DOI: 10.2478/agri-2020-0006 Original paper

IDENTIFICATION OF BREAD AND DURUM WHEATS FROM THEIR DIPLOID ANCESTRAL SPECIES BASED ON CHLOROPLAST DNA

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HAIDER, N. – NABULSI, I.: Identification of bread and durum wheats from their diploid ancestral species based on chloroplast DNA. Agriculture (Poľnohospodárstvo), vol. *66*, 2020, no. 2, pp. 56 – 66.

Species that have been identified as the genome donors to cultivated polyploid durum and bread wheats (*Triticum durum* L. and *T. aestivum* L., respectively) are potential gene sources for the breeding of these two crops. Therefore, their accurate identification facilitates their use in the improvement of these crops. Based on chloroplast DNA analysis (*rpL2* and *rps16* introns, *psbC-trnS*, *trnT-L*, and *trnL-F*) using polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP), an attempt was made in 2018 (Department of Molecular Biology and Biotechnology/AECS) to identify durum and bread wheats from each of their proposed diploid ancestral species (i.e., *T. monococcum*, *T. urartu*, *Aegilops speltoides,* and *Ae. tauschii*). The use of two PCR markers (*psbC-trnS* and *trnL-F*) and three PCR-RFLP locus-enzyme combinations (*rps16* intron-Tru 1I, *rpL2* intron-Taq I, and *trnT-L*-Taq I) allowed the identification of all species involved. Reliable and accurate identification of diploid ancestors of durum and bread wheats using these candidate species-specific cpDNA markers will be useful for wheat breeding programs, *in situ* and *ex situ* conservation efforts, verification of seed purity in commercial seed stocks, and ensuring identity and integrity of accessions held within a collection does not change through unwanted gene flow or by genetic drift after regeneration by seed.

Key words: Ancestral species, chloroplast DNA, PCR-RFLP, *Triticum*

Wheat (*Triticum* L.) is an annual plant that belongs to the grass family *Poaceae,* tribe *Triticeae*, and subtribe *Triticineae*. It is the oldest and most widely grown grain crop (17% of the crop acreage worldwide) (FAOSTAT). For about 40% of the world population, wheat is the main food (Konopatskaia *et al.* 2016) and is used to produce various end-use products like bread, biscuits, and pasta (Patil *et al.* 2011).

Wheat species form a classical polyploid series at three ploidy levels of diploid einkorn [2n=14, AA, *T. monococcum* L. subsp. *monococcum* (syn. *T. monococcum* L.)] and *T. urartu* Tumanian ex Gandilyan), emmer and timopheevi tetraploid (2n=28, AABB and AAGG, respectively), and hexaploid (2n=42, AABBDD) species complexes (Provan *et al.* 2004). Domesticated einkorn wheat is regarded as one of the oldest crops in the world and part of the Neolithic package that started emerging ~10,400 calibrated years before present (BP) in the Near East (Zohary & Hopf 2000; Haldorsen *et al.* 2011).

Emmer [*T. turgidum* L. subsp. *dicoccum* (Schrank ex Schübl.) Thell. or *T. turgidum* L. subsp. *dicoccum* Schrank ex Schübler] (syn. *T. dicoccum* Schrank ex Schübler) is a tetraploid wheat (AABB; 2n=4x =28) which is a domesticated form of *T. turgidum* subsp. *dicoccoides* (wild emmer wheat). Durum or

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macaroni wheat (*T. turgidum* L. subsp. *durum* Desf. or *T. turgidum* L. subsp. *durum* (Desf.) Husn.) (syn. *T. durum* Desf.) is a tetraploid wheat (AABB; 2n= 4x=28) originated from the domesticated emmer (Arzani & Ashraf 2017).

Among the more ancient, and now less frequently cultivated species are *T. monococcum*, *T. dicoccum*, and the 6x spelt (*T. aestivum* L. subsp. *spelta* (L.) Thell., syn. *T. spelta* L., AABBDD) (Shewry & Hey 2015).

Of special cultural and economic importance are the tetraploid wheat *T. durum* (durum, which means "hard" in Latin, or macaroni wheat) and the hexaploid bread wheat (common or soft wheat) *T. aestivum* L. subsp. *aestivum* (syn. *T. aestivum* L., Baum *et al.* 2009). *T. aestivum* is the major wheat species grown throughout the world, accounting for about 95% of the 700 + million tonnes of wheat which are grown annually. Durum is the only tetraploid wheat species that is considered important commercially and is widely cultivated today; about 35 – 40 mt are grown each year of *T. durum*. This species is adapted to the hot dry conditions surrounding the Mediterranean Sea and similar climates in other regions (Shewry & Hey 2015).

