



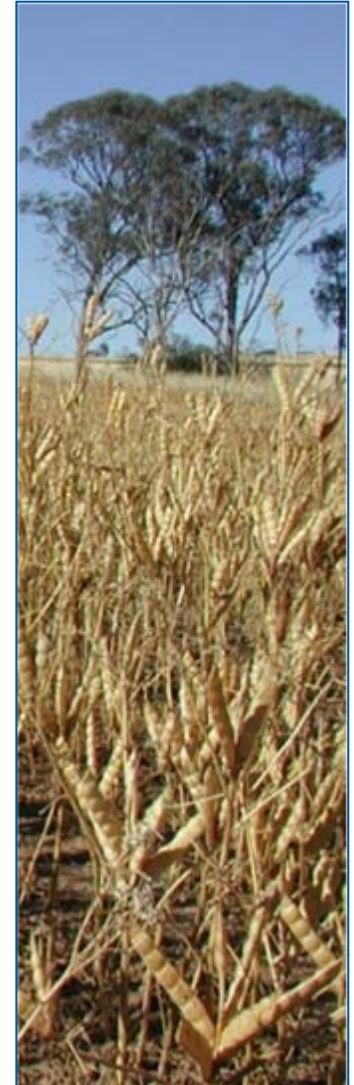
# Estimation of genetic diversity in albus lupin (*Lupinus albus*) using DArT and genic markers

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# Why Measure Genetic Diversity?

- Understand relatedness of current cultivars, crossing parents & breeding lines
- Broaden genetic base of breeding program
- Complement mapping work
  - Map density
  - Marker discovery



# Genic markers

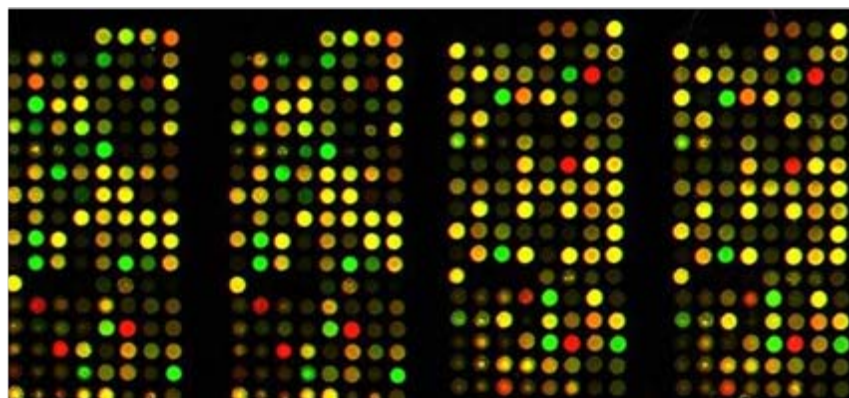
- Albus lupin map\*
  - Kiev-mutant x P27174
  - Anthracnose-resistance
  - 28 linkage groups ( $2n=50$ )
  - 220 AFLPs
  - 105 ITAPs



\* Phan *et al.* (2007)

# DArT markers

- DArT discovery chip
  - 96 contributing genotypes
  - 11 lupin species
  - 15,000 genomic DNA fragments
  - Unmapped



Lupin species	Genotype number
angusifolius	59
albus	7
luteus	5
mutabilis	4
cosentinii	3
atlanticus	4
digitatus	1
hispanicus	4
princei	1
pilosus	6
palaestinus	2

# First Stage



## “Core” genotype set

- Kiev-mutant Ukraine, standard Aus. cultivar
- Rosetta New Australian cultivar
- Lucky-1 Single plant from French cultivar
- P27174 Ethiopia, anthracnose-Res
- P25758 Crete, Pleiochaeta Root Rot-Res
- P27593 Azores, Pleiochaeta Root Rot-Res
- XA100 Dwarf, French breeding line
- Start Russian cultivar

