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DIVERSITY, MARKER ASSISTED SELECTION IN DURUM WHEAT IMPROVEMENT IN ALGERIA

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Dedications

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List of abreviations

ms : dry matter basis

DNA :Deoxyribonucleic acid

RFLPs:Restriction fragment length polymorphism

AFLPs : Amplified Fragment Length Polymorphism

SSRs : Simple Sequence Repeats

SNPs: Single Nucleotide Polymorphisms

PCR:Polymerase chain reaction

°C: degree celsius

Tm :melting temperature

PAGE : polyacrylamide gel electrophoresis

A /G/C/T : adenine / guanine / cytosine / thymine

InDels: insertions and deletions

CAPS : Cleaved Amplified Polymorphic Sequences

QTL : Quantitative trait loci

MAS : Marker-Assisted Selection

FHB : Fusarium head blight

FCR : Fusarium crown rot

DON : deoxynivalenol

INRA : Institut National de la Recherche Agronomique

CARASE : the Algerian Center for Agronomic, Scientific, and Economic Research

CIMMYT: the International Maize and Wheat Improvement Center

FAO :the Food and Agriculture Organization

IDGC : The Field Crops Development Institute

PNAB: The National Wheat Improvement Program

PIC : the Polymorphism Information Content

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Résumé

importance, la culture du blé dur en Algérie est freinée par divers stress biotiques et abiotiques, impactant le rendement et la qualité. Les récentes avancées en biologie moléculaire et en biotechnologie ont introduit la sélection assistée par marqueurs (MAS) comme une stratégie efficace pour l'amélioration des cultures. Cette étude examine l'application de la MAS pour améliorer la résilience et la productivité du blé dur en Algérie, structurée en quatre chapitres. Le premier chapitre fournit un aperçu du blé dur, y compris son importance historique, ses caractéristiques biologiques et ses pratiques agronomiques, en mettant l'accent sur son importance mondiale et locale. Le deuxième chapitre explore les marqueurs moléculaires et leur application en agriculture, détaillant les SSR, les SNP et les principes de la MAS. Il aborde également les stress biotiques et abiotiques spécifiques affectant la culture du blé dur en Algérie. Le dernier chapitre intègre ces concepts, en se concentrant sur les applications pratiques et les études de cas réussies de la MAS pour améliorer la résistance aux stress, le rendement et la qualité du blé dur.

Mots clés:

Blé dur, Algérie, sécurité alimentaire, sélection assistée par marqueurs, amélioration des plantes.

Abstract

Durum wheat (Triticum durum Desf.) is a cornerstone of Algeria's agriculture, playing a critical role in food security and cultural heritage. Despite its importance, the cultivation of durum wheat in Algeria is hold back by various biotic and abiotic stresses, impacting yield and quality. Recent advancements in molecular biology and biotechnology have introduced Marker Assisted Selection (MAS) as an effective strategy for crop improvement. This study examines the application of MAS in enhancing the resilience and productivity of durum wheat in Algeria, structured into four chapters. The initial chapter provides an overview of durum wheat, including its historical significance, biological traits, and agronomic practices, emphasizing its global and local relevance. The second chapter explores molecular markers and their application in agriculture, detailing SSRs, SNPs, and the principles of MAS. It also addresses the specific biotic and abiotic stresses affecting durum wheat in Algeria. The final chapter integrates these concepts, focusing on practical applications and successful case studies of MAS in improving durum wheat's stress resistance, yield, and quality.

Keywords:

Durum wheat, Algeria, food security, Marker Assisted Selection, plant breeding.

ملخص

القمح الصلب (Triticum durum Desf.) هو حجر الاساسي في الزراعة الجزائرية، حيث يلعب دورًا حاسمًا في الأمن الغذائي والتراث الثقافي. على الرغم من أهميته، فإن زراعة القمح الصلب في الجزائر تتعرض لعدة ضغوط حيوية وغير حيوية تؤثر على الإنتاجية والجودة. شهدت السنوات الأخيرة تقدمًا في علم الأحياء الجزيئي والتكنولوجيا الحيوية، مما أدى إلى تقديم الانتقاء بمساعدة العلامات (MAS) كاستراتيجية فعالة لتحسين المحاصيل. تستكشف هذه الدراسة تطبيق MAS في تعزيز مقاومة وإنتاجية القمح الصلب في الجزائر، وتضم أربعة فصول. يوفر الفصل الأول نظرة عامة على القمح الصلب، بما في ذلك أهميته التاريخية، وخصائصه البيولوجية، والممارسات الزراعية، مع التركيز على أهميته العالمية والمحلية. يستكشف الفصل الثاني العلامات الجزيئية وتطبيقاتها في الزراعة، مع تفصيل SSRs و SNPs ومبادئ MAS. كما يتناول الضغوط الحيوية وغير الحيوية المحددة التي تؤثر على زراعة القمح الصلب في الجزائر. يدمج الفصل الأخير هذه المفاهيم، ويركز على التطبيقات العملية والدراسات الحالة الناجحة لاستخدام MAS في تحسين مقاومة القمح الصلب للضغوط، والإنتاجية، والجودة.

الكلمات المفتاحية :

القمح الصلب ، الجزائر، الأمن الغذائي، الاصطفاء المدعوم بالمؤشر، تحسين النوع النباتي

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INTRODUCTION

Durum wheat (Triticum durum Desf.) holds significant economic and cultural importance in Algeria, a country where it serves as a staple crop integral to food security and traditional cuisine. However, the cultivation of durum wheat faces numerous challenges, including biotic and abiotic stresses that threaten yield and quality. In recent years, advances in molecular biology and biotechnology have opened new avenues for crop improvement, with Marker Assisted Selection (MAS) emerging as a powerful tool for enhancing durum wheat resilience and productivity. This study explores the application of MAS in the improvement of durum wheat in Algeria, structured into four comprehensive chapters.

The first chapter provides a broad overview of durum wheat, covering its historical significance, biological characteristics, and agronomic practices. It discusses the global and local importance of durum wheat, particularly in the Mediterranean region, where it is predominantly grown. The chapter also addresses the genetic diversity of durum wheat, highlighting the traditional and modern varieties cultivated in Algeria and their respective roles in agriculture.

Chapter two delves into the scientific principles underlying molecular markers and their application in agriculture. It explains the different types of molecular markers, such as SSRs (Simple Sequence Repeats), SNPs (Single Nucleotide Polymorphisms), and their utility in plant breeding. The concept of Marker Assisted Selection (MAS) is thoroughly examined, showcasing how it enables the precise selection of desirable traits, thereby accelerating the breeding process and improving crop characteristics. This chapter focuses on the specific biotic and abiotic stresses that impact durum wheat cultivation in Algeria. Biotic stresses include diseases and pests prevalent in the region, while abiotic stresses encompass drought, salinity, and extreme temperatures. The chapter reviews current research on the resistance and tolerance of durum wheat varieties to these stresses, providing a foundation for understanding the critical challenges faced by Algerian farmers.

The final chapter integrates the concepts discussed in the previous chapters, focusing on the practical application of molecular markers in the improvement of durum wheat in Algeria. It highlights successful case studies and ongoing research projects that utilize MAS to enhance stress resistance, yield, and quality of durum wheat.

1 .CHAPTER 01:OVERVIEW OF DURUM WHEAT

Durum wheat, also known as pasta wheat or macaroni wheat (scientifically referred to as Triticum durum or Triticum turgidum subsp. durum), is a tetraploid wheat species. Despite being the second most cultivated wheat species globally, it accounts for only 5% to 8% of the total wheat production. The origins of durum wheat can be traced back to the artificial selection of domesticated emmer wheat strains that were once cultivated in Central Europe and the Near East around 7000 BC. Over time, these strains evolved into a naked, free-threshing form. (Tidiane Sall. and al ,2019)

Similar to emmer wheat, durum wheat possesses awns (bristles) and is the predominant wheat variety in the Middle East. The term "durum" originates from Latin and translates to "hard," reflecting the hardness of this wheat species. (Belderok,B.and all, 2000) This hardness is particularly associated with the resistance of the grain during milling, specifically the starchy endosperm. As a result, dough made from durum wheat flour is characterized as weak or "soft." This unique trait makes durum wheat well-suited for the production of semolina and pasta. However, it is less practical for flour compared to hexaploid wheats like common bread wheats, as the latter requires more effort in processing. (Helguera, M.and al,2020)

Despite its high protein content, durum wheat does not confer strength to dough in the traditional sense of forming a robust gluten network. Durum wheat contains approximately 27% extractable wet gluten, which is about 3% higher than common wheat (Triticum aestivum L.) (Faltermaier, A.,and al.2014)

1.1 Classification

1.1.1 Botanic Classification

Durum wheat, scientifically known as Triticum durum, is a type of wheat that is well-suited for making pasta and certain types of Mediterranean bread. It belongs to the Poaceae family and the Triticum genus (Yousefi Javan, I. 2012). The classification of durum wheat is as follows:

Category	Description
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Monocots
Clade	Commelinids
Order	Poales
Family	Poaceae
Subfamily	Pooideae
Genus	Triticum
Species	T. durum
Binomial Name	Triticum durum

Table 1 : Botanical classification (*Durum*, n.d.2024)

Durum wheat is characterized by its hard texture, high protein content, and amber-colored kernels. It is primarily cultivated in regions with a Mediterranean climate, where it thrives in hot and dry conditions. The durum wheat grains are ground to produce semolina, which is a key ingredient in pasta and couscous (Punia, S.,and al.2022)

1.1.2 Genetic classification

Wheats form a complex in which many species have been named. For a long time, botanists tended to give a species name to each morphological variant (Clark, J. A.,1922).

Since the beginning of the 19th century, wheat has been the subject of numerous cytogenetic studies, and we now know that it is classified in a polyploid series. They differ in the number of chromosomes they have and in the make-up of their genome. (Gupta, P. K. 1991).

Some are diploid (they have two sets of chromosomes) and share a genome called AA; others are tetraploid (four sets of chromosomes) and have the formula AA BB. One group is hexacordic (six sets of chromosomes) and has the formula AA BB DD. Finally, endemic wheats from Georgia form a parallel series with AA GG and AA AA GG genomes, as shown in Table II. (Muenchrath, D.,2023)

Within each group, the forms are interfertile, whereas the hybrids between groups are highly sterile. In addition, a very small number of genes are responsible for the spectacular differences between wild forms (with fragile rachis) and cultivated forms (with solid rachis) or between dressed grains (with glumes and glumellae adhering to the grain). And naked grains. Modern authors (Mac Key, 1966) believe that it is these natural groups that should be given species status.

1.2 History and eco-geographical distribution

Durum wheat is an important crop used for the production of pasta, couscous, and in some areas of the world, various types of bread (Quaglia 1988). Durum wheat occupies approximately 20-30 million hectares worldwide, spread over many countries, accounting for 8% of total world wheat production (Bozzini 1988). More than half of the total cultivation lies in the Mediterranean area including southern Europe, North Africa and Southwest Asia, where tetraploid wheats were domesticated around 10,000 to 15,000 BC (Bozzini 1988, Srivastava et al 1988).

The annual world durum wheat production in a three-year period (1991-93) was estimated to be 25.6-34.4 million metric tons (International Wheat Council, London). Historically, the yield level of durum wheat is 80% of bread wheat, which has been attributed partly to less favorable crop- growing environments and management practices (Srivastava et al 1988). However, new high yielding semidwarf durums have been produced that have yield potential equal, or even superior to the highest yielding bread wheat in some areas (Breth .,1975). As the price of durum is often higher than that of bread wheat, it is a promising and viable alternative crop.

