Influence of seed colour on seed vigour in *Brassica napus*

Anja B. Neubert, Wilfried Lühs*, Roland Baetzel and Wolfgang Friedt
Institute of Crop Science and Plant Breeding I, Justus-Liebig-University,
Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany; Phone: +49-641-99-37446,
Fax: +49-641-99-37429, wilfried.luehs@agrar.uni-giessen.de

ABSTRACT

Yellow seed colour in *Brassica napus* is a desired trait and has a strong impact on the quality of Canola and the processed products from the seed. The trait is accompanied with a thinner seed coat when compared to black seeds. For the model plant *Arabidopsis thaliana*, different mutants have been characterised showing the transparent testa phenotype to be associated with light seed colour due to the lack of anthocyanins. Regarding dormancy, viability and germination, the function of the seed coat has been investigated based on *Arabidopsis* mutants affected in seed pigmentation and/or testa structure (Debeaujon et al. 2000, Plant Physiol. 122, 403-413). In rapeseed (*B. napus*) the same effects are of interest, as in breeding nurseries reduced field emergence of yellow-seeded genotypes has often been observed. In relation to reduced thickness of the seed coat and altered pigmentation, this effect of impaired seed vigour may be explained differently: 1) reduction of resistance against mechanical injury caused by threshing and seed processing, 2) decrease of resistance against fungal pathogens due to seed injury, breakage and altered pigmentation, 3) increase of sensitivity against agrochemicals, 4) alteration in secondary plant metabolism, e.g. flavonoids, 5) reduced seed-coat related dormancy.

Key words: *Brassica napus* - transparent seed testa - seed vigour - germination - field trial

INTRODUCTION

Extracted rapeseed meal contains about 40% protein with a well-balanced amino acid composition. Besides other anti-nutritional compounds, including glucosinolates, phytate, sinapine, phenolic acid and tannins, crude fibre (and substances enclosed by them) affect adversely the usability of rapeseed meal (cf. Friedt and Lühs 1999). Yellow *B. napus* seeds are characterised by thinner seed coats than black seeds (Fig. 1). They are also associated with a reduction of crude fibre content and a concomitant increase of digestible energy and protein content (Theander et al. 1977, Slominski 1997). Regarding the introgression of genes encoding seed pigmentation, e.g. from related *Brassica* species, the situation in *B. napus* is complex due its polyploid genetic constitution, multiple gene control, predominantly maternal determination and environmental impact (Van Deynze et al. 1993, Getinet and Rakow 1997, Lühs et al. 2000, Heneen and Brismar 2001, Rahman et al. 2001, Baetzel et al. 2003). Both seed testa pigmentation and formation of crude fibre components (e.g. lignin) are related due to the biochemistry of phenylpropanoids including a wide range of plant phenolic compounds, such as flavonoids, stilbenes, cumarins and tannins, as well as cell wall constituents, like lignin. Flavonoids are present at high levels in most seeds and grains and they appear to play vital roles in defence against pathogens or pests and

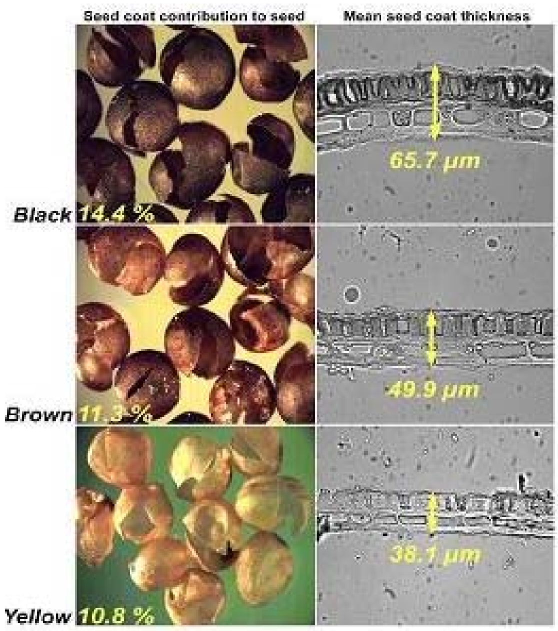

Figure 1. As in *Arabidopsis thaliana*, the yellow seed colour trait in *Brassica* results from a thinner, more transparent testa.

contribute to physiological functions, such as seed maturation, dormancy, viability and seedling vigour as well as protection against ultraviolet (UV) light. At the same time, particular subclasses of flavonoids, such as the proanthocyanidins (condensed tannins), negatively impact the use of seeds and grains in animal feed and can add undesirable qualities to food products for human consumption (Weisshaar and Jenkins 1998, Whetten et al. 1998, Winkel-Shirley 1998, Debeaujon et al. 2000, Peer et al. 2001). For *A. thaliana* at least 21 different mutants have been identified or in a some cases have already been cloned showing the *transparent testa* (*tt*) phenotype which is associated with yellow or ochre seed colour due to lack of anthocyanin (Koornneef 1990, Shirley et al. 1995, Focks et al. 1999, Nesi et al. 2000, 2001, Debeaujon et al. 2000, 2001, Winkel-Shirley 2001, Sagasser et al. 2002, Shikazono et al. 2003).

