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Antimicrobial and Other Oligopeptides of Grapes

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Abstract—Structures and functions of about 700 oligopeptides of various plants are presently known. However, only one polypeptide has been isolated from grapes and characterized. At the same time, tens of thousands of uncharacterized amino acid sequences have been revealed in this plant, among which there can also be precursors of oligopeptide regulators. Due to the scientific and practical importance of innate immunity of agricultural plants, we have undertaken structural and functional investigation of these sequences to identify new regulatory oligopeptides including antimicrobial agents. For this purpose, we elaborated a special method of computer analysis enabling comparison of primary structures of putative precursors of grape oligopeptides with amino acid sequences of known oligopeptides of other plants. Structural similarity served as the basis for prediction of potential functional properties. As a result, over 20 new structures of antimicrobial and other grape oligopeptides have been found.

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It is known that natural oligopeptides containing from 2 to 50 amino acid residues [1, 2] are involved in practically all regulatory processes [3, 4]. Chemical structures are now known for over 9000 oligopeptides found in more than 1600 living organisms representing all biological kingdoms [5]. The spectrum of their functional activity is very broad. Among them about 700 plant oligopeptides are described. It became clear that oligopeptides of similar primary structure can exhibit different functions. Thus, antimicrobial oligopeptides defensin J1-1 and J1-2 from pepper *Capsicum annuum* fruits [6] are homologous to α-amylase inhibitors SIa-2 and SIa-3 from *Sorghum bicolor* seeds [7], toxin zeathionin γ-1 of *Zea mays* seeds [8], translation inhibitor γ-hordothionin of barley *Hordeum vulgare* endosperm [9], synthesis inhibitors purothionins γ-1 and γ-2 from seeds and endosperm of wheat *Triticum aestivum* [10], and other oligopeptides exhibiting similar functions. At the same time, such homology is usually not considered in available structural—functional classifications of antimicrobial oligopeptides [11, 12].

Only one oligopeptide has been isolated from grape and characterized so far [13]. It is highly active in the plants' struggle against diseases caused by pathogenic fungi. In particular, this oligopeptide very efficiently prevents wilt developed in response to such pathogens as *Fusarium oxysporum* and *Verticillium dahliae*.

However, the protein databases contain information concerning an enormous number of the grape amino acid sequences that are not characterized in most cases and, by analogy with polypeptide structures of other plants, can be precursors of regulatory oligopeptides.

Since oligopeptides play an important role in various types of regulation (including innate plant immunity [14]), they are of great interest for biotechnology [15], and obtaining new information about their structures and functions is therefore important. Keeping this in mind, we carried out the computer structural—functional investigation of all known amino acid sequences of grape *Vitis vinifera* to identify new oligopeptide structures exhibiting antimicrobial and other functions.

METHODS OF INVESTIGATION

A method of computer analysis was elaborated for this investigation. All amino acid sequences of grape and of all known plant oligopeptides were compared using

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Abbreviations: ESTs, expressed sequence tags.
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programs designed by the authors. Information contained in the protein database Swiss-Prot/TrEMBL (http://www.expasy.ch/sprot/) on 55,628 amino acid sequences of grape from over 7 million records was used.

Amino acid sequences of known plant oligopeptides are contained in this and other specialized peptide databases. Thus, the base PhytAMP (http://phytamp.pfba-lab-tun.org/statistics.php) was elaborated only for antimicrobial peptides (231 sequences), APD2 (http://aps.unmc.edu/AP/main.php) for all antimicrobial peptides (1726), and AMSDb (http://www.bbcm.univ.trieste.it/~tossi/antimic.html) for all oligopeptides of eukaryotes (895). There are also other databases containing data on antimicrobial peptides like ANTIMIC (http://research.i2r.a-star.edu.sg/Templar/DB/ANTIMIC/) and APPDb (http://ercbinfo1.ucd.ie/APPDb/), but recently access to them has been difficult. In our study we have used the EROP-Moscow database (http://erop.inbi.ras.ru/, over 8300 records) that contains more (compared to other databases) amino acid sequences of peptides both of all plants (720) including 232 antimicrobial and of all antimicrobial (1915).

In the course of the investigation, uncharacterized grape oligopeptide amino acid sequences from Swiss-Prot/TrEMBL recorded in FASTA format were compared step-by-step with amino acid sequences of oligopeptides stored in the EROP-Moscow database in special format. In the case of similarity criterion no less than 30%, homologous structures were revealed (with a less strict criterion informational noise sharply increases). In this case the free program SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/) was used, which makes it possible to determine the site of signal peptide cleavage with high (up to 93%) probability. Then possible functions of the found grape structures were comparatively analyzed, and in this way their functional potentials were determined.

RESULTS AND DISCUSSION

Our investigations revealed in the grape amino acid sequences numerous regions structurally similar to known oligopeptides of many different plants. It was found that 35 of them are homologous to oligopeptides exhibiting functions of antimicrobial agents, enzyme inhibitors, or toxins.

As shown in Fig. 1, the amino acid sequence with identification number E07213 is fully identical to the primary structure of the unique known grape oligopeptide exhibiting antifungal activity (1.1) [13]. Also, it appeared that structures of 51 other oligopeptides with different extent of similarity (from 39.1 to 91.5%) are its homologs. All of these contain cysteine residues evenly distributed along the peptide chain. In most structures eight cysteine residues are located in such manner, and only in one case (1.51) there are four such residues.

Figure 2 shows amino acid sequences of the grape polypeptide regions that can exhibit regulatory functions. The similarity of each detected region is shown only for one known oligopeptide, maximally similar structurally, but the same could be observed for a greater number of homologs. Among found structures there are nine whose precursors were previously characterized as different chitinases [16, 56, 62, 65, 66-70].

In addition to the described oligopeptides, we have also obtained data concerning structures homologous to the different functional class oligopeptides, but their characteristics require special analysis.

All revealed amino acid sequences (35 oligopeptides shown in the Fig. 2) can be initially sorted out using the precursor cross-reference proposed by the Swiss-Prot/TrEMBL data base. The first group includes peptides whose precursors are homologous to defensin of invertebrates, γ-thionin, and γ-purothionin. ESTs (expressed sequence tags) of precursors of the group 1 peptides are associated with different chromosomes.

The next three groups contain one structure each. The oligopeptide 5 precursor is homologous to wound-inducible basic proteins, and precursor of oligopeptide 6 is homologous to proteins fulfilling the following functions: chitin binding, cell wall catabolic process, defense response to bacteria, and defense response to fungi. The cross-reference of precursor 7 is distinguished by cysteine protease inhibitor activity. ESTs of precursors of these oligopeptides were also identified on different grape chromosomes (table). It is remarkable that ESTs of precursors 6 and 7, associated with chromosomes 14 and 8, respectively, are located in the nucleotide binding sites of genes involved in formation of resistance to diseases [16].

The other 27 oligopeptides are combined as precursor homologs (group 5) carrying out functions of chitin binding, chitinase activity, and cell wall and chitin catabolism. However, this representative group disintegrates at least to two parts on the basis of putative precursors (chitinases of class I and IV). Localization on the grape chromosome 5 is characteristic of EST precursors of 10 members of this group.

It should be noted that works of two independent research groups on decoding of the complete *Vitis vinifera* genome contributed to the database with information concerning precursors of grape oligopeptides. The first group (Jaillon et al.) used shotgun cloning followed by computer analysis, and in this case sequence overlapping made it possible to analyze the genome in 8.4-fold repetition [16]. Another group of authors (Velasco et al.) used both shotgun cloning (reading repetition 6.5-fold) and sequencing by synthesis (reading repetition 4.2-fold) to increase accuracy of analysis [56]. The coincidence of sequences in the abovementioned works and in previous studies on analysis of chitinase gene fragments attracts attention [62, 64, 66, 67, 69]. cDNAs were used for sequence analysis in works of 1997-2006.

