH (n=45)	p
Age (years)	57.4±1.6	50.7±2.8	0.03
Weight (kg)	85.9±2.5	97.2±4.7	0.02
BMI (kg/m²)	29.1±0.6	34.9±1.7	0.001
Total Cholesterol (mg/dL)	200±5	222±8	0.02
LDL Cholesterol (mg/dL)	116±5	138±7	0.01
ALT (U/L)	39±2	31±4	0.05
AST/ALT	1.4±0.1	1.1±0.1	0.01
TSH (mU/L)	1.4±0.1	2.2±0.2	0.001
SCCA-IgM (ua/mL)	9.2±1.8	31.7±7.2	0.007

Table 1. Characteristics of NAFLD cohort

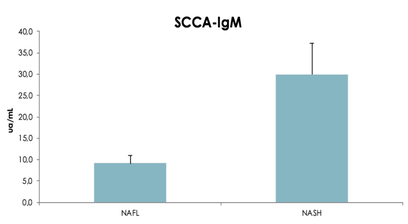


Figure 1. SCCA-IgM levels in patients with NAFL and NASH

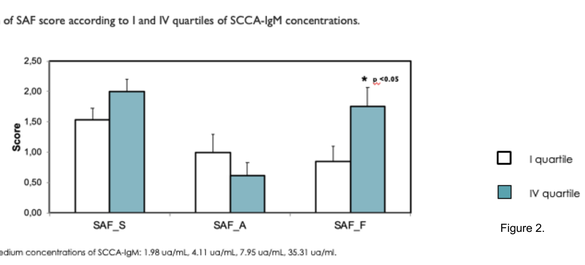


Figure 2.

	No liver disease (n=865, 33.7%)	NAFLD F0-F2 (n=1,176, 45.8%)	NAFLD F3-F4 (n=297, 11.6%)	NAFLD HCC (n=226, 8.9%)	p value
Age	44 ± 6	42 ± 16	58 ± 14	69 ± 9	<.0001
Sex, M	455 (52.6)	677 (57.6)	171 (57.6)	178 (78.8)	<.0001
T2D, yes	8 (0.9)	238 (20.2)	169 (56.9)	145 (64.2)	<.0001
BMI, Kg/m²	25.3 ± 5.0	32.7 ± 8.6	30.7 ± 5.1	30.2 ± 5.6	<.0001
PRS-HFC	0.266 (0.128-0.402)	0.392 (0.130-0.522)	0.457 (0.329-0.631)	0.459 (0.329-0.662)	<.0001
PRS-5	0.233 (0.065-0.394)	0.329 (0.128-0.459)	0.421 (0.256-0.597)	0.399 (0.266-0.660)	<.0001

Table 2. Characteristics of NAFLD cross-sectional cohort

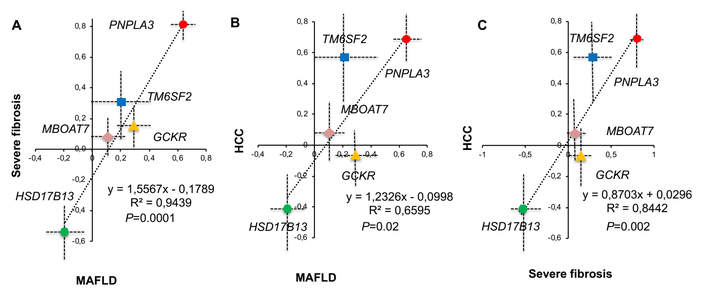


Figure 4. Mendelian randomization

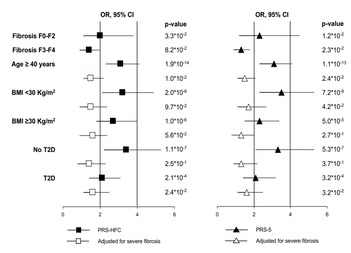
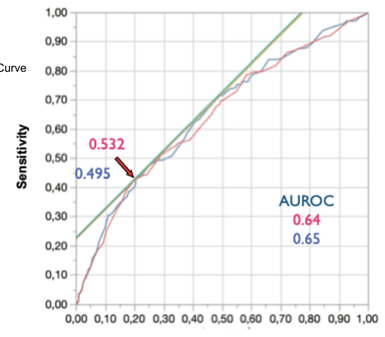


Figure 6. Mendelian randomization

Figure 5. ROC Curve



	PRS-HFC	PRS-5
OR	3.0	2.9
95% c.i.	(2.2-3.9)	(2.1-3.8)
p value	$3.7*10^{-14}$	$8.1*10^{-13}$
Sensitivity	43%	43%
Specificity	80%	79%
PPV	0.17	0.16
NPV	0.93	0.94
LR+	2.13	2.06
LR-	0.71	0.72

CONCLUSIONS:

In patients with NAFLD, SCCA-IgM levels were associated with the diagnosis of NASH and histological severity of hepatic fibrosis . To the best of our knowledge, the role SCCA-IgM in NAFLD has never been evaluated, therefore any accurate cut-off is available in this clinical setting. Further prospective studies will be crucial to confirm these findings.

In NAFLD cross-sectional cohort, our data are consistent with a causal association between genetically determined hepatic fat and HCC development, which was mainly - but not exclusively - mediated by predisposition to severe fibrosis. High PRS had a quite good specificity (≈80%) for detection HCC, and may be useful to stratify NAFLD-HCC risk and guide surveillance in patients with metabolic risk factors for HCC, independently of fibrosis severity. The role of PRS in NAFLD-HCC risk stratification should be evaluated in prospective studies.

Acknowledgement:

Professor L. Valenti and Medical Doctor C. Bianco, *Translational Medicine – Department of Transfusion Medicine and Haematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan*, for their support, statistical analysis, and data sharing.

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UNIVERSITÀ DEGLI STUDI DI UDINE
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Technological interventions driving structural design for improving food functionalities

Formulation and process strategies to create a new generation of food products

AIMS/OBIETTIVI:

The modern food system is called to create a new generation of food products able to satisfy not only consumer requirements, but also socioeconomic and environmental trends. The aim of this research activity was to design novel food structures with enhanced technological and nutritional functionalities. To this purpose, different innovative formulation and processing strategies were applied.

APPLICATIONS/AMBITO DI APPLICAZIONE:

This PhD research activity proposes a robust and effective method to develop food products with the desired healthy, sustainable and technological attributes. The "food design approach" applied is an iterative process consisting of the food optimization throughout its design, development and production (Fig 1). The proposed approach could be a useful tool not only for the research hub, but also for food industries.