The early domesticated tetraploid durum wheat and bread wheat were founded by the wild emmer *T. dicoccoides* (Dvorak *et al.* 2006). It is worth noting here that there is no wild hexaploid progenitor of bread wheat in nature (Salamini *et al.* 2002). *T. durum* has been nominated as the tetraploid parent and the cytoplasm donor of *T. aestivum* (Feldman 2001; Matsuoka & Nasuda 2004).

Regarding the A genome of durum and bread wheats, the diploid *T. monococcum* was frequently suggested as the A genome donor of polyploid wheats (e.g., Sourdille *et al.* 2001). It was revealed, however, by other studies that the A genome of *T. urartu* is closer to polyploid wheats than *T. monococcum* genome, and therefore *T. urartu* has generally been accepted to be the A genome donor of polyploid wheats (Rajpal *et al.* 2016).

Aegilops speltoides Tausch (2n=2x=14, SS) (*Aegilops* L., Poaceae) was proposed to be the B genome donor or the most closely related diploid species to the B genome of tetraploid and hexaploid wheats (Haider 2012). In her review, Haider (2013) attempted for the first time to review the immense literature on the origin of the genomes of wheat, with a particular emphasis on the controversial B genome. The hybridisation between species proposed as A and B genomes donors created the species *T. turgidum* (including durum wheat).

The donor of the D genome of bread wheat has generally been accepted to be the Asian wild goatgrass *Ae. tauschii* Coss. (2n=2x=14, DD, syn. *Triticum tauschii*, *Ae. squarrosa* auct. non L.), and particularly subsp. *strangulata* (Dvorak *et al.* 1998), which is a diploid self-pollinating species. Hybridization of *Ae. tauschii* with tetraploid wheat *T. turgidum,* about 7,000 years ago, led to the development of hexaploid *T. spelt* from which bread wheat evolved (Dvorak *et al.* 1998).

Species that have been identified as the genome donors to polyploid wheats are potential gene sources for wheat breeding. The explotation of genetic diversity in these species and discovery of novel variant alleles may provide opportunities for further wheat genetic improvement.

Durum wheat is easily used to improve bread wheat by interspecific hybridization with homologue chromosome pairing and recombination (Wang *et al.* 2005) because they both share the A and B genomes. Synthetic amphiploids have been used for the transfer of genes for disease resistance from diploid to tetraploid or hexaploid wheats. In majority of cases of gene transfer from 2x species via synthetic amphiploids, durum has been used as one of the buffering or bridging species for making synthetic amphiploids for their ultimate hybridization with bread wheat cultivars. Combinations of durum − *T. monococcum*, durum − *T. urartu*, durum − *Ae. tauschii*, and *Ae. speltoides* − *T. monococcum* have been used as synthetic amphiploids for gene transfer into bread wheat. The generated hybrids are known as synthetic wheat (Tuberosa *et al.* 2014). Hence, the synthetic hexaploids contain genomes from a tetraploid wheat (e.g., *T. durum*) and from a diploid species (e.g., *T. monococcum, T. urartu,* or *Ae. tauschii*) (Gorham 1990). For instance, a series of durum − *Ae. tauschii* synthetic amphiploids were developed at the International Maize and Wheat Improvement Center (known by its Spanish acronym, CIMMYT) in Mexico to transfer disease and insect resistance and quality traits from *Ae. tauschii* into bread wheat (Rajpal *et al.* 2016).

T. urartu and *T. monococcum* harbor useful variability for many economically important genes that can be used for hexaploid wheat improvement although they have not been used to the level of the D genome progenitor (Yao *et al.* 2007; Rajpal *et al.* 2016). Several disease resistance genes have been transferred from *monococcum* and *urartu* to cultivated wheat (e.g., leaf rust, stem rust, stripe rust, root rot, scab, septoria tritici avenae, nodorum, Hessian fly and aphid, and powdery mildew) (see Rajpal *et al.* 2016). In a study carried out by Alvarez *et al.* (2013), the Glu-A1x alleles in *T. urartu* showed clear differences to the bread wheat alleles. This variation could enlarge the high-quality genetic pool of modern wheat and be used to diversify the bread-making quality in durum and common wheats.

