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REGULAR ARTICLE

Breeding strategy for improvement of colour quality and carotenoid levels in dry pea seeds

Eszter Nemeskéri

Agrona Ltd. Debrecen, Domokos M. Kert 72, H-4002, Hungary.
E-mail: agrona@chello.hu

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ABSTRACT

The purpose of this study was to show the relationship between the yellow pigments of pea (*Pisum sativum* L.) seeds and climatic conditions, and to identify effective selection methods for improving seed colour quality. Three dry pea cultivars with different yellow hues of seeds and leaves and their progenies were grown in non-irrigated field experiments. A colour scale from 1 to 9 was created to measure seed colour. Drought during seed development caused a significant decrease in the xanthophyll content of pea seeds. Based on heritability estimates, the potential for selection for increased xanthophyll content in seeds (h^2=0.78) was greater than for carotene (h^2=0.32). The "yellow index," defined as the ratio of carotene and xanthophylls, expressed the intensity of yellow colour of the seeds. The weak relation between seed colour values and yellow index (R^2=0.445) should allow simultaneous selection for seeds with deep-yellow colour and higher carotene content.

Key Words: *pea; carotene; xanthophylls; heritability.*

INTRODUCTION

Colour and appearance of food products are important considerations for consumers. Dry peas can supply the required nutrients to various age groups owing to their high protein content and favourable composition of amino acids (Győri et al., 1998) and low trypsin inhibitor levels (Kochar et al., 1988; Ferrasson et al., 1997; Nemeskéri, 1997). The most important carotenoids in agricultural crops are β-carotene, α-carotene, β-cryptoxanthin, lutein and lycopene. The deep-red colour of crops is associated with high level of lycopene, while high β-carotene content causes the orange colour (Davis, 1976). Colour characteristics of and variation among cultivars are essentially under genetic control (Goodwin, 1986), but they are affected by environmental factors, such as temperature (Helyes et al., 2002).

The advancement of global warming requires development of breeding strategies for increasing food quality, particularly in grain legumes because of their sensitiveness to water stress. There is insufficient knowledge about the content of carotenoids in mature pea seeds produced under drought as well as about pigments determining colour intensity.

The objectives of this study were to investigate the yellow pigments of dry pea seeds and the effect of climatic conditions on seed colour. We analysed properties that were related to consumption quality for possible use as a selection criterion.

MATERIAL AND METHODS

From 1999 to 2003, three different yellow hue-seeded dry pea cultivars and breeding lines were grown under non-irrigated conditions in field experiments. The experiments were conducted on a chernozem soil at Agrona Ltd in Debrecen situated in eastern part of Hungary. The soil of the experiment field had neutral pH (7.38) and a humus content of 1.8%. The afila type, represented by cultivar Y228, had light-yellow seeds whereas the cultivars of the conventional groups, such as Bohatyr, had medium-yellow seeds; Auralia pea cultivar had deep-orange seeds.

To study the relationship between seed colour intensity and content of carotenoids in different generations, cultivar Auralia was crossed with Y228 and Bohatyr. The parents and the selected strains were sown in the same field experiments between the 1^{st} and 4^{th} of April of 1999 through 2003. The plot size was 6 m^2. Individual plants were harvested from the F_2 and F_3 generations to determine the distributions of colour and seed size and shape. Twenty plants were taken from selected F_4, F_5 and F_6 breeding lines and parent cultivars. Genotypes were selected for deep-orange colour and round seeds from F_2 to F_6 generations.

The seed colours of pea strains were identified via the UPOV system (OMMI, 1996) used for visual assessment in comparison with standard cultivars, such as Bohatyr and Profi. A colour scale from 1 to 9 was created to measure hue of seed colour, where 1=green, 2=yellow-green and 3=yellow; and the range between 5 and 9 represented the following hues of colour: 5=light-yellow, 7=medium-yellow, and 9=deep-orange. The above scale was used to measure hue colour of seeds of the individual plants of breeding lines. The strains were classified as follows: group 1 (3.5-4.5 code) included the white-yellow seeded strains; group 2 (4.6-5.5 values), light-yellow seeds; group 3 (5.6-7.5 values), medium-yellow seeds; and group 4 (7.6-9.0), deep-orange seeds. Thousand-seed weight and weight of seed coat were recorded to determine the percentage of seed coat in genotypes. The precipitation and temperature were also recorded from sowing to harvest.