1	A7QBX4	Uncharacterized (grape)	...aeaRTCESQSHRFKGTCVRQSNCAAVC QTEGFH GGNCRG F RRRCFCTK HC	100,0	[12]	
1.01	E07213	Antifungal peptide Vv-AMP1 (grape)	RTCESQSHRFKGTCVRQSNCAAVC QTEGFH GGNCRG F RRRCFCTK HC	100,0	[9]	[9]
1.02	E07394	Antimicrobial peptide γ-Thionin (European chestnut)	RTCESQSHRFKGPCVRKSNCASVC QTEGFH GGQCRG F RRRCFCTK HC	91,5	[13]	
1.03	E07395	Defensin PpDFN1 (pearch)	RTCESQSNRFKGTCVSTSNCASVC QTEGFP GGHCRG F RRRCFCTK HC	87,2	[14]	
1.04	E03980	γ-Thionin homolog At2g02100 (mouse-ear cress)	RTCESQSHRFKGTCVSASNCANVC HNEGFV GGNCRG F RRRCFCTR HC	85,1	[15]	
1.05	E03978	γ-Thionin homolog PPT (violet-flowered petunia)	RTCESQSHRFHGTCVRESNCASVC QTEGFI GGNCRA F RRRCFCTR NC	85,1	[16]	
1.06	E07400	Thionin-like antimicrobial peptide (common tobacco)	RTCESQSHRFKGPCSRDSNCATVC LTEGFS GGDCRG F RRRCFCTR PC	80,9	[17]	
1.07	E07399	Defensin (bell pepper)	RTCESQSHRFKGVCASETNCASVC QTEGFS GGDCRG F RRRCFCTR PC	78,7	[18]	
1.08	E07396	Defensin EGAD (African oil palm)	RTCESQSHKFQGTCLRESNCANVC QTEGFQ GGVCRG V RRRCFCTR LC	78,7	[19]	
1.09	E03011	Defensin J1-2 (bell pepper)	RTCESQSHRFKGLCFSKSNCGSVC HTEGFN GGHCRG F RRRCFCTR HC	78,7	[20]	[20]
1.10	E04851	Antimicrobial peptide γ-Thionin tgas118 (tomato)	RTCESQSHRFKGPCVSEKNCASVC ETEGFS GGDCRG F RRRCFCTR PC	78,7	[21]	
1.11	E02424	Antimicrobial peptide Pseudothionin St1 (potato)	RHCESLSHRFKGPCTRDSNCASVC ETERFS GGNCHG F RRRCFCTK PC	76,6	[22]	[23]
1.12	E07398	Defensin (native tobacco)	RTCESQSRRFRGLCFSKSNCGSVC HTEGFN GGHCRG F RRRCFCTR HC	74,5	[24]	
1.13	E04246	Defensin SD2 (common sunflower)	RTCESQSHKFKGTCLSDTNCANVC HSERFS GGKCRG F RRRCFCTT HC	74,5	[25]	
1.14	E07402	Kunitz type trypsin inhibitor (runner bean)	RVCESQSHGFKGACTGDHNCALVC RNEGFS GGNCRG F RRRCFCTK IC	74,5	[26]	
1.15	E03982	γ-Thionin homolog At2g02100 (mouse-ear cress)	RTCESKSHRFKGPCVSTHNCANVC HNEGFG GGKCRG F RRRCYCTR HC	74,5	[27]	
1.16	E07387	Defensin Ec-AMP-D2 (annual weed barnyard grass)	RECQSQSHRYKGACVHDTNCASVC QTEGFS GKCVG F RGRCFCTK HC	73,9		[28]
1.17	E07401	Kunitz type trypsin inhibitor (sweet potato)	RMCESASSKFKGPCSRDSNCATVC HTEGFT GGDCKG F RRRCFCTK PC	72,3	[29]	
1.18	E07397	Putative defensin 1 (columbine)	RDCESQSHKFKGTCIRKSNCASVC QSEGFN GGHCRG V TRRCYCTA KC	72,3	[30]	
1.19	E03979	Enzyme inhibitor Cp-Thionin (cowpea)	RVCESQSHGFKGACTGDHNCALVC RNEGFS GGNCRG F RRRCFCTL KC	72,3		[31]
1.20	E07386	Defensin Ec-AMP-D1 (annual weed barnyard grass)	RECQSQSHRYKGACVHDTNCASVC QTEGFS GKCVG F RGRCFCTK AC	71,7		[28]
1.21	E03981	γ-Thionin homolog At2g02100 (mouse-ear cress)	RTCASQSQRFKGKCVSDTNCENVC HNEGFP GGDCRG F RRRCFCTR NC	70,2	[32]	
1.22	E07257	Defensin Tk-AMP-D6 (wheat)	RDCRSQSKTFVGLCVSDTNCASVC LTEHFP GGKCDG Y RRCFCTK DC	63,0		[33]
1.23	E07258	Defensin Tk-AMP-D6.1 (wheat)	RECRSQSKQFVGLCVSDTNCASVC LTEHFP GGKCDG Y RRCFCTK DC	63,0		[33]
1.24	E06142	Proteinase inhibitor P322 (soybean)	RVCESQSHGFHGLCNRDHNCALVC RNEGFS GGRCKR S RRCFCTR IC	63,0	[34]	
1.25	E07256	Defensin Tk-AMP-D5 (wheat)	RECRSESKKFVGLCVSDTNCASVC LTERFP GGKCDG Y RRCFCTK DC	60,9		[33]
1.26	E07255	Defensin Tk-AMP-D4 (wheat)	RDCTSQSHKFVGLCLSDRNCASVC LTEYFT GGKCDH RRCVCTK GC	60,0		[33]
1.27	E07252	Defensin Tk-AMP-D1.1 (wheat)	RDCESDSHKFHGACFSDTNCANVC QTEGFT AGKCVG V Q RHCHCTK DC	59,6		[33]
1.28	E07260	Defensin TAD1 (wheat)	RTCLSQSHKFKGTCLSNSNCAAVC RTENFP DGECNTHL V E RKCYC KRTC	59,2	[35]	
1.29	E07254	Defensin Tk-AMP-D3 (wheat)	RDCKSDSHKFHGACFSDTNCANVC QTEGFT RGKCDGIH CHCIK DC	55,6		[33]
1.30	E03018	Protein translation inhibitor γ-Hordothionin (barley)	RICRRRSAGFKGPCVSNKNCAQVCMQ EGWG GGNCDGPL RRCKC MRRC	55,3		[36]
1.31	E03017	Protein synthesis inhibitor γ-2 Purithionin (wheat)	KVCRQRSAGFKGPCVSDKNCAQVCLQ EGWG GGNCDGPF RRCKC IRQC	55,3		[37]
1.32	E07253	Defensin Tk-AMP-D2 (wheat)	RTCESQSHKFKGPCFSDSNCATVC RTENFP RGQCNQHH V E RKCYC ERSC	55,1		[33]
1.33	E03016	Protein syntheses inhibitor γ -1 Purithionin (wheat)	KICRRRSAGFKGPCMSNKNCAQVCQQ EGWG GGNCDGPF RRCKC IRQC	53,2		[38]
1.34	E01571	α-Amilase inhibitor SIa-2 (sorghum)	RVCMGKSAGFKGLCMRDQNCAQVCLQ EGWG GGNCDG VM RQCKC IRQCW	52,1		[39]
1.35	E03983	γ-Thionin homolog At2g02100 (mouse-ear cress)	RTCESPSNKFQGVCLNSQSCAKAC PSEGFS GGRCSS L RCYCSK AC	51,1	[40]	
1.36	E07259	Defensin Tm-AMP-D6.2 (wheat)	RTCQSQSHKFKGACFSDTNCASVC RTENFP RGQCNQHH V E RKCYC ERDC	51,0		[33]
1.37	E03010	Defensin J1-1 (bell pepper)	KICEALSGNFKGLCLSSRDCGNVC RREGFT DGSCIG FRL Q CFCTK PCA	51,0	[20]	[20]
1.38	E03021	α-Amylase inhibitor SI alpha-2.1 (sorghum)	RVCRRRSAGFKGLCMSDHNCAQVCLQ EGWG GGNCDG VM RQCKC IRQC	51,0		[40]
1.39	E03019	Toxin γ-1 Zeathionin (maize)	RVCRRRSAGFKGVCMSDHNCAQVCLQ EGYG GGNCDG IM RQCKC IRQC	51,0		[41]
1.40	E01572	α-Amilase inhibitor SIa-3 (sorghum)	RVCRRRSAGFKGLCMSDHNCAQVCLQ EGWG GGNCDG VI RQCKC IRQCW	50,0		[38]
1.41	E07251	Defensin Tk-AMP-D1 (wheat)	RTCQSQSHKFKGACFSDTNCDSVC RTENFP RGQCNQHHV E RKCYC ERDC	49,0		[33]
1.42	E03682	Antimicrobial peptide γ-Thionin (Norway spruce)	RTCKTPSGKFKGVCASSNNCKNVC QTEGFP SGSCD FHVAN RKCYC SKPCP	48,0	[42]	
1.43	E04088	Defensin Psd2 (garden pea)	KTCENLSGTFKGPCIPDGNCNKHCRNNEHLI SGRCRDDF RCWCTN RC	46,8		[43]
1.44	E07385	Defensin Lc-def (cultivated lentil)	KTCENLSDSFKGPCIPDGNCNKHC KEKEHLLSGRCRDDF RCWCTR NC	46,8	[44]	[44]
1.45	E03431	Antimicrobial peptide Fabatin-1 (broad bean)	LLGR CKVKSNRFHGPCLTDTHCSTVC RGEGYK GGDCHG L RRRCMC LC	45,8		[45]
1.46	E03432	Antimicrobial peptide Fabatin-2 (broad bean)	LLGR CKVKSNRFNGPCLTDTHCSTVC RGEGYK GGDCHG L RRRCMC LC	45,8		[45]
1.47	E03006	Antifungal peptide AX1 (sugar beet)	AICKKPSKFFKGACGRDADCEKACDQ ENWP GGVCV PF L RCECQR SC	41,3		[46]
1.48	E04847	Defensin-like peptide NaD1 (Persian tobacco)	RECKTESNTFPGICITKPPCRKAC ISEKFT DGHCSKIL RRCLCTK PC	40,4	[47]	[47]
1.49	E04250	Antimicrobial peptide γ-Thionin FST (common tobacco)	RECKTESNTFPGICITKPPCRKAC ISEKFT DGHCSKLL RRCLCTK PC	40,4	[48]	
1.50	E04849	γ-Thionin-like peptide TPP3 (tomato)	QQICKAPSQTFPGLCFMDSSCRKYC IKEKFT GGHCSKL Q RKCLCTK PC	39,6	[49]	
1.51	E02423	Defensin So-7 (spinach)	GIFSSRKCKTPSKTFKGYCTRDSNCDTSC RYEGYPA GD	39,5		[50]
1.52	E06935	Defensin (mung bean)	RTCMIKKEGW GKCLIDTTCAHSC KNRGYI GGNCKGMT RTCYCLV NC	39,1	[51]	