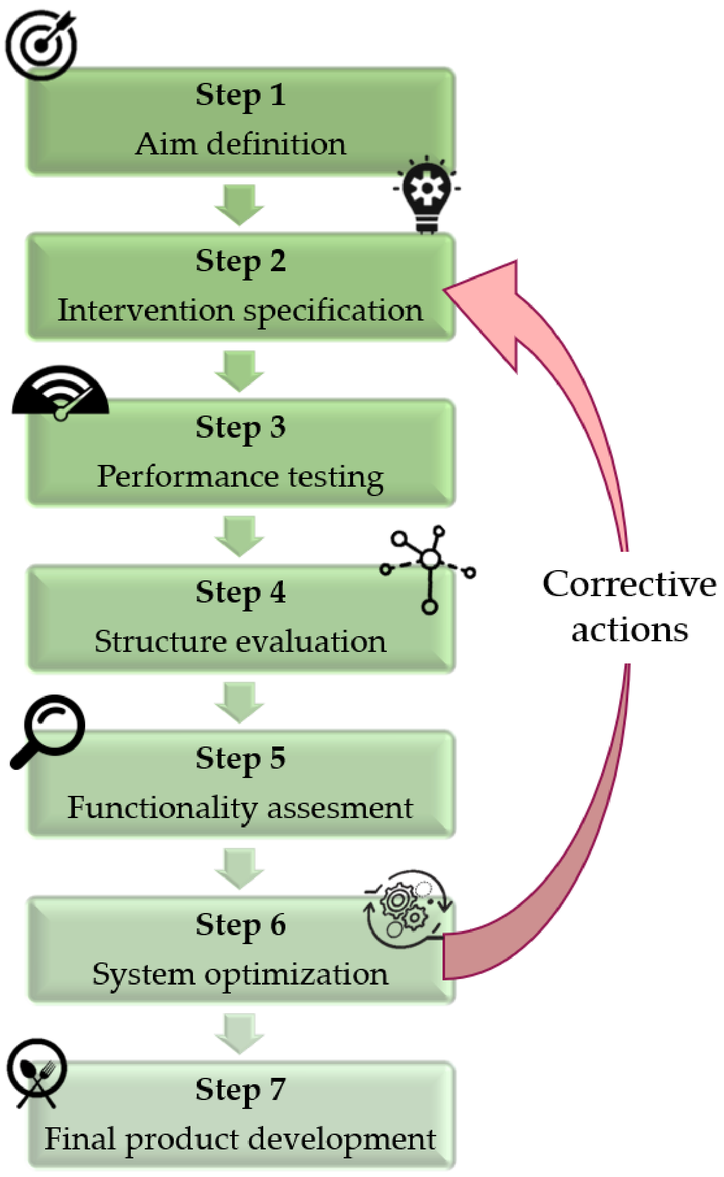
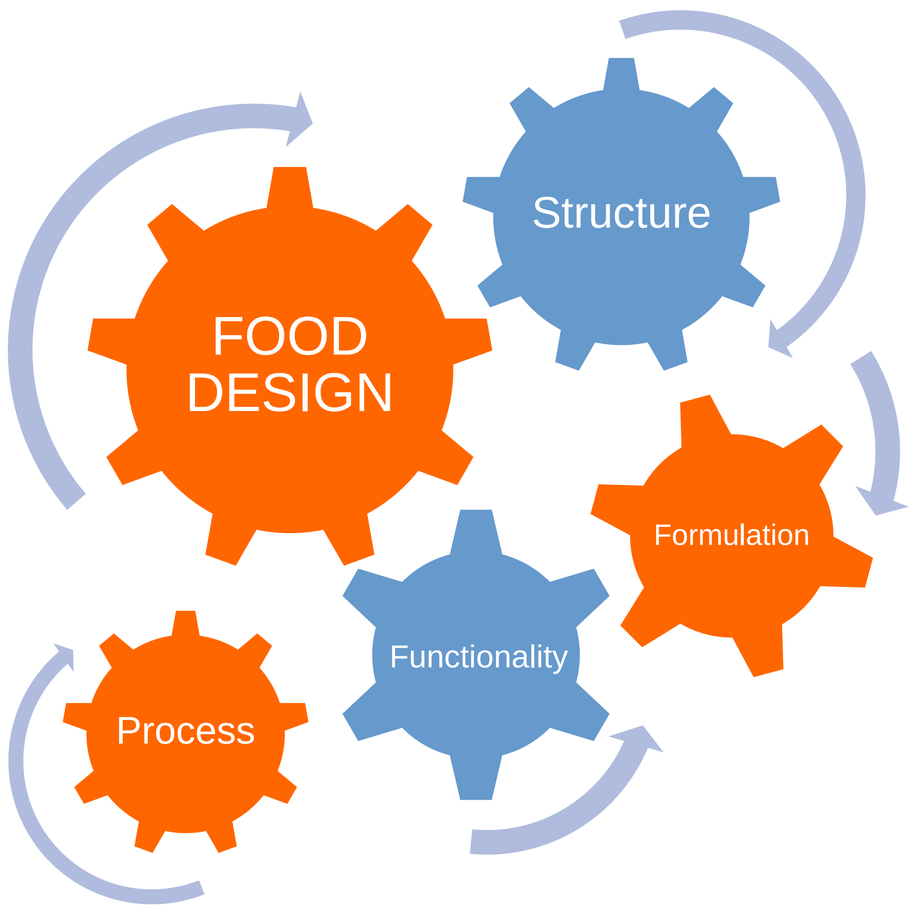


Fig. 1 The «food design approach»



RESULTS/RISULTATI:

During the PhD activity, the proposed "food design approach" allowed to:

- ✓ Design delivery systems for probiotic bacteria. The ability of different biopolymers and lipids to protect probiotics during process, storage and *in vitro* digestion was evaluated. Among all, monoglyceride-based gels containing milk were the best performing systems due to both their structural organization and formulation that guarantee physical protection and nutrients to probiotics (Fig 2). These systems were used to develop novel functional foods (i.e. ricotta cheese)
- ✓ Shape biopolymer functionality. The potentialities of novel and green technologies (high-pressure homogenisation, dense phase CO_2, pulsed electric fields) were used to improve both technological and nutritional functionalities of vegetable proteins and starch. Biopolymers thereof obtained could be used as novel ingredients for specific technological applications or for target population segments.

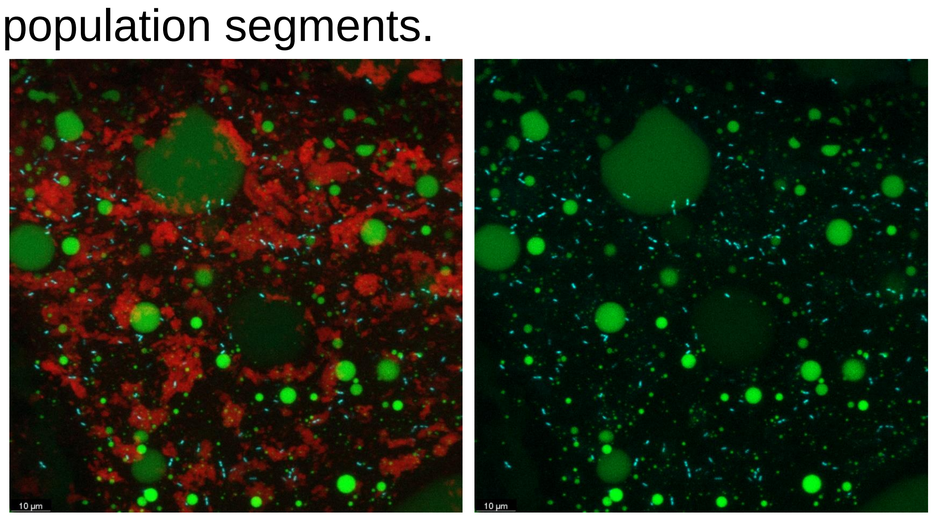


Fig. 2 Confocal laser scanning micrographs of monoglyceride-based gel containing milk (Red: proteins; Green: lipid phase; Cyan: microorganisms)

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IDENTIFICATION OF HISTOLOGICAL INDICATORS OF LARVAE AND JUVENILES QUALITY IN MARINE FISH SPECIES

A novel Multiparametric Semi-quantitative Scoring System for the quality assessment in gilthead sea bream and European sea bass larvae

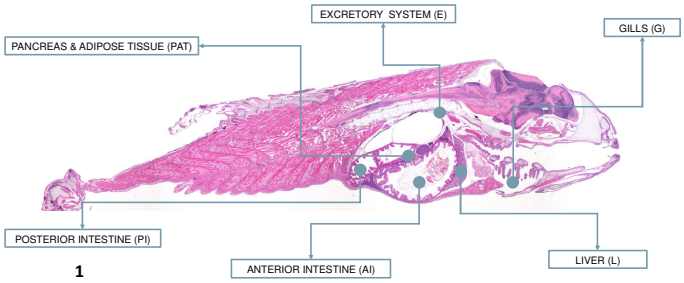
PerformFISH

AIMS

This PhD project is conducted within the framework of the Horizon2020 PerformFISH project, which aims at increasing the sustainability and the competitiveness of Mediterranean gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) farming. The objective of this research is to validate prognostic histological indicators of larval/juvenile quality allowing to promptly recognise health problems in a fish batch and help farmers to solve critical limits in the hatchery phase.

