

## ESTIMATION OF GENETIC DIVERSITY IN ALBUS LUPIN (*LUPINUS ALBUS* L.) USING DArT AND GENIC MARKERS

R. Raman, D.J. Luckett and H. Raman

EH Graham Centre for Agricultural Innovation (an alliance between NSW Department of Primary Industries and Charles Sturt University), Agricultural Institute, PMB, Wagga Wagga NSW 2650, Australia

Corresponding author's email: david.luckett@dpi.nsw.gov.au

### ABSTRACT

Ninety four accessions of white lupin (*Lupinus albus* L.) representing local and foreign cultivars, landraces and advanced breeding lines were analysed for allelic diversity with Intron-Targeted Amplified Polymorphisms (ITAPs), SSR motifs, and DArT markers developed from *Lupinus* species. A total of 295 and 50 alleles were identified using DArT and ITAP markers respectively. The number of ITAP/SSR alleles per locus ranged from one to eight. A principal coordinate analysis indicated a high level of genetic diversity in the germplasm and showed that both the genic and the DArT markers used in this study are suitable for estimation of genetic diversity. Landrace accessions from Ethiopia formed a distinct and separate grouping. Australian cultivars and breeding lines were clustered and tended to be distinct from European landraces. These findings will allow us to select appropriate parents, and broaden the genetic base of the white-lupin breeding program.

### KEY WORDS

white lupin, molecular diversity, germplasm, molecular markers, DArT

### INTRODUCTION

White Lupin (*Lupinus albus* L.;  $2n = 50$ ), a member of the Leguminosae family, is an annual grain-legume crop widely grown in Australia and other parts of the world. In Australia, it is grown as an important rotational crop and is useful in controlling diseases and weeds in a crop rotation in a mixed farming system. Due to its association with *Bradyrhizobium* bacteria, lupin fixes atmospheric nitrogen and improves soil fertility. White lupin is an efficient scavenger of phosphorus due to the presence of proteoid roots which secrete organic acids and make phosphorus more available (Dinkelaker, Romheld *et al.* 1989; Gardner, Parbery *et al.* 1982; Neumann and Martinoia, 2002). Lupin seeds are utilised as feed for livestock, and food for human consumption (Barnevelda, 1999; Cowling, 1998; Kattab, 1986; Patterson, 1998).

White lupin and other *Lupinus* species have been fully domesticated only recently when compared with most crops (Noffsinger and Santen, 2005). Genetic

variability especially for extreme temperatures and drought tolerance, and disease and insect resistance in the cultivated germplasm is very low. In order to provide insurance against erosion of the genetic base, lupin germplasm has been conserved at different genetic resource centres in various countries *ex situ*. This resource can be exploited to identify novel alleles, and to broaden the genetic base of lupin breeding programs.

The genetic diversity of white lupin and other species of *Lupinus* has been characterised using morphological and agronomical attributes (Gonzalez-Andres, Casquero *et al.* 2007), isozymes/proteins (Vaz, Pinheiro *et al.* 2004) and molecular markers including randomly amplified polymorphism DNAs, amplified fragment length polymorphism and inter simple sequence repeats (Gilbert, Lewis *et al.* 1999; Talhinhas, Neves-Martins *et al.* 2003). Assessment of genetic diversity on the basis of morphological traits is not very reliable, as it may be influenced by the environment, and the number of traits with known inheritance is small. Molecular markers have the distinct advantages of being independent of climatic variables, and very numerous.

Recently, Phan *et al.* (2007) has located 105 gene-based markers in a mapping population of white lupin. These markers are based on Intron-Targeted Amplified Polymorphisms (ITAPs), and EST-SSR motifs (Nelson, H.T.T., Phan *et al.* 2006). The majority of these markers are locus-specific. The usefulness of these markers in the assessment of genetic diversity has not been determined. However, SSR markers are known for their high levels of polymorphism and suitability for high-throughput analysis in various crops.

Diversity Array Technology-based markers [DArTTM, Jaccoud *et al.* (2001)] are becoming an alternative to the conventional marker technologies, particularly in wheat and barley. These markers are also amenable for high-throughput genotyping and are cost-effective per data point and therefore are suitable for screening large number of individuals. DArT markers have been utilised for germplasm characterisation, molecular mapping of different genomes and development of trait-marker associations in wheat, barley, rice and cassava (Akbari, Wenzl *et al.* 2006; Jaccoud, Peng *et al.* 2001; Stodart, Raman *et al.* 2007; Wenzl, Carling *et al.* 2004; Wenzl, Li *et al.* 2006;

Xia, Peng *et al.* 2005). More recently, DArT P/L developed a platform for molecular analysis of *Lupinus* species (Killian, pers. comm.). The application of the DArT technology for the assessment of genetic diversity in white lupin has not been previously reported. The availability of a suite of markers based upon structural and functional genes (Nelson, H.T.T. Phan *et al.* 2006; Phan, Ellwood *et al.* 2007) plus a DArT lupin chip provided an opportunity to assess the genetic diversity in the germplasm available to the *albus* breeding program at Wagga Wagga, and to compare the two approaches.

