

The plants that capture the atmospheric nitrogen
The “nodulazione” of the leguminoses through *Rhizobium leguminosarum*
Logbook

The context

The activity has been realised in class II Á of the Secondary School I Degree “Giovanni XXIII” in Cava de’ Tirreni(Sa), in the branch school of Passiano village.

The class is composed of 21 pupils, 8 girls and 13 boys. The learning level is middle-tall. The pupils are vivacious and available to the learning and are actively involved in the school activities.

The work has been done with the support of doctor Beatrice Senatore (training SICSI, graduate in biological chemistry with consolidated experiences in the biological-molecular sector) that has developed activity of search dealing herself besides with the nodulazione of leguminoses through genetically modified rizobi.

The run has been developed according to the methodology CLIL (Content and language Integrated Learning) conferring to the experience an “additional value” because it has allowed the students to approach English language in a natural way, exploiting the curiosity that they generally manifest towards the experiments of sciences.

Past significant experiences

The class has a certain custom to the experimentation:

- during their first year of this School the boys participated in the experimentation on the organisms model (plain ISS), preparing an aquarium for the reproduction of zebrafish.
- they usually build and design robot (RCX Lego brick Mindstorms).

Prerequisites

Characteristics of the living beings, classification and scientific name, the reproduction, structure and function of the roots, chlorophyll photosynthesis.

Input test

			punteggio
The vegetable cell shows the same characteristics of the animal one	T	F	1
The stem is the organ of support of the plant and through the vases conductors, allows the circulation of the sap	T	F	1
During the photosynthesis carbonic anhydride is produced	T	F	1
What’s the name of the science that studies the living beings?			1
Through which biochemical process do the pants produce their nourishment?			2
Complete the following sentences setting the lacking terms in the blanks: DEATH-ORGANISM-ASEXUAL-SPORULAZIONE-EMBRYO-FERTILIZATION- GEMMATION - BIRTH The reproduction..... can happen for binary division, for....., for..... The vital cycle goes from the.....moment to the.....moment			2

Complete the following sentences setting the lacking terms in the blanks: VASE - SOIL-WIND - MINERAL SALTS-ANCHOR- STEM-AIR The roots..... firmly the plant to the..... and they prevent that it is brought away by the..... besides they absorb water and..... from the ground and they carry them in the.....	2
Set in order the following groups of living beings starting from that more narrow to that ampler: phylum order kind family class species kingdom	2
The chlorophyll photosynthesis requires: - Kinetic power - Luminous power - Thermic power	2
The roots are the part of the plant specialized for the absorption - of the water and of the mineral salts. - of the organic substance - of the micro organisms in the soil	1

U.A. "Ecology" Aims

To define the components of an **ecosystem** and the relationships between them

To describe as the different populations can interact in a **community**

To describe the **biogeochemical cycles** in the their biotic and non biotic components

To recognize the **interactions** among the different components of the various levels

Cultivation of the leguminoses. Local habits

The large majority of the pupils has the grandparents or the uncles that cultivate the earth. The teacher asks the pupils to interview one of them to get information about the local habits.

- Which are the leguminoses plants?
- When they are sowed?
- What's the treatment during the cultivation? (times of growth, period of harvest, use of fertilizer/pesticides)
- What happen to the plants after the harvest?

It emerges that all the boys know the leguminoses plants and they mention for instance broad beans, peas, beans, lentils, chick peas, someone mention the "cicerchie" ("chichierchie" in their dialect, legumes that seem an intersection between the broad bean and the chick peas). During the discussion the boys mention other dialect terms that in some cases don't have the correspondent term in Italian language. With the help of the Italian teacher the meanings are defined.

Phases of the experimental run

Observation of plants of broad bean, cultivation in vase and classification.

Washing of the roots and radical nodules unsheltered.

Questions Stimulus: What are the nodules of the leguminoses for? *What do they contain?*

The teacher propose to extract their content and to cultivate it on plate.

30 January

Extraction of the bacterias and seeding on plate

The activity is done in the classroom; four groups are formed, a card of job is distributed in which is explained-in English-the procedure to be followed for the seeding in plate. The boys in the time of 20' read the text, translate it and helped in the understanding from the figures and the dictionary, they infer a scheme of the various phases.

In the meantime on an empty desk the required materials are prepared: broad bean plants, handles and throw-away gloves, natural mineral water, Petri capsules previously prepared with the ground of crop (TYR: Triptone - Yeast extract - Rhizobium), a small mortar with pestle.

Students use the plants of broad bean put in vase in the month of December.

After having washed the roots, They detach some nodules gently and put them in the mortar with some water, crushing them with the pestle. Under conditions of sterility, with the gloves and with a throw-away handle, every group withdraws a drop of suspension and effects the graze on plate.

Peas seeds cultivation

Every group prepares two envelopes, one of these will be infected with the bacterias as soon as it sprouts the little root, the other one will be used as check. The groups alternate to place the seeds of peas in the previously prepared envelopes. Everything has to happen under conditions of absolute sterility: it is necessary put the gloves or dip the fingers in ethylic alcohol and let evaporate it (shaking the hands or blowing).

