DNA extraction from strawberry (*Fragaria ananassa*) as a practical tool for teaching genetic content

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ABSTRACT
Genetics is an important curricular component of the Biological Sciences discipline and a great study tool for scientists. The aim of this study was to verify the existence of DNA molecules in strawberry. To carry out the practice, DNA extraction was performed from the strawberry. A tangle of white strands can be seen between the solution and the alcohol, forming a 3-phase mixture. It can be concluded that DNA extraction from the strawberry is an easy practice to perform.

Keywords: Genetics, Plants, Botany.

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INTRODUCTION

The origin of experimental classes dates back more than a hundred years, influenced by the work developed in universities, with the objective of improving the learning of scientific content, since students learned the theory, but did not know how to apply the learned content in practice. Thus, learning does not take place only by being a listener in a classroom and written in the notebook, but by a theoretical-practical relationship, not only for comparison purposes, but also to arouse students' interest, generate discussions, and improve their performance in class (ALMEIDA, MANNARINO, 2021).

For the teaching of Genetics content as part of the Biology discipline in high school, there is a greater difficulty in reconciling practical classes, since it often requires advanced and expensive equipment.

DNA (deoxyribonucleic acid), whose structure model was published by the American biologist James Dewey Watson and the English physicist Francis Harry C. Crick (1952), is formed by two parallel chains of nucleotides joined in sequence, forming a double helix. Each nucleotide is formed by the combination of three components: phosphate, nitrogenous base (puric: adenine and guanine; pyrimidics: thymine and cytosine) and the sugar deoxyribose (FRANÇA, AUGUSTO, 2021).

Fragaria ananassa (strawberry) is of European origin, it is soft and easy to homogenize, it also produces pectinases and cellulases, which are enzymes that degrade pectin and cellulose (respectively), present in the cell walls of plant cells.

Nowadays, there is a lot of praise for DNA extraction in various researches, being of paramount importance for biotechnology, medical and forensic areas. In view of this, it is important to inform and bring to the forefront of society, with this study, the relevance of DNA extraction, the way it is present within the daily life of the population and to what extent it influences and reflects on the life of each individual, in addition to being essential in the scope of citizens in the globalized society, in which science and technology play an increasingly important role.

In view of the above, the present study aimed to verify the existence of DNA molecules in strawberries (*Fragaria ananassa)* through visualization with the naked eye and to understand and describe the physical and chemical transformations that occur at the end of the experiment, in addition to evaluating the feasibility of performing this practice in the school context.

THEORETICAL FRAMEWORK

GENETICS

Since the dawn of humanity, there have been questions about the similarities between puppies and their parents; diseases present in the parents and that have appeared in the children; the emergence of plants through seeds, and so on. As an explanation, the theory of mixture was presented, since it responds to the combination of factors to form a single compound (GRIFFITHS, 2019).

However, this theory was gradually becoming scarce to answer all the questions. It was then that Gregor Mendel, an Austrian monk, began his experiments in the cross-pollination of pea seeds, where he identified that the factors (today, known as genes) do not mix, but rather, are passed on intact from generation to generation. (GRIFFITHS, 2019).

In the experiment, Mendel proposed that each pea had two copies of the factor that controls the color of the flowers, which would be somatic cells. But when it enters the reproductive mode, forming gametes (eggs and sperm), only one copy of the gene enters these reproductive cells. At the end, there is a new individual formed, which has two copies of the flower color gene in each cell (GRIFFITHS, 2019).

In addition, Mendel identified two gene variants, the recessive and dominant alleles, which are responsible for the genetic combinations and possibilities of individuals. Even with an incredible discovery, Mendel's work of 1865 was forgotten for a long time, and it was only in 1900 that the British biologist William Bateson came into contact with the copy of the article, studied it and was enchanted by the discovery of the deceased. In 1905, he introduced the term genetics, which means the study of genes, as well as providing an advance for the subject, which to this day remains with incessant discoveries with the help of increasingly advanced technologies (GRIFFITHS, 2019).

PRACTICAL METHODS FOR TEACHING GENETICS

Genetics is the field of Biology characterized by the study of heredity, as well as the structure and functions of genes, that is, it studies the genetic material, its components and the way in which the transmission of hereditary characteristics occurs over generations. Thus, it elucidates how the characteristics present in each organism are transmitted to its successors (GRIFFITHS, 2019).

