

Optimizing Canola Seed Secondary Metabolism for Aquaculture Feed Formulations

Abdelali Hannoufa

AAFC-Saskatoon Research Centre



Agriculture and
Agri-Food Canada

Agriculture et
Agroalimentaire Canada

Demand for plant-based fish feed

- Continuing expansion of the aquaculture industry
- Global scarcity of current constituents of fish feed ingredients
- Environmental and health concerns associated with the use of fish meals (e.g. PCBs...etc)



Canola protein as fish feed

- Good balance of essential amino acids, low antigenicity, high digestibility
- A CPC could replace 100% protein derived from fish meal (Higgs *et al.*, 1995)
- CanPro-IP65 replaced up to 75% of fish meal protein (Maenz *et al.* 2003)

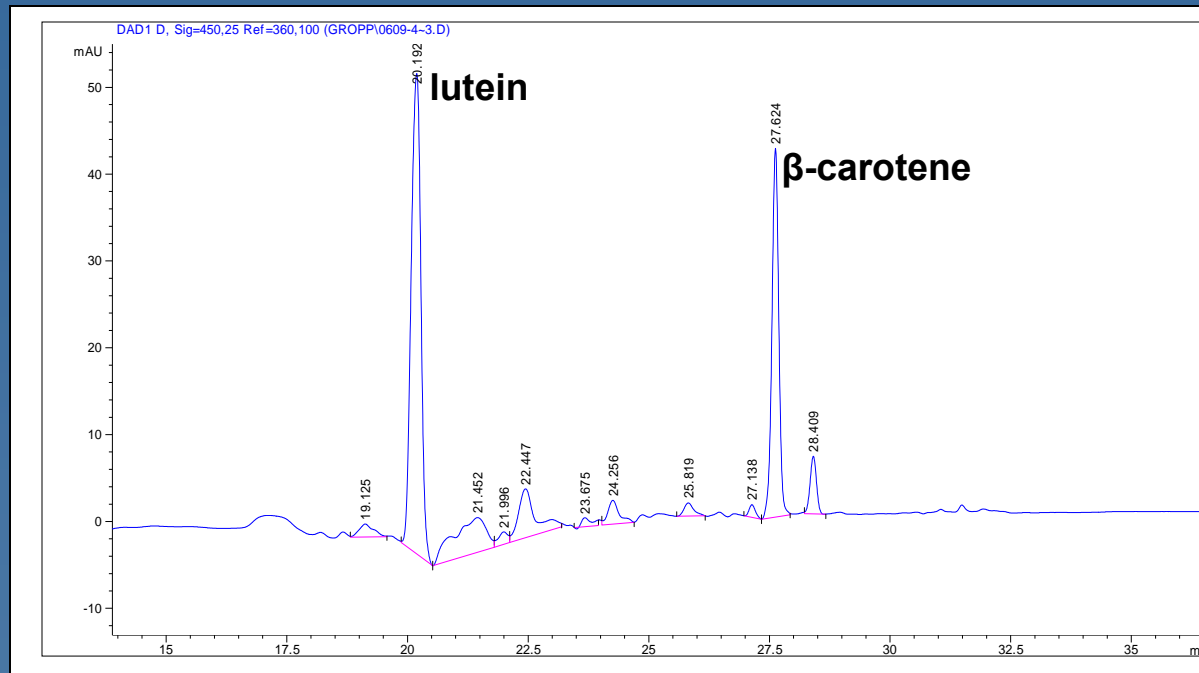


Canola meal

- Reduce levels of antinutritional factors, e.g. sinapine, fibre and phytate
- Enhance levels of carotenoids and xanthophylls



Carotenoid and xanthophyll content of canola seed



lutein	20.13 ug/g
β-carotene	2.6 ug/g
zeaxanthin	u.d.
canthaxanthin	u.d.
astaxanthin	u.d.



Astaxanthin ...

