High School Students for Agricultural Science Research

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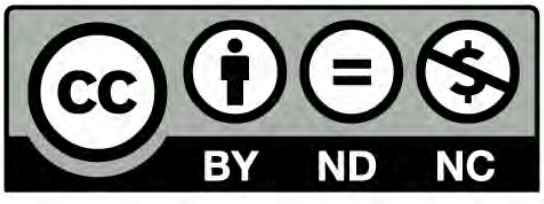

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PREFACE

Ninguna especie viva practica tantas actividades distintas como la nuestra. Muchas son universales, y entre ellas, las esenciales para la supervivencia de los organismos y de la especie, "mantenencia" y "juntamiento", como decía el Arcipreste; su fuerte determinación genética deja pocas decisiones al sistema nervioso, cuando lo hay. Los genes determinan las bases del conocimiento, la producción de objetos y la comunicación sonora, actividades prominentes, pero no exclusivas, de *Homo sapiens*; para alcanzar niveles altos tenemos que aprender, sea por imitación o por enseñanza activa, modificando nuestro cerebro a largo plazo. El método científico es una adquisición única de nuestra especie, tan reciente que la evolución biológica no ha podido afectarla específicamente y tan improbable que ha requerido la acumulación de pasos sucesivos; es difícil de aprender y debemos preguntarnos si todos deberíamos intentarlo y a qué edad convendría empezar.

La Estación Experimental del Zaidín ha abierto una experiencia modélica para iniciar en el método científico a jóvenes adolescentes. Aprovecha la presencia en las enseñanzas preuniversitarias de muchos docentes experimentados, competentes y entusiastas, sin tiempo ni medios para practicar habitualmente la investigación científica.

Ocho grupos de jóvenes expusieron en el "Congreso Caos" y colgaron en la Red los resultados de sus trabajos dirigidos al alimón por científicos y profesores. Han sido actividades muy variadas, tanto por sus fines, producir conocimiento (Ciencia) o mejorar el mundo (Técnica), como por sus métodos, que incluían el acopio de información preexistente y la aplicación de protocolos biológicos, físicos, químicos e informáticos. Me han sorprendido las nuevas habilidades, la capacidad de comunicación y el entusiasmo demostrados por muchos jóvenes. Sugiero que también se aplique a esta experiencia al método científico siguiendo el rastro dejado en ellos.

Me quedan dudas sobre si siempre se intentó aplicar el método científico, cuyo rigor puede inducir sequedad ascética, o se cayó en los juegos de la ciencia recreativa. Las imperfecciones del inglés usado por varios participantes dificultó la comunicación sin mejorar el nivel lingüístico de los oyentes.

Sospecho por de pronto que se lo han pasado muy bien y certifico que yo me lo he pasado muy bien en su Congreso.

Enrique Cerdá Olmedo

Sevilla, 2022-05-17

No living species carries out so many different activities as ours. Many of them are universal, and among them, those essential for survival of the organisms and the species, "sustenance" and "adjoining", as the Arcipreste said; their strong genetic determinism leaves few decisions to the nervous system, when there is one. Genes determine the basis for learning, the production of objects and sound communication, prominent activities –though not exclusive– of Homo sapiens*. To reach high levels we must learn, be it by imitation or by active teaching, modifying our brain in the long term. The scientific method is a unique acquisition of our species, so recent that biological evolution has not been able to influence it specifically yet, and so unlikely that it has needed an accumulation of sequential steps. It is difficult to learn and we must ponder if all of us should try, and at what age it would be best to start.*

The Estación Experimental del Zaidín has started a model experience to acquaint teenagers with the scientific method. It takes advantage of the presence in pre-university studies of experienced, able, and enthusiastic teachers, without the time or means to carry out scientific research on a routine basis.

Eight groups of students presented at the "CAOS Meeting", and published on the web, the results of their projects, supervised jointly by scientists and teachers. The activities have been very varied, both in their goals –producing knowledge (Science) or improving the world (Technology)- and in their methods, which included collecting preexisting information and applying biology, physics, chemistry and informatics protocols. I have been surprised by the new abilities, the communication capacity and the enthusiasm shown by many youngsters. I suggest to apply the scientific method also to this experience, following up the imprint left in them.

I still have some doubts as to whether the scientific method –whose rigour may lead to ascetic harshness– was always sought, or if there were instances of recreational science games. Imperfections in the English used by some participants made communication difficult without improving the language level of listeners.

I suspect in any case they had a lot of fun, and I certify I had a lot of fun in their Meeting.

Enrique Cerdá Olmedo (*Translation: M. Espinosa*)

Seville, 2022-05-17

Identifying novel retrons by bioinformatic analysis

Celia Romero-Rodríguez[1*], Emma Xuerou Pérez-Gil[1*], D. Cabrerizo[2], José Julian Fernández[1,3], Francisco Martínez-Abarca[2#]

[1]*IES Joanot Martorell C. de la Ciutat de l'Aprenent, 2, 46017 Valencia, Spain.*

[2]*Department of Soil Microbiology and Symbiotic Systems. Estación Experimental del Zaidín. CSIC. Profesor Albareda 1, 18008 Granada, Spain.*
[3]*Department of Biology and Geology. IES Joanot Martorell C. de la Ciutat de l'Aprenent, 2, 46017 Valencia, Spain.*

*All these students contributed equally to this work
#Corresponding author: fmabarca@eez.csic.es

Highlights: *Students have learned to identify genes using bioinformatics tools and understood how analyze and compare DNA and protein molecules. With these tools, they have been able to identify novel retrons in the genome of bacteria non described so far.*

Summary

Students have learned how to manage DNA sequence data using different bioinformatics tools. Once have a practice we put our interest in specific groups of prokaryotic retroelements, named as retrons that recently have been described as novel phage defense systems. They consist in tripartite elements composed by two encoded protein genes: a Reverse Transcriptase and an effector protein altogether with a ncRNA structured molecule. Students identify and compare all parts of several examples of these elements of a particular subgroup of retrons, the III-A3 type. These comparisons allowed to identify the presumably structure of the ncRNA and consequently the msDNA formed. The complete description of non-described previously retrons in bacteria of the genera *Pseudomonas* provide a basis and a strategy 'in silico' to identify this type of molecules.

Keywords: Clone manager, RNAfold, Retrons, bacterial genomes, phage defense islands, sRNAs.

INTRODUCTION

Viral infection causes detrimental of bacterial cells killing them at the end of the infection cycle. Once attached to the cell surface, phages inject their nucleic acids to produce the new progeny. Bacterial defense strategies can be divided in three main types (Figure 1; Tal & Sorek 2022). (i) Degradation of phage nucleic acids. The most abundant defense systems in

the microbial world. Among others, restriction-modification (RM) systems cleave phage DNA upon recognition of specific sequence motifs. Enzymes of the host recognizes unmodified motifs in the DNA of the viral invader and acts to cleave it. CRISPR-Cas systems act through recognition and interference with phage nucleic acids through adaptive immune memory. (Figure 1, left).

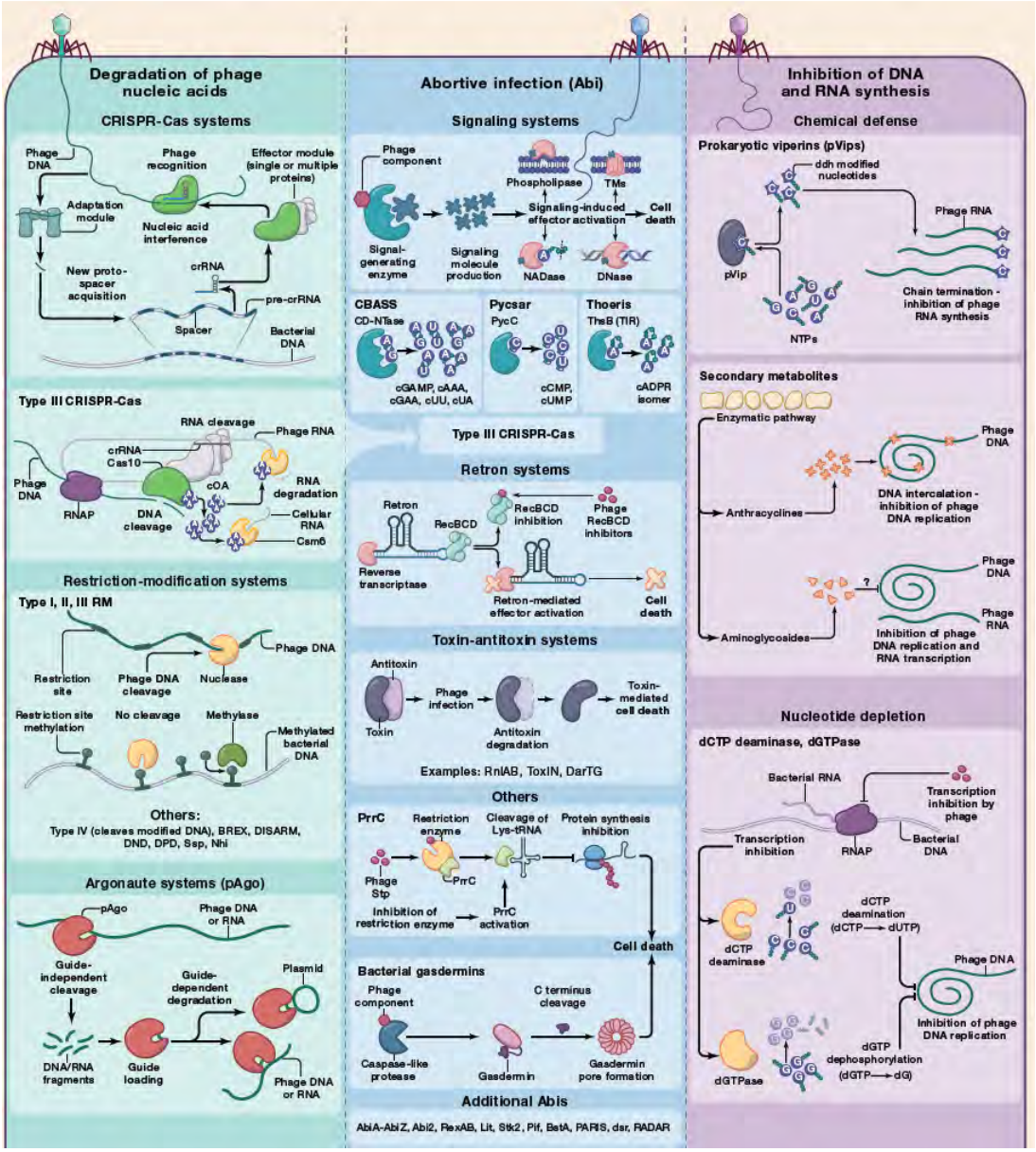


Figure 1: **Bacterial Immunity**. Examples of the three main classes of bacterial defense against viruses. (left) Mechanisms that degrade the Nucleic acid of the invader. (Center) Systems that provoke an abortive infection by inducing cellular death. (Right) Enzymes and secondary metabolites that interfere with the invader replication machinery (more information in Tal & Sorek 2022).

Another group of systems seems to inhibit DNA and RNA systhesis (right of figure 1) bacterial cells act via chemical defense producing small metabolites that poison the synthesis of nucleic acids. Examples of these systems are the production of aminoglycoside antibiotics or defensive enzymes that deplete deoxynucleotides avoiding the ability of the phage to replicate. A final group of systems (the middle of the Fig. 1) consist in those generating of

Abortive infection just before the phage begin to replicate, thus protecting the bacterial community (Lopatina, A., et al. 2020). These Abi systems are described from 1950s, but a great variety of mechanisms are studied. Among them, recently has been described those based in so called "retrons" (Millman et al 2020).

Retrons, discovered at the end of 80's past century, consist in a single transcriptional unit containing a promoter, an msr and a reverse transcriptase gene (Figure 2). The msr part includes a conserved priming guanosine residue and self-complementary regions that cause the msr-msd transcript folds in a characteristic structured RNA molecule. The final mature Retron msDNA consists in a hybrid DNA-RNA in which the DNA molecule is covalently bounded to the priming guanosine via a 2'-5' linkage (Simon et al 2019).

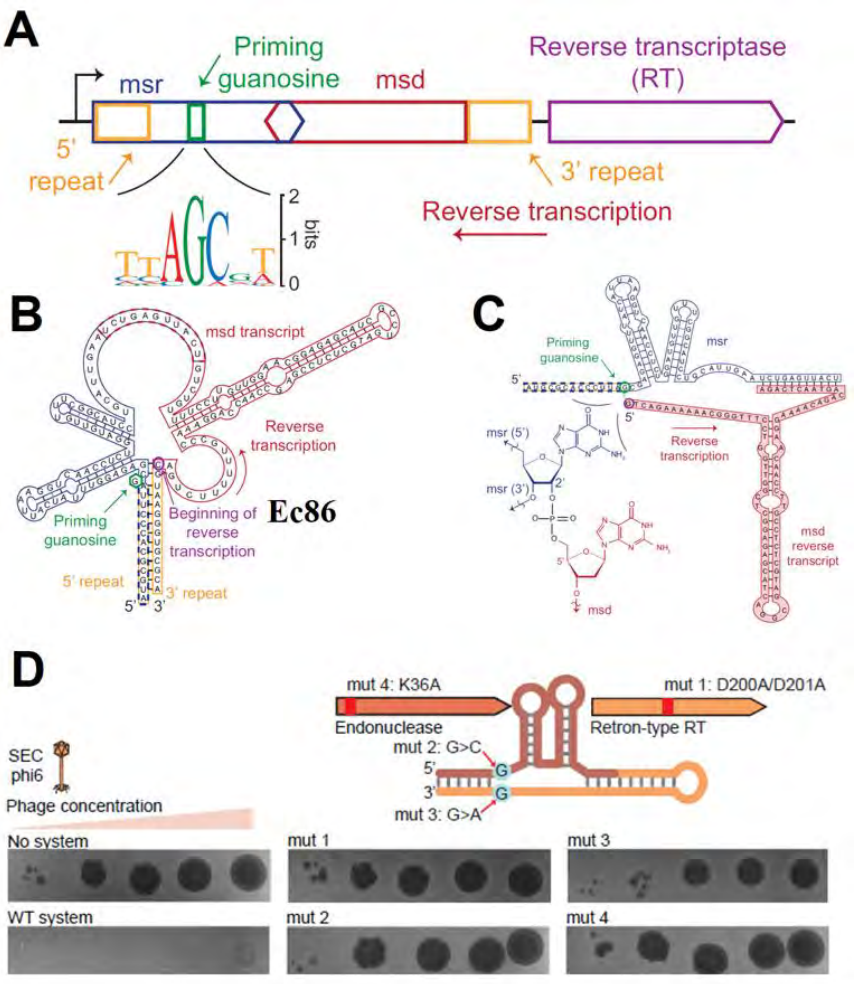


Figure 2: **Retron structure, organization and biological role**. (A) Retrons are encoded as a single transcriptional cassette see details in the main text (B) Structure of a representative msr-msd transcript encoded by Retron-Eco1 (Ec86) (C) Structure of the mature Retron-Eco1 (Ec86) msDNA. (D) Functional example of the retron system as antiviral defense. 10-fold serial dilution plaque assays against a mutational analysis of elements within the defense system. Compare the strain with the wild-type system to strains with mutated versions indicated as the above scheme of the domain organization of the gene cassette. (Adapted from Simon et al. 2019; and Millman et al. 2020).

All these components together with an effector gene are essential to defense against viruses. As example in a recent work (Figure 2D; Millman et al 2020), authors demonstrate that mutations in critical parts of the retron and in adjacent genes affect their resistance against a bacteriophage in plaque assays in vivo. Only when the system is complete no phage plaques appear. A recent classification of at least 13 types of retrons has been proposed mainly based on the characteristics of the associated effector gene (Figure 3, Mestre et al 2020) indicating that an important number of groups remain to be studied in detail.

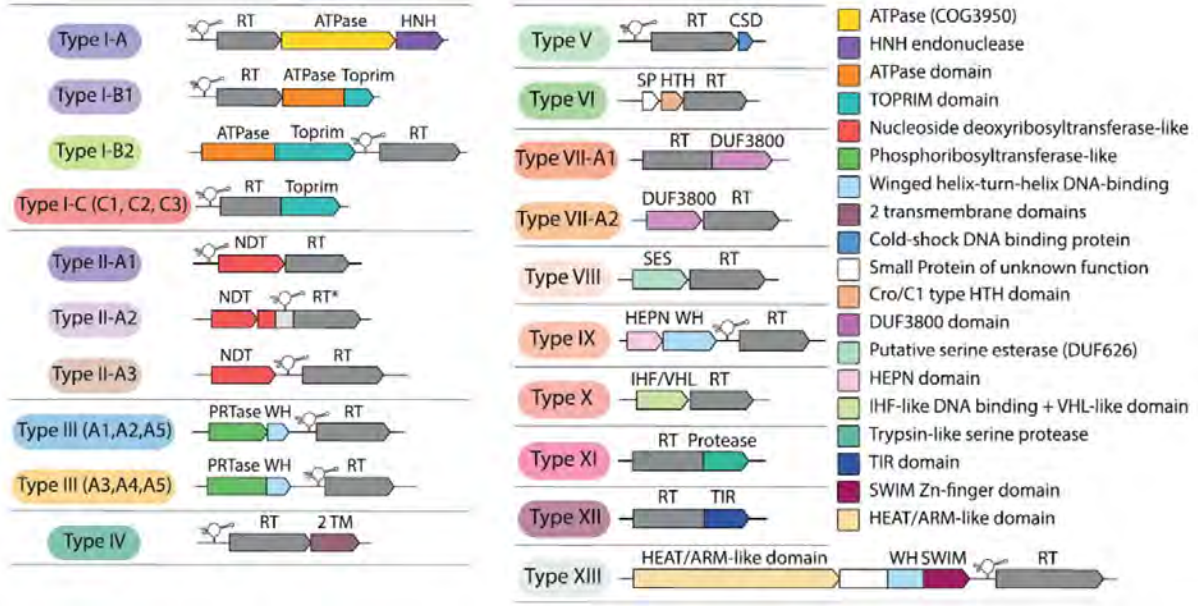


Figure 3: **Current classification of Retron diversity Systems.** Schematic diagram of the genomic organization of the 13 different types/variants of retron systems (Adapted from Mestre et al. 2020).

One of these novel groups, is the Type III A3 System (Figure 3). It is defined by the presence of an effector gene containing a Phosphoribosyl Transferase domain joined to a Winged Helix DNA binding domain (Mestre et al 2020). In fact, although a predicted structured RNA is suspected, none experimentally validated retron within this group has been described so far.

Our study has consisted to define and characterize bioinformatically novel retrons belonging to type III-A3 System.

MATERIAL AND METHODS

1. Bacterial genome data set.

A collection of 31 DNA entries (of 5-10 kb size) showed in the 1828 Retron dataset described in Mestre et al 2020 has been selected in order to analyze and define describe the Type IIIA3 Retron Systems by the students (Table 1). Around 3 kb upstream and downstream of the RT annotated gene were selected for further bioinformatics analysis.

2. Clone manager program

Clone Manager Professional Suite version 6.0 for sequences treatments was the bioinformatics program used to familiarize with the visualization of DNA sequences. This program allows, based in any DNA sequence, to get its reverse, to search immediately any DNA sequence within.

Table 1: DNA collection of 31 entries of selected Type IIIA3 Retron Systems used in this study.

	Entry	RT/Clade	Retron	NCBI Accession	Species/strain
A	1352	9	Type IIIA3	WP_061377473.1	Salmonella enterica subsp. enterica serovar Telelkebir
	1353	9	Type IIIA3	WP_002108265.1	Acinetobacter baumannii TG27387
	1354	9	Type IIIA3	WP_080314647.1	Serratia marcescens
	1355	9	Type IIIA3	WP_028302093.1	Oceanospirillum beijerinckii DSM 7166
	1356	9	Type IIIA3	WP_006955932.1	Idiomarina baltica OS145
	1334	9	Type IIIA3	WP_131706093	Rhizobium leguminosarum strain GLR69
	1335	9	Type IIIA3	WP_083231682.1	Blastomonas sp. RAC04
	1357	9	Type IIIA3	WP_009043910.1	Pseudomonas chlororaphis subsp. aureofaciens 30-84
	1358	9	Type IIIA3	WP_081076837.1	Burkholderia cepacia
	1359	9	Type IIIA3	MBS97888.1	Oceanospirillaceae bacterium
	Entry	**RT/Clade**	**Retron**	**NCBI Accession**	**Species/strain**
B	1325	9	Type IIIA3	WP_010517219.1	Komagataeibacter oboediens 174Bp2
	1326	9	Type IIIA3	WP_062609423.1	Rhizobium sp. Leaf453
	1327	9	Type IIIA3	WP_083463522.1	Prosthecomicrobium hirschii
	1328	9	Type IIIA3	PBC19310.1	Mesorhizobium sp. WSM4311
	1329	9	Type IIIA3	EKY24482.1	Brevundimonas diminuta
	1330	9	Type IIIA3	WP_091916648.1	Mesorhizobium sp. YR577
	1331	9	Type IIIA3	WP_063292689.1	Pseudovibrio sp. Ad5
	1332	9	Type IIIA3	WP_062603027.1	Rhizobium sp. Leaf386
	1333	9	Type IIIA3	WP_082786070.1	Acetobacter tropicalis
	Entry	**RT/Clade**	**Retron**	**NCBI Accession**	**Species/strain**
C	1296	9	Type IIIA3	WP_029240877.1	Pseudomonas amygdali pv. tabaci str. 6605
	1297	9	Type IIIA3	WP_047286524.1	Pseudomonas fluorescens
	1298	9	Type IIIA3	WP_081041775.1	Pseudomonas fluorescens
	1299	9	Type IIIA3	WP_090430556.1	Pseudomonas guguanensis
	1300	9	Type IIIA3	WP_093099634.1	Pseudomonas sp. Z003-0.4C(8344-21)
	Entry	**RT/Clade**	**Retron**	**NCBI Accession**	**Species/strain**
D	1336	9	Type IIIA3	WP_028626024.1	Pseudomonas plecoglossicida
	1337	9	Type IIIA3	WP_029528615.1	Pseudomonas aeruginosa
	1338	9	Type IIIA3	WP_017702484.1	Pseudomonas syringae
	1339	9	Type IIIA3	WP_024648491.1	Pseudomonas syringae pv. pisi str. PP1
	1340	9	Type IIIA3	WP_031687304.1	Pseudomonas aeruginosa
	1341	9	Type IIIA3	WP_057724478.1	Pseudomonas orientalis
	1342	9	Type IIIA3	WP_101208573.1	Pseudomonas sp. 43NM1

1st Column. Nomenclature of entries corresponding to entries of table S1 in Mestre *et al.* 2020
4th Column: NA accession number in NCBI database (https://www.ncbi.nlm.nih.gov/nucleotide/)

To have a DNA sequence implies to have a protein sequence (generally a gene) due to the universal genetic code that changes the code of four letters (G,A,T,C) to a code of 20 letter corresponding to the amino acids present in a particular protein [Caskey 1970]. This information can be visualized with the program (see further examples). Clone manager program predict genes present in a DNA sequence using the tool ORF search (Figure 4).

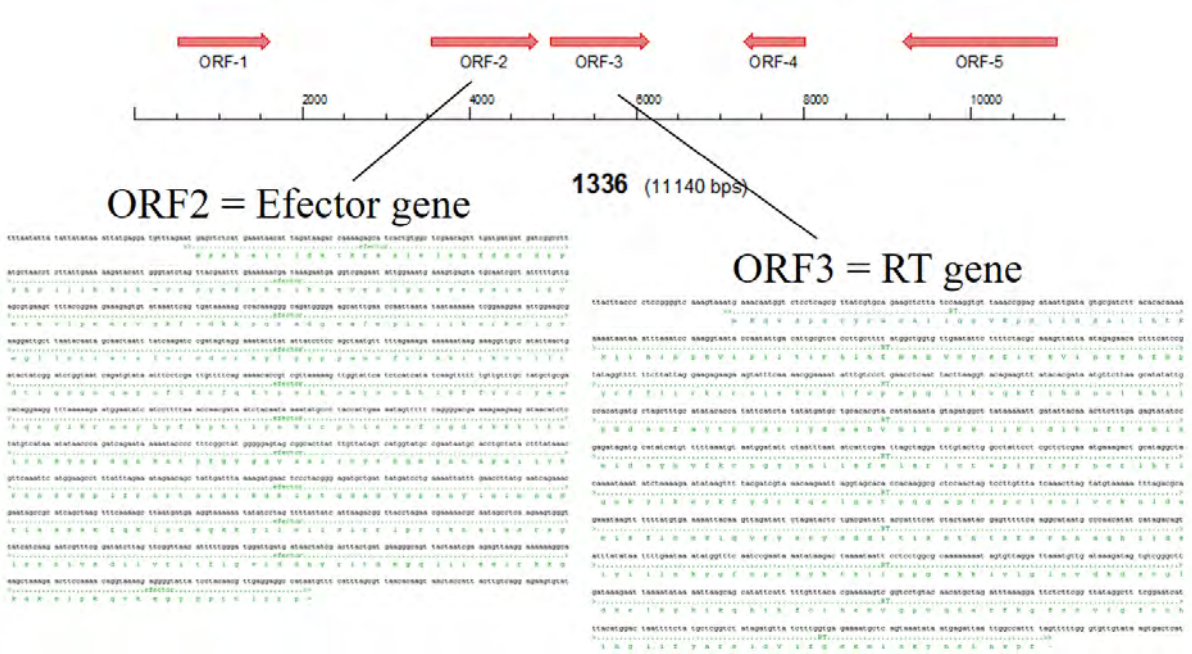


Figure 4: Example of the results obtained with ORF search tool. This figure is an example of the use of the tools 'ORF search', 'Translate' allowing the identification of the two genes (Effector and RT) for every entry of the Table 1.

A unique DNA sequence can be translated into 6 different forms. '*ORF search*' help to identify the gene, generally, considering that an open reading frame has at least 100 aa, starting by an ATG and following in frame (sets of three nucleotides) without none of the three different stop codons: TAA, TAG, TGA. The results will appear in a scrolling list box and a location map display as can be visualized in the example of Figure 4. The use also of the tools: '*translate*' for change the sequence of a gene to a sequence of a protein and '*align*' to compare protein sequences was the methodological base of this study on these 31 DNAs stretch containing the Retron System.

3. RNAfold Server program

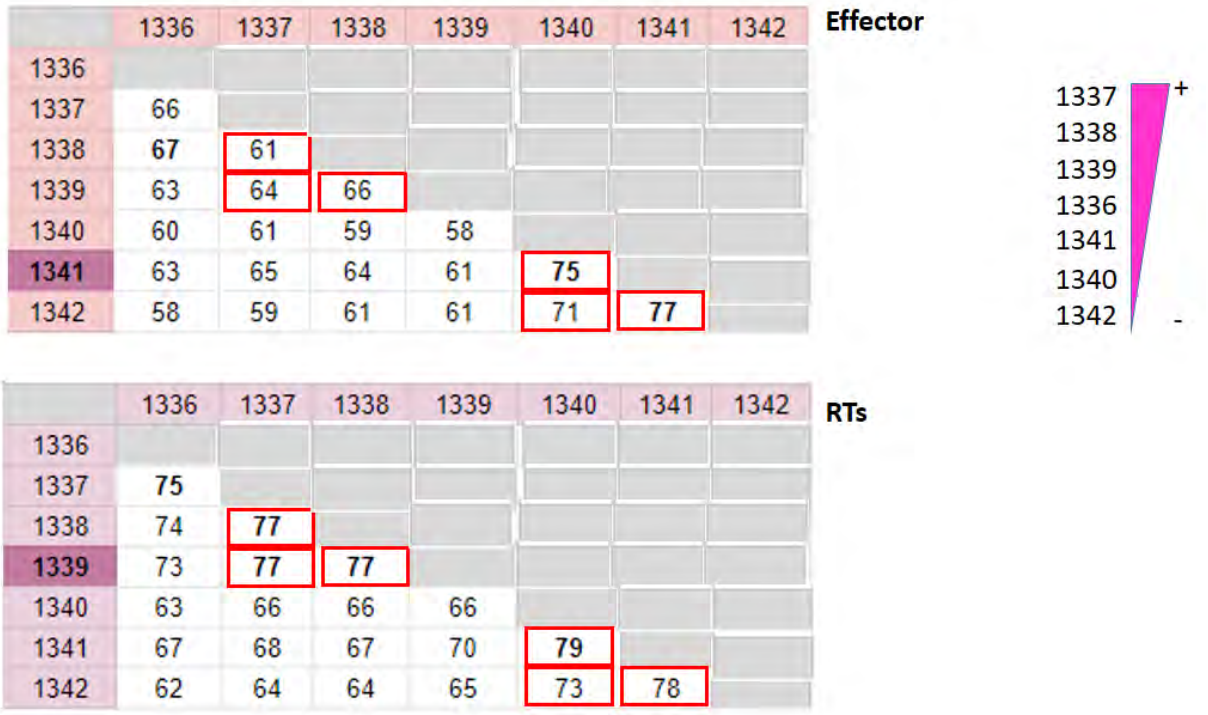
The RNA fold server of the Vienna university (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi; Lorenz et al 2011) to understand and infer the structure of the RNA molecule. Also students used the option to search for RNA structure of an alignment (see below).

RESULTS & DISCUSSION

1. Paralelism in the divergency of the efector and Reverse Trancriptase tandem genes

Using the ORF search tool we identified the corresponding effector and RT genes of every DNA locus of the sequence of the table 1 (Figure 4). Once, translate the DNA sequence to protein we performed the corresponding alignments which allow to determine the distance among those genes as percentage of identity (exemplified in Figure 5). Based on this

percentage we can obtain a distance matrix to order the different systems from closer to more distantly related. We can observe a parallelism between the distances of both genes for every group of sequences.



Effector

	1336	1337	1338	1339	1340	1341	1342
1336							
1337	66						
1338	**67**	61					
1339	63	64	66				
1340	60	61	59	58			
1341	63	65	64	61	**75**		
1342	58	59	61	61	71	**77**	

RTs

	1336	1337	1338	1339	1340	1341	1342
1336							
1337	**75**						
1338	74	**77**					
1339	73	**77**	**77**				
1340	63	66	66	66			
1341	67	68	67	70	**79**		
1342	62	64	64	65	73	78	

Figure 5: Example of Phylogenetic matrix distances of the efector and RT tandem genes of the entries corresponding to the D group of the table 1. Red squares indicate the two groups of closest entries within the group.

This parallelism predicts a close physical interaction of both genes (Pazos and Valencia 2008).

2. Analysis of the DNA sequence between effector and RT gene (Group D sequences)

Once both genes are ordered in a distance matrix, we determine if exist any conservation in the intergenic DNA region and studying if a msRNA-DNA structured retron can be formed (Figure 6). The alignment of the DNA region from the last part of the Effector gene (last 20 nt) and the first part of the RT (first 20 nt) revealed an important conservation in some regions (Figure 6A). This alignment can be used as a guide to study conserved self-interactions of the corresponding RNA molecules with the tool RNAfold of the Viena University. This guide revealed RNA interactions suggesting a conserved ncRNA structure (Figure 6B).

Thus, the interaction a1 and a2 (as a zipper for all the structure) can be clearly identified. Also, three additional stems are predicted. C and D, more conserved, and b1/b2, less conserved, as expected from the structure of other retrons described (Simon et al 2019). Moreover, this structured RNA identified clearly the two Gs Branching and Opposing critical in the formation of the cDNA retron molecule. The identification of these 'Gs' allow to propose a probably final structure for all retrons of this group non described so far.

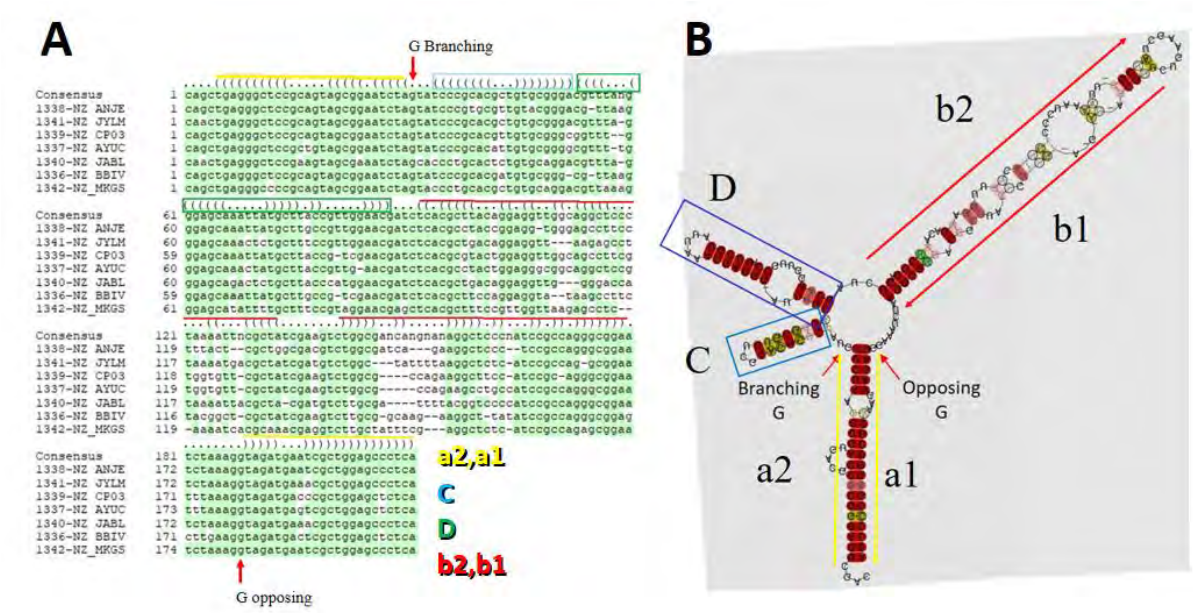


Figure 6: Result derived of the RNAalifold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAalifold.cgi) of the intergenic region (220 bp approx.) of the alignment of putative retron loci of group D. A) Nucleotide alignment of the seven entries. Above the alignment is shown the Vienna code for folding interactions illustrated as a colour code. Two red arrows indicate the positioning of the G branching and opposing characteristic of every retron. B) Folding structure for the retron of the D group entries. Red background indicates conserved interactions. Coloured lines and boxes are according to lines indicated on figure A.

3. Inferring the final structure of msDNA Pa95 retron

Understanding the retron msDNA synthesis (Simon et al 2019) allows to predict the final retron structure. The identification of the G branching where the RTs extend the DNA using as first nucleotide template de G opposing define the beginning of de cDNA that finish short nucleotides after de b2/b1 branch. As example, the final structure predicted for the retron found in the isolate corresponding to one of the entries: the 1337 is presented in Figure 7.

This retron, found in one isolate of the bacteria *Pseudomonas aeruginosa* (table 1, entry 1337) has been named as msDNA-Pa95. Consisting in a cDNA of 95 nt joined to an RNA molecule of 86 nt. Similar structures ranging from 93 to 95 nt of cDNA can be predicted for all the entries of this group D giving a confidence in the prediction of this retron structure. This work resembles a previous description of msDNAs corresponding to retrons found in pathogenic bacteria (Das et al 2011).

