INSECT RESISTANT TR PLANTS

BTC704

Advances in Transgenic Plant Utilization in Agriculture

Prof. ÖKTEM

İLAÇLAMA MASRAFLARI

BİTKİ	İNSEKTİSİT TUTARI (Milyar $)
MEYVE-SEBZE	2,465
PAMUK	1,870
ÇELTİK (PİRİNÇ)	1,190
MISIR	620
DİĞER	1,1965
TOPLAM	8,110

İLAÇLAMAYA RAĞMEN OLUŞAN KAYIPLAR

BİTKİ	KAYIP (Milyar $)	KAYIP (%)
ÇELTİK	45,000	27
MEYVE	20,000	6
SEBZE	25,000	9
MISIR	8,000	12
TOPLAM	98,000	

Insecticides

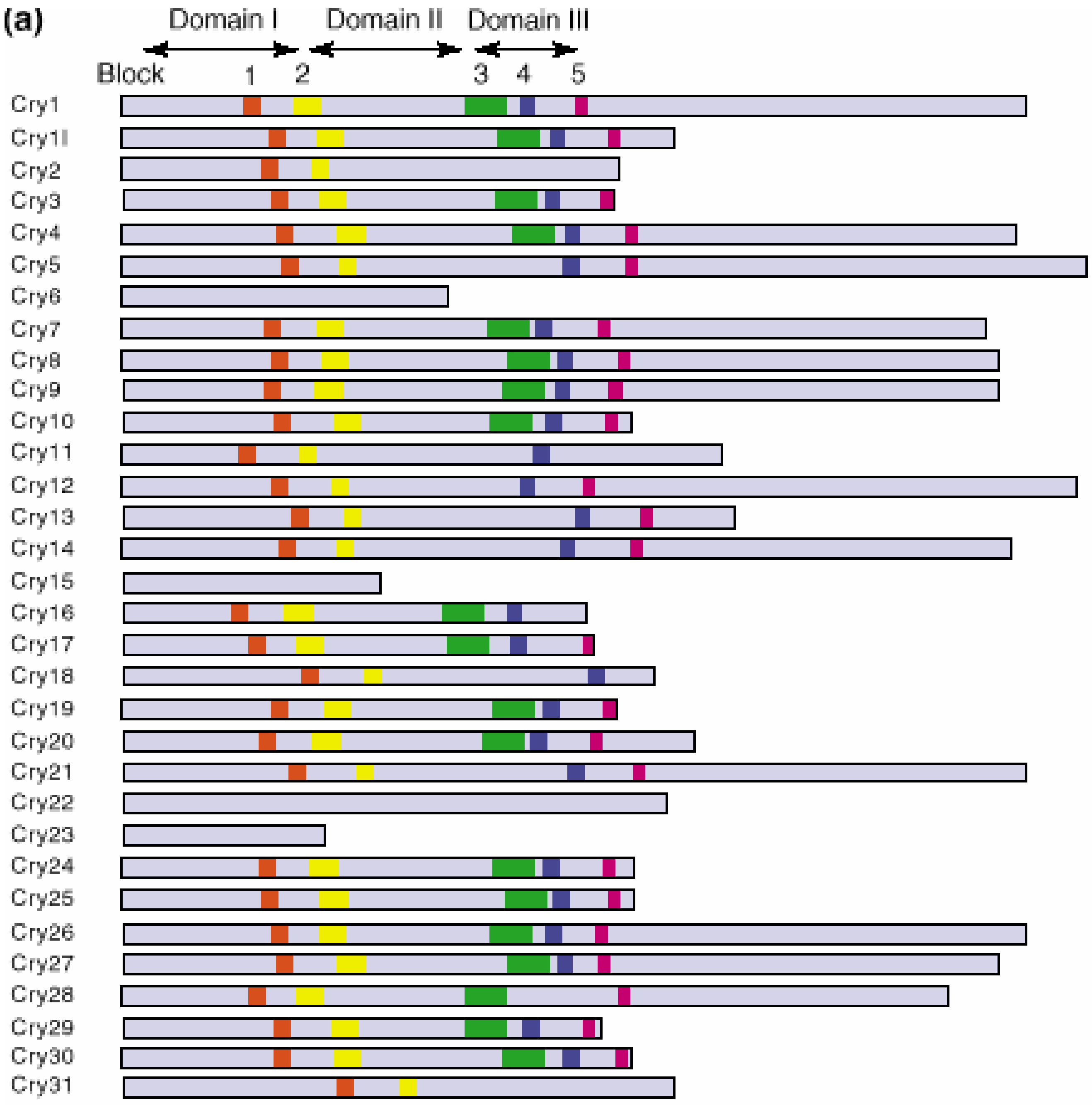
Active ingredient	Acres treated		Lbs AI	
	#	%	Per acre	Total
Aldicarb	102	8	2,79	284
Azinphos- methyl	100	8	0,52	52
Carbaryl	40	3	1,06	42
Carbofuran	248	20	0,93	230
Diazinon	12	1	1,53	18
Dimethoate	344	28	0,74	254
Disulfoton	25	2	2,44	61
Endosufan	196	16	0,96	188
Esfenvalerate	141	11	0,04	6
Ethoprop	26	2	4,86	126
Imidacloprid	275	22	0,16	44
Methamidophos	370	30	1,20	445
Methyl Parathion	18	1	1,42	25
Oxamyl	6	1	0,82	4
Permethrin	88	7	0,12	10
Phorate	242	20	2,66	644
Propargite	51	4	1,92	98
				2531

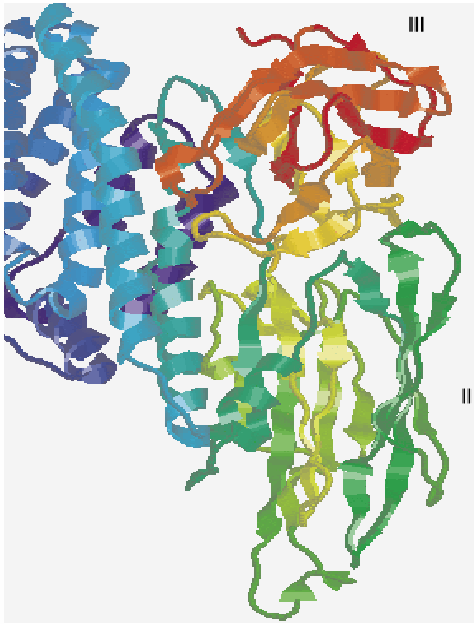
INSECT RESISTANCE STRATEGIES: Overwiev

- *Bacillus thuringiensis* δ-Endotoxins
- Chitinase
- Lectins
- Proteinase inhibitors
- α-Amylase Inhibitors
- VIP Protiens from Bacillus cereus & thuriengis
- Cholesterol oxidase
- Avidin-streptavidin

Bacillus thuringiensis δ-Endotoxins

- Gram pozitif bir bakteri olan *Bacillus thuringiensis* (Bt) ilk olarak 1902 yılında Japonya'da keşfedilmiştir. Daha sonra 1911'de bir un güvesi popülasyonundan, Berlier tarafından Almanya Thüringen'de yeniden izole ve karakterize edilmiştir. Bu bakteriler sporülasyon esnasında insektisidal etki gösteren bazı yapılar oluşturmaktadırlar. Bu kristalize yapılar delta-endotoksin olarak adlandırılan pro-toksinlerden oluşmaktadırlar. Bt suşları sınırlı konukçu profili olan farklı kristalize (Cry) proteinler üretmektedirler.
- Günümüzde farklı Bt izolatlarından 130'un üzerinde Bt endotoksin geni izole edilmiş ve sekanslanmıştır (Höfte ve Whiteley, 1989; Crickmore *ve ark*., 1998). Ayrıca, Bt toksinin bilimsel adlandırılması için bazı web adreslerinden de bilgi elde edilebilir (örnek: *http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/ index.html*). Bt endotoksin proteinleri yapısal özellikleri ve konukçu profillerine göre Cry-I, Cry-II, Cry-III ve Cry-IV olmak üzere dört farklı grupta kategorize edilmektedirler. Bunlardan **Cry-I toksinleri Lepidoptera** üzerinde etki gösterebilirken, **Cry-III toksinleri Koleoptera Cry-IV ise Diptera** üzerinde etkili olabilmektedir. **Cry-II toksinlerinin ise hem Lepidoptera hem de Diptera** üzerinde etkin oldukları bilinmektedir.
- Bt delta-endotoksinleri, pro-toksin halinde 130 kDa büyüklüğündeki proteinlerden oluşmaktadır. Toksinler, böcekler tarafından alındığında, orta bağırsak (midgut) bölgesinde çözülmekte ve proteolitik aktivite sonucu 65-70 kDa büyüklüğündeki aktif formlarına dönüşmektedirler


(a)
Domain I
Domain II
Domain III
Block
1
2
3
4
5
Cry1
Cry1I
Cry2
Cry3
Cry4
Cry5
Cry6
Cry7
Cry8
Cry9
Cry10
Cry11
Cry12
Cry13
Cry14
Cry15
Cry16
Cry17
Cry18
Cry19
Cry20
Cry21
Cry22
Cry23
Cry24
Cry25
Cry26
Cry27
Cry28
Cry29
Cry30
Cry31


III
II

What happens when ingested by insect?

Solubilization & Activation

may depend on high pH of insect midgut ~10-11
inactive protoxins are proteolytically cleaved

Binding to Midgut Receptors

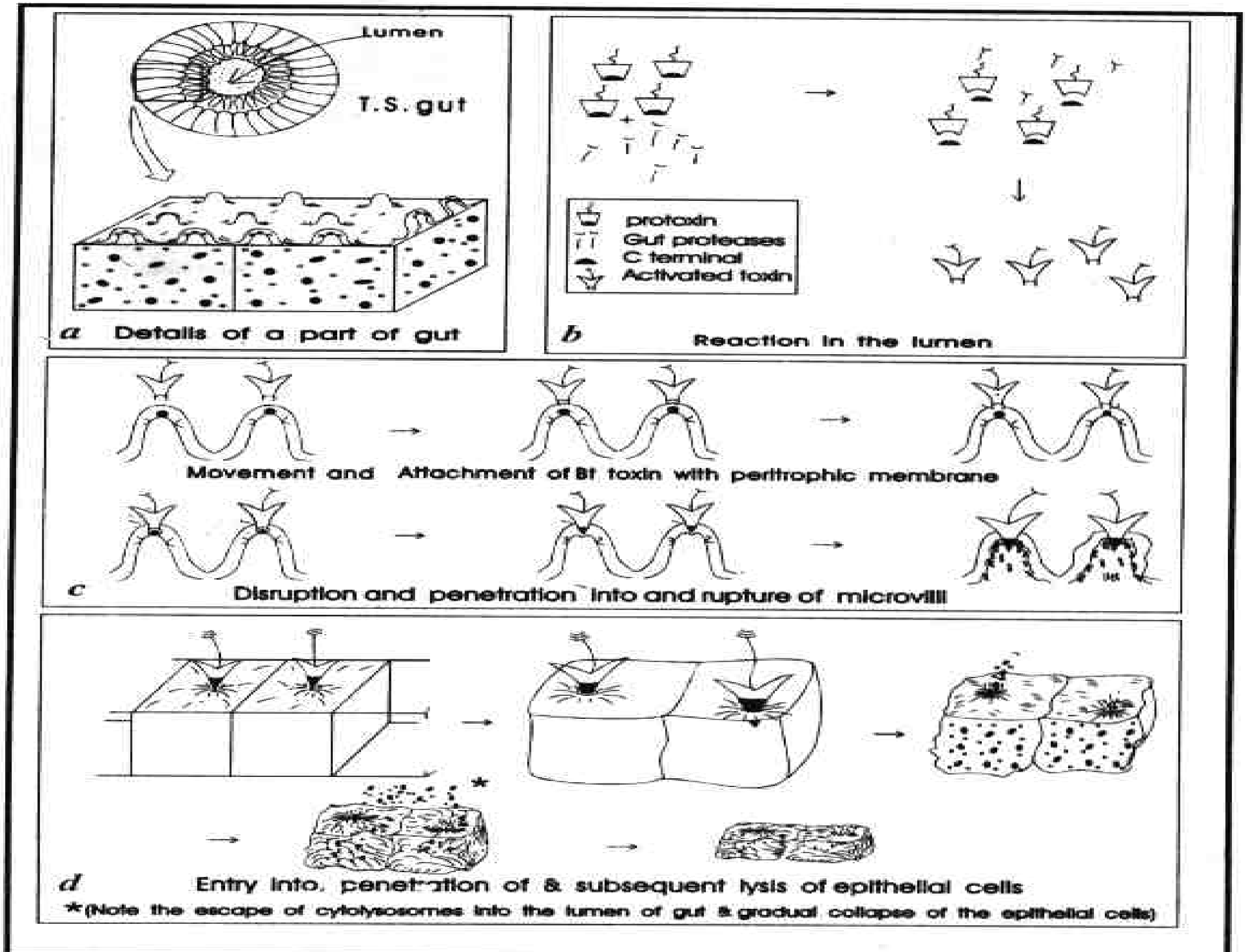
specificity to Cry & insect type

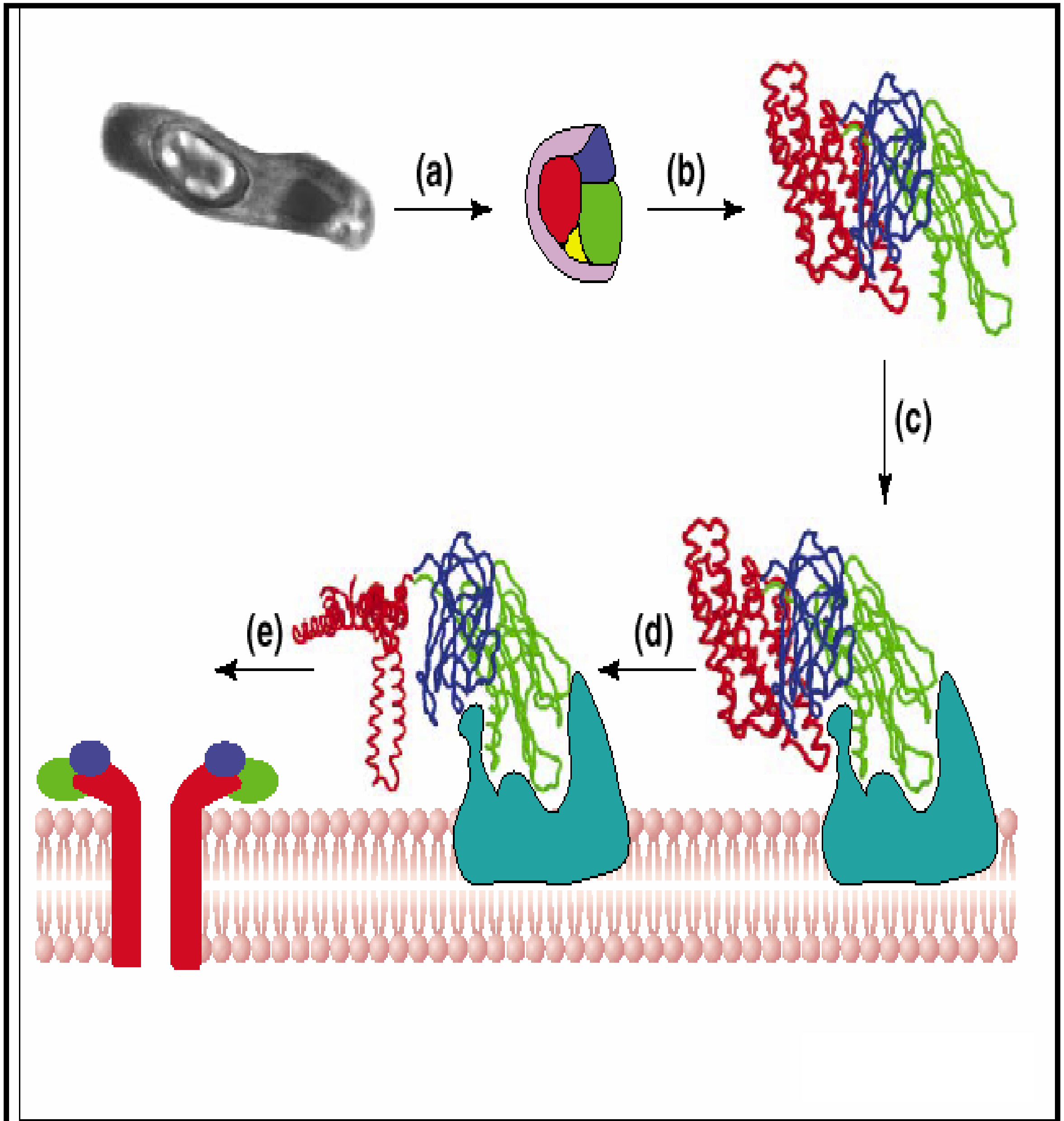
Formation of Pore

leading to disruption of electrical, K^+ and pH gradients

Death from starvation

Schematic presentation of the mode of Bt toxin action on the microvilli of insect leading to its death



