توظيف التقنيات الاحيائية في تحسين تحمل النبات للتقلبات البيئية وظروف الاجهاد

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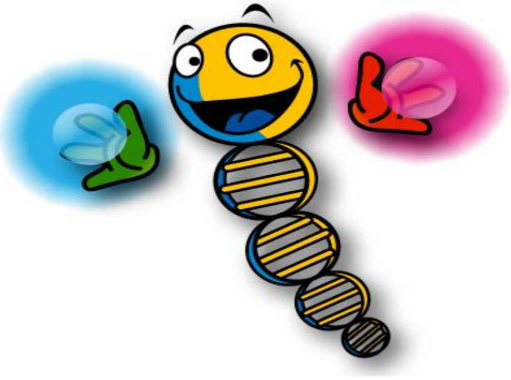
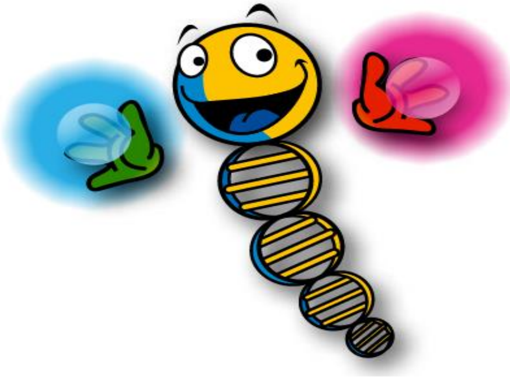


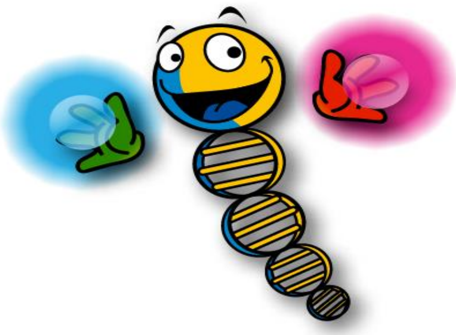
The Application Of Biotechnology to improve Salt stress tolerance in Wheat

Responses and Approaches to Mitigate stress

Dr Mohammed Hamdan Al-Issawi

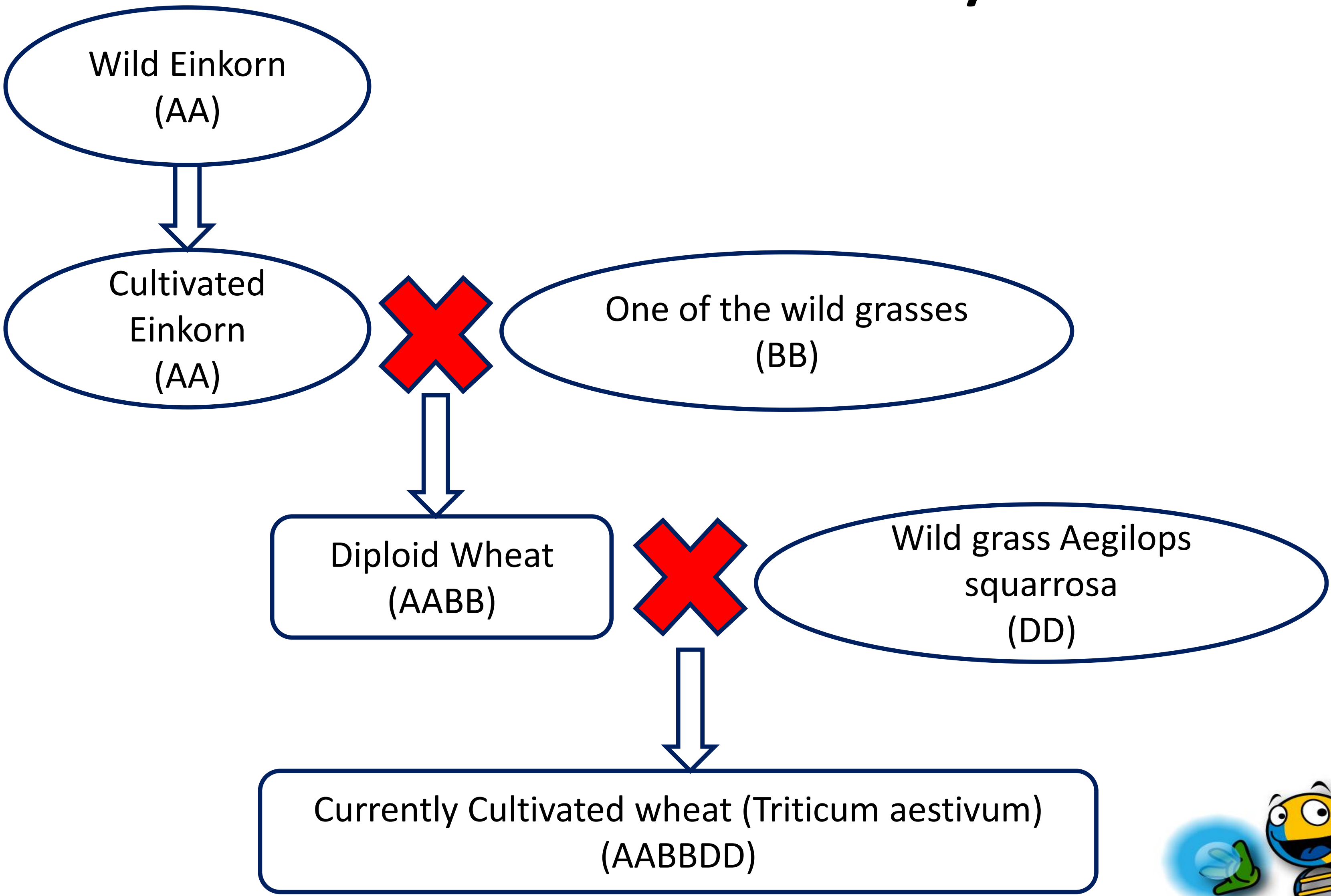


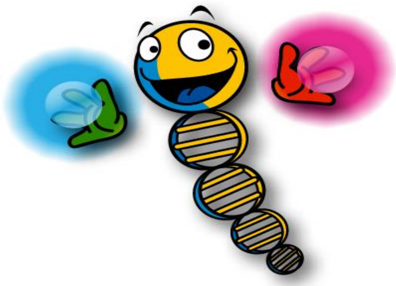
Outlines



- Wheat: history, types and importance.
- Salinity: effects and mechanisms
- Wheat response to salinity
- Biotechnology approaches to increase salt stress tolerance in wheat.
- Summary and Conclusions