- Provides typical pink red color to salmon, trout, shrimp and lobster
- Protects eggs from damage by UV-radiation
- Improves growth and survival rates of juveniles
- A potent antioxidant with immunostimulant functions in humans

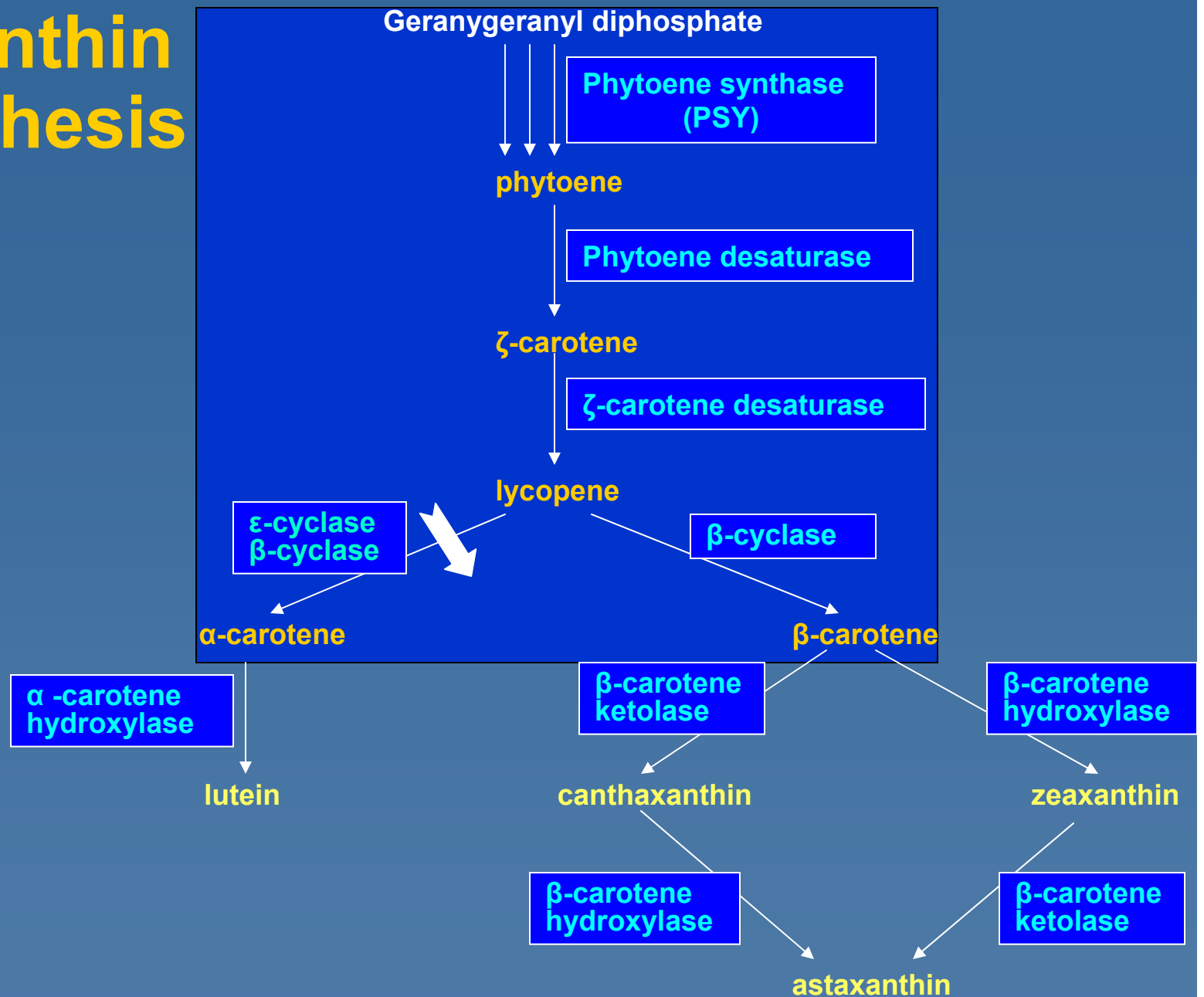


Astaxanthin ...