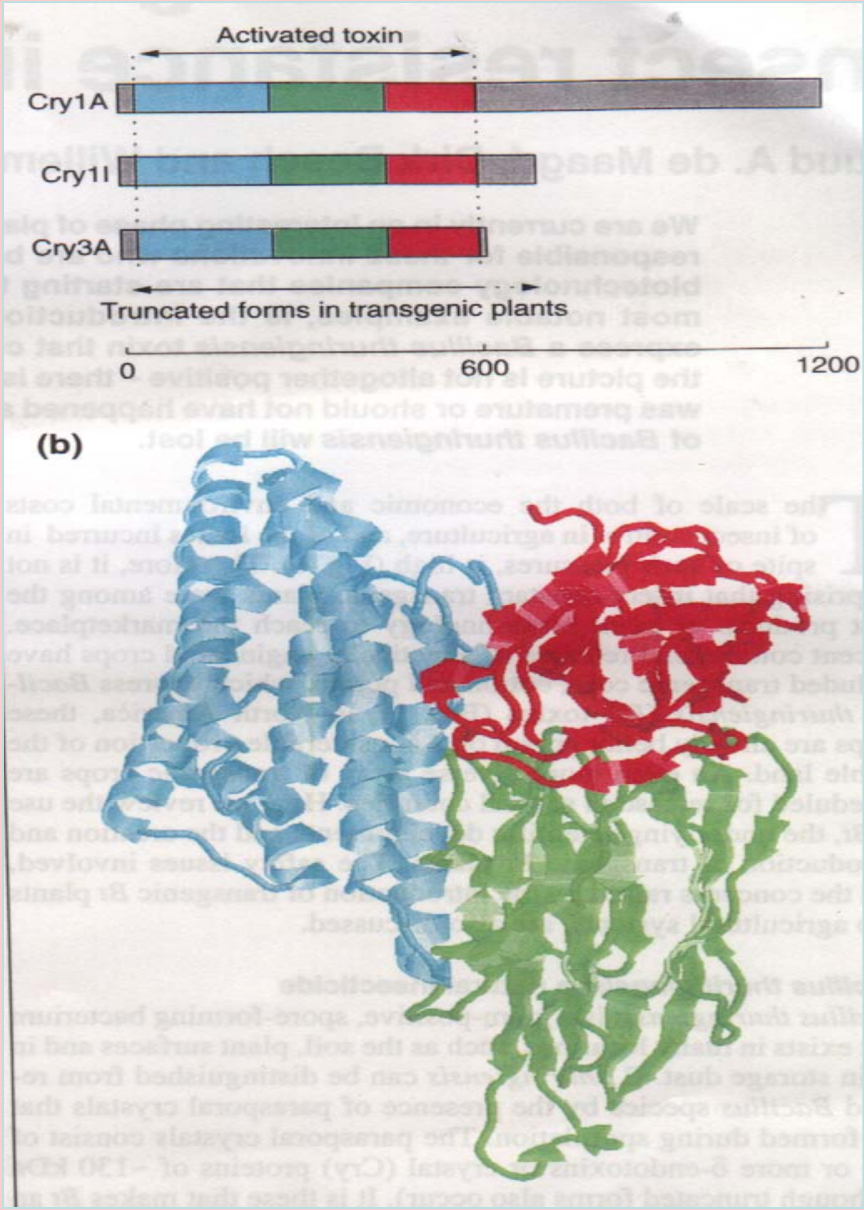


ACTION MECHANISM

a) The bacterium produces the toxin
b) The inactive toxin in crystal form attains the active form inside the insect gut
c) It binds to a receptor on the gut cell membrane
d) The toxin undergoes structural rearrangement
e) It is inserted into the membrane as a pore

The gut epithelium cells burst and the insect dies...

Structure of *Bacillus thuringiensis* delta-endotoxsins (cry proteins)

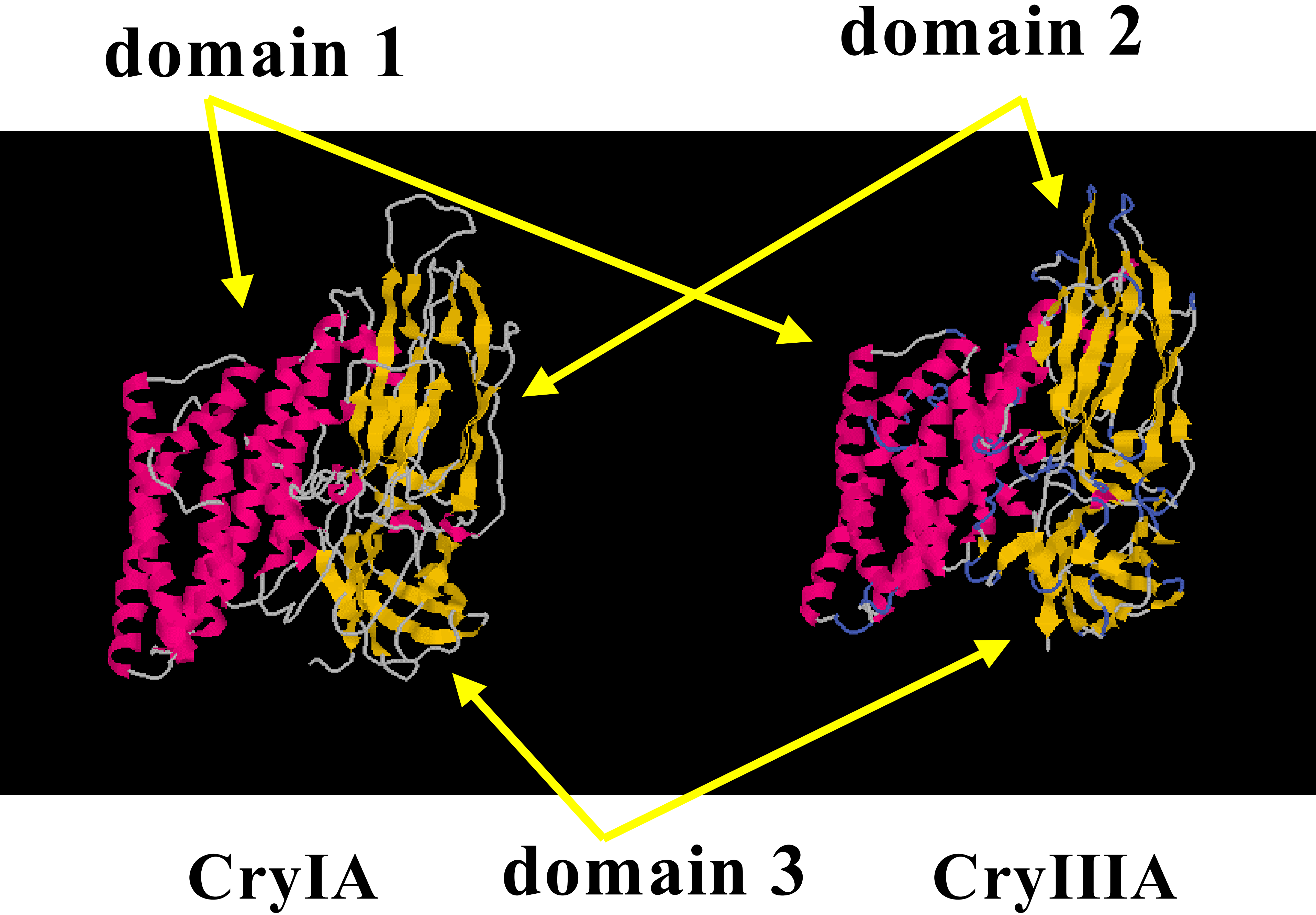


3D structure of Bt-delta endotoxins (Cry1Aa).

Trimed regions (grey) by insect gut proteases.

Domain 1 (blue): membrane insertion and pore formation;

Domain 2 (green) and Domain 3 (red): Receptor recognition and binding.


domain 1
domain 2
domain 3
CryIA
CryIIIA

Bacillus thuringiensis δ-Endotoxins

Most widely used, effective at 50-500 ng/ml

cry1Ab, cry1Ac, cry3A

cotton, maize, rice, potato, tomato, chickpea, peanut, conifer ....1994- (TABLE-LINK)

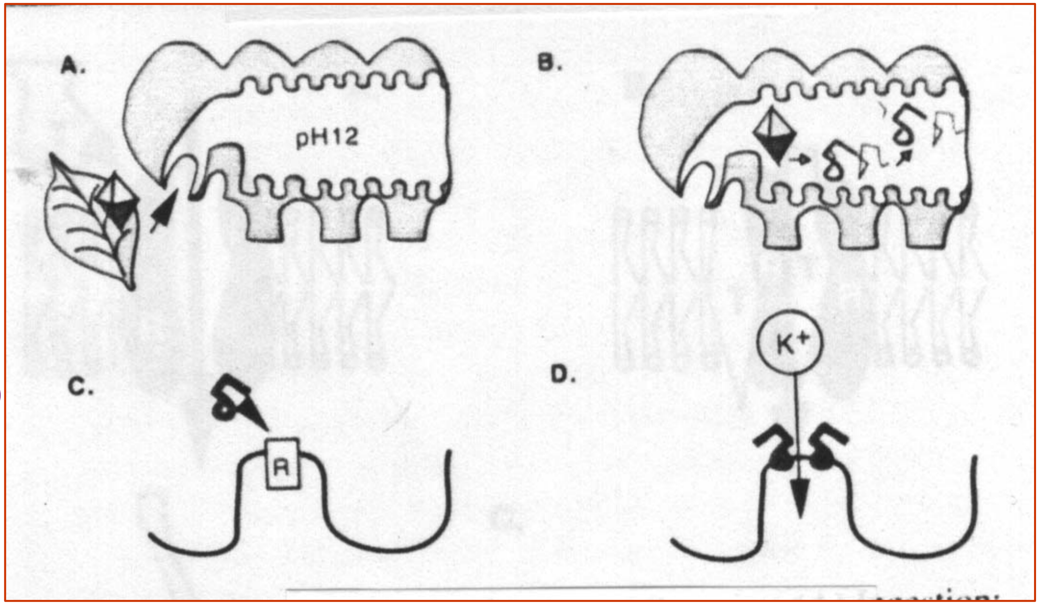
Action Mechanism

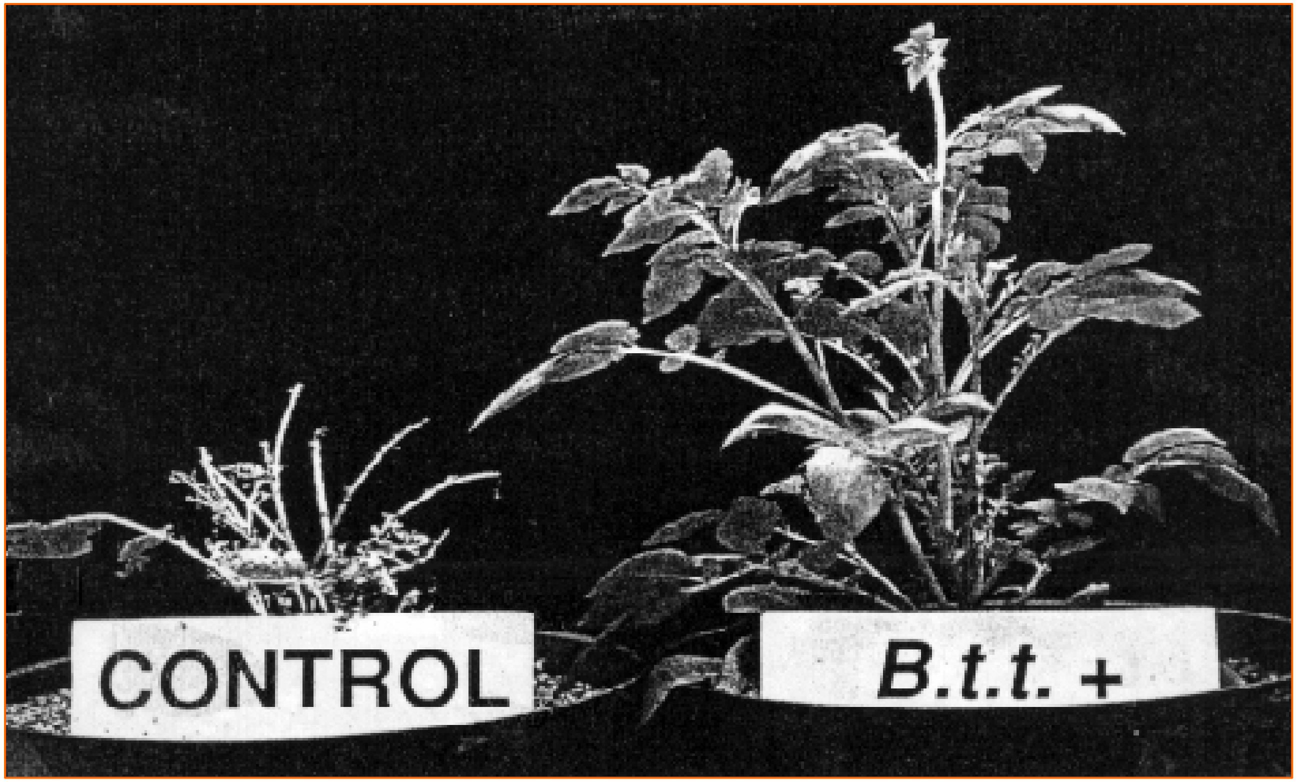
a. ingestion

b. solubilisation & activation

c. binding to the receptor

d. toxic effect (columlar cell lysis)





cryIIIA delta endotoksin geni transfer edilmiş (B.t.t. +) ve normal (control) patates bitkilerinde patases böceği tarafından oluşturulan zarar. Fotoğraf böcek uygulamasından 10 gün sonra alınmıştır.

