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ORIGINAL ARTICLE

Taxonomical classification and origin of Kamut® wheat

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Abstract Bioagriculture and healthy lifestyle are trends of the twenty-first century. Bioagriculture involves the breeding of crops without using modern synthetic substances. Kamut brand wheat is one of the popular biocereals grown mainly in the USA and Europe. This cereal has the status of ancient wheat, not only because it has been grown since the era of the ancient Egyptian civilization, but also for its properties favorable for modern breeding programs and modern food marketing. In spite of Kamut's® interesting history and stable place in the market, it is not a common subject of genetic studies. It is also interesting that it has not been successfully taxonomically classified yet. There are a few studies which classify this tetraploid wheat as *Triticum polonicum* L., *T. turanicum* Jakubz., *T. turgidum* L. and *T. durum* Desf. These studies are based on cytological and comparative methods. We chose molecular (transposable element resistance gene analog polymorphism, diversity arrays technology, sequencing of genes *SBEIIa,* and *ψLpx-A1_like*) and statistical methods to classify Kamut® wheat. According to our experiments we suggest that Kamut brand wheat originated as a natural hybrid between *Triticum dicoccon* conv. *dicoccon* and *T. polonicum* and is not original ancient Egyptian wheat. We suggest that Etruscan wheat has the same parents as Kamut®.

Keywords Kamut® · Molecular taxonomy · Origin · TERGAP · DArT

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Introduction

The Kamut brand wheat (Kamut®) is a tetraploid wheat commonly known as King Tut's or Khorasan wheat (also nomenclature for *T. turanicum*). The nomenclature "kamut" means "wheat" in ancient Egyptian language. Egyptologists claim the root meaning of this word is "soul of the Earth". Since 1990, a registered trademark (Kamut®) has been used in marketing products of the protected cultivated *T. turanicum* variety "QK-77" (Quinn 1999).

The origin of this wheat is intriguing. During World War II an American airman claimed to have taken a few grains of some cereal from an ancient Egyptian tomb near Dashare. The story tells that he gave these grains to a farmer, who grew plants from them and resurrected a long-forgotten cereal. This story is just a modern legend and as a fact it has to be rejected, for most scientists believe that it probably survived the years as an obscure grain kept alive by the diversity of crops common to peasant farmers, perhaps in Egypt or Asia Minor. It is thought to have evolved contemporarily with the free-threshing tetraploid wheat.

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Besides its unusual history, this crop is interesting for its properties that are due to isolation from modern breeding. Kamut® has not been in contact with synthetic substances commonly used in modern breeding programs (Hammer et al. 2000). Kamut brand wheat is of ≈127 cm height and has two to three times larger grains than other wheat cultivars. The grains are narrow, vitreous and flinty with a characteristic hump (Vavilov 1951). The grain contains 20–30 % more proteins, higher levels of eight out of nine minerals, more lipids and up to 65 % more amino acids than other wheat cultivars. Alleles for prolamin, related to good pasta quality, were identified (Rodríguez-Quijano et al. 2010). Since lipids present more energy than carbohydrates, Kamut® is characterized as high-energy wheat. Kamut® products are marketed mainly through health food outlets. Due to its sweet taste, it plays a special role in bakeries as there is no need to add any sugar to pastries produced from Kamut® flour (Quinn 1999).

In the past it was believed that Kamut® wheat did not induce as strong allergy as other wheat in patients suffering from gluten intolerance (Quinn 1999). This information was refuted by the discovery that Kamut brand wheat causes the same allergic reactions as *T. durum* (Simonato et al. 2002). That means that, in spite of many marketing affirmations, products made of Kamut® wheat are not suitable for celiac disease patients. The most recent study on Kamut brand wheat showed that this grain protects organisms from oxidative stress better than *T. durum* (Benedetti et al. 2012).

The growing of this special wheat is exclusively managed by license agreements and requires organic certification of the crop. It is grown mainly in the USA and in a limited area of Austria (Grausgruber et al. 2004).

There are still disagreements about the nearest relatives of Tut's wheat. First, it was classified as *T. polonicum* and it was also believed that Kamut® was a natural hybrid between *T. durum* and *T. polonicum* (Kuckuck 1959). Some groups classify this wheat as *T. turanicum* (Percival 1921) or *T. turanicum* var. *notabile* (Perciv.) Gökg. (Gowayed 2009) and others as *T. turgidum* or *T. durum* (Brouwer 1972).