Currently, there are large markets for durum wheat grown in traditional areas, both for domestic consumption and for export to developing countries where there is a greater demand for food due to increasing populations and improving standards of living. (Cereal Chem, 1996)

1.3 Origin of durum wheat

Geographical :

According to Vavilové in Erroux, durum wheat has two origins: Abyssinia and North Africa. Where as for Grignac, the Middle East is the generating centre of durum wheat, where it has differentiated in three regions: the western basin of the Mediterranean, southern Russia and the Near East (Syria and northern Palestine). (Nacera, M. H. 2012).

Genetics :

Observation of the behaviour of chromosomes during meiosis and the results of hybridisation experiments have shown that the genomes (complete sets of genetic material) of grasses can often be grouped into two distinct types. Each type has been given the name A, B or D.

Grasses that share the same genome will be more or less inter-related and can be treated by botanists as a single species. (Chase, M.1993)

Identifying genome types is an interesting tool for identifying hybridisation, for example. If two diploid plants hybridise to give a new polyploid form (an allopolyploid), the two original genomes will be present in this new form. (Taylor, S. A., and Larson, E. L. 2019).

Determining the origin of each of the wheat genomes is difficult because of the evolution of species.

Current knowledge of the origin of wheat genomes has been acquired through cytological studies, but the development of molecular tools has made it possible to refine and complete this knowledge (Rayburn and Gill, 1985).

Sakamura (1918) distinguished three major natural groups: emekorn or field seeds, emmer or starch plants and dinkel or spelt, which had 14, 28 and 42 chromosomes respectively. Kihara (1919) et al (in Cauderon, 1979) showed that allopolyploidy played a fundamental role in the evolution of wheat.

Allopolyploidy is a phenomenon resulting from the hybridisation of a group of species of the same genus with different degrees of ploidy (Gorenflot et al., 1990).

Allopolyploids fall into two categories: genomic allopolyploids derived from crosses between species with distinct genomes, and segmental allopolyploids resulting from crosses between more closely related species with partial homology of their genomes (Gorenflot et al., 1990).

Despite a certain affinity, the homeologous chromosomes of genomes A, B and D do not pair normally at meiosis. Pairing occurs only between homologous chromosomes. All wheat species, even polyploids, have only bivalents at meiosis in prophase I and disomic-type inheritance.

According to Gorenflot (1990), the existence of bivalents in certain interspecific hybrid polyploids to which the tetraploid and hexaploid Triticum species belong is not related to homology of the genomes invol

We can deduce that the A, B and D wheat genomes are relatively close from a phylogenetic point of view and that their chromosomes are homeologous, i.e. partially homologous (Gorenflot et al., 1990).

According to Bernard (1992), it has been possible to create natural polyploid species by increasing the number of basic chromosomes (x).

According to Auriau 1978 (in Gallais et Bannerot, 1992), tetraploid wheats with 28 chromosomes are the result of hybridisation followed by chromosomal duplication between Triticum Urartu and a wild grass Aegilops of the Sitopsis section with a base chromosome number of 7. Wheats with 28 chromosomes are allotetraploids and wheats with 42 chromosomes result from a second hybridisation between tetraploid wheats and Aegilops squarrosa after chromosomal duplication.

1.4 Origin of the A genome

The work of Kihara (1924) cited by Felix (1966) made it possible to attribute the origin of the A genome to Triticum monococcum var. boeticum or var. urartu.

A recent study based on the polymorphism of repeated sequences has established that Triticum urartu, which is a close relative of Triticum boeticum but not inter-fertile, is the donor of the A genome for all polyploid wheats (Dvorak, 1988).

1.5 Origin of the B genome

Many hypotheses have been put forward as to the origin of the wheat B genome: Table I summarises the plausible explanations.

Author	Year	Possible origin of the B genome
Pathak	1940	Aegilops speltoides
Sarkar et Stebbins	1956	Aegilops speltoides
Johnson	1975	Triticum urartu
Konarev et al	1976	Aegilops longissima
Feldman	1978	Aegilops searsii
Kushnir et Halloran	1981, 1983	Aegilops sharonensis

Lange et Balkema boomstra	1988	Aegilops, Viz de la section Sitopsis

Table 2: Possible origins of the B genome (Kerby and Kuspira, 1987).

According to this table, the origin of the B genome remains uncertain (unidentified source) and controversial. It is present in most tetraploid wheats and is similar to Aegilops speltoides. Thus, six species have been given or proposed as potential donors and Aegilops searsii seems to be the most likely donor (Kerby and Kuspira, 1987).

1.6 Origin of the D :

Mc Fadden (1926) showed that the species Aegilops taushii (Aegilops squarrosa) is the origin of the D genome in hexaploid wheats, giving them greater resistance to cold and certain distinct morphological characteristics. (Feldman, M., & Levy, A. A. 2015).

Cauderon (1979) points out that it took almost 30 years to find out the origin of the D genome. He indicates that genomic analysis by crossing 2 wheats T. aestivum and T. turgidum and 3 species of Aegilops; Aegilops . cylindrica , Aegilops. caudata and Aegilops . squarrosa led to the conclusion that the species Aegilops squarrosa is the origin of the D genome. (Ceoloni,. 2006).

Each of the A, B, and D genomes comes from a different diploid ancestral species, and the three species themselves are thought to be descended from a common diploid ancestor.

This origin has undoubtedly given it this flexibility of adaptation, hence its cultivation in many regions of the world (Piccard, 1988).

The A and B genomes generally control the architecture, resistance, and fertility of the species, and the D genome gives soft wheat its aptitude for bread-making technology (Piccard, 1988).

1.7 Structure and composition of the durum wheat kernel

According to (FEILLET, 2000) a wheat grain is made up of 3 regions (figure 1):

- The albumen, made up of the starchy albumen and the aleurone layer (80-85% gluten).

- The seed and fruit coats, made up of six different tissues: nucellar epidermis, seminal tegument or test (seed coat), tubular cells, cross cells, mesocarp and epicarp (13-17%).

- The germ (3%), consisting of an embryo and the scutellum. Compared with other cereals, maize and rice in particular, the wheat kernel has a furrow resulting from an invagination of the teguments towards the inside of the kernel, along its entire length and on the side of the germ; the nourishing bundles of the seed during its development are located at the bottom of this furrow. Its presence determines the way in which the albumen and husks are separated to extract the flour. (Lintas, C., & Mariani-Costantini, A. 1991).

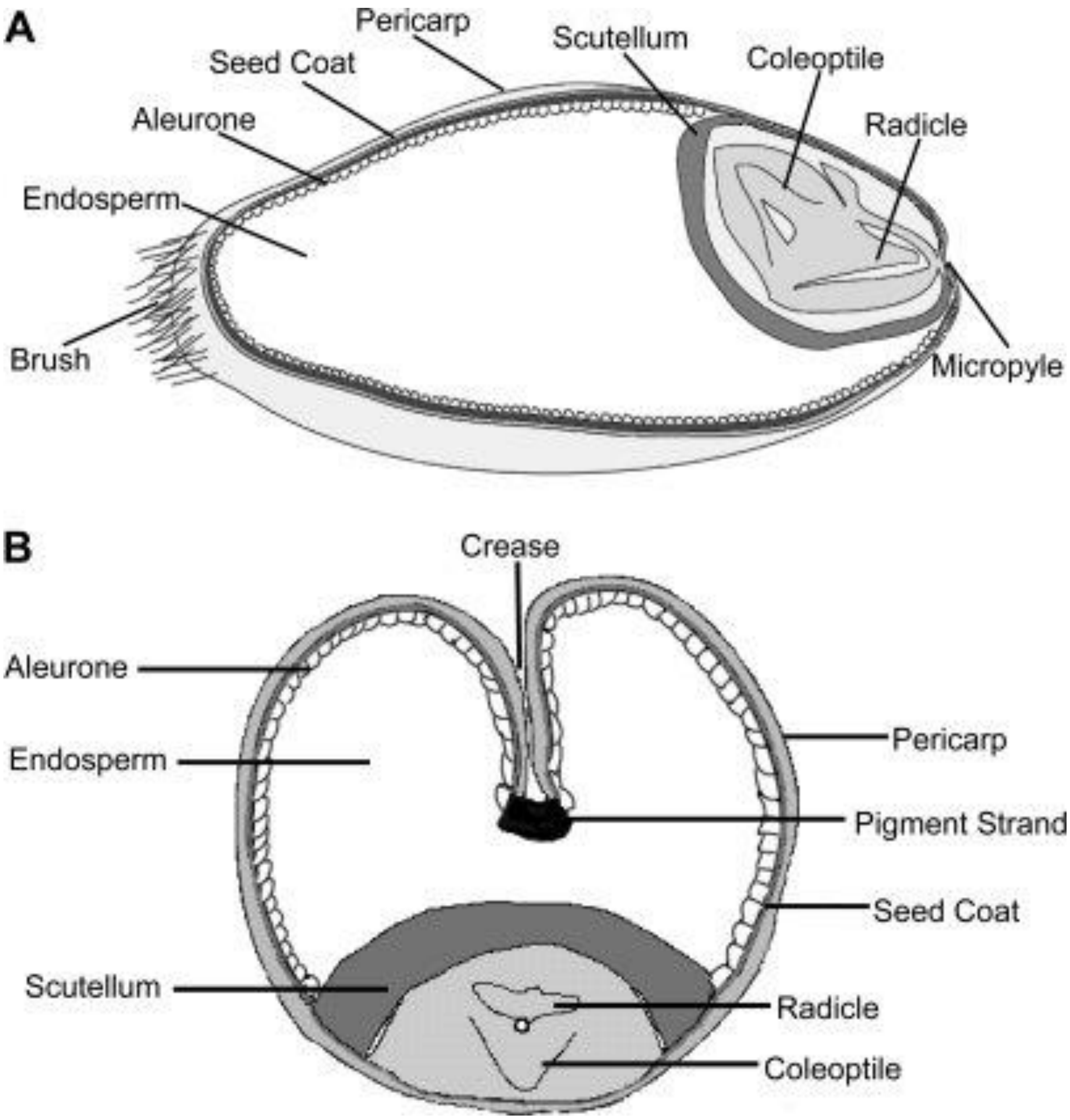

Figure 1: Histological diagram of a longitudinal section of a wheat grain (Bounneche, 2015)

The composition of wheat grains is highly complex. It depends not only on the species and variety of wheat but also on the climate, growing methods, and storage conditions. The grain is mainly made up of starch (around 70%) and proteins (10 to 15%, depending on the variety and growing conditions), with the other constituents, which are minor by weight (only a few percent), being lipids, cellulose, free sugars, minerals, and vitamins (Mahiddine, T., & al., 2022). (Table 03)

Nature of components	Content (% ms)
Protein	10-15
Starch	67-10
Pantosans	8-10
Cellulose	2-4
Free sugar	2-3
Lipids	2-3
Mineral matter	1.5-2.5

table3 :chemical composition of wheat grain (usual limits of Variation) (FEILLET, 2000)

1.8 Systematic position of durum wheat

Durum wheat is a self-pollinating cereal belonging to the order Graminnales and/or Poales, family Graminaceae and/or Poaceae (HABBI, Oumelkhair, et al 2018). A detailed classification is given in the table below

Phylum	Spermaphytes
Subphylum	Angiosperms
Class	Monocotyledons
Super Order	Commeliniflorales
Order	Poales
Family	Gramineae
Tribe	Triticeae
Subtribe	Triticinae
Genus	Triticum
Species	Triticum durum Desf

Table 4 : Botanical classification of durum wheat (Hennouni, 2012).