Ae. tauschii and *Ae. speltoides* are also very important genetic resources for the breeding of cultivated wheat (Haider & Nabulsi 2008). *Ae. tauschii* represents a rich reservoir of disease resistance, productivity traits, and abiotic stress resistance. The wide genetic variation in *Ae. tauschii* has been exploited by various groups around the world for cultivated wheat improvement. For example, *Ae. tauschii* shows resistance to Hessian fly (*Mayetiola destructor,* Amri *et al.* 1992) and green bug (*Schizaphis graminum,* Raupp *et al.* 1988). It has also been considered as the main contributor to bread-making properties of bread wheat (Hsam *et al.* 2001). *Ae. speltoides* has depicted very high levels of disease resistance for leaf rust and stripe rust (Rajpal *et al.* 2016).

In durum and bread wheats breeding, it is essential to identify the parent species of pre-breeding program before performing any cross-pollination (Tuberosa *et al.* 2014). For morphological differentiation between tetraploid (*T. turgidum*) and hexaploid (*T. aestivum*) wheat accessions, Geleta and Grausgruber (2013) recorded the number of spikelet per spike (NPS), spike length (SPL), awn length (AWL) and thousand kernel weight (TKW) after harvest. The modern cultivated wheats can be differentiated from their progenitors by several morphological features that are related to spike morphology (shape, shattering, and thresh ability, Konopatskaia *et al.* (2016)). Other diagnostic characters that are used at different stages of plant development for the traditional identification of these species are those of leaf (the type of pubescence, ciliation of the blade margin, color and ciliation of auricles, and shape of the leaf tip), stem nodes, and glumes.

Although *Aegilops* is morphologically highly distinct from *Triticum*, with rounded glumes rather than keeled glumes, diploid *Aegilops* species are characterised by relatively narrow ranges of morphological variation (Zohary & Feldman 1962). Above all, species identification using morphological characters possess several disadvantages like large space requirement, seasonal dependence, and environmental influence. It is also tedious and time-consuming.

Based on 1) what stated above, 2) the fact that varieties of durum and bread wheats are similar with respect to growth, morphology, and yield-related characteristics (Patil *et al.* 2011), 3) ambiguity between spike morphology of certain morphotypes of 4x and 6x wheat and their ploidy level (Belay *et al.* 1994), diagnosis of durum from bread wheat and diagnosis of each of these species from its 2x ancestral species using morphological characters is a difficult task for the non-specialists, and not always accurate. Hence, there is a need to develop DNA-based markers for an unambiguous identification of these species.

Because several studies have been carried out for identification of durum from bread wheat (e.g., Pasqualone *et al.* 2007; Casazza *et al.* 2012), an attempt was made here based on chloroplast DNA (cpDNA) data to identify durum and bread wheats from each of their proposed diploid ancestors (i.e., *T. monococcum*, *T. urartu*, *Ae. speltoides,* and *Ae. tauschii*). Since *Ae. speltoides* has similar genome and morphology to the other four species (*Ae. searsii* Feldman & Kislev ex Hammer, *Ae. bicornis* (Forssk.) Jaub. & Spach*, Ae. longissima* Schweinf. & Muschl., and *Ae. sharonensis* Eig) in the section *Sitopsis* of the genus *Aegilops*, a marker that separates *Ae. speltoides* from these four species has been also developed.

MATERIAL AND METHODS

Plant material

Seeds of four landrace accessions representing four *Triticum* species and six accessions representing six *Aegilops* species were provided in 2010 by the Genetic Resources Unit (GRU) at the International Centre for Agricultural Research in the Dry Areas

(ICARDA), Aleppo, Syria. There were also seeds of five accessions for each of *T. urartu* and *Ae. tauschii* species, and four accessions of each of *T. monococcum* and *Ae. speltoides*. Accessions of each species were selected to represent as much of the geographic range of each species as possible. All seeds were grown and allowed to germinate in a heated glasshouse (minimum temperature 11°C). One accession, which was selected randomly (Haider & Nabulsi 2008), of each of the 10 species to be studied was used for molecular analysis (Table 1).

DNA extraction

For DNA extraction, fresh leaves were collected from plants 3 − 4 weeks after seed germination. DNA was isolated according to the method of Dorokhov and Klocke (1997). Recovered DNA pellets were dried under the laminar flow and then resuspended in 150 ml of doubled distilled and sterilised water. DNA was quantified using Gene Quant Spectrometer (Amersham Biosciences) and the concentration of all samples was set at 10 ng/μL.

