



## Characterization and Advancement of Microsatellite (SSR) Markers for Various Stresses in Wheat

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### ABSTRACT

Wheat is a basic diet in most countries so it fulfills 20% of global caloric requirements. But the population of the world is increasing day by day and to achieve the requirements of this growing population. We need almost 60% more wheat and to gain this we need high-yielding varieties which can be obtained by developing the trait management and use of modern biotechnological apparatuses like that SSRs marker and genetic engineering. Molecular markers are short segments of DNA that are used to identify the different traits of interest in species of plants. These can be transformed while developing novel varieties of crops. Wheat is the major staple food of many countries, so it is required in large quantities to fulfill the requirement of the large population of the world. Microsatellite markers are repetitive DNA sequence that occurs in many places within the plant genomes. These consist of more mutations than other parts of DNAs. They are also called simple sequence repeats. These markers are used for DNA profiling; they are used for mapping places within the genome, especially for genetic linkage study Microsatellite markers are assisting the plant breeding. By using these markers, we can develop high-yielding varieties which will be resistant to biotic and abiotic stress.

**Key words:** Wheat, SSR marker, Microsatellite Marker, Biotic and Abiotic stress.

### INTRODUCTION

Wheat is an important grass cultivated for its seed and a cereal grain which cultivated worldwide staple food. So, most countries use it as a staple food and at this time the population of the world is increasing and land area is decreasing in the world. At this time, we need varieties that show high tolerance to biotic stress and abiotic stress and ultimately, we can get a high yield that will be sufficient to fulfill the requirement of this world (Anderson *et al.*, 2001). One of the most critical challenges facing wheat improvement is the evaluation of genetic potential in wild wheat relatives and the application of that information for wheat improvement. Although previously, microsatellites (SSR markers) were shown to be more informative due to their high levels of chromosome specificity, reproducibility, co-dominant inheritance and polymorphism but now SSR markers show even greater

levels of chromosome specificity, reproducibility, co-dominant inheritance and polymorphism. Triticum and Aegilops species with A, B, D, G and S genomes have been effectively studied using microsatellite markers, but the same success has not been found for species with other genomes. Studies showed that wheat microsatellites were successfully used to perform molecular characterization of Aegilops and Triticum-Aegilops lines, both of which include introgressions from the C and U chromosomes. Despite this, however, microsatellite markers for Aegilops species, including those that have C and U genomes, remain undiscovered (Arfan *et al.*, 2007).

So, to get a good yielding crop and get good results, we need a tool that can assist and for this purpose, there is a need for the development of microsatellite markers for abiotic and biotic stress in wheat so that high-yielding and resistant varieties can developed in wheat. This will give us the edge to fulfill the 60% caloric requirement of the world

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(Gill *et al.*, 1993). Microsatellite markers are DNA markers or small-tandem repeat or polymorphic DNA-loci containing repetitive nucleotide sequences which will be 2-7 nucleotides per unit. The length is the same for the majority of the repeats within an individual-microsatellite locus so many of them may differ so its results are alleles of variable size. Microsatellite study consists of PCR amplification markers using fluorescently labeled primers. Microsatellite instability (MSI) is a type of genomic instability that arises from decreased reliability through the repetition of repeated DNA which happens in tumor cells (Bennett *et al.*, 2016).

Microsatellite markers have a great role in the projects of plant breeding and genetics. So, as in plants, a large number of SSR markers have been identified. Some of them are recently validated in the laboratories of the world. The purpose of our study was to develop microsatellite-markers for biotic and abiotic stresses in wheat for the study we collected different research articles and studied them thoroughly. ISSR study is a process used for recognizing entities, usually in plant species also other herbs ornamentals &, some flowers, etc. Short tandem repeats (STRs), also known as microsatellites, are polymorphic-DNA-loci distributed throughout the genome and are commonly utilized in several research fields, including for the discovery of genetic markers for linkage mapping, for studies of association, and for identifying the species (Bittner *et al.*, 2015). Abiotic stress: The stress which is due to environments that include temperature, relative humidity, drought and many others. Yield-related traits are targeted primarily for improvement of crop performance under abiotic stresses. New target traits related to drought resistance are developed through wheat breeding by certain genomic technologies. Traits related to drought resistance are complex and mutagenic. Microsatellite markers, SSR, STRs and SSLP start in prokaryotes and eukaryotes. These are widely spread in the whole genome, particularly in the eukaryotes' euchromatin, coding and non-coding nuclear and organelles (Gill *et al.*, 1996).