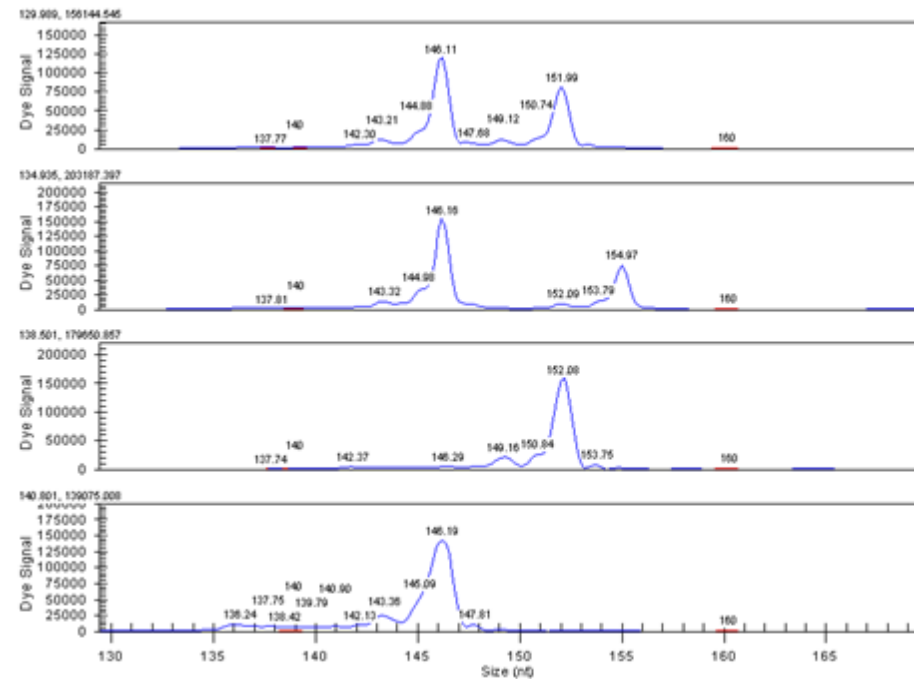
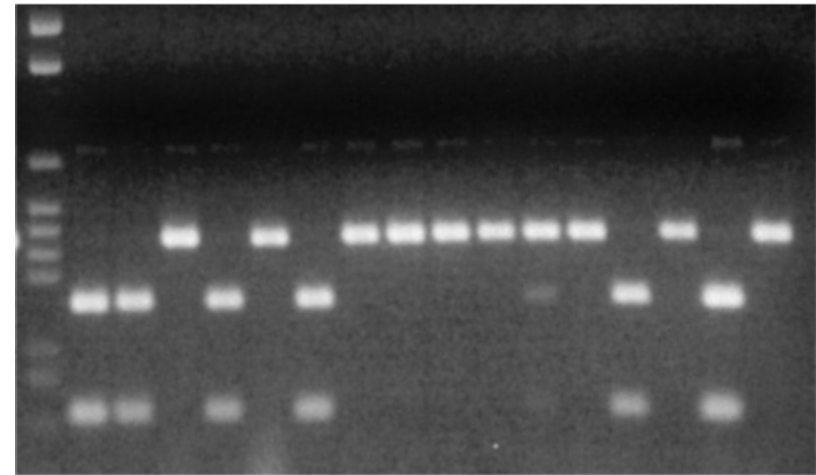
# Analysis

- Leaves – 10 plants/genotype
  - pooled DNA sample
  - capture all variability
  - not all genotypes uniform
  - especially landraces



# Marker choice (ITAPs)

- 63 ITAPs
- Easy to use & cheap
- Polymorphic
- Visualised
  - Gel
    - agarose or PAGE
  - Genotyper machine
    - M13 fluorescent tag
- Scored as dominant
  - DARwin & Anderson software