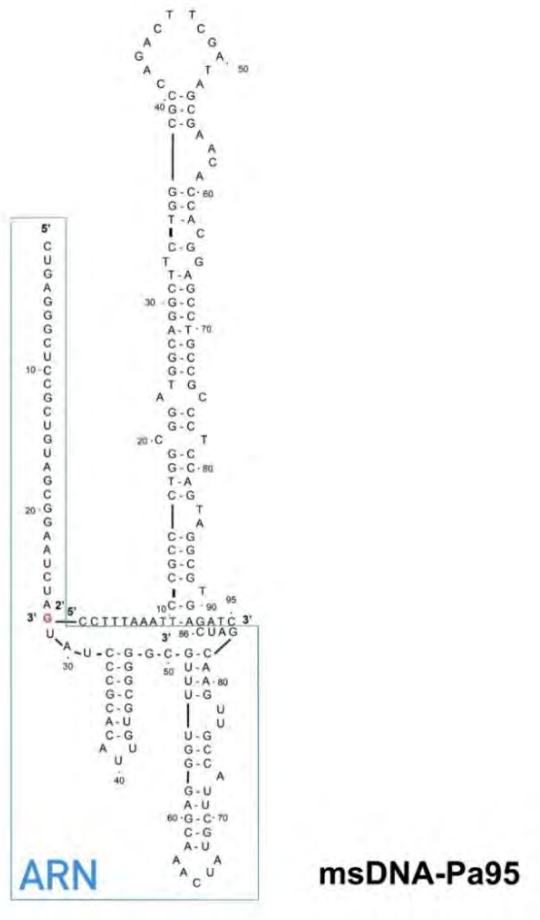


Figure 7: Putative secondary structure of multicopy single-stranded DNA (msDNA) msPa95 from *Pseudomonas aeruginosa* (entry 1337 in Table 1). (a) The branching guanine base (G) residue at position 28 in RNA portion of msDNA is indicated as red letter and forming a 2′, 5′-phosphodiester bond. Both the DNA and RNA secondary stem loop structures were suggested on the basis of their RNAalifold prediction. The RNA portion was boxed and the numbers of RNA and DNA were begun from 5′ ends.

CONCLUSIONS

Several conclusions can be obtained for this work. We can confirm that comparisons of particular set of effector and RT tandem genes suggest a concert evolution in type II-A Retron system and probably can be extended to the all retron systems in which several genes act in concert. Our work has determined A probable final structure for retrons (ncRNA-msDNA) for a group of the bacteria of Pseudomonas genera (the D group of this work). And finally, a method for the identification of the retron (msDNA) for every locus of these type of elements could be applied for other groups of retrons.

Acknowledgements

This work was performed in the Microbiology and Symbiotic Systems department in the Estación Experimental del Zaidín – Consejo Superior de Investigaciones Científicas and at student's home. It was supported by research projects BIO-2017-82244-P; PID2020-113207GB-I00 and FGCCLG-2021-0015 from la Fundación General del CSIC (Cuenta la Ciencia Program). Also we appreciate the coordination of the project of teacher Javier Julian Fernández of the Secondary School Institute Joanot Martorell in Valencia. We are particularly indebted with Antonio Quesada Ramos for his help in developing our blog: https://dna4letters.blogspot.com/.

References

[1] Caskey CT. The universal RNA genetic code. Q Rev Biophys. 1970 Aug;3(3):295-326.

[2] Das R, Shimamoto T, Arifuzzaman M. A Novel msDNA (Multicopy Single-Stranded DNA) Strain Present in Yersinia frederiksenii ATCC 33641 Contig01029 (2011) Enteropathogenic Bacteria with the Genomic Analysis of It's Retron. J Pathog. 2011:693769.

[3] Lopatina, A., Tal, N., and Sorek, R. (2020). Abortive Infection: Bacterial Suicide as an Antiviral Immune Strategy. Annu. Rev. Virol. 7, 371–384.

[4] Lorenz, R., Bernhart, S.H., Höner zu Siederdissen, C. et al. (2011) ViennaRNA Package 2.0. Algorithms Mol Biol 6, 26.

[5] Mestre MR, González-Delgado A, Gutiérrez-Rus LI, Martínez-Abarca F, Toro N. Systematic prediction of genes functionally associated with bacterial retrons and classification of the encoded tripartite systems. Nucleic Acids Res. 48(22):12632-47.

[6] Millman A, Bernheim A, Stokar-Avihail A et al. Bacterial retrons function in anti-phage defense. Cell 2020;183:1551-1561.e12.

[7] Pazos F, Valencia A. Protein co-evolution, co-adaptation and interactions. EMBO J. 2008 Oct 22;27(20):2648-55.

[8] Simon AJ, Ellington AD, Finkelstein IJ. Retrons and their applications in genome engineering. Nucleic Acids Res 2019;47:11007–19.

[9] Tal N, Sorek R. SnapShot: Bacterial immunity. Cell. 2022 Feb 3;185(3):578-578.e1.

MY OWN IDEAS

Celia Romero Rodríguez (1 Bachiller)

From my point of view, participating in this project has been a very interesting and rewarding experience. In the first place, we learned to use the bioinformatic program Clone Manager in different videocalls with Paco. The first reunion was a little overwhelming because we didn't understand very much and we thought it was going to be very difficult to use the program. In the next reunions Paco sent us some test sequences to practice and, little by little, we managed to correctly use the different tools of the program, for example the tool "align", which is used to compare genes and see its percentage of similarity.

In the second place, we started working with the sequences that we were going to investigate, which were divided in 4 groups from A to D. We searched the effector and RT genes using the ORF Search tool. We had some problems finding these genes because some of them didn't start with the typical start codon, but by changing some settings of the ORF search tool we managed to do it. In the third place we compared the genes and we ordered them in a chart by percentage of similarity. We observed a parallelism between the RT and the effector genes that showed an interaction between them. In the fourth place, we started working with the intergenic DNA region of each sequence and we wondered if a putative retron and a conservation existed between them. With the RNAFold program of the Vienna University we observed that the intergenic region generated a very characteristic structure which represented the final mature retron. We copied this structure letter by letter in a drawing, making the necessary modifications, and we obtained the structure of a type of retron never described before.

The last step was going to Granada to the Estación Experimental del Zaidín to present our project in the CAOS Congress. It was an experience that we will never forget because we had never done something like that before.

I think that the results of this project could be useful for future investigations which could contribute to the development of genetic engineering. In fact, it has been discovered recently that retrons can be used to edit genes, just as CRISPR systems. In addition, during this project we were able to see how it is to do research work, which will be useful for when we need to do a final degree project in university. For all of this I'm very happy that I was able to participate in this project.

To finish I would like to give some acknowledgements. To start I would like to thank our parents for always supporting us and doing the effort of taking us to Granada so we could enjoy this experience. I would also like to thank Javier for getting us in touch with Paco, and for helping us with every question we had. Last but not least, I would like to thank Paco for giving us the opportunity of participating in such an interesting project which has made us learn so much, and for his patience while explaining to us such complex topics like the genetics of retrons.

Para mí, participar en este proyecto ha sido una experiencia muy interesante y enriquecedora. En primer lugar, aprendimos a utilizar el programa bioinformático Clone Manager en diferentes videollamadas con Paco. La primera reunión fue un poco abrumadora ya que no entendíamos demasiado y pensábamos que nos iba a costar mucho utilizar el programa. En las siguientes reunions Paco nos envió secuencias de prueba para practicar, y poco a poco, conseguimos utilizer correctamente las diferentes herramientas del programa,

como por ejemplo la herramienta align, para comparar genes y ver su porcentaje de coincidencia.
En segundo lugar, empezamos con las secuencias a investigar, divididas en 4 grupos de la A a la D. Buscamos los genes efector y RT de cada secuencia con la herramienta ORF Search. Tuvimos problemas para encontrar algunos genes ya que no empezaban por el codón de inicio habitual, pero cambiando algunos ajustes de la herramienta ORF Search conseguimos hacerlo. En tercer lugar comparamos los genes y los ordenamos en una tabla por porcentaje de similitud. Observamos un paralelismo entre los RT y los efectores que indicaba una interacción entre ellos. En cuarto lugar, empezamos a trabajar las zonas intergénicas de cada secuencia, y nos preguntamos si había conservación y un posible retrón en ellas. Con el programa RNAFold de la universidad de Viena observamos que la zona intergénica generaba una estructura muy característica que representaba el retrón maduro final. Copiamos letra por letra esta estructura en un dibujo, haciendo las modificaciones necesarias, y obtuvimos la estructura de un nuevo tipo de retrón jamás descrita anteriormente. El último paso fue ir a Granada a la Estación Experimental del Zaidín a presentar nuestro trabajo en el Congreso CAOS, una experiencia para recordar ya que nunca habíamos hecho algo así. Creo que los resultados de este trabajo podrían servir para investigaciones futuras que contribuyan al desarrollo de la ingeniería genética. De hecho, recientemente se ha descubierto que los retrones pueden utilizarse para editar genes al igual que los sistemas CRISPR. Además, durante el transcurso de este proyecto hemos podido observar de primera mano cómo es realizar un trabajo de investigación, cosa que nos será útil para cuando necesitemos hacer un trabajo final de grado en la universidad. Por todo esto me alegro mucho de haber podido participar en este proyecto.
Por último me gustaría dar algunos agradecimientos. Para empezar me gustaría dar las gracias a nuestros padres por apoyarnos siempre y por hacer el esfuerzo de acompañarnos hasta Granada para que pudiéramos disfrutar de esta experiencia. También me gustaría agradecer a Javier por ponernos en contacto con Paco y ayudarnos con nuestras dudas. Y por supuesto, quiero agradecer a Paco por darnos la oportunidad de participar en un proyecto tan interesante y que tanto nos ha hecho aprender, y por su paciencia a la hora de explicarnos temas tan complejos como son la genética de los retrones.

Emma Xuerou Pérez Gil (1 Bachiller)

It has been a unique experience. In the beginning, we were lost and the development of the project was difficult because we were working with retrons, which we have never heard of. However, with the passage of time and practice, we gradually became familiar with it and achieved a good result.
As in all experiments, there are usually unpredictable things. An example was the discovery of start codons which were different to the ones we had learnt at secondary school. It follows that some sequences start by GTG, instead of ATG, which is more common.
After finding all the open reading frames (effector and RT genes) of each sequence with the ORF Search tool and aligning them correctly according to their similarity, all was easier. We found every intergenic region and annotated their parts. As this region has a higher degree of conservation than the rest of the sequence, it could harbor a possible retron. So we could name the different retrons of the D group, a group without too many anomalies, and make the structure of the retron with the structure generated by the RNA Fold program of the University of Vienna from the intergenic region sequence. Do this was not easy. We spent a lot of time on

it because we had to place each nucleotide manually, looking at the sequence of the Clone Manager at the same time. But although we had some problems with the format (some parts were displaced and made the structure wrong), the result was optimum.
Finally, we presented our project and results at the CAOS Congress that was held at the Estación Experimental del Zaidín in Granada. It was a new experience and we were able to learn from other projects. It is something I will never forget.
Even if we had different problems, this project has been very interesting, but above all enriching. This project about retrons can help to study them in detail and as its function seems like CRISPR-Cas9's, it can revolutionize the biotechnology and improve human's lives.
To finish, I want to give some acknowledgements. Thanks to our researcher Paco, who was patient with us and taught us about the retrons, and Javier, our teacher and tutor of our research project who told us about retrons. Thanks to Celia, who is a very good friend, but also a great teammate and a great support for me. Finally, I am grateful to my parents, who support me every day and made it possible to attend the congress.

Ha sido una experiencia única. Al principio, estábamos perdidas y el desarrollo del proyecto avanzaba lento y pesado. Al fin y al cabo, estábamos trabajando con retrones, de los cuales nunca habíamos oído hablar. Pero con el paso del tiempo y la práctica, nos fuimos familiarizando poco a poco y conseguimos un buen resultado.
Como en todos los experimentos, hay cosas que no se pueden predecir. Un ejemplo fue el descubrimiento de codones de inicio diferentes a los que habíamos aprendido en el instituto. Resulta que hay algunas secuencias que empiezan por GTG, en vez de lo más común que es ATG.
Después de encontrar correctamente todos los marcos abiertos de lectura (genes efector y RT) de cada secuencia con la herramienta ORF Search y alinearlos según su similitud, todo fue mucho más sencillo. Encontramos cada región intergénica y anotamos todas sus partes. Como esta región posee un mayor grado de conservación que el resto de la secuencia, podría albergar un posible retrón. Así pudimos nombrar a los diferentes retrones del grupo D, un grupo sin demasiadas anomalías, y construir el dibujo del retrón con la estructura que generaba el programa RNA Fold de la Universidad de Viena a partir de la secuencia de la región intergénica. Hacer la estructura final no fue fácil. Le dedicamos mucho tiempo porque había que colocar cada nucleótido a mano, fijándonos en la secuencia del programa Clone Manager al mismo tiempo. Aunque tuvimos algunos problemas con el formato (algunas partes se desplazaban y no cuadraba el dibujo), al final el resultado fue óptimo.
Finalmente, presentamos nuestro proyecto y resultados en el Congreso CAOS que se hacía en la Estación Experimental del Zaidín en Granada. Fue una experiencia nueva y pudimos aprender de otros trabajos. Es algo que nunca olvidaré. Pese a los diferentes contratiempos, pienso que ha sido un trabajo muy interesante, pero sobre todo enriquecedor. Este trabajo sobre retrones puede ayudar a estudiarlos más a fondo y como su función se asemeja a la del CRISPR-Cas9, revolucionar la biotecnología y mejorar la vida de las personas.
Para acabar, quiero dar unos agradecimientos. Gracias a nuestro investigador Paco, por su paciencia y sus grandes lecciones sobre retrones, y a Javier nuestro profesor y tutor de nuestro trabajo de investigación, quien nos introdujo en el mundo de los retrones. Gracias a Celia, que a parte de una gran amiga es una gran compañera de equipo y un gran apoyo para mí. Por último, estoy muy agradecida con mis padres, quienes me apoyan cada día e hicieron posible la asistencia al congreso.

Javier Julián Fernández (Teacher)

Years ago, when I studied genetics at university, retrons were unkown. Almost 20 years later, I had the opportunity to meet Francisco Martínez-Abarca, an expert in these bacterial genetic elements, who suggested that my high school students work together with him to describe novel retrons. Without any doubt, I said 'yes'.
Retrons have a function similar to the famous CRISPR, which has revolutionized genetic engineering and been the subject of a Nobel Prize. I thought that retrons would not end up being used in genetic engineering and it would take many years to be used. However, a recent newspaper report about a scientific article where CRISPR and retrons were used together brings to me the conclusion that our research could form part of a next revolution in genetic engineering.
The two best students of the institute, Emma and Celia, from 1st year of Bachelor, carried out a laborious and meticulous work, with the tutoring and guidance of Francisco and after several months of bioinformatics work, they managed to understand knowledge about genetics at university level and carry out a work at the level of a Final Degree Project, in addition, they managed to describe a never before described retron and they exhibited their entire project at the CAOS congress in Granada.
For me, this project has been a fresh breath of knowledge and an incentive to coordinate with scientific researchers who are at the forefront of work with science and scientific knowledge. It has been a success and very satisfying for everyone, but especially for the students who have seen first-hand how they could work in the future. Thank you for this great opportunity Paco.

Hace años, cuando estudié genética en la universidad no se conocían lo que eran los retrones. Casi 20 años más tarde, tuve la ocasión de conocer a Francisco Martínez-Abarca, expero en estos mecanismos genéticos bacterianos, que propuso que mis alumnos del instituto trabajaran conjuntamente con él para describir retrones, sin pensarlo dije que sí.
Los retrones tienen una función similar al famoso CRISPR, que ha revolucionado la ingeniería genética y sido el motivo de un premio Nóbel. Creí que los retrones no acabarían utilizádose en ingeniería genética ya que estaba CRISPR o que se tardarían muchos años en ser utilizados, pero tras una noticia de periódico reciente sobre un artículo científico donde se utilizaban conjuntamente CRISPR y retrones, llegué a la conclusión que podríamos estar trabajando con el mecanismo en el que se basará la próxima revolución en ingeniería genética.
Las dos mejores alumnas del instituto, Emma y Celia, de 1º de Bachiller, realizaron un trabajo laborioso y minucioso, con la tutorización y guía de Francisco y al cabo de varios meses de trabajo de bioinformática, consiguieron comprender conocimientos sobre genética a nivel universitario y realizar un trabajo a nivel de Trabajo de Final de Grado, además, consiguieron descibir un retrón jamás descrito y expusieron todo su proyecto en el congreso CAOS en Granada.
Para mí este proyecto ha supuesto un aliento fresco de conocimiento y un incentivo para coordinarnos con investigadores científicos que están en primera línea de trabajo con la ciencia y el conocimiento científico. Ha sido un éxito y muy satisfactorio para todos pero sobretodo para las alumnas que han conocido de primera mano cómo podrían trabajar ellas en un futuro. Gracias por esta gran oportunidad Paco.

Plants can be used as electrical batteries

Luis José Fernández Cirre[1], Jorge Plaza de Bruijn[1], Lucía Rodríguez Moreno[1], Daniel Illescas Vílchez[1], Jaime Ortega Martín[1], Franco Marlon Loza Alcocer[1], Marcos López Perea[1], Lucía Morcillo Pérez[1], Emilio Padilla Méndez[1], Germán Tortosa Muñoz[2#]

[1]*I.E.S. Padre Suárez, Gran Vía de Colón, 61, 18001 Granada, Spain.*

[2]*Department of Soil and Plant Microbiology. Estación Experimental del Zaidín. CSIC. Profesor Albareda 1, 18008 Granada, Spain.*

#Corresponding author: german.tortosa@eez.csic.es

Summary

In the rhizosphere, lots of chemical reactions occur while root plants are transforming the soil organic matter and assimilating nutrients. These redox conversions produce an electrons flux similar to a battery, which can be captured by an electrode device; it's the so-called biophotovoltaic energy. In this project, we have evaluated onions growth to produce it and the electric potential difference (or voltage) was measured every week during 89 days. According to our results, the voltage of pots with plants was notable higher than in pots with soil but without plants, which meant that roots stimulated organic matter degradation and the electrical flow. During this trial, the voltage became higher when plants start growing properly and then, onion roots were able to produce similar voltage as a zinc-carbon battery.

Keywords: *Allium cepa,* bio-waste, compost, electricity, onions, organic matter, substrate, voltage.

INTRODUCTION

Soil is essential for the development of life on our planet, especially for plants. Organic matter is one of its basic components, which can be defined as a group of carbon-based compounds and molecules. It is well known that the organic matter can improve the physical, chemical and biological properties of soils [1]. Therefore, the addition of organic matter to soil is beneficial for the biological process of nutrient conversion and its assimilation by the root system. For that reason, soil organic matter brings important ecological and production benefits for agriculture and environment.

When the organic matter is added to a soil, several chemical compounds oxidations and reductions occur and a flow of electrons similar to a battery is created. This electrical energy can be used to generate electricity; it's the so-called Biophotovoltaic energy [2]. This technology is based on installing anodes and cathodes in the soil rhizosphere (where biological activity and redox reactions occur) to generate an electric potential difference (or voltage) that can produce electrical energy [3, 4 and 5].

According to that, the objective of this project was to demonstrate if plants can be used as a common battery.

MATERIAL AND METHODS

1. Growing substrates

In this experiment, 1 kg of a pre-sieved soil was collected from an agricultural location of La Vega de Granada (Churriana de la Vega, Spain). Also, bio-waste compost was used as an organic matter amendment. Briefly, compost was made by mixing chopped tree pruning, fresh cut grass and food waste from Estación Experimental del Zaidín (EEZ-CSIC). The composting procedure and characteristics can be consulted in [6].

2. Plant experiment.

At the beginning of the assay, several 0.1 l pots were filled with growing substrates according to these treatments (Figure 1):

- Agricultural soil (S): 150 g.
- Bio-waste compost (C): 150 g.
- Mixture 1:1 (v/v) of soil and compost (SC): 75 g of S and 75 g of C.

Then, pots were watered with 50 ml of tap water every week, and after 19 days, pre-germinated onions (*Allium cepa*) were transplanted in each pot (four pots per treatment). A set of pots with only soil were used as a Control treatment.

Plants were grown at the I.E.S. Padre Suárez facilities under environmental conditions. After 89 days, onions were harvested and plant heights were registered. Also, soil pHs were measured by using a portable pH-meter (pH PCE-PHD 1-PH) after 1:20 (w:v, weight to volume) aqueous extraction at both, the beginning and the end of the experiment.

2. Electrical device and voltage measurement.

Copper tubes (120 and 15 mm of length and diameter) and galvanized screws (60 mm of length) were used as cathodes and anodes, respectively (Figure 1). Both electrodes were inserted in each pots substrate (40 mm under soil surface) at the beginning of the experiment and the electric potential difference (or voltage) was recorded by using a professional digital multitester (AoKoZo 21D Polimetro Digital 6000 Cuentas, TRUE RMS). Every week and just after watering, the voltage was both measured individual and collectively, the later by connecting pots as a series circuit (with cable connectors), as it is shown in Figure 2.

Figure 1. Pots used in this experiment: S (soil), C (compost) and SC (soil and compost at 50%). In each pot, a copper tube and galvanized screws were used as a cathode and anode, respectively.

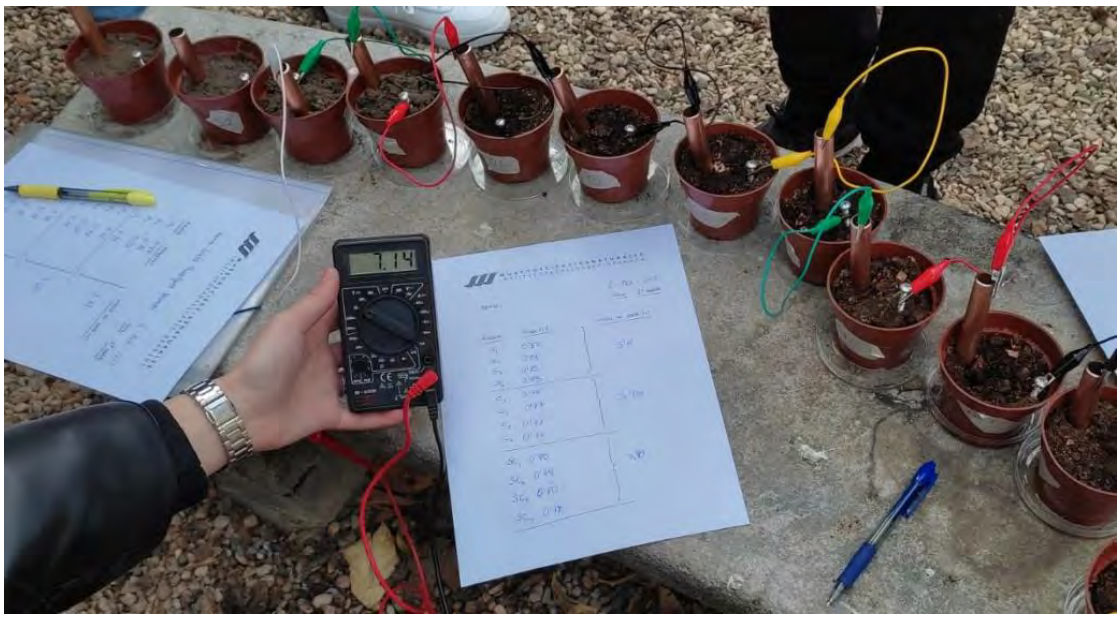


Figure 2. Electric potential difference (or voltage) measurement done by using a digital multitester.

RESULTS AND DISCUSSION

The evolution of voltage during the experimentation is shown in Figure 3. According to these data, three periods were found:

The first phase was related to pots without plants and lasted 14 days. The voltages were ranged between 2.6 and 3.2 V, in which the voltage values were S>SC>>C.

The second one (from 14 to 69 days) lasted 55 days. This period began when the onions were transplanted. During this period, voltage values and their behavior were similar that those obtained in the first period (S>SC>>C).

Finally, the third phase (from 69 to 89 days) was characterized by an increase in the voltage values in all treatment assayed compare to pots with only soil (Control treatment). This behavior could be explained due to plants start to grow significantly. This fact was confirmed with onion tallness results: SC showed the highest onion height recorded (45 cm) compare to S (37 cm) and C (31 cm) treatments, respectively (Figure 5). According to that, compost promoted onions growth only when it was mixed with soil at 50%.

Just before transplanting, substrate pHs were measured and pH values (7.9, 8.5 and 8.1 for S, C and S+C treatments) showed an inverse tendency compare to voltage (Figure 4). As much higher the pH was, the lower voltage was registered. However, this behavior was not found at the end of experimentation, with alkaline pH values ranged between 8.5 an 9.0 for all treatments.

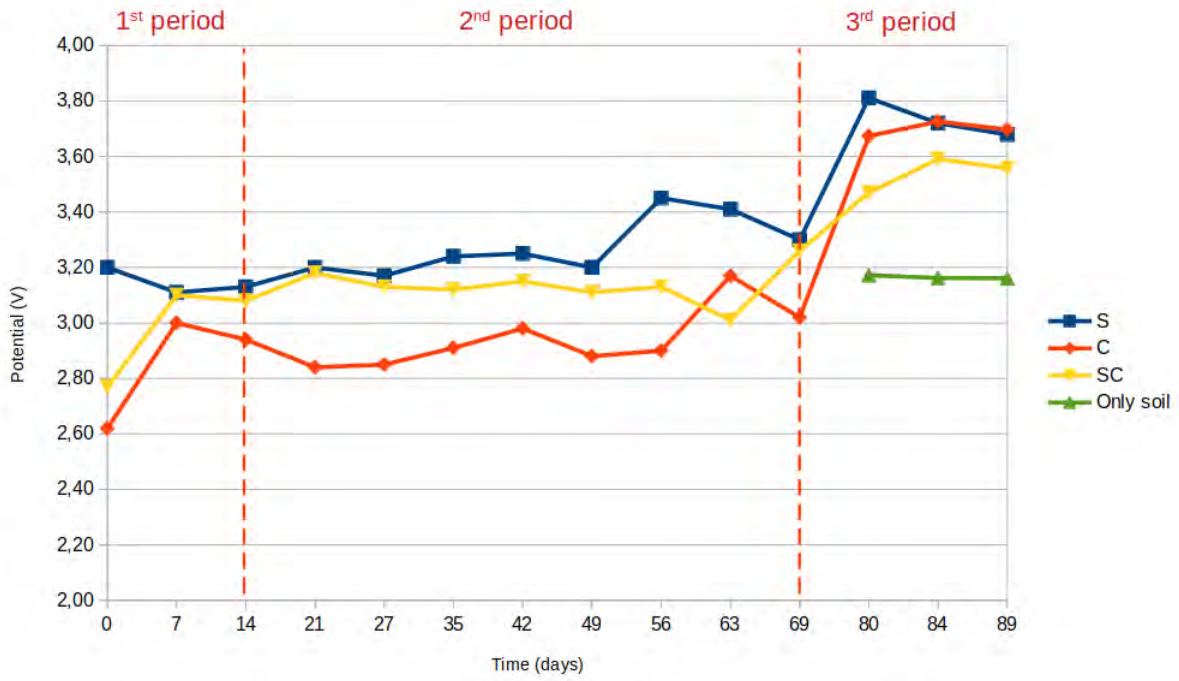


Figure 3. Evolution of electric potential difference (V) during the experimentation: S (soil), C (compost) and SC (soil and compost at 50%).

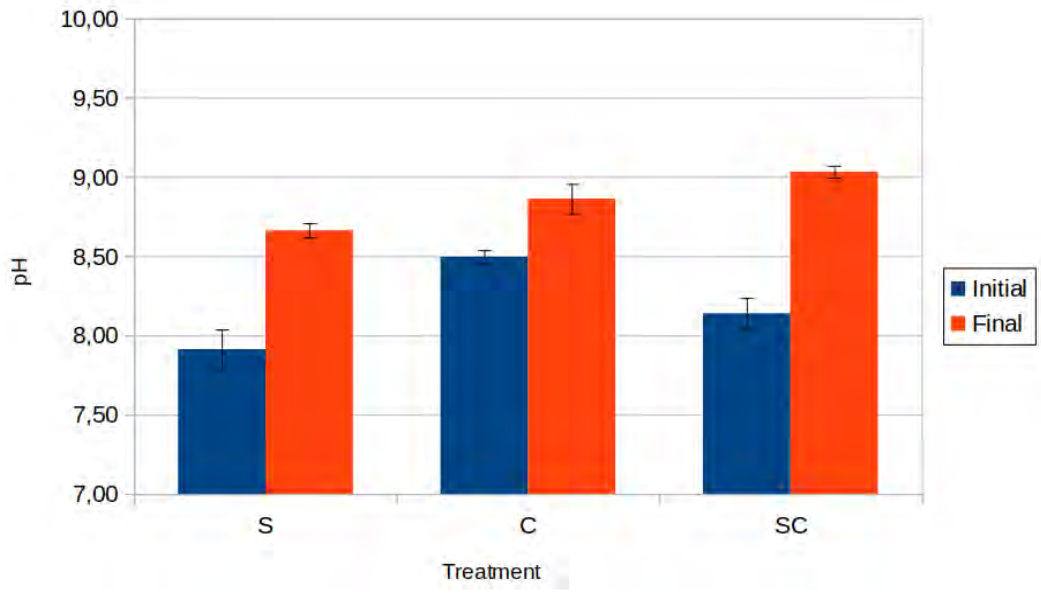


Figure 4. Soil pH of pots measured at initial and final stages of the experiment. Treatments: S (soil), C (compost) and SC (soil and compost at 50%).

According to these data, it can be concluded that the voltage with onion pots was higher compare to control pots without plants, and voltages became higher when plant starts growing. On the other hand, it was confirmed that compost promoted plant growth with apparently no influence on biophotovoltaic energy production. This could be explained due to volume pots were so small (only 0.1 L) or even electrodes sizes and configuration were not optimal. Further research needs to be done in order to address these issues and to obtain electrical energy flow from this system.

Acknowledgements

We are very grateful to Loli Riesco Conde for her kindly and useful help with the watering while we cannot do it.

References

[1] Bot A., Benites, J. 2005. The importance of soil organic matter. Key to drought-resistant soil and sustained food production. FAO Soils Bulletin, Nº 80, ISBN 92-5-105366-9. Available in: http://www.fao.org/3/a0100e/a0100e.pdf

[2] Tschörtner J., Lai B, Krömer JO. 2019 Biophotovoltaics: Green Power Generation From Sunlight and Water. Front Microbiol 10:866. doi: 10.3389/fmicb.2019.00866

[3] Salgado Gutiérrez MC, Zepeda Hernández JA, Cid del Prado Martínez M., Luna Villalobos J., Cruz Rodríguez R.I. 2019. Celda biofotovoltaica para la generación de energía eléctrica a través de plantas vivas. Academia Journals, 2019. Ebook Online. ISBN: 978-939982-52-0.

[4] Zapien-Rodríguez JM, Solorio de Jesús BA, Ballesteros-Pacheco JC, Núñez-Ayala FL. 2019. Generación Eléctrica a Partir de la Fotosíntesis Natural; ¿Una Realidad Escalable? Revista de Energías Renovables. Vol. 3, Nº1 1-6. DOI: 10.35429/JRE.2019.10.3.1.6

[5] Bombelli P et al. 2016 Electrical output of bryophyte microbial fuel cell systems is sufficient to power a radio or an environmental sensor. R Soc open sci 3: 160249. http://dx.doi.org/10.1098/rsos.160249

[6] Díaz JM, Moro C, Coletti Y, de la Torre A, de la Torre J, Rolland A, Ledesma D, Bedmar EJ, Tortosa G. 2020. Biological insights of Estación Experimental del Zaidín (EEZ) bio-waste composting. High School Students for Agricultural Science Research. Vol. 9, Estación Experimental del Zaidín (EEZ-CSIC), ISSN: 2340-9746. Availbale in: https://www.ucc.eez.csic.es

MY OWN IDEAS

Luis José Fernández Cirre

It has been an unforgettable experience since we have learned things that we normally do not study in our institute. It has been very interesting to know how an investigation should be carried out. We have used instruments that we had not used before.
Going to a congress and presenting in front of scientists is something incredible and makes me want to participate in new research projects more.

Jorge Plaza De Bruijin

I really enjoyed participating in this project with my colleagues. I've learned things I didn't know about plants, like how you can get electricity from them. That has surprised me.
During these last months we have worked very well and I hope that this project will support other new ones and that this method of obtaining electrical energy will be used more often in the future. I think it would be better for everyone.

Jaime Ortega Martín

This research project has been very interesting. I have learned many things about plants and about the scientific method.
Participating in a project like this, attending the final congress at the Estación Experimental del Zaidín (EEZ-CSIC) in Granada and presenting our results in front of such a large audience, are experiences that have been worthwhile and I recommend other colleagues to participate in the future.

Lucía Rodríguez Moreno

I really liked the experience. Learning science by doing research is very interesting. I have learned to face a large audience, larger than normal to what I am used to. I have increased my creativity, working in a group with my colleagues and doing a process that is not easy, but in the end every effort has its reward.

Daniel Illescas Vílchez

I really liked participating in this project, since we have promoted learning outside the classroom. I have learned how to do scientific research, learning new concepts and handling new instruments.

Efficacy of different face masks against bacteria

Jorge Contreras[1], Carolina Tarazaga[1], Claudia Donaire[1], Lucía Graciano[1], Cristina Gutiérrez[1], Javier Juguera[1], Antonio Ontiveros[1], Marta Victoria[1], Manuel Espinosa[2], Dolores Bernal[1#]

[1]*Department of Biology and Geology. IES Francisco Ayala, Granada, Spain.*

[2]*Department of Environmental Protection. Estación Experimental del Zaidín. CSIC. Granada, Spain.*

#Corresponding author: lbernal5@hotmail.com

Summary

In this work we have intended to evaluate the effectiveness of different types of face masks against bacterial penetration from the outside or the inside, to simulate the posibility of becoming infected or of transmitting an aerosol-borne bacterial infection. Besides introducing students to some aspects of basic microbiology, one objective of this project was to allow them to test first-hand the importance of correctly wearing and handling a face mask, and the differences between masks. Now, students have confirmed by themselves that face-mask negationists are on the wrong side, despite their noise in social networks.

Keywords: *Escherichia coli*, surgical mask, FFP2, nanofibres, aerosols, air-borne bacteria

INTRODUCTION

The pandemic caused by the SARS-CoV-2 virus has modified many of our daily habits. One of the most persistent changes has been the routine use of face masks, which have been required in public places in Spain from March 2020 until April 2022. In the first period of the pandemic, many people made their own fabric masks due to the lack of factory-made ones, while later on surgical masks and (less often) FFP2 masks have been the most frequently used. Commercial fabric masks have also been available, with or without different kinds of filters. At the same time, social movements in some countries against the mandatory use of masks could be seen in the news. Although these have been minoritary, "negationism" and the variety of available mask types with different mechanisms of filtration [1] offer a good opportunity to put the scientific method into practice and test first-hand the utility of masks.

For this project we have focused on air- or aerosol-borne bacteria, with the following objectives:

- The filtering efficiency of different masks against bacteria present in the air
- The potential protection of masks against simulated respiratory infections
- Factors that can affect the efficiency of masks: color, humidity and time of use.

MATERIAL AND METHODS

1. Masks

The following mask types have been used:

Fabric masks: these are not standardized and their efficiency could depend on the type of material used, the presence or absence of a filter, and the type of filter.

