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Molecular Characterization of *TaMATE1* Homoeologues Genes and The Analysis of OAs Genes Expression Under Al Stress in Bread Wheat

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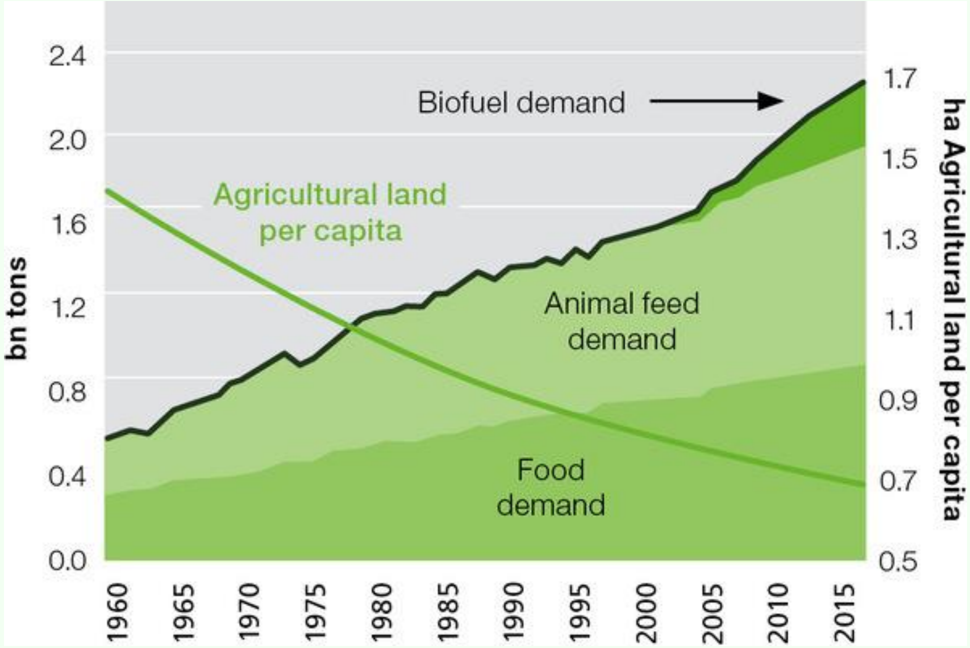
Abiotic Stress Tolerance: Crop Productivity

Global climate changes and burgeoning human population pressure are seriously affecting the arable land availability.

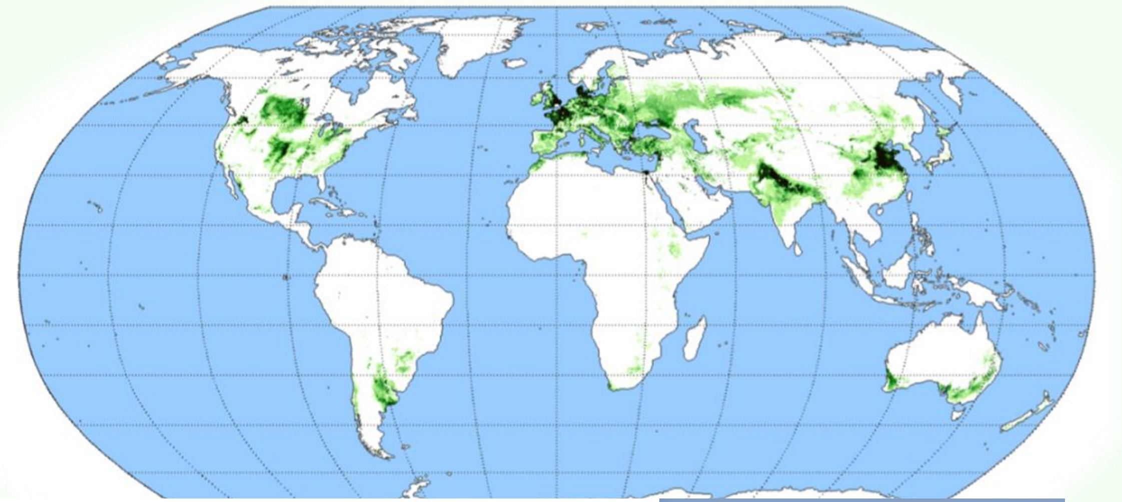


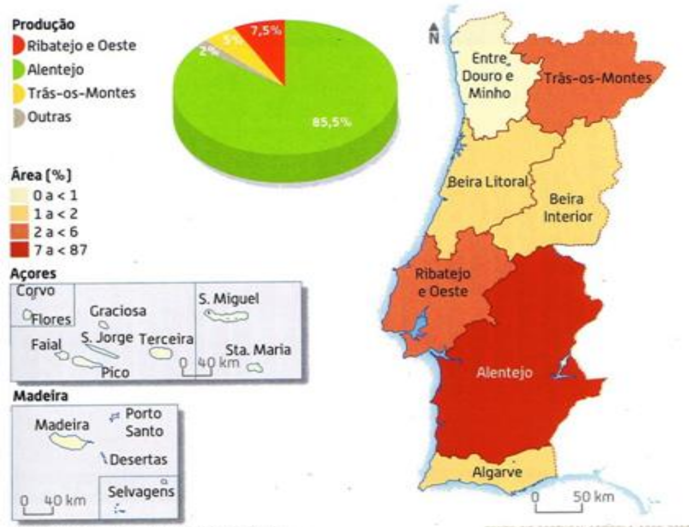
Utilization of less favorable soils by plants tolerant to abiotic stresses provides the greatest potential for agricultural expansion.

