RESEARCH

Transfer of Soft Kernel Texture from *Triticum aestivum* to Durum Wheat, *Triticum turgidum* ssp. *durum*

Craig F. Morris,* Marco C. Simeone, G. E. King, and Domenico Lafiandra

ABSTRACT

Durum wheat (*Triticum turgidum* ssp. *durum*) is a leading cereal grain whose primary use is the production of semolina and pasta. Its rich culinary relationship to humans is related, in part, to its very hard kernel texture. This very hard texture is due to the loss of the *Puroindoline* genes that were eliminated during the allopolyploid formation of *T. turgidum* approximately 0.5 million years ago. In the present report, we describe the transfer of the *Puroindoline* genes through *ph1b*-mediated homoeologous recombination. *Puroindoline a* and *Puroindoline b* were successfully recombined (translocated) from chromosome 5D of the soft wheat (*T. aestivum*) variety Chinese Spring into cv. Langdon durum using a Langdon 5D(5B) disomic substitution line. Although initial recombination lines were highly unstable, recurrent backcrossing into Svevo durum cultivar produced stable lines that segregated in a normal 1:2:1 soft:heterozygous:very hard ratio. The final backcross (BC_3) Svevo line produced uniformly soft grain (Single Kernel Characterization System hardness of 24 ±14). The transfer of this fundamental grain property to durum wheat will undoubtedly have an expansive and profound effect on the way that durum grain is milled and on the products that are made from it. As such, our interaction with this important food species will continue to evolve.

C.F. Morris, USDA-ARS, Western Wheat Quality Lab. (USDA ARS WWQL), E-202 Food Science & Human Nutrition Facility East, P.O. Box 646394, Washington State Univ., Pullman, WA 99164-6394; M.C. Simeone, Dep. of Agrobiology and Agrochemistry, Univ. of Tuscia, Via S. Camillo De Lellis, Viterbo, Italy 01100 (formerly postdoctoral research associate at USDA-ARS WWQL); G.E. King, Dep. of Food Science and Human Nutrition, Washington State Univ., E-202 Food Science & Human Nutrition Facility, Pullman, WA 99164-6394 (assigned to the USDA-ARS WWQL); D. Lafiandra, Dep. of Agrobiology and Agrochemistry, Univ. of Tuscia, Via S. Camillo De Lellis, Viterbo, Italy 01100.

Received 27 May 2010. *Corresponding author (morrisc@wsu.edu).

Abbreviations: mt, metric tonnes; HI, hardness index; PCR, polymerase chain reaction; SKCS, Single Kernel Characterization System.

The world produced 2.34×10^9 metric tonnes (mt) of cereal grains in 2007 (FAO, 2008). Of this, wheat (*Triticum* spp.) represented 26% (6.00×10^8 mt). But of the cereals consumed for food, wheat represented nearly half (4.21×10^8 mt of 9.49×10^8 mt total, data from 2003) (FAO, 2008). The success of wheat as a crop results from three main factors: (1) its culture is adapted to a wide range of environments, (2) its dry grain is easily stored and transported, and (3) its grain can be processed into a limitless variety of healthy and appealing foods.

Two principal species comprise nearly all the wheat produced in the world (FAO, 2008). The vast majority is the allohexaploid (*2n* = 42 = AABBDD), *T. aestivum* L.; durum wheat (*T. turgidum*

Published in Crop Sci. 51:114–122 (2011).
doi: 10.2135/cropsci2010.05.0306
Published online 22 Nov. 2010.

ssp. *durum* [Desf.(Husn.)]) is an important albeit minor crop worldwide (Bonjean and Angus, 2001; Fabriani and Lintas, 1988). Annual world production of durum wheat stands at about 3.5×10^7 mt. Durum wheat is an allotetraploid ($2n = 28$ = AABB) and its ancestor, *T. turgidum* ssp. *dicoccoides* (Körn. ex Asch. & Graebn.), is considered one of the progenitors of hexaploid wheat, having contributed two of the three sets of the seven haploid homoeologous chromosomes. World durum production is limited owing to a generally more restricted range of adaption and the very specialized nature of the foods prepared from durum grain, primarily pasta and couscous.

From the human perspective of grain utilization (processing and end-uses), the single most important trait in wheat is the physical texture of the caryopsis (kernel texture or "grain hardness"). "Soft" *T. aestivum* wheats are used for biscuits, cakes, confections, and some types of noodles and steamed breads, whereas "hard" *T. aestivum* wheats are used for yeast-leavened breads and other styles of steamed breads and noodles (Morris and Rose, 1996). The genetic basis for the soft and hard kernel texture classes are the two puroindoline genes, *Puroindoline a* and *Puroindoline b* (*Pina* and *Pinb*, respectively), which are coded at the *Hardness* locus on the distal portion of chromosome 5DS (reviewed in Morris, 2002; Bhave and Morris, 2008a,b). PINA and PINB function in some unknown manner to create the soft kernel phenotype. If either puroindoline gene is perturbed by mutation (nucleotide base changes, deletions, or insertions), the softening effect is greatly reduced and the hard kernel texture phenotype results. Soft and hard wheats mill very differently and produce flours of markedly different particle size, starch damage, and other end-use properties. Durum wheat is completely lacking in the *Ha* puroindoline genes and consequently its kernel texture is considered "very hard," notably harder than "hard" *T. aestivum* wheat. Because of this very hard kernel trait, durum wheat requires a specialized milling process that produces as its primary product coarse "semolina," as opposed to finer-textured "flour." The U.S. Code of Federal Regulations provides definitions for each.

The forerunner of modern durum wheat, *T. turgidum* ssp. *dicoccoides* ('wild emmer') originated some 0.5 to 3 million years ago (refs. cited in Chantret et al., 2005) through the hybridization of two wild grasses, *T. urartu* (Tumanian ex Gandilyan) ($2n = 14$ = AA) and a member of the *Sitopsis* section of *Aegilops* (an unidentified diploid, $2n = 14$, with a genome designated "BB" mostly similar to *Ae. speltoides* [Tausch.]). Analysis of these and other related wild diploid members of the Triticeae tribe indicate that all possess forms of the *Pina* and *Pinb* genes (reviewed in Morris, 2002).

