Review of the PhD thesis by András Patyi

Structure of the PhD thesis

The PhD thesis by András Patyi is composed of a summary (in English, Polish, and Hungarian), introduction (4 pages), aims (1/2 page), research hypotheses (1 1/2 pages), materials and methods (5 pages), results and discussion (5 pages), and conclusions & future prospects (2 pages). The thesis also includes two first-author publications describing the PhD candidate's research in detail.

Quality and originality of the research

The PhD thesis describes research aimed at accelerating the breeding of white lupin with respect to the content of toxic quinolizidine alkaloids (QAs) as well as susceptibility to the devastating fungal disease known as anthracnose. The research is original and the results are impactful, with immediate and clear applications in lupin breeding.

Disclaimer by the reviewer: as a chemist and molecular biologist specialized in plant natural product biosynthesis in plants, I am better suited to reviewing the part related to the quinolizidine alkaloids (QAs) compared to the part about anthracnose resistance.

On the side of the QAs, the PhD candidate identified a completely new genomic region responsible for the low-QA phenotype of a sweet white lupin type represented by the cultivar Dieta. This type of sweetness was only recently shown to be different than the more widespread sweetness caused by the *pauper* gene, which is known to encode an acyltransferase involved in early QA biosynthesis. The identification of this new genomic region is remarkable and promises novel insights into QA biosynthesis. While the exact causal gene (and mutation) remains to be determined, the work by the PhD candidate narrows this search down to around 10 genes, none of which is an obvious biosynthetic gene candidate (making it very exciting to find the causal gene).

Moreover, the strategy used to find this new genomic region was brilliant in that it also enabled assessing the impact of stacking both sweet genes on top of each other (the Dieta one and *pauper*). Namely, the PhD candidate obtained crosses of Dieta and *pauper* types and then carried bulk segregant analysis on the progeny. Excluding individuals that were homozygous for the known *pauper* gene allowed the PhD candidate to identify said genomic region confidently, while the crossing pipeline also yielded individuals carrying both sources of sweetness in a homozygous state. Remarkably, these individuals were extremely sweet, namely, around 10 times lower (sweeter) than the generally accepted threshold for food safety of 0.02%.

I only have two small remarks about this part of the PhD thesis. Regarding candidate gene Lalb_Chr18g0051511, the PhD candidate highlights it as a plausible causal gene due to a similar annotation compared to the known *pauper* gene. However, Lalb_Chr18g0051511 is annotated as a putative aminoacyltransferase, and, by contrast, the *pauper* gene encodes a BAHD acyltransferase. While both types of enzymes catalyze acylation reactions, aminoacyltransferases generally use ATP for activation, where one example of their biological function is the loading of amino acids onto tRNAs. However, BAHD acyltransferases generally use coenzyme A-activated substrates, for example acetyl-, benzoyl-, or cinnamoyl-CoA, and are typically involved in natural product biosynthesis.

A second, minor comment relates to the particular biochemical step at which the gene causing sweetness in Dieta may be acting. Based on a much-reduced ratio between 13-hydroxylupanine and lupanine, the PhD candidate suggests via the published paper that the genetic determinant in Dieta may be involved in the conversion between lupanine and 13-hydroxylupanine. While this is a perfectly plausible hypothesis, other explanations are also plausible. Most importantly, a very low

accumulation of lupanine may itself have a decisive impact on the downstream pathway flow purely due to enzyme kinetics. Generally, enzymes tend to act more slowly when their substrate(s) are present at very low levels (Michaelis-Menten kinetics). Thus, it is plausible that the very low levels of lupanine are the cause of the reduced, downstream metabolic flux.

The progress made by the PhD candidate on anthracnose resistance is also exciting. While multiple studies have reported genetic determinants of anthracnose resistance and suggested associated molecular markers, the transferability of these reports to unrelated white lupin panels has been very limited (and so has their application in non-associated breeding programs). The PhD candidate tested nine previously published markers in multiple panels and environments. While seven of these were shown to be panel-specific, two markers showed consistent associations across multiple panels. Moreover, the PhD candidate identified five additional SNP markers through re-analysis of GWAS datasets and also developed a genomic prediction model based on 42 SNP loci. The model reached high prediction accuracy in the training population but re-training was needed to maintain performance in unrelated panels.

Quality and impact of the publications

Both publications are of high quality, as evidenced by the reputation of the journals they were published in, namely, BMC Plant Biology and Molecular Breeding. While the impact factor of Molecular Breeding (3.6) is lower than that of BMC Plant Biology (5.6), Molecular Breeding is known for publishing timely and impactful stories that will have practical application in the field of crop breeding. Accordingly, both the QA and anthracnose advances are expected to find immediate application in white lupin breeding. Moreover, the insight that stacking two different sweet genes can have an additive effect is a remarkable result that was by no means obvious to those skilled in the art. Consequently, after the publication of the BMC Plant Biology paper, several lupin breeding programs have planned to stack sweet genes in a similar way, including in other lupin species such as narrow-leafed lupin.

Conclusions

The PhD thesis by András Patyi describes high-quality, original research that has been published in two reputable journals with the PhD candidate as first-author. In addition, the research has real-life implications in the field of lupin breeding, as exemplified by the stacking of sweet genes giving rise to ultra-low alkaloid lines. Thus, I recommend awarding the candidate a PhD with distinction.



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