

Fig. 1. M3: Descendants of chlorophyll mutant which appeared after treatment with chloramphenicol.

CAROTENOID COMPOSITION AND PROVITAMIN A VALUES OF F₂ SEEDS FROM THE CROSS OF WL 3527 (orc) x Wt 11145 (Orc)

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The composition and content of carotenoids was investigated in dry and fresh seeds of lines Wt 3527 (orc I- yellow cotyledons), Wt 11145 Orc 1 - orange cotyledons), and in dry F₂ seeds of the hybrid Wt 3527 x Wt 11145. F₂ seeds were divided into five groups: yellow, dark yellow, light orange, orange, brick (see [1]).

The seeds were ground and hydrolyzed (KOH in alcohol) prior to extraction with hexane. Chromatographic separation of carotenoids was done on an MgO column using hexane as the mobile phase followed by 10 and 20% solutions of acetone in hexane.

Moreover, the separation of pigments was investigated by TLC on silica gel using solvent mixture of hexane-ethylacetate-methanol (75:20:7) as the mobile phase.

The content of a given carotenoid was estimated on the basis of a spectrophotometric assay using tabular values of $E_{1\%}^{1\text{cm}}$, viz: beta-carotene 2592; beta-cryptoxanthin 2386; lutein 2550.

For the determination of the provitamin A value, the biological activity of beta-carotene was taken as 100% beta-cryptoxanthin as 57%, and lutein as 0%.

Column chromatography of pigment extracts from dry seeds of line Wt 3527 showed the presence of two fractions migrating in 10% and 20% solution of acetone in hexane. But carotenoids from dry seeds of line Wt 11145, fresh seeds of line Wt 3527, and all F_2 recombinants had three fractions, eluted in turn by hexane, 10% and 20% solution of acetone in hexane. Fresh seeds of line Wt 11145 did not contain the carotenoid eluted from the column by 20% solution of acetone.

Fresh

The TLC method showed that the fraction eluted from the column by 20% solution of acetone contained not only lutein but also two additional carotenoid pigments (Fig. 1). The following substances were identified in solutions obtained by separation of carotenoids by column chromatography:

Dry

Further examination of the pigment in the 20% fraction which migrated below lutein on the TLC chromatogram (Fig. 1d) had absorption maxima in the following wave lengths (nm): 420, 445, 472, corresponding to taraxanthin. The lowest migrating carotenoid was not identified (Fig. 1e).

Wt 11145 had two to four times more total carotenoids than the other lines, but 90% of this was lutein (Table 1). Lutein was the predominant carotenoid in dry seeds. Fresh seeds contained greater amounts of 6-carotene and beta-cryptoxanthin. The amount of beta-cryptoxanthin in dry seeds was the least variable constituent: 94-110 mkg/100g.

The highest provitamin value was found in dry seeds of an F_2 from cross Wt 3527 x Wt 11145 having dark yellow cotyledons (Table 2). Other segregants had less than half the provitamin A.

1. Blixt, S., and W. K. Swiecicki. 1983. PNL 15:9-10.

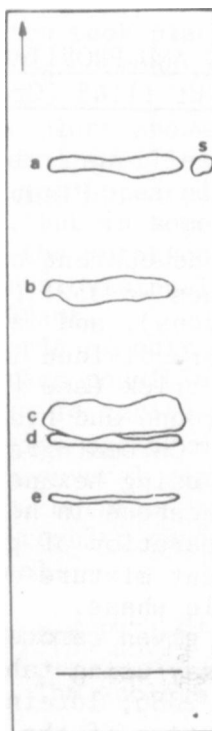


Fig. 1. Carotenoid separation extracted from seeds of hybrids WT 3527 and WT 11145.
 a) beta-carotene;
 b) beta-cryptoxanthin;
 c) lutein;
 d) taraxanthin;
 e) unidentified;
 s) standard all-trans-beta-carotene.

Table 1. Carotenoid content isolated from pea seeds by column chromatography. Fresh and dry seeds of parents and dry seeds of selected F₁'s.

Stage of seeds maturity	Wiatrowo line number	Color of dry cotyledons	Carotenoid (g/100g)			
			-carotene	-crypto-xanthin	Lutein	Total
Fresh	(P ₁) Wt 3527	yellow	107	68	76	224
	(P ₂) Wt 11145	orange	47	177	-	251
Dry	(P ₁) Wt 3527	yellow	-	105	305	410
	(P ₂) Wt 11145	orange	25	99	964	1086
	(F ₂) Wt 3527 x Wt 11145	yellow	29	106	204	339
	(F ₂) Wt 3527 x Wt 11145	dark yellow	154	94	390	638
	(F ₂) Wt 3527 x Wt 11145	light orange	14	98	340	452
	(F ₂) Wt 3527 x Wt 11145	orange	24	110	466	600
	(F ₂) Wt 3527 x Wt 11145	brick	40	110	255	405

Table 2. Provitamin A value of pea seed (in g of -carotene/100g)

Stage of seeds maturity	Wiatrowo line number	Color of dry cotyledons	Provitamin A carotenoids (g/100g)	Provitamin A value
Fresh	(P ₁) Wt 3527	yellow	175	146
	(P ₂) Wt 11145	orange	224	148
Dry	(P ₁) Wt 3527	yellow	105	60
	(P ₂) Wt 11145	orange	122	79
	(F ₂) Wt 3527 x Wt 11145	yellow	135	89
	(F ₂) Wt 3527 x Wt 11145	dark yellow	248	208
	(F ₂) Wt 3527 x Wt 11145	light orange	112	70
	(F ₂) Wt 3527 x Wt 11145	orange	134	87
	(F ₂) Wt 3527 x Wt 11145	brick	150	103