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The Important of SNPs as Molecular Markers in Plant Pathology Branch, Targets and Applications

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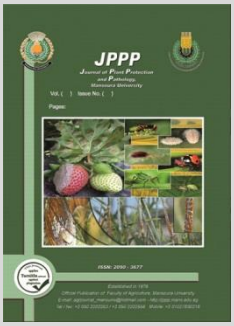

ABSTRACT

Using molecular markers in the improvement of plant sciences research is considered one of the most promising fields, as it shows accurate analysis of genetic variation, understanding of biological phenomena in plants and building a correlation between genomic and phenotypes. The single nucleotide base polymorphism method, which is abundant through DNA genomic is one of the molecular markers used in differentiation or variation and identification studies. SNPs are a very important molecular marker, as it uses in important studies such as mapping, tagging of economic genes, genotyping, diversity analysis and evaluation. Many tools were used in the identification of SNPs, every tool has its advantages and limitation, choice of a certain tool depends on consideration of the research and the availability of the resources. This review shows the important reasons for the use SNPs in the research field and the different tools used in the detection or identification of SNPs with a wide range of facilities ranging from low-cost equipment to high-cost technology.

Keywords: molecular markers, single nucleotide polymorphism (SNPs), genotype.

INTRODUCTION

Genetic variation in organisms needs accurate analysis to identify and investigate genetic structures. Increasing resistance to both abiotic and biotic stresses to improve the genetic crop species. Plant breeder looking for plants/individuals with desirable characteristics. The first attempt to improve plant crops use the phenotype of a plant for a specific character as a tool for selection, which is called the conventional method, which gave an idea about the genotype. This genotype contains quantitative genes influenced by the environment, which alter the genetic phenotypic agronomic traits. So, it probably did not give a correct vision of the genetic makeup of plants. Depending on phenotypic markers, which call in the same time dominant markers. They are so limited due to changes in environmental conditions that make genes under stress not express well and results differ when scored under different breeders. Another group of markers biochemical markers, such as proteins and enzymes which are known as codominant markers . These biochemical markers are considered the end product of gene expression, so they could be affected by environmental factors, These group of biochemical markers gave just an idea about the genetic makeupbiochemical markers are codominant markers, such as proteins and enzymes. They are products of gene expression, so they could be affected by environmental factors. They just gave an idea about the genetic makeup, and variation (of existing or absent molecular bands) it can be determined by the electrophoretic mobility of proteins.

There are other molecular markers groups or DNA markers, they are very accurate and contain information about the genotype. They are more advanced than phenotypic and biochemical markers. A new class of molecular markers called single nucleotide polymorphism (SNPs) has been utilized as an important tool in genetic studies. Jehan and Lakhanpaul 2006.

Such as genotyping, diversity analysis, tagging genes, and evaluation among and between plant species. SNP is defined as a change in a single base of DNA, due to occurring polymorphism between different DNA samples. A map of some plants variation in sequence were containing one SNP every 70 bp in maize, while in some loci detected in a crop such as wheat the average rate of SNP was one bp for every twenty bp. In spite of, the high cost of SNPs markers compared with other groups of molecular markers it is prefer to use, because they already have the following features.They are abundant in the genome which makes them useful for creating a high-density genetic map. They have a lot of information content, a high level of polymorphisms, are highly amendable to automation, non-gel based, so less time-consuming and the frequency rate of SNP is low accompanied with other markers, which makes them evolutionarily stable. Sometimes SNP existing in coding regions (CSNPs), as it may have resulted in a change in amino acid causes the altered phenotypic appearance, increasing the influence of this molecular marker.

Due to the abundance of SNPs in the genome, it's become high informative genotype assay, as it is important to know the different assays used in determining SNPs. Many tools were used to detect SNPs in different plant species. Every assay has its own advancement and dis advancement character as described below Brookes 1999; Bhattramakki (*et al.*,) 2000; Sachidanandam (*et al.*,) 2001;(Agarwal *et al.*, 2008 and Milee *et al.*, 2008)

Data bases and Bioinformatics

Data base established enables researchers to benefit from a publicly available map using SNPs as markers. Researchers can get the information of data through keywords

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like (gene, SNP, genotype data, allele frequency and SNP linkage maps). Some examples of public SNP data bases:
1.http://www.ibc.wustt.edu/SNP/
2.http://www.genome.wi.mit.edu/SNP/human/index.html.
(Syvane *et al.*, 1999; Zubritsky 1999 and Thorisson *et al.*, 2003)

