

Exploiting genetic resources of the genus *Beta* to broaden the genetic variation in beet breeding

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Improving a cooperation network between actors involved in conservation and utilization of *Beta* genetic Resources, Venice, 19.-20.6.2018



Plant Breeding
Institute



Structure

- Gene bank at the PBI Kiel
- Structured populations
- Genes controlling life cycle regime
- *B. corolliflora* addition lines
- Procumbentes species as donors for cyst nematode resistance

Accessions of the genus *Beta* available at the PBI Kiel

Species name	ssp.	conv.	prov.	Type	# accessions	Diversity set
<i>Beta vulgaris</i>	vulgaris	vulgaris	altissima	Sugar beet	390	250
	vulgaris	crassa	conditiva	Red beet	78	30
	vulgaris	crassa	crassa	Fodder beet	41	20
	vulgaris	crassa	cycla	Leaf beet	60	30
	maritima			Wild species	5610	137
	adanensis			Wild species	8	8
<i>B. patula</i>				Wild species	1	10
<i>B. macrocarpa</i>				Wild species	6	10
<i>B. corolliflora</i>				Wild species	2	2
<i>B. macrorhiza</i>				Wild species	1	1
<i>B. lomatogona</i>				Wild species	0	1
<i>B. nana</i>				Wild species	1	1
monosomic addition lines						
					56	
					164	
					23	
					351	
					77	
total					6479	250

Haplotype variation of flowering time genes of sugar beet and its wild relatives and the impact on life cycle regimes

major flowering time regulators *BTC1*, *BvBBX19*, *BvFT1* and *BvFT2*

Beta diversity panel

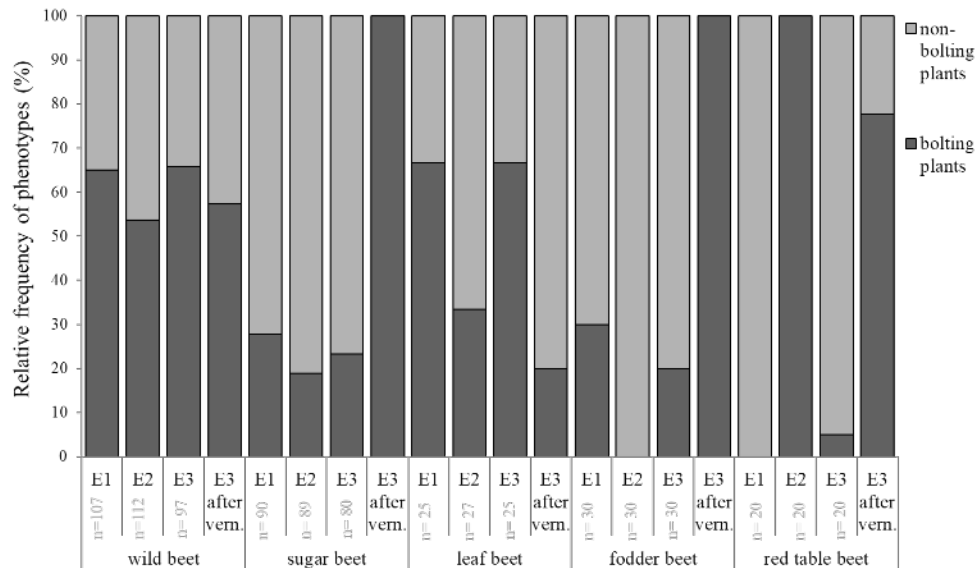
Haplotype analysis of *BTC1*, *BvBBX19*, *BvFT1* and *BvFT2*

accessions from northern latitudes flowered significantly later → strong impact of latitude of origin on life cycle

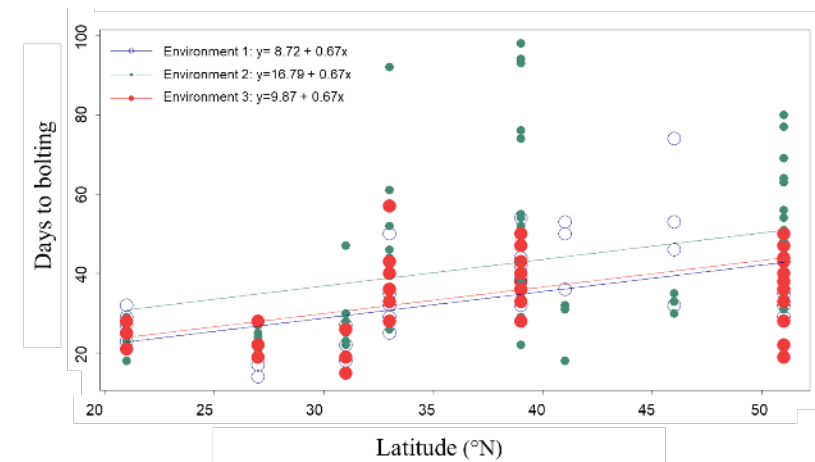
We identified sequence variation which may impact life cycle adaptation in beet.

importance of *BTC1* in the domestication process of cultivated beets

distribution and adaptation of *Beta* species to different life cycle regimes in response to different environments.



