

IS OPAQUE-2 STILL TEACHING US SOMETHING NEW IN REGULATING GENE EXPRESSION IN *ZEA MAYS*?

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The Opaque2 (O2) is a bZIP transcription factor specifically expressed in maize (*Zea mays*) endosperm during seed development and controls the expression of several genes. Among different opaque-2 (*o2*) mutations so far characterized by us, the *o2-It* allele produces two polypeptides: the former, referred to as long (o2-ItL or mutant-defective), migrates in SDS-PAGE as the wild-type protein; the latter, referred to as short (o2-ItS) is a mutant-truncated protein. The *o2It* allele carries a deletion of 10 nucleotides in the fifth exon that generates a frame-shift and gives rise to a premature stop codon 45 base pairs downstream. Interestingly, the o2It-L polypeptide is detected by the Ab-O2 carboxy-terminal antiserum, suggesting that its carboxy-terminal region must be conserved despite the presence of the in frame shift mutation.

To clarify the mechanism that underlines the presence of the two polypeptides in the *o2-It* line, we conducted an extensive analysis of *o2-It* transcripts. Indeed, we found that *o2-It* produces two transcript isoforms in a nearly 1:1 ratio. One isoform matches the *o2-It* genomic sequence, whereas the second isoform lacks five additional nucleotides. This further deletion re-establishes the correct coding reading frame and explains the nature of the o2-ItL polypeptide. The presence of two *o2* mRNA isoforms strongly suggest that the 10 nucleotide deletion alters the splicing activity during pre-mRNA maturation, likely by a different recruitment of splicing factors, as suggested by ESE finder tool. This mutation may be considered as further mechanism of allele variation that might generate a novel transcriptional factor with new regulatory function.