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IN SILICO AND GENETIC ANALYSIS RELATED TO TILLERING ABILITY IN MAIZE

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SUMMARY

Maize developed from its ancestor, teosinte, about 10,000 years ago. The evolution has gone from teosinte with multiple tillers to single-tiller maize plants. An investigation took place to identify and sequence genes related to tillering ability in maize and perform *In silico* analysis. Mating proceeded by manual pollination between the commercial hybrid SC2031 of maize (*Zea mays* L.) and the teosinte genotype Domiata (*Durra rayyana*). The parents, F_1 hybrids, and their F_2 progenies gained evaluation for tillering ability. The SC2031 (low or no-tillers) exhibited fragments ranging from 75 to 420 bp. The fragment observed with 262 bp size was in the F_1 and nine out of 10 low-tillering F_2 progenies. The Domiata parent, a high-tillering variety, exhibited three fragments ranging from 82 to 534 bp. The fragment with 445 in size occurred in nine out of 10 high-tillering F_2 progenies. The fragment of 262 bp, which may be responsible for the low tillering ability, and the fragment of 445 bp, for the high tillering ability, were eluted from the gel, sequenced, and submitted to GenBank. The predicted protein, T1-L, of the DNA sequence from the low-tillering parent has a molecular weight of 6.69 kD and pI of 6.53, belonging to the GRAS family, which plays an important role as transcriptional factors required for the adequate pattern of radial rooting and shooting. The predicted protein, T1-H, of the DNA sequence from the high-tillering parent has a molecular weight of 14.08 kD, and pI of 7.97, belonging to the TEOSINTE BRANCHED 1, CYCLOIDEA, PCF1 (TCP) family that plays an essential role as transcriptional factors required for apical dominance.

Keywords: Tillering, maize, GRAS protein, TCP protein, transcriptional factors, *In silico*

Key findings: The fragment of 262 bp might be responsible for low tillering ability and produced a protein with 6.69 kD belonging to the GRAS family. In comparison, the fragment of 445 bp might be responsible for high tillering ability and produced a protein with 14.08 kD belonging to the TCP family.

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INTRODUCTION

Maize developed from its ancestor, teosinte, about 10,000 years ago (Doebley *et al*., 2006). The evolution has gone from teosinte with multiple tillers to maize plants with single or low tillers (Doebley *et al*., 1997). Sweet corn and popcorn maize differ from most commercial maize varieties in terms of the extent to which tillers and ears develop (Poethig, 1993). Sweet and popcorn maize usually has two or three tillers with ears.

Genetic analysis methods are beneficial for assessing genetic stability, establishing the genetic identity of different germplasm, and evaluating genotypes and mutants for improved characteristics. Bennici *et al*. (2004) demonstrated the efficiency of cytological and random amplified polymorphic DNA (RAPD) molecular analyses in assessing the genetic stability of organogenesis and somatic embryogenesis-derived fennel plants. RAPD, inter simple sequence repeat (ISSR), and amplified fragment length polymorphisms (AFLP) were widely used for these purposes (Zein *et al*., 2012; Khaled *et al*., 2015; Azzam and Khalifa, 2016; Abdalla *et al*., 2018; Khaled *et al*., 2018; Azzam *et al*., 2019a,b; Hamada *et al*., 2019). Ribosomal DNA (rDNA) was also used for genotype identification as Ismael *et al*. (2018) used 16S rDNA for identifying Rhizobium genotypes, and Salah *et al*. (2021) used 26S rDNA for the identification of Pichai genotypes. Start Codon Targeted (SCoT) markers have advanced efficiency in characteristic studies (Al-Taweel *et al*., 2019; Abou-Sreea *et al*., 2021; Azzam *et al*., 2022; Khaled *et al*., 2022a), and simple sequence repeats (SSRs), which were also used in quantitative trait loci (QTLs) mapping (Azzam *et al*., 2022; Khaled *et al*., 2022b).