Variety	Species name	Seed code	Geographical Origin	Latitude (0°N)
wild beet	<i>B. vulgaris ssp. maritima</i>	080287	Ireland	53.0
		080461	Denmark	56.0
		080468	Egypt	27.0
		080437	Pakistan	31.0
		080418	India	21.0
		100539	Germany	51.0
		991971	Greece	39.0
		080260	Netherlands	52.0
		930034	Spain	40.0
		112787	France	46.0
sugar beet	<i>B. vulgaris ssp. vulgaris</i>	112823	Great Britain	54.0
		080538	Great Britain	54.0
		090023	Germany	51.0
		930176	Germany	51.0
		130333	Germany	51.0
		100043	Germany	51.0
		001684	Germany	51.0
		080394	Iran	32.0
		930181	USA	45.0
		080384	Turkey	39.0
fodder beet	<i>B. vulgaris ssp. vulgaris</i>	091645	Germany	51.0
		080281	Germany	51.0
		080313	Greece	39.0
		080396	Iran	32.0
red table beet	<i>B. vulgaris ssp. vulgaris</i>	092312	Russia	60.0
		080339	France	45.5
leaf beet	<i>B. vulgaris ssp. vulgaris</i>	080238	Iraq	33.0
		081845	China	35.0
		092459	Italy	42.0



Höft N et al (2018) Haplotype variation of flowering time genes of sugar beet and its wild relatives and the impact on life cycle regimes. *Frontiers in Plant Science* 8 (2211).

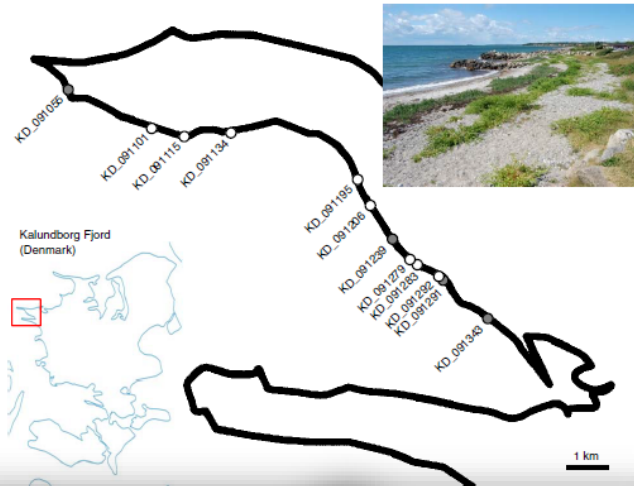
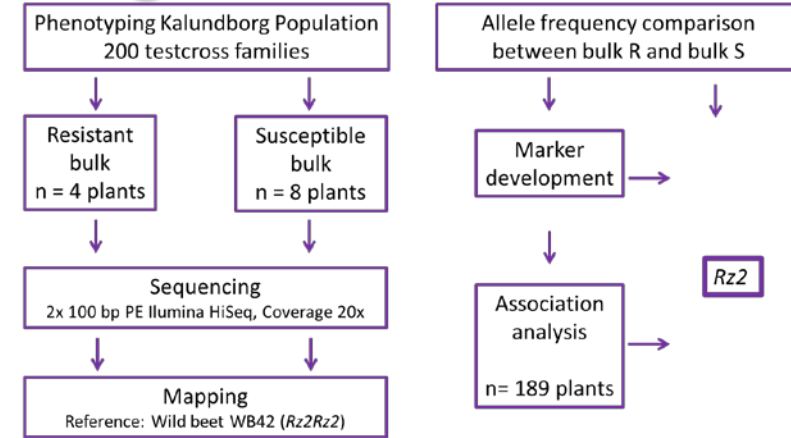
Cloning the *Rhizomania* resistance gene *Rz2*

