

# Pollination, phytochemistry and food on table: how and whether environmental health can impact on human nutrition

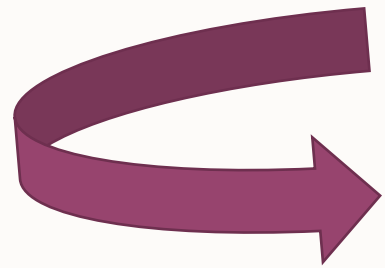
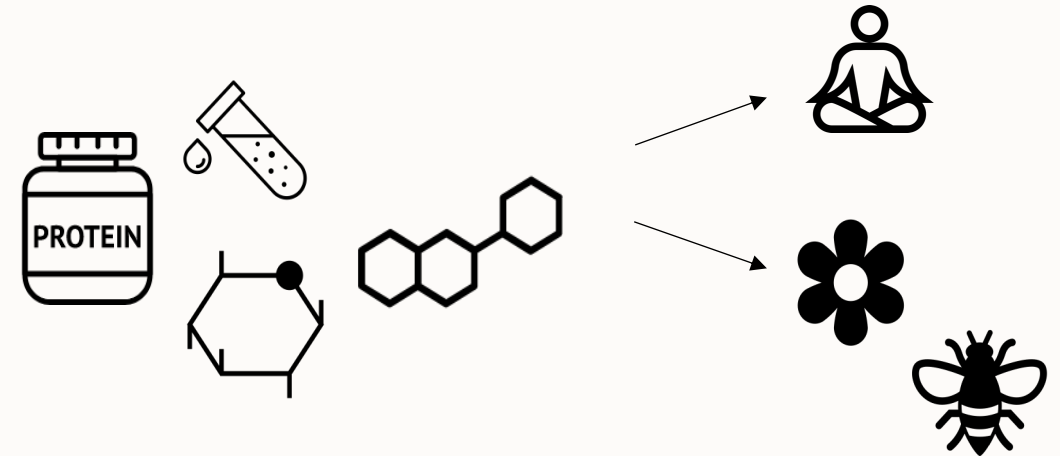
LORENZO GUZZETTI

19<sup>th</sup> December 2022



# NUTRITIONAL ECOLOGY AND PHYTOCHEMISTRY

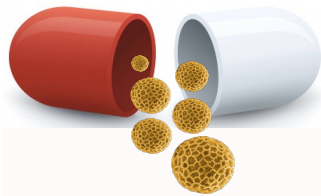
NUTRIENTS PLAY AN IMPORTANT ROLE NOT ONLY FOR HUMAN HEALTH BUT ALSO FOR ECOSYSTEM INTEGRITY



INVESTIGATION WORKFLOW AT OUR LAB:



RESOURCE QUALITY



POLLINATORS HEALTH

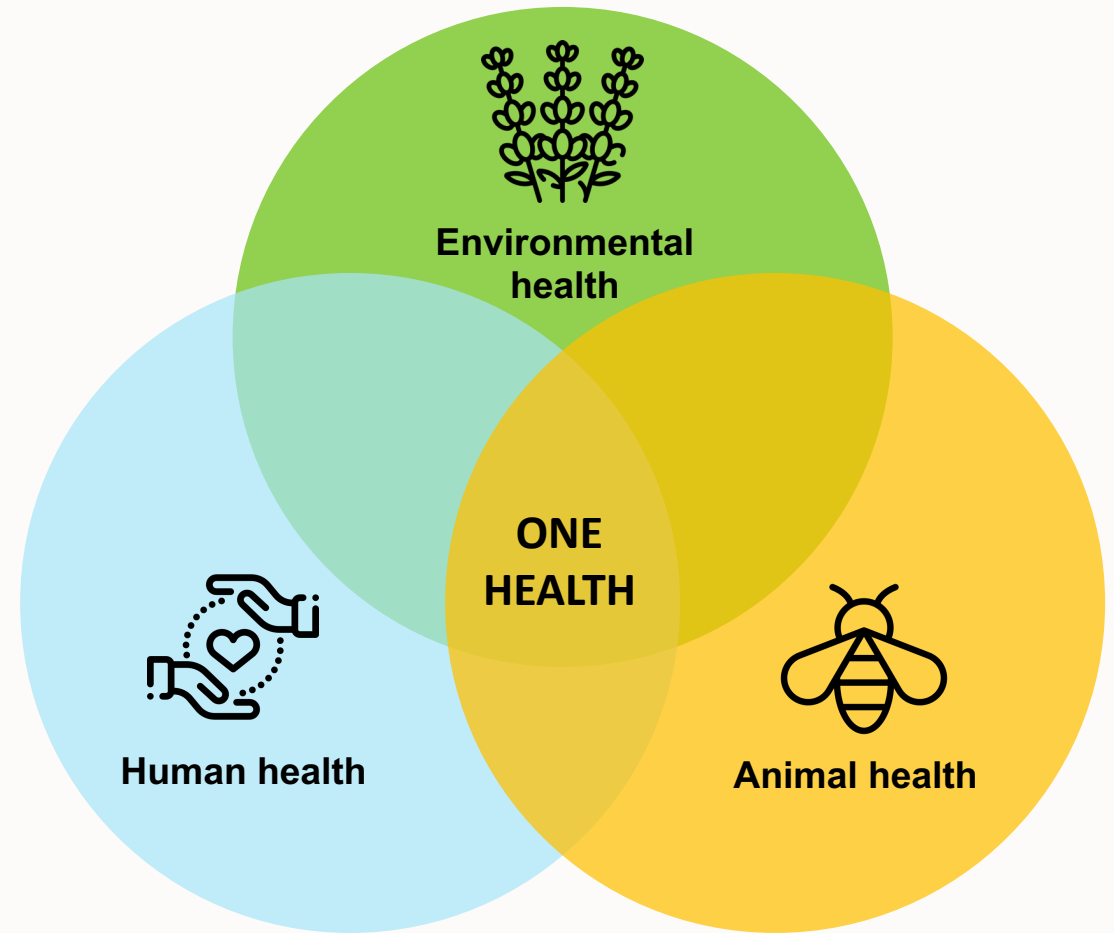
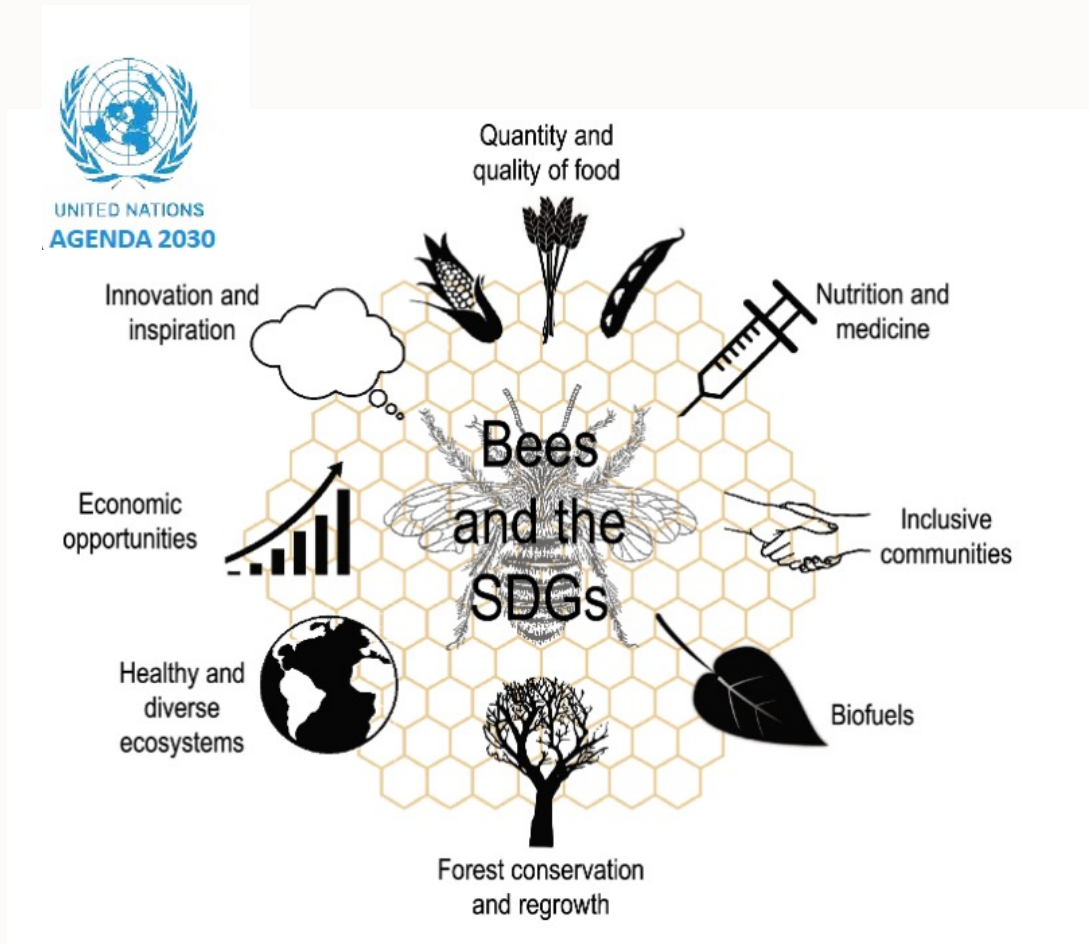


