Use of Diversity Array Technology (DArT) for Genotyping of *Humulus lupulus* L.

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**Abstract**

Diversity Arrays Technology (DArT - www.diversityarrays.com) is a microarray based DNA marker technique for genome wide discovery and genotyping of genetic variation. DArT potentially allows simultaneous scoring of thousands of restriction site polymorphisms between genotypes and does not require DNA sequence information or site specific oligonucleotides. An international consortium (Australia, USA, UK, Slovenia) screened 86 (cultivated, wild; female, male) accessions of hop from Europe, North America, Asia and Australia, including examples of *Humulus lupulus* var. *lupuloides*, *H. lupulus* var. *pubescens* and *H. lupulus* var. *neomexicanus*, using DArT. The accessions included key current and historical, high and low α-acid, aroma, triploid, dwarf, and powdery and downy mildew resistant and susceptible cultivars. DArT identified 730 polymorphic markers, with reproducibility approaching 100%, and a high call rate. Preliminary studies of the data indicate that a large proportion of the markers reflect differences between wild and cultivated material. Distinction can be made between and within material of European, North American and Asian origin, with related accessions, and accessions from a single cultivar known by several synonyms clustering together. Initial investigation suggests that the reproducibility of DArT markers, the level of genetic variation detected with this system in hop, and the resolution of known genetic affinities are positive signs that DArT will provide the platform for future exploration of marker-trait associations to develop marker assisted selection.
technologies in hop. Based on this study, the DArT platform provides a reproducible, accurate, high throughput genotyping tool for hop. In the future this work will be developed to increase the number of markers available for hop using DArT, and to generate linkage maps and identify quantitative trait loci for key traits in a number of families.

INTRODUCTION

The genus *Humulus*, (*Cannabinaceae, Urticales*), consists of three species: *H. lupulus*, *H. japonicus* and *H. yunnanensis*; and is indigenous throughout much of the northern hemisphere (Neve, 1991). Original wild populations of the cultivated hop may be further classified into taxonomic varieties; var. *lupulus* for European wild hops and cultivars, var. *cordifolius* for Japanese wild hops, and var. *neomexicanus, pubescens* and *lupuloides* for North American hops (Small, 1978). Commercially grown hop is propagated clonally, and often cultivars have poor adaptability outside the environment in which they were selected. Therefore, hop breeding programs, operating largely independently, have been important in the development of hop production in Europe, North America, South Africa, Australia and New Zealand. Several of these breeding programs have been successful in producing triploid seedless cultivars.

Historically, assessing genetic variation in hop relied upon morphological markers, biochemical markers such as essential oils and flavonoids, or combinations of biochemical and agronomic traits. Key studies using molecular markers (random amplified polymorphic DNA - RAPD, amplified fragment length polymorphisms - AFLP, sequence tagged sites - STS, inter-simple sequence repeats - ISSR) to assess genetic diversity in hop were summarised by Henning (2004), who found that few studies had included genetic material from native North American populations. Subsequently, microsatellite data have been used to investigate the relationship between wild genotypes and cultivars (Jakse et al., 2004), wild genotypes from the throughout the natural distribution of the genus *Humulus* (Murakami et al., 2006a), and DNA sequences from the nuclear ribosomal DNA spacer region and noncoding regions of hop chloroplast DNA were used for phylogenetic reconstruction in wild hop (Murakami et al., 2006b).

Diversity Arrays Technology (DArT) is a molecular marker system with the ability to detect and type DNA variation similar to AFLP markers at several hundred genomic loci in parallel without relying on sequence data (Jaccoud et al., 2001; Wenzl et al., 2004). Since proof of concept studies in rice (Jaccoud et al., 2001), the DArT system has been employed for marker discovery (*Manihot esculenta*, cassava - Xia et al., 2005; *Cajanus cajan*, pigeonpea - Yang et al., 2006; *Solanum lycopersicum*, tomato - Van Schalkwyk et al., 2008) and linkage mapping (*Hordeum vulgare*, barley - Wenzl et al., 2004; *Arabidopsis thaliana* - Wittenberg et al., 2005; *Triticum aestivum*, wheat [hexaploid] - Akbari et al., 2006).