Genetics is a subject that involves mathematics, text interpretation, logic, among other areas, that is, it is a transdisciplinary content. As a result, many students see Genetics as something difficult, especially in the first experiences, and also in many cases point out difficulties in the process of learning such content. In addition, the themes within Genetics address many representations that seek to characterize the processes that take place in nature. These representations can cause a distancing from reality, affecting the appropriation of the content by students (BORGES *et al*., 2020).

Several studies have been carried out with the purpose of analyzing what knowledge and perception young people have about genetics at the end of the years of compulsory schooling and how they perceive the issues that have been generated by the application of new genetic technologies in different contexts. However, the results achieved are worrisome, as they show that, many times, not even the basic notions of genetics are understood (GAMBIN; SCHEID; LEITE, 2021).

Similarly, studies that seek to identify the adversities encountered by teachers, both at the beginning of their teaching work, during initial training, and during their professional careers, present as problematic the issues related to the teaching of genetics and its technologies (OLIVEIRA NETO *et al.*, 2018).

These inquiries lead to a reflection on the development of the training of Biological Sciences teachers. Probably, one of the obstacles in the teaching-learning process is the positivist view of science, still very present, which imprints a technical rationality that makes the teacher responsible for the possession of discovered truths, which leads to their students as ready, unquestionable (OLIVEIRA NETO *et al*., 2018).

The History of Science, framed in scientific education, may provide an opportunity to guide students in the appropriation of a conception of science as a human exercise, built on the interaction between the knowing subject (capable of knowing), the object to know and the "state of knowledge". In this way, the teacher can act as a mediator between historians of science and students by using History and Science as a tactic to problematize conceptions and content of science (TAKAHASHI, 2018).

Ludwik Fleck's conception becomes proficient in the interpretation of reports on the evolution of scientific knowledge that culminated in the proposition, for example, of the double helix model for the DNA molecule and its acceptance by the scientific community (TAKAHASHI, 2018).

In this sequence, Fleck, based on considerations about the understandings and practices established by medical science, introduces the concepts of style of thought and collective of thought, stating that the act of knowing is an activity that is unified to the social and cultural conditions of the subject belonging to a collective of thought. This collective can be understood as a community of individuals who share practices, conceptions, traditions, and guidelines. It is worth noting that each collective of thought has a unique way of seeing the object of knowledge and relating to it, determined by the style of thought it has (TAKAHASHI, 2018).

That said, the inclusion of the History of Science, that is, the history of the construction of knowledge, can be a facilitator of scientific education, when the assumption is the dynamic aspect of scientific knowledge. When it is used in the teaching of genetics, it should lead the student to conceive that science is a socio-historical-cultural construction. From another perspective, it can also help in the understanding of the fundamental concepts of the discipline. (TAKAHASHI, 2018).

In view of this, the recommendation that the History of Science should be incorporated into teacher training courses contributes to a new way of conceiving science as dynamic knowledge, produced by human beings with limitations, using devices that are not always so perfect, because "a scientific fact cannot be understood outside the context of the history of thought and results from a determined style of thought" (RODRIGUES *et al*., 2020).

By allowing the exploration of a more adequate view of the production and evolution of scientific knowledge, the History of Science will be able to bring important contributions to the area of scientific education, especially in the area of genetics, which is in a rapid process of knowledge renewal. It is also worth emphasizing that, most of the time, the contents are presented without connection to the students' daily lives in a fragmented and decontextualized way, compromising the quality and effectiveness of the teaching and learning process (SIMÃO, 2018).

Following the example of the lack of understanding of the relationship that exists between the gene (as a fragment of DNA with a specific location on the chromosome) and the transmission of the genetic information that this gene transmits (which determines the precise information of the gene product), the effective understanding of topics related to inheritance within the teaching of Genetics is inaccessible (GAMBIN; SCHEID; LEITE, 2021).

Consequently, it is difficult for the student to understand the concept of alleles and their direct connection in the transmission of characteristics between individuals, leading students to disconnected ideas and the understanding of the whole process would be compromised. Therefore, for the understanding of the general concepts of genetics, it is essential to have a prior and concrete understanding of what a gene is; where it is located (physical location – tissue – organ – cell – DNA) and the expression of the information contained in these genes (GAMBIN; SCHEID; LEITE, 2021).