Materials and methods

Samples of tetraploid wheat

The analyzed samples belonged to the group of species with GGAA genomes: *T. timopheevii* (Zhuk.) Zhuk. (TIM), *T. araraticum* Jakubz. (ARM); and species with BBAA genomes: *T. dicoccoides* (Körn. ex Asch. et Graebn.) Schweinf. (DCS), *T. dicoccon* Schrank (DIM, for European samples *T. dicoccon* convar. *dicoccon* EUR abbreviation was used), *T. turgidum* L. (TRG), *T. carthlicum* Nevski (CAR), *T. durum* Desf. (DUR), *T. turanicum* Jakubz. (TRN), *T. polonicum* L. (PLN), *T. ispahanicum* Heslot (ISP), one sample of Kamut® wheat, and one sample of Etruscan wheat (BVAL 212017). We used one sample of *Aegilops speltoides* Tausch (SPE, SS genome) and one sample of *T. urartu* Thum. ex Gandil. (URA, AA genome) as outgroups. Wheat nomenclature in this article is according to Dorofeev et al. (1979) for naked wheat and Szabó and Hammer (1996) and Hammer et al. (2011) for hulled wheat.

Isolation of DNA samples

In our experiments, we analyzed 57 tetraploid wheat samples. DNA samples were isolated using CTAB extraction buffer [200 mmol l^{-1} Tris–HCl (pH 8.0), 1.4 mol l^{-1} NaCl, 20 mmol l^{-1} EDTA, 2 % (w/v) CTAB, 0.2 % (v/v) 2-mercaptoethanol] and High Pure PCR Product Purification Kit (Roche) from wheat grains.

Transposable element resistance gene analog polymorphism

Isolated DNA was analyzed using the TERGAP method. To avoid non-specific products, PCR was performed using hot start polymerase TrueStart *Taq* (Fermentas). The reaction mixture was composed of: 1× PCR buffer mixture 1:1 (GoTaq, Promega; TrueStart, Fermentas); 1 U *Taq* polymerase (TrueStart, Fermentas); 2.0 mM $MgCl_2$; 0.25 mM dNTP; 0.4 μM primers and 30 ng of template DNA in total volume 12.5 μl. We designed 17 combinations of primers. Primer sequences and combinations are summarized in Tables 1 and 2. We optimized PCR cycle: starting denaturation 95 °C (2 min); 34 cycles with temperatures 94 °C (45 s), 54 °C (45 s), 72 °C (2 min); final polymerization 72 °C (7 min). Amplification results were evaluated by 1.5 % agarose electrophoresis gel; fragments were separated over 2.5 h in constant voltage 100 V (3.5 V/cm of electrode distance) and stained with ethidium bromide.

Diversity arrays technology polymorphism (DArT)

Isolated DNA samples were sent to Triticarte™ whole-genome genotyping service for wheat DArT analysis, which is based on hybridization of DNA with special probes. These probes are designed strictly for wheat and carry up to 2,500 polymorphic markers. Binary data are output of this analysis.

Sequencing of genes *SBEIIa* and *ψLpx-A1_like*

Some of isolated DNA samples were used for sequencing of genes *SBEIIa* and *ψLpx-A1_like*. Each PCR reaction

Table 1 Sequences of primers used in TERGAP reactions

Primer sequences	
Primer name	Sequence (5′–3′)
M1fwd1	CAG GCT CCA GAA ATG CTT CA (Civáň unpublished)
M1rev1	GCT TGC AAA TGA AGT GCA GA (Civáň unpublished)
Jeli	CCT AGG AAC ATA GCT TCA TC (Civáň et al. in press)
Xa1LRF	CTC ACT CTC CTG AGA AAA TTA C (Civáň unpublished)
Ptokin1	GCA TTG GAA CAA GGT GAA (Civáň unpublished)
NLRRfor	TAG GGC CTC TTG CAT CGT (Civáň unpublished)
CLRRfor	TTT TCG TGT TCA ACG ACG (Civáň unpublished)
NLRR-INV1	TGC TAC GTT CTC CGG G (Civáň unpublished)
RLRRfor	CGC AAC CAC TAG AGT AAC (Civáň unpublished)
As1-INV	CCT AAC GGT GAT CGC AAC (Civáň unpublished)
Xa1NBS-R	CTC TGT ATA CGA GTT GTC (Civáň unpublished)