Associated with the allotetraploidization event that produced *T. turgidum* ssp. *dicoccoides*, both puroindoline genes from the A and B genomes were eliminated (Chantret et al., 2005; Li et al., 2008). The subsequent allohexaploidization event between *T. turgidum* ssp. *dicoccon* (emmer) and *Ae. tauschii* (Coss.) ($2n = 14$ = DD) some 7000 to 9500 yr ago was accompanied by a restoration of the puroindoline a and b genes from *Ae. tauschii*, and with them, soft kernel texture. Consequently, these two polyploidization events in the evolution of wheat had a profound impact on the processing properties of durum and *T. aestivum* wheat and influenced the culinary relationship that humankind developed with them.

A key to the success of durum and *T. aestivum* wheats as polyploid species lies in the control (i.e., prevention) of pairing and recombination of their homoeologous chromosomes by *Ph1* (*Pairing homeologous*) located on the long arm of chromosome 5B (Okamoto 1957; Sears and Okamoto 1959; Riley and Chapman 1958), effectively making them function as diploids. Although durum and *T. aestivum* wheats can be intermated, there is little if any chance for gene exchange from the D genome (including the puroindoline genes on the short arm of chromosome 5D).

Artificially induced loss-of-function mutations in the *Ph1* gene in *T. aestivum* cv. Chinese Spring (*ph1b*) (Sears, 1977) and the durum cultivar Cappelli (*ph1c*) (Giorgi, 1983) relieve the suppression of homoeologous pairing. Although homoeologues may pair in the absence of functional *Ph1*, the frequency and occurrence can vary greatly among different chromosomes and genetic backgrounds and is largely unpredictable (Bonafede et al., 2007; Jauhar et al., 1999; Jauhar and Doramaci, 2008; Luo et al., 2000). Nevertheless, the use of *ph1* mutations is an effective means of chromosome engineering and wheat improvement (Ceoloni et al., 2005; Qi et al., 2007).

A second important feature of the allopolyploid nature of durum and *T. aestivum* wheats is their capacity to tolerate aneuploidism, including homoeologous substitution. Joppa produced a set of disomic substitution lines wherein each of the durum chromosome pairs were replaced by the homoeologous D-genome pair from Chinese Spring, for example 1D(1B) (Joppa, 1973; Joppa and Williams, 1983; Joppa et al., 1983). In general, durum wheat is markedly less tolerant of aneuploidy than is *T. aestivum*.

The manipulation of kernel texture in wheat and other cereals is of great technological importance. Numerous naturally occurring mutations in the puroindoline genes have been documented in *T. aestivum*, but all result in harder kernel texture (reviewed in Morris, 2002; Bhave and Morris, 2008a,b; Morris and Bhave, 2008).

To create soft(er) *T. aestivum* wheat, additional copies of the endosperm-softening puroindolines have been introduced by means of cytological approaches. The *Ha* locus from *T. monococcum* L. (nearly the entire short arm of $5A^m$) was transferred to Chinese Spring (Luo et al., 2000; Tranquilli et al., 2002). Bonafede et al. (2007) reduced this $5A^m$ chromatin to only 6.3 cM using *ph1b*. Pshenichnikova et al. (2010) transferred *Ha* from *Ae. speltoides* into 5A of

T. aestivum cv. Rodina, thereby softening this hard grain variety. See et al. (2004) crossed disomic substitution lines $5A^m$(5A) and $5S^s$(5B) in Chinese Spring and recovered progeny with both substitutions. Lines carrying the $5A^m$ and/or $5S^s$ chromosomes were softer than Chinese Spring; progeny with both substitutions were softest of all (the $5S^s$ were from *Ae. searsii* Feldman & Kislev ex K. Hammer).

Durum wheat can be made soft by similar means. Liu et al. (1995) showed that the 5D(5A) and 5D(5B) D-genome disomic substitution lines produced by Joppa and Williams (1988) using Chinese Spring were dramatically softer in grain hardness. Drawbacks regarding the subsequent use of these lines for crop improvement are that (1) in future crosses with durum plants, the 5D chromosomes will not pair and thus will be inherited without recombination, and (2) the 5D(5B) substitution line is unstable owing to the absence of chromosome 5B and thus a functional *Ph1*. Consequently, one strategy would be to reduce the 5D chromatin in the 5A substitution using *ph1*.

Preliminary reports indicated the successful transfer of *Ha* from *T. aestivum* into the durum genome with the result being a soft kernel phenotype (Gazza et al., 2002, 2003, 2008; Simeone et al., 2003). Both groups took similar approaches, that is, *ph1*-mediated recombination involving the *T. aestivum* 5D chromatin containing the *Ha* locus and its homoeologous counterparts of durum wheat. However, in none of these reports is the development of stable lines adequately described, nor is there sufficient description of how these lines could be used in future crosses with durum parents. The present report describes in detail the production of durum lines with soft kernel texture via homoeologous recombination (translocation), and their stable use in crossing and transfer of the soft kernel texture trait to other durum cultivars.

MATERIALS AND METHODS

Taxonomy follows the naming conventions established by van Slageren (1994). Other terminology follows the definitions of Rieger et al. (1976).

The initial putative soft durum recombination lines were developed by Dr. Leonard Joppa. For completeness and because this work is not described elsewhere, the genetics stocks and general scheme are presented here. Beginning stocks included 'Langdon-16', a selection of Langdon durum (*T. turgidum* ssp. *durum* cv. Langdon [Desf.] Husn., CItr13165); Chinese Spring (*T. aestivum*; CI14108); 'Chinese Spring disomic substitution line 5D(5B)' (equivalent to Chinese Spring Nullisomic 5B/Tetrasomic 5D [N5B/T5D]); 'Chinese Spring *ph1b/ph1b*' (Chinese Spring carrying the *ph1b* mutation); 'Langdon double ditelosomic-5B' (an aneuploid stock of Langdon wherein the 5B complete chromosomes of Langdon have been replaced with pairs of the corresponding telocentric 5BS and 5BL chromosomes) (Joppa and Williams, 1977), 'Langdon-47-1', a line of Langdon carrying the *Pairing homoeologous-1* mutation (*ph1b*) (pedigree = 'Chinese Spring *ph1b/ph1b*/⋆2 Langdon-16//2⋆Langdon-47-1) (*NB*: this line was maintained in the heterozygous condition such that F_2 seed were employed); 'Langdon 5D(5B)', a substitution line of Langdon wherein the 5B chromosomes of Langdon have been replaced with a pair of 5D chromosomes from Chinese Spring (pedigree = Chinese Spring 5D(5B)/⋆12 Langdon-16) (Joppa et al., 1978). Chromosome staining and pairing analysis were conducted using pollen mother cells at metaphase I of meiosis or root tip meristems (acetocarmine squashes) as appropriate.