PCR-RFLP analysis

All species samples were subjected to PCR-RFLP analysis for which universal primers that target the *rpL2* intron (Haider & Wilkinson 2011) and the two chloroplast intergenic spacers *trnT-L* and *trnL-F* (Taberlet *et al.* 1991) were used. PCR was performed in 0.2 ml microtubes (Greiner Bio-One-USA) using Eppendorf thermocycler as described by Haider and Nabulsi (2008). PCR reactions were subjected to one cycle of 94°C for 5 min. This was followed by 35 cycles each of which had the following temperatures: 1) 94°C for 30 s for DNA denaturation, 2) 52 − 58°C for 1 min for annealing of primers, and 3) 72°C for 1 min for extension of the target chloroplast region. PCR reactions were then subjected to 72°C for 5 min for the final extension.

For the visualization of PCR products, 2 μL of each PCR product was loaded into 1.5% agarose gel that was run at 120 V for 30 min. Restriction digests were performed on PCR amplicons of the four chloroplast regions analyzed to generate species-specific PCR-RFLP markers. Each marker exploited a potentially variable single nucleotide polymorphism (SNP) positioned within the amplicon and in which one allelic state coincided with a recognition site of a restriction enzyme. PCR products (1 − 5 μL) were digested according to manufacturer (Fermentas) instructions using Taq I for *trnT-L* and *rpL2* intron. Digested fragments were separated by electrophoresis on 2% agarose gel that was run at 100 V for 2 h in 1x TAE buffer and visualized under UV lights using Gel Documentation System (GDS8000, UVP).

For the identification of *T. durum* and *T. aestivum* from *A. speltoides* and the latter from the remaining four species in the section *Sitopsis*, two noncoding regions of cpDNA were amplified in these sev-

Table 1

Accessions of *Triticum* and *Aegilop*s species used in this study

Codes or numbers refer to species	Genus/species	ICARDA accession used
M1	*T. monococcum* subsp. *monococcum*	IG. 132870, Georgia
U1	*T. urartu*	IG. 115815, Jordan
D1	*T. turgidum* subsp. *durum*	IG. 76488, Ethiopia
T2	*T. aestivum* subsp. *aestivum*	IG. 43818, Yugoslavia
22	*Ae. tauschii*	IG. 47259, Syria
R4	*Ae. speltoides*	IG. 48764, Lebanon
86	*Ae. searsii*	IG. 47293, Jordan
112	*Ae. bicornis*	IG. 46854, Turkey
105	*Ae. longissima*	IG. 48613, Palestine
121	*Ae. sharonensis*	IG. 47098, Union of Soviet Socialist Republics

en species using two sets of the universal primers designed by Nishizawa and Watano (2000). These are *rps16* intron and *psbC-trnS*. PCR amplifications were carried out in a total volume of 25 µL containing 10 ng of template DNA, 0.5 µM of each primer, 2.0 mM of $MgCl_2$, 0.1 mM of dNTPs, 2.5 µL of 10 × reaction buffer and 0.5 unit of Taq DNA polymerase (Roche Diagnostics, Germany). Amplification was carried out with one cycle at 94°C for 5 min and 35 cycles each of which consists of 1) 30 sec at 94°C, 2) 1 min annealing at 55°C (for *rps16* intron) or 58°C (for *psbC-trnS*), and 3) 2 min at 72°C, and one cycle at 72°C for 5 min. PCR products were visualized by electrophoresis in 1.5% agarose (SIGMA, Germany) gels that were run at 120 V for 30 min, and then the gels were photographed under ultraviolet illumination.

Detection of species-specificity of markers developed PCRs and PCR-RFLP locus-enzyme combinations that were useful in generating PCR and PCR-RFLP markers that aid the discrimination of targeted species were applied on accessions of each of those species (Table 2).

RESULTS

When the three primer pairs that target *rpL2* intron, *trnT-L*, and *trnL-F* were applied to template DNAs from the 10 species analyzed in this study, single-band amplicons of the appropriate size were generated (480 bp for *rpL2* intron, 655 bp for *trnT-L*, and 437 bp for *trnL-F* in 8/10 species). There was no size variation, estimated visually, among those species for 2/3 regions amplified. The exception to this rule was the amplicons generated using the *trnL-F* primers, where *T. monococcum* and *T. urartu* yielded an identical and distinctive

Table 2

Accessions used for detection of intraspecific conservation of PCR and PCR-RFLP markers developed