## MATERIALS AND METHODS

### PLANT MATERIAL AND DNA EXTRACTION

Seeds of 94 accessions of white lupin (Table 1) representing local and foreign cultivars, landraces and advanced breeding lines were collected from lupin breeding program, located at the New South Wales Department of Primary Industries, Wagga Wagga, Australia. Seeds were grown in row-plots in an insect-proof greenhouse for morphological observations. One leaflet from each of 10 phenotypically-uniform plants per accession/genotype was taken for DNA isolation. The pooled, or bulk, sampling procedure (Gilbert, Lewis *et al.* 1999) was followed as an efficient way to determine genetic diversity. Total genomic DNA was isolated from young leaf tissue from all the accessions, as described by Raman *et al.* (2005).

### PCR BASED MARKER ANALYSIS

Initially a diverse subset of eight genotypes, 'Kiev-mutant', 'Rosetta', 'Lucky-1', 'P27174', 'P25758', 'P27593', 'XA100' and 'Start' was tested for polymorphisms. Sixty three primers were selected from linkage map of *Lupinus angustifolius* L. (Nelson, H.T.T. Phan *et al.* 2006). Polymorphic primers were further used to screen ninety-four accessions of white lupin. Molecular analysis was performed following the recommended PCR profiles (Nelson, H.T.T. Phan *et al.* 2006). Primers generating amplicons below 400 base pairs were tailed with the M13 sequence (Schuelke, 2000). SSR genotyping was performed using labelled M13 primers as described by Raman *et al.* (2005). Amplified DNA fragments were separated on CEQ8000 DNA sequencer (Beckman Coulter Inc.) and their sizes measured using fragment analysis software as recommended by the manufacturer (Beckman Coulter Inc.) (Raman, Raman *et al.* 2005). Primers generating amplicons over 400 bp were used as standard oligos (untailed) and PCR products were separated by electrophoresis on 2.5% (w/v) agarose or on 8% polyacrylamide gels. CAPS analyses were done as described by Nelson, H.T.T. Phan *et al.* 2006. The digested products were resolved on 2% (w/v) agarose gels. All gels were stained with ethidium bromide and visualised under ultraviolet transilluminator.

### DART MARKER ANALYSIS

A *Lupinus* species DArT chip was employed for molecular diversity analysis of the set of 94 genotypes of *Lupinus albus* L. The array comprises 15,000 clones derived from the metagenome of 100 diverse genotypes of *Lupinus angustifolius*, *L. albus* and *L. luteus*. DArT analysis was conducted at DArT P/L (Canberra, Australia). General procedures have been described in Jaccoud *et al.* (2001).

### DATA ANALYSIS

The SSR and CAPS allele data were converted to a presence (1)/absence (0) matrix and was combined with the binary dataset from DArT markers. Polymorphic information content (PIC) index for DArT markers was calculated according to Anderson *et al.* (1993).

Dissimilarity matrices (1000 bootstraps) were calculated for single data based on presence/absence of alleles using the Jaccard coefficient as implemented in DARwin 5 software (Perrier and Jacquemoud-Collet 2006). Cluster analysis was performed using unweighted Neighbour-joining method (Saitou and Nei, 1987) and 1000 bootstraps. A cophenetic correlation was calculated to compare the dissimilarities and the distances as represented in the dendrogram. Principal Coordinate (PCO) analysis was conducted to visualise the genetic relationships among lupin accessions as described by Anderson *et al.* (2003). The first two axes representing the largest components of genetic variance were extracted to generate a scatter plot.

## RESULTS AND DISCUSSION

The markers were intentionally chosen for their ease of marker assaying and scoring (Nelson, H.T.T. Phan *et al.* 2006). Twenty markers were found to be polymorphic (Table 1) among 8 diverse lines.

These were subsequently employed to estimate genetic diversity among 94 accessions of white lupin. A total of 50 alleles were identified by 20 ITAP/SSR markers (data not shown). The most informative locus was PT1, at which 8 alleles were amplified among the 94 accessions. 295 DArT markers were polymorphic with a call rate of more than 80%. The PIC values of DArT markers ranged from 0.04 to 0.50. Given that DArT markers are dominant, the maximum PIC value was 0.5. The large number of DArT marker loci which have high PIC values indicates that the DArT approach is very suitable for assessing genetic diversity in white lupin germplasm.