B. vulgaris ssp. *maritima* population of 200 wild beets, sampled in their natural habitat

mapping-by-sequencing: generating a draft genome sequence of the wild beet

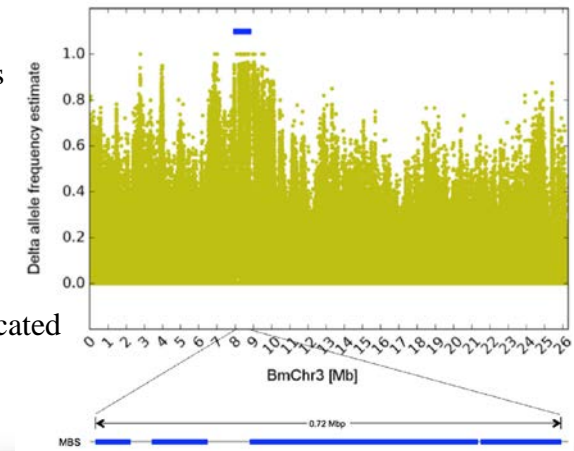
Rz2 encodes a CC-NB-LRR protein, verified by RNAi

great potential of wild beets for rapid gene discovery



MBS with two pools of homozygous resistant and susceptible wild beet

wild beet population of *B. vulgaris* ssp *maritima* located along the coastal line of the Kalundborg fjord in Denmark



B. vulgaris x maritima F2-populations



93161 x 960037



960037 x 070325

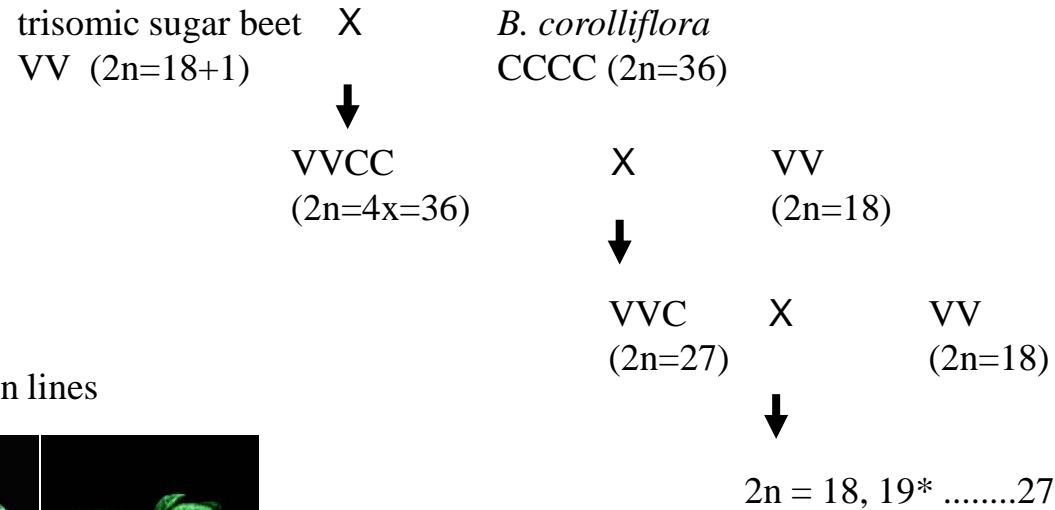


93161 x 980319

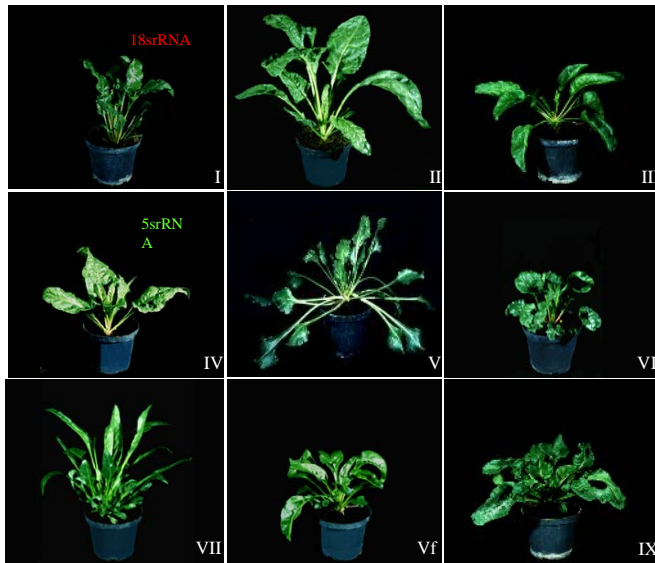


960037 x 000358

Sugar beet x *B. corolliflora* monosomic addition lines



B. corolliflora-addition lines



- I. 970005/8
- II. 970025/2
- III. M12/2
- IV. 970015/32
- V. 983x44/13
- Vf. 970014/58
- VI. 970001/4
- VII. 970026/39
- IX. 98-6812/12

