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Working Group on Chitinases

Plant Chitinase Genes

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The plant endochitinases (chitinases, E.C. 3.2.1.14) are a diverse group of enzymes that differ in primary structure, isoelectric point and cellular localization (Collinge et al., 1993; Meins et al., 1992; Mikkelsen et al., 1992; Stinzi et al., 1993). Chitinases have been grouped into three classes (I-III) (Shinshi et al., 1990) on the basis of amino-acid sequence similarity to the tobacco (*Nicotiana tabacum*) enzymes. More recently, three additional classes of chitinases have been identified: class IV (Collinge et al., 1993; Margis-Pinheiro et al., 1991), class V represented by the precursor of stinging nettle (*Urtica dioica*) lectin (Lerner & Raikhel, 1992), and class VI represented by tobacco chitinases showing amino-acid sequence homology to bacterial exochitinases (Melchers et al., 1994). Most chitinase genes have been identified and classified by sequence similarity to genes described earlier. Few have been unequivocally identified by demonstrating that the proteins encoded have chitinase activity.

We propose classifying chitinase genes into families based on the nomenclature for the proteins. New chitinase genes would be assigned to existing families or new families on the basis of sequence similarity. When possible, tobacco was chosen as a "standard" species because of

the abundance of information at the protein and DNA level available. The scheme proposed retains the nomenclature for chitinase classes already used widely, provides simple criteria for naming new genes, and is sufficiently flexible to add new structural classes without reevaluation of the entire classification system.

Guidelines

Chitinase classes I-III

The families *Chi1* to *Chi3* correspond to chitinase classes I-III (Meins et al., 1992) and are defined as:

Chi1. Amino acid sequence >50% identical to tobacco class I chitinase, which contains a conserved N-terminal, cysteine-rich lectin domain also found in cereal lectins (Raikhel & Lee, 1993).

Chi2. Amino acid sequence >50% identical to tobacco class II chitinase; amino acid sequence >50% identical to tobacco class I chitinase, but lacking the lectin domain of the class I enzymes.

Chi3. Amino acid sequence >30% identical to tobacco class III chitinase/lysozyme (Lawton et al., 1992) with no sequence similarity to the class I and II tobacco enzymes.

Class IV chitinases

The family *Chi4* corresponds to class IV chitinase (Mikkelsen et al., 1992; Collinge et al., 1993) with an amino acid sequence >50% identical to the *Phaseolus vulgaris* PR 4 chitinase (Margis-Pinheiro et al., 1991). This family differs from the *Chi1* family in having a deletion in the lectin domain, several deletions of approximately 22 amino acids in the catalytic domain, and a truncated C-terminal end.

Class V chitinases

The family *Chi5* corresponds to class V chitinase with an amino acid sequence >50% identical to the stinging nettle (*Urtica dioica*) lectin precursor and having a duplicated N-terminal lectin domain (Lerner & Raikhel, 1992).

Class VI chitinase

The family *Chi6* corresponds to class VI chitinase with an amino acid sequence >50% identical to the tobacco endochitinases showing signifi-

cant amino-acid sequence similarity to the bacterial exo-chitinases from *Bacillus circulans*, *Serratia marcescens*, and *Streptomyces plicatus*, but no sequence similarity to the class I-V proteins (Melchers et al., 1994).

New families of chitinases

Newly reported genes are assigned to existing and new families using amino acid sequence similarity of 50% as a criterion. New families are numbered consecutively in order of discovery. By analogy to the *Chi1* and *Chi2* families, groups of genes with similar sequences but differing in the presence or absence of a conserved lectin domain would be classified into different families.

Chitinases of uncertain affiliation

Chitinase genes that cannot be assigned unambiguously to one family would be designated *Chi0* genes, *e.g.*, the *Arachis* chitinases (Herget et al., 1990).

Comments and Examples

Representative chitinases with a consensus sequence (viz. *Chi1*, *Chi2*, *Chi4* and *Chi5*) are shown in Fig. 1. The recommended names and classification of chitinase genes in individual plants are shown in the print-out of *Mendel* beginning on p. 89.