Fig. 1. Structural family of different plant regulatory oligopeptides obtained by the alignment procedure. There are in succession structure number, identification numbers of records in databases Swiss-Prot/TrEMBL and EROP-Moscow (always begins with letter E), the name of the regulatory oligopeptide together with functional property, primary structure with deletions and insertions, similarity percent, and literature sources for translated and experimentally obtained structures. Amino acid residues retaining their position upon transfer from one structure to another are shown in bold, while residues localized beyond oligopeptide sequence are shown by small letters. Names of oligopeptides and plants correspond to international designations.

It is necessary to explain the great number of sequences whose precursor EST are included in the same supercontig 72 on chromosome 5 (see table). Since *Vitis vinifera* is a paleohexaploid organism [16], it is characterized by a high copy number of genes encoding proteins that are involved in synthetic processes important for the organism. Thus, stilbene synthetase genes are represented by 43 copies, while genes of terpene synthetases are represented by 89 copies of functioning genes and 27 copies of pseudogenes [16]. Therefore genes of chitinases belonging to the group of pathogenesis-related proteins [66] can be also represented in a higher number of copies. Works on cloning chitinase 4 genes (VvCki4A and VvCki4B) are also indicative of possible multiple copy number of chitinase genes [69].

Analysis of oligopeptide structural similarity made it possible to better regulate sequence sorting (Fig. 3). First of all, it revealed fully identical structures. This can be explained by gene duplication and variations in the precursor amino acid sequence beyond the oligopeptide region. Besides, in some cases coincidence of structures is explained by the fact that the same precursors were obtained by different research groups. Two identical structures appeared six times, and in one case coincidence was observed three times. As a result, seven structurally homologous families containing from 1 to 13