Development of Putative Recombination ("Translocation") Lines

Development of homoeologous 5D/5B translocation lines were accomplished using the following scheme. All procedures were conducted in a glasshouse.

Step 1. Langdon 5D(5B) crossed with Langdon-47-1 (*Ph1/ph1b*).

Step 2. F_1 plants with $2n = 28$ chromosomes were selected and grown, those with 13 bivalents + 2 univalents were assumed not to carry *ph1b*, whereas those with variable pairing were assumed to carry *ph1b*; plants with variable pairing that carried *ph1b* were selected.

Step 3. F_2 seeds were harvested; plants with variable pairing (*ph1b*) were selected. Plants in which some cells had at least one of the chromosomes failing to form ring bivalents were identified, as this may indicate that one of the chromosomes was a recombination (translocation) between 5B and 5D.

Step 4. Selected F_2 plants were crossed to Langdon-16 (considered backcross 1, "BC_1").

Step 5. BC_1F_1 plants with 13 pairs of chromosomes at metaphase I of meiosis and variable pairing were selected and self-fertilized.

Step 6. BC_1F_2 plants were grown, emasculated, and "test crossed" by crossing to Langdon double ditelosomic-5B (a test for 5B/5D recombination vs. whole chromosome substitution).

Step 7. F_1 progeny were grown and plants with at least some meiocytes with 13 bivalents + 1 telomeric (heteromorphic) bivalent + 1 telocentric univalent (suggestive of a 5B/5D recombination) were selected; plants with 13 bivalents + 1 trivalent comprised of one whole chromosome and 2 telocentric univalents, one for each arm all paired together (indicating that the 5B chromosome was present) were discarded; and plants with 13 bivalents + 1 univalent + 2 telocentric univalents (indicating the presence of a 5D disomic substitution) were discarded.

Step 8. Selected plants were crossed to Langdon 5D(5B) substitution line; seeds were harvested and germinated, and cytological analysis identified those parental plants in which only one of the chromosomes failed to form ring bivalents (an indication but not proof of recombination). These selected parents were grown and self-fertilized.

The pedigree of the resulting putative recombination progeny is: Chinese Spring(*ph1b*)/2⋆Langdon-16//2⋆Langdon-471/3/Chinese Spring 5D(5B)/⋆12 Langdon-16/4/Langdon double ditelosomic-5B/5/Chinese Spring 5D(5B)/12 Langdon-16.

Table 1. F_3–derived putative soft-kernel Langdon durum recombination lines, seed production, and result of crossing to Svevo durum cultivar.

F_3–derived recombination line	F_3:F_4 seeds produced	F_3:F_5 seeds produced	Used for crossing to Svevo	F_1 seeds produced with Svevo	F_1 plants that produced seed for SKCS†	No. (%) of soft progeny in crosses with Svevo‡
	n	g				
674	35	–	Yes	21	21	3 (< 1)
675	16	17.3	Yes	48	45	17 (38)
677	20	10.2	Yes	No	–	–
678	55	23.3	Yes	10	10	3 (30)
679	22	0.2	Yes	No	–	–
680	14	–	No	No	–	–
681	8	–	No	No	–	–
682	34	–	No	No	–	–
683	24	36.2	Yes	1	1	0 (0)
684	13	–	No	No	–	–
685	17	4.3	Yes	6	6	0 (0)
686	41	0.6	Yes	7	6	1 (17)
687	27	2.2	Yes	No	–	–
688	76	1.9	Yes	78	78	17 (22)

†SKCS, Single Kernel Characterization System.

‡"Soft" defined as HI < 39.

Glasshouse and field plot plant culture and crossing were conducted using common practices. All crosses were conducted in a glasshouse. DNA extraction, polymerase chain reaction (PCR) protocols, and puroindoline primers followed methods of Gautier et al. (1994). Kernel texture ("hardness") testing employed the Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Inc., Springfield, IL) following AACC International Approved Method 55-31 (AACCI, 2000). The SKCS instrument crushes individual kernels and transforms a "force-crush profile" and kernel weight into a unitless numerical hardness index (HI). Very generally, soft wheats center around HI of 25 and hard wheats around HI of 75 (Morris et al., 2001). (The SKCS unitless scale was developed to emulate previous near infrared reflectance spectroscopy technology.)

RESULTS

Development of Putative Soft Kernel Homoeologous Recombination Lines

The preliminary work of Dr. Joppa involved (1) using the Chinese Spring N5B/T5D line of Sears (1966) to produce a 5D(5B) disomic substitution in cv. Langdon durum and (2) transferring the *ph1b* mutation from Chinese Spring *ph1b/ph1b* to Langdon. The disomic substitution, since it lacks *Ph1*, was maintained with a 5B monosome and used as male in crosses since the monosome rarely transmits through the pollen. The Langdon *ph1b/ph1b* ('Langdon-47-1'), which also lacks a functional *Ph1*, was maintained and used as a heterozygote. Of note, Joppa also produced the Langdon 5D(5A) disomic substitution, but the progeny were nearly sterile, and even when maintained with a 5A monosome the plants were quite sterile. Consequently the 5D(5B) substitution was the sole option. The first cross resulted in F_1 plants that were monosomic for both 5D and 5B, with some progeny expected to carry *ph1b*. Plants with *ph1b* were expected to have homoeologous pairing and crossing over between 5D and 5B; they were also expected to have considerable genome instability. F_3 seeds were obtained from Dr. Joppa.

Transfer of the Soft Kernel Trait to Svevo Italian Durum Cultivar

The parental plants identified in the final Step 8 produced F_3 plants that were rather weak and partially sterile, and some exhibited defects in chromosome pairing. F_3–derived F_4 seeds were harvested from 14 plants; seed yields are shown in Table 1 and ranged from $n = 8$ to 76 per plant. These plants represented the primary putative homoeologous Langdon recombination lines.