Wheat: History





World Wheat Production (June 2017) (USDA)

Country		Production in metric ton
1	European unions	150,754,000
2	China	131,000,000
3	India	96,000,000
4	Russia	69,000,000
5	United State	49,642,000
6	Canada	28,350,000
7	Pakistan	26,200,000
8	Ukraine	25,000,000
9	Australia	25,000,000
10	Turkey	18,000,000
11	Argentina	17,500,000
12	Iran	15,000,000
13	Kazakhstan	13,000,000
14	Egypt	8,100,000
15	Uzbekistan	7,200,000
16	Morocco	5,800,000
17	Brazil	5,600,000
18	Afghanistan	5,000,000
19	Ethiopia	4,200,000
20	Iraq	4,025,000

More Food Is Needed!

- Technology increased the production of wheat by 41 kg ha^{-1} _ **NOT ENOUGH**
- Population by 2050 is estimated to be 9-10 billion.
- Current production of wheat is 600 million metric ton
- By 2020 we would require 1 billion metric tons

What is the stress

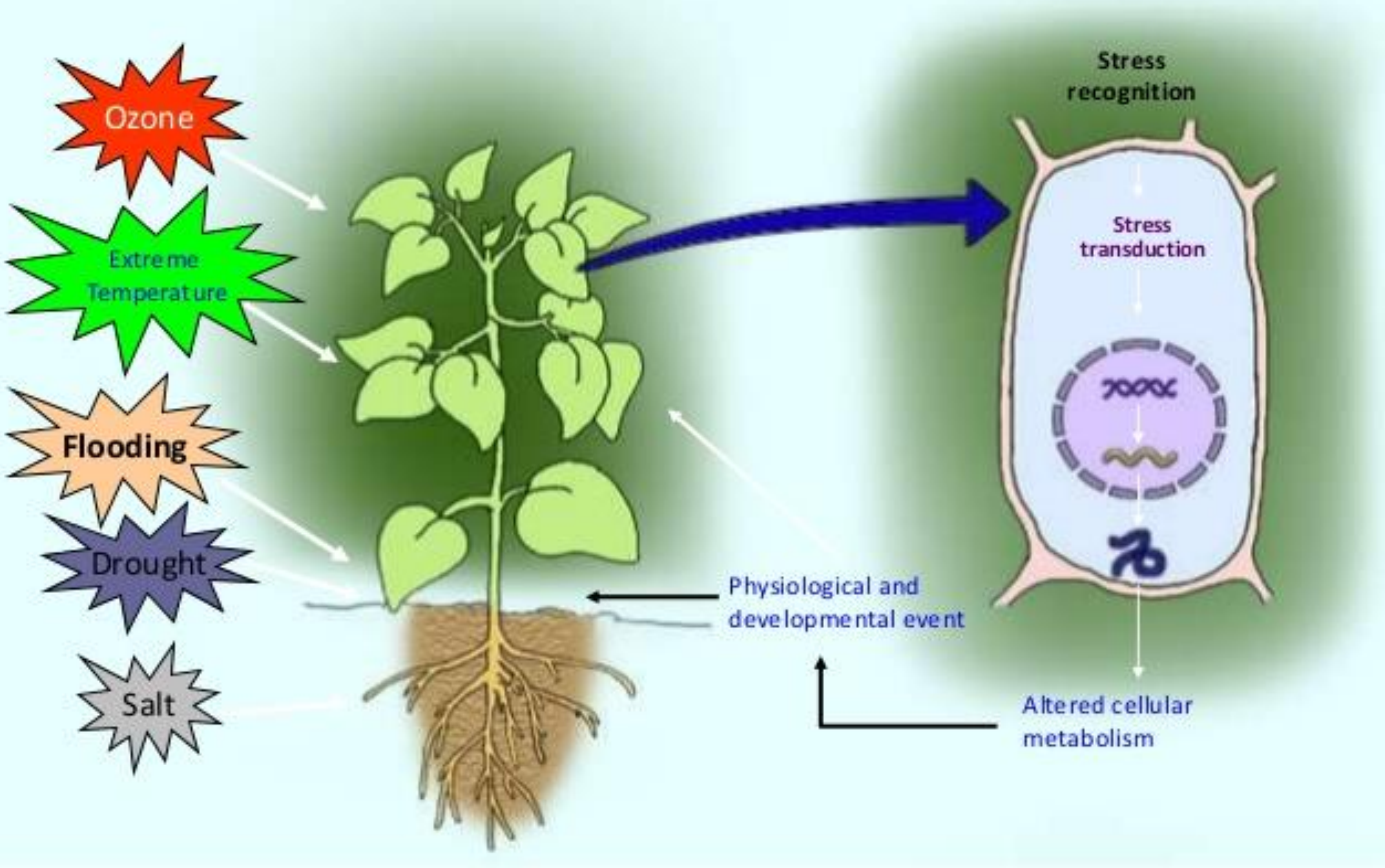
- Stress is the external condition that adversely affect growth, development and/or productivity.
- Stress triggers wide range of plant responses:

  - altered gene expression

  - cellular metabolism

  - changes in growth rates and crop yield

Response of Plants to Various Stresses

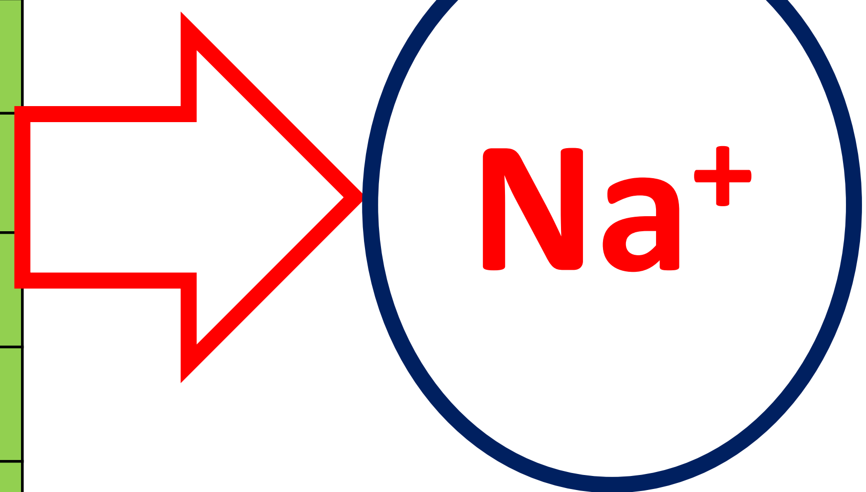


What is Salinity

- The use of bad quality irrigation water
- Bad drainage systems
- Mixing with sea water
- Accumulation of salt next to root of plant due to Evaporation