GENETIC ANALYSIS

Genetic analysis was carried out by using the mean and variance of seed colour values based on frequency of colour distribution. The heritability of attributes that influenced the intensity of seed colour was estimated via the ratio of genetic progress and selection difference (Singh and Chaudhary, 1979) as follows:

$$h^2 = \frac{x_1 - x_0}{x_S - x_0}$$

where h^2 is heritability, x_1 is the mean of F_6 lines descended from F_5 selected lines, x_0 is the mean of F_4 population, and x_S is mean of F_5 lines selected from F_4 population.

CHEMICAL ANALYSIS

The chemical analysis of pea seeds was carried out at the Central Laboratory of the University of Debrecen Centre of Agricultural Sciences. The dry seeds were milled and dry-matter, protein, carotene and xanthophyll contents were determined. The measurement of seed protein was made with the Kjel-Foss method (AOAC, 1995). The total carotene and xanthophyll contents were determined with chromatography following extraction with hexane/petroleum ether-acetone. The measurements were made at 450 nm on a spectrophotometer and the content of carotene and xanthophylls calculated as mg kg^{-1}. The data were analysed by analysis of variance. Regression analysis was applied to study the relationships between the seed colour values and carotenoids and protein contents.

RESULTS AND DISCUSSION

We investigated the effects of drought on seed colour stability of parent cultivars and strains. The total carotene content of yellow seeds in pea was rather low (0.32 mg kg^{-1}), but the total xanthophyll content was relatively high (10.20 mg kg^{-1}). During seed development, 12 days before harvesting, the lowest carotene content was measured under the cool rainy weather conditions of 2001. The role of precipitation in the accumulation of xanthophylls in the seeds was not clear, because the xanthophyll content of the seeds was significantly lower in the drought year of 1999 and under cool rainy weather conditions in 2001 than in warm or wet years (Table 1). The carotenoid levels depended on the genetic background of cultivars as well as climatic conditions. In the dry warm year of 1999, both pigment levels in afila type Y228 with light-yellow seeds were the lowest of all cultivars studied. However, the middle-yellow and deep-orange-seeded cultivars, such as Bohatyr and Auralia, also contained low concentrations of xanthophylls in seeds; the carotene content was 2.5-fold higher (0.33 mg kg^{-1}) than that of light-yellow seeded cultivars (0.13 mg kg^{-1}).

Table 1. Climatic conditions during 12 days before harvesting and seed quality at the harvest dry pea.

Year	Precipitation mm	Tmax[a] (°C)	Tmin (°C)	Seed coat (%)	Seed colour values	Carotene (mg kg^{-1})	Xanthophylls (mg kg^{-1})	Carotene /Xanthophylls (%)	Protein (%)
1999	1.0	32.6	17.1	8.09	6.84	0.26 bc*	5.66 c	4.56 a	19.80 b
2000	2.0	27.9	11.7	8.33	6.67	0.36 ab	11.29ab	3.19 b	22.31 a
2001	41.1	24.8	14.5	7.70	7.36	0.16 c	8.07 c	2.09 bc	20.00 b
2002	26.4	30.3	16.1	7.17	5.96	0.43 a	12.17 a	3.54 a	20.13 b
2003	6.4	27.6	13.3	7.20	7.13	0.26 bc	11.84 a	2.30 bc	22.41 a

* The values in a row having different letter are significantly different at the 0.05 probability level according to Duncan's multiple range test.

[a] Tmax=average of temperature maximum, Tmin= average of temperature minimum.

The high ratio of carotene and xanthophylls (4.05%) was due to higher carotene content of deep-orange seeds in the case of Auralia cultivars and the low ratio (2.52%) was related to the light-yellow colour, e.g., afila type Y228 (Table 2). The middle-yellow seed colour of Bohatyr cultivar was attributed mainly to the high xanthophyll content, although its carotene content was similar to that of the Auralia cultivar with deep-orange seeds. These results suggested that the ratio of carotene and xanthophylls had an influence on the yellow hue of seeds expressed by colour values. We determined this ratio as "yellow index" and expressed it as a percentage. There were no significant differences in protein content of seeds among cultivars (Table 2).