(Perlak, F.J., Plant Mol. Biol, 22, 313-321, 1993)

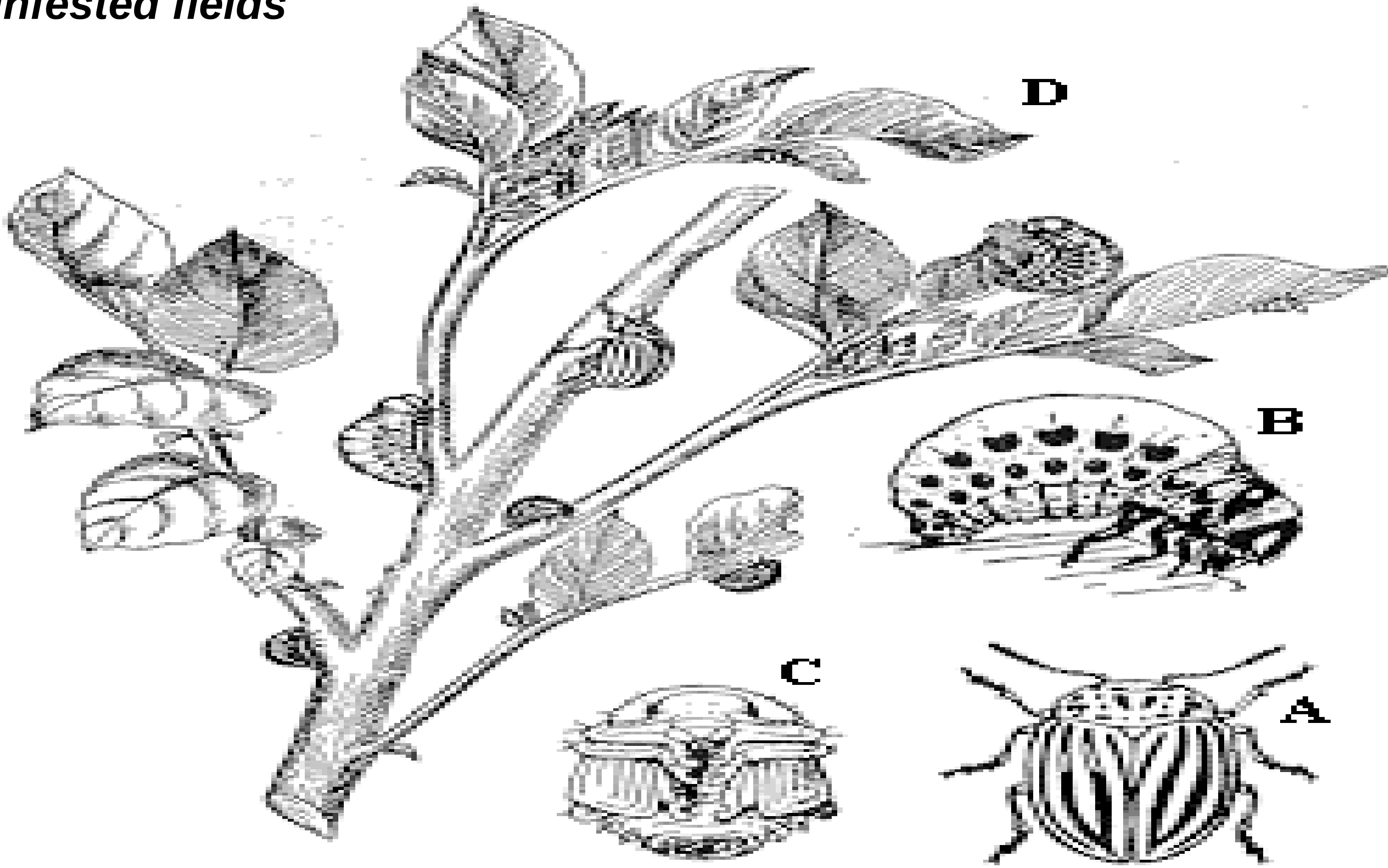
Leptinotarsa decemlineata

infest a wide variety of plants including tomato, potato,eggplant, pepper...

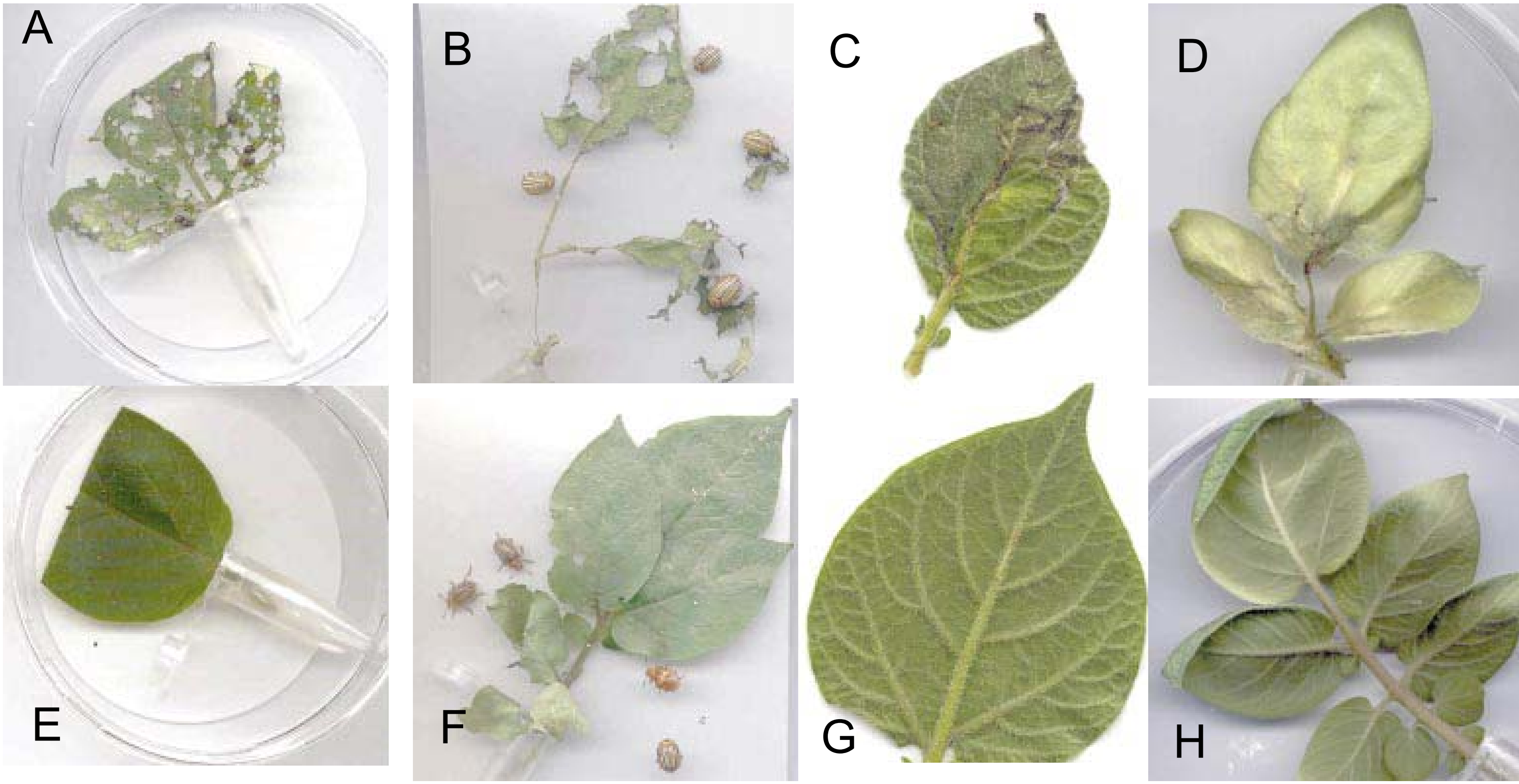
% acres affected: 100%

Yield loses: Up to 100%

in severely infested fields



Colorado potato beetle. A, Adult. B, Larva. C, Pupa. D, Damage.



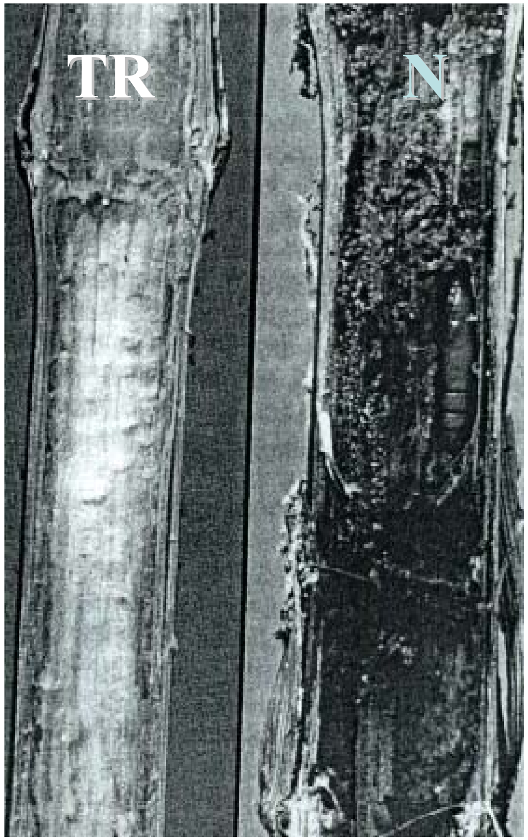
Leaf feeding assays comparing control lines (A–D) and SN19-expressing (E–H) line 11 (expressing 0.23% of total soluble protein).
A, E: CPB larvae; B, F: CPB adults; C, G: PTM larvae; D, H: ECB larvae.

Samir Naimov, Stefan Dukiandjiev and Ruud A. de Maagd
A hybrid Bacillus thuringiensis delta-endotoxin gives resistance against a coleopteran and a lepidopteran pest in transgenic potato
Plant Biotechnology Journal (2003)**1**, pp. 51–57.

Transgenik (Bt) tütün bitkilerinin *Heliothis armigera* larvalarına karşı gösterdikleri tepki.

KONTROL

TRANSGENİK



B.thuringiensis **delta endotoksin proteinlerini sentezleyebilen transgenik ve normal mısır saplarının “European Core Borer” zararlısına karşı gösterdiği tepki.**
(Perlak F.J., 1994)

Mısır kurdu zararı sonrası transgenik (TR) ve Kontrol (C) bitkilerden elde edilen koçanlar. Iowa State University, Image Galery, http://www.ent.iastate.edu/imagegallery/

Field trials:. Location- Adana-TURKEY

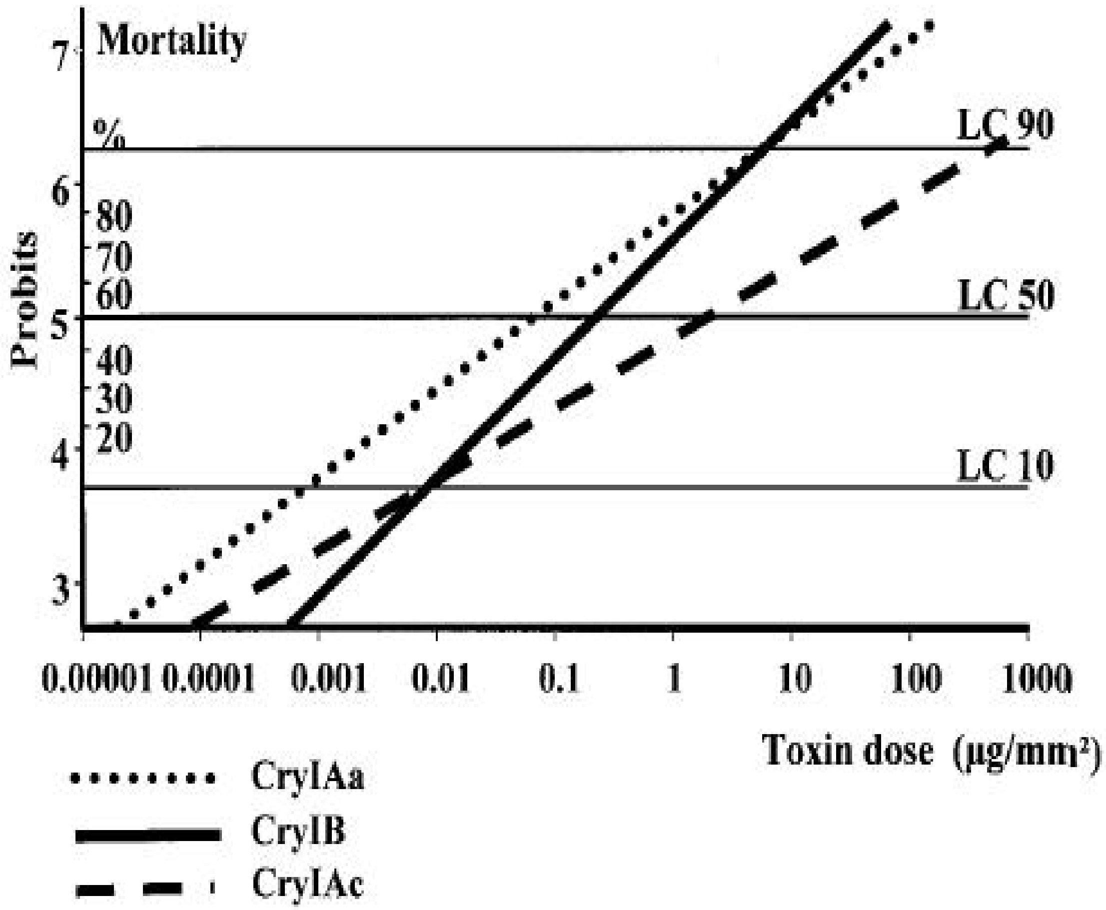
Striped Stem Borer

➔Asian origin insect

➔Affects rice production in southern Europe

➔Yield losses 15-20%

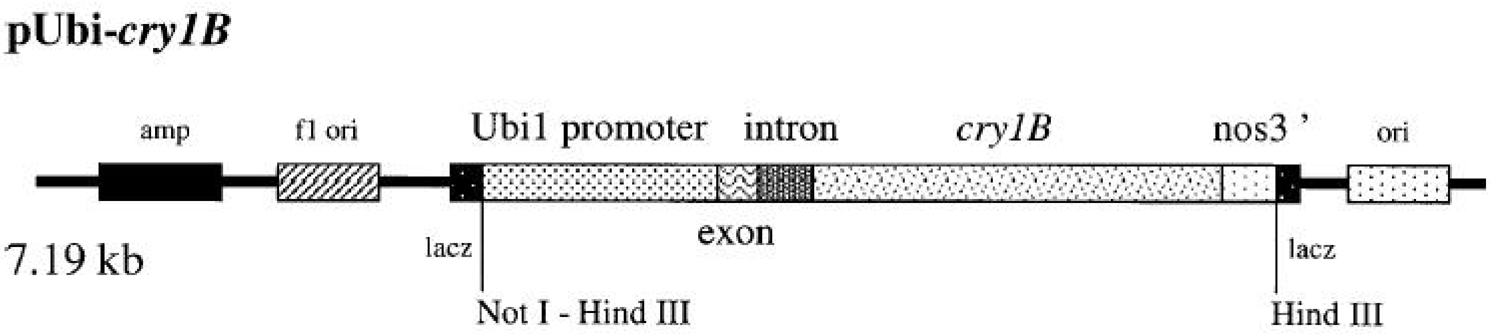
➔A diet incorporation assay involving seven *Bt* toxins has shown that the toxin exhibiting the highest toxycity towards SSB larvae is Cry1Aa, followed by Cry1B and Cry1Ac.



