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Identification and diversity analysis of leafy vegetable crops consumed in Malaysia

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Introduction

Micronutrient deficiency, especially Iron deficiency, is a major health concern in Malaysia (Khor, 2005). Leafy vegetable crops are a major source of micronutrients like Iron (Singh et al, 2001). Ambiguous vernacular nomenclature and massive phenotypic variability make it essential to ascribe these crops to their respective taxonomic status before any germplasm enhancement programme can be undertaken.

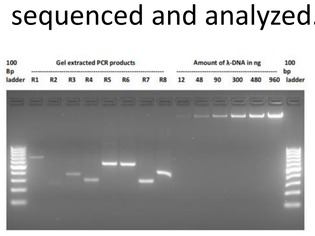
Morphological variability in Sawi and Sawi Pahit (1,2,3,4,5)
Both are called *B juncea* (6,7)

Methodology

DNA barcoding as a method for identifying the botanical status of a crop will be deployed. Using a test crop, protocols for DNA barcoding will be operationalized with due modifications based on local requirements and resources. A limited survey will be carried out, across varying agro-climatic regions of Malaysia, for locally consumed leafy vegetable crops. The surveyed crops will be identified using DNA barcoding and molecular markers. Genetic relationships across these crops will be explored using molecular and morphological parameters.

Results

A commonly consumed local leafy crop, Pak Choy, was taken up as a test crop. 15 PCR primer-sets, covering 10 genomic regions, were chosen and procured. Protocols for DNA barcoding were tested out on Pak Choy. Due protocol modifications were made. All 15 PCR primer-sets were found to give good PCR amplifications. PCR products for 11 out of 15 primer-sets, covering 9 genomic regions, were sequenced and analyzed.



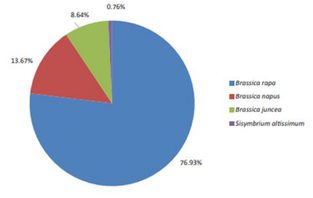
Legends

PCR product	Primer set	PCR product	Primer set
R1	rbcla-F/rbcla-R	R2	YCF5-1f/YCF5-4r
R3	psbA/trnH2	R4	trnH(GUG)/psbA
R5	rpoC1-1f/rpoC1-3r	R6	rpoB-2f/rpoB-4r
R7	accD-1f/accD-4r	R8	ndhJ-2f/ndhJ-4r

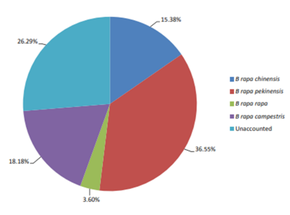
Gel purification of PCR products for DNA barcoding (R1 to R8) and PCR product quantification

PCR product	Sequence
R1	TGGGTGCTTAAAGAGTATAAATTAAATTATTATACTCCTGAATATGAAACCAAGGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGA GTTCCACCTGAAGAAGCAGGGGCTGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGCCTTGACGGTT ACAAAGGACGATGCTACCACATCGAGCCCGTTCCAGGAGAAGAAACTCAATTTATGCGTATGTAGCTTACCCATTAGACCTTTTGAAGAAGGGTCT GTTACTAACATGTTTACCTCAA
R3	TCATGCTGTTGAGGCTCCATCTATAAATGGATAATTCTTTAGTGTTAGTCTAGACCTAGTTTAGTAATATTAAAAACGAGCGATTATAAGCCTTATTTTTA AGGCTTATATCGCTCGTTTTTTCTATAAAACGGAACAAATCATTTTTTTTATATAATTTTTTCTATTATATAATAAAATAGAAAAAATACTATTATAAATTT ATAATTTTTTTTTATCAAAAAAAATATTGCTGCGTTTTTATTTTAGACAATACAAACAAGATATGATGTATAGTATAGTAGGGGCGGATGTAGCCAAG TGGATTAAGGCAGTGGATTGTGAATCCACCAGGGCGGAA
R4	CACCATCATATCTTGTTGTATTGTCTAAATAAAAACGCAGCAATATTTTTTTTGATAAAAAAAAAATTATAAATATATAATAGTATTTTTTCTATTTTATA TATAATAGAAAAAATTATATATAAAAAAATGATTTGTTCCGTTTTATAGAAAAAACGAGCGATATAAGCCTTAAAAATAAGGCTTATATCGCTCGTTTT TAATATTACTAAACTAGGTCTAGACTAACACTAAAGAATTATCATTGTAGTGGGGAGCTTCAA
R5	AGGGGATGTCATAATAAGTTTACAAGTCATTTTCAGATGTAATTGAAGGAAAAGAGGTAAAGATTTCGCGAGACTCTGCTTGGCAAACGGGTCGAT TATTCGGGGCGTTCGGTGATTGTCGTTGGACCCTCACTTTCATTACATCGCTGTGGATTGCCTCGCGAAATAGCAATAGAGCTCTTCCAGACATTTGTA ATTCGTGGTCTAATTAGACACATCTCGCTTCGACATAGGAGTTGCTAAGAGTCAAATTCGTGAAAAAAGCCGATGTCTCGGGAAATCCTTCAAGA AGTTATGCAGGGGCATCCCGTATTACTGAATAGAGCACCTACTCTACATAGATTAGGCATACAGTCATTCCAACCCATTTTAGTGGAAGGACGCACTA TTTGTTTACATCCATTAGTTTGTAAGGGGTTCAATGCAGACTTTGATGGGGATCAAATGGCTGTTCATGTGCCTTTATCTTTAGAAGCTCAAGCAGAGG CCCGTTTACTTATGTTTTCTCAA

DNA sequences of genomic regions represented by DNA barcodes R1, R3, R4 and R5



Species level discrimination



Subspecies level discrimination

Conclusions

By doing a BLAST search of the obtained DNA sequences in the NCBI (National Center for Biotechnology Information) DNA sequence database, studying the evolutionary history of the candidate crop hits and by comparing the morphological characters of the test crop with crop candidates as revealed by DNA barcoding, it was possible to identify the test crop, Pak Choy, as *Brassica rapa*. However, the subspecies status of the test crop could not be determined. Homology score as obtained by matching the DNA barcode sequences against NCBI sequence database is represented as %age in the above picture. DNA barcoding gave promising information for further studies to identify the subspecies status of the test crop. The use of molecular markers and reference morphotypes in future studies would further resolve the taxonomic status of crop morphotypes under study.

References

Khor, G. (2005). Micronutrient status and intervention programs in Malaysia. *Food & Nutrition Bulletin*, *26*(2), 281–285.

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