APPLICATIONS

In marine aquaculture, the hatchery phase still represents one of the main bottlenecks; for this reason, the interest of farmers and researchers in identifying new methods to evaluate and improve larval quality is high.The histological tools and knowledge deriving from this research can be effective for promptly recognize problems in larval batches and provide farmers with information about the impact of their managerial practices. Moreover, they can act as support both for hatcheries, as internal quality control at different developmental stages, and for on-growing farms for the control of incoming fry.



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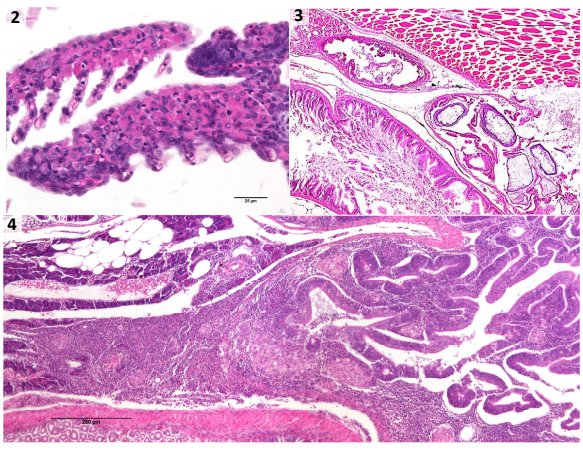
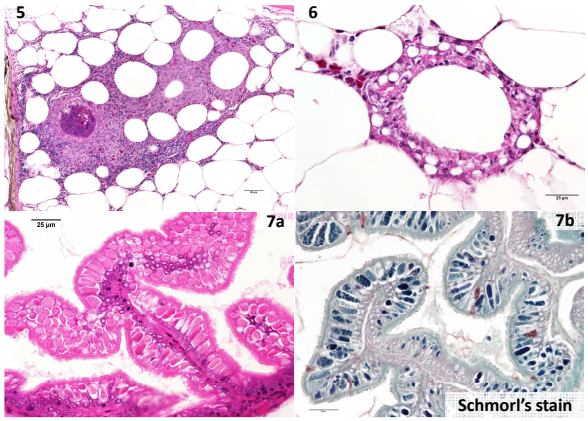
RESULTS

An original 5 points Multiparametric Semi-quantitative Scoring System (MSSS) was built. It includes 18 descriptors belonging to 6 organ districts (Fig.1). A weighted sum allows to obtain a single Cumulative Histological Index (CHI) that returns general information on larval batch quality. The use of the MSSS on a large number of samples from different EU hatcheries allowed to identify histopathological lesions providing farmers with useful information to detect managerial issues, such as inadequate physico-chemical water parameters (chloride cells hyperplasia Fig.2; nephrocalcinosis Fig.3) or problems in food management (inflammatory lesions involving intestine, pancreas and adipose tissue, Fig. 4,5). At the same time, immunohistochemical and histochemical investigations were conducted on supranuclear vacuoles (Fig.7a,b) and crown like structures (Fig.6).





This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727610.

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Biochemical, metabolic and molecular characterization of pear-apple hybrids: PEARAPPLE-Omics.

AIMS

Apple and pear are economically important fruit crops well known for their unique textures, flavours and nutritional qualities. Both genera are characterised by a specific pattern of secondary metabolites, which directly affect not only the resistance or susceptibility towards certain diseases, but also have significant impact on flavour and nutritional value of the fruits. The similar chromosome number, genome size, their recent divergence date, together with DNA-markers have led to the assumption that apple and pear genomes are highly co-linear.

Since hybrids between apple and pear provide a unique germplasm resource for genomic, transcriptomic and metabolic profiling studies, the main task of this project is to understand whether putative apple-pear hybrids available from Edmund Mach Foundation (FEM) (San Michele all'Adige (TN), IT), Plant & Food Research (PFR) (Palmerston-North, NZ) and University of Bologna (UniBo), are true hybrids or not. This research work is in cooperation with University of Udine.

This PhD project utilizes comparative genomic approaches; high resolution melting (HRM) SNP analysis, simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) SNP-chip analysis to identify genetic differences among the putative hybrids and its offspring. Furthermore, the PhD will describe the genus-specific metabolite pattern and will assess the resistance or susceptibility to Fire Blight, Apple Scab and Pear Scab in these plant materials. The use of genomics and other -omics technologies (metabolomics, transcriptomics) will provide insight into the genetic reorganization of the putative hybrids. In addition, it will enhance and accelerate the breeding process for the development of crops with improved characteristics for both producers and consumers, by introducing desired traits from the pear gene pool into apple and *vice versa*.

APPLICATIONS

Leaves were sampled from two sources: the published hybrid 'Zwintscher's Hybrid' (Fischer, *et al*. 2014) and its F2 progeny held at FEM, 32 putative apple-pear hybrids obtained from crosses of 'Cox's Orange Pippin' x 'Old Home' developed at PFR and three putative hybrid plants developed at Unibo (one from crossing of 'Abate' x 'Fuji' and two of 'Decana' x 'Murray').

For the SSR analysis of FEM samples, DNA was extracted from leaves of the four available parental accessions in the pedigree of 'Zwintscher's Hybrid' specified above, as well as 'Zwintscher's Hybrid' and the five putative F2 individuals using the NucleoSpin Plant II® Macherey Nagel kit. The DNA quality and quantity was evaluated with the NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™). For the SSR analysis of PFR samples and for HRM experiments for FEM and PFR plant material, DNA was extracted from milled freeze-dried leaves using a modified cetyltrimethylammonium bromide method (Doyle and Doyle, 1987; C. Kirk, pers. comm.). For SSR analysis of UniBo the DNA was extracted from freeze-dried young leaf material using the DNeasy® Plant Mini Kit (Qiagen, http://www.qiagen.com/). The DNA quality and quantity of the two later extractions were evaluated with the Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies Corporation).

At least twenty-one published SSR markers (Liebhard, *et al.* 2002; Silfverberg-Dilworth, *et al.* 2006; Yamamoto, *et al.* 2001; Yamamoto, *et al.* 2002 and Yamamoto, *et al.* 2007) mapping to different chromosomes were selected to check the degree of relationship between the hybrids and their parents. The SSRs data were analysed using GeneMarker, GenAlex and Whichparents software.

Prior to metabolomics analysis, fresh leaf samples of FEM materials (500 mg), PFR plants (500 mg) and UniBo *in vitro* plants (100 mg) were accurately weighed into a 15 mL plastic tube and extracted with 80% methanol in a ratio of 1/40 (w/v) for 24 h at 4˚C in the dark, after which the resulting supernatant of each sample was filtered into UPLC vials for analysis.