**Table 1.** Accessions used for assessment of genetic diversity in white lupins using molecular markers. Countries of origin identified using ISO 3-letter country codes. \*indicates a single plant selection origin from the cultivar.

Name	Origin	Type			
97B031-3	AUS	Breeding line	P28752	UKR	Cultivar
AMIGA	CHL	Cultivar	P28753	UKR	Cultivar
ANDROMEDA	AUS	Cultivar	P28754	UKR	Cultivar
ASTRA	FRA	Cultivar	P28974	RUS	Breeding line
ESTA-1**	ZAF	Cultivar	P28975	UKR	Breeding line
FP21	DEU	Breeding line	P28978	UKR	Breeding line
HAMBURG	DEU	Cultivar	P28979	UKR	Breeding line
IDA	DEU	Cultivar	P28980	RUS	Breeding line
KIEV-MUTANT	UKR	Cultivar	P28981	RUS	Breeding line
LA300-SD	FRA	Breeding line	P28983	ESP	Breeding line
LAGO-AZZURRO	AUS	Cultivar	P28984	ESP	Breeding line
LUBLANC-1	FRA	Cultivar	P28985	ITA	Breeding line
LUCKY-1	FRA	Cultivar	P28986	DEU	Breeding line
LUTOP-1	FRA	Cultivar	P28989	GRC	Landrace
LUXOR	AUS	Cultivar	P28990	MAR	Landrace
MADEIRA	PRT	Cultivar	P28991	ISR	Landrace
MAGNA	AUS	Cultivar	P28993	SDN	Breeding line
MINIBEAN	AUS	Cultivar	P28994	UKR	Breeding line
MINORI	DEU	Cultivar	P28995	RUS	Breeding line
MULTOLUPA-2	DEU	Cultivar	P28996	ESP	Landrace
NEULAND	DEU	Cultivar	P28997	ESP	Landrace
P20913	EGY	Cultivar	P28998	ESP	Landrace
P25758	GRC	Landrace	P28999	POL	Breeding line
P25863	GBR	Breeding line	P29000	NLD	Breeding line
P26734	HUN	Landrace	P29002	USA	Landrace
P26777	GRC	Landrace	P29003	ARG	Breeding line
P26791	SYR	Cultivar	P29005	TUR	Landrace
P27154	ESP	Landrace	P29017	POL	Breeding line
P27172	ETH	Landrace	P29021	GER	Breeding line
P27174	ETH	Landrace	ROSETTA	AUS	Cultivar
P27277	ITA	Landrace	START	RUS	Cultivar
P27279	ITA	Landrace	TYPTOP	CHL	Cultivar
P27393	SYR	Landrace	ULTRA	DEU	Cultivar
P27433	SYR	Landrace	VLADIMIR	RUS	Cultivar
P27441	SYR	Landrace	WALAB2008	AUS	Breeding line
P27593	PRT	Landrace	WK134	AUS	Breeding line
P27662	TUR	Landrace	WK147	AUS	Breeding line
P27663	TUR	Landrace	WK163	AUS	Breeding line
P27664	TUR	Landrace	WK172	AUS	Breeding line
P27840	SYR	Landrace	WK188	AUS	Breeding line
P28199	DZA	Landrace	WK212	AUS	Breeding line
P28233	ETH	Landrace	WK236	AUS	Breeding line
P28507	ETH	Landrace	WK290	AUS	Breeding line
P28552	ETH	Landrace	WK302	AUS	Breeding line
P28561	ETH	Landrace	WK320	AUS	Breeding line
P28573	ETH	Landrace	WK325	AUS	Breeding line
			WK338	AUS	Breeding line
			XA100	FRA	Cultivar

Neighbour-Joining trees constructed using 295 DArT and 20 gene-based markers (Figs 1 and 2) showed a high level of genetic diversity in the



None of the DArT markers used here have yet been mapped and validated for their location on the genetic map of white lupin. However, DArT markers are chip-based and several thousand markers can be screened, more efficiently and economically than PCR-based markers. SSR markers are amenable for high-throughput genotyping, but their use is relatively costly. The CAPS markers are also costly, time-consuming and are not suitable for high-throughput analysis. In the present study, it is possible that certain alleles in pooled samples may have remained silent/unamplified. Nevertheless, our results suggest that molecular markers based upon ITAP and DArT are suitable for assessment of genetic diversity in white lupin. Findings will allow us to increase the efficiency of assessment of germplasm for various traits of interest.

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