

Supplementary Material

1 Supplementary Figures and Tables

Figure S1. Setup of growth temperatures in greenhouse chambers and experimental design.

Figure S2. High temperatures affect growth parameters of *Brassica* flowering plants.

Figure S3. The number of ovules and the pollen development are not affected by high temperatures.

Figure S4. Embryo development is accelerated by high temperatures.

Figure S5. High temperatures affect embryo development. Original pictures presented in Figure 4.

Figure S6. Silique growth rate is reduced at elevated temperatures.

Table S1. Primers used in qPCR analysis and LOC number of amplified genes.

Table S2. Pearson correlation coefficient between the length of the main inflorescence stem, the flowering time, and the number of flowers.

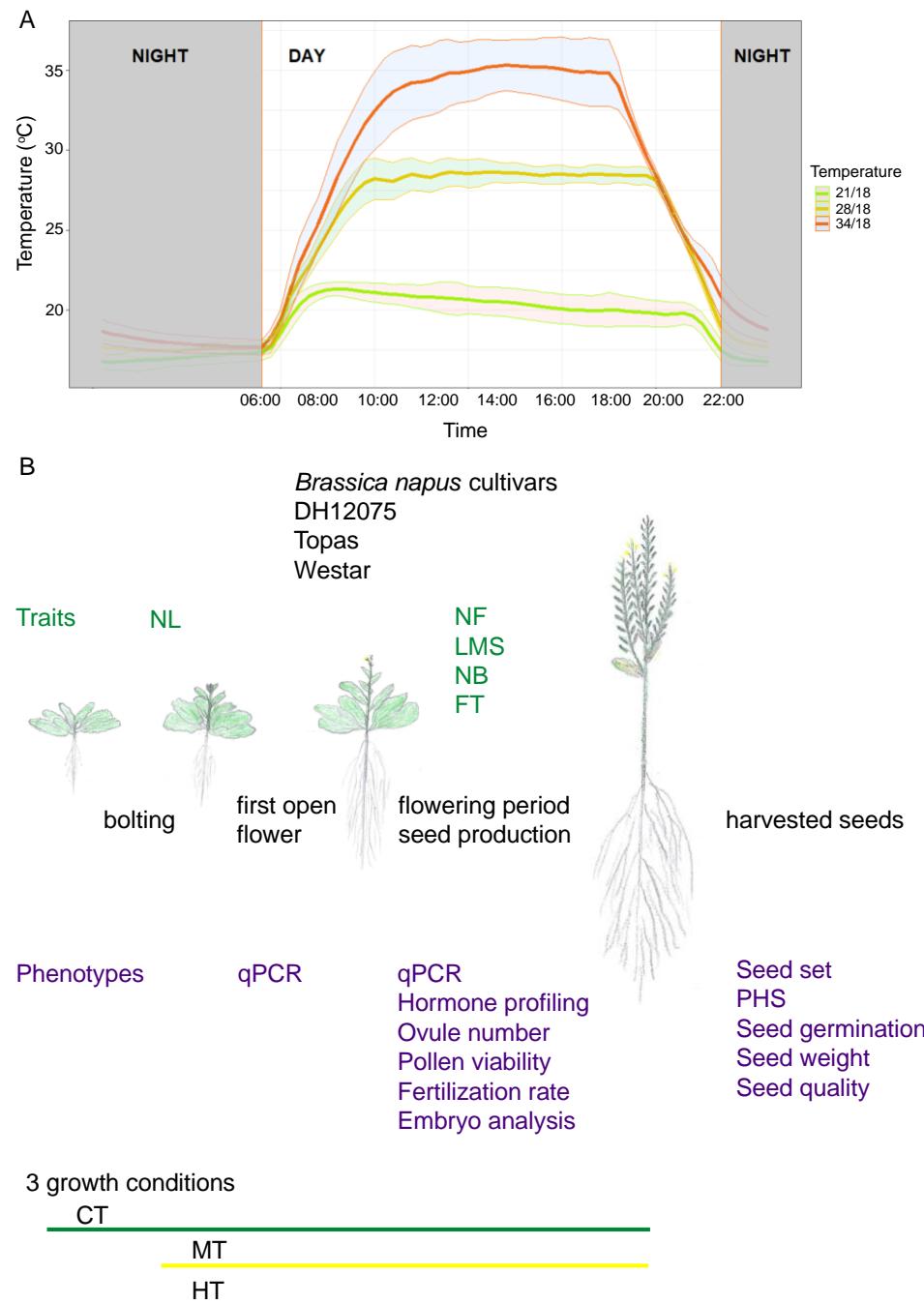
Table S3. Auxin and auxin metabolites measurements (source data of Figures 2 and 7).

Table S4. ABA measurements (source data of Figure 3).

Table S5. Pearson correlation coefficient between the siliques growth rate, the seed number per silique, and the growth temperatures.