A complex genetic network controls the development of maize tillers. A critical gene, *tb1*, encoding the Teosinte-branched 1/Cycloidea/Proliferating (TCP) protein, was responsible for the transition from branched teosinte to single-stalk maize (Studer *et al*., 2011). The *grassy tillers1* (*gt1*) gene acts as a repressor, and high expression of *gt1* inhibits tiller bud growth (Whipple *et al*., 2011). The *gt1* gene can respond to shade through homeodomain protein encoding related to the expression of *tb1*. The gene *tassels replace upper ears1* (*tru1*) that *tb1* targets directly through encoding an ankyrin-repeat domain protein also has a tiller repression effect. The axillary bud formations in maize get suppressed by the high transcription of *tru1* (Dong *et al*., 2017). The *sugary1* affected the balance of carbohydrate metabolism and may enhance tiller bud formations (Kebrom and Brutnell, 2007). On the other hand, the major QTL of tiller number in maize, *tin1*, participates in many pathways. The *tin1* represses the tiller-related genes, *gt1* and *Laba1/An-2*, and regulates the tiller formation through interactions with some TOPLESS proteins (Zhang *et al*., 2019). This investigation aimed to identify and sequence genes related to maize tillering and perform *In silico* analysis.

MATERIALS AND METHODS

The plant materials used in this investigation were commercial maize (*Zea mays* L.) hybrid SC2031 and the teosinte genotype Domiata (*Durra rayyana*). Mating proceeded by manual pollination to produce the F_1 hybrids, and the F_1 plants were left to self-pollinate to produce the F_2 progenies. All genotypes (parents, F_1, and F_2) evaluated ensued in a Randomized Complete Block Design with three replications in plots. Each plot was a single row by 6 m long and 0.8 m wide. Recording of the number of tillers per plant (N.T./P.) was on 20 chosen plants per plot for all genotypes.

Molecular analysis and gene expression

DNA extraction and SSR amplification

DNA isolation from the meristems used the method of Khaled and Esh (2008) as described by Khaled *et al*. (2015). The target genomic regions for tillering ability amplification used constructed primer pairs (Table 1).

With the PCR mixture prepared, the reaction was performed. The performed amplification used a GTC 96S thermocycler (Cleaver -UK). The condition setting was as follows: 94 °C/1 min for denaturation (one cycle), followed by 35 cycles of 94 °C/20 sec, 55 °C/35 sec, and 72 °C/45 sec, and a final extension adjusted to 72 °C/45 sec (one cycle). The product was run on 2% agarose gel and visualized under UV light. The agarose gel piece, including the amplified target genomic region, was extracted from the gel and purified according to Downey (2003), then sequenced using an ABI sequencer by the GATC Company with appropriate primers.

Table 1. Forward and reverse primers constructed to detect sequences related to tillering ability.

No.	Code	Forward primer	Reverse primer
1	Til1	TTCCCCACTTTGCTCTTACC	GAATGTGGGGCCAATACTTT
2	Til2	CTGATTAGATGCGCTGGGTA	GCAGATGGATCTTGGATGG

Table 2. The number of tillers for SC2031and Domiata parents and their progenies.

Genotypes	SC2031	Domiata	F_1	F_2 High	F_2 Low	L.S.D $_{0.05}$
N.T./P.	1	6	9	8	2	0.98

N.T./P: Number of tillers per plant

***In silico* analysis**

The DNA sequence was translated online on https://web.expasy.org/translate/ to predict the protein sequence. DNA and predicted protein sequences alignment with BLAST (nih.gov) programs progressed for the homology study. The protein analysis estimates used the ProtParam tool (ExPASy, Switzerland).

RESULTS AND DISCUSSION

Evaluation of the number of tillers

The F_1 plants surpassed the parents and F_2 progenies in the number of tillers (Table 2), depending on their hybrid vigor. F_1 plants had nine tillers, while their parent had one and six tillers. F_2 plants got screened for their number of tillers, with the two extreme plants chosen for the conduct molecular analysis. In F_2, the lowest value was 2 N.T./P., and the highest was 8 N.T./P. (Table 2).

The amount of branching and the number, size, and placement of the female inflorescences (ears) changed substantially during maize domestication due to a profound rise in apical dominance (Doebley *et al*., 1997). A tassel caps the numerous, long lateral branches of teosinte plants. There are collections of a few tiny ears at each node along these lateral branches. In contrast, the maize plant has fewer lateral branches (typically only two), each terminated by a single big ear, as opposed to a tassel, as is the case with teosinte. Landrace types of maize rarely produce more than six ears per plant, and most modern commercial varieties develop just one or two ears per plant.