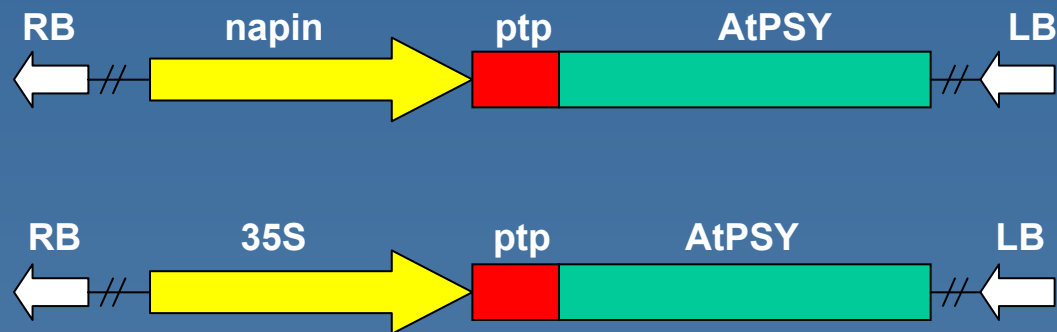
- Obtained from crustaceans, yeast, microalgae and chemical synthesis:
 - limited yield
 - costly preparation
- Accounts for ~20% of the total feed cost
- Biochemical precursor, β -carotene, naturally present in *B. napus* seed



