

Melanin formation in barley grain occurs within plastids of pericarp and husk cells

Olesya Yu. Shoeva^{1*}, Sergey R. Mursalimov¹, Natalya V. Gracheva², Anastasiya Yu. Glagoleva¹,
Andreas Börner³, Elena K. Khlestkina^{1,4}

¹ *Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia*

² *Volgograd State Technical University, Volgograd, Russia*

³ *Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

⁴ *N.I.Vavilov All-Russian Research Institute of Plant Genetic Resources, Saint-Petersburg, Russia*

*e-mail: olesya_ter@bionet.nsc.ru

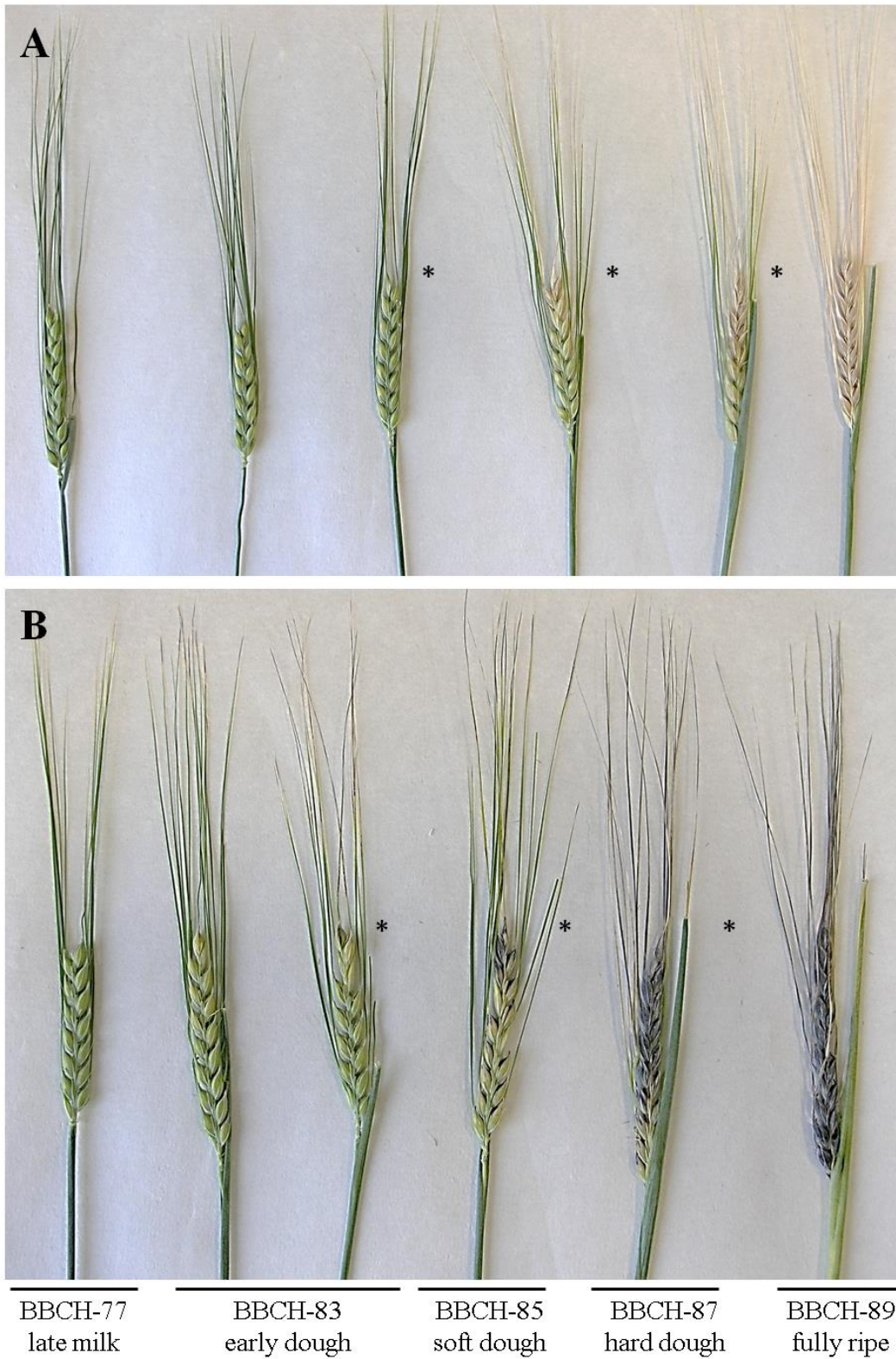


Fig. S1. Development of the spike in cv. Bowman (A) and the *i:BwBlp* NIL (B). Asterisks mark the grain developmental stages chosen for sampling.

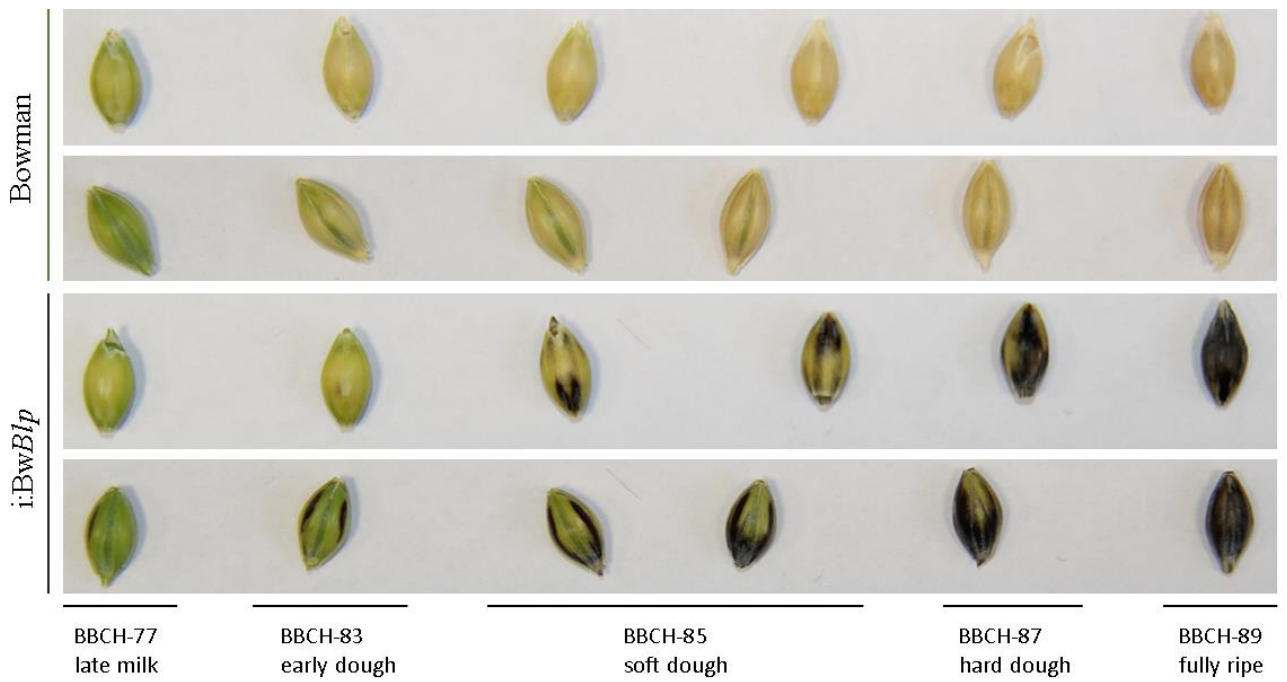


Fig. S2. Development of the grain set by cv. Bowman plants and those of the *i:BwBlp* NIL.