ECOSYSTEM SERVICES

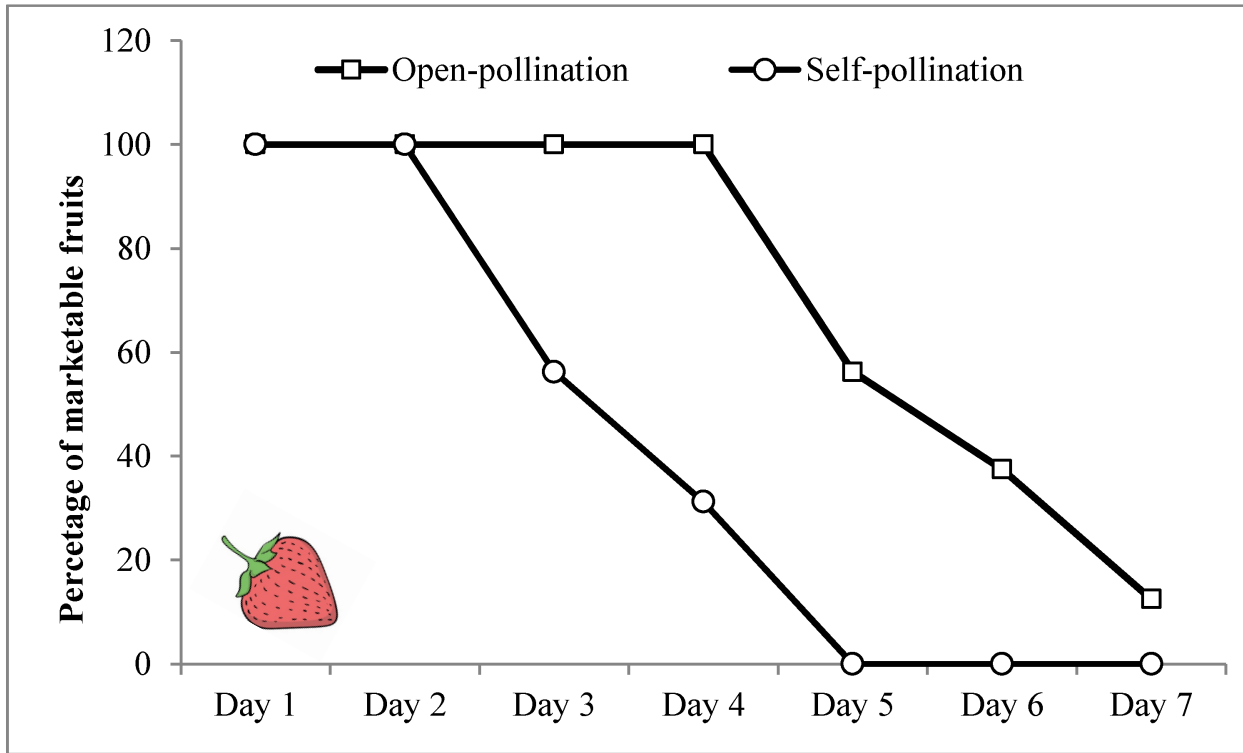


FOOD QUALITY

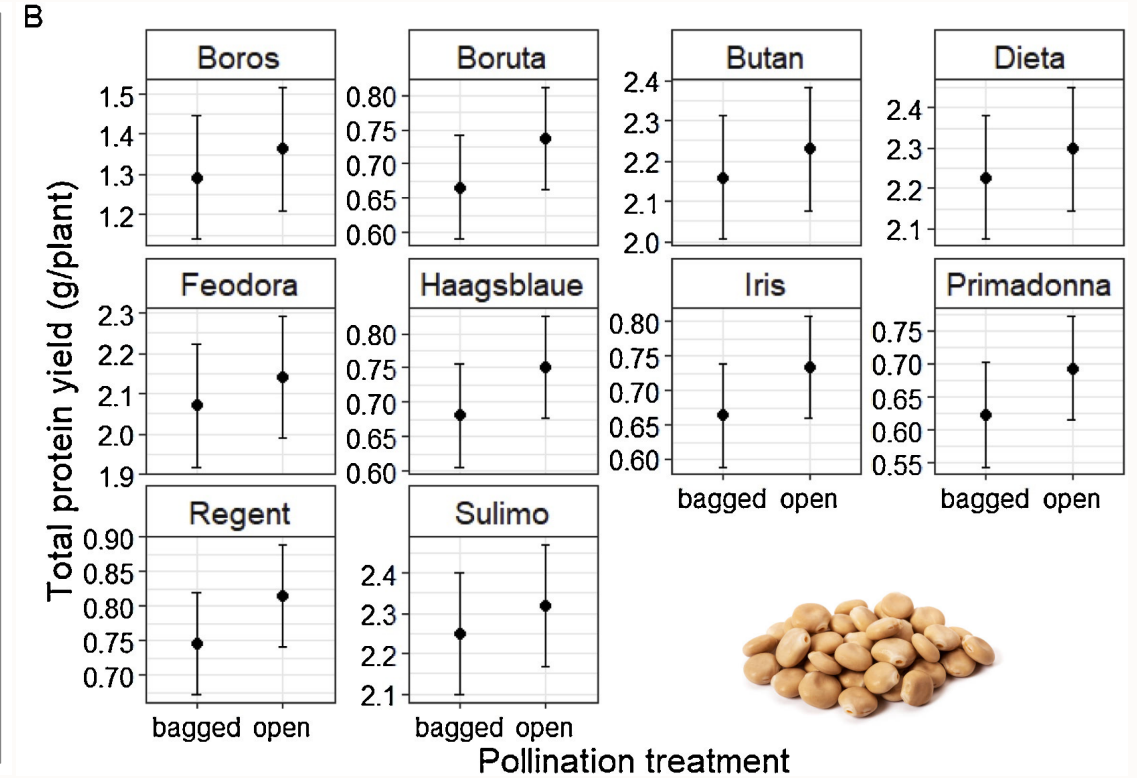
# POLLINATORS AND AGENDA 2030: THE *ONE HEALTH* CONCEPT



# POLLINATORS SHAPE CROP YIELD AND QUALITY



Anees et al., 2022 «Impact of bee and fly pollination on Physical and biochemical properties of strawberry fruit» *Horticulturae*, 1-10