DNA content of 'Zwintzscher's Hybrid, 'Murray', 'Gala', 'André Desport', 'Abate' x 'Fuji', 'Decana' x 'Murray' 1 and 'Decana' x 'Murray' 2 was measured according to Plant Cytometry Services (Schijndel, The Netherlands).

Bioinformatics analysis to detect SNP variants unique to the apple or pear genomes, respectively, was performed prior to designing HRM 'apple/pear' primers. The LightCycler® 480 System was used to screen DNA samples that included 'Cox's Orange Pippin', 'Old Home', 'Zwintscher's Hybrid', its parents and various mixtures, to determine the efficiency of each primer. A set of 39 informative primers distributed along the genome were identified and screened over 47 putative hybrids.

RESULTS

HRM analysis confirmed again that 'Zwintscher's Hybrid' is a hybrid and also indicated the 32 putative apple-pear hybrids from 'Cox's Orange Pippin' x 'Old Home' apple-pear hybrids were true hybrids, with 9-15 primers per genotype providing evidence for hybridity. One to six primers per genotype provided evidence for hybridity of the five 'Zwintscher's Hybrid' F2 progeny held at FEM. Figure 1 shows an exemplar HRM result, where Melting Peaks and Normalized Melting Curves of both parents demonstrate homozygosity, (blue: apple 'Cox's Orange Pippin', red: pear 'Old Home'). Green peaks represent six of the 32 apple-pear hybrids as heterozygotes with double peaks.

The HRM results suggest that the apple and pear parents did not appear to contribute equally to the genomes of the progenies of 'Cox's Orange Pippin' and 'Old Home', unlike the 'Zwintscher's Hybrid', where the parents did contribute equally to the hybrid genome.

This result was confrmed by SSR and metabolomics analysis (Figure 2A). Furthermore, SSR analysis confirmed that the 'Abate' x 'Fuji' progeny and 'Zwintscher's Hybrid' were true hybrid, but the two from 'Decana' x 'Murray' and the five 'Zwintscher's Hybrid' F2 progeny were not (Figure 2). As result of population assignment, the lower log-likelihood value for the apple parents and grandparents ('Fuji', 'Murray', 'Cox's Orange Pippin', 'Kalco') on the X axis indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents and grandparents ('Abate', 'Decana', 'Old Home', 'Williams Christ' and 'Andre` Desportes') on the Y axis indicates population 2 as the most likely for pear.

The 'Abate' x 'Fuji' hybrid was located between the two parents. Moreover, the result of Whichparents analysis highlighted the presence of null alleles at five loci, probably due to the inability of apple markers to determine the presence of pear allele. The genetic results correlate with the metabolomics analysis, which confirmed that the putative hybrid 'Abate' x 'Fuji' and 'Zwintscher's Hybrid' accumulate the genus-specific secondary metabolite phloridzin from *Malus* ('Cox's Orange Pippin') and arbutin from *Pyrus* ('Williams Christ' and 'Old Home'). The two putative pear-apple hybrids from 'Decana' x 'Murray' accumulated only arbutin, the 32 putative apple-pear hybrids from 'Cox's Orange Pippin' x 'Old Home' and the five 'Zwintscher's hybrid' F2 progeny accumulated only phoridzin (Figure 3).

Significant differences (p=0.0001) in absolute DNA content of the *Malus* and *Pyrus* genotypes, as well as for the putative hybrids were found by flow cytometry. The DNA content of apples 'Gala' and 'Murray' was on average 1.51 pg/2C in comparison with pear cultivars at 1.12 pg/2C. The DNA content of 'Zwintscher's Hybrid' and the 'Abate' x 'Fuji' hybrid was 1.30 pg/2C, which is intermediate between the DNA content of the *Malus* and *Pyrus* parents. The two other putative hybrids ('Decana' x 'Murray' 1, 'Decana' x 'Murray' 2) have a DNA content closer to pear (Table 1).

Our results suggest that the putative hybrid from 'Abate' x 'Fuji' is a true hybrid, as is 'Zwintscher's Hybrid' (Fischer, *et al*. 2014).

This method can be used to identify apple/ pear hybrids and to use these in breeding to develop novel crops by introducing desired traits from the pear gene pool into apple and *vice versa*.

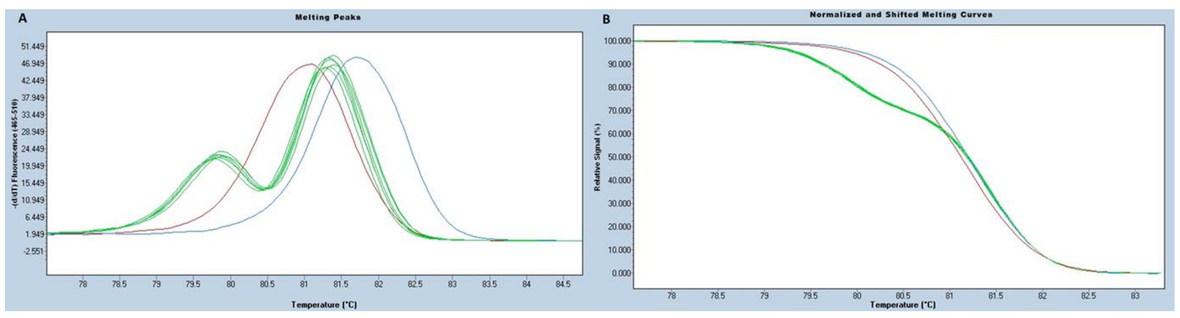

Figure 1: HRM result showing melting peaks (A) and normalized melting curves (B). Both parents are homozygous for this marker, the blue curve is apple, 'Cox's Orange Pippin', and red curve is the pear parent, 'Old Home', while the green curves represent the heterozygous nature of six apple-pear hybrids (CO 10-15) with double peaks in Figure 1A.

