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Characterization and Molecular Mapping of a Soft Glume Mutant in Diploid Wheat (*Triticum monococcum* L.)

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Diploid wheat *T. monococcum* L. is a model plant for wheat functional genomics. A soft glume mutant was identified during manual screening of EMS-treated M_2 progenies of a *T. monococcum* accession pau14087. The seeds in the mature spike of the mutant could be easily threshed manually. The soft glume mutant with high sterility, tapering and broader spikes had also tougher rachis than the wild type parent. Genetic analysis of crosses of the mutant with wild type indicated that the mutant was monogenic recessive. To map the soft glume mutant, a mapping population was developed by crossing the soft glume mutant with wild *Triticum boeoticum* acc. pau 5088, having tough glumes and hard threshing. The soft glume mutant was mapped between SSR markers Xgwm473 and Xbarc69 on $7A^mL$ chromosome of *T. monococcum,* with a genetic distance of 1.8 cM and 8.3 cM, respectively. The soft glume mutant mapped on $7A^mL$, being distinct from a previously mapped soft glume mutant in wheat, has been designated as *sog2*. The work on fine mapping of *sog2* gene is in progress.

Keywords: ethyl methane sulfonate, molecular mapping, microsatellite marker, soft glume mutant – *sog2, Triticum monococcum* L.

Introduction

The diploid wheat *Triticum monococcum* L. (einkorn) was one of the first crops domesticated by humans in the Fertile Crescent 10,000–12,000 years ago which is being still cultivated for its edible seeds in the mountainous regions of Germany, Switzerland and Italy. *T. monococcum,* previously considered as the A genome donor, is most closely related to the A genome donor of cultivated wheat, *T. urartu* (Kilian et al. 2007). It is tall with tough rachis and hard threshing and has mostly single large and bold seed per spikelet. It is an

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ideal material for induced variability which could be characterized and transferred to cultivated wheat. *T. monococcum* (2 n= 14) with smaller genome size of 5,700 Mb, the existence of a very high level of polymorphism for DNA based markers with its wild progenitor *T. boeoticum* (Singh et al. 2007), conservation of colinearity and synteny with other cereal crops, availability of a BAC libraries and resistance against various wheat diseases makes this species an attractive diploid model for gene discovery in wheat (Wicker et al. 2001). Using the diploid wheat mapping populations, high density molecular maps have already been developed (Dubcovsky et al. 1996; Taenzler et al. 2002; Singh et al. 2007) and a number of mutants have been mapped (Kuraparthy et al. 2007; Sood et al. 2009; Ansari et al. 2012) and genes for disease resistance have been introgressed into bread wheat cultivars (Chhuneja et al. 2008; Singh et al. 2010).

Free threshing is an important domestication trait in wheat as the free-threshing cultivars could be easily harvested and threshed. All the wild relatives of wheat have tough glumes and hard threshing habit whereas most of the cultivated wheat varieties are free-threshing with soft glumes. Two genetic loci are known to govern the threshability in bread wheat. The *Q* gene located on chromosome 5AL and tenacious glume *(Tg)* genes located on homoeologous group-2 chromosomes seem to interact to produce free-threshing spikes (Faris et al. 2005). The soft glume *(sog)* gene of diploid wheat and tenacious glume *(Tg)* gene of hexaploid wheat have been characterized and mapped on the short arms of chromosomes $2A^m$ and 2D, respectively (Sood et al. 2009). A spontaneous free threshing mutant of *T. monococcum* subsp. *monococcum* referred to as *T. sinskajae* mapped on the short arm of chromosome $2A^m$ (Taenzler et al. 2002) was found allelic to *sog* reported by Sood et al. (2009).

EMS mutagenesis in *Triticum monococcum* is a powerful tool for map-based cloning of genes for important traits (Yan et al. 2003, 2004). Keeping in view the various aspects of EMS mutagenesis for functional genomics in diploid wheat, the present investigation was carried out to map an EMS induced soft glume mutant of *Triticum monococcum* using morphological, histological and molecular studies.

