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ORIGINAL PAPER

Strong presence of the high grain protein content allele of *NAM-B1* in Fennoscandian wheat

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Abstract Grain protein content in wheat has been shown to be affected by the *NAM-B1* gene where the wildtype allele confers high levels of protein and micronutrients but can reduce yield. Two known non-functional alleles instead increase yield but lead to lower levels of protein and micronutrients. The wildtype allele in hexaploid bread wheat is so far mainly known from historical specimens and a few lines with an emmer wheat introgression. Here we report a screening for the wildtype allele in wheats of different origin. First, a worldwide core collection of 367 bread wheats with worldwide origin was screened and five accessions carrying the wildtype *NAM-B1* allele were found. Several of these could be traced to a Fennoscandian origin and the wildtype allele was more frequent in spring wheat. These findings, together with the late maturation of spring wheat, suggested that the faster maturation caused by the wildtype allele might have preserved it in areas with a short growing season. Thus a second set consisting of 138 spring wheats of a northern origin was screened and as many as 33 % of the accessions had the wildtype allele, all of a Fennoscandian origin. The presence of the wildtype allele in landraces and cultivars is in agreement with the use of landraces in Fennoscandian wheat breeding. Last, 22 spelt wheats, a wheat type previously suggested to carry the wildtype allele, were screened and five wildtype accessions found. The wildtype *NAM-B1* accessions found could be a suitable material for plant breeding efforts directed towards increasing the nutrient content of bread wheat.

Introduction

Bread wheat, *Triticum aestivum* spp. *aestivum*, is a major crop with an annual production of over 650 million tonnes (Food Outlook, http://www.fao.org). The majority of wheat is used for human consumption making it one of the most important sources of not only carbohydrates, but also vegetable protein in human food. This has led to a large interest into the genetic background of grain protein content (GPC) in wheat. Wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) was already in the 1970s suggested as a potential gene source for improving protein content in wheat (Avivi 1978). Crosses between wild emmer wheat and durum wheat (*T. turgidum* ssp. *durum*) were made and substitution lines with high GPC identified (Joppa and Cantrell 1990). Joppa et al. (1997) reported that a quantitative trait locus (QTL) explained 66 % of the variation in

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GPC in the substitution lines. The QTL was later shown to be a simple Mendelian locus, *Gpc-B1* (Chee et al. 2001; Olmos et al. 2003; Distelfeld et al. 2004; Uauy et al. 2006a). Uauy et al. (2006a) positionally cloned *Gpc-B1* showing it to be a No Apical Meristem protein (NAM) belonging to the NAC transcription factor family and therefore renaming it *NAM-B1*.

The increase in GPC caused by the functional *NAM-B1* allele has been found to be very stable through different environments and genetic backgrounds, including both tetra- and hexaploid wheats, containing a *Gpc-B1* introgression from *T. turgidum* spp. *dicoccoides* (e.g. Joppa and Cantrell 1990; Joppa et al. 1991, 1997; Cantrell and Joppa 1991; Steiger et al. 1996; Mesfin et al. 1999; Chee et al. 2001; Uauy et al. 2006b; Distelfeld et al. 2007; Brevis and Dubcovsky 2010; Brevis et al. 2010). Pleiotropic effects on yield and grain weight are not always pronounced, but in some genetic backgrounds and environments grain weight decrease when the functional allele is present (Joppa et al. 1991; Uauy et al. 2006b; Brevis and Dubcovsky 2010; Brevis et al. 2010). The negative effect on grain weight appears stronger in tetraploids than in hexaploids (Brevis et al. 2010). Additionally, the functional *NAM-B1* allele accelerates flag leaf senescense and efflux of N, Fe, Zn and Mn from flag leaves to the developing grains (Uauy et al. 2006a, b; Distelfeld et al. 2007). In contrast, in RNAi knockouts targeting NAM genes on all chromosomes plant senescence can be delayed by as much as 3 weeks (Uauy et al. 2006a; Waters et al. 2009). This difference in length of the grain filling period could well explain the effects of grain weight. Taken together, the different studies paint an overall picture where the functional allele contributes to higher protein and mineral content and more rapid senescence, but could have negative influence on seed size whereas null alleles have the opposite effects.

