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Microsattelites assisted selection for yellow rust resistance in Algerian bread wheat *(Triticum aestivum* L.)

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Dedication

We dedicate this humble work to both our families, parents especially, for all the sacrifices they went through in order for them to get us here.

To my parents Abdelouahab and Habiba for supporting me through the whole journey without giving me any kind of pressure, for always being supportive no matter what I do, to let me make my own decisions, to my sister Hiba for always having my back and guiding me whenever I feel lost, To my grandma who took me to school for the first time and always looked after me Rbiha, to my friends.

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Mouad L.

Abstract

Wheat is currently one of the most important cultivated crops, considering its contribution in the global economy and food security; it is also one of the most consumed cereal crops in Algeria. In the year 2004, the eastern region of Algeria had a considerable damage in wheat fields due to yellow rust that is caused by a fungus called *Puccinia striiformis*. Leading to taking serious measures regarding that disease, especially research and wheat development. In this study, we aimed to find the resistance genes in seven Algerian bread wheat varieties, three sensitive and four resistant. The research was conducted using eight microsatellite markers, of which we can mention Wmc44, Barc119, and Wms533…etc. Using a CTAB DNA extraction method then followed by PCR amplifications and an electrophoresis migration. The results we obtained indicated that the markers did show polymorphism in multiple occasions, however after the PIC calculation and establishing a dendrogram, we have to conclude that the markers revealed different random wheat characteristics, although there were not clear links to the yellow rust resistance.

Key words: microsatellite, yellow rust, wheat, marker assisted selection and resistance.

الملخص

يعتبر القمح من أهم المحاصيل الزراعية في العالم، نظرا لمساهمته الاقتصادية واهميته لتحقيق الأمن الغذائي. القمح من أكثر محاصيل الحبوب الأكثر استهلاكا في الجزائر. في عام 2004، تعرضت المنطقة الشرقية من الجزائر لأضرار جسيمة في حقول القمح بسبب الصدأ الأصفر الناجم عن عدوى فطرية *Puccinia striiformis*. مما اوجب اتخاذ تدابير وقائية تجاه هذا المرض، ومحاولة ايجاد حلول بالبحث العلمي. كان الغرض الاساسي من هذه الدراسة هو إيجاد جينات المقاومة في 7 أصناف من القمح اللين الجزائري، 3 منها حساسة اتجاه الصدأ الاصفر و4 مقاومة. تم إجراء البحث باستخدام 8 من مؤشرات التتابعات المتكررةSSR، نذكر منها Wmc44، Barc119، Wms533 ... إلخ. تم استخراج الحمض النووي بتقنية CTAB متبوعة ب PCR ثم هجرة كهربائية. أشارت النتائج التي حصلنا عليها إلى أن المؤشرات ذات تنوع مورفولوجي، ولكن بعد حساب PIC وتحليل مخطط القرابة، ورغم أن المؤشرات اظهرت أليلات عشوائية مختلفة، الا اننا لم نتوصل الى وجود روابط واضحة بين النتائج المحصل عليها وجينات مقاومة الصدأ الأصفر.

الكلمات المفتاحية: المؤشرات الوراثية المتكررة، القمح، الصدأ الأصفر، الانتقاء باستخدام المؤشرات الجزيئية، مقاومة الامراض.

Résumé

Le blé est actuellement l'une des cultures cultivées les plus importantes, compte tenu de sa contribution économique à la sécurité alimentaire mondiale. C'est l'une des cultures des céréales les plus dominantes en Algérie. En 2004, la région de l'Est algérien a subi des dégâts considérables dans les champs de blé tendre en raison de la rouille jaune causée par un champignon appelé *Puccinia striiformis*. Conduisant à prendre des mesures sérieuses concernant cette maladie, en particulier la recherche et le développement de nouvelles variétés du blé tendre. Dans cette étude, nous avons cherché à trouver les gènes de résistance dans 7 variétés de blé panifiable algérien, 3 sensibles et 4 résistantes. La recherche a été menée à l'aide de 8 marqueurs Microsatellites, parmi lesquels on peut citer, Wmc44, Barc119, Wms533...etc. A l'aide d'une méthode d'extraction d'ADN CTAB, suivie par amplification d'ADN par PCR, et puis d'une migration par électrophorèse. Les résultats que nous avons obtenus ont indiqué que les marqueurs présentaient un polymorphisme à plusieurs reprises, mais après le calcul du «PIC» et l'établissement du dendrogramme, nous avons conclu que les marqueurs utilisés révélaient différentes caractéristiques aléatoires chez les 7 génotypes du blé tendre étudiés, bien qu'il n'y ait pas de liens clairs avec la résistance à la rouille jaune.

Mots clés : Microsatellite, Rouille jaune, Blé, Sélection assistée par marqueurs et Résistance.

Abbreviations

- **A** : Anthesis
- **AFLP**: Amplified fragment length polymorphism
- **Bc**: Before christ
- **BGF**: Biggining of grainfilling period
- **bp**: Base pair
- **CF**: Final Cincentration
- **Cimmyt**: The International Maize and Wheat Improvement Center
- **CNCC** : Le Centre National de Contrôle et de Certification des semences
- **CTAB**: Cetyl trimethyl ammonium bromide)
- **DR** : Double ridge appearance
- **E** : Emergence
- **F**: Forward
- **G** : Germination
- **GS** : Browth stage
- **HST** : Host specific toxins
- **ID**: Identity
- **INRAA**: L' institut National de la Recherche Agronomique d'Algérie.
- **ITGC**: L'Institut Technique des Grandes Cultures
- **NCBI**: The National Center for Biotechnology Information
- **PCR**: polymerase chain reaction
- **PIC :** Polymorphism information content
- **PM** : Bhysiological maturity
- **PNAB**: National wheat improvement program
- **R**: Reward
- **RAPD**: Random amplified polymorphic DNA
- **RFLP**: Restriction fragment length polymorphism
- **R-gene:** Resistance gene
- **rpm**: Revolutions Per Minute
- **S**: Sowing
- **SSR** : Single sequence repeated
- **TE**: -EDTA (Ethylenediamine Tetraacetic Acid; buffered solution)
- **TS** : Terminal spikelet initiation

- **UV**: Ultra violet
- **V**: Volume
- **Akh**: Akhamoukh
- **Ben**: Benmabrouk
- **Bou**: Boumerzoug
- **Hd:** Hidhab
- **Mas**: Massine
- **Mo**: Morocco
- **Tid**: Tidis

Table of contents

List of figures

List of tables

Annexes List

Introduction

Introduction

Wheat is one of the very first vegetation to be exploited by humans in multiple fields, going from their own consumption as it can be converted to a variety of nutritional products, to rearing cattle. It has a long rich history of spreading and evolving regarding its adaptation to each area of the world it settled at in a defined period.

Wheat becoming an integral part of the present diets on a global spectrum, many countries across the world have adopted not only it's cultivating but also developing it into varieties of better quality in terms of production quantities, biotic and abiotic stress resistance and nutritional value. in time, there was an appearance to organizations and centers aiming for that purpose, most of them were resulted by the collective, collaborative efforts of multiple countries, from which we can mention «International maize and wheat development center» CIMMYT, that has research projects in over 50 countries around the world (CIMMYT, 2021).

Algeria is a very good example for a North African country that is highly reliant on cereals and wheat in specific for its population's nutritional consumption. The country is considered the largest in Africa by a land that covers over 2 million square kilometers, with 80% of that being a desert occupying most of the middle and the whole south, and proving to be very much of a hindrance in the way of agriculture in general, pushing it towards the north. Almost half of the agriculture (40% of it) is cereal based, annually covering 3 to 3.5 million ha of the total land dedicated to agriculture only 245,000 ha of it is irrigated (Benbelkacem, 2014). Leading the country to be very dependent on imports by 45% of its food consumption and being unable to achieve a complete food security (Benbelkacem, 2014).

It is correct that their contribution is neither as sufficient nor significant as it's needed to be, but both agriculture and cereal development research against stresses are still crucial for the overall improvement of the lastly mentioned numbers. For that, since independence 1962, Algeria gave great importance to agricultural research and cereal development in the pursuit of eliminating that deficit in wheat production.

Researchers have settled development programs aiming for the selection of wheat varieties most adapted to the local climate to begin with and then are using methods

and strategies to create optimal varieties, resistant to common diseases in the area such as rusts. Creating these varieties could be aided by classic selection methods and the collected genetic data of both the disease and wheat, especially that wheat's genome has been completely sequenced in 2018, combining these efforts with the use of molecular and biotechnological tools such as molecular marker, it is not far to witness unimaginable progress in the very near future.

The purpose we're pursuing and aiming to achieve from this study, can be described as the detection of yellow rust resistance genes in a number of Algerian bead wheat varieties (*Triticum aestivum* L.), then comparing our results to earlier published works.

Chapter I : Literature review

I. Wheat

1. History and origin of wheat

1.1. Geographical history of wheat

For one to discuss the geographical journey wheat had pathed throughout history, the Fertile Crescent ought to be mentioned. It is where Archaeologists agree with evidence that agriculture in general, including wheat agriculture, has first began around 9000 and 7000BC (Murray, 1970; Bell 1987). It's domestication and cultivation however, started between 7500-6500BC as (Feldman 1976; Bell 1987) stated and indicated some cultivated wild varieties that existed in the fertile crescent back in that era, worth mentioning : *Triticum boeoticum, T. dicoccoides and T. araraticum* (Bell 1987).

wheat then encountered a movement of spreading towards the southeastern Europe with the appearance of farmers, declaring the beginning to the European farming in Greece around 6000BC (Murray, 1970; Bell 1987), the spreading of agriculture from Greece to Europe was mainly through the Initial Colonization Cultures - the Starcevo-Koros resulting an important distribution of it.

In the other side of the world wheat agriculture was introduced to China and east Asia around 7000BC, and The only part of Africa to have it around that time was Egypt due to the close interaction with Asia and fertile crescent, whilst to rest of Africa followed roughly after 3000BC (Bell 1987).

1.2. Genetic origin and botanical description of bread wheat

1.2.1. Genetic structure and origin

Wheat also known as *Triticum* classified into three types based on their ploidy level:

1- Diploid with 14 chromosomes (einkorn wheat).
2- Tetraploid with 28 chromosomes (emmer wheat).
3- Hexaploid with 42 chromosomes (bread wheat).

This distinction led to new classification of wheat genomes (A, B and D) which is the classification used nowadays (Benjamin and *al.*, 2010).

According to Haider (2013), *Triticum aestivum* (commonly known as bread wheat) is a hexaploid with the 3 different genomes (**AABBDD**) that was resulted from

hybridizations that at least one of them includes *Agilops* (DD) and the tetraploid *T. turgidum* (AABB).