Table 2 Review of primer combinations used in TERGAP reactions

Primer combinations		
Claudia retrotransposon + RGA primer	Jeli retrotransposon + RGA primer	Single RGA primer reaction
M1fwd1 + Xa1LRf	Jeli + Xa1LRf	–
M1rev1 + Ptokin1	Jeli + Ptokin1	–
M1fwd1 + NLRRfor	Jeli + NLRRfor	–
M1fwd1 + CLRRfor	Jeli + CLRRfor	–
M1fwd1 + NLRR-INV1	M1fwd1 + NLRR-INV1	NLRR-INV1
M1fwd1 + RLRRfor	Jeli + RLRRfor	–
M1fwd1 + As1-INV	Jeli + As1-INV	–
M1fwd1 + Xa1NBS-R	Jeli + Xa1NBS-R	–

mixture was composed of 1× PCR buffer (HotFirePol), 1 U *Taq* polymerase (HotFirePol, Solis BioDyne), 1.6 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM primers and 40 ng of template DNA in a total volume 50 µl. Primer sequences are summarized in Table 3. We optimized the PCR cycle as: starting denaturation 95 °C (12 min); 35 cycles with temperatures 95 °C (45 s), 59 °C (45 s), 72 °C (45 s); final polymerization 72 °C (7 min). The PCR products were purified using High Pure PCR Product Purification Kit (Roche). The products were sequenced using ABI 3730xl sequencing machine (Applied Biosystems). The sequences were aligned by software Mega5 (Tamura et al. 2011).

Table 3 Sequences of primers used for sequencing of genes *SBEIIa* and *ψLpx-A1_like*

Primer sequences	
Primer name	Sequence (5′–3′)
SBEII_fwd	CCTGTTTCTGGTCTGATGGTC
SBEII_rev	ATGGGAGATCCCTACAATGC
ψLpx-A1_like_fwd	CCAACGACGTGAGTGATCCTTTTGC
ψLpx-A1_like_rev	AGCGCGAACCGTCATCTCGAA

Construction of phylogenetic trees

DNA banding patterns were visualized using Vilber Lourmat Gel Documentation system, and detected bands were sorted according to their size in bp with Bio-1D software (Vilber Lourmat, France). A discrete-state data matrix in binary format was constructed manually from the Microsoft Excel output of Bio-1D software, assigning value 1 to band presence and value 0 to band absence for each observed band size value. The phylogenetic analysis was performed using the programs included in the PHYLIP package, version 3.69 (Felsenstein 2005). A total of 500 bootstrap replicates of binary data matrix generated by the SEQBOOT program were analyzed by the PARS program with Wagner parsimony method using randomized input order of taxons with 100 times to jumble. An extended majority rule consensus tree was constructed using the CONSENSE program. The resulting phylogenetic tree was visualized with the Mega5 program with ClustalW algorithm (Tamura et al. 2011).

Statistical evaluation of data

For estimating population average pairwise differences, we used Arlequin software (version 3.5) in which we chose distance method pairwise difference. We computed Nei's average number of differences between populations (Nei and Li 1979).

For visualization purposes, we used multidimensional scaling technique (MDS) that is one of the dimension reduction methods (techniques that can reduce any multi-dimensional space to lower dimensional space). The main advantage is that it tries to preserve the original distances between pairs of objects (but similar to other dimension reduction techniques, there is also some distortion in the final projection). It takes dissimilarity matrix as the input, so any kind of data where one can compute dissimilarities between objects can be used. In our case we first used two dimensions to make the final 2D MDS projection (Fig. 1).

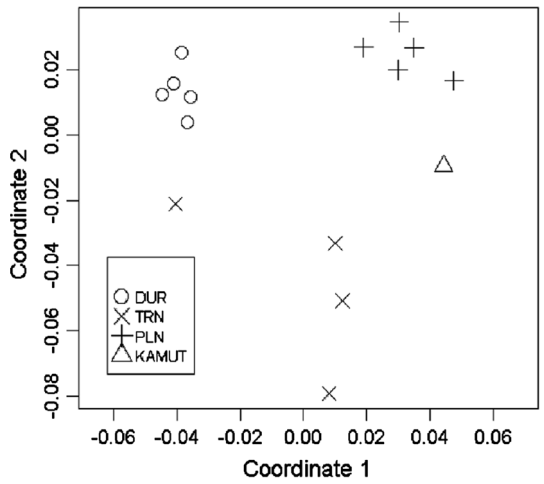

Fig. 1 MDS distance comparison of Kamut brand wheat and three genetically close species

The hybrid status of samples was evaluated by means of STEM-hy program (Kubatko et al. 2009). This is a program to perform maximum-likelihood analysis for estimation of the species tree from multilocus data under the coalescent process. Coalescent trees for each of the sequenced genes were constructed by the Mesquite program (Maddison and Maddison 2011). The most probable gene tree from 10,000 trees was chosen by the COAL program (Degnan and Salter 2005).