A subset of approximately 170 F_3–derived F_4 plants from the 14 putative recombination lines were grown in a glasshouse. Nearly 50% died of low vigor or were sterile and set no seed. Conversely, some plants were very vigorous, similar to Svevo and Langdon. Progeny vigor was independent of the F_3 line from which the plant was derived. Plants were allowed to self-pollinate (seed yields in Table 1). From some of these plants pollen was harvested and used in crossing to Svevo (indicated in Table 1).

The two putative recombinant F_3 lines with the most well-developed seed (678 and 683) were selected for further characterization. Ten seeds from each of 10 self-pollinated progeny from each of these two lines were bulked and subjected to SKCS kernel texture analysis. Single Kernel Characterization System results showed both kernel samples were highly heterogeneous. Mean HI and standard deviations were 34 ±31 and 41 ±33 for lines 678 and 683, respectively. The SKCS instrument also produces a four-class histogram with HI limits of ≤33, 34–46, 47–59, and ≥60. For line 678

the percentages of kernels in each class were 59, 5, 6, and 30, and for line 683 the percentages were 48, 7, 7, and 38. These data clearly indicated that as a population of kernels, the kernel texture distributions for both lines were highly bimodal, with about half the kernels soft (i.e., HI ≤33).

From crosses with Svevo, 176 F_1 kernels were obtained and all were planted; about 96% of the plants set seed. Height was recorded on the F_1 plants and exhibited a marked bimodal distribution. Langdon is a tall "standard" height cultivar, whereas Svevo is semi-dwarf. The observed ratio was 74 short:97 tall. This unexpected phenotypic observation indicated the highly unstable cytological nature of these lines. Seed was harvested from each plant and subjected to SKCS kernel texture analysis. The HI should be viewed as the kernel texture phenotype of a subset of the progeny from a particular plant. Seed (F_2) from nearly all plants (n = 166) was evaluated using the SKCS. The HI ranged from 5 to 82, with standard deviations from 7 to 35. Again, these data are not consistent with normal $F_{1:2}$ phenotypes generally encountered. From general experience, soft and hard hexaploid (*T. aestivum*) varieties delineate HI around 45 to 50, with respective means centered on about 25 (soft) and 75 (hard) (Morris et al., 2001). Further, for "pure" grain lots of uniform genetics from a single environment, the standard deviation (SD) rarely exceeds approximately 16 to 18; values greater than this are indicative of heterogeneous grain lots, in other words, physical mixtures or segregating populations.

Plots of the SKCS HI SD vs. HI for the progeny of each putative recombination line are presented in Fig. 1. A visual inspection and comparison of the plots facilitated some phenotypic groupings based on HI and HI SD distributions, and the aforementioned empirical experience with means and HI SD in hexaploid and durum wheat. For the progeny of all but one cross, delineations at 39 and 68 HI provided empirical phenotypic groupings based on kernel texture. The distribution of progeny of line 688, which produced the greatest number of fertile F_1 plants, suggested a slightly different separation but was nonetheless similar to the other crosses (Fig. 1F, cf. Figs. 1A–E). Other "gaps" in the HI distributions could be envisaged but were not as consistent across crosses. Similarly, a delineation in HI SD was usually apparent in most of the crosses, and a line could be drawn at HI SD of 18 (a reasonable value based on prior experience) (Fig. 1A–F). Certainly this delineation was not without some subjectivity, and others could be justified or valid. On the basis of these phenotypic classifications, each population was divided into six possible sectors. In typical, monogenic, two-allele, biparental crosses, typical of the *Hardness* locus in *T. aestivum*, only three of these sectors would be populated, those being (1) a "soft" parental class of low HI and low HI SD, (2) a "hard" parental class of high HI and low HI SD, and (3) a "heterozygote" class wherein seeds are segregating on the spike of a heterozygous plant producing an intermediate mean HI but a high HI SD, reflecting the multiple kernel haplotypes associated with that plant. The expected ratio of these classes is 1:2:1, soft:intermediate:hard. None of the durum putative recombinant line–Svevo crosses conformed to this model (Fig. 1), indicating that cytologically these lines were highly unstable.

In all six crosses except 686, progeny with HI greater than 68 and HI SD less than 18 were observed (no progeny with HI greater than 68 and HI SD greater than 18 were observed). These progeny were assumed to lack the *Hardness* locus from chromosome 5D such that they were uniformly hard at a level similar to Svevo and Langdon and other durum wheats and were judged as possessing no recombination involving the *Hardness* locus.

Surprisingly, progeny in the 39 to 68 HI range mostly had lower HI SD (less than 18), indicating that the kernels from each plant were similar in texture. In most crosses, the prevalence for HI SD greater than18 was with progeny having HI less than 39. But again, this was not consistently so, and there were progeny with HI less than 39 that also had HI SD less than 18. Consequently, it was difficult to adequately interpret these results, that is, to make some model for the mode of inheritance that would be consistent with these phenotypic distributions. Kernel texture follows the triploid double fertilization additive gene model for endosperm texture (Bettge et al., 1995). Without extensive further analysis, no explanation could be provided to adequately explain these observations. Male gamete transmission distortions were certainly a possibility. A cursory study, however, provided the following results.

Eight F_1 plants from the Svevo/675 cross were selected with HI from 20 to 48 and HI SD in the range of 10 to 24. One F_2 plant was grown from each and self-fertilized. Two to five F_3 plants from each F_2 were grown to maturity and harvested, and the seed was used to plant an F_4–derived row at the Washington State University Spillman Agronomy Farm. Twenty-five plant rows in total were grown, and the F_5 seed were assayed for SKCS kernel texture on a per-row basis (Fig. 2). The distribution of kernel texture phenotype was more in keeping with a single locus–two allele model wherein those lines with HI greater than 65 and HI SD less than 16 apparently lacked a 5DS-derived *Hardness* locus and were uniformly hard, those with HI from about 39 to 65 and HI SD greater than 18 were heterogenous (ostensibly segregating), and those with HI less than 39 were candidate carriers of a homozygous *Ha* locus and were soft. In this group of soft-kernel plants, the HI SD ranged from 11 to 18. Svevo was located in the "hard" group.