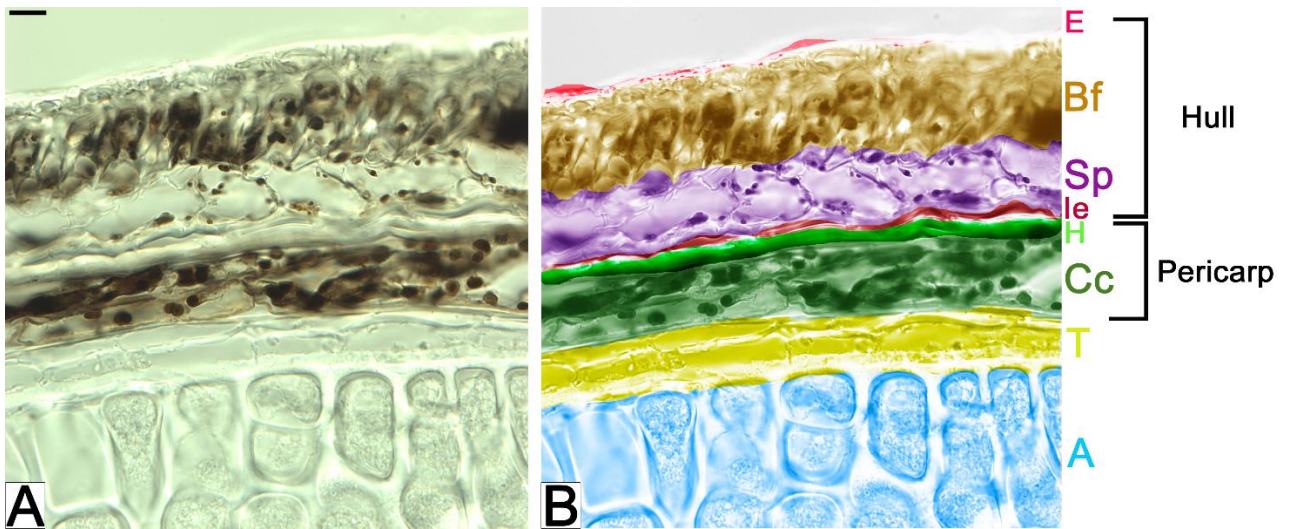


Fig. S3. Cross-section of dehusked grain of the *i:BwBlp* NIL sampled at the hard dough stage (A) with manual dyeing of the grain layers using Photoshop CS2 software (Adobe Systems Incorporated) (B). A: aleurone, E: epidermis, Bf: bast fibres, Cc: cross cells, H: hypodermis, le: inner epidermis, Sp: spongy parenchyma, T: testa. Scale bar: 10 μ m.

Table S1. Solubility tests of the black pigment purified from the i:BwBlp NIL. Published protocols used to solubilize anthocyanins and tannins, were ineffective in solubilizing the dark pigment from the i:BwBlp husk and pericarp. Based on ability of the extracted material to dissolve in alkali solutions, discolor under the influence of strong oxidizing agents (H₂O₂, KMnO₄), interaction with FeCl₃ and resistance in common organic solvents, that have been reported as hallmarks of melanin [21], the dark pigment was referred to melanins.

Solubility	
Water and acid	
Water	Insoluble
76% H ₂ SO ₄	Partially soluble
Alkali solutions	
0.5% NaOH	Soluble
Organic solvents	
Ethanol	Insoluble
Isopropanol	Insoluble
Hexane	Insoluble
Petroleum ether	Insoluble
Ethyl acetate	Insoluble
Hydroxymethylformamide	Swell
Oxidizing agents	
10% H ₂ O ₂	Decolored
0.1 N KMnO ₄	Decolored
Interaction with chemicals	
0.5-1.0 mg/ml FeCl ₃	Precipitation at low concentration and dissolving at high concentration

References

Sava VM, Yang S-M, Hong M-Y, Yang P-C, Huang GS (2001) Isolation and characterization of melanic pigments derived from tea and tea polyphenols. Food Chem 73: 177–184.