A. Origin of A-genome

The specie named *T. urartu* is a diploid (2n = 14, AA) that is believed to be the first origin of this genome, and that includes the A genome founded in *T. turgidum* that we mentioned earlier (Haider, 2013).

B. Origin of B-genome

According to Haider (2013), the origin of this genome was unknown, until 2018, where a transcriptomic study has highly suspected that the B-genome actually originated from the *S* genome of the species *Ae. speltoides* (Yuka Miki and *al.*, 2019).

C. Origin of D-genome

The very first donor of genome D is believed to be *Aegilops tauschii*, which was crossed with *Triticum dicoccum* creating the very first hexaploid (Grine, 2015). (Luo and *al.*, 2017).

1.2.2. Botanical classification

Bread wheat (T*riticum aestivum* L.), or typically called common wheat, is an autogamous, mid-tall, annual cereal of the Gramineae (Poaceae) family (Bálint et *al.*, 2000*)*. It has been classified based on its morphology and have been proposed by multiple authors (Kornicke, 1885 in Grignac, 1965; Dalhgreen et Clifford, 1985; Sanah, 2015) then by geographical origin, like Vavilov.

Table 1 : Taxonomic hierarchy for bread wheat
(https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=42237#null)

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridi plantae – green plants
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida Superorder Lilianae – monocots, monocotyledons, monocotylédones
Order	Poales Family Poaceae – grasses, graminées
Genus	*Triticum* L. – wheat
Species	*Triticum aestivum* L. – common wheat, wheat

2. Wheat characteristics

2.1. Wheat morphology

The plant can be morphologically divided into six main parts.

2.1.1. The stem

A central stem is made up of repeating segments, called phytomers and from which leaves emerge (Thell, 2008).

2.1.2. The leaf

The leaf is made of the sheath and leaf blade, these two are joined at the leaf blade base where the ligules and auricles are situated (Kirby, 2002; Thell, 2008). Leaves are organized in an alternative and opposite manner on the sides of the stem, then are numbered evenly on each side (Setter & Carlton 2000; Thell 2008) (*fig. 1*).
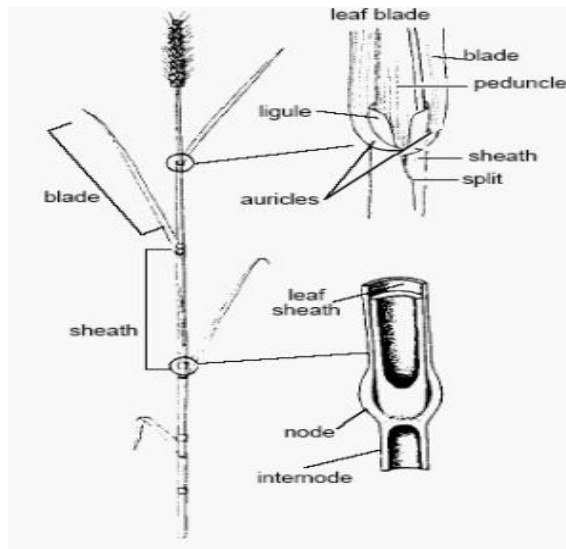
Figure 1: The stem and leaf structure (Setter & Carlton 2000; Thell 2008)

2.1.3. Tillers

From the main stem, emerge lateral branches which we call Tillers (Kirby2002; Thell 2008). Exactly like the main stem, they also produce leaves on opposite sides and, ears at their terminal (Setter & Carlton 2000; Thell 2008).

2.1.4. The roots

There are two types of roots, seminal which are the first emerge with the grain, then the nodal, that emerge at the tillers development (Kirby 2002; Thell 2008).

2.1.5. The ear

It consists of two rows of spikelets that contain florets and have a similar arrangement to the leaves. These florets are composed of the carpel and three stamen and anthers (Setter & Carlton 2000; Thell 2008) (*fig. 2*).
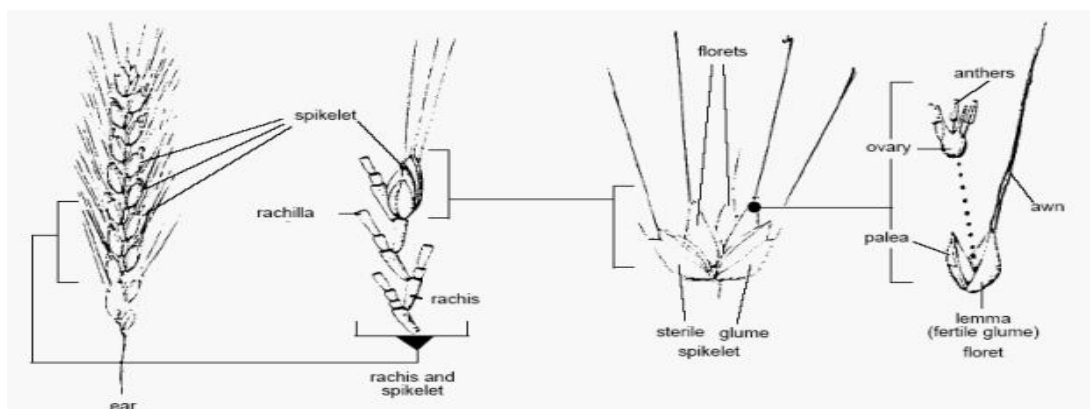
Figure 2: The structure of spikelets and florets in wheat ear (Setter & Carlton 2000; Thell 2008)

2.1.6. The caryopsis

The caryopsis is the wheat grain, which is made of three parts (Thell, 2008):

- **Bran coat:** It is outer shell of the wheat grain and makes up around 14.5 percent of its weight. It consists of trace minerals and a little protein, but primarily insoluble fiber.

- **Endosperm:** This is the bulk of the grain, about 83 percent of its total weight. The endosperm stores the starch and protein important to plant development (Setter & Carlton, 2000; Thell, 2008). It is also a source of soluble fiber.

- **Germ :** the embryo makes only 2.5% of the total weight, yet contain the root radical and the shoot apex, all surrounded by coleoptile(Setter & Carlton, 2000; Thell, 2008).

2.2. Wheat development phases

2.2.1. Germination to emergence (E)

For the grain to germ it needs to first, contain amounts of water equivalent to 35-45% of its weight (Evan et *al.*, 1975; Curtis, 2002), then a temperature between 4° and 37°C, bigger seeds are advantageous in terms of growth and number of fertile tillers (Spilde, 1989; Curtis, 2002). They also gave better results when grown under stresses (Mian and Nafziger, 1994; Curtis, 2002). During germination, the seminal roots are the first to show, then the coleoptile that protects the emergence of the first leaf (Kirby, 1993; Curtis 2002) (*fig. 3*).
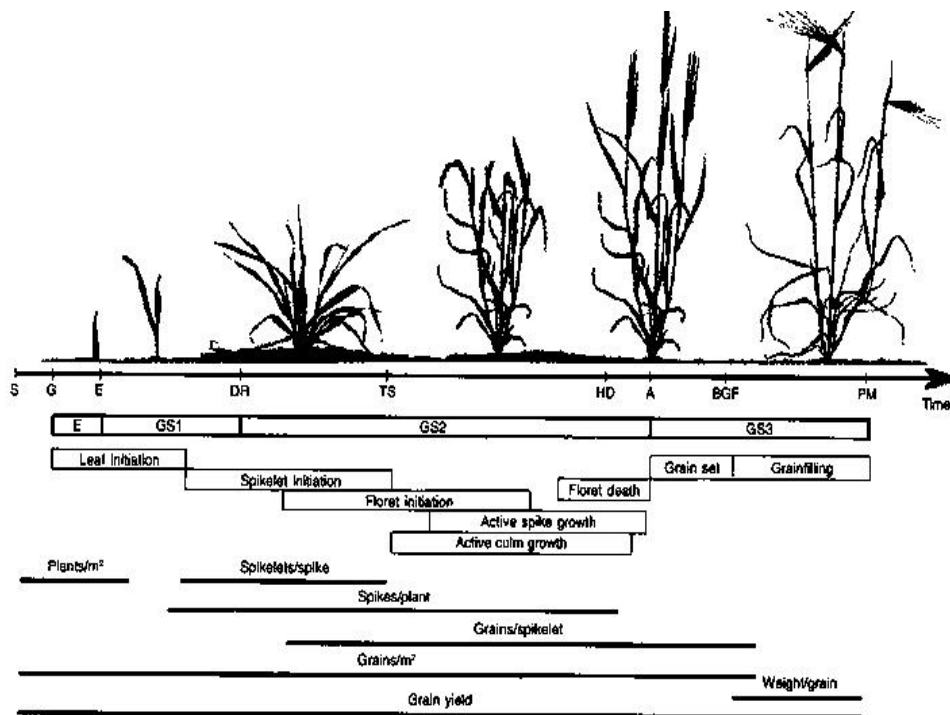
Figure 3: Wheat growth and developement stages (Slafer and Rawson, 1994; Curtis, 2002).

2.2.2. Emergence to double ridge (GS1)

It is where the bud differentiation into tillers begins then wheat tillers appear and grow from the axil of the main shoot leaves (Baker and Gallagher, 1983; Curtis, 2002). The usual number of fertile tillers per plant in favorable conditions is around one and one-half, even with that, not all tillers produce spikes and many abort before anthesis (Gallagher and Biscoe, 1978; Curtis, 2002).

GS1 is also called the vegetative stage, and varies in duration from 60 to 150 days all depending on leaf appearance rates, and occurrence of floral differentiation, which are cause by vernalization and photoperiod (Curtis, 2002).

- **Vernalization**

It is dedicated to wheats which are responsive to it, and its results (flowering phase) only show after the chilling conditions are met. It differs in the two main flowering wheat type's winter-type wheats and spring-type wheats (Flood and Halloran, 1986; Curtis, 2002).

Vernalization can occur in these three growth stages: germination, vegetative phase and seed formation in mother plant (Flood and Halloran, 1986; Curtis, 2002). In temperatures differing from spring-type at 7°C to 18°C, and winter-type at 0°C to 7°C

(Evans et *al*., 1975; Curtis, 2002), it has been observed that vernalization stimulates cell divisions in winter-types (Manupeerapan et *al*., 1992; Curtis, 2002).

- **Photoperiod**

Occurs after vernalization, in plants that need lengthy day times to flower, most cultivated wheats flower faster as the longer the day goes, however, they day length isn't a crucial factor to induce flowering (Evans et *al*.,1975; Major and Kiniry,1991; Curtis, 2002).

Usually one single leaf is all it requires to sense photoperiod and induce flowering, and once the photoperiod ends, floral induction follows right after, and initiates the reproductive stage (Major, 1980; Boyd, 1986; Curtis, 2002).