QUESTIONS STIMULUS

What do the seeds need to germinate?

Why they cannot be watered directly pouring the water on them?

If water goes down to the bottom of the envelope, how does it reach the seeds?

Where to put the box? Over the water, what does a plant need?

Why have the seeds to stay in the dark?

When all the groups have deposed the seeds of peas and watered, the box is set on the window-sill of the classroom, covered with a black cloth.

The Jensen solution is also prepared that contains all the mineral salts the plant need except the nitrogen.

2 February

The seeds show a little root of 2-3 cms, so the teacher decides to proceed with the infection.

Students withdraw a colony using a sterile handle and put in suspension again in natural mineral water. Then with the so prepared bacterial solution, they bath the roots of peas which are in the envelopes marked "inoculated."

Immediately after, the boys water all the envelopes. From now onwards it will be necessary to check that in the envelopes there is always around 2 cms of water. Furthermore once a week they will add 10 mls of Jensen solution to every envelope.

The result of the experiment is tied up to the ability not to contaminate the peas of control with the Rhizobiums contained in the inoculated envelopes. To this purpose separate pipettes will be used for the two groups of envelopes.

The following questions are set, drawn by the protocol Pascucci-Forni:

What has happened to the bacterias and the seeds after 4 days?

The bacterias.....

The seeds.....

When the root has reached the length of 3-4 cms we have infected the seeds with the bacterias.

What do you think it will happen?.....

The most greater part of the pupils believe that the plants infected with Rhizobium grow more quickly, someone says that they will be greener and more luxuriant. Nobody forecasts on a possible difference in the development of the flowers (reproductive apparatus of the plant).

In the classroom they keep on reasoning, driving the discussion with some questions: With infection/without: does it grow before/after? *Does it grow better / worse?*

In the following days the boys observe the growth of the seedlings (roots, little stems and leaves)

Thursday February 7 th

The boys observe the development of the roots trying to individualize - with the help of a lens of enlargement-possible already present nodules. The teacher proposes to affix a number with a pen in

correspondence of the probable nodules individualized so that to follow their evolution in the following days. In the following observations the possible new nodules in progressive way will be numbered for also having a dating of their appearance.

QUESTIONS STIMULUS

What's the function of the nodules?

Why the nodules are developed in different times?

What does it depend from a greater or a smaller number of nodules?

In which cases does the plant need a greater number of nodules?

Which benefits does it bring rhizobium to the plant?

Which nitrogen do the nitrogen-fixers bacterias use?

9 February

What happened 7 days after the infection?

Pupils can observe the development of the roots and of the aerial part of the seedlings.

Then water is added in all the envelopes (control and inoculated).

They also observe the phenomenon of the phototropism ("the seedlings move themselves and tilt toward the light of the sun").

14 February

All the seedlings have side roots and well visible radical hair . Students check what of the points marked on February 7 are possible nodules.

The boys of the various groups measure the height of the stems and the roots and they compile a card of observation.

Variability and distribution of frequency

Students observe the variability among the two groups of plants: inoculated and control.

They can confirm that the height of the seedlings inoculated (calculated on the average) it is greater of that of the seedlings used as check. The calculation of the average is done after having brought the values in a excel sheet using the function of automatic sum.

In a second moment the attention of the boys focuses on the fact that also within the same group there is a reasonable variability: the individual biological variability within the same kind. The boys are stimulated to think to other examples such as the different height of the people. Then the histogram of the frequencies is drawn (gaussiana).

16 February

The stems start to bend and to twist themselves risking to break themselves and the extraction of the envelopes from the box becomes more and more difficult. They discuss how to solve this problem: conforming to the systems used by farmers, a light structure of support it is created with straws and a little net.

Then they observe the envelopes to follow the course of the development of nodules. In the marked points in the preceding days the presence of small nodules is confirmed.

What has happened 14 days after the infection?....

"It has happened that the roots and the plants have lengthened and the leaves have opened.

The roots are very long and a lot of secondary roots have appeared. I have also noticed that a stem that seemed broken is healed.

In the infected plants the stem is more strong, long and straight enough, while in the control it is thin and more fragile." (Valeria)

Saturday 23 February

What has happened 21 days after the infection?....

The infected plants show very more ramified roots. The stems are decidedly longer.

There is no difference in the colour and in the consistence of the leaves.

The boys trace a graph of the heights of the stems respectively considering the tallest plant of the group CONTROL and INOCULATED. They observe that between the second and the third week they can appreciate a difference in the height of the stems.

Saturday March 8 th

35 days after the infection

Circle time - Discussion on the results of the experience and evaluation of the run.

The boys have experimentally verified that, thanks to the nitrogen-fixers bacteria that live in symbiotic relationship on the roots, leguminosae plants are able to fix the atmospheric nitrogen, that enters the synthesis of the proteins and of the Dna.

In fact making to grow two groups of plants in a checked environment:

- in absence of bacteria >> there is no formation of the nodules >> fragile and little developed seedlings
- with inoculated bacteria >> there is the formation of the nodules >> taller and with more strong stem seedlings.