Surgical masks: designed to protect from infectious droplets in clinical sttings. They are based on mechanical filtration with pores between 10-20 microns. The usual ones with blue (external) and white (internal) sides were routinely used. Additional experiments were done with white masks provided by Junta de Andalucía

FFP2 masks: particles are trapped by electrostatic filters. They protect against smaller aerosols, but they hamper breathing after prolonged use.

CSIC-designed masks: these are made with a nanofibers-based material, which increases the filtration capacity with respect to surgical masks and do not hamper breathing. They were purchased from Proveil.

2. Bacteria, media and growth conditions

Escherichia coli W3110 was used to simulate bacterial infections. This strain is a well-characterized derivative of *E. coli* K-12 [2] and is non-hazardous and non-pathogenic, routinely used in laboratory research. *E. coli* was grown overnight at 37°C with orbital shaking in Luria-Bertani (LB) medium [3]. For aerosol preparation, the culture was diluted 1:1000 in sterile distilled water. Petri dishes of Ø 50 mm or 90 mm with LB + agar (1% w/v) were used in the experiments.

3. Experimental setup for airborne microorganisms

To study the differences in the levels of airborne microorganisms, triplicate Petri plates (Ø 90 mm) were set in different spaces of the high school and left open for 30 min., with or without being covered by a face mask. After that time, plates were closed and incubated at 25°C for 24h. The number of colonies was then counted.

4. Experimental setup for simulated respiratory infection

To analyze the efficacy of masks against aerosol-borne bacteria (as may be present in a sneeze), open Petri plates (Ø 50 mm or Ø 90 mm) were placed on a polystyrene cube with pins simulating ears and nose. The plate was immediately covered by a face mask that was attached to the cube using the ear rubber bands (Figure 1). An *E. coli* aerosol was sprayed on the device (which we named Evaristo), at a distance of around 25 cm. Plates were covered

and incubated overnight at 30°C. Controls without mask or sprayed with water were also tested.

Figure 1. Experimental setup for testing aerosol bacteria ("Evaristo").

The same setup was used to test the filtration efficiency of each side of the face mask, and the effect of humidity on filtration efficiency, by spraying with water before spraying with the bacterial suspension. To simulate the influence of longer use or repeated exposure, a second spray on fresh plates was done after the first one with bacteria.

5. Accumulation of microorganisms on mask surfaces

Pieces of 1 cm^2 (corresponding to the areas around the nose and mouth) were cut from masks worn during the previous day, and placed on Petri dishes for 5 min each side. Then the pieces were removed, plates incubated overnight at 30°C, and the number of colonies counted, distinguishing between bacteria and fungi.

RESULTS AND DISCUSSION

Airborne microorganisms in the high school environment.

The first analysis was to assess the number of airborne microorganisms present in different locations of our high school, by counting the number of colonies on Petri plates left open for 30 minutes. Results are presented in Figure 2. Surprisingly, the highest number of colonies was obtained on plates placed in the library. This suggests that the space does not receive sufficient ventilation and/or cleaning and could be a source of contagion. The playground also showed relevant number of microorganisms, which my reflect the fact that it is a common area where a larger number of students accumulates, compared to other areas tested.

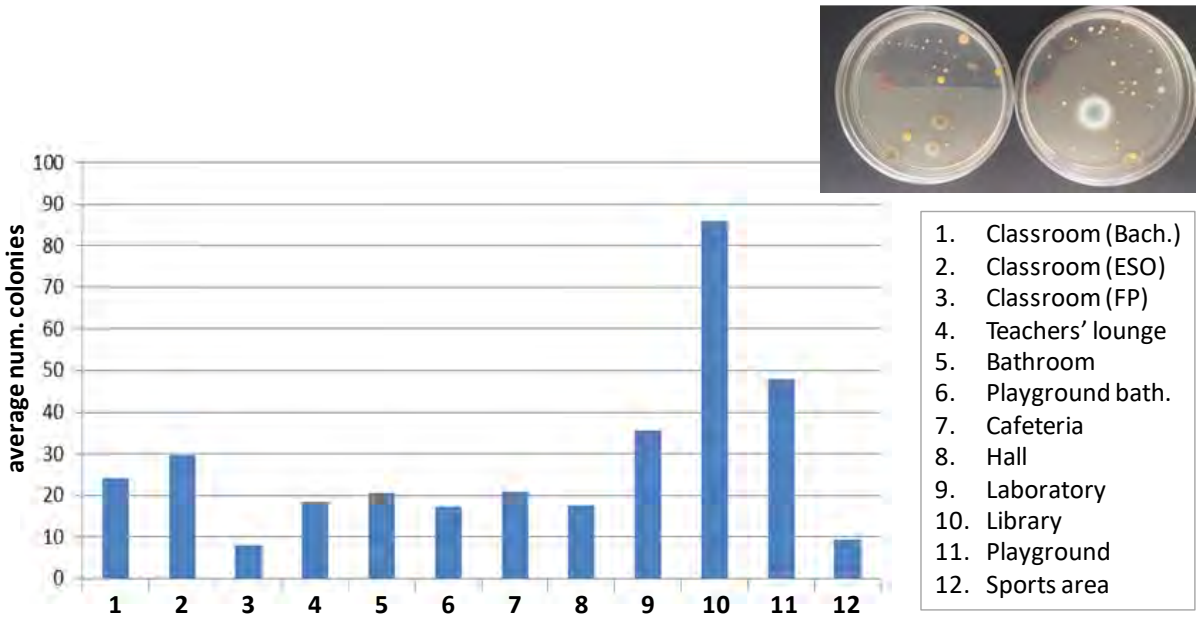


Figure 2. Presence of airborne microorganisms in different areas of IES Francisco Ayala high school. Examples of plates are also shown (top right image).

Mask efficiency against airborne microorganisms.

Next, the efficiency of surgical and FFP2 masks in filtering these airborne microorganisms was tested. The results are presented in Figure 3. In both cases, a notable reduction in the number of colonies was observed, with the FFP2 mask having a higher filtering capacity (93% reduction, vs. 82% for the surgical mask). These results support the efectiveness of face masks in reducing the risk of airborne contagion, and the higher protection offered by FFP2 masks.

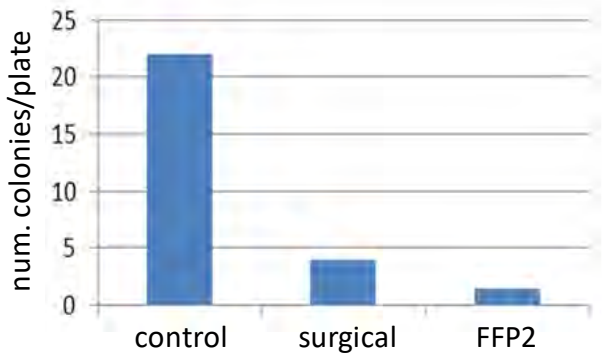


Figure 3. Average number of colonies on plates after exposure to classroom air for 40 min. without (control) or with face masks covering the plate.

Mask efficiency against aerosol-borne bacteria.

We went on to test the performance of different types of masks against a simulated bacterial infection coming from sneezes (aerosol-borne bacteria). In this series of experiments we questioned the possibility of being infected (filtration efficiency for bacteria coming from the outside, i.e. external side of the mask), and of infecting (filtration efficiency for bacteria coming from the inside, i.e. internal side of the mask).

In a first experiment, three types of masks were used: surgical, FFP2, and nanofibers (CSIC technology) to test their efficiency against external infection by aerosols. Since it is not uncommon for people to use masks of different colors, we also questioned if this could influence the efficiency, and included white and black FFP2 masks in the assay. Results are shown in Figure 4. The data indicate a clear advantage of the CSIC masks over other types, both in terms of the risk of being infected (aerosol challenging the external side of the mask) and of infecting others (internal side of the mask). Again, the FFP2 masks showed better performance than the surgical ones, as expected. An intriguing result is the fact that black masks seem more effective than white ones in protecting from the outside, while the opposite is true from the inside. The large difference in numbers between the external and internal sides is likely due to the fact that different plate sizes were used in each of these experiments. To better compare both results, data were normalized with respect to the area. Values per cm^2 were calculated and are presented in Figure 5. This graph shows that in general masks are more efficient filtering from the outside than from the inside, and therefore offer higher protection to the person wearing it, while we are less protected if people around us wear a mask and we don't.

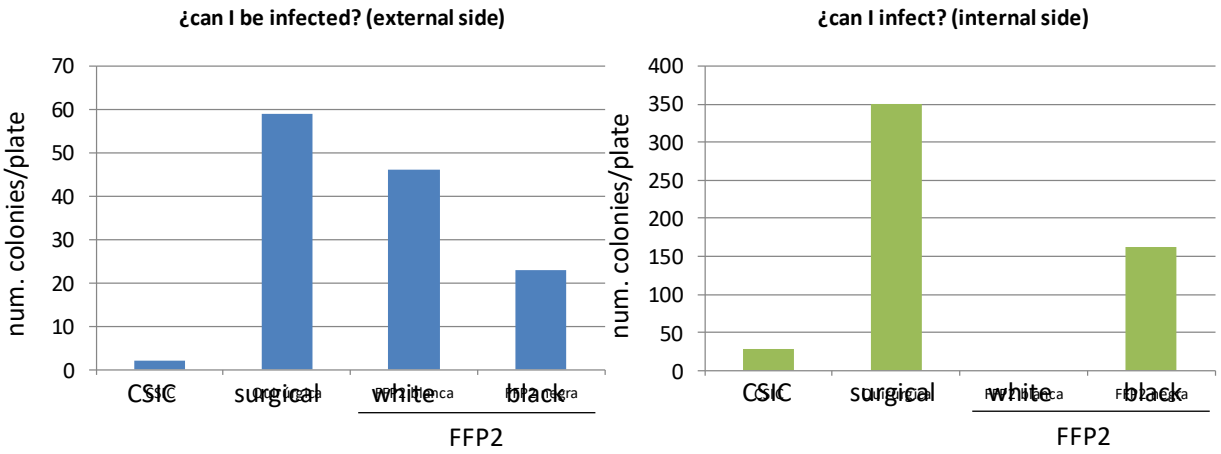


Figure 4. Filtration efficiency of the two sides of face masks against aerosol-borne bacteria.

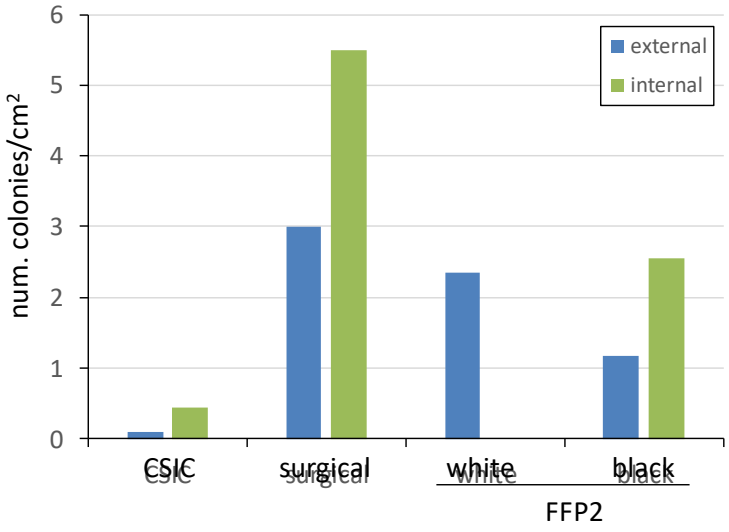


Figure 5. Normalized filtration efficiency of masks.

Finally, we tested also a mask made of two fabric layers separated by a filter. Interestingly, this type of mask was clearly more effective than surgical masks, with filtration values similar to those of FFP2 masks (data not shown).

Factors affecting efficiency

We tested two situations that could affect the filtration efficiency: humidity and repeated exposure to aerosols containing bacteria. Results are shown in Figures 6 and 7. In these experiments we compared two types of surgical masks (external side blue and all white) and FFP2 masks. Masks were first sprayed with water and then with the bacterial suspension (Figure 6), or twice sprayed with the bacterial suspension (Figure 7). Results show in both cases a reduction in efficiency, although with a lot of variability between masks. Humidity can reduce filtration between 2 and 22 times, whereas a second "sneeze" can result in a reduction ranging from 23 to 1.5 times. Again, the FFP2 mask better protection in all the conditions tested.

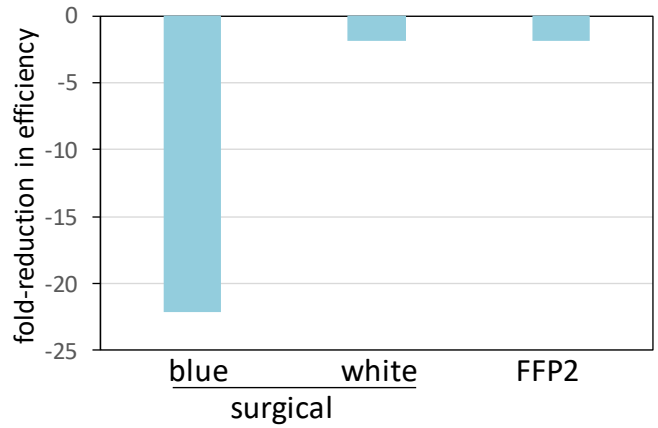


Figure 6. Reduction in filtration efficiency due to humidity: number of colonies, after spraying with water and then with bacteria relative to the number of colonies without previous spray with water (external side of the mask).

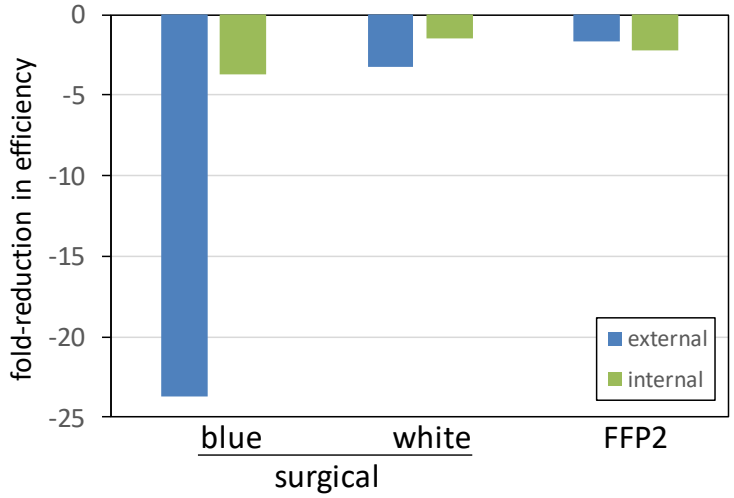


Figure 7. Reduction in filtration efficiency of each side of the mask after repeated exposure to aerosol-borne bacteria: number of colonies after the second spray relative to the number of colonies after the first spray.

Microorganisms retained in masks after prolonged use

One of the important recommendations for the use of face masks is to avoid excessive use. As seen above, accumulated humidity and repeated exposure can reduce their efficiency. We decided to check the type and number of microorganisms retained in masks after prolonged use by different individuals. Results are shown in Figure 8, and indicate a large variability depending on the user. Overall, there are no significant differences between the nose and mouth areas or between the internal and external sides, once we remove the bias introduced by the two individuals where very high numbers of colonies appear. In general, bacteria seem to be more abundant in these samples than fungi. Colonies were also observed under a stereomicroscope, and representative images are shown in Figure 9.

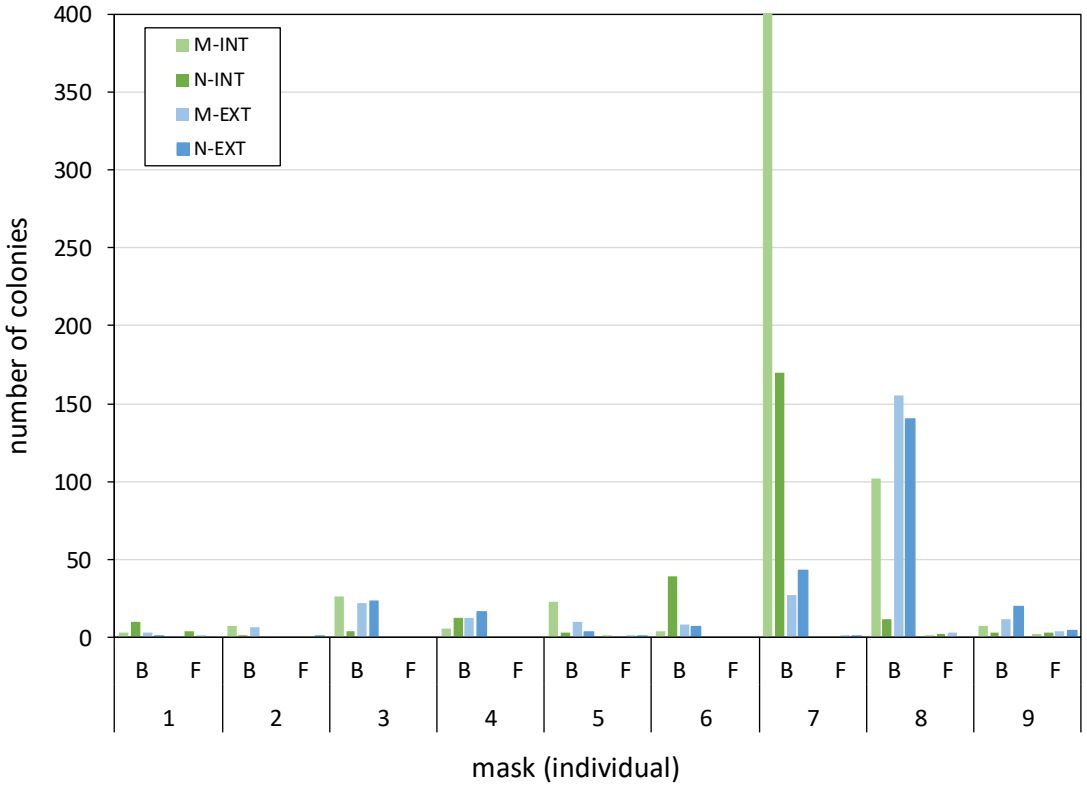


Figure 8. Bacteria (B) and fungi (F) recovered from 9 different masks, corresponding to the internal (green bars) and external (blue bars) sides, from the mouth (light color) or nose (darker color) areas.

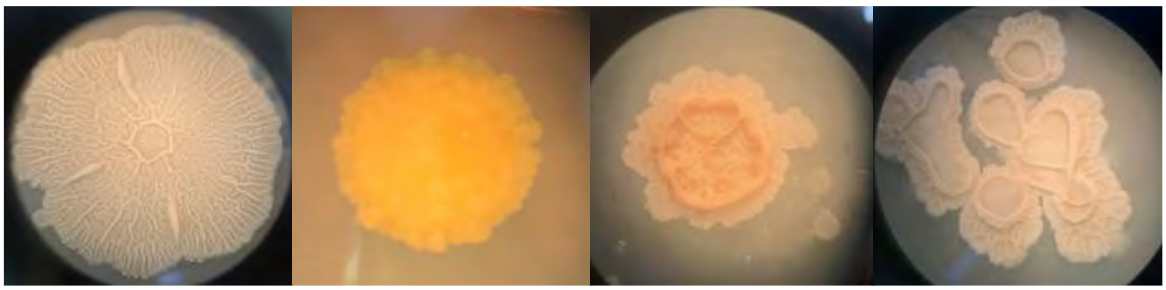


Figure 9. Images of representative colonies isolated from face masks.

CONCLUSIONS

This work confirms that face masks are useful to protect against airborne microorganisms and aerosols containing bacteria, although their efficiency varies depending on the type of mask and factors such as humidity and repeated exposure to aerosols. The nanofiber-based masks developed by CSIC show the best performance of all the types tested, but FFP2 and fabric masks containing a filter are also a good alternative against bacteria.

References

[1] Tcharkhtchi A, Abbasnezhad N, Zarbini Seydani M, Zirak N, Farzaneh S, Shirinbayan M (2021) An overview of filtration efficiency through the masks: Mechanisms of the aerosols penetration. Bioactive Mat 6: 106-122

[2] Bachmann BJ (1972) Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol Rev 36: 525–557

[3] Lennox ES (1955) Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1: 190–206.

MY OWN IDEAS

Javier Juguera

In this project we have studied the efficiency of different types of face masks, at first it wasn't easy as we had to think how we will develop our investigation. While we were planning our experiments a lot of challenges appeared and that was an excellent opportunity to let our creativity freedom to solve them, that also let us share different point of views, as one problem could be solve in different ways. With this project we have learnt perfectly the scientific method developing a way to simulate a real case of sneezing between two people using simple materials.l am so glad to be part of this project as it has made me learn many different things about bacteria and microorganism more specifically about the E. coli, the experience was amazing and our first congress in front of experts was unforgettable. This could not have been possible without the help of Manolo (CSIC scientist) and Lola (our anatomy teacher), they taught us a lot about how an investigation works and that made the experience easier and more enjoyable for us. If I was given the opportunity to participate again, I would do so without a doubt.

Jorge Contreras

First of all, I would like to thank the CSIC, our researcher Manuel Espinosa and our professor Lola Bernal, for giving us the opportunity to participate in this project.
This project has meant a change in my way of thinking, since, by doing so, we have been able to verify the effectiveness of something so important in these years of pandemic, verifying its effectiveness, and testing different cases that concern us day by day, as a result of This my critical thinking is developed, since it has given me the opportunity to rethink the use of the mask, its operation and how such a small thing can affect our lives.

This project has allowed me to improve my techniques in the field of microbiology, in addition to allowing me to see how an investigation is developed, looking for those reasons and those methods to carry out said project, this has served as an impetus to determine to study biology and be a researcher, since this project gives you the opportunity to participate in unknown or remote areas for a Baccalaureate student. Projects like these condition students and in some cases, if not most, encourage students to become researchers or dedicate themselves to science. Last but not least, this project shows you that teamwork and accuracy and precision in the processes are very important for the proper development of research and this leads to applying many things learned in different high school subjects giving a meaning and use for this.
Finally, I would like to say that I hope that the CAOS project will continue to be carried out for many years and continue to motivate students like us in the fields of science.

Carolina Tarazaga

Thanks to the project we had the oppotunity of having a unique experience, since we have learnt to do experiments in a professional way. The most important thing is that my classmates and I have learnt techniques that can be useful in the future if we still have interest on a scientific career. We have worked collectively and thanks to that we have been able to do many experiments that we could have not thought about alone, since everyone offered different ideas.
The meeting was one of the things I liked most, because we could see other interesting projects and get an idea of how the world of professional science is. It was a great opportunity, all the projects were spectacular and thanks to the great conference we could see how science is in reality and the vocation we must have to follow that path.
Finally, thanks to the researcher, Manuel Espinosa, and our teacher, Lola Bernal, for this opportunity, where we have opened to the world of Microbiology. It was very interesting for me and very up to date given the use of face masks in these years.

Cristina Gutiérrez

Thanks to the CAOS project we have learnt a lot about different aspects of bacteria, culturing, the different colonies we can find in masks, etc. We have also done different experiments to analyze and understand the results of our project. This work has sparked interest in science in many of us and thanks to it we have a basis that impulses us to continue studying this area in the future.
The meeting we attended was one of the best parts, since there we could present our work and the results in front of a scientific audience.
Besides, we have to thank the researcher who accompanied us, Manuel Espinosa, and our teacher Lola Bernal for teaching and leading us in each step of the project, since they have given us a unique opportunity to learn about the world of Microbiology.

Claudia Donaire

My experience has been great, because I have improved and expanded my little knowledge about Microbiology. Besides that, all together we have obtained results that led us to think and study in depth the reasons for our work.
Finally, the meeting we attended was the best opportunity to really learn about research, and to me, listening to all the wonderful projects presented by students from other schools, is without a doubt what makes me want to participate again in this great work.

Lucía Graciano

I think for me and my classmates this project has been a great step towards scientific research, since in it we have had a first contact with all this implies. Preparing the project, thinking by ourselves how we could solve the problems that appeared, doing the practices where we have learnt to use different techniques to culture bacteria, etc. We have also learnt to explain what we have done in our project in a more professional way, and present ir in a meeting in which we could learn a lot. Our researchers has also been of great help, he has directed us during our project and has counselled us. I think in a near futur this experience will be very useful to those who want to work in scientific research areas, since we have learnt no only scientific knowledge but also how to work as a team.

Marta Victoria

The CAOS project has been a unique experience in which my classmates and me have had the luck of participating, and I am sure that if we had the chance we would repeat this great experience. Thanks to the CAOS project my classmates and me have been able to expand our knowledge about science and put it in practice. Besides, we have been able to see a little of how the Biotechnology degree would bem since we have worked with bacteria, this has been a preview of how the degree would be and if we would really like studying it or not.
Working with bacteria we have bee able to learn the different techniques that are used nowadays in science and we have practiced them. Besides, we have discovered the different necessary utensils, what they serve for and how to use them. Besides, we have not only learnt the theory but how to work as a team and respect the other classmates, which is equally important.
Finally, thanks to the conference at the Meeting we could see how there are people who reach their dream of becoming scientist, and that you can do it too.

Antonio Ontiveros

Firstly, I am very grateful for the opportunity to participate in this project, in which I have learnt not only about microorganisms but also to cooperate among classmates forming groups, and a little also about research (which is one of the things I like most).
The main goal of our project was to test the efficiency of face masks, but we did not only do that. At the beginning, to get into the world of microorganisms we did a series of experiments where we created culture media, we grew in them air microorganisms and we tried to differenciate them.
After that we started the most fun part of the project, to test the efficiency of face masks. We did several experiments with different masks and in different ways, ever we repeated some to confirm the results.
All the project has been very entertaining and gratifying, but without a doubt the best part was the creation of "Evaristo", a dummy we invented to be our test subject and that now all of us love.
I must say at the beginning the project seemed difficult, since we were going to work with beings that we cannot see, at least with the naked eye, but with the help of my classmates, our researcher and our teacher, everything was easier.

Regarding agriculture in Mars: Isolating and identifying chlorate resistant bacteria

Iván Molina Morillo[1], Rosalía Magdalena Anglada Osorio[1], Julia Gómez Molina[1], Alma Luque Barrio[1], Almudena Urbano Azpeitia[1], Pablo Álvarez Rodríguez[1], Lucía Cardaldas Fornieles[1], Antonio Quesada Ramos[1#], María Antonia Molina Henares[2], Manuel Espinosa Urgel[2#].

[1] Departament of Biology and Geology. IES. Zaidín Vergeles.Granada, Spain. Primavera 26, 18008 Granada Spain

[2]Department of Biotechnoloy and Environmental Protection. Estación Experimental del Zaidín. CSIC. Granada, Spain.

#For correspondence: quesadaramos@gmail.com; manuel.espinosa@eez.csic.es

Summary

Mars is a inhospitable planet for life. Liquid water only would exist as brines, solutions with high a concentration of salts like chlorates and perchlorates that seem ubiquitous in the soils of the red planet. This compounds are extremely toxic for living beings making difficult growing plants in Mars in case of a hypothetical human settlement or in desert locations on Earth where they have been detected too. A possible solution is the use of microorganisms. Some strains of bacteria can contribute to agriculture by bioremediation, removing toxic salts from soils, or improving the germination of seeds and the development of seedlings.

In order to assess this possibility, four strains of microorganisms have been isolated from samples of soils taken by high school students and identified by RNA 16S sequencing as *Planococcus rifietoensis*, *Pseudomonas xanthomarina*, *Peribacillus simplex* and *Bacillus cereus*. The review of literature shows that some strains of those bacteria are able to remove toxic products from soils or produce substances that promote the germination and development of plants.

The next step of this research will be to assess the potential beneficial effects of these microorganisms in plants cultivated in martian soil simulants added with potassium chlorate, resembling the conditions in the red planet.

Keywords: Mars, chlorates, perchlorates, bioremediation, *Planococcus rifietoensis, Pseudomonas xanthomarina, Peribacillus simplex, Bacillus cereus.*

INTRODUCTION

Mars is an inhospitable planet with a gravity that is one third of that in Earth, extreme low temperatures, an atmosphere whose pressure that is more than one hundred times lower than Earth's but with a high proportion of carbon dioxide and high levels of radiation on the surface. One of the most important consequences of this environment is the scarcity of liquid water on Mars and the difficulty of living beings to develop there.

Liquid water, however, would exist as brines, saturated solutions with salts like chlorates and perchlorates due to its high cryoscopic effect. This compounds have been detected in situ on Mars. Phoenix spacecraft made the first detection in the northern polar region and they have been found too by Curiosity rover in crater Gale; indeed, a re-evaluation of data from Viking instruments suggests that these compounds could be present in Chryse Planitia and Utopia Planitia, the places were those spacecrafts landed [1]. Chlorates and perchlorates, consequently, seem to be ubiquitous on Mars and liquid water could exist, at least, in middle latitudes with mild temperatures. Considering human habitability of the red planet, these salts could be relevant in terms on in situ resource utilization as oxygen can be obtained from them [2] but on the contrary, they are extremely harmful for living beings, including humans [3, 4, 5]. These salts are not exclusive of Mars, they are present in Earth and probably throughout the Solar System [6, 7].

A hypothetical future human settlement in Mars would require to develop systems that provide oxygen, water and food for human beings, and plants can supply them. In this sense, it is important to depend on in-situ Resource Utilization (ISRU), in other words, the use of existing material at the site whenever possible. Regarding growing plants on Mars, examples of in situ resources in the red planet are light, water, carbon dioxide or regolith [8]. However, chlorates and perchlorates present in soils, although essential for the existence of liquid water, could make them inappropriate for agriculture.

This has been confirmed with some studies assessing agriculture on Mars developed with Martian soil analogues. Oze et al. (2021) [9] reported different results with several simulants. While some of them like JSC-Mars 1 (Johnson Space Center 1) or MMS (Mars Mojave Simulant) were able to support plant growth, seeds could not germinate in others like MGS-1 (Mars Global Simulant). In any case, the addition of perchlorates to these soil analogues did not support plant growth. Experiments carried out in our laboratory with a soil simulant elaborated from volcanic scoria [10, 11] also revealed the difficulty of Arabidopsis thaliana to grow in this analogue [12], while additioned with potassium chlorate showed the toxic effect of this salt either the germination or the development of Zea mays and Vigna unguiculata [13].

In this scenario, there are some possibilities in order to grow plants in the red planet. The first one is artificial selection, the selection of plants with efficient mechanisms to support the stress conditions due to those salts. In this sense, differences in development have been observed between Zea mays and Vigna unguiculata grown in perlite or a Martian analogue suplemented with potassium chlorate [13]. The second one is the genetic modification of plants with the introduction of genes that make them more tolerant to salt excess. Also related to plants, phytoremediation has been demonstrated as an effective method for removing perchlorates from soils through mechanisms like phytoaccumulation, phytodegradation and rhizodegradation [5]. Another possibility is with microorganisms; on one side, they could help plants to grow; on the other side by microbial bioremediation, removing toxic products from soils [14].

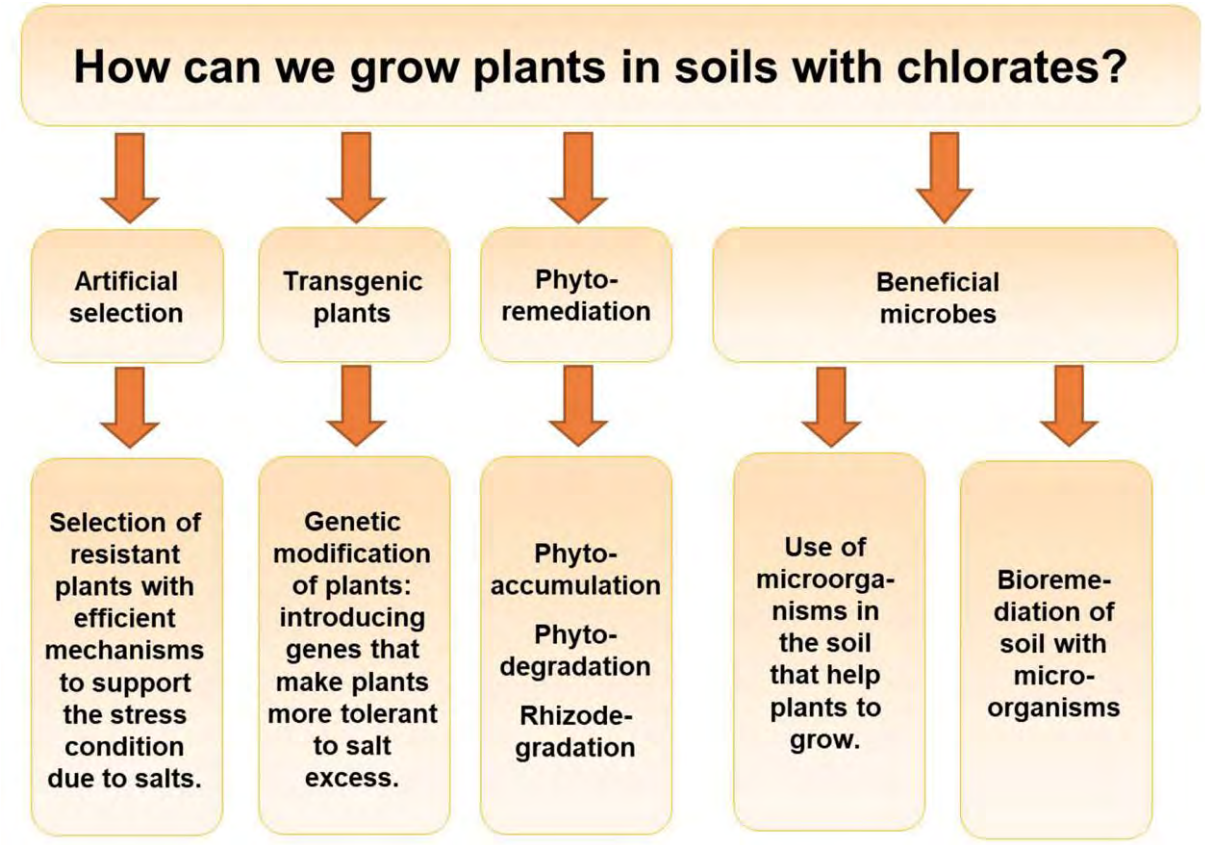


Figure 1. Methods for growing plants in soils contaminated with salts.

Considering the mentioned alternatives with microorganisms, the main objective of this research is to isolate and identify bacterial strains tolerant to chlorates from Earth soils samples in order to test in a future project their potential use for improving hypothetical crops in Mars. Taking into account that this is an educational project carried out by high school students, potassium chlorate is a safer option for the experiments than perchlorate. On other side, a recent reseach suggests that under the conditions prevailing on Mars the production of chlorates can be orders of magnitude higher than those of perchlorates, highlighting the importance of chlorates in the surficial environments and habitability of modern Mars compared with perchlorates [15].

MATERIAL AND METHODS

Soil samples were collected by the students from different places: seashores, gardens, farmland and even pots. Seashore soils have special interest due to the close contact with marine salt water, so it seems likely that salt tolerant microorganisms could be isolated from there.

Aliquots of one gram of soil were taken ant put into bottles separating them into different sets. To one set, ten mililiters of sterile saline solution were added; to the other, ten milliliters of a solution of potassium chlorate at 0,4 % (weight/volume), that is 0.33 M. Both samples were incubated for 24 hours at room temperature. Then, 50 microliters of every suspension were inoculated on Petri dishes with Triptone Soy Agar medium and incubated for 48 hours at 28ºC. In order to test the effect of potassium chlorate, both number and morphology of colonies were studied.