Gao D et al. (2001) Monosomic addition lines of *Beta corolliflora* in sugar beet: cytological and molecular marker analysis. Theoretical and Applied Genetics 103:240-247

Gao D, Jung C (2001) Monosomic addition lines of *Beta corolliflora* in sugar beet: plant morphology and leaf spot resistance. Plant Breeding 121:81-8

Gao D et al. (2000) Molecular characterization and chromosomal distribution of species specific repetitive DNA sequences from *Beta corolliflora*, a wild relative of sugar beet. Genome 43:1073-1080

* frequency of monosomic additions: 10.4%

H. schachtii resistance and tolerance genes

- All cultivated beets are susceptible against *H. schachtii*
- wild beets of genus *Patellifolia*
 - complete resistance: no cyst formation
 - J2 larvae invade roots, no females (catch crops)
 - small syncytia can be found
- tolerance: *B. vulgaris* ssp. *maritima* WB242

Wild beet chromosomes carrying genes for nematode resistance or tolerance

	Chromosome			
	1	5	7	8
<i>P. procumbens</i>	<i>Hs1 pro-1</i> <i>Hs1-2 pro-1</i>		<i>Hs2 pro-7</i>	<i>Hs3 pro-8</i>
<i>P. patellaris</i>	<i>Hs1 pat-1</i>		---	---
<i>B. vulgaris</i> ssp. <i>maritima</i> WB242 ¹		<i>HsBvm-1</i>		

¹ Stevanato, P. et al. Plant Molecular Biology Biology Reporter (2014).

Nematode resistant plant materials

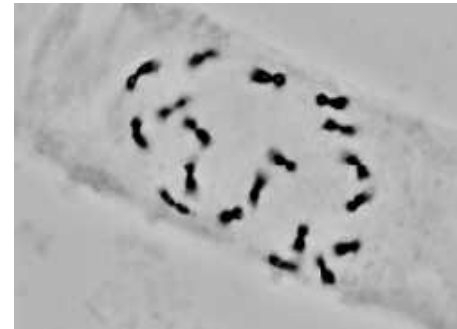
Wild beets

- *P. procumbens*
- *P. patellaris*
- (*B. webbiana*)

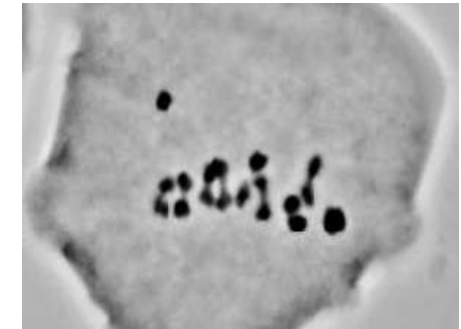
Resistant sugar beet

- $2n=19$ (Monosomic addition lines)
- $2n=19$ (Monosomic fragment addition lines)
- $2n=18$ (Translocation lines)
- Segregating populations