### Wheat Scenario

Wheat is a very important cereal crop worldwide; it is used as a staple food in Pakistan. Wheat is directly used for chapatti making sometime it should be used as a by-product like baking industries etc. It gives 25%–50% of the caloric requirement of the rising human population. Wheat gives basic calories to humans. Wheat flour has many proteins they have gluten gladden glutenin (Czembor *et al.*, 2003). They are present in seed sometime it will present in endosperm and also aileron layer is also present. Nonetheless, development in world wheat output and production has remained stagnant for many years as a result of several factors. wheat output and productivity have increased significantly over the past half-century, due to mainly the use of plant resistance genes to various biotic and abiotic stressors, as well as to plant dwarfness and photoperiod insensitivity. Since this increased productivity is only achievable by genetic enhancement of wheat for various abiotic stressors, such as salt stress, heat stress, and drought stress, increased productivity is only possible by continuing to genetically enhance wheat (Biswal and Kohli, 2013). Wheat is an important grass cultivated for its seed and a cereal grain which cultivated worldwide staple

food. So, most countries use it as a staple food and at this time the population of the world is increasing and land area is decreasing in the world. At this time, we need varieties that show high biotic and abiotic stress resistance and ultimately we can get a high yield that will be sufficient to fulfill the requirement of this world (Avalbaev *et al.*, 2010). One of the major difficulties in wheat is assessing wild wheat relatives' genetic potential and applying the results to wheat improvement.

### Biotic Stresses

In the case of biotic stress, wheat cultivar is affected by various diseases and pests. Sometimes pathogens may cause different types of diseases. In wheat, rust has affected the plants and may also decrease the yield some varieties have the ability to perform well in different environments. (Czembor *et al.*, 2003). In wheat, common diseases are leaf rust stem rust & some wilts are also damaged the crop verticillium wilt and fusarium wilt. Due to these diseases, plants can be gone into a permanent wilting condition so yield may also decrease. Due to diseases crops may also decrease long-term remaining capacity so it decreases yield and other important parameters. In biotic stress, crop may affect some insects' pests like aphid jassid, parrilla, etc. The most common is leaf rust of wheat. A yield loss from this is due to the reduced number of grains per head and lesser grain weight and it may reach in prone cultivar (Gao *et al.*, 2004).

Microsatellite markers are very efficient in detecting the biotic stresses in different species of wheat. Microsatellite markers are linked with different diseases pathogens, insects, & pests. The identification of these biotic factors with the help of microsatellite markers can be controlled. For example, novel, microsatellite markers have revealed the origin of *Botryosphaeria dothidea*. These pathogens cause the disease which is called Chinese grape wine trunk disease in a different host (Korzun *et al.*, 1997).

### Leaf Rust (*Puccinia triticina*)

Rust that affects wheat leaves is the most common and widely distributed. Both Canada and the US conduct annual research on wheat leaf rust virulence traits. The 70 distinct races are identified in the US by a collection of isogenic Thatcher wheat 37. France has 30-50 races a year. (Kota *et al.*, 1993). Infectious of the rust resistance races rise very rapidly in reply to extensive practice of wheat variabilities with race precise resistance genes. Leaf rust population is much great, so random mutation occurs in virulent races in an adequate amount to yield new virulence-free races. Leaf rust resistance genes Lr9 Lr11, Lr18, Lr24, and Lr26, are found in soft red winter wheat from southeastern.

### Wheat Stem Rust (*Puccinia graminis*)

Usually, wheat stem rust is present in North America. Many yield losses occurred in wheat during heavy epidemics in different years like 1916, 1935, 1937, 1950-1954. In North America and many areas of the world it can be successfully controlled due to high resistant cultivar and by removing the alternate host Barberry. The number of stem rust can be reduced by the effective size of the *P. graminis* population and chances of virulence mutation are permanently reduced in the adopted *P. graminis* cultivar (Ashraf, 2009).

