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## Webinar

Anna Paola Frascini

# 1 General info

**Nome:** Annapaola Frascini;

**Università:** Università Cattolica del Sacro Cuore (PC);

**Corso di laurea:** Scienze e tecnologie Agrarie – Produzioni vegetali e difesa integrata delle colture;

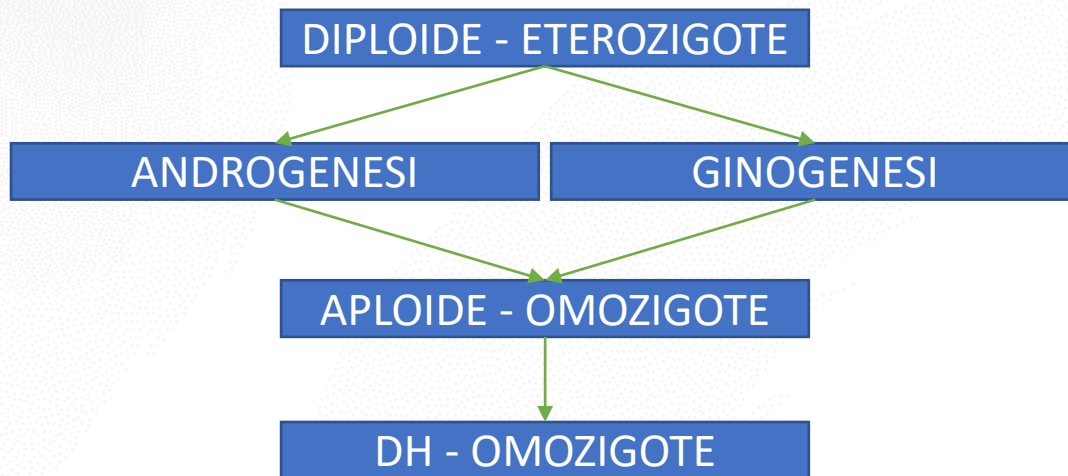
**Internship in ISI Sementi:** da 11/18 a 11/19.

**Supervisors:** Busconi Matteo (UCSC) – Alice Brunazzi (ISI Sementi);

**Titolo elaborato:** «Ottenimento di una linea omozigote DH in cucurbita pepo ssp. pepo da coltura in vitro»



# Introduction and Objectives



Linea omozigote **BREEDING CLASSICO**: 8 – 9 ANNI.

Linea omozigote **IN VITRO**: 1 ANNO.

**SCOPO DELLA TESI**: redigere un protocollo valido per la produzione di una linea DH di zucchini mediante androgenesi e ginogenesi.

