Study of polymorphism DNA-sized microsatellites in order to determine the distribution of Périgord Black Truffles genome (*Tuber melanosporum* Vitt. 1831) in space and time within a burn-out zone. (up to June 2014)

This project of genetic analysis is part of the school genome project initiated by the Observatoire de Paris in 2011th. This prospection work on the truffle is done with the help of a supervisor : M. C.MURAT research engineer of INRA Nancy.

The goal of this project is to follow the genomes evolution of Périgord truffles genome in burn-out in space and time. The use of SSRs (Simple Sequence Repeats) as a marker allows the realization of this work. In fact, the genetic rules may be used at this marker as any other individual genes.

This work needs to have a ''truffière'' (truffles field), considering how long it takes to obtain truffles from mycorhise's trees, the Périgord's farmers truffles federation, our collaborator, give the truffles production of three trees.

But, so as to be self-dependant in the next years, we implanted a truffle field (nine trees) into the school enclosure.

Our work on this subject has several steps:

- Maintenance and follow up the high school field truffle
- Knowledge of this fungus : *Tuber melanosporum* (Tm) and study of the relationship between the tree and the fungus : the mycorhises,
- Genetic analysis of the truffles.

1. Maintenance and follow up the high school truffles field

- a) In April 2013, we implanted a truffle field into our high school.
- b) In September 2013, we did the maintenance and the follow up of the trees:

  - ventilating the soil,uprooting the weeds,
  - measuring the height and the neck trees diameter to follow up their growth.

(a)

(b)

2. Knowledge of this fungus.

2.1. Generality

Tuber melanosporum belong to the *fungi* kingdom, *Ascomycota* division, gender : *Tuber*. In this gender, this fungus:

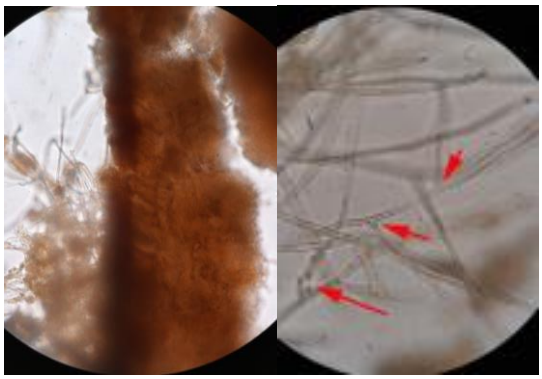
- has a hypogeous fructification,
- is symbiotic to the trees, they form the mycorhises,
- has an annual cycle, the harvest period begins in November and finishes in March.
- lives in the calcareous soil (pH > 7).

2.2. Our work

In this project, we analyzed the mycorhisal status of selected trees in the experimental truffle field station of ''Glane''. After the harvest of the roots (with or without mycorhises), we tried to identify them.

A Tm's mycorhise has two essentials characteristics :

- a) The coat cells are puzzle- shaped
- b) The mycelia produced from the mycorhise presents a right-angle connection.



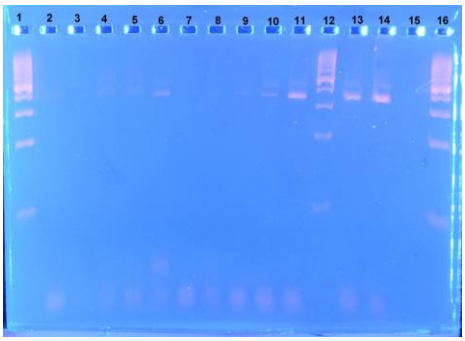
(a) (b)

We analyzed eleven trees, with two roots samples by tree. This result has been obtained:

- For three trees, two samples are identified Tm,
- For one tree, one sample out of two is identified Tm,
- Eight samples aren't identified,
- Three samples are identified as belonging to the puffball's group,
- Four samples aren't analyzed.

3. Truffles genetic analysis

The four analyzed truffles have been harvested under the p27 tree (no new harvest since the 2012 season). On these truffles, we made the DNA extraction using the **Kit QUIAGEN DNeasy Plant Mini Kit®**, we made the amplification of the SSR Tm22 and we analyzed the amplicons by agarose (4%) gel electrophoresis, we obtained the following results.



agarose gel electrophoresis

SSR: Tm22

1 : ladder
2 : n37 1b
3 : n37 2b
4 : n37 3b
5 : n37 4b
6 : n37 5b
7 : n37 6b
8 : n37 7b
9 : n37 8b
10 : p27 1b
11 : p27 2b
12 : ladder
13 : p27 3b
14 : p27 4b
15 : blanc
16 : ladder

3.1. Homozygosis/Heterozygosis

3.1.1. Generality

The truffles have a diploïd state at one moment of their annual cycle. After the meiosis, the half part of the ascopores produced contains the first allele of the gene we studied, and the other half contains the second allele of this gene.

We use the polymorphism of size of the SSRs for the genetic analysis of the truffles. For the analysis of one SSR :

- If the truffle is homozygote, only one allele is present. The analysis of the electrophoresis gel show only one smear.
- If the truffle is heterozygote, two alleles are present. The analysis of the electrophoresis gel show two smears.

3.1.2. Results

The analysis of the amplicon on the agarose electrophoresis gel for the SSR Tm22 shows the presence of only one smear for each truffle.
We conclude that the four truffles analyzed are homozygote for the SSR Tm22.

3.2. Studying the polymorphism of size of the SSR Tm22 on truffles

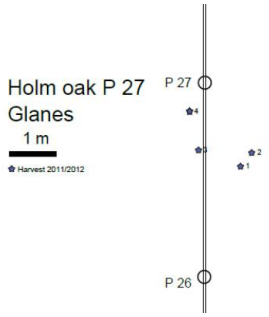
After the gel standardization by using the ladders, we realize a linear regression to obtain the equation : distance of smear migration = f($\log_{10}$(number of bases pairs), so we determine the size of the amplicon and after we calculate the number of repetitions of the Tm22's base sequence ($^{5'}$CCTCAT$^{3'}$) for each smear . Our results are presented in the follow table:

Reference of analysed truffle	Amplicon's size (bp)	Lenght of primers (pb)	Sequence size of Tm22 microsatellite (bp)	Number of basis repeated	Repetition number
	a	**b**	c	**d**	e
p27/1b	**399**	**39**	360	**6**	60
p27/2b	**397**		358		60
p27/3b	**387**		348		58
p27/4b	**367**		328		55
	c = a - **b**			e = c / **d**	

The result analysis shows the presence of three different SSR Tm22. For the truffles one and two, the SSR Tm 22 is repeated 60 times. For the truffles three and four this SSR is repeated respectively 58 and 55 times.
These harvested truffles at the bottom of the p27 tree present a polymorphism in their size, which enables us to tell the difference between these three truffles lineage for this SSR Tm22.

3.3. Geographic repartition of the truffles under the p27 tree

In 2012, we have realized the cartography of the harvested truffles under the p27 tree. When we report our result on this map, we can observe that the truffles one and two are relatively close, while the truffles three and four are far from each other and geographically opposed to the truffles one and two.



4. Conclusion

This first results shows :

- The four truffles analyzed are homozygote for the SSR Tm22,
- The existence of a genetic polymorphism in the species *Tuber melanosporum*. This polymorphism can be used to follow the evolution of the truffle genome in the burned-out area in space and in time.

In the future, we will try to :

- Improve these results by using other SSRs and check the reproducibility of these experiments,
- Analyze the truffle production of other trees (n35 and n37), analyze a new truffle production.