### Wheat Stripe Rust (*Puccinia striiformis*)

Yellow rust of wheat is also known as wheat stripe rust. In cooler environments, wheat stripe rust is principally found as one of three wheat rust diseases. These are located in the cooler season and also in different locations in northern latitudes. This disease will be attacked on crops in different reproductions stages during this temperature range between 2 and 15 C other favorable conditions includes high humidity and rainfall for creating the infection on both leaf and leaf sheath, leaf blade and also in spike. When present in epidemic form shows symptoms of stunted growth (Hart *et al.*, 1993).

### Leaf and Stem Rust Resistance in Wheat

Leaf rust was caused by *Puccinia triticina*. It's far more common to see wheat leaf rust than stem rust or stripe rust in wheat fields worldwide. Yield losses in wheat from the fungal pathogen *P. triticina* infections can be attributed to reduced grain weight and fewer grains per head. In the mid of 20<sup>th</sup>-century yield losses by stem, rust reached 20-30% in Australia, China and many other countries including Eastern and Central Europe (Hazen *et al.*, 2002).

### Abiotic Stress

Wheat germplasm are adversely influenced by environmental stressors including salinity, humidity, micronutrient and temperature nutrition supply. To severe droughts in rain-fed zones of the world, year-round exposure to high temperatures could have been preferable. The rapid rate of evaporation from topsoil leads to a severe salt solution that may occur if too high temperatures are present. One study of 1,700 studies found that each 2°C rise in temperature lowers wheat yields by the roughly same amount in tropical and temperate locations (Cregan *et al.*, 1994). According to a recent study, a 6% decrease in wheat yields occurs for every 1-degree Celsius increase in temperature. This would correspond to a reduction of about 42 million metric tonnes of wheat on a global scale. In other words, the major issue of today's agriculture is to keep crop production levels consistent to meet the rising demand for food as a result of a growing population in the given amount of capital. Not only is conventional breeding a period and labor-intensive undertaking, but it also involves relatives of different genes that help control the physiological and molecular processes. Because of the significance of molecular plant breeding in the improvement of crops for stress tolerance, it has a significant role in molecular plant breeding. Abiotic stress effect crops severely so plant growth will be affected due to climatic conditions. So, yield of the crop will decrease due to unfavorable conditions plant damage occur effect on the grain of wheat. Grain may shrive so wait may also decrease. Abiotic stress also affects the reproductive stage of the crop. Temperature also affects plants very severely, due to high-temperature water loss will be more evaporation will be more plant uptake water fastly so the plant has a deficiency of water it cannot take food properly. Photoperiod also affects the crop in the presence of light Plants can make their food when nighttime food cannot be prepared due to the absence of light. Light will likewise disturb the growth and development of the plant. Plant uptake CO<sub>2</sub> and release O<sub>2</sub> for human beings (Hazen *et al.*, 2002).

In addition to disease-causing microorganisms, the most significant threats to the wheat crop are rust pathogens. They are detected by wheat producers from all over the world, resulting in enormous harm to the global wheat harvest. These diseases have a restricted host range, and they are parasites in nature. The rusts have been documented in favorable conditions to harm yields in several areas. Rust infections have an estimated worldwide cost of 15-20%, which is roughly 20-30 million tons of grain. Certain diseases can influence plant physiology, causing plant growth to be hindered. Crop yield decrease due to a severe attack of the pathogen if an attack is severe, we should try to discard the damaged plant immediately, otherwise, it will spread to the whole crop (Hart *et al.*, 1993).

Microsatellite markers may an important role in the identification of abiotic stresses. A large number of microsatellite markers are located on DNA sequences of the heat stress library of wheat. These markers are also found in wheat libraries of salinity, drought, cold stress, etc.

### Heat Stress

Winter wheat is mostly grown. When the optimum temperature is 15 degrees Celsius, for every degree over this, yields drop by 3 to 4 percent. Though, the world's average temperature has risen by 0.18 °C per ten years during the last century. Also, because of this, increasing interest has recently been placed on the effect of heat stress on wheat. The adverse effect of heat stress injury on cellular structure and metabolic pathways, especially photosynthesis, membrane thermal stability, and starch synthesis pathways, is due to the fact that heat stress leads to a higher temperature, which causes an increase in temperature and stress. The denaturation of proteins and improvements in the phases of unsaturated fatty acids by heat stress causes a disturbance in the movement of water, ions, and organic solutes, which impedes cross-membrane movement and, in turn, how much cellular function is retained (Leonova *et al.*, 2003). When it comes to plant development and growth, the temperature has a noticeable impact. Agronomic and agricultural characteristics are also affected by heat stress, however, earlier growth stages (such as germination, which takes place prior to blooming) are more vulnerable to high temperatures. Heat tolerance is quantified, with many genes and QTL controlling this trait (Apel and Hirt, 2004).