Salt sources in soil

NaSO4	SO_4^{2-}
NaHCO3	CO_3
MgCl2	Ca^{2+}
CaCO3	Cl^-
MgSO4	Na^+
	Mg^{2+}



- **Makes Soil Sodic**
- **More challenging because: Poor architecture and this prevents filtration as well as drainage**

Water Salinity categories

- Fresh water: **0.6 ds m^{-1}**
- Relatively Saline: **0.6-1.5 ds m^{-1}**
- Little Saline: **1.5-3 ds m^{-1}**
- Average Saline: **3-8 ds m^{-1}**
- Saline: **8-15 ds m^{-1}**
- High Saline: **more than 15 ds m^{-1}**

1 ds m^{-1}= 10 mM

Salinity Effects

- Lack in P, K and Ca absorption

- Ion poisoning because of Na^+, Cl^- and $SO4^-$

- Increasing in osmotic pressure leading to hindering water absorption

Wheat and salinity

- Wheat (semi dwarf) can tolerate up to **8.6 ds m^{-1}**
- Wheat (Durum) can tolerate up to **5.9 ds m^{-1}**
- Wheat (Triticum aestivum) can tolerate up to **6 ds m^{-1}**
-

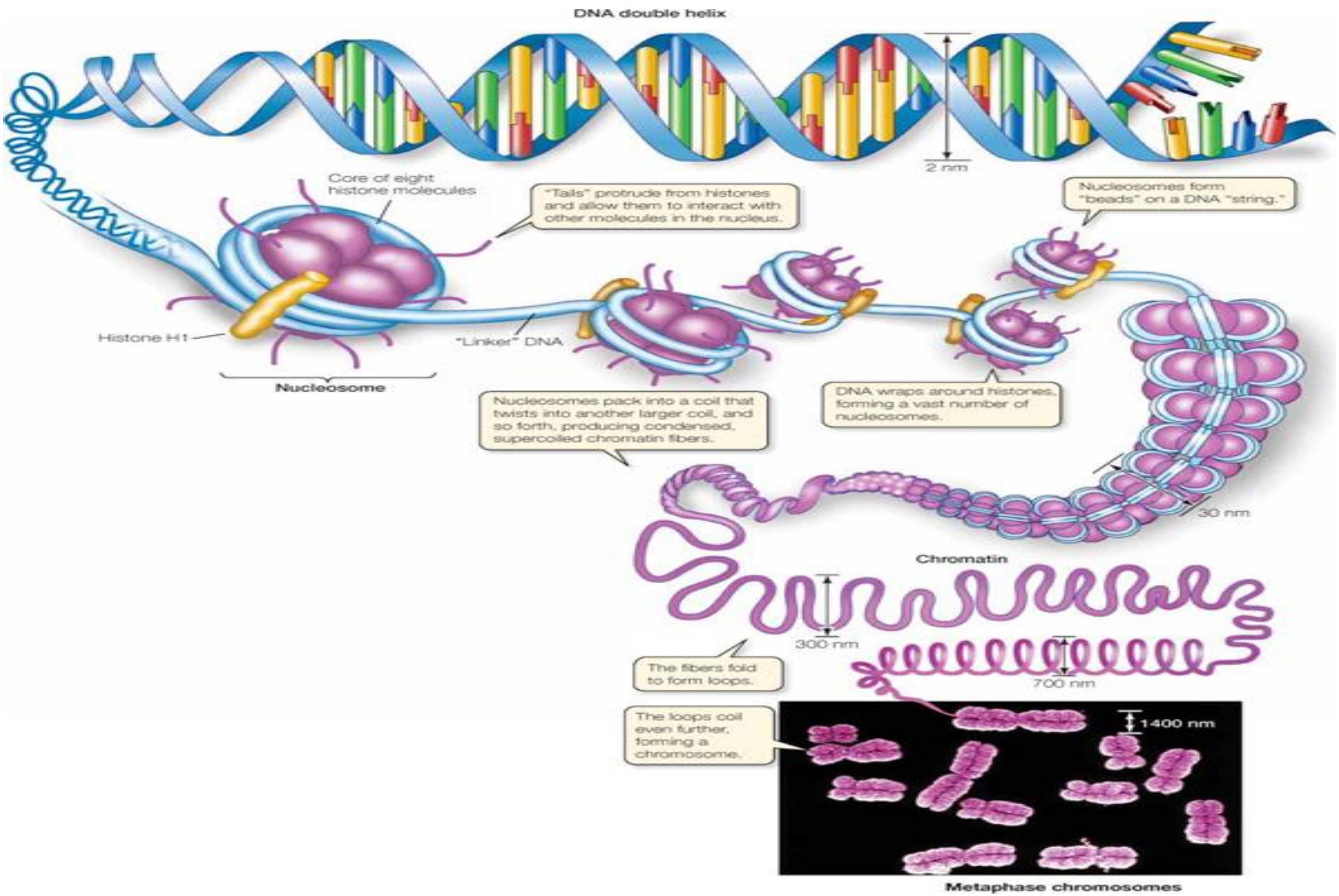
Mechanisms of salt tolerance in wheat

- Deep root with high efficiency in water uptake
- High ability for Homeostasis (Osmoses adjustment), therefore cells can do their functions in proper way.
- Ability of regulating growth under salt stress (phenotypic and anatomical characteristics)
- High water use efficiency in order to get high dry matter