Fijen et al., 2021 «Pollination increases white and narrow-leaved lupin protein yields but not all crop visitors contribute to pollination» *Agr Ecosyst Environ*, 1-7



**LACK OF INFORMATION ABOUT FUNCTIONAL FOOD ASPECTS (e.g., nutraceutical value)**



# GREENHOUSE EXPERIMENTS

## DRY FRUIT

*Vigna unguiculata* L. Walp.



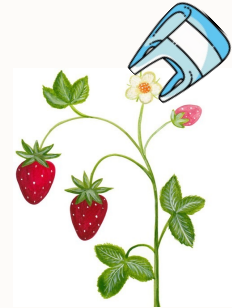
AUTOGAMY



OPEN  
POLLINATION

## FLESHY FRUIT

*Fragaria vesca* L.



AUTOGAMY



ALLOGAMY

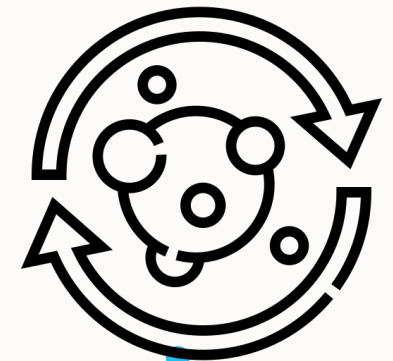


OPEN  
POLLINATION

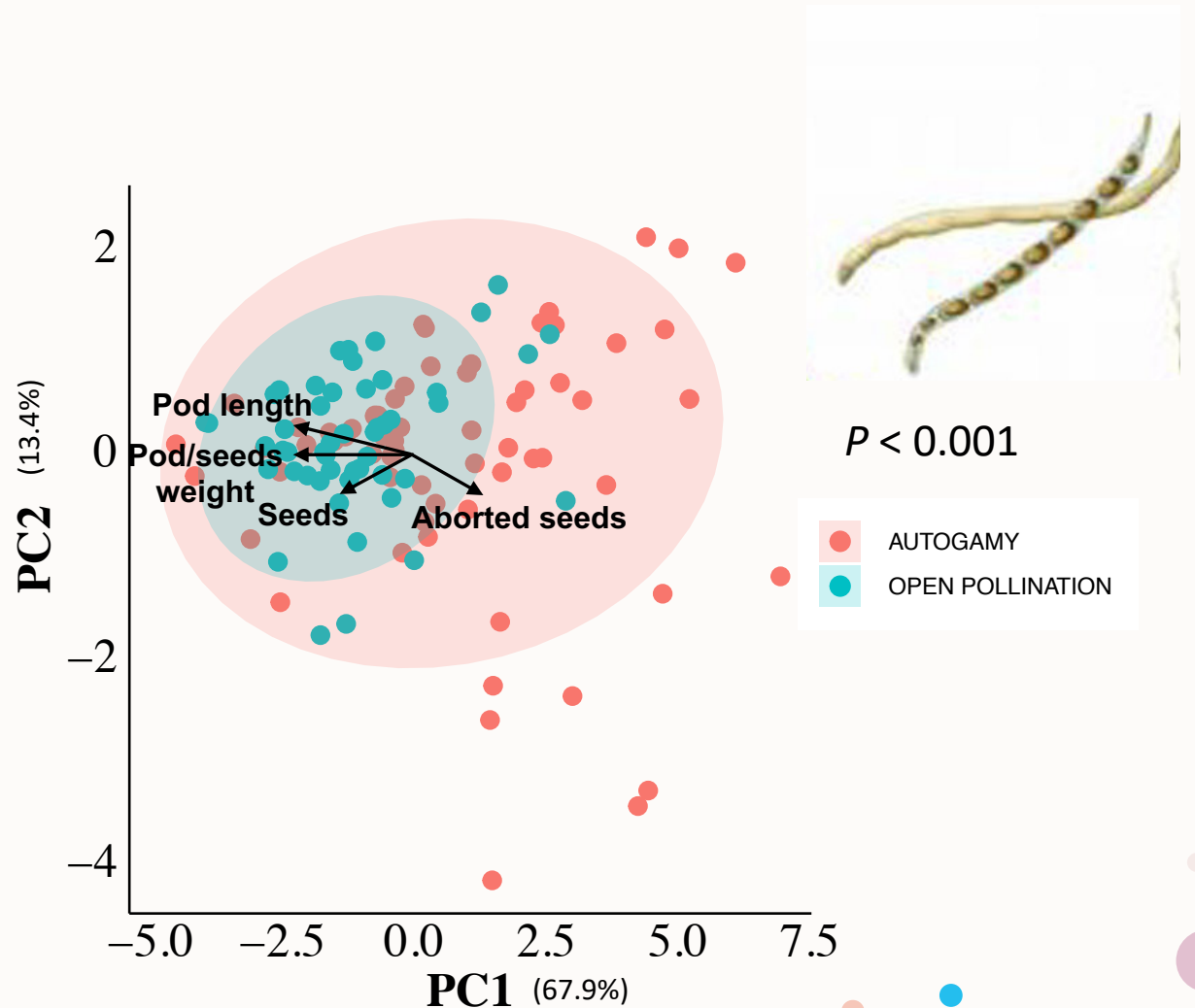
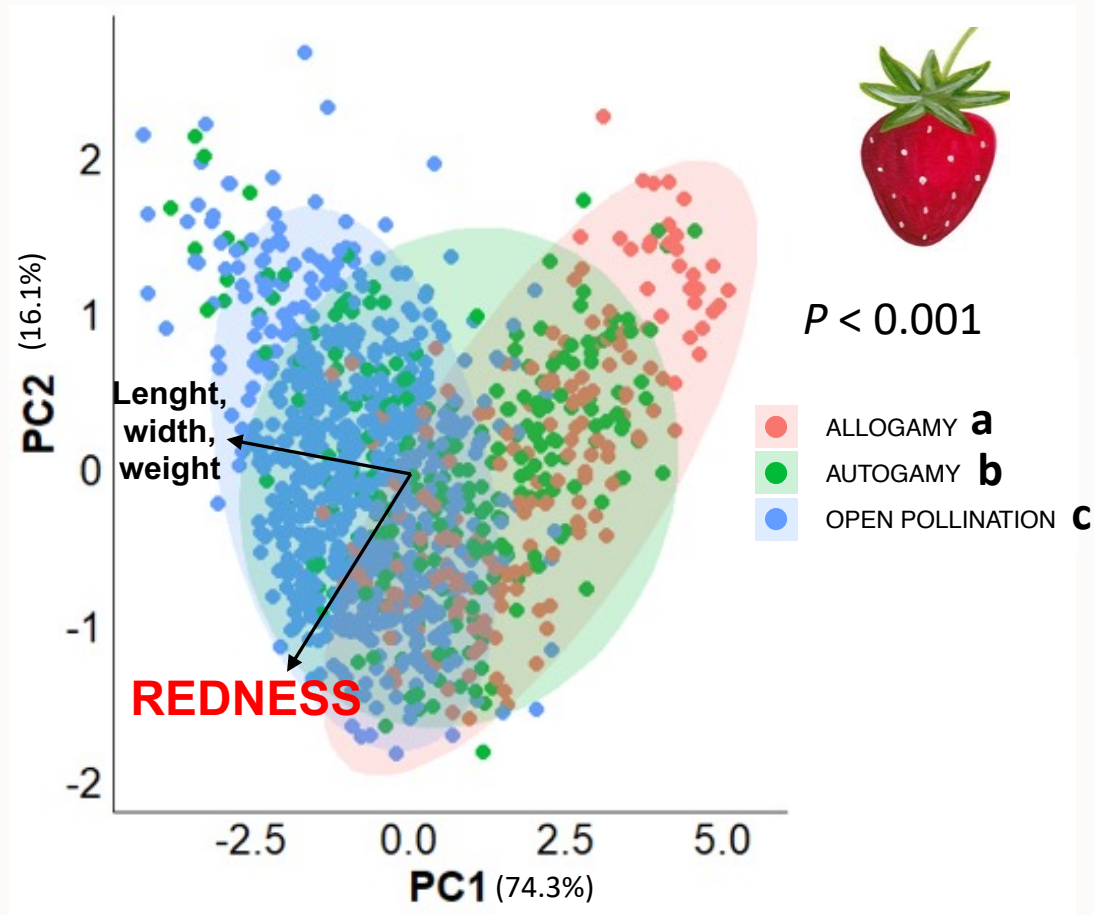
Impact of pollination mechanism  
on fruit and seeds metabolism