Parental populations of Kamut® variety were estimated from the binary data obtained from DNA polymorphism of DArT type by means of the HIest program (Fitzpatrick 2012). This program uses likelihood to estimate ancestry and heterozygosity. The *threeway* module of this program enables the maximum-likelihood estimates of ancestry for a sample of hybrid in a three-way hybrid zone, i.e., from three potential parental lineages. The output parameters s1, s2 and s3 of this program mean the proportion of alleles derived from parental lineages P1, P2 and P3.

Results

TERGAP analysis

TERGAP reactions were analyzed by agarose gel electrophoresis. During gel analyzing, several specific polymorphisms were identified. These genome-specific and species-specific polymorphisms were crucial for phylogenetic tree construction. Nine B genome-specific and three G genome-specific polymorphisms were identified. These polymorphic markers included three length polymorphisms and seven band presence/absence-based polymorphisms. Species-specific polymorphisms were identified for *T. polonicum*, *T. turgidum*, *T. dicoccoides* and *T. timopheevii* species. We also found polymorphism specific for *T. polonicum* in Kamut's® genotype. Two polymorphisms were specific for multiple taxons: one was common for *T. dicoccon* and *T. ispahanicum*, and the other one was common for *T. durum, T. turanicum* and *T. polonicum* species.

Phylogenetic tree

In the phylogenetic tree constructed by the PHYLIP programs, *GGAA* and *BBAA* lines were divided into two major clusters. Hulled and naked wheat was also distinguished (Fig. 2). Most samples from the same species were grouped into separate clusters. GGAA group was clearly divided into *T. timopheevii* and *T. araraticum* species. In contrast, the BBAA group was not differentiated as clearly as the GGAA group. There were no individual clusters for *T. dicoccon*, *T. dicoccoides* and *T. ispahanicum* (which formed a common cluster) and for *T. turanicum* and *T. polonicum* samples, which also clustered together. The sample of Kamut® wheat was integrated into one cluster with *T. turanicum* and *T. polonicum* samples.

Population average pairwise differences

Statistical analysis of species sample groups revealed the most significant resemblance of Kamut® wheat and *T. polonicum* samples, which was demonstrated by a value of 8.5. This number shows the lowest value of Kamut's® distance from each analyzed species (Table 4). In Table 4 it is necessary to pay attention to the diagonal line. Values above the diagonal reflect the average number of pairwise differences between populations (sample groups in this case), diagonal elements mirror the average number of pairwise differences inside populations and values below the diagonal show the corrected average pairwise differences. The highest diversity presented by the highest value of average number of pairwise diversity was detected inside the *T. turanicum* sample group. In contrast, the *T. araraticum* sample group seemed the most compact. The highest diversity between groups of samples was detected between *T. araraticum* and *T. turanicum* groups. While evaluating Table 4, it is not possible to estimate the columns "SPE, BVAL, KAMUT, CYL, URA" as groups/populations, as we used only one sample for each genotype.

Multidimensional scaling

In this method, we compared Kamut® wheat with its three genetically close species (*T. polonicum*, *T. turanicum* and *T. durum*). The status of Kamut® on the multidimensional scaling graph (Fig. 1) proves our theory that Kamut® is the most related to *T. polonicum*, which also proves the results obtained by the pairwise difference method (Table 4).