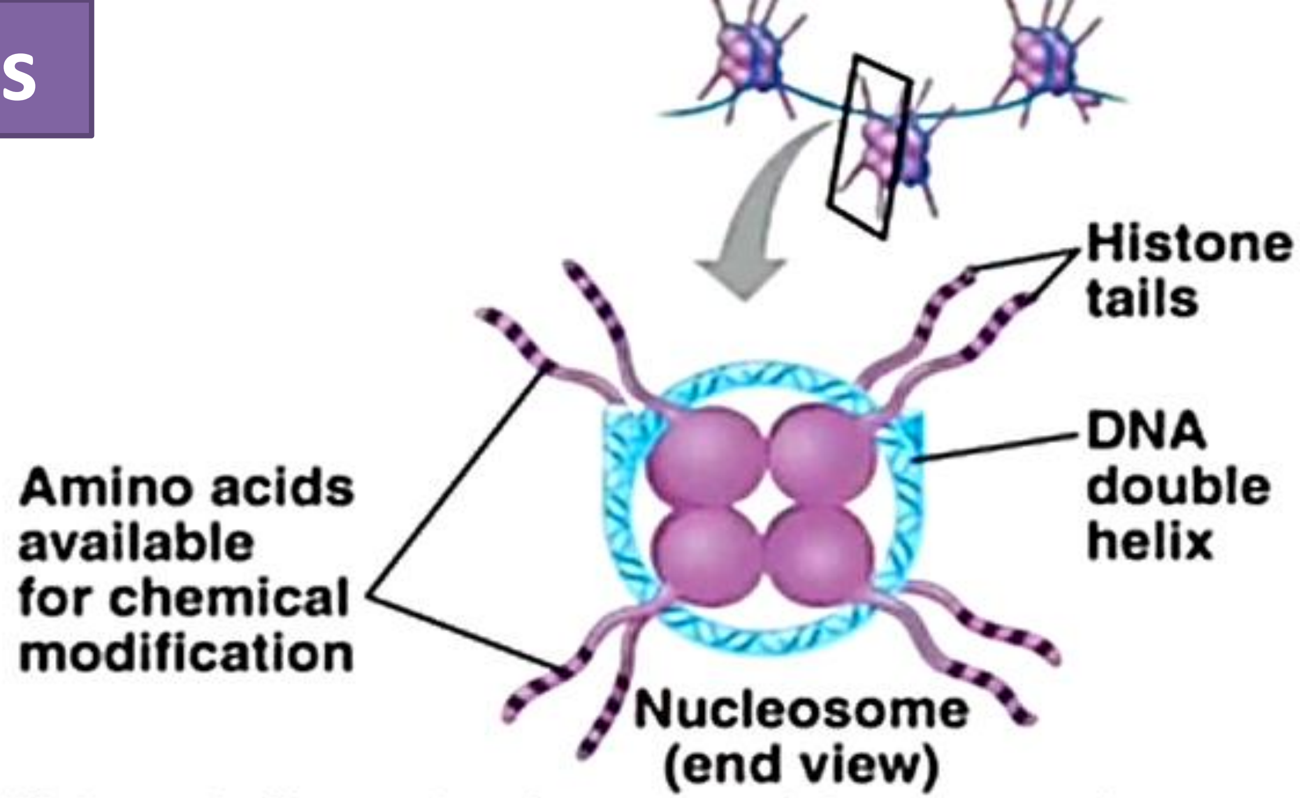
Moreover all mechanisms involve:

- Na^+ exclusion from the transpiration stream
- Sequestration of Na^+ and Cl^- in the vacuoles of root and leaf cells
- Any other processes that promote fast growth despite the osmotic stress of the salt outside the roots

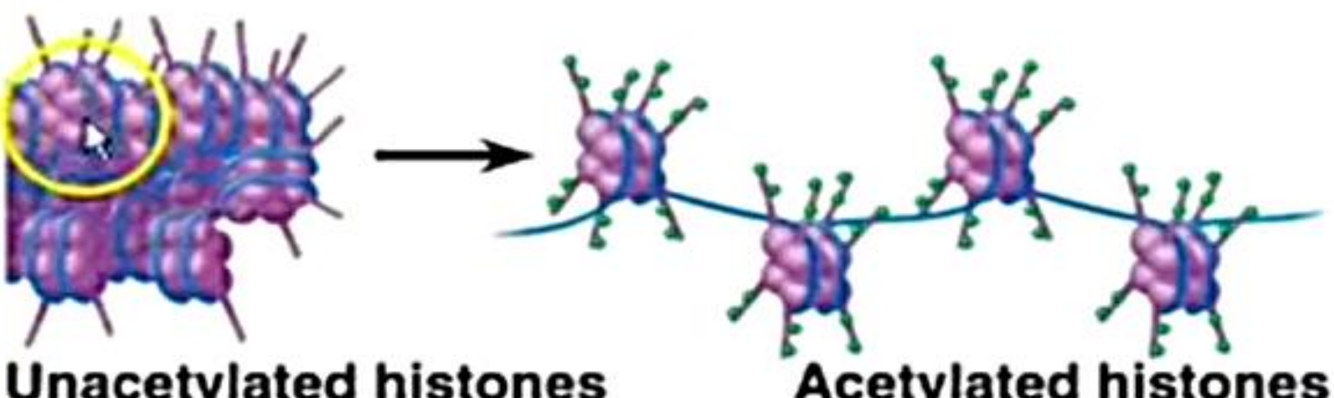
What is Gene


DNA double helix
2 nm
Core of eight histone molecules
"Tails" protrude from histones and allow them to interact with other molecules in the nucleus.
Nucleosomes form "beads" on a DNA "string."
Histone H1
"Linker" DNA
Nucleosome
Nucleosomes pack into a coil that twists into another larger coil, and so forth, producing condensed, supercoiled chromatin fibers.
DNA wraps around histones, forming a vast number of nucleosomes.
30 nm
Chromatin
300 nm
The fibers fold to form loops.
700 nm
The loops coil even further, forming a chromosome.
1400 nm
Metaphase chromosomes

