Article name: Copy number and haplotype variation at the *VRN-A1* and central *FR-A2* loci are associated with frost tolerance in hexaploid wheat

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Fig. S1 CAPS markers used to distinguish *FR-A2* haplotypes

Lane M: DNA marker. Lane 1: *CBF-A12* band type for 'FR-A2-S'. Lane 2: *CBF-A12* band type for 'FR-A2-T' (*CBF-A12* was digested with *Zra*I). Lane 3: *CBF-A15* band type for 'FR-A2-S'. Lane 5: *CBF-A15* band type for 'FR-A2-T' (*CBF-A15* was digested with *Sal*I).

Table S1 List of the 65 winter wheat accessions in the 'winter panel'

FR-A2 Haplotype	N	Origin	Varieties
FR-A2-S	7	France	Alma (PI 174592),
		Denmark	Viking (PI 316424)
		Germany	Bussard (PI 300949)
		Poland	Mira (PI 338396)
		USA	ORFW
		Serbia	Sava (PI 323638)
		Unknown	Malakov [a] (PI 351207)
FR-A2-T	58	USA	AGS2000 (PI 612956), Arthur (CItr 14425), Bavaria (PI 606754), Boundary (PI 603039), Chukar (PI 628641), Centurk 78 (CItr 17724), Crimson (PI 601818), Daws (CItr 17419), Eltan (PI 536994), Expedition (PI 629060), Endurance (PI 639233), Frankenmuth (CItr 17830), Freedom (PI 562382), Geneva (PI 505819), Harding (PI 608049), Hopewell (PI 595678), IDO621, Jagger (PI 593688), Jerry (PI 632433), Karl (PI 527480), Millennium (PI 613099), Minhardi (CItr 5149), Moro (CItr 13740), OH552, Redwin (CItr 17844), SD97250, Seward (PI 508289), Simon (PI 636132), Stephens (CItr 17596), Tiber (PI 517194), Trego (PI 612576), Wahoo (PI 619098), Wanser (CItr 13844), Wesley (PI 605742), Xerpha (PI 645605)
		Russia	Alabasskaja (PI 262636), Albatros Odesskii (PI 565418), Bezostaja 1 (PI 345685), Kavkaz (PI 361879), Ilichevka (PI 410425), Inna (PI 591957), Odesskaja117 (PI 592065), Purkov (CItr 8381)
		Ukraine	Mironovskaja 808 (PI 392080), Volgogradskaja 84 (PI 565410)
		Finland	Olympia (PI 175514), Vakka (PI 265483)
		Canada	CDC Kestrel (CN 18797), CDC Clair (CN 106345), Chernova, Norstar (CItr 17735)
		Romania	F26-67 (PI 367713), Fundulea 158-69 (PI 519086), Fundulea 133 (PI 519268), Fundulea 174-71 (PI 519097), Fundulea 63-70 (PI 519128), Moldova (PI 350311)
		Switzerland	Munstertaler (PI 351176)

[a] Malakov: provenance listed as former USSR, likely originated from Crimea.

Table S2 List of the 81 spring wheat accessions in the 'spring panel'