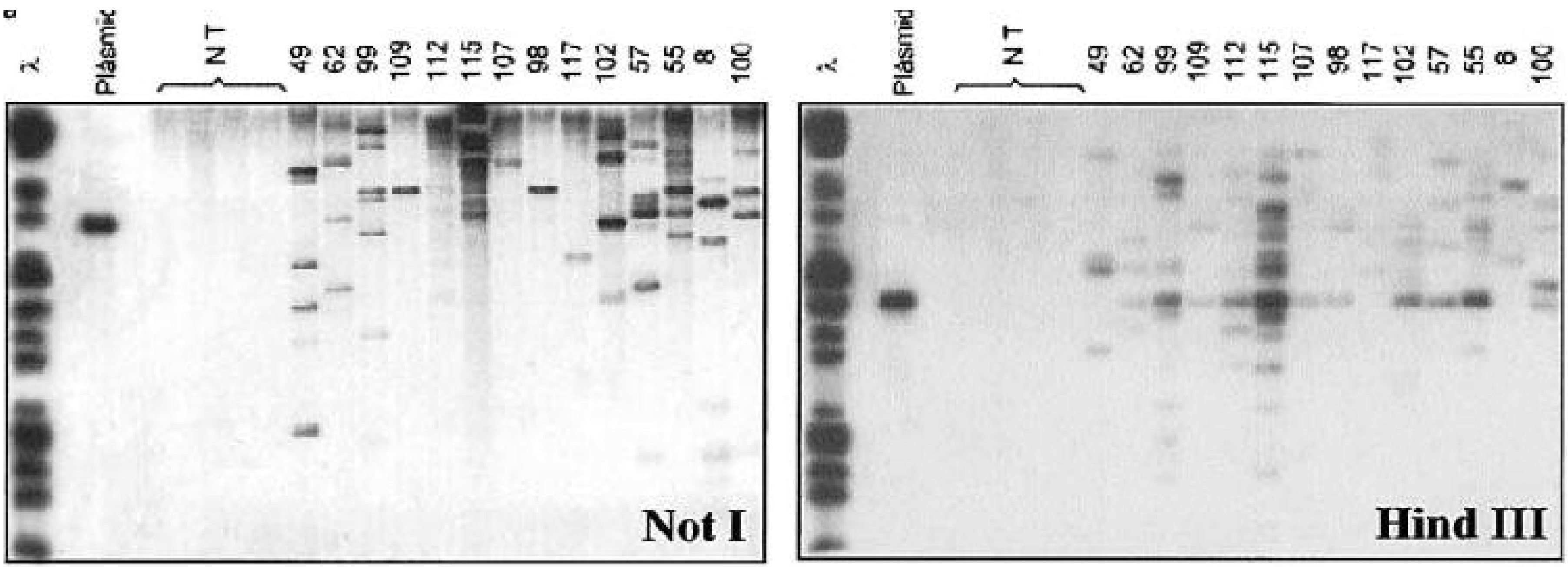
Lethal concentration (LC)50 curves of L2 SSB larvae in diet incorporation assay.

The effect of Cry1B (0,34 μg/cm^2) and Cry1Aa (0,1μg/cm^2) are higher than that of Cry1Ac(3,3μg/cm^2). Cry1Aa has low toxin dose in the LC10, while Cry1B and Cry1Ac have similar toxin dose which is higher than Cry1Aa. The LC90 of Cry1Aa and Cry1B appear to be similar and lower than Cry1Ac.

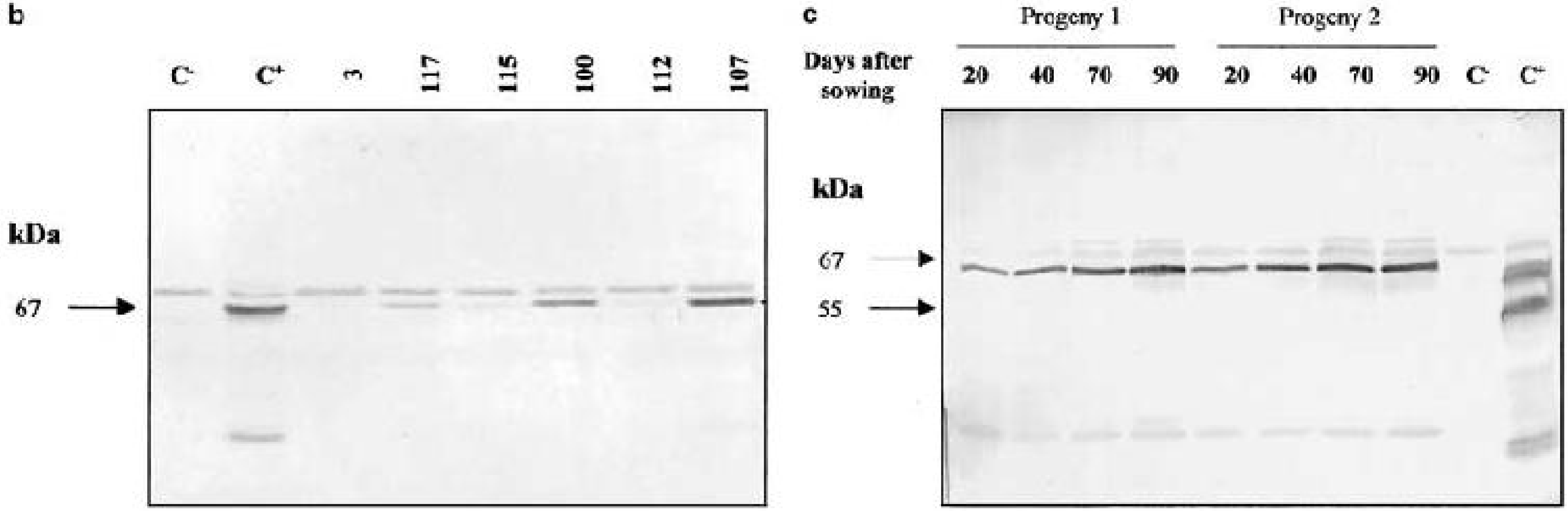
Diagrammatic representation of the pUbi-*cry1B* plasmid



- Synthetic cry1B GC content of 58%
- Clone under maize ubiquitin promoter first intron first exon regions
- Transferred to two commercial Mediterranean rice cultivars, Ariete and Senia



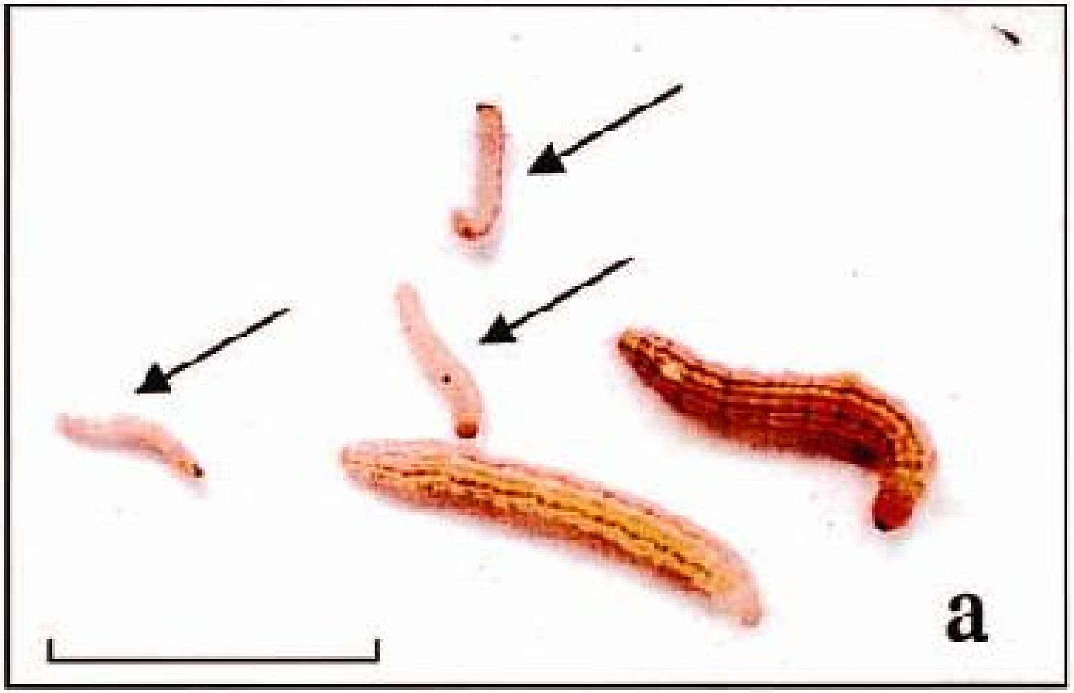
Southern blot analysis of total genomic DNA of T0 plants of cv. Senia digested by *Not*I (single cut) and *Hin*dIII (double cut releasing the gene cassette of the pUbi-*cry1B* plasmid) and hybridized with the *cry1B* probe.



•Immunoblot analyses of 6 T0 events of cv. Senia (C- protein extract of leaf tissue of an untransformed rice plant, C+ protein extract of leaf tissue of a transformed rice plant shown to accumulate Cry1B at 0,4% in a reconstruction assay).

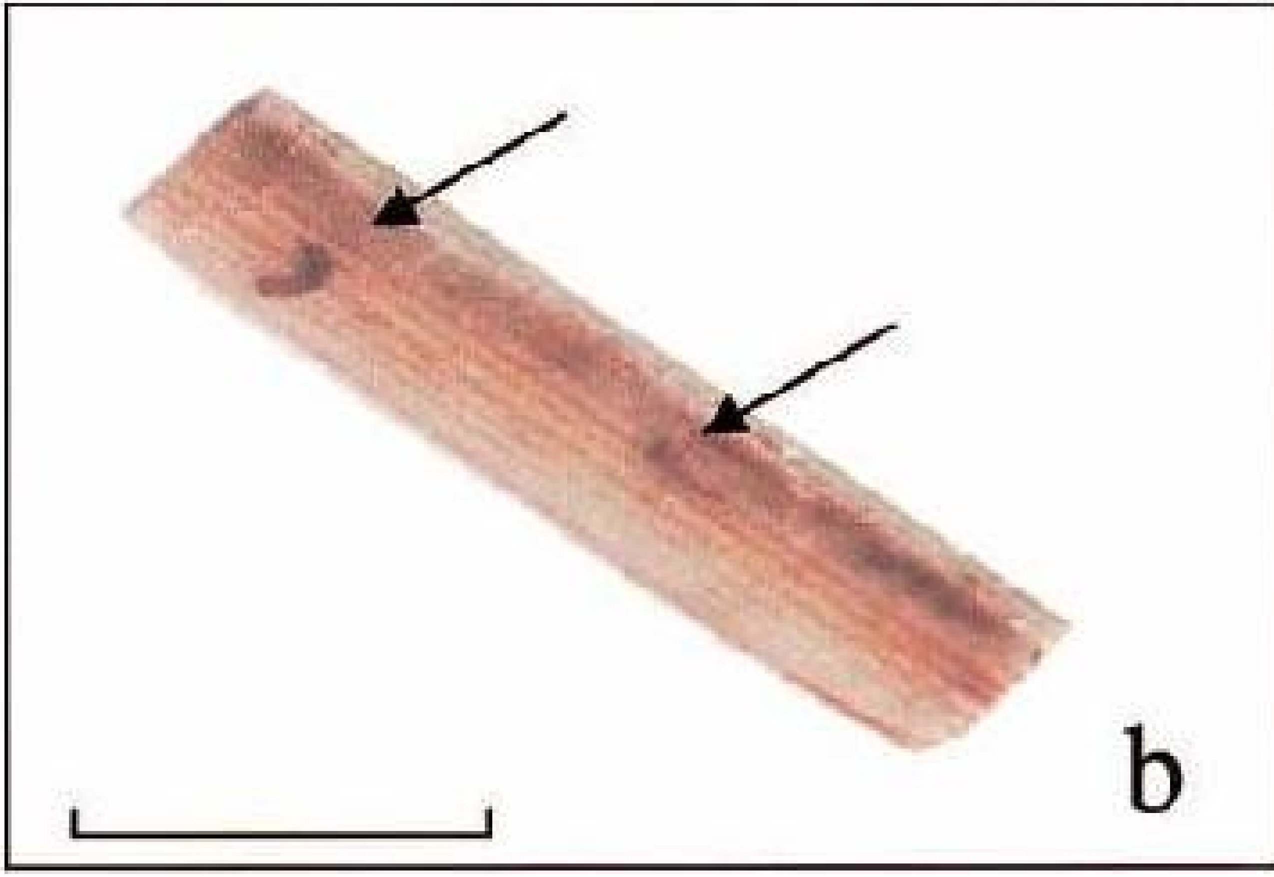
•Accumulation of the Cry1B toxin in the antepenultimate leaf blade tissue of 2 T3 progeny Ariete plants of event, 20, 40(tillering stage), 70(heading stage) and 90(grain filling stage) days after sowing (C+ 0,1% of Cry1B purified toxin in rice leaf protein extract)

Comperative damage observed on transformed (left) and control (right) plants 15 days after their infestation with L2 larvae.



Detailed view of the few SSB larvae recovered alive on Ariete (arrows) plants compared to those recovered on control plants, 7 days after release.

The L2 SSB larvae infested with T0 plants showed the high mortalitiy, most of the larvae were found to be dead and the survivals had not gained weight. From this plants, it was observed that Cry1B satbly accumulated at 0,4% of the total soluble proteins in T1-T4 progenies.



Detailed view of SSB larvae recovered dead (arrows) on a leaf sheath of rice plant, 7 days after release.

Bacillus thuringiensis delta-endotoxsins (cry proteins)

Transgenic (TR) and wild (W) type chrysanthemum plant responses against beet armywarm larvae.