1	A7QBX4	Uncharacterized (grape)	...aeaRTCESQSHRFKGTCVRQSNCAAVCQTEGFHGGNCRGFRRRCFCTKHC	100,0	[12]	
	E07213	Antifungal peptide Vv-AMP1 (grape)	RTCESQSHRFKGTCVRQSNCAAVCQTEGFHGGNCRGFRRRCFCTKHC	100,0	[9]	[9]
2	A5BBI9	Uncharacterized (grape)	...seaRVCESQSHKFEGACMGDHNCALVCRNEGFSGGKCKGXRRRCFCTKLC	100,0	[52]	
	E07402	Kunitz type trypsin inhibitor (runner bean)	RVCESQSHGFKGACTGDHNCALVCRNEGFSGGNCRGFRRRCFCTKIC	85,1	[26]	
3	A7QR44	Uncharacterized (grape)	...seaRVCESQSHKFEGACMGDHNCALVCRNEGFSGGKCKGLRRRCFCTKLC	100,0	[12]	
	E07402	Kunitz type trypsin inhibitor (runner bean)	RVCESQSHGFKGACTGDHNCALVCRNEGFSGGNCRGFRRRCFCTKIC	85,1	[26]	
4	A7QMI0	Uncharacterized (grape)	...teaRLCESQSHWFRGVCVSNHNCAVVCRNEHFVGGRCRGFRRRCFCTRNC	100,0	[12]	
	E03980	γ-Thionin homolog At2g02100 (mouse-ear cress)	RTCESQSHRFKGTCVSASNCANVCHNEGFVGGNCRGFRRRCFCTRHC	76,6	[15]	
5	A7QX91	Uncharacterized (grape)	MIYDVNSPLFRSFLSQKGGSSDKRKTEEQKPKEQRPKASENKPVMTE	100,0	[12]	
	E03355	Wound-induced basic peptide (kidney bean)	MIYDVNSPLFRSFLSQKGGSSDKRKTEEQKPKEHRPKASENKPIMTE	95,7	[53]	
6	A7QVP7	Uncharacterized (grape)	...aaaQQCGRQAGGRTCANNLCCSQYGYCGTTAEYCSPSQSCQSNCQSGGqss...	100,0	[12]	
	E03350	Fungal growth inhibitor Pseudo-hevein (rubber tree)	EQCGRQAGGKLCPNNLCCSQYGWCGSSDDYCSPSKNCQSNCKGGG	71,1		[54]
7	A7PTC9	Uncharacterized (grape)	...qhqSEWQVVPTNDPEVQDAANHVVKSIQMRSNSIFRYELLEILLAKAKVIeqs...	100,0	[12]	
	E07353	Enzyme inhibitor Cystatin 2 (rice)	...ghePGWRDVPVHDPVVKDAADHAVKSIQQRSNSLFPYELLEIVRAKAEVVedf...	63,8	[55]	
8	A5AHS9	Uncharacterized (grape)	...leaKVCQRPSKTWSGFCGSSKNCDRQCKNWEGAKHGACHAKFPG VACFCYFNC	100,0	[12, 52]	
	E03013	Defensin Ct-AMP1 (butterfly pea)	NLCERASLTWTGNCGNTGHCDTQCRNWESAKHGACH KR GNWKCFCYFNC	63,3		[56]
9	A7R0S4	Uncharacterized (grape)	...isaQQCGRQASGKRCAGGLCCSQYGYCGSTRPYCGVGCQSQCRqqa...	100,0	[12]	
	E03438	Antifungal peptide PN-AMP2 (morning glory)	JQCGRQASGRLCGNRLCCSQWGYCGSTASYCGAGCQSQCR	77,5		[57]
10	Q9FS45	Chitinase (grape)	...aqeQCGRQAGGALCSGGLCCSQYGYCGSTSAYCSTGCQSQCPSqqs...	100,0	[12, 58]	
11	A5AT01	Uncharacterized (grape)	...aqeQCGRQAGGALCSGGLCCSQYGYCGSTSAYCSTGCQSQCPSggs...	100,0	[52]	
	E06093	Antifungal peptide pnAMP-h2 (morning glory)	JQCGRQASGRLCGNGLCCSQWGYCGSTAAYCGAGCQSQCKS	75,6	[59]	
12	A7R0S0	Uncharacterized (grape)	...qqeQCGSLAGGALCSGGLCCSQYGYCGSTPAYCSTGCQSQCTSqqs...	100,0	[12]	
	E06092	Antifungal peptide pnAMP-h1 (morning glory)	JQCGSQARGRLCGNGLCCSQWGYCGSTAAYCGAGCQSQCKS	73,2	[59]	
13	Q9ZTK4	Class I extracellular chitinase	...aqeQCGRQAGGALCSGGLCCSQYGYCGSTSAYCSTGCQSQCPCqqs...	100,0	[60]	
	E06093	Antifungal peptide pnAMP-h2 (morning glory)	JQCGRQASGRLCGNGLCCSQWGYCGSTAAYCGAGCQSQCKS	73,2	[59]	
14	A3QRB6	Chitinase class I basic (grape)	...saeQCGGQAGGRVCPGGACCSKFGRCGNTADYCGSGCQSQCSStqd...	100,0	[61]	
15	A3QRB7	Chitinase class I basic (grape)	...saeQCGGQAGGRVCPGGACCSKFGRCGNTADYCGSGCQSQCSStgd...	100,0	[61]	
	E06092	Antifungal peptide pnAMP-h1 (morning glory)	JQCGSQARGRLCGNGLCCSQWGYCGSTAAYCGAGCQSQCKS	65,9	[49]	
16	P51613	Basic endochitinase (grape)	...saeQCGGQAGGRVCPGGACCSKFGWCGNTADYCGSGCQSQCSStqd...	100,0	[62]	
17	Q546P8	Chitinase class I (grape)	...saeQCGGQAGGRVCPGGACCSKFGWCGNTADYCGSGCQSQCSStgd...	100,0	[12, 52, 58, 63]	
	E06092	Antifungal peptide pnAMP-h1 (morning glory)	JQCGSQARGRLCGNGLCCSQWGYCGSTAAYCGAGCQSQCKS	65,9	[59]	
18	Q7XAU6	Class IV chitinase (grape)	...aavAQNC GCASGLCCSKYGYCGTGSDYCGDGCQSGPCDSsqs...	100,0	[12, 64]	
19	O24530	Class IV endochitinase (grape)	...aavAQNC GCASGLCCSKYGYCGTGSDYCGDGCQSGPCDSsgs...	100,0	[65]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	62,2		[66]
20	A5AK36	Uncharacterized (grape)	...aavAQNC GCASDQCCSKYGYCGTGNDYCGNTCQSGPCYSsqq...	100,0	[12, 52]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	62,2		[66]
21	A5BSD1	Uncharacterized (grape)	...pvlAQNC GCNAGLCCSQYGYCGTGNDYCGTGCQAGPCYSsps...	100,0	[52]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	59,5		[66]
22	A7QAP2	Uncharacterized (grape)	...pvlAQNC GCDAGLCCSQYGYCGTGNDYCGTGCQAGPCYSpps...	100,0	[12]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	59,5		[66]
23	A7QAP8	Uncharacterized (grape)	...cysGQTC GCDASLCCSQYGYCGTGDDYCGTGCQAGPCYSsqq...	100,0	[12]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	59,5		[66]
24	O24531	Class IV endochitinase (grape)	...aavAQNC GCASGLCCSKYGYRGTGSDYCGDGCQSGPCDSqsq...	100,0	[65]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	59,5		[66]
25	A5AJB3	Uncharacterized (grape)	...stvAQDC GCSSDQCCSQWGYCGTGDDYCGTGCQSGPCTStsn...	100,0	[12, 52]	
26	Q7XB39	Class IV chitinase (grape)	...stvAQDC GCSSDQCCSQWGYCGTGDDYCGTGCQSGPCTStsn...	100,0	[67]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	56,8		[66]
27	A7QAP5	Uncharacterized (grape)	...fvlAQNC RCDAGLCCSQYGYCGTGNDFCGTGCQAGPCYSsqq...	100,0	[12]	
28	A5BSD2	Uncharacterized (grape)	...fvlAQNC RCDAGLCCSQYGYCGTGNDFCGTGCQAGPCYSsgg...	100,0	[52]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	56,8		[66]
29	A7QAO0	Uncharacterized (grape)	...savLSQDC GCGAGLCCSKFGYCGTGKEYCGTGCQAGPCDSsss...	100,0	[12]	
	E05812	Antimicrobial peptide Avesin (oat)	WSG CSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	56,8		[66]
30	A7QAO2	Uncharacterized (grape)	...palAQNC GCSASVCCSQYGYCGTTKDYCGAGCLAGPCYSsss...	100,0	[12]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	56,8		[66]
31	A5BSD0	Uncharacterized (grape)	...qvvGQDC GCAADLCCSRWGYCGTGDDYCGTGCQEGPCNPaps...	100,0	[52]	
32	A7QAP0	Uncharacterized (grape)	...gvvGQDC GCAADLCCSRWGYCGTGDDYCGTGCQEGPCNPaps...	100,0	[12]	
33	A7QAP6	Uncharacterized (grape)	...gvvGQDC GCAADLCCSRWGYCGTGDDYCGTGCQEGPCNPpps...	100,0	[12]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	54,1		[66]
34	A5C2S9	Uncharacterized (grape)	...fvlAQNC GCAAGLCCSQYGYCGTGNDFCGTGCQAGPCYSsqq...	100,0	[52]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	54,1		[66]
35	A7QAO7	Uncharacterized (grape)	...stvAQNC GCASGLCCNKFGYCGTGNAYCGDGCQAGPCYSsqq...	100,0	[12]	
	E05812	Antimicrobial peptide Avesin (oat)	WSGCSPCPGNECCSKYGYCGLGGDYCGAGCQSGPCYG	54,1		[66]

Fig. 2. Structures of all revealed regions of the grape amino acid sequences together with maximally similar regulatory oligopeptides of other plants. Symbol J designates pyroglutaminyl formed from glutamine residues at the *N*-terminus of amino acid sequences [72, 73]. Other explanations as in Fig. 1.

members were formed of 27 different amino acid sequences. The most representative was family I whose structures with extent of similarity from 54.1 to 62.2% are homologous to the known antimicrobial oligopeptide from seeds of the common oat *Avena sativa* L. avesin [70]. Almost all members of the family, as in the case of structure 1, contain eight cysteine residues each, with the exclusion of two (5 and 7) from which these residues are absent.