Thus, the use of didactic models as a methodological resource for the teaching of Genetics in the initial training of teachers becomes of paramount importance, as they enable that from the instrumentalization of knowledge about the methodology and its possibilities, the graduates can make use of these resources in their practice.

DNA: STRUCTURE AND REPLICATION

The structure of DNA consists of Watson and Crick's model, which postulates that DNA is composed of an antiparallel double helix, nucleotides, and four nitrogenous bases: adenine, cytosine, guanine, and thymine. This structure suggested that the nucleotide sequences present in the two strands of DNA were responsible for the standardized formation of an organism and the idea of complementarity showed that the sequence of one strand determines how the sequence of the other will be. Thus, the genetic data present in DNA can pass from the mother cell to the daughter cell, so that each strand will serve as a template for the replication of a new double-stranded DNA (GRIFFTHS *et al*., 2019).

The nitrogenous bases present in DNA (adenine, thymine, cytosine and guanine) are composed of puric and pyrimidic bases, so adenine and guanine chemically derive from the purine substance and their structures form two aromatic rings. Whereas thymine and cytosine originate from pyrimidine and make up only one aromatic ring. Thus, it was observed that DNA was composed of bonds between nitrogenous base, deoxyribose and phosphate. These bonds are made from the so-called hydrogen bonds and the phosphodiester bond, and from there, the nucleotides bond with each other until they form DNA (MENCK, 2017).

Based on these precepts, rules were established that were related to the composition of the DNA double helix. The first provided that the total amount of purine bases would be equal to the total amount of pyrimidic bases. The second showed that the amount of adenine and thymine would always be equal, since one was bound to the other, and the amount of cytosine and guanine would also be equal, because they also did, although it would not be necessary for the amount of adenine + thymine to be equal to the amount of cytosine + guanine, and vice versa (MENCK, 2017).

Regarding DNA replication, the accepted hypothesis is the semiconservative model, which was also proposed by Watson and Crick. This replication model conceptualizes that the DNA double helix is unwound and each strand serves as a template for the assembly of new complementary bases that follow the rule of binding between adenine and thymine, guanine and cytosine. This conception of semi-conservative replication was due to the fact that the new double helix is formed from a conserved strand of the previous double helix, so in each replication, one strand will be conserved and a new one will be generated from it (GRIFFTHS *et al*., 2019).

DNA replication in eukaryotes happens with the help of some enzymes, so the one responsible for recognizing the origins of replication and assisting in the binding of the helicase is called ORC (origin recognition complex), after which comes the helicase, responsible for breaking the hydrogen bonds of the DNA. After unwinding the double helix, the single strand of DNA is stabilized by replication factor A, then topoisomerase removes the kinks that arise on the strand. After that, the synthesis of RNA primers begins, which are made by the DNA polymerase delta-primase complex and thus inserts the DNA polymerase that elongates the DNA. The next step is the removal of the RNA primers and in their place the DNA bases are added, this process is carried out by the enzyme FEN1, while DNA polymerase replaces the RNA primers with DNA. Finally, DNA ligase I binds Okazaki fragments, which are fragments of spaces formed after the removal of primers (GRIFFTHS *et al*., 2019).

EXTRACTION OF DNA FROM PLANTS

Much has been used of proposals for practical activities to facilitate the teaching of genetics, as in the work of Gonçalves (2021) who used simple and low-cost materials for the extraction of DNA (deoxyribonucleic acid) from the cells of common fruits on the Brazilian table, such as tangerine (*Citrus reticulata*) and mango (*Mangifera indica*).

According to Moreschi *et al.* (2021), the practice of extracting DNA from vegetables, as it is low-cost, was chosen to be worked on by the teachers, due to the possibility of students being able to reproduce the practice at home, using the fruit and the onion bulb, presenting the chemical reactions and steps, such as maceration, density, separation of liquids and solids, and finally, the definition of what DNA is.