INSECT RESISTANCE STRATEGIES:

- **Other Insectisidal Proteins**

  Effective at μ/ml level (requires higher expression)

  - ✓ **Chitinase**
  - ✓ **Lectins**
  - ✓ **Proteinase inhibitors**
  - ✓ **α-Amylase Inhibitors**

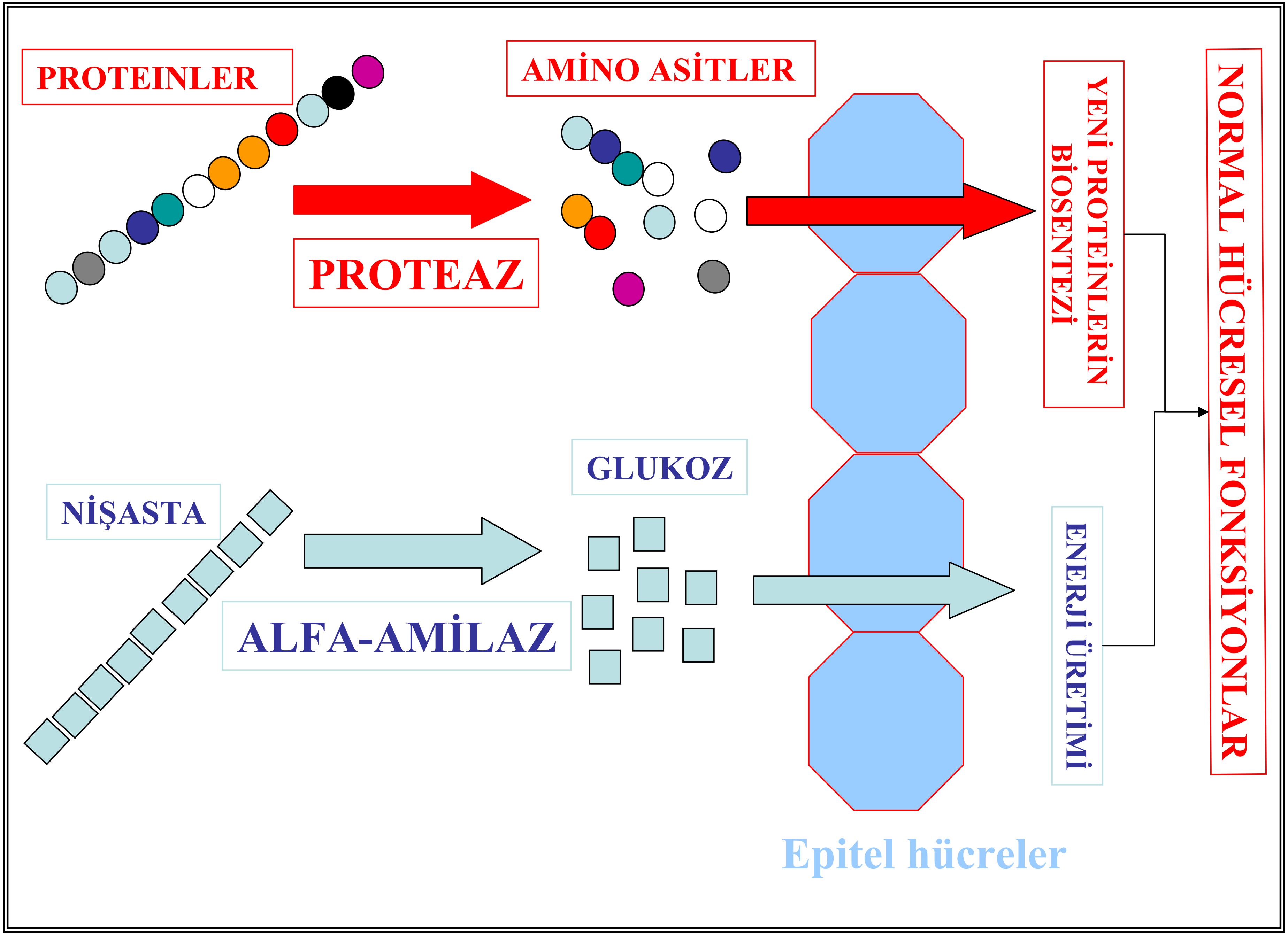
- **New Generation Proteins**

  Effective at ng/ml level

  - ✓**VIP Protiens from *Bacillus cereus & thuriengis***
  - ✓**Cholesterol oxidase**

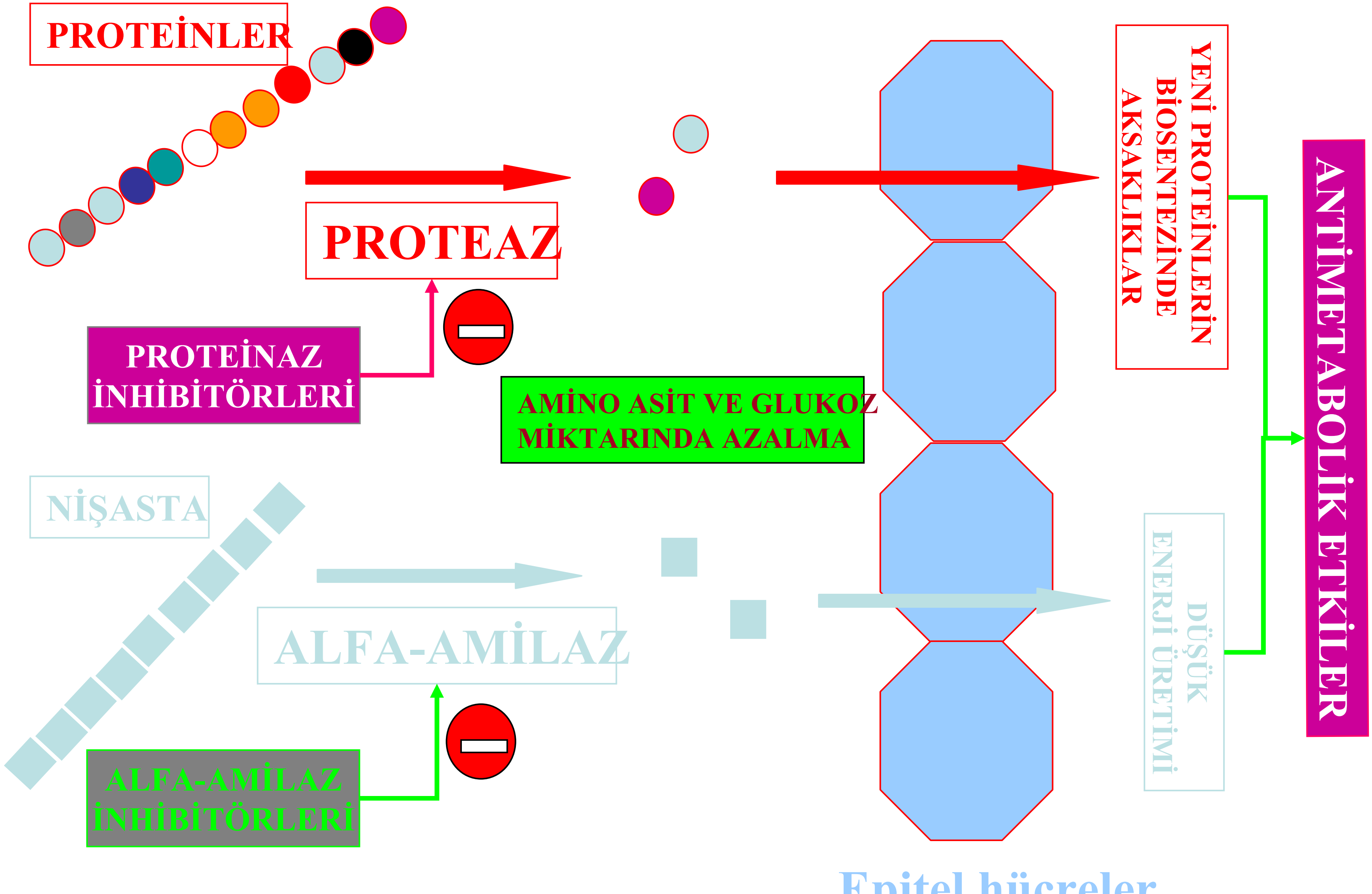
PROTEİNAZ İNHİBİTÖRLERİ

- 40-180 amino asit içeren polipeptidler.
- Oldukça stabil yapıya sahip moleküller.
- Bitkilerin doğal savunma mekanizmalarında görev almakta.
- Etki mekanizması: Böceklerin orta bağırsağında sindirimden sorumlu proteaz enzimlerini bloke ederek antimetabolik etki yaratmakta.
- Afidlere dirençli TR bitkilerin geliştirilmesinde ümitvar sonuçlar gözlenmekte.



PROTEINLER
PROTEAZ
AMİNO ASİTLER
YENİ PROTEİNLERİN BİOSENTEZİ
NORMAL HÜCRESEL FONKSİYONLAR
NİŞASTA
ALFA-AMİLAZ
GLUKOZ
ENERJİ ÜRETİMİ
Epitel hücreler

Bitki	Gen	İnhibitor Tipi	Hedef Böcek	Kaynak
Tütün	*CpTI*	Cowpea serin PI	*Manduca sexta* (L)	Hidler *ve ark*., 1987
	PPI-II *TPII*	Patates serin PI Domates serin PI	*Manduca sexta* (L)	Johnson *ve ark*., 1989
	CpTI	Cowpea serin PI	*Heliothis virescens* (L)	Gatehouse *ve ark*., 1992
	OCI	Çeltik sistein PI (Oryzacystatin)	?	Masoud, 1993
	PPI-II	Patates serin PI	*Chrysodeixis eriosoma* (L)	McManus *ve ark*., 1994
	MS-PI	*Manduca sexta* PI	*Bemisia tabaci*	Thomas *ve ark*., 1995a
	CB-PI	Mısır PI	?	Masoud *ve ark*., 1996
	SpTi-I	Tatlı patates PI	*Spodoptera litura* (L)	Yeh *ve ark*., 1997
	Na-PI	*Nicotiana alata* PI	*Helicoverpa armigera* (L)	Charity *ve ark*., 1999
	SKTI	Soya (Kunitz) tripsin PI	*Spodoptera litura*	McManus *ve ark*., 1999
Çeltik	*OCI*	Çeltik sistein PI	?	Hosoyoma *ve ark*., 1994
	CpTI	Cowpea serin PI	*Seramina inferens* (L)	Duan *ve ark*., 1996
	CC	Mısır cystatin PI	*Sitophilus zeamais*	Irie *ve ark*., 1996
	PPI-II	Patates serin PI	*Seramina inferens* (L), *Chilo suppressalis* (L)	Xu *ve ark*., 1996
	SKTI	Soya (Kunitz) tripsin PI	*Nilaparvata lugens*	Lee *ve ark*., 1999
	WTI-1B	Fasulye tripsin PI	*Chilo suppressalis* (L)	Mochizuki *ve ark*., 1999
Buğday	*BTI-CMe*	Arpa tripsin PI	*Sitotroga cerealella*	Altpeter *ve ark*., 1999
Pamuk	*MS-PI*	*Manduca sexta* PI	*Bemisia tabaci*	Thomas *ve ark*., 1995b
	SKTI	Soya (Kunitz) tripsin PI	*Heliothis armigera*	Wang *ve ark*., 1999
Patates	*OCI*	Çeltik sistein PI	?	Benchekroun *ve ark*., 1995
	CpTI	Cowpea serin PI	*Lacnobia oleracea* (L)	Gatehouse *ve ark*., 1997
	OCI	Çeltik sistein PI	*Leptinotarsa decemlineata* (C)	Lecardonnel *ve ark*., 1999
Bezelye	*Na-PI*	*Nicotiana alata* PI	*Helicoverpa armigera* (L)	Charity *ve ark*., 1999



PROTEİNLER
PROTEAZ
PROTEİNAZ İNHİBİTÖRLERİ
AMİNO ASİT VE GLUKOZ MİKTARINDA AZALMA
NİŞASTA
ALFA-AMİLAZ
ALFA-AMİLAZ İNHİBİTÖRLERİ
YENİ PROTEİNLERİN BİOSENTEZİNDE AKSAKLIKLAR
DÜŞÜK ENERJİ ÜRETİMİ
ANTİMETABOLİK ETKİLER
Epitel hücreler

α-Amylase

- Seeds contain high amounts of starch and the insects feeding on these seeds contain amylolytic enzymes to exploit these starch reserves.

- α-Amylases catalyze the hydrolysis of internal α-(1$\rightarrow$4) glucosidic linkages.

α-Amylase Inhibitor (αAI)

- One secondary metabolite, peoteinaceous α-amylase inhibitor (αAI), is widely distributed in seeds of most cereal crops and some grain legumes, and it is assumed to be responsible for biochemical defense, especially against insects,
- Bean αAI is a glycoprotein that forms a one to one complex with insect and mammalian α-amylases but is not active against plant and bacterial α-amylases.

- Transfer of the cDNA encoding αAI from common bean *Phaseolus vulgaris* L. to either pea, or azuki bean, clarifies the role of this protein.

- Seeds of these transgenic grain legumes resist infestation by two bruchids azuki bean weevil and the cowpea weevil which also cannot infest common bean seeds.

- Bean αAI inhibits α-amylase in the midgut of coleopteran-stored product pests of the genera *Callosobruchus* and *Bruchus* and blocks larval development.

Molecular Characteristics Of α-Amylase Inhibitor

- αAI is a glycoprotein, synthesized as an initial translation product that is co-translationally and post-translationally modified to yield a holoprotein consisting of two subunits of α and β, which form a complex.

- The processing step which gives rise to the two polypeptides occurs after protein folding has occurred, either in the trans-Golgi or in the vacuoles; it is most unlikely that the two polypeptides will again dissociate and associate.

- The molecular weight of the holoprotein has ranged from 20000 Da to 60000 Da

- It seems likely that there are two types of molecular structure: $\alpha\beta$ and $\alpha_2\beta_2$ and $\alpha\beta\gamma$ in which one of the $\alpha\beta$ subunits remains unprocessed. This unprocessed subunit is referred to as the γ-subunit and it is thought to be responsible from the heat lability of the protein.

- Whether this γ-subunit is the product of the same gene that produces the processed α and β subunits, or the product of a different gene is not yet clear.

- Some studies disclosed for the first time that a legume α-amylase inhibitor has a tetrameric structure, $\alpha_2\beta_2$, which is essential for the inhibitory activity.

- The aminoacid sequences established for the two kinds of subunits, α and β, of αAI wild common bean were highly homologous to those of αAI from white kidney bean.

- Polypeptide molecular weight of αAI wild common bean, considered together with the sequence molecular weights of the subunits, α and β, demonstrated that αAI from wild common bean is also a tetrameric complex, $\alpha_2\beta_2$, analogous to αAI from white kidney bean.

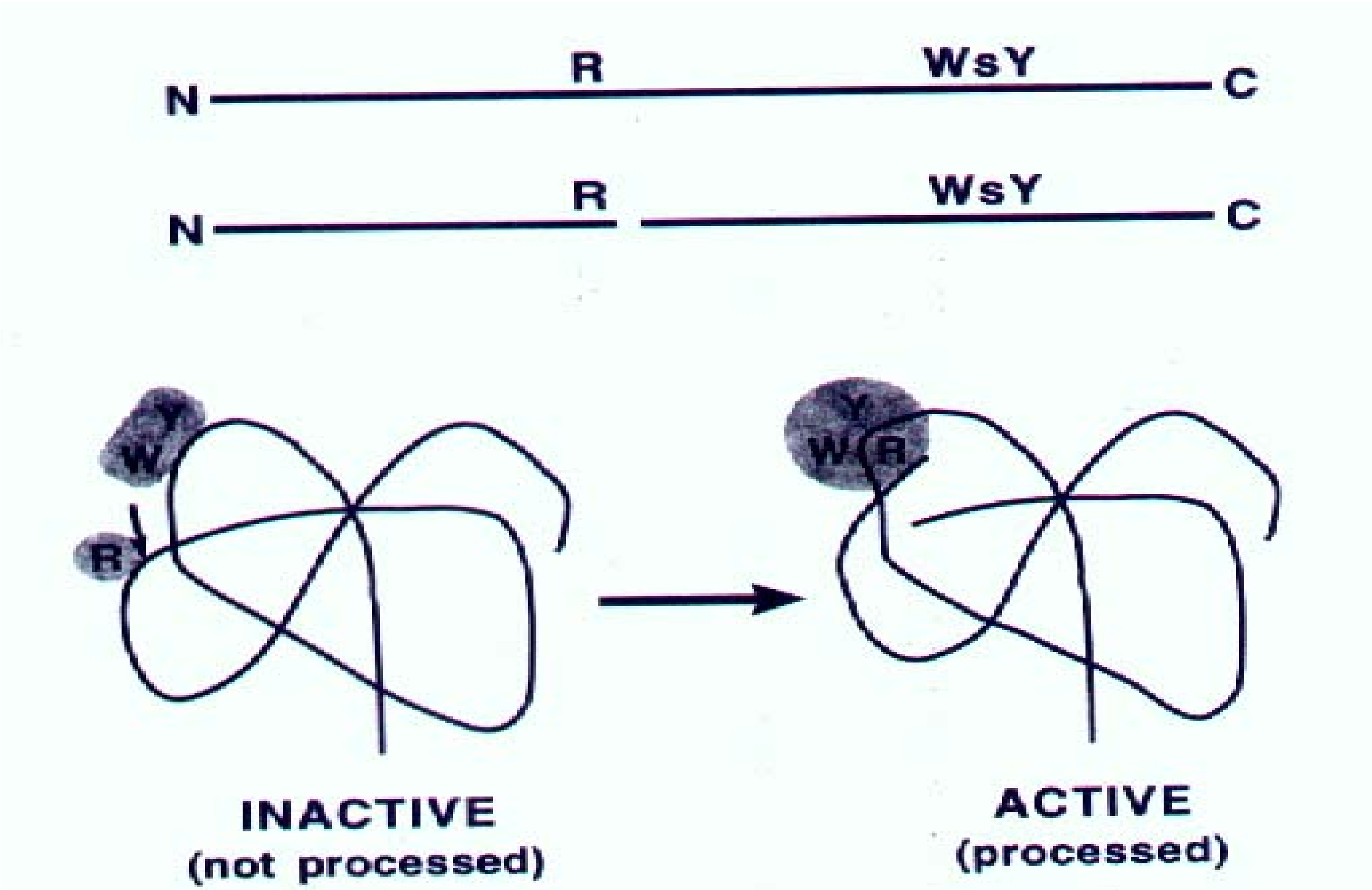
- This suggests that the tetrameric structure is common to leguminous α-amylase inhibitors.