Our results showed that the xanthophylls were the predominant carotenoids in dry pea cultivars. The deep-orange coloured seeds of Auralia cultivar contained 0.36 mg kg^{-1} of carotene and 9.3 mg kg^{-1} of xanthophylls where the colour was controlled by more dominant alleles (Nemeskéri, 2005). There was no significant difference in the carotene content of the seeds between the Bohatyr cultivar with middle-yellow coloured seeds and the Auralia cultivars with deep-orange seeds, but significant differences were found in the xanthophyll content. Swiecicki (1998) and Swiecicki et al. (2000) reported that multiple dominant alleles of the Orc locus in *Pisum* controlled orange colour of cotyledons and carotenoids, whereas the recessive orc allele produced yellow cotyledons. We supposed that the middle-yellow colour was attributed to more recessive alleles than dominant ones and light yellow colour was controlled by recessive alleles.

Table 2. Consumption quality of dry seeds in the pea cultivars.

Character	Cultivar			
	Y228	Bohathyr	Auralia	LSD5%
Thousand grain weight (g)[a]	203.80c*	238.80b	281.30a	24.50
Seed coat (%)	7.97a	8.86a	6.22b	1.65
Seed colour values	4.42c	6.19b	7.55a	0.99
Carotene (mg kg^{-1})	0.25	0.40	0.36	ns
Xanthophylls (mg kg^{-1})	10.59a	12.70a	9.30b	2.11
Ratio of carotene and xanthophylls (%)[b]	2.52	3.43	4.05	ns
Protein (%)	21.57	21.51	22.26	ns

* The values in a row having different letter are significantly different at the 0.05 probability level; *ns* = not significantly different.
[a] The values of the average of four years of experimental data.
[b] named as yellow index.

Years had no significant effect on thousand-grain weight and the percentage of seed coat (Table 1). We analysed the relationship between seed colour and levels of yellow pigments to see if this could be used as a selection criterion to improve seed colour quality. Contrary to our expectation, the relation between seed colour value and carotene content was low (R^2=0.269, P<0.10). Group 1 plants (4-5.5 colour values) related to low carotene content included the genotypes with light-yellow seeds. Higher colour values were somewhat associated with higher carotene content. These findings showed that selection on the basis of colour values for genotypes with high carotene content of the seed would not be successful.

Afila genotypes segregated for leaf type in the F_3 generation and many of them had low seed colour values. The frequency of green and green-yellow seeds expressed by 1 and 2 colour values decreased the mean colour values of strains. However, the seed colour of parents was yellow; we found that the percentage of green seeds of their progenies was different. In those F_2 generations where the maternal cultivar was afila type, such as Y228, the ratio of green seeds was higher (7.4%) than in the progenies that originated from Auralia maternal cultivar with normal leaves (2.7%) (Nemeskéri, 2005). Weeden et al. (1998) showed that the i allele accounted for green colour of cotyledons and the af allele controlled the semi-leafless, i.e., development of tendrils instead of leaves. Both genes were in the same linkage group. We supposed that this could explain the larger percentage of green seeds in the progenies originated from Y228 maternal cultivar, which depended on the genetic structure of maternal cultivar of afila type.

In the cool and rainy weather in 2001, a significant relationship existed between seed colour values and xanthophyll content of the seeds (R^2=0.738; Figure 1). Under these climatic conditions, selection was performed for high colour values (5.6-9.0) among the F_4 strains; then the F_5 and F_6 generations were grown without selection. The selected F_4 strains with middle-yellow and deep-orange seeds ranging between 6.6 and 8.0 proved that the high seed colour values related to high xanthophyll contents of seeds in the F_6 generation (r=0.929) (Figure 1). This confirmed the results of genetic analysis where we found high heritability of the xanthophyll content of seeds (h^2=0.78) (Table 3). The heritabilities of carotene content (h^2=0.32) and yellow index (h^2=0.21) were rather low.

Table 3. Consumption quality of dry seeds in different pea generations 2001-2003.