Photoperiod, vernalization, and adaptive mechanisms to environmental changes.

2.2.3. Floral initiation (double ridge) to anthesis (GS2)

Wheat plants have 4-8 leaves in the main shoot when the growing apex changes from the vegetative to the reproductive stage. The glume and lemma primordium stages follow. Complete sterility may happen if the temperature surpasses 30°C during floret formation (Owen, 1971; Saini and Aspinal, 1982; Curtis, 2002). Each spikelet up to 12 floret primordia in the center of the spike. The basal and distal spikelets have from six to eight florets, and roughly less than half of these florets complete anthesis, while the rest abort. (Kirby, 1988; Kirby and Appleyard, 1987; Hay and Kirby, 1991; Curtis, 2002).

In this stage, the terminal spikelet formation happens, where the number of spikelets per spike is determined varying from 20 to 30 (Allison and Daynard, 1976; Curtis, 2002). Once formed the spike growth stage follows, with elongation in the stem and the appearance of the leaf prior to the flag leaf (Kirby and Appleyard, 1987; Curtis, 2002). The spike growth begins slowly then greatly increases in speed once the ligule of the flag leaf shows. (Kruum et *al*., 1990; Curtis, 2002).

2.2.4. Anthesis to physiological maturity (GS3)

The anthesis is initiated in the central part of the spike then towards the basal and apical parts. A spike consists of one sole spikelet per rachis node and a spikelet has 3-6 potentially fertile florets (Kirby and Appleyard, 1984; Curtis, 2002).

Cellular division happens at high rate after floret fertilization, here happens the formation the endosperm cells and amyloplast, known as the lag phase, the phase that follows is the phase of cell growth and differentiation and starch deposition in the endosperm. Both the last phases are the one responsible for grainfilling period, while the embryo is formed at the time of endosperm growth (Jones et *al*., 1985; Curtis, 2002).

3. Wheat diseases in Algeria

Like all living organisms, wheat is also threatened by different kinds of pathogens (bacteria, viruses and fungus) that can cause massive economic losses.

The most common wheat diseases in Algeria such as Oidium, Septoria and Rusts are mainly caused by mycosis. These diseases can cause severe losses to not only the Algerian economy but also the completely global economy in general. Since these diseases such as Septoria that affected lot of wheat fields in North Dakota in the United States of America (Andrew and Zhaohui, 2021). Or like yellow rust which is very common wheat disease especially in Asian countries such as : Pakistan which is one of the ten largest exporters of wheat, with a volume of 25.247 thousand tons produced 2020 (FAO, accessed on June 10, 2022), 2.88% of total wheat crop in Punjab-Pakistan was infected by yellow rust (Uferah Shafi and *al.,* 2022).

Some of these diseases can travel thousands of miles by the wind that makes it difficult to control their spreading.

3.1. Oidium

A disease that affects both *Triticum durum* Desf. and *Triticum aestivum* L*.,* mainly caused by a fungus known as *Blumeria graminis***.**

Oidium also known as powdering mildew may cause severe losses in the field specifically on *Triticum durum* when it is cultivated in Mediterranean conditions, which is considered the most favorable agroclimatic condition for this pathogen.

The powdering mildew can show up on leaves as white spots (*fig. 4*). Germination may happen when humidity level is relatively high with a lack of water in its liquid form (Tomas and Solis, 2000).

Figure 4: Powdering mildew spots on infected wheat leaf

(https://agrichem.dz/detailfleu/62/l-oidium-du-ble-et-de-l-orge/)

3.2. Septoria

Septoria can be caused by two main pathogens *Septoria tritici* and *Septoria nodorum*. Septoria can cause annual yield losses around the globe. This fungal pathogen produces at least three toxins called HST that causes yellow and brown spots on leaves (*fig. 5*).

Figure 5: Septoria infection symptom on wheat leaves.

(https://fr.wikipedia.org/wiki/Septoria#/media/Fichier:Septoria-tritici.jpg)

Some spots can grow and appear as a tan, the spots however show up as a brown to dark small spots surrounded by bigger yellow spots, other common symptoms can appear on the seed coat, a symptom commonly known by American farmers as "the red smudge" (*fig. 6*).

Spores grow by spring, travel through the wind, and finally gets attached to wheat plants to start a new infection (Andrew and Zhaohui, 2021).

Figure 6: Red smudge infection in wheat grains.

(https://www.topcropmanager.com/managing-red-smudge-in-durum-wheat-1459/)

(https://webapp.agron.ksu.edu/agr_social/m_eu_article.throck?article_id=612)

(https://www.apsnet.org/edcenter/disandpath/fungalasco/pdlessons/Pages/TanSpot.aspx)

3.3. Rusts

Rust refers to a group of diseases caused by fungal infection by a species called *Puccinia* sp which is a pathogen fungus that affects wheat crops.

Rust is one of the most significant diseases of wheat, its ability to move long distances and its high virulence to multiple varieties makes it hard to manage its spreading.

There are mainly three kinds of wheat rusts depending on which *Puccinia* species that causes the infection:

3.3.1. Black rust (Stem rust)

Caused by *Puccinia graminis,* has been one of the farmer's worst nightmares since decades. Thankfully, it does not show up so frequently, but recently it was recorded in Ireland for the first time in decades (Ayako and *al*., 2021).

According to Singh et *al*., 2011, the highly virulent race Ug99 showed up in Uganda in the ear 1998, a specie that threatened 80% of the world's wheat varieties which were all vulnerable to this one race (in: Ayako and *al*., 2021).

The reappearance of this disease in Ireland after all these years, and in multiple locations makes the Irish farmers anxious and makes the whole world's crops under the threat, for such matters wheat breeders are always seeking to develop new resistant varieties to prevent such threats in future.

Favorable conditions for this pathogens growth are in wet warm seasons, with temperature from 15° to 35°C, symptoms show up as brown to black stains on the stems and leaves (mostly on stems). Infection usually ends up by destroying the whole

plant few weeks before the harvest by breaking the stems and shriveling the grains (Aqsa and *al*., 2018) (*fig. 7*).

Figure 7: Wheat stem infected by Black rust

(https://www.natureasia.com/en/nmiddleeast/article/10.1038/nmiddleeast.2017.154)

3.3.2. Brown rust (Leaf rust)

Appears due to an infection by *Puccinia triticina* and can cause some serious yield losses, easily detected by oval or circular orange pustules that shows up on the surface of the infected leaves. Favorable temperature for this fungus is between 10°C to 30°C (Aqsa and *al.*, 2018) (*fig. 8*).

Figure 8: Leaf rust symptoms on *Triticum.*

(https://www.cropscience.bayer.us/learning-center/articles/wheat-rust-diseases)

3.3.3. Yellow rust (Stripe rust)

One of the most damaging wheat diseases mainly caused by *Puccinia striiformis*, widely spread threatening crops around the world, Stripe rust of wheat has been reported in more than 60 countries and in all continents except Antarctica (Chen, 2005 in: Alma and *al.,* 2021).

In the year 2004, the eastern region of Algeria had a considerable damage in wheat fields, especially to the HD1220 variety, that turned out to be highly sensitive to yellow rust. Since the yield of affected crops reached 70 to 100% of this particular

variety (Zaidi, 2018), which makes it one of the most dangerous diseases that farmers and breeders have to deal with.

When *Puccinia striiformis* infects the wheat spores start to appear on the leaves as yellow to orange stains. The spore's size is around 20μm to 30μm, yet they have the capacity to cause a complete destruction to the economy and even the ecosystem.

The infectious fungus that spreads quickly across continents has been a threat to wheat crops since 8000 years ago (Robert, 2009). The favorable conditions for this biotrophic mycosis are cool temperature (7°C to 12°C) and moisture.

A *Puccinia* first starts as uredinio-spores carried by the air. After finding a host, the infection starts by formation of vessels from the attached uredinio-spore that penetrate the plant tissue to reach inside the cells and absorb the nutriments needed for its multiplication. More spores will be produced and they will erupt from the surface of the leaf, the spreading of spores will eventually show up as an elongated chlorotic or necrotic spots (*fig. 9*).
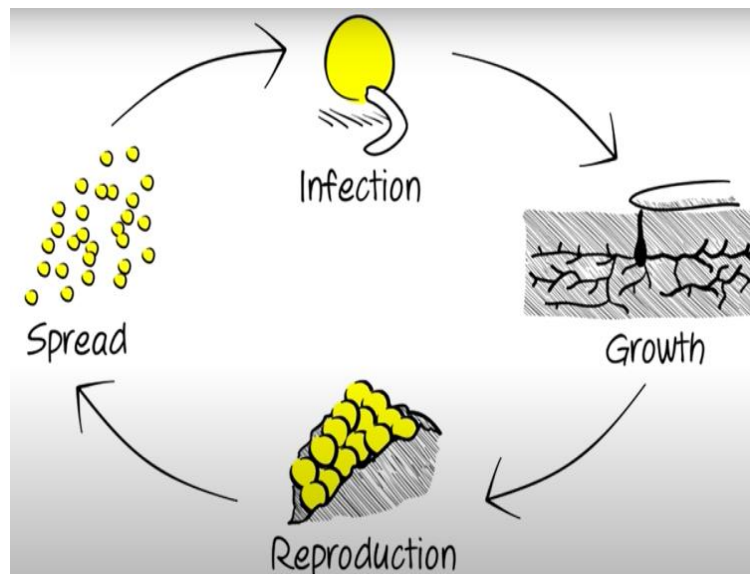
Figure 9: Diagram shows how yellow rust spreads.
(https://www.youtube.com/watch?v=raIIBFrcLuQ)

Germination can take 3h, first symptoms appears 6-8 days after the infection (*fig. 10*). Sporulation however happens 12 to 14 days after infection (Wamquan et *al.*, 2014), thus farmers have to keep on checking their crops, if any symptoms showed up it would be better to start using fungicides to limit its spreading (Ciara Beard and *al.*, 2021).

Figure 10: Yellow rust infection spotted in *Triticum aestivum* L. (Benmabrouk variety,. Baaraouia El-Khroub 2022).

II. Molecular markers

A molecular marker is a DNA fragment that is used to detect a specific genes in a large genome, mainly through PCR amplification, which is essential processes for disease detection, phylogeny researches, genome mapping, resistance and sensitivity toward biotic stresses, tolerance of abiotic stresses, diversity analysis… etc.

Nowadays the fields that we mentioned previously are very crucial for a modern sustainable agronomic development, such as developing new plants verities that can resist diseases, tolerate the climatic stresses, and provide good quality and quantity.

1. Types of molecular markers

There are two main categories of molecular markers:

1.1. Dominant markers

1.1.1. RAPD Markers

Short for Random Amplified Polymorphic DNA, (also known as RAPID). These markers does not require any knowledge of the targeted DNA, which is technically a random DNA amplification. RAPD markers are mainly used for genetic diversity analysis (NCBI, 2022).