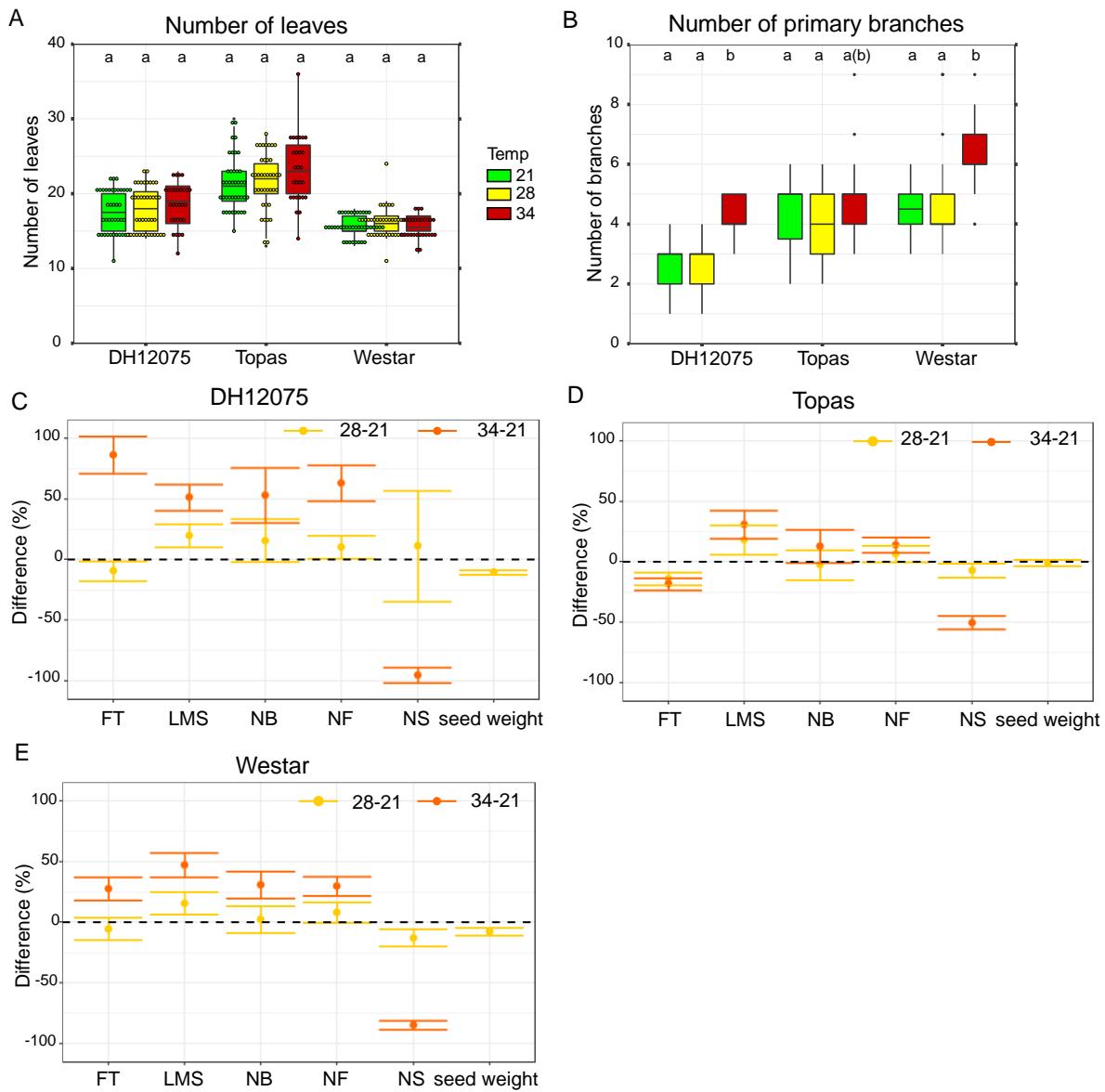
Table S6. Glucosinolates, nitrogen, and seed oil measurement (source data of Figure 9).

