Alternative splicing of *β*-carbonic anhydrase genes and implications for the evolution of C_4 photosynthesis in the grass subtribe Neurachninae

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The Neurachninae subtribe is a useful model group for studying the molecular evolution of C_4 photosynthesis in grasses. Out of the 11 Neurachninae species two are C_4 and one is C_2, while the remainder are currently classified as C_3. Phylogenetic analyses suggest multiple evolutionary origins of C_4 photosynthesis in this lineage.

cDNAs encoding four distinct *β*-carbonic anhydrase (CA) isoforms were characterized in four Neurachninae species: *Neurachne alopecuroidea* (C_3), *N. minor* (C_2), *N. munroi* (C_4), and *Paraneurachne muelleri* (C_4). Alignment with genomic DNA sequences revealed that these cDNAs were encoded by two genes in each species (*CA1* and *CA2*), with each gene having two different splice forms (a and b) encoding CA polypeptides with different N-terminal regions. RT-qPCR analysis of leaf tissue extracts using splice form-specific primers showed that in all four species the majority of *β*-CA transcripts were transcribed from the *CA1* gene. However, the proportion of each *CA1* splice form present varied between species. *CA1a* transcripts were more abundant than *CA1b* in all species except *P. muelleri*, where the reverse was true. Subcellular localization experiments using GFP fusion constructs showed that *CA1b* encodes a cytosolic isoform in all four species, while *CA1a* encodes a chloroplastic isoform in all species except *N. munroi*, where it is cytosolic.

These findings suggest that increased expression of a cytosolic *β*-CA associated with C_4 photosynthesis in the Neurachninae occurred via two different methods: alteration of a chloroplast transit peptide, and altered regulation of alternative splicing.