1) Direct sequencing:

Sequencing is considered a direct method for identifying SNPs. DNA can be sequenced by using different methods, but direct sequencing is costly and time-consuming; besides, the error rate of sequencing one base for every 100 bp would identical to the frequency rate at which SNPs are occurring. The direct sequence of plant species for heterozygotes or polyploidies just gave an idea about the base difference. In another method, SNP analysis assay can be useful for the non-model organisms, in which the direct sequence of AFLP bands, enables us to isolate SNP, as sufficient sequence data is not available, that it can be used locus-specific primers. Using a convenient method for the discovery of SNPs such as EST databases with partial CDNA clones by using the genome of the organisms. Detecting SNPs by comparison genomic sequences by make aligning the sequence of different genotypes from the same locus. The most disadvantage observed when using this assay, is using preexisting sequence data. As EST databases program included high sequence error, which lead to false positives identification. The databases used in SNP identification must be verified Nilsson *et al.*, 1997.

Single strand conformation polymorphism (SSCP):

This strategy base on detects polymorphisms by observing the behaviour of DNA samples in different electrophoretic migrations. The electrophoretic mobility in a non-denaturing gel is dependent on both the size and the structure of a single DNA molecule stranded. A single base difference between the sequences can alter the secondary and tertiary conformations.

This difference is due to the influence of various parameters such as the length of the strand, sequence, location, and the number of loci of base pairing. In the primary sequence, a mutation at a particular nucleotide position can change the conformation of the molecule. Direct sequencing could detect the change in the nucleotide, beside reduce the total number of samples demanded sequencing.

Many modifications and improvements to the SSCP technique were done; for example, both of the methods Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE). Both of them could detect SNP in an ordinary laboratory as it is an easy, not very expensive and very precise method. Sunnucks *et al.*, 2000; Gasser *et al.*, 2006 and Robin *et al.*, 2006.

Cleavase fragment length polymorphism (CFLP):

This assay is based on DNA single strands folding on themselves, they assume a secondary structure that is a highly specific feature of the DNA, which is called hairpin molecular structures. The Cleavase I enzyme is working on a specific structure, thermostable , after the enzyme recognized the hairpin region it cleaves the junctions between single and double-stranded regions.

locus-specific oligonucleotide probes (LSOPS) method depends on PCR amplified of target DNA, then heated PCR products to denature the molecule. Transfer the DNA single stranded molecule to cooling temperature which allows to generating the secondary intrastrand structures, then samples treated with an enzyme create the cleavase, and after separating or resolving by electrophoresis it can be detected Fig (1).

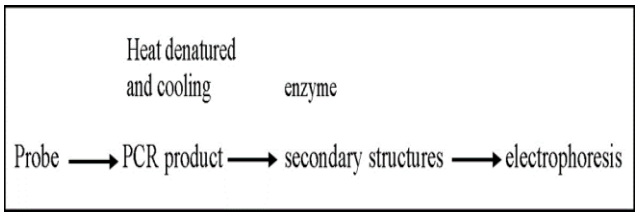

Fig. 1. This diagram summarizes the steps of Cleavase fragment length polymorphism (CFLP) method.

Finally, barcodes appear and any change in one single base nucleotide (SNP) leads to a change in the conformations and generated a special feature of a barcode. CFLP is used in identifying sequence polymorphism in large DNA fragments. This assay depends on enzymatic cleavage rather than electrophoretic mobility (Edward *et al.*, 2001).

Invader assay:

It is a new technology, which identify the SNP genotype depending on the activity of thermostable endonuclease enzyme. The endonuclease enzyme is considered one of FEN family (cleavase) called archaebacterial flap. This endonuclease enzyme cleaves structure-specific in instead of sequence-specific sites.

Designed two oligonucleotide probes, one is called an invader oligonucleotide, while the other is called a signal probe. Target DNA forms a duplex with the signal oligonucleotide prbo , while the invader oligonucleotide probe invades the duplex, forming an overlap at this point. The segment of DNA upstream of the SNP site has sequence homology with the Invader oligonucleotide. The signal nucleotide at its 3´ end has sequence homology with the target DNA, while the segment at its 5^{-} end does not have homology with the target DNA.

Flap endonuclease degrades the overlapping (unpaired) locus on the 5´ end of signal oligonucleotide named flap and allows detection of the specific DNA target.