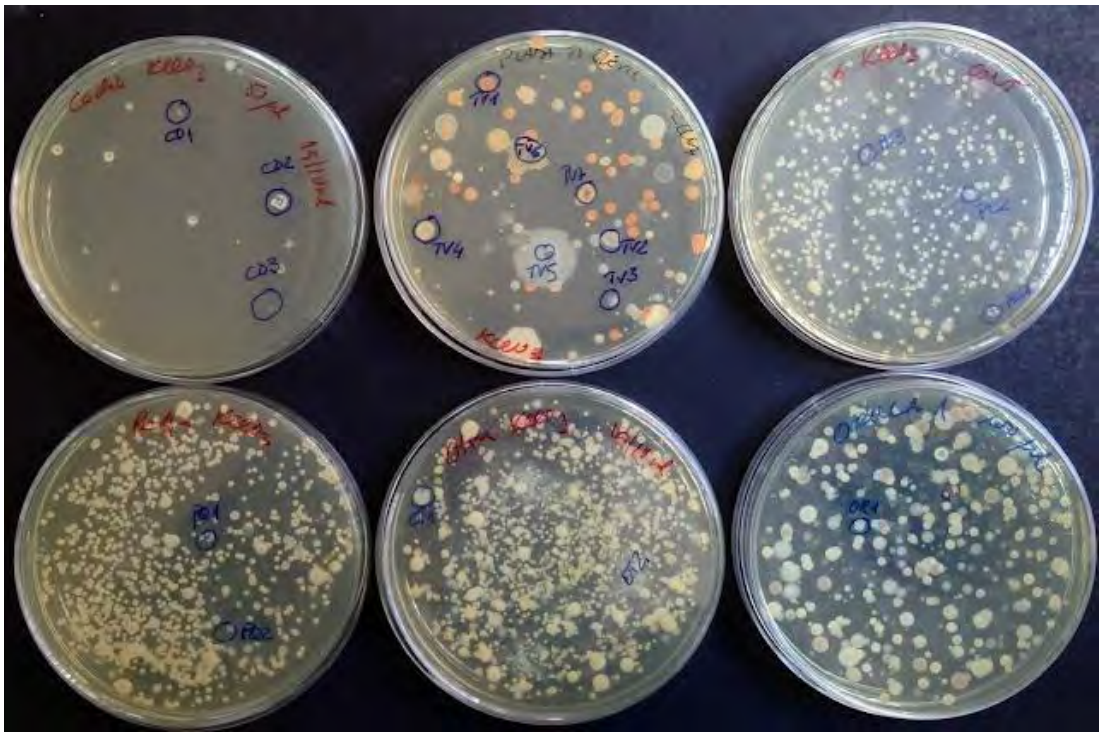


Figure 2. Selected colonies for genetic identification from plates with $KClO_3$

Seventeen colonies were isolated and six of them were chosen for genetic identification (Figures 2, 3). Fragments encoding for 16S ribosomal RNA were amplified by PCR using biomass from the colonies and universal 16S primers containing conserved sequences. The PCR conditions were as follows:

Initial denaturing	95°C	5'	
| Denaturing	95°C	30''	|
| Annealing	48°C	30''	| × 30 cycles
| Extension	72°C	1' 30''	|
Final extension	72°C	5'	

Amplification was successful only in four of the six clones analyzed. PCR products were sequenced at Instituto de Biomedicina y Parasitología López Neyra (CSIC). Bacteria were identified with the Basic Local Alignment Search Tool (BLAST).

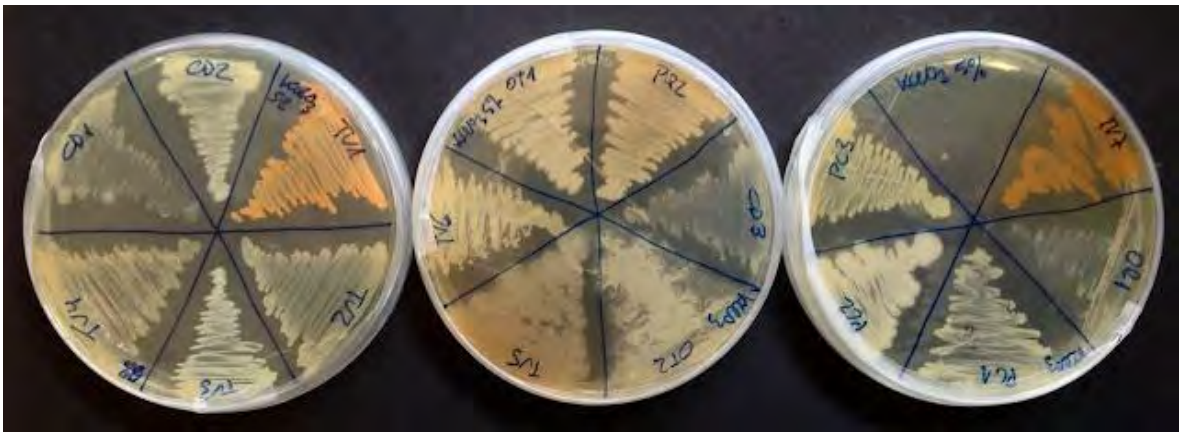


Figure 3. Isolates from different strains for genetic identification.

RESULTS

Potassium chlorate had revealed as a toxic product for microorganisms. Plates showed important differences, both in the number and type of colonies (Figure 4). In those with potassium chlorate there were less colonies and, regarding to morphology, less variety of them. Presence of potassium chlorate in culture media, reduces both the survival and diversity of microorganisms.

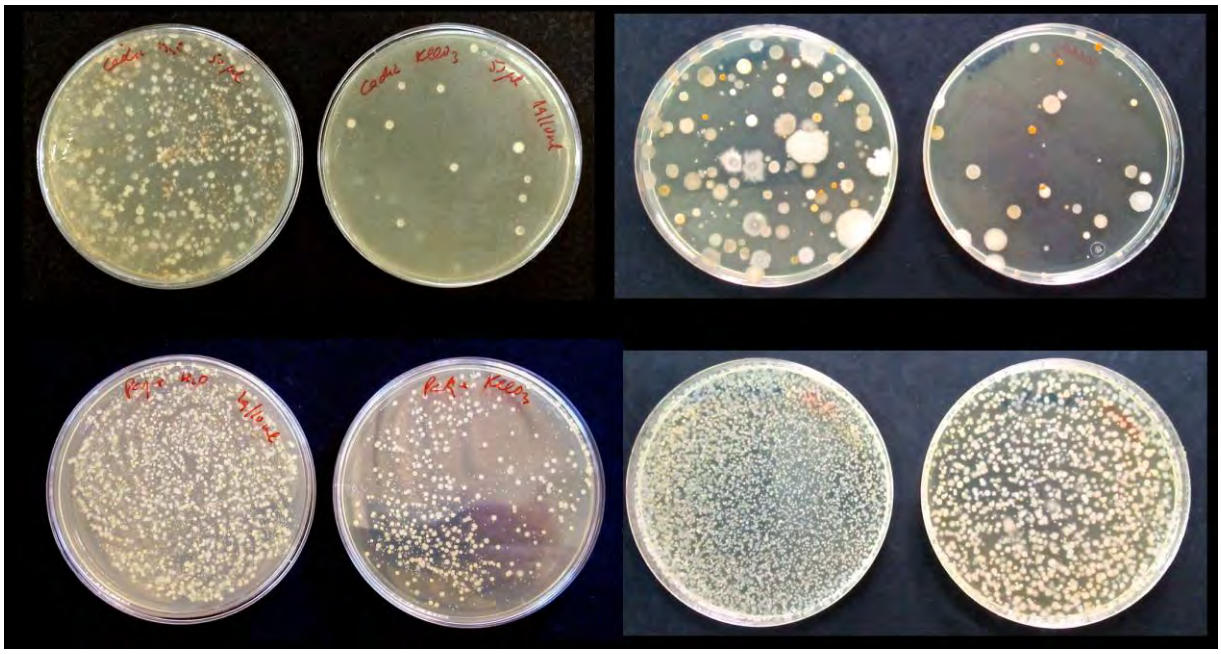


Figure 4. Effect of $KClO_3$ on abundance and diversity of microorganisms.

From all the isolated microorganisms, PCR was carried out in six. Only four sequences amplified and were sequenced, two from Playa del Cura (Torrevieja, Alicante) and two from a

local park near the high school in Granada (Figure 5). Chlorate tolerant strains were identified with BLAST as *Planococcus rifietoensis* (TV1), *Pseudomonas xanthomarina* (TV2), *Peribacillus simplex* (PQ2) and *Bacillus cereus* (PC2) (Figure 6).

Sample TV1 (isolated from Playa del Cura. Torrevieja, Alicante).

CTAATACATGCAAGTCGAGCGGAGATAGTGGAGCTTGCTCCATTATCTTAGCGGCGGACGGGTGAGTA
ACACGTGGGCAACCTGCCCTGCAGATCGGGATAACTCCGGGAAACCGGTGCTAATACCGAATAGTTTG
CGGCCTCTCATGAGGCTGCACGGAAAGACGGTTTCGGCTGTCACTGCAGGATGGGCCCGCGGCGCATT
AGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA
CACTGGGACTGATACACGGCCCAGACTCCTACGGCACGCAGCAGTAGGGAATCTTCCG

Sample TV2 (isolated from Playa del Cura. Torrevieja, Alicante).

GGCCTACACATGCAAGTCGAGCGGATGAAGAGAGCTTGCTCTCTGATTCAGCGGCGGACGGGTGAGTA
ATGCCTAGGAATCTGCCTGATAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCT
ACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGG
TGAGGTAATGGCTCACCAAGGCAGAC

Sample PQ2 (Isolated from a local park near the high School).

TACTGCAAGTCGAGCGAATCAGATGGGAGCTTGCTCCCTGAGATTAGCGGCGGACGGGTGAGTAACAC
GTGGGCAACCTGCCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATACGTTCTTTT
CTCGCATGAGAGAAGATGGAAAGACGGTTTACGCTGTCACTTATAGATGGGCCCGCGGCGCATTAGCT
AGTTGGTGAGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACAGCCCACACTCCTACGGAGGCA

Sample PC2 (Isolated from a local park near the high School).

GCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGA
GTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAAC
ATTTTGAACCGCATGGTTCGAAATATGAAAGGC

Figure 5. DNA encoding RNA 16S sequences from isolated microorganisms.

Description	Total Score	Query Cover	E value	Per. Ident	Acc. Len
Planococcus rifietoensis strain M8 16S ribosomal RNA, partial sequence	582	100%	6e-166	99.09%	1531
Planococcus citreus strain NBRC 15849 16S ribosomal RNA, partial sequence	582	100%	6e-166	99.09%	1476
Planococcus columbae strain PgEx11 16S ribosomal RNA, partial sequence	582	100%	6e-166	99.09%	1481
Planococcus citreus strain NCIMB 1493 16S ribosomal RNA, partial sequence	546	100%	4e-155	96.99%	1432

Description	Total Score	Query Cover	E value	Per. Ident	Acc. Len
Pseudomonas xanthomarina strain KMM 1447 16S ribosomal RNA, partial sequence	404	98%	2e-112	99.56%	1464
Pseudomonas vancouverensis strain DhA-51 16S ribosomal RNA, partial sequence	398	98%	1e-110	99.12%	1492
Pseudomonas moorei strain RW10 16S ribosomal RNA, partial sequence	398	98%	1e-110	99.12%	1459

Description	Total Score	Query Cover	E value	Per. Ident	Acc. Len
Peribacillus simplex NBRC 15720 = DSM 1321 16S ribosomal RNA, partial sequence	526	99%	4e-149	98.69%	1522
Peribacillus simplex NBRC 15720 = DSM 1321 16S ribosomal RNA, partial sequence	526	99%	4e-149	98.69%	1476
Peribacillus simplex strain LMG 11160 16S ribosomal RNA, partial sequence	526	99%	4e-149	98.69%	1503
[Brevibacterium] frigoritolerans strain DSM 8801 16S ribosomal RNA, partial sequence	523	99%	5e-148	98.36%	1503

Description	Total Score	Query Cover	E value	Per. Ident	Acc. Len
Bacillus cereus strain IAM 12605 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1486
Bacillus cereus strain JCM 2152 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1474
Bacillus tropicus strain MCCC 1A01406 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1509
Bacillus paramycoides strain MCCC 1A04098 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1509
Bacillus nitratireducens strain MCCC 1A00732 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1509
Bacillus luti strain MCCC 1A00359 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1509
Bacillus albus strain MCCC 1A02146 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1509
Bacillus cereus strain CCM 2010 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1535
Bacillus cereus strain NBRC 15305 16S ribosomal RNA, partial sequence	296	99%	6e-80	99.40%	1476

Figure 6. Genetic identification of DNA sequences of chlorate tolerant bacteria.

DISCUSSION

A hypothetical future human settlement on Mars would require systems to provide in situ substances like oxygen, water and food for the metabolic needs of humans beings and also systems for transforming and recycling waste products [16], and plants and microorganisms would be the best option for them.For growing plants on Mars it would be pertinent, as much as possible, to depend on in-situ resource utilization (ISRU), i.e. the use of materials present there. In case of Mars, the carbon dioxide from the atmosphere, light, soils and liquid water, if it exists. It has been suggested that liquid water only could exist in Mars as brines, saturated solutions of salts present in soils. The deliquescence of salts like chlorides, chlorates and perchlorates could provide a transient source of liquid water available for plants and microorganisms, as has been reported in Atacama desert [17] but extremely toxic for them.

Previous experiments carried out in our laboratory revealed that a martian soil simulant added with sodium chloride or potassium chlorate clearly inhibited the development of plants with nutritional value like *Zea mays* and *Vigna unguiculata* [13]. Similarly, present experiments demonstrate that potassium chlorate is harmful for some microorganisms.

Several procedures have been proposed in order to grow plants in saline soils. Some of them involve plants, like the selection of naturally or genetically modified plants to tolerate salts or phytoremediation. Especially interesting are those that imply microorganisms as they could be able to remove toxic products from soils or even help plants to grow [14].

Regarding the possibility of growing plants in soils rich in toxic salts as those of Mars, a first step in our project has been to isolate bacteria tolerant to high concentrations of potassium chlorate. The choice of this salt is justified, on one side because a recent research points that the production of chlorates may be higher than that of perchlorates in the recent history on Mars and, in the other side, because chlorates are less harmful than perchlorates and the research has been carried out by high school students.

Four microorganisms were identified with BLAST: *Planococcus rifietoensis* TV1 and *Pseudomonas xanthomarina* TV2 (isolated from Playa del Cura, Torrevieja, Alicante), *Peribacillus simplex* PQ2, and *Bacillus cereus* PC2 (isolated from a city park, near the high school). The next step was to review literature in order to assess if there were described strains of these microorganisms either capable of bioremediation or supporting the development of plants.

Planococcus rifietoensis is described as a rod-shaped, gram positive bacterium, that grows in orange colored colonies, and is able to grow at high sodium chloride concentration. Some strains reduce the toxic effect of salinity in soils and improve the germination and growth of wheat [18]; Zhou et al. (2017) [19] described greater seed germination and plant biomass with higher photosynthetic capacity in sugar beet plants inoculated with *P. rifitoensis*.

Peribacillus simplex is a rod-shaped, gram positive microorganism. Most strain are strictly aerobic. They form spores under adverse environmental or nutritional conditions. *P. simplex* has been described as one of the most promising biofertilizer for canola crops [20].

Pseudomonas xanthomarina are gram negative, rod shaped, non fluorescent bacteria. Some strains have been considered a possible resource for agriculture and bioremediation of contaminated soils [21, 22].

Bacillus cereus is Gram-positive, rod- shaped, facultatively anaerobic, motile and spore forming bacteria. Zhou et al. (2021) [23], described strain YN917 which can promote seed germination and seedling plant growth in rice plants along with disease prevention factors. Regarding bioremediation, *B. cereus* T-04 is an efficient crude oil degradating bacteria, isolated from soils contaminated with crude oil [24].

The review of the available literature about the species of the isolated microorganisms has shown promising results: there are strains of these species capable to promote germination and development of plants and bioremediation. The next step in our research should be to test the posible promoting effect of our isolated microorganisms on the germination and growth of plants cropped in Martian soils simulants added with chlorates as well as their capacity of removing those toxic salts from soils.

Acknowledgements

The following students have also participated in this project, and their contribution is acknowledged: Carlos Balderas González, Martín Benítez Muñoz, Noel Calabria Ceballos, Yurena Carretero Ballesteros, Lucia Cubillas Robles, Paula Durán Guerrero, Antonio Encinas Camacho, Irene Espínola Rodríguez, Juan Cruz Gamarra Única, Carla Gamaza Ferrer, Maribel González Fernández, Yerai Lens Herrera, Irene Sala Zornosa, Candela Torres Gurrea and Natalia Viviescas Hernández.

References

[1] Clark J, Sutter B, Archer PD, Ming D, Rampe E, McAdam A, Navarro-González R, Eigenbrode J, Glavin D, Zorzano MP, Martin-Torres J, Morris R, Tu V, Ralston SJ, Mahaffy P. (2021) A review of sample analysis at mars-evolved gas analysis laboratory analog work supporting the presence of perchlorates and chlorates in Gale crater, Mars. Minerals, 11(5), [475]. https://doi.org/10.3390/min11050475

[2] Gayen P, Sankarasubramanian S, Ramani VK. (2020) Fuel and oxygen harvesting from Martian regolithic brine. PNAS, 117(50), 31685–31689. https://doi.org/10.1073/pnas.2008613117

[3] Li H, Zhang X, Zeng X, Nie C. (2006) Effect of potassium chlorate on peanut growth. Chin J Plant Ecol, 2006, 30(1): 124-131.

[4] Li H, Zhang X, Lin C, Wu Q. (2008) Toxic effects of chlorate on three plant species inoculated with arbuscular mycorrhizal fungi. Ecotoxicology and environmental safety, 71(3), 700–705. https://doi.org/10.1016/j.ecoenv.2008.01.027

[5] Misra G, Smith W, Garner M, Loureiro R. (2021) Potential biological remediation strategies for removing perchlorate from Martian regolith. New Space, 9:217-227.

[6] Calderón R, Palma P, Parker D, Molina M, Godoy FA, Escudey M. (2014) Perchlorate levels in soil and waters from the Atacama Desert. Archives of environmental contamination and toxicology, 66(2), 155–161. https://doi.org/10.1007/s00244-013-9960-y.

[7] Jackson WA, Davila AF, Sears DWG, Coates JD, McKay CP, Brundrett M, Estrada N, Böhlke JK. (2015) Widespread occurrence of (per)chlorate in the Solar System. Earth and Planetary Science Letters, 430:470-476.

[8] Eichler A, Hadland N, Pickett D, Masaitis D, Handy D, Perez A, Batcheldor D, Wheeler B, Palmer A. (2021) Challenging the agricultural viability of martian regolith simulants, Icarus, 354,114022. https://doi.org/10.1016/j.icarus.2020.114022.

[9] Oze C, Beisel J, Dabsys E, Dall J, North G, Scott A, Lopez AM, Holmes R, Fendorf S. (2021) Perchlorate and agriculture on Mars. Soil Systems, 5(3):37. https://doi.org/10.3390/soilsystems5030037

[10] López-Galindo A, Jiménez de Cisneros C, Quesada-Ramos A. (2019) Simulating Martian soil as part of an educational project in Astrobiology with high school students. High School Students for Agriculture Research, 8:1-8.

[11] Quesada-Ramos A, Jiménez de Cisneros C, López-Galindo A. (2020) Simulando suelo marciano en el aula. Astronomía, 249:32-38.

[12] Quesada-Ramos A, Ruiz C, Espinosa-Urgel M, Palma JM. (2019) Growing plants in Mars: not so easy. High School Students for Agriculture Science Research, 8:38-46.

[13] Barroso N, Carrascosa M, Duro P, Gutiérrez N, Mansour M, Manzano V, Martínez J, Martínez E, Molina S, Ortíz A, Reyes L, Rodríguez E, Ruiz A, Villoslada A, Zamora A, Quesada A, Espinosa M. (2021) Growing plants in saline worlds: Earth vs. Mars. High School Students for Agriculture Science Research, 10:21-42.

[14] Arora S, Vanza M. (2017) Microbial Approach for Bioremediation of Saline and Sodic Soils. In: Arora S., Singh A., Singh Y. (eds) Bioremediation of Salt Affected Soils: An Indian Perspective. Springer, Cham. https://doi.org/10.1007/978-3-319-48257-6_5.

[15] Qu SY, Zhao YYS, Cui H, Yin XZ, Jackson WA, Nie X, Wu ZC, Wang JH, Zhou DS, Qi C, Li XY, Liu JZ. (2022) Preferential formation of chlorate over perchlorate on Mars controlled by iron mineralogy. Nat Astron. 6, 436–441 (2022). https://doi.org/10.1038/s41550-021-01588-6

[16] Monje O, Stutte GW, Goins GD, Porterfield DM, Bingham GE. (2003) Farming in space: environmental and biophysical concerns. Advances in space research : the official journal of the Committee on Space Research (COSPAR), 31(1), 151–167. https://doi.org/10.1016/s0273-1177(02)00751-2

[17] Davila AF, Duport LG, Melchiorri R, Jänchen J, Valea S, de Los Rios A, Fairén AG, Möhlmann D, McKay C P, Ascaso C, Wierzchos J. (2010) Hygroscopic salts and the potential for life on Mars. Astrobiology, 10(6), 617–628. https://doi.org/10.1089/ast.2009.0421.

[18] Rajput L, Imran A, Mubeen F, Hafeez FY. (2013) Salt-tolerant PGPR strain *Planococcus rifietoensis* promotes the growth and yield of wheat (*T. aestivum* L.) cultivated in saline soil. Pak. J. Bot., 45(6): 1955-1962.

[19] Zhou N, Zhao S, Tian C. (2017) Effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet (*Beta vulgaris* L.) under salt stress. FEMS Microbiology Letters, 364(11), 10.1093/femsle/fnx091.

[20] Martínez-Hidalgo P, Flores-Félix JD, Sánchez-Juanes F, Rivas R, Mateos PF, Regina IS, Peix Á, Martínez-Molina E, Igual JM, Velázquez E. (2021) Identification of canola roots endophytic bacteria and analysis of their potential as biofertilizers for canola crops with special emphasis on sporulating bacteria. Agronomy, 11(9). https://doi.org/10.3390/AGRONOMY11091796.

[21] Sopeña F, Laiz L, Morillo E, Sanchez-Trujillo MA, Villaverde J, Jurado V, Saiz-Jimenez C. (2014) Phenanthrene biodegradation by *Pseudomonas xanthomarina* isolated from an aged contaminated soil. Clean Soil Air Water, 42: 785-790. https://doi.org/10.1002/clen.201300247

[22] Crovadore J, Cochard B, Calmin C, Chablais R, Schulz T, Leforta F. (2016) Whole-genome sequence of *Pseudomonas xanthomarina* strain UASWS0955, a potential biological agent for agricultural and environmental uses. Genome announcements, 4(5), e01136-16.

[23] Zhou H, Ren ZH, Zu X, Yu XY, Zhu HJ, Li XJ, Zhong J, Liu EM. (2021) Efficacy of Plant Growth-Promoting Bacteria *Bacillus cereus* YN917 for Biocontrol of Rice Blast. Frontiers in Microbiology, 12, 684888.

[24] Zhenshan D, Yingying J, Kaikai C, Fei G, Xiaodong L. (2020) Petroleum depletion property and microbial community shift after bioremediation using *Bacillus halotolerans* 1-1 and *Bacillus cereus* T-04. Frontiers in Microbiology, 11, 1-9.

MY OWN IDEAS

Iván Molina Morillo

Mars in our lab is the first scientific project in which I have participated and it has been an unforgettable experience. It all started with the research we carried out on the geology of Mars to learn about its conditions. Later, we isolated the microorganisms from the substrate samples that we brought and finally we amplified the DNA of four of them. Each of these stages has been a new challenge in which we have learned all the phases in which a project is divided. From the initial research phase on Mars, the realization of the methods to isolate the microorganisms and their sequencing to the phase of the analysis of the results. They were all very complex and required knowledge of different fields of science such as geology, genetics, technology... And that has been one of the things that I liked the most.

Now we must continue with the investigation based on the results obtained. This may be the most difficult phase of all. We have isolated four bacteria resistant to chlorates present on Mars. The next step is to experiment with these microorganisms to find out if they are effectively capable of bioremediating contaminated soils and helping plant growth, as the bibliography we have found says. On the one side, we would grow plants in substrates with chlorates and inoculate them with the isolated bacteria to see if they help their growth; On the other side, we would analyze if the concentration of chlorates in these soils decreases with the presence of these bacteria.

This project has opened our minds to the part of science that is not taught in textbooks: the investigation. I feel proud to be part of something that very few students have the opportunity to do and I want to thank the teachers and researchers who have made it possible. Thank you for this great experience.

Lucía Cardaldas Fornieles

Mars in our lab, es el proyecto en el que llevamos trabajando todo el curso. Durante el tiempo que lleva el proyecto hemos realizado numerosos experimentos, para ver si se podían cultivar plantas en marte, para conocer su geología y en estos últimos meses en concreto, hemos trabajado con unas sales llamadas cloratos y percloratos los cuales pueden hallarse en Marte en las salmueras, que por lo que sabemos es la única manera de encontrar agua líquida en Marte. Para trabajar en esto, nos hemos implicado cogiendo sustrato, y conforme avanzaba el proyecto hemos aprendido a utilizar numerosos utensilios del laboratorio, algo que me ha llamado mucho la atención y me ha entusiasmado a seguir con este proyecto. Y también, a aislar bacterias y a cultivarlas en el medio, de forma que hemos visto poco a poco cómo crecían las colonias y la diferencia entre las que tenían cloratos y las que no.

Este proceso ha sido una oportunidad de conocer otra parte de la biología que no solemos apreciar en los centros educativos y que nos acerca mucho más a ella y con la que creo que todos hemos aprendido muchísimo y aún más si le añadimos las experiencias que el proyecto nos ha dado con gente externa del centro y de la que podemos sacar muchísimas cosas interesantes.

Con lo que llevamos hemos visto los efectos de los cloratos en las bacterias y comprobado que con ellos crecen menos bacterias, ahora bien, una vez aisladas las bacterias que toleran medios con clorato potásico y secuenciadas de forma que ya sabemos de qué bacterias se tratan buscamos ideas para poder continuar con el proyecto.

Si recordamos, nuestra principal función era comprobar si estas bacterias que resisten a los cloratos afectarían al crecimiento de las plantas y cómo y a su vez al suelo. Por lo que algo interesante sería ver cómo crecerían algunas plantas con la presencia de las bacterias que secuenciamos, y así analizar su crecimiento comparándolo con uno de un planta en condiciones normales. Si finalmente vemos que no crecen, habría que buscar soluciones para que pudieran desarrollarse. En clase comentamos algo que sería muy interesante y me llamó mucho la atención, aunque lo veo bastante complejo, y es la modificación genética de las plantas para aumentar su tolerancia a los cloratos.

Assessment of the quality and origin of honeys from the region of Andújar (Jaén, Spain).

Lima-Cabello E.[1*], Salas M.[1*], de Torres S.[2], Aguilar J.[2], Alarcón A.[2], Álvarez C.[2], Benítez J.A.[2], Chércoles D.[2], Fuentes D.[2], García-Bravo A.[2], García-Mena N.[2], García-Rodríguez M.[2], Herrada L.[2], Lázaro E.M.[2], Lópiz A.[2], Lorenzo R.L.[2], Lorite C.[2], Martínez M.[2], Meco L.[2], Moreno M.[2], Muñoz A.[2], Puig F.J.[2], Rascón E.[2], San Eufrasio A.[2], Torrellas E.[2], Berral J.M.[1] & Alché, J.D.[1#]

[1]*Plant Reproductive Biology and Advanced Imaging Laboratory (PReBAIL). Department of Biochemistry, Cell and Molecular Biology of Plants. Estación Experimental del Zaidín. CSIC. Profesor Albareda 1, 18008 Granada, Spain.*

[2]*IES Jándula, San Vicente Paul 32, 23740 Andújar, Jaén, Spain.*

*Contributed equally to this work
#Corresponding author: juandedios.alche@eez.csic.es

Summary

Honey from different botanical sources and collection times presents a great variability in chemical composition, physicochemical properties, and even sensorial attributes. The objective of this study was to evaluate the main physicochemical characteristics and the palynological footprint of a representative number of honeys produced in Andújar (Jaén, Spain). Samples included both putatively multi-floral and monofloral honeys. Physicochemical parameters analyzed included water and sugar content, pH and electrical conductivity as well as color determinations using the Pfund scale. Physicochemical analyses showed a good standard of quality for all honeys, with parameters indicating positive attributes and absence of unlabeled origins or processes involved in their preparation. Moreover, palynological analyses showed pollen grains from a large number of contributing species in the case of multi-floral honeys, and an accurate and univocal origin for monovarietal ones, fully matching the declared origin. This is to our knowledge the first report of such characteristics corresponding to honeys from this area, with a large tradition in honey production.

Keywords: color, collection, conductivity, honey, humidity, monofloral, multi-floral, pollen, sugars.

INTRODUCTION

Honey is a very sweet and viscous fluid produced by bees of the genus *Apis*, mainly the domestic honey bee, from the nectar of flowers or from secretions of living parts of plants or excretions of plant-sucking insects (aphids). These substances are collected by the bees, then transformed by combining them with their own substances, deposited, dehydrated and stored in the honeycombs for maturation. The intervention of man in the process of exploiting the honeycombs of the hive is known as beekeeping.

Honey has been used for millennia throughout the world, either as a flavoring agent for drinks, as a food or medicine. Its composition is variable, however its main components are carbohydrates in the form of monosaccharides such as fructose and glucose, as well as disaccharides such as maltose. These ingredients are responsible for the intense honey sweetness. In addition, it contains oligosaccharides such as panose, enzymes such as amylase, amino acids, some vitamins like those of the B complex, vitamin C, niacin and folic acid, minerals such as iron and zinc, and antioxidants.

In order to preserve que quality of honeys and to determine their precise geographical origin, preventing the occurrence of frauds, new labelling requirements have been recently determined by the Spanish food authorities, in accordance with EU rules [1]. Research has to be carried out in order to provide precise tools to identify and assess the origin and quality of honeys.

The Sierra de Andújar Natural Park is a great producer of high-quality honey. Just in the province of Jaén, approximately 36,000 hives have been registered, from which *ca.* 21,000 are located in the area of Andújar [2], generating a large production of honey. The main objectives of the present study are to characterize most relevant physical and chemical parameters of representative samples of honeys from this region as well as to confirm their botanical origin by means of pollen analysis in comparison with a core pollen collection. Such determinations are highly relevant to evaluate the commercial quality of this product.

MATERIALS AND METHODS

1. Origin of honeys.

Honey samples were supplied by beekeepers or purchased at commercial stores in the geographical area of interest (Fig. 1). Samples were stored in Falcon vials at 4ºC in the dark until their analysis.

Figure 1: Origin of honey samples in the area of Andújar. Samples analysed include multi-floral (2 samples), orange blossom, eucalyptus, rosemary (2 samples) holm oak, thyme and a undetermined sample.

2. Determination of physicochemical parameters.

The physicochemical parameters determined in the honey (moisture, electrical conductivity, pH) were carried out following the official analysis methodology for honey [1, 3-4].

b. Humidity and sugar content.

The water content in the honey was calculated by the value of the refractive index, determined by using a specific commercial refractometer for honey with a triple scale (%Brix, °Bé, %Water). A drop of honey from each sample was placed directly on the prism of the refractometer and a refraction value was obtained for each sample. The measurement was made at 20 ºC.

c. Conductivity and pH.

An individual amount of honey was weighed for each sample, depending on the water content, according to the next formula:

$$m = \frac{20 \times 100}{(100-A) \times 4}$$

being "m" the grams of honey needed and "A" the percentage of moisture in the honey sample. Next, the honey was dissolved in distilled water, recently boiled and the volume completed up to 25 ml in a volumetric flask. This solution was poured into a 50 ml beaker and placed in a thermostatic bath at 20± 0.5 ºC, allowing it to stabilize at that temperature. The measuring electrode of the conductivity/pH meter was introduced into the honey solution and the readings were taken in mill siemens/pH units.

d. Color determination.

Five grams of honey were weighed and poured into a 50 ml beaker. Three ml. of distilled water were added and honey dissolved with a glass rod. Volume was brought to 10ml with distilled water, and the prepared solution was placed in the cuvette of a spectrophotometer and let stand for 10 to 15 minutes, before taking the reading. Honey with impurities due to a bad processing was filtered with filter paper, and left 10 or 15 minutes of rest before taking the reading. Absorbance was read in a spectrophotometer at 635 nm (A_{635}), previously setting the absorbance to zero with distilled water. To obtain the mm Pfund from the absorbance value, the following formula and Table 1 was used:

$$\text{Mm Pfund} = -38.70 + 371.39 \times \text{absorbance}$$

Table 1. Classification of honey colors according to optical density (OD) and Pfund scale (mm)

Color name	Pfund scale (mm)	A_{635}
water white	>9	0.0945
Extra white	9-17	0.189
white	18-34	0.378
extra clear amber	35-50	0.595
clear amber	51-85	1.389
amber	86-114	3.008
dark amber	>114	---

e. Palynological analysis.

For pollen grains qualitative analysis, 10 g of honey were weighed and dissolved with distilled water to a final volume of 30 ml. It was then centrifuged at 4,500 rpm (3,383 g) for 10 minutes (spin I). The supernatant was removed with a pipette, and again brought to a volume of 30 ml with distilled water and centrifuged at 4500 rpm for 5 min (centrifugation II). Once the second centrifugation was finished, the supernatant was decanted again, 500 µl of distilled water were added and the sediment was shaken to homogenize it.
A heating plate was then heated to 60 °C on which the slides were deposited. A volume of 100 µl was taken, which was placed on a properly labeled slide on the heating plate and allowed to dry. A coverslip with a drop of fuchsine staining solution and a drop of gelatin-glycerin mounting medium was placed on each slide. The preparations were analyzed in a Motic light microscope and systematic photographic documentation of the samples was performed at least at two different magnifications (using 20x and 100X objectives).

RESULTS & DISCUSSION

1. Water content of the different samples of honey

Water content in most honey samples varied from 15.8% to 16.9%, with an exceptionally low content observed in the eucalyptus sample (14.6%) (Figure 2).

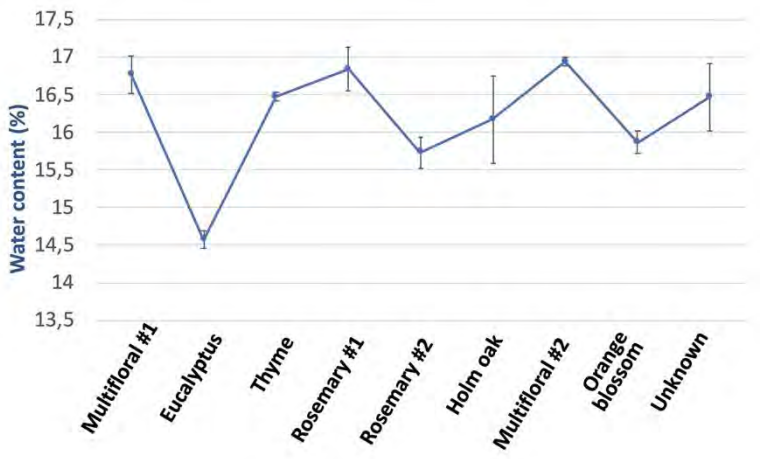


Figure 2: Water content of the nine samples of honey analyzed.