Three allelic variants of *NAM-B1* are known. In addition to the functional wildtype allele present in wild and cultivated emmer wheat Uauy et al. (2006a) found the low GPC allele of *NAM-B1* in all 57 durum wheat varieties screened. These durum wheat accessions had a 1-bp insertion causing a frame-shift mutation making it non-functional. In the 37 accessions of hexaploid wheat screened by Uauy et al. (2006a) five carried the 1-bp insertion allele and in another 31 accessions the *NAM-B1* gene seemed to be partially or fully deleted. The size of the deletion is not known, but seems to be at least 100 kb (unpublished data). In none of the durum and only one of the hexaploid wheats screened by Uauy et al. (2006a) was the wildtype allele recovered. The wheat with the wildtype allele was assumed by Uauy et al. (2006a) to result from a wild emmer wheat chromosome segment introgression.

The lack of examples of the wildtype *NAM-B1* allele in durum and bread wheat suggested that it had been lost during wheat domestication (Uauy et al. 2006a; Dubcovsky and Dvorak 2007). However, most of the varieties screened were developed in the US during the twentieth century. Asplund et al. (2010) instead focused on obsolete nineteenth century wheat cultivars from before the era of modern plant breeding and showed, using historical specimens, that the wildtype allele was still present in some widely cultivated varieties at that time. They suggested that if the wildtype allele had indeed been lost it had been so during the last century of plant improvement rather than during domestication.

It is, however, also possible that the wildtype allele is still present in extant wheat accessions stored in genebanks. For example, the shorter maturation time conferred by the *NAM-B1* wildtype may have contributed to its preservation in areas with a short growing season. The objective of the study was to investigate the presence of the *NAM-B1* wildtype allele in wheats of different origin. Finding the high GPC allele in wheats adapted to different climatic conditions and end uses would be very valuable for worldwide wheat breeding. Therefore, we explored the presence or absence of the *NAM-B1* wildtype allele in extant wheat accessions by screening 527 wheat accessions for the three *NAM-B1* alleles described by Uauy et al. (2006a). These accessions were of different cultivation status and registration dates and from different geographic areas, covering most of the regions where wheat is presently cultivated.

Materials and methods

Plant material

Three sets of materials were used: (1) a set of 367 worldwide bread wheat accessions belonging to the INRA core collection. This collection captures more than 98 % of the total allelic diversity observed at 38 SSR polymorphic loci in a sample of 4,000 bread wheat accessions and has been selected to represent the genetic diversity present in cultivated wheat (Balfourier et al. 2007); (2) Spring wheats from Northern latitudes (in total 138 accessions), 43 accessions provided by INRA Genetic Resource Center—Clermont-Ferrand, 88 accessions from the Nordic Genetic Resource Center (NordGen) and seven accessions available as historical seed samples in the KSLA seed collection (Leino et al. 2009); and (3) Spelt wheats (*T. aestivum* spp. *spelta*, 22 accessions), provided by INRA and NordGen. All accessions are described in online resource 1.

Genotyping

DNA was extracted from a leaf from a single individual of each accession using either the DNeasy® Plant Mini Kit or

DNeasy 96 Plant Kit from Qiagen or the E-Z 96 Plant DNA Kit from Omega Bio-Tek. All extractions were carried out according to the manufacturers' instructions using mechanical grinding of frozen shoots to homogenize the plant tissue prior to DNA extraction.

PCR amplification of *NAM-B1* was carried out as in Asplund et al. (2010) to genotype the 527 wheat accessions for the three described *NAM-B1* alleles (wildtype, 1-bp insertion and deletion). DNA integrity was determined by amplifying the microsatellite marker Xgwm518 (Röder et al. 1998). Fragment lengths of the *NAM-B1* amplicon were analysed using ABI 3130xl Genetic Analyzer and GeneMapper version 4.0 to discriminate between the wildtype and the 1-bp insertion alleles. Accessions amplifying Xgwm518, but not *NAM-B1,* in two consecutive amplification attempts were considered to have the deleted version of *NAM-B1*. Positive amplifications in the *NAM-B1* gene in the accessions of the core collection were confirmed by direct sequencing of the 500 first nucleotides of the gene, containing the site of the 1-bp insertion.