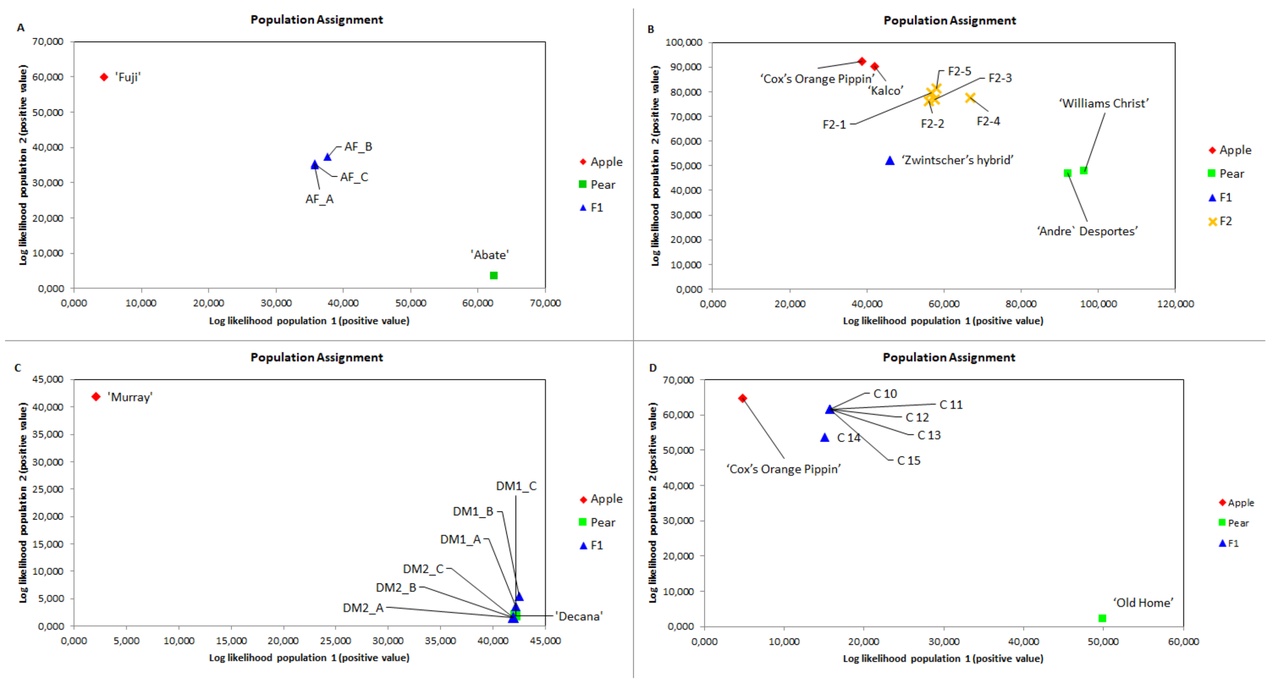

Figure 2: Population assignment of putative hybrids as deduced from the SSR marker analysis. Chart represents the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Fuji', 'Murray', 'Cox's Orange Pippin', 'Kalco') X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents ('Abate', 'Decana', 'Old Home', 'Williams Christ' and 'Andre` Desportes') on Y axes indicates population 2 as the most likely for pear. The three replicates of the putative hybrid from 'Abate' x 'Fuji' (AF) (A) and the 'Zwintscher's Hybrid' (B) were located between the two parent groups, the three replicates of two putative pear-apple hybrids from 'Decana' x 'Murray' (DM1, DM2) were located near pear (C),the five 'Zwintscher's Hybrid' F2 (F2) progeny were located near apple (B), and the 6 of 32 putative apple-pear hybrids from 'Cox's Orange Pippin' x 'Old Home' (CO) were located near apple (D).

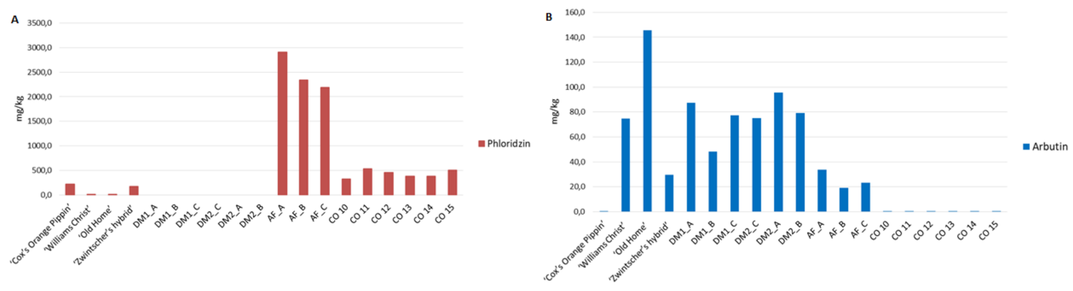

Figure 3: Metabolomics analysis. The three replicates of the putative hybrid from 'Abate' x 'Fuji' (AF) and 'Zwintscher's hybrid' accumulated both genus-specific secondary metabolites phloridzin from *Malus* (A) and arbutin from Pyrus (B), while the three replicates of two putative pear-apple hybrids from 'Decana' x 'Murray' (DM1, DM2) accumulated only arbutin, and six apple-pear hybrids from 'Cox's Orange Pippin' x 'Old Home' (CO 10-15) accumulated only phloridzin.

Table 1: Cellular DNA content.

Cultivar	Mean DNA content [pg] ± SD
'Zwintzscher's Hybrid'	1.30 ± 0.01
'Murray' (apple)	1.54 ± 0.01
'Gala' (apple) in vitro	1.49 ± 0.01
'André Desport' (pear)	1,12 ± 0.01
'Abate' x 'Fuji'	1.29 ± 0.01
'Decana' x 'Murray' 1	1.19 ± 0.01
'Decana' x 'Murray' 2	1.20 ± 0.01

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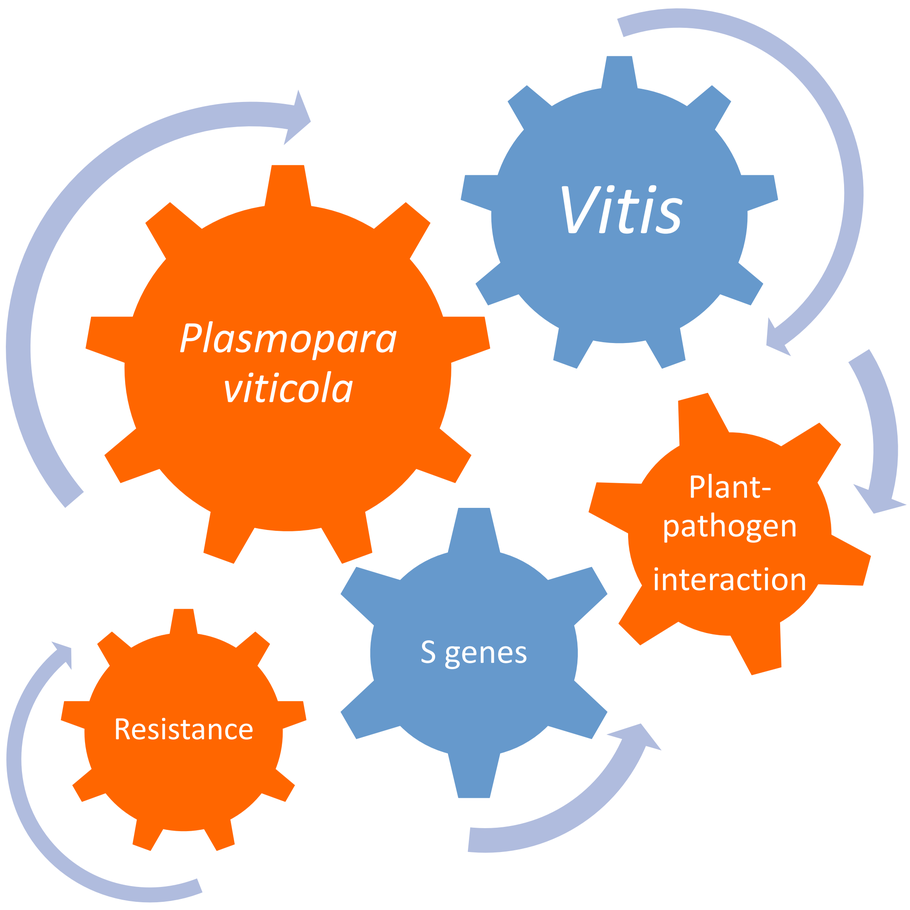
Exploring the role of downy-mildew susceptibility genes in *Vitis* by studying the genetic variation and the *in-vivo* function

DMR (Downy Mildew Resistant) and DLO (DMR-like Oxygenases) are susceptibility (*S*) genes to downy mildew (DM) in several herbaceous plant species.

AIMS:

DM is one of the most widespread diseases in viticulture, caused by the Oomycete *Plasmopara viticola*. A groundbreaking research was pursued in the discovery of *DMR* and *DLO* gene roles in *Vitis* by pairing the genetic investigation of natural mutants with the functional characterization of the *Vvdmr6.1* gene Knock out. Aim of the project was to evaluate the impact of natural and induced mutations in *VvDMR6.1* from a phenotypic point of view.