Species name	Origin and code of accession	Sample code
T. monococcum	IG. 132870, Georgia	M1
	IG. 44852, Turkey	M2
	IG. 45110, Turkey	M3
	IG. 45231, Albania	M4
T. urartu	IG. 115815, Jordan	U1
	IG. 45281, Syria	U2
	IG. 110726, Syria	U3
	IG. 46071, Turkey	U4
	IG. 45477, Iran	U5
Ae. tauschii	IG. 46802, Turkey	21
	IG. 47259, Syria	22
	IG. 49123, Iran	23
	IG. 48747, Armenia	24
	IG. 46645, Pakistan	25
Ae. speltoides var. *speltoides*	IG. 48764, Lebanon	R1
Ae. speltoides	IG. 48433, Syria	R2
Ae. speltoides var. *speltoides*	IG. 49008, Iraq	R3
Ae. speltoides var. *ligustica*	IG. 48847, Iran	R4

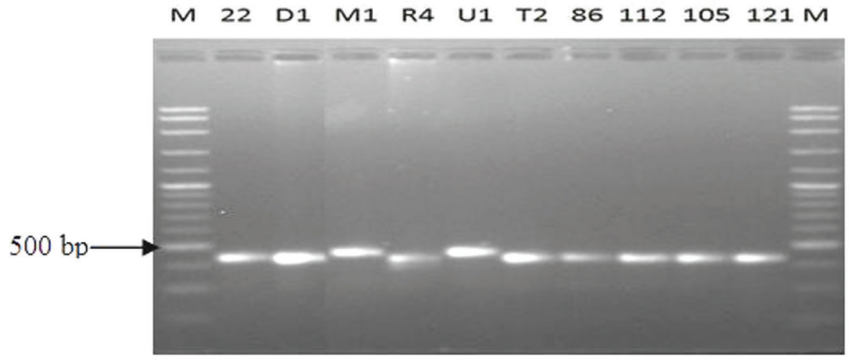

Figure 1. PCR products generated for *trnL-F* in species analyzed. M (100 bp DNA marker), 22 (*Ae. tauschii*), D1 (*T. turgidum* subsp. *durum*), M1 (*T. monococcum* subsp. *monococcum*), R4 (*Ae. speltoides*), U1 (*T. urartu*), T2 (*T. aestivum* subsp. *aestivum*), 86 (*Ae. searsii*), 112 (*Ae. bicornis*), 105 (*Ae. longissima*), 121 (*Ae. sharonensis*)

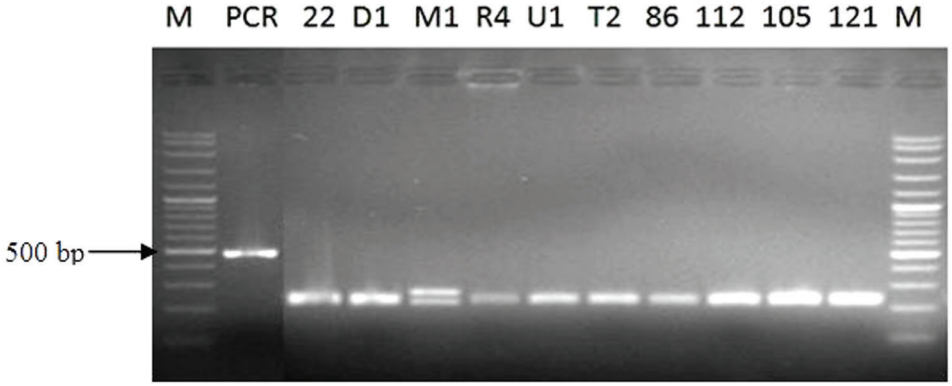

Figure 2. PCR-RFLP restriction profiles generated for species targeted using the combination *rpL2* intron-Taq I. M (100 bp DNA marker), 22 (*Ae. tauschii*), D1 (*T. turgidum* subsp. *durum*), M1 (*T. monococcum* subsp. *monococcum*), R4 (*Ae. speltoides*), U1 (*T. urartu*), T2 (*T. aestivum* subsp. *aestivum*), 86 (*Ae. searsii*), 112 (*Ae. bicornis*), 105 (*Ae. longissima*), 121 (*Ae. sharonensis*)