DNA access

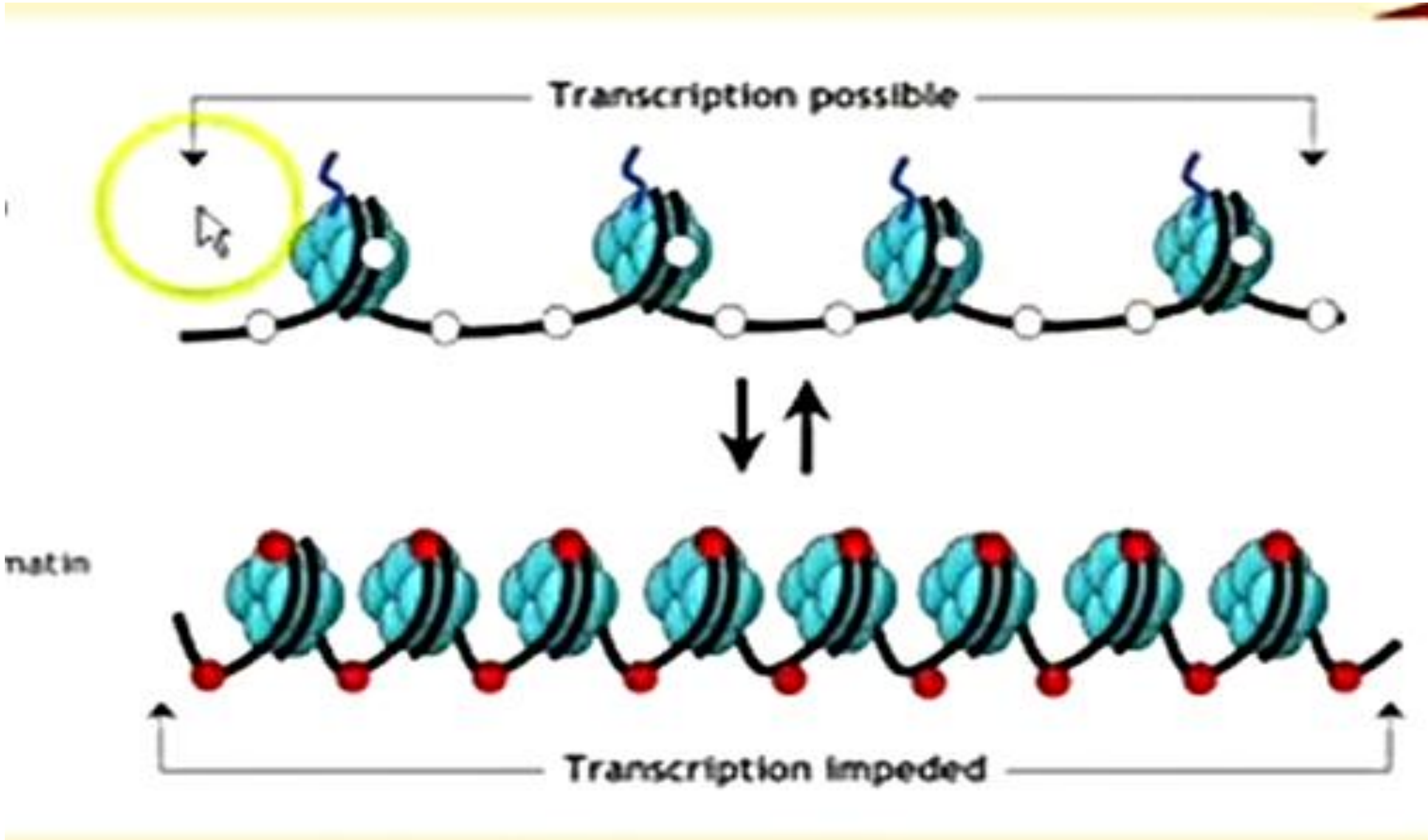


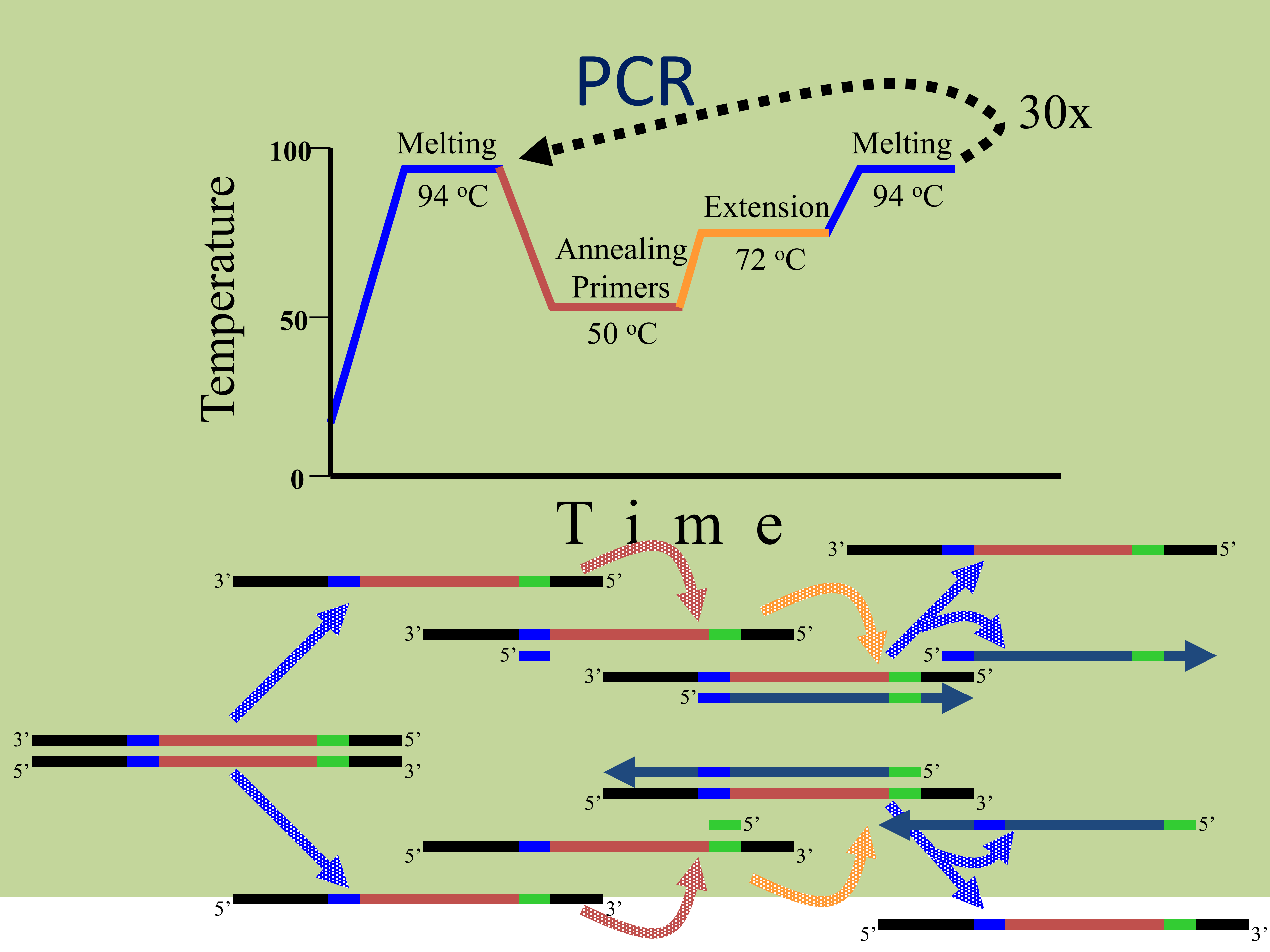
(a) Histone tails protrude outward from a nucleosome