Finding a great number of oligopeptide homologs in the same biological species, including plants, is not rare. For example, the structure-homologous family of antimicrobial oligopeptides found in the West-Australian violet *Hybanthus floribundus* consists of 11 members [74]. These oligopeptides were obtained from combined material of the above-ground part of plant, i.e. from its various organs. In the case of grape, oligopeptide structural polymorphism can also be explained by the fact that, as follows from data in the table, their precursors were found in leaves, berries, and young shoots. Besides, although in all studies only one grape species *Vitis vinifera* was used, its varieties (both with light and dark fruits), reared during a long time on different continents were different. Among these varieties there is grape cultivated in Europe [16, 62, 65-68, 71], North America [56], and Australia [64, 69]. At the same time, absolutely identical amino acid sequences are preserved in different varieties such as structures 18 and 19 obtained by different research groups from European [16, 68] and Australian [69] varieties. However, the reliability of identification of single amino acid residues in three studied oligopeptide structures can be doubtful.

In oligopeptide 24 from family I, an arginine residue is in position 18, where, in accordance with homology of

Some characteristics of precursors containing structures of grape regulatory oligopeptides

Cross reference	No.	Swiss-Prot accession number	Precursor source	Grape cultivar	Material used	Refe-rence
1	2	3	4	5	6	7
1	1	A7QBX4	Chromosome chrl scaffold_75, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
	2	A5BBI9	Putative uncharacterized protein	Piont Noir clone ENTAV 115	young shoot	[56]
	3	A7QR44	Chromosome undetermined scaffold_147, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
	4	A7QMI0	Chromosome chrl9 scaffold_126, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
	8	A5AHS9	Putative uncharacterized protein (Chromosome chr18 scaffold_l, whole genome shotgun sequence)	Pinot Noir 40024 genotype Piont Noir clone ENTAV 115	n.i. young shoot	[16] [56]
2	5	A7QX91	Chromosome undetermined scaffold_215, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
3	6	A7QVP7	Chromosome chrl4 scaffold_190, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
4	7	A7PTC9	Chromosome chr8 scaffold_29, whole genome shotgun sequen	Pinot Noir 40024 genotype	n.i.	[16]
5	9	A7R0S4	Chromosome undetermined scaffold_319, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
	10 10	Q9FS45	Chitinase (Chromosome undetermined scaffold_319, whole genome shotgun sequence), gene chit lb	Pinot Noir 40024 genotype Ugni Blanc	n.i. berry, leaf	[16] [62]
	12	A7R0S0	Chromosome undetermined scaffold_319, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
	11	A5AT01	Putative uncharacterized protein	Piont Noir clone ENTAV 115	young shoot	[56]
	13 13	Q9ZTK4	Class I extracellular chitinase, gene chit 1b	Sultana	leaf, berry	[64]
	13 13			Shiraz	leaf, berry	[64]
				Cabernet Sauvignon Semillon	leaf, berry	[64] [64]
	14 15	A3QRB6 A3QRB7	Chitinase class I basic	n.i.	n.i.	[65] [65]
	16	P51613	Basic endochitinase EC=3.2.1.14, gene chit 1b	Pinot Noir	leaf	[66]
	17	Q546P8	Chitinase class I (Putative uncharacterized protein)	Pinot Noir	leaf	[66]

Table (Contd.)

1	2	3	4	5	6	7
	17 17		(Chromosome chr3 scaffold_8, whole genome shotgun sequence), gene chit la	Ugni Blanc	berry, leaf	[62]
	17			Pinot Noir 40024 genotype	n.i.	[16]
	17			Piont Noir clone ENTAV 115	young shoot	[56]
				Cardinal	berry	[67]
	18	Q7XAU6	Class IV chitinase (Chromosome chr5 scaffold_72, whole genome shotgun sequence)	Pinot Noir 40024 genotype	n.i.	[16]
	18			Pinot Noir	berry	[68]
	19	024530	Class IV endochitinase, EC=3.2.1.14, gene VvChi4A	Shiraz	berry	[69]
	24	024531	Class IV endochitinase, EC=3.2.1.14, gene VvChi4A	Shiraz	berry	[69]
	26	Q7XB39	Class IV chitinase, gene Chi4C	n.i.	n.i.	[71]
	21	A5BSD1	Putative uncharacterized protein	Piont Noir clone ENTAV 115	young shoot	[56]
	28	A5BSD2				[56]
	31	A5BSD0				[56]
	34	A5C2S9				[56]
	20	A5AK36	Putative uncharacterized protein (Chromosome chr5 scaffold_72, whole genome shotgun sequence)	Pinot Noir 40024 genotype	n.i.	[16]
	20			Piont Noir clone ENTAV 115	young shoot	[56]
	25	A5AJB3	Putative uncharacterized protein (Chromosome chr5 scaffold_72, whole genome shotgun sequence)	Piont Noir clone ENTAV 115	young shoot	[56]
	25			n.i.	n.i.	[16]
	22	A7QAP2	Chromosome chr5 scaffold_72, whole genome shotgun sequence	Pinot Noir 40024 genotype	n.i.	[16]
	23	A7QAP8				[16]
	27	A7QAP5				[16]
	29	A7QAQ0				[16]
	30	A7QAQ2				[16]
	32	A7QAP0				[16]
	33	A7QAP6				[16]
	35	A7QAQ7				[161

Note: n.i., not indicated.

all the other disulfide-bonded structures, a cysteine residue should be present. Our analysis of the cDNA-corresponding sequence has shown that the arginyl nucleotide triplet, following the tyrosine residue triplet TAC, was read as CGT, whereas it can be one of the cysteine triplets TGT. It is possible that during the automatic analysis of sequence using built-in sequencer programs, C-base duplication occurred due to a signal ambiguity, and point C substitution for T caused arginyl triplet translation instead of the cysteine codon.

In oligopeptide 13 from family II, the *C*-terminus of the amino acid sequence revealed a cysteine residue in