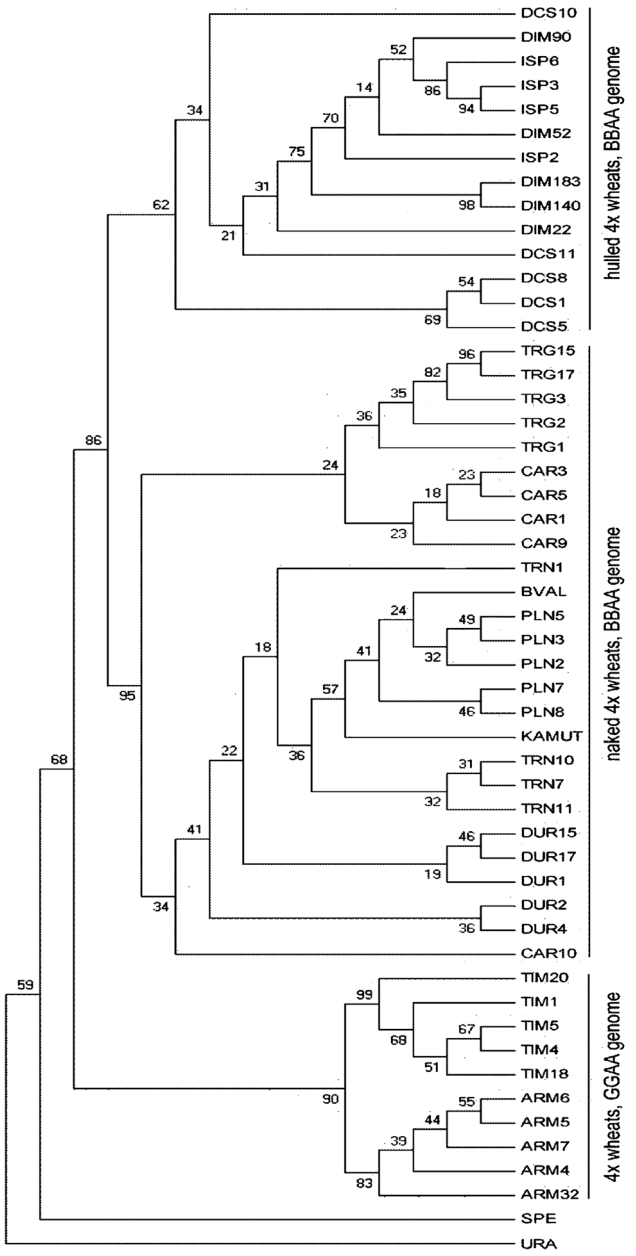

Fig. 2 Phylogenetic tree constructed by PHYLIP 3.69; sample URA was used as an outgroup. Samples are divided into two main clusters, one with BBAA genomes and the other with GGAA genomes. We could also distinguish hulled and naked wheat. Kamut® wheat was integrated right on the edge between *Triticum turanicum* and *T. polonicum* species

Haplotypes *ψLpx-A1_like*

Sequencing of *ψLpx-A1_like* gene divided our samples into four haplotypes (Fig. 3). According to these results, the Kamut® sample carries the same haplotype as some European samples of *T. dicoccon* convar. *dicoccon*, which indicates that Kamut® is most probably a hybrid wheat, because it reflects similarity both with naked (*T. polonicum*) and hulled taxons (*T. dicoccon*).

STEM-hy analyses

The results of sequencing of *ψLpx-A1_like* and *SBEIIa* have pushed us forward into proving that our hypothesis about the hybrid origin of Kamut® is right. According to the four haplotypes obtained by sequencing of *ψLpx-A1_like*, we chose five samples for STEM-hy analyses (Kubatko et al. 2009), which was designed to estimate hybrid samples (Table 5). These five samples (DCS 11, Kamut, PLN 1, EUR 42, EUR 132) are typical representations of four haplotypes. The results for sample DCS 11 are not given in Table 5 because this sample is ancestral and thus we do not expect its hybrid origin. In this table, Kamut® sample has the highest values of likelihood, which suggests that this sample is a hybrid. Another output of STEM-hy software is a hybrid tree (Fig. 4) that shows which one of the tested samples has the largest amount of genetic information common with Kamut®. This is presented by branch localization; the branch nearest to the tested sample is genetically most similar to this sample and it may also indicate the sample's parental ancestors in some cases. In hybrid tree, Kamut® is localized on the same branch as the reticulated sample EUR 42, indicating that these two samples are probably the progeny of the same crossing. This result signifies that one of Kamut's® parents is the European genotype of *T. dicoccon* convar. dicoccon, which is very similar to the EUR 42 sample.

HIest analysis

Phylogenetic tree and MDS diagram constructed by using TERGAP binary data have indicated *T. polonicum* to be one of Kamut's® parental genotypes. HIest software was used to confirm this hypothesis using three potential parental populations: P1 (DUR 16, DUR 21, DUR 24, DUR 30, DUR 38), P2 (PLN 1, PLN 2, PLN 3, PLN 5, PLN 11, PLN 14) and P3 (EUR 132, EUR 387, EUR 189). This software operated with DArT binary data and calculated ancestry indices s1, s2 and s3 from 125 diagnostic markers which are specific for each parental population. Each ancestry index (s) shows the proportion of alleles derived from a particular parental lineage (P). The highest values of ancestry index were detected in population P2 (Table 6), which supports our hypothesis that Kamut® also has a naked ancestor within *T. polonicum*. As shown in Table 6, this model is more probable when a hybrid sample of Kamut® was included in the calculation of parental frequencies of *T. polonicum* for each of the 125 diagnostic markers. This is proved by a higher value of likelihood (−54.861).