### Drought Stress

A time in which the plant water content has reduced enough to interfere with the plant mechanism is characterized as a drought. roughly 70% of potential yield damages throughout the world are caused by hostile environmental factors, such as water scarcity. Sindh and Baluchistan are the most impacted areas in Pakistan. Drought is a naturally occurring phenomenon, and as it persists, its effects on the local population slowly increase. A sustainable and economically practical method to increasing crop production and ensuring food security for the expanding human population is referred to as establishing crop varieties with better drought tolerance. (Achard *et al.*, 2006).

### Osmotic Stress

Drought is a worldwide problem, happening in almost any wheat-making zones can create simple osmotic strain.

Guesses show that drought strain in the United States is accountable for yield shortage and enhancing the stress on worldwide food security. Examples of drought become more common and enhancing determined due to universal warming elevation the likely to threaten yields soil salinization is another basis of simple osmotic strain that threatens 20% of the cultivated area (Lin *et al.*, 2006).

### Temperature Stress

Temperature causes drought and dehydrated plants so these plants will not survive and finally these plants will die or they will give less yield. Similarly, humidity and high wind also affect the yield of the crop because if their concentration increases the required level or decreases the required level it will obviously prove harmful to the crop and ultimately the yield of the plant will be decreased (Hohmann *et al.*, 1994).

As temperature rises, plant growth and development are strongly impacted. Wheat is widely grown because it can tolerate a broad range of temperatures. Since wheat can tolerate temperatures between 30 C and 35 C, it can grow well at either of those temperatures. As the temperature rises, plant development and growth will reach a new plateau. As of ambient soil temperature, the sprouting of seed is vulnerable to temperature strain and seed continuity disruptions as a result of metabolic processes changing. Disruption in germination rate can result in the density of plants changing, as well as their crop emergence. The early-season consequences of this can include an appearance of high temperatures during anthesis and seed set, which in turn results in significant crop losses (Leonova *et al.*, 2003).

### Salinity Stress

The salinity of the soil is a major factor restricting agricultural plant cultivation. Of the entire land area, around 800 million hectares (or 6% of the total) are impacted by salt. In maximum cases, salinity outcomes from natural reason's salt accumulate for an extensive duration of a period. In addition, a share is producing farming land is become saline due to deforestation or the availability of more water and fertilizer in the soil. Present estimates show that 25% of the roughly 230 million hectares of moistened soil is affected by salinity. Therefore, one and only of the third food productivity comes from irrigated land so salinity becomes a thoughtful problem for yield plant growth and development. Salinity affects crops at different stages if excess salts are in the soil so the plant doesn't uptake water properly so nutrient uptake will be less so plant yield may decrease. Salinity also affects the reproductive growth of the plant. If soil is saline plant water uptake cannot work properly so it also affects the yield of the crop and also damages the crop. Due to salinity pathogens can easily attack the host plant and may damage the crop (McCouch *et al.*, 2002).

### Plant Adaptations to Different Abiotic Stresses

Escape, Avoidance and Tolerance are the strategies through which crop plants resist to stress conditions.

#### Escape

Escape depends on the effective achievement of the reproduction phase before the start of severe strain that is due to developing plasticity, attained by initial flowering and brief growth period.

#### Avoidance

The restriction or reduction of the influence of strains in plant itself is an example of avoidance. This may be seen in halophytes, which tend to exhibit higher water-uptake rates and lower water-loss rates.

#### Tolerance

Tolerance depends on the inherent capability of the plant to withstand growth and development in conditions that are inappropriate for the plant to perform its basic metabolic processes. This approach contains coordination of physical and chemical changes at the cellular and molecular stages such as osmotic adjustment.

### Different Technologies are used to Study Plant Response to Abiotic Stress Including

These techniques are Genomics, Proteomics and Metabolomics.