Wheat: Global & Portugal Perspective

- Globally, wheat is grown on more land area than any other commercial crop and ranks second, after rice, as main human food crop

- Wheat is a staple food in Portugal





in LOBATO, Cláudia - Geografia 11, Parte 1, Porto, 2008

FONTE: INE, PORTUGAL AGRÍCOLA, 1960-200

Global Perspective of Acid Soils Prevalence

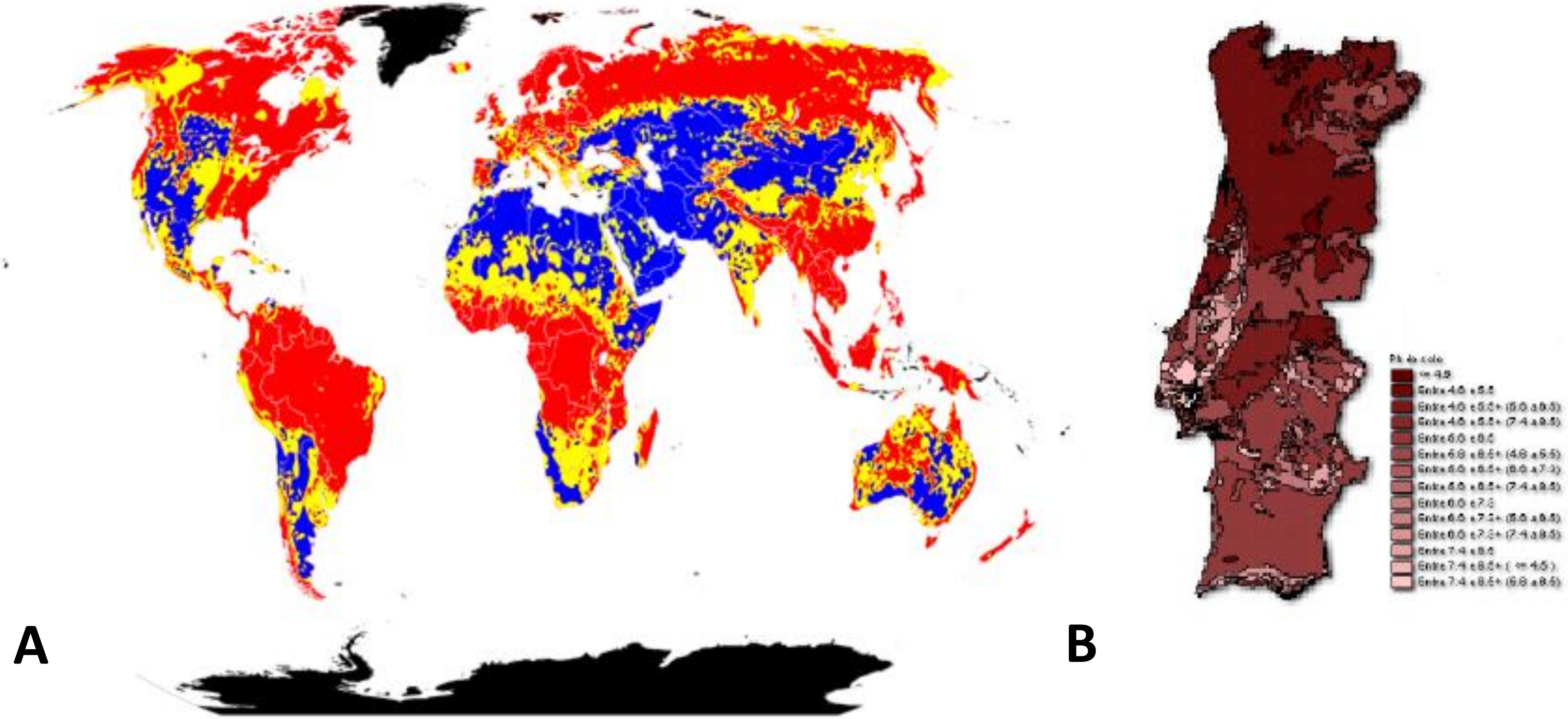


Figure: Distribution of acid soils (red colour) around the world (A) and Portugal (B). Source: von Uexkúll and Mutert, 1995; http://www.iambiente.pt/

Bread Wheat & Soil Acidity

- In acidic soils, Al toxicity induces severe damages in plant root system and it interferes in the uptake of water and nutrients (Ca, P, Fe)

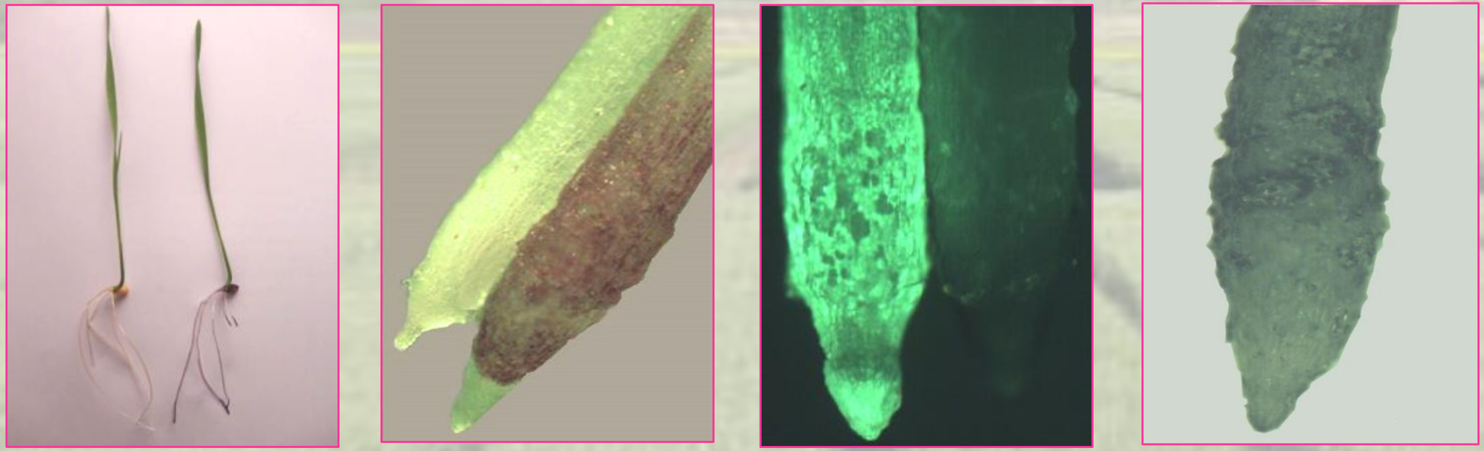


Figure - Al accumulation and cell membrane injuries in the root treated with Al

(Garcia-Oliveira *et al.*, 2014 submitted)

- Prominent abiotic stress tolerance mechanisms include:

  - Altered gene expression
  - Facilitated membrane transport

Molecular Basis of Al Tolerance in Plants

So far, natural variation for Al tolerance has been explained by mainly two genes in different plant species :

- ***ALMT1*** (*Al-Activated Malate Transporter 1*)

  - Wheat (Sasaki *et al.*, 2004).