Table 4 Average numbers of pairwise differences by distance method pairwise difference

	ARM	TIM	DCS	DIM	ISP	TRG	CAR	DUR	TRN	PLN	SPE	BVAL	KAMUT	URA
ARM	*5.8*	24	43.9	51.1	59.3	50.6	53.9	51.6	58.1	57.5	63.8	59.4	57.8	84.8
TIM	17.9	*6.4*	55.2	60.5	66.6	60.9	62.6	60.4	66.5	63.2	64	65.2	65.2	89.4
DCS	33.3	44.3	*15.4*	23.9	30.3	42	41	36.9	42.7	41.8	72.6	47.4	38.2	86.8
DIM	40.3	49.4	8.3	*15.8*	19.9	43.4	39	40.2	42.1	46.6	64.4	52.4	39.6	89.8
ISP	50.8	57.8	16.9	6.4	*11.2*	51.2	46.2	45.4	48.3	45.1	72.3	52.8	44.3	96.3
TRG	42.4	52.4	29	30.2	40.3	*10.6*	15.6	20.4	26.4	28.3	75.8	27.8	29.8	81.2
CAR	46.2	54.6	28.5	26.3	35.8	5.5	*9.6*	17.2	23.4	26.7	69.4	28.2	27	80.8
DUR	45	53.6	25.6	28.7	36.2	11.5	8.8	*7.2*	19.2	17.3	77.6	18	19.6	83.4
TRN	47	55.1	26.8	26	34.5	12.9	10.4	7.4	*16.3*	20.3	72.5	23.5	18	88.5
PLN	50.5	55.9	29.9	34.6	35.4	18.9	17.8	9.6	8	*8.2*	82.6	12.2	12.6	94.4
SPE	60.9	60.8	64.9	56.5	66.7	70.5	64.6	74	64.3	78.5	*0*	86	76	91
BVAL	56.5	62	39.7	44.5	47.2	22.5	23.4	14.4	15.3	8.1	86	*0*	18	91
KAMUT	54.9	62	30.5	31.7	38.7	24.5	22.2	16	9.8	*8.5*	76	18	*0*	93
URA	81.9	86.2	79.1	81.9	90.7	75.9	76	79.8	80.3	90.3	91	91	93	*0*

Values above diagonal average number of pairwise differences between populations. *Diagonal elements* average number of pairwise differences inside populations. *Values below diagonal* corrected average pairwise differences. The highest values show the highest distances inside or between populations

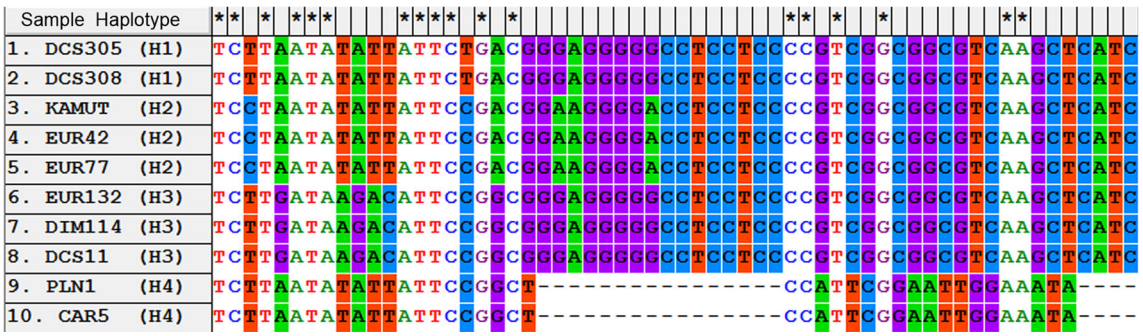

Fig. 3 Variable position of *ψLpx-A1_like* gene for selected tetraploid wheat samples, which represent four haplotypes (*H1*, *H2*, *H3*, *H4*). The majority of conservative positions were deleted

Table 5 Values of likelihood and AIC of hybrid trees calculated by STEM-hy for postulated hybrids