1.1.2. AFLP Markers

The Amplified Fragment Length polymorphism is a type of markers that relays on the enzymes restriction site polymorphism. AFLP markers can be used in fingerprinting, varieties identification and phylogeny, genome mapping, cloning (Grine, 2015).

1.2. Co-dominant markers

1.2.1. RFLP Markers

Restriction Fragment Length Polymorphism is a molecular marker specific to a single clone restriction enzyme combination (NCBI, 2022).

These markers can reveal the variation size or length of the DNA fragment resulted from a restriction enzyme digestion. RFLP markers can be used to follow the DNA inheritance through families (Shurjo, 2022).

1.2.2. SSR Markers (Microsatellites)

SSR or Single Sequence Repeated is DNA sequence that contains a repeated 1-6 combination of nucleotides. Microsatellites are single locus markers, that are highly polymorphic and co-dominant that can distinguish between a homozygote and heterozygote individuals, which makes them (SSR markers) very useful in many researches such as diseases resistance (Grine, 2015), genetic adaptation and genetic diversity (Gueraiche, 2016), analyzing varieties characteristics (Nouar and Benabdelkader, 2018) and population diversity (Houmer, 2021).

Chapter II: Material and Methods

I. Vegetal material and collection sites

In our selection of bread wheat (*Triticum aestivum* L.) varieties needed for the study, we took an approach based on three main steps, the first was checking online to make a list of accessible near varieties (sensitive & resistant to yellow rust), «Le bulletin des variétés de Céréals autogames» CNCC, 2018 was of great help in that.

The second was contacting **Mr. Abdelkader Benbelkacem** (in charge of the national wheat improvement program**) (**PNAB)**,** who guided us in their disease experiment site in INRAA, El Baaraouia, El Khroub, where we visually analyzed for the existence of yellow rust patterns and ended up picking samples of 7 different varieties (some have symptoms and others intact) (*fig. 11*).

The last step was a detailed data collection for each variety and which can be summarized in (table.2).

Figure 11: Picturing the visual analysis, before picking samples (Baaraouia-Elkhroub-15 Mai 2022).

A: Intact sample for the variety Tidis in field (intact);

B: Intact sample for the variety Massine in field (intact);

C: The presence of apparent yellow rust patterns in a Benmabrouk leaf;

D: Intact sample for the variety Boumerzoug;

E: The presence of slight patterns of yellow rust in a Morocco sample.

Table 2: Data concerning the selected varieties

Variety name	Origine	Pedigree	Yellow rust resistance	Reference
Akhamokh	CIMMYT (Mexico)	IRENA/BABAX//PASTOR	Resistant	Ayadi, 2019
Benmabrouk	ITGC	A local native variety that belongs to the southern oases	Highly sensitive	Salemi, 2018
Boumerzoug	CIMMYT (Mexico)	KAUZ/PASTOR	Resistant	Zaidi & *al*., 2018
Hidhab (HD1220)	CIMMYT (Mexico)	D1220/*Kal/NalCM 40454	Sensitive	Zaidi & *al*., 2018
Massine	CIMMYT (Mexico)	PFAU/SERI-82//(SIB)BOBWHITE	Resistant	Zaidi & *al*., 2018
Morocco	CIMMYT	Moroccan origine	Sensitive	Zaidi & *al*., 2018
Tidis	CIMMYT (Mexico)	BUCKBUCK/FLICKER//MYNA/VULTURE	Resistant	Zaidi & *al*., 2018

The collected samples were soaked in liquid nitrogen then were kept in -80C° for conservation purposes.

II. DNA Extraction and SSR markers analysis

1. DNA extraction Protocol

The method applied in this extraction was based on a modified CTAB taking the steps that follows:

- Preheating the buffer **CTAB x2 with added Beta-Mercaptoethanol** in a water bath at 65C°
- Cryogenic grinding in liquid nitrogen with mortar and pestle to turn the vegetal tissue into fine dust
- Transferring the dust of each sample to Eppendorf tubes then numbering them with two repetition for each sample based on a varietal coding.
- Add **900µl** of **CTAB x2 with added Beta-Mercaptoethanol** preheated in a water bath at 65C°, in each tube

- Use a vortex to homogenize the tube contents
- 60min of incubation in a water bath at 65C° with agitation every 10min
- Centrifuge for 15min at 10000 rpm at 4C°
- Capturing 800µl of the supernatant into a new set of tubes (eppendorf 2ml tubes) and conserving the old tubes away from light at 4C° (for backup cases)
- Add 800µl of **Chloroform/isoamyl (24:1)**
- Agitate for 45min at low speed (100 to 150rpm) on an agitation table (caution for leaks)
- Centrifuge for 15min at 10000rpm at 4C°
- Retrieve the upper aqueous phase using a micropipette P1000 and place it into new eppendorf tubes
- Re-add 800µl of **Chloroform/isoamyl (24:1)**
- Agitate for 45min at low speed (100 to 150rpm) on an agitation table (caution for leaks)
- Centrifuge for 15min at 10000rpm at 4C°
- Retrieve the upper aqueous phase using micropipette 1000p and place it in new Eppendorf tubes (avoid retrieving the white layer in the middle)
- Add 3 to 5µl of RNase A (10mg/ml), agitate then incubate for 30min at 37C°
- Add 200 to 400µl (2/3Vol) of **cold Isopropanol** (-20C°)
- Inverse the tubes gently until a white residuum appears
- Leave to precipitate at 20C° for 5 to 10min
- Centrifuge for 10min at 10000rpm at 4C°
- Delicately eliminate the supernatant
- Add 500µl of the **washing solution 1**
- Incubate for 15min in an ambient temperature
- Centrifuge 5min at 10000rpm at 4°
- Delicately Eliminate the supernatant 500 and add 500ul of **washing solution 2**
- Do not incubate for more than 5min.
- Centrifuge 5min at 10000rpm at 4°
- Eliminate the supernatant and dry the DNA in free air or using DNA Speedvac
- Suspend the DNA residuum in 50µl of **TE 0.1 X**
- Store the DNA for a few hours at 4°C

2. DNA quantification and quality test

- **DNA quantification:**

We incubated the tubes for 10min at 60C° then passed them by a short centrifugation and finally used the NanoDrop2000 machine to determine the exact amount of DNA in 2μl from each tube.

- **Quality test:**

We prepare an agarose gel 0.8%, then set it with the samples to an electrophoresis for 20min, then concluded by an analysis in the UV test chamber leaving us with the following results:

3. SSR Markers Analysis

To select the most suitable SSR markers for this study, we used related articles, and the *graingene* database.

We first went through each of the 82 existing yellow rust resistance genes in the database, and examined their details for the commonly used SSR markers associated to them, and had findings sorted in a list.

To continue, we analyzed the Map location of every marker in that list; and compared the distances between the marker and the yellow rust R-gene, then classified and filtered them according to that. Which left us with 14 good SSR markers. The following screenshots taken from the database clarify the approach we have taken systematically.

Screenshot 1: entry page for first step   **Screenshot 2:** Step 2

Screenshot 3: Step3

Locus Associated with Gene	Yr29		Locus for Probe	wmc44
Type	Trait		Type	Microsatellite
Chromosome	1B		Map	Wheat, Yr genes and QTL 1B
Chromosome Arm	1BL			Wheat-2014-Consensus-Ug99-Update_1B
Map	Wheat, Yr genes and QTL 1B			Wheat_Consensus_2014_Ug99_Rust_1B
	Wheat-Composite2004-1B			Wheat_Yr_2021-1B ⟵ (examining the distance between the SSR marker and Yr Rgene locations)
	[Show Nearby Loci]			[Show Nearby Loci]
In QTL	QYr.cim-1BL_Pastor (Lr46/Yr29) Yr29/Lr46		In QTL	QStsp.emmer2013-1B
Map Data	Wheat, Yr genes and QTL		Map Data	Wheat, Yr genes and QTL
Species	Triticum aestivum			Wheat, Yr Update, 2021
Probe	WMC44 ⟵ (SSR Marker)			Wheat-2014-Consensus-Ug99-Update
Linked QTL	Yr29/Lr46			Wheat_Consensus_2014_Ug99_Rust
Associated Gene	Yr29 (Triticum)		Probe	WMC44
Gene Class	Reaction to Puccinia striiformis Westend.		Possible Orthologs	Xwmc44
Reference	Lan CX et al. (2014) QTL characterization of resistance to leaf rust and stripe rust			Xwmc44-1B
Data Source	Soria, Marcelo A.			wmc44-1B
Remark	Trait marker Xwmc44-1B			
	Nearby marker on Composite map: Xwmc44			
	Position on Composite map estimated by GrainGenes.			

Screenshot 4: Step 4　　　　　　　　　　**Screenshot 5:** Step 5
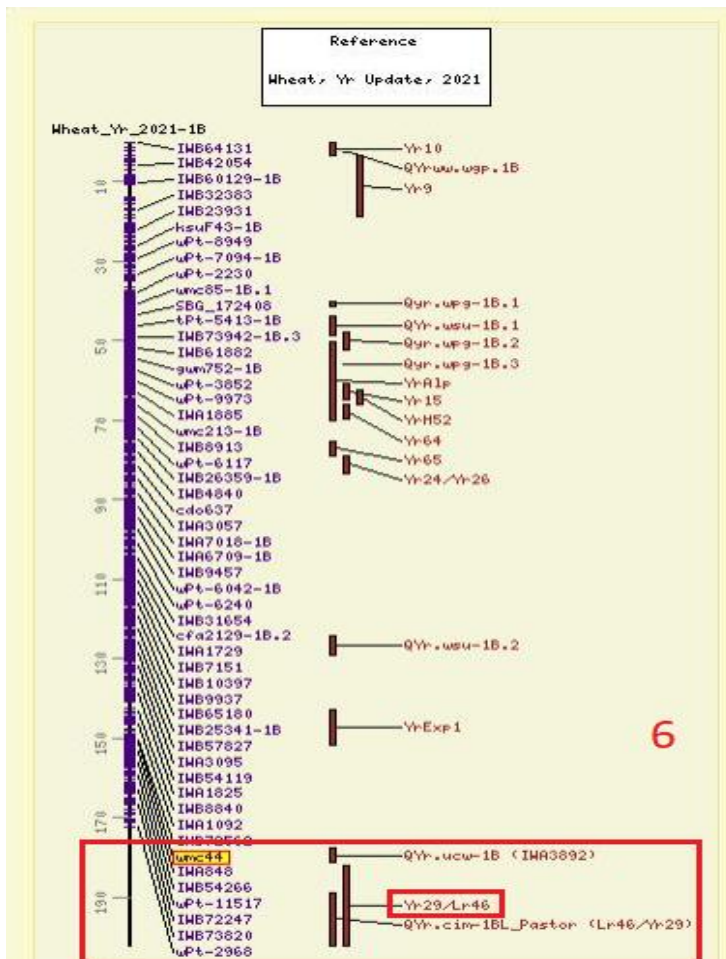
Screenshot 6: Step 6

The past screenshots represent the steps we took in order to examine yellow rust R-genes data in the database *graingene*, and analyzing their maps. (The red squares highlight the procedure of research).