Pedigree analysis

To track ancestry, origin and release year of accessions the wheat pedigree on line resource (http://genbank.vurv.cz/wheat/pedigree/) was used as well as the SESTO database from NordGen (http://www.nordgen.org/index.php/en/content/view/full/344/). Additional data for early Fennoscandian (Danish, Finnish, Norwegian or Swedish) cultivars were found in Åkerman (1948) and of origin of spelt wheats in Siedler et al. (1994) and references therein.

Statistical analysis of spatial and temporal distribution of alleles

The software R 2.14.2 (R Development Core Team 2012) was used to compare the proportions of the two non-functional allele types in the different cluster groups described by Horvath et al. (2009). Independence of allele type and cluster group was tested with Pearson's Chi-squared test, and standardized residuals were computed according to Agresti (2007).

The presence of the wildtype allele over time was analysed for spring wheats of Fennoscandian origin. The software JMP 9.0.0 (SAS Institute Inc. 2010) was used to perform a binomial logistic regression of allele against year of release, where the wildtype allele was one response and deletion and insertion alleles were combined for the other response. Cultivars and landraces (not breeding lines) were included, and in the absence of release year all landraces were assigned to year 1890. The Pearson χ^2/df did not indicate overdispersion. The plot was made with R 2.14.2 (R Development Core Team 2012).

Results

Worldwide distribution of the *NAM-B1* alleles

To explore the distribution of the three *NAM-B1* alleles (wildtype, 1-bp insertion and deletion) across the range of wheat cultivation, and to survey the importance of the wildtype allele globally, we screened the accessions of the INRA core collection comprising 124 spring and 243 winter wheat accessions from all over the world.

Of the 367 accessions 104 (28 %) had the 1-bp insertion. All accessions amplified for DNA integrity controls suggesting that 244 accessions (66 %) had the deleted allele (online resource 1). For 14 accessions different individuals within the same accession showed different genotypes (1-bp insertion and deleted, respectively) also when confirming the genotype with additional markers. These accessions may be genotype mixtures and they were thus excluded from further analysis.

Only five accessions (1 %) were found to have the functional wildtype allele (Table 1). Four of these were spring wheats and one was a winter wheat, and the accessions differed widely in origin: Israel, France, Finland and Canada (Table 1). Closer examination of the ancestry of these accessions showed the Israeli accession to be a cross with emmer wheat (known to carry the wildtype allele). The French cultivar Mars de Suède Rouge Barbu and the Finnish cultivar Tähti are both derived from Finnish and/or Swedish landraces while the Canadian cultivar Stanley resulted from crosses of Red Fife and Ladoga, both suggested to be related to Galizian wheat (Symko 1999), previously shown to carry the wildtype allele (Asplund et al. 2010). The sequences obtained confirmed the wildtype genotype, and no other sequence variation was found except a A/C change in the second intron of the two French accessions.

We also analysed the distribution of the two non-functional alleles in relation to the five clusters described for this core collection by Horvath et al. (2009) (Western European wheats; Asian wheats; Eastern European and North American wheats; Nepalese wheats; and wheats from the CIMMYT and ICARDA breeding programs). The proportions of the non-functional alleles differed significantly between the cluster groups ($\chi^2_{(4)} = 29.98$, $p < 0.0001$). The standardized residuals (not shown) showed that group 3, Eastern European and North American wheats, deviated the most from the expected distribution, with an excess of the 1-bp insertion allele. This group also contained all but one of the wheats carrying the wildtype allele (Table 2). In group 1 (Western European wheats) and group 4 (Nepalese wheats), the deletion allele was more common than expected and the deletion allele was almost fixed in the Nepalese wheats (Table 2).