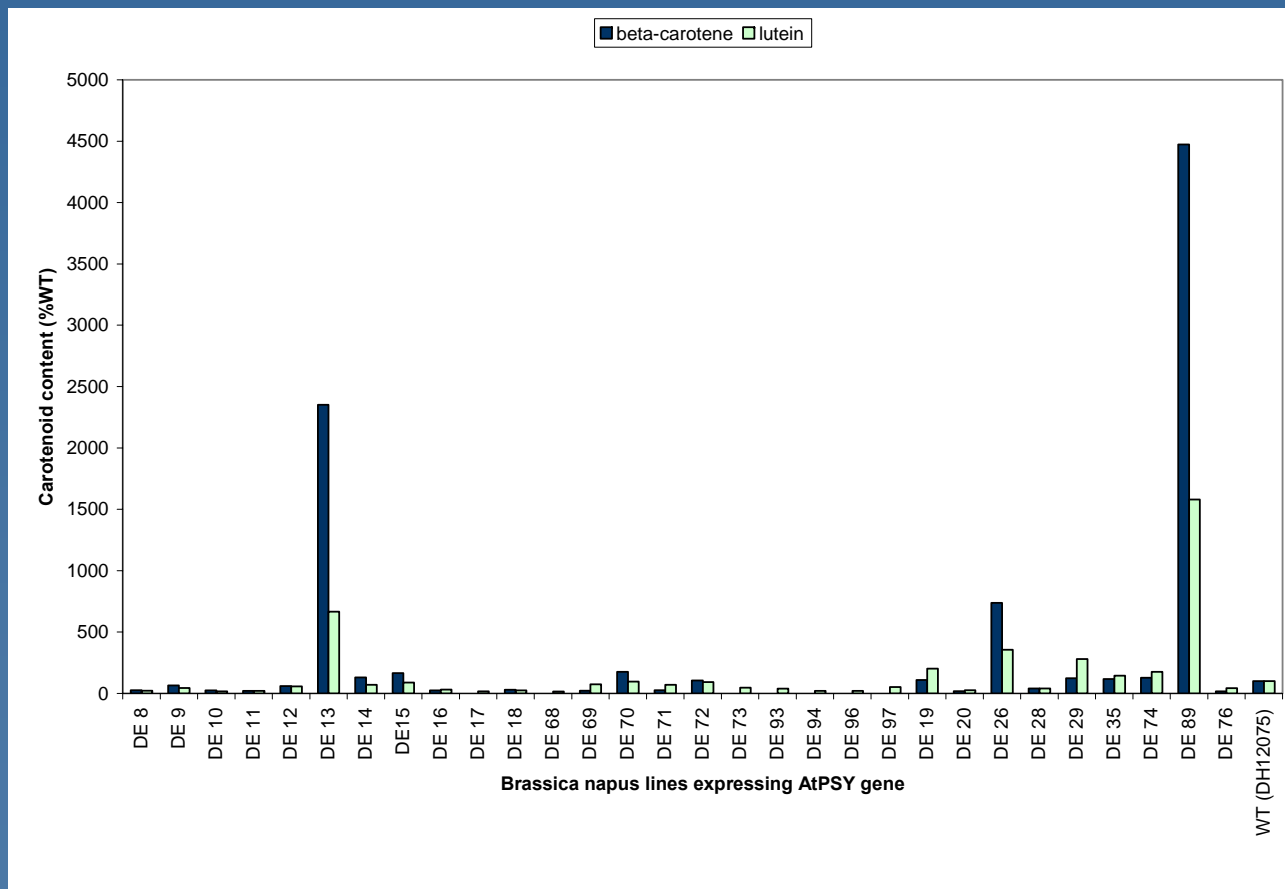
Astaxanthin biosynthesis



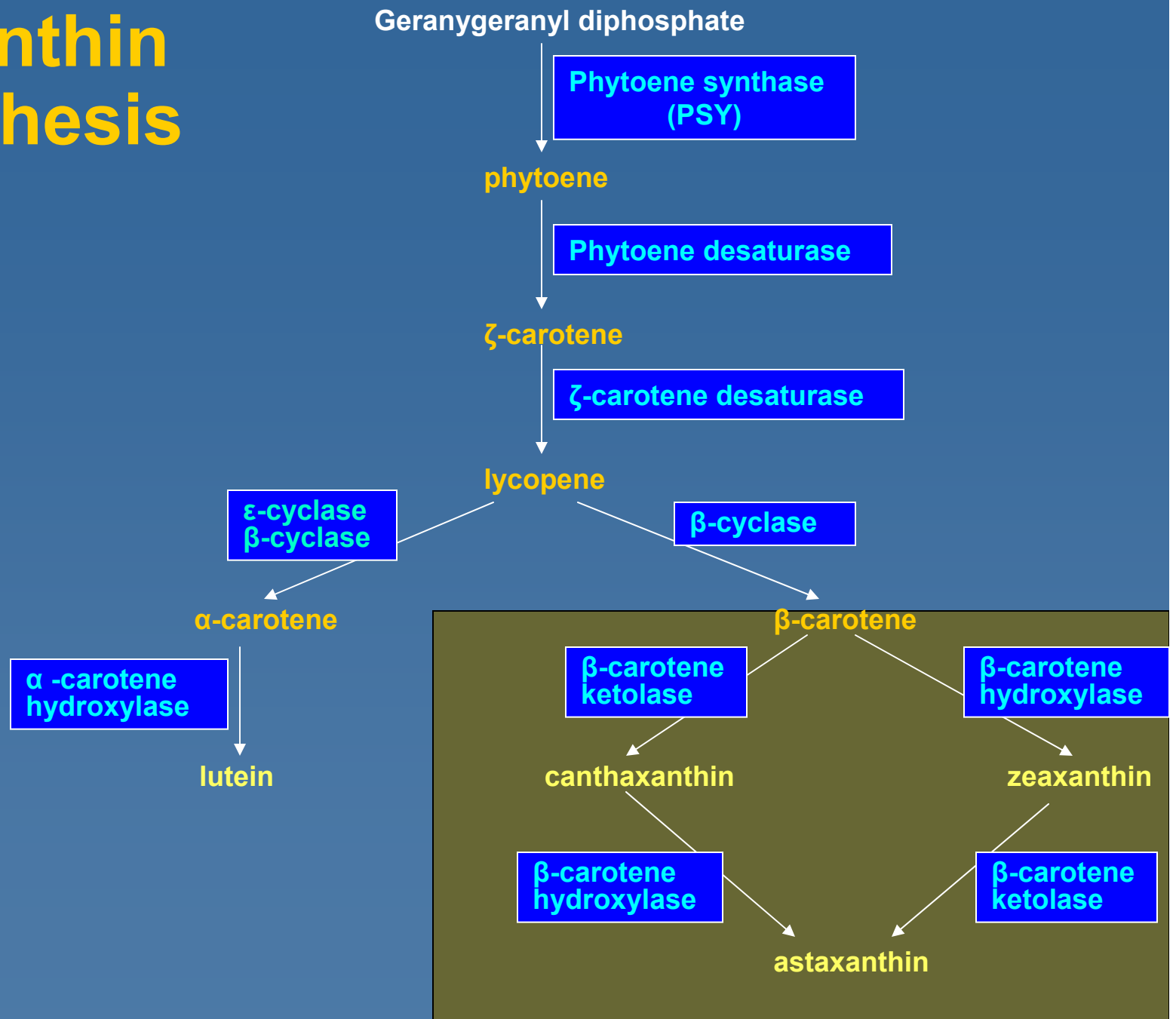
Expression of *AtPSY* gene in *B. napus*