Most honeys show percentages of water around 17-18%. Such values are in contrast with that of nectar, which normally displays a much higher percentage of water (around 70-80%). Desiccation of nectar mainly occurs at hives, thanks to the displacement of air produced by bees with their wings. Once the appropriate level of humidity is reached, bees seal cells with wax.

The water content of honeys is highly dependent on the season and the climatic conditions of the time of its formation [5]. Thus, honeys collected in spring and autumn display higher contents of water than those collected in summer, which may sometimes have percentages of water close to 16%. This is likely the case of several honeys in the present study, whereas eucalyptus honey probably has suffered an additional process of dehydration.

Low water content notably reduces the probability of fermentation, which occurs in some low-quality honeys.

2. Sugar content of the different samples of honey

Analysis of honeys showed the percentages of sugar displayed in Figure 3, with values ranging between 81.5 and 83.9. Overall, such data are complementary with those of water content. Thus, eucalyptus honey sowed the higher level of sugar.

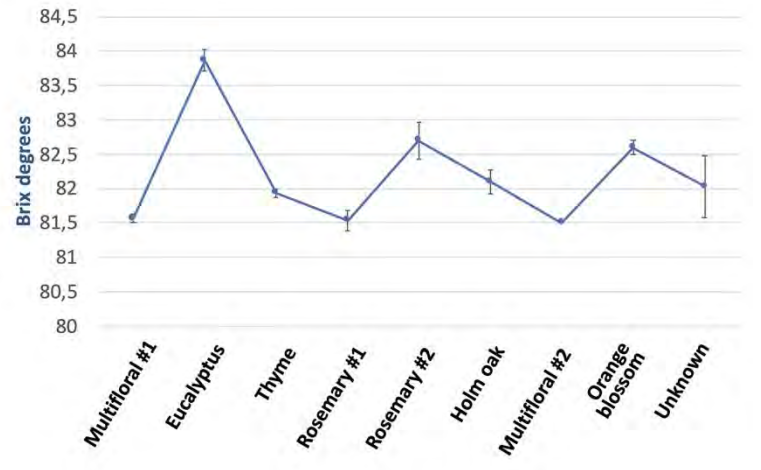


Figure 3: Sugar content of the nine samples of honey analyzed.

Average content in honeys is about 82%. Sugar components mainly correspond to fructose (28-44 %), glucose (22-40 %), maltose (2-16 %), sucrose (0,2-7 %) and other sugars (0,1-8 %). Specific sugar composition of the honeys analyzed here will be determined in upcoming studies, as parameters like the fructose/glucose ratio and water content are determinant for important characteristics of honeys at commercial level, like its crystallization tendency [6].

3. pH and conductivity of the different samples of honey

All honeys analyzed present acidic pHs with values ranging 3.5 to 4.4, as represented in Figure 4.

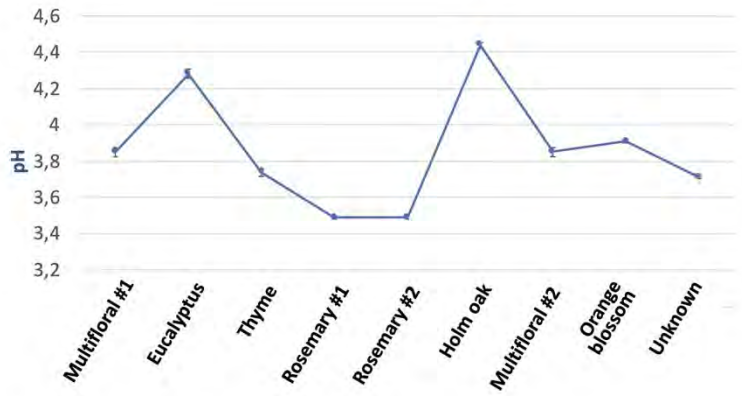


Figure 4: pH of the nine samples of honey analyzed.

Normal pH values of honeys range between 3.2 and 4.5, therefore our samples are well between normal values. Such acidic pH, together with the high osmolarity present in honeys is enough to hinder the growth of microbes [7]. High pH values are indicative of the addition of hydrolyzed syrups or fructose syrups for fraudulent purposes. In our study these values do not exceed values of 4.5, and are probably associated with botanical origin. Adulteration would not be suspected since the values of sugars, fructose and glucose, fall within normal limits.

Both pH and conductivity of honeys are depending on their dilution rate [8]. The values obtained in the present study for conductivity (Figure 5) ranged between 1200 and 1580 mS (Figure 5).

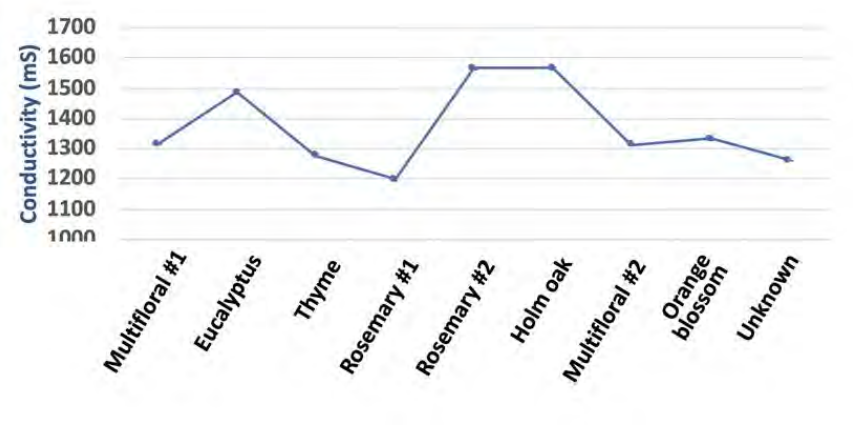


Figure 5: conductivity of the nine samples of honey analyzed.

As indicated by [8], the electrical conductivity of the honey is closely related to the concentration of mineral salts, organic acids and proteins and proved useful for discriminating honeys of different floral origins. That is why many authors have suggested the measurements of electrical conductivity as an alternative for other time-consuming methods like gravimetry. Thus, they can be considered as an indirect technique to determine the mineral content in some foods, and have been particularly investigated as a possible tool for classification [8].

4. Color of the different samples of honey

Figure 6 shows the absorbance values at 635 nm (A_{635}) of honeys analyzed in the present study, correlated with the Pfund scale (Table 1). Most honeys were classified as white or clear amber.

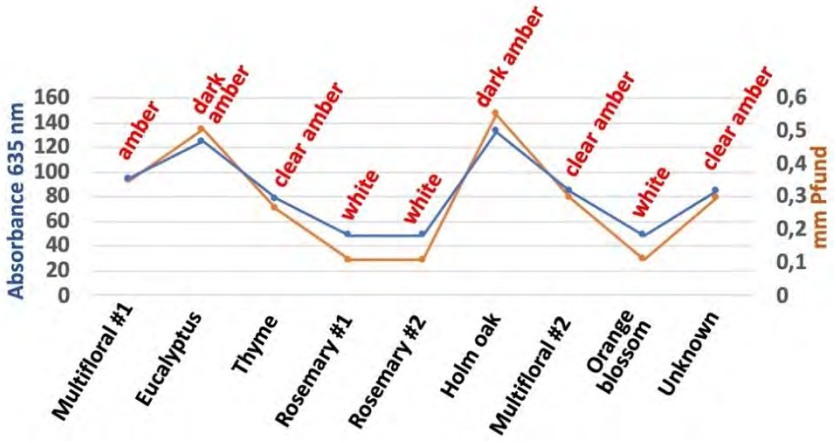


Figure 6: color of the nine samples of honey analyzed.

The color of honey is strictly associated with nectar composition, and many chemical compounds of honey can be correlated. Diverse authors [9-10] detected significant correlations between color parameters and the presence of chemical compounds and other

physicochemical parameters like pH, free and total acidity, proline, hydroxymethyl furfural, diastase activity, and conductivity.

Color measurement can be achieved by other methods. Thus, the use the CIELab system is a representation of the color quite close to that perceived by the human eye. The classification of honey based on visual evaluation of professional tasters established that the honey considered clear by the tasters has a value of luminosity greater than 50 units of CIELab [9]. There is some kind of agreement in that light-colored honey such those analyzed here may have an additional economic value, since the light-colored honey is very appreciated and valorized by consumers of many countries [10].

5. Palynological assessment of the different samples of honey

Pollen isolation from honey samples succeeded in the generation of palynological fractions highly enriched in pollen grains (Figures 7 and 8).

Figure 7 shows pictures of a high number (>10) of pollen species present in multi-floral honeys #1 and also in multi-floral #2 (not shown) with a large variety of sizes, shapes and palynological characteristics (i.e. different exine patterns, number of apertures…). Also, pollinia were observed (Figure 7F). Precise identification of the plant species originating these pollen grains is yet to be carried out, with the help of different reference articles [11-18] and databases [19-23].

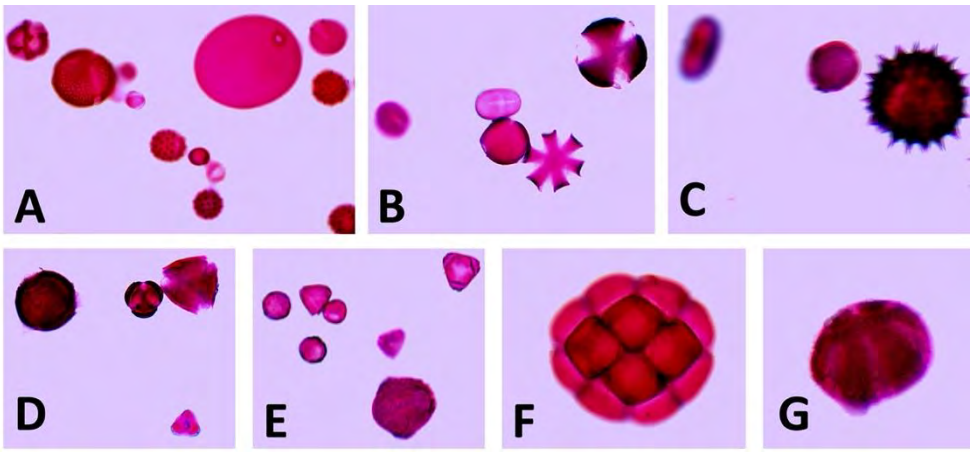


Figure 7: palynological analysis of multi-floral honey #1 used in this assay.

Palynological analysis of several monofloral honeys is displayed in Figure 8. Thyme honey (Figure 8A) presents a very high percentage of isopolar, hexazonocolpate pollen grains of *ca.* 35 µm, and regulate exine, features which are highly compatible with previous descriptions of pollen grains of *Thymus caespititius* Brot. (*Lamiaceae*) and *T. citriodorus* in the databases mentioned above and [24]. Other pollen grains with different characteristics have also been observed, representing a minority (<5%) of pollen grains.

Figure 8B corresponds to eucalyptus honey, where the higher proportion of pollen grains (>95%) are triangular, spheroid to optically slightly oblate, isopolar, scabrate, with narrow colpi that usually unite at the poles and a size of *ca.* 35 µm. Such features are highly

compatible with previous descriptions for *Eucalyptus globulus, E. ficifolia, E. globulus* pollen grains and other eucalyptus species (*Myrtaceae*) in the above-mentioned pollen databases.

Figure 8C corresponds to rosemary honey #1 (highly similar to rosemary honey #2, not shown), with a 100% of pollen grains of *ca.* 30-50 µm, isopolar, prolate/oblate reticulate and hexacolpate, highly compatible with previous descriptions for *Rosmarinus officinalis* (*Lamiaceae*) in the above-mentioned pollen databases.

Finally, Figure 8D shows palynological isolate of orange blossom honey. In this case, pollen grains were 100% of a size of *ca.* 30µm, spheroidal, isopolar, reticulate and tetracolporate, with a high compatibility with previous descriptions of pollen for species like *Citrus aurantium*, or *C. aurantiifolia* (*Rutaceae*).

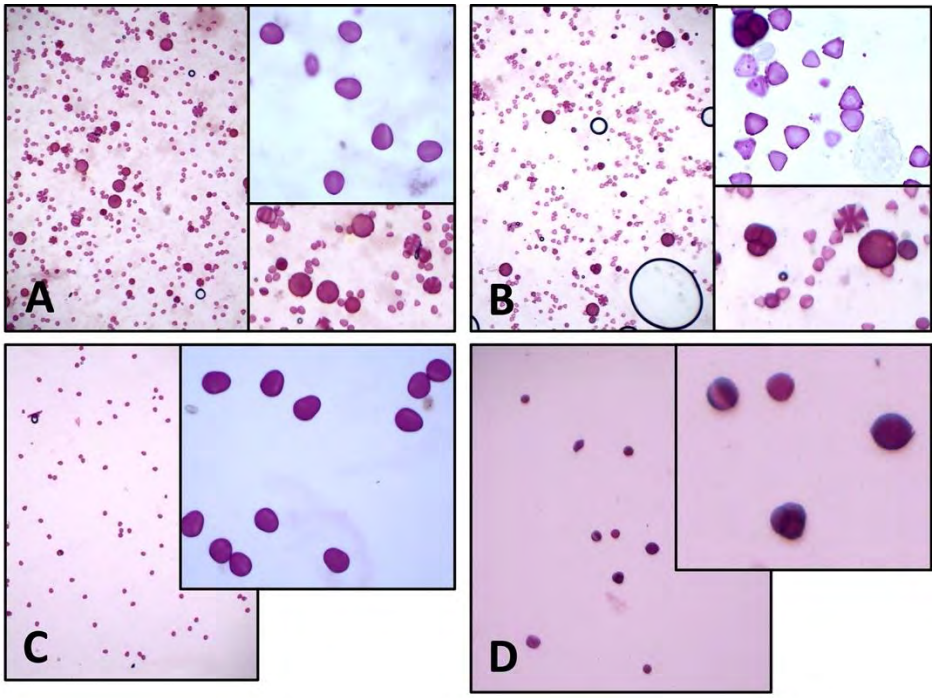


Figure 8: palynological analysis of four monofloral honeys used in tis assay. A: thyme, B: eucalyptus, C: rosemary, D: orange blossom.

In the view of the descriptions indicated above, honeys analyzed were perfectly labelled as multi-floral in the case of multi-floral #1 and #2 and monoflorals in the remaining honeys. Palynologycal analysis also confirmed the accuracy of the botanical origin of the honey samples analyzed.

CONCLUSIONS

- The artisan honeys collected in the Andújar region have exceptional levels of quality, with very low humidity levels and a high sugar content.
- The remaining physicochemical characteristics analyzed (pH, conductivity) also fit well between the standard of quality for honeys.

- There is a wide variability in the botanical origin of honey, which gives it differential characteristics. Among these characteristics, color stands out, as well as a wide range of organoleptic characteristics that must be further analyzed in detail.
- The declared origin of the artisan honeys collected in the region corresponds unequivocally to the pollen composition determined in this study.

Acknowledgements

This work was performed at the Department of Biochemistry, Cell and Molecular Biology of Plants of the Estación Experimental del Zaidín – Spanish Council for Scientific Research (CSIC)- and at student's home. The work was supported by research projects MCIN/AEI/PID2020-113324GB-100, JA/PAIDI/P18-RT-1577, JA/PAIDI/PYC20 RE009CSIC.EEZ y JA/PAIDI/ UMA20-FEDERJA-029, and FGCCLG-2021-0015 from la Fundación General del CSIC (Cuenta la Ciencia Program), all of them co-funded by ERDF program of the EU.

References

[1] Real Decreto 523/2020, de 19 de mayo, por el que se modifica el Real Decreto 1049/2003, de 1 de agosto, por el que se aprueba la Norma de calidad relativa a la miel. BOE 173, 22 June 2020, pp. 43006-43008.

[2] https://www.coagjaen.es/etiquetado-miel/

[3] Orden de 12 de Junio de 1986, por la que se aprueban los métodos oficiales de análisis para la miel. BOE 145, 18 June 1986, pp. 22195-22202.

[4] Real Decreto 473/2015, de 12 de junio, por el que se modifica el Real Decreto 1049/2003, de 1 de agosto, por el que se aprueba la Norma de calidad relativa a la miel. BOE 147 20 June 2015, pp. 51558-51559.

[5] https://www.maeshoney.com/humedad-de-la-miel/

[6] Escuredo I, Dobre I, Fernández-González M, Seijo MC. 2014. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chem 149:84-90.

[7] Mandal MD, Mandal Sh. 2011. Honey: its medicinal property and antibacterial activity. Asian Pacific J Tropic Biomed 1,154:160.

[8] Acquarone C, Buera P, Elizalde B. 2007. Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. Food Chem 101:695-703,

[9] González-Miret ML, Terrab A, Hernanz D, Fernández-Recamales MA, Heredia FJ. 2005. Multivariate correlation between color and mineral composition of honeys and by their botanical origin. J Agric Food Chem 53:2574–2580.

[10] Scholz MBS, Quinhone Júnior A, Delamuta BH. et al. 2020. Indication of the geographical origin of honey using its physicochemical characteristics and multivariate analysis. J Food Sci Technol 57:1896–1903.

[11] Punt W. (1976). The Northwest European Pollen Flora. Ed. Elsevier, T I, Amsterdam, The Netherlands. 145pp.

[12] Moore PD, Webb JA. 1978. An illustrated guide to pollen analysis. Hodder and Stoughton, London-Sydney-Auckland-Toronto, 133 pp.

[13] Punt W, Clarke GCS. 1984. The Northwest European Pollen Flora. Ed. Elsevier, T VI, Amsterdam, The Netherlands, 369 pp.

[14] Walter HL, Prathibha V, Vincent E. 1983. Airborne and allergenic pollen of North America. Johns Hopkins Series University Press, Univ Michigan, USA, 288 pp.

[15] Valdés B, Díez MJ, Fernández I. 1987. Atlas polínico de Andalucía Occidental. Instituto de Desarrollo Regional. Univ Sevilla, Spain, 450 pp.

[16] Punt W, Clarke GCS. 1984. The Northwest European Pollen Flora. Ed. Elsevier, T VI, Amsterdam, The Netherlands, 369 pp.

[17] Punt W, Blackmore S, Clarke, GCS. 1988. The Northwest European Pollen Flora. Ed. Elsevier, T VI, Amsterdam, The Netherlands, 154 pp.

[18] Punt W, Blackmore S. 1991. The Northwest European Pollen Flora. Ed. Elsevier, T VI, Amsterdam, The Netherlands, 275 pp.

[19] http://www-saps.plantsci.cam.ac.uk/pollen/pollen/index.htm

[20] https://www.paldat.org/search/A

[21] https://sites.google.com/site/microscopiabarrido/

[22] http://pollen.tstebler.ch/MediaWiki/index.php?title=Artenliste

[23] http://www.patrimoniocultural.gov.pt/static/data/patrimonio_arqueologico/larc/pollenatlasofportugal.pdf.

[24] Terrab A, Pontes A, Heredia FJ, Díez MJ. 2004. A preliminary palynological characterization of Spanish thyme honeys, Bot J Linnean Soc 146:323–330.

MY OWN IDEAS

Lucía Herrada

One day, our teacher told us about starting a new project with some scientists from Granada. For us, it was something new, interesting and of course we were looking forward to starting to work. The project that we are involved in is about trying to find out the quality of honeys, their characteristics and their origins.

The first meeting, was in our High School, we started to analyze some samples of honey and making photos of their pollens.

The second meeting was in The Zaidin, the place where the scientist work. It was such a great opportunity to go there, because apart from continuing doing the project we have immersed ourselves in a scientist life.

After doing the project we are more conscious about buying natural honeys to our most near beekeepers, because honey has many properties that we would take advantages of.

We are so thankful of having participate in this project, because we have discovered that science is something magic that could allow us to discover incredible things.

Carla Álvarez

Hello, my name is Carla and I am going to explain my work on honey research. Last year our teacher encouraged us to participate in a project in which we analyzed honey from different areas and textures. Some people explained this project at a science fair and others presented it at a conference. The best thing was going to Granada and doing the project with professionals, it was a very good experience!

Ángela Lópiz Garzón

At first, I thought that this project would be good to learn things about plants, their respective pollen and the pollination process. I never would have thought that this project would be so fun and interesting at the same time. Learning about it with our friends and talking about this project was great too.
This experience was incredible because we were able to investigate and learn with professional material. I felt like I was a real researcher, although I still have a lot to learn.
I feel very lucky to have had the opportunity to live an experience like this.

Álvaro Muñoz Moreno

This incredible experience started with the proposition of our teacher to collaborate with a group of scientists from Granada in a project focused on honeys, to find out their characteristics and quality. The project started by collecting honeys from different flowers and origins.
The first meeting with the scientists from Granada was in our institute where we were analysing the different honeys we had collected. The second meeting with them was in El Zaidín, where the scientists were working. In addition to following the project, we were able to see the facilities and other projects they are carrying out there.
At the end of the project we learned about the many properties of honey and how beneficial it is to buy natural honey from the beekeepers in our area.
We found this project to be an incredible opportunity where we discovered that science can teach us amazing things and for this reason we are grateful to have participated in the project.

Esther Torrellas López.

Not everyone has the opportunity of meeting scientists in order to put into practice everything we have learnt previously in class by using the book. Fortunately, my class has enjoyed this chance thanks to our Biology teacher, who has made us live this amazing experience.
Scientists from Zaidín (Granada) have travelled to our secondary school with the aim of providing us all kind of laboratory instruments with the main objective of analyzing our surroundings' honey.
The next time we met them, we went to Zaidín in order to learn how they usually work daily at their laboratories. We have seen every type of microscopes, from the cheap ones, to the most expensive ones.
Thanks to this project, we have learnt how to use a big variety of laboratory instruments, as well as uncountable facts about honey, bees, pollen, microscopes, etcetera. If you get the chance of going to a laboratory, don't miss it!

Lucía Meco Solís

Last year our biology teacher proposed us to do a project with some scientists of Granada. This project was about honey; we had to investigate it's features, it's origin… We were so excited to work with scientists! They came to our high school and we started to make experiments. In addition, they helped us with the new material and all the different types of honey. Other day my partners and I went to Granada by bus. We finished the project in the Experimental Station of the Zaidín. From my point of view, I enjoyed a lot this experience and I would repeat it if I could.

Assessment of organoleptic and nutritional features of pepper fruits subjected to different time and temperature conditions: a preliminary study

Elsa Cárdenas-El Mejdoubi[1], Candela Cortés-Moya[1,] Rocío Fernández-Rodríguez[1], Miguel R. Higueras-Raya[1], Lin-Oliver Martín-Contreras[1], Claudia Moreno-Villegas[1], Patricia Rodríguez-De la Rosa[1], Sebastián Chaparro[1], Carolina Domek-Águila[1], María Gómez-Molina[1], Ángel Navarra-Villalba[1], Arturo Ruiz-Joya[1], María Jesús Campos[2], Carmelo Ruiz[2], Antonio Quesada[1,#], José M. Palma[2,#]

[1]*IES Zaidín-Vergeles, Primavera 24-26, 18008 Granada, Spain.*

[2]*Department of Stress, Development and Signaling in Plants. Estación Experimental del Zaidín. CSIC. Profesor Albareda 1, 18008 Granada, Spain.*

#Corresponding authors: quesadaramos@gmail.com; josemanuel.palma@eez.csic.es

Summary

A set of experimental conditions was used in this work in order to seek for the best storage practices to maintain and/or improve the nutritional properties of sweet pepper fruits. Thus, fruits from Dulce italiano variety were incubated at room temperature, 8ºC and -10ºC and sampling was done at 0, 1, 2, and 4 weeks. Different nutritional and organoleptic parameters were, then, analyzed, including fresh and dry weight, water loss, acidity, soluble sugars, protein and vitamin C contents, and profiles of photosynthetic pigments (chlorophylls and carotenoids). Our results indicate that storing in plastic bags at room temperature seems to be the most appropriate practice to maintain the best organoleptic and nutritional properties of Dulce italiano pepper fruits.

Keywords: Acidity, Brix, carotenoids, chlorophyll, pepper, vitamin A, vitamin C, water loss

INTRODUCTION

Pepper (*Capsicum annuum* L.) is an annual herbaceous species which belongs to the Solanaceae, a family that also includes a number of important crops for the human diet, such as tomato (*Lycopersicum esculentum*), potato (*Solanum tuberosum)*, and aubergine (*Solanum melongena*) [1,2]. There are a great number of pepper varieties differentiated according to their colour, size, shape, organoleptic features and culinary uses, these latter not only interesting for gastronomy purposes but also for health [3,4].

Due to their high content in water and fibre, pepper fruits are low in calories, but they are also a good source of antioxidants such as vitamin C (ascorbic acid, ascorbate), vitamin A (β-carotene as a precursor), flavonoids, polyphenols, capsaicinoids and diverse minerals (K, Fe, Mn, Cu, P) [4,5]. Vitamin C, which might be considered as the paradigm of the low molecular weight antioxidants, is essential in animals and humans since it participates in many metabolic pathways and in the prevention of a number of pathologies [6-9]. Therefore, pepper fruit might be postulated as a potential nutraceutical food [10,11].

Peppers, as other vegetables and fruits, undergo changes in nutrient composition and organoleptic features during prolongued postharvest periods, and this makes them unappropriated and undesirable for consumption. Besides the time lapse from the harvest to the market, pepper fruits are usually stored at home until their consumption, mainly at low temperatures (the fridge or even the freezer), but also at room temperature, without paying attention to those potential changes in quality. Storage must not only preserve food but keep it in appropriate conditions for maximum quality. In this respect, little has been investigated about the storage conditions of peppers fruits at home, and their possible loss of organoleptic, nutritional and health properties through time [12,13].

Some of the most important factors that negatively impacts pepper fruit during storage are water loss, chilling injury and susceptibility to pathogens. These processes result in fruit softening and reduced shelf life and it varies depending on the cultivars and the storage conditions [14-16]. Low temperature and high relative humidity are primary factors to maintain the products' quality during storage, and also reduced temperatures decrease physiological, biochemical and microbiological processes that may lead to alteration of attributes like flavour, texture, colour and nutritive value [17].

Another important consequence of storage is the loss of vitamins. For example, ascorbic acid is highly sensitive to temperature, and it is used as a marker for product quality deterioration [10,14]. Vitamin A is also lost in a high proportion when fruits and vegetables are kept at room temperature [12]. The biological value of proteins is usually little affected, but fats usually undergo quality deterioration during prolonged storage due to oxidation and rancidity, mainly caused by moisture and water. On the contrary, carbohydrates seems relatively stable to storage.

In this framework, the main objectives of this research were to assess the influence of storage conditions on the dehydration of pepper fruits, to follow the time course of certain biomolecules like soluble sugars or proteins, and to evaluate changes throughout time in ascorbate (vitamin C) and carotenoid (source of vitamin A) levels. In summary, this work was aimed at assessing the best storage conditions for pepper fruit in order to preserve its organoleptic and nutritional features.

MATERIAL AND METHODS

1. Plant material

Sweet pepper (*Capsicum annuum* L.) fruits, type Dulce italiano (Spring season, 2022), were purchased from the local market at immature green ripening stage. As this is a preliminary study with an educational approach, carried out with high school students and some logistics limitations, only one fruit was used for each of the condition assessed in this work.

2. Experimental design

Organoleptic and nutrient changes over time and storage conditions of fruits were tested along four weeks. Ten pepper fruits were distributed in three groups with three fruits each, and stored inside black plastic bags: one group was kept at room temperature; another one was maintained in the fridge at 8ºC; and the last one was store in the freezer (-10ºC). Another pepper fruit was used as a control and analyzed at the beginning of the experiment. All fruits were weighted the first day (day 0). Then, measurements of fresh and dry weights, soluble sugars content, pH, vitamin C concentration and photosynthetic pigments levels were taken from the control fruit and the respective groups at weeks 1, 2 and 4. The experimental design is shown in Figure 1.

		Day 0 (23/2)			Day 7 (2/3)			Day 14 (9/3)			Day 28 (23/3)			
ROOM TEMP	Pep. 1	Total weight	Fresh weight	Brix, pH, vit C. extracts		Dry weight								
	Pep. 2	Total weight			Total weight	Fresh weight	Brix, pH, vit C. extracts		Dry weight					
	Pep. 3	Total weight						Total weight	Fresh weight	Brix, pH, vit C. extracts				
	Pep. 4	Total weight									Total weight	Fresh weight	Brix, pH, vit C. extracts	Dry weight

		Day 0 (23/2)			Day 7 (2/3)			Day 14 (9/3)			Day 28 (23/3)			
FRIDGE	Pep. 5	Total weight			Total weight	Fresh weight	Brix, pH, vit C. extracts		Dry weight					
	Pep. 6	Total weight						Total weight	Fresh weight	Brix, pH, vit C. extracts				
	Pep. 7	Total weight									Total weight	Fresh weight	Brix, pH, vit C. extracts	Dry weight

		Day 0 (23/2)			Day 7 (2/3)			Day 14 (9/3)			Day 28 (23/3)			
FREEZER	Pep. 8	Total weight			Total weight	Fresh weight	Brix, pH, vit C. extracts		Dry weight					
	Pep. 9	Total weight						Total weight	Fresh weight	Brix, pH, vit C. extracts				
	Pep.10	Total weight									Total weight	Fresh weight	Brix, pH, vit C. extracts	Dry weight

Figure 1. Experimental design where sampling time (Day 0-28), storage conditions (Room temp, Fridge, Freezer) and analyses performed in each fruit (Pep. 1-10) are indicated.

3. Dry weight and weight loss percentage

Fresh pepper fruits were weighed and then oven-dried at 50ºC for a week. Then, samples were weighted again and the loss of water was calculated by subtraction. Dry weight was expressed as the percentage with respect to the original fresh weight value.

For weight loss percentage, pepper fruits were weighed at day zero and in each sampling day as indicated in Figure 1. The difference between initial and final weights was considered as total weight loss during the storage periods (weeks 1, 2 and 4), and expressed as percentage.

4. Total soluble sugars (Brix) and pH

Pepper juice was extracted by grinding the fruits in a mortar with a pestle and then was filtered with the help of a plastic syringe and nylon tissue. Total soluble solids in filtrates was determined with an Atago refractometer and referred to ºBrix. At 20ºC the ºBrix is considered equivalent to the percentage of soluble sugars in the solution.

The pH was measured directly in pepper juice with pH indicator strips pH 0-14 (Dosatest).

5. Vitamin C determination

Vitamin C (ascorbic acid, ascorbate) content of pepper fruits was evaluated by a titration method with a lugol solution and starch as indicator [10]. Basically, iodine of the lugol solution is able to react with starch resulting in a dark blue colour. Ascorbic acid prevents the appearance of such colour as it reacts with iodine. The higher ascorbic acid concentration, the higher volume of the lugol solution to stain starch is necessary.

A standard curve was prepared as follows. Test tubes with 2 mL of starch 0.5% (w/v) were added 1 mL of ascorbic acid solutions of known concentrations. Then, with the help of a pipette, increasing volumes of the lugol solution were added until the appearance of the dark blue colour in the test tube. The concentration on the known ascorbate concentrations were plotted against the lugol volumes used.

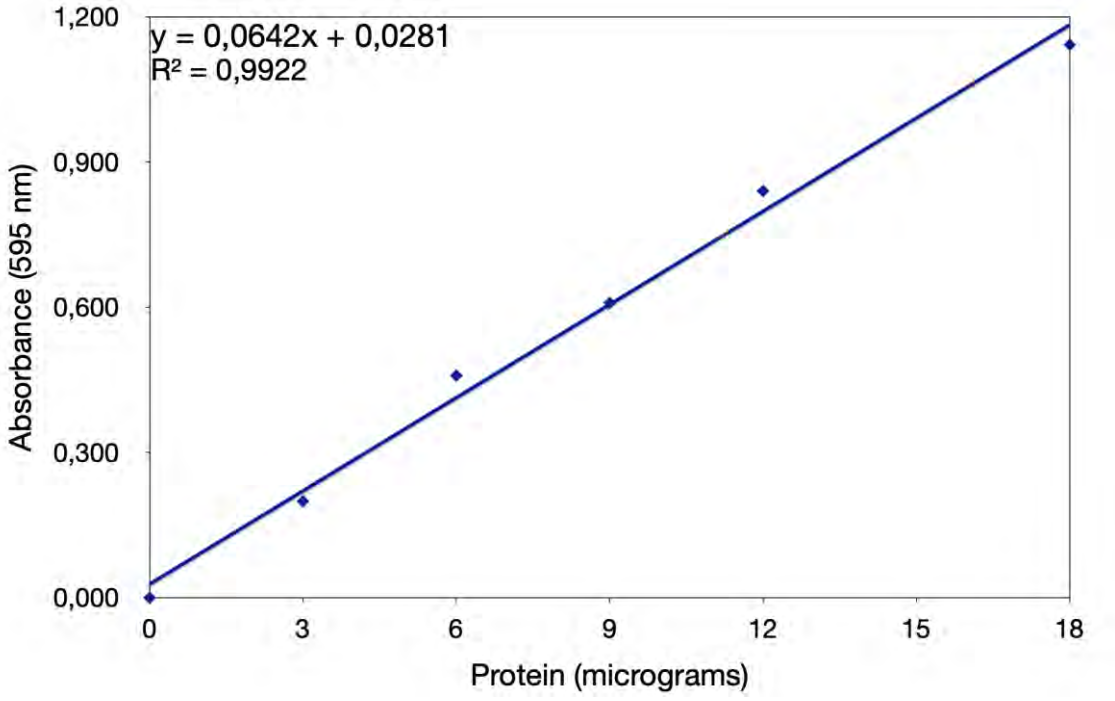
For the determination of ascorbate in samples, 3 g of pepper fruits were ground in a mortar with a pestle with 3 mL of Tris-HCl 50 mM, pH 8.0. The homogenate was centrifuged at 5000 g for 5 min. Then, 1 mL of homogenate was added to 2 mL of 0.5% (w/v) starch solution and evaluated with the procedure described above. Volumes of lugol used were then extrapolated to the standard curve. An average of three measurements were used for calculation of vitamin C concentration in peppers.

6. Protein determination

It was performed following the colorimetric method of Bradford [19]. A standard curve was prepared with different dilutions of a bovine serum albumin (BSA, 0.6 mg/ml) solution in a final volume of 800 µL. Then, 200 µL of Bradford reactive (Bio-Rad Protein Assay Dye Reagent) were added, as shown in Table 1. The optical density of the dilutions was measured at 595 nm in a Shimadzu UV 120-02 spectrophotometer, and a standard curve was obtained as shown in Figure 2.

Table 1. Procedure for the preparation of the standard curve for protein determination. BSA, bovine serum albumin.

Tube number	0	1	2	3	4	5
µL BSA (0.6 mg/mL)	-	5 (3 µg)	10 (6 µg)	15 (9 µg)	20 (12 µg)	30 (18 µg)
µL H_2O	800	795	790	785	780	770
µL Bradford reagent	200	200	200	200	200	200



igure 2. Standard curve for protein determination.

Samples from pepper fruit homogenates were plotted in this standard curve in order to know their protein concentrations. They were assayed using 5 µL of pepper extracts plus 795 µl of distilled water and 200 µl of the Bradford reagent.