Lastly, we took the list to the lab and picked the available markers which gave us a final list of the following eight SSR markers:

Table 3: List of the eight SSR markers selected for the study

SSR Markers	Primers	Resistance genes	Chromosome locus
GWM2 F	5' CTGCAAGCCTGTGATCAACT 3'	*Yr79*	3A
GWM2 R	5' CATTCTCAAATGATCGAACA 3'		
BARC8 F	5' GCGGGAATCATGCATAGGAAAACAGAA 3'	*Yr15*	1B
BARC8 R	5' GCGGGGGCGAAACATACACATAAAAACA 3'		
WMC44 F	5' GGTCTTCTGGGCTTTGATCCTG 3'	*Yr29*	1B
WMC44 R	5' TGTTGCTAGGGACCCGTAGTGG 3'		
BARC119 F	5' CACCCGATGATGAAAAT 3'	*Yr64*	1A
BARC119 R	5' GATGGCACAAGAAATGAT 3'		
WMS124 F	5' GCCATGGCTATCACCCAG 3'	*Yr29*	1B
WMS124 R	5' ACTGTTCGGTGCAATTTGAG 3'		
WMC488 F	5' AAAGCACAACCAGTTATGCCAC 3'	*Yrxy1*	7A
WMC488 R	5' GAACCATAGTCACATATCACGAGG 3'		
Wms533F	5' AAGGCGAATCAAACGGAATA 3'	*Yr-3b*	3B
Wms533R	5' GTTGCTTTAGGGGAAAAGCC 3'		
CFA2040 F	5' TCAAATGATTTCAGGTAACCACTA 3'	*Yr67, Yr52*	7A
CFA2040 R	5' TTCCTGATCCCACCAAACAT 3'		

III. PCR amplification and electrophoresis

1. PCR amplification

In order to reach faster results, increase specificity and avoid the redesigning of primers, we used three PCR programs, one of them was performed with the Touchdown technique allowing us to include two different annealing temperatures at once (61C° and 57C°).

To prepare for the PCR we had set a total 20µl volume of DNA, Primer and Master Mix mixture with concentrations based on table 4:

Table 4: PCR mixture dosage

Mixture	Volume (µl)	Concentration
Master Mix 2X	10	1X
Primer F	0,2	0.1-1µM
Primer R	0,2	0.1-1µM
Genomic DNA	4	20ng/µl
H_2O QSF 20µl	5,6	#

● **PCR program n°1 (Touchdown):** this program was dedicated to the markers: wmc488, cfa2040, wmc44, wmc533, wmc124

94°C 4mn
94°C 1mn
61°C 1mn   — 10 Cycles
72°C 1mn
94°C 1mn

57°C 1mn
72°C 1mn   — 30 Cycles
72°C 10mn

● **PCR program n°2:** the second was dedicated to : barc119, gwm2

94°C 4mn
94°C 1mn
51°C 1mn   — 35 Cycles
72°C 1mn
72°C 10mn

- **PCR program n◦3:** was solely for barc8

94°C 4mn
94°C 1mn
57°C 1mn
72°C 1mn
72°C 10mn

45 Cycles
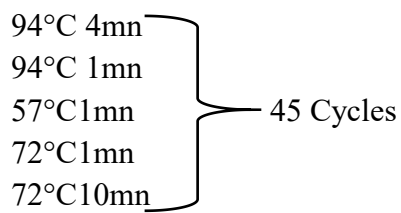
2. Electrophoresis

For the result analysis, we separated the samples on two groups and executed the migration of each group in different horizontal electrophoresis units.

The first was performed in a midi horizontal gel tank with a 3% agarose gel in 100V for 2h.

The second was realized in a mini horizontal get tank with a 1.5% agarose gel in 100V for 30min.

After the migration, each gel was exposed to UV in order to visualize the sample's migration with Ebox™ VX2 imaging system.

The PhotoCapt v12.6 software was used to process the images generated by the UV Ebox and measure the length of amplified bands.

Chapter III: Results and discussion

I. DNA quantification and purity test

1. DNA quantification

In order for us to homogenize extracted DNA concentrations between the samples, we needed to first determine the amounts of DNA obtained from each sample. For that, we used a NanoDrop2000 giving us the results in table 5. Then made the dilution calculations for each sample and noted the final concentration (100ng/µl).

Table 5: Dosage of DNA extracted from the samples and dilution results.

Sample ID	Nucleic Acid Concentration (ng/µl)	Ratio 260/280	Ratio 260/230	Final Concentration CF (ng/µl)
Akhamokh	841.3	1.85	1.97	100
benmabrouk	704.3	1.89	2.03	100
Boumerzoug	1235	1.92	1.93	100
Hidhab	1758.3	1.85	1.86	100
Massine	2919.1	1.80	1.72	100
Morocco	1330.9	1.90	1.90	100
Tidis	350.8	1.87	1.97	100

The results show that we have extracted sufficient amount of DNA for the PCR experiment.

Absorbance ratio (260/280) allows us to know if there is a protein contamination (Values between 1.8 and 2 means that there is no contamination), in this case, the ratio varies between 1.85 and 1.9 that indicates that the samples are not contaminated.

The 230/260 ratio however is used measure the nucleic acids purity (compared to phenolic compounds). According to Desjardins and Conklin, (2010) if there is no contamination in samples the ratio 260/230 should be around 1.8 to 2.2 (In: Nouar and Benabdelkader, 2018), by comparison to our samples we can deduce they are pure (1.9-2.03), except for Massine where there was a slight contamination.

2. DNA purity test
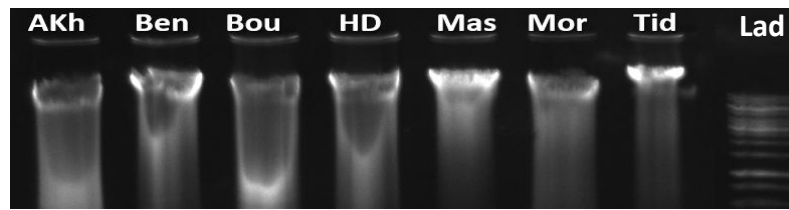
Figure 12: Pre-diluted Extracted DNA analysis for seven varieties of *Triticum aestivum* L. with electrophoresis in 0.8% agarose gel at 100V for 20 min.
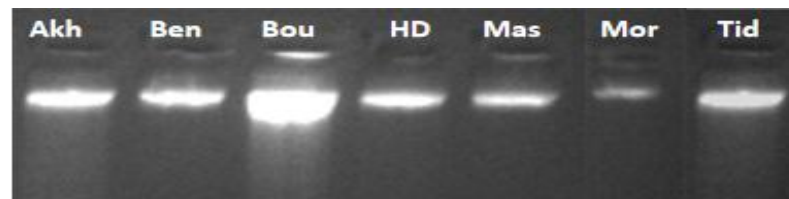
Figure 13: Electrophoretic analysis of 5µl extracted genomic DNA from the seven varieties after dilution (100ng/µl) in 3% agarose gel at 100V for 2h.

Based the results shown in table 5 and the electrophoresis migration figures 12 and 13, the DNA was well extracted with good quality and high concentration that makes it qualified for a PCR experiment with 20ng/µl.

II. Analysis and interpretation of electrophoresis results

1. Gwm2 amplification results

After analysis, it is not hard to observe the existence of 2 to 3 bands for each variety. Two bands are common in all the seven varieties, the size of the first is around 385±15bp, while the second band has a size of 200±10bp. The third band however, was present exceptionally in the variety Tidis with the size of 231bp (*fig. 14*).

Figure 14: Electrophoresis migration for an SSR reaction amplified by Xgwm2 primer pair, using 3% agarose gel (100V for 2h).

The Xgwm2 locus is situated in the chromosome 3A and from its cartography is close to the yellow rust resistance gene *Yr79* (Graingenes, 2021). In the gel, we observe two common bands in all the varieties showing the possible existence of that gene in all of them, and by looking back to the sensitive varieties (Benmabrouk, Morocco and Hidhab).

The polymorphism information content (PIC) was calculated and estimated to 0.61, which means that it is an informative marker (PIC>0.44) (Carl et *al.,* 1992).

2. Barc8 amplification results

This marker's amplification allowed us to discern 1 to 4 bands for each variety majority are nonspecific amplification (monomorphic), one common band in all the varieties, of a size that goes between 227bp for Tidis and 251bp for Boumerzoug. The second band has the size of 469±10bp, and is not present in just the three varieties Boumerzoug, Massine and Hidhab. Then, for the third band that is around 323±10bp, it is also absent in the three varieties Akhamoukh, Boumerzoug and Massine. To continue, there are 3 other bands that stand out, 2 of them are exceptionally present in the variety Boumerzoug, the first by a size of 495bp and the second 374bp the third band appears only in the variety Tidis with a size of 590bp (*fig. 15*).

Figure 15: Electrophoresis migration for an SSR reaction amplified by Barc8 primer pair, using 3% agarose gel (100V for 2h).

The marker barc8 is located in the chromosome 1B along with Wmc44 and Wms124, however barc8 does not share the same resistance gene with them, the nearest resistance gene to it is *Yr15* (Graingenes, 2021).

The marker shows a common band in all the varieties and other non-specific amplified bands which means that this marker is mono-morph. The PIC value of this marker is zero, which means that this marker is not informative.

3. Wmc44 amplification results

The amplification of Wmc44 shows 1 to 4 bands per variety, one band common in all seven varieties and has the size of 192±10bp, the second band, by the size of 246±10bp, is shared by the varieties Akhamoukh, Benmabrouk, Massine. Then two bands, both are common in the same varieties, the first band has a size of 770±10bp, while the second is 820bp (*fig16*).

Figure 16: Electrophoresis migration for an SSR reaction amplified by Wmc44 primer pair, using 3% agarose gel (100V for 2h).

This marker Wmc44 is located in chromosome 1B which is the same as Wms124 and Barc8, linked to the resistance gene *Yr29*. The gel results show three heterozygous varieties (Akhamoukh, Benmabrouk and Massine), the rest are homozygous, but although that Benmabrouk is sensitive, here it shares the same profile as the resistant variety Massine, the same case applies to Morocco and Hidhab (sensitive) and Tidis and Boumerzoug (resistant).