Carotenoid content of seeds of *B. napus* lines expressing *AtPSY* gene



Astaxanthin biosynthesis

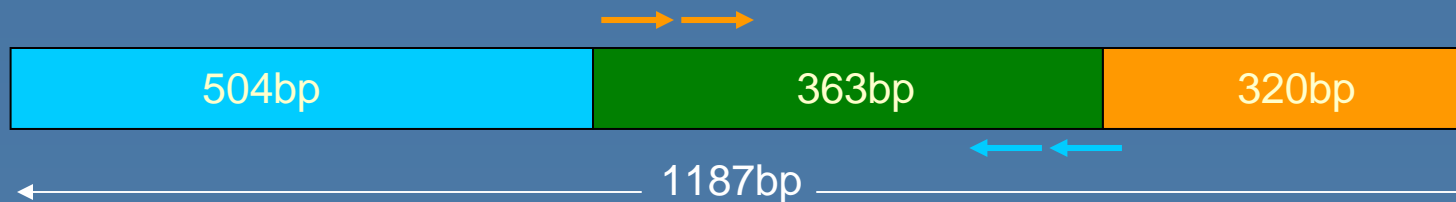


Adonis aestivalis



Cloning of β -carotene hydroxylase

LeCrtR-b2	VGA AVGMEFWARWAHKALWHASLWHMHE..SHHKPREGPFELNDV	193
AsCrtZ	VVAIVLVMELTAYSVHRWIMHGPLGCGWHKSHHEEHDHALEKNDL	51
AtHX	VGA AVGMEFWARWAHRALWHASLWNMHE..SHHKPREGPFELNDV	169
CHX1	VGA AVGMEFWARWAHKALWHASLWHMHE..SHHRPREGPFELNDV	193
HpHX	VGGALGMEYARYAHKAIWHESPLGWL LHKSHHTPRIGPFEANDL	207
LeCrtR-b1	FGA AVGMEYWARWAHRALWHASLWHMHE..SHHRPREGPFEMNDV	197
Consensus	shh e nd	
LeCrtR-b2	FAITNAVPAIALLN YGFFHKGLIAGLCFCAGLGITVFGMAYMFVH	238
AsCrtZ	YGVVFAVLA TITLFTV GAYWWPVLWWIALGMTVYGLIYFIL...H	92
AtHX	FAIVNAGPAIGLLSYGFFNKGLVPGLCFGAGLGITVFGIAYMFVH	214
CHX1	FAIINAVPAIALLSFGFFHKGLVPGLCFGAGLGITVFGMAYMFVH	238
HpHX	FAIINGLPAMLLCTFGFWLPNVLGAACFCAGLGITLYGMAYMFVH	252
LeCrtR-b1	FAITNAVPAIGLLSYGFFHKGI V PGLCFGAGLGITVFGMAYMFVH	242
Consensus	a l g g h	
LeCrtR-b2	DGLVHKRFPVGPVANVPYLRKVAAAHSLHHSEK	271
AsCrtZ	DGLVHQRPFRYIPRRGYFRRLYQAHRLHHAVE	125
AtHX	DGLVHKRFPVGPIADV P YLRKVAAAHQLHHTDK	247
CHX1	DGLVHKRFPVGPIADV P YFRRVAAAHQLHHS DK	271
HpHX	DGLVHRREP TGP IAGLPYMKRLIVAHQLHHS GK	285
LeCrtR-b1	DGLVHKRFPVGPIANVPYFRRVAAAHQLHHS DK	275
Consensus	dglvh r p y ah lhh	