(b) Acetylation of histone tails promotes loose chromatin structure that permits transcription

DNA methylation: Methyl groups added to DNA, inactivates DNA




PCR
30x
Melting
94 ºC
Annealing
Primers
50 ºC
Extension
72 ºC
Melting
94 ºC
100
50
0
Temperature
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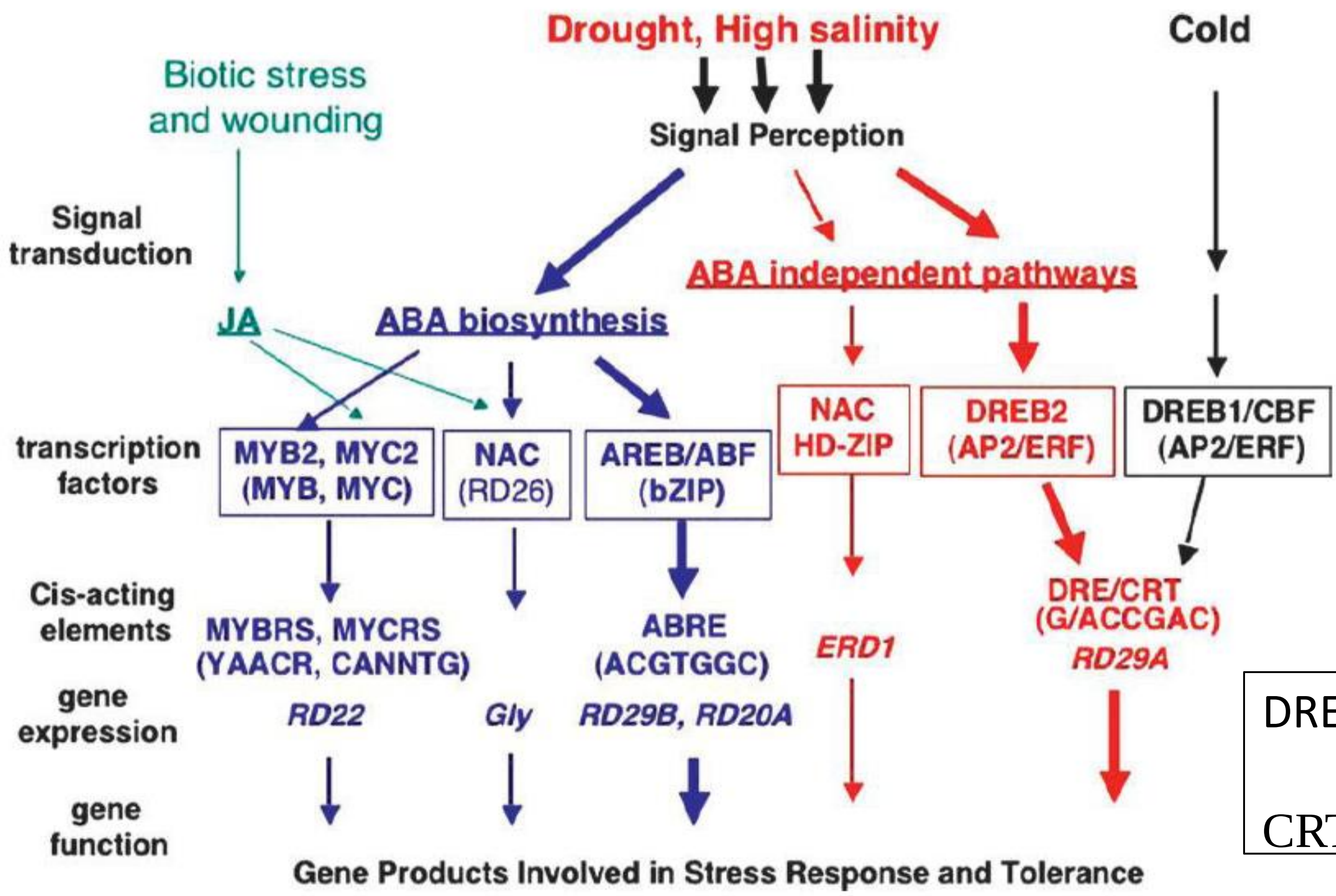
The Abiotic Stress Transcription Factors



