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Defensins of Triticum urartu and T. monococcum subsp. aegilopoides seed.

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Plants have evolved diverse mechanisms to combat fungal and bacterial infections. The most important among them are the reinforcement of plant cell walls and the release of different components with antimicrobial properties. They comprise the reactive oxygen species, phytoalexins, and PR-proteins including antimicrobial peptides (AMPs) (Selitrennikoff 2001; Garcia-Olmedo et al. 2001).

Defensins are the most conserved cysteine-rich AMPs which were found in nearly all taxa of living organisms: invertebrates, vertebrates, plants and fungi (Thomma et al. 2002). Plant defensins are small (45–54 amino acid residues), basic peptides with four disulphide bridges. Despite a conserved scaffold, the amino acid sequences of defensins vary considerably with only eight cysteine residues being conserved. Variation in amino acid sequences most likely accounts for diverse biological functions displayed by different members of the family. By *in vitro* studies, defensins were shown to exhibit antifungal/antibacterial and insecticidal activities, some of them inhibit enzymes, others act as ion channel

blockers (Broekaert et al. 1995; Lay and Anderson 2005). Defensins were demonstrated to be associated with resistance to abiotic stress (Koeke et al. 2002; Mirouze et al. 2006). Some defensins are constitutive components of plant cells, while others are induced upon challenge with pathogens or stressful abiotic factors. Defensins show promise for creation of resistant plants and the development of new drugs in medicine as an alternative to conventionally used antibiotics and antimycotics.

In our previous studies, we studied defensins from seeds of *T. kiharae*, a synthetic allopolyploid produced by crossing *T. timopheevii* with *Ae. tauschii*, and related *Triticum* and *Aegilops* species (Egorov et al. 2005; Odintsova et al. 2006). We have focused our attention on defensins of *T. monococcum* subsp. *aegilopoides* and *T. urartu*, the presumable A-genome donors to polyploid wheats and compared their structure and complexity with defensins from *T. kiharae*.

Materials and Methods. The species used in this study were *T. monococcum* subsp. a*egilopoides* from Azerbaidzhan, *T. urartu* from Syria, and *T. kiharae*. Flour was extracted with a mixture of two acids (1 M HCl and 5% HCOOH) for 1 h at room temperature and desalted on an Aquapore RP300 column. Freeze-dried acidic extract was subjected to chromatography on Heparin Sepharose. Proteins and peptides were eluted with a stepwise NaCl gradient. The 100-mM NaCl fraction was collected, desalted as described above and separated on a Superdex Peptide HR 10/30 column (Amersham, Pharmacia, Biotech, Uppsala, Sweden). Proteins and peptides were eluted with 0.05% TFA, containing 5% acetonitrile at a flow rate of 250 µl/min, and monitored by absorbance at 214 nm. The peptide fraction was further separated by RP-HPLC on a Vydac C18 column (4.6 x 250 mm, particle size 5 µm) with a linear acetonitrile gradient (10–50%) for 1 h at a flow rate of 1 ml/min and 40°C. Peptides were detected at 214 nm. Mass spectra were acquired on a model Reflex III mass spectrometer (Bruker Daltonics, Bremen, Germany). N-terminal amino acid sequences were determined by automated Edman degradation on a model 492 Procise sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol.

Results and Discussion. For the isolation of defensins from the diploid species, we followed the procedure earlier developed for the isolation of *T. kiharae* defensins (Egorov et al. 2005; Odintsova et al. 2006). Acidic extraction of flour was followed by subsequent separation of the protein-peptide extract by a combination of different types of HPLC (affinity, size-exclusion and reversed-phase). Defensins were identified on the basis of their retention time from the RP-HPLC column, mass-spectrometric analysis and, in some cases, N-terminal sequencing of the reduced and alkylated peptides. In *T. monococcum* subsp. *aegilopoides* seeds, the following defensins were found: D1.1, D1.2, D2, and a D3 homologue; its N-terminal amino acid sequence coincided with that of D3, although its molecular mass was different (Table 1).

Table 1. Defensins identified in *T. monococcum* subsp. *aegilopoides* seed.

RP-HPLC fraction number	N-terminal amino acid sequence	Molecular mass (Da)	Peptide
1	RDCESDSH	5130	Tk-AMP-D1.1
2	RTCQSQSH	5692	Tk-AMP-D1.2
3	RTCESQSHKF	5692	Tk-AMP-D2
4	RDCKSDSHKFHGACF	4859	Tk-AMP-D3 homologue

Table 2. Defensins identified in *T. urartu* seed.

RP-HPLC fraction number	N-terminal amino acid sequence	Molecular mass (Da)	Peptide
1	RDCESDSH	5130	Tk-AMP-D1.1
2	RTCQSQSH	5736	Tk-AMP-D1
3	RTCESQSHKF	4980	Tk-AMP-D4
4	RDCKSDSHKFHGACF	5151	Tk-AMP-D5

The molecular mass analysis of the main fractions obtained by size-exclusion chromatography of *T. urartu* samples from Syria revealed the molecular masses characteristic of D1, D1.1, D4, and D5. The identity of these peptides to the above-mentioned defensins was confirmed by sequencing (Table 2).

Analysis of the data obtained showed that *T. monococcum* subsp. *aegilopoides* defensins differed considerably both from those of *T. urartu* and *T. monococcum*. In this species, we discovered D1.1, D1.2, D2, and a D3 homologue. Defensin D3 and its homologues were not found in *T. monococcum* and *T. urartu*, they we identified earlier in the species of the *Aegilops, Ae. tauschi* and *Ae. speltoides*, respectively (Odintsova et al. 2007).

In summary, our data on the array and amino acid sequences of D defensins provide new evidence for the closer relationship between the polyploid wheat *T. kiharae* and *T. urartu* than with *T. monococcum* subsp. *aegilopoides*. Of particular interest are that the amino acid sequences of D defensins are highly conserved and persisted for about 10 thousand years that followed from the origin of polyploid forms. This observation provides strong evidence in favor of vital functions of this AMP family in plants.

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ITEMS FROM THE REPUBLIC OF SOUTH AFRICA

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Triticale breeding.

Widespread stem rust and leaf rust infections occurred during 2007. All our established commercial cultivars proved to be highly susceptible and are being phased out. However, the recently released cultivar US2007 remained completely resistant. Another advanced line (to be named AgBeacon) that also has complete resistance and excellent yield potential is being multiplied for release in 2009 and has the pedigree: Massa/Nimir 3/3/Yogui 1/Tarasca 87 3// Hare 212/4/Ibis/8/Ibis/7/Hare 212/3/Champlain/Aronde 68//VPM/Moisson/4/Juanillo 100/5/ANDAS'S'/6/Durum wheat/Balbo//BOK'S'/3/ANDAS'S'//TJ/BGL'S'.

Wheat recurrent mass selection.

New material developed in each phase of the program included approximately 60,000 new, potentially different F_1 genotypes. For the second year, an F_7 nursery consisting of 204 pure lines was distributed to local breeders (PANNAR, SGI, Monsanto, Cengen, and Afgri-Seed). The same material was evaluated in Uganda for resistance to the UG99 stem rust virulence. A genotyping system (microsatellite and AFLP loci) to distinguish F_6 inbred lines from one another and from released commercial cultivars was tested and found to discriminate among the majority of lines.