#### Genomics

Refer to the study of the genome (total genetic content like genes or genetic sequences and studies related to that like sequences and studies related to that like sequence, analysis recombinant DNA, mapping alleles, bioinformatics, etc.)

#### Proteomics

Large scale study of proteomes (the entire set of proteins) →variable under different conditions →purifications, structures, compositions, mass spectrometry, activity pattern and modifications methods, microarrays, etc.

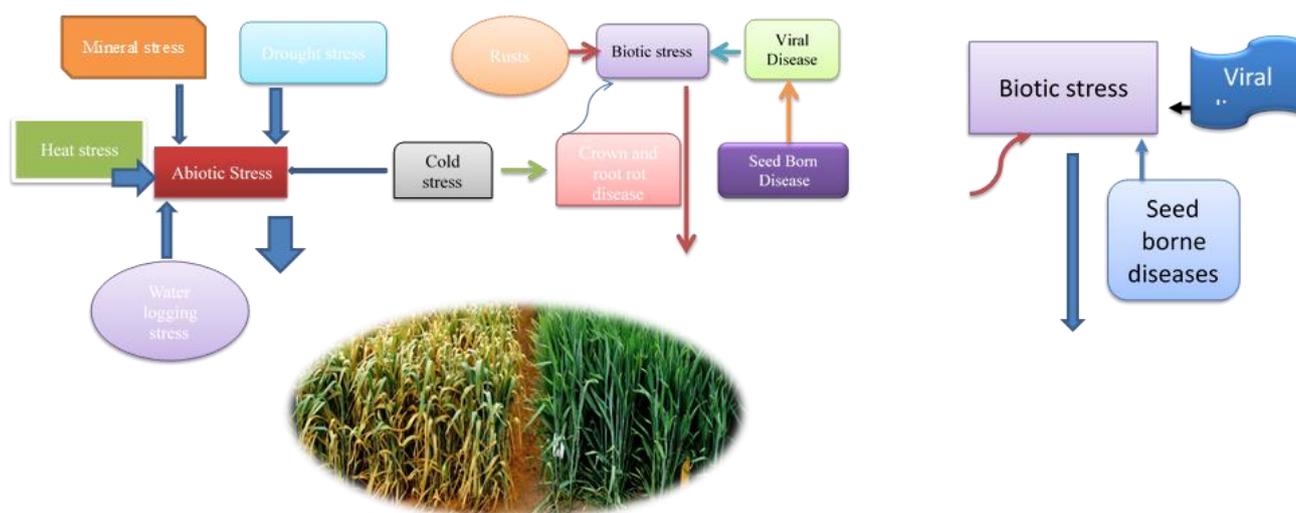
#### Metabolomics

The scientific study of chemical processes involving metabolites →chemical fingerprints that cells leave at a certain time.

### What are Molecular Markers?

The world population is increasing day by day and in order to cope with it, there is a need for high-yielding, resistant to biotic and abiotic stresses varieties so that the need could be satisfied. The plant breeders are working on it and they developed varieties by crossing the existing cultivar with such lines that have the desired trait. This method of developing novel varieties is very laborious and time-consuming. There was a need for such a method which requires less time so molecular markers were identified in the plants. Molecular markers are fragments of DNA that are associated with certain locations present within the genomes of different species of plants. Molecular markers are now used to locate the desired trait in the genomes of different species. These markers have some linkage to such agronomic and disease resistance traits. Major molecular markers which are used are the following.

- 1) RFLP (Restriction fragment length polymorphism).
- 2) AFLP (Amplified fragment length polymorphism).
- 3) RAPD (Random amplification of polymorphic DNA).
- 4) Microsatellite markers. The development of such types of markers can be done either by constructing the genomic libraries or by random PCR amplification of genomic DNA (Kim and Ward, 2000).



### Utilization of Different Molecular Markers

Molecular markers have their uses in different fields. Mostly molecular markers are used in the field of biotechnology and biology. The purpose of their utilization is to identify the particular sequence of DNA in a pool of unknown genomes. Genetic markers can help the scientist while developing novel traits which can be involved in the production of MAS. Molecular markers can be used in the identification of the fingerprinting of genotypes. The molecular markers can be used to estimate the distance between different species and their offspring. Molecular markers have also it used in marker-assisted selection (MAS). Molecular markers have vast applications in the field of fisheries and aquaculture (Delaney *et al.*, 2008).