1.9 Ecological Requirements of Durum Wheat

Durum wheat exhibits distinct ecological requirements compared to soft wheat. It necessitates high levels of sunlight, has low tolerance to cold and moisture, typically yields average outputs (generally lower than those of soft wheat, except for recent varieties), and is more susceptible to certain fungal diseases compared to soft wheat. (Moayedi, A. A. 2010).

In Morocco, the cultivated area for durum wheat was approximately 1.1 million hectares in the 2003-2004 period. While durum wheat can be grown in all agricultural regions of Morocco, significant rainfall during the maturation phase can impact grain quality. (Tadesse, W.,2017)

Durum wheat thrives in well-drained, healthy soils, but it should not be overly prone to water stress, particularly during the grain accumulation period. Planting durum wheat in poorly draining soils renders it more vulnerable to fungal diseases such as smut and fusarium. The quality of grains can be adversely affected by substantial rain during the maturation phase.

1.10 Production

Durum wheat (Triticum turgidum ssp. durum) ranks as the 10th most cultivated cereal globally, contributing to a total production of approximately 38 million tons (Rabti, A. B.,and al.2020). The predominant type grown today is amber durum, characterized by grains with an amber hue owing to additional carotenoid pigments. These grains are notably larger than those of other wheat varieties. Durum possesses a yellow endosperm, imparting its distinctive color to pasta. When durum undergoes milling, the endosperm is transformed into semolina, a granular product. Semolina derived from durum is prized for premium pastas and breads, and it is unique as one of the few flours intentionally oxidized for enhanced flavor and color. Additionally, there is a red durum variant primarily used for livestock feed.

Cultivating durum proves advantageous in regions with low precipitation, yielding higher outputs compared to other wheat varieties. While irrigation can enhance yields, it is seldom practiced. Historically, durum cultivation was widespread in Russia during the first half of the 20th century. In West Asia, where durum is a vital food crop, the wheat variety is diverse but not extensively grown, necessitating imports. Canada's West amber durum, predominantly used for semolina and pasta, is also exported to Italy for bread production. (Marti, J., & Slafer, G. A. 2014).

In the Middle East and North Africa, half of the durum consumption is attributed to local bread-making, with some flour imports. European countries significantly contribute to durum production. In India, durum constitutes about 5% of the country's total wheat production, utilized for products such as rava and sooji. (Alsberg, C. L. 1939).

Durum wheat undergoes a four-step processing sequence: cleaning, tempering, milling, and purifying. Cleaning removes foreign material and defective kernels, while tempering adjusts moisture levels to toughen the seed coat for efficient bran and endosperm separation. Milling is a complex procedure involving repetitive grinding and sieving. Proper purifying ensures maximum semolina yield with minimal bran powder. (Patel, A.,2019)

For bread production, durum wheat is ground into flour, mixed with water to form dough, and fermented with yeast to create a gas-retaining gluten network, resulting in fluffy bread. The quality of the bread relies on gluten's viscoelastic properties, protein content, and composition. Durum wheat contains approximately 12% total protein in defatted flour, with 27% extractable, wet gluten, outperforming common wheat in both aspects. (Patel, A.,2019)

1.11 Growing durum wheat

Worldwide

Wheat ranks first in world production and second after rice as a source of food for human populations, providing 15% of their energy needs. Wheat is grown mainly in the arid and semi-arid countries of the Mediterranean basin, where agriculture is in the worst shape. These regions are characterised by rising temperatures coupled with falling rainfall, in addition to desertification and drought (Borlaug, N. E.2003).

In Algeria

Durum wheat (Triticum durum Desf) is the leading cereal grown in the country. It occupies more than one million hectares annually. The national production of durum wheat is still low, covering only 20–25% of the country's needs, with the rest being imported. The main reason for the low level of durum wheat production in Algeria is the low level of productivity (yield) obtained, i.e. 9 to 11 quintals/hectare, which is itself due to abiotic constraints (especially rainfall), biotic constraints (especially weeds) and human constraints (technical itineraries applied, etc.) (Amira Djahida, F. M. 2013).

More than two-thirds of these areas are located inland, on the high plateaux. Constraints linked to the soil, climate, varieties adopted, and farmers' know-how limit grain yield expectations in this region. Climate variability is the main factor affecting durum wheat production, which varies from 9 to 20 million quintals depending on the year. (Hami, S. 2018)

1.12 Use of wheat

Durum wheat production covers 60 to 70% of the population's needs. The Algerian cereal industry is heavily focused on the production of semolina for pasta ‘Tableau 1’ (Romayssa, A., et al., 2021).

Country	Pasta (%)	Couscous (%)	Bread (%)	Others (%)
ITALY	60	-	40	-
FRANCE	60	-	40	-
SPAIN	70	-	30	-
ENGLAND	80	-	20	-
BENELUX	100	-	-	-
TUNISIA	30	50	15	5
ALGERIA	30	40	10	20
MOROCCO	7	5	85	3
EGYPET	100	-	-	-

Tableau 2 : Main uses of durum wheat worldwide (Cherdouh , 1999)

1.13 Biology and development cycle of wheat

1.13.1 Morphological characteristics

The grain :

The wheat kernel is a caryopsis. It is a dry, indehiscent fruit. It is amber to purplish yellow in colour, depending on the species (durum wheat or soft wheat) and variety. It has a flat (ventral) part and a slightly rounded dorsal part. The enlarged base contains the germ, and the top is covered with small hairs (the brush). The ventral part is split by a furrow that penetrates deep into the grain; however, the dorsal part has a more or less pronounced ridge (Gondé, 1968). The longitudinal section reveals the following parts from the outside inward: the husks, the germ, and the albumen, or kernel (Figure 2). (Punia, S.,2022)

Fruit husks :

These represent 14 to 16% of the weight of the kernel. From the outside in, they are made up of :

- The pericarp: the walls of the ovary, comprising the epicarp, mesocarp and endocarp.

- The tegument: the seed coat, comprising the seminal tegument and the hyaline band.

- The protein base: which represents 60% of the weight of the envelopes and is made up of aleurone cells, rich in protein (Soltner, 1987).

The germ :

This represents 2.5 to 3% of the grain and comprises :

- The cotyledon or scutellum, separated from the kernel by a diastatic bed intended for future digestion of the albumen for the benefit of the seedling.

- The seedling, with its sheath-covered gemmule, the coleoptile, its short stem and its sheath-covered radicle, the coleorhiza.

The germ is very rich in fat, nitrogen and vitamins A, E and B (Soltner, 1987).

The albumen or kernel :

This represents 83 to 85% of the weight of the grain and is made up of 70% starch and 7% gluten. In durum wheat, the albumen is horny and glassy, rather like that of rice.

The endosperm plays an essential role in the composition of the seed; it serves as a reserve and will not be fully used until germination (Guergah, 1997).

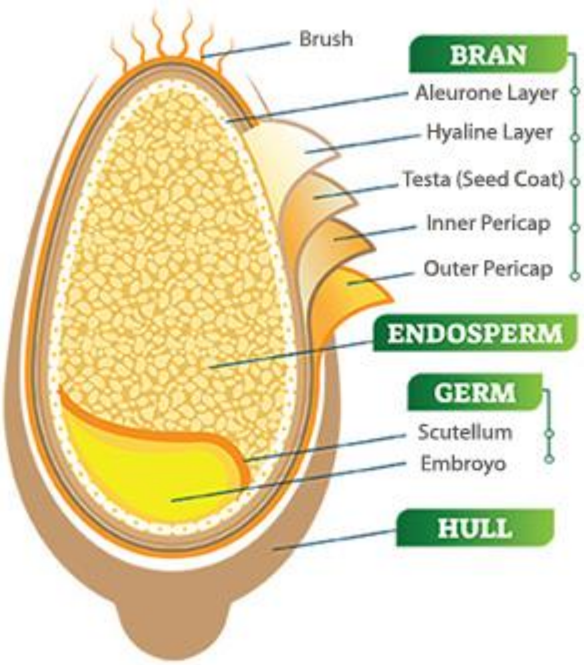

Figure 2 : Cross-section of wheat grain (Hadidi, M et al.,2023)

1.13.2 The egetative system

The vegetative system comprises the aerial system and the root system.

The root system :

It is of the fasciculated type, with two systems forming during the plant's development:

A primary system (seminal roots): this root system functions from germination to the branching of the plant, i.e., tillering. These roots are of embryonic origin but are associated in the seed with different parts of the embryo:

a main root resulting from the elongation of the radicle.two pairs of lateral roots.an epiblastic root (Grignac, 1965).

Secondary system (adventitious roots) this is a coronal root system or tillering root system. It is formed at tillering and replaces the seminal system in parallel (Grignac, 1965).

The aerial system

The aerial system is made up of a certain number of units corresponding to tillers, starting from an area at the base of the plant called the tillering plateau. Once the plant has fully developed, each tiller is made up of a stem and leaves.

Wheat stubble is a cylindrical stalk formed by knots separated by more or less protruding nodes. Each node is the attachment point for a leaf.

The wheat leaf is simple, elongated, alternate, and parallel-veined; it consists of two parts:

The lower part, which surrounds the young shoot and is called the sheath.

The upper part, blade-shaped, is the leaf blade.

1.13.3 The reproductive system

The spikelet is a small cluster of one to five flowers, each enveloped by two glumes (lower and outer). The cluster is enclosed between two bracts, or glumes, and the flowers are attached to the rachillet. Each flower generally has 3 stamens and an ovary; the flowers are hermaphroditic, and wheat is a self-pollinating plant; pollen from one flower pollinates the ovary of the same flower (“Sciences Le blé”,2024) (Figure 3).

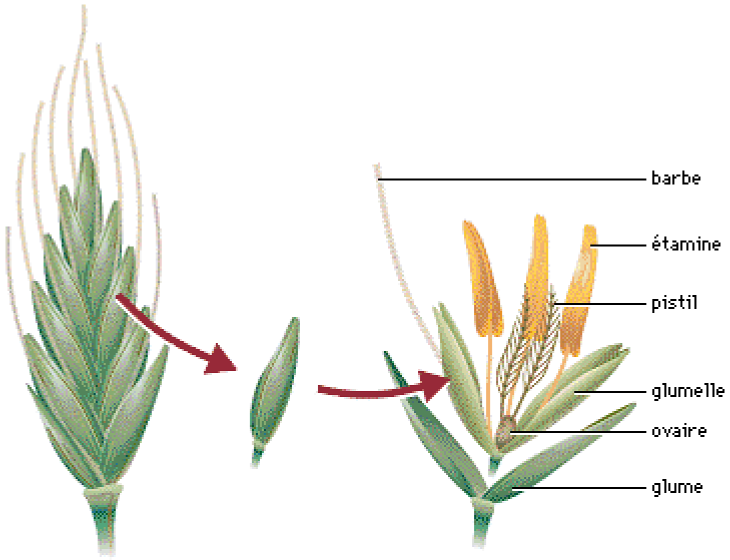

Figure 3 : Structure of a wheat ear and spikelet (“Sciences Le blé”,2024)

1.14 Reference stages and phases

In order to characterise the wheat cycle, various rating scales have been developed, based either on changes in external appearance or on changes in the internal appearance of the reproductive organs.

The Jonard et al. scale (1952) was used to recognise stages based on changes in external appearance (emergence, bolting, etc.).

The Zadoks et al. scale (1974), used to recognise stages by changes in internal appearance (differences in the ear: 1 cm ear stage, etc.),. (Gate, 1995).