FR-A2 haplotype	N	Origin	Varieties
FR-A2-S	53	Australia	Baart (PI 5078), Bobin (PI 106120), Cleveland (PI 89191), Clubhead (PI 116224), Dundee (PI 89424), Eden (PI 224658), Falcon (PI 292578), Florence (PI 38349), Gabo (PI 155431), Gluclub (PI 67326), Gular (PI 113489), Insignia (PI 210975), Steinwedel (PI 41081), Yandilla King (PI 42120)
		North America	24IBWSB#67 (From CIMMYT), 775 (PI 601334), Borlaug M95 (From CIMMYT), Cajeme 71 (PI 412955), Fielder (CItr 17268), Gabo 55 (PI 583713), Hope (CItr 8178), Inia 66 (PI 412973), Kenya (PI 192099), Kentana 48 (CItr 12980), Louise (PI 634865), Marquis (CItr 3641), Prospur (CItr 17408), Prelude (CItr 4323), Spring field (CItr 14589), Sonora 64 (CItr 13930), Sterling (CItr 17859), Tadinia (PI 494096), Thatcher (PI 168659), Timstein (CItr 12347), Yaqui 50 (PI 210890), Yaqui 54 (CItr 13218), Yecora Rojo (CItr 17414)
		Europe	Ladoga (CItr 4795), Peragis (PI 184582), Sol (CItr 6009)
		Africa	Kenya 58 (CItr 12471), Kenya 324 (PI 283840), Kenya C 9906 (PI 351682)
		South America	Americano 44D (PI 191937), Barleta (CItr 8398), Chino (CItr 12601), Klein 157 (PI 161825), Klein Lucero (CItr 14047), Napo 63 (PI 337711), Tezanos Pintos Precoz (PI 345731)
		Asia	Aka-daruma (PI 325843), Gehun (PI 116066), NP876 (PI 322271)
FR-A2-T	28	Australia	AUS 90168 (PI 422410), Currawa (PI 42105), Federation (PI 41080), Fife (PI 283820), Gallipoli (PI 55857), Hard federation (PI 41079), Indian F (PI 93986), Ranee (PI 67875)
		North America	Alpowa (PI 566596), Lerma 52 (PI 210887), Lerma rojo (CItr 13651), Lerma rojo 64 (CItr 13929), Lerma rojo 64A (PI 342642), Little club (CItr 4066), Red fife (PI 348919), Siete cerros 66 (PI 338921), Sonora 64A (PI 320109), White fife (CItr 4412), Yaktana 54A (PI 351913)
		Europe	Heines Kolben (CItr 11772), Lutescens 62 (PI 74489), Mentana (CItr 12448), Noe (CItr 5015), Squarehead (PI 51694)
		Africa	Chile 1B (PI 320098), Red Egyptian (CItr 12345)
		South America	Frontana (CItr 12470)
		Asia	Aka komugi (PI 45234)

Table S3 Genomic specific primers and PCR conditions used to amplify and sequence the *CBF12*, *CBF14* and *CBF15* homoeologs from the A, B and D genomes of hexaploid wheat

***CBF* Gene**	**Forward Primer (Forward 5'-3')**	**Reverse Primer (Reverse 5'-3')**	**Product Length (bp)**	**Touch down PCR annealing temperatures**
A12	GAGGGAAGCTGCCTTTTCTT	ATGCCCATCATGGGTCCACAC	1188	65-58 ºC, 1 ºC decline /cycle 58 ºC, 32 cycles
A14	CACCGCATGCAGCTAAACT	CAATGAACGAGCACATATGGA	819	65-58 ºC, 1 ºC decline /cycle 58 ºC, 32 cycles
A15	CAAGCATAACCAACACTCCT	ATCAAGCAGCGATGTCCTTT	1017	65-58 ºC, 1 ºC decline /cycle 58 ºC, 32 cycles
B12	GGCAAGCCACTGGTTTACTG	GGCCTTCATATGTCCAGGAG	1065	70-62 ºC, 1 ºC decline /cycle 62 ºC, 34 cycles
B14	TCAACGCAGCAGCTAAACAC	CAATGAACGAGCACATACGG	842	70-62 ºC, 1 ºC decline /cycle 62 ºC, 34 cycles
B15	CAAGCATAACCAACACTCCT	TCCATAGATCGACCTTGGCT	971	70-62 ºC, 1 ºC decline /cycle 62 ºC, 34 cycles
D12	TACGTACGACCGCCTAGCTT	CAGACTTGGCATGTCCAGAA	957	70-62 ºC, 1 ºC decline /cycle 62 ºC, 34 cycles
D14	GATCGATCGATGGACGCCGT	ATATCCCCATCTGTTTCCTCCCG	725	65-55 ºC, 1 ºC decline /cycle 55 ºC, 30 cycles
D15	CTCCATAGATCGACCTCGCT	CAAAGCAGCTGGCTGGAATA	804	60-50 ºC, 1 ºC decline /cycle 50 ºC, 30 cycles

Table S4 Primers and probes used in the Taqman® assays for the CNV analysis at *VRN-A1* and the central cluster of the *FR-A2* locus. *VRN-A1* and *CO2* assays adapted from Díaz et al. (2012).