APPLICATIONS:

Since its emergence, many have been the attempts to control DM in viticulture. In the last decades, in order to avoid an ever-increasing use of fungicides, many resistant varieties were developed taking advantage of resistance (*R*) loci, which are now showing to be easily overcome by the pathogen in few years. Since *S* genes don't directly interact with pathogen effectors, their inactivation brings to a broad-spectrum resistance, which coupled with the use of a lower amount of fungicides and/or *R* genes could provide a stable level of protection against DM.

RESULTS:

The integration of the results obtained from the two lines of investigation allowed to draw a broad framework which needs however further investigation. The genetic study was able to scavenge the presence of natural-occurring mutations on *S* genes. Some of these mutations likely impact on the protein function and in a few cases the mutation was related to disease resistance. This association needs however further validation. The functional analysis of the edited plants, highlighted that more members of this gene family are functionally integrated in response to DM. These results pave the way for the exploitation of a new source of resistance to DM in grapevine.

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Metabolomics as a tool for the assessment of agronomical parameters of Ribolla Gialla for the production of sparkling wine

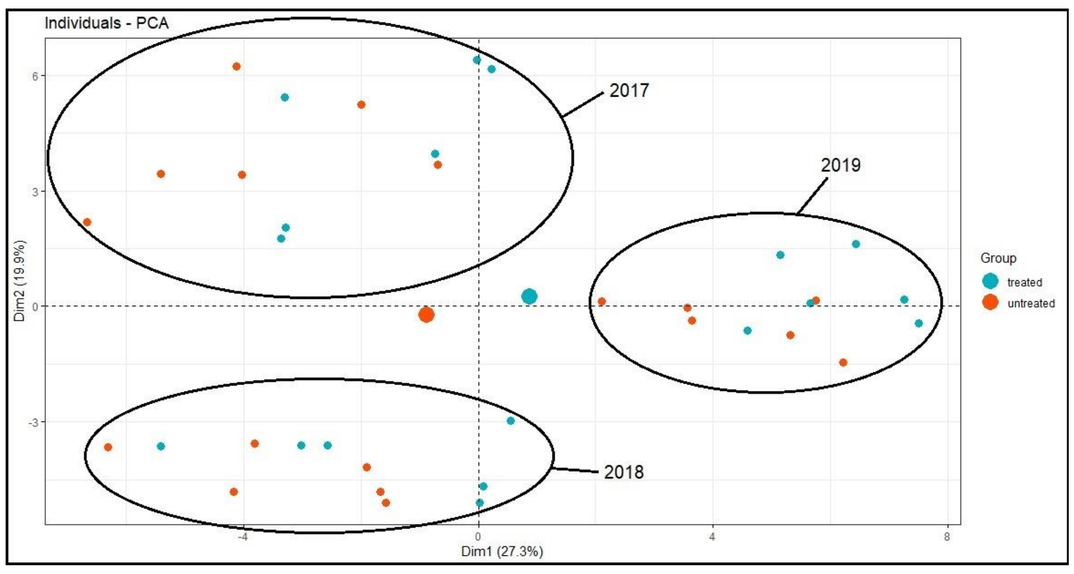
AIMS:

Ribolla Gialla is an indigenous and an old white grape variety mostly cultivated today in North-Eastern Italy (in the Friuli Venezia Giulia region), in Slovenia and on the Ionian Islands in Greece. Despite the increasing area of cultivation with Ribolla Gialla, there is little information about the effects of cluster thinning used in the vineyard on the quality of monovarietal sparkling wines. Using the metabolomic approach during wine production can therefore help to improve chemical and sensorial characteristics of the aforementioned wines.

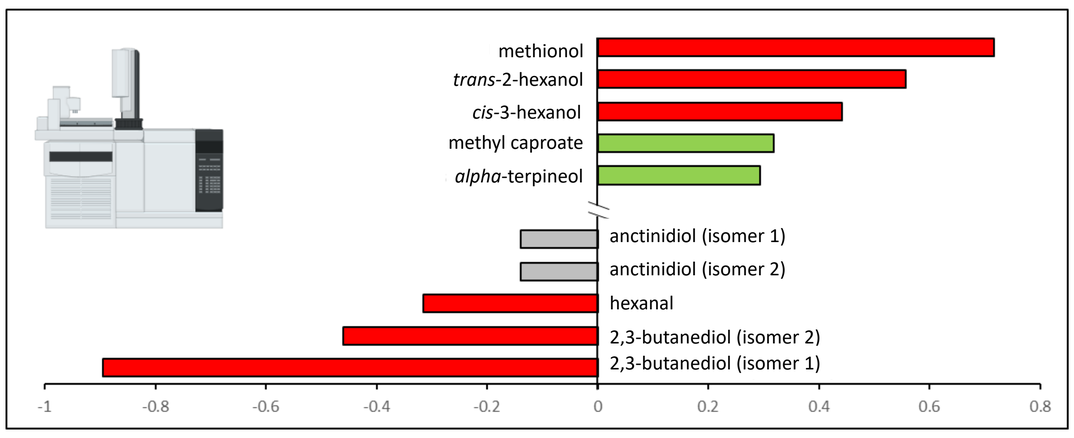
METHODOLOGY:

The viticultural trial was carried throughout 2017-2019 growing seasons in two commercial vineyards in different DOC (Denominiazione di Origine Controllata) districts (Corno di Rosazzo and Casarsa della Delizia) in the Friuli Venezia Giulia region in North-East Italy. The vines were divided into two treatments in each vineyard site: no thinning (U), and 20% thinning (T) at the veraison. At the optimal phenological and technological maturation, the grapes were harvested, crushed and pressed, and all the musts obtained were immediately inoculated with selected *Saccharomyces cerevisiae* yeast. The sparkling wines were produced by the Charmat method, where the base wine is left to undergo a second alcoholic fermentation in sealed autoclaves. By using SPME-GC-MS/MS, and UHPLC-MS/MS methods, the content of volatile compounds[1], the metabolites of aromatic amino acids[2], and the lipids[3] were determined in the sparkling wines.

RESULTS:



In the data obtained, a strong vintage effect initially emerged after PCA analysis, which was subsequently normalized to the standard deviation 1 and the mean 0 (z-scale) in order to facilitate the examination of grape yield reduction impact.



Log2 fold change analysis represents the logarithm of the average concentrations of volatile compounds in sparkling wines, comparing the effect of cluster thinning respect to control. Positive numbers indicate that the concentration in the treated samples is increasing while negative numbers indicate the opposite. The different colors in the histogram represent a positive (green) or negative (red) contribution to the wine's aroma.