- ***MATE1/AACT1*** (*Multidrug and Toxic compound Exudation Al-Activated Citrate Transporter 1*)

  - Sorghum (Magalhães *et al.*, 2007)

  - Barley (Furukawa *et al.*, 2007)

Objectives:

- **Cloning and mapping of *MATE1* gene in bread wheat**
- **Molecular characterization of *TaMATE1* homoeologues**
- **To investigate the upstream variation of *TaMATE1* and *TaALMT1* genes in selected diverse genotypes**

Phenotypic Variability Related to Al Tolerance

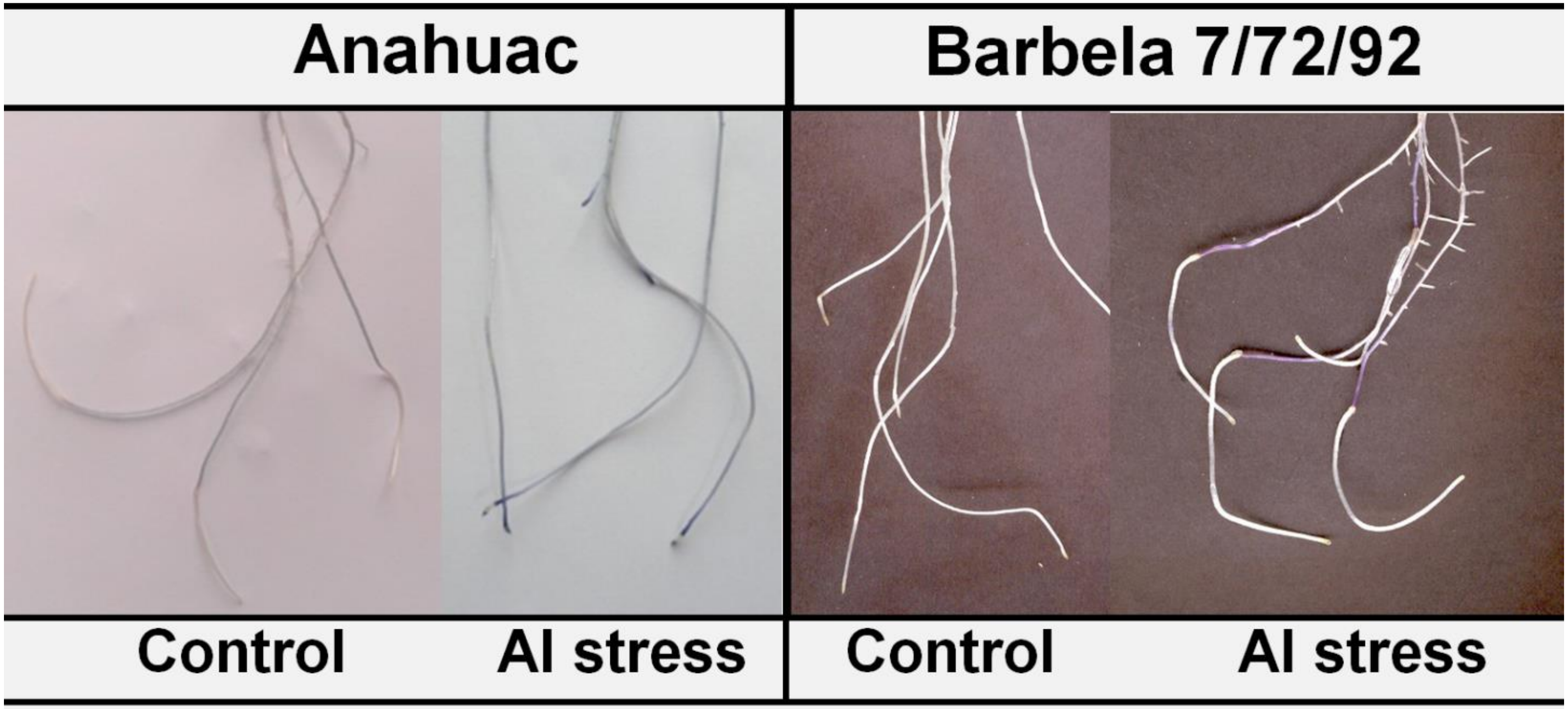
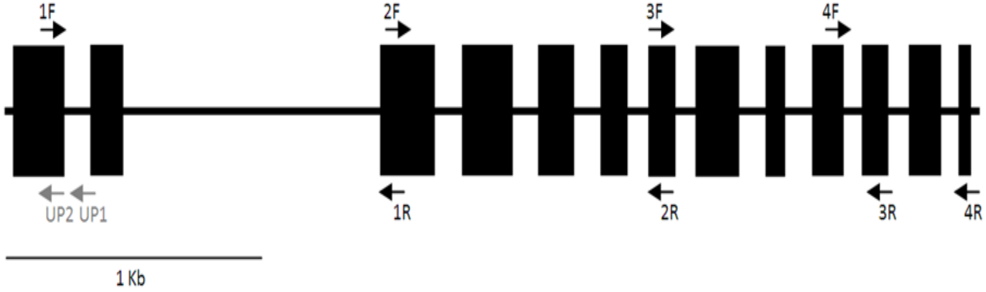
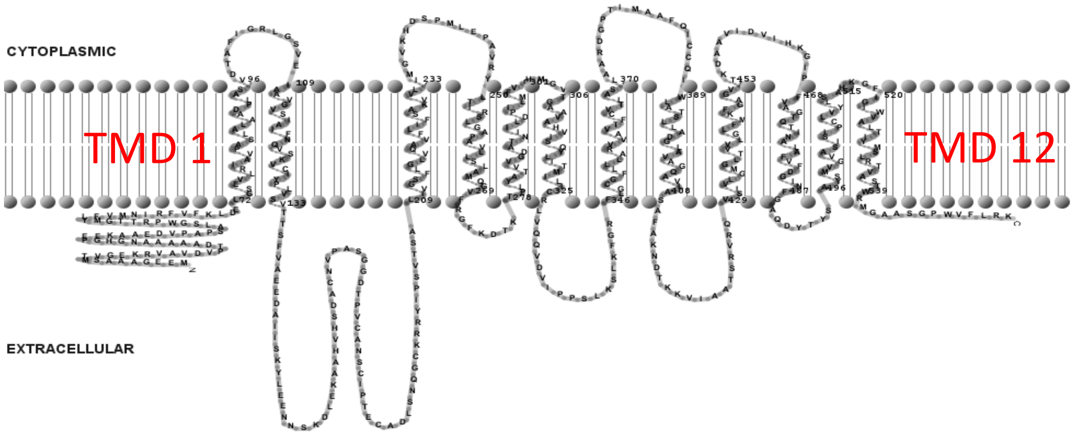


