Anthocyanin profiles of colored wheat crosses via HPTLC

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Highlights

- Method development for anthocyane analysis of wheat crosses → simple extraction of the wheat → challenging matrix
- Pronounced differences in the anthocyanin fingerprints of colored wheat extracts
- Fast visual comparison of combinations of blue aleurone and purple pericarp genotypes
- Further characterization via derivatizations, evaluation of anthocyanin sugar components and HPTLC-UV/Vis-MS of anthocyanins

1. Anthocyanin fingerprints

Milled colored wheat grains were suspended in methanolic hydrochloric acid, 0.5% (0.1 g/1.5 mL), mixed, ultrasonicated (0.5 h), centrifuged and membrane filtered. Anthocyanins were separated on either HPTLC silica gel 60 NH_2 F_{254} with ethyl acetate - 2-butanone – water - formic acid 7:3:1.5:1 or RP-18 $WF_{254}s$ with water - *n*-propanol - formic acid 9:4:0.6 [1].
The profiles on the amino phases (Fig. 1) clearly differentiated purple- (no. 1-13) *versus* blue-grained (no. 14-19) varieties as well as combinations of the different genetic backgrounds of blue aleurone and purple pericarp genotypes. [1,2].

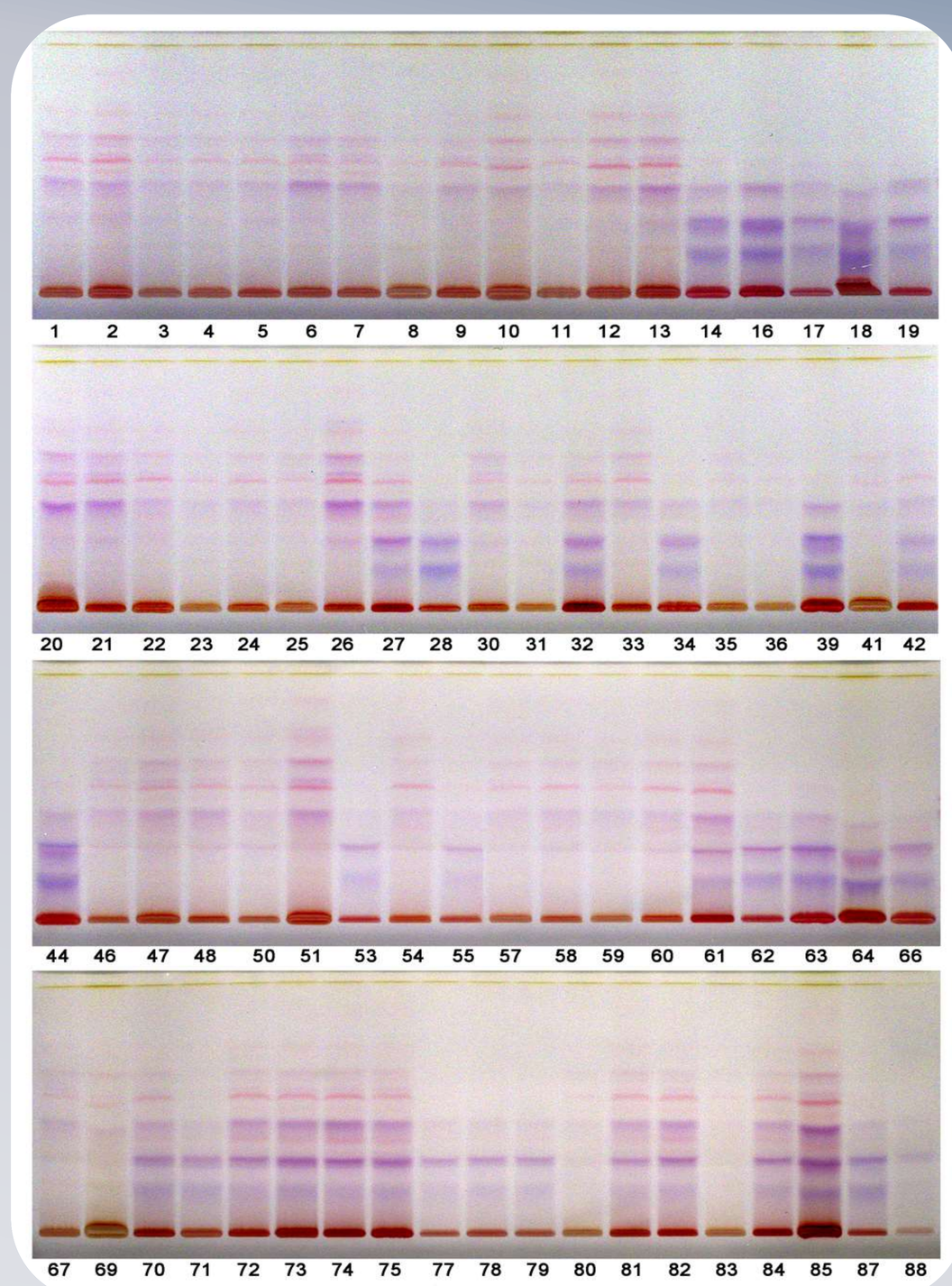

Fig. 1 HPTLC chromatograms of 88 colored wheat extracts showing purple- (no. 1-13) *versus* blue-grained (no. 14-19) varieties