Effects for human nutrition and well-being



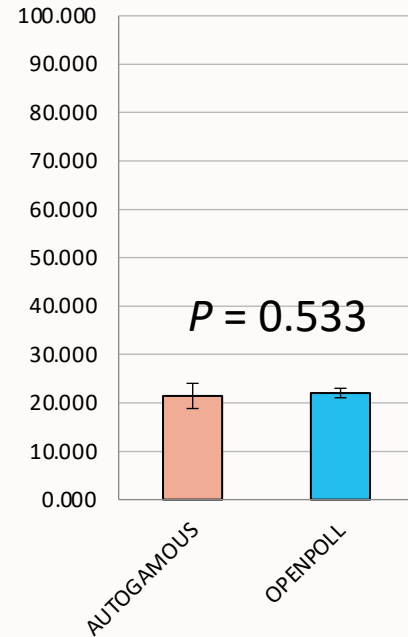
# MORPHOLOGICAL TRAITS (season 2021)



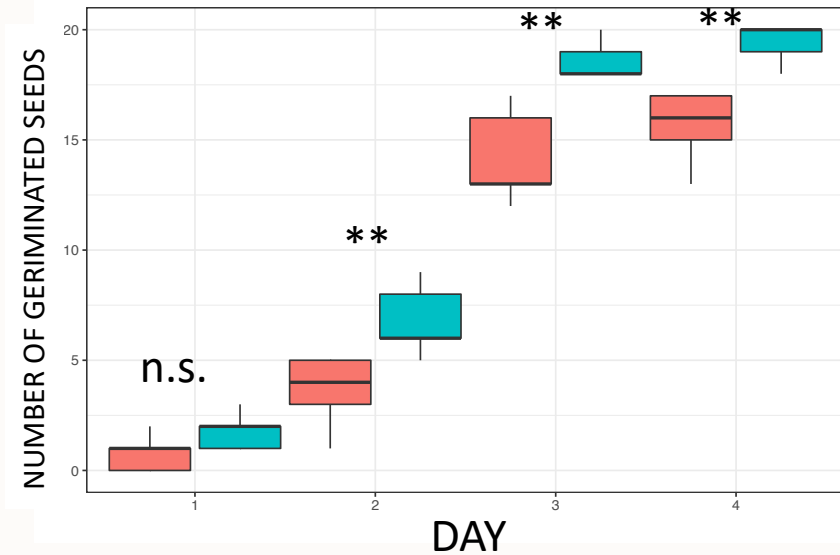
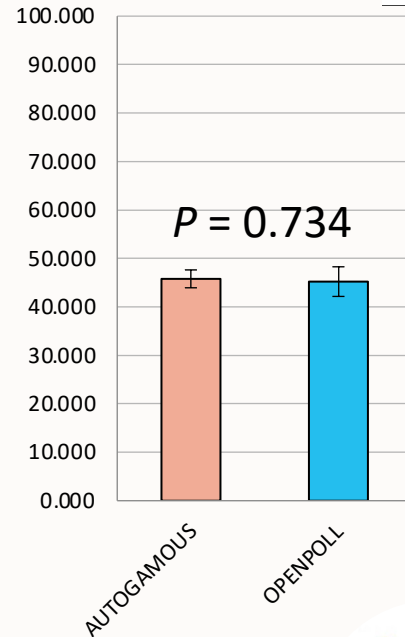
# PRIMARY METABOLISM AND GERMINATION IN *V. UNGUICULATA* (2021)



% proteins (dry weight)

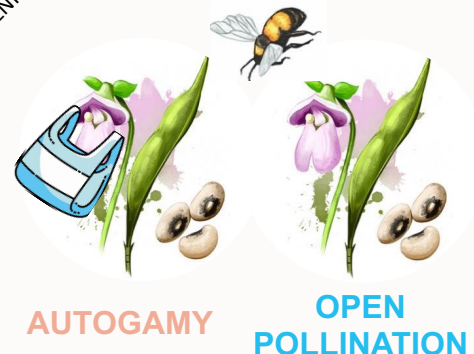


% starch (dry weight)



● AUTOGAMY  
● OPEN POLLINATION

$n = 80$



# HRMS ANALYSES PROCEDURE



## EXTRACTION PROCEDURE

0.5 g dry powder agitated for 15 min in 10 mL of a hydro-alcoholic solvent (MeOH 70% v/v pH 3.5) twice.

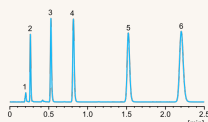


## ISOLATION OF PIGMENTS

Solid Phase Extraction (SPE) through C<sub>18</sub> column.  
Elution with EtOH:H<sub>2</sub>O 1:1 v/v + 0.1% HCOOH

## HPLC-PDA-QTOF-MS<sup>n</sup>

Column: Zorbax SB-C18 (100 x 2.1 mm) 3.5 μm  
Mobile Phases: A: H<sub>2</sub>O + 0.1% HCOOH, B: MeCN + 0.1% HCOOH.  
Gradient elution.



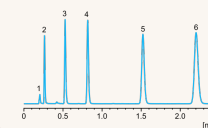
## EXTRACTION PROCEDURE

0.5 g dry powder ultrasonicated for 10 min in 10 mL of a hydro-alcoholic solvent (EtOH 50% v/v) three times.