1.1 Supplementary Figures



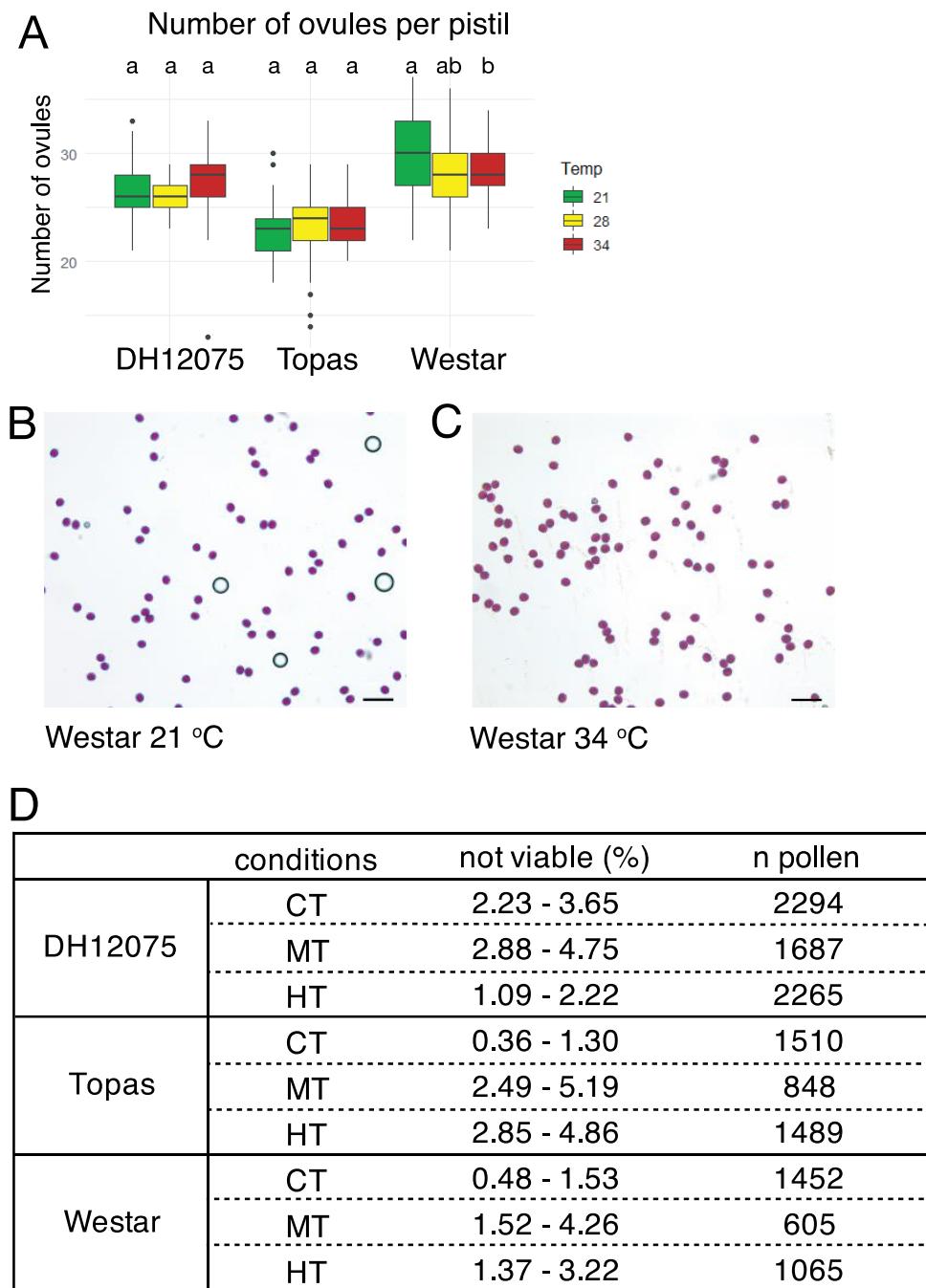
Supplementary Figure 1. Setup of growth temperatures in greenhouse chambers and experimental design. (A) Three conditions were selected for the temperature stresses and control conditions. Temperature condition for control (CT, 21/18, green), mid (MT, 28/18, yellow), and high temperature (HT, 34/18, red) chambers were set to 21 °C, 28 °C, and 34 °C, respectively, with ramping of the temperature up and down by 4 °C per hour from night temperatures set at 18 °C for all conditions. Day period was set between 6:00 and 22:00 (light grey). The graph shows the mean temperatures (bold line)

± 95 % confidence interval in each greenhouse chamber measured throughout the experiment. **(B)** Experimental design. Plants from three *Brassica napus* cultivars (DH12075, Topas, and Westar) were grown until bolting at CT growth temperatures, then kept at CT or moved to MT or HT until seed harvest. Traits are measured at bolting (NL, number of leaves) and during the flowering period and seed production: NF (number of flowers), LMS (length of the main flowering stem), NB (number of branches,) and FT (flowering time). Phenotyping experiments are also performed: expression analysis by qPCR on leaves, pistils, and seeds, hormonal profiling, ovule number, pollen viability, fertilization rate, embryo phenotyping, seed set, PHS (pre-harvest sprouting), seed germination assay, seed weight, and quality.