Postulated hybrid	Likelihood	AIC
KAMUT	−5,791.83	11,593.663
PLN 1	−5,900.83	11,811.662
EUR 42	−5,791.83	11,593.663
EUR 132	−5,853.13	11,716.276

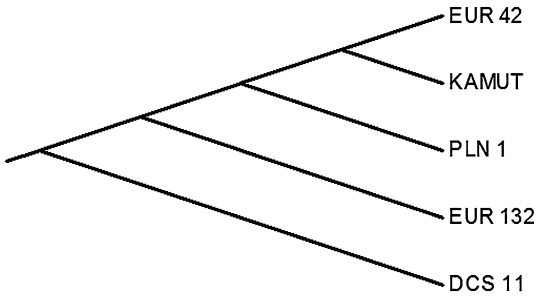

Fig. 4 Cladogram produced by STEM-hy software

Similarity analysis of DArT polymorphism

Similarity analysis was chosen to estimate the most probable *T. polonicum* parental genotype of Kamut®. This polymorphism included binary data for 2,571 markers. The similarity of sample pairs was estimated by similarity coefficient *S* (McGraw-Hill Science & Technology Dictionary), computed by pattern $S = n_s/(n_s + n_d)$, where n_s represents the number of positive features shared by two samples, and n_d represents the number of features positive for one sample and negative for the other. From the total of

Table 6 Analysis of ancestry by HIest program

s1	s2	s3	Likelihood	Hybrid sample KAMUT
0.33	0.48	0.19	−62.842	Not included in the calculation of parental frequencies of *T. polonicum*
0.17	0.67	0.15	−54.861	Included in the calculation of parental frequencies of *T. polonicum*

P1, P2, P3 possible parental populations (samples from *T. durum*—P1, *T. polonicum*—P2 and *T. dicoccon* convar. *dicoccon*—P3); *s1, s2* and *s3* ancestry indices calculated for KAMUT from 125 diagnostic markers

20 analyzed samples of *T. polonicum*, the highest value of similarity coefficient *S* was detected between Kamut® and PLN 1 sample (0.872), meaning that the most probable naked parent of Kamut® was PLN 1-like sample.

Discussion

TERGAP technology seems to be appropriate for evolution studies. Electrophoretic analysis of PCR fragments revealed polymorphic markers specific for both B and G genomes, and also for some analyzed species. We could identify nine polymorphisms specific for the B genome and three specific for the G genome. Three of these were length polymorphisms, and seven were polymorphisms based on the presence or absence of specific bands. These polymorphic markers caused an almost exact clustering of the same species samples.

Phylogenetic tree constructed on the basis of binary data obtained from electrophoretic analyses was logically divided into two major clusters. These clusters represented the GGAA line and BBAA line taxons and were statistically cogently divided. Samples from the GGAA line were strictly clustered into *T. timopheevii* and *T. araraticum* species.

BBAA line taxons were not clustered as strictly as GGAA taxons, but naked and hulled wheat was clearly distinguished. *T. durum*, *T. turgidum* and *T. carthlicum* samples were divided into separate clusters, except the CAR10 sample, which is regarded to be a hybrid genotype. Hulled wheat samples (*T. dicoccoides, T. dicoccon* and *T. ispahanicum*) clustered together with no further division. *T. dicoccon* and *T. dicoccoides* wheats are very closely related, which is probably the reason for their common grouping in the tree. *T. dicoccon* samples were mixed with *T. ispahanicum* samples, which might be due to a close geographic location during their evolution. The close relationship of these two taxa has been confirmed by other experiments (unpublished data). In naked wheat clusters, *T. turgidum* and *T. carthlicum* samples grouped into common subcluster and *T. turanicum, T. polonicum* and *T. durum* samples formed another subcluster. *T. durum* species was sorted into a separate group in the mentioned subcluster. Samples of *T. turanicum* and *T. polonicum* were mixed in a common subcluster as they are closely related, which is seconded by their very similar morphology. Kamut® wheat sample was included in this group, right on the edge between *T. turanicum* and *T. polonicum* species. According to our dendrogram, it is probable that sample TRN 1 is phylogenetically the oldest one of the *T. turanicum* and *T. polonicum* samples, and during evolution other *T. turanicum* samples used in this study differentiated followed by Kamut® wheat and *T. polonicum* genotypes.