The Marker is polymorphic with a PIC value estimated to 0.86, meaning that this marker is highly informative.

4. Barc119 amplification results

In this amplification, realized using the Barc119 marker, we distinguish 4 to 7 bands differing from a variety to another. Two bands existing in all the seven varieties, one of 120±9bp and the other of approximately 500±15bp. Then two other bands common in six varieties, the first of these bands is around 222±6bp and excluded the variety Boumerzoug, the second band is 246±5bp and excluded the variety Benmabrouk. In addition to these, we note two other bands, both appearing in the same 5 varieties and being absent in the 2 Akhamoukh and Tidis, one band 675±9bp and the other around 425±5bp. Lastly, there's one more band, apparent exceptionally in the varieties Boumerzoug, Hidhab and Morocco and is of the size 815±4bp (*fig. 17*).

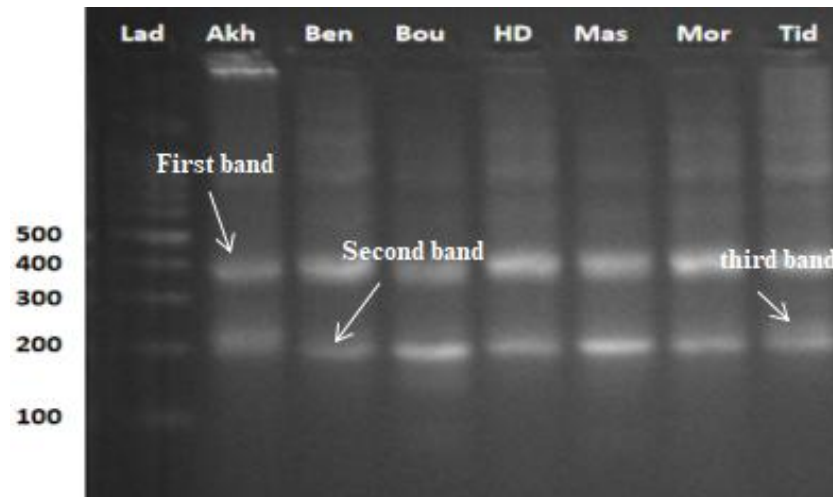**Figure 17:** Electrophoresis migration for an SSR reaction amplified by Barc119 primer pair, using 3% agarose gel (100V for 2h).

The marker Barc119, which is linked to the resistance gene *Yr64* located in chromosome 1A, shows a very high polymorphism with the appearance of seven different bands. Revealing two bands indicating heterozygous in all varieties except for Benmabrouk where only one band appeared indicating homozygous.

The PIC value was 0.65, meaning that this marker is significantly informative

5. Wms124 amplification results

The result of amplifying with the Wms124 maker, make us notice one single explicit band in all the varieties, and it is almost identical in size which is around 187±5bp (*fig. 18*).

Figure 18: Electrophoresis migration for an SSR reaction amplified by Wms124 primer pair, using 3% agarose gel (100V for 2h).

The marker Wms124 located in chromosome 1B, linked to the resistance gene *Yr29*, shows only one sole band, indicating that all the varieties are homozygous, and that no polymorphism in that marker.

The PIC calculations show that the marker is non-informative (PIC = 0).

6. Wmc488 amplification results

This amplification here, revealed 1 to 3 bands on each variety, a first band with a size of 110±10bp appearing in all the varieties except for Hidhab and Tidis. The second band is also common in all the varieties but Boumrezoug, and has a size of 140±6bp. The last band is 65±10bp and only appears in all the varieties (*fig. 19*).
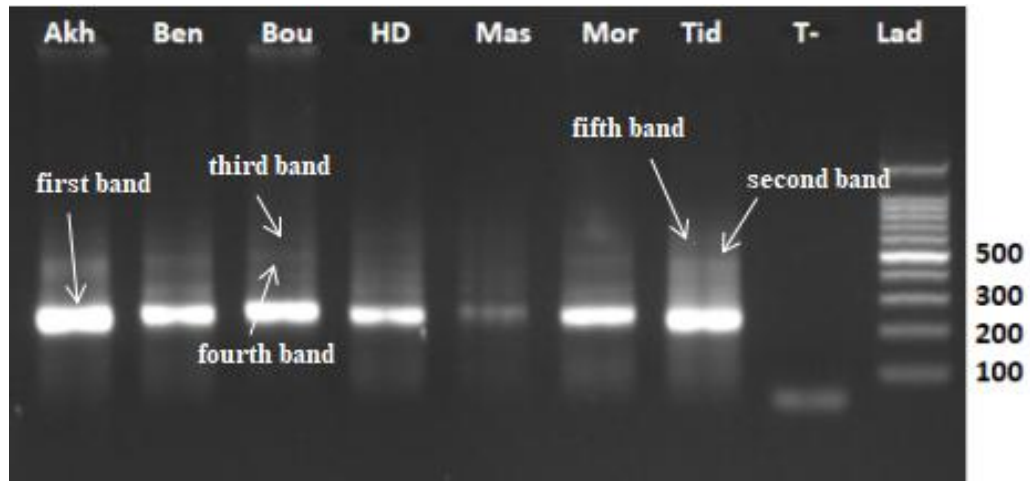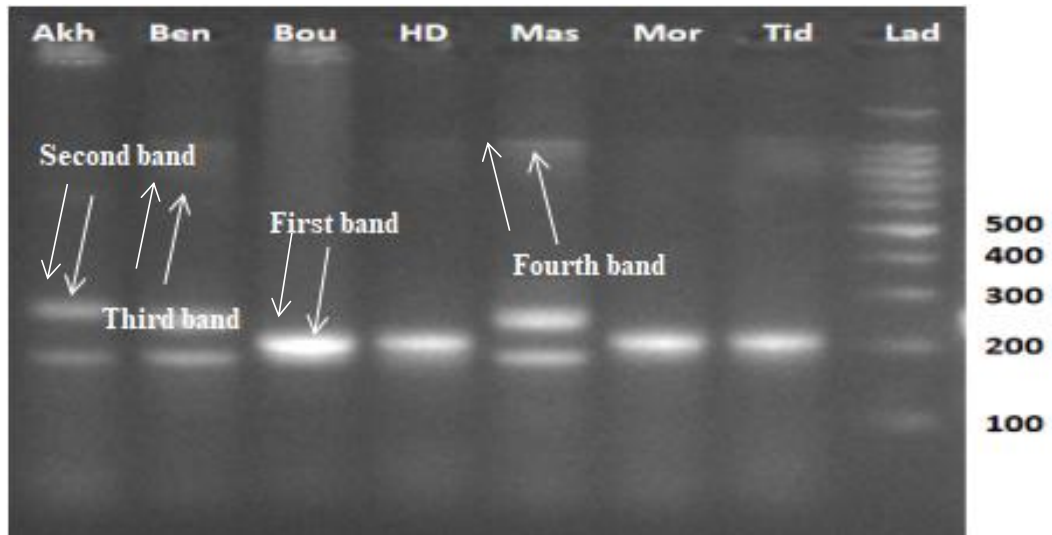

Figure 19: Electrophoresis migration for an SSR reaction amplified by Wmc488 primer pair, using 3% agarose gel (100V for 2h).

The marker Wmc488 is linked to the resistance gene *Yrxy1-QTL*, it is located in chromosome 7A linked shared with marker Cfa2040, reveals a polymorphism by the appearance of 3 different bands, two bands in all of the varieties except for Boumerzoug, indicating homozygousy in Boumerzoug and heterozygousy in the rest.

The PIC was estimated to 0.53 meaning that the marker is informative.

7. Wms533 amplification results

The analysis of that amplification opens the door to noting 1 to 5 bands per variety; the first is around 121±10bp and appears in all of varieties. The second and third bands exist in four varieties but not in Akhamoukh, Benmabrouk and Boumerzoug. The size of the second is 261±7bp while the third is 537±7bp. The fourth, fifth and sixth bands were exceptional, as the fourth with 340bp and the fifth with 695bp only

appear in the variety Massine. The sisxth band only shows in Benmabrouk with a size of 288bp (*fig. 20*).
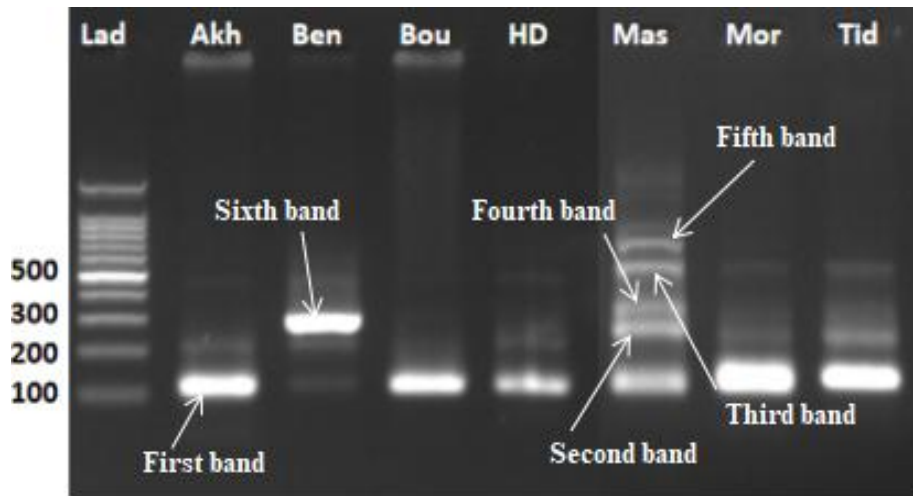
Figure 20: Electrophoresis migration for an SSR reaction amplified by Wms533 primer pair, using 3% agarose gel (100V for 2h).

Wms533 linked to *Yr-3B* located in chromosome 3B, appears as a highly polymorphic marker. The first band which common among all of the varieties except for the second sample, indicating a significant difference between Benmabrouk and the rest of the varieties, yet there is band, that was amplified. This difference might lead us to think that maybe this is the difference between resistance and sensitivity toward yellow rust, but since the other DNA samples from sensitive didn't behave differently from the resistant samples leads us to two assume that benmabrouk is sensitive to a different *Puccinia. st* race.

The PIC value was estimated about 0.84, which means that this marker is highly informative.

8. Cfa2040 amplification results

In that one, there also are 2 to 3 bands per variety; we can notice one common band between all the varieties excluding Boumerzoug, by the size of 257±5bp. The second band shows common appearance in the five varieties, Akhamoukh, Benmabrouk, Boumerzoug, Massine and Morocco, and its size goes around 236±4bp. For the third band, it has the size of 300±10bp and is absent in the two varieties Akhamoukh and Benmabrouk, while appearing in all the rest. Now to close the observation, we mention the last band, which appeared exceptionally in Akhamoukh with the size of 333bp (*fig. 21*).