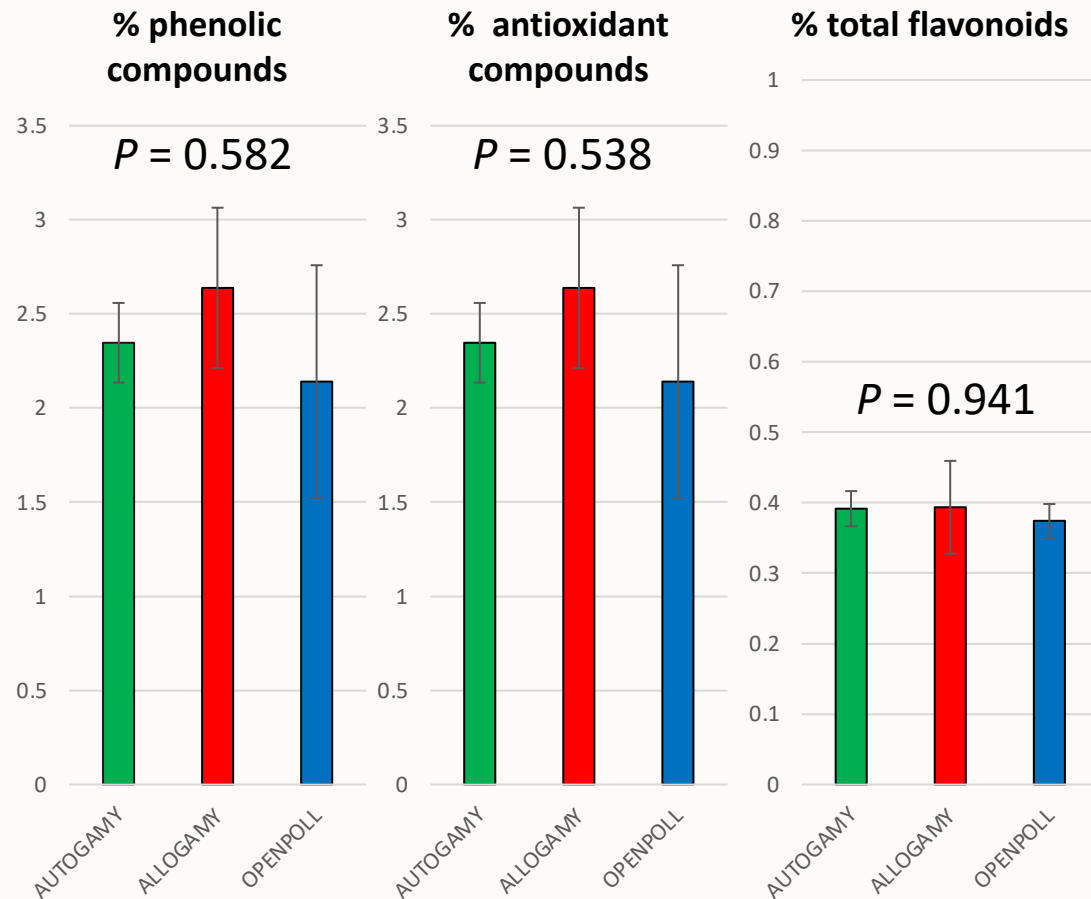
## HPLC-PDA-QTOF-MS<sup>n</sup>

Column: Zorbax SB-C18 (100 x 2.1 mm) 3.5 μm  
Mobile Phases: A: H<sub>2</sub>O + 0.1% HCOOH, B: MeCN + 0.1% HCOOH.  
Gradient elution.





# TOTAL ANTIOXIDANTS COMPOSITION



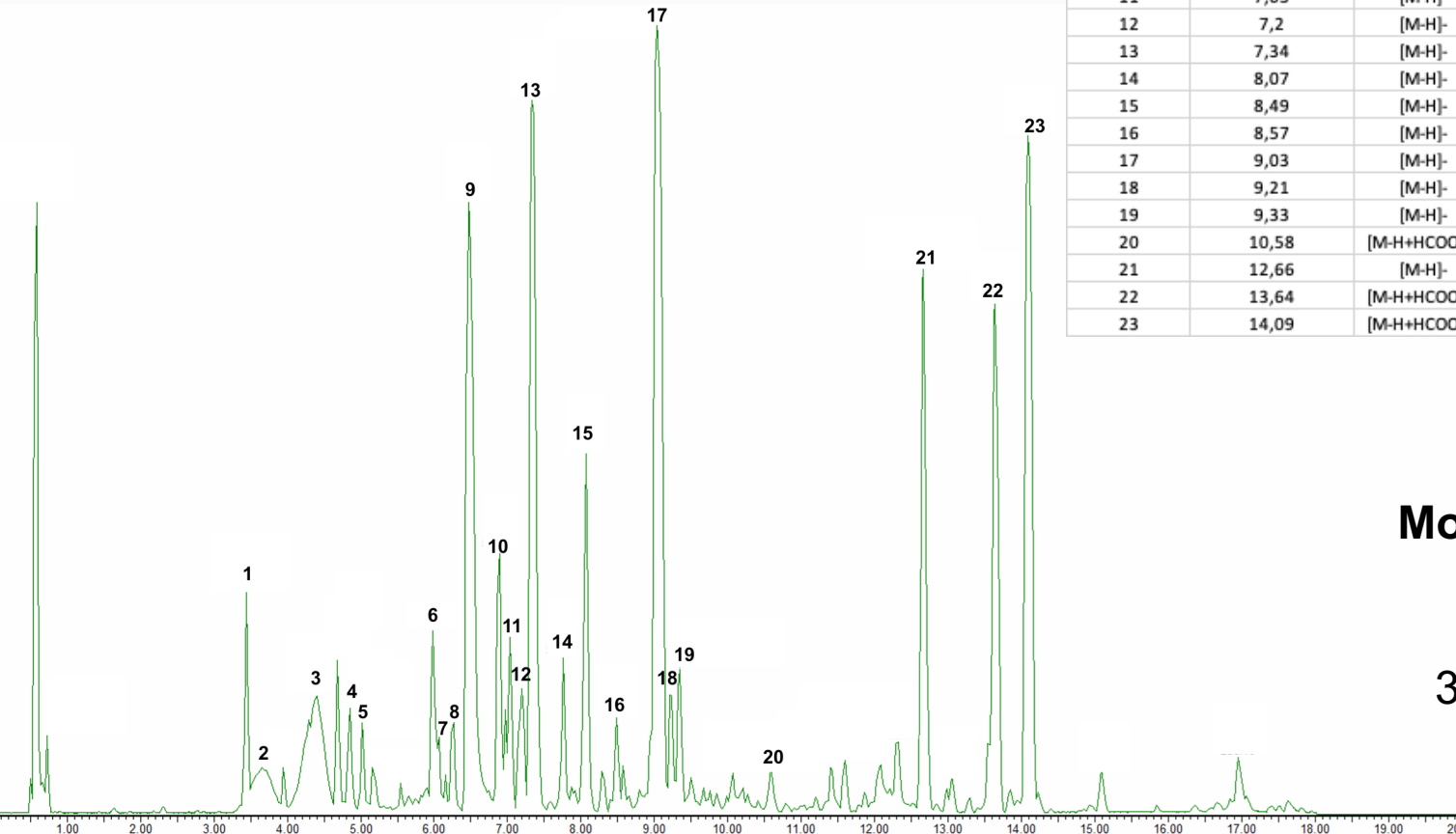
NO SIGNIFICANT DIFFERENCES IN TERMS OF OVERALL ANTIOXIDANT COMPOUNDS BUT NO INFORMATION AT THE METABOLITES LEVEL