DRE: TA**CCGAC**AT

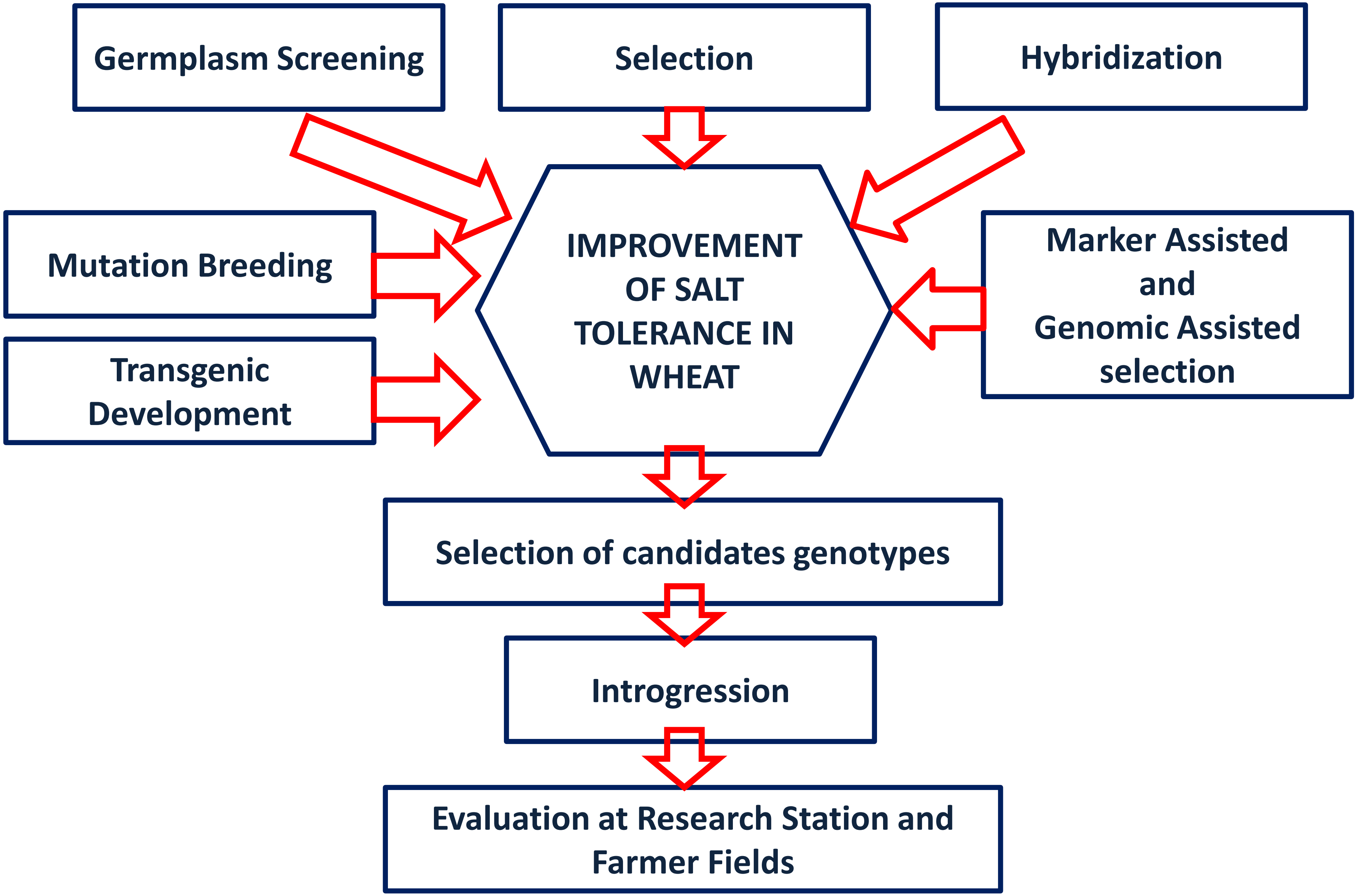
CRT:TGG**CCGAC**

Changes in gene expression to stress

- A stress response is initiated when plants recognizes stress at the cellular level
- Stress recognition activates signal transduction pathways that transmit information within the individual cell and throughout the plant
- Changes in gene expression may modify growth and development and even influence reproductive capabilities

Gene expression results in:

- Increase amounts of specific mRNA
- Enhance translation
- Stabilize proteins
- Altered protein activity
- A combination of the above



BIOLOGICAL PRACTICES FOR IMPROVEMENT OF SALT TOLERANCE (Adapted)

Screening for salt tolerant wheat germplasm

- **Wheat Species comprised of a large number of varieties, strains, cultivars and lines.**

- **There are groups even within species have genetic differences for numerous parameters**

- **Salt tolerance is one of the parameters for which lot of genetic differences are present within species.**

- **Screening must be done for parameter related with the ultimate objective of plant breeder which the yield.**

Statistics

- Biometrical tools assist in assessment of genetic variability among wheat germplasm: (Analysis of variance, **mean comparison tests**, basic summary statistics, **metroglyph analysis**, D2 statistics, **principle component analysis** and biplot graphical analysis.

- Salt tolerance indices are profusely used for efficient screening of germplasm in different crop plants.

Stress susceptibility index	**Geometric mean productivity**
Mean productivity	**Harmonic mean**
Tolerance index	**Stress tolerance index**
Yield index	**Yield stability index**
Ranking index	**Integrated selection Index**
Integrated scoring	

- **Therefore, screening of previously available wheat germplasm for Salt resistance proved to be resource efficient biological strategy**.

Conventional breeding strategies

- Creation of genetic variability and novel gene combination through intercrossing of targeted parents is one of the practices used to develop tolerant genotypes

- followed by appropriate selection scheme enables to develop an ideotype plant that is suitable for environment specific cultivation

- Higher genetic variability, high heritability, and higher selection intensity empower the breeder to make appropriate selection in the germplasm

Conventional breeding methods rely on conducting multilocation, multiyear and multiseason yield trials for evaluation of stability in the performance against salt stress

Time and efforts consuming

Pushs us for the biotechnology……

Marker-Assisted and Genomic-Assisted Breeding

- Tolerance is complex feature governed by large number of traits, and these traits are controlled by large number of chromosomal regions known as quantitative trait loci (**QTLs**)
- Parents with contrasting phenotypic expression are crossed to develop segregating progenies
- Segregating populations are screened with the help of DNA markers like, **RAPD**, **RFLP**, **AFLP**, **SSR**, and **SNPs**

Continue…

- Exploitation of DNA-based markers for identification of QTL mapping linked with morphological, physiological, and biochemical traits could be targeted by breeder for salt resistance improvement

- After identification of QTLs linked with traits, Salt tolerance can be improved by **introgression** of these QTLs into modern promising cultivars