# Materials and Methods

## 1. SCELTA DEL MATERIALE: VARIABILITÀ FENOTIPICA



ISI\_1 F1



ISI\_2 F1



ISI\_3 F1



ISI\_4 F1



ISI\_5 F1

TRAITS: RUSTICITÀ, ADATTABILITÀ, VIGORIA, RESISTENZE

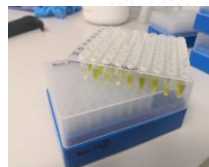
## 2. SEMINA



## 3. NUMERAZIONE PIANTE



## 4. CAMPIONAMENTO, ESTRAZIONE DNA E ANALISI MOLECOLARE



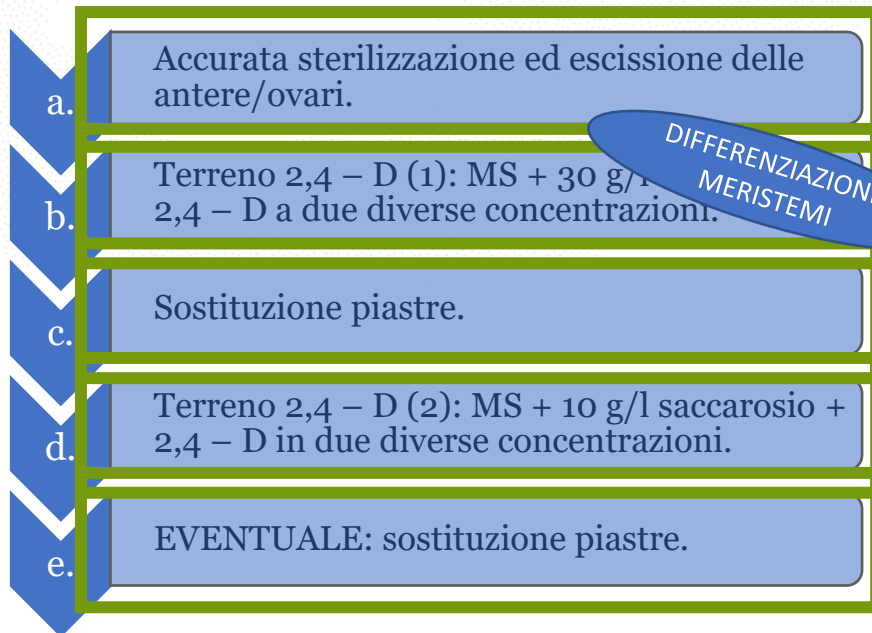
## 5. RACCOLTA INFIORESCENZE



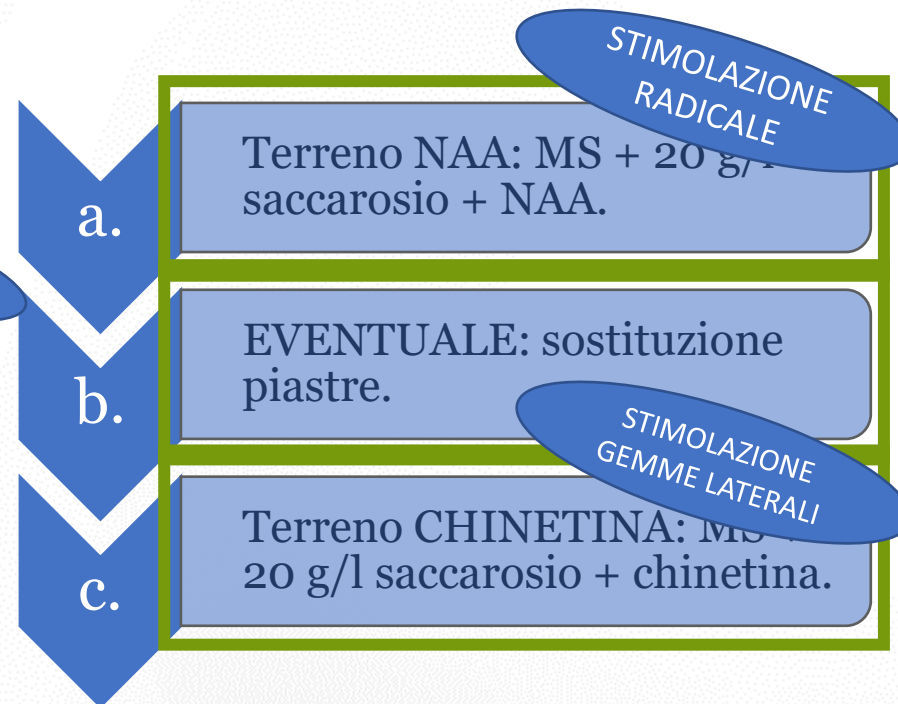
## 6. TRATTAMENTO AL FREDDO



## 7. STERILIZZAZIONE, TAGLIO, PRIMA FASE MANTENIMENTO (AL BUIO)



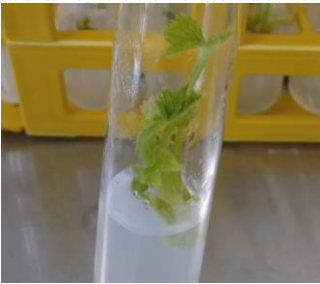
## 8. SECONDA FASE MANTENIMENTO (ALLA LUCE)



MUTAGENO!