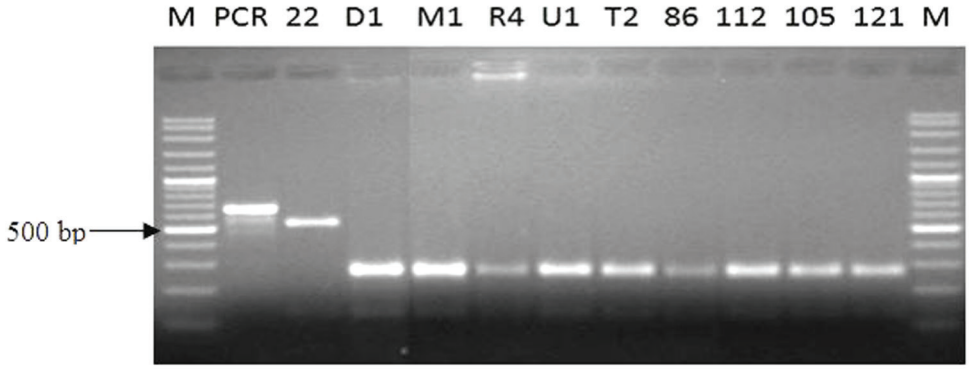

Figure 3. PCR-RFLP restriction profiles generated for species targeted using the combination *trnT-L*-Taq I. M (100 bp DNA marker), 22 (*Ae. tauschii*), D1 (*T. turgidum* subsp. *durum*), M1 (*T. monococcum* subsp. *monococcum*), R4 (*Ae. speltoides*), U1 (*T. urartu*), T2 (*T. aestivum* subsp. *aestivum*), 86 (*Ae. searsii*), 112 (*Ae. bicornis*), 105 (*Ae. longissima*), 121 (*Ae. sharonensis*)

amplicon of around 471 bp, whereas those generated from remaining *Triticum* and *Aegilops* species were all approximately 437 bp (Figure 1).

PCR-RFLP using the combination *rpL2* intron-Taq I generated a unique restriction profile for *T. monoccocum* (Figure 2). Similar scenario was observed when *trnT-L* was restricted with Taq I where *Ae. tauschii* had a unique restriction profile (Figure 3). Using the universal primers that target *rpl2* intron and *trnT-L*, PCR-RFLP had diagnostic utility for 3/10 species examined. These are *T. monococcum*, *T. urartu* and *Ae. tauschii.* No polymorphisms were detected either in terms of the presence of restriction or in the profile generated when any of the three variable markers were applied to multiple accessions of each of these three species.

The Nishizawa and Watano (2000) primer pairs used on template DNAs from durum and bread wheats and the five *Sitopsis* species generated single-band amplicons of the appropriate size (300 bp for *rps16* intron, and 210 or 240 bp for *psbC-trnS*). Restriction of *rps16* intron with Tru 1I separated all the five species of *Sitopsis* from durum and common wheats (Figure 4).

Size variation, estimated visually, was observed for the amplicons generated using the *psbC-trnS* primers, where all *Sitopsis* species apart from *Ae. speltoides* yielded an identical and

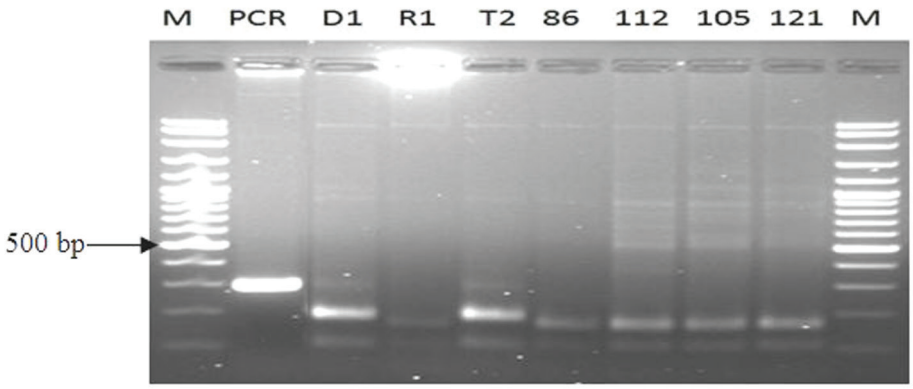

Figure 4. Restriction profiles generated for *Sitopsis* species and durum and bread wheats using the combination *rps16* intron-Tru 1I. M (100 bp DNA marker), D1 (*T. turgidum* subsp. *durum*), R1 (*Ae. speltoides* var. *speltoides*), T2 (*T. aestivum* subsp. *aestivum*), 86 (*Ae. searsii*), 112 (*Ae. bicornis*), 105 (*Ae. longissima*), 121 (*Ae. sharonensis*).