When the DNA target and the probe are not complementary, the cleavage rate is wildly decreased whereas the overlap is rejected. The discrimination of SNP analysis depends on the cleavage rate of the enzyme for overlapped regions including perfect match and imperfect match.

Invader squared essay contains more than one step reaction. The flap comes from the primary or initial cleavage reaction joined in the second cleavage reaction as an invader probe, which will be against containing fluorescent target among fluorescent reporter and quencher. On cleavage, the target releases a fluorescent signal and it's approximately squaring the amount of cleavage produced when compared with the invader probe in one reaction.

The advantage of this method:

It's highly specific in identifying polymorphisms, accurate and it's precise in discriminating single base differences.
- Does not involve PCR so it's free from contamination.
- Does not need special instruments and the recent fluorescent signal used. But it's a uniplex method as only one genotype can be detected per assay. Griffin *et al.*, 1999; Lyamichev *et.al.*, 1999 and Lyamichev *et al.*, 2000

PNA Directed PCR Clamping:

This method is consisting of a Peptide Nucleic Acid (PNA) which contain a (N-2-aminoethyl glycine backbone with

nucleoside bases attached to the backbone by methylene carbonyl groups). It is a synthetic analogue of (DNA), it is similar in performance to sequence-specific hybridization. This PNA can identify and connect with the complementary nucleic acid sequence, but it is different from corresponding DNA oligonucleotides in the high thermal stability, it is more stable. DNA-DNA duplexes unstable under high temperature compared with PNA/DNA duplexes in common by 1 C per base pair, PNAs use in identification process in case that target DNA has substitution one base pair, this is considered an efficient tool in the identification genotype of SNPs Fig (2), Fig (3) and Fig (4). (Syvanen et al., 1999 and Thorisson & stein, 2003)

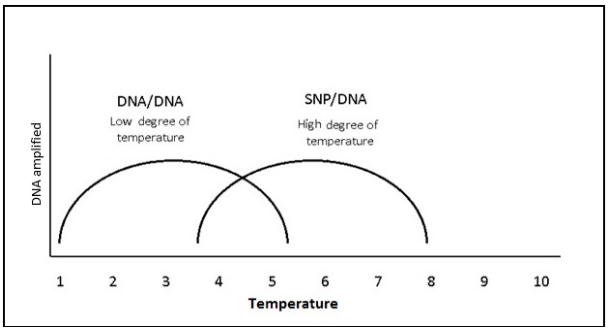

Fig. 2. This diagram clarify that it can be detect the SNP exciting in sample under the optimal temperature in separate tubes.

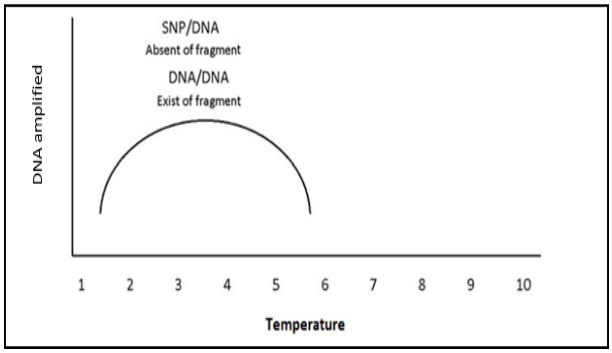

Fig. 3. This diagram clarify that it can be not detect the SNP exciting in sample under the same temperature (not the optimal temperture) in separate tube.

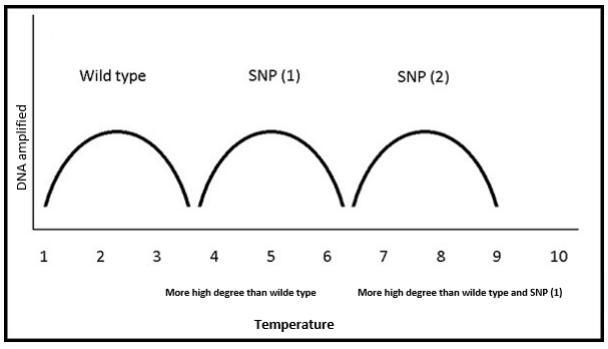

Fig. 4. This diagram clarify that it can be detect more than one SNP exciting in samples under different optimal temperature in separate tubes. When detecting more than one SNPs in the individual tube with different thermal degrees.