Figure: Physiological differences between Anahuac (Al sensitive) and Barbela 7/72/92 (Al tolerant) roots with and without Al stress (Eriochrome root re-growth assay)

TaMATE1 Genomic, Transcript & Putative Protein Structure





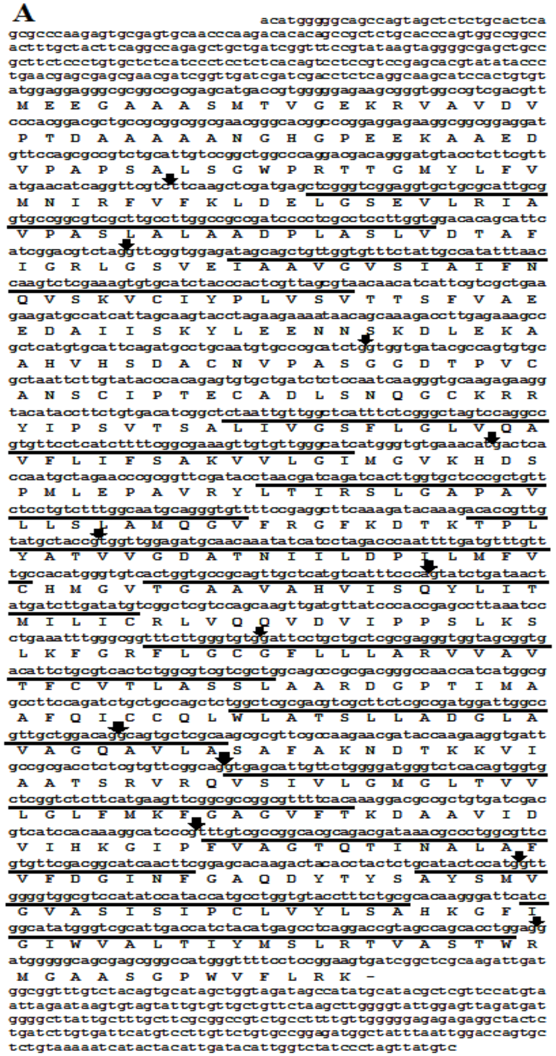


Figure: Structure of the genomic sequence of *TaMATE1* (upper left), TaMATE1-4D transcript (right) and putative protein structure (below left)

Phylogenetic Analysis of MATE1 Proteins

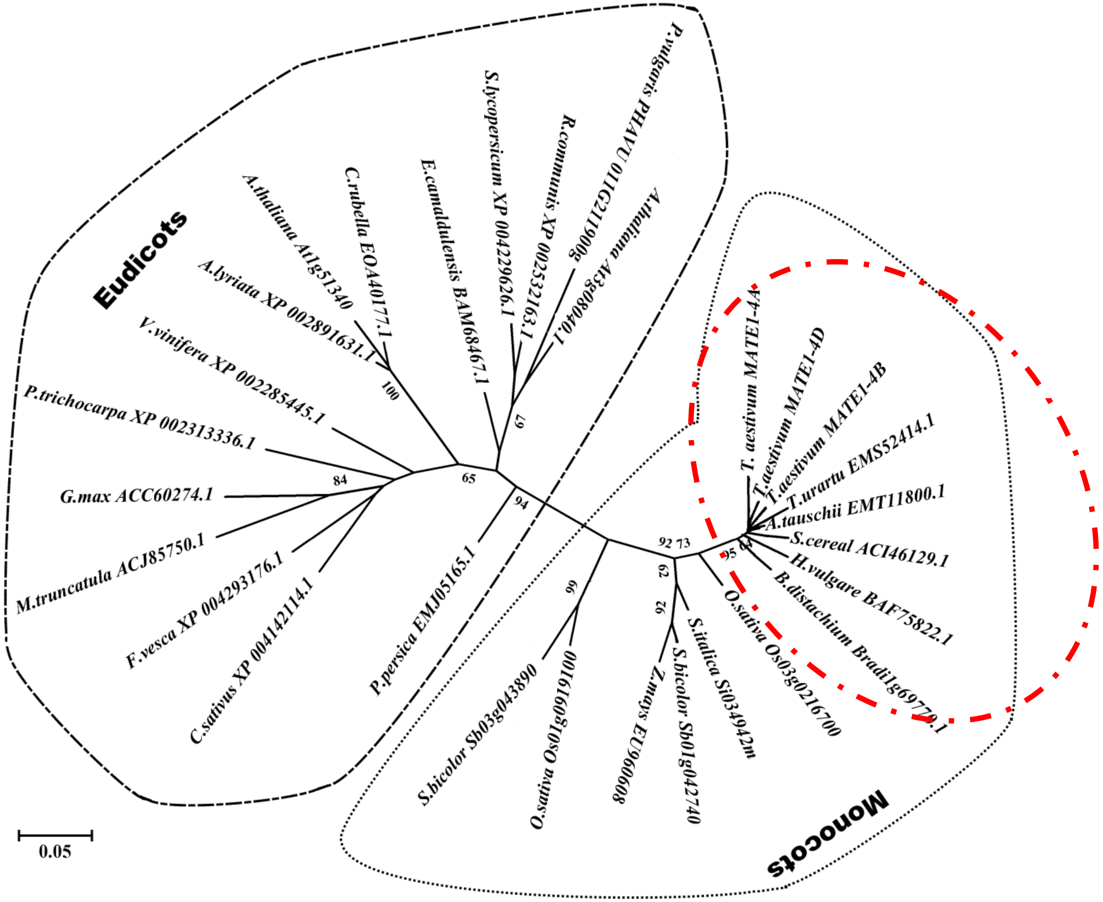


Figure: Phylogenetic tree constructed on the basis of a.a. sequences of known and putative MATE1 genes