7. Chlorophyll and carotenoids concentration

Chlorophyll *a* and *b* and carotenoids content were determined spectrophotometrically as described by Pompelli et al. [20]. Fresh pepper fruits (0.6 g) were ground in a mortar with a pestle with 12 mL of ethanol (96%, v/v), incubated for 20 h and centrifuged at 5000 g for 10 minutes. Supernatants were used for the analyses. The absorbance of the extracts was measured at 664, 649 and 470 nm in a Shimadzu UV 120-02 spectrophotometer. Pigment content, expressed as µg/g fresh weight of the extracts, was calculated from the following equations:

Chlorophyll *a* (µg/mL) = 13.36 · A664 – 5.19 · A649

Chlorophyll *b* (µg/mL) = 27.43 · A649 – 8.12 · A664

Total carotenoids (µg/ml) = [(1000 · A470 – 2.13 · (chlorophyll *a*) – 97.64 · (chlorophyll *b*)]/209

RESULTS

Pepper fruits were stored in black plastic bag in order to reduce the water loss and photooxidation of biological compounds. Figure 3 shows the fresh weight loss of pepper fruits in the different conditions assayed along the experiment. Thus, after four weeks, the

weight loss percentage was higher in peppers stored at room temperature (88.27% of the initial weight), while there were no relevant differences in those fruits stored at 8ºC in the fridge (95.20%) or at -10ºC in the freezer (95.90%). Taking into account that this reduction is mainly due to dehydration of fruits, peppers kept at room temperature lost about 11.73% of water; whereas the weight loss of those stored at low temperature, either 8ºC or -10ºC, was below 5%. On the other hand, no substantial changes were observed in the dry weight evolution throughout time (Figure 4).

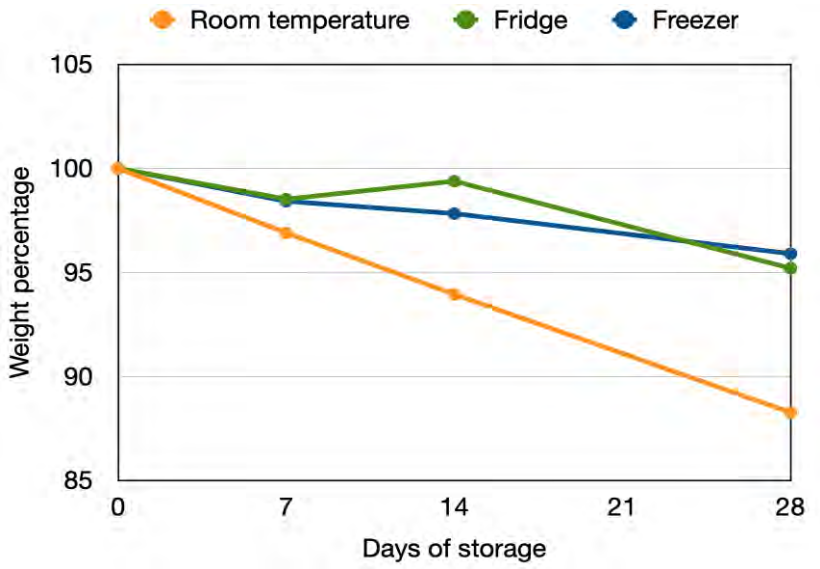


Figure 3. Pepper fruits weight changes over time in different storage conditions. Values were calculated as referred to pepper fruit fresh weight at time 0. Fruits were stored at room temperature, 8ºC (Fridge) and -10ºC (Freezer).

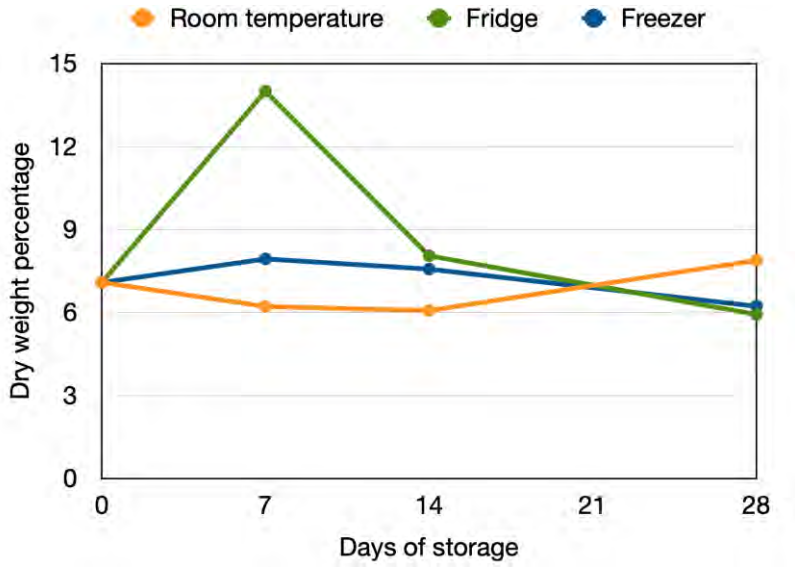


Figure 4. Dry weight evolution of pepper fruits in different storage conditions. Fruits were stored at room temperature, 8ºC (Fridge) and -10ºC (Freezer). Values were calculated as referred to the fresh weight of each fruit.

The soluble sugar content of pepper fruit decreased throughout the four weeks, mainly in those fruits stored in the fridge or the freezer (Figure 5). At day zero, Brix was 6%, whereas the final values ranged from 3,8% - 5,2%.

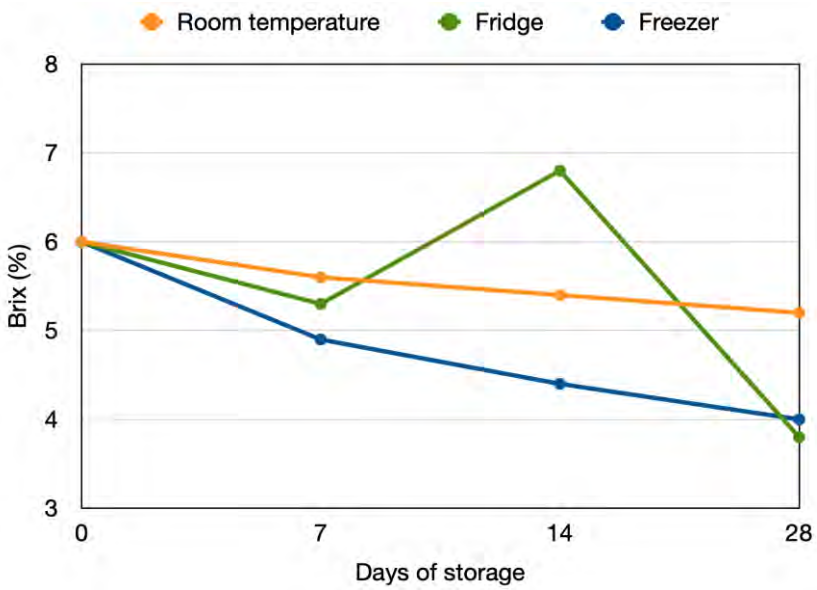


Figure 5. Soluble sugars content (Brix, %) changes over time on pepper fruits stored at different conditions. Fruits were stored at room temperature, 8ºC (Fridge) and -10ºC (Freezer).

Regarding acidity, extracts from pepper fruits stored at room temperature showed the most relevant pH changes, with values from 5,5 - 4,5. No meaningful variation was observed in those fruits kept a lower temperatures (Figure 6).

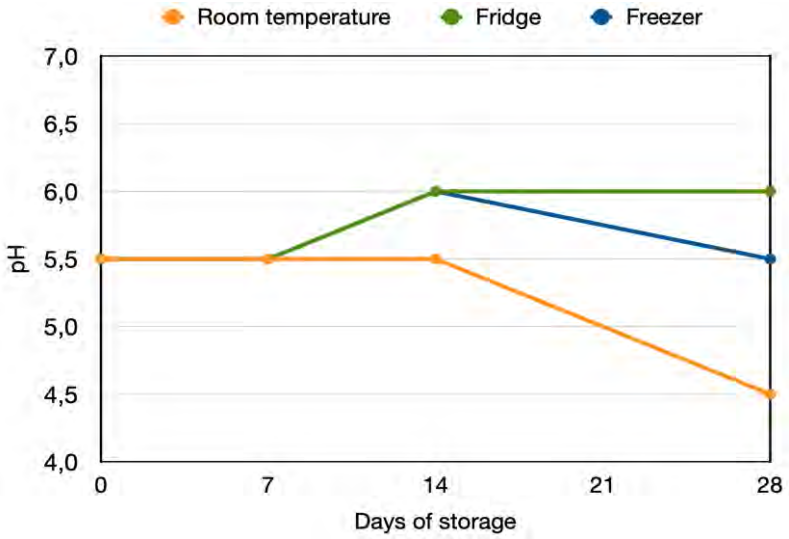


Figure 6. pH changes under different storage conditions of fruits. Fruits were stored at room temperature, 8ºC (Fridge) and -10ºC (Freezer).

Figure 7 shows the changes of protein concentration in pepper fruits subjected to different conditions over time. The higher concentration was observed in fruits stored in the fridge; on the contrary, those fruits maintained in the freezer showed the lowest levels. No relevant changes could be detected after the second week for each treatment.

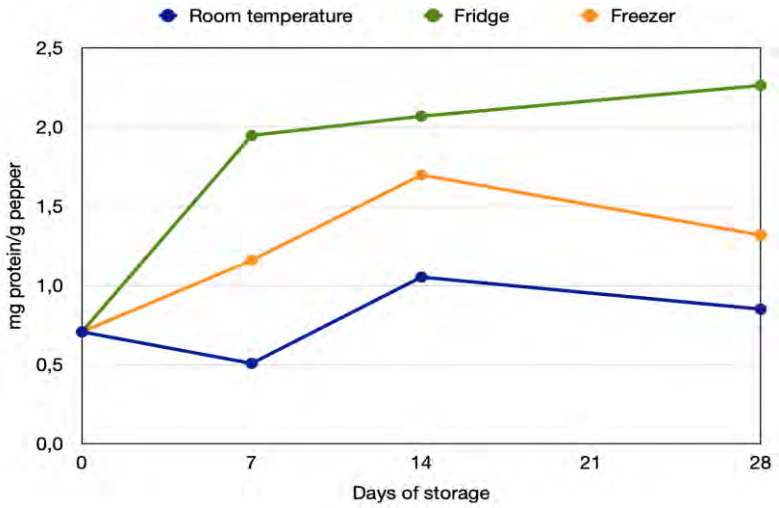


Figure 7. Changes over time in protein content of pepper fruits subjected to different incubation conditions. Fruits were stored at room temperature, 8ºC (Fridge) and -10ºC (Freezer).

With respect to the ascorbate content, peppers kept at room temperature showed the highest values, while those stored in the freezer had the lowest ones. Considering the evolution of this metabolite over time, a decrease in ascorbate concentration has been observed in pepper fruits frozen or stored at 8ºC. Similarly, fruits kept at room temperature showed a decrease in vitamin C concentration during the first two weeks, but an increase was observed in the last two weeks of the assay (Figure 8).

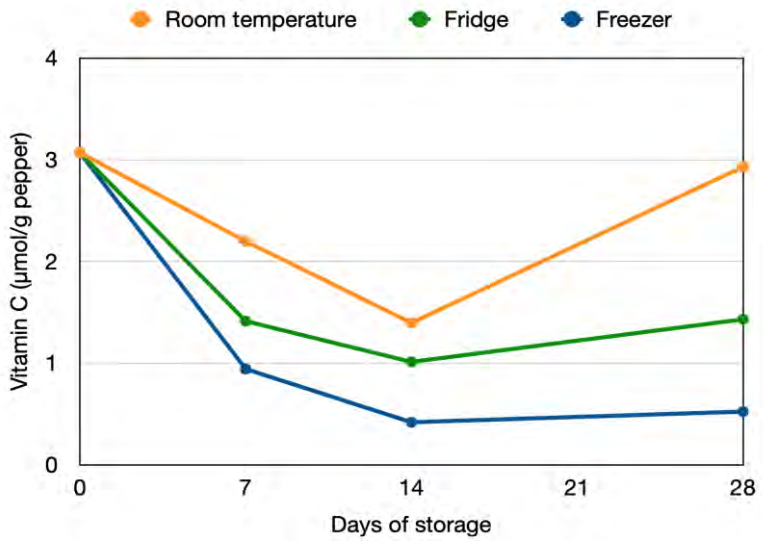


Figure 8. Changes in ascorbate concentration in fruits regarding storage conditions. Fruits were stored at room temperature, 8ºC (Fridge) and -10ºC (Freezer).

Changes in pigment concentration over time were also analyzed in pepper fruits subjected to the reported conditions. At first sight, the most noticeable changes were observed in fruits stored at room temperature as they shifted to red color as a consequence of ripening (Figure 9).

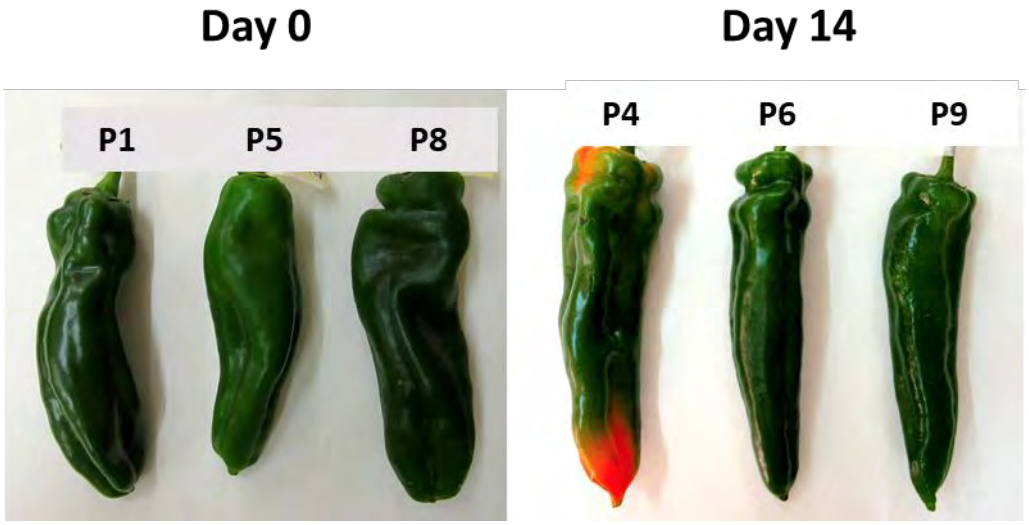


Figure 9. Changes in colour observed in pepper fruits after two weeks of storage at room temperature. The photo was taken using fruits P1, P4, P5, P6, P8 and P9 as designated in Fig. 1.

In sweet pepper extracts, chlorophyll *a* showed higher concentration than chlolorphyll *b*, both of them being above carotenoids concentration at the beginning of the experiment. As a consequence of ripening, chlorophylls were completely absent after four weeks in fruit maintained at room temperature. On the contrary, carotenoids showed the highest concentration at this time (Figure 10). These results are consistent with the color change observed in pepper fruits.

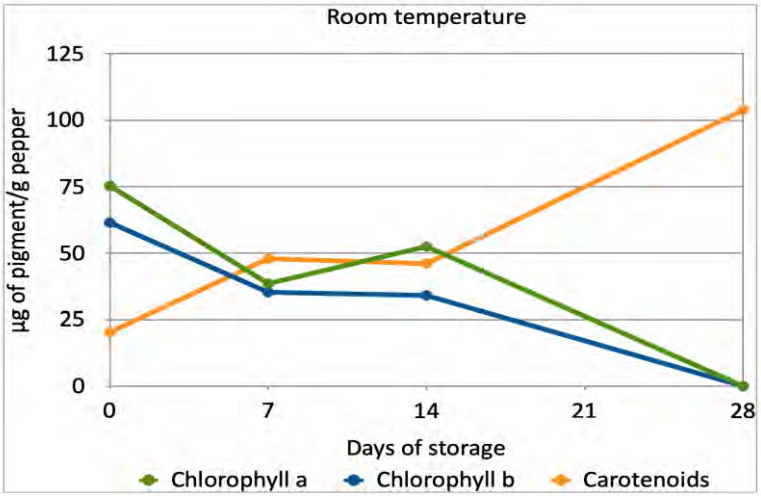


Figure 10. Changes in chlorophylls and carotenoids content over time in peppers fruits stored at room temperature.

On the other hand, pepper fruits stored in the fridge and in the freezer remained green. No significant changes in pigment concentration were observed in those fruits (Figure 11).

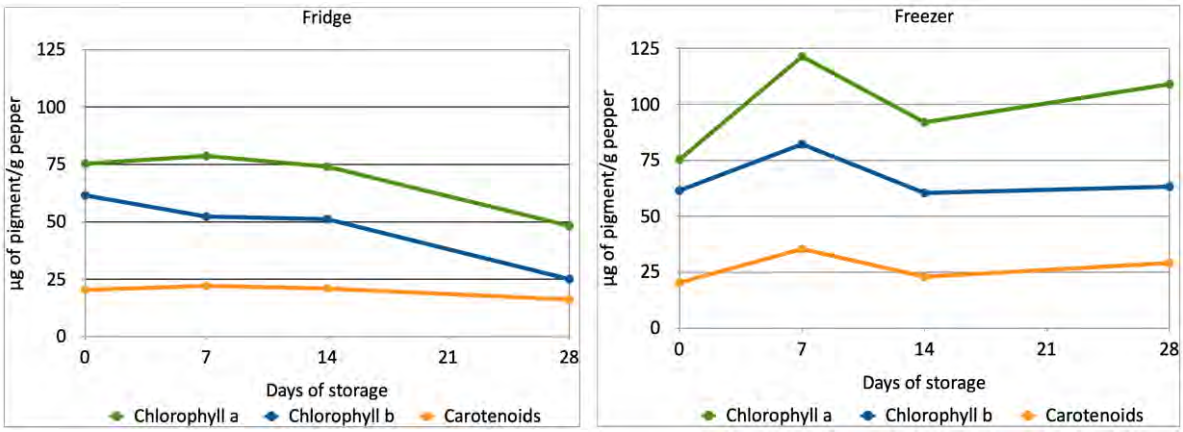


Figure 11. Changes in chlorophylls and carotenoids content over time in peppers fruits stored in the fridge (left) and the freezer (right).

DISCUSSION

The main goals of this research were to assess the changes in some biological parameters from sweet pepper fruits incubated in different storage conditions over time. As fruits were obtained at inmature green stage, changes observed at room temperature may be associated either with the own ripening process (they became red at the end of the experiment), the storage conditions or both. In order to assess properly our results, it is necessary to emphasize that this is a preliminary study carried out by high school students and that there was only one sample for each one of the studied conditions, as indicated above.

Water loss has been described as one of the most important factors that negatively affect pepper fruits during storage and this influences the consumer acceptance of vegetables [17]. In order to prevent dehydration and to preserve fruits from light, they were stored in black plastic bags. Regarding water loss, our data showed that water content progressively decreased in all samples, but mainly in those stored at room temperature. The difference in moisture content with those stored in the fridge is mainly due to the lower temperature, although Bernardo et al. [3] already reported a progressively decrease in water content during ripening of pepper fruits.

No important changes in fruits firmness were observed in fruits stored in the fridge over time. This trait slightly decreased over time in peppers kept at room temperature throughout the four weeks, and this mainly related to water loss. Notwithstanding, the fruits looked nearly acceptable for consumption as fresh vegetables. On the contrary, those fruits kept in the freezer were firmless when they were thawed; consequently they were not appropriate for salads or for consumption as fresh, only for cooking.

Regarding soluble sugars concentration, Brix decreased in all treatments, mainly in pepper stored at low temperatures, either in the fridge or in the freezer. Our results disagree with those described in literature [3,17]. These authors observed an increase of soluble sugars

during ripening associated to compositional changes in cell wall carbohydrates as a consequence of the action of wall degrading enzymes or starch degradation, and the magnitude of those changes varied among cultivars. Therefore, the decrease over storage observed in this work may be explained by the respiratory degradation of sugars by fruit cells. Our experiments did not show any increase of soluble sugars content associated with the ripening process in peppers stored at room temperature. The lower Brix values of observed in our experiments on peppers kept in fridge or freezer could be explained by the reduced activity of enzymes that degrade both starch or cell wall carbohydrates.

pH of fruits extracts is related to the amount of organic acids present in fruits and also to the content of sugars. Our results on peppers stored at room temperature agree with those reported earlier [3] where a decrease of this parameter has been described during the ripening process. On the contrary, no variation or even a slight increase was observed in peppers kept at low temperaturas, either 8ºC or -10ºC. Perhaps, since fruits were stored into plastic bags with little exposition to air, fermentative processes could explain the formation of organic acids. Low temperatures would prevent that changes. But this needs to be explored in more depth.

Regarding protein content, peppers stored at low temperatures (fridge or freezer) showed higher values than those stored at room temperature. Dadango [12] found that high storage temperature causes that aminoacids, like lysine, chemically bind to simple sugars affecting the nutritional value of food. No important changes were observed over time in the present work in agreement to other previous studies [2,12].

Ascorbic acid is highly sensitive to storage conditions and it is used as a marker for product quality deterioration [18]. In our experiments, pepper fruits stored at room temperature showed the highest concentrations of vitamin C while the minimum was measured in those kept in the freezer. An increase of vitamin C content during pepper ripening has been described [16,17]. Bernardo et al. [3] also reported an increase by a factor ranging from 1,2 to 4. These authors concluded that red ripe fruits are more beneficial in supplying the vitamin C recommended daily allowance. Probably, this production of ascorbate during ripening could explain the increase observed during the last two weeks of our experiments, just when our fruits maintained at room temperature became red.

The process of ripening that underwent peppers stored at room temperature was confirmed by the analysis of photosynthetic pigments. No variations over time were observed in those kept either in fridge or freezer with chlorophylls concentrations higher that carotenoids. On the contrary, on peppers kept at ambient temperature, chlorophylls were degraded and they were replaced by carotenoids. According to this change, an increase of vitamin A could be associated with the higher concentration of carotenoids over time.

Storage at low temperature has been proposed as the most efficient method to keep quality of fruits and vegetables, mainly due to its effects in reducing respiration rate, ethylene production, ripening and senescence [16,17]. Taking into consideration a wide view of the present work, we can conclude from our data that storing in plastic bags at room temperature seems to be the most appropriate practice to maintain the best organoleptic and nutritional properties of Dulce italiano pepper fruits.

Acknowledgements

This research was supported by European Regional Development Fund (ERDF)-cofinanced grants from the Ministry of Science and Innovation (PID2019-103924GB-I00) and Junta de Andalucía (P18-FR-1359), Spain. The students participating in this work want to thank our teacher Antonio Quesada for proposing projects like this that will allow us to have a much better personal development and to see Science from other points of view. We also acknowledge our high High School for accepting proposals of this type and Pepe Palma for giving us the opportunity to be even closer to a biological experiment, and for being so kind and helpful.

References

[1] Gebhardt C. 2016. The Historical Role of Species from the Solanaceae Plant Family in Genetic Research. Theor Appl Genet 129:2281–2294.

[2] Li W, Li J, Zhao J, He C. 2015. Evolutionary Developmental Genetics of Fruit Morphological Variation within the Solanaceae. Front. Plant Sci 6:248.

[3] Bernardo A, Martínez S, Álvarez M, Fernández A, López M. 2008. The composition of Two Spanish Pepper Varieties (Fresno de la Vega and Benavente-Los Valles) in Different Ripening Stages. J Food Quality 31:701-716.

[4] Palma JM, Corpas FJ, Ruiz C, Molina T, Campos-Ramos MJ, Juanena A, Torreira JR. 2016. Los Pimientos de las Variedades Padrón, Piquillo y Alegría Riojana: Una Buena Fuente de Macro y Microelementos para Nuestra Dieta. Horticultura 323:60-64.

[5] Guevara L, Domínguez-Anaya MA, Ortigosa A, González-Gordo S Díaz, C, Vicente F, Corpas FJ, Pérez del Palacio J, Palma JM. 2021. Identification of Compounds with Potential Therapeutic Uses from Sweet Pepper (*Capsicum annuum* L.) Fruits and Their Modulation by Nitric Oxide (NO). Int J Mol Sci 22: 4476.

[6] Grosso G, Bei R, Mistretta A, Marventano S, Calabrese G, Masuelli L, Giganti MG, Modesti A, Galvano F, Gazzolo D. 2013. Effects of Vvitamin C on Health: A Review of Evidence. Front Biosci 18:1017–1029.

[7] Gordon DS, Rudinsky AJ, Guillaumin J, Parker VJ, Creighton KJ. 2020. Vitamin C in Health and Disease: A Companion Animal Focus. Top Comp Anim Med 39:100432.

[8] Rowe S, Carr AC. 2020. Global Vitamin C Status and Prevalence of Deficiency: A Cause for Concern? Nutrients 12:2008.

[9] Palma JM, Seiquer I. 2020. To be or not be . . . an antioxidant? That is the question. Antioxidants 9:1234.

[10] Entrena-Aranda P, Paula Entrena-Aranda, Roldán-Martín M, Mengíbar-López I, Rodríguez-López L, Moreno-Duperón AY, De la Torre-Amat E, Shan Vida-Lara J, Quesada A, Ruiz C, Campos MJ, Palma JM. 2021. Analysis of vitamin C content of fruits from five pepper varieties showing different pungency levels and antimicrobial potentiality of capsaicinAnalysis of vitamin C content of fruits from five pepper varieties showing different pungency levels and antimicrobial potentiality of capsaicin. High School Stud Agric Sci Res 10:51-65.

[11] Palma JM, Pérez del Palacio J, Rodríguez-Ruiz M, Salvador González-Gordo S, Díaz C, María C Ramos, Cautain B, Vicente F, Corpas FJ. 2022. Pepper Fruit as a Nutraceutical Food with Anti-Proliferative Activity Against Tumor Cells Potentiated by Nitric Oxide (NO). In: Muntane J., Morbidelli L., Bonavida B. (eds.) Therapeutic Applications of Nitric Oxide and Derivatives. Springer, in press.

[12] Dadango MA. 2009. Changes in Nutrients During Storage and Processing of Food: A Review. Techno Sci Afri J 3:24-27.

[13] Tsegay D, Tesfaye B, Mohammed A, Yirga H, Bayleyegn A. 2013. Effects of Harvesting Stage and Storage Duration on Postharvest Quality and Shelf Life of Sweet Bell Pepper (*Capsicum annuum* L.) Varieties under Passive Refrigeration System. Int J Biotech Mol Biol Res 4:98-104.

[14] Lownds NK, Banaras M, Bosland PW. 1994. Posthavest Water Loss and Storage Quality of Nine Pepper (*Capsicum*) Cultivars. HortScience 29:191-193.

[15] Smith D, Stommel JR, Fung RWM, Wang CY, Whitaker BD. 2006. Influence of Cultivar and Harvest Method of Postharvest Storage Quality of Pepper (*Capsicum annuum* L.) Fruit. Postharv BiolTechnol 42:243-247.

[16] Palma JM, Freschi L, Rodríguez-Ruiz M, González-Gordo S, Corpas FJ. 2019. Nitric oxide in the physiology and quality of fleshy fruits. J Exp Bot 70:4405–4417.

[17] Samira A, Woldetsadik K, Workneh TS. 2013. Postharvest Quality and Shelf Life af Some Hot Pepper Varieties. J Food Sci Technol 50:842-855.

[18] Martínez S, López M, González-Raurich M, Bernardo A. 2005. The Effects of Ripening Stage and Processing Systems on Vitamin C Content in Sweet Peppers (*Capsicum annuum* L.). Int J Food Sci Nutr 56:45-51.

[19] Bradford MM. 1976. A rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem 72:248–254.

[20] Pompelli MF, França SC, Tigre RC, Oliveira MT, Sacilot M, Pereira EC. 2013. Spectrophotometric Determinations of Chloroplastidic Pigments in Acetone, Ethanol and Dimethylsulphoxide. Res Bras Bioci. 11:52-58.

MY OWN IDEAS

Candela Cortés Moya

My own opinion according to this experimental project couldn't be better. When I first listened about this activity I was so excited. I've always dreamed about being a scientist and manipulate laboratory instruments, but this has certainly gone further than I could ever imagine.

We were given the choice of working with several of the best researchers of Spain, and obviously, this was an incredible advantage. Moreover, the purpose of the investigation was extremely interesting owing to the fact that a research in the nutritional characteristics of peppers could teach some new data to the citizenship. So, this project is not just for ourselves, it was thinking for the bast majority of the population, and for me, that is one of the best awards that we could have.

On the other hand, talking about what this project has given to me, I would say that this experience is designed not only for searching information, but also is designed for giving us the opportunity of develop new skills in the scientist area. I've learned new specific vocabulary which I cannot use on my daily basis but obviously in a scientific environment. Moreover, I'm so grateful for having an incredible group which have taught me how to cooperate with others, cause this is an magnificent thing.

So, to sum up, this project was the first scientist activity in which I took part, and I'm totally sure that it is not going to be the last, cause it was brilliant.

Lin-Oliver Martín Contreras

I still remember when all I knew about these projects was what our teacher Antonio Quesada would tell us when we entered the lab and asked him about the Martian diorama that occupied one of the tables. This year I've had the opportunity of experiencing it myself. This project has taken me out of my comfort zone in so many ways. I have learned to be part of a working group where we all had to cooperate and still give our best, and I have also ended up speaking at a congress in front of experts in the field, which is something I would have thought impossible just a few months ago.
When we finished our presentation at the congress, we were asked several question, and one of them was asked by Dr. Francisco Martínez Abarca. He asked us why we had not made our measurements with the dry weight of the peppers. I thought this was a good idea that maybe we can explore next year, because it eliminates the small differences that can be caused by the presence or loss of water in the pepper fruit.
Thanks to this project, and of course to all the people that have made it possible, I have finally begun to understand the true meaning of scientific research. Being in the lab all these days, taking measurements and drawing conclusions, has taught me so many things that I can hardly believe it has only been a year since the beginning of it.

Elsa Cárdenas El Mejdoubi

When Pepe Palma was explaining to us the previous projects I could only think "I want that". I couldn't contain the urge to ask "When will start ours?". Then I waited impatiently for them to confirm the start of the investigation and throughout the whole project I was excited and enthusiastic.
I have always seen scientists working and obtaining results and I knew that was my aspiration for the future. What I didn't know was that, at the age of 15, I would be investigating something that no one had ever done before.
As I see it, this has been a very enriching experience with which we have learnt more than in a common class. For instance, I was the one in charge of the spectrophotometer, a machine which existence was invisible to me until I was in front of it. Moreover, we have worked with different laboratory techniques and learnt how to organize and distribute tasks.
One of my favourite parts was analysing and interpreting the results, seeing how our effort was worth it. As well the congress, exchanging information with other schools, people who were listening and interested in what we were saying.
Overall, it has been very satisfying watching the project evolve and I am completely glad we had this opportunity.

Rocío Fernández Rodríguez

A principio de curso nuestro profesor nos proporcionó la opción de poder realizar un proyecto superándonos día a día, realizando descubrimientos que nunca pensamos que podríamos llegar a hacer.
Esta actividad nos ha ayudado a saber que todas las barreras se pueden superar con esfuerzo y ganas, además de que nos ha ayudado a conocer los instrumentos que nuestros científicos utilizan día tras día.

Realmente este proyecto ha sido posible gracias a la buena organización que hemos tenido en clase, cada uno dominábamos algo pero a la vez sabíamos un poco de todo, después de hacer los experimentos y descubrimientos tuvimos que interpretar los resultados, lo que nos resultó algo más difícil por que era la primera vez que tuvimos que hacer algo parecido y algo en donde no podíamos equivocarnos ya que luego podría tener importancia a nivel internacional.
Lo más difícil para mi fue tener que dejar mis miedos de lado y enfrentarme a muchas personas, pero dirigirme a ellas en inglés, uno de mis mayores miedos. Pero gracias a esto, me he dado cuenta de que todo se puede conseguir con dedicación y esfuerzo. Esta experiencia sin duda ha sido para mi un crecimiento personal increíble porque me ha hecho ver que si que puedo y que no es tan difícil afrontar eso que no te deja seguir creciendo como persona.
Nos haría mucha ilusión que nos volvieran a dar otra oportunidad como esta para poder realizar una segunda parte de nuestro proyecto, ya que creo que podemos conseguir algo innovador con los conocimientos que hemos adquirido en este proceso.

Carolina Domek Águila

This project has consisted of being able to observe the evolution of some peppers subjected to different characteristics, this work was divided into various groups within the class and not all of us were able to enjoy the entire procedure although we were able to both understand it and observe it, although in general we all have collaborated the same, for this reason I am going to talk about my contribution to the project. Our group was in charge of extracting the extract from the peppers through a continuous and not very complex process. This extract was analyzed by other colleagues to get the brix and the pH.
Throughout the project we have met several scientists that I personally could take as an example in the future, we enjoyed numerous talks given by the experts, which is an honor to be able to listen to, talks that will later be useful for us to carry out the project. .
In addition, at our young age, we learned to handle numerous laboratory utensils and know how to move freely in it.
After, at the end of the project, we had the opportunity to present our work in front of expert scientists on the subject so that they could see our work and give their point of view, I personally could not attend the congress and I could not live that experience, however some of my colleagues do, and they never cease to surprise the public as much as the simple fact of presenting the work, in short, a very beautiful and unique experience
In general, we are very grateful, both professors, scientists and students have fulfilled our work correctly and we have obtained a friendly and comfortable work environment to learn in a very nice way.

Arturo Ruiz Joya

At the beginning of the course they told us about a project that the 1st year high school students did the previous year and they offered us to do another research project. In this case we had to measure some values such as pH or proteins of the peppers to see how the vegetable was degrading over the days. They divided us into groups so that each one did a task and in this case it was to cut the peppers. The first thing we look at is the alteration in the texture of the peppers, since as the days went by the peppers changed depending on where they were.

At room temperature the peppers remained smooth until the end of the second week approximately. At this time some parts of the pepper began to rot and these were not used to measure parameters as they would harm the result. In addition to the texture, the vegetables did change their color, so this meant that they continued to ripen even once cut from the plant.
In the refrigerator, the peppers were smooth throughout the project, but in contrast, they did not ripen and therefore did not change their color from green to characteristic red. In the freezer the peppers did have a big change. Once they thawed, it was observed with the naked eye that they lost a lot of water and did not stay smooth. These also did not ripen since they did not change their color.
Apart from the research project, we were able to learn some processes that are carried out in laboratories because most of the time we were doing practical work and observing the different experiences with our own eyes.

Claudia Moreno Villegas

The day we were told that we were going to participate in a research project, I really liked the news, and at the same time it generated a lot of intrigue. And when I found out what our work was going to focus on, I was very excited because I liked the idea of ??the studio.
One of the positive experiences of having done this work is having met people who are dedicated to research and knowing the difficulties involved in carrying out this type of study. However, when I found out that we had to defend the results of the work in a congress and that I was going to be one of them, which at first seemed good to me, as the weeks went by and the date of the defense approached, my nerves and the fear of exposure made me quite uncomfortable. Even so, I realized that it wasn't such a big deal once I defended this work at the CSIC. Except for this exception, I really liked participating in the processing, obtaining and analysis of the results.
In general, it has been a very positive experience and if I could participate in another job, I would be delighted to do so.