The aim of the present international collaboration was to assess the ability of DArT to identify highly polymorphic and reproducible molecular markers in hop. If successful, evidence from other systems suggests that DArT can provide a cost effective, high throughput genotyping tool. The present paper reports some basic parameters of the DArT markers discovered in hop and examines the molecular variation uncovered in hop using DArT in relation to previous studies.

MATERIALS AND METHODS

An international consortium (Australia, USA, UK, Slovenia) screened 86 (cultivated, wild; female, male) accessions of hop from Europe, North America, Asia and Australia, including examples of *Humulus lupulus* var. *lupuloides*, *H. lupulus* var. *pubescens* and *H. lupulus* var. *neomexicanus*, using DArT. The accessions included key current and historical, high and low α-acid, aroma, triploid, dwarf, and powdery and downy mildew resistant and susceptible cultivars. Samples were sourced from the collections held by John I Haas, USDA ARS National Genetic Resources Program.
Germplasm Resources Information Network (GRIN - Beltsville, Maryland. http://www.ars-grin.gov/cgi-bin/npgs/html/site.pl?COR, 2008) and the Slovenian Institute of Hop Research and Brewing, Žalec. Pedigree information (where available) for many of the commercial cultivars and breeding lines has been published previously (Brady et al., 1996; Šuštar-Vozlič and Javornik, 1999; Seefelder et al., 2000; Patzak, 2001).

The processes of DArT array development and marker discovery have been described previously (Jaccoud et al., 2001; Wenzl et al., 2004; Wittenberg et al., 2005; Xia et al., 2005; Akbari et al., 2006; Yang et al., 2006; Van Schalkwyk et al., 2008). Reproducibility, call rates and polymorphism information content (PIC - Anderson et al., 1993) were estimated by DArT. Principle Co-ordinates analysis (PCoA) was used for preliminary analysis of genetic variation.

RESULTS AND DISCUSSION

Prior to this study the DArT system had demonstrated a virtually constant (low) genotyping error rate across genomes with a threefold difference in ploidy level and an up to 150-fold variation in size (Akbari et al., 2006). To date, DArT has identified 730 polymorphic clones (markers) in the hop accessions supplied. The reproducibility and call rates of DArT markers in hop appears to be similar to those found in other species (barley - Wenzl et al., 2004; Arabidopsis - Wittenberg et al., 2005; pigeonpea - Yang et al., 2006). The high frequency of markers with PIC values above 0.31 appears similar to that found in DArT studies of pigeonpea (Yang et al., 2006) and barley (Wenzl et al., 2004).

Preliminary PCoA showed a large divergence between North American wild accessions and those accessions of European origin, whether wild or cultivated, with most cultivars and breeding lines more closely related to each other than to wild North American material. The major identifiable groups included i) European and Asian wild and cultivated (cultivars and breeding lines) accessions in one group, ii) most of the cultivars known to be derived from hybridisation between European and North American genotypes in another group, and iii) the wild North American genotypes in the third group. The structure of variation observed agreed well with previous studies of the molecular variation within wild (Murakami et al., 2006a,b), among wild and cultivated genotypes (Jakse et al., 2004), and within hop cultivars (Hartland Seefelder, 1998; Seefelder et al., 2000; Patzak, 2001).

Future work on this data set will include more in-depth data analysis to construct a dendrogram, perform principle co-ordinates analysis and, while recognising the limitations of this data set due to the number of cultivars in certain groups, investigation of levels of genetic variation and population differentiation observed between and within groups, sexes and cultivated and wild accessions in this subset genetic variation of hop.

CONCLUSIONS

This international collaboration has been based on the concept that marker discovery and validation are pre-competitive research with benefits to all parties involved. It is hoped that the present co-operation leads to future collaboration between those involved, but it is recognised that with validation of the methods and discovered markers, individual members of the collaboration are free to use the technology in isolation if they wish. The molecular variation identified thus far is consistent with what is known from previous studies. Examination of the discriminatory power of DArT markers in hop is currently underway, with an expanded set of polymorphic markers being developed in order to genotype 3 crosses for linkage mapping and QTL identification. Currently the experimental development of DArT markers for hop is being conducted by the original consortium, with the addition of HortResearch (New Zealand) who are providing genetic material for the second round marker discovery and linkage mapping.
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Literature Cited

