A GENETIC LINKAGE MAP FOR WHITE LUPIN (*LUPINUS ALBUS* L.)

Dănuţ SIMIONIUC[1], Mădălina BURLACU-ARSENE[1], Violeta SIMIONIUC[1], Florin LIPSA[1]
E-mail: simion@uaiasi.ro

Abstract

We report about the constructing of the linkage map of white lupin (*Lupinus albus* L.). For constructing of this map only RAPD markers were used. Sixty arbitrary primers (10-meres) from firma Roth (Germany) were used for testing RAPDs polymorphism in the F2 recombinant inbred line population. The F2 population of the Kiew X Lublanc cross population was selected for mapping studies. The software MAPMAKER version 3.0 was used for the multi-point analysis with LOD ≥ 3. Statistical analysis provided the map position of 48 RAPDs loci. The length of the generated linkage map is close to 620 cM with an average distance between markers close to 14 cM. The markers are distinguished in 17 linkage groups.

Key words: linkage map - RAPD markers - white lupin

Lupin species have a relatively short domestication history compared with most crops. Focused breeding efforts began in Germany during World War I due to a need for high-protein pulse crops adapted to temperate conditions. Subsequent breeding has concentrated on the introduction of key traits such as early flowering, reduced pod-shattering, soft seed and anthracnose disease resistance (Phan et al., 2007). Lupin grain is high in protein (30– 40%) and in dietary fibre (30%), low in oil (6-12%), and contains minimal starch (Lindbeck et al. 2001, Noffsinger et al. 2005). Lupin has the lowest Glycaemic Index of any commonly consumed grain (www.glycemicindex. com), which has significant implications for Western societies with an increasing incidence of obesity and associated risk of diabetes and cardiovascular disease (Uauy et al. 1995). Lupin fibre acts as a soluble fibre and drops total cholesterol levels without affecting beneficial HDL cholesterol (Hall et al. 2004). White lupin itself is used as feed for livestock and has established a growing market for human consumption due to the development of low alkaloid varieties with a lack of protease inhibitors. (Hamama et Bhardwaj 2004). In addition, white lupin has become an illuminating model for the study of plant adaptation to extreme phosphorus (P) deficiency (Dinkelaker et al. 1989, Massonneau et al. 2001, Neumann et Artinoia 2002).

Nowdays, in Europe exists the tendency to avoid some of the negative effects caused by the hyperintesivity of agriculture such as the genetical erosion. The white lupin due to its characteristics (content and quality of proteins very good, nitrogen fixing attributes, allowing phosphate mobilisation, capacity to be use in integrated farming systems and environmentally sustainable agriculture) could become a very interesting specie for the farmers. Based on our 15 years researches regarding white lupin, we want to use modern methods based on molecular markers (RAPD, AFLP and SSR) for complex genetic analysis of existing germplasm.

The objective of this study was to realise a skeletical genetic map using RAPD markers for a F2 population resulted from a cross between the white lupin genotypes Kiew and Lublanc.

MATERIAL AND METHOD

For constructing of this map only RAPD markers were used. Sixty arbitrary primers (10-meres) from firma Roth (Germany) were used for testing RAPDs polymorphism in the F2 recombinant inbred line population. The F2 population of the Kiew X Lublanc cross population was selected for mapping studies. To produce the F2 population, Kiew was used as the female parent and Lublanc as the male. Total genomic DNA was extracted as previously described by Doyle and Doyle (1990).

RAPD (*Random Amplified Polymorphic DNA*) was descovered by Williams et al. in 1990. The method is fast, relatively cheap and well adapted to obtain an unradioactive genetic fingerprint (Welsh et McClelland 1990). It use a single primer (with a low number of nucleotids, usually 10) in an amplification reaction (Williams et al. 1990). Were reported some problems regarding the de reproductibility of the method and some software erorrs for markers evaluations (Demeke et al. 1997, Karp et al. 1997).