A reduction in the number of branches and inflorescences per plant is a consequence of apical dominance. However, when inflorescences appear, they have more or larger fruits or seeds. Therefore, enhanced apical dominance can provide simpler harvestability by lowering the number of inflorescences harvested without resulting in a corresponding decrease in yield per plant. In addition, bigger seeds enable more robust growth after germination, when seedlings may experience fierce competition from weedy plants. Moreover, because there are fewer but larger inflorescences, they all mature within a smaller window, allowing for the simultaneous collection of all fruit and seed from a plant.

Molecular analysis

The PCR products of the two parents and their progenies amplified using the primers were run on agarose, visualized, and analyzed. The SC2031 parent, with low or no-tillers, exhibited three fragments ranging from 75 to 420 bp. The fragment with 262 bp size showed in the F_1 and nine out of 10 low-tillering F_2 progenies (Figure 1). The Domiata parent, chosen as a high-tillering variety, exhibited three fragments ranging from 82 to 534 bp. The fragment with 445 bp in size came out in nine out of 10 high-tillering F_2 progenies (Figure 1). The 262 bp fragment may be responsible for low tillering ability and the 445 bp fragment may be responsible for high tillering ability. These DNA fragments were eluted from the gel, sequenced, and submitted to GenBank, then assigned accession numbers LC739768.1 and LC739767.1, respectively (Table 3).

***In silico* analysis**

The 262 bp fragment produced a 243 bp sequence and was designated as T1-L, while the 445 bp fragment produced a 400 bp sequence, then assigned as T1-H (Table 3). The T1-L and T1-H functioned as query sequences for alignment using BLAST in NCBI databases. T1-L showed very similar to the accession LC713218.1, which refers to the *Zea mays* gene for protein, dwarf, and low-tillering ability, with 100% identity and 90% query

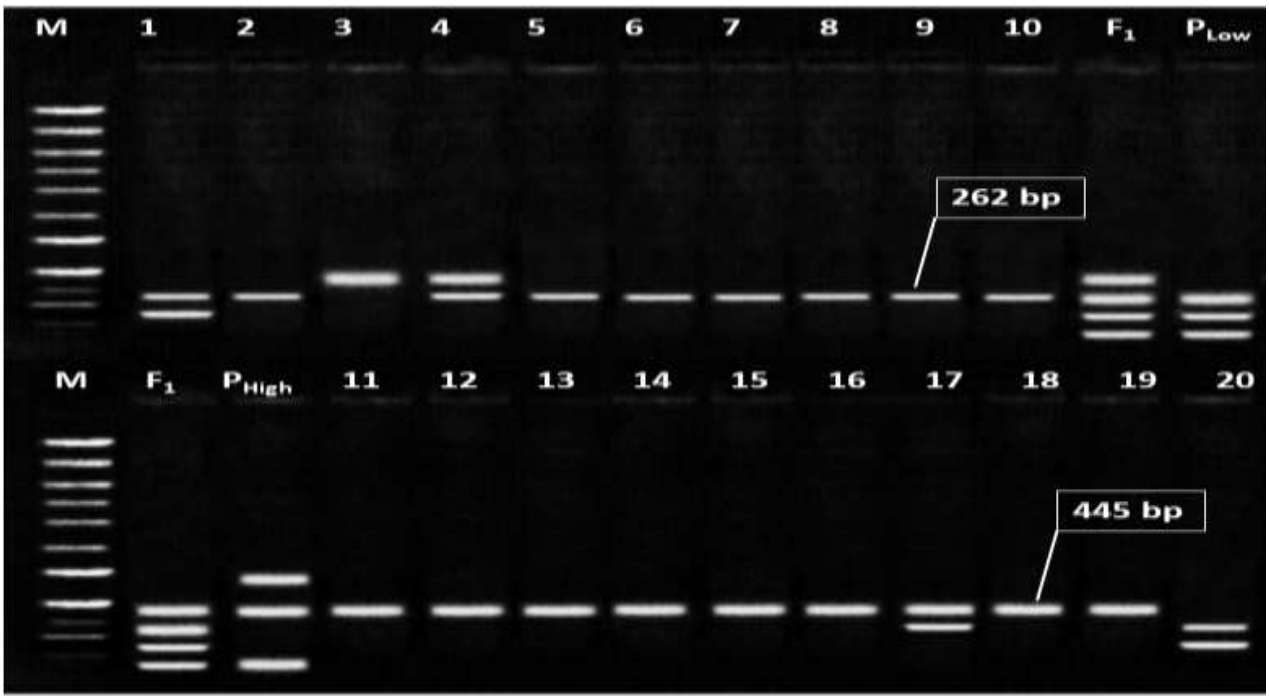

Figure 1. Patterns of SC2031 (*Zea mays* L.); Domiata (*Durra rayyana*); their F_1 and F_2 progenies against specific primers. M: DNA ladder, P_{Low}: the SC2031 parent, P_{High}: the Domiata parent, 1-10: the low tillering ability F_2 progenies, and 11-20: the high tillering ability F_2 progenies.