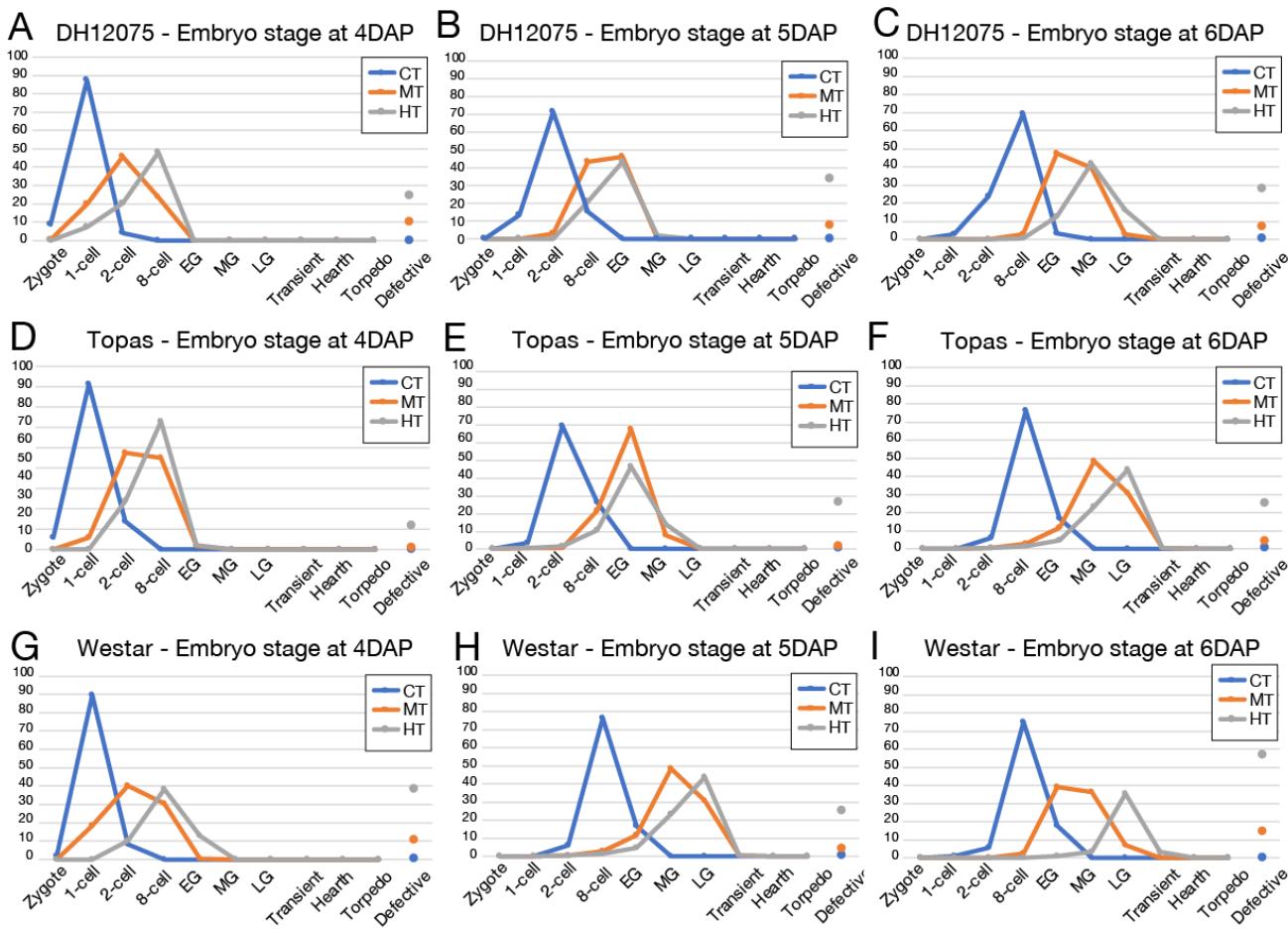
Supplementary Figure 2. High temperatures affect growth parameters of *Brassica* flowering plants. (A, B) The number of leaves(A), and the number of primary branches (NB) (B) were quantified in DH12075, Topas, and Westar cultivars at CT (21/18 °C, green), MT (28/18 °C, yellow), and HT (34/18 °C, red). Growth parameters are presented as boxplots (the box represents the interquartile range and the line inside the box represents the median). Each dot is an observation(A), or only outliers (B). The Pearson correlation coefficient between LSM, FT and NF is shown in **Supplementary Table S2**. Boxes with the same letters (a, b) within each cultivar do not differ significantly ($p < 0.05$). (C-E) Graphs display the differences between CT and MT (yellow), and CT and HT (orange) in flowering time (FT, days), length of the main stem (LMS, cm), number of branches (NB), number of flowers (NF), number of seeds (NS) and seed weight in DH12075 (D), Topas (E) and Westar (F). Dots represent

the mean values of the differences and error bars, the 95 % confidence interval. Data on FT, LMS and NF are presented in Figure 1. Data on NS and seed weight are presented in Figure 5.