Although the primary Svevo–recombinant line F_1 results could not be immediately accounted for, most crosses, nevertheless, produced "soft" kernel durum plants, which was the primary interest and goal of this work. Consequently, the progeny of greatest interest were those with HI below ~39. This level of kernel texture is not encountered in *T. turgidum* ssp. *durum* and is in the range of soft *T.*

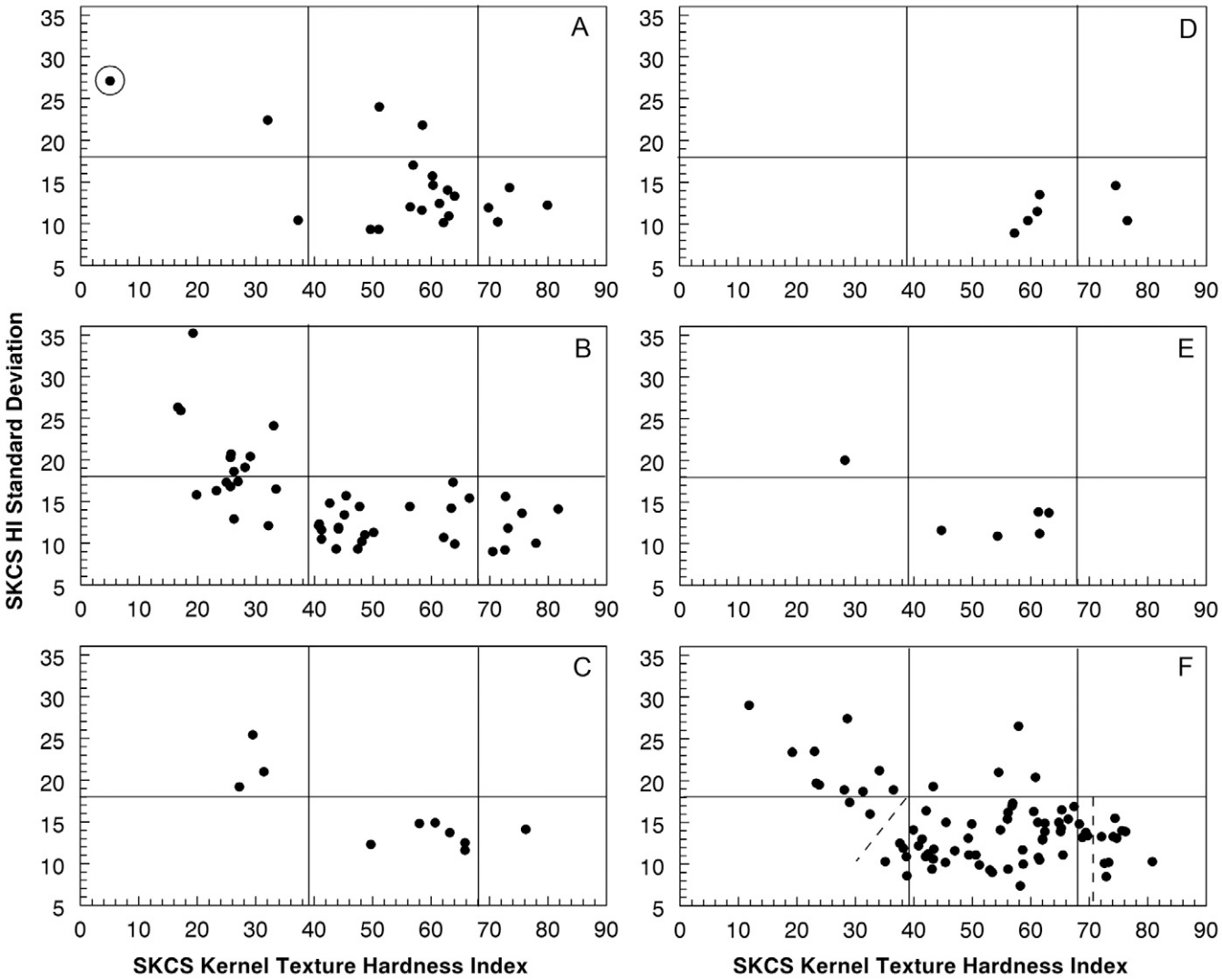

Figure 1. Plot of Single Kernel Characterization System (SKCS) kernel texture hardness index (HI) standard deviation versus SKCS HI of F_2 kernels harvested from individual F_1 plants (*n* indicated) derived by crossing the Italian durum wheat (*Triticum turgidum* ssp. *durum*) cultivar Svevo with Langdon durum putative homoeologous recombination lines. Graphic lines indicate suggested delineations in kernel texture phenotype classes. $F_{3:5}$ Langdon putative recombinant plants were used as male. (A) Putative recombinant line '674' (*n* = 21); the circled symbol identifies line 'No. 152' that was selected for backcrossing. (B) Putative recombinant line '675' (*n* = 45). (C) Putative recombinant line '678' (*n* = 10). (D) Putative recombinant line '685' (*n* = 6). (E) Putative recombinant line '686' (*n* = 6). (F) Putative recombinant line '688' (*n* = 78).

aestivum wheats. Line 674 produced three fertile F_1 progeny plants with Svevo in this soft range (Fig. 1A). One F_1 plant, in particular, was very soft (HI = 5, designated line 'No. 152'). The other crosses produced from 0 to 17 progeny with HI less than 39, proportionally 0 to 38% of progeny (mean of 25% over all F_1 plants) (Table 1).

The softest line (No. 152) from the Svevo/674 cross with HI = 5 (Fig. 1A) was selected for further backcrossing. DNA was extracted from distal half seeds (*n* = 10) and assayed using *Pina* and *Pinb* primers in PCR for the presence of these two genes. Six of the 10 seeds were positive for both *Pina* and *Pinb*. The remaining embryo halves were germinated and the plants grown, emasculated, and backcrossed to Svevo as female (representing BC_1). Resultant progeny were assayed for *Puroindolines* via PCR as before (10 of 19 seeds were positive for both genes); positive progeny were selected, propagated, and used for BC_2. The process was repeated for BC_3. *Puroindoline* PCR-positive progeny were grown in the field near Viterbo, Italy, and allowed to self-pollinate. Four to seven BC_3F_2 seeds from each of these BC_3F_1 plants were subjected to SKCS kernel texture analysis on an individual plant basis (Fig. 3). A total of 92 plants were evaluated along with 2 plants of Svevo. From these results the most promising plant (identified as 'No. 88') was selected as having a high probability of being uniformly soft and carrying the *Ha* locus. Line No. 88 was advanced through two more cycles of self-pollination to produce BC_3F_4 seed.