Table 3. Sequences of targeted genes related to tillering ability in maize.

Sequence Name	Sequence	Size (bp)	Access. No.
Low tillering	CATTCCCCACTTTGCTCTTACCGGCCTTCCCCCACGGCATGTCCGCCGCCGCCGCTG GCCCGTCGCTGGCCGTGGCCAAGAAAGAGGAGTTCAGCAAGTCGCCGTCCAACTCG CCGGCCTCATCGGGTGGCACGGACGGTGGCTCGTCGGCTGTCCCGTGGCCGGAGCA GATCCACGCGCAGAACAGCAGCCCGACGCTGTTAGAACCGAGTCGCCAAGTATATAC AAGTATAAAGTAAGAACCGAGTCGCCAAGTATTTGG	243	LC739768.1
High tillering	CAAGATCCATCTGCTGATTAGATCAAGATCCATCTGCTGATTAGATGCGCTGGGTAAA GCTTGCTTCAGCAATTGACGTGGAGGCGGCGGCTGCCTCGGGGCCGAGCGACAGGC CGAGCTCGAACAATTTGAGCCACCACTCATCGTTGTCCGTGCTGCCGCTGAATTGGA GGAGAGGGAGAGGTGTTCATCAGCTCTCAGCAATAGATCAGCAGGTAGGATGCAAGA AATCACAGGGGCGAGCGACGTGGTCCTGGGCTTTGGCAACGGAGGAGGAGGATACG GCGACGGCGGCGGCAACTACTACTGCCAAGAGCACGCTTCTACTGAACACTACGGGC GCACTAGGTACTAGAACTACTCTTTCGACTTACATCTATCTCCTTTCCCTCAACGTGAG CTTCCATGTTTCTTTGCCCATCCAAGATCCATCTGCTGATTAGAT	445	LC739767.1

covering. The T1-H revealed very similar to the accession LC713219.1, which refers to the *Zea mays tb1* gene for teosinte branched 1, with 100% identity and 89% query covering.

The translated protein of T1-L analysis used the ProtParam tool (ExPASy, Switzerland). The results indicate that T1-L protein has 66 open reading frames (ORF), with a molecular weight of 6.69 kilodaltons (kD) and an isoelectric point (pI) of 6.53. The T1-L protein helped as a query sequence for alignment in the UniProt protein database. The results indicated that the T1-L protein is very similar to the UniProt accession A0A804M0C0_MAIZE (https://www.uniprot.org/uniprotkb/A0A804M0C0/entry), which belongs to the GRAS family (GRAS domain-containing protein) (Figure 2). This accession contains 50 amino acids out of 136 that comprises the GRAS protein. GRAS proteins essentially function as transcriptional factors required for the suitable pattern of radial rooting and shooting in Arabidopsis (Scheres *et al*., 1995; Fukaki *et al*., 1998).