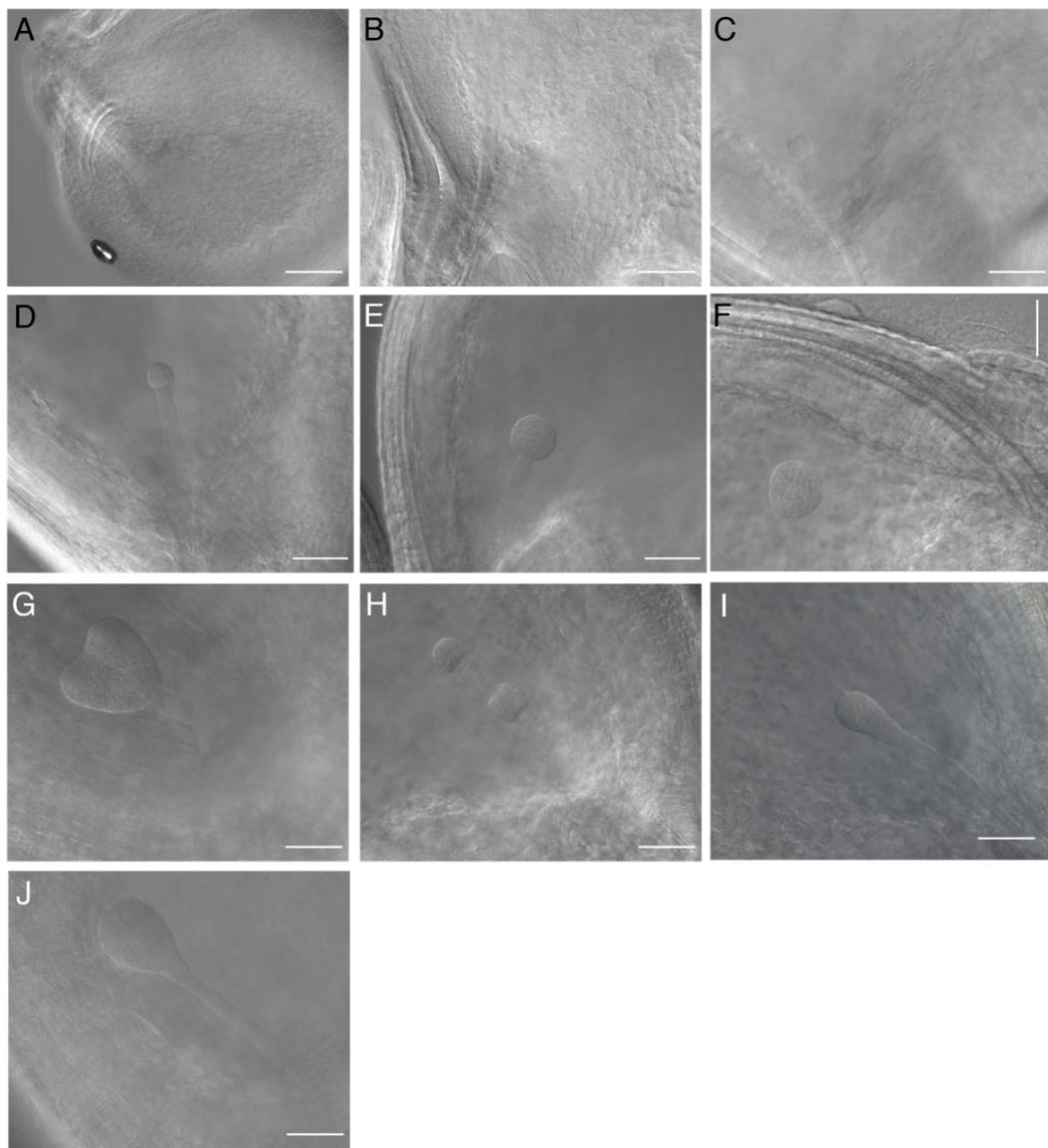


Supplementary Figure S3. The number of ovules and the pollen development are not affected by high temperatures. (A) Graph displaying the number of ovules per pistil in DH12075, Topas and Westar cultivars at CT (21/18 °C, green), MT (28/18 °C, yellow) and HT (34/18 °C, red). Relates to Table 1. The number of ovules per pistil is presented as boxplots (the box represents the interquartile range and the line inside the box represents the median). Each dot indicates outliers. Boxes with the same letters (a, b) within each cultivar do no differ significantly ($p < 0.05$). (B, C) Pollen grain development is not affected by our growth conditions. Pollen grains of Westar were assayed for viability with Alexander staining. No differences were observed in pollen viability from plants grown at 21 °C (B) and 34 °C

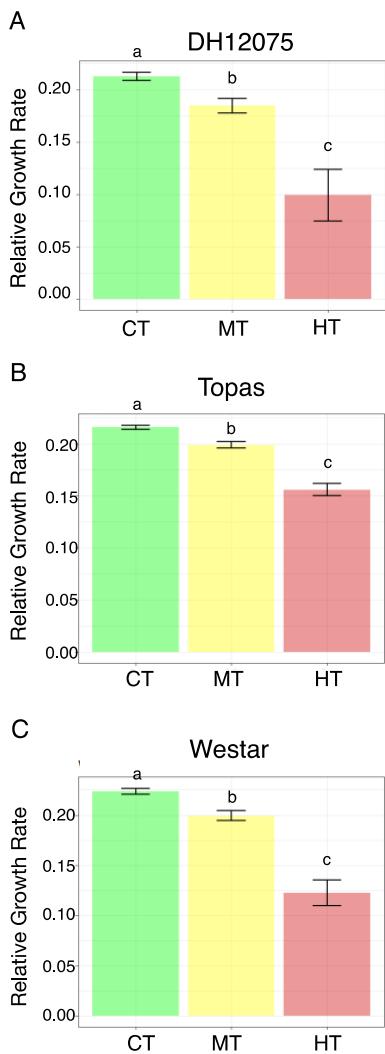
(C). Scale bars represent 100 µm. **(D)** Table with the count of viable pollen grains by growth conditions per cultivar. The 95% confidence interval is given as a percentage.



Supplementary Figure S4. Embryo development is accelerated by high temperatures. Graphs displaying the distribution (as a percentage) of embryonic development stages per siliques at 4 DAP (A, D, G), 5 DAP (B, E, H) and 6 DAP (C, F, I) in DH12075 (A-C), Topas (D-F), and Westar plants (G-I) grown at CT (21/18 °C, blue), MT (28/18 °C, orange) and HT (34/18 °C, grey). EG, early globular; MG, mid-globular; LG, late globular embryos. Relates to Table 2.



Supplementary Figure S5. High temperatures affect embryo development. Original pictures are presented in Figure 4. **(A-G)** Embryos from plants grown at CT (21/18 °C). **(H-J)** Range of defective embryos observed in *B. napus* plants grown at MT (28/18 °C) and HT (34/18 °C) between 6 and 8 DAP. Scale bars represent 100 µm.



Supplementary Figure S6. Growth rate of siliques is reduced by growth at elevated temperatures. Graphs displaying the relative growth rate of siliques between 0 DAP to 11 DAP in DH12075 (**A**), Topas (**B**) and Westar (**C**) plants grown at CT (green), MT (yellow) and HT (red). Shown are barplots with 95 % confidence intervals. Bars with the different letters (a, b, c) within each cultivar differ significantly ($p < 0.05$).

1.2 Supplementary Tables

Supplementary Table S1. Primers used in qPCR analysis and LOC number of amplified genes