# Second Stage

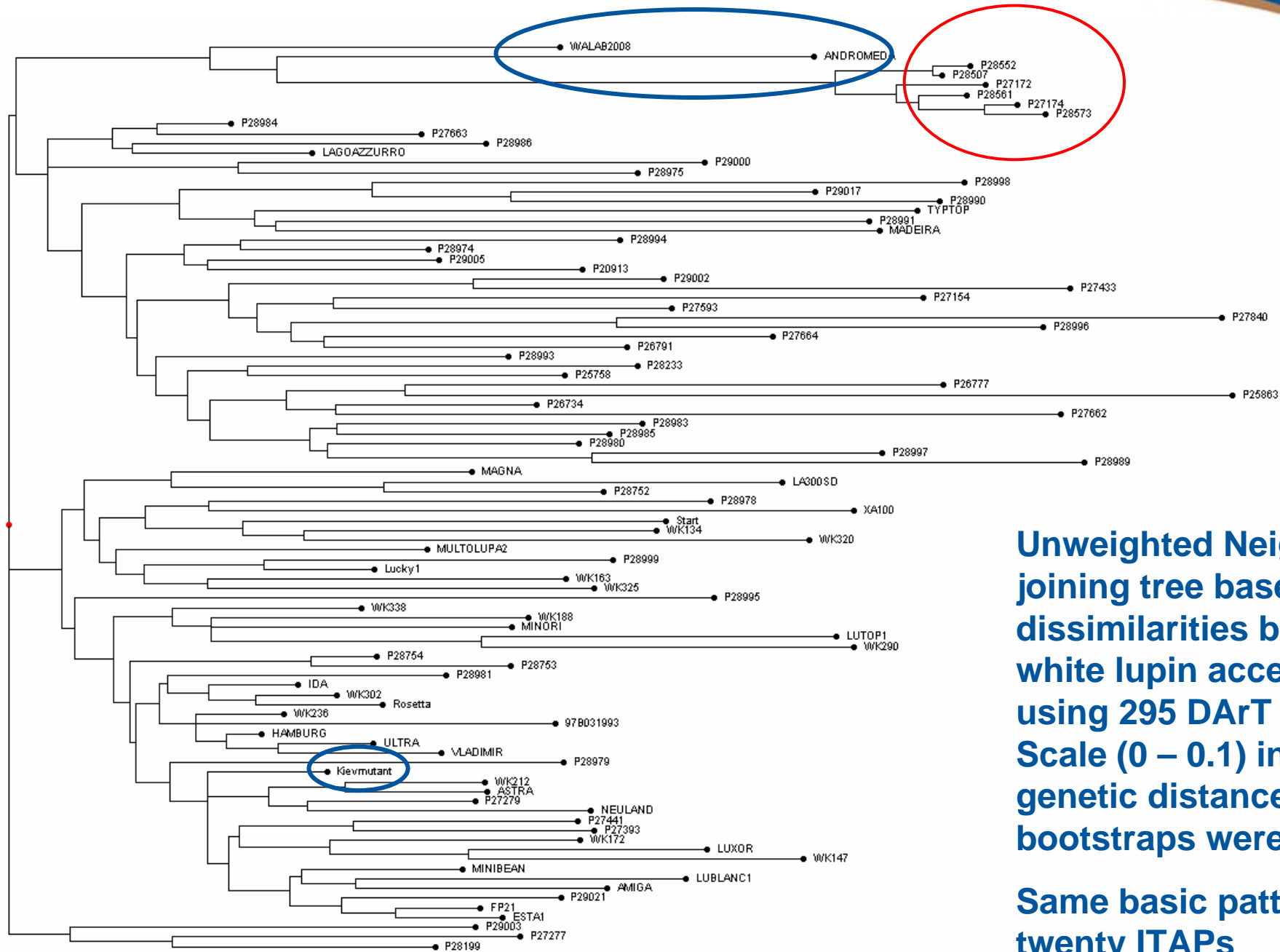


- Extra 86 genotypes (94 total)
- Smaller set of ITAP markers – 20/63
  - gave 50 alleles
  - PT1 gave 8 alleles
- 295 polymorphic DArT markers
  - 2% of total clones on chip