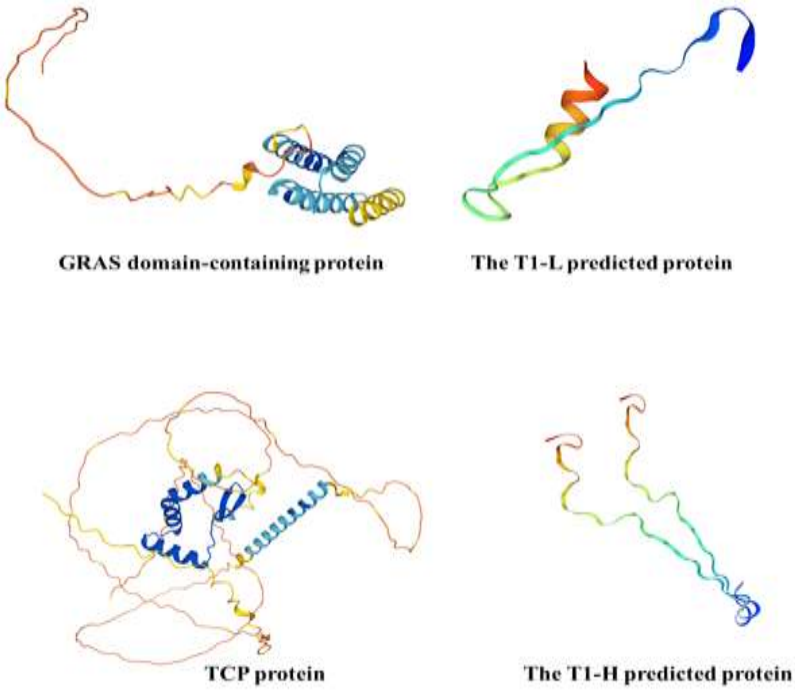

Figure 2. Protein structure alignment of T1-L and T1-H predicted protein related to tillering ability.

The first three members of the GRAS gene family are Gibberellic Acid Insensitive (GAI), Repressor of GA1 (RGA), and SCARECROW (SCR) (Pysh *et al*., 1999). These have a variety of functions in phytochrome A signal transduction, gibberellic acid (GA) signaling, and root and shoot development (Bolle, 2004). Leucine heptad repeat I (LHRI), VHIID motif, leucine heptad repeat II (LHRII), PFYRE motif, and SAW motif are the 5-carboxy-terminal motifs present in all GRAS proteins (Gallagher and Benfey, 2009).

Maize contains 86 GRAS genes that have gained further description using phylogenetic analyses, genomic locations, and expression patterns. The phylogenetic analysis helped to classify the 86 GRAS genes into eight groups: SCL3, HAM, LS, SCR, DELLA, SHR, PAT1, and LISCL. Most maize GRAS genes (80.23%) only have one exon, and members of the phylogenetic tree with close kinship share similar structures and motif compositions (Guo *et al*., 2017).

The translated protein of T1-H analysis ensued with the ProtParam tool (ExPASy, Switzerland). The results indicate that T1-H protein has 132 ORFs with a molecular weight of 14.08 kD and a pI of 7.97. The T1-H protein is very similar to the UniProt accession Q93WI2 (TB1_MAIZE) (https://www.uniprot.org/uniprotkb/Q93WI2/entry), which belongs to the TEOSINTE BRANCHED 1, CYCLOIDEA, PCF1 (TCP) family (Figure 2). The *tb1* is a major QTL of the evolution from the teosinte to the maize morphology. TCP proteins play a crucial role as transcriptional factors required for apical dominance, repressing the growth of lateral branches, enabling the formation of female inflorescences, and regulating the number and length of axillary branches.

It is well-established that the TCP genes are essential for developing and patterning plant organs. The TCP gene family may or may not contribute to the maize's ability to withstand drought stress, a significant barrier to maize production. In this study, 46 *ZmTCP* genes revealed in the maize genome and their evolutionary relationships and synteny with TCP genes from rice, sorghum, and Arabidopsis received systematic examination. The roles of the 46 *ZmTCP* genes in the response of maize to drought stress resulted in an investigation of their expression in various tissues during drought conditions. Notably, genetic differences in *ZmTCP32* and *ZmTCP42* depended on seedling drought resistance (Ding *et al*., 2019).

CONCLUSIONS

The 262 bp fragment of the low-tillering SC2031 parent showed in the F_1 and nine out of 10 low-tillering F_2 progenies. The 445 bp fragment of the high-tillering Domiata parent occurred in nine out of 10 high-tillering ability F_2 progenies. The DNA fragments associated with low and high tillering abilities were eluted from the gel, sequenced, and submitted to GenBank. The sequence from the low-tillering parent produced a predicted protein, TL-1, with 66 ORFs, a molecular weight of 6.69 kD, and pI 6.53. It belonged to the GRAS family (GRAS domain-containing protein), playing an essential role as transcriptional factors required for sufficient radial rooting and shooting patterns. The sequence from the high-tillering parent produced a predicted protein, T1-H protein, with 132 ORFs, a molecular weight of 14.08 kD, and a pI of 7.97. It belonged to the TCP family, significantly functioning as transcriptional factors required for apical dominance.

List of abbreviations

ORF: open reading frame, Tm (°C): annealing temperature, GC%: the number of G's and C's in the primer as a percentage of the total bases, BLAST: Basic Local Alignment Search Tool, BLASTn: Nucleotide BLAST, BLASTp: Protein BLAST.

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