Chromosomal Mapping on *TaMATE1* Homoeologues

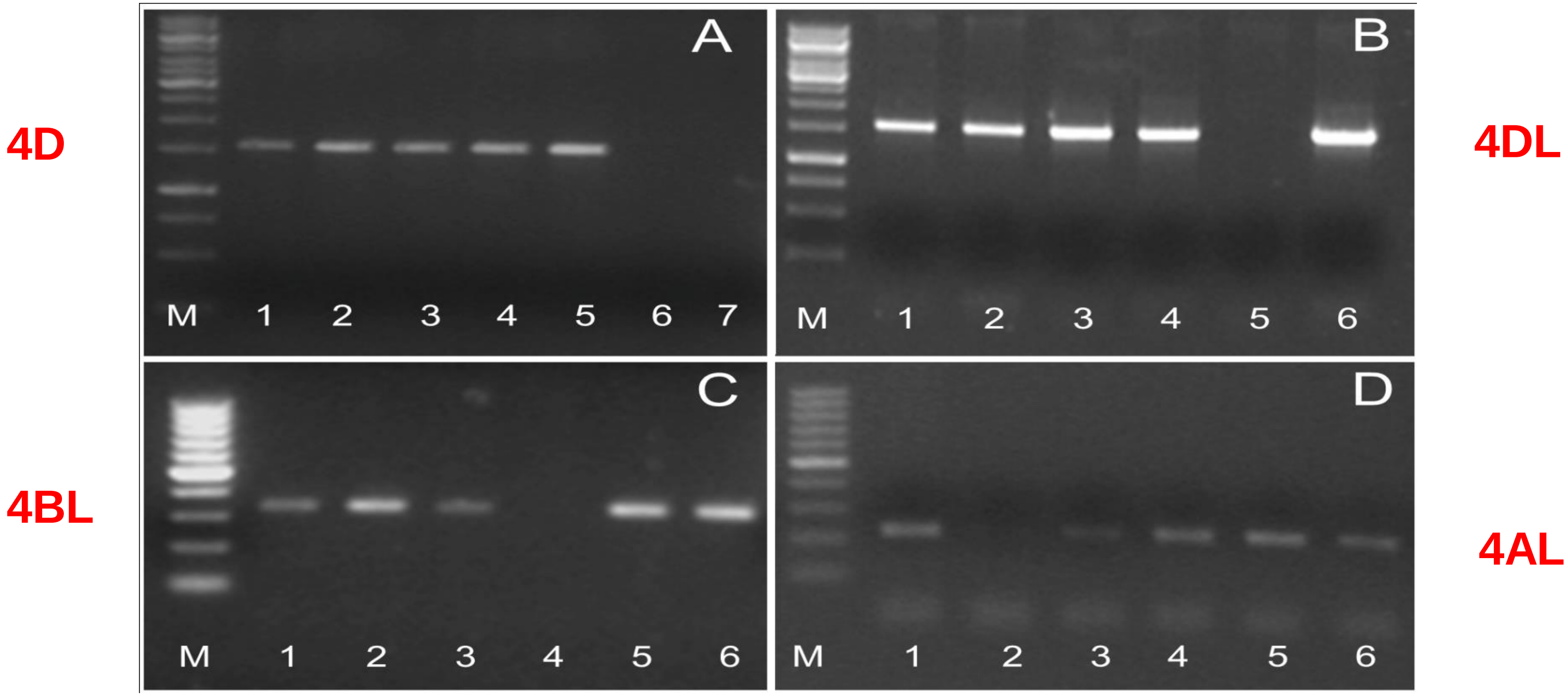


Figure TaMATE1 mapping on wheat chromosome from genome D using nullitetrasomics lines [A]. **M** (Marker); **1** ('*Chinese Spring*' control); **2** (N4AT4B); **3** (N4A4D); **4** (N4BT4A); **5** (N4BT4D); **6** (N4DT4A); **7** (N4DT4B)

Arms localization of *TaMATE1-4D* [**B**], *TaMATE1-4B* [**C**] and *TaMATE1-4A* [**D**]. M (Marker); **1** ('*Chinese Spring*' control); **2** (Dt4AS); **3** (Dt4AL); **4** (Dt4BS); **5** (Dt4DS); 6 (Dt4DL)

Relative Quantification of TaMATE1 Homoeologues in Root & Shoot Tissues

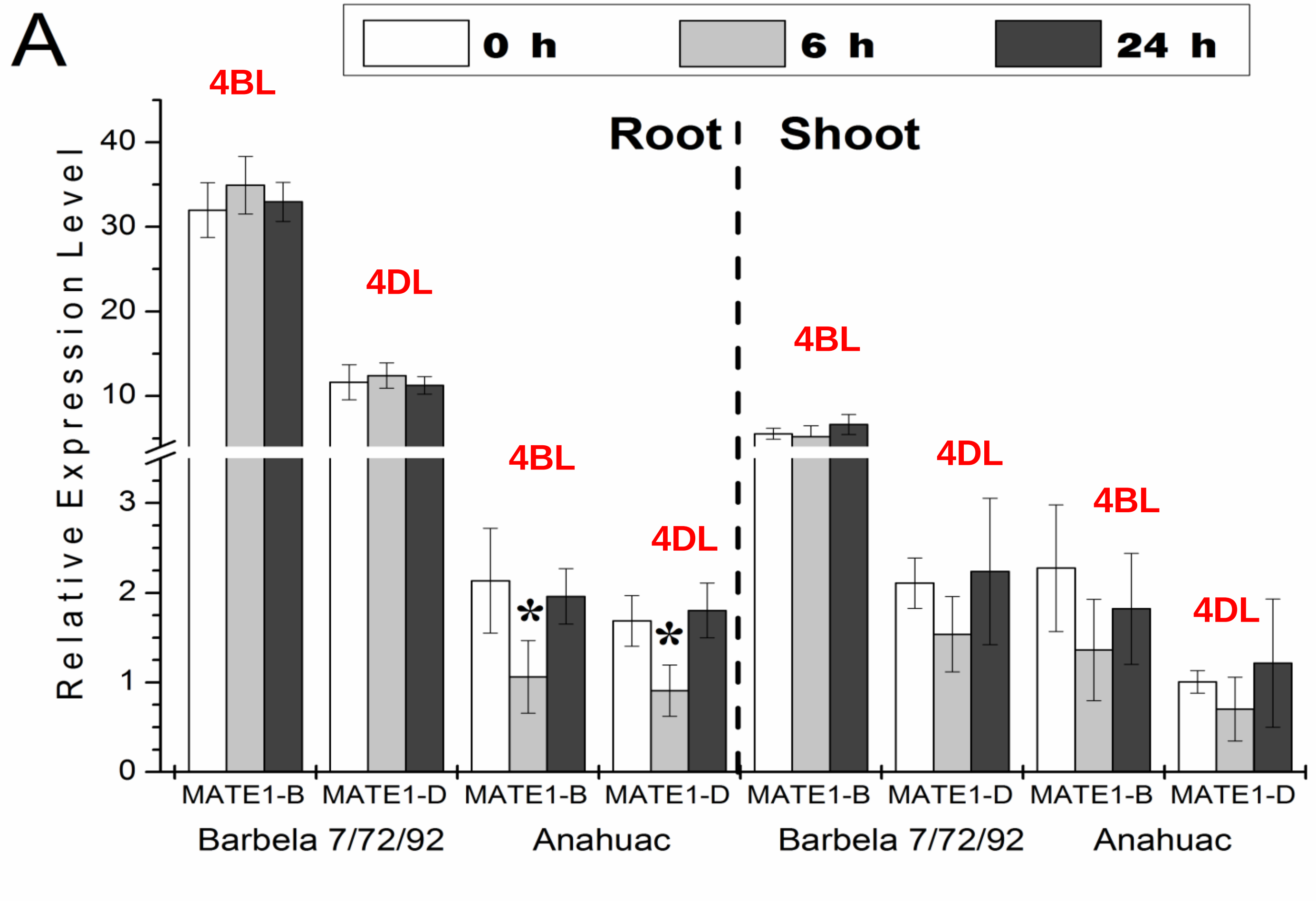
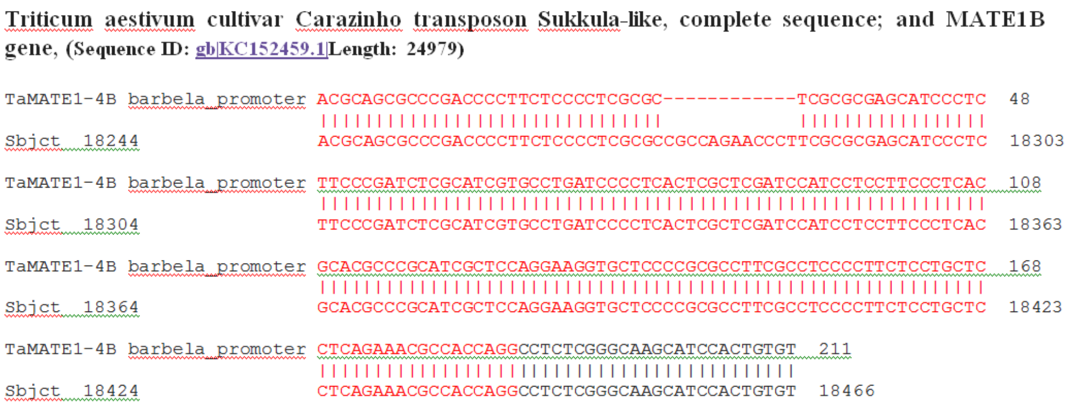