Materials and Methods

Plant materials

The soft glume (SG) mutant used in the present study was isolated from diploid wheat *T. monococcum* accession pau14087 at the Punjab Agricultural University, Ludhiana, after seed treatment with 0.25% EMS. This mutant was identified during manual screening in the EMS- treated M_2 population in the field. The seeds of the mutant, the wild type (WT) parent and an accession pau5088 of *T. boeoticum* (the wild and hard threshing progenitor of *T. monococcum*) were planted at the Indian Institute of Technology (IIT), Roorkee in November, 2005. A recombinant inbred lines (RILs) population $>F_{10}$ of *T. boeoticum* pau5088 × *T. monococcum* pau14087 derived from F_2 was also planted in the field (Singh et al. 2007) and some RILs were chosen at random to develop a universal negative bulk for mapping this and other EMS induced mutants. The soft glume mutant

was crossed with both wild type (WT) parent, *T. monococcum* and its wild progenitor *T. boeoticum* for developing F_2 populations for inheritance and mapping studies, respectively. The F_2 populations were planted at IIT, Roorkee in 2007-08 in 2 m rows with row-to-row distance of 30 cm and plant to plant distance of 10 cm following the standard package of agronomic practices for wheat cultivation.

Phenotypic analysis

Threshability of individual spikes was recorded after harvest. In the F_2 populations, spikes of the plants were scored for threshability. Each spike was hand threshed and scored as either hard or free-threshing.

Histological observations

For histological examination, the rachis of spike, were excised to fix in FAA fixative, dehydrated in a graded ethanol series and finally xylene following the protocol of Ansari et al. 2012). For sectioning, the tissues were embedded in paraffin wax (Sdfine) at 60°C, and sectioned to 10 µm thickness on a rotary microtome. The tissues were stained with toluidine blue O (0.06%, w/v) dye, as suggested by Johansen (1940). Longitudinal sections of the rachis of the soft glume mutant and *T. monococcum* were observed under a light microscope (*Axiostar plus 1169-151,* Carl Zeiss Co., Oberkochen, Germany) at different magnifications.

Genomic DNA isolation and PCR amplification

The genomic DNA from parents and the F_2 population was extracted following the CTAB method, as described by Saghai-Maroof et al. (1984). The anchored molecular markers from a map developed in a RIL population between *T. monococcum* acc. pau14087 and *T. boeoticum* acc. pau5088 (Singh et al. 2007) were used for mapping the soft glume mutant. The primers for anchored SSR markers at about 10 cM from each of the diploid wheat chromosomes and polymerase chain reaction (PCR) protocols were carried out in a thermo cycler (Applied Biosystems, Singapore) according to methods described by Singh et al. (2007). The PCR products were separated on 2.5% agarose and stained with ethidium bromide.

Inheritance and molecular mapping

To study the inheritance of SG mutant, it was crossed with its wild parent *T. monococcum* and *T. boeoticum* for molecular mapping. The F_1s were advanced to F_2 generation. The phenotypic data on soft glume (hard vs free threshing) was recorded on each plant F_2 population. Chi-square test was applied to test the goodness of fit to the segregation ratio.

For bulk segregant analysis (BSA), positive bulk of 12 SG mutant plants was made from homozygous mutant F_2 soft glume plants from the respective F_2 mapping population of *T. monococcum* × *T. boeoticum*. A universal negative bulk was prepared from 15 RILs of *T. monococcum* pau5088 × *T. boeoticum* pau14087 cross without any mutant in the parents for bulk segregant analysis of SG and other EMS induced mutants so that the time to

select homozygous F_3 plants for negative bulk could be saved. An equal amount of DNA from each plant / RIL of both the bulks was pooled along with the parents. The bulks along with the parents were used to identify putative SSR markers linked to the SG mutant. Genotyping of the debulked F_2 plants was done with the putatively linked markers Xgwm473, Xwmc96 and Xbarc69 (as per the diploid wheat map of Singh et al. 2007). These three SSR markers were used to genotype individual plants of the F_2 mapping population. A linkage map was constructed with MAPMAKER/EXP version 3.0 (Lander et al. 1987; Lincoln et al. 1993) according to the linkage data of the SG mutant and polymorphic SSR markers in the F_2 mapping population.