Several authors have described the development cycle of wheat by breaking it down into two periods: a vegetative period and a reproductive period. Others consider that maturation constitutes a third period. Morphological changes are the result of both growth and development processes.

These two processes are complementary and inseparable. They lead to the production of dry matter, resulting from the transformation of environmental resources via aerial sensors (leaves: photosynthesising surfaces) and underground sensors (roots: water and mineral element sensors).

Growth consists of an irreversible increase in the size and weight of the plant's various constituent organs. It is a quantitative concept.

Development involves the appearance of new organs or the plant moving on to a different but complementary stage. This is a qualitative concept (Papadakis, 1938). Growth and development are measured using several scales, including the Feekes scale (Large et al., 1954). One scale, based on a description of the morphology of the Master Shoot, is used to characterise the reference stages.

- Physiological processes related to the different phenological stages

Germination – emergence:

Germination is characterised by the imbibition of the seed. Enzymes are reactivated and the reserves assimilated by the embryo are broken down. The radicle emerges from the seminal envelopes, followed by the establishment of the number of plants per square metre; the soil is then pierced by the coleoptile, which is a protective sheath for the first leaf. Emergence is noted when 50% of the seedlings have emerged from the soil. During this phase, young plants are sensitive to lack of water, which causes a reduction in their numbers. (Karou et al., 1998).

Tallage:

This phase begins from the fourth leaf. The beginning of tillering is marked by the appearance of the tip of the first leaf of the primary lateral tillers, then other tillers are born successively in the axils of the 2nd and 3rd leaves of the central stem, the whole remaining short-knotted, forming a tillering plate just at ground level. These primary tillers can then produce secondary tillers, which in turn produce tertiary tillers (Gate, 1995). Late tillering marks the end of the vegetative period and the start of the reproductive phase, conditioned by the photoperiod and vernalisation, which allow the internodes to elongate (Gate, 1995).

Setting – swelling:

From the 1 cm ear stage, the first internode elongates. This stage can be seen once the ear of the main shoot reaches 1 cm in height from the crown or tillering plateau (Gate, 1995).

This stage is sensitive to low temperatures ranging from +4 to 0°C. According to Baldy (1984), bolting is the most critical phase in wheat development. Any hydric or thermal stress during this phase reduces the number of ears rising per unit area. This phase ends when the ear takes on its final shape inside the sheath of the swelling flag leaf (swelling stage).

Heading – flowering :

Heading is determined by the appearance of the ear outside the sheath of the last leaf. Unsheathed ears generally flower between 4 and 8 days after heading (Bahlouli et al., 2005). Low temperatures during this stage greatly reduce the fertility of the ears (Abbassenne et al., 1998).

Grain filling :

The first stage involves the multiplication of cells in the young, green grain, supported by assimilates from photosynthesis in the flag leaf and non-structural carbohydrates stored in the ear's neck. During this phase, the grain's water content stabilizes, a condition referred to as the hydric plateau. High temperatures during this period can halt the migration of reserves from the leaves and stem to the grain, resulting in grain scald. Following this is the drying phase, where the grain loses moisture and achieves its final dry weight (Wardlaw, 2002).

Yield components, such as the number of grains per ear and the average grain weight (often measured as the weight of 1000 grains), are determined at different stages. The number of ears is established during the bolting stage, followed by the number of grain sites per ear at the heading stage. Grain weight is finalized during the active filling phase. These components are influenced by various growth conditions and compensatory effects between them (Abbassenne et al., 1998). In the Mediterranean region, the best yields are determined primarily by the number of ears and grains produced per unit area. Simane et al. (1993) highlight that in water-limited environments, the slow improvement in yields is partly due to the compensatory effects between yield components based on moisture availability. This compensation mechanism helps restore grain yield after stress (Blum, 1996).

Increasing the number of ears per unit area can reduce their fertility (Bendjemaa, 1977), and there is no direct relationship between tillering capacity and the number of ears per unit area (Blum and Pnuel, 1990). The best grain yields in durum wheat in semi-arid zones are achieved through a combination of genetic capacity to produce more ears per unit area and good fertility (Abbassenne et al., 1997a). Simane et al. (1993) found that the number of grains per spike directly contributes to grain yield, with fertility being the most crucial yield component (Ledent, 1978). Couvreur (1981) observed that average grain weight, formed latest, is negatively associated with the number of grains per unit area.

Grain weight has minimal impact on yield variation in local varieties under stress, as these tall varieties support grain growth by transferring reserves from stems under stress (Blum et al., 1989). The size and average weight of the grain contribute to the production stability of a cultivar and depend on post-anthesis growth conditions, photosynthetic activity during grain filling, and the number of endosperm cells (Benlaribi, 1984). Wardlaw (2002) noted that grain filling time significantly affects average grain weight, with the filling rate per grain better explaining differences in weight than the duration of the filling phase (Abbassenne et al., 1997a). Simane et al. (1993) found all direct effects of yield components to be positive, indicating that maintaining other components constant would result in yield increases.

Richards et al. (1997) pointed out that high biomass is desirable in semi-arid environments, and Siddique et al. (1989) noted that high biomass leads to higher yields in recent varieties. A longer development cycle favors the production of high-quality above-ground biomass. Abbassenne et al. (1997b) found that dry matter weight in the ear at the heading stage is not a reliable predictor of final grain yield. Dakheel et al. (1993) observed a positive correlation between the harvest index and grain yield across different environments. Under non-limiting growth conditions, the harvest index is about 50%, but it drops to 35% in arid regions, casting doubt on whether improving this index would enhance grain yields under water stress. Achieving high above-ground biomass, which predominantly forms as grains, is essential.

2 .CHAPTER 02: MOLECULAR MARKER AND MARKER ASSISTED SELECTION IN AGRICULTURE

2.1 The concept of Molecular Markers

Molecular markers are indispensable tools in modern agriculture, providing insights into the genetic composition of crops that are essential for breeding programs and crop improvement efforts. These markers, which are essentially identifiable DNA sequences scattered throughout the genome, serve as landmarks for specific genes or genetic variations within populations of plants. By deciphering the genetic makeup of crops, researchers can gain a deeper understanding of traits of interest, such as disease resistance, yield potential, and environmental adaptation (Varshney et al., 2019).

2.2 Types of Molecular Markers

There are three major types of genetic markers: (a) morphological markers (also called "classical" or "visible" markers) which are phenotypic traits, (b) biochemical markers, which are called isozymes, including allelic variants of enzymes, and (c) DNA markers (or molecular markers), which reveal sites of variation in DNA . Morphological markers are usually identified visually (eg, traits such as flower color, seed shape, growth habits or pigmentation). Isozymes are allelic variants of enzymes that are detected by electrophoresis and specific staining. They are able to detect diversity at functional gene level and have simple inheritance.(Varshney et al., 2019)

The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant . However, despite these limitations, morphological and biochemical markers have been extremely useful to plant breeders . DNA markers are the most widely used type of marker predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA . These markers are selectively neutral because they are usually located in non-coding regions of DNA. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant (Varshney et al., 2019)

Several types of molecular markers are employed in agricultural research, each offering unique advantages and applications. such as Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs).(Nadeem et al,2018)

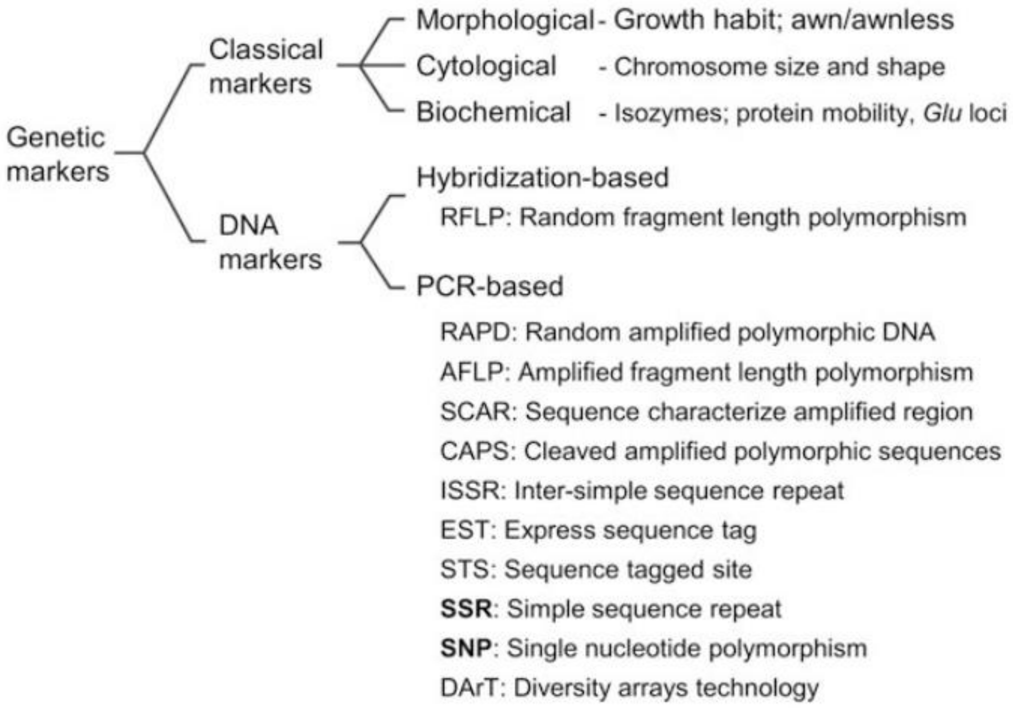

Figure 4 :Classification of genetic markers. Highlighted in bold are markers widely used in MAS in wheat(Nadeem et al,2018)

2.2.1 Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) is a powerful method for amplifying particular segments of DNA, distinct from cloning and propagation within the host cell. This procedure is carried out entirely biochemically, that is, in vitro. PCR was invented by Kary Mullis in 1983. (Aryal, 2022)

Steps in PCR :

There are three major steps involved in the PCR technique: denaturation, annealing, and extension.

In step one, the DNA is denatured at high temperatures (from 90 - 97 degrees Celsius). In step two, primers anneal to the DNA template strands to prime extension. In step three, extension occurs at the end of the annealed primers to create a complementary copy strand of DNA. This effectively doubles the DNA quantity through the third steps in the PCR cycle. To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA. Next, an enzyme called "Taq polymerase" synthesizes - builds - two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on, and so on (Joshi et al., 2011).