2. Characterization via MS

For each genotype, major anthocyanin zones were further analyzed via HPTLC-ESI$^+$-MS (Fig. 2). The purple-grained variety (no. 6) showed four zones (hR_F ≥42; A-D) and the blue-grained variety (no. 16) three zones (hR_F ≤42; A-C). The zone at hR_F 42 was found in both varieties (no. 6D and 16A). Mass spectra of both zones showed the molecular ion of cyanidin $[M]^+$ at *m/z* 287, suggesting a cyanidin-backbone. With the cyanidin-3-glycoside standard this was confirmed via comparison of colors and hR_F values.

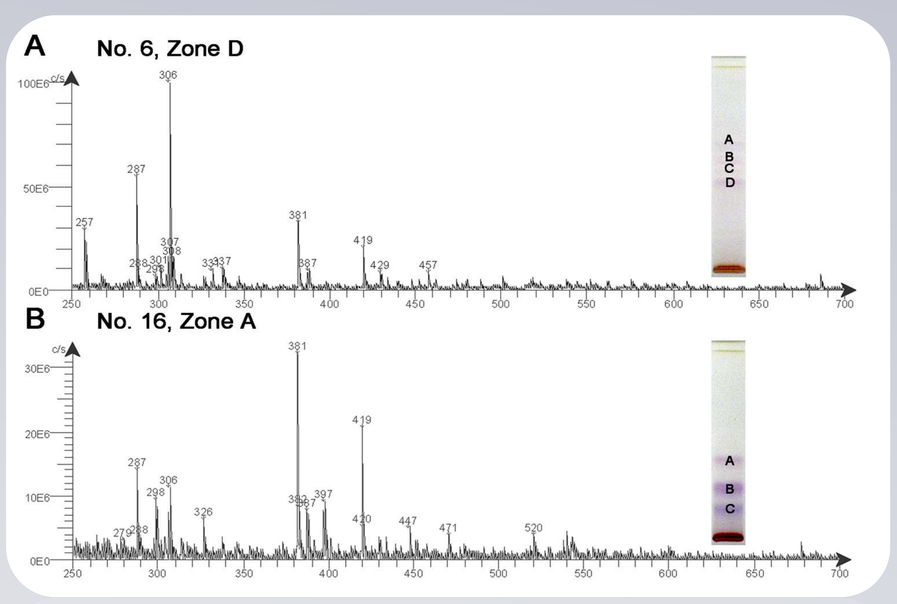

Fig. 2 HPTLC-MS via the elution head-based interface of two anthocyanin zones

3. Multi-detection → matrix

The chromatograms were detected using various derivatization reagents to obtain further information on matix compounds (Fig. 3).

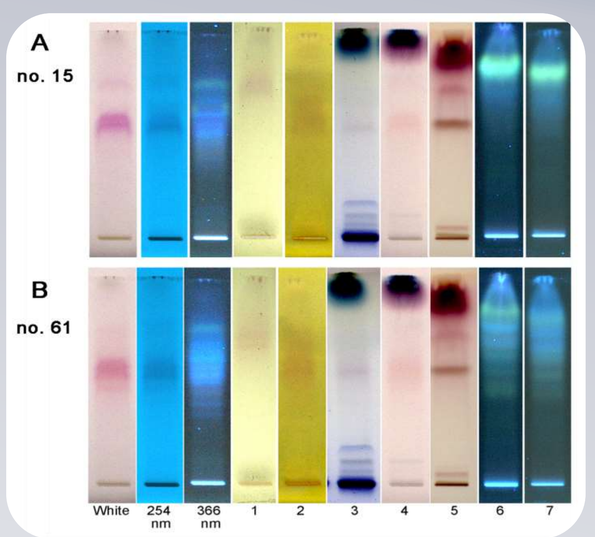

Fig. 3 Multi-detection of samples no. 15 and 61 at white light, UV 254 nm, UV 366 nm and after derivatization with fast blue salt B (1), Dragendorff (2), anisaldehyde sulfuric acid (3), diphenylamine aniline phosphoric acid (4), ninhydrine (5), all at white light, as well as after natural product reagent A (6) and aluminum chloride (7) at UV 366 nm [1]

Literature [1] S. Krüger, G.E. Morlock, J. Chromatogr. *A*, in submission. [2] S.N.S. Jaafar, J. Baron, S. Siebenhandl-Ehn, T. Rosenau, S. Böhmdorfer, H. Grausgruber, Plant Breed. 132 (2013) 546-552

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