Results

Identification of soft glume mutant

The spikes of soft glume (SG) mutant were more compact and tapering towards tips and had lax glumes. The seeds in the mature spike could be easily threshed manually as compared to the wild type (WT) parent. During removal of individual spikelets from the spike near maturity, the rachis remained intact in the SG mutants (Fig. 1B) whereas in

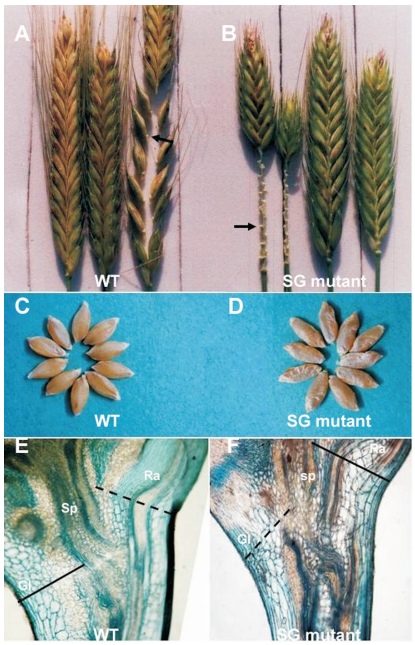

Figure 1. Comparative morphology, seed shape and histology of wild type (WT) *T. monococcum* and its soft glume (SG) mutant, A: Spike and brittle rachis(arrow) of *T. monococcum*, B: Spike and tough rachis (arrow) of SG mutant, C: Seeds of *T. monococcum*, D: Slightly shrivelled seeds of soft glume mutant, E: Longitudinal section of rachis of *T. monococcum,* —— tough glume, - - - - - brittle rachis sections, F: Longitudinal section of rachis of soft glume mutant, - - - - - soft glume and spikelet breaking section, —— non-brittle rachis section. Ra = Rachis, Sp = Spikelet, Gl = Glume

T. monococcum the spikelets separated with some force leaving a wedge of rachis fragments without intact rachis (Fig. 1A) indicating that SG mutant had a pleiotrophic effect on the rachis, making it slightly tough in the mutant plants. In the SG free-threshing mutants, the glumes were softer, longer and broader (Fig. 1B). There was significantly reduced seed set in the SG mutants and the seeds were also slightly smaller and shrivelled (Fig. 1C, 1D).

Histological analysis of SG mutant

To determine, any alterations in cell morphology and cell arrangements in the soft glume mutant at the site of separation of spikelet from the rachis as compared to WT, longitudinal sections of the spikelet of the mutant and wild type plants through rachis were developed and histochemically stained with toludine blue. It was found that in the WT, there was continuous vascular supply from rachis to different parts of spikelet with more sclerenchymatous tissues at the spikelet and glume attachment rachis (Fig. 1E). However, in SG mutant this vascular supply was interrupted by some irregularly placed cells not longitudinally placed in vascular bundles and making an abscission layer like structure (Fig. 1F). Loosely attached spikelets and glumes separate from the rachis in SG mutant from this part only after slight mechanical pressure on the spikelet while rachis becomes hard by more development of sclerenchymatous tissues (Fig. 1F).

Inheritance and mapping of the SG mutant

The F_1 obtained from the cross of *T. monococcum* × SG mutant was like the wild type parent, indicating that the mutant was recessive. Out of 170 F_2 plants, 129 plants had hard threshing and 41 had free threshing due to soft glume, chi-square value (0.072) gave a good fit ($P = 0.05$) to 3:1 ratio indicating that the SG mutant was monogenic recessive.