### Microsatellites Markers

Microsatellite markers are categorized as DNA markers and these markers are based on Polymerase chain reaction (PCR). These markers actually have a higher level of Polymorphism and they are present in the wheat genome. Firstly, these SSR markers were studied in humans, and later on these markers were also analysed in mammals, fish, insects, yeast and several monocot and dicot species. SSR markers have a unique attribute that makes them valuable is their high level of allelic diversity. SSR Markers have unique sequences which border the Motifs of markers and they provide a template for primers to be amplified. Microsatellite markers are more informative than other classes of markers so they provide great information to the researcher. DNA markers are needed to create a genetic map or to find out the quantitative trait loci (QTL) for a specific trait that is needed to study (Levinson and Gutman, 1987). This trait could be important for any aspect of research. The same markers could be used for the purpose to incorporate genes into any specific variety. The markers play a very important role in determining the position of genes and that gene can be used for targeted traits and novel varieties can be developed by using this genetic makeup. Gene can be of any type; the markers can detect that type of gene. In bread wheat, the detection of polymorphism by Restriction fragment polymorphism (RFLP), AFLP and RAPD is very limited and is at low level so there is need of such markers which have high level of detection of polymorphism and good results. The microsatellite markers in wheat have such

characteristics which can be used for the detection of polymorphism and can give good results (Gastier *et al.*, 1995). The microsatellite markers in wheat have good stability, easy visualization, and co-dominance to genes and most importantly these markers are highly polymorphic. The examination of genes in wheat could be performed more efficiently by using these markers. For this purpose, we need good development and mapping of these markers in wheat so that genes related to abiotic and biotic factors could be detected and then further can be processed (Akkaya *et al.*, 1992).

### Why Microsatellite's Markers are Preferred than others

Microsatellites markers are a unique type of markers. These markers are preferred than other molecular markers because of several unique types of characters. These characters are the following. Microsatellite markers have a high level of allelic diversity. This character makes these markers very valuable and worth full. Microsatellite markers have a high potential for use in evolutionary studies. These markers are also preferred because they have a vast level of polymorphism. These markers are inherited in co-dominant manners. Microsatellite markers are preferred due to the reason that is they are chromosome-specific (Kostia *et al.*, 2011).

### Development of Microsatellite Markers

For the development and mapping of microsatellite markers in wheat against biotic and biotic factors, first the genomic libraries will be constructed, and these genomic libraries will have different genomes and genetic makeup. The purpose of these libraries will be the isolation of microsatellite markers. The preliminary genetic analysis and map could be constructed for this purpose (Endo and Gill, 1996). The mapping and development of Informative markers could be very difficult and time-consuming in wheat because the reason behind this is the large genomic size, the polyploidy effect and there is high level of repetitive sequences in the genome of wheat. The primer could be designed and further evaluation of these primers would be done for development of markers. The genetic and physical mapping of markers is two important components in the development and the total identified markers would be used for this purpose. Mapping should be handled carefully and well planned so that there will be

no any type of problem regarding the ambiguity. Some markers are very specific so these markers should be carefully handled (Dubcovsky *et al.*, 1997).

#### **Development of Microsatellite Markers from Genomic DNA Libraries**

Wheat genetic libraries can be constructed for screening and sequencing of DNA which is desired that is for abiotic and biotic stresses. Libraries can be constructed by the isolation of microsatellites. One group of the library can be constructed by the method of selective hybridization, in this method microsatellite are isolated which contains portions of the DNA by using the hybridization process with the help of a specific repeats probe. Another method of developing a genetic library is the primer extension method, in this method microsatellite markers are selectively amplified by using the specific primers. This method actually depends on the foundation of the primary genetic library. In this method, the process of ligation is also performed.

The non-enriched genomic library can also construct for the microsatellite DNA markers. In the process of construction of the library, the DNA is restricted, ligated into a very specifically designed vector and this DNA is transformed so that genomic DNA library can be constructed (Lincoln and Lander, 1993).