- Further a comperision of the aminoacid sequences of these inhibitors and other *P. vulgaris* defense proteins suggested that the post-translational processing of the precursors to form an active tetramer needs an Arg residue close to the processing site.

Action Mechanism of α-Amylase Inhibitor

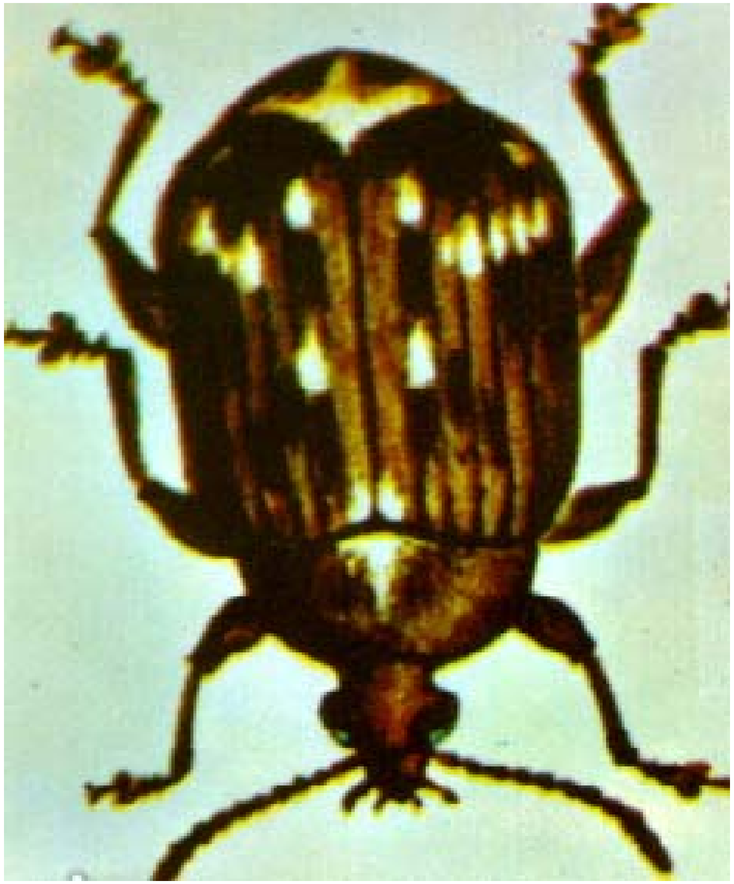
- It is of particular interest that the αAI protein has no inhibitory activity until it is proteolytically processed at Asn^{77} which could bring about a small conformational change that creates an active site and allows αAI to bind its target enzyme.

- **Figure 1.4** Schematic representation of αAI and its active site consisting of R, W and Y.



- Complex formation has a pH optimum around 5.5 and for this reason, αAI inhibits amylases in the acid midgut of *Coleoptera,* but not in the alkaline midgut of *Lepidoptera.* αAI is therefore particularly well suited for defense against the starch-eating bruchids.

Bruchus pisorium



Baklagil tohumlarında önemli zarar oluşturan ergin *Bruchus pisorum* böceği.

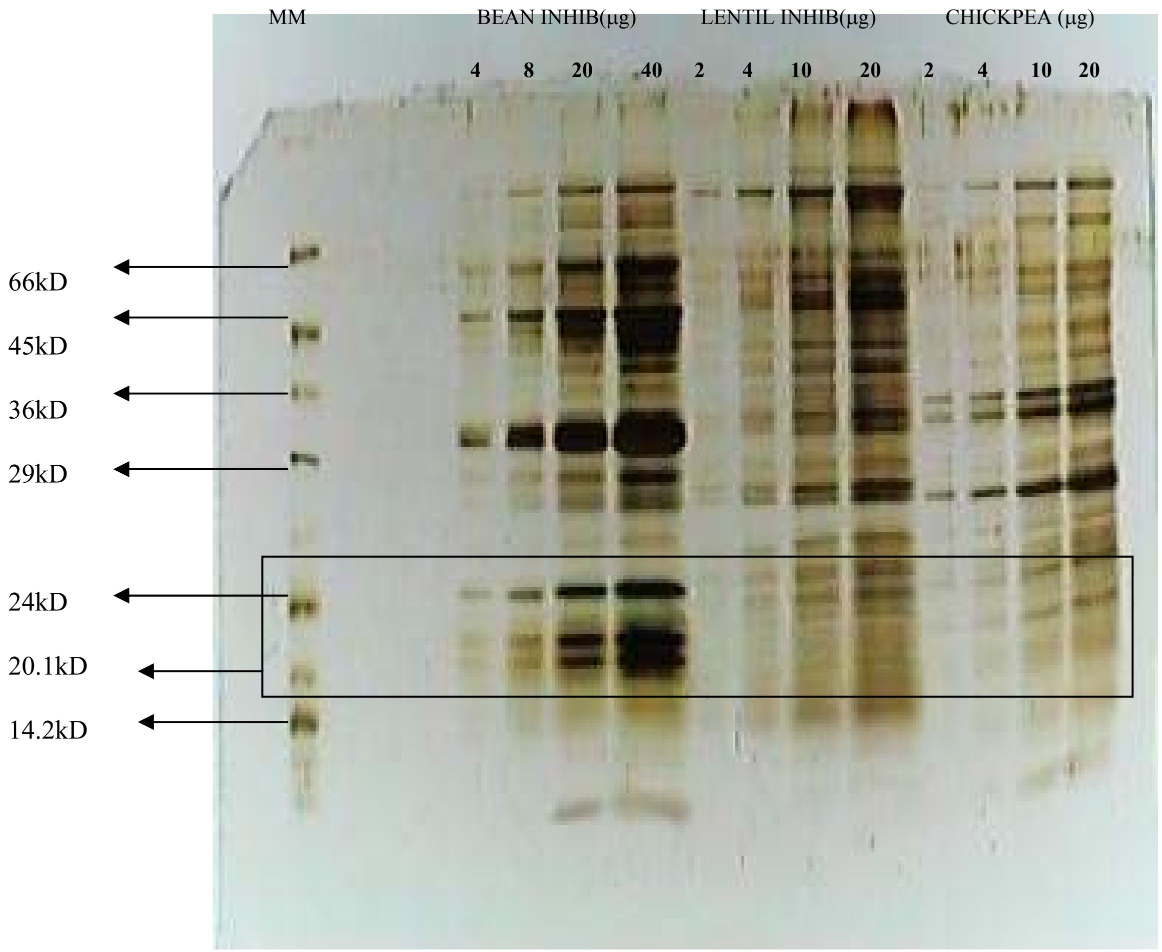
Chickpea damage

Nohutta bir tohum böceği olan *Callosobrucus chinensis* tarafından oluşturulan zarar.

Damage caused by Peeweevil

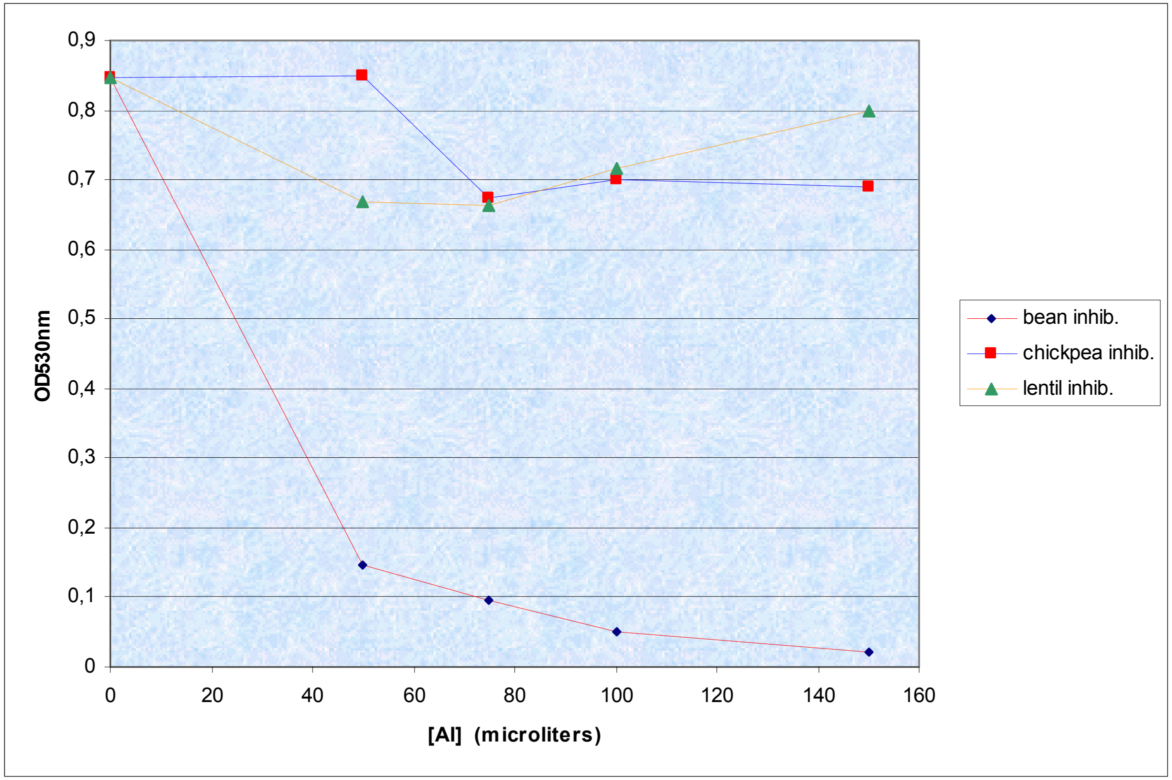
Damage caused by bean beetle

Isolation Of α-Amylase Inhibitors from Various Legumes



SDS PAGE analysis of the αAI inhibitors extracted from bean, lentil and chickpea.

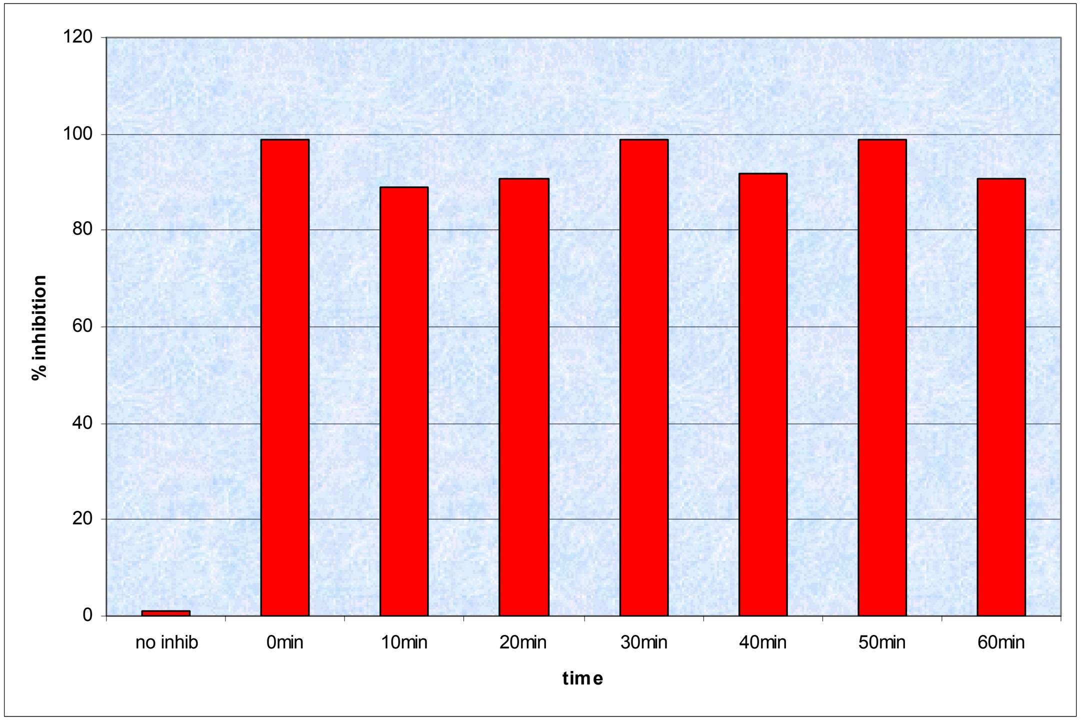
Comparison of the Activities of Inhibitors from Different Legume Sources



The inhibitory action of αAI isolated from bean, chickpea and lentil

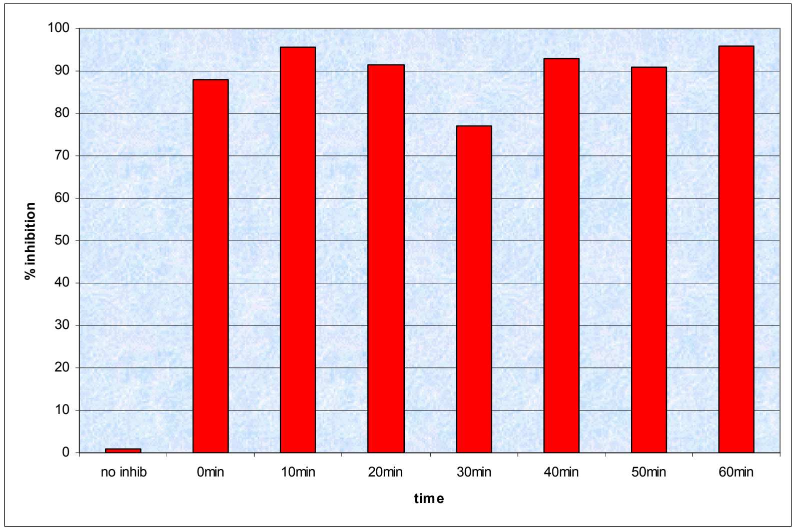
- Protein concentrations for the inhibitor extracts were 4 μg protein/μl for bean and 2 μg protein/μl for lentil and chickpea.