	Generations			Sd[a]	h^2 [b]
Characters	F_4	F_5	F_6		
Seed colour values	7.36ab*	6.09c	7.42ab	–1.27	0.05
Yellow pigments (mg kg^{-1})	8.23b	12.29a	11.28b	4.06	0.75
Carotene (mg kg^{-1})	0.16c	0.44a	0.25bc	0.28	0.32
Xanthophylls (mg kg^{-1})	8.07b	11.86a	11.03a	3.79	0.78
Yellow index (%)	2.09c	3.64ab	2.41bc	1.55	0.21
Protein (%)	20.00b	22.25a	22.50a	2.25	

* The values in a row having different letter are significantly different at the 0.05 probability level according to Duncan's multiple range test.
[a] Sd= selection difference, [b] h^2 = heritability.

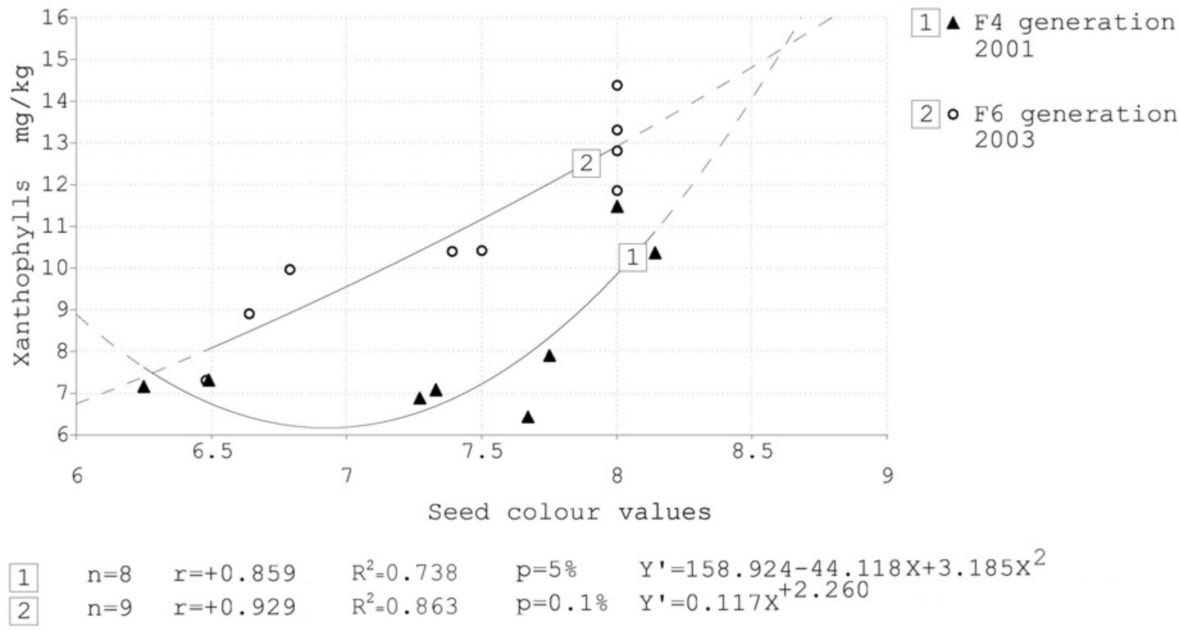

Figure 1. Relationship between xanthophylls content of the seeds and seed colour values in pea strains.

Under rainy weather conditions, such as year 2001, protein content of dry seed in pea was rather low, ranging from 18.4 to 21.35%. Selection was performed for high colour values (above 6.0 values) and also high seed protein (above 19%) among the F_4 strains. There was no significant relationship (r=0.379) between seed colour value and protein content of seed in cool and rainy weather. Contrary to our results, Reichert and MacKenzie (1982) found that peas with low protein content had a bleached yellow seed colour, whereas the peas with high protein content had a bright-yellow seeds and xanthophylls were predominant in these pea cultivars. A significant relationship (r=0.516) between seed colour values and protein content was only shown in the selected F_5 strains grown under optimal conditions (unpublished data).

Several authors have reported that the biosynthesis of carotenoids depended on temperature (Koskitalo and Ormrod, 1972; Terjung and Garab, 1998; Brandt et al., 2003) and water stress (Iturbe-Ormaetxe et al., 1998). Lers et al. (1990) suggested that the activation of specific genes and protein synthesis were necessary for the biosynthesis of β carotene because the light-activated synthesis of β carotene can be halted by the use of transcriptional inhibitors of protein synthesis. Orset and Young (2000) also reported that the accumulation of β carotene was closely linked to an increase in irradiance.