#### **Development of Microsatellite Markers by using PCR Based Markers**

The microsatellite markers can be also developed by the utilization of molecular markers such as RAPD, ISSR and AFLP. While developing the microsatellites by using the RAPD markers, the nitrocellulose membrane is used onto it the fingerprinting and blotting of RAPD amplicons is done which is followed by the screening. The development of microsatellites can be done by the cloning and sequencing of ISSR amplicons. The primers are also designed for this method of development. AFLP markers also play its role in the development of microsatellites by using the amplification, re-amplification and cloning of these types of markers (Marino *et al.*, 1996).

#### **Others Methods of Development of Microsatellites**

The other methods for the development of microsatellite markers include the SAM (selectively amplified Microsatellites) in this method selective amplification of restricted fragments along with primers sequence, tags, and probes is done. The other method is RAM (random amplified microsatellite) in this method SSR bands are converted to markers. MAL (microsatellite amplified library) approach is also used for the development of microsatellite markers which include constructions of DNA library and the hybridization is avoided (Devos *et al.*, 1995).

#### **Development by using Next-generation Sequencing Technologies**

Next-generation sequencing Technologies is used for the development of microsatellites. In this technique a large volume of sequencing data is generally created which is later on screen out with the help of tools of Bioinformatics. These tools are used for the identification of microsatellite repeats. In this method there is no need of constructions of

DNA and this is very rapid approach for the constructions of libraries (Bloem *et al.*, 1992).

#### **Mapping of the Microsatellites Markers**

Microsatellite markers can be mapped by using two methods. These two methods are following;

##### **Genetic Mapping**

The mapping of the microsatellite markers can be genetically done in wheat by using a different inbred lines which can be derived from the cross of synthetic wheat with any line having desired traits. The collected data can be put in to a framework map. Genetic mapping can be done by using the 222 BARC microsatellite loci which was shown as polymorphic in the respected population and these genetic maps will help out in the process of development of markers (Gill *et al.*, 1996).

##### **Physical Mapping**

Physical mapping is generally used to estimate the distance between specific sequences in a chromosome. This distance is generally measured by counting the number of base pairs. The procedure of physical mapping is as follows, first of all there is preparation of multiple copy of a genome and later on these copies is cut in to smaller pieces. In the next steps these pieces are put on a microchip to analyze them the electric current is generally passed across the chip. This is done to straighten out and line up the parallel strands of DNA. The genetic mapping may be used to analyze the physical distribution of recombination, which then governs each chromosome's recombination and the physical distances between loci.

In wheat, the Physical mapping can be processed by 432 BARC loci and it will also include the 222 loci which were mapped genetically so these physical maps will give us an idea about the biotic and abiotic stresses. PCR amplification is also used in this process of physical Mapping (Apse and Blumwald, 2011).