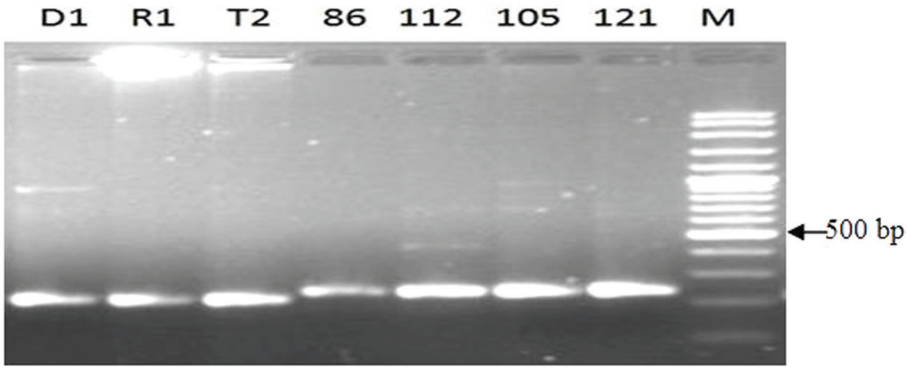

Figure 5. PCR products generated for *psbC-trnS* in *Sitopsis* species and durum and bread wheats. M (100 bp DNA marker), D1 (*T. turgidum* subsp. *durum*), R1 (*Ae. speltoides* var. *speltoides*), T2 (*T. aestivum* subsp. *aestivum*), 86 (*Ae. searsii*), 112 (*Ae. bicornis*), 105 (*Ae. longissima*), 121 (*Ae. sharonensis*).

distinctive amplicon of around 240 bp, whereas those generated for the later and the two *Triticum* (*durum* and *aestivum*) species were approximately 210 bp (Figure 5). This implies that *PsbC-trnS* PCR marker identified *Ae. speltoides* from the remaining four species in the section *Sitopsis*, and restriction of *rps16* intron with Tru 1I separated *Ae. speltoides* from *T. durum* and *T. aestivum*.

DISCUSSION

Many attempts were made to study the genetic diversity of *Triticum* species and subspecies. For instance, Wang *et al.* (1997) investigated genetic diversity among plasmons of *Triticum* species based on PCR-single strand conformational polymorphism (PCR-SSCP) analyses. At the subspecies level, Talbert *et al.* (1992) assessed repetitive DNA variation among accessions of hexaploid and tetraploid wheats. *Triticum* subspecies were also the focus of diagnosis studies since several attempts have been made to distinguish them. For example, Laidò *et al.* (2013) were able to distinguish the durum wheat from the other tetraploid subspecies based on SSRs and DArT markers.

The precise distinctions between *Triticum* species are, however, not always clear. Most efforts in this regard were carried out to discriminate between durum and aestivum based on 1) the electrophoretic pattern for endosperm peroxidases (Asins *et al.* 1981), 2) amplification of Dgas44 that is present only in bread cultivars but absent in all durum cultivars (Ibrahim *et al.* 2011), and 3) RAPD (Patil *et al.* 2011).

Sequencing of cpDNA regions has been the method of choice for the identification of many plant species (e.g., Yao *et al.* 2015). During sequencing, however, there can be no guarantee that an identified SNP will be suitable for use as a molecular marker. However, if a SNP occurs within the recognition site of a restriction enzyme, it is much easier to use PCR-RFLP markers. When Yousefzadeh *et al.* (2014) compared the efficiency of PCR-RFLP and sequencing techniques in recognition of different species of Tilia from Hyrcanian forest, a high similarity was observed between the results of both techniques. This besides the fact that PCR-RFLP is less expensive and less time consuming made the authors recommend PCR-RFLP as a simple and economical method for the diagnosis of plant species. Shavrukov (2016) believes that the presence of highly polymorphic genetic regions containing SNPs allows the simple development of PCR-RFLP in small-scale experiments. Hence, an attempt was made in this study to discriminate *T. aestivum* and *T. durum* from their proposed diploid ancestor species using PCR-RFLP technique.

In congruence with the observations of Ogihara and Tsunewaki (1988) that *T. urartu* has the same chloroplast genome as that of *T. monoccocum* based on the RFLP analysis of the isolated cpDNA, the two species were revealed in this study to have identical cpDNA haplotype.

The morphological similarity of characters of the first leaf (color and ciliation of auricles, short or lacking pubescence) in *T. urartu* and *T. monococcum* suggests a common ancestor of the two diploid species. Ogihara and Tsunewaki (1988) revealed that *T. urartu* has the same chloroplast genome as that of *T. monoccocum* based on the RFLP analysis of the isolated cpDNA. In congruence with these observations, results generated here allowed separation of the two species from other species analyzed based on *trnL-F* PCR-based marker. The high similarity between the cpDNA of the two species did not, however, prevent us from detecting a SNP for their differentiation.