# ITAP markers used

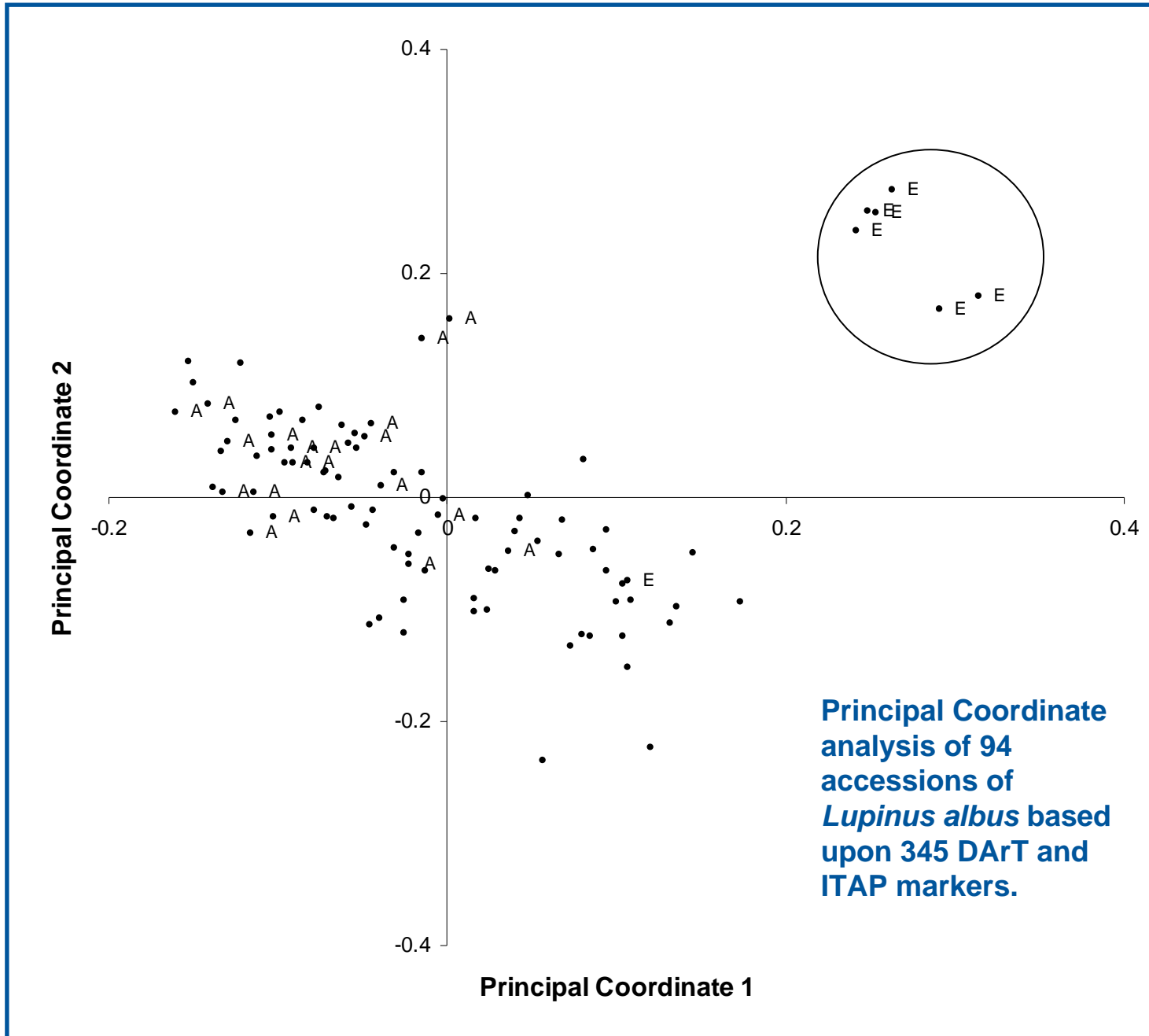
Marker	Assay	Restriction enzyme	Linkage Group*
Lup124	Agarose	Apol	LG15
Lup104	Agarose	HpaII	LG13
Lup195	Agarose	HpyCH4V	LG20
Lup229	Agarose	TasI, TspRI	LG5
Lup264	Agarose	RsaI	LG19
Lup269	Agarose	Apol	LG15
Lup272	Agarose	PsiI	LG12
Lup275	Agarose	Apol	LG7
CHS9	Agarose	-	?
Lup125	Agarose	-	LG27
Lup146	Agarose	-	LG21
AnMtS13	PAGE	-	LG12
GLNA	PAGE	-	LG8
Lup109	PAGE	-	?
Lup197	PAGE	-	LG13
Lup243	PAGE	-	?
Lup273	PAGE	-	LG16
LSSR14	M13	-	?
AnTjNBSM1	M13	-	?
PT1	M13	-	LG21





**Unweighted Neighbour-joining tree based on dissimilarities between 94 white lupin accessions using 295 DArT markers. Scale (0 – 0.1) indicates genetic distance. 1000 bootstraps were used.**