[1] University of Agricultural Sciences and Veterinary Medicine, Iaşi

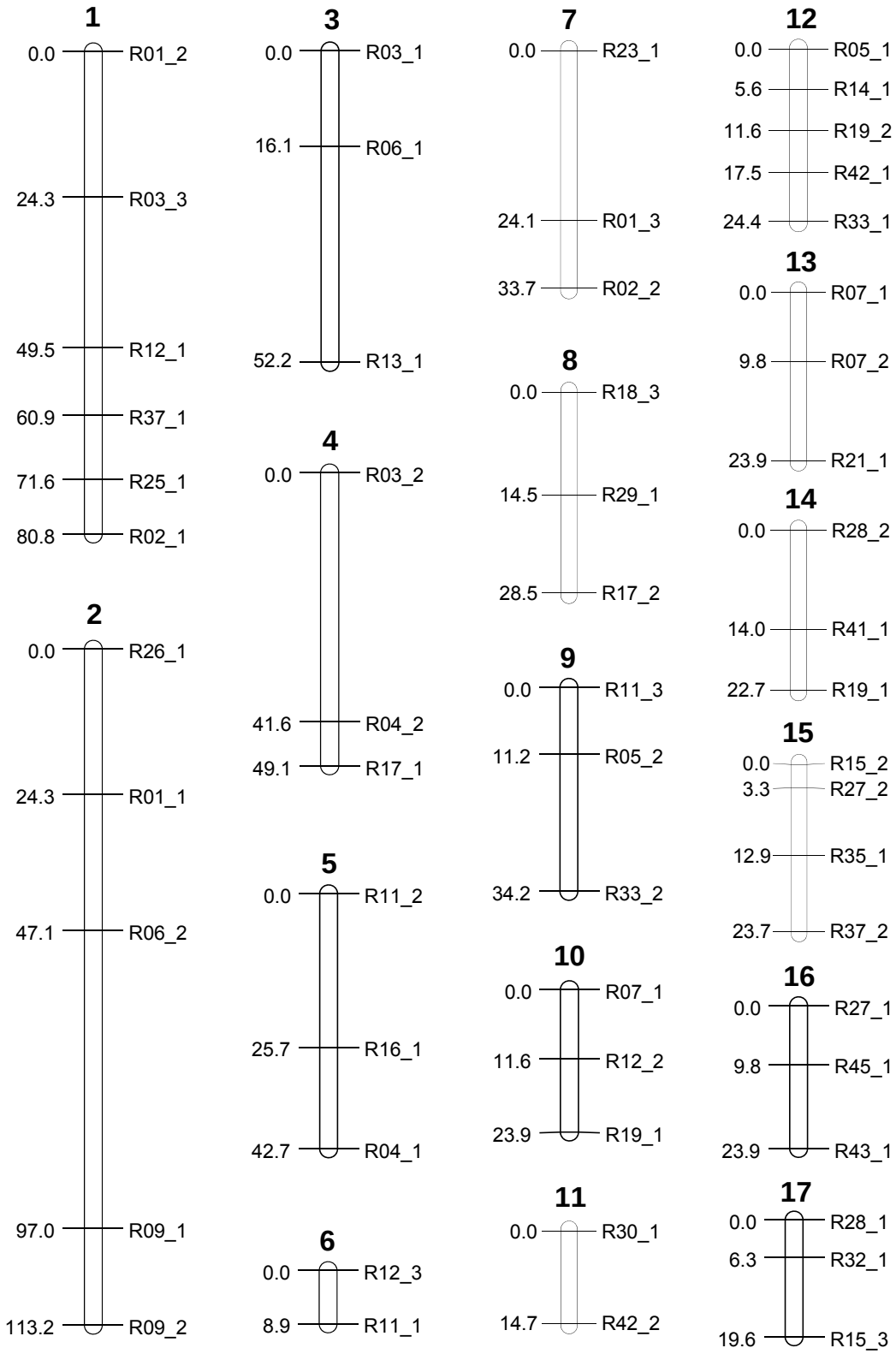

Figure 1 **A genetic linkage map of Lupinus albus based on 48 loci in 17 linkage groups**

A single primer made of ten nucleotids will identify homologs sequences in analysed DNA and will cause the amplifications in different areas of the genome with the length from 200 up to 2000 kb which could be found between two complementary copies of it. Statistic, the identification chance of an homologue sequence is around 1/1000000 bp.

During PCR reaction will be generated a set of fragments of different sizes and, because the fragments are amplified, will exist enough DNA which could be seen by staining with ethidium bromid. Generally, for a medium size genome will be generated between 5 and 10 fragements. Could be used different primers and are practically an unlimited number of RAPDs in one genome. The method is available without a previous known of the analysed genome and could be used universal primers (Bardakci, 2001).

DNA amplified fragments are separated by electrophoresis in an agarose gele. The staining will be made with ethidium bromid, and the DNA stripes will be seen by exposure of the gele which contain DNA marked at a UV sources.

Images will be obtain which will be scanned and lately processed with the help of a specific software.