**9.** DIPLOIDIZZAZIONE CON COLCHICINA;



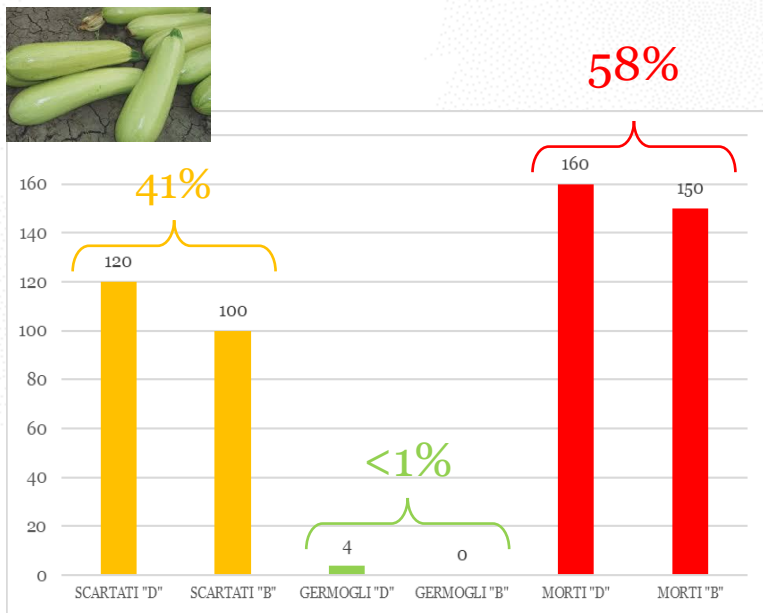
**10.** ACCLIMATAMENTO

Cp_SNP1_F/R	Cp_W2_F/R+Pr obe_Cp_W2_Sr ev AMPL	Cp_MSP14_F/R	NOTE
AB	AB	<u>AB</u>	
AB	BB	<u>AB</u>	REJECTED
AB	AB	<u>AB</u>	
AB	BB	AB	REJECTED
AB	AB	AB	