María Gómez Molina

The day that this great project was proposed to us, the whole class was not knowing where to start since we hardly had any knowledge in the laboratory, the 1st day that specialists such as Pepe Palma, Marichu came, they explained to us and introduced us to the world of the laboratory that we all had a great desire to carry out this project since it seemed quite interesting to us.
The work consisted of measuring the vitamin, ph, brix, pigments of 10 peppers measured at different temperatures for 4 weeks, with this we could see the difference of such, also one thing that caught our attention and important is the color change they had the peppers over the weeks
They put all of us to work, once your work was awarded, that would always be yours throughout the project, to make you a "specialist" and thus be able to make the minimum mistake. My group had to crush the peppers so that the stratum was obtained and that the next group could measure the pH, brix, vitamins, pigments...
Carrying out this work has been fascinating since it is a clear example that with work, perseverance, desire and enthusiasm good results come out, on a personal level I think it has

helped us in many ways since it has given us a wider range of laboratory knowledge, and in this project we have had a lot of camaraderie.
I must also point out that our project has been known by several scientists, institutes through a conference in which the project and its results were presented, in addition Canal Sur has disseminated our project throughout the community, which makes the project known more.

Patricia Rodríguez de la Rosa

I'm really proud of the development of this project and all the things that I've learned about the scientific method and the investigation process. As our project was very experimental I've tested that in the investigation there aren't wrong data or conclusions that involve a failed project, because at the beginning of this we didn't know what we were going to find, so every piece of data is significant and even if they aren't as we expected, they can help us to change the perspective of the project or the method. This is what I love about investigation, so thanks to this amazing experience I have decided that I want to dedicate myself to the investigation in my profesional future.
Other thing that I love about this project is that everything I've learned has been in a practical way, nowadays I know more things that I would learn just by studying a text book, because no matter how much you study, the experience in a laboratory is something that you only learn by handling it.

Sebastián Chaparro Díaz

At first, I thought that it was going to be a boring project. Not gonna lie I didn't have hope for this. It didn't make sense to me. Peppers! My teacher said so happily. I was really confused, nevertheless as soon as we started.
The project kick-off made sense after some time. My teammates and I examined the tampon of this and we cut peppers into small pieces. Normal peppers, cold peppers, and frozen peppers, and crush the peppers in a bowl.
I've never imagined doing this in front of my partners with such a weird feeling as if we were cooking on a tv show.
I hope for the greatest things for the future. Maybe we can change the molecular biology of the pepper to don't have to expend water in their growth. Or what if we make gigantic healthy peppers. Who knows what the future will hold for us? That's the only thing I cannot answer to anyone or myself because the future is unstable.

Ángel Navarra Villalba

For the beginning of the project, certain groups have begun to be created with the function of organizing the different works of the project
Each group carried out specific steps. My group focused on extracting the extract from the peppers and calculating the chlorophyll a, b, and final carotenoids. With this we made the project more entertaining since we all had to do something.
I think the project has enriched us as people because we have been able to receive information and work hand in hand with a series of well-known scientists in the world of science.
We have seen that a simple pepper has very interesting things and a lot of work to develop.

Las plantas del Mariana: un proyecto educativo para despertar la curiosidad y la vocación científica en estudiantes de la ESO y Bachillerato.

(The plants of the "Mariana": an educational project to arouse curiosity and scientific vocation in high school students)

Estudiantes de Educación Especial, ESO y Bachillerato, Teresa Arroyo[5], Lidia Díaz[7], Jerónimo Fernández[8], Margarita García[6], Carmen García[13], Juanjo García[1], Sergio González[13], Virginia González[4], Nerea Ibañez[12], Alejandro Jiménez[10], Sonia Laguna[9], Marta Moreno[12], Rafael Navarro[12], Joaquín Ortega[14], Cecilio Pérez[3], Francisco Fernández[3], Blanca Rodríguez[11], Isabel Rodriguez[5], Raul Rojano[2], Juan Antonio López Raéz[15], María Eugenia Ramos-Font[15], Carlos Muñoz[2#].

[1]Departamentos de Educación Plástica y Visual, [2]Biología y Geología, [3]Economía, [4]Inglés, [5]Francés, [6]Griego y Latín, [7]Matemáticas, [8]Música, [9]Educación Física, [10]Física y Química, [11]Religión, [12]Educación Especial y [13]Tecnología, [14]IES Mariana Pineda. C/ Beethoven 4. 18006 Granada, España.
[15]Estación Experimental del Zaidín. CSIC. Profesor Albareda 1, 18008 Granada, España.

#Autor de contacto: carlos.munoz@iesmarianapineda.net

Resumen

"Las plantas del Mariana" es un proyecto de educación y sensibilización ambiental que utiliza como recurso el estudio y la observación de las plantas del I.E.S. Mariana Pineda (Granada) para la realización de proyectos educativos de carácter integrador. Doce departamentos del Instituto plantearon distintas actividades relacionadas con las plantas que tuvieron una gran acogida entre los estudiantes. Además, varias de las actividades fueron planteadas conjuntamente entre varios departamentos, incluido el Aula de Educación Especial, favoreciendo la convivencia entre los/as estudiantes de dicha aula y los de la ESO. Gracias a este proyecto los/as estudiantes han fomentado su curiosidad y actitud crítica y han trabajado de forma colaborativa y creativa. Ha habido una mayor concienciación sobre el respeto a otros seres vivos y la valoración del compromiso ciudadano con el bien común, y se ha reflexionado sobre el papel fundamental de la ciencia en la sociedad, impulsando, especialmente entre las alumnas, el interés por la ciencia, la cooperación y la comunicación como parte esencial de las metodologías de trabajo científico. Este proyecto podría servir de inspiración para cualquier otro centro de secundaria o de primaria que desee despertar la curiosidad y la vocación científica de sus estudiantes.

Palabras clave: STEAM, aprendizaje activo, jardín y huerto escolar, bilingüismo, trabajo por proyectos**.**

Summary

"The plants of the Mariana" is an education and environmental sensibilization project that uses as a resource the study and observation of plants in the I.E.S. Mariana Pineda (Granada), to carry out educational projects of an integrative nature. Twelve departments of the High School designed different activities in relation to plants, which were very well received by students. Besides, some activities were organized jointly by several departments, including the Special Education Classroom, thus favouring the relationship between special education and ESO students. Thanks to this project, students have increased their curiosity and critical attitude, and have worked in a collaborative and creative way. There has been a greater awareness about respecting other live beings and valuing the commitment of citizens with the common goods. There have also been considerations about the fundamental role of science in society, stimulating –particularly among female students- the interest for science, and cooperation and communication as essential parts of the methodology of scientific work. This project could serve as inspiration for any other elementary or high school whishing to arouse the curiosity and scientific vocation of their students.

Keywords: *STEAM, active learning, school garden and orchard, bilinguism, project-oriented work.*

INTRODUCCIÓN

"Las plantas del Mariana" es un proyecto de educación y sensibilización ambiental que utiliza como recurso el estudio y la observación de las plantas del I.E.S. Mariana Pineda (Granada) para la realización de proyectos educativos de carácter integrador, mediante procesos de investigación que culminan con productos finales presentados por las/los estudiantes ante sus compañeros/as.

El proyecto comenzó en abril de 2021 a sugerencia de varios/as estudiantes de 1ºESO. El profesorado de las asignaturas de Tecnología Aplicada, Biología y Geología y Competencia Digital propusieron al alumnado realizar un eBook de algunas de las plantas del instituto. Para ello se observarían distintas plantas del centro y se recabaría información sobre ellas, que sería incluida en dicho eBook.

Figura 1. Índice y primera página del eBook creado por el alumnado de 1º de la ESO.

Nuestro proyecto tuvo su punto de inflexión tras la lectura de dos magníficos libros:

- **"Esto no estaba en mi libro de botánica" (Ed. Alfaguara. 2020)**, de la Dra. en Bioquímica y Biología Molecular Rosa Porcel. La obra está estructurada en cuatro partes, siendo la primera la que nos dio la idea del proyecto, ya que nos presenta a las plantas no tanto desde un punto de vista biológico sino de forma muy transversal. Nos introduce en el origen evolutivo de las plantas y en la estrecha relación de éstas con el ser humano: desde la domesticación de especies y la aparición de la agricultura, pasando por los usos curativos y como veneno, hasta su presencia en la cultura diaria, mitología y leyendas.
- **"La evolución de Calpurnia Tate" Ed. Henry Holt and Company, 2009**. El libro trata de una niña de 12 años, Calpurnia Tate, que vive en un pueblo de Texas en el año 1899. Callie es la única chica de siete hermanos. Su madre tiene para ella preparado un futuro en el que tocar el piano, coser y cocinar sea lo único a lo se debe dedicar. Sin embargo, ella está más interesada en lo que ocurre tras la puerta de la biblioteca o en el laboratorio de su abuelo.

Figura 2. Portadas de los libros que inspiraron este proyecto.

Ambos libros inspiraron el propósito de este proyecto: fomentar que los/as estudiantes del centro descubran la naturaleza de primera mano y en un futuro podamos despertar vocaciones científicas.

El proyecto

Una vez que empezó el curso 2021/2022, se dio a conocer al claustro de profesores los objetivos del proyecto para que cada cual propusiera, desde su asignatura, las actividades que estimasen convenientes a sus alumnos/as de diferentes edades.

El carácter integrador de este proyecto quedó reflejado mediante la implicación de la mayoría de los Departamentos didácticos, que desarrollaron diferentes actividades vinculando las plantas con su área de conocimiento. A continuación se detallan algunos ejemplos de las actividades realizadas y que fueron recogidas en el blog del proyecto (https://lasplantasdelmariana.blogspot.com/), alojado en la página web del centro (https://www.iesmarianapineda.net/).

1. **Economía 1º Bachillerato.** Los/las estudiantes de 1º de Bachillerato, realizaron trabajos sobre la importancia económica de los cultivos de aloe vera, olivo, eucalipto, orégano, tabaco, naranjo y flor cortada (ver algunos ejemplos en el siguiente link).
2. **El aula de Educación Especial** tuvo un rol fundamental en el proyecto, puesto que sus estudiantes son los responsables del cultivo y cuidado del huerto escolar a lo largo del curso. Además, este proyecto ha permitido una convivencia periódica de los/las estudiantes de Educación Especial con compañeros/as de otros cursos, lo que ha favorecido el desarrollo personal y humano de todo el alumnado. Asimismo, el alumnado del Aula de Educación Especial junto con otros/as estudiantes de la ESO cosecharon distintas verduras (espinacas, lechugas, brócoli) que, posteriormente, utilizaron para la elaboración y degustación de distintas recetas saludables. Durante esta actividad se habló sobre el valor nutritivo de los alimentos y se establecieron vínculos entre los/las estudiantes gracias al ambiente distendido que se generó. Por último, los estudiantes del Aula de Educación Especial participaron en dos talleres: uno sobre poda y otro sobre riegos.

Figura 3. Estudiantes del Aula de Educación Especial cosechando verduras del huerto escolar del IES Mariana Pineda (izquierda) y parcela de cultivo del huerto en otoño (derecha).

Figura 4. Estudiantes del Aula de Educación Especial y de la ESO degustando las verduras cocinadas por ellos.

Figura 5. Alumnos/as del Aula de Educación Especial y de la ESO en los talleres de poda y riego.

3. **Tecnología**. Los/as estudiantes de 4ºESO realizaron un proyecto sobre la aplicación de una pequeña corriente eléctrica en el suelo para estimular el crecimiento de las plantas en el huerto escolar, mediante la colocación de pequeños dispositivos eléctricos en el suelo, cerca de las plantas. Esta fue una actividad interdisciplinar en la que los/as estudiantes pudieron aplicar conocimientos de distintas áreas como: tecnología, biología, física, química y matemáticas.

Figura 6. Fotografías sobre el experimento de Tecnología de aplicación de corriente eléctrica en el suelo.

4. **Biología y Geología**. Cada grupo de estudiantes de 1º, 3º y 4º ESO seleccionó una especie vegetal sobre la que debía investigar y elaborar un trabajo. Los/as estudiantes de 1º y 4º ESO realizaron trabajos monográficos para el inventario digital de "Las plantas del Mariana", publicado en nuestro blog (https://lasplantasdelmariana.blogspot.com/p/nuestras-plantas.html), que presentaron a sus compañeros/as en el jardín con apoyo de algunas diapositivas. Por su parte, los/as estudiantes de 3º seleccionaron plantas comestibles de las cuales debían obtener información sobre sus propiedades nutritivas y su impacto sobre la salud de las personas, dependiendo de las condiciones físicas y de salud de éstas. Además, debían elaborar una receta de cocina con dicha planta y grabar un vídeo durante la elaboración.

Figura 7. Estudiantes de 1º ESO explicando sus proyectos a otros estudiantes de cursos superiores.

Como parte de su trabajo de investigación, plantearon distintas preguntas a través de un formulario de google, a las cuales trató de dar respuesta la Dra. María Eugenia Ramos Font, bióloga especialista en botánica de la Estación Experimental del Zaidín.

Para ello, María Eugenia Ramos visitó a las/los estudiantes para resolver sus dudas, e intercambiar experiencias y conocimientos con ellos. Fueron unas charlas muy dinámicas en las surgieron planteamientos y reflexiones muy interesantes, fruto de la curiosidad despertada por la observación periódica y minuciosa de las plantas asignadas.

Finalmente, este Departamento organizó dentro de la III Semana de la Ciencia una charla titulada "Jardinería Ecológica", impartida por la Dra. María Eugenia Ramos.

Figura 8. Resolución de dudas sobre botánica y propiedades nutritivas de las plantas con los/las estudiantes de la ESO, por parte de María Eugenia Ramos Font (bióloga del CSIC).

Figura 9. Portada de la charla de Jardinería ecológica.

5. **Matemáticas**. Los estudiantes de 1º ESO aplicaron sus conocimientos sobre el sistema métrico decimal mediante la medición del área de hojas de tres especies de árboles del itinerario botánico del instituto con ayuda de papel milimetrado. Esto permitió realizar una aproximación a las estimaciones y los errores en los análisis estadísticos.

Figura 10. Estudiantes de 1º ESO realizando mediciones de hojas sobre papel milimetrado (izquierda) y ejemplo de uno de los ejercicios utilizando una hoja de plátano de paseo (derecha).

6. **Latín y griego**. Dentro de la III Semana de las Ciencias y las Letras del IES Mariana Pineda, los/las estudiantes de 1º de Bachillerato recopilaron información sobre la etimología de los nombres científicos de las plantas del centro y sobre los mitos que se les atribuyen.

Figura 11. Estudiantes de 1º de Bachillerato explicando a sus compañeros/as sus trabajos sobre la etimología y mitos de los nombres científicos de las plantas del IES Mariana Pineda.

7. **Religión**. Los/las estudiantes de religión de 1ºESO-B y C, presentaron dentro de la III Semana de las Ciencias y las Letras el proyecto titulado "Las plantas de la Biblia", centrado en tres plantas muy representativas e importantes en la Biblia: la vid, el trigo y el olivo. Se intentó transmitir cómo los frutos de estas plantas mencionadas en la Biblia han sido la base de la alimentación de todo el Mediterráneo. En ella se han utilizado para explicar una realidad más profunda, trascendente y poder comprender el Reino de Dios, que se hace presente en este mundo en los elementos de la Naturaleza. Así, **la vid** simboliza a Jesucristo y los sarmientos son sus seguidores. De su fruto se saca el vino, que se transformará en la sangre de Cristo en el sacramento de la Eucaristía. **La espiga de trigo** simboliza la unión de todos los cristianos y, una vez triturados sus granos, serán el pan cotidiano y el cuerpo de Cristo en la Eucaristía (alimento espiritual). **El olivo**, común en toda Palestina, es símbolo de prosperidad. Sus ramas han simbolizado la Paz tanto en el Antiguo Testamento como en el Nuevo Testamento. De su fruto se saca el aceite, usado como "oleum" en los sacramentos del Bautismo, Confirmación, Orden sacerdotal y Unción de enfermos. En definitiva, se trató de explicar cómo la observación de la realidad nos puede hacer trascender hacia una realidad superior que nos une a Dios.

Figura 12. Estudiantes de 1º ESO presentando su proyecto "Las plantas en la Biblia" durante la III Semana de la Ciencia y las Letras.

8. **Música y Biología y Geología**. El alumnado de 1ºESO interpretó "The photosynthesis song", como parte de las actividades de la III Semana de la Ciencia y las Letras. Para ello contaron con un bajista, dos guitarristas, un batería, un piano e instrumentos de lámina percutida. La canción fue acompañada de una coreografía creada por ellos mismos previamente y el resultado de la combinación de música y danza fue muy satisfactorio.

Figura 13. Estudiantes de 1º ESO interpretando "The photosynthesis song".

9. **Física y Química**. Dentro de la III Semana de la Ciencia, los/las estudiantes de Ciencias aplicadas a la actividad profesional de 4º ESO prepararon un taller sobre cómo elaborar jabón casero a partir de aceite de girasol y oliva reciclado.

Figura 14. Taller de elaboración de jabón con estudiantes de 4º ESO.

10. **Inglés**. Los/las estudiantes de 1ºESO-A y B buscaron información sobre la flor o el árbol representativo de cada uno de los estados de Estados Unidos. Recortaron en una cartulina la silueta del estado correspondiente e incluyeron los nombres de la flor o el árbol y los dibujos. Por último, se dibujó un gran árbol y se colocaron los dibujos a modo de hojas que colgaban de las ramas. Como actividad final, los/las estudiantes hicieron de manera voluntaria una pequeña exposición en clase sobre la flora del estado en cuestión.

Figura 15. Ejemplos de los trabajos realizados por los estudiantes sobre las plantas representativas de los distintos estados de Estados Unidos en la asignatura de Inglés.

11. **Francés.** El 1 de mayo las calles de Francia y Bélgica se inundan de puestos con *muguet (Convallaria majalis* L.*)*. El *muguet* es una flor blanca pequeña con forma de campanilla. Esta flor se regala para desear suerte y trabajo. Esta tradición procede de la Edad Media. El rey Carlos IX en 1560 hizo un viaje a Le Dauphiné. Allí se le ofreció un ramo de *muguet*. Le gustó tanto la flor que tomó por costumbre regalar *muguet* a las damas de la corte. En Le Dauphiné esta tradición se hacía desde tiempos muy antiguos. Para sus habitantes esta flor atraía lo bueno y expulsaba lo malo. Con el pasar de los años, el 1º de mayo también se instauró como el día del trabajador. Por este motivo, en la actualidad, regalar *muguet* no sólo es un deseo de suerte y salud sino también de trabajo.

Figura 16. Profesoras de Francés y Latín y Griego mostrando las actividades de la Semana de las Letras

Finalmente, se llevaron a cabo dos actividades transversales realizadas de manera colaborativa entre distintos departamentos:

12. **Educación Física, Inglés y Biología y Geología.** Se diseñó una gymkhana bilingüe de plantas en el patio del centro para estudiantes de 1º ESO. La Figura 16 muestra algunas de las pruebas planteadas.

Figura 16. Ejemplos de alguna de las pruebas diseñadas para la gymkhana bilingüe.

13. **Departamentos de Tecnología y Biología y Geología**. Varios/as estudiantes de 4ºESO junto con sus profesores acudieron a la XX Feria de la Ciencia de Sevilla a presentar el proyecto SCRATCH "Misión Espacial Arca de Noé". En el mismo se planteaba un escenario en el que la Tierra se había hecho inhabitable y se necesitaba crear una colonia en otro planeta. A lo largo del juego los/las estudiantes tenían que ir clasificando correctamente diferentes seres vivos, entre ellos plantas.

Figura 17. Imagen de una de las pruebas que tenían que resolver los estudiantes en el proyecto SCRATCH "Misión Espacial Arca de Noé".

CONCLUSIONES

El proyecto "Las plantas del Mariana" ha sido una herramienta muy eficaz para fomentar el desarrollo de la curiosidad, la actitud crítica, la alfabetización científica, el conocimiento del entorno, el desarrollo de hábitos saludables, el consumo responsable, el cuidado del medioambiente, el respeto a otros seres vivos y la valoración del compromiso ciudadano con el bien común entre nuestro alumnado.

También ha permitido que el alumnado valore el papel fundamental de la ciencia en la sociedad. Se impulsó, especialmente entre las alumnas, las vocaciones científicas, la solidaridad y el trabajo en equipo y se promovió el perfeccionamiento lingüístico, al ser la cooperación y la comunicación parte esencial de las metodologías de trabajo científico.

Además, se animó al alumnado a utilizar diferentes formatos y vías para comunicarse y cooperar. El trabajo grupal ha sido una herramienta para la integración social de estudiantes con dificultades. La naturaleza científica de este proyecto ha contribuido a despertar en el alumnado el espíritu creativo y emprendedor, que es la esencia misma de todas las ciencias. La investigación mediante la observación de campo, así como la experimentación y la búsqueda en diferentes fuentes para resolver cuestiones o contrastar hipótesis de forma tanto individual como cooperativa son elementos constituyentes de este proyecto. El proyecto fomentó el uso responsable y crítico de las tecnologías de la información y la comunicación.

Sin embargo, no ha sido fácil ejecutar dada la dedicación de tiempo que requería para coordinar, animar a la participación y difundir los resultados. Por tanto, recomendamos y nos planteamos en un futuro seguir ampliando el proyecto en la medida de nuestras posibilidades de tiempo, de esfuerzo y económicas con la idea de concluir con un Inventario Digital de todas las Plantas del Mariana en el que los/as futuros/as estudiantes del centro puedan aprender de los trabajos de sus compañeros/as y colaborar en la ampliación y perfeccionamiento del mismo. Finalmente, creemos que este proyecto podría servir de inspiración para cualquier otro centro de secundaria o de primaria que desee despertar la curiosidad y la vocación científica de sus estudiantes.

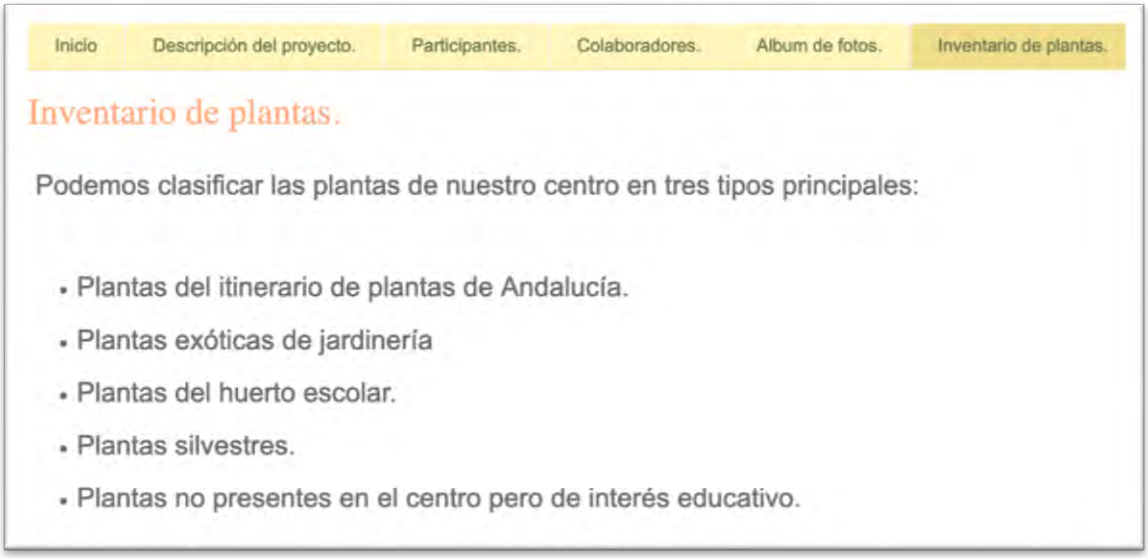


Figura 18. Página web del inventario de plantas.

MY OWN IDEAS

Blanca Verdugo. 1ºESO-A

El Viernes 29 de mayo, expusimos unos trabajos de plantas a nuestros compañeros/as de 3º de E.S.O. Nos bajamos al patio, para ponernos al lado de nuestra planta, que en nuestro caso la nuestra es el roble. A las componentes de mi grupo nos gustó mucho está experiencia, por exponer delante de nuestros compañeros/as, al exterior…Nos repartimos las diapositivas del trabajo, para que cada una explicara una, y así que el trabajo no se hiciera muy pesado. De vez en cuando, le dábamos a nuestros compañeros/as alguna hoja o bellota del roble para que la pudieran observar. Por ejemplo, cuando hablábamos de las hojas les dábamos algunas para que las tocasen, y las comparasen por ejemplo con la de la encina, que es una especie de la misma familia, y tiene las hojas puntiagudas, mientras que las del roble son suavitas. Así que les dimos las hojas para que las comparen. Esta experiencia nos gustó mucho a todos, y nos gustaría que se volviera a repetir más adelante.

Inés Palma Valero. 1ºESO-A.

In my opinion, this project is a very good method to learn about the plants of our high school and also to learn how to speak in public. Also, this project has helped us to know how to investigate and get information from different sources.

Rodrigo Erik Rea Soto. 1ºESO-B.

I have loved the activities of the Plants of the Mariana, how many activities can be done with the plants and I was amazed. For me the activities that I have liked are the one of the exhibitions of choosing a plant and explaining it and the one of the gymkana They gave you a description of a Mariana plant and you had to know which one it was. It was fun, as the English can do different types of activities and hopefully repeat them the following year. Also the photosynthesis song that is very catchy and drawing the flowers of your plant, I really liked this year in biology with plants using a lot of English.

Agustina Rodríguez Salazar. 1ºESO-B

En mi opinión yo creo que ha sido interesante el trabajo de las plantas porque aprendes más cosas de las plantas, además he aprendido a expresarme mejor, con más fluidez y con mejor interpretación. Además, he aprendido a relacionarme con más gente que no conocía. Para cuidar más el medio ambiente, porque gracias a muchas plantas nos podemos alimentar de ellas y saber cómo cuidarlas mejor. Así podemos saber si algunas plantas son venenosas y cuáles no, también podemos saber si algunas plantas dan medicamentos y así también podemos saber si algunos frutos son venenosos y cuales podemos comer y cuáles no.

Candela Sánchez López. 1ESO-B

In my opinion, I think this job can help a lot to those who do it. You learn a lot of new plants that you didn't even know and apart from that you learn a lot of new things about the plant that you have touched. Apart from all the information and new things you learn, you also meet new people by exposing it. I think this job is a very good idea.

Sandra Escudero Abellán. 1º ESO-C.

En mi opinión este trabajo es muy importante, ya que buscas información sobre un árbol y puedes encontrar datos curiosos e impresionantes que nunca podrías haberte imaginado, también buscas su familia, y descubres que se parecen mucho unos a otros. Los insectos también tienen un papel importante en los árboles, ya que hay algunos que les ayudan, y otros que les perjudican. Este trabajo también es importante porque te mantienes informado, y estás pendiente de cualquier mínimo detalle que le pueda pasar a tu árbol, como por ejemplo en cada estación del año, cambian mucho. Sin los árboles ni los humanos ni ningún ser vivo podría sobrevivir, ya que dan oxígeno, se pueden hacer muchos materiales gracias a los árboles, y también a veces puede haber algún nido de pájaro. Yo he aprendido mucho con este proyecto, y me lo he pasado muy bien, espero que también lo haya sido para todos mis compañeros.

Juan Pablo Rodríguez Linares. 3ºESO-B.

Un resumen de la charla elaborado por Juan Pablo Rodríguez: "La charla sobre jardinería ecológica ha sido muy interesante. He aprendido muchas cosas ya que yo no sabía que existían los jardines ecológicos. La charla ha sido impartida por la botánica Mariu, la misma que nos dió la charla sobre la importancia de las plantas en nuestra dieta. Ella comenzó la charla preguntando la definición de jardín. Nos dijo que la palabra jardín viene del francés jardin, huerto. Es una zona de terreno donde se cultivan distintas especies vegetales con posibles elementos de decoración como fuentes, esculturas, etc. Después nos definió la palabra ecológico: que defiende y protege el medio ambiente. Y por último nos definió la jardinería ecológica: es aquella que aprovecha los medios que la propia naturaleza ofrece para la autorregulación de los procesos naturales. También nos preguntó para qué servía un jardín. Varios compañeros contestaron que sirve como decoración, para crear belleza, una forma de entretenerse, … Mariu nos recomendó que las plantas que se suelen emplear en un jardín ecológico son hierbas aromáticas como lavanda, salvia, romero,menta, ruda, albahaca, tomillo, estragón, … Algunos de los consejos que dió para tener un jardín ecológico son: instalar un sistema de riego para ahorrar agua, aprovechar el agua de la lluvia para reducir costes, sembrar y cultivar especies locales, usar fertilizantes orgánicos o pesticidas ecológicos y usar nuestros residuos orgánicos para abonar el jardín. Son muchos los beneficios de los jardines ecológicos ya que combaten el calentamiento global y sus efectos, mantienen la salud del suelo, favorecen la calidad y el ahorro de agua y contribuyen al bienestar de los animales. Nos orientó de que tenemos que tener cuidado a la hora de seleccionar las especies de nuestro jardín ya que éstas condicionarán en gran medida que el jardín sea sostenible. Por ejemplo podemos utilizar especies autóctonas, ya que están más adaptadas a las condiciones locales y son más resistentes a las plagas. Los depredadores naturales ayudarán a mantener el jardín a salvo. Por ejemplo las aves insectívoras, lagartijas, arañas, mariquitas, ciempiés, e incluso los sapos. Para atraerlos al jardín se pueden instalar algunos elementos como comederos, cajas nido, pequeños montones de piedras y también se pueden sembrar gran variedad de flores, árboles y arbustos . Estos setos se utilizan no sólo de decoración sino con un objetivo funcional como arbustos cortavientos.No hay que olvidar que es fundamental la importancia del agua. En conclusión decir que me ha parecido un tema muy interesante, ya que he aprendido cosas que no sabía y sobretodo estos tipos de jardines generan un gran ahorro de energía y de agua muy importante para contribuir a la preservación del medio ambiente.

Jorge Tapia. 3ºESO-B

From the subject of biology and geology and for more than a month, we have done various activities and practical tasks on plants, food and nutrition. Specifically, we have done the following: we have known the plants that we have in the garden of our school; choosing some of them, we had to develop a recipe, and learn and explain different things about food as nutrients that form it, the amount of that product that is recommended to eat, for which people are suitable and for whom not, etc.. But before this, we went to the supermarket to find out in practice what nutrients are in the food. In addition, we have learned more about the foods we chose and we have been able to clarify all our doubts thanks to the talk that María Eugenia Ramosgave us. Also with her, we have learned about organic gardening, in which she gave us tips on the best plants we can plant, how to take care of them and water them in a way that we can help to take care of the environment. It is always easier to learn when we do practical things, and when we have to research on our own. And the activities we have done help with this. In addition and with all these activities, and also with the work we did in physical education, I have become aware of how important it is to introduce vegetables in our diet (something that is very difficult for me), to prevent diseases and to feel better. But I also think that sometimes we have too many activities and work to do at the same time in this subject and in the rest of the subjects along with exams, exercises, etc.. And that makes that sometimes, at least for me, I can't prepare them better or enjoy doing them more.

Gloria Díaz. 3ºESO-C

Este viernes a última hora, mi clase y yo bajamos al patio a ver los trabajos sobre las plantas que los/as estudiantes de primero habían realizado. A mi solamente me dio tiempo a ver 3 de todos los puestos que había, uno sobre bichos palo, el de la azufaifa y el acebo. Esta actividad me parece muy interesante, porque ayuda a los/las estudiantes que la realizan a aprender más de cerca sobre la naturaleza y las plantas. Y no solamente aprenden ellos, aprendemos también nosotros, los visitantes.

María Fernández Vargas. 3ºESO-C

En nuestro curso hemos estado realizando algunas recetas con las plantas que hay en nuestro instituto, tanto en el huerto de los chicos de educación especial, como en los árboles de los alrededores. Cada uno ha cogido una o varias de estas plantas y hemos buscado una receta que las contenga, también hemos buscado la información nutricional de los ingredientes, en qué son más ricos, que funciones realizan en nuestro cuerpo, la procedencia de estos, si han viajado o si son productos nacionales, y cuáles se deben omitir, sustituir o disminuir si tenemos alguna enfermedad o dieta especial. También hemos ayudado a los chicos de educación especial en algunas ocasiones y con los que hemos trabajado algunas recetas. En mi opinión creo que esta actividad es necesaria para nuestra generación ya que nos hemos vuelto muy dependientes de la comida precocinada y de la comida rápida. Debemos aprender a valernos por nosotros mismos y un ejemplo sería aprender a cocinar comida saludable. Además, está actividad también sirve para romper los estereotipos de género de que solo las mujeres tienen que cocinar y mantener a su familia. Asimismo, hemos descubierto qué ingredientes son más saludables y debemos agregar a nuestra dieta y consumirlos más a menudo, y cuáles menos y debemos disminuir su cantidad en nuestra dieta y comerlos en ocasiones señaladas. Creo que

ha sido muy enriquecedora para todos nosotros y esperamos que se repita otros años para que otros cursos también aprendan.

Lucía Marín. 4ºESO-B

Durante estos dos trimestres y el que nos queda por delante, hemos llevado a cabo un proyecto de investigación acerca de las plantas. Cada uno tenía una distinta y buscamos información sobre su genética (esto solo lo hicimos los de 4º ESO debido a que es el temario que se da en biología) sobre sus hojas, flores, tallo y corteza. Además, nos informamos de las cadenas tróficas y aprendimos un montón ya que eran más grandes de lo que pensábamos y aprendimos la importancia que tienen los insectos para que no se desestructuren cuando muchos de nosotros pensábamos que eran irrelevantes. Nos sorprendimos sobre la cantidad de enfermedades que pueden poseer las plantas solo con el hecho de cómo las regamos y esto hizo que nos diésemos cuenta de lo importante que era hacerlo de la manera correcta. A todos nos encantó buscar curiosidades sobre ellas ya que salían cosas inesperadas y que nos llamaron la atención. Lo mejor del proyecto ha sido ver cómo mes a mes van creciendo las plantas que cultivamos a principio de curso y como iban cambiando según los meses y las estaciones. Ha sido un proyecto en el que hemos aprendido mucho y en el que todavía nos queda mucho por el que aprender. Realizar el proyecto "las plantas del Mariana" me ha hecho ver que cada planta es un mundo totalmente diferente. Iba con las expectativas de que no iba a aprender nada y que pocas cosas nuevas iba a poder aprender sobre las plantas y cuando me puse a investigar sobre mi planta me di cuenta de la cantidad de información que hay y de todo lo que no se sabe todavía acerca de esta e incluso de todo el trabajo que han tenido que realizar los científicos para conocer todas sus características. También cuando los compañeros nos contaron información acerca de su planta me sorprendió mucho ver lo diferentes que eran y a la vez iguales unas plantas con otras. Por supuesto llevar esta investigación a todas las asignaturas me ha hecho ver las relaciones existentes entre la ciencia y las letras y ver que podemos llevar a cabo investigaciones en conjunto. En conclusión, creo que haber tenido la oportunidad de poder realizar este proyecto y más tarde exponerlo en un congreso ha sido una experiencia increíble e inolvidable.

Jaime Espín. 4ºESO-B

This has been a very interesting activity that has allowed us to learn about things that I have never thought about, but they are very interesting. We have learned the origin of names of the plants, their nutritive characteristics, their genetics, how they grow, their different parts and much more about the plants that we can find in our high school. We also learned how to do scientific research to find the information we need. All of these to finally teach it to the rest of the students of our high school. With this activity we can understand better how the scientific method works. And how the scientific method says the last step is to show the results in a congress, in this case the CAOS congress.