The method is used mainly to identify genetic diversity and phylogeny studies (Simioniuc et al. 2002).

The software MAPMAKER version 3.0 (Lander et al., 1987) was used to view the consistency of marker orders across population.The Kosambi mapping function (Kosambi, 1944) was used to convert recombination frequency into genetic map distance. Linkage was considered significant if the LOD score was ≥3.0.

RESULTS AND DISCUSSIONS

A total of 48 polymorphic markers (RAPD-Marker) covering 620.1 cM were localized in the genetic map for F2 population (figure 1). Linkage mapping identified 17 major linkage groups named continuously from 1 to 17.

The mean size of chromosomes is 36.5 cM, and this corresponds to an average marker distance of 12.9 cM and 3 markers per chromosome. The largest linkage group (2) with 113.2 cM consists of 5 RAPD markers and the smallest (6) has 8.9 cM and consists of 2 RAPD markers.

CONCLUSIONS

We present a new genetic map of white lupin that will serve as a strong foundation for future genomic research in this important grain legume species. 46 polymorphic Markers (126 AFLP and 50 SSR-Marker) covering 620.1 cM were localized in the genetic map for F2 population.

AKNOWLEDGEMENTS

This work was supported by CNCSIS - UEFISCU, project number PNII – IDEI 2033/2008 (contract no. 1108/2009).

BIBLIOGRAPHY

Bardakci, F., 2001 – *Random amplified polymorphic DNA (RAPD) markers*. Turk. J. Biol. 25:185–196.

Demeke, T., Adams, R. P., Chibbar, R., 1992 - *Potential taxonomic use of random amplified polymorphic DNA (RAPD): A case study in Brassica*. Theor. Appl. Genet. 84: 990-994.

Dinkelaker, B., Romheld, V., Marschner, H., 1989 - *Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (Lupinus albus L.),* Plant Cell Environ., 12, 285–292.

Doyle, J.J., Doyle, J.L., 1990 - *Isolation of plant DNA from fresh tissue,* Focus, 12: 13-15.

Hall, R.S., Johnson, S.K., Baxter, A.L., Ball, M.J., 2004 - *Lupin kernel fibre-enriched foods beneficially modify serum lipids in men,* Eur. J. Clin. Nutr., 59: 325–333.

Hamama, A.A., BHARDWAJ, H.L., 2004 - *Phytosterols, triterpene alcohols, and phospholipids in seed oil from white lupin,* J. Am. Oil Chem. Soc., 81: 1039–1044.

Kosambi, D. D., 1944 - *The estimation of map distances from recombination values*, Ann. Eugen. 12:172-175.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly M.J., 1987 - *MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations*, Genomics **1**:174-181.

Lindbeck, K.D., Murray, G.M., Priest, M., Dominiak, B. C., Nikandrow, A., 2001 - *Survey for anthracnose caused by Colletotrichum gloeosporioides in crop lupins (Lupinus angustifolius, L. albus) and ornamental lupins (L. polyphyllus) in New South Wales, Australasian* Plant Pathology, 27: 259–262.

Massonneau, A., Langlade, N., Leon, S., 2001 - *Metabolic changes associated with cluster root development in white lupin (Lupinus albus L.): relationship between organic acid excretion, sucrose metabolism and energy status,* Planta, 213: 534–542.

Neumann, G., Artinoia, E., 2002 - *Cluster roots—an underground adaptation for survival in extreme environments,* Trends Plant Sci., 7: 162–167.

Noffsinger, S.L., Van Santen, E., 2005 - *Evaluation of Lupinus albus L. Germplasm for the Southeastern USA,* Crop Sci, 45: 1941–1950.

Phan, H.T.T., Ellwood, S.R., Adhikari, K., Nelson, M. N., Oliver, R.P., 2007 - *The First Genetic and Comparative Map of White Lupin (Lupinus albus L.): Identification of QTLs for Anthracnose Resistance and Flowering Time, and a Locus for Alkaloid Content* DNA Res 14: 59-70.

Simioniuc, D., Uptmoor, R., Friedt, W., Ordon, F., 2002 - *Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs*, Plant Breeding 121: 429-435.

Uauy, R., Gattas, V., Yanez, E., 1995 - *In Simopoulos, A. M. (Ed.). World Review of Nutrition and Dietetics.* Karger, Basel, Vol. 77: 75–88.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V., 1990 - *DNA polymorphisms amplified by arbitrary primers are useful as genetic markers*. Nucleic Acids Research 18: 6531-6535.

******* - www.glycemicindex.com.