MATERIALS and METHODS

The rapeseed material (Tab. 1) consisted of doubled haploid (DH) lines derived from a cross between the yellow-seeded double-low line 'T25629' and the dark-seeded high-erucic acid doubled-haploid line 'DH 26-96' as well as one inbred line ('Askari' x 'T25629'), the yellow-seed line '1012-98' (other origin than 'T25629') and the cultivar 'Express' (Baetzel et al. 2003). Field plots (plot size 3.0 x 1.25m) were harvested in 2001 either manually or by using a plot combine (Hege Maschinen GmbH, Waldenburg/Germany). In the variants with coating 100 g seed material were treated with 0.65 ml suspension of the fungicide 'TMTD' (thiram, 700 g l^{-1}) and 2.0 ml suspension of the insecticide 'Chinook' (imidacloprid and beta-cyfluthrin, each 100 g l^{-1}). The trial for the evaluation of field emergence was carried out in autumn 2001 at the experimental field station Rauischholzhausen (nearby Marburg/Germany) in a completely randomised block design with four replications (plot size 1.0 x 1.25m). Field emergence was determined 18 d after sowing.

Table 1. Pedigree and seed colour of rapeseed lines used in the field trial

Genotype	**Pedigree**	**Seed colour**
Express'	Cultivar	Black
'DH 4-34'	T25629 (yellow) x DH 26-96 (black)	Black
'DH 4-40'	T25629 (yellow) x DH 26-96 (black)	Brown
'DH 4-245'	T25629 (yellow) x DH 26-96 (black)	Brown
'DH 4-143'	T25629 (yellow) x DH 26-96 (black)	Light brown
'Inbred line'	Askari (black) x T25629 (yellow)	Yellow-brownish
'1012-98'	1663/94 HK 3G3	Yellow

RESULTS and DISCUSSION

Flavonoid content and composition of the seed coat have been shown to influence dormancy, viability and germination as well as storability and quality of the seeds (Winkel-Shirley 1998, 2001, Debeaujon et al. 2000). In rapeseed (*B. napus*) these are agronomically important traits, not only with regard to human and animal nutrition. In breeding nurseries reduced seed vigour and field emergence of yellow-seeded genotypes has often been observed. Our laboratory germination tests (data not shown) revealed that dark seeds imbibe and germinate later than yellow seeds, probably due to their thicker seed coat and phenolic compounds affecting permeability (cf. Debeaujon et al. 2000, Ye et al. 2001, Rahman et al. 2001). In the course of a preliminary field trial involving breeding lines of different seed colour we observed that the seed vitality or emergence of light-coloured seed material was more affected by mechanical injury than the corresponding features of dark seeds. However, seed coating treatment using the fungicide 'TMTD' (thiram) and the insecticide 'Chinook' (imidacloprid and beta-cyfluthrin) showed an improving effect on emergence, especially in the case of light-coloured seeds (Fig. 2). Although coated yellow seeds never gained the same emergence capacity as dark seeds. Yellow *B. napus* seeds seem to possess reduced dormancy, imbibe and germinate much faster, suffer more imbibition damage, and therefore survive a shorter time in the soil than dark seeds.