Figure: Quantification of TaMATE1 homoeologues using qRT PCR.
*** Significant P value < 0.05**

Detection of Sukkula-like Transposon (SLT) in *TaMATE1-4B* promoter



Triticum aestivum cultivar Carazinho transposon Sukkula-like, complete sequence; and MATE1B gene, (Sequence ID: gb|KC152459.1|Length: 24979)

```
TaMATE1-4B barbela_promoter ACGCAGCGCCCGACCCCTTCTCCCCTCGCGC------------TCGCGCGAGCATCCCTC  48
                            |||||||||||||||||||||||||||||||            |||||||||||||||||
Sbjct  18244                ACGCAGCGCCCGACCCCTTCTCCCCTCGCGCCGCCAGAACCCTTCGCGCGAGCATCCCTC  18303

TaMATE1-4B barbela_promoter TTCCCGATCTCGCATCGTGCCTGATCCCCTCACTCGCTCGATCCATCCTCCTTCCCTCAC  108
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18304                TTCCCGATCTCGCATCGTGCCTGATCCCCTCACTCGCTCGATCCATCCTCCTTCCCTCAC  18363

TaMATE1-4B barbela_promoter GCACGCCCGCATCGCTCCAGGAAGGTGCTCCCCGCGCCTTCGCCTCCCCTTCTCCTGCTC  168
                            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18364                GCACGCCCGCATCGCTCCAGGAAGGTGCTCCCCGCGCCTTCGCCTCCCCTTCTCCTGCTC  18423

TaMATE1-4B barbela_promoter CTCAGAAACGCCACCAGGCCTCTCGGGCAAGCATCCACTGTGT  211
                            |||||||||||||||||||||||||||||||||||||||||||
Sbjct  18424                CTCAGAAACGCCACCAGGCCTCTCGGGCAAGCATCCACTGTGT  18466
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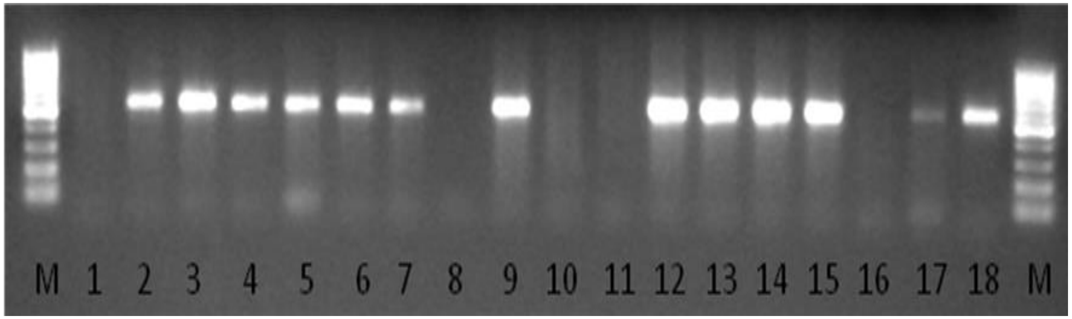
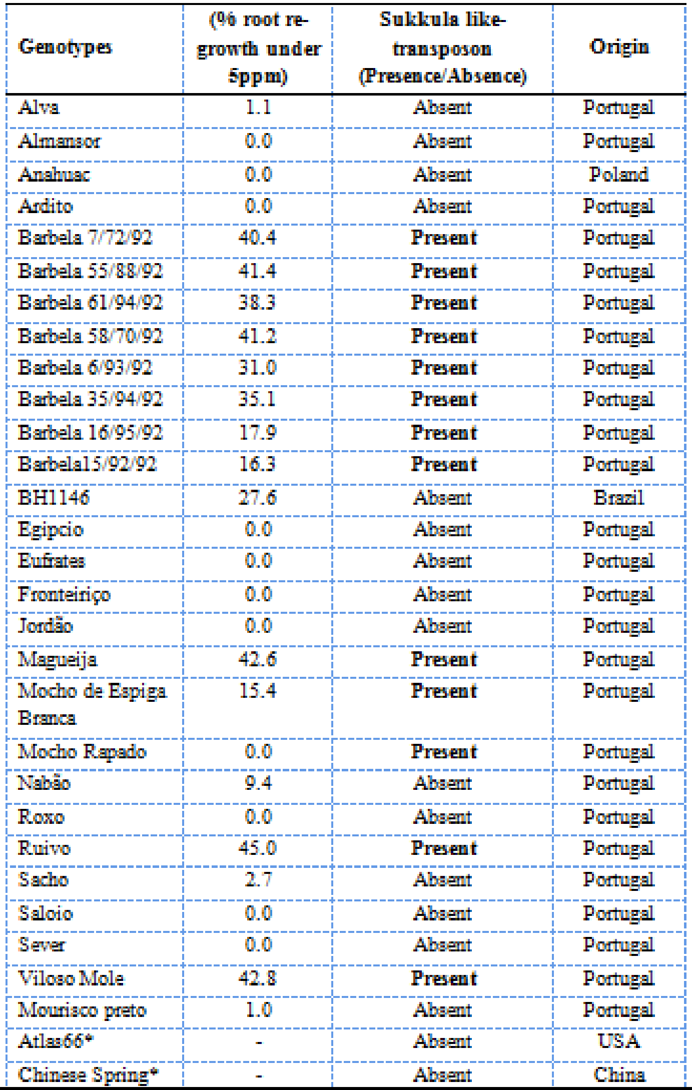


Figure- Identification of SLT in the promoter of *TaMATE1-4B* homoeologue in bread wheat (upper left) Confirmation in diverse bread wheat genotypes (lower left and right)