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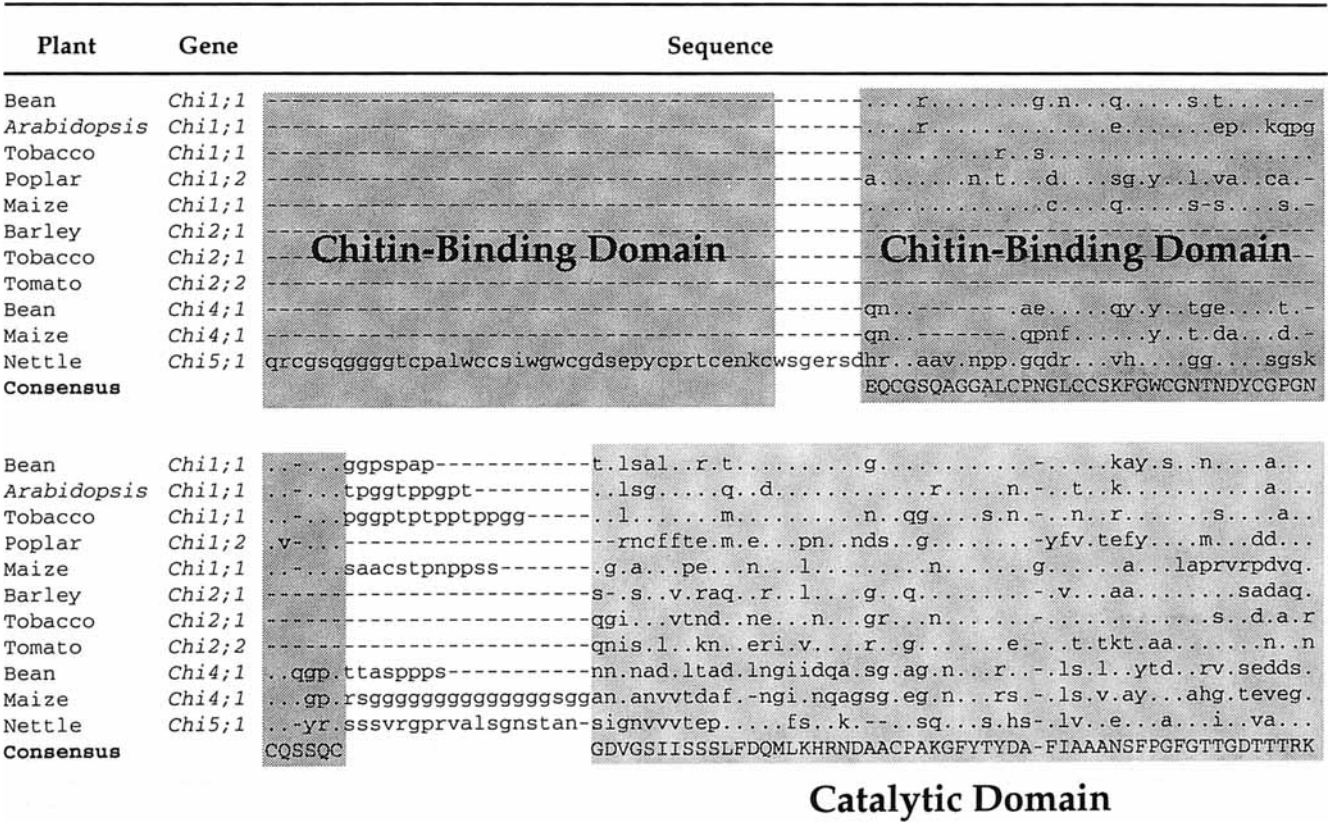
Plant
Gene
Sequence
Bean Chi1;1
Arabidopsis Chi1;1
Tobacco Chi1;1
Poplar Chi1;2
Maize Chi1;1
Barley Chi2;1
Tobacco Chi2;1
Tomato Chi2;2
Bean Chi4;1
Maize Chi4;1
Nettle Chi5;1
Consensus
Chitin-Binding Domain
Chitin-Binding Domain
EQCGSQAGGALCPNGLCCSKFGWCGNTNDYCGPGN
Catalytic Domain

Plant	Gene	Sequence
Bean	*Chi1;1*	......lg.....v..rnp---.t..sa--tp.f.....qq.....i.....qc....
Arabidopsis	*Chi1;1*	k.v....g.....s.....q..npa--....e.--.at....s..r.....m.....lc....
Tobacco	*Chi1;1*	.....wl....s.--g...t.--.g.....r..f.....i.h.....c....
Poplar	*Chi1;2*	..l.....q..s.rsiige.a.ft....lvn.lnpn--.....ktk.sy..va--d.....l..r.....lc.ddl
Maize	*Chi1;1*	..l...l.....r-r.....e....a.gp...e.--.a.....a.....pra..i.i.i.i.....ps
Barley	*Chi2;1*	..v...l.....af.....q.r.as--....t.--.a.....r.....h.....
Tobacco	*Chi2;1*	k.....g.....sls.--e.ftg....v.qndq----------sdr.....tnqn..ek..n..
Tomato	*Chi2;2*	k.....l.....s....yn....s.--g...as--.q.....f.....i.y.....a..s..
Bean	*Chi4;1*	.....a..hft...----------ghf.yie.id.a..-....ee.ia.y..sss.g.h.....f.....s.n
Maize	*Chi4;1*	.....hvt...----------ghf.yis.in--ksna...a-.nr.....a.q.....l.i.....d.
Nettle	*Chi5;1*	..v...l.hi.qa.s.ersdven-.h...l.hintttvten-.f.t---..d....a....sp.....th.f...l..q..
Consensus		REIAAFFAQTSHETTGGWATAPDGPYAWGYCFKREQGGPSKSDYCDP-SSSQWPCAPGKKYYGRGPIQLSWNYNYGPAGRAI