A set of 133 anchored SSR markers polymorphic between *T. monococcum* and *T. boeoticum* (Singh et al. 2007) were used for bulk segregant analysis (BSA). The marker Xgwm473 and Xbarc69 located on chromosome $7A^mL$ showed polymorphism between the positive and negative bulk of SG mutant (Fig. 2A). The negative bulk developed from 15 RILs of *T. monococcum* × *T. boeoticum* without mutant was heterogeneous for both the alleles of the SSR markers Xgwm473 and Xbarc69. Debulking of positive bulk plants also showed close linkage of the SG mutant with markers Xgwm473 as none of the 15 plants had its WT allele followed by Xbarc69 with 3/15 plants with its WT. To map the SG mutant, F_2 mapping population developed from the cross of the mutant with *T. boeoticum* acc. pau 5088, a hard threshing and brittle rachis wild progenitor of *T. monococcum,* was used.

A total of 170 individual F_2 plants were phenotyped for free threshing and genotyping for the putatively linked markers Based on the recombination frequency between the mutant and the markers Xgwm473 and Xbarc69 the SG mutant was mapped on chromosome $7A^mL$ at 1.8 cM and 8.3 cM from the markers, respectively (Fig. 3).

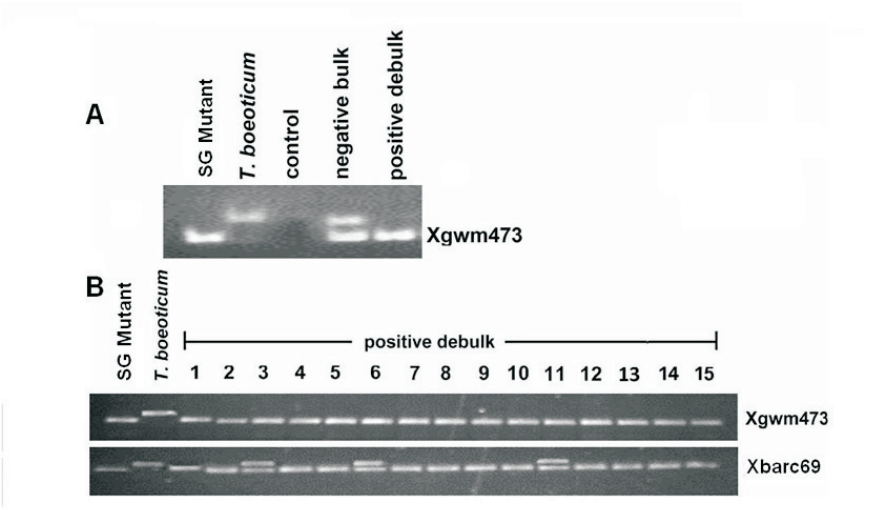

Figure 2. Identification of putative linked SSR markers with SG mutant through BSA with SSR marker Xgwm473; A: Lane 1: SG mutant, 2: *T. boeoticum,* 3: Control, 4: Negative bulk, 5: Positive bulk of soft glume mutant; B: PCR amplification of debulks of positive bulks of F2 plants using putatively linked markers Xgwm473 and Xbarc69 identified in BSA

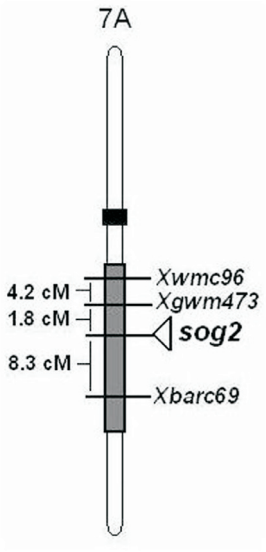

Figure 3. Mapping of the soft glume mutant *(sog2)* on chromosome 7AL of *Triticum monococcum*

Discussion

In the present study, the EMS induced SG mutant of *T. monococcum* was used for its phenotypic and molecular characterization. The SG mutant was monogenic recessive. Such a mutant probably could not have been recovered in polyploid wheat because of its dominant orthologous loci on other homoeologous genomes unless and until multiple mutants are induced at all the loci or certain loci had been silenced during evolution. The vernalization genes *VRN1* and *VRN2* were cloned in wheat by using the naturally existing variants in *T. monococcum* (Yan et al. 2003, 2004). The tillering mutant *tin3* and soft glume mutant *sog* from the EMS induced mutant collections of *T. monococcum* (Dhaliwal et al. 1987) were mapped on chromosome arm 3A^{m}L and 2A^{m}S, respectively (Kuraparthy et al. 2007; Sood et al. 2009).