# METABOLITES COMPOSITION

Compounds occurring in the enriched fraction were tentatively identified by comparison with available literature

Peak number	Retention time	Ion current	Precursor ion	Fragments	Tentative identification	References
1	3,4	[M-H]-	289	245, 123, 109	(-)Epicatechin	Del Bubba, 2012
2	3,68	M+	449	287	Cyanidin hexoside	Del Bubba, 2012
3	4,39	M+	433	271	Pelargonidin hexoside	Del Bubba, 2012
4	4,87	M+	563	463, 301, 286	Peonidin methyl malonyl hexoside	Del Bubba, 2012
5	5,01	[M-H]-	449	269, 193, 165	Ferulic acid hexose derivative	Sun et al., 2014
6	5,99	[M-H]-	447	301, 300	Ellagic acid rhamnoside	Del Bubba, 2012
7	6,07	M+	519	271	Pelargonidin-malonylglucoside	Del Bubba, 2012
8	6,2	[M-H]-	447	300	Ellagic acid rhamnoside	Sun et al., 2014
9	6,46	[M-H]-	655	301, 229, 201, 129	Ellagic acid derivative	Sun et al., 2014
10	6,88	[M-H]-	521	359	Tetramethylellagic acid hexose	Sun et al., 2014
11	7,03	[M-H]-	463	300, 179, 151	Quercetin-3-O-glucoside	Sun et al., 2014
12	7,2	[M-H]-	521	359, 344	Tetramethylellagic acid hexose	Sun et al., 2014
13	7,34	[M-H]-	435	305, 285	Taxifolin 3-O-arabinofuranoside	Sun et al., 2014
14	8,07	[M-H]-	447	327, 285, 255, 227	Kaempferol 3-O-glucoside	Sun et al., 2014
15	8,49	[M-H]-	477	315, 314, 299, 285, 271, 257, 243	Methylellagic acid hexose	Sun et al., 2014
16	8,57	[M-H]-	519	315, 300	Methylellagic acid acetyl hexoside	Sun et al., 2014
17	9,03	[M-H]-	461	315, 300	Methylellagic acid rhamnoside	Sun et al., 2014
18	9,21	[M-H]-	489	447, 327, 285, 255	Kaempferol acetyl hexoside	Del Bubba, 2012
19	9,33	[M-H]-	461	315, 300	Methylellagic acid rhamnoside	Sun et al., 2014
20	10,58	[M-H+HCOOH]-	711	503	Triterpene glycoside	Spinola et al., 2019
21	12,66	[M-H]-	593	447, 307, 285	Kaempferol 3-coumaroylglucoside	Sun et al., 2014
22	13,64	[M-H+HCOOH]-	695	487	Triterpene glycoside	Lorenço et al., 2021
23	14,09	[M-H+HCOOH]-	695	487	Triterpene glycoside	Lorenço et al., 2021



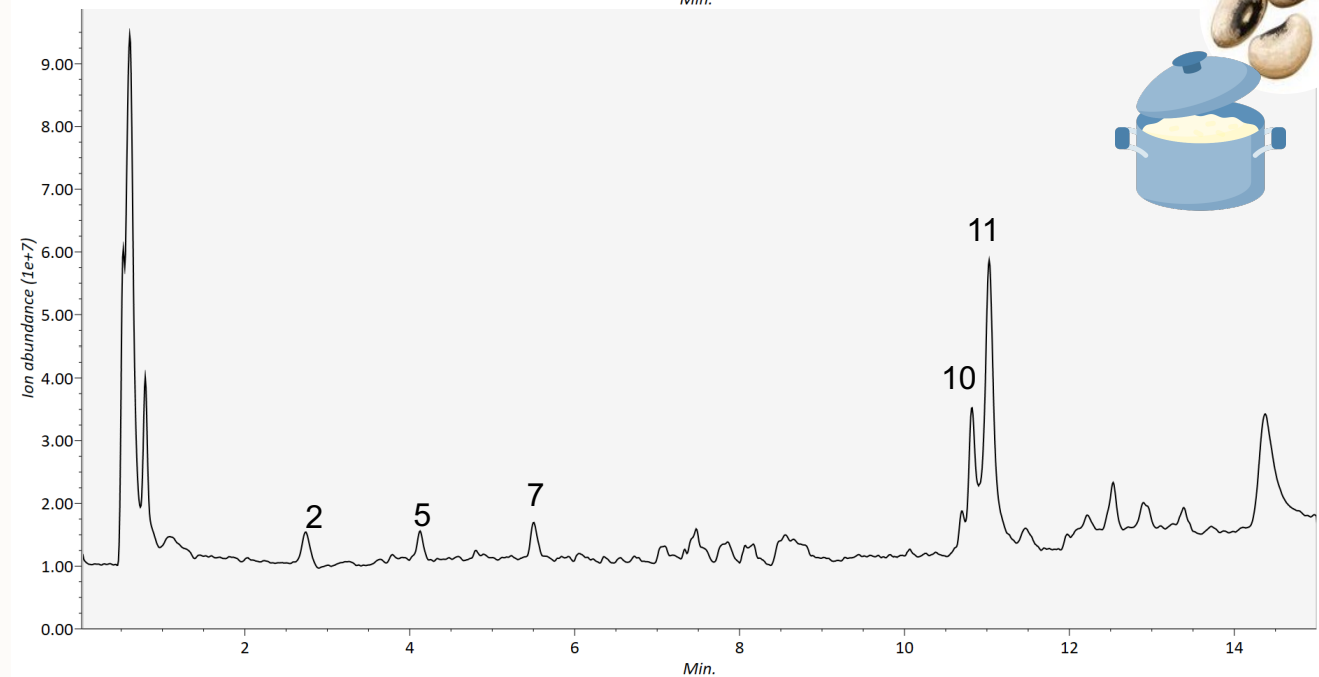
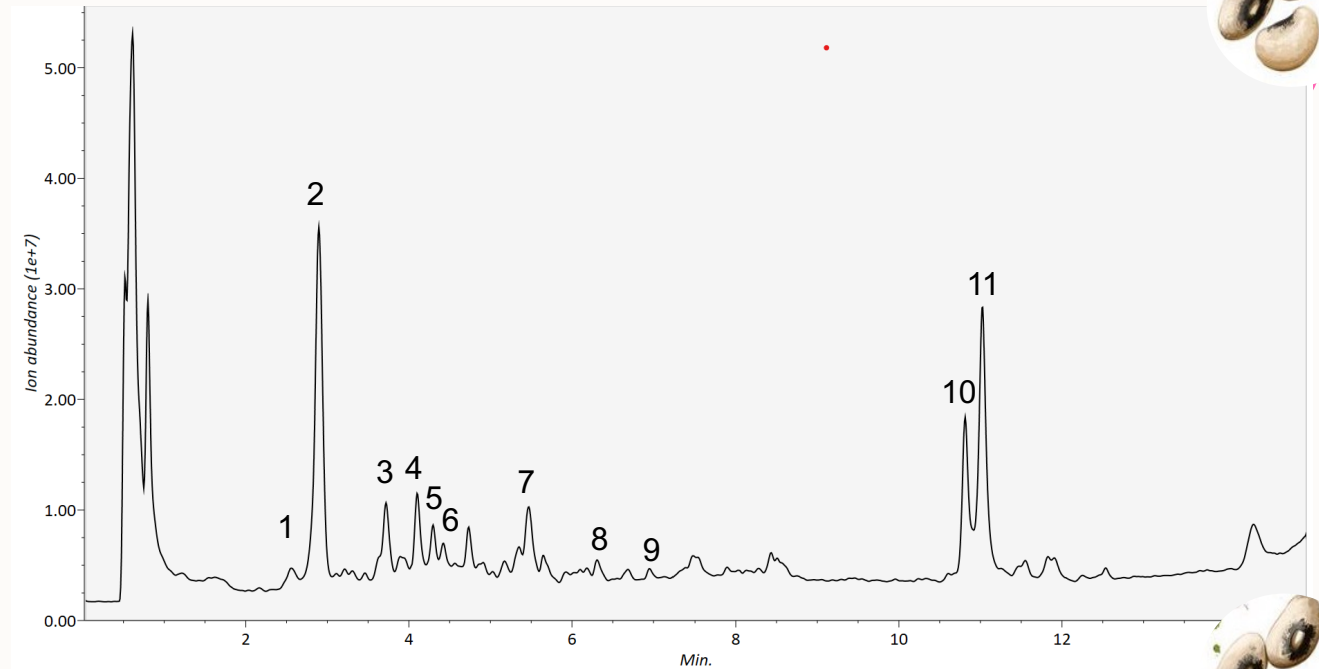
**Most frequent compounds:**