Table 1 Wheat accessions carrying the *NAM-B1* wildtype allele

Data set	Genebank	Accession number	Type	Name	Origin	Cultivation code	Release year	Ancestry^{a}
INRA 372 CC	INRA	4482	s	M708//G25/N163	ISR	BL		Cross with emmer wheat
INRA 372 CC	INRA	4645	s	Mars de Suede Rouge Barbu	FRA	CV	1922	Selection of Swedish landrace
INRA 372 CC	INRA	6846	s	Tähti	FIN	CV	1972	**Ruskea** and Finnish landraces in pedigree
INRA 372 CC	INRA	7011	w	Touzelle-Blanche-Barbue	FRA	L		French landrace
INRA 372 CC	INRA	8097	s	Stanley	CAN	CV	1890	Ladoga × Red Fife
Northern accessions	NGB	42	s	Horsmanaho ME0201; Timantti	FIN	L		Landrace from Eastern Finland
Northern accessions	NGB	120	s	Sarkalahti ME0101	FIN	L		Landrace from Eastern Finland
Northern accessions	NGB	121	s	Haarajärvi ME0102; Apu	FIN	L		Landrace from Eastern Finland
Northern accessions	NGB	130	s	ME8008070302B, Timantti, Paavo	FIN	L		Landrace from Eastern Finland
Northern accessions	NGB	131	s	Järvenkylä ME0302 SEP A; Timantti	FIN	L		Landrace from Eastern Finland
Northern accessions	NGB	349	s	Ruso	FIN	CV	1967	**Ruskea** and Finnish landraces in pedigree
Northern accessions	NGB	351	s	Ulla	FIN	CV	1975	**Tammi**^{b} × TA332
Northern accessions	NGB	353	s	Taava	FIN	CV	1978	Derived from **Ruso**
Northern accessions	NGB	357	s	Luja	FIN	CV	1981	Svenno × (**Hopea**^{b} × **Tammi**^{b})
Northern accessions	NGB	359	s	Touko	FIN	CV	1950	**Diamant** × **Hopea**^{b}
Northern accessions	NGB	2125	s	Børsum	NOR	L		Landrace from Norway
Northern accessions	NGB	2126	s	Fram I	NOR	CV	1936	Crosses of Norwegian landraces
Northern accessions	NGB	2127	s	Fram II	NOR	CV	1938	Crosses of Norwegian landraces
Northern accessions	NGB	2128	s	Kr Finset, Eikesdal	NOR	L		Landrace, likely from Mid-Norway
Northern accessions	NGB	2129	s	Landvårkveite	NOR	L		Landrace from Norway
Northern accessions	NGB	2130	s	Lanor	NOR	CV	1970	**Norrøna** × Lade
Northern accessions	NGB	2132	s	Nora	NOR	CV	1959	**Fram II** × **Sopu**
Northern accessions	NGB	2133	s	Norrøna	NOR	CV	1958	**Fram II** × **Sopu**
Northern accessions	NGB	2138	s	Skirne	NOR	CV	1937	Gelchsheimer × Norwegian landrace selection
Northern accessions	NGB	2139	s	Snøgg I	NOR	CV	1939	0843 × **Ås**
Northern accessions	NGB	2140	s	Snøgg II	NOR	CV	1940	0834 × **Ås**
Northern accessions	NGB	2141	s	Tautra	NOR	CV	1950	Not known
Northern accessions	NGB	2142	s	Trym	NOR	CV	1948	Fylgia × Huron
Northern accessions	NGB	2143	s	Ås	NOR	CV	1926	Norwegian landrace selection
Northern accessions	INRA	4959	s	Moystad	NOR	CV	1966	Kärn II × (**Fram II** × **Sopu**)
Northern accessions	NGB	6409	s	Halland	SWE	L		Landrace from Southern Sweden
Northern accessions	NGB	6410	s	Dalarna	SWE	L		Landrace from Mid-Sweden
Northern accessions	INRA	6634	s	Sopu 7	FIN		1935	Marquis × **Ruskea**
Northern accessions	NGB	6673	s	Lantvete från Dalarna	SWE	L		Landrace from Mid-Sweden
Northern accessions	NGB	6678	s	Rubin	SWE	CV	1921	Kolben × **Dalavete**