Primer name	Primer sequence	Targeted LOCs in <i>B. napus</i>	Homologous genes in <i>Arabidopsis</i>
BnaMYB34-FW	ACGTCGATTCTCCGACCAA	LOC106374996	<i>MYB34</i>
BnaMYB34 REV	TATGAAACCGCTTGACGCTG	LOC106434612	<i>At5g60890</i>
BnaCYP79B-FW	CTTGGAAAGTTGCCTGAGAATG		<i>CYP79B2</i>
BnaCYP79B-REV	GAACGATGCTCTGGGAGTC	LOC106439341	<i>At4g39950</i> <i>CYP79B3</i> <i>At2g22330</i>
BnaDAOs-FW	TTTGTGGATGCTAACATCCG	LOC106414697 LOC106430970 LOC106369131 LOC106399859 LOC106380301 LOC106382334 LOC106414671 LOC106369133 LOC106362101 LOC106379949	<i>DAO1</i> <i>At1g14130</i>
BnaDAOs-REV	TAAGCTTGAGAGCTTCTCCATC		<i>DAO2</i> <i>At1g14120</i>
BnaGH3.9s-FW	ACGTGGTTCTAACGATCGAC	LOC106392343	<i>GH3.9</i>
BnaGH3.9s-REV	GCGAGGAATGCCTGTTTC	LOC106396677 LOC106392367 LOC106450413	<i>At2g47750</i>
BnaACGH3.1-FW	AGTTGTTAGTGAGGGACGGA	LOC106409452	<i>GH3.1</i>
BnaACGH3.1-REV	CGGTAAACCGAGTTCAACGA	LOC106432954	<i>At2g14960</i>
BnaGH3.5s_FW	AAGAACGCAATGACACACCT	LOC106409348	<i>GH3.5</i>
BnaGH3.5s_REV	TGGCCAGGGATAGAACTTGT	LOC106376087 LOC106440950 LOC106445777	<i>At4g27260</i>
BnaNCED9-FW2	GTGACGGTAAGTTCGGAGGA	LOC106354557	<i>NCED9</i>
BnaNCED9-REV2	GATCTCACATTTCTCGTCGT	LOC106354700 LOC106441091	<i>At1g78390</i>
BnaABA1-2 FW1	TTCGGACAAGAAGGCGG		<i>ABA1/ZEP</i>
BnaABA1-2 REV1	AATTCAACCGTCGGTTACTCAA	LOC106390346	<i>At5g67030</i>
BnaPHYA-FW1	TTGTGTCAGAACAGCAGCTCAG	LOC106400740	<i>PHYA</i>
BnaPHYA-REV1	TCCAGATCCAAGCACCCTTC	LOC106346267 LOC106417700 LOC106420085	<i>At1g09570</i>
BnaELF4-FW1	GGAGCAGGGAGGAGAAGATC	LOC106420605 LOC106394477 LOC106394476 LOC106438048 LOC106399222 LOC106454592	<i>ELF4</i>
BnaELF4-REV1	GGTGATTGTCGTTGACCTGC	LOC106399325	<i>At2g40080</i>

BnaFT-FW2	GAGGTGACAAATGGGTTGGA	LOC106402982 LOC111213971 LOC111207300 LOC106426212	<i>FT</i> <i>At1g65480</i>
BnaFT-REV2	GCTAGGACTTGGAACATCTGG		<i>TSF</i> <i>At4g20370</i>
BnaRNPII37c-FW1 BnaRNPII37c-REV1	TAACGACAAGGGAAGGCTGT GTGTTCCCTCATCCTCTGCCT	LOC106377472 LOC106353095	<i>HSP70</i> <i>At3g12580</i>
BnaSCL30A-FW2 BnaSCL30A-REV2	AACGATCAAGGGGAAGGACAA CCTGTAGACTGGCGAACGT	LOC106412002 LOC106452514	<i>SCL30A</i> <i>At3g13570</i>
BnaSLU7-FW1 BnaSLU7-REV1	GTCGTGGTGGAAAGGATCAAC CTTCCTCAGCCGCCTCTATC	LOC106446239 LOC106453449	<i>SMP1</i> <i>At1g65660</i>
BnaFBA6-FW2 BnaFBA6-REV2	GTTGAAGACTTGGGGAGGGA TCAGAGTTAGCCTTGCACCT	LOC106391482 LOC106395718 LOC106367801 LOC111206734 LOC106367922 LOC106452433 LOC106428297 LOC106445170	<i>FBA6</i> <i>At2g36460</i>
BnaDRM2-FW2 BnaDRM2-REV2	TCTCACCCAAACTCTCCCAC TCACCAGCATGTCACTCCAT	LOC106451187 LOC106347199 LOC106437808	<i>DRM2</i> <i>At2g33830</i>
BnaTMA7-FW1 BnaTMA7-REV1	TTCCTGTGTTTATCCATGTAGCC CAGTCACTCTCCTACGAACATGATAG	LOC106452133	<i>TMA7</i> <i>At1g15270</i> <i>At3g16040</i>
BnaACT7-FW1 BnaACT7-REV1	GAGCAGCATGAAGATCAAGGT CTTCGAGATCCACATCTGTTGG	LOC106382989 LOC106426760 LOC106426759	<i>ACT7</i> <i>At5g09810</i>
BnaeIF5A-FW1 BnaeIF5A-REV1	ATCTCAGCGCTCTGATGAAGA ACTATTGGTTACTGCCACC	LOC106389545	<i>eIF5A</i> <i>At1g13950</i> <i>At1g26630</i> <i>At1g69410</i>

Supplementary Table S2. Pearson correlation coefficient between the length of the main inflorescence stem, the flowering time duration and the number of flowers.