- 1) Flavonoids
- 2) Anthocyanins
- 3) Ellagic acid derivatives
- 4) Triterpenes

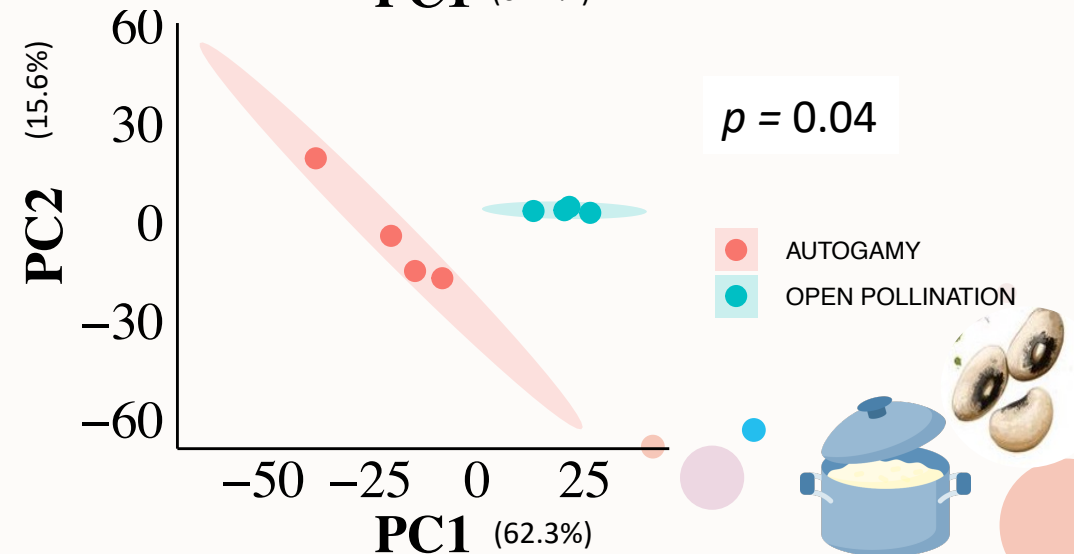
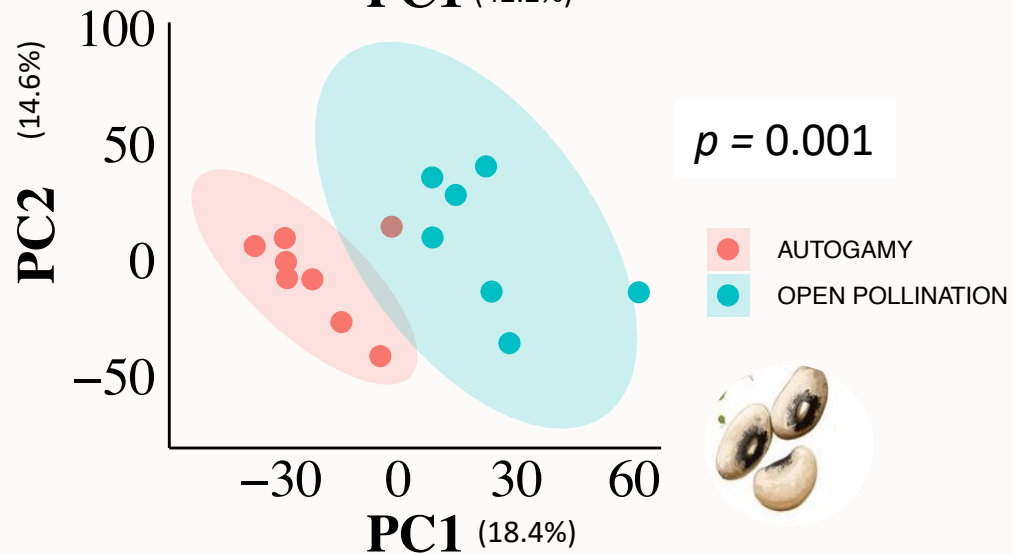
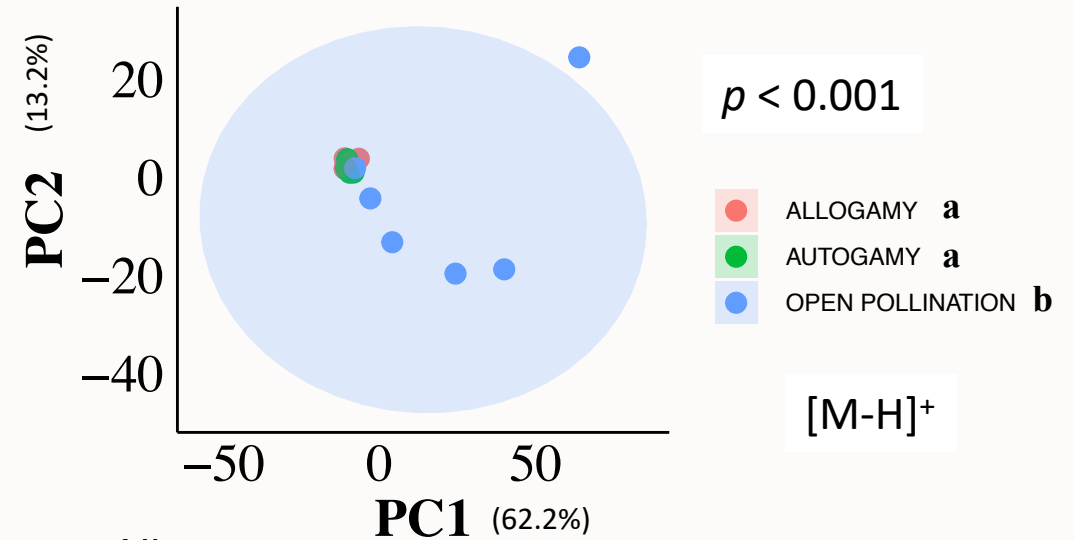
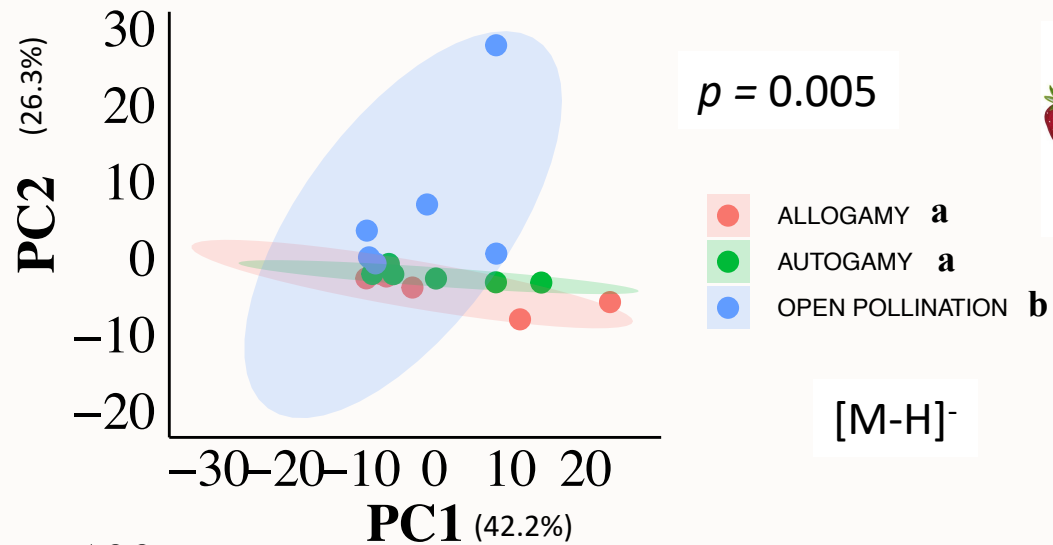
# METABOLITES COMPOSITION