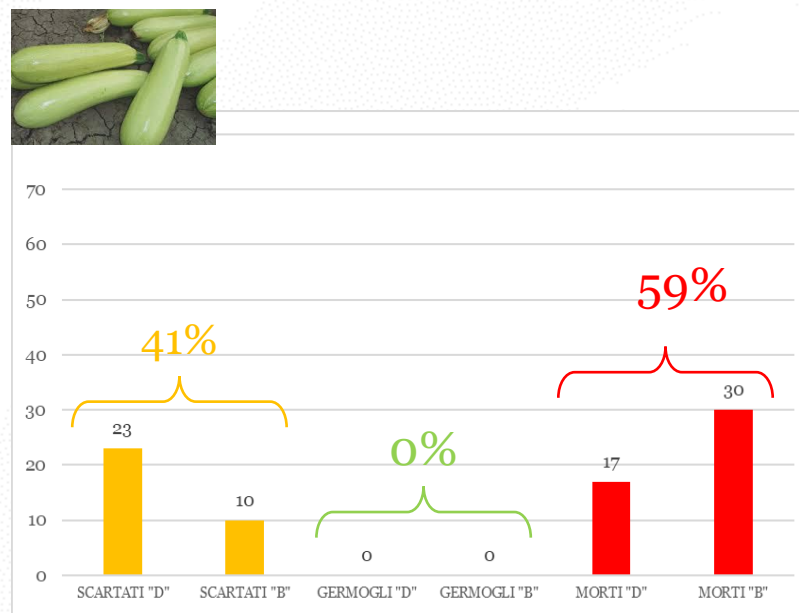
CAMPIONI ANALIZZATI: 400

INDIVIDUI SCARTATI: 3

## ISI\_1 F1 - ANDROGENESI



## ISI\_1 F1 - GINOGENESI



Terr. D: 1,25 ml/l 2,4 - D  
Terr. B: 0,25 ml/l 2,4 - D

STERILIZZAZIONE  
BLANDA

ESCISSIONE  
TROPPO FITTA

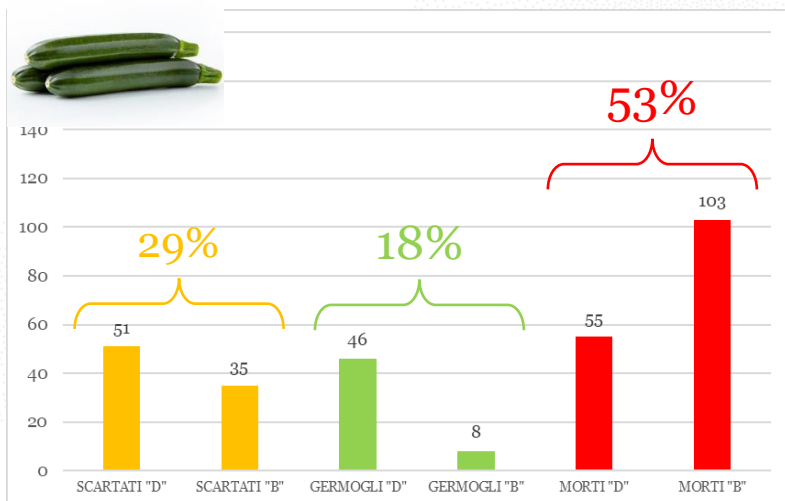
ERRATA GESTIONE  
MANTENIMENTO

PRECOCE  
ESPOSIZIONE ALLA  
LUCE

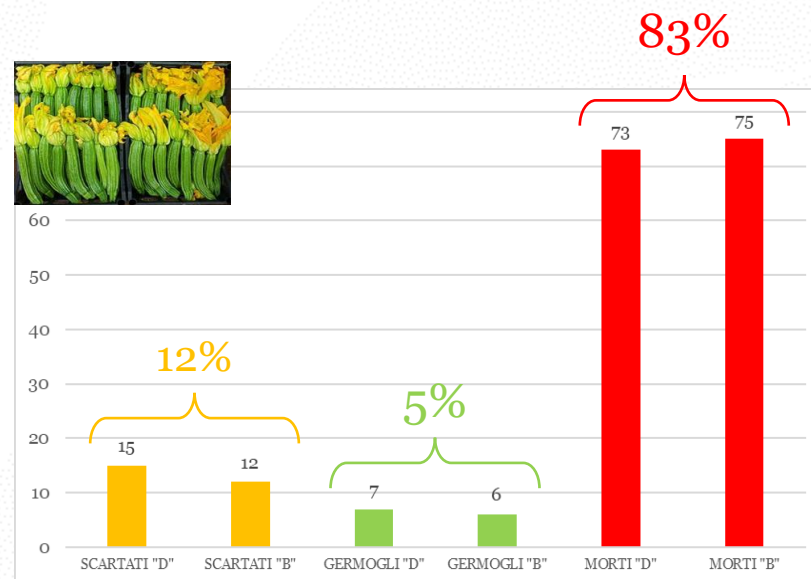


# Results

## ISI\_2 F1 - ANDROGENESI



## ISI\_3 F1 - ANDROGENESI



Terr. D: 1,25 ml/l 2,4 - D  
Terr. B: 0,25 ml/l 2,4 - D

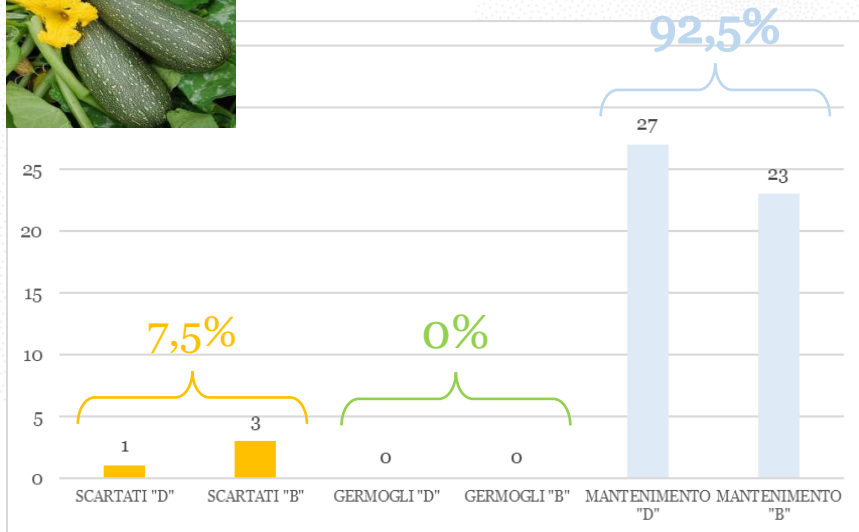
STERILIZZAZIONE  
MIGLIORATA

ESCISSIONE  
MENO FITTA

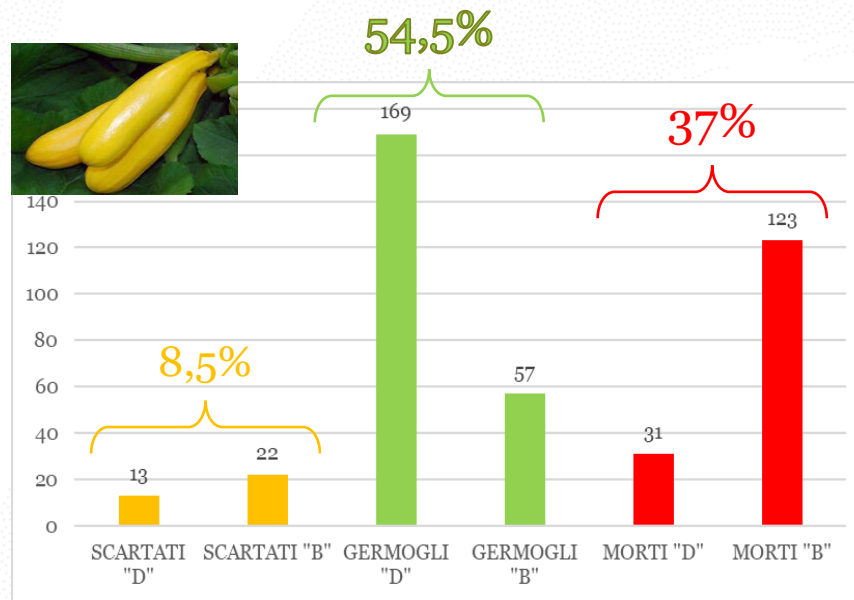
ESPOSIZIONE AL  
BUIO

PERIODO DI  
SEMINA ERRATO

## ISI\_4 F1 - GINOGENESI



## ISI\_5 F1 - ANDROGENESI



Terr. D: 1,25 ml/l 2,4 - D  
Terr. B: 0,25 ml/l 2,4 - D

STERILIZZAZIONE  
AGGRESSIVA

TRATTAMENTO AL CALDO  
POST ESCISSIONE  
(ANDROGENESI)

CAMBI TERRENO PIÙ  
FREQUENTI

- ANALISI MOLECOLARE;
- NON UTILIZZABILE PER PROPAGAZIONE MASSALE;
- INFLUISCONO SUL RISULTATO: TECNICHE, QUANTITÀ ORMONE INIZIALE, GENOTIPO, PERIODO DI SEMINA;
- PROTOCOLLO NON DEFINITIVO.



**IMPORTANCE OF DOUBLED 1 HAPLOID IN SUMMER SQUASH I**

HANDLING A.1, CRESCENDO L.11, CORNICO M.11, BUCCONI M.11, SERIE I A.M.11, BRUNAZZO A.11  
 TUTTOSENTO D'ITALIA, PAPA PAPA L.11, SQUASH ITALIA S.11, BAMBINO DI ITALIA S.11, BAMBINO DI ITALIA S.11, BAMBINO DI ITALIA S.11, BAMBINO DI ITALIA S.11, BAMBINO DI ITALIA S.11

**Summer squash (*Cucurbita pepo* L.) is a very important outdoor vegetable. It is an annual plant which can nearly reach 5 m in height, but it is generally harvested in lower height, the fruit is picked when it measures almost 20 cm in length. It is a diploid, monoecious plant which produces male and female flowers separately on the same plant.**

**AIM OF THE EXPERIMENT**  
 Explore the main factors affecting the production of doubled haploid plants from pollen and ovule plants.