	LMSxFT			LMSxNF			FTxNF		
	21	28	34	21	28	34	21	28	34
DH12075	0.88	0.63	0.78	0.77	0.43	0.77	0.76	0.57	0.23
Topas	0.80	0.79	0.41	0.81	0.90	0.44	0.79	0.77	0.37
Westar	0.81	0.63	0.60	0.81	0.74	0.22	0.76	0.58	0.22

Strong correlation 1-0.5, medium 0.5 -0.3, small < 0.3

Supplementary Table S3. Auxin and auxin metabolites measurements (source data of Figures 2 and 7). One representative biological replicate is presented.

sample		average		S.D.	av.	S.D.	av.	S.D.
type	temp	TRP		IAOx			ANT	
Pistil	21 CT	26 006.7	± 5 356.7 RSD 21%	194.9	± 42.5 RSD 22%	408.5	± 113.7 RSD 28%	
	34 HT	43 330.1	± 4 248.1 RSD 10% ** / 0.00203	155.8	± 23.8 RSD 15% - / 0.18946	436.4	± 160.6 RSD 37% - / 0.78438	
5 DAP	21 CT	79 755.2	± 18 901.1 RSD 24%	34.4	± 2.5 RSD 7%	410.9	± 193.1 RSD 47%	
	34 HT	31 326.1	± 8 433.4 RSD 27% ** / 0.00158	<LOD		357.4	± 154.2 RSD 43% - / 0.69579	
26 DAP	21 CT	9 491.1	± 7 234.0 RSD 76%	14 728.9	± 1 560.1 RSD 11%	NQ		
	34 HT	26 807.7	± 11 659.5 RSD 43% * / 0.03558	6 946.5	± 541.2 RSD 8% *** / 0.00007	NQ		

sample		average		S.D.	av.	S.D.	av.	S.D.
type	temp	IPyA		IAM		IAN		
Pistil	21 CT	12 271.6	± 2 001.4 RSD 16%	1 123.2	± 241.9 RSD 22%	92 234.3	± 4 992.8 RSD 5%	
	34 HT	15 655.5	± 3 640.1 RSD 23% - / 0.14192	2 550.0	± 766.7 RSD 30% * / 0.01595	217 595.1	± 14 508.7 RSD 7% *** / 0.00000	
5 DAP	21 CT	7 435.3	± 802.1 RSD 11%	270.6	± 15.3 RSD 6%	17 592.6	± 1 011.9 RSD 6%	
	34 HT	6 755.5	± 961.2 RSD 14% - / 0.30912	260.6	± 53.3 RSD 20% - / 0.75714	28 585.4	± 2 034.0 RSD 7% *** / 0.00005	
26 DAP	21 CT	14 813.1	± 2 759.9 RSD 19%	NQ		33 648.6	± 2 111.6 RSD 6%	
	34 HT	7 990.2	± 992.5 RSD 12% ** / 0.00164	NQ		12 648.1	± 984.5 RSD 8% *** / 0.00000	

sample		average		S.D.	av.	S.D.	av.	S.D.
type	temp	IAA		oxIAA			IAAsp	
Pistil	21 CT	429.7	± 171.1 RSD 40%	292.9	± 43.4 RSD 15%	6.6	± 0.8 RSD 12%	
	34 HT	311.0	± 11.2 RSD 4% - / 0.20350	298.9	± 18.4 RSD 6% - / 0.81269	4.5	± 1.3 RSD 29% - / 0.05361	
5 DAP	21 CT	2 746.6	± 108.8 RSD 4%	11 409.7	± 603.0 RSD 5%	43.8	± 2.4 RSD 6%	
	34 HT	2 132.2	± 83.1 RSD 4% *** / 0.00006	15 045.6	± 914.7 RSD 6% *** / 0.00052	23.1	± 2.1 RSD 9% *** / 0.00001	
26 DAP	21 CT	14 152.9	± 371.3 RSD 3%	110 339.3	± 6 979.5 RSD 6%	87.5	± 6.1 RSD 7%	
	34 HT	3 904.8	± 585.7 RSD 15% *** / 0.00000	44 577.9	± 3 869.0 RSD 9% *** / 0.00000	2 087.7	± 172.8 RSD 8% *** / 0.00000	

sample		average	S.D.	av.	S.D.	av.	S.D.
type	temp	IAGlu		IAA-Glc		oxIAA-Glc	
Pistil	21 CT	<LOD		143.3	± 29.7 RSD 21%	2 923.0	± 169.6 RSD 6%
	34 HT	<LOD		150.8	± 18.2 RSD 12% - / 0.69455	1 690.0	± 83.5 RSD 5% *** / 0.00000
5 DAP	21 CT	62.9	± 6.4 RSD 10%	876.5	± 57.3 RSD 7%	43 524.6	± 2 863.5 RSD 7%
	34 HT	54.4	± 9.0 RSD 17% - / 0.20301	478.4	± 68.5 RSD 14% *** / 0.00008	43 785.4	± 1 864.8 RSD 4% - / 0.88867
26 DAP	21 CT	17.2	± 1.8 RSD 11%	NQ		788 378.5	± 46 734.7 RSD 6%
	34 HT	96.1	± 5.8 RSD 6% *** / 0.00000	NQ		150 035.7	± 11 672.8 RSD 8% *** / 0.00000

NQ: not quantified; <LOD: below the limit of detection; CT: control temperature; HT: hight temperature; av.: average; S.D.; standard deviation; RSD: relative standard deviation (ratio of the standard deviation over the average). Asterisks indicate statistically significant difference in HT in a paired Student's t-test (t-test; *, **, and *** correspond to P-values of $0.05 > p > 0.01$, $0.01 > p > 0.001$, and $p < 0.001$, respectively). All measurements are as pmol/g FW.