Temperature Modulated Heteroduplex Assay (TMHA):

This method called (Denaturing High Performance Liquid Chromatography) (DHPLC); this method can describe the difference in physical properties between mismatched DNA of homoduplexes and heteroduplexes happened during the annealing for both of wild type and mutant DNA; so, we can distinguish between homoduplexes and heteroduplexes. Many experimental studies must be done to determine the optimal temperature used to distinguish between homoduplexes and heteroduplexes by heating a mixture of DNA from wild type and variant DNA followed by slow cooling. Due to single base pair mismatching particle denatures result in different chromatographic patterns**.**

The advantages of this technique:

- Does not need labels for detection.
- Allows multiplexing.
- Automated genotyping of SNPs.
- This approach may not be useful when the species have a degree of high polymorphisms. It's just screening for a sequence variant without any prior information about the nature of this mutation, it only detects the presence of the mutation, which could be followed by sequencing for SNPs genotypes (Orum *et al.*, 1993; Kota *et al.*, 2001) and (Nicod & Largiader, 2003).

SNP genotyping scoring platforms mass spectrometry:

Matrix Assisted laser Desorption Ionization Time of Flight (MALDT-TOF) Mass Spectrometry (MS).

It's one of the most promising approaches in detecting SNP. It is utilizing from discovering previously unknown and known SNPs. This analysis by (MALDT-TOF) Mass Spectrometry (MS) depends on the intrinsic property like charge/ration (m/z), making the method more accurate than use neither electrophoresis nor hybridization-based method. Besides, this process does not affected by forming secondary structures of nucleic acid.

MALDI-IOF-MS includes many SNP analysis detections such as enzymatic DNA sequencing or mini-sequencing or invader assay or PNA assay.

This method takes milliseconds in detection, as it depends on both ionization and separation by size.

There are some limitations to this method such as,

1) Increasing DNA size has an impact on signal intensity and mass resolution, this depends on the fragmentation process of DNA for the phosphodiester backbone through (MALDI) procedure.
2) Deficiency in specificity of mass peaks can be resolved by utilizing from nucleotide-specific tagging containing stable isotopes like C13, N15 and H2 C^{13}, N^{15} and H^{2} (Hillenkamp, 1991 ; Butler, 1996; Fu *et al.*,1998; Kirpekar *et al.*,1998; Yates, 1998;) (Griffin & Smith, 2000); (Carlson *et al.*, 2001 and Paris *et al.*, 2001).

Flow cytometry-based genotyping:

Combined Mini-sequencing with Flow Cytometry improves the platform of SNP scoring. The concept of this assay can be summarizer in both SNP sites and single biotinylated oligonucleotides are annealed immediately adjoining to each other, adding fluorescent ddNTPs and DNA polymerase to elongate one base. After the extension, biotinylated primers were captured by streptavidin-coated with microsphere after that measured the fluorescence signal. Flow cytometry instrument does not need washing or separation step for purification of PCR products, it is describing the intrinsic resolution excitedly between both free and particle fluorescence bound.

The advantages of this method.

- Is multiparameter detection.
- This program can label each one of the four ddNTPs with a different fluorophore and determine them in the same

reaction like multiplex analysis, besides it is a sensitive and efficient method.

One of the limitations of this assay:

The primer heterodimer is formed and false priming results; but this can be overcome by selecting a suitable primer (Cai, 2000).

Fluorescence resonance energy transfer-based method:

The principle of this method depends on fluorescent energy transfer by using real-time PCR.

It consists of two parts TaqMan assay and Molecular Beacons (MBs). The probe included one part called fluorescent reporter molecule and the other part called quencher at its 5^- end and 3^- end, respectively. After complementary to amplicon and hybridization to DNA template Taq polymerase $5^- \rightarrow 3^-$ exonuclease activity degraded DNA at its 5 ends. Separating the reporter from the quencher allows for signals to emit (Tyagi & Kramer, 1996, Tyagi *et al*., 2000 and Milee *et al*., 2008).

Alpha screen method:

This technique has two beads one called Donor (D) and the other called Acceptor (A).

(D) Contains phthalocyanine, a photosensitizer that gave oxygen signal or irradiation at 680 nm and it is accepted by (A), which contains a mixture of chemiluminescent and fluorophores when it is activated, emits signal light at 600 nm.

This method is used now in detecting nucleic acid. It consists of two parts Allele-Specific Amplification (ASA), and Allele-Specific Hybridization (ASH).