Genotypes	(% root re-growth under 5ppm)	Sukkula like-transposon (Presence/Absence)	Origin
Alva	1.1	Absent	Portugal
Almansor	0.0	Absent	Portugal
Anahuac	0.0	Absent	Poland
Ardito	0.0	Absent	Portugal
Barbela 7/72/92	40.4	**Present**	Portugal
Barbela 55/88/92	41.4	**Present**	Portugal
Barbela 61/94/92	38.3	**Present**	Portugal
Barbela 58/70/92	41.2	**Present**	Portugal
Barbela 6/93/92	31.0	**Present**	Portugal
Barbela 35/94/92	35.1	**Present**	Portugal
Barbela 16/95/92	17.9	**Present**	Portugal
Barbela15/92/92	16.3	**Present**	Portugal
BH1146	27.6	Absent	Brazil
Egipcio	0.0	Absent	Portugal
Eufrates	0.0	Absent	Portugal
Fronteiriço	0.0	Absent	Portugal
Jordão	0.0	Absent	Portugal
Magueija	42.6	**Present**	Portugal
Mocho de Espiga Branca	15.4	**Present**	Portugal
Mocho Rapado	0.0	**Present**	Portugal
Nabão	9.4	Absent	Portugal
Roxo	0.0	Absent	Portugal
Ruivo	45.0	**Present**	Portugal
Sacho	2.7	Absent	Portugal
Saloio	0.0	Absent	Portugal
Sever	0.0	Absent	Portugal
Viloso Mole	42.8	**Present**	Portugal
Mourisco preto	1.0	Absent	Portugal
Atlas66*	-	Absent	USA
Chinese Spring*	-	Absent	China

Citrate Efflux Quantification

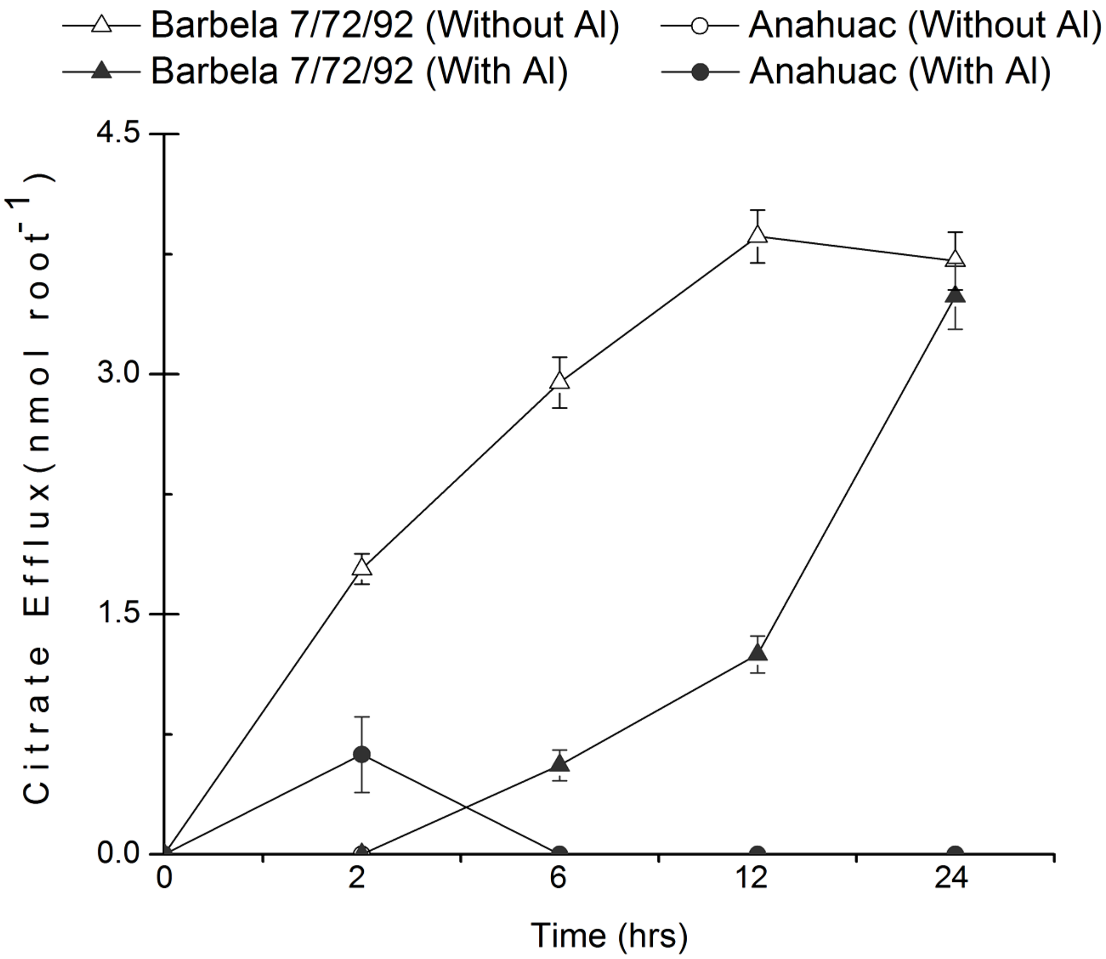
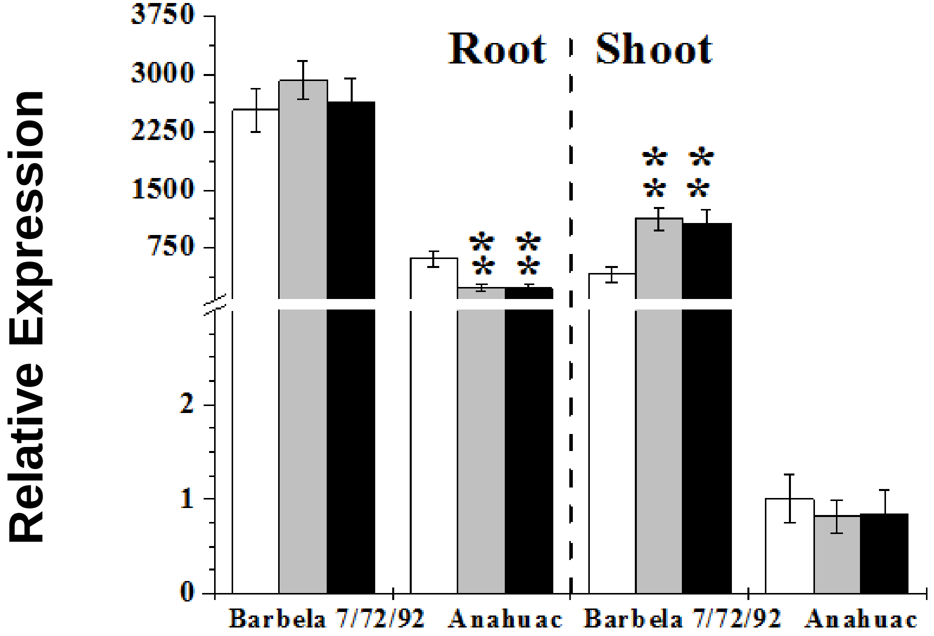


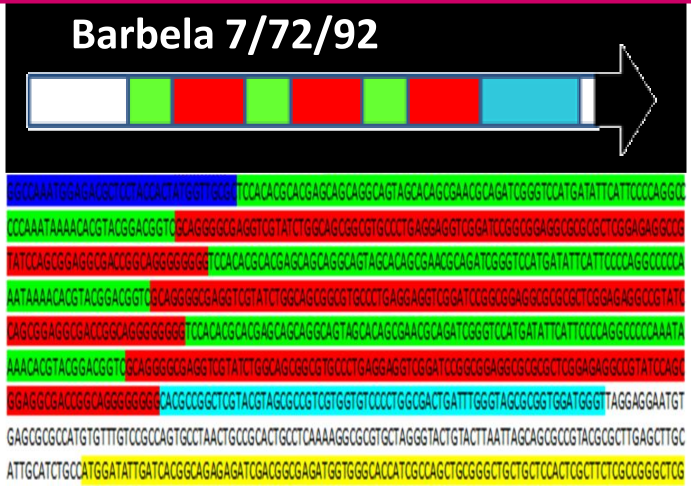
Figure: Time course of citrate efflux from intact roots of two bread wheat genotypes

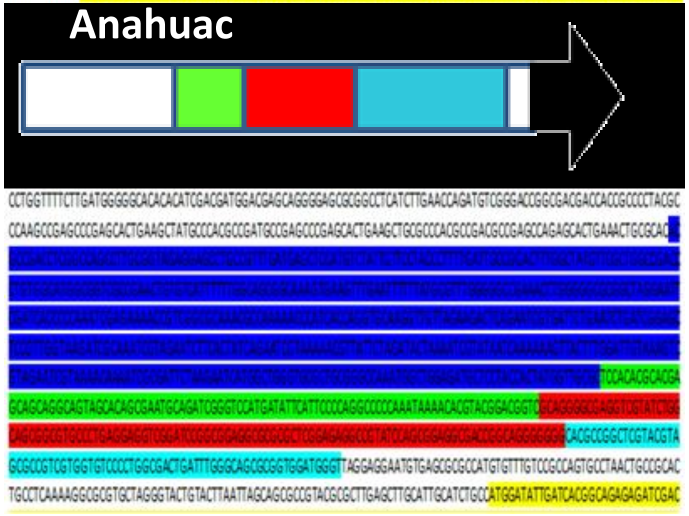
Relative Quantification of TaALMT1 and its Promoter Analysis



*Statistically different P value < 0.01