The annealing phase happens at a lower temperature, 50-60°C. This allows the primers to hybridize to their respective complementary template strands, a very useful tool to forensic chemistry. The newly-formed DNA strand of primer attached to template is then used to create identical copies of the original template strands desired. Taq polymerase adds available nucleotides to the end of the annealed primers. The extension of the primers by Taq polymerase occurs at approx 72°C for 2-5 minutes. DNA polymerase cannot be used to elongate the primers as one would expect because it is not stable at the high temperatures required for PCR. The beauty of the PCR cycle and process is that it is very fast compared to other techniques and each cycle doubles the number of copies of the desired DNA strand. After 25-30 cycles, whoever is performing the PCR process on a sample of DNA will have plenty of copies of the original DNA sample to Y X conduct experimentation. Assuming the maximum amount of time for each step, 30 cycles would only take 6 hours to complete. (Joshi et al., 2011)

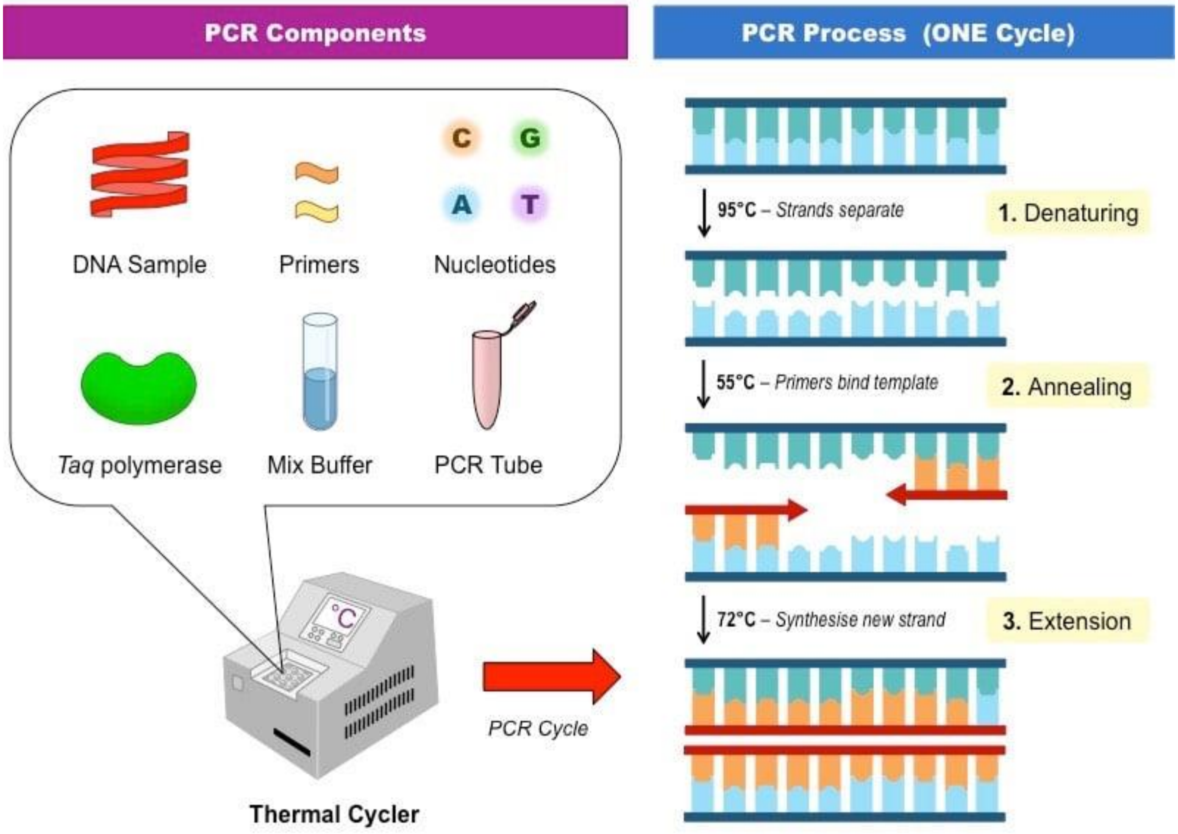

Figure 5 : The polymerase chain reaction (PCR) (Aryal, 2022)

2.2.1.1 PCR Primers

PCR primers, essential components in the PCR process, initiate the synthesis of DNA strands. The efficacy of primers significantly influences the sensitivity and success rate of PCR experiments. Several factors dictate primer efficiency: (Aryal, 2022) the stability of primer-template duplex formation and dissociation during annealing and extension phases, (Joshi et al., 2011) the resistance of the duplex to mismatches, and (3) the polymerase's ability to identify and extend the mismatched duplex. Critical features such as primer length, GC content, melting temperature (Tm), specificity of the 3' end, and stability of the 5' end profoundly impact primer performance. Careful primer design is paramount for successful PCR outcomes; even a minor imbalance in primer characteristics can disrupt or yield false results in PCR experiments. Optimal primer length typically ranges from 18 to 30 nucleotides, while a melting temperature (Tm) within 52–58°C generally yields favorable results. Moreover, maintaining a GC content between 45% and 60% is pivotal for primer efficiency (Aryal, 2022) .

2.2.2 -Restriction fragment length polymorphism (RFLP)

RFLP was the first molecular marker technique and the only marker system based on hybridization. Individuals of same species exhibit polymorphism as a result of insertion/deletions (known as InDels), point mutations, translocations, duplications and inversions. Isolation of pure DNA is the first step in the RFLP methodology. This DNA is mixed with restriction enzymes which are isolated from bacteria and these enzymes are used to cut DNA at particular loci "known as recognition sites".(Nadeem et al,2018). This results in a huge number of fragments with different length. Agarose or polyacrylamide gel electrophoresis "PAGE" is applied for the separation of these fragments by producing a series of bands. Each band represents a fragment having different lengths. Base-pair deletions, mutations, inversions, translocations and transpositions are the main causes for the variation resulting in the RFLP pattern. These variations lead to the gain or loss of recognition sites, resulting in fragments of various length and polymorphism. The restriction enzymes will not cut the fragment if a single base-pair variation occurs in the recognition site. However, if this point mutation occurs in one chromosome but not the other, it is called heterozygous for the marker, as both bands are present .(Nadeem et al,2018)

2.2.3 Single nucleotide polymorphism (SNP)

Single nucleotide variations within an individual's genome are classified as SNPs, representing the most common type of genetic variation . SNPs encompass transitions "e.g., C/T or G/A" and transversions "e.g., C/G, A/T, C/A, or T/G", reflecting substitutions between nucleotides (Brookes, A. J. 1999). Additionally, SNPs may include single base insertions/deletions (InDels), further contributing to genomic diversity. As the fundamental unit of inheritance, SNPs offer a straightforward and abundant source of genetic markers, facilitating diverse applications across plants and animals . The frequency of SNPs in plant genomes typically ranges from 1 SNP per 100–300 base pairs (Rafalski, A. 2002).

Widely distributed throughout genomes, SNPs can be found within coding or non-coding regions of genes, as well as in intergenic regions, each exhibiting distinct allelic frequencies. Various genotyping methodologies have been devised to detect SNPs, leveraging different techniques of allelic discrimination and detection platforms. Notably, Restriction Fragment Length Polymorphism (SNP–RFLP) and Cleaved Amplified Polymorphic Sequences (CAPS) markers represent straightforward and accessible approaches for SNP detection, based on the presence or absence of restriction enzyme binding sites.(Rafalski, A.,2002)

2.2.4 Random amplified polymorphic DNA (RAPD)

The RAPD technique, pioneered independently by Williams et al. and Welsh and McClelland, revolutionized genomic analysis by enabling the amplification of diverse DNA fragments. Utilizing PCR with single, short (10 nucleotides) and randomly chosen primers, genomic DNA is amplified, with amplification occurring when two hybridization sites align in opposite directions (Williams, J. G., et al. 1990). The resulting amplified fragments depend on the lengths and sizes of both the target genome and the primer employed . Optimal primer selection is crucial, with a minimum GC content of 40% recommended to withstand the annealing temperature (72°C), where DNA elongation by DNA polymerase occurs (McClelland, M., et al. 1995).

Following amplification, the PCR product is visualized by separation in an agarose gel stained with ethidium bromide . Polymorphisms, either at or between primer binding sites, are discerned during electrophoresis by confirming the presence or absence of specific bands . However, achieving reproducible RAPD markers hinges on several critical factors, including the quantity and quality of DNA, PCR buffer composition, magnesium chloride concentration, annealing temperature, and the type of DNA polymerase (Taq DNA polymerase) utilized .(McClelland, M., et al.1995)

2.3 Classification of Molecular Markers

Molecular markers can be classified based on various criteria, including their origin, detection methods, or functional relevance. From a functional perspective, markers are often categorized as either functional or non-functional. Functional markers directly affect phenotypic traits or gene function and are therefore of direct interest to breeders. In contrast, non-functional markers are merely linked to traits of interest without directly impacting their expression (Pandey et al., 2020). This classification facilitates the selection of markers based on their utility in breeding programs, with functional markers being prioritized for their immediate impact on trait improvement.

Feature and description	RFLP	RAPD	AFLP	SSR	SNP
Genomic abundance	High	High	High	Moderate to high	Very high
Genomic coverage	Low copy coding region	Whole genome	Whole genome	Whole genome	Whole genome
Expression/inheritance	Co-dominant	Dominant	Dominant/co-dominant	Co-dominant	Co-dominant
Number of loci	Small (< 1000)	Small (< 1000)	Moderate (1000s)	High (1000s–10,000s)	Very high (> 100,000)
Level of polymorphism	Moderate	High	High	High	High
Type of polymorphism	Single base change, indel	Single base change, indel	Single base change, indel	Changes in length repeat	Single base change, indel

Cloning and/or sequencing	Yes	No	No	Yes	Yes
Type of probes/primers	Low-copy DNA or cDNA clones	10 bs random nucleotides	Specific sequence	Specific sequence	Allele-specific PCR primer
PCR-based	Usually no	Yes	Yes	Yes	Yes
Radioactive detection	Usually yes	No	Yes or no	Usually no	No
Reproducibility/reliability	High	Low	High	High	High
Amount of DNA required	Large (5–50 µg)	Small (0.01–0.1 µg)	Moderate (0.5–1.0 µg)	Small (0.05–0.12 µg)	Small (> 0.05 µg)
Genotyping throughput	Low	Low	High	High	High
Cost	Moderate to high	Low	Moderate	Moderate to high	High
Marker index	Low	Moderate	Moderate	Moderate to high	Moderate
Time demanding	High	Low	Moderate	Low	Low
Number of polymorphic per loci	1.0–3.0	1.5–5.0	20.100	1.0–3.0	1.0
Primary application	Genetic	Diversity	Diversity and genetic	All purposes	All purposes

Tableau 3 : Comparison of most widely used DNA marker system in plants(Pandey et al., 2020)

2.4 Advantages and Limitations of Molecular Markers

The adoption of molecular markers in agricultural research has brought about a paradigm shift in crop improvement strategies, offering several advantages over traditional breeding methods. These advantages include increased precision in trait selection, accelerated breeding cycles, and the ability to introduce desirable traits from wild relatives or exotic germplasm

(Saxena et al., 2022). Furthermore, molecular markers enable breeders to overcome limitations associated with phenotypic evaluations, such as long generation times and environmental variability. However, challenges such as marker saturation, cost constraints, and the need for specialized expertise in marker analysis must be addressed to fully harness the potential of molecular markers in agriculture (Yang et al., 2023). Collaborative efforts between researchers, breeders, and policymakers are essential to overcome these obstacles and maximize the impact of molecular markers on crop improvement.

2.5 Applications of Molecular Markers in Durum Wheat Research

2.5.1 Construction of linkage maps and QTL mapping

Quantitative trait loci (QTL) are regions of DNA that are associated with variations in a particular trait or characteristic within a population. These traits can be continuous, such as height or weight, and are influenced by multiple genes and environmental factors. QTLs are identified by correlating molecular markers (*Quantitative Trait Locus*, n.d.),

One of the main applications of DNA markers in agricultural research is the construction of linkage maps for different types of crops. Linkage maps are used to identify chromosomal regions that contain single gene traits (controlled by a single gene) and quantitative traits using QTL analysis . In other words, in order to use the genetic information that has been provided by molecular markers, it is important to know the relative location of molecular markers on chromosomes. (Kordrostami et al.,2015)

Linkage map can be considered as a "road map" of chromosomes from two different parents . Genetic linkage maps show the relative distances between markers along the chromosomes which are similar to the symptoms and signs along the highway. The most important application of linkage maps is the identification of chromosomal regions containing QTLs and genes associated with desired traits. Such maps can be named QTL maps. In other words, the process of making linkage maps and QTL analysis to identify genomic regions associated with desirable traits known as QTL mapping . QTL mapping is based on this fact that genes and markers are segregating through chromosomal recombination (chromosomal crossover) during meiosis (sexual reproduction), which permit to analyze them in the progenies . Genes or markers which are extremely close to each other or tightly linked, are transferred from parent to progenies together. This is more common than the other genes or markers that have been separated(Kordrostami et al.,2015).