It uses Bridging probes connect to a common nucleic acid target, Both A and D beads are linked to the surface of oligos nucleotide sequences. The bridging probes Hybridize to the target, and both A and D beads become nearness in space and emit signal immediately after thermal cycling analysis and obtaining the genotype.

Alpha screen ASA:

ASA using a 3^- end base of the primer in clarification polymorphisms and to increase the specificity of amplification reaction can add the penultimate position of the allele-specific primer for each allele in separate wills.

The disadvantages of this method can be included:

1) The percentage of false positive results is high.
2) The high cost of this method, so it's still needed more research and studies (Ullman *et al.,* 1994; Griffin & Smith, 2000 and Beaudet *et al.,* 2001).

Padlock probes

This assay depends on padlock probes, which included two target-complimentary fragment connected by a non-complementary ligate sequence. fragments connected by a linker sequence non-complementary to each other. In hybridised position between probes and the DNA target the two ends of these linear probes come in juxtaposition and are joined together by DNA ligase appearing in the circular shape of the DNA molecule.

If the form shape of a circular DNA probe is integrated into the target sequence of DNA afford signal, but if the mismatch happened, the two ends of the probe do not connect by ligase, and no signal appears.

This method has some advantages such as:

1) High-specific detection of nucleic acids
2) Stability of the probes bounded to the target strand, so it can be resisting extreme washing which reduces non-specific signals.
3) In the state of the perfect match, the two ends of the probes will ligate by DNA ligase, so it will allow efficient distinct among sequence variants. (Nilsson *et al.,* 1994) and (Nilsson *et al.,* 1997), (Baner et al., 1998 and Lizardi *et al.,* 1998).

Genetic Bit Analysis (GBA):

This method is called "genetic bit"; it is an applied method which can detect genetic material under the condition that the sequence of target nucleic acid already known, it consists of two primers one called "primer guided" used in detect SNP, which is known the sequence of nucleic acid polymorphism; and the second primer is phosphorothioate modified primer. When a duple strand of DNA converted to a single strand by treated nucleic acid with T7-gene exonuclease. The complimentary happens between guided primer and target loci, it does not include the site of SNP but is immediately adjacent to the target loci of DNA. In individual wells of a microtitre plate, by using hybridization of PCR product where captured sites were immobilized to oligonucleotide primer.

To obtain DNA fragments including the variation site of SNP, the four ddNTPs, one biotin, one fluorescein and two unlabeled ddNTPs were added to DNA polymerase, after terminating the extension of chain which included the polymorphic sites, conjugate by enzyme-linked done to detect SNP through colourimetry. This is considered a high flexible method (Schmid *et al.,* 2002 and Nicod & Largiader, 2003).

Capillary assay electrophoresis (CAE):

This assay likes the method used before with DNA sequencing microsatellite genotyping which uses target DNA (specific allele) in PCR amplification with labelled primer. Utilize capillary array loader to load samples in channels tubes coated with gel call (capillary), after the run of samples completed in the buffer between two electrical potentials. The labelled DNA fragments were separated and detected on microplates by the fluorescent scanners (Kheterpal *et al.,* 1996); (Ronaghi *et al.,* 1998) and (Shi *et al.,* 1999).

Pyrosequencing:

It's a new DNA-sequencing method that benefits from the reaction of a coupled enzyme to release pyrophosphate (PPi) during nucleotide incorporation.

When inorganic phosphate is released, detection can be done by luminometric . It allows sequence for short segments; typically, 20 nucleotides. It is perfect to identify SNPs as it needs only (1-5 bp).

Designed primers from sequences flanking SNP sites and PCR-amplified products were used to change DNA to single strands.

Combined single strands of DNA, DNA polymerase, primer, ATP sulphurate, luciferase and apyrase and dNTP when in the cyclic set. Nucleotides of a base pair were created.

Generate detectable light from Luciferin benefits from ATP. utilize this ATP to generate detectable light and this light could be estimated by a luminometer. This detectable light can be seen as a peak in the pyrogram which translated to a conjugated number of dNTP.

The excess of unconjugated nucleotide for every cycle will degrade by apyras. If the DNA template cannot form base pair with the nucleotide, in this manner light will not be detected.