Peak	Ion current	Ion	Fragments	Tentative identification	Formula	References
1	[M-H]-	305	167, 175, 137, 125, 109	(-) Gallo catechin	C <sub>15</sub> H <sub>14</sub> O <sub>7</sub>	Seyit Yuzuak, 2018
2	[M-H]-	451	289	Catechin 3-O-glucoside	C <sub>21</sub> H <sub>24</sub> O <sub>11</sub>	Seyit Yuzuak, 2018
3	[M-H]-	577	451, 425, 407, 289	Procyanidin dimer	C <sub>30</sub> H <sub>26</sub> O <sub>12</sub>	Ismael Ivan Rockenbach, 2012
4	[M-H]-	289	245, 221, 125, 109	(+) Catechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	Seyit Yuzuak, 2018
5	[M-H]-	865	695, 577, 543, 407, 451	Procyanidin trimer	C <sub>45</sub> H <sub>38</sub> O <sub>18</sub>	Ismael Ivan Rockenbach, 2012
6	[M-H]-	387	225, 207	Medioresinol (Lignan)	C <sub>21</sub> H <sub>24</sub> O <sub>7</sub>	Razgonova Mayya P. 2022
7	[M-H]-	625	301, 300, 463	Quercetin dihexoside	C <sub>27</sub> H <sub>30</sub> O <sub>17</sub>	Leonnard O. Ojwang, 2012
8	[M-H]-	463	301, 300, 271, 151	Quercetin hexoside	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	Junfeng Hao, 2021
9	[M-H]-	801	625, 301, 300	Quercetin-3-(6''-feruoyl)-glucoside	C <sub>31</sub> H <sub>28</sub> O <sub>15</sub>	Leonnard O. Ojwang, 2012
10	[M-H]- [M-FA]-	941/987	-	Soyasapogenol A	C <sub>30</sub> H <sub>50</sub> O <sub>4</sub>	Razgonova Mayya P. 2022
11	[M-H]-	957	-	Soyasapogenol B	C <sub>30</sub> H <sub>50</sub> O <sub>3</sub>	Razgonova Mayya P. 2022



# POLLINATION AND METABOLISM (2021)



# IMPACT ON HUMAN NUTRITION?

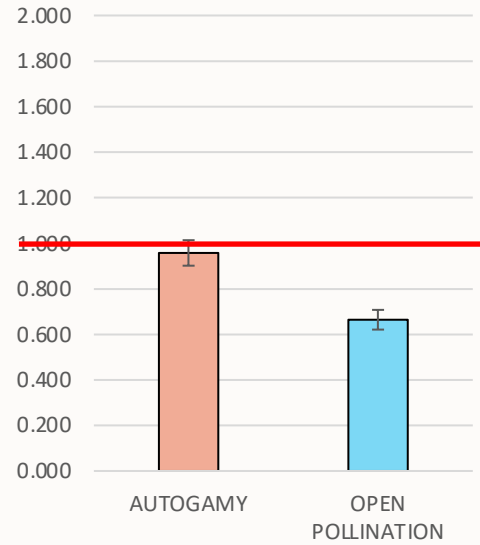


CCD841: HEALTHY COLON CELL LINE

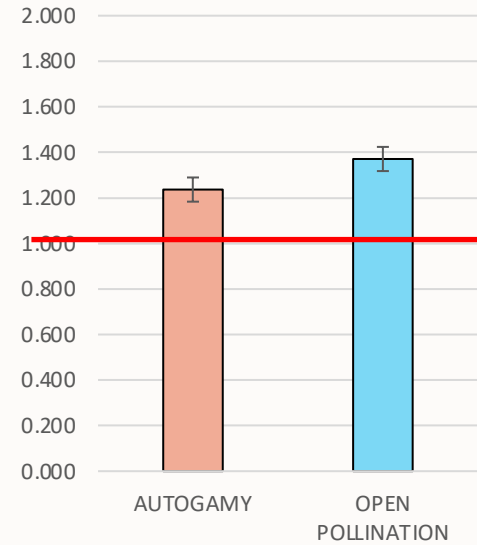


E705: TUMOR COLON CELL LINE

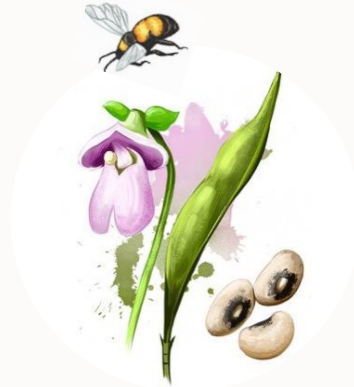
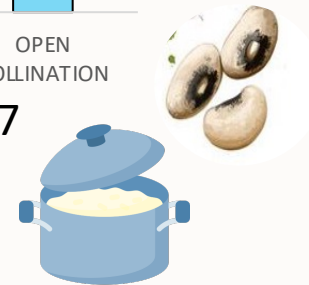
VIABILITY RATIO  
E705/CCD841 CELL LINES



$P < 0.001$



$P = 0.117$



OPEN POLLINATION



AUTOGAMY

# NEXT STEPS...



Identification of the metabolites mainly influenced by pollination treatments both in *Vigna* and *Fragaria* sp.



Screening of the bioactive properties of strawberries from different pollination treatments on human *in vitro* models



Confirm or deny of the identified patterns by the 2022 growth season

**THANK YOU FOR  
THE ATTENTION!**

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