**GENERAL**  
 Four hybrid varieties, some considered for the experiment: "S\_1\_1\_1", "S\_1\_1\_2", "S\_1\_1\_3", "S\_1\_1\_4". The "S\_1" seeds were manually sown in pots in greenhouse during winter season (2018-19) (latitudes: 41°52'9.63"N 12°2'5'68"E).

**POLLINIZATION**  
 The pollination process was carried out by hand using a brush to transfer pollen from male to female flowers.

**ANALYTIC**  
 For the initial analysis are used 31 SNPs, the molecular basis of most haplotypes. It is essential to add only 17 plants which are heterozygous for these three markers. We need more information to verify one or more other alleles in 510 plants. We do not know precisely if the plant which originates from the callus came from the pollen, ovule or from other sources.

**RESULTS**  
 After 12 months, the plants were harvested and analyzed. The results showed that the doubled haploid plants were obtained from both pollen and ovule plants.

**FINAL VALIDATED PROTOCOL**  
 12 months

**CONCLUSIONS**  
 Doubled haploid in summer squash is not a common technique, but it is very useful for the future. The protocol has to be perfected, but the results obtained in this experiment are promising.



**PREMIO "CONVEGNO SIGA 2019"**

assegnato a  
**ANNAPAOLA FRASCHINI**  
 per la presentazione poster "Importance of doubled haploids in summer squash" at LXIII Convegno Annuale SIGA

IL SEGRETARIO  
 PROF. EDGARDO FILIPPONE

IL PRESIDENTE  
 PROF. MARIO PEZZOTTI

Napoli, 12 Settembre 2019

# GRAZIE PER L'ATTENZIONE!