The only free threshing mutant of *T. monococcum* with soft glumes, investigated in this study, was distinct from the previously mapped tenacious or soft glume mutants of *T. aestivum* and *T. monococcum* (Nalam et al. 2007; Salamini et al. 2002; Sood et al. 2009), as SG mutant mapped on 7AL and hence not orthologous to those. It will, therefore be appropriate to designate the soft glume mutant of *T. monococcum* as *sog2* to differentiate it from the already characterized a soft glume mutant in *T. monococcum*. Furthermore, the soft glume, *sog2* mutant in addition to free threshing also has tough rachis which remains intact during maturity like that of free threshing wheat with Q gene (Faris et al. 2002) whereas in *T. monococcum* with tough rachis, the rachis breaks as a wedge with individual spikelet leaving no intact rachis. Slightly higher rachis toughness of the *sog2* mutant over the tough rachis of its wild type parent *T. monococcum* may be due to its pleiotropic effect similar to that of the free threshing *Q* allele over its non-free threshing *q* allele.

A spontaneous free threshing mutant of *T. monococcum* subsp. *monococcum* referred to as *T. sinskajae* (Filatenko and Kurkiev 1975 cited by Gonchariov et al. 2002), controlled by a single recessive gene *sog,* has been mapped on the short arm of chromosome 2A^m (Taenzler et al. 2002). Sood et al. (2009) by comparative mapping with other cereals, mapped soft glume *(sog)* region of diploid *Triticum monococcum* L. and tenacious glume *(Tg)* region of hexaploid *T. aestivum* on chromosome 2A^{m}S and 2DS, respectively.

In polyploid wheats, a polygenic system along with modifier genes is known to govern rachis fragility and glume tenacity (MacKey 1966). Rachis fragility is primarily controlled by genes present on the homoeologous group-3 chromosomes (Watanabe and Ikebata 2000; Nalam et al. 2006; Li and Gill 2006). All the wild *Triticum* and related *Aegilops* species have a brittle rachis leading to shattering of either the whole spike or individual spikelets (Li and Gill 2006). The first cultivated wheat had non-brittle rachis (mutant *br*) with tough glumes and thus was non-free-threshing. The *Tg* gene controlling tenacious glumes in wheat is present on short arm of the group 2 chromosomes (Kerber and Rowland 1974; Chen et al. 1999; Taenzler et al. 2002; Nalam et al. 2007). A major modifier gene for domestication related traits (*q* gene) is located on the long arm of chromosome 5A (MacKey 1966; Muramatsu 1986; Faris et al. 2002, 2005). Subsequent mutations at these loci during domestication (Salamini et al. 2002) led to the modern free-threshing wheats with geno-

type *brbrtgtgQQ*. Among these three genes, only *Q* has been cloned and found to be a member of the *APETALA2* family of transcription factors (Simons et al. 2006).

Simonetti et al. (1999) in a RIL population derived from a *T. turgidum* subsp. *durum* × *T. turgidum* subsp. *dicoccoides* cross found four QTL influencing the threshability trait on 2BS, 5AL, 5AS, and 6AS. The 2BS and 5AL QTL corresponded to the orthologous genes *Tg* and *Q* of hexaploid wheats, respectively. Furthermore, their study suggested the complexity of free-threshing trait in tetraploid wheat where major genes, *Tg2* and *Q* along with several minor genes are required for the complete expression of the free-threshing trait. The ortholog of tough glume gene, *tga1(teosinte glume architecture 1)* on chromosome 4 (Dorweiler et al. 1993) of maize has been implicated to be on group 7 chromosomes of wheat (Sood et al. 2009) but no soft glume mutant on chromosome 7 of wheat has been reported. A free threshing naked caryopsis mutant *(nud)* in barley, involving a distinct mechanism from that of *tga1, Tg* and *sog* genes, has been mapped on chromosome 7HL (Taketa et al. 2008), which is homoeologous to wheat group 7 chromosomes. The possibility whether the *sog2* on 7A^{m}L of *T. monococcum* is orthologous to *tga1* of maize and *nud* of barley is worth exploring.

