# ABSTRACT

Plant height is one of the most important agronomic traits in crop breeding program. In the Green Revolution, the introduction of dwarf/semi dwarfing mutation caused huge yield increases in several different crop species, such as wheat and rice. In maize several dwarf mutants had been obtained, and the underlying dwarf genes were isolated and functionally characterized. By screening EMS mutants from inbred line Ye478 background, a dwarf mutant M34 was obtained by our previous work. In this study, we aim to phenotypic characterize this mutant M34 and investigate their physiological and/or morphological basis of dwarf phenotype. Furthermore, the candidate gene underlying the dwarf mutant was map-based cloned and the cause of gene mutation were identified accordingly. The main results are presented as below:

1. The phenotypic evaluation was performed between wild type Ye478 and the dwarf mutant M34 when they were grown in the field at the day of 21, 48, 78 and 90 after sowing. It showed that M34 exhibited a reduced plant height up to 60-70% compared to Ye478, mainly due to the shortened internodes up to 28.9-45.5%. However, the number of internodes remained unchanged. In addition, the dwarf mutant presented a small leaf in terms of length and width.
2. By examining the cellular size between wild type Ye478 and mutant M34, the stomata cells were investigated by cytological analysis. The result revealed that stomata guard cell of Ye478 had 37.8 μM in length and 4.4μM in width. By contrast, M34 had a small cellular size with 34.4μM in length and 2.3μM in width. Thus, the dwarf phenotype in M34 probably resulted from the reduced cell size rather than cell number.
3. The gibberellin (GA) sensitivity of M34 had been analyzed between wild type Ye478 and mutant M34. It showed that plant height of both WT and mutant plants could enhance by GA application as well as the distance from 1st to 2nd node. Therefore, the isolated mutant M34 was insensitive to GA, and then assigned into group of GA-insensitive dwarf mutant group.
4. To investigate the genetic basis of M34 mutant, M34 mutant was crossed with B73 and generated a F2 population. Phenotypic analysis of dwarf plants revealed an approx. 3:1 ratio of normal: dwarf, indicating that a single recessive gene was responded to the dwarf phenotype.
5. The primary mapping was undertaken for M34 mutant using bulk-segregation-analysis (BSA) strategy. By screening 3072 SNPs using custom-designed maize SNP chips, 1153 polymorphic SNPs were identified between two bulks. Further analysis showed that between SNPs marker PZE-101181259 and PZE-101256289 a set of Ye478 alleles were only presented in dwarf-pool, indicating that the caused gene was localized in this genomic region at chromosomal 1.
6. The fine-mapping process was performed using an enlarged F2 population from the crosses between M34 and Wu312. Four polymorphic SSR were identified, and further analyzed within the population. According to the recombinants, the candidate gene was further narrowed down between SSR maker umc2189 and umc1553, ranging from 269,963,414-287,039,764 bp at chromosomal 1.
7. A candidate gene for dwarf mutant M34 was predicted, which was previously cloned as *ZmVP8*. To confirm the cause mutation in *ZmVP8* gene, we cloned and sequenced, and further identified a single nucleotide alteration at codon 1606 in the seventh exon (G mutated to A), resulted in an amino acid sequences substituted (amino acid E to K). The protein domain analysis by the SMART databased further suggested that this mutation led to the switch of SPEC domain to XPGN domain as a result of a-helix to [β-sheet](http://baike.baidu.com/view/81824.htm#2_2) changed.

Taken together, this work described a phenotypic and genetic characterization of dwarf mutant M34 in maize, and discovered an amino acid substitition in ZmVP8 as cause effect for the dwarf performance. These results not only provide the better understanding of mechanism of maize dwarf, but also provide valuable genetic material for breeding high yielding varieties.

**Key Words:** Maize, Plant height, Dwarf mutant, *ZmVP8*