Table 1 continued

Data set	Genebank	Accession number	Type	Name	Origin	Cultivation code	Release year	Ancestry[a]
Northern accessions	NGB	6679	s	Diamant	SWE	CV	1928	Kolben × **Stenings vårvete**
Northern accessions	NGB	6681	s	Diamant II	SWE	CV	1938	**Diamant** × Extra Kolben
Northern accessions	NGB	6684	s	Rival	SWE	CV	1952	Extra Kolben × **Diamant**
Northern accessions	NGB	6688	s	Prins	SWE	CV	1962	**Diamant II** × Kärn II
Northern accessions	NGB	6689	s	Amy	SWE	CV	1971	**Prins** × (SV01281 × SV55460)
Northern accessions	NGB	7210	s	KVL 8063	DNK	L		Unclear origin, donated from Gatersleben
Northern accessions	NGB	8922	s	Østby	DNK	L		Probably Norwegian landrace
Northern accessions	NGB	9710	s	Ås II	NOR	CV	1945	Crossings with Norwegian landraces, **Ås** and **Diamant**
Northern accessions	NGB	11709	s	Manu	FIN	CV	1993	**Ruso** × Runar
Northern accessions	NGB	13346	s	Sopu	FIN	CV	1935	Marquis × **Ruskea**
Northern accessions	NGB	13441	s	Västergötland	SWE	L		Landrace from Southwestern Sweden
Northern accessions	INRA	36018	s	Ruskea	FIN	CV	1919	Probably selection from Swedish landrace
Northern accessions	NM	25	s	Svalöf 0880	SWE	BL		Kolben × **landrace from Dalarna**
Northern accessions	NM	37	s	Lantvårvete fr Dalarna	SWE	L		**Landrace from Dalarna**
Northern accessions	NM	51	s	Emma-vårvete 0201	SWE	BL		Probably pure line from Dutch Emma (Wilhelmina × Essex)
Northern accessions	NM	8	s	Stenings vårvete fr Halland	SWE	L		**Landrace from Halland**
Spelt	INRA	847	w	Albin	BEL	CV	1979	von Rechbergs Früher Dinkel × Ardenne
Spelt	INRA	885	w	Altgold	CHE	CV	1952	Oberkulmer × Sandmeier
Spelt	INRA	20294	w	Rouquin	BEL	CV	1979	(Lignée 24 × Ardenne) × **Altgold**
Spelt	INRA	24151		Landrace	IRN	L		
Spelt	INRA	24208		Landrace	TKM	L		

s spring wheat, *w* winter wheat, *CV* cultivar, *BL* breeding line, *L* landrace

[a] Wheat accessions known to carry the *NAM-B1* allele are given in bold

[b] Tammi and Hopea are genotype mixtures where some individuals carry the wildtype allele

Table 2 Results of *NAM-B1* genotyping of the INRA core collection

Total	Deletion		+1 bp		Wildtype	
	242		103		5	
Total	Spring 86	Winter 156	Spring 28	Winter 75	Spring 4	Winter 1
Group 1 Western Europe	8	69	1	16		
Group 2 Asia	34	9	10	6		
Group 3 Eastern Europe and North America	9	42	3	45	3	1
Group 4 Nepal	1	21	1			
Group 5 CIMMYT and ICARDA	34	15	13	8	1	

High frequency of the *NAM-B1* wildtype allele in northern latitude spring wheats

NAM-B1 accelerates the rate of senescence in wildtype plants leading to a faster maturation of the wheat. We noted that three of the five wildtype accessions in the INRA 372 core collection could be traced back to spring wheats from a northern latitude (Polish, Finnish or Swedish). Although the frequency of the wildtype was too low to allow for statistical testing we noted a much higher proportion of the wildtype allele in spring wheats (4/119) than winter wheats (1/234). Consequently, as spring wheat also is the cereal type that has the latest maturation date we hypothesised that a faster maturation time in wildtype spring accessions had made them favourable as crops in northern latitudes where the growing season is shorter.

To explore this hypothesis we genotyped 138 spring wheats with a northern origin (Russia, Canada, Mongolia, Japan and Fennoscandia). These included both landraces and commercial varieties with release dates from across the twentieth century as well as some of the earliest commercial wheat breeding lines and cultivars produced in Sweden, available only as nineteenth century seeds.

In this set of accessions 23 had the 1-bp insertion allele and 69 accessions amplified only the DNA integrity control and thus probably have the deleted allele (online resource 1). The remaining 46 accessions all had the wildtype allele (Table 1). The northern accession set thus had a frequency of the wildtype allele of 33.3 % compared with the 1.4 % in the INRA core collection. Closer examination of the northern accession set showed that all wildtype accessions originated in Fennoscandia (Tables 1, 3). Of the Fennoscandian accessions in the northern set 14/49 of the Swedish, 16/20 of the Norwegian, 2/6 of the Danish and 14/29 of the Finnish had the wildtype allele (Table 3).