Thermostability Test



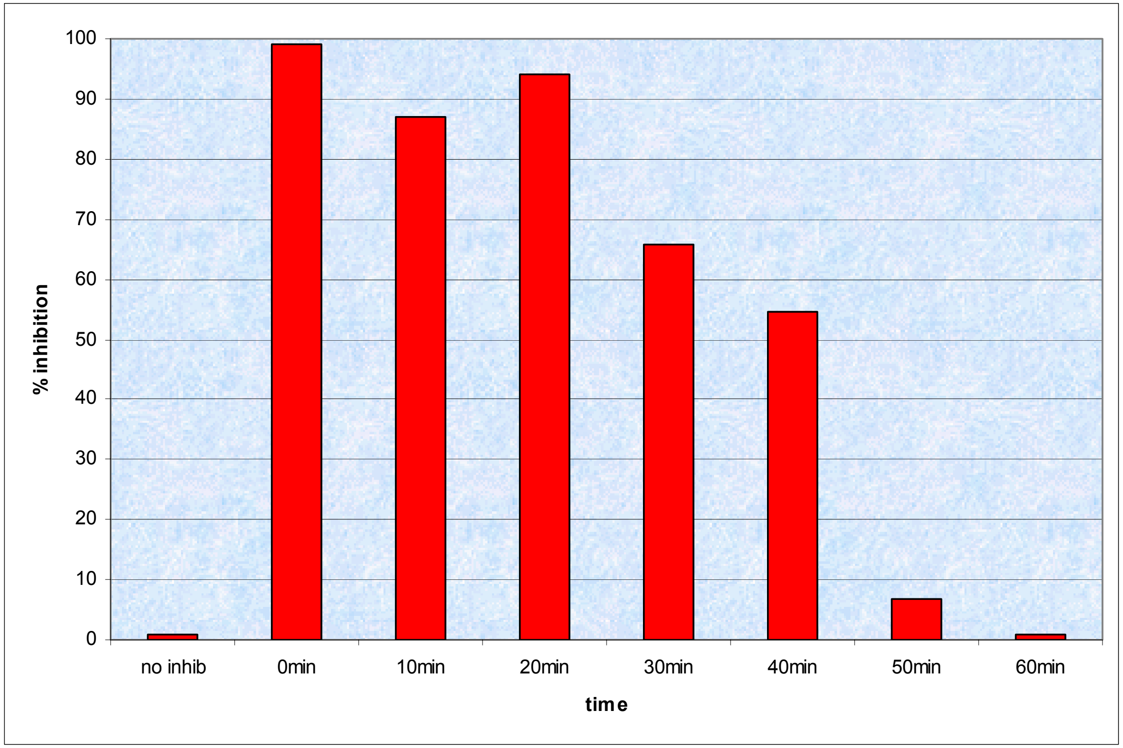
Stability analysis of αAI at 50^{o}C.

- 200μg of αAI extract was incubated at 50^{o}C for different durations.
- Reducing sugar assay with 2 units of porcine α-amylase was performed.



Stability analysis of αAI at 60ºC.

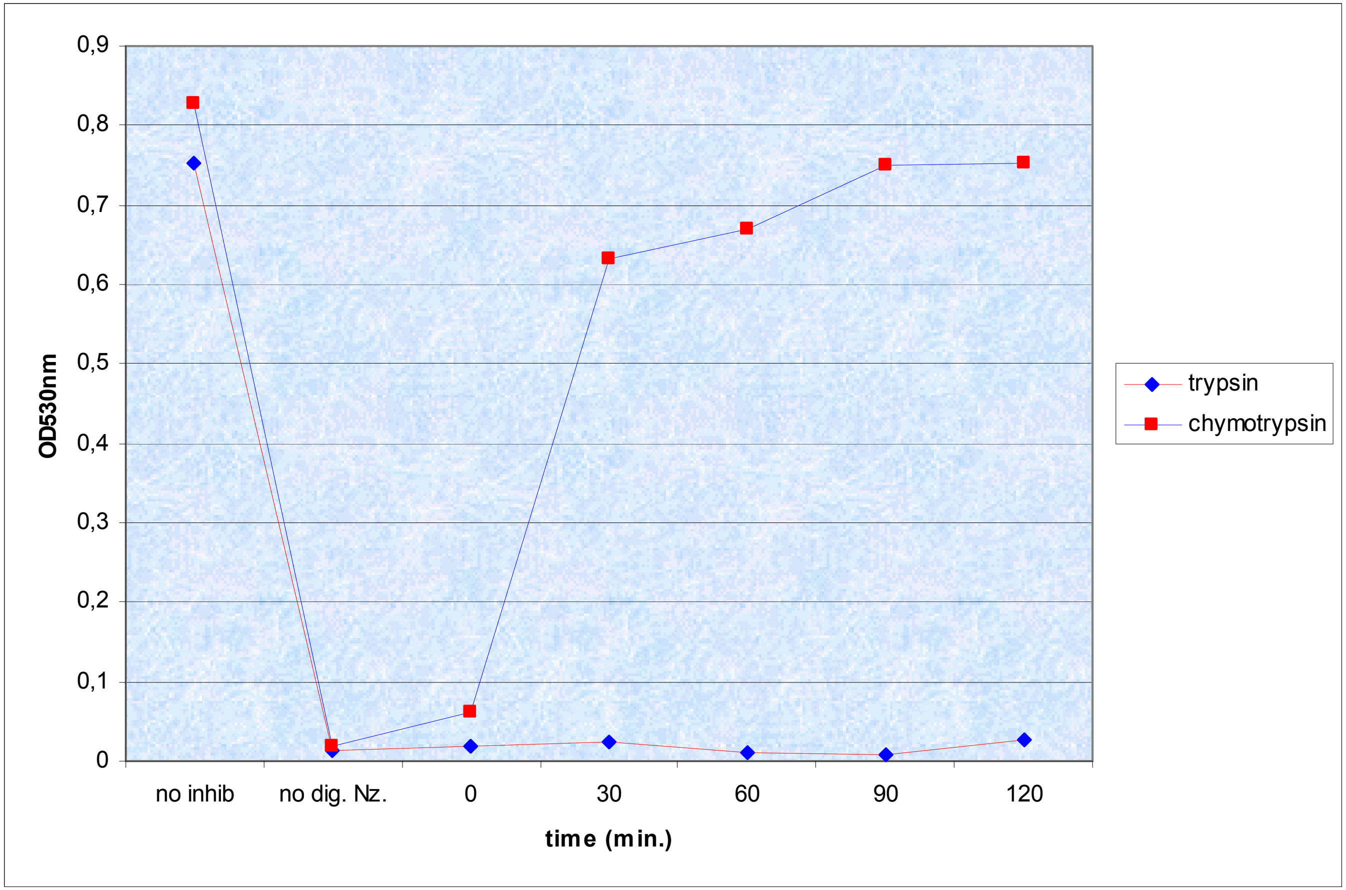
- 200μg of αAI extract was incubated at 60ºC for different durations
- Reducing sugar assay with 2 units of porcine α-amylase was performed.



Stability analysis of αAI at 70^{o}C.

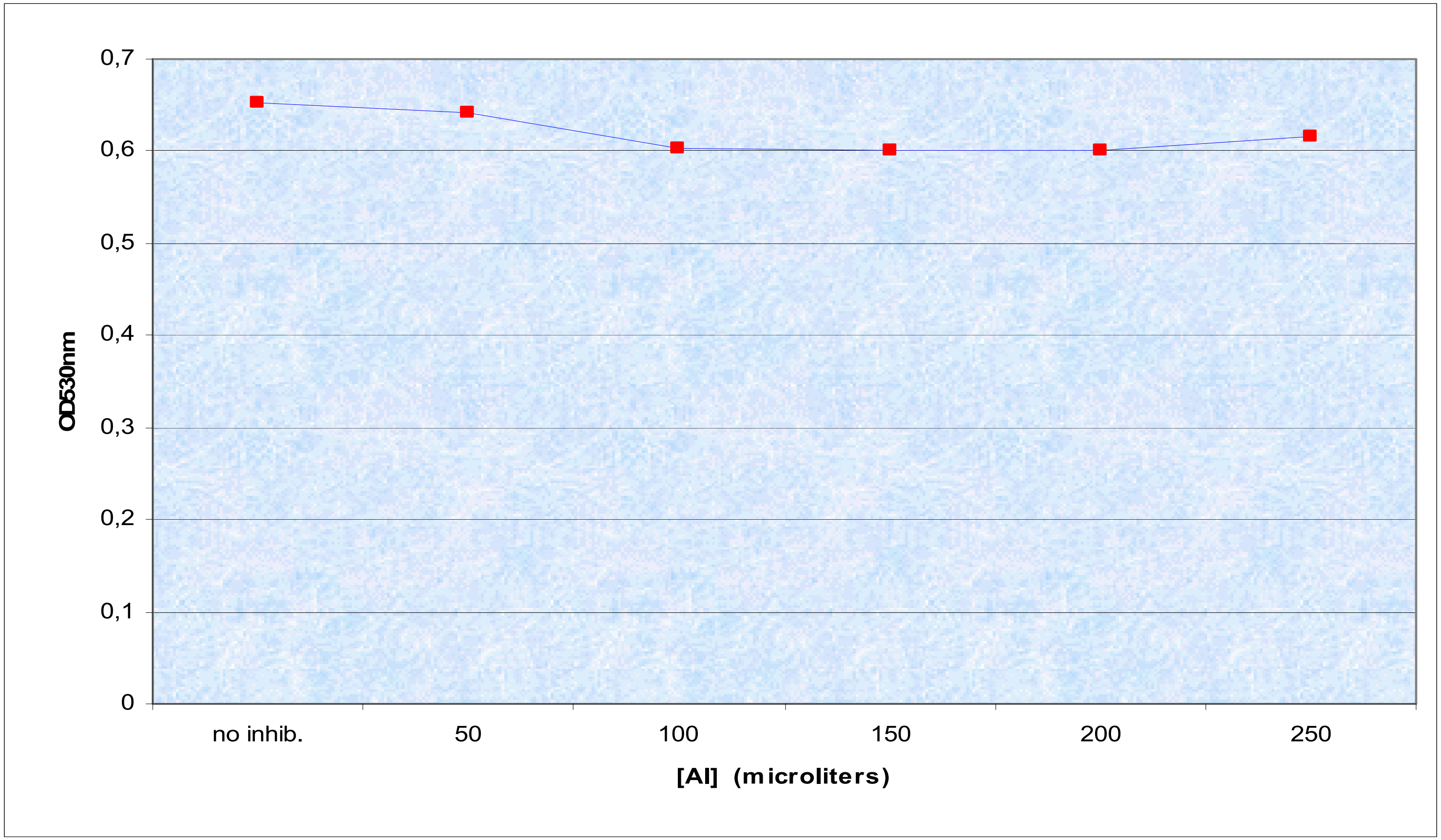
- 200μg of αAI extract was incubated at 70^{o}C for different durations
- Reducing sugar assay with 2 units of porcine α-amylase was performed.

Stability of bean αAI to trypsin and chymotrypsin digestion



- 200 μg of the inhibitor extract was treated with 2.5 units of trypsin or 2.5 units of chymotrypsin for indicated time durations at 37°C and 25°C respectively.

Effects of αAI on α-Amylases from Different Sources

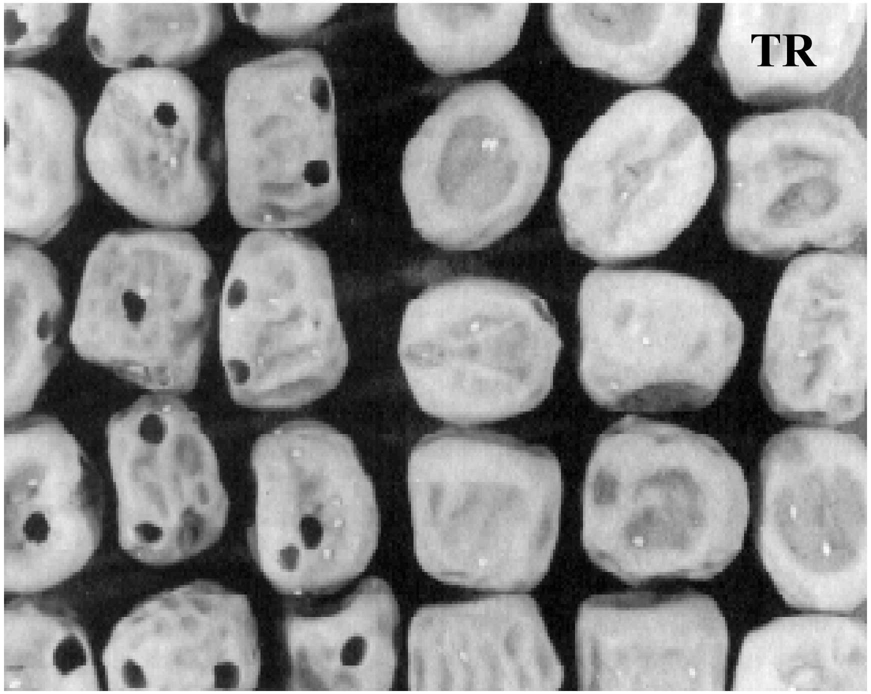


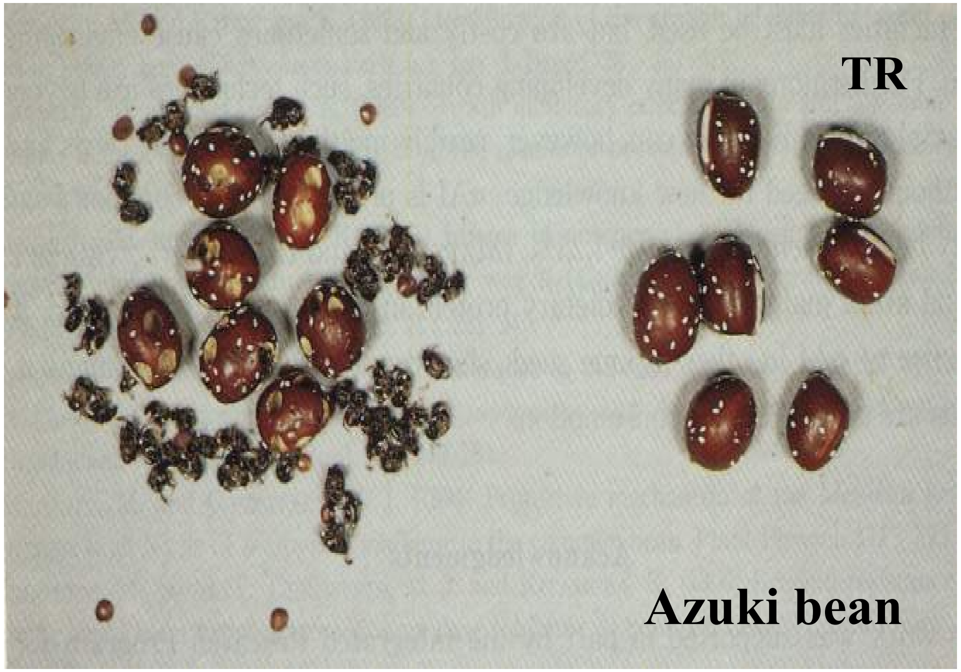
Inhibitory activity of αAI from common bean, on bacterial (*Bacillus* species) α-amylase.

- Bacterial α-amylase was incubated with different quantities of the inhibitor extract (4 μg protein/μl) and assayed by reducing sugar assay.