2.5.2 Marker-Assisted Selection (MAS)

Marker-assisted selection (MAS) can be defined as the manipulation of genomic regions that are involved in the desirable trait of interest through DNA markers , and their potential use in crop improvement begins a new era of molecular breeding . The MAS has an edge over the visual phenotypic selection because the trait of interest is linked with a molecular marker which increases the selection efficiency of the targeted trait . (Kumawat, G.et al .,2021).The MAS has comprehensively been used in plant breeding to characterize germplasm, diversity analysis, trait stacking, gene pyramiding, multi-trait introgression, and genetic purity of different cereals, pulses, oilseeds, and fiber crops, etc. Mapping studies pointed out several markertrait associations from different crop species, which specifies the potential application of MAS in accelerating crop improvement. (Jiang, G.-L.,2013)

The prerequisite for an efficient MAS program is reliable markers, quality of DNA extraction method, genetic maps, knowledge of marker-trait association, quick and efficient data processing, and availability of high throughput marker detection system . Marker development pipeline adapted from Collard and Mackill, 2008, in Figure 1 explain that how marker assisted selection imposed from development of population through various steps (Kumawat, G.et al .,2021)

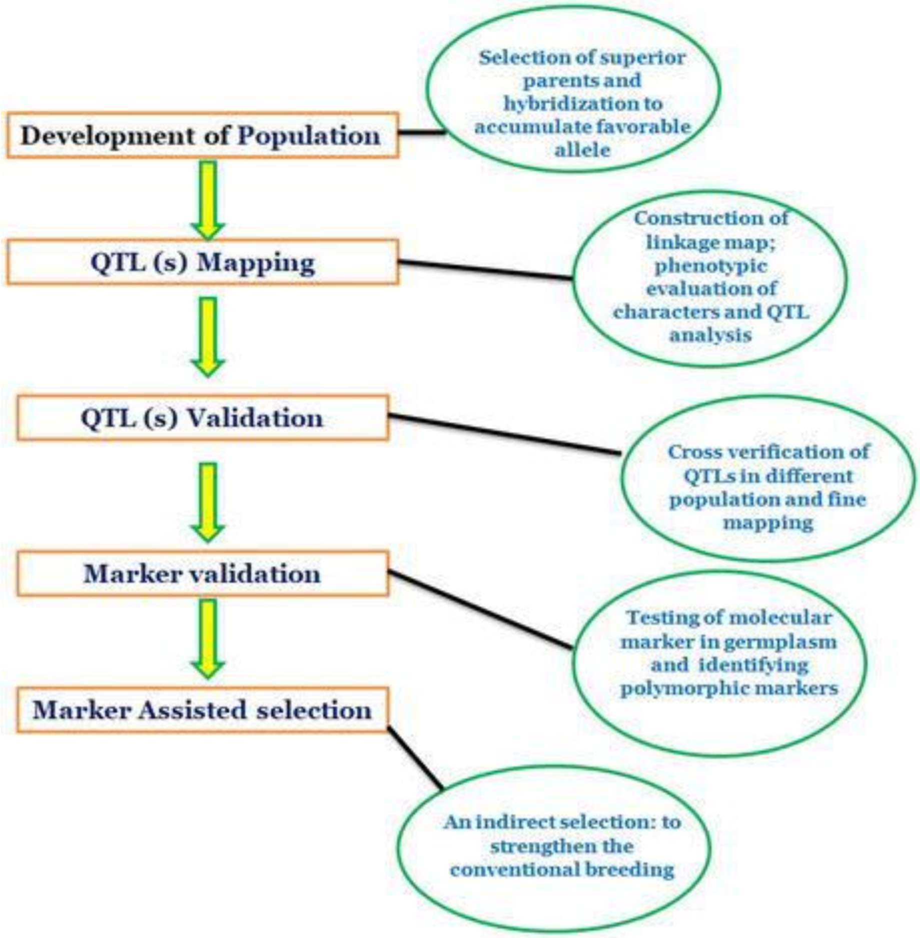

Figure 6 : Marker development flow chart (Kumawat et al ., 2021)

2.5.3 Application of QTL Mapping for disease resistance in wheat diseases

Using QTL mapping for disease resistance in wheat is a promising approach in modern agriculture. By identifying and mapping quantitative trait loci (QTL) associated with disease resistance, researchers can better understand the genetic basis of resistance to various wheat diseases (Collard et al., 2005). This knowledge allows for the development of wheat varieties with improved resistance through marker-assisted selection (MAS) or genomic selection (Peleman, 2003).

2.5.4 Exampels of the most disease related to durum wheat :

2.5.4.1 Fusarium head blight :

Fusarium head blight (FHB) is a significant disease in wheat-growing countries, caused mainly by Fusarium graminearum, contaminating grains with mycotoxins.

Key QTLs for FHB resistance include Fhb1 on chromosome 3BS and Fhb2 on chromosome 6BS, with Fhb1 reducing disease severity significantly.(Vagndorf et al.,2018)

Resistance gene	Marker type	Marker name	Location
Fhb1	SSR	Xgwm493, Xgwm533	3BS
Fhb2	SSR	Xgwm133, Xgwm644	6BS
Fhb4	SSR	Xhbg226, Xgwm149	4B
Fhb5	SSR	Xgwm304, Xgwm415	5A
Fhb6	KASP	Wg1s_snp1	1AS
Fhb7	SSR	XsdauK66, Xcfa2240	7DS

Tableau 4 : Overview of the FHB-resistant genes identified in wheat using molecular markers.(Vagndorf et al.,2018)

2.5.4.2 Wheat stripe rust (yellow rust)

Wheat stripe rust, caused by Puccinia striiformis, leads to significant yield losses annually, affecting cereals and Berberis spp. Breeding efforts since 1905 have identified over 70 Yr genes for resistance, with adult plant resistance considered more durable than seedling resistance. Molecular markers like SSRs used to identify FHB-resistant genes in wheat breeding programs.Some widely used Yr genes like Yr17, Yr27, and Yr31 have lost effectiveness against yellow rust, emphasizing the need for continuous research and breeding efforts.(Vagndorf et al.,2018)

Resistance gene	**Marker type**	**Marker name**	**Location**
Yr5	SSR	Xgwm501	2BL
Yr7	SSR	Xgwm526	2BL
Yr15	SSR	Xbarc8, Xgwm493	1BS
Yr18	CAPS	Cssfr6	7D
Yr36	SSR	Xgwm508, Xbarc136	6BS
Yr60	SSR	Xwmc776	4AL
Yr76	SSR	Xwmc11, Xwmc532	
Yr78	SNP	IWA7257	6BS

Tableau 5 : A selection of the genes conferring YR resistance identified by molecular markers.(Vagndorf et al.,2018)

3 .CHAPTER 03: BIOTIC AND ABIOTIC STRESSES IN ALGERIAN DURUM WHEAT

3.1 Concept of Stress

The concept of stress in agriculture refers to the disruption of normal physiological processes in plants caused by various external factors. Stress can be defined as any condition that imposes a potentially harmful constraint on the living organism, leading to changes in physiological processes that may result in damage, injury, inhibition of growth, or developmental impairment. Stress can manifest at cellular levels and impact the overall health and productivity of the plant. It is often characterized by a deviation from the normal functioning of plant physiology and can be induced by both biotic and abiotic factors (Hopkins et al.,1995).

3.2 Main Constraints Related to Wheat Production in Algeria

Wheat production in Algeria confronts multifaceted challenges, hindering its productivity and long-term sustainability. Abiotic factors, notably water scarcity, temperature fluctuations, and soil salinity, significantly impede optimal crop growth and yield. Algeria's semi-arid climate, characterized by erratic rainfall patterns and high evaporation rates, poses a substantial constraint on wheat cultivation, with water scarcity often limiting agricultural output (FAO, 2020). Moreover, temperature extremes, ranging from scorching summers to chilling winters, can adversely affect wheat development and yield potential, particularly during critical growth stages (Nouar et al., 2018). Soil salinity, exacerbated by improper irrigation practices and inadequate drainage systems, further restricts wheat productivity by impeding nutrient uptake and root development (Belkhodja et al., 2016).

Limited access to essential resources and inputs presents another significant hurdle for wheat farmers in Algeria. Smallholder farmers, who comprise a substantial portion of the agricultural workforce, often face challenges in acquiring quality seeds, fertilizers, and pesticides due to financial constraints and inadequate infrastructure (Zennouche et al., 2017). Insufficient mechanization and outdated farming techniques further exacerbate these resource limitations, impeding efficient wheat production and reducing overall crop yields (Belabid et al., 2020). The lack of modern equipment and machinery not only increases labor requirements but also hampers the adoption of more sustainable and productive agricultural practices.

In addition to abiotic challenges and resource constraints, biotic stresses pose significant threats to wheat cultivation in Algeria. Fungal diseases, such as wheat rusts and powdery mildew, can cause substantial yield losses if left unmanaged, particularly in regions with conducive environmental conditions (Benbelkacem et al., 2019). Weed infestations represent another pervasive issue, competing with wheat plants for resources and reducing crop vigor and yield potential (Derridj et al., 2018). Limited access to effective agrochemicals and integrated pest management strategies further complicates the control of biotic stresses, leaving farmers vulnerable to crop losses and economic hardships.

3.3 Types of Stress

3.3.1 Abiotic Constraints

Abiotic constraints, encompassing environmental factors, pose significant challenges to durum wheat cultivation in Algeria. These constraints include hydric stress, thermal stress, and salinity stress, each presenting distinct obstacles to crop growth and productivity. Hydric stress, often induced by erratic rainfall patterns or limited access to irrigation water, can lead to water scarcity during critical growth stages, impairing physiological processes and ultimately reducing yields. Thermal stress, exacerbated by high temperatures and heatwaves, can adversely affect durum wheat development, causing heat-induced sterility, reduced photosynthetic activity, and accelerated plant senescence. Additionally, salinity stress, arising from the accumulation of salts in the soil or irrigation water, can impair water uptake and nutrient absorption by durum wheat plants, leading to osmotic imbalance, ion toxicity, and reduced growth and yield.(Hamoud, A et al.,2021)

These abiotic constraints not only limit durum wheat production but also necessitate the implementation of adaptive strategies to mitigate their adverse effects. For instance, the adoption of drought-tolerant durum wheat varieties, improved water management practices, and the utilization of soil amendments to mitigate salinity stress can enhance crop resilience and productivity in the face of these environmental challenges.(Hamoud, A et al.,2021)

3.3.1.1 Hydric Stress

Hydric stress, commonly known as water stress, is a prevalent abiotic constraint affecting wheat cultivation in Algeria. This stress arises when plants encounter either a deficiency or an excess of water, leading to physiological disruptions and limitations in growth. Prolonged drought conditions or excessive rainfall can both induce hydric stress, negatively impacting plant water uptake, photosynthesis, and nutrient absorption (Ammar et al., 2020).