A 500-g sample of the BC_3F_4 seed of Line No. 88 was provided to Kim Shantz, WestBred LLC, Yuma, AZ,

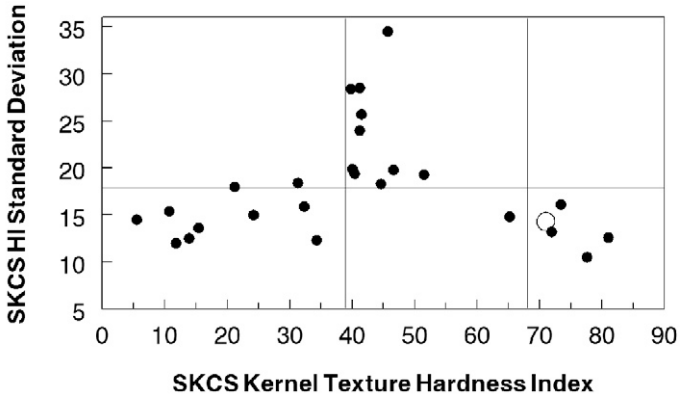

Figure 2. Plot of Single Kernel Characterization System (SKCS) kernel texture hardness index (HI) standard deviation versus SKCS HI of F_6 kernels harvested from individual F_5 plants (n = 25) derived by self-pollinating the progeny from crosses involving three different Langdon durum putative homoeologous recombinant lines and Svevo Italian durum cultivar (filled circles). Svevo (larger open circle) parent value is shown. Graphic lines indicate suggested delineations in kernel texture phenotype classes.

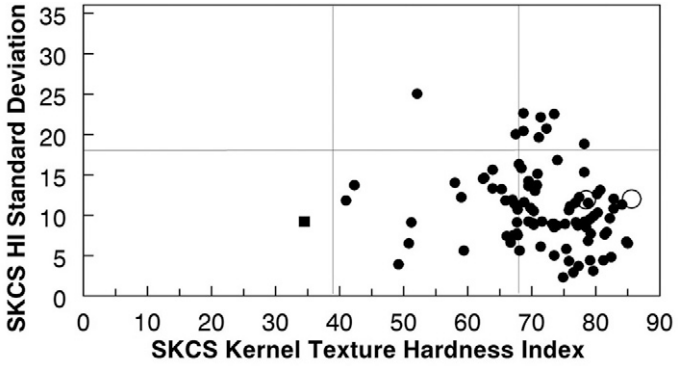

Figure 3. Plot of Single Kernel Characterization System (SKCS) kernel texture hardness index (HI) standard deviation versus SKCS HI of BC_3F_2 kernels harvested from individual BC_3F_1 plants (n = 91) derived from Svevo/674 line 'No. 152' (filled circles and filled square). The selected progeny (line 'No. 88') (filled square) is shown, as are two Svevo (larger open circles) parents.

for field increase during the 2007–2008 crop season. The line was morphologically and developmentally indistinguishable from Svevo growing at the same location (K. Shantz, personal communication). Approximately 135 kg of BC_3F_5 seed was harvested in 2008. The kernel texture of this field increase was HI 24 ±14, indicating that it was uniformly soft. Seed of this line and this specific grain lot was deposited with the American Type Culture Collection as Accession No. PTA-10087. Other work has confirmed a stable transfer of the soft kernel trait to five other improved durum cultivars (data not shown).

Preliminary studies have since been conducted to characterize the milling quality of this novel soft-textured durum grain (Morris et al., 2009). In that study, break flour yield of the soft durum (Miag Multomat Breaks 1, 2, and 3; see Posner and Hibbs, 1997) equaled 21.8%, Break 1 produced 10.0% flour yield, and straight grade flour yield was 74.9%. Comparable yields for Xerpha soft white winter and Blanca Royale hard white spring varieties also milled on the Western Wheat Quality Lab's Miag Multomat as part of the 2009 Pacific Northwest Wheat Quality Council collaborative were 24.4% break flour, 9.5% Break 1 yield, and 74.7% straight grade yield, and 15.8% break flour, 4.9% Break 1 yield, and 73.5% straight grade yield, respectively. Durum wheat has not been milled on this mill, but would produce dramatically different results.

DISCUSSION

Polyploidization has been a highly successful evolutionary event in many plant species, including wheat, but apparently carries the risk of gene elimination (Feldman et al., 1997; Feldman and Levy, 2005). Such a loss of genetic material occurred with the allotetraploidization of *T. turgidum*. Interestingly, the *Hardness*-bearing loci were eliminated from both the A- and B-genomes (Chantret et al., 2005). From the standpoint of humankind's interaction with this species (perhaps for tens of thousands of years), a key feature has been the very hard kernels that *T. turgidum* ssp. *durum* produces. The two species (wheat and human) have influenced each other's evolutionary trajectories: on the one hand, domestication and then selection for free-threshing forms in *T. turgidum*, and on the other the development of sedentary agriculture and a largely cereal-based diet. It would be fair to state that where cultures have traditionally eaten pasta, they have done so because of a long interaction with this very hard-kernel wheat.

The soft kernel trait of *T. aestivum* resulted from *Ae. tauschii*, and similar to durum, the cultural and culinary relationships that formed between *T. aestivum* and humankind are intimately intertwined with its kernel texture. In the case of *T. aestivum*, however, humans have, in the last 100 to 150 yr or so, actively and preferentially selected (albeit unknowingly) mutations in the kernel softening genes, *Puroindoline a* and *Puroindoline b*, preferring the milling and processing properties of harder-kernel wheats that are more compatible with the steel roller mill and production of yeast-leavened breads (Wiley, 1884).