A specific pattern (pyrogram) can be observed for a combination of each allele. The advantage of this technique is rapid as it can be analyzing one SNP every 6 seconds, and it

is suitable for large-scale studies. Avoiding the use of either labelled primers or labelled nucleotides, this technique does not need gel electrophoresis. The limitation of this method, it is requiring more time and cost to prepare PCR before SNP analysis. In addition, the single-stranded template needs to sequence and this increase the cost and time for this procedure Fig (5), Fig (6) and Fig (7). (Ronaghi et al., 1998 and Ahmadian et al., 2000).

$$\text{Primer} + \text{dNTP} \xrightarrow{\text{Polymerase}} \text{primer} + 1\text{N} + \text{PPi} \quad (1)$$

Fig. 5. This diagram summarizes the steps of Pyrosequencing method.

The nucleotide bases incorporate by DNA polymerase and pyrophosphate will consequently be released.

$$\text{APS} + \text{PPi} \xrightarrow{\text{Sulphyrase}} \text{ATP} \quad (2)$$

Fig. 6. This diagram summarizes the steps of Pyrosequencing method.

Utilize from pyrophosphate to convert 5^{-} aminophsulfonate (APS) into ATP-by-ATP sulphyrase.

$$\text{Luciferin} + \text{ATP} \xrightarrow{\text{luciferase}} \text{Oxyluciferins} + \text{light} \quad (3)$$

Fig. 7. This diagram summarizes the steps of Pyrosequencing method.

Microarrays:

It consists of DNA chips connected with a semiconductor coated with an agarose layer containing streptavidin to separate the biological materials, which are oligonucleotide immobilized on a solid surface used in hybridization to detect SNP. The DNA target was amplified and labelled. Poured oligonucleotide molecules (probes) in chips and immobilized them. After complementarity between bases is completed the unbound DNA removes by washing. Tested the four oligonucleotides in these chips, after the complimentary between oligomers was done one only nucleotide will appear to be different in this set. A number of these columns were arranged, and each of these columns consists of four arranged sites, oligomer which consists of these sites were different for each one, these sites or position different in one oligomer termed substitution position and this assay is called (Tilling strategy) (Pease *et al.*, 1994); (Chee *et al.*, 1996; Sosnowski *et al.*, 1997); (Cho *et al.*, 1999 and Shi *et al.*, 1999).

Single Nucleotide Polymorphism Hybridization Arrays:

A strategy termed (Population-Based Quality Filtering of SNP Hybridization Data) is utilized to differentiate between patterns of genuine deletions from failure in a technical process. The parameter Presence-Absence Variation (PAV) of data show significate quantitative resistance to disease in *Brassica napus*. Increasing the value of missing data which happened during the use of the SNP array technique and this can be solved by using the method Single Nucleotide Absence Polymorphisms (SNaPs). Besides, improving the accuracy of genetic mapping in many economic crop species, the rate of a parameter such as false-positive marker-trait associations were decreased, and some applied models were utilized like a combined linear model to control population substructure.

The genome structural variation was observed during the study of the diploid (*B. napus*) organism. When studying the allopolyploid genome, the results indicated high homology through the diploid ancestor genome A and B subgenomes. SNP filtering data might be the appropriate approach for the major phenomena of many crop plants like pangenomes, this phenomenon can be recognized in many diploid and polyploidy genomes for economic crop plants such as maize, sorghum, cotton, wheat and others.

Combination the technique of Single Nucleotide Absence Polymorphism (SNAP) markers with the analysis of Genome-Wide Association Studies (GWAS) to determine the quantitative resistance to both off-blackleg disease and sclerotinia stem rot, studies indicated a significant increase in the trait associations. The frequent localization of new QTL loci affected by Presence-Absence Variation (PAV) confirms the principle that PAV has particular relevance on disease resistance. The rule of Presence-Absence Variation (PAV) emphasizes the principle that (PAV) has a certain reflection on disease resistance when determining frequent localization for the locus of new QTL influenced by Presence-Absence Variation (PAV). The high rates of Presence-Absence Variation and included structural variations for resynthesized *B. napus* have great importance to use in the resistance breeding program of the Offspring. (Gabur et al., 2018)

Genotyping by Multiplexed Sequencing (GMS) combined with SNP markers consider a new protocol, it has a great opportunity for flexibility of data genotype information. Some advantages of this protocol, it could be included in a single sequencing run, PCR-based can directly sequence depending on the interested markers with a great precise analysis when dealing with multiplexing hundreds of markers, and it is simple to deal with a group of markers by modifying interested species. SNPs spread through the genome in both genic and non-genic regions, the first category has a great importance and is considered a valuable marker for both breeders and researchers, as it correlates with the defining marker and phenotypic traits, while the second category is considered not valuable marker as it has no influence on the phenotypic of the organisms and this retrain to, there is no correlation between the defined marker and the phenotypic. SNPs are often bi-allelic with a low mutation rate and this makes them ideal for most studies interested in Mapping, Genomic Studies and Marker Assisted Selection (Eagle et al., 2021).