##### **Identification of QTL by using Microsatellite Markers**

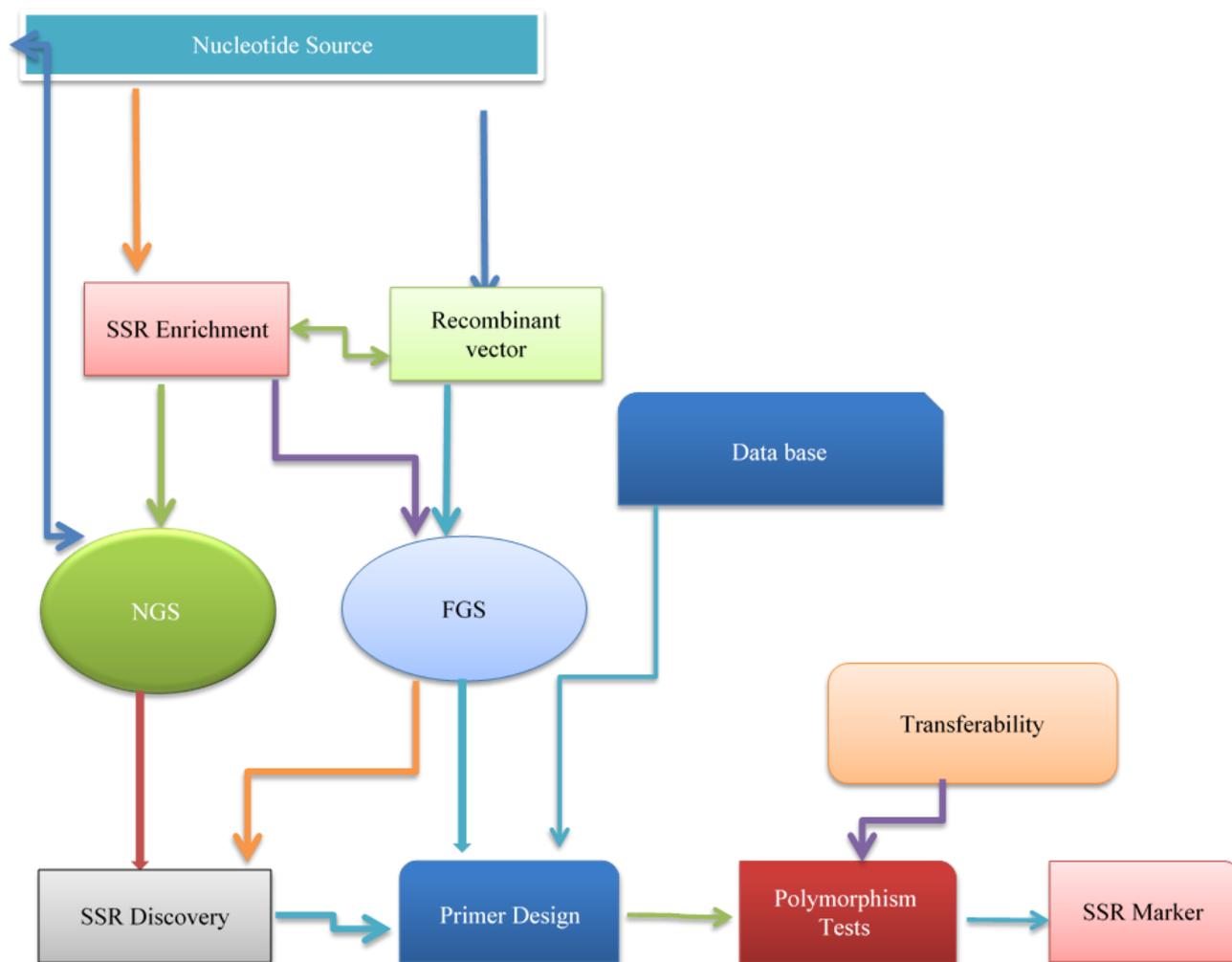
QTL (quantitative trait loci) can be identified by the process of mapping. The mapping can be done genetically and physically to identify them.

##### **Role of Microsatellite Markers in Marker-assisted Selection**

Marker-assisted selection is a novel technique in which indirectly selection is done and trait of interest is selected on the base of molecular markers. Microsatellite markers play an important role in marker-assisted selection. These markers can easily recognize all the phenotypes of different species. Microsatellite markers can be used efficiently in marker-assisted selection because they have very high allelic diversity and this property make them very beneficial (Blum *et al.*, 1989).

##### **Conclusion**

Wheat is a very important crop in the world and it is attacked by many biotic and abiotic factors so there is a need to control these factors so that the production of wheat can be increased. Biotic stresses cause major loss in wheat because a large number of diseases and pathogens affect it. Abiotic stresses such as salinity, cold stress, drought and



heat stress also have a major effect on the production of wheat. Different microsatellite markers are linked to these stresses so the identification of the desired traits can be done and by using the novel techniques of biotechnology, the stresses can be controlled. In the situation of biotic stress, wheat cultivars are exaggerated by various infections and pests. Sometimes pathogens may basis altered types of diseases. In wheat, rust is affected the plants and may also decrease the yield some varieties have the capability to perform well in different environment. In case of abiotic stress, wheat germplasm is affected by strain due to wetness content humidity, salt, temperature and micronutrient, like insect pests. Yearly involvement in excess temperatures in rain-fed areas of the globe may have favored drought stress. Microsatellite markers are considered as DNA markers and these markers are based on Polymerase chain reaction (PCR). These markers truly have a higher level of Polymorphism and they are present in the wheat genome. The mapping and progress of Instructive markers could be very problematic and time overriding in wheat because the reason behind this is the large genomic size, the polyploidy effect and there is the high level of tedious arrangements in the genome of wheat.

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