In the present report we describe in detail the development of soft kernel durum wheat through homoeologous transfer of the puroindoline genes and the *Hardness* locus from *T. aestivum*. A unique complication in this work is the involvement of homoeologous group 5 chromosomes, as they carry not only *Ha* but also *Ph1*. Of further note, *Vrn1B* is located on this chromosome; durum is intolerant of nullisomy of 5B. In total, the use of *Ph1*, aneuploid, and other cytological stocks, although by no means trivial, was successful in accomplishing the desired recombination. The initial recombinant lines in Langdon and first generations of crossing and backcrossing were highly unstable. Nevertheless, by the third backcross, phenotypic segregations were in keeping with single locus bi-allele segregation. We have used line No. 88 in subsequent

crosses and backcrosses to five other improved North American durum varieties and have consistently obtained single-locus, bi-allele segregation (data not shown).

It is most probably accurate to state that with the exception of whole chromosome substitutions (Liu et al., 1995; Joppa and Williams, 1988), soft-kernel durum wheat has never existed before, and certainly has not been at the disposal of plant breeders and food technologists. As such this development has the potential to alter the future relationship that humankind has with this species, hopefully to the betterment of both.

Acknowledgments

Dr. Leonard Joppa, formerly of the USDA, Agricultural Research Service, Northern Crop Sciences Laboratory, Fargo, ND, was instrumental in this research, as he developed the cytological stocks and produced the initial putative recombination lines. At his behest, he declined co-authorship on the present manuscript. The assistance of Stacey Sykes, Shawna Vogl, and Chris Hoagland are gratefully acknowledged. Profs. Kulvinder Gill and Adam Lukaszewski are thanked for their reviews and helpful discussions; the extensive edits and suggestions of an anonymous reviewer are greatly appreciated.

References

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th ed. The Association, St. Paul, MN. Method 55-31.

Bettge, A.D., C.F. Morris, and G.A. Greenblatt. 1995. Assessing genotypic softness in single wheat kernels using starch granule-associated friabilin as a biochemical marker. Euphytica 86:65–72.

Bhave, M., and C.F. Morris. 2008a. Molecular genetics of puroindolines and related genes: Allelic diversity in wheat and other grasses. Plant Mol. Biol. 66:205–219.

Bhave, M., and C.F. Morris. 2008b. Molecular genetics of puroindolines and related genes: Regulation of expression, membrane binding properties and applications. Plant Mol. Biol. 66:221–231.

Bonafede, M., L. Kong, G. Tranquilli, H. Ohm, and J. Dubcovsky. 2007. Reduction of a *Triticum monococcum* chromosome segment carrying the softness genes *Pina* and *Pinb* translocated to bread wheat. Crop Sci. 47:819–826.

Bonjean, A.P., and W.J. Angus (ed.). 2001. The world wheat book: A history of wheat breeding. Lavoisier Publishing, New York.

Ceoloni, C., P. Forte, S. Gennaro, S. Micali, R. Carozza, and A. Bitti. 2005. Recent developments in durum wheat chromosome engineering. Cytogenet. Genome Res. 109:328–334.

Chantret, N., J. Salse, F. Sabot, S. Rahman, A. Bellec, B. Laubin, I. Dubois, C. Dossat, P. Sourdille, P. Joudrier, M.F. Gautier, L. Cattolico, M. Beckert, S. Aubourg, J. Weissenbach, M. Caboche, M. Bernard, P. Leroy, and B. Chalhoub. 2005. Molecular basis of evolutionary events that shaped the *Hardness* locus in diploid and polyploid wheat species (Triticum and Aegilops). Plant Cell 17:1033–1045.

Fabriani, G., and C. Lintas (ed.). 1988. Durum chemistry and technology. American Association of Cereal Chemists, St. Paul, MN.

FAO. (2008) FAOSTAT © FAO Statistics Division [Online]. Available at http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID = 567#ancor (accessed 15 Aug. 2008).

Feldman, M., B. Liu, G. Segal, S. Abbo, A.A. Levy, and J.M. Vega. 1997. Rapid elimination of low-copy DNA sequences in polyploid wheat: A possible mechanism for differentiation of homoeologous chromosomes. Genetics 147:1381–1387.

Feldman, M., and A.A. Levy. 2005. Allopolyploidy—a shaping force in the evolution of wheat genomes. Cytogenet. Genome Res. 109:250–258.

Gautier, M.F., M.E. Aleman, A. Guirao, D. Marion, and P. Joudrier. 1994. *Triticum aestivum* puroindolines, two basic cystine-rich seed proteins: CDNA sequence analysis and developmental gene expression. Plant Mol. Biol. 25:43–51.

Gazza, L., A. Niglio, E. Mei, E. De Stefanis, D. Sgrulletta, and N.E. Pogna. 2002. Production and characterization of durum wheat (*Triticum turgidum* ssp *durum*) lines containing puroindolines A and B. p. 285–288. *In* M.G. D'Egidio (ed.) Proc. 2nd International Workshop on Durum Wheat and Pasta Quality: Recent Achievements and New Trends. Instituto Sperimentale per la Cerealicoltura, Rome.

Gazza, L., A. Niglio, F. Nocente, and N.E. Pogna. 2003. Evidence of a gene coding for grain softness protein (*GSP-1a*) on the long arm of chromosome 5D in *Triticum aestivum*. p. 457–460. *In* D. Lafiandra, S. Masci, and R. D'Ovidio (ed.) Proc. 8th International Gluten Workshop. The Royal Society of Chemistry, Cambridge, UK.

Gazza, L., L. Zanella, and N.E. Pogna. 2008. Development of durum wheat (*Triticum turgidum* ssp *durum*) lines with soft kernel texture by chromosome engineering. p. 339–341. *In* R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntyre, and P. Sharp (ed.) Proc. 11th International Wheat Genetics Symposium, Vol. II. Sydney Univ. Press, Sydney.

Giorgi, B. 1983. Origin, behaviour and utilization of a *PH1* mutant of *durum* wheat, *Triticum turgidum* (L.) var. *durum*. p. 1033–1040. *In* S. Sakamoto (ed.) Proc. 6th International Wheat Genetics Symposium. Kyoto Univ., Kyoto.

Jauhar, P.P., A.B. Almouslem, T.S. Peterson, and L.R. Joppa. 1999. Inter- and intragenomic chromosome pairing in haploids of durum wheat. J. Hered. 90:437–445.

Jauhar, P.P., and M. Doramaci. 2008. Chromosome pairing in durum wheat haploids with and without *ph1b* of bread wheat. Euphytica 159:353–358.