For the creation of innovative and diversified methodologies, aiming at the promotion and construction of knowledge, Santos *et al.* (2020) developed a pedagogical workshop called "Playing geneticist: discovering DNA" with the objective of identifying and recognizing the DNA molecule of two vegetables: bananas and strawberries, in addition to understanding the importance of this molecule for life and stimulating the search for concepts and allowing students from public schools and low-income to have the opportunity to have an active attitude towards the learning process.

To make the class more participatory on the part of the students, through the methodology of teaching by research, in order to understand the contents of genetics, Fagundes *et al.* (2022) proposed an activity in which they could set up their own DNA extraction experiment, where they were able to relate the importance of genetics in its broadest sense, within the reality of each one to show that DNA is in all forms of life (FAGUNDES *et al.*, 2022)

Aiming at access, democracy and inclusion, Ferreira *et al.* (2021) proposed a natural sciences workshop in a remote environment, through the Moodle platform, the application of the investigative method for teaching what life is, explaining concepts such as DNA and pigments, aimed at students of Elementary School II and High School, and one of these experiences was the extraction of DNA from plants in general, through manipulative actions by the students themselves. Low-cost materials were used and guidance was provided through the platform to help students in the process of building knowledge.

The authors stated that, with these activities, they aim to give students the opportunity to create hypotheses, discuss among other students, develop solid and convincing argumentation and the ability to recognize errors, in addition to taking photos of the experiment and sharing it in the presentation forum (FERREIRA *et al.,* 2021).

Matta *et al.* (2020) carried out an intervention in a public school in Natal-RN, developing experimental DNA extraction classes and finally, created and developed a *Quiz* to collaborate in the teaching-learning of these students, in addition to stimulating the taste for experimentation and the development of scientific thinking. Five stages were carried out, namely: the first, a questionnaire to survey previous knowledge; the second, the dialogue and discussion of the theme and explanation of the activity; the third, the extraction of the DNA itself, from the tomato fruit; the fourth, a learning verification questionnaire and the fifth, and last, the development of questions asked by the students themselves in the *Kahoot application,* showing that the students had a great commitment to the proposed activities and were able to discuss the reagents taught and the results obtained, thus requiring the use of activities that make the knowledge more meaningful.

MATERIAL AND METHODS

The practice was developed at the Cytogenetics and Mutagenesis Laboratory (LABCIM) of the University Center of Patos de Minas, present in block M. 6 strawberries were used to perform the experimental procedure; 100 ml of distilled water; 5g of NaCl (table salt); 60 ml of alkylbenzene sulphonate (neutral detergent) and 100 ml of ice-cold 70% ethyl alcohol. The equipment used was: crucible and pistil, used in the maceration process; 100 ml beaker and cylinder, used to measure and store the contents. On the other hand, the use of the stick, the filter paper and the glass funnel served to assist in the management of some solutions and filtration. While the water bath at 60°C was used to heat the liquid acquired from the extraction of the strawberries with the solution of alkylbenzene sulfonate and NaCl and the styrofoam box with ice, to assist in the cooling of the filtrate.

Magalhães and Muller (2022) developed a project to apply active methodologies for teaching and learning Science in Elementary School, in which one of them was the extraction of DNA from a vegetable, done at the student's home, during the COVID-19 pandemic. The students posted photos of their experiments, showing the DNA precipitated between the alcohol and the filtered material, with the pectin in the supernatant. This work became, according to the authors, the most attractive classes, generating satisfactory results in the teaching-learning process and favoring engagement around science, research and studies.

Based on Magalhães and Muller (2022), the development of the experiment followed the following steps: 1- cut and macerate the strawberries (about 6 units) with the help of the crucible and pistil (figure 1); 2- prepared the solution of NaCl and alkylbenzene sulfonate, both of which were dissolved in a beaker containing 100 ml of distilled water (Figure 2); 3- added the strawberries to this solution; 4- put this mixture in a water bath for 15 timed minutes (Figure 3); 5- removed the solution from the bain-marie and added it to the Styrofoam with ice at the same time for 5 minutes (Figure 4); 6- filtered this solution with the aid of filter paper and a glass funnel in a 250 ml Erlenmeyer (Figure 5); 7- passed this filtered solution to a glass beaker of approximately 250 ml (taking into account the amount, in ml, that it was possible to filter); 8- added 100 ml of ice-cold 70% alcohol to this solution, slowly and along the walls of the beaker, observing the result (Figure 6); 9- With the help of the clean stick, circular movements were made in this solution and the phases that were formed were mixed; 10- The acquired content was observed and the analyses were performed.