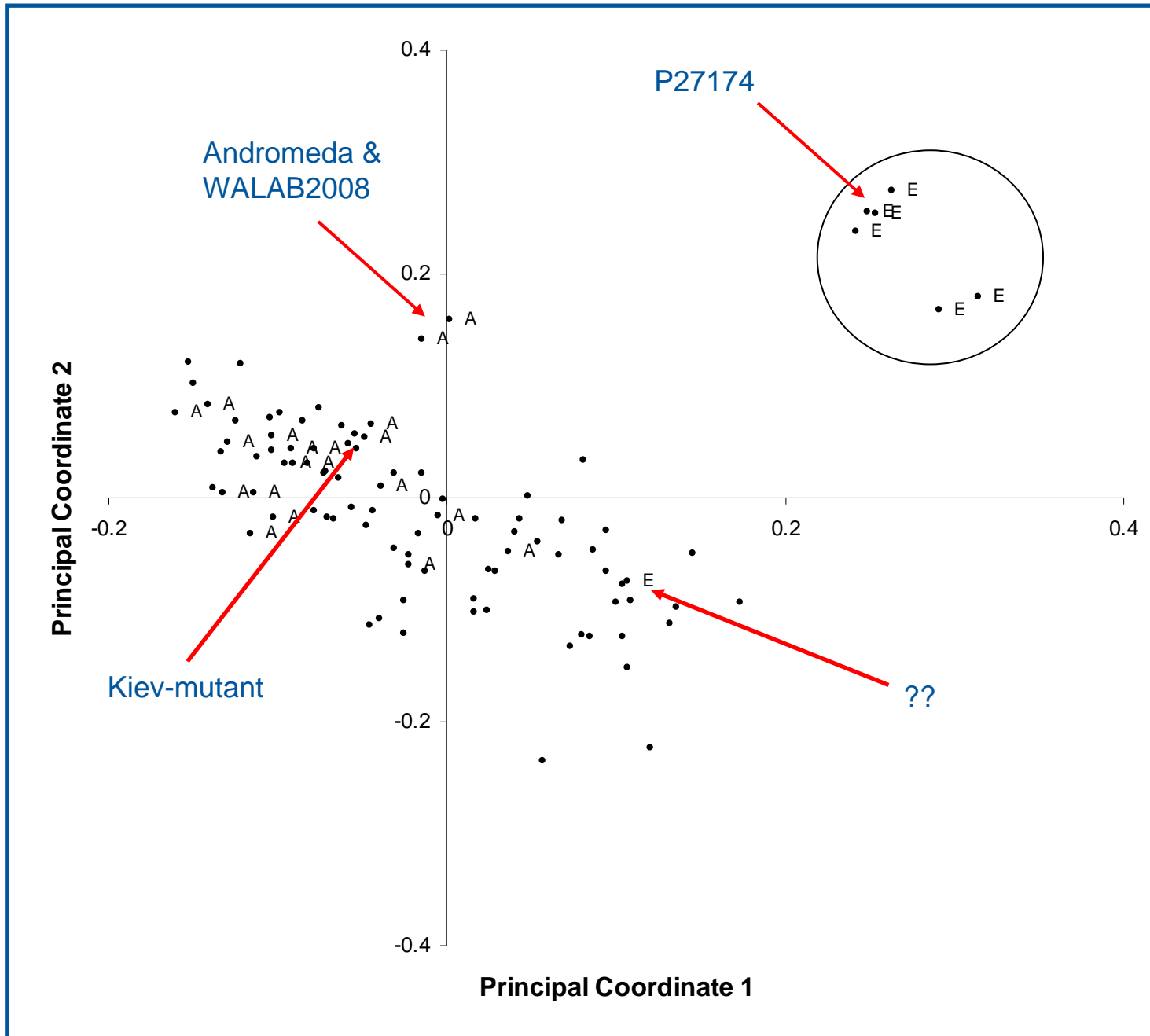
**Same basic pattern as with twenty ITAPs**