Gene	F primer (5'-3')	R primer (5'-3')	Taqman® Probe
VRN-A1	GCAGCCCACTTTTGGTCTCTA	TCTGCCCTCTCGCCTGTT	FAM-TGTGTTCGCTTTGGTTGTGCAGCA-BHQ1
CBF-A12	GCCAGACAATGCCAGCTC	ACATGTCGTGGCACAATGC	FAM-TCGGCGGCGGCGGCCCACTCACA-BHQ1
CBF-A14	TTGAATGAGCACTGGTTTGG	CACCGAGCTCAAAGTCTTCC	FAM-TCCATGAACTCCGAGTAGCA-BHQ1
CBF-A15	GTCGTCCATGGAAAATACCG	ATGTGTCCAGGTCCATTTCC	FAM-TCGTCGTCGTCGTCCCTACCTTCT-BHQ1
CO2	TGCTAACCGTGTGGCATCAC	GGTACATAGTGCTGCTGCATCTG	CY5-CATGAGCGTGTGCGTGTCTGCG-BHQ3

Table S5 *T. dicoccoides* accessions assayed and their FR-A2 haplotype.

***T. dicoccoides* sub-population**	***FR-A2* haplotype**	**N**	**Accessions**
Northern (Iraq, Turkey)	S	4	UH42, UH43, UH44, PI 428017
	T	9	UH41, PI 428020, PI 428028, PI 428036, PI 428041, PI 428047, PI 428055, PI 428058, PI 428061
Southern (Israel, Lebanon)	S	8	UH7, UH9, UH11, UH17, UH19, UH24, UH30, UH33
	T	4	UH1, UH23, UH27, UH32

Accession numbers are either PI numbers from the GRIN database, or correspond to 'Location–Genotype' identification numbers from the University of Haifa (UH) wheat germplasm collection (Nevo and Beiles 1989; Peleg et al. 2005).

Table S6 qRT-PCR primers used in this study and their amplification efficiencies

Target	Forward primer (5' – 3')	Reverse primer (5' – 3')	Efficiency (%)
CBF12	GCCAGACAATGCCAGCTC	ACATGTCGTGGCACAATGC	92.3
CBF14	CCACCAAATATGGGAGGAAA	GCTTTCACAATGAACGAGCA	93.8
CBF15	CATGTTCGAGCTGGATATGTCCGGGG	CAGCTTCGGTTTGTTCCATGC	96.4
ACTIN	ACCTTCAGTTGCCCAGCAAT	CAGAGTCGAGCACAATACCAGTTG	99.1

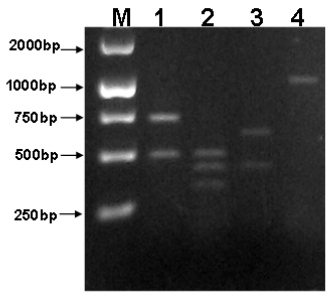

Fig. S1 CAP markers used to distinguish *FR-A2* haplotypes. Lane M: DNA marker

Lane 1: *CBF-A12* band type for 'FR-A2-S'. Lane 2: *CBF-A12* band type for 'FR-A2-T' (*CBF-A12* was digested with *Zra*I). Lane 3: *CBF-A15* band type for 'FR-A2-S'. Lane 5: *CBF-A15* band type for 'FR-A2-T' (*CBF-A15* was digested with *Sal*I).