Looking for the transmission of the pungency trait among pepper plants. Design of an innovative breeding and educational project.

Ignacio Mengíbar-López[1], Leonardo Otero-Galadí[1], Navarro Weis, N., Paula Entrena-Aranda[1], Laura Rodríguez-López[1], Hussein Kaj Hussein-Mohamad[1] Elena De la Torre-Amat[1], Julia Shan Vida-Lara[1], Francisco Javier Martínez-Domingo[1], María Jesús Campos[2], Carmelo Ruiz[2], Antonio Quesada[1,#], José M. Palma[2,#]

[1]*IES Zaidín-Vergeles, Primavera 24-26, 18008 Granada, Spain.*

[2]*Department of Stress, Development and Signaling in Plants. Estación Experimental del Zaidín. CSIC. Profesor Albareda 1, 18008 Granada, Spain.*

#Corresponding authors: quesadaramos@gmail.com; josemanuel.palma@eez.csic.es

Summary

It is a general farmers' feeling and belief that hot peppers "contaminate" their pungency to sweet peppers when they are grown in the same terrain. From a scientific point of view, it has been reported some data that support the capsaicin transmission throughout the soil or as response to factors present in soils which activate the pungency trait in pepper fruits. On the other hand, this contagion may be due to genetics events, as a consequence of cross-pollination between hot and sweet peppers. However, very little little dedication by the scientific community has been paid to this issue, in spite of the relevance for breeding and crop purposes. In this work, a pioneer experiment carried out in the field with several pepper varieties containing different pungency levels, and the proposal of some experimental models to explain how the pungency trait is trasmitted from hot to sweet peppers is reported.

Keywords: capsaicin, *Capsicum*, experimental design, hot/sweet pepper fruits, hypothesis, proposal, pungency

INTRODUCTION

Pepper (*Capsicum annuum*) is an annual herbaceous plant originating from South America. Its fruit is one of the most consumed horticultural products worldwide [1] with hundred of varieties displaying different shapes, sizes, colours, flavours and tastings. Despite of this diversity, all of them can be divided into two main categories according to culinary and gastronomic purposes: sweet and hot peppers, with the last ones being characterized by their pungency. This property is due to substances like capsaicin and related compounds called capsaicinoids. While sweet peppers do not contain molecules of such family, a huge number of hot peppers varieties are classified according to the levels of those chemicals.

Thus, the pungency trait is measured by the Scoville scale, expressed as Scoville Heat Units (SHU). Bell peppers are not pungent (SHU=0), while some varieties can reach to more than 5 million SHU. In this scale, the highest level, 16,000,000 is assigned to pure capsaicin. It has been reported that hot peppers are among the diet likes of some consumers, whilst there are people that do not like them at all [2]. It has been suggested that genetic factors may explain those differences in pungency preferences [3], but there are also some cultural and geographic issues which are associated to the cultivation and consume of the varieties worldwide.

It is a general farmers' feeling and belief that hot peppers "contaminate" their pungency to sweet peppers when they are grown in the same terrain. Furthermore, they usually assert that this "contagion" takes place in the same season when both pepper types are cultivated at once. According to this observation, there are two main possibilities to explain that pattern: The first one implies the transmission of this trait (pungency) throughout the soil due to the connection among roots and or/ transport of capsaicin from one specimen to another. Thus, if for irrigation purposes hot peppers are placed at the beginning of the water source in the terrain, and sweet peppers are located some distance later, the last ones usually become pungent. According to this opinion, some substances, like capsaicin o whatever metabolite capable of promote their synthesis, are released by hot peppers, are able to move through the soil, dragged by water, absorbed by roots of sweet pepper plants, and transported through the xylem until reaching the fruits. The second alternative, perhaps less probable, includes spreading through air, perhaps via pollination, but it would need some more complex processes.

With this *in agro* background, but from a scientific point of view, it has been reported some data that support the capsaicin transmission throughout the soil or, at least, that highlights the importance of factors present in soils to "switch on"/activate the pungency trait in pepper fruits. Das et al. [4] reported the importance of soils' composition in the capsaicin content, the pungency, and the capsaicin synthase activity; several genes involved both in processes of biosynthesis and accumulation of capsaicin are upregulated in alluvial soils rather that in lateritic soils. Additionally, Antonious [5,6], reported higher concentrations of capsaicin and dihydrocapsaicin in peppers grown in soils treated with sewage sludge than in soils added with other recycled waste. In a similar study it was also found that the growth, yield and fruit quality of pepper plants were improved when sanitized sewage sludges was used for their culture [7].

On the other hand, these changes may be due to genetics events, as a consequence of cross-pollination between hot and sweet peppers. Hot and sweet peppers botanically belong to the same or closely related species; so they can cross one to another. This species, as many angiosperms, presents flowers with male and female reproductive organs (hermaphrodite species) and self-fertilization is the most frequent reproductive event. However, in different cultivars, the cross-pollination rate is highly variable, ranging from 2 to 90% [8]. In a simple model, if sweet peppers become hot (according to the farmers' observation), and not the opposite, the pungency could be considered as a dominant trait; but this genetic control must be more complex as there are several genes implied in the capsaicin biosynthesis. Justino et al. [8] confirmed that the pungency can be transmitted through pollination as they used the *Pun1* gene, a codominant gene associated with the accumulation of capsaicin in pepper fruits, as a molecular marker, in order to estimate the natural cross-pollination rate in

Capsicum annuum. Accordingly, pungency can be transmitted from hot pepper plants to sweet ones in a insect-depending manner, but the effects would not be observed in fruits until the next generation. This is because the genetic hybridation takes place once cross-pollination occurs and seeds are formed. Thus, if the seeds collect the genes codifying the synthesis of capsaicin from a former generation, the pungency will not be detected until fruits of the next generation blow up. To investigate the pungency transmission between pepper varieties should then require the study of this trait along several generations.

Overall, this is a very interesting issue with still little dedication by the scientific community. However, with this little information, High School Students from the IES Zaidín-Vergeles (Granada, Spain) have dived into this subject to make an atractive proposal to be properly addressed in the future. This is the first time that teenager students design a real scientific project and propose how to accomplish it with potential benefits to farmers and horticultural companies. To this purpose, this work will be scrolled down through chapters including the hypothesis and main objective of this work, the preliminary experiments done by the own students in the field, and the pioneer obtained results which have driven to set an improved experimental design to address the objective.

BACKGROUND INFORMATION, HYPOTHESIS, AND MAIN OBJECTIVE

This project arises in the context of a wider educational research about healthy properties of peppers carried out by High School Students of the First Course of *bachillerato* from the IES Zaidín Vergeles (Granada, Spain), supervised by scientists from the Estación Experimental del Zaidín (CSIC, Granada). Issues such as vitamin C content of several varieties of hot and sweet pepper fruits, the antimicrobial properties of these fruit extracts [9], consumption habits and knowledge about healthy properties of pepper fruits in Spain [2] and changes of organoleptic and nutritional properties with respect to storage conditions [10] have been the platform to address the present work.

Thus, with the hypothesis that capsaicin is transferred from hot pepper to sweet fruits, as observed by some of the authors' relatives and other farmers, the aim of this research was to investigate whether the transmission of pungency among pepper plants is carried out either through the soil or by cross-pollination. For that purpose, a preliminary experimental design to be implemented in the field was set up.

MATERIAL AND METHODS

1. Farm location and features

The experiments were carried out from Spring-Summer 2021, in a farm located in Camorro mountain slope, Cuevas de San Marcos, Málaga, Spain (37º15'47''N, 4º24'18''W; Figure 1). Regarding to soil characteristics, the terrain consisted of a flat ground with a clay soil, 1-meter depth and one-year fallow. With respect to climatology, winters are characterized by mild temperatures (5º-20ºC) and hot summers (30º-40ºC). The average rain is about 517 L/m^2 a year.

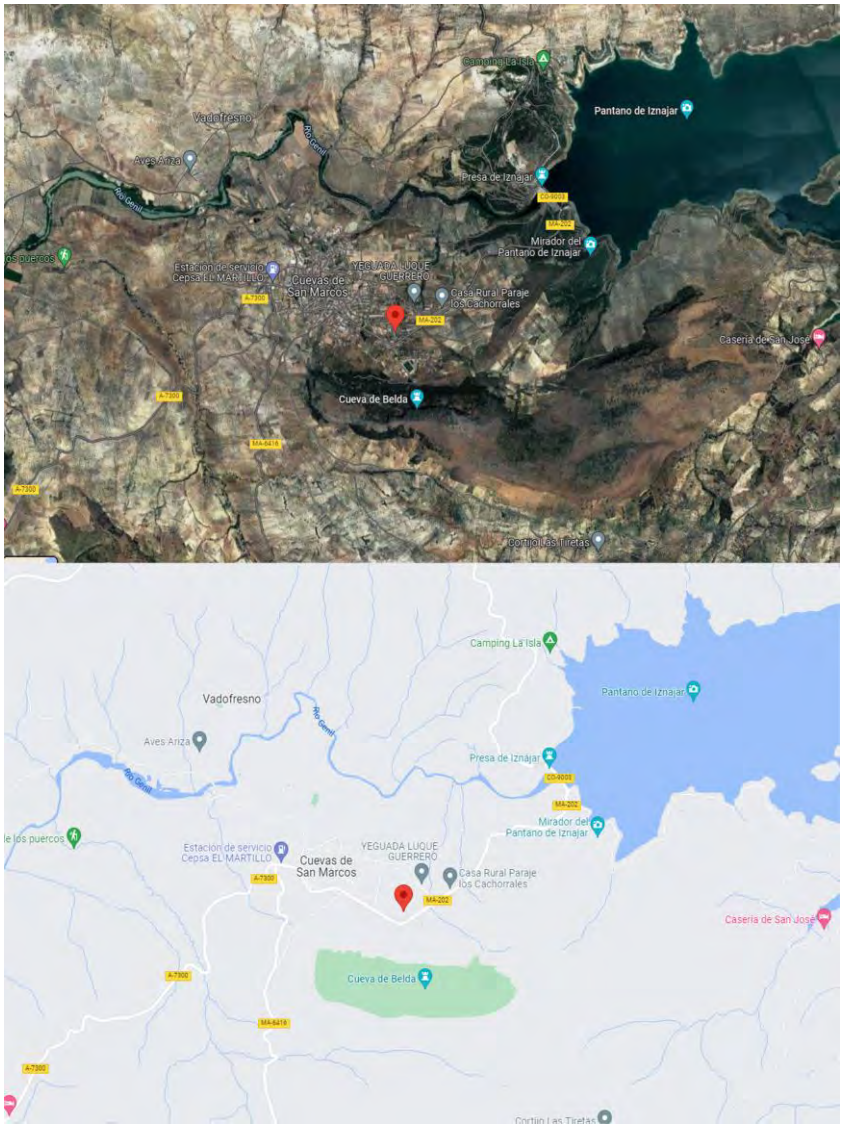


Figure 1. Farm location in Málaga province, Spain.

2. Pepper varieties and experimental design

Pepper varieties used in this work were those from local origin, commonly used for cooking purposes: Guindilla (G), Corneta (C), Ñora (Ñ), Guindilla del palmito (GP), and Morrón (M) (Figure 2).

Figure 2. Fruits of varieties used in this work.

Pepper seeds were sown in early May. Afterwards, seedlings were grown in one row and arranged as shown in Figure 3. Hot (H) peppers alternated with sweet (S) ones, with 6 Guindilla (H) plants cultivated, 13 Corneta (S), 3 Ñora (S), 1 Guindilla del palmito (H) and 3 Morrón (S).

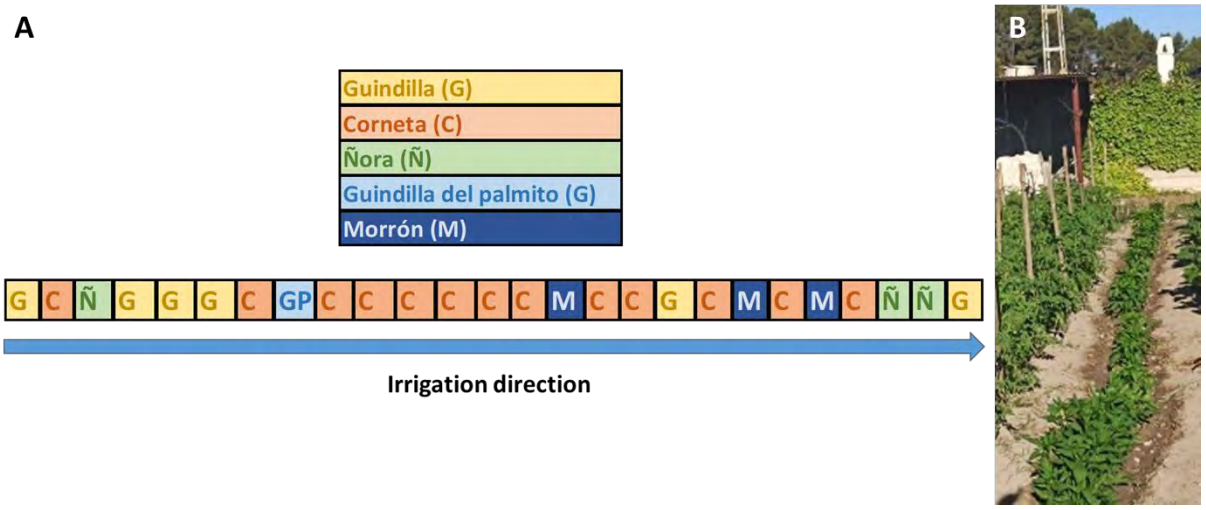


Figure 3. Distribution of the five pepper varieties (A) in only one row (B). The blue arrow indicates the water flow in the field culture.

Throughout the summer season, data regarding plant height, and number of flowers and fruits were measured from the crops in order to assess properly future results. Finally, a tasting panel of peppers from the different varieties was set composed by 10 local people.

RESULTS

As shown in Figures 4-6, the Guindilla variety plants had the fastest growth rate and also the highest average number of flowers and fruits per plant every week.

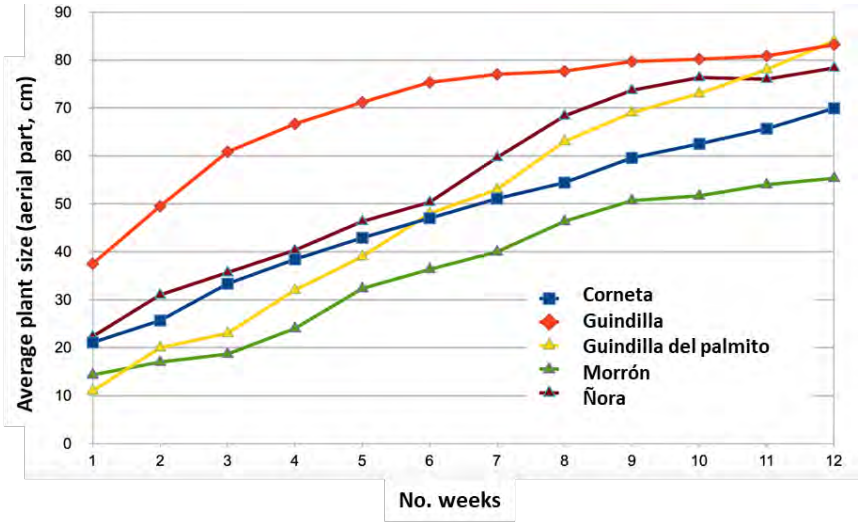


Figure 4. Growth of the different pepper varieties.

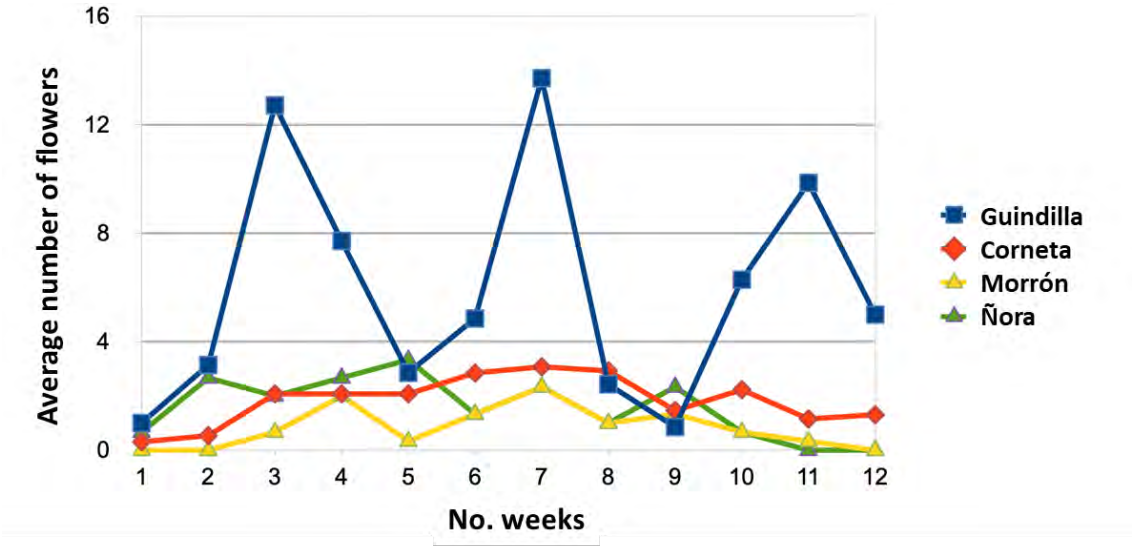


Figure 5. Average number of new flowers per plant in different pepper varieties.

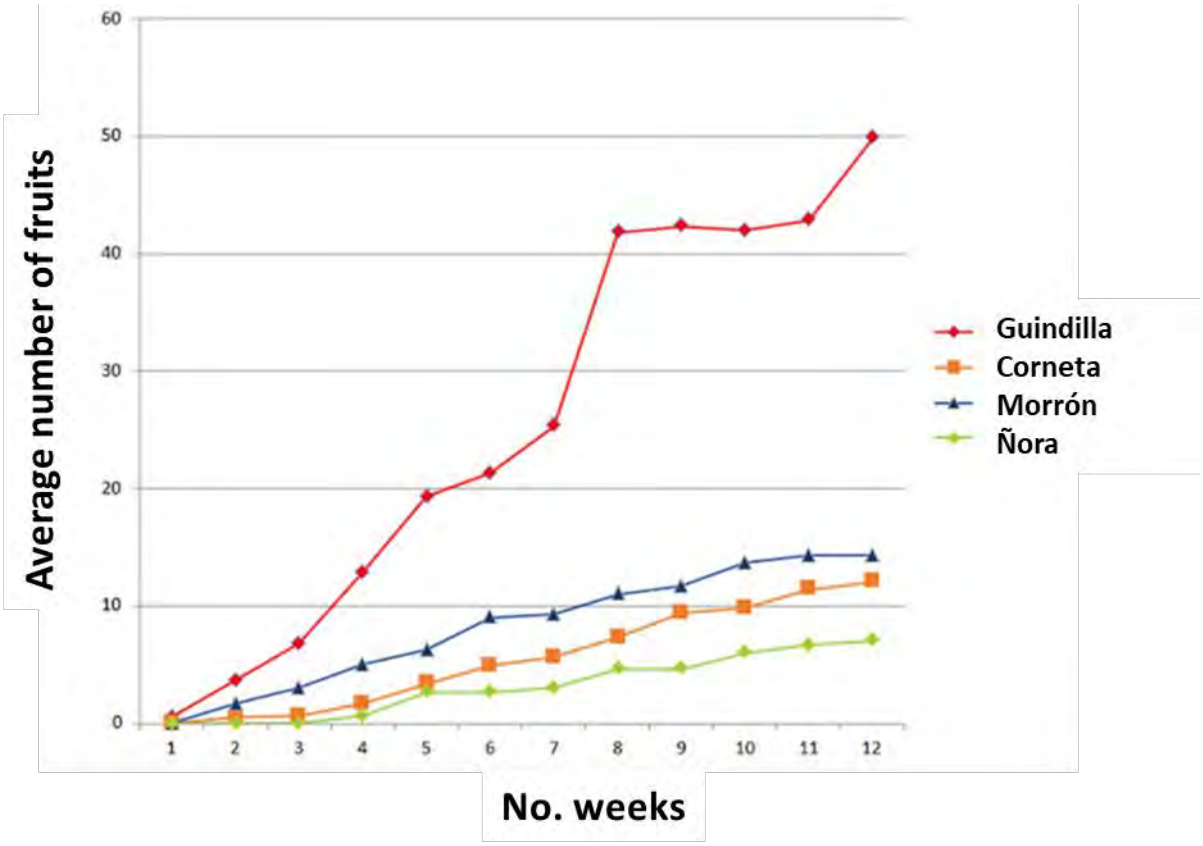


Figure 6. Average number of fruits per plant in different pepper varieties.

After harvest, the panel of 10 people tasted the fruits from the five varieties assayed in this work (Fig. 7), and they do not find differences in the pungency trait. None of the sweet varieties of peppers became pungent.

Figure 7. Tasting of the harvested peppers.

PROPOSED EXPERIMENTAL DESIGN

As a consequence of the negative results obtained in the field experiments, a new design has been devised in order to consider the two possible ways of pungency transmission among the different pepper varieties. From a theoretical point of view, we establish several models:

A. Considering that transmission occurs through the soil (Figure 8).

Model A.1 Model A.2

Figure 8. Experimental approach in order to test pungency transmission through the soil. Model A1 requires that hot and sweet peppers grow in different pots, in order to avoid transmission through soil. This could be the control conditions. Model A2 is carried out by growing hot and sweet pepper plants closely placed one to another in the ground.

B. Considering that transmission occurs through pollination (Figure 9).

Figure 9. Experimental approach in order to test pungent transmission through pollination. Model B1 requires that hot and sweet peppers grow in different pots and one of them is protected by a transparent cover, in order to avoid transmission through cross-pollination. Model A2 is carried out by growing hot and sweet pepper plants closely place one to another in the ground and both protected by a transparent cover.

Regarding the expected results, in case the that pungency transmission is through the soil, sweet peppers will become hot in models A.2 and B.2. Alternatively, if the transmission is through cross-pollination, positive results can be expected in models A1 and A2, but they will be only detected in the next generation. If transmission is possible through both ways, positive results will be only observed in model A.2.

Acknowledgements

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References

[1] Palma JM, Terán F, Contreras-Ruiz A, Rodríguez-Ruiz M, Corpas FJ. 2020. Antioxidant Profile of Pepper (*Capsicum annuum* L.) Fruits Containing Diverse Levels of Capsaicinoids. Antioxidants 9:878.

[2] Otero-Galadí L, Maqueda-Garrido FJ, Martín-Payán JD, Martínez-Domingo FJ, Quesada A, Ruiz C, Campos MJ, Palma JM. 2021. Pepper Fruits: Healthy Properties and Consumption in Spain. A Survey in a High School Environment. High School Stud. Agric. Sci. Res. 10:66-77.

[3] Lillywhite JM, Simonsen JE, Uchanski ME. 2013. Spicy Pepper Consumption and Preferences in the United States, HortTechnol, 23:868-876.

[4] Das S, Sarkar S, Das M, Banik P, Bhattacharya SS. 2021. Influence of Soil Quality Factors on Capsaicin Biosynthesis, Pungency, Yield, and Produce Quality of Chili: An Insight on *Csy1*, *Pun1*, and *Pun12* Signaling Responses. Plant Physiol Biochem 166:427–436.

[5] Antonious GF. (2013). Enzymatic Activity and Half-Lives of Capsaicin, Dihydrocapsaicin, and Pentadecanoic Acid Methyl Ester in Soil Sprayed with Hot Pepper Extract. J Environ Sci Health, Part B, 48:836-843.

[6] Antonious GF. 2015. Elevating Concentrations of Capsaicin and Dihydrocapsaicin in Hot Peppers Using Recycled Waste. J Environ Sci Health. Part. B, 50:523–532.

[7] Pascual I, Azcona I, Aguirreolea J, Morales F, Corpas FJ, Palma JM, Rellán-Álvarez R, Sánchez-Díaz M. 2010. Growth, Yield and Fruit Quality of Pepper Plants Amended with Two Sanitized Sewage Sludges. J Agric Food Chem 58:69-51-6959.

[8] Justino EV, Fonseca MEN, Ferreira ME, Boiteux LS, Silva PP, Nascimento WM. 2018. Estimate of Natural Cross-Pollination Rate of *Capsicum annuum* Using a Codominant Molecular Marker Associated with Fruit Pungency. Gen Mol Res 17:gmr16039887.

[9] Entrena-Aranda P, Paula Entrena-Aranda, Roldán-Martín M, Mengíbar-López I, Rodríguez-López L, Moreno-Duperón AY, De la Torre-Amat E, Shan Vida-Lara J, Quesada A, Ruiz C, Campos MJ, Palma JM. 2021. Analysis of vitamin C content of fruits from five pepper varieties showing different pungency levels and antimicrobial potentiality of capsaicinAnalysis of vitamin C content of fruits from five pepper varieties showing different pungency levels and antimicrobial potentiality of capsaicin. High School Stud Agric Sci Res 10:51-65.

[10] Cárdenas-El Mejdoubi E, Cortés-Moya C, Fernández-Rodríguez R, Higueras-Raya MR, Martín-Contreras LO, Moreno-Villegas O, Rodríguez-De la Rosa P, Chaparro S, Domek-Águila C, Gómez-Molina M, Navarra-Villalba A, Ruiz-Joya R, Campos MJ, Ruiz C, Quesada A, Palma JM. 2022. Assessment of Organoleptic and Nutritional Features of Pepper Fruits Subjected to Different Time and Temperature Conditions: A Preliminary Study. High School Stud Agric Sci Res, this volume.

MY OWN IDEAS

Ignacio Mengíbar López

This project begins with a small comment about a very common phenomenon in the world of agriculture: When we plant hot peppers near sweet peppers, they become hot.
In the summer, an experimental design was carried out to determine the factors responsible for said transmission, measurements of the peppers plants, number of flowers and fruits per week. But our experimental model failed to convey the spiciness.
This year we have dedicated ourselves to collecting information about this phenomenon, and it has questioned and proposed new experimental designs that are more objective.
We determinate that the transmision of capsaicin may be by the pollination or through the soil. And we designed different experimental models with hot and sweet peppers: first, the plants will be placed in individual pots with a certain proximity (it will determine if it is by pollination), second, the peppers plants will be isolated, but both will be in the same pot (it will determine if it is through soil), and thirdly, sweet plants will be taken and placed in a row (it will determine if the transmission is genetic, applying Mendelian laws of genetics).
This summer we are going to take answers from our experimental design.

Laura Rodríguez López

The variability of the pungent flavor is in the capsaicin levels (within the same variety of plant) depending on the growing conditions of the plant. If the plant is of a variety capable of synthesizing capsaicin it appears that the growing conditions of the plant may help to increase capsaicin synthesis and thus the pungency. The production of capsaicin within a species is influenced by the type of soil, better a little poor, irrigation, with scarce irrigation periods, temperature, with periods of temperatures higher than its ideal, and other factors that produce uncomfortable conditions for the plant. It seems that stress produces greater production of capsaicin.
On the other hand when non-hot pepper plants are planted near plants of very hot pepper species it sometimes appears that hot peppers grow out of normal pepper plants. This could be understood if, during pollination, pollen from pungent plants could be crossed with non-pungent ones. Most pepper flowers are self-pollinating but it cannot be avoided in the open field that the wind causes the pollen pollination of other flowers if they are planted together hot and non-hot, and of course insects can pollinate them with pollen of other flowers.
The study could be:
1) Plant non-hot peppers near hot peppers and during flowering, see how pollination affects them. Perhaps by subjecting them to different pollinating agents.
If it is the effect between plants, and the two are planted together (or both groups, spicy and not spicy) and have the same conditions of soil, irrigation, food and temperature. See only the effect of different types of pollination: (wind, insects, types of insects...). Knowing these, it is possible to study the influence of environmental factors added to that of pollination (more or less poor soils, with more or less irrigation, more or less temperature...)
2) Time: you can also see if as time goes by the pepper plant varies the effect and is more spicy.
3) Reversibility or duration. You can see if this effect somehow changes to the original plant. That is, if we then isolate it, we leave it alone, the plant (removing the spicy plant) would only produce sweet peppers or in some way the plant has been influenced so that it produces more capsaicin.

Elena de la Torre Amat

In this project, where we have investigated the transition from hot to sweet peppers, I have learned that the transition from hot to sweet peppers can be due to several factors.
This project has taught me fellowship with my class, looking for questions that need experimentation, research, or expert help. We were lucky enough to meet Pepe, about a year ago, thanks to his help we have been able to move forward with some doubts that have arisen over the days.
This doubt arose from the grandfather of one of my classmates, in which they both did different experiments, to find out if the spicy is transferred through the water with which the pepper is watered or through the air. As a result of this question, different proposals were made to carry it out. such as separating the pots to check if the spicy is transferred through the air or water, we also check if the spicy can be passed to other types of fruits or vegetables such as a tomato or an aubergine, when carrying out that experiment, we verified that the spicy is indeed transferred.
For me, this project has taught me the most is that the data does not always have to come out well, nor the expected ones, sometimes the results are not what we expect, but that does not mean that these data are not useful or worthwhile.

Julia Shan Vida Lara

The project has been a pleasant experience, we started the project without knowing how it was going to end, raising things like the level of capsaicin, we were lucky to have the help of Pepe Palma, who facilitated the experiments, in addition to the grandfather de Nacho contributed with the most experimental part of the project, and even without knowing how the project will end, at the moment, we are investigating the way in which the spice is transferred between them, we have the theory that it may be below land, through water, or through the air due to pollination

Leonardo Otero Galadí

This project is very enriching not only in our knowledge of peppers, but also in our notion of how plants can relate to each other in many ways to give rise to particularly rare and curious phenomena. The project is not finished yet, there are still several unknowns to be solved and a growing medium to be analyzed.
I personally believe that these two years of research have yielded many interesting results through science. I am very grateful for the opportunity to participate in this great work that we have done with so much patience and effort.
Thanks to my teacher Antonio Quesada, Pepe Palma, Marichu and all my classmates who have done such a splendid job.
I'm really looking forward to finishing the project and to know which way do the peppers transmit the pungency through.

Paula Entrena Aranda

The continuity of the project started last year has meant for me a concern and a need to know more and more about science. Our main objective was to investigate the transmission of pungency between pepper plants through the soil or through the air.

Among the various experimental models proposed, I would highlight the model in which we take into account the transmission as a whole both by air and by land.
Thanks to this project I understand that science is a very extensive subject in which more can always be investigated. Well-qualified people capable of working as a team are needed.
Through this project we managed to apply the theory learned in the classroom such as genetics or the transmission of substances to real cases such as pepper bushes.
Science is something so variable that when one question is closed, others are opened. So surely we can find other questions that have not yet been answered, and are there, waiting to be answered

Hussein Mohamad Hussein Kaj

In this document, I´m going to explain my experience in this project, ideas that I´ve had during this year and the conclusions that we have all reached.
The project continued after the summer in October 2021, where the hot peppers were planted nearthe sweets to see if the heat could be transmitted. We had a presentation of the data from Nacho and we spoke with José Manuel Palma, our direct contact with CAOS. In the end, more unknowns were opened than expected, but the investigation didn´t stop. The following month, we had information of the 5 varieties of peppers that were planted, and the growth of each was averaged. Finally, we observe that the "cornetas", "pimientos de asar" and "ñoras" have a similar growthr, but that of chillies is much greater, and the growth of his height compared to others is greater too. Also, it was more logical that more fruits came out of the chilli pepper because it was the one that had the most
In December we came to the conclusion with the help of Manuel's grandfather and the internet: the spicy could be transmitted by air or ground. We didn't really know what to go on with, but we came up with some theories. And we finished in January with a magnificent talk by Nacho's grandfather and his mother, where they explained to us a little about what the countryside is like, and how they did the experiments. All of his people found out that he was participating in this experiment and they also helped.
During this long project, I haven't been able to come up with my own hypotheses very well, but what I am sure of is that we aren't going to meet many people who have thought of doing an experiment as unusual as this one. Even on the internet there isn't as much information as there should be, so we arelooking at something unknown that could really become something big. Another problem is that every time we hypothesized something, more possibilities came out and we were never going to finish. But a hypothesis that I would like to check is whether other foods with a strong flavor, such as ginger, lemon or onion, can transmit their itching, acidity or sweetness to diferent ones, as with peppers. Checking everything would be crazy, but surely we would discover something new.
And to conclude, I would like to say that 2 years ago I did not expect to carry out a project with a national institute like CSIC. When we started we thought it was going to be a small job of 1 or 2 months. But when we saw who we were dealing with, we realized that this was going to take a long time. The whole course has been involved in the project and we have drawn conclusions from real data about something simple like a vegetable plantation. One of my conclusions is that we can make science of everything, even of what seems impossible. In the past, proving that the earth was round was practically impossible, today you can literally take a picture of the planet, and that's what I like about science, that you can always prove something impossible. Another conclusion is that the research work is very cool, but it requires a lot of effort and work;

It took us months just to find time to think of a hypothesis while studying the rest of the subjects, butwe do it.
To sum up, I think that if we had the necessary resources, we could have shown more things than we thought. There are still thousands of possibilities that we have not thought of and that could be done.

Francisco Javier Martínez Domingo

The project started when we had doubts about capsaicin, and if the spiciness could pass from a hot bell pepper plant to a sweet bell pepper plant.
In order to solve the doubt we turned to Nacho and his grandfather, and thanks to them we were able to make an experimental model to test it on their land in Malaga.
After waiting for a long time to see the results, Nacho gave us a presentation and together with his grandfather he showed us the results of the experiment, which consisted of planting several bell pepper plants, hot and sweet, in an area of land and see if the spiciness was transmitted.
Seeing the results we can highlight the fruits obtained from the chili peppers that stand out above the others, in addition to the number of flowers, which in the first weeks was also the one that had the most.
Also, according to Nacho, there were several factors that had a negative influence on the insects, since they do not use insecticide, but for the most part everything went well. It is worth noting that in the graph you can see how the roasting bell pepper was the one that produced the least fruit and also the one that took the longest to start flowering while the chili pepper was the most exponential with respect to the others.
The conclusion was that the hotness was not transmitted to the sweet peppers, which was a surprise since we had been doing this for quite some time.
But the results were not a problem, on the contrary. We were able to raise hypotheses on which to base a future experimental model:
The main or most talked about hypotheses were about water and pollination.
The first would be to see if capsaicin can be transmitted through water, this would consist of a hot bell pepper plant being watered next to a hot bell pepper plant so the water could supposedly transmit the hotness to the sweet peppers.
The second model would be to plant two bushes, one hot and one sweet, in different pots, so they would not be watered with the same water, and then you could test whether it is transmitted by air, pollination or some other factor, which would not be water.
But I came up with a third hypothesis related to the planting area or geographical area, which would mix the two previous hypotheses. With this it could be understood that the passage of hotness from some peppers to others in one year if it worked and the following year it did not. This could be explained by the influence of soil type, temperatures or insects. To check if this is true, a model similar to a greenhouse system could be used.
In this model we would propose different organizations of plants in a greenhouse in which all factors such as irrigation or temperature would be controlled, in the greenhouse we could test this experimental model and see if the conditions around the plants really influence the experiment.


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