As the heritabilities of carotene and xanthophyll content of pea seeds are influenced by climatic factors, we assumed that the ratio of carotene and xanthophylls, measured as "yellow index," expressed the impact of environmental conditions on these pigments and could be used as a selection criterion. The relationship between yellow index and colour values was exponential in the case of parent cultivars, and the determination coefficient was rather low (R^2=0.330, P<0.10). The large seed colour values of F_6 generations was significantly related to low yellow index (R^2=0.445; Figure 2). Although we expected that the high seed colour values were related to high seed carotene contents, we found that by using yellow index for selection the errors caused by environmental effects were reduced. This presumably related to the carotene synthesis that was inhibited by low temperature or the increase in xanthophyll levels of seeds, which might be caused by oxidation of carotene under high temperatures.

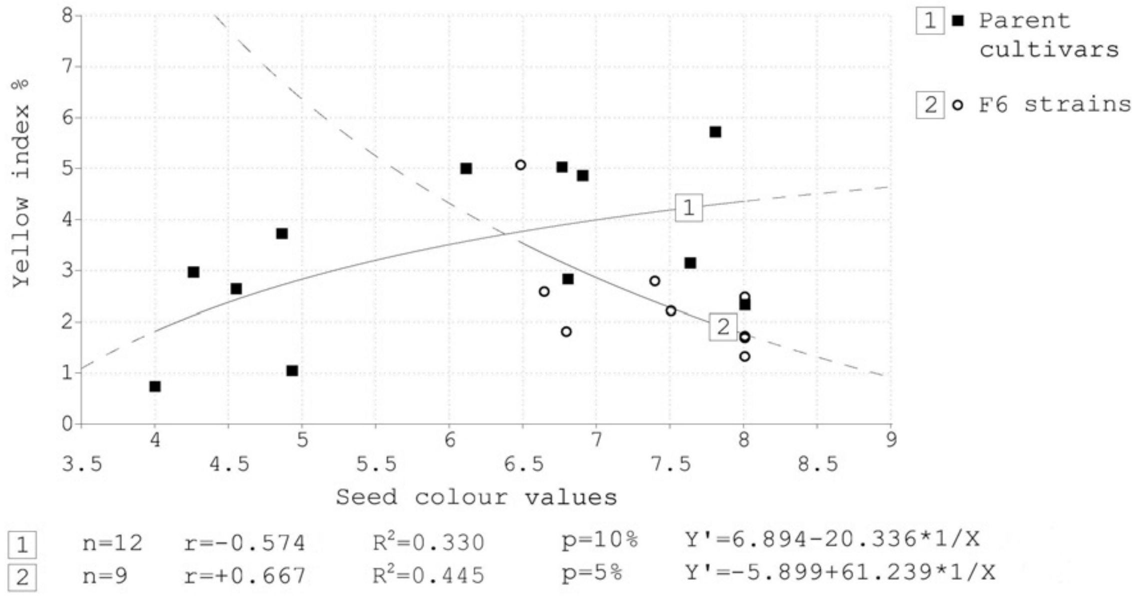

Figure 2. Relationship between the yellow index and seed values in pea cultivars and F_6 generations.

CONCLUSIONS

Because the thickness of seed coat did not affect the estimation of colour values, we could carry out the selection based on seed colour values to improve the yellow colour intensity and the pigment contents of the seeds. Under cool and rainy weather conditions the selections for high colour values from the F_4 generation resulted in increased carotene and xanthophylls content of the seeds in F_5 strains. The selection seemed to be more efficient for xanthophyll than for carotene levels. These findings were confirmed by the genetic progress between the selected strains and the basic populations; xanthophylls had high heritability (h^2=0.78) and carotene had low heritability (h^2=0.32). Those deep-orange-seeded genotypes, produced with intensive selection based on colour values, showed uniformity in colour and genetic stability in carotenoids in the F_6 generation. The selection for high seed colour values could more effectively increase the intensity of yellow colour of seeds under rainy weather conditions than under warm and drought weather conditions. Yellow index can also be used to select genotypes with light-yellow colour seeds; however, these have high xanthophyll and rather low carotene contents (in the 3.5-5.5 range of colour values).

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