Monosomic addition lines

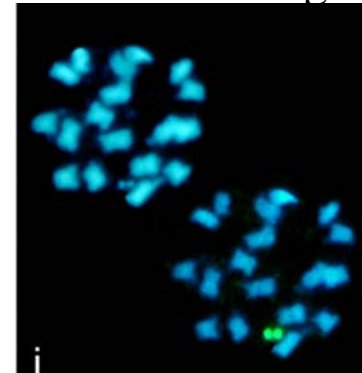


Mitotic metaphase

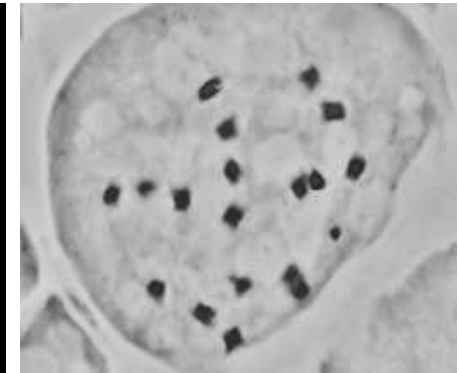


Meiotic metaphase

Monosomic fragment addition lines

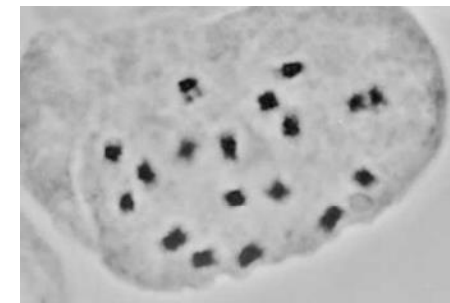


Mitotic metaphase, in situ hybridization

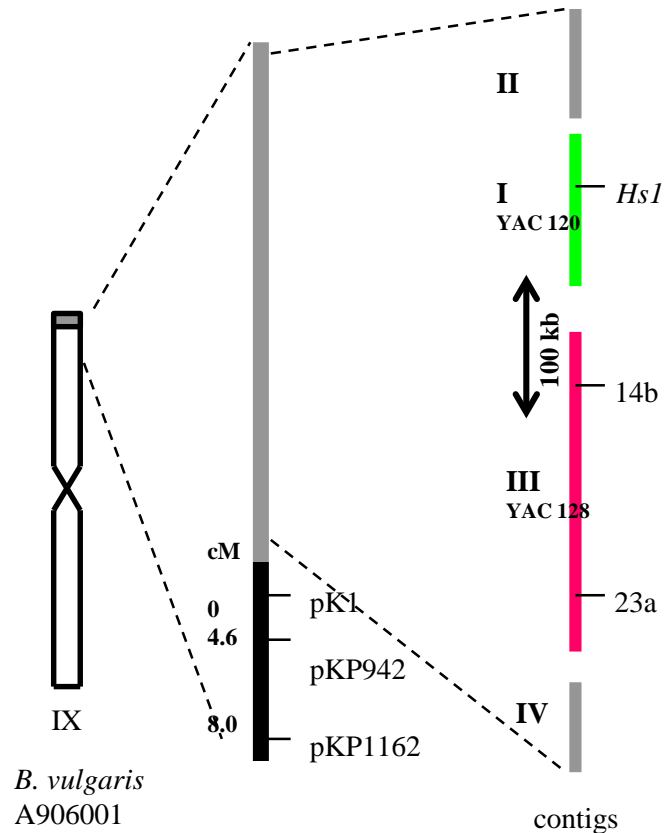


Mitotic metaphase

Diploid translocation lines Mitotic metaphase



The *P. procumbens* translocation TR520 in sugar beet



In situ hybridization with two YACs as probes, pachytene chromosomes
Desel, C., Jung, C., Cai, D., Kleine, M. & Schmidt, T. High-resolution mapping of YACs and the single-copy gene *Hs1^{pro-1}* on *Beta vulgaris* chromosomes by multi-colour fluorescence *in situ* hybridization. *Plant Molecular Biology* **45**, 113-122 (2001).

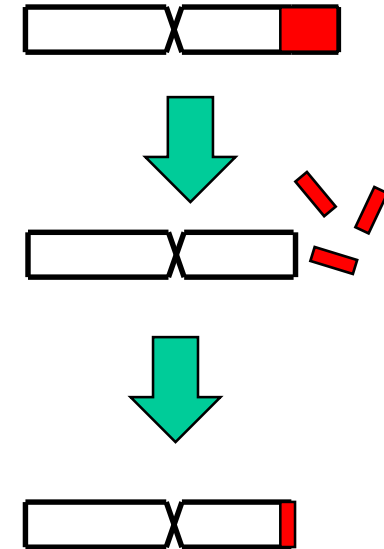


Features of the TR520 translocation:

- Size: 2.2 Mbp (0.3% beet genome)
- Number of ORFs: 320
 - Retrotransposons: 13%
 - unclassified proteins: 24 %
 - similarity to known proteins: 32 %
 - no significant similarity: 31 %

Selection of new mutants derived from the translocation line TR520

- Doses for gamma irradiation: 100gy, 200gy, 400gy
- Number of seeds irradiated: 4700
- Number of M2 families screened: 1400
- Markers assisted selection:
- Features of new translocation mutants:
 - susceptible against the BCN
 - smaller-sized translocation as compared to the resistant ones



Current work to clone the second nematode resistance gene *Hs1-2*

NGS of the resistant line TR520 → complete sequence available

Translocation breakpoint identified

Sequence annotation within the critical region → candidate genes identified

Functional analysis: hairy root transformation in combination with CRISPr-Cas mutation induction

Alignment of wild beet translocations on sugar beet chromosome 9 after physical mapping and whole genome sequencing



Patellifolia procumbens

• *Susceptible*

• *resistant*

Beta vulgaris

■ 'critical region': present only in resistant translocation lines → genome region housing *Hs1-2*