Figure 1: Cutting and steeping of strawberries.

Source: Authors

Figure 2: NaCl and alkylbenzene sulfonate solution.

Source: Authors

Figure 3: Mixtures in a water bath.

Source: Authors

Figure 4: Mixtures on ice.

Source: Authors

Figure 5: Solution filtering.

Source: Authors

Figure 6: Addition of 100ml of ice-cold 70% alcohol.

Source: Authors

RESULTS AND DISCUSSION

At the end of the experiment, the strawberry DNA was obtained in a solution with three visible phases, the most precipitated being the aqueous solution, the medial phase the DNA and the most superficial phase containing the alcohol. This is because ice-cold alcohol is less dense than aqueous solution and DNA is also less dense than aqueous solution, but denser than ethanol (Figures 7, 8 and 9).

The strawberry needs to be macerated to increase the contact surface and homogenize the plant tissue. On the other hand, the addition of detergent (alkylbenzene sulfonate) is due to the emulsification of lipids, destructuring the lipid bilayer and disrupting the plasma membrane of the cells. Regarding the fact that it heats the solution, it is due to the increase in temperature that provides an increase in kinetic energy, the rupture of membranes and the denaturation of proteins and enzymes, such as histones and DNAs, inhibiting their interaction with DNA.

Thus, the addition of sodium chloride to the solution, which is due to the contribution of Na+ cations to neutralize phosphate groups in DNA and Cl- anions to neutralize histones, favors DNA agglutination. There is also the importance of the water bath and the sudden drop in temperature leading the solution to ice, which is due to the aid in the maintenance of the proteins in their denatured character, disfavoring their interaction with the DNA molecule. Regarding filtration, it is important to carry it out so that there are no cellular debris, except for DNA. Regarding alcohol, it is understood that the colder it is, the less soluble the DNA is in the solution, since DNA is not soluble in ethanol.

Silva (2018) performed the DNA extraction of *Allium cepa* (onion) and discussed the importance of the practical class for learning the concepts of genetics. With the onion, he had the same result with the whitish strands representing the DNA, between the cold alcohol and the filtered compound, not forming pectin in the supernatant, since this compound is common in fruits such as strawberries and bananas. With the practical class, the students showed interest and presented good performance in the processes, being encouraged to bibliographic research and reading the content.

Cavalcante *et al.* (2018) performed the DNA extraction of *Aloe vera* (aloe vera) in order to carry out a differentiated and innovative class. The DNA was extracted in a simple, adapted and easily accessible way, forming a kind of "white cloud", which was pulled to better visualize the deoxyribonucleic acid of the aloe vera, making the extraction of this DNA an important didactic resource for genetics classes and attractive to the student, thus facilitating the teaching-learning in Biological Sciences.

Fagundes *et al.* (2022) carried out five activities, in which the third was the extraction of DNA with different fruits, such as papaya, banana, tomato, lemon, among others, and the students would bring from home what they were going to assemble and explain, contributing to the students attributing new meanings to scientific knowledge and practices. As a result, the students had good performance, good interest, full participation and full integration with what was proposed.

Santos and Pereira Júnior (2019) went further, in a public school in Cametá-PA, performed DNA extraction using alternative materials and extracted DNA from tomatoes, onions, and the saliva of volunteers to carry out the practice. The authors were able to conclude that experimentation in Science represents an excellent tool for students to make a connection between theory and practice, making them more apt to solve problems that arise during their school life, in addition to leading teachers working in the classroom to a new way of teaching. Even in a public school, with needy students and few resources to carry out a practical class.

Figures 7, 8 and 9: DNA extracted from strawberry in three phases, the densest of aqueous solution, the intermediate of DNA and the least dense of ethyl alcohol.

Source: Authors

CONCLUSION

In the experimental conditions outlined in this study, it can be concluded that the DNA was visualized in the intermediate phase of the final solution, identifying its characteristics during the experiment and the reactions between the substances. In addition, it was noticed that this practice can be carried out in public schools lacking physical resources or even in the students' own homes, since the materials used can be easily found.

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