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References

Ansari, M.J., Kumar, R., Singh, K., Dhaliwal, H.S. 2012. Characterization and molecular mapping of EMS-induced brittle culm mutants of diploid wheat (*Triticum monococcum* L.). Euphytica **186**:165–176.

Chen, Q.F., Yen, C., Yang, J.L. 1999. Chromosome location of the hulled character in the Tibetian weedrace of common wheat. Genet. Resour. Crop. Evol. **46**:543–546.

Chhuneja, P., Kaur, S., Garg, T., Ghai, M., Kaur, S., Prashar, M., Bains, N.S., Goel, R.K., Keller, B., Dhaliwal, H.S., Singh, K. 2008. Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. Theor. Appl. Genet. **116**:313–324.

Dhaliwal, H.S., Multani, D.S., Sharma, S.K., Singh, M. 1987. Induction of useful variability in *T. monococcum* L. Crop Improv. **14:**1-5.

Dorweiler, J., Stec, A., Kermicle, J., Doebley, J. 1993. *Teosinte glume architecture 1:* A genetic locus controlling a key step in maize evolution. Science **262**:233–235.

Dubcovsky, J., Luo, M.C., Zhang, G.Y., Bainsteitter, R., Desai, A., Kilian, A., Kleinhofs, A., Dvorak, J. 1996. Genetic map of diploid wheat *T. monococcum* L. and its comparison with maps of *H. vulgare* L. Genetics **143**:983–999.

Faris, J.D., Gill, B.S. 2002. Genomic targeting and high-resolution mapping of the domestication gene Q in wheat. Genome **45**:706–718.

Faris, J.D., Simons, K.J., Zhang, Z., Gill, B.S. 2005. The wheat super domestication gene Q. Wheat Info. Serv. **100**:129–148.

Gonchariov, N.P., Kondratenko, E., Kawahara, T. 2002. Inheritance of dense spike in diploid wheat and *Aegilops squarossa*. Hereditas **137**:96–100.

Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill Book Co., New York, USA, 523 pp.

Kerber, E.R., Rowland, G.G. 1974. Origin of the free-threshing character in hexaploid wheat. Can. J. Genet. Cytol. **16**:145–154.

Kilian, B., Ozkan, H., Walther, A., Kohl, J., Dagon, T., Salamini, F., Martin, W. 2007. Molecular diversity at 18 loci in 321 wild and 92 domesticated lines reveal no reduction in nucleotide diversity during *Triticum monococcum* (Einkorn) domestication: Implications for the origin of agriculture. Mol. Biol. Evol. **24**: 2657–2668.

Kuraparthy, V., Sood, S., Gill, B.S. 2007. Genomic targeting and mapping of tiller inhibition gene *(tin3)* of wheat using ESTs and synteny with rice. Funct. Integr. Genomics. **8:**33–34.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., Newburg, L. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:**174–181.

Li, W., Gill, B.S. 2006. Multiple genetic pathways for seed shattering in the grasses. Funct. Integr. Genomics **6**:300–309.

Lincoln, S.E., Daly, M.J., Lander, E.S. 1993. Constructing genetic maps with MAPMAKER/EXP version 3.0: A tutorial and reference manual. Whitehead Inst. Biomed. Res. Tech. Rpt. 3rd edn. Whitehead Institute for Biomedical Research, Cambridge, UK, 97 pp.

MacKey, J. 1966. Species relationship in Triticum. In: Proceedings of the 2nd International Wheat Genetics Symposium (Lund) 1963, Sweden. Hereditas (suppl.) **2**:237–276.