Swaminathan *et. al.*, interested with the *Fusarium virguliforme* which is a soil born root pathogen causes sudden death syndrome (SDS) in soybean lead to death of plants. Control management for SDS are limited and the most effective method for protecting the crop from *F. virguliforme* depend on breeding resistant of varieties, even SDS resistance related with breeding program is partial as it encoded by a large number of genes and each one contributing with small effect; beside there are interaction between SDS resistance and most of QTL for SDS resistance or SNPL identified for foliar SDS.

F. virguliforme is a root pathogen and causes damages to the infected roots. Studies for identified soybean genotypes carrying both of foliar SDS and root rot resistance are limited. Which make research for both symotymes of foliar and root rot a goal for identify soybean germplasm. Looking for both of foliar SDS and root rot resistance can be providing protection against *F. virguliforme.*

The majority of SDS resistance QTL constricted mapped depend on crosses of progenies for bi-parental. Genome Wide Association Studies (GWAS) have identified 30 single nucleotide polymorphic loci (SNPL) linked to foliar SDS resistance, part of them were co mapped to SDS resistance OTL that were already identified previously through bi-parental crosses.

Study GWAS indicated that, their are 19 SNPL that linked with 14 genomic regions and eight SNPL linked with seven genomic regions encoding for both of foliar SDS and root rot resistance respectively.

Beside, identify 27 SNPL, six SNPL for foliar SDS resistance and two SNPL for root rot resistance, co-mapped previously and were identify by QTL for SDS resistance, and identify 13 SNPL associated with eight novel genomic regions included foliar resistance genes and six SNPL with five novel regions for root – rot resistance, from 27 SNPL five nonsynonymous mutations in five genes, three were previously mapped depend on QTL for foliar SDS resistance and two were novel regions for root rot genes. The three genes identify previously which containing nonsynonymous mutations, two encode LRR-receptor proteins and one encodes an unknown protein. These five genes identify by 27 SNPL has changes the amino acid residues in five proteins. The research interested in determine if the nonsynonymous mutations have changes any of the conserved amino acid residues involved in gene functions.

Comparison between target proteins and homologous protein sequences indicated that these mutations lead to changes in either of protein structure nor post- translation modification, which has influence on expression of SDS resistance in the three genes, the mutation altered the conserved amino acid residues serine or histidine for phosphorylation, which could be has an effect on SDS susceptibility.

The results identify the five genes carry nonsynonymous mutations were three genes mapped for resistance against foliar SDS, they were Glyma.13g035700, Glyma.13g079100 and Glyma.14g035000, they were encoded for proteins LLR-receptor protein which the mutant serien residue substituted with arginine residue, LLR-receptor protein which the mutant lysine residue substituted with glutamate residue and novel protein with unknown function the mutant proline residue substituted with leucine residue, respectively.

While the two gens mapped were resistance against root rot SDS they were Glyma.o1g222900 and Glyma.10go58700 and they were encoded for the proteins LEA hydroxyproline-rich glycol protein family which mutant serine residue substituted with asparagine residue and heparan-alpha – glucosaminide N-acetyl transferase was mutant histidine residue substituted with arginine residue, respectively.

These mutations can change both of structures and functions of the two proteins. The evaluation of these genes needs to determine their role in SDS resistance by genetic mapping studies or by these generated mutants in candidate SDS resistance genes.