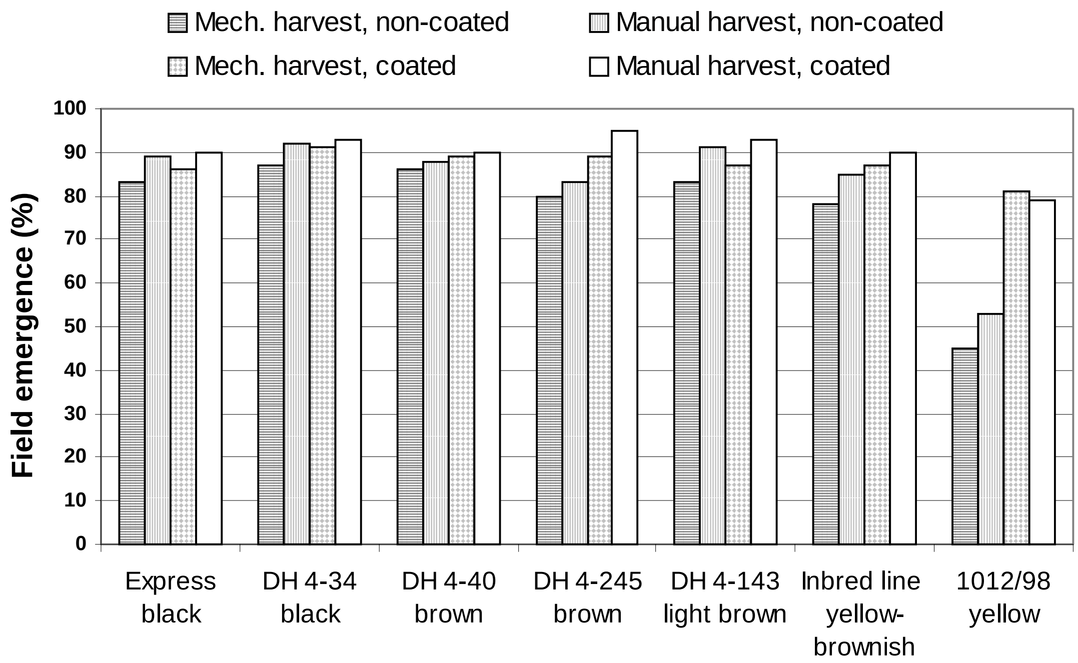

Figure 2. Effect of mechanical seed coat injury during harvest and coating treatment (TMTD+Chinook) on field emergence of winter rapeseed differing in seed colour.

CONCLUSIONS and OUTLOOK

As biochemical pathways leading to lignin and seed testa pigmentation are considered to coincide along the phenylpropanoid pathway, interest exists to investigate if any of the known *Arabidopsis tt* mutants correspond to one or more of the *B. napus* loci encoding yellow seededness or if novel sources for this agronomically important trait could be exploited for breeding in rapeseed. However, the biochemical lesions leading to the lack of pigmentation or the effects on germination in *B. napus* have not been identified, yet. Therefore, exploiting the knowledge about *Arabidopsis* genomics (Schmidt 2002, Lotz et al. 2003) in improving the nutritional value of rapeseed through the development of yellow-seeded *B. napus* varieties would have a strong impact on the production of oilseed rape. As germination problems may be managed by seed coating (Falloon and Fletcher 1983) the short-time survival of yellow seeds lost during harvest could reduce the problems caused by volunteer plants.

ACKNOWLEDGEMENTS

The authors thank Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (Hohenlieth, Germany) for providing the coating suspension for seed treatment as well as Rosa Allerdings, Petra Degen, Horst Schaub and Mechthild Schwarte for their excellent technical assistance.

REFERENCES

Baetzel, R., W. Lühs, A.G. Badani, and W. Friedt, 2003: Development of segregating populations in the breeding of yellow-seeded winter rapeseed (*Brassica napus* L.). 11th Intern. Rapeseed Congr., 6-10 July 2003, Copenhagen, Denmark (Poster).

Debeaujon, I., K.M. Léon-Kloosterziel, and M. Koornneef, M., 2000: Influence of the testa on seed dormancy, germination and longevity in Arabidopsis. Plant Physiol. 122, 403-413; Erratum: 125, 1139-1141.

Debeaujon, I., A.J.M. Peeters, K.M. Léon-Kloosterziel, and M. Koornneef, 2001: The *TRANSPARENT TESTA12* gene of Arabidopsis encodes a multidrug secondary transporter–like protein required for flavonoid sequestration in vacuoles of the seed coat endothelium. Plant Cell 13, 853–872.

Falloon, R.E., and R.H. Fletcher, 1983: Increased herbage production from perennial ryegrass following fungicide seed treatment *Lolium perenne*, New Zealand. New Zeal. J. Agric. Res. 26 (1), 1-5.

Focks N., Sagasser M., Weisshaar B., Benning C., 1999: Characterization of *tt15*, a novel *transparent testa* mutant of *Arabidopsis thaliana* (L.) Heynh. Planta, *208*: 352-357.

Friedt, W., and W. Lühs, 1999: Breeding of rapeseed (*Brassica napus*) for modified seed quality - synergy of conventional and modern approaches. In: Proc. 10th Intern. Rapeseed Congr., 26-29 Sept. 1999, Canberra, Australia (http://www.regional.org.au/au/gcirc/4/440.htm).