I	18; 19	Q7XAU6; O24530	Class IV chitinase; Class IV endochitinase	AQNCGCASGLCCSKYGYCGTGSDYCGDGCQSGPCDS	100,0	[12, 64; 65]
	24	O24531	Class IV endochitinase	AQNCGCASGLCCSKYGYRGTGSDYCGDGCQSGPCDS	97,2	[65]
	20	A5AK36	Uncharacterized	AQNCGCASDQCCSKYGYCGTGNDYCGNTCQSGPCYS	83,3	[12, 52]
	35	A7QAQ7	Uncharacterized	AQNCGCASGLCCNKFGYCGTGNAYCGDGCQAGPCYS	83,3	[12]
	34	A5C2S9	Uncharacterized	AQNCGCAAGLCCSQYGYCGTGNDFCGTGCQAGPCYS	80,6	[52]
	21	A5BSD1	Uncharacterized	AQNCGCNAGLCCSQYGYCGTGNDYCGTGCQAGPCYS	80,6	[52]
	22	A7QAP2	Uncharacterized	AQNCGCDAGLCCSQYGYCGTGNDYCGTGCQAGPCYS	80,6	[12]
	25; 26	A5AJB3; Q7XB39	Uncharacterized; Class IV chitinase	AQDCGCSSDQCCSQWGYCGTGDDYCGTGCQSGPCTS	75,0	[52; 12; 67]
	27; 28	A7QAP5; A5BSD2	Uncharacterized; Uncharacterized	AQNCRCDAGLCCSQYGYCGTGNDFCGTGCQAGPCYS	75,0	[12; 52]
	29	A7QAQ0	Uncharacterized	SQDCGCGAGLCCSKFGYCGTGKEYCGTGCQAGPCDS	75,0	[12]
	23	A7QAP8	Uncharacterized	GQTCGCDASLCCSQYGYCGTGDDYCGTGCQAGPCYS	72,2	[12]
	30	A7QAQ2	Uncharacterized	AQNCGCSASVCCSQYGYCGTTKDYCGAGCLAGPCYS	69,4	[12]
	31; 32; 33	A5BSD0; A7QAP0/6	Uncharacterized; Uncharacterized; Uncharacterized	GQDCGCAADLCCSRWGYCGTGDDYCGTGCQEGPCNP	69,4	[52; 12; 12]
II	10; 11	Q9FS45; A5AT01	Chitinase; Uncharacterized	QCGRQAGGALCSGGLCCSQYGYCGSTSAYCSTGCQSQCPS	100,0	[12, 58; 52]
	13	Q9ZTK4	Class I extracellular chitinase	QCGRQAGGALCSGGLCCSQYGYCGSTSAYCSTGCQSQCPC	97,7	[60]
	12	A7R0S0	Uncharacterized	QCGSLAGGALCSGGLCCSQYGYCGSTPAYCSTGCQSQCTS	90,0	[12]
	9	A7R0S4	Uncharacterized	QCGRQASGKRCAGGLCCSQYGYCGSTRPYCGVGCQSQCR	75,0	[12]
	16; 17	P51613; Q546P8	Basic endochitinase; Chitinase class I	QCGGQAGGRVCPGGACCSKFGWCGNTADYCGSGCQSQCSS	67,5	[62; 12, 52, 58, 63]
	14; 15	A3QRB6; A3QRB7	Chitinase class I basic; Chitinase class I basic	QCGGQAGGRVCPGGACCSKFGRCGNTADYCGSGCQSQCSS	65,0	[61; 61]
III	6	A7QVP7	Uncharacterized	QQCGRQAGGRTCANNLCCSQYGYCGTTAEYCSPSQSCQSNCQSGG	100,0	[12]
IV	1	A7QBX4	Uncharacterized	RTCESQSHRFKGTCVRQSNCAAVCQTEGFHGGNCRGFRRRCFCTKHC	100,0	[12]
	2	A5BBI9	Uncharacterized	RVCESQSHKFEGACMGDHNCALVCRNEGFSGGKCKGXRRRCFCTKLC	68,1	[52]
	3	A7QR44	Uncharacterized	RVCESQSHKFEGACMGDHNCALVCRNEGFSGGKCKGLRRRCFCTKLC	68,1	[12]
	4	A7QMI0	Uncharacterized	RLCESQSHWFRGVCVSNHNCAVVCRNEHFVGGRCRGFRRRCFCTRNC	66,0	[12]
V	8	A5AHS9	Uncharacterized	KVCQRPSKTWSGFCGSSKNCDRQCKNWEGAKHGACHAKFPGVACFCYFNC	100,0	[12, 52]
VI	5	A7QX91	Uncharacterized	MIYDVNSPLFRSFLSQKGGSSDKRKTEEQKPKEQRPKASENKPVMTE	100,0	[12]
VII	7	A7PTC9	Uncharacterized	SEWQVVPTNDPEVQDAANHVVKSIQMRSNSIFRYELLEILLAKAKVI	100,0	[12]

Fig. 3. Structural families of putative grape regulatory oligopeptides. Family numbers are designated by Roman numerals, and numbers of oligopeptides are designated by Arabic numerals. Symbol ";" is used to note which literature sources correspond to identification numbers of protein sequences in the Swiss-Prot/TrEMBL database.

addition to the even number already present and occupying proper positions. In all the other oligopeptides of this family a serine residue is located in this position. As in the previous case, TCT (serine) triplet replacement by TGT (cysteine) could happen. This can also be confirmed by data showing that the noted residue is included in the region of the loop joint connecting chitin-binding (cysteine-rich) and catalytic domains of this oligopeptide precursor [62]. The presence in such mobile structural fragment of a cysteine residue, as a rule forming a bond with another analogous residue in the polypeptide structure, is unlikely.

Appeal to the cDNA sequence for oligopeptide 2 from family IV makes it possible to elucidate the significance of uncertainty of amino acid residue X in position 37. In the CYT triplet, encoding the abovementioned residue, the second base was not identified. The coincidence of all residues of oligopeptides 2 and 3, with the exclusion of one, points to the possibility of the unread triplet interpretation as CTT, i.e. as encoding leucine.

Factors responsible for substitutions in cDNA sequence can be both errors of RNA- and DNA-dependent polymerases during mRNA amplification and errors in signal interpretation during sequencing. The problem of possible erroneous substitutions during DNA sequencing presently attracts special attention, because a significant number of nucleotide sequences containing false point replacements accumulated in opened databases. Owing to this, methods are developed for identification of real mutations and their distinctions from the false ones [75, 76], the use of which will with time allow correction of most errors in nucleotide sequencing.

Of course, real existence in grape of predicted oligopeptide structures requires experimental confirmation. The final proof will be studying proteolysis of revealed oligopeptide precursors in living cells as well as direct isolation and sequencing of oligopeptides.

However, it is also possible to adduce at this stage additional arguments in favor of their natural existence apart from homology to structures of known oligopeptides and coincidence of the cysteine residue system. Thus, the *C*-termini of precursors of oligopeptides 1-4 from family IV and 8 from family V coincide with their homologs obtained from different plants, while in oligopeptide 5 from family VI and its precursor the first residues of the *N*-terminus coincide as well (Figs. 2 and 3). In all these cases in base sequences the codon of the last amino acid residue is followed by a stop-codon. Besides, pre- and post-oligopeptide regions of precursors of these and some other obtained grape structures are homologous to the same regions of precursors from other plants.

The beginning of the oligopeptide amino acid sequence, i.e. position of the *N*-terminal residue, can in some cases be substantiated. Its position is easy to estimate using present-day program software such as the popular program SignalP 3.0 [77]. As an example, Fig. 4 shows such estimation for oligopeptide 9 of family II, from which it follows that, most probably, the signal peptide cleavage occurs between residues Gln19 and Gln20. Thus, the remaining part of the amino acid sequence begins with the oligopeptide predicted by us.

It is shown in the same figure that cleavage of the precursor can also happen in other places but at lower

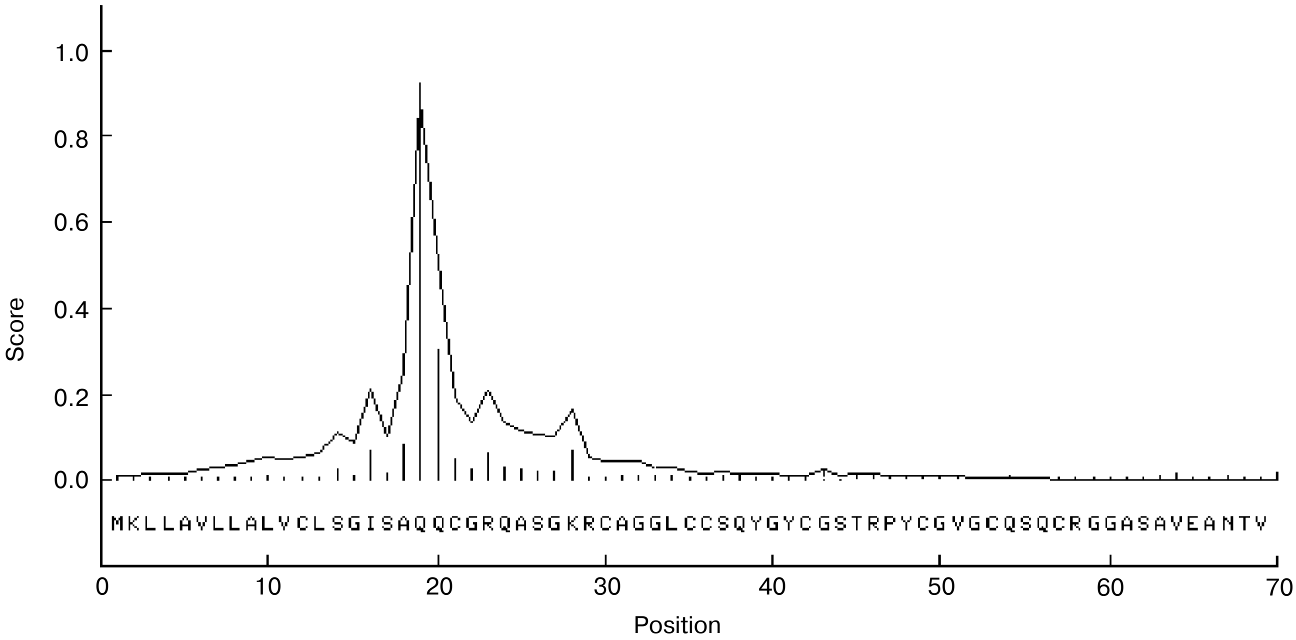

Fig. 4. Site of signal peptide cleavage in structure 9 (A7R0S4) obtained using the SignalP 3.0 program. The amino acid sequence of the initial part of the regulatory oligopeptide precursor is given below, while vertical lines point to the last possible residue of the signal peptide. Lengths of these lines characterize relative probability of the site of signal peptide cleavage.