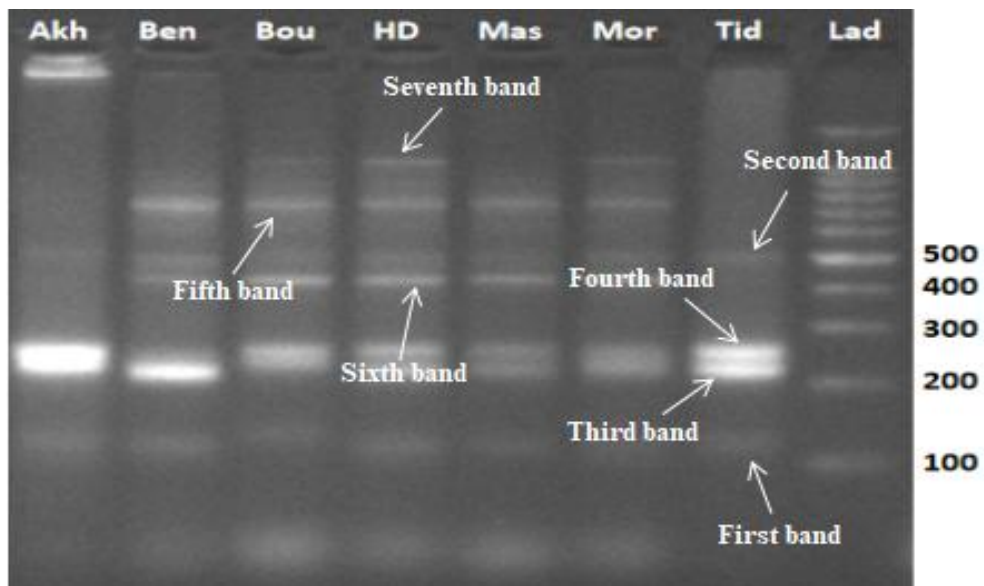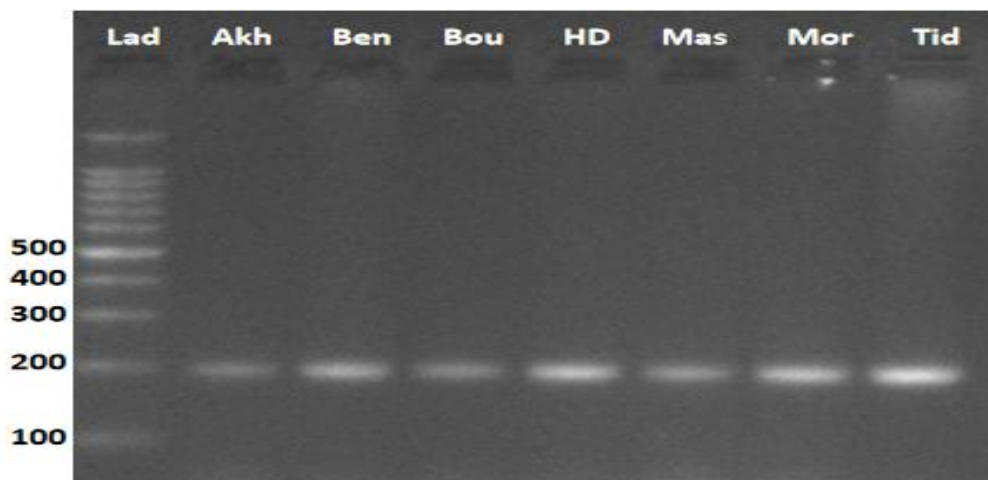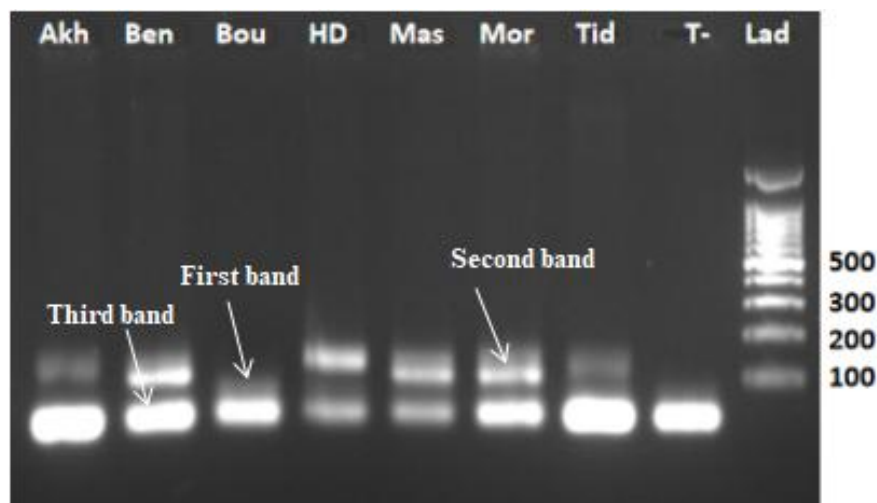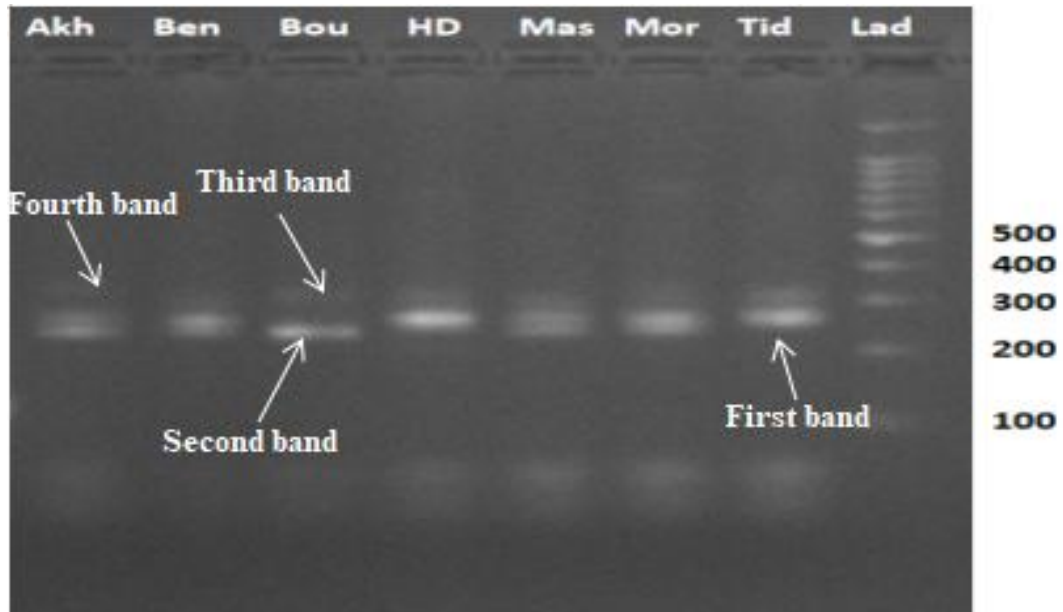**Figure 21:** Electrophoresis migration for an SSR reaction amplified by Cfa2040 primer pair, using 3% agarose gel (100V for 2h).

The Marker Cfa2040 is linked to the resistance genes *Yr67* and *Yr52* located in chromosome 7A. The marker is polymorphic, we can see the first two bands appears in all varieties indicating that they are heterozyous, except for Boumerzoug and Hidhab where only one band appeared meaning that they is homozygous. Another faded bands might be seen (3ed and 4th band) as well but they appear in both resistant and sensitive, which means that the only thing we can get from this migration result is that Boumerzoug in this particular essay has a different genotype different than the other samples.

The PIC value for this marker is 0.43 (close to 0.44), meaning that we can say that this marker is moderately informative. (Carl et *al.*, 1992).

The results we acquired were reformed into Polymorphism Information Content (PIC), which indicates a different interpretation depending on its value. If PIC = 0, the marker is probably a monomorphic (1 allele). On contrary if PIC = 1, that means that the marker is polymorphic with an infinite number of alleles. PIC value less than 0.37 probably has only two alleles. However when PIC =0.44 the marker is polymorphic and we can say that it is moderately informative. Finally, if PIC is higher than 0.7 that means that the marker is highly informative (the higher the PIC is the more alleles we have) (Carl.E et *al,* 1992).

III. Hierarchical ascendant classification (Cluster analysis)

Hierarchical clustering is an algorithm that groups similar objects into groups called clusters. The endpoint is a set of clusters, where each cluster is distinct from each other cluster, and the objects within each cluster are broadly similar to each other.
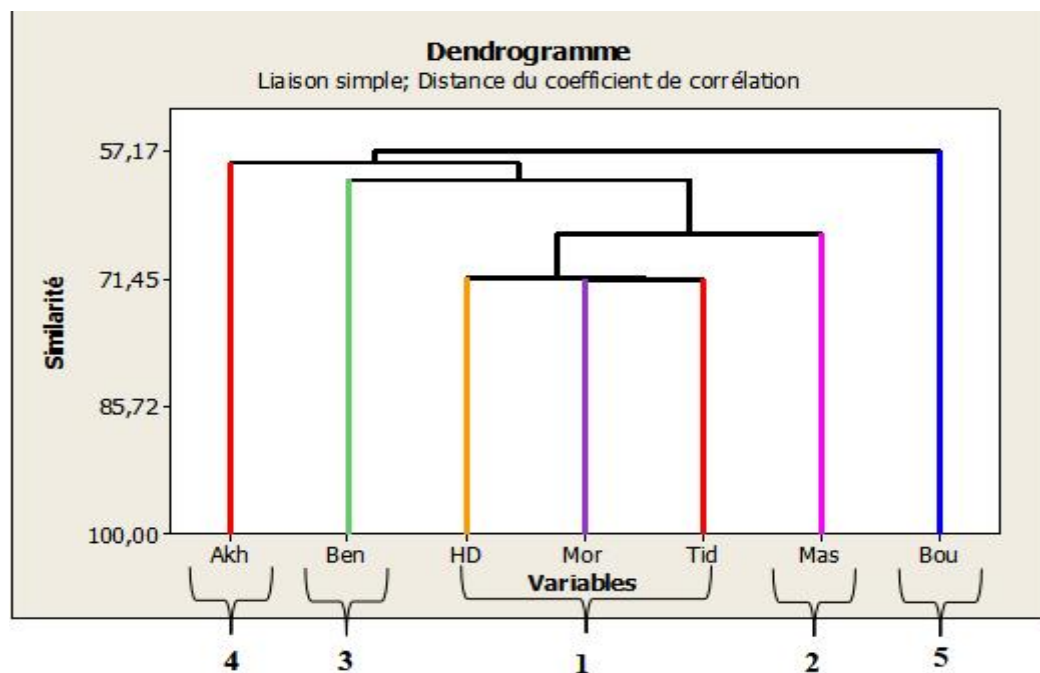
Figure 22: Dendrogram showing the similarity relation between the seven varieties of bread wheat (established using MiniTab software).