We traced the ancestry of all the cultivar accessions carrying the wildtype allele (Table 1). In almost all cases the pedigree led back either to accessions that themselves carried the wildtype allele or to landrace wheats from Fennoscandia. Among the landraces tested 53 % had the wildtype allele, which was abundant among landraces with Finnish, Swedish and Norwegian origin. The two Danish landraces with the wildtype allele have unclear passport data and one case is probably an accession with Norwegian origin.

Table 3 Results of *NAM-B1* genotyping of spring wheat accessions with a northern origin

Total	+1 bp 23	Gene deleted 69	Wildtype 46 (33.3 %)	Total 138
Country				
CAN	1	8	0	9
DNK	2	2	2 (33.3 %)	6
FIN	6	9	14 (48.3 %)	29
DEU	0	4	0	4
JPN	2	6	0	8
KOR	1	0	0	1
MNG	1	1	0	2
NOR	1	3	16 (80 %)	20
POL	3	0	0	3
RUS	3	4	0	7
SWE	3	32	14 (28.6 %)	49

For the spring wheats with a Fennoscandian origin we used a binomial logistic regression to study how the frequency of accessions with the wildtype alleles has changed during the twentieth century. The presence of the wildtype allele decreased from late nineteenth century to late twentieth century ($\chi^2_{(1)} = 4.20$, $p = 0.04$) (Fig. 1). Of the preserved landraces genotyped in this study 53 % had the wildtype allele, while none of the genotyped accessions released during the 1990s carried it.

Presence of wildtype allele in both landraces and cultivars of spelt wheat

Asplund et al. (2010) also reported the *NAM-B1* wildtype allele in widely cultivated nineteenth century spelt wheats. We thus genotyped spelt wheats of Eurasian origin to also explore the presence of the *NAM-B1* wildtype allele in extant spelt wheat. Of the 22 spelt wheats genotyped five

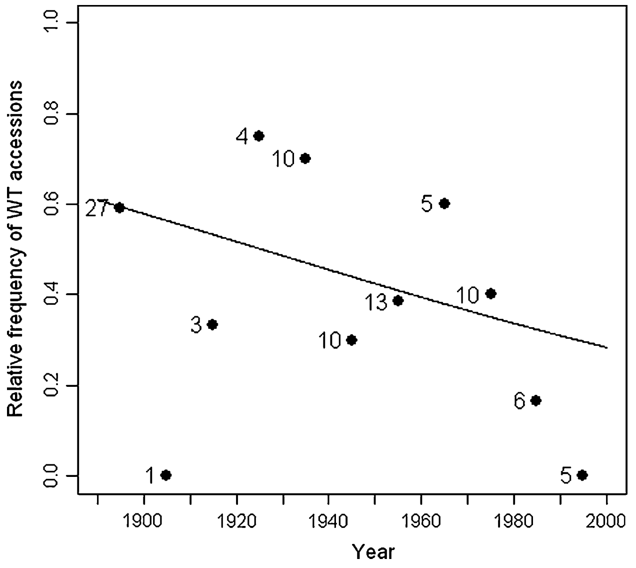

Fig. 1 Relative frequency of WT accessions among Fennoscandian spring wheats, fitted line and observed proportions per decade. *Numbers* indicate total number of accessions analysed each decade

were found to carry the wildtype allele with an additional five having the 1-bp insertion and the remaining 12 failing to amplify for the *NAM-B1* locus (Table 1; online resource 1). In the spelt wheats none of the wildtype accessions had a northern origin. The two wildtype landraces originated in Iran and Turkmenistan, respectively, while the wildtype cultivars were from Belgium and Switzerland. These cultivars originate in Swiss and Belgian landraces (Table 1; Siedler et al. 1994).

