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Defining the molecular pathways that result in phi thickening formation and mechanical strengthening of the Brassica root

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Background:

The phi thickenings in gymnosperm and angiosperm roots species have been enigmatic since their discovery in the nineteenth century because they have no known function. These structures are thick rings of lignified, secondary wall that loop around root cortical cells, a location where only a thin, primary cell wall would normally be found. Because the phi thickening bands align between adjacent cells, they form a complex scaffold wrapping around the root's central vasculature. We speculate that thickenings mechanically strengthen the growing root tip, aiding root penetration through the soil [1], a process critical for seedling establishment. We previously demonstrated that jasmonic acid (JA) mediates phi thickening induction in Brassica oleracea integrating both osmotic and mechanical signals [2].

Objective:

We aim to understand the molecular pathways involved in developing phi thickenings in Brassica roots by linking the genetic variation in JA-induced thickenings and SNP markers.

Methods:

We induced phi thickenings by growing seedlings on agar plates supplemented with 1 µM JA and then quantified lignified thickenings with fluorescence microscopy. A subset of ~250 B. napus lines from the ERANET-ASSYST (BnASSYST) diversity panel was screened and genome-wide association analysis (GWAS) was performed to identify loci associated with phi thickenings. Additionally, a panel of 25 Brassica lines representing parental lines of 14 mapping populations, a part of the Australian Brassica Germplasm Improvement Program, was also screened. Based on thickening phenotypes, an F2 population (241 lines) of B. rapa was analysed to detect associated QTLs. We are validating the candidate genes identified at linked loci using RT-qPCR.

Results:

The GWAS identified nine QTLs linked to JA induction of thickenings on eight separate chromosomes, with the most significant loci on chromosomes A06, A07, C02 and C07. The B. rapa F2 progeny screen identified a genomic region (~4 Mb) on chromosome A07 linked to thickening formation that did not overlap with the B. napus A07 QTL identified from the GWAS set. RT-qPCR comparing gene expression in the B. napus lines Darmor (non-inducing in JA) and Yudal (inducing) has identified several genes showing differential expression including positive controls (secondary wall cellulose synthase genes). Importantly, B. napus orthologues of two Arabidopsis thaliana genes, the secondary cell wall master regulator transcription factor NST2 and the JA response gene JAZ10, showed a very strong induction but only in the phi thickening forming accession.

Conclusions:

The varied phi thickening induction responses of Brassica cultivars in response to JA have allowed us to start dissecting the molecular pathway leading to the formation of these enigmatic structures. We are currently continuing with RT-qPCR to better define this pathway, and experiments have commenced to clarify the function(s) of these unusual cell walls in the Brassica root tip, and to determine whether they allow the root to penetrate soil more successfully.

References:

1. Aleamotu'a et al., (2019) J. Exp. Bot. 70, 4631

2. Aleamotu'a et al., (2022) J. Exp. Bot. 73, 756