From the dendrogram (*fig. 22*), we can group the varieties into two main groups; the first group containing all the varieties except Boumerzoug, meaning that Boumrzoug has proven to possess exceptional overall characteristics based on four loci wmc44, barc119, wmc488 and Cfa2040. We then can also separate them into four clusters based on their distance in similarity:

- **First cluster:** assembles the varieties Hidhab, Morocco and Tidis
- **Second cluster:** consists of the variety Massine
- **Third cluster:** only contains the variety Benmabrouk
- **Fourth cluster:** Composed of the variety Akhamokh

Worth mentioning that the 1st, 2nd and 3rd clusters exist within the range of a common large group, which means on a closer distance, while the clusters containing

Akhamokh and Boumerzoug are situated on the edges, further from the rest, meaning they're remotely different from the other varieties.

Although the 3 varieties Hidhab, Morocco and Benmabrouk are supposed to be sensitive, we observe that only two of them belong to the same cluster while Benmabrouk showing difference and being in a cluster of its own, which may mean that they're not resistant to the same races of yellow rust.

Conclusion

Conclusion

This study was oriented to detect the yellow rust resistance genes in Algerian wheat varieties, using a set of Microsatellite markers to show their potential in selecting among seven different Algerian wheat varieties. The used Microsatellites are all known and with prior experiences on bread wheat.

The PIC values for our eight markers varied from 0 to 0.86. PIC was 0 for wmc124 and barc8 deeming them to being completely non-informative for our purpose, while in Cfa2040, Wmc488, barc119 and Xgwm2, PIC was moderately informative with values respectively 0.43, 0.53, 0.65, 0.61, finally it was highly informative in wmc44 and wms533 with values of 0.86 and 0.84.

From the gel results, PIC values and dendrogram we could get to the conclusion that the markers revealed alleles for different random characteristics for wheat in general, however, they did not show any clear or genuine connection to yellow rust resistance or sensitivity, as there were no matches with our first collected data.

The first possible explanation to these results can be that our samples possess the targeted resistance genes; however, they were not specific to this particular race of *Puccinia striiformis*.

The second possibility can be that the resistance genes in the resistant varieties are recessive.

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Annexes

Annexes

Annexe 1: Extracted DNA dosage for yellow rust resistance essay- May 2022.

Sample ID	DNA]	A260	A280	260/280	260/230	Sample Type	Factor
1	**841.3**	16.827	9.074	1.85	1.97	DNA	50
2	**2838.2**	56.763	31.007	1.83	1.87	DNA	50
3	**704.3**	14.085	7.461	1.89	2.03	DNA	50
4	**423**	8.46	4.564	1.85	1.57	DNA	50
5	**477.1**	9.542	5.104	1.87	1.66	DNA	50
6	**792.6**	15.851	8.625	1.84	1.69	DNA	50
7	**313.5**	6.271	3.36	1.87	1.73	DNA	50
1'	**786.9**	15.737	8.502	1.85	1.88	DNA	50
2'	**1235**	24.701	12.855	1.92	1.93	DNA	50
3'	**3711.4**	74.227	40.063	1.85	1.87	DNA	50
4'	**1758.3**	35.166	18.993	1.85	1.86	DNA	50
5'	**2919.1**	58.382	32.41	1.8	1.72	DNA	50
6'	**1330.9**	26.619	14.041	1.9	1.9	DNA	50
7'	**350.8**	7.016	3.757	1.87	1.97	DNA	50

Annexes

Annexe 2: Gwm2's marker table resulted by PhotoCapt

Gwm2							
Valeurs P.M.	Akh	Ben	Bou	HD	Mas	Mo	Tid
Bande 1	370	385	379	397	400	394	400
Bande 2	209	193	193	198	202	203	231
Bande 3							207

Annexe 3: Barc8's marker table resulted by PhotoCapt

Barc8							
Valeurs P.M.	Akh	Ben	Bou	HD	Mas	Mo	Tid
Bande 1	458	474	495	316	244	469	590
Bande 2	232	320	374	239		323	463
Bande 3		241	251			236	330
Bande 4							227

Annexes

Annexe 4: Barc119's marker table resulted by PhotoCapt

Barc119							
Valeurs P.M.	Akh	Ben	Bou	HD	Mas	Mo	Tid
Bande 1	526	667	819	815	681	819	511
Bande 2	244	492	674	681	511	681	246
Bande 3	228	432	511	504	432	511	221
Bande 4	121	216	425	425	246	428	120
Bande 5		119	242	251	219	244	
Bande 6			129	217	118	227	
Bande 7				116		119	

Annexe 5: Wmc44's marker table resulted by PhotoCapt

Wmc44							
Valeurs P.M.	Akh	Ben	Bou	HD	Mas	Mo	Tid
Bande 1	257	820	192	204	820	200	820
Bande 2	180	780			770		770
Bande 3		236			246		200
Bande 4		180			180		

Annexes

Annexe 6: Wmc124's marker table resulted by PhotoCapt

Wmc124							
Valeurs P.M.	Akh	Ben	Bou	HD	Mas	Mo	Tid
Bande 1	192	192	192	187	187	183	183

Annexe 7: Wmc488's marker table resulted by PhotoCapt

Wmc488							
Valeurs P.M.	Akh	Ben	Bou	HD	Mas	Mo	Tid
Bande 1	113	146	110	140	137	135	135
Bande 2		104		65	100	103	77
Bande 3					64		

Annexe 8: Wms533's marker table resulted by PhotoCapt

Wms533							
Valeurs P.M.	Akh	Ben	Bou	Hd	Mas	Mo	Tid
Bande 1	324	257	315	309	267	700	309
Bande 2	255	238	232	253	236	262	260
Bande 3	234					236	

Annexes

Annexe 9: Cfa2040's marker table resulted by PhotoCapt

cfa2040							
Valeurs P.M.	Akh	Ben	Bou	Hd	Mas	Mo	Tid
Bande 1	333	251	306	303	294	306	303
Bande 2	260	236	232	253	257	262	260
Bande 3	232				236	236	
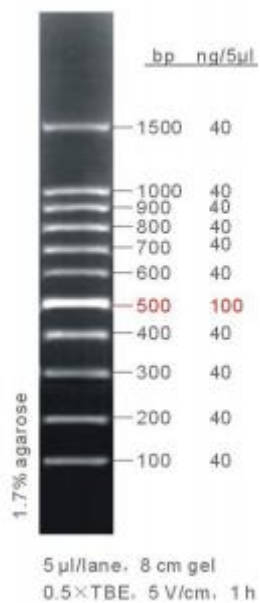
Annexe 10: Biomatik 100bp DNA Ladder

Annexe 11: Polymorphism of the seven varieties of bread wheat using eight Microsattellite markers linked to yellow rust resistance genes, June 2022

Marker	Band	Varieties						
		Akh	**Ben**	**Bou**	**HD**	**Mas**	**Mor**	**Tid**
GWM2	400	0	0	0	1	1	1	1
	385	1	1	1	0	0	0	0
	230	0	0	0	0	0	0	1
	203	1	0	0	1	1	1	1
	193	0	1	1	0	0	0	0
BARC8	590	0	0	0	0	0	0	1
	495	0	0	1	0	0	0	0
	466	1	1	0	0	0	1	1
	374	0	0	1	0	0	0	0
	323	0	1	0	1	0	1	1
	251	0	0	1	0	0	0	0
	241	0	1	0	0	1	0	0
	232	1	0	0	1	0	1	1
BARC119	819	0	0	1	1	0	1	0
	671	0	1	1	1	1	1	0
	526	1	0	0	0	0	0	0
	511	0	0	1	1	1	1	1
	492	0	1	0	0	0	0	0
	428	0	1	1	1	1	1	0
	244	1	0	1	1	1	1	1
	228	1	0	0	0	0	1	0
	217	0	1	0	1	1	0	1
	129	0	0	1	0	0	0	0
	118	1	1	0	1	1	1	1
WMC44	820	0	1	0	0	1	0	1

	775	0	1	0	0	1	0	1
	257	1	0	0	0	0	0	0
	231	0	1	0	0	1	0	0
	204	0	0	0	1	0	1	1
	192	0	0	1	0	0	0	0
	180	1	1	0	0	1	0	0
WMC124	190	1	1	1	1	1	1	1
WMC488	146	0	1	0	1	0	0	0
	135	0	0	0	0	1	1	1
	113	1	0	1	0	0	0	0
	100	0	1	0	0	1	1	0
WMS533	720	0	0	0	0	1	0	0
	610	0	0	0	0	1	1	1
	540	0	0	0	1	0	0	0
	400	0	0	0	0	1	0	0
	360	0	1	0	0	0	0	0
	290	0	0	0	0	1	0	0
	230	1	0	0	1	0	0	1
	180	0	0	0	0	0	0	1
	165	0	0	0	0	0	1	0
	140	1	0	1	1	1	0	0
Cfa2040	333	1	0	0	0	0	0	0
	303	0	0	1	1	1	1	1
	262	1	0	0	0	1	1	1
	251	0	1	0	1	0	0	0
	234	1	1	1	0	1	1	0

Année universitaire : 2021-2022	Présenté par : LAHMER Mouad Mouhamed Salah M'HENNI Omar

Mémoire pour l'obtention du diplôme de Master en Biotechnologie et Génomique Végétale

Intitulé : **Microsattelites assisted selection for yellow rust resistance in Algerian bread wheat *Triticum aestivum* L.**

Abstract:

Wheat is currently one of the most important cultivated crops, considering its contribution in the global economy and food security; it is also one of the most consumed cereal crops in Algeria. In the year 2004, the eastern region of Algeria had a considerable damage in wheat fields due to yellow rust that is caused by a fungus called *Puccinia striiformis*. Leading to taking serious measures regarding that disease, especially research and wheat development. In this study, we aimed to find the resistance genes in seven Algerian bread wheat varieties, three sensitive and four resistant. The research was conducted using eight microsatellite markers, of which we can mention Wmc44, Barc119, and Wms533…etc. Using a CTAB DNA extraction method then followed by PCR amplifications and an electrophoresis migration. The results we obtained indicated that the markers did show polymorphism in multiple occasions, however after the PIC calculation and establishing a dendrogram, we have to conclude that the markers revealed different random wheat characteristics, although there were not clear links to the yellow rust resistance.

Key words: microsatellite, yellow rust, wheat, marker assisted selection and resistance.

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