Muramatsu, M. 1986. The super vulgare gene, Q: Its universality in durum wheat and its phenotypic effects in tetraploid and hexaploid wheats. Can. J. Genet. Cytol. **28**:30–41.

Nalam, V.J., Vales, M.I., Watson, C.J.W., Kianian, S.F., Riera-Lizarazu, O. 2006. Map-based analysis of genes affecting brittle rachis character in tetraploid wheat (*Triticum turgidum* L.). Theor. Appl. Genet. **112**: 373–381.

Nalam, V.J., Vales, M.I., Watson, C.J.W., Johnson, E.B., Riera-Lizarazu, O. 2007. Map-based analysis of genetic loci on chromosome 2D that affect glume tenacity and threshability components of free-threshing habit in common wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **116**:35–145.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, A.R., Allard, R.W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. **81:**8014–8018.

Salamini, F., Ozkan, H., Brandolini, A., Schafer-Pregl, R., Martin, W. 2002. Genetics and geography of wild cereal domestication in the Near East. Nat. Rev. Genet. **3**:429–441.

Simonetti, M.C., Bellomo, M.P., Laghetti, G., Perrino, P., Simeone, R., Blanco, A. 1999. Quantitative trait loci affecting free-threshing habit in tetraploid wheats. Genet. Res. Crop Evol. **46**:267–271.

Simons, K.J., Fellers, J.P., Trick, H.N., Zhang, Z., Tai, Y.S., Gill, B.S., Faris, J.D. 2006. Molecular characterization of the major wheat domestication gene Q. Genetics **172**:547–555.

Singh, K., Ghai, M., Garg, M., Chhuneja, P., Kaur, P., Schnurbusch, T., Keller, B., Dhaliwal, H.S. 2007. An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* × *T. monococcum* RIL population. Theor. Appl. Genet. **115**:301–312.

Singh, K., Chhuneja, P., Singh, I., Sharma, S.K., Garg, T., Garg, M., Keller, B., Dhaliwal, H.S. 2010. Molecular mapping of cereal cyst nematode resistance in *T. monococcum* L. and its transfer to genetics background of cultivated wheat. Euphytica **176**:213–222.

Sood, S., Kuraparthy, V., Bai, G., Gill, B.S. 2009. The major threshability genes soft glume *(sog)* and tenacious glume *(tg),* of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. Theor. Appl. Genet. **119**:341–351.

Taenzler, B., Esposti, R.F., Vaccino, P., Brandolini, A., Effgen, S., Heun, M., Schafer-Pregl, R., Borghi, B., Salamini, F. 2002. Molecular linkage map of einkorn wheat: Mapping of storage-protein and soft-glume genes and bread-making quality QTLs. Genet. Res. Camb. **80**:131–143.

Taketa, S., Amino, S.Y., Tsujino Sato, T., Saisho, D., Kakeda, K., Nomura, M., Suzuki, T., Matsumoto, T., Sato, K., Kanamori, H., Kawasaki, S.,Takeda, K. 2008. Barley grain with adhering hulls is controlled by an ERF family transcription factor gene regulating a lipid biosynthetic pathway. Proc. Natl. Acad. Sci. **105**: 4062–4067.

Watanabe, N., Ikebata, N. 2000. The effects of homoeologous group 3 chromosomes on grain colour dependent seed dormancy and brittle rachis in tetraploid wheat. Euphytica **115**:215–220.

Wicker, T., Stein, N., Albar, L., Feuillet, C., Schlagenhauf, E., Keller, B. 2001. Analysis of a contiguous 211 kb sequence in diploid wheat (*Triticum monococcum* L.) reveals multiple mechanisms of genome evolution. Plant J. **26**:307–316.

Yan, L., Loukoianov, A., Tranquilly, G., Helguera, M., Fahima, T., Dubcovsky, J. 2003. Positional cloning of the wheat vernalization gene VRN1. Proc. Natl. Acad. Sci. **100**:6263–6268.

Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., San-Miguel, P., Bennetzen V., Dubcovsky, J. 2004. The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science **303:**1640–1644.