Supplementary Table S4. ABA measurements (source data of Figures 3 and 6)

sample		average		S.D.
type	temp.	ABA		
Pistil	21	1 101.6	±	77.8
	CT	RSD		7%
	34	513.6	±	30.3
	HT	RSD		6%
5 DAP	21	1 329.7	±	32.0
	CT	RSD		2%
	34	1 372.4	±	63.8
	HT	RSD		5%
26 DAP	21	6 750.6	±	283.6
	CT	RSD		4%
	34	2 567.6	±	100.3
	HT	RSD		4%
		***	/	0.00000

NQ: not quantified; CT: control temperature; HT: high temperature; S.D.; standard deviation; RSD: relative standard deviation (ratio of the standard deviation over the average). Asterisks indicate a statistically significant difference in HT in a paired Student's t-test (t-test; *** correspond to P-values of $p < 0.001$).

Supplementary Table S5. Pearson correlation coefficient between the siliques growth rate, the seed number per siliques and the growth temperatures

Cultivars	CT (21°C)	MT (28°C)	HT (34°C)
DH12075	0.83	0.96	0.99
Topas	0.63	0.92	0.92
Westar	0.67	0.92	0.96

Strong correlation 1-0.5, medium 0.5 -0.3, small < 0.3

Supplementary Table S6. Glucosinolates, Nitrogen and seed oil measurement (source data of Figure 9).

sample		av.		S.D.		av.		S.D.		av.		S.D.	
Cult.	temp	GSL in 9% humidity				Nitrogen compound				Oil in dry matter			
Topas	21 CT	10.61 RSD	±	0.46 4.36%		21.11 RSD	±	0.63 2.97%		42.33 RSD	±	1.55 3.65%	
	28 MT	12.77 RSD **	±	2.05 16.05% / 0.01008		26.71 RSD ***	±	0.70 2.61% / 0.00000		40.14 RSD *	±	1.73 4.30% / 0.01664	
Westar	21 CT	8.61 RSD	±	1.07 12.38%		20.94 RSD	±	0.52 2.48%		43.94 RSD	±	1.20 2.74%	
	28 MT	11.05 RSD **	±	1.87 16.89% / 0.00540		24.78 RSD ***	±	0.65 2.64% / 0.00000		39.00 RSD ***	±	0.83 2.12% / 0.00000	
DH12075	21 CT	11.51 RSD	±	0.79 6.91%		20.30 RSD	±	0.72 3.54%		42.27 RSD	±	1.97 4.66%	
	28 MT	16.80 RSD ***	±	1.35 8.02% / 0.00000		24.12 RSD ***	±	0.99 4.12% / 0.00000		36.24 RSD ***	±	1.49 4.11% / 0.00000	

sample		av.		S.D.		av.		S.D.		ODP		LDP	
Cult.	temp	Palmitic acid				Stearic acid				ODP		LDP	
Topas	21 CT	4.23 RSD	±	0.17 4.04%		2.40 RSD	±	0.19 7.99%		27.3895		31.2718	
	28 MT	3.71 RSD ***	±	0.17 4.60% / 0.00002		2.50 RSD -	±	0.28 11.29% / 0.42912		24.9661		27.8822	
Westar	21 CT	4.03 RSD	±	0.16 3.90%		2.30 RSD	±	0.12 5.12%		23.1731		28.9365	
	28 MT	3.90 RSD -	±	0.16 4.19% / 0.11280		2.21 RSD -	±	0.16 7.36% / 0.20737		20.3773		25.7554	
DH12075	21 CT	4.09 RSD	±	0.16 4.02%		2.21 RSD	±	0.21 9.68%		25.0497		31.1685	
	28 MT	4.20 RSD -	±	0.12 2.86% / 0.17945		2.55 RSD **	±	0.12 4.76% / 0.00129		22.1162		26.4949	

sample		av.		S.D.		av.		S.D.		av.		S.D.	
Cult.	temp	Oleic acid				Linoleic acid				Linolenic acid			
Topas	21 CT	66.63 RSD	±	0.56 0.84%		17.27 RSD	±	0.63 3.63%		7.86 RSD	±	0.30 3.79%	
	28 MT	69.49 RSD ***	±	1.29 1.86% / 0.00003		16.67 RSD -	±	0.90 5.42% / 0.14231		6.45 RSD ***	±	0.33 5.11% / 0.00000	
Westar	21 CT	70.63 RSD	±	0.96 1.36%		15.14 RSD	±	0.77 5.08%		6.16 RSD	±	0.33 5.39%	
	28 MT	74.00 RSD ***	±	0.66 0.89% / 0.00000		14.06 RSD **	±	0.70 5.00% / 0.00990		4.88 RSD ***	±	0.22 4.53% / 0.00000	
DH12075	21 CT	68.34 RSD	±	1.16 1.70%		15.72 RSD	±	0.80 5.10%		7.12 RSD	±	0.37 5.26%	
	28 MT	70.93 RSD ***	±	1.09 1.53% / 0.00029		14.81 RSD -	±	1.15 7.76% / 0.08300		5.34 RSD ***	±	0.30 5.60% / 0.00000	

CT: control temperature; MT: mid temperature; av.: average; S.D.; standard deviation; RSD: relative standard deviation (ratio of the standard deviation over the average); ODP: Oleic desaturation proportion; LDP: Linoleic desaturation proportion. Asterisks indicate statistically significant difference in MT compared to CT in a paired Student's t-test (t-test; *, **, and *** correspond to P-values of $0.05 > p > 0.01$, $0.01 > p > 0.001$, and $p < 0.001$, respectively). GSL is quantified as $\mu\text{mol/g}$ FW. Nitrogen compound and total oil in dry matter are quantified as % of FW. Fatty acids are quantified as % of total oil content.