It was observed that the concentration of *alpha*-terpineol, an important monoterpene that is characterized as “floral” feature in wines, was increased in the treated samples. However this represents one of the few positive contributors to the flavor profile in sparkling wines, while compounds such as methionol, *trans*-2-hexanol and *cis*-3-hexanol represent less pleasant aromas, as they are described using sensory descriptors respectively as "burnt milk", “fat" and, "astringent".

a)

Aromatic amino acid metabolites	T / U
L-tyrosine	40%
phenylalanine	-29%
tryptophan	-13%
kynurenic acid	10%
nicotinamide	-20%
L-Tryptophan ethyl ester	-30%
L-Tyrosine ethyl ester	-16%
N-acetyl-L-tyrosine ethyl ester	46%
tyrosol	0%
hydroxytyrosol	-2%
phenylacetic acid	-9%
tryptophol	7%
indole-3-acetic acid	-39%
indole-3-lactic acid	9%
indole lactic acid glucoside	-5%
N-acetylserotonin	-3%
phenylacetic acid	4%
tryptophol-SO3H	-17%
anthranilic acid	-3%
abscisic acid	10%
abscisic acid glucoside	-7%

b)

Lipids	T / U
lupeol	-21%
ergosterol	0%
ethyl stearate	-50%
linoleic acid	3%
behenic acid	0%
linolenic acid	0%
stearic acid	-4%
palmitoleic acid	-24%
lignoceric acid	-4%
arachidic acid	0%
oleic acid + *cis*-vaccenic acid	-2%
myristic acid	-2%
palmitic acid	-3%
miristoleic acid	2%
margaric acid	-5%

Percentual change in the concentration of tryptophan metabolites (a) and lipids (b) in the thinning treatment (T) compared to the control (U) as an average of three years and two locations. Green to red hues represent changes in concentration from more negative to more positive.

CONCLUSIONS:

The cluster thinning showed a minimal positive effect on the volatile composition of the wines produced and a positive effect related to the concentration of aromatic amino acid metabolites, which means a reduced likelihood of atypical aroma formation in the later stages of wine aging. Moreover, the cluster thinning had also limited effect on the presence of the lipids, considering their contribution to the wine aroma complexity.

***References*:**

[1] S. Carlin, U. Vrhovsek, P. Franceschi, C. Lotti, L. Bontempo, et al.; Food Chem. 208 (2016) pp 68–80.
[2] P. Arapitsas, G. Guella, F. Mattivi; Sci. Rep. 8 (2018) pp 1–13.
[3] A. Della Corte, G. Chitarrini, I.M. Di Gangi, D. Masuero, E. Soini, F. Mattivi, U. Vrhovsek; Talanta. 140 (2015) pp 52–61.

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MOUNTAIN PRODUCTS AND ECOSYSTEM SERVICES: ASSESSMENT METHODS AND ENHANCEMENTS STRATEGIES

The added value of mountain products

AIMS:

The gradual abandonment of traditional activities and the difficulties faced in promoting local productions, are challenging mountain cultural and natural heritage.
This study aims to identify and quantify ecosystem services in mountain dairy farms in Easter Alps (Veneto, Trentino Alto Adige, Friuli Venezia Giulia and Carinthia), in order to add a hidden value into a broader food quality concept.

METHODS:

Seventy-five dairy cattle farms conferring milk to 10 cooperative dairies were sampled.
The following methodological approaches were applied:

- environmental footprint (Life Cycle Assessment, LCA);
- animal welfare (animal-based measures - ABM- assessed complying with the EFSA adapted protocol on small-scale dairy farms);
- biodiversity (analysis of species in the grasslands managed by each farm).

Correlations among the different indicators were tested to analyze synergies and trade-offs in a multi-indicators approach.

RESULTS:

- The impact categories calculated with LCA approach evidenced that the traditional managing using pasture and summer farms, do not worsen the environmental footprint indicators but greater milk productivity resulted in a lower impact per unit of milk.
- Quartiles were identified for all ABM in order to suggest critical/achievable levels of animal welfare. Good performance was found for what concern body condition score, cleanliness and clinical indicators such as nasal discharge.
- The results highlights a decrease in species richness and phytosociological classes botanical composition due to the increase milk production and forage self-sufficiency.

The innovative approach allows not only to support the high-quality value of the mountain products but to improve the external quality attributes by adopting effective communication strategies and meeting consumer decision.

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OVEREXPRESSION AND ANALYSIS OF THE MOLECULAR TARGET OF APPLE PROLIFERATION PHYTOPLASMA EFFECTOR

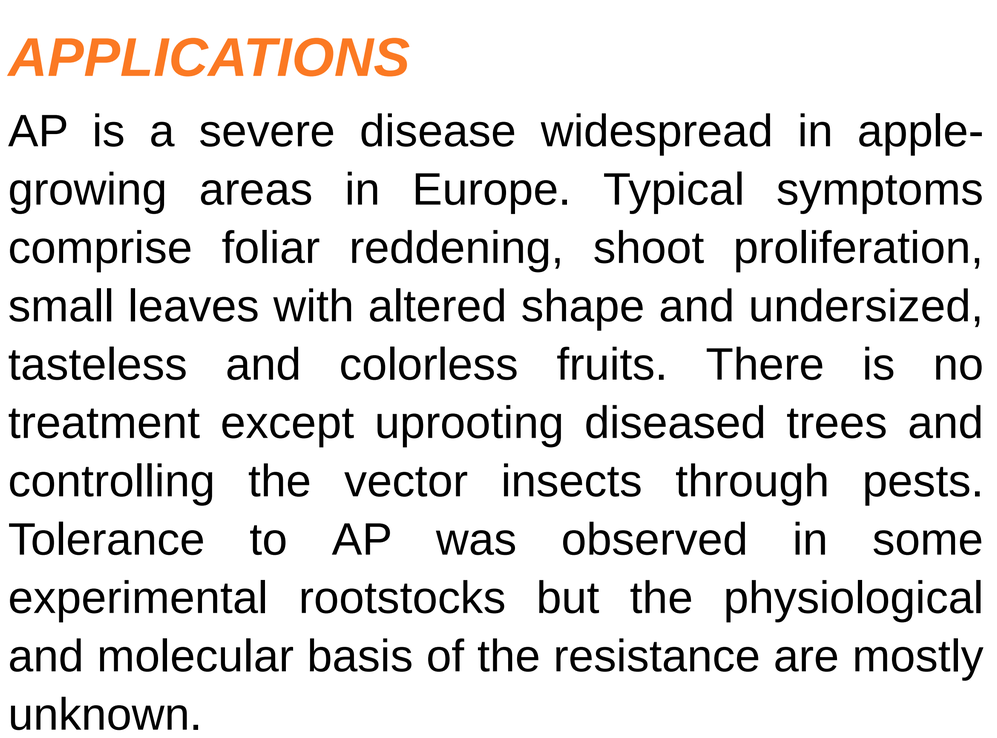
A study on Apple Proliferation (AP) phytoplasma 'Candidatus phytoplasma mali' (Ca. P. mali') effector target genes