Discussion

In our screen of 505 bread wheat accessions we found that, with a few exceptions, most wildtype accessions originated from countries in northern latitudes. The vast majority originated from the Fennoscandian countries (Denmark, Norway, Sweden and Finland). The very rare presence of wildtype alleles in non-Fennoscandian accessions explain why it was never detected among the wheat accessions studied by Uauy et al. (2006a). Interestingly, a close relationship between Galizian spring wheat, carrying the wildtype allele (Asplund et al. 2010) and some Swedish and Norwegian landraces (Halland; Hallandsvete; Børsum) was suggested already by Åkerman (1948). If this is the case this would mean an even more restricted origin of the wildtype allele. The wheat from Galizia (in present Poland) was in the nineteenth century recognized for its excellent qualities and cultivated in large parts of Europe and Northern America (Körnicke and Werner 1885; Symko 1999). In spite of this extensive cultivation it seems as if Galizian wheat has not contributed to any extensive spread of the wildtype allele, possibly with the exception of the cultivar ‘Stanley’ (see below).

The INRA core collection has been selected to cover the vast majority of the genetic diversity in wheat and our results should be a good indicator of the worldwide presence of such a rare allele as the wildtype allele of *NAM-B1*. It is clear that the wildtype phenotype has been disfavoured in most cultivation environments. In contrast, it seems the short growing season in Fennoscandia has favoured the faster maturation time of accessions with the *NAM-B1* wildtype allele and helped preserve it in landraces to much higher frequences (15 out of 33) in the northern accessions than elsewhere (1 out of 91 in the INRA core collection). However, a possible advantage of early maturation has not been so large that accessions with the wildtype allele have been universally preferred in countries with short growing season. The wildtype allele is absent from wheats from northern latitudes outside of Fennoscandia, and neither is there any indication that the short growing season in, e.g. the Mediterranean area has preserved the wildtype allele. The pleiotropic effects of *NAM-B1* on yield, GPC and senescence are complex and largely dependent on genetic background and environment (Uauy et al. 2006b; Distelfeld et al. 2007; Brevis and Dubcovsky 2010). Possibly, the advantageous effects have been more pronounced in the Fennoscandian cultivation area. We note, however, that our number of accessions with a Mediterranean origin is rather low and it is possible that further investigations will reveal wildtype accessions among wheats of a Mediterranean origin or in other areas with a short growing season.

With the clear Fennoscandian presence of the wildtype allele we also explored the geographical distribution of the *NAM-B1* deletion and the 1-bp insertion allele. Different geographic origin of the two non-functional alleles and subsequent spread of the wheats from there could give rise to geographically different distributions of different alleles, such as that seen for the *Ppd-H1* alleles in barley (Jones et al. 2008; Lister et al. 2009). We could detect some geographic patterns in the distribution of the two derived *NAM-B1* alleles such as a higher frequency than expected of the 1-bp insertion allele in the group 3 accessions of Horvath et al. (2009), corresponding to wheats from Eastern Europe and North America. Additionally, Western European and Nepalese wheats seemed to have a slight excess of the deletion allele. These patterns were clear both from the testing independence of allele type and cluster group with Pearson’s Chi-squared test and from factorial correspondence analysis (data not shown). To detect clearer patterns of geographic distribution, however, it will most likely be necessary to study landrace wheats with a geographically certain origin, or archaeological remains, rather than a material containing also varieties developed from crosses between accessions from different countries, such as the ones studied here.

When systematic plant breeding began in Fennoscandia in the late nineteenth century the starting material was primarily the locally cultivated landraces. Åkerman (1948, 1951) describes how the landrace populations were used initially for pure line selections and subsequently in crossings, often with foreign cultivars. In the Fennoscandian spring wheat breeding programs high GPC was early considered a very important trait, and landraces were recognized for their high GPC and good baking quality as well as their fast maturation. In all likelihood this lead to the *NAM-B1* wildtype allele being systematically, although unknowingly, bred from local landraces into some key cultivars such as Ruskea, Ås, Diamant, Fram II and Sopu that were subsequently and extensively used to develop new cultivars.

In later decades fewer and fewer cultivars with the wildtype allele have been released (Fig. 1). This could be a result of selection for yield taking precedence over GPC, use of other genes to maintain baking quality or a pure dilution effect when Fennoscandian landraces and accessions developed from them were repeatedly crossed to germplasm of other origin not carrying the wildtype allele. For a period, during the 1940s and 50s, inherent landraces were used in Swedish spring wheat breeding to improve quality (Svensson 1997), which could explain the increase in wildtype allele frequency in cultivars released during the 1950s and 60s (Fig. 1).