Figure: Relative transcript level of TaALMT1 and its promoter analysis in Barbela 7/72/92 and Anahuac





TaALMT1 promoter analysis exhibited that *Barbela 7/72/92* contains three tandem repeats of a 205 bp block (type VI promoter), whereas Al-sensitive genotype Anahuac showed only a single block (type I promoter)

Conclusion

- TaMATE1, a citrate transporter gene localized on the long arms of homoeologous group 4 chromosomes in bread wheat.
- Expression profiling identified **biased transcript expression** of TaMATE1 homoeologues in bread wheat.
- In Barbela 7/72/92, the presence of Sukkula-like Transposon in the upstream of TaMATE1-4B seems to play a **major role** in altering the transcript expression of *TaMATE1-4B* homoeologue which could play major role in citrate efflux.
- TaMATE1-4B allele having Sukkula-like transposon is widespread among Al tolerant Portuguese bread wheat genotypes which was considered as rare allele.

Conclusion

- The high basal level of TaMATE1- 4B and TaALMT1 in Barbela 7/72/92 **shoots** indicates that **internal detoxification mechanisms** of Al tolerance **may also play an important role in this genotype**

- Our results also indicate that the origin of similar transposon in Brazilian bread wheat cv Carazinho goes back to **Portuguese bread wheat germplasm** which might be primarily introduced in Brazil by Portuguese navigators during the early colonial period.

- Finally, we found a genotype were **both genes ALMT1 and MATE1 are acting together** to lead a genotype more tolerant to Al stress.

Acknowledgements

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