probability, the highest of which is shown for the pair Gln20 and Cys21. Thus, formation of a shortened oligopeptide is also possible. Cases of existence of such oligopeptide multiforms cleaved from the same precursor are well known, including those in plants. For example, two substructures of trypsin inhibitor (seeds of melon *Cucumis melo*), shortened at *N*-terminus by 1 and 2 amino acid residues, were found [78]. Obviously, this type of structural polymorphism is also possible in grape oligopeptides.

Functional ambiguity of structurally homologous oligopeptides, shown in Fig. 1, should be also noted. Among structurally similar oligopeptides, along with antimicrobial agents, there are toxins, and inhibitors of enzymes, translation, and protein synthesis. However, only a single type of activity was studied for each of them. At the same time, numerous data show that many oligopeptides are polypotent. Thus, antimicrobial oligopeptides of the skin of the frog *Amolops loloensis* also have functions of toxins and hormones [79]. Obviously, functional properties of plants should be studied in different tests, although this is usually not done.

It should be noted in conclusion that in plants, including grape, only a small number of putative regulatory oligopeptides have been identified, and those belonging to yet unknown structural families can be among those still not revealed. In the huge number of uncharacterized grape amino acid sequences there can be regions consisting of oligopeptides now having no samples for comparison. Therefore, further accumulation of data concerning structure and functions of regulatory oligopeptides of various plants will make possible more complete description of functional abilities of still unstudied grape regulatory oligopeptides, and these data might be used in practice, in particular in the struggle against different diseases of this and other plants.

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REFERENCES

1. Zamyatnin, A. A. (1984) *Ann. Rev. Biophys. Bioeng.*, **13**, 145-165.
2. Zamyatnin, A. A. (1991) *Prot. Seq. Data Anal.*, **4**, 49-52.
3. Sewald, N., and Jakubkem, H.-D. (2002) *Peptides: Chemistry and Biology*, Wiley-VCH Verlag GmbH, Weinheim.
4. Zamyatnin, A. A. (2007) in *Peptides 2006* (Rolka, K., Rekowsky, P., and Silberring, J., eds.) Kenes International, Geneva, pp. 384-385.
5. Zamyatnin, A. A., Borchikov, A. S., Vladimirov, M. G., and Voronina, O. L. (2006) *Nucleic Acids Res.*, **34**, D261-D266.
6. Meyer, B., Houlne, G., Pozueta-Romero, J., Schantz, M. L., and Schantz, R. (1996) *Plant Physiol.*, **112**, 615-622.

7. Bloch, C., and Richardson, M. (1991) *FEBS Lett.*, **279**, 101-104.
8. Castro, M. S., Fontes, W., Morhy, L., and Bloch, C. (1996) *Prot. Pept. Lett.*, **3**, 267-274.
9. Mendez, E., Moreno, A., Colilla, F., Pelacez, F., Limas, G. G., Mendez, R., Soriano, F., Salinas, M., and de Haro, C. (1990) *Eur. J. Biochem.*, **194**, 533-539.
10. Colilla, F. J., Rocher, A., and Mendez, E. (1990) *FEBS Lett.*, **270**, 191-194.
11. Garcia-Olmedo, F., Rodriguez-Palenzuela, P., Molina, A., Alamillo, J. M., Lopez-Solanilla, E., Berrocal-Lobo, M., and Poza-Carrion, C. (2001) *FEBS Lett.*, **498**, 119-122.
12. Silversten, A., Moskal, W. A., Wu, H. C., Underwood, B. A., Graham, M. A., Town, C. D., and VandenBosch, K. A. (2007) *Plant J.*, **51**, 262-280.
13. De Beer, A., and Vivier, M. A. (2008) *BioMed. Central Plant. Biol.*, **8**, 1-16.
14. Ganz, T. (2003) *Nature Rev. Immunol.*, **3**, 710-720.
15. Tavares, L. S., Santos, M. O., Viccini, L. F., Moreira, J. S., Miller, R. N. G., and Franco, O. L. (2008) *Peptides*, **29**, 1842-1851.
16. Jaillon, O., Aury, J. M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., et al. (2007) *Nature*, **449**, 463-467.
17. Schafleitner, R., and Wilhelm, E. (2001) *Submitted to the GenBank database*, Access Number: AAL15885.1.
18. Bassett, C. L., Wisniewski, M. W., Artlip, T. S., and Webb, R. P. (2002) *Submitted to the GenBank database*, Access Number: AAL85480.1.
19. Yu, D. Y., Quigley, F., and Mache, R. (1992) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Number: Q39182.
20. Karunanandaa, B., Singh, A., and Kao, T. H. (1994) *Plant Mol. Biol.*, **26**, 459-464.
21. Takemoto, D., and Kawakita, K. (1999) *Submitted to the GenBank database*, Access Number: BAA95697.1.
22. Do, Y. M., Lee, S. C., Jung, H. W., Sohn, K. H., and Hwang, B. K. (2004) *Plant Sci.*, **169**, 1297-1305.
23. Tregear, J. W., Morcillo, F., Richaud, F., Berger, A., Singh, R., Cheah, S. C., Hartmann, C., Rival, A., and Duval, Y. (2002) *J. Exp. Bot.*, **53**, 1387-1396.
24. Meyer, B., Houlne, G., Pozueta-Romero, J., Schantz, M. L., and Schantz, R. (1996) *Plant Physiol.*, **112**, 615-622.
25. Van den Heuvel, K. J. P. T., Hulznik, J. M. R., Barendze, G. W. M., and Wullems, G. J. (2001) *J. Exp. Bot.*, **52**, 1427-1436.
26. Stiekema, W. J., Heidekamp, F., Dirske, W. G., van Beckum, J., de Haan, P., ten Bosch, C., and Louwerse, J. D. (1988) *Plant Mol. Biol.*, **11**, 255-269.
27. Moreno, M., Segura, A., and Garcia-Olmedo, F. (1994) *Eur. J. Biochem.*, **223**, 135-139.
28. Roda, A., and Baldwin, I. T. (2003) *Submitted to the GenBank database*, Access Number: AAS13434.1.
29. Urdargarin, M. C., Norero, N., Broekaert, W. F., and de la Canal, L. (2000) *Plant Physiol. Biochem.*, **38**, 253-258.
30. Weterings, K., Apuya, N. R., Fischer, R. L., Harada, J. J., and Goldberg, R. B. (2000) *Submitted to the GenBank database*, Access Number: AAG17880.1.
31. Yu, D. Y., Quigley, F., and Mache, R. (1992) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Number: Q9ZUL7.
32. Odintsova, T., Rogozhin, E. A., Baranov, Y., Musolyamov, A. K., Yalpani, N., Egorov, T. A., and Grishin, E. V. (2008) *Biochimie*, **90**, 1667-1673.
33. Huang, D. J., and Lin, Y. H. (2004) *Submitted to the GenBank database*, Access Number: AAS65426.1.
34. Whittall, J. B., Medina-Marino, A., Zimmer, E. A., and Hodges, S. A. (2006) *Mol. Phylogenet. Evol.*, **39**, 124-134.
35. Melo, F. R., Rigden, D. J., Franco, O. L., Mello, L. V., Ary, M. B., Grossi de Sa, M. F., and Bloch, C., Jr. (2002) *Proteins*, **48**, 311-319.
36. Yu, D. Y., Quigley, F., and Mache, R. (1992) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Number: Q41914.
37. Odintsova, T. I., Egorov, T. A., Musolyamov, A. Kh., Odintsova, M. S., Pukhalsky, V. A., and Grishin, E. V. (2007) *Biochimie*, **89**, 605-612.
38. Choi, Y., Choi, Y. D., and Lee, J. S. (1993) *Plant Physiol.*, **101**, 699-700.
39. Koike, M., Okamoto, T., Tsuda, S., and Imai, R. (2002) *Biochem. Biophys. Res. Commun.*, **298**, 46-53.
40. Mendez, E., Moreno, A., Colilla, F., Pelacez, F., Limas, G. G., Mendez, R., Soriano, F., Salinas, M., and de Haro, C. (1990) *Eur. J. Biochem.*, **194**, 533-539.
41. Colilla, F. J., Rocher, A., and Mendez, E. (1990) *FEBS Lett.*, **270**, 191-194.
42. Bloch, C., and Richardson, M. (1991) *FEBS Lett.*, **279**, 101-104.
43. Yu, D. Y., Quigley, F., and Mache, R. (1992) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Number: Q9ZUL8.
44. Nitti, G., Orru, S., Bloch, C., Morhy, L., Marino, G., and Pucci, P. (1995) *Eur. J. Biochem.*, **228**, 250-256.
45. Castro, M. S., Fontes, W., Morhy, L., and Bloch, C. (1996) *Prot. Pept. Lett.*, **3**, 267-274.
46. Sharma, P., and Lonneborg, A. (1996) *Plant Mol. Biol.*, **31**, 707-712.
47. Almeida, M. S., Cabral, K. M., Zingali, R. B., and Kurtenbach, E. (2000) *Arch. Biochem. Biophys.*, **378**, 278-286.
48. Finkina, E. I., Shramova, E. I., Tagaev, A. A., and Ovchinnikova, T. V. (2008) *Biochem. Biophys. Res. Commun.*, **371**, 860-865.
49. Zhang, Y., and Lewis, K. (1997) *FEMS Microbiol. Lett.*, **149**, 59-64.
50. Kragh, K. M., Nielsen, J. E., Nielsen, K. K., Dreboldt, S., and Mikkelsen, J. D. (1995) *Mol. Plant-Microbe Interact.*, **8**, 424-434.
51. Lay, F. T., Brugliera, F., and Anderson, M. A. (2003) *Plant Physiol.*, **131**, 1283-1293.
52. Gu, Q., Kawata, E. E., Morse, M.-J., Wu, H.-M., and Cheung, A. Y. (1992) *Mol. Gen. Genet.*, **234**, 89-96.
53. Milligan, S. B., and Gasser, C. S. (1995) *Plant Mol. Biol.*, **28**, 691-711.
54. Segura, A., Moreno, M., Molina, A., and Garcia-Olmedo, F. (1998) *FEBS Lett.*, **435**, 159-162.
55. Chen, J.-J., Chen, G.-H., Hsu, H.-C., Li, S.-S., and Chen, C.-S. (2003) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Number: Q6T418.
56. Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D. A., Cestaro, A., Pruss, D., Pindo, M., FitzGerald, L. M., et al. (2007) *PLoS ONE*, **12**, e1326-e1326.