One early cultivar that figures frequently in the pedigrees of Fennoscandian wheats with the wildtype allele is Hopea. Our genotyping of several individuals both from INRA and NordGen showed that the cultivar is heterogenous in respect of *NAM-B1* with individuals either with the deleted allele, 1-bp insertion or the wildtype allele. It is likely that Hopea inherited the wildtype allele from its maternal parent Ruskea but that the allele was not fixed. Similarly, Tammi seem to be a mixture of the wildtype and the 1-bp insertion allele.

The only identified bread wheat accessions with the wildtype allele not having a clear Fennoscandian or emmer wheat origin were the French landrace Touzelle-Blanche-Barbue and the obsolete Canadian cultivar Stanley. Unfortunately, no background data on Touzelle-Blanche-Barbue are available and its relationship to other accessions with the wild-type allele is unclear. The cultivar Stanley was derived from a cross between Red Fife and Ladoga in the 1880s (Symko 1999). However, neither Red Fife nor Ladoga was found to carry the wild-type allele in our screen. The suggested progenitor to Red Fife, Galizian Halychanka, as well as Ladoga was repeatedly reintroduced into Canada from Germany, Ukraine and Russia in the nineteenth century (Symko 1999). The parents crossed to Stanley and the accessions tested by us may well be genetically different. A Galizian origin of either or both parents of Stanley is compelling as Asplund et al. (2010) found the wildtype allele in a historical sample of Galizian wheat.

We have identified five new spelt wheat accessions with the wildtype allele, three European cultivars and two landrace accessions from Iran and Turkmenistan, respectively. The genetic diversity within European spelt cultivars is narrow (Siedler et al. 1994; Bertin et al. 2001) and all three European wildtype spelt cultivars share a similar origin. In addition, all European landrace spelts lacked the wildtype allele further suggesting a restricted distribution of the wildtype allele in spelt.

Recent gene flow from emmer wheat (Dvorak et al. 2012) or different selection effects on *NAM-B1* alleles in this subspecies compared with bread wheat may explain the presence of the wildtype alleles in the cultivars and landraces found in this and other studies (Asplund et al. 2010). Spelt wheats are generally recognized for high grain protein content but low yield in comparison with bread wheat (Bertin et al. 2001 and references therein). The effect of the different *NAM-B1* alleles on GPC, yield and senescence in spelt has, to our knowledge, not been investigated.

The green revolution during the 1960s and 70s saw huge improvements in wheat yield, but nutrient content did not increase along with yield. An analysis of wheat tested in England 1845–2005 showed that nutrient content in wheat remained the same from 1945 to mid 1960s, but decreased sharply thereafter (Fan et al. 2008). At the same time the mineral content of the soil remained constant. Similar decreases in nutrient content could be seen in hard red winter wheat varieties released in different years in USA. Garvin et al. (2006) found negative correlations for micronutrient content and both year of release and yield of the varieties for some micronutrients and localities.

NAM-B1 is a strong candidate gene for improving the nutrient content of wheat and indeed, extensive effort has been made to cross the wildtype allele of *NAM-B1* into the genetic background of elite cultivars. So far, wild emmer wheat, *T. turgidum* ssp. *dicoccoides*, has been the only source of *NAM-B1* wildtype alleles and a limited number of elite germplasm lines with the wildtype allele introgressed is available (Brevis and Dubcovsky 2010). Our results here show that the wildtype allele is common in a large number of Fennoscandian spring wheats. A positive effect on GPC of the wildtype allele, similar to the one consistently detected in previous studies (see references above), can be expected also in Fennoscandian wheats. The negative pleitropic effects have proved to be far more variable with respect to environment and genetic background and the analysis of the wildtype wheats reported here will make an important addition to our understanding of the effects of the *NAM-B1* gene. Additionally, these wheats should be explored as a potential material for crossing the *NAM-B1* wildtype allele into high-performance genetic backgrounds. The presence of the wildtype allele in the winter type wheat INRA7011 (Touzelle Blanche Barbu) should likewise be useful for direct backcrosses with elite winter wheat lines.

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