A unique chloroplast PCR-RFLP profile was also observed for *Ae. tauschii* when *trnT-L* was restricted with Taq I. A similar finding was observed by Haider and Nabulsi (2008) based on sequencing information of *orf62* and *trnL-F*. This also agrees with earlier observations of Bowman *et al.* (1983).

It was also possible to separate durum and aestivum from *Ae. speltoides,* which was very frequently nominated as their cytoplasm donor (see Haider 2013 for review). Because this species has been reported to be distinct from the remaining four *Sitopsis* species based on cpDNA (Ogihara & Tsunewaki 1988; Wang *et al.* 1997; Haider & Nabulsi 2008), it was possible in this study to develop a species-specific marker for diagnosis of *Ae. speltoides* from remaining four *Sitopsis* species. Based on mitochondrial SSR haplotypes identified among common wheat and its ancestral species,

Ae. tauschii and *Ae. speltoides* species (and *T. monoccocum*) showed their own specific haplotypes (Ishii *et al.* 2006).

The PCR and restriction fragment patterns of Emmer and bread wheats generated in this study were identical; supporting the hypothesis that Emmer wheat is the cytoplasm donor of *T. aestivum* (Kihara 1944; Graur *et al.* 1989; Matsuoka & Nasuda 2004). In two earlier studies, Tsunewaki (1996) and Ogihara and Tsunewaki (1988) revealed that bread wheat and domesticated Emmer share identical chloroplast genome type with wild Emmer. In 2006, Ishii *et al.* reported that Emmer and common wheats also share the same mitochondrial DNA haplotype.

Due to the great risk of loss of the genetic variability of cultivated wheats and their wild relatives in response to changing environmental conditions and cultural practices, accurate identification of species analyzed is essential for the maintenance of their germplasm collections and in particular the wild relatives of wheat crops that are threatened and endangered to extinction. Bachmann *et al.* (1999) believe that identification of mislabelled or unintentionally duplicated material within and between germplasm collections is a fundamental issue for germplasm conservation. Furthermore, when germplasm collections are stored in genebanks as seeds, the characterisation of seeds and checking the seeds contamination is widely viewed as important (Ford-Lloyd *et al.* 1997).

Reliable identification of species studied here is also important for *in situ* conservation efforts (conservation of species within their natural habitats). There is increasing interest in this strategy particularly to protect wild relatives of wheat crops.

Maxted *et al.* (1997) define *ex-situ* conservation as the maintenance of components of biological diversity outside their natural habitats. This helps minimising genetic erosion on crops gene pools. A comprehensive international *ex-situ* program aims to conserve crop and wild germplasms in genebanks [as accessions (an accession is a group of similar plants received from a single source at a single time)] and botanical gardens (contain representatives of populations lost in the wild) (Brush 1995). The accurate PCR- or PCR-RFLP identification of species targeted here helps checking the true identity of material entering the genebank.

The use of DNA marker technology for wheat identification has also found commercial application for verification of seed purity in seed stocks. Specifically, there is an interest in the quantification of contaminating weed seeds or of seeds belonging to other crops in the production of commercial hybrids, and for evaluating seed purity in a commercial F_1-hybrid cultivars (i.e., absence of parental lines).

CONCLUSIONS

Due to the importance of durum and bread wheats in the food industry, there is a great need for their improvement. The genetic resources of the two species include the cultivated species and their ancestral diploid species that contributed the A, B, or D genomes. In wheat breeding, it is essential to accurately identify the parent species of the pre-breeding program prior to performing any cross-pollination. PCR and PCR-RFLP applied in this study on cpDNA regions provided sufficient variation for the identification of durum and bread wheats and their proposed diploid ancestors. Species-specific cpDNA markers developed here help breeders identify parent species at any stage of development and for any organ of the plant (seeds, leaves, etc.). Other applications of these markers include seed industry and conservation.

Acknowledgements. We would like to thank the Director General of AECS and the Head of Department, for their support. We also thank Dr. Jan Valkoun at GRU in ICARDA, Aleppo, Syria for providing seeds of acce ssions used in this study. The technical assistance of N.S. Eldeen and Q. Ganimeh is much appreciated.

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Received: February 26, 2020
Accepted: June 5, 2020