Gang Li et. Al., (2019) Studied the genetic resistance level toward Verticillium wilt of cotton and other hosts with difficult to find resistance genes against this pathogen, as these genes are very rare. Group of resistance genes (R) , candidate genes involved in the interaction between plants and pathogens, determined was done by Genome Wide Association Study (GWAS) for Verticillium wilt resistance. Utilizing from a panel of *Gossypium hirsutum* to improve the immunity resistance responses against target pathogen invasion. The majority of disease resistance genes in plants encode (nucleotide blinding site, leucine-rich repeat) encoded NBS-LRR proteins, GhDSC1 is a protein cloned from resistance gene of cotton and it is encoding a protein sharing homology with TIR-NBS-LRR receptor – like defense protein DSC1 in *Arabidopsis Thaliana*. This protein nuclear localization and expression with high levels as a result to the treatments with Verticillium wilt or Jasmonic acid (JA) in resistant cotton cultivars. This resistance bakes to accumulation and increased expression of JA-signaling of reactive oxygen species (ROS) of related genes. The results indicate that Verticillium wilt resistance candidate gene GhDSC1 encodes a typical TIR-NBS-LRR protein structure. Confirming the results of GhDSC1 gene transfer it to *Arabidopsis thaliana* and indicted the Verticillium wilt resistance in *A. thaliana*. The results of disease indices of the resistant germplasm were 20 compared with 60 for the susceptible germplasm beside the open reading frame (ORF) of GhDSC1 homologues were amplified, sequenced and alignments for all 18 cotton germplasm accessions. The results show that GhDSC1 homologues were exciting among the 18 cotton accessions except for 11 single nucleotide polymorphisms (SNPs) at 673 bp was a guanine (G) in resistant accessions, while it was cytosine (C) in susceptible accessions. The sequence analysis indicate that nonsynonymous mutation occurred in P-loop motif influence on function of TIR-NBS-LRR proteins thus GhDSC1 gene could be distinguished by the nonsynonymous mutation between the resistant and susceptible cotton GhDSC1 gene sequences.

Garcia and Ramirez in 2022 were used Genome-wide Single Nucleotide Polymorphisms (SNPs) in study the diversity between guava. The results indicate that there is no linkage between genetic groupings and geographic origins of guava when analysing the principal component and structure analyses, these molecular studies often depend on local germplasm. In case of including accession of guava from different countries, a clear relationship could be recognized between (genetic diversity and geographic origin). Besides, large population sizes are required to determine both of functional categories and gene families. This germplasm study of guava was done by using the procedure Genome-Wide Molecular Markers through Genotyping by Sequencing.

The goal of this research was to identify and characterize the polymorphisms related to climate adaptation that could be used in genomic selection for traits like future thermal resistance in sheep, because changes in biological molecules as a result of heating stress are considered cytotoxic. This heat stress is altering the shape of the cells, such as disrupting cell activities, affecting metabolic responses, and causing oxidative leading to cell damage and others. This study indicated that the polymorphism of (17 sheep breeds) originating from different climatic changes were determined by (51 SNPs) of (29 genes) participated in thermo-tolerance, Astuti *et al.*, 2022.

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الاختلافات المظهرية لقاعدة نيوكلتيدة واحدة ، الأهداف و التطبيقات فى علوم أمراض النباتات

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المخلص

استخدام المعلمات الوراثية فى تحسين علوم البحوث النباتية يعتبر أحد المجالات الواعدة ، حيث انه يوضح الأختلافات أو التباينات الوراثية بصورة دقيقة و بالتالى يمكن تفسير الظواهر البيولوجية فى امراض النباتات و بالتالى القدرة على بناء علاقة بين الجينوم و الشكل المظهرى . و يعتبر تكنيك الأختلافات المظهرية لقاعدة نيوكلوتيدة واحدة و المنتشرة خلال جينوم الدنا أحد المعلمات الوراثية المستخدامة فى دراسة التباينات أو التعريفات.و الأختلافات المظهرية لقاعدة نيوكلوتيدة واحدة أحد أهم المعلمات الوراثية حيث يستخدم فى ترقيم الجينات الأقتصادية المهمة و دراسة التراكيب الوراثية و تحليل و تقيم التباين.و قد تم استخدام العديد من الطرق فى التعرف على الأختلافات المظهرية لقاعدة نيوكلوتيدة واحدة و كل طريقة لها مميزاتها و محدداتها و اختيار طريقة معينة يعتمد على اعتبارات مثل نوع الدراسة ووجود موارد متاحة .هذا البحث التجمعى يوضح أهمية استخدام طريقة الأختلافات المظهرية لقاعدة نيوكلوتيدة واحدة فى المجال البحثى و الطرق المختلفة المسخدامة فى الكشف أو التعرف على الأختلافات المظهرية لقاعدة نيوكلوتيدة واحدة مع وجود مدى واسع من المستلزمات المستخدمة بواسطة اجهزة منخفضة التكلفة الى استخدام تكنولوجى عالى و متطور و مكلف.