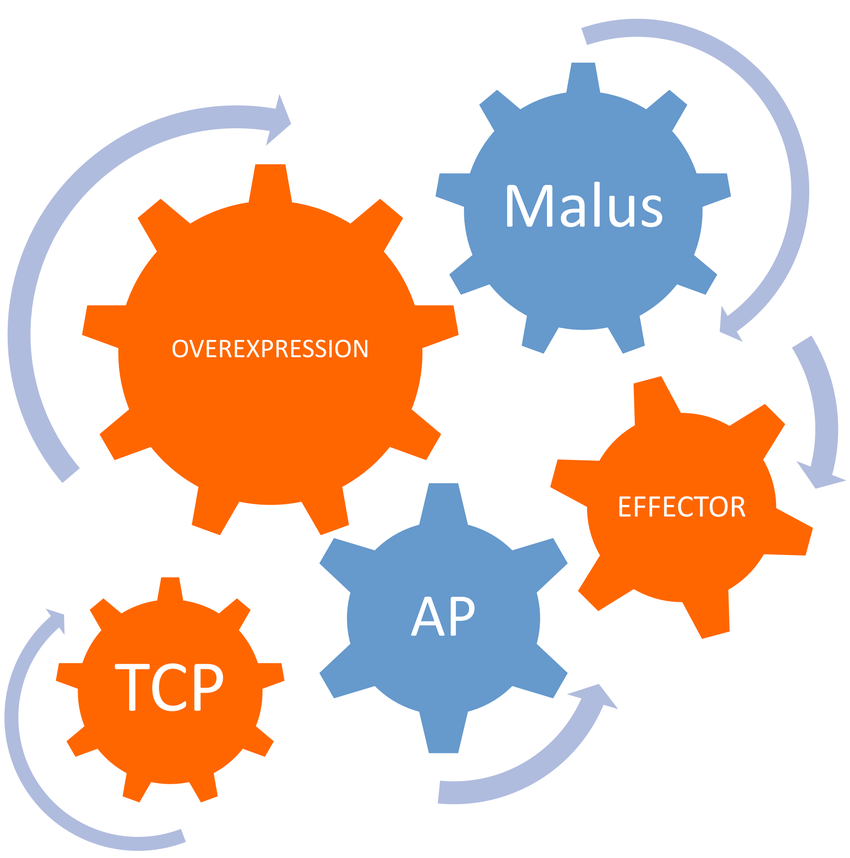
AIMS

The genome analysis of different strains of '*Ca*. P. mali', causal agent of AP, revealed the presence of one effector, $SAP11_{CaPM}$, which has been observed to bind and deactivate at least two apple CIN-class II TCP transcription factors. Goal of this work was to induce a stable overexpression of a target gene in apple to shed light on the role of this gene in the apple physiology and in the development of the disease. Moreover, the sequences of the target genes from susceptible and resistant Malus have been compared to elucidate the role of $SAP11_{CaPM}$ and its targets in AP tolerance.

APPLICATIONS

AP is a severe disease widespread in apple-growing areas in Europe. Typical symptoms comprise foliar reddening, shoot proliferation, small leaves with altered shape and undersized, tasteless and colorless fruits. There is no treatment except uprooting diseased trees and controlling the vector insects through pests. Tolerance to AP was observed in some experimental rootstocks but the physiological and molecular basis of the resistance are mostly unknown.
A deeper understanding of these phenomena would help develop environmentally sustainable ways to control the disease.





RESULTS

Interestingly, some rare amino acid substitutions exclusively present in one of the target genes of AP tolerant *Malus x domestica* could be observed. After the sequencing of other AP-tolerant plants it was found that 13 out of 15 plants carry the same mutations, thus indicating a potential role of these mutations as marker of tolerance. A direct role of these alleles in the tolerant behavior was investigated but didn't provided conclusive results yet.
In the graph below, the expression levels of the gene of interest in 4 transgenic lines compared to the Wild Type are displayed.
As visible in the picture below, overexpressing plants display loss of apical dominance, smaller leaves and shorter stem compared to the Wild Type in the first 6 months after acclimation; the plants have now been infected with the phytoplasma and an extensive analysis of their expression is being carried out.

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GLIAL FIBRILLARY ACIDIC PROTEIN AS A PREDICTIVE MARKER OF CHILD'S NEURODEVELOPMENT AFTER CARDIAC SURGERY

Circulating GFAP >0.44 ng/mL during cardiac surgery resulted in being a significant marker of acute neurological injury, since it was associated to abnormal neurodevelopmental assessment at 4-8 years of age, even in the presence of a normal IQ

AIMS

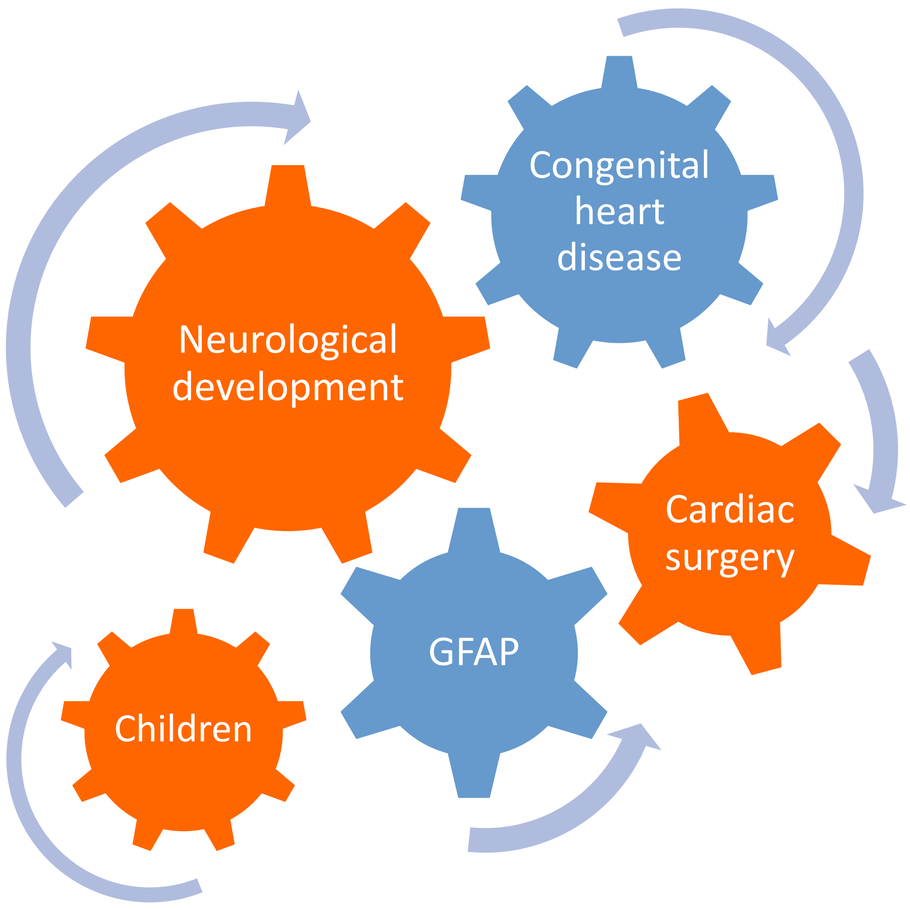
Adverse neurodevelopmental outcome is frequent in children with congenital heart disease (CHD).
The underlying cause is due to multiple factors, both preventable and not. Research need to focus on the potential onset of brain injury during and after cardiac surgery.
The aim of the study is to identify a reliable biomarker of brain injury, which can predict neurodevelopmental anomalies in infants with congenital heart disease undergoing surgical repair.

APPLICATIONS

Neurodevelopmental disorder in children with CHD may involve multiple domains and abnormalities discovered in early infancy may persist into childhood with a global reduction of quality of life.

An increasing number of studies analyzed the GFAP blood values during and after surgery in children with CHD, but only a few have investigated its potential role in predicting any adverse neurodevelopmental outcome.

Neurological development was assessed at 4-8 years of age using tests and questionnaires to assess cognitive, neuropsychological, and psychopathological functioning.



RESULTS:

We enrolled 38 patients. Mean GFAP peak value was 1.42 ± 1.69 ng/mL (median of 0.95 ng/mL, IQR 0.44-1.57) and 70% of children showed pathological GFAP peak value (i.e. greater than 0.44 ng/mL).
IQ scores fell within the normal range for most subjects, whereas 58% of them showed an abnormal NDI, with the greatest impairments in the psychopathological area.
GFAP >0.44 ng/ml showed a sensitivity of 90.9% and a specificity of 56.3% to detect an abnormal NDI.
Regression analysis showed that GFAP max >0.44 ng/mL was the only independent predictor of an abnormal NDI (OR: 12.8; 95% CI 2.2-74.5).
Surgical phases characterized by body temperature changes seem to be the most dangerous for developing an acute neurological injury.