Getinet, A., and G. Rakow, 1997: Repression of seed coat pigmentation in Ethiopian mustard. Can. J. Plant Sci.77, 501-505.

Heneen, W.K., and K. Brismar, 2001: Maternal and embryonal control of seed colour by different *Brassica alboglabra* chromosomes. Plant Breeding 120, 325-329..

Koornneef, M., 1990: Mutations affecting the testa colour in Arabidopsis. Arabid. Inf. Serv. 27, 1-4.

Lühs, W., R. Baetzel, and W. Friedt, 2000: Genetic analysis of seed colour in rapeseed (*Brassica napus* L.). Czech. J. Genet. Plant Breed. 36, 111-115.

Lotz, T., R. Snowdon, R. Horn, G. Dewal, B. Weisshaar, W. Friedt, H. Belcram, M. Caboche, and B. Chalhoub, 2003: Molecular analysis of *Arabidopsis thaliana* transparent testa (*tt*) genes in *Brassica napus*. 11th Intern. Rapeseed Congr., 6-10 July 2003, Copenhagen, Denmark (Poster).

Nesi, N., I. Debeaujon, C. Jond, G. Pelletier, M. Caboche, and L. lepiniec, 2000: The *TT8* gene encodes a basic helix-loop-helix domain protein required for expression of *DFR* and *BAN* genes in Arabidopsis siliques. Plant Cell 12**,** 1863–1878.

Nesi, N., C. Jond, I. Debeaujon, M. Caboche, and L. Lepiniec, 2001: The Arabidopsis TT2 Gene Encodes an R2R3 MYB Domain Protein That Acts as a Key Determinant for Proanthocyanidin Accumulation in Developing Seed. Plant Cell 13, 2099-2114.

Peer, W.A., D.E. Brown, B.W. Tague, G.K. Muday, L. Taiz, and A.S. Murphy, 2001: Flavonoid accumulation patterns of transparent testa mutants of Arabidopsis. Plant Physiol. 126, 536-548.

Rahman, M.H., M. Joersbo, and M.H. Poulsen, 2001: Development of yellow-seeded *Brassica napus* of double low quality. Plant Breeding 120, 473-478.

Sagasser, M., G.-H. Lu, K. Hahlbrock, and B. Weisshaar, 2002: *A. thaliana* TRANSPARENT TESTA 1 is involved in seed coat development and defines the WIP subfamily of plant zinc finger proteins. Genes & Development 16 (1), 138-149.

Schmidt, R., 2002: Plant genome evolution: lessons from comparative genomics at the DNA level. Plant Mol. Biol. 48, 21-37.

Shikazono, N., Y. Yokota, S. Kitamura, C. Suzuki, H. Watanabe, S. Tano, and A. Tanaka, 2003: Mutation rate and novel *tt* mutants of *Arabidopsis thaliana* induced by carbon ions. Genetics 163, 1449-1455.

Shirley, B.W., W.L. Kubasek, G. Storz, E. Bruggemann, M. Koornneef, F.M. Ausubel, and H.M. Goodman, 1995: Analysis of Arabidopsis mutants deficient in flavonoid biosynthesis. Plant J. 8 (5), 659-671.

Slominski B.A., 1997: Developments in the breeding of low fibre rapeseed/canola. J. Anim. Feed Sci., *6*: 303-317.

Theander, O., P. Aman, G.E. Miksche, and S. Yasuda, 1977: Carbohydrates, polyphenols, and lignin in seed hulls of different colors from turnip rapeseed. J. Agric. Food Chem. 25, 270-273.

Van Deynze, A.E., W.D. Beversdorf, and K.P. Pauls, 1993: Temperature effects on seed color in black- and yellow-seeded rapeseed. Can. J. Plant Sci. 73, 383-387.

Weisshaar B., Jenkins G.I., 1998: Phenylpropanoid biosynthesis and its regulation. Curr. Opin. Plant Biol. 1, 251-257.

Whetten, R.W., J.J. MacKay, and R. Sederoff R., 1998: Recent advances in understanding lignin biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol., *49*: 585-609.

Winkel-Shirley, B., 1998: Flavonoids in seeds and grains: Physiological function, agronomic importance and the genetics of biosynthesis. Seed Sci. Res. 8, 415-422.

Winkel-Shirley, B., 2001: Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol. 126, 485-493.

Ye, X.L., J.N. Li, Z.L. Tang, and S. Li, 2001: Dynamic studies on difference of seed coat pigments and related characters between black- and yellow-seeded rapeseed lines (*Brassica napus* L.) in the developmental stages of embryo. Proc. Intern. Symp. On Rapeseed Science, pp. 73-84. Science Press, New York.