INSECT RESISTANT TR LEGUMES

Pea





Fasulyeden izole edilen alfa-amilaz inhibitörlerinin sentezinden sorumlu geni taşıyan transgenik ve normal bezelye tohumlarının *Bruchus* böceklerine karşı gösterdikleri tepki.

(Shade R.E. et al., BIO/TECHNOLOGY, 12,793-796, 1994)

LEKTİNLER

- Protein yapısında moleküller.
- Polisakarit, glikoprotein ve glikolipidler yüksek afinite ile bağlanma özelliği göstermekteler.
- Bazı lektinlerin insektisidal aktivite gösterdiği bilinmekte
- Etki mekanizması tam olarak bilinmemekte.
- En yaygın kullanılan lektin GNA (*Galanthus nivalis*)
- Afidlere dirençli TR bitkilerin geliştirilmesinde ümitvar sonuçlar gözlenmekte.

Bitki	Lektin	Lektin Orijini	Hedef Böcek	Kaynak
Tütün	GNA	Kardelen	*Myzus persicae*	Hidler *ve ark*., 1995
	GNA	Kardelen	*Aulacorthum solani*	Down *ve ark*., 1996
	GNA	Kardelen	*Heliothis armigera*, *Myzus persicae*	Wang ve Guo, 1999
Patates	GNA	Kardelen	*Lacanobia oleracea*, *Myzus persicae*	Gatehouse *ve ark*., 1996; Gatehouse *ve ark*., 1997
	ConA	Fasulye	*Lacanobia oleracea*, *Myzus persicae*	Gatehouse *ve ark*., 1999
Domates	GNA	Kardelen	*Myzus persicae*	Wu *ve ark*., 2000
Pamuk	P-Lec	Bezelye	*Heliothis armigera*	Wang *ve ark*., 1999
Buğday	GNA	Kardelen	*Sitobion avena*	Stoger *ve ark*., 1999
Çeltik	GNA	Kardelen	*Nephotettix virescens*	Foissac *ve ark*., 2000
Mısır	WGA	Buğday	*Ostrinia nubilalis*	Cavalieri *ve ark*., 1995

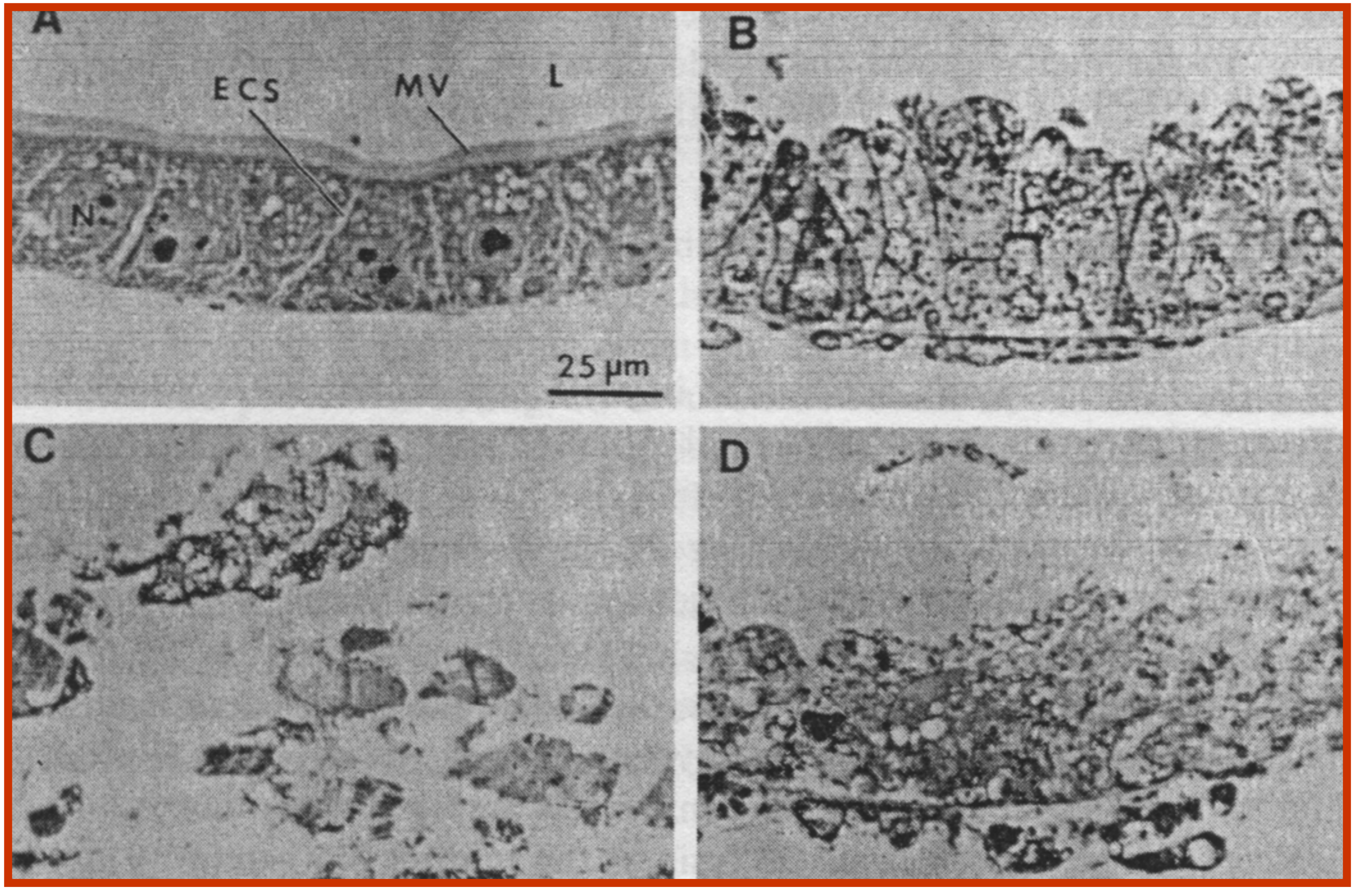
Chitinase

- Doğrusal bir homopolimer olan kitin (β(1-4)2-deoxy-2acetamido-D-glucopyranosyl /N-asetilglukozamin) önemli bir yapısal molekül olmanın yanısıra, böceklerde peritrofik zarların yapısında da yer almaktadır. Buna ek olarak kitin, fungus ve alglerin duvar zarlarının yapılarında da bulunmaktadır. Birçok farklı organizmada yapısal işlevi olan kitin doğada en yaygın rastlanan biyopolimerdir.
- Kitinazlar (EC 3.2.1.14) kitin homopolimeri üzerinde hidrolitik aktivite gösteren enzimlerdir. Bitki orijinli kitinazların hastalıklara dayanıklılıktaki önemi net olarak gösterilmektedir (Graham ve Sticklen, 1994). Bitki ve mikroorganizma orijinli değişik kitinaz genleri aktarılarak geliştirilen transgenik bitkilerin fungal etmenlere karşı dayanıklılık gösterdiği değişik araştırıcılar tarafından belirtilmişitr

Cholesterol oxidase

- Kolesterol oksidaz farklı mikroorganizmalar tarafından üretilebilen bir enzim olup 3-hidroksiteroidlerin ketosteroidlere ve hidrojen perokside oksitlenmesini katalizlemektedir. Kolesterol oksidazın insektisidal aktivitesi ilk olarak Purcell *ve ark*. (1993) tarafından 10.000 kadar mikrobial fermantasyon filtratlarının yapay diyetle böcek besleme testlerinde gösterilmiştir.

ACTION OF CHOLESTEROL OXIDASE ON EPITHEL CELL MEMBS



Stroptomyces'**den izole edilen kolesterol oksidaz enziminin *Heliotis zea* larvalarının ortabağırsak epitel hücreleri üzerindeki etkisi. A) Kontrol larvaların orta bağırsak bölgesinin normal histolojisi. ECS: Hücre dışı bölge, N: hücre çekirdeği, MV: mikrovilüs.**

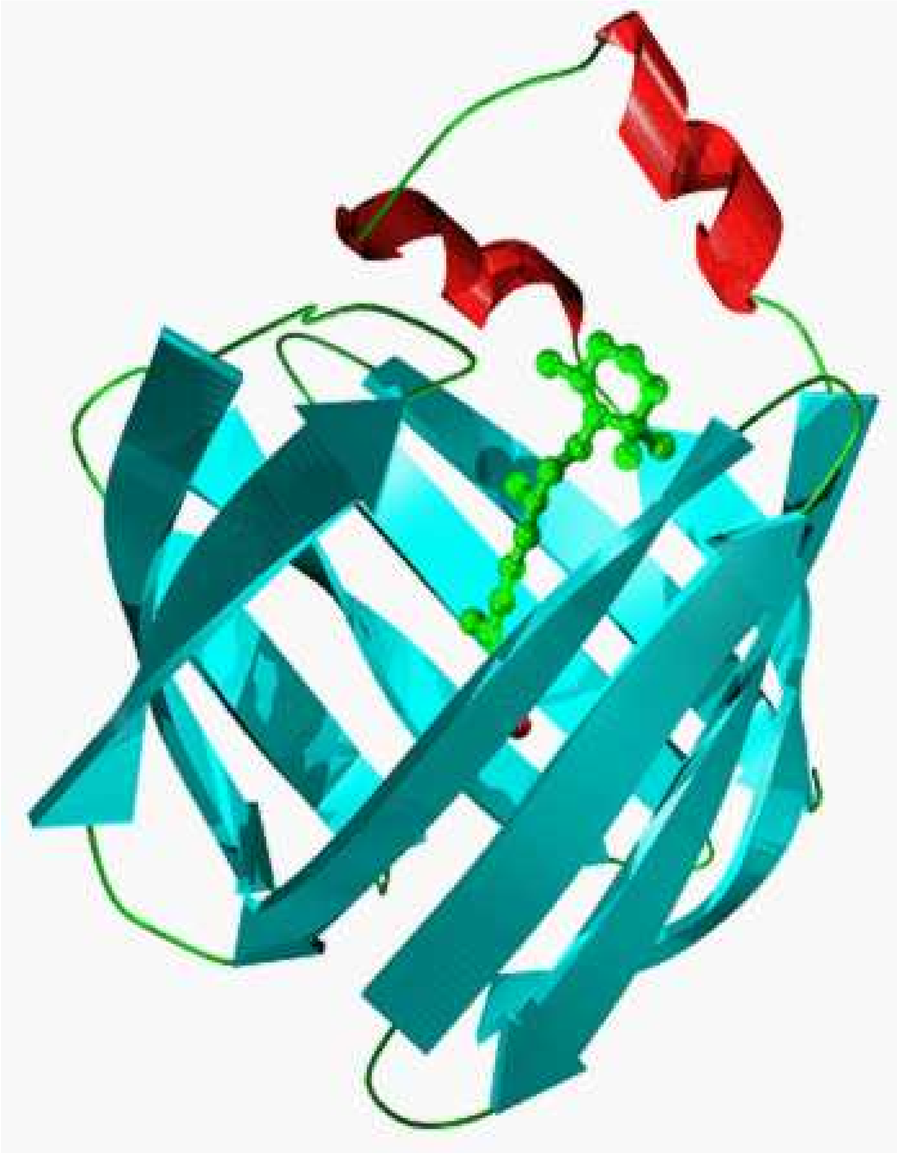
B) 10 µg/ml kolesterol oksidaz içeren diyetle beslenen böceklerin orta bağırsak bölgesinin görünümü. Bu dozun MV'nin ve hücrelerin yapısını etkilediği açıkca gözlenmektedir.

C-D) Sırası ile 30 ve 100 µg/ml kolesterol oksidazın epitel hücre yapısını tamamen etkilediği gösterilmektedir (Purcell, 1997'den alınmıştır).

AVIDIN : AN EGG – CITING INSECTICIDAL PROTEIN

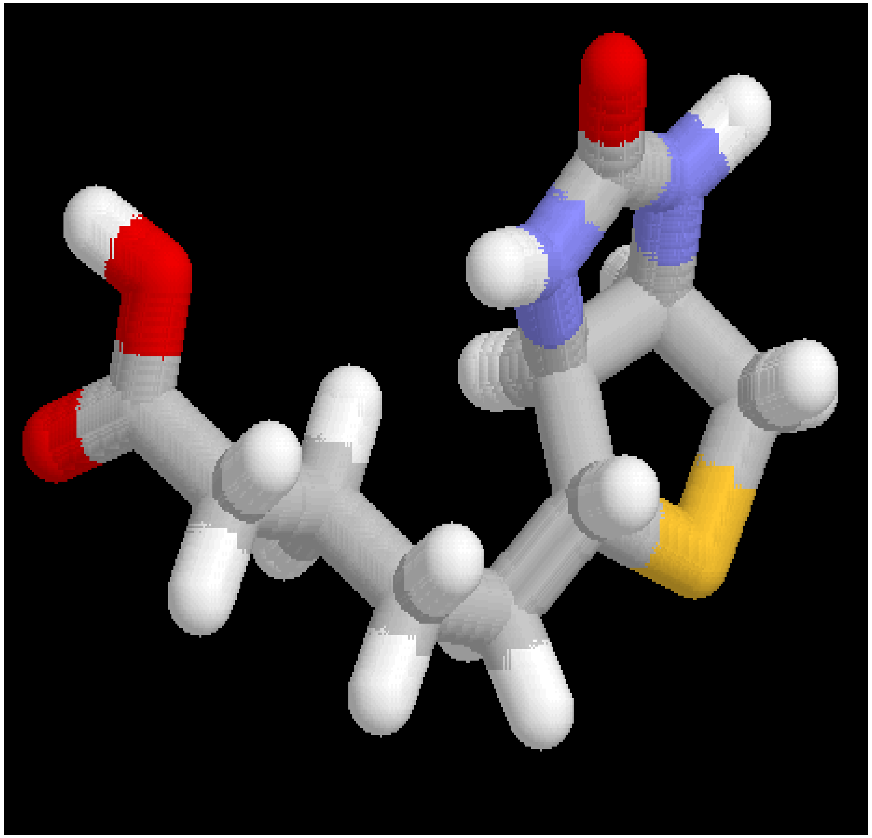
WHAT IS AVIDIN

- A tetrameric protein composed of 4 subunits
- Each subunit has 1 binding site to Biotin
- Very stable against heat and pH
- Stable for months at 4°C
- Highly solube in water and salt solution at pH 7,5 – 8,0
- Found in egg whites
- Lethal to many pesticides
- Has a neutral bacterial analog , called Streptavidin (Streptomyces avidinii)



WHAT IS BIOTIN

- One kind of the Vitamin B
- Also known as Vitamin H
- Essential for the breakdown of fatty acids and carbohydrates and converts them into energy
- For human organism , hairloss , depression and eczema may result from Biotin insufficiency



THE STRATEGY

- Isolate the gene , coding Avidin
- Put a promoter
- Transfer into corn
- Avidin binds to Biotin in insect
- Restricts the availibality of Biotin
- Insect stops developing and dies

BIOPESTICIDE TESTS

- When kernels of Avidin corn were infested with Angounois grain moths , most of the larvaes died inside kernels contained at least 20 ppm of Avidin
- Most of the insects fed corn with 100 ppm Avidin were unable to grow and develop
- About half of the insects were killed by concentration as low as 30 ppm Avidin
- Because of male sterility , only half of the kernels in the corn contained Avidin
- One species of stored – grain pests , Prostephanus truncatus , escaped death

CONCLUSIONS

- Products made from Avidin will have long shelf life
- Unlike chemical insecticidal sprays that can be washed off by rain or inactivated by UV rays , Avidin works regardless of the weather