3.3.1.1.1 Effect of Hydric Stress

The impact of hydric stress on wheat cultivation in Algeria is profound, significantly affecting both yield and grain quality. Prolonged water deficits can result in stunted growth, reduced tillering, and diminished grain filling, ultimately culminating in yield losses. Conversely, waterlogging and excessive soil moisture can lead to root suffocation, nutrient leaching, and heightened vulnerability to diseases such as root rot. Effective water management strategies, such as irrigation scheduling and the utilization of drought-resistant cultivars, are imperative for mitigating the adverse effects of hydric stress on wheat production (Belkhodja et al., 2016).

3.3.1.2 Thermal Stress

Thermal stress, induced by extreme temperatures, represents another significant abiotic constraint impacting wheat cultivation in Algeria. Elevated temperatures during the growing season can expedite crop development, decrease grain yield, and compromise grain quality. Conversely, frost events occurring during critical growth stages can inflict frost damage, resulting in yield losses and quality deterioration. Management strategies to alleviate thermal stress, including the selection of heat-tolerant cultivars and the implementation of cultural practices to mitigate temperature extremes, are crucial for sustaining wheat production amidst climate variability (Nouar et al., 2018).

3.3.1.2.1 Effect of Thermal Stress on Wheat Cultivation

The effect of thermal stress on wheat cultivation in Algeria is multifaceted, with temperature extremes posing significant challenges to crop growth and development. High temperatures can accelerate crop phenology, leading to shortened growth cycles and reduced grain filling periods. Conversely, cold temperatures during critical growth stages can induce physiological damage, reduced tillering, and poor grain set, ultimately resulting in yield losses. Selecting heat-tolerant cultivars, implementing appropriate crop management practices, and adopting climate-resilient technologies are essential strategies for mitigating the adverse effects of thermal stress on wheat production (Nouar et al., 2018).

3.3.1.3 Salinity Stress

Salinity stress, arising from the accumulation of salts in the soil, is a prevalent abiotic constraint affecting wheat cultivation in Algeria. Elevated soil salinity levels impede plant water uptake, disrupt nutrient balance, and interfere with physiological processes, thereby inhibiting growth and reducing yield. Management strategies for salinity stress, such as soil amendments, crop rotation, and the utilization of salt-tolerant cultivars, are indispensable for minimizing its impact on wheat productivity (Belkhodja et al., 2016).

3.3.2 Biotic Stresses

Biotic stresses pose significant challenges to wheat production in Algeria, with fungal diseases and weed infestations being prominent among them. Fusarium crown rot (FCR) and Fusarium head blight (FHB), caused by various Fusarium species, are particularly prevalent fungal diseases that have detrimental effects on wheat crops, resulting in yield losses and decreased grain quality (Boutaoui et al., 2019). FCR affects the crown and roots of wheat plants, leading to root rot and impaired nutrient uptake, while FHB primarily affects the spike, causing bleaching of the kernels and mycotoxin contamination, which poses risks to human and animal health (Gharsallah et al., 2018).

Weed infestations further compound the challenges faced by wheat farmers in Algeria, especially with the emergence of herbicide-resistant weed species. Weeds compete with wheat plants for essential resources such as nutrients, water, and sunlight, thereby reducing crop yields and impeding overall productivity. Moreover, weed interference can hinder harvesting operations and increase production costs for farmers (Rezig et al., 2020). The spread of herbicide resistance among weed populations exacerbates this issue, limiting the effectiveness of chemical control methods and necessitating alternative approaches for weed management.

3.3.2.1 Fusarium Crown Rot (FCR)

Fusarium crown rot (FCR), caused primarily by Fusarium spp., is one of the most devastating diseases affecting durum wheat worldwide (Akinsanmi et al., 2019). The disease manifests as a rot of the crown and roots, leading to stunted growth, wilting, and ultimately plant death. The pathogen infiltrates the plant through the roots and colonizes the crown and lower stem tissues, causing necrosis and compromising the plant's ability to absorb water and nutrients (Kazan and Gardiner, 2018).

3.3.2.1.1 Symptoms of Fusarium Crown Rot

Symptoms of FCR typically appear during the later stages of plant development, often becoming apparent during the flowering and grain-filling stages. Infected plants exhibit wilting, yellowing of foliage, and premature senescence.figure(07) Additionally, brown discoloration and rotting of the crown and root tissues can be observed upon closer inspection (Sarver et al., 2020).

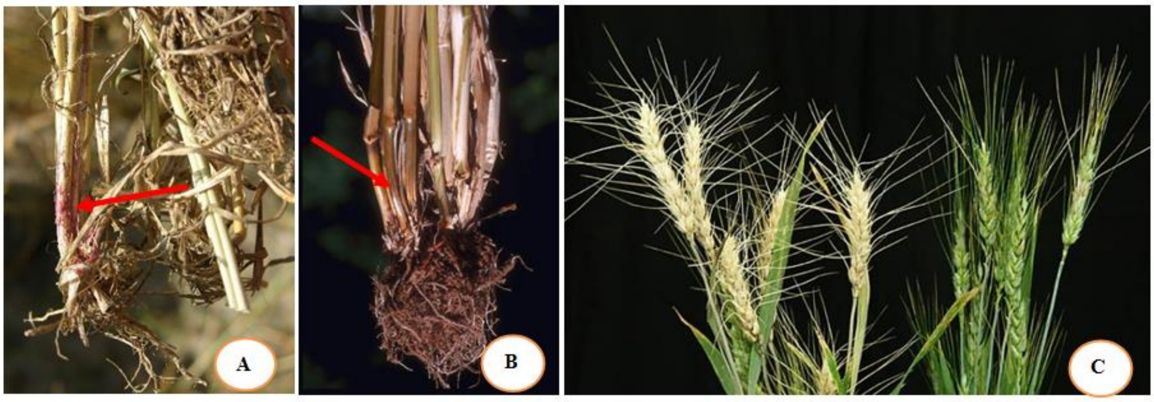

Figure7: Head blight symptom on wheat and barley caused by Fusarium (FCB).
(A,B) Pink/red fungal mycelium and discoloration of stem bases, Photo by SIMMYT.
(C) Whitehead symptom on spike right: non infected, left: infected (White head), Photo by Guihua Bai. (Matny ON.,2015)

3.3.2.2 Fusarium Head Blight (FHB)

Fusarium head blight (FHB) stands as a formidable threat to durum wheat production globally, attributed primarily to Fusarium graminearum and Fusarium culmorum (Bai and Shaner, 2004). This disease strikes durum wheat during the critical flowering stage, leading to substantial yield losses, compromised grain quality, and contamination with mycotoxins, particularly deoxynivalenol (DON), which poses significant risks to human and animal health (Goswami and Kistler, 2004).

3.3.2.2.1 Symptoms of Fusarium Head Blight

The onset of Fusarium head blight (FHB) is characterized by distinctive symptoms observable on wheat heads. Affected spikelets often exhibit premature bleaching or a distinct white discoloration, earning them the moniker "white heads." Furthermore, infected spikelets may display additional symptoms such as the development of pink to orange fungal growth, commonly referred to as "tombstone" symptoms, indicative of colonization by Fusarium spp. (Bai and Shaner, 2004).

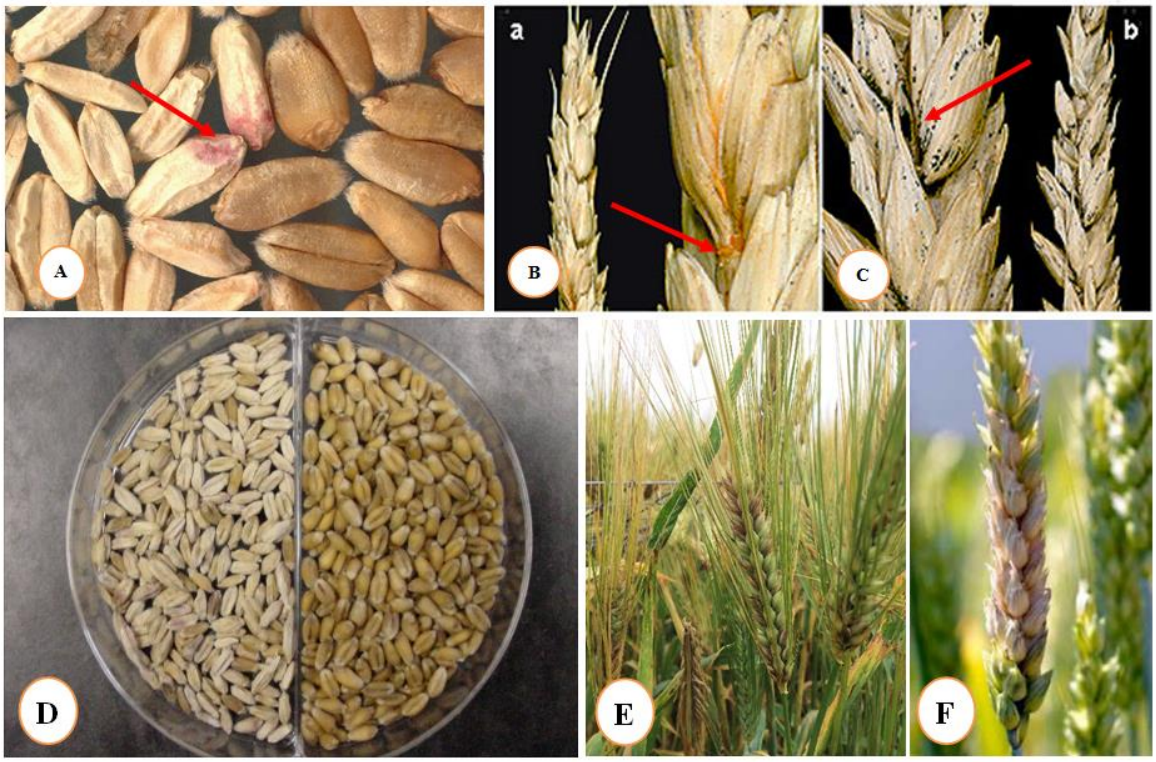

Figure 8 :Head blight symptom on wheat and barley caused by Fusarium (FHB). (Matny ON.,2015)

(A,D)small, shriveled pale white appearance and sometimes pink, infected (left)and healthy (right)

(B,C) Orange sporodochia and Bluish black perithecia are formed at the base of the glumes.

(E,F) Premature bleaching of Barley and wheat spikes.