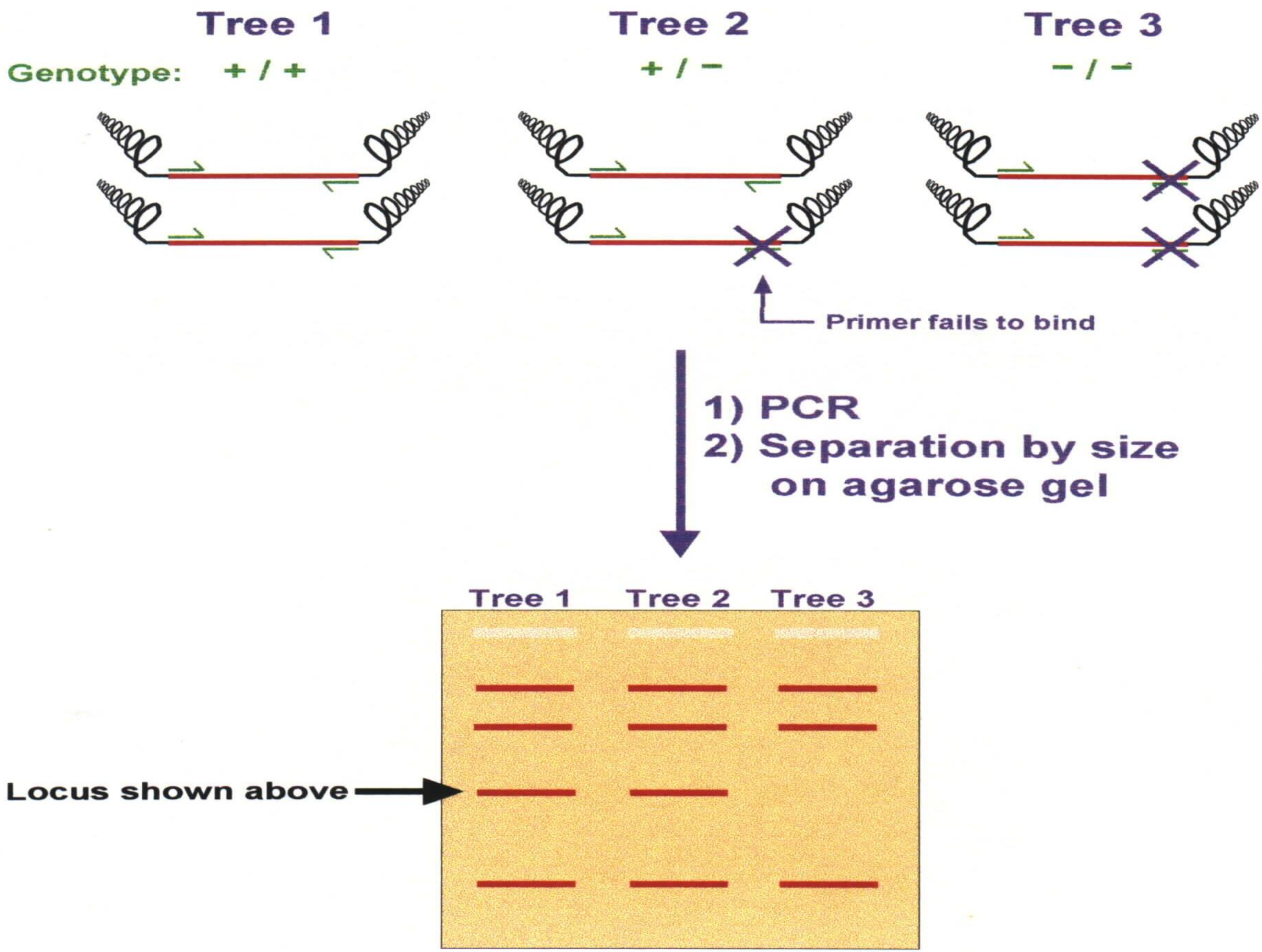
Genetic diversity using RAPD *techniques*

Variation DNA detected by RAPD is due to the loss of RAPD loci. The loss of RAPD loci is caused by:

a) **change of sequence at primer annealing site in the genomic DNA**

b) **deletion of primer annealing site in the genomic DNA**

c) **large insertion in between two primer annealing sites**


Tree 1
Tree 2
Tree 3
Genotype: + / +
+ / −
− / −
Primer fails to bind
1) PCR
2) Separation by size on agarose gel
Tree 1
Tree 2
Tree 3
Locus shown above

Simple Sequence Repeats _ SSRs

- SSRs are loci that are comprised of highly variable arrays of tandemly repeating DNA sequences 2 to 6 base pairs long. SSRs are useful molecular markers because they are:
- **(1) abundant;**
- **(2) uniformly distributed;**
- **(3) highly polymorphic;**
- **(4) co-dominant;**
- **(5) rapidly produced by PCR;**
- **(6) relatively simple to interpret; and**
- **(7) easily accessed by other laboratories via published primer sequences**

Transgenic Wheat Development

Genes which can be manipulated in genetic engineering are categorized into four main classes:

(1) genes involved in transcriptional and signal transduction pathways,

(2) genes involved in protection of cellular membranes and biosynthesis of stress-responsive proteins,

(3) genes involved in uptake of ions and water-like ion transporters and aquaporins

(4) genes involved in cellular metabolism e.g., free amino acids, proline, soluble sugars, polyols, and glycinebetaine

Conclusions and Summary

- Salinity is a major abiotic stress which hinder wheat productivity across the world
- Wheat is a very important crop worldwide and salinity effects on it are prevalent from germination to harvest maturity.
- All wheat genotypes are not equally affected by salinity stress due to high level of genetic variability in genetic backgrounds of this crop.
- Salt-tolerant wheat genotypes maintain their growth and development along with economical grain yield under salt stress.
- In contrast; susceptible genotypes maybe lacking of the adaptive mechanisms and facing sever damage in terms of growth and development and grain yield.
- Salt tolerance is very complex mechanism which is collaboratively conferred by **OSMOTIC ADJUSMENT, PLANT GROWTH REGULATORS, ANTIOXIDATIVE DEFENSE, STRESS RESPONSIVE PROTEINS, WATER CHANNEL PROTEINS, TRANSCRIPTION FACTORS and SIGNAL TRANSDUCTION PATHWAYS**

As demand of wheat is increasing day by day and global warming is also increasing therefore

- There is a dire need to improvement of wheat against salt stress:
- E.g. Managerial strategies and Biological strategies

- Managerial strategies involve the usage of water resources and adoption of saving agronomic practice

- Biological strategies deal with manipulation of genetic backgrounds of wheat for improvement against salt stress.

- Biological strategies are preferred over managerial practices due to long term and economical effectiveness.

- Biological strategies can be practiced in different forms.

- Available wheat germplasm has lot of genetic variability which can be exploited for higher salt tolerance through effective screening tools.

- Conventional breeding, Mutation breeding, marker assisted and genomic assisted breeding, and development of salt tolerant transgenic wheat are numerous strategies which are enlisted under the heading of **BIOLOGICAL STRATEGIES**

Mission: Possible

http://www.economist.com/node/5323362

Points to take:

- What are the significances of wheat crop?
- ما الذي يجعل الحنطة محصول استراتيجي مهم؟
- What is the salinity and its effects on wheat?
- ما هي الملوحة وما هي تاثيراتها على محصول الحنطة؟
- What the source of salinity?
- ما هي مصادر الملوحة؟
- What are the biotechnologies that can be used in enhancement of wheat salt tolerance
- ماهي التقنيات الاحيائية التي من شأنها زيادة تحمل الحنطة للشد الملحي؟