Cloning of β -carotene ketolase

Psp BKT	FIIAHDAMHGSSVVPGRPRANAAMGQLVLWLYAGFSWRKMIVKHMA	105
CrtO	FITTHDAMHGTTIAMRNRQLNDFLGRVCISLYAWFDYNMLHRKHWE	179
Gv BKT	FITTAHDAMHRTVFPANHRINDWLGTAAVGLYAFMPYRELLIKHQ	118
Hp 39982 BKT	FITTHDAMHGTTIALRHRQLNDLLGNICISLYAWFDYSMLHRKHWE	170
Nsp BKT	FITTAHDAMHGVVYPKNPRINNFIGNKLTLLIYGLLPYKDILLKHWL	120
Consensus	fitahdamhgtv p n r nd lg lya f y l khw	
Psp BKT	HHRHAGTDDDPDFDHGGPVRWYARFIGTYFGWREGLLLPVIVTVY	150
CrtO	HHNHTGEVKGDPDFHRGNPGIVPWFASFMSYMSMWQFARLAWWT	224
Gv BKT	HHRFPATGKDPDYHDGEHSGFFQWYLKFMKDYMESRNTPFLLIAGM	163
Hp 39982 BKT	HHNHTGEVKGDPDFHKGNPGLVVPWFASFMSYMSLWQFARLAWWA	215
Nsp BKT	HHGHPGTDLDPDYNGHPQNFLLWYLHFMKSYWRWTQIFGLVMIF	165
Consensus	hh h gt dpd hgg g wf fm sym q l	
Psp BKT	ALILGDRWYVVFWPL...PSI...LASI.QLFVFGTWLPH	184
CrtO	VVMQLLGAPMANLLVFMAAAP.I...LSAFRLFYFGTYMPH	261
Gv BKT	AVVFGVCTWLMGVPLVNLALFWLLPLVLSLQLFYFGTYLPH	205
Hp 39982 BKT	VVMQMLGAPMANLLVFMAAAP.I...LSAFRLFYFGTYLPH	252
Nsp BKT	HGLKNLVHIPENNLIIIFWMIPSI...LSSVQLFYFGTFLPH	203
Consensus	v l n l a p i l s qlfyfgtylph	

5'

369bp

3'



Summary

- Three *B. napus* lines that hyper-accumulate β -carotene in the seeds
- Full length cDNA clone of β -carotene hydroxylase
- Partial cDNA sequence of β -carotene ketolase
- Co-expression of β -carotene ketolase and β -carotene hydroxylase in seeds of *B. napus* lines having enhanced levels of β -carotene
- Crossing to *B. napus* lines having reduced levels of anti-nutritional factors



Acknowledgements

Bianyun Yu
Ricci Schäfer
Gordon Gropp

Funding

AAFC-Crop Genomics Initiative
Saskatchewan Agricultural Development Fund



Agriculture and
Agri-Food Canada

Agriculture et
Agroalimentaire Canada