Our multiple statistical analyses classified naked Kamut® wheat to be most closely related to naked *T. polonicum* (Tables 3, 6; Fig. 1), which favors our molecular analyses conclusions and also the earlier hypothesis of Kuckuck (1959). Our results are in contrast to two other studies which had classified Kamut® as the *turanicum* genotype (Kokindova and Kraic 2003; Grausgruber et al. 2004).

Population average pairwise difference analysis suggested the highest interspecific distances between *T. araraticum* and *T. turanicum* sample groups. The highest intraspecific distances were detected inside the *T. turanicum* group and by contrast we identified the *T. araraticum* group to be the most compact and least diversified (Table 3). The highest variability of genetic distances inside the *T. turanicum* group is probably caused by the original classification of Kamut® and BVAL samples into this species. If these two samples are hybrids, it is highly probable that this may induce increased variability in the *T. turanicum* sample group.

These results led us to another experiment to clarify our theory about the hybrid origin of Kamut®. Detection of four haplotypes in *ψLpx-A1_like* gene (Fig. 3) illustrated that the Kamut® sample was most similar to the European *T. dicoccon* convar. *dicoccon* (EUR) samples. This result is interesting, because Kamut® is naked wheat and *T. dicoccon* species has hard glumes, so we suggest it has to be a hybrid sample. Using STEM-hy software we tested the hypothesis that one of four samples listed in Table 5 is a hybrid. Likelihood values indicate that the samples of Kamut® and EUR 42 are most probably hybrid samples, because likelihood values are highest for these two samples' hybrid trees. The relevancy of this model is supported by the AIC criterion (Akaike Information Criterion—a measure of the relative quality of a statistical model for a given set of data), whose values for these samples' hybrid trees are lowest in comparison with alternative hypothesis that PLN 1 and EUR 132 samples are hybrids. This analysis results in the conclusion that Kamut® and EUR 42

(European material from *T. dicoccon* convar. *dicoccon*) resulted from the same crossing.

Using the DArT binary data and HIest software, we proved our previous hypothesis that *T. polonicum* is one of Kamut's® parents. HIest software compared ancestry indices for 125 diagnostic markers of Kamut® with three potential parental lineages P1 (*T. durum*), P2 (*T. polonicum*) and P3 (European *T. dicoccon* convar. *dicoccon*). The values of ancestry indices (s2—Table 6) were highest for Kamut® in the parental lineage of *T. polonicum* (P2). Computing of similarity coefficient *S* revealed that Kamut® was most similar to PLN 1 sample, that is why we suggest that the parents in hybrid crossing were *T. polonicum*, most probably PLN 1-like sample with origin in western Turkey (near Balikesir city) and some genotype of *T. dicoccon* convar. *dicoccon* samples (EUR 42-like). According to microsatellite analysis, Khlestkina et al. (2006) also suggested that *T. polonicum* from the Fertile Crescent could be one of the Kamut's® parents.

Although our results indicate that *T. polonicum* is one of Kamut's® parents, Kamut® is morphologically very similar to *T. turanicum* var. *notabile*. This discrepancy between botanical and genetical approaches to classification of Kamut® may be caused by the hybrid origin of *T. turanicum* species as it was hypothesized by Percival (1921) and Kuckuck (1970). They supposed that *T. turanicum* originated from the hybridization between *T. polonicum* and *T. durum*. According to Dorofeev et al. (1979), *T. turanicum* var. *notabile* is geographically distributed in the countries of Central Asia, Fertile Crescent and in Egypt, Libya and Sudan. It is probable that Kamut® was introduced from the country of its origin to Egypt together with samples of *T. turanicum*.

It is interesting that the sample named BVAL (Etruscan wheat, BVAL 212017) is almost identical to Kamut®. This sample had the same haplotype in the *ψLpx-A1_like* sequence as Kamut®. Within DArT polymorphism, Kamut® and BVAL differed only in the values of 8 markers from the 2,571 analyzed (similarity coefficient 0.9968, unpublished data). We achieved sample BVAL 212017 from the National Inventory of Plant Genetic Resources in Austria (Linz) and its origin is unknown. We could only hypothesise whether this sample was brought to Europe by Etruscans. According to our results we suggest that Etruscan wheat has the same parents as Kamut®. In our statements we allege that these parents were PLN 1-like and EUR 42-like genotypes. Samples PLN 1 and EUR 42 have origin in Turkey, so we might suggest that Kamut® and Etruscan wheat also originate from Turkey. If Etruscan wheat really originates in western Turkey, it could indicate that Etruscans also originates from this area as Greek philosopher Herodotos claimed.

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