Catalytic Domain

Bean	*Chi1;1*	......k.....s.....s.....a....-..s.....s.....s..v..r.l..y.tv.....r.q.....
Arabidopsis	*Chi1;1*	.....n.a..a..a.i.....a.p..-....a..a.q.q..d.....l..y.....r.q.g..a
Tobacco	*Chi1;1*	.....s.....-.....i...q...g....n.l.....r.t.....
Poplar	*Chi1;2*	klp..qe.ek.....l..ea.....n.h.tga....e....e.s..e..ie...k....ml....tn.g..tkdgkt.q.
Maize	*Chi1;1*	apgi.a.....r.hrvvrrpsgsg.....-...t..m..q.....t....l..y..v...s.....a....a
Barley	*Chi2;1*	.....a.....atvg....i.....a.p..-..s.a..a.q.s..g.....i.....q....a
Tobacco	*Chi2;1*	rq..v.....at.....i.....dn.-..s....i.s.....qs.n.a..c.....i...v.pnaa.e
Tomato	*Chi2;2*	..n.....n.a.v.....a.q..-..a.....s..v..s.p.....m..ns.snalmd
Bean	*Chi4;1*	nf.g.ga.et.sn.v.v.....y..qhvr.----------vinq...atira...a...dganptt..
Maize	*Chi4;1*	.fng.ad.nr..q.a..a.....nnvhg----------vmpq...atira...a...ngnnpaqmn
Nettle	*Chi5;1*	.e..iq.....ek..i.....sqhdn.-.....ivln------.ns..n.i.nk...g...srafghddfavrss-
Consensus		GVDLLNNPDLVATDPVISFKTALWFWMTPQSPK-PSCHDVITGRWTPSAADRAAGRVPGFGVITNIINGGLECGHG-DSRVQ

Catalytic Domain

Plant	Gene	Sequence
Bean	*Chi1;1*	.....fk....l...gy.n.....s.t...nslllsdlvtsq*
Arabidopsis	*Chi1;1*	......q...n.f..n..g.......s.vnglleaai*
Tobacco	*Chi1;1*	.........s..........g...s..ngllvdtm*
Poplar	*Chi1;2*	n..dy.l....m.q.d.....y.d..et.edngllkmvgtm*
Maize	*Chi1;1*	......k....l....y.....a..t.n.*
Barley	*Chi2;1*	......k.......gy.n.....s....a*
Tobacco	*Chi2;1*	....y.....gm.n.a...........n.aqg*
Tomato	*Chi2;2*	n.........q....d..n....a......*
Bean	*Chi4;1*	a.vny.te..rq...at....t.*
Maize	*Chi4;1*	a.v.y.kq..qq.r.d..p..i.*
Nettle	*Chi5;1*	-s....k....m.....y.hd.kywfdntpssefqriqmrvaa*
Consensus		DRIGFYRRYCDILGVSPGDNLDCYNQRPFG

Catalytic Domain

Fig. 1. Examples of amino acid sequences of chitinase gene families with a consensus sequence. Individual genes are identified by their mnemonic and member number separated by ";". References: Bean *Chi1;1*, (Broglie et al., 1986); *Arabidopsis Chi1;1* (Samac et al., 1990); tobacco *Chi1;1* (Shinshi et al., 1987); poplar *Chi1;2* (Parsons et al., 1989); maize *Chi1;1* (A.L. Kriz & S. Wu, unpublished); barley *Chi2;1* (Leah et al., 1991); tobacco *Chi2;1* (Linthorst et al., 1990); tomato *Chi2;2* (Danhash et al., unpublished; bean *Chi4;1* (Margis-Pinheiro et al., 1991); maize *Chi4;1* (Huynh et al., 1992); nettle *Chi5;1* (Lerner & Raikhel, 1992).

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