Joppa, L.R. 1973. Development of D-genome substitution lines of durum wheat (*Triticum turgidum* L.). p. 685–690. *In* E.R. Sears and L.M.S. Sears (ed.) Proc. 4th International Wheat Genetics Symposium. Univ. of Missouri, Columbia, MO.

Joppa, L.R., J.A. Bietz, and N.D. Williams. 1978. The anueploids of durum wheat: D-genome addition and substitution lines. p. 420–426. *In* S. Ramanujam (ed.) Proc. 5th International Wheat Genetics Symposium. Indian Society of Genetics & Plant Breeding, New Delhi.

Joppa, L.R., C. Josephides, and V. Youngs. 1983. Chromosomal location of genes affecting quality in durum wheat. p. 297–301. *In* S. Sakamoto (ed.) Proc. 6th International Wheat Genetics Symposium. Kyoto Univ., Kyoto, Japan.

Joppa, L.R., and N.D. Williams. 1977. Mono-telo-disomics of durum wheat and the production of ditelosomics. p. 60. *In* The 69th Annual Meeting American Soc. Agron. Abstracts. ASA, Madison, WI.

Joppa, L.R., and N.D. Williams. 1983. The Langdon durum D-genome disomic-substitutions: Development characteristics and uses. p. 68. *In* The 75th Annual Meeting American Soc. Agron. Abstracts. ASA, Madison, WI.

Joppa, L.R., and N.D. Williams. 1988. Langdon durum disomic substitution lines and aneuploid analysis in tetraploid wheat.

Genome 30:222–228.

Li, W., L. Huang, and B.S. Gill. 2008. Recurrent deletions of puroindoline genes at the grain *Hardness* locus in four independent lineages of polyploid wheat. Plant Physiol. 146:200–212.

Liu, C.Y., A.J. Rathjen, K.W. Shepherd, P.W. Gras, and L.C. Giles. 1995. Grain quality and yield characteristics of D-genome disomic substitution lines in 'Langdon' (*Triticum turgidum* var. *durum*). Plant Breed. 114:34–39.

Luo, M.C., Z.L. Yang, R.S. Kota, and J. Dvorak. 2000. Recombination of chromosomes $3A^m$ and $5A^m$ of *Triticum monococcum* with homeologous chromosomes *3A* and *5A* of wheat: The distribution of recombination across chromosomes. Genetics 154:1301–1308.

Morris, C.F. 2002. Puroindolines: The molecular genetic basis of wheat grain hardness. Plant Mol. Biol. 48:633–647.

Morris, C.F., and M. Bhave. 2008. Reconciliation of D-genome puroindoline allele designations with current DNA sequence data. J. Cereal Sci. 48:277–287.

Morris, C.F., D.A. Engle, A.D. Bettge, M.C. Simeone, D. Lafiandra, and K. Shantz. 2009. Milling behavior of a soft durum wheat line [Online]. Cereal Foods World 54:A24. Available at http://www.aaccnet.org/meetings/2009/abstracts/o09ma45.htm (verified 8 Oct. 2010). 2009 AACCI Annual Meeting, Baltimore, MD. 13–16 Sept. 2009.

Morris, C.F., G.E. King, R.E. Allan, and M.C. Simeone. 2001. Identification and characterization of near-isogenic hard and soft hexaploid wheats. Crop Sci. 41:211–217.

Morris, C.F., and S.P. Rose. 1996. Chapter 1.Wheat. p. 3–54. *In* R.J. Henry and P.S. Kettlewell (ed.) Cereal Grain Quality. Chapman Hall, London.

Okamoto, M. 1957. Asynaptic effect of chromosome V. Wheat Inform. Serv. 5:6.

Posner, E.S., and A.N. Hibbs. 1997. Wheat flour milling. American Association of Cereal Chemists, St. Paul, MN.

Pshenichnikova, T.A., A.V. Simonov, M.F. Ermakova, A.K. Chistyakova, L.V. Shchukina, and E.V. Morozova. 2010. The effects on grain endosperm structure of an introgression from *Aegilops speltoides* Tausch. into chromosome 5A of bread wheat. Euphytica. (Published online DOI 10.1007//s10681–010–0168–1) Euphytica 175:315-322.

Qi, L., B. Friebe, P. Zhang, and B.S. Gill. 2007. Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res. 15:3–19.

Rieger, R., A. Michaelis, and M.M. Green. 1976. Glossary of genetics and cytogenetics. Springer-Verlag, Heidelberg.

Riley, R., and V. Chapman. 1958. Genetic control of cytologically diploid behavior of hexaploid wheat. Nature 182:713–715.

Sears, E.R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. p. 29–45. *In* R. Riley and K.R. Lewis (ed.) Chromosome Manipulations and Plant Genetics. Oliver & Boyd, Edinburgh.

Sears, E.R. 1977. An induced mutant with homoeologous pairing in common wheat. Can. J. Genet. Cytol. 19:585–593.

Sears, E.R., and M. Okamoto. 1959. Intergenomic chromosome relationships in hexaploid wheat. p. 258–259. *In* Proc. 10th International Congress of Genetics. Univ. of Toronto, Toronto, ON.

See, D.R., M. Giroux, and B.S. Gill. 2004. Effect of multiple copies of puroindoline genes on grain softness. Crop Sci. 44:1248–1253.

Simeone, M.C., D. Lafiandra, and C.F. Morris. 2003. Development of soft durum lines by means of cytogenetical approach. p. 1391–1393. *In* N.E. Pogna, M. Romano, E.A. Pogna, and G. Galterio (ed.) Proc. 10th International Wheat Genetics Symposium. Instituto Sperimentale per la Cerealicoltura, Rome.

Tranquilli, G., J. Heaton, O. Chicaiza, and J. Dubcovsky. 2002. Substitutions and deletions of genes related to grain hardness in wheat and their effect on grain texture. Crop Sci. 42:1812–1817.

van Slageren, M.W. 1994. Wild wheats: A monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Wageningen Agricultural Univ., The Netherlands.

Wiley, J.W. 1884. Report of the Chemist. p. 19–22. *In* Report of the Commissioner of Agriculture for the Year 1884. U.S. Gov. Print. Office, Washington, DC.