57. Sheng, J., and Mehdy, M. C. (1993) *Plant Physiol.*, **101**, 1409-1409.
58. Soedjannatmadja, U. M., Hofstenge, J., Jeronimus-Stratingh, C. M., Bruins, A. P., and Beintema, J. J. (1994) *Biochim. Biophys. Acta*, **1209**, 144-148.
59. Sasaki, T., Matsumoto, T., Yamamoto, K., Sakata, K., Baba, T., Katayose, Y., Wu, J., Niimura, Y., Cheng, Z., Nagamura, Y., Antonio, B. A., Kanamori, H., Hosokawa, S., Masukawa, M., et al. (2002) *Nature*, **420**, 312-316.
60. Osborn, R. W., de Samblanx, G. W., Thevissen, K., Goderis, I., Torrekens, S., van Leuven, F., Attenborough, S., Rees, S. B., and Broekaert, W. F. (1995) *FEBS Lett.*, **368**, 257-262.
61. Koo, J. C., Lee, S. Y., Chun, H. J., Cheong, Y. H., Choi, J. S., Kawataba, S.-I., Miyagi, M., Tsunasawa, S., Ha, K. S., Bae, D. W., Han, C.-D., Lee, B. L., and Cho, N. J. (1998) *Biochim. Biophys. Acta*, **1382**, 80-90.
62. Robert, N., Roche, K., Lebeau, Y., Breda, C., Boulay, M., Esnault, R., and Buffard, D. (2002) *Plant Sci.*, **162**, 389-400.
63. Koo, J. C., Chun, H. J., Park, H. C., Kim, M. C., Koo, Y. D., Koo, S. C., Ok, H. M., Park, S. J., Lee, S. H., Yun, D. J., Lim, C. O., Bahk, J. D., Lee, S. Y., and Cho, M. J. (2002) *Plant Mol. Biol.*, **50**, 441-452.
64. Jacobs, A. K., Dry, I. B., and Robinson, S. P. (1999) *Plant Pathol.*, **48**, 325-336.
65. Cardoso, H., Balde, A., and Pais, M. (2006) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Numbers: A3QRB6, A3QRB7.
66. Busam, G., Kassemeyer, H. H., and Matern, U. (1997) *Plant Physiol.*, **115**, 1029-1038.
67. Romero, I., Sanchez-Ballesta, M. T., Maldonado, R., Escribano, M. I., and Merodio, C. (2006) *Postharvest. Biol. Technol.*, **41**, 9-15.
68. Manteau, S., Bezier, A., Mazeyrat-Gourbeyre, F., Lambert, B., Legendre L., and Baillieul, F. (2002) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Number: Q7XAU6.
69. Robinson, S. P., Jacobs, A. K., and Dry, I. B. (1997) *Plant Physiol.*, **114**, 771-778.
70. Li, S.-S., and Claeson, P. (2003) *Phytochemistry*, **63**, 249-255.
71. Bezier, A., Lambert, B., and Baillieul, F. (2002) *Submitted to the EMBL/GenBank/DDBJ databases*, Access Number: Q7XB39.
72. Dimarchi, R. D., Tam, J. P., Kent, S. B. H., and Merrifield, R. B. (1982) *Int. J. Pept. Prot. Res.*, **19**, 88-93.
73. Abracham, G. A., and Podell, D. N. (1984) *Mol. Cell. Biochem.*, **38**, 181-190.
74. Simonsen, S. M., Sando, L., Ireland, D. C., Colgrave, M. L., Bharathi, R., Goransson, U., and Craik, D. J. (2005) *Plant Cell*, **17**, 3176-3189.
75. Gilks, W. R., Audit, B., de Angelis, D., Tsoka, S., and Ouzounis, Ch. (2002) *Bioinformatics*, **18**, 1641-1649.
76. Gajer, P., Schatz, M., and Salzberg, S. L. (2004) *Nucleic Acids Res.*, **32**, 562-569.
77. Bendtsen, J. D., Nielsen, H., von Heijne, G., and Brunak, S. (2004) *J. Mol. Biol.*, **340**, 783-795.
78. Nishino, J., Takano, R., Kamei-Hayashi, K., Minakata, H., Nomoto, K., and Hara, S. (1992) *Biosci. Biotechnol. Biochem.*, **56**, 1241-1246.
79. Lu, Y., Li, J., Yu, H., Xu, X., Liang, J., Tian, Y., Ma, D., Lin, G., Huang, G., and Lai, R. (2006) *Peptides*, **27**, 3085-3091.