**E = Ethiopian landrace**

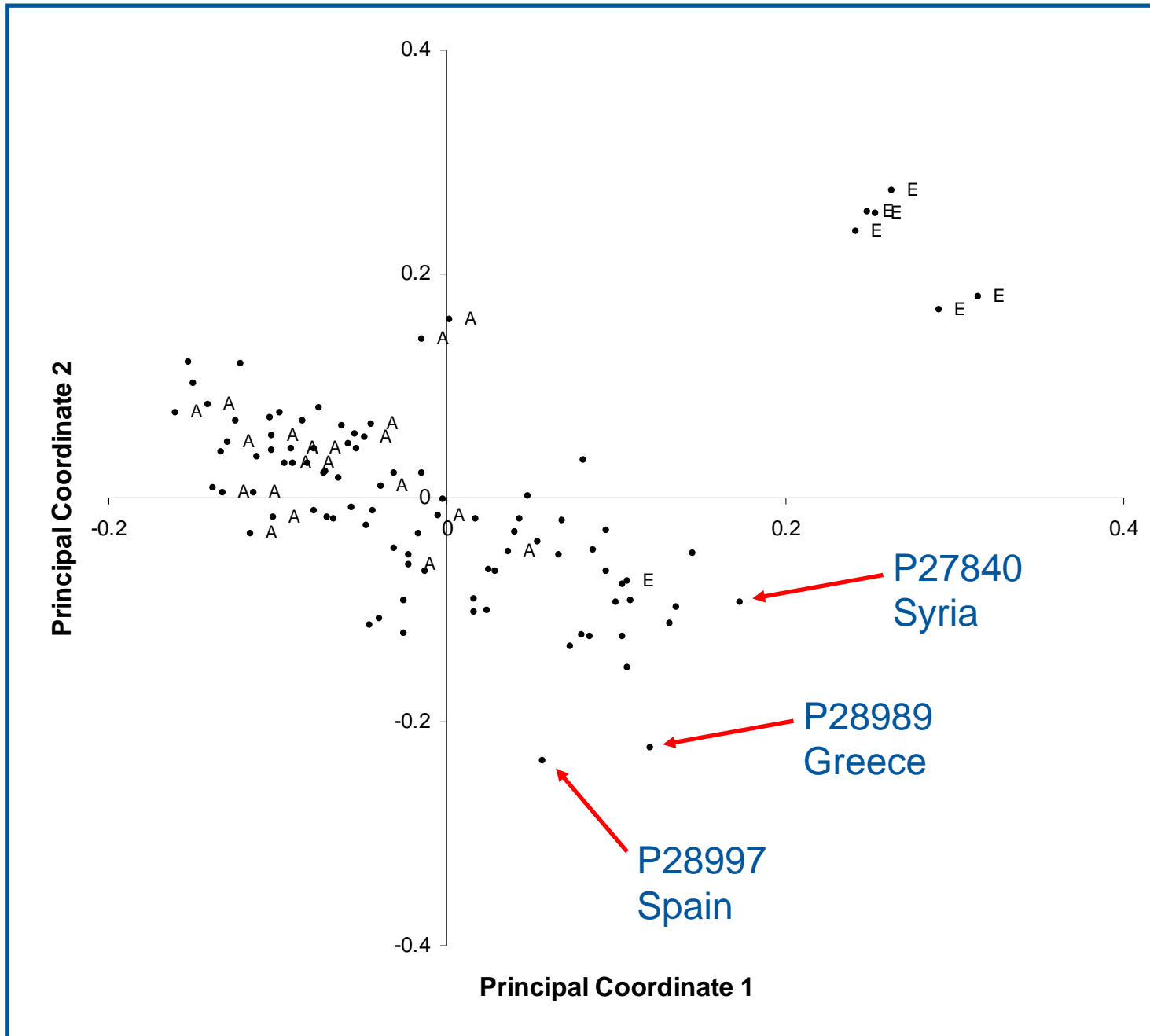
**A = Adapted Australian breeding line or cultivar**

**PC axis 1 – 17%**  
**PC axis 2 – 13%**



**E = Ethiopian landrace**  
**A = Adapted Australian breeding line or cultivar**

**Combined ITAPs and DArT**



Some other  
genotypes are  
quite unusual in  
this small  
collection

# Results

- Considerable genetic diversity
- Ethiopian material is distinct
  - centre of secondary origin?
- Breeding material similar
  - still scope for broadening base
  - to bring new alleles into breeding program



# Future Work

- Screen more germplasm
- Increase map density
  - Map DArT markers
  - SNPs, or albus-specific DArT chip
- Marker-trait associations
  - Locate QTLs
  - Linked markers
    - Phomopsis
- Map second population
  - Kiev-mutant x P25758
    - Pleiochaeta Root Rot





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*an alliance between Charles Sturt University & NSW Department of Primary Industries*