3.3.2.2.2 Life Cycle of Fusarium Head Blight (FHB) and Fusarium Crown Rot (FCR)

The causal agent for FCR and FHB is interplay. Infection occurs when spores land on susceptible wheat and barley heads. Heads are most susceptible at early flowering and infection may occur up to the soft dough stage, although severity is greatly reduced. If the flowers are infected just after their emergence kernels will not develop. Florets that are infected later will produce tombstones. Kernels that are infected by the pathogen during late kernel development may appear healthy, but be contaminated with mycotoxins. *Fusarium* crown and root rot can develop in the following season if infected seed is planted. Also maize is one of the crop that infected by same *Fusarium* spp that infected wheat and barly, so cultivation wheat or barly near or after maize rotation will increase inoculation which leaded to hight disease incidence (Matny,. 2015)) figure (9)

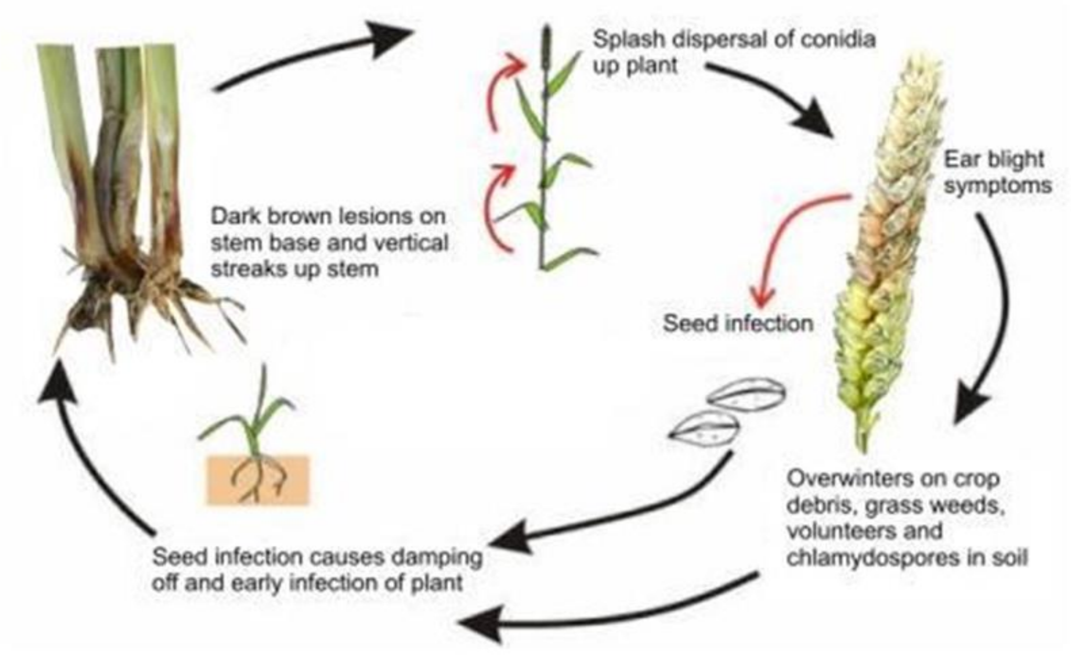

Figure 9 :The typical life cycle of Fusarium in wheat (Matny ON.,2015)

3.4 Weed Infestations and Herbicide Resistance

Weed infestations pose a formidable challenge to durum wheat production, not only diminishing crop yields but also imposing significant economic burdens on farmers. These invasive plants compete vigorously with durum wheat for crucial resources such as water, nutrients, and sunlight, thereby hampering crop growth and development. The unchecked proliferation of weeds can lead to severe yield reductions, jeopardizing food security and agricultural sustainability. Moreover, weed infestations necessitate labor-intensive and costly weed management practices, further adding to the production costs and operational challenges faced by farmers (Heap, 2021).

3.4.1 Utilization of Herbicide Resistance

In light of the escalating prevalence of herbicide-resistant weed biotypes, the development and adoption of herbicide-resistant durum wheat cultivars have emerged as a promising strategy to combat weed infestations effectively. These genetically modified cultivars are equipped with specific genetic traits that confer tolerance or resistance to particular herbicides, enabling targeted weed control while minimizing environmental impact and preserving crop yield potential. By integrating herbicide-resistant traits into durum wheat varieties, farmers can implement more efficient and sustainable weed management strategies, thereby enhancing productivity and profitability in durum wheat cultivation (Busi et al., 2018).

The utilization of herbicide-resistant durum wheat cultivars represents a significant advancement in agricultural biotechnology, offering farmers a valuable tool to mitigate the detrimental effects of weed infestations on crop production. Through the strategic deployment of herbicide-resistant traits, farmers can achieve effective weed control, reduce reliance on conventional herbicides, and optimize resource utilization, ultimately contributing to the long-term sustainability and resilience of durum wheat farming systems (Busi et al., 2018).

4 .CHAPTER 04 : THE USE OF MOLECULAR MARKERS IN DURUM WHEAT IMPROVEMENT IN ALGERIA

4.1 Historical background

durum wheat breeding program in Algeria began with France, until the 1960s, production and research programs have mainly been conducted in Algeria, Morocco, and Tunisia. In 1930, breeding work was conducted in Algeria by Ducellier,who identified 29 local cultivars or populations of durum wheat. Wheat research was performed and coordinated by the Institut National de la Recherche Agronomique (INRA) in Montpellier until Algeria's independence in 1962, the Algerian Center for Agronomic, Scientific, and Economic Research (CARASE) took over the selection work, maintaining existing collections and testing different varieties. The Ministry of Agriculture (MOA) later evaluated new, improved, and high-yielding germplasm, mainly from the International Maize and Wheat Improvement Center (CIMMYT) (Abdelkader,2014)

4.2 Cereals Project and Field Crops Development Institute

In 1971, the "Projet Céréales" (Cereals Project) was created to integrate different elements within a technological package, involving the Food and Agriculture Organization (FAO) and the Central Cash of the European Community. This project marked the beginning of a new research-for-development process targeting cereal production intensification and dryland field crop diversification. The Field Crops Development Institute (IDGC) was established in 1974 to organize the development of cereals, forages, and food legumes within the country.Varietal Achievements and Breeding Approaches .The IDGC's germplasm enhancement program targeted good adaptation to diverse conditions, high fertility/productivity, disease and pest resistance, and good end-use quality.This result to good variations table (08) (Abdelkader,2014). The institute conserved botanical collections, utilized local landraces, and introduced new genotypes from abroad for adaptation trials and crosses. Collaboration with CIMMYT and the International Center for Agricultural Research in the Dry Areas (ICARDA) contributed significantly to Algeria's field crops research development (Abdelkader,2014)

Durum Wheat		Bread Wheat	
Chougrane	Boussellem	Hodna	Aïn Abid
Rahouia80	Simeto	Zidene	Orion
Guemgoum Rkhem	Ofanto	Nesser	Almirante
Sebaou	Gur/Dur.	Mimouni	ElWiffak
Arbs	Eider	Soummam	Hamam-1
Righa	Carioca	Chelif	Anapo
Sahel	Cirta	Anza	Tiddis
Chen'S'	Orjaune	Tessalah	Massine
Bibans	Poggio	Rhummel	Boumerzoug
Khroub76	Wahbi	Sidi Okba	Akhamokh
Z bans	Beni Mestina	Ziad	Yacine
Tassili	Sigus	Isser	
Hoggar	Aïn Lehma	Arz	
Kebir03	Ammar 6	Hidhab	
Belikh2	5115		
OumRabi	Megress		
Sham3	Tejdid		

Tableau 6 : List of new varieties released since 1975 in Algeria. (Abdelkader,2014)

4.3 Current Status and Future Directions

Today, Algeria's wheat production is focused on maximizing yield potential under favorable rain-fed production conditions. Breeding efforts concentrate on tolerance to major biotic and abiotic stresses, such as drought and cold. The National Wheat Improvement Program (PNAB) was established in 2005, merging the wheat improvement programs of the ITGC and INRAA. The program involves a participatory approach with farmers to better target and promote new products

4.4 Current use of molecular markers

In Algeria, the utilization of molecular markers in durum wheat improvement represents a significant stride towards more efficient breeding practices. These markers serve as invaluable tools for understanding genetic diversity, characterizing germplasm, and pinpointing alleles linked to desirable breeding traits. (Bousba et al., 2020)

4.5 Case Studies on the Use of Molecular Markers in Durum Wheat in Algeria

One notable study (Younes, 2009) explores the genetic variability in drought resistance among various wheat cultivars grown in Algeria. The study focuses on Simple Sequence Repeat (SSR) molecular markers located on chromosome 7A, which are known to be associated with drought resistance.

The study utilizes SSR (Simple Sequence Repeat) markers, specifically Xwmc9, Xwmc596, and Xwmc603, which are located on chromosome 7A of wheat. These markers have been identified in previous studies to have a connection with drought resistance (Younes, 2009). The research includes 14 wheat cultivars from Algeria. The methodology employed involved a DNA extraction protocol followed by PCR amplification of DNA using primers specific to the SSR markers on chromosome 7A. Electrophoresis was then used to evaluate the PCR products on 2% agarose gels, comparing the electrophoretic profiles to identify polymorphisms among the cultivars. The study found significant polymorphism among Algerian wheat cultivars for the SSR markers studied.

A similar study conducted by (Bousba et al.,2013) also explores the use of molecular markers to assess genetic diversity and drought tolerance in durum wheat genotypes. The study used the same type of molecular marker but examined the broader genetic and phenotypic associations across multiple genotypes. The researchers used 26 different SSR markers to screen a collection of 40 durum wheat genotypes, including landraces, old, moderate, and improved varieties. Genetic diversity was evaluated via the Polymorphism Information Content (PIC) values, determining allelic variations and polymorphic information content to assess genetic diversity and relationships among genotypes. The analysis revealed significant variation in all the measured parameters, with high levels of polymorphism recorded for SSR markers, confirming the high genetic diversity of the genotypes (Bousba et al., 2013).

Furthermore, (De Trigo et al.,2018) explored the genetic diversity of durum wheat varieties in Algeria using microsatellite markers. This study aimed to assess genetic diversity with implications for breeding and genetic conservation. The methodology included DNA extraction followed by PCR amplification using microsatellite primers, and electrophoresis was used to evaluate the PCR products. Genetic diversity was assessed using Polymorphism Information Content (PIC) values and clustering analysis to determine genetic relationships among varieties. The study revealed substantial genetic diversity within the examined varieties, grouping them based on genetic similarities, which aids in identifying distinct genetic pools. The results support the effective use of microsatellite markers in the management and conservation of genetic resources in durum wheat breeding, enhancing efforts to improve yield, stress tolerance, and disease resistance.

In another study,(Boudour and Chaib,2016) analyzed the morphological, phenological, and genetic variation among twenty Algerian durum wheat accessions. The study's goal was to analyze the morphological, phenological, and genetic variation among these accessions. The methodology combined morphological and phenological data collection with genetic analysis. DNA extraction was followed by PCR amplification using specific primers, and electrophoresis was used to separate PCR products and assess polymorphisms. Significant variation was observed in both morphological and genetic traits, with the accessions grouped into clusters based on phenotypic and genotypic similarities, indicating rich diversity among the studied accessions. This comprehensive understanding of genetic and phenotypic diversity in Algerian durum wheat is crucial for breeding and conservation efforts aimed at developing improved wheat varieties.

Additionally, (Bousba et al., 2020) assessed the genetic diversity among various durum wheat genotypes using RAPD markers. The study aimed to evaluate genetic diversity to inform breeding and conservation strategies. The methodology involved DNA extraction followed by PCR amplification using RAPD primers. Electrophoresis was used to evaluate the PCR products, and genetic diversity was assessed using cluster analysis to determine genetic relationships among genotypes. The study found significant genetic variability among the genotypes, demonstrating the utility of RAPD markers in revealing genetic diversity, which can be harnessed in breeding programs to enhance durum wheat varieties (Bousba et al., 2020).

These studies collectively advance our understanding of the genetic landscape of durum wheat in Algeria, leveraging various molecular markers to assess genetic diversity and trait associations. Despite differences in methodologies and specific focuses, they all contribute valuable insights for breeding programs aimed at improving the resilience and productivity of durum wheat in the region.

5 CONCLUTION

Durum wheat is a staple crop with significant importance in agriculture and economic trade worldwide. It provides a substantial portion of the world's population with food. This means that any stress from biotic (living) and abiotic (non-living) factors can constrain the yield and growth of the plant, having a devastating impact. Algeria, like many Mediterranean countries, bases its consumption on durum wheat. This reliance leads to significant efforts to cultivate durum wheat. Using marker-assisted selection techniques, we can enhance the precision in breeding for more efficient, desirable traits that can withstand harsh climates, resist diseases, and improve yield and quality.

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