



Diversity array technology (DArT) for the rice breeding program

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IN A RICE HULL

- ▶ Diversity array technology (DArT) provides a powerful tool for measuring genetic diversity among breeding lines and cultivars at the molecular level – the technology provides a means for "fingerprinting" rice varieties
- ▶ DArT can be used in breeding programs to compare the DNA of parent lines and their progeny
- ▶ Diversity panels (the means of setting out all the genetic information) have been generated and analysed using DArT for 243 diverse rice varieties from around the world, including 50 varieties used extensively in the breeding program at Yanco
- ▶ A sub-set of 69 semi-dwarf lines were phenotyped for seedling vigour from a specific cross, and DArT analysis of the highest and lowest vigour lines showed a total of 61 DArT markers that varied – raising the prospect of using DArT analysis to select for specific DNA fragments associated with traits needing improvement, such as seedling vigour

Rice has been an important crop in the development of biotechnology. Much research has focused exclusively on rice, and outcomes include a wide range of molecular markers and the sequencing of the entire rice genome.

The development and use of molecular markers for simply inherited traits which are influenced by one or two genes is relatively straightforward, and the rice breeding program at Yanco Agricultural Institute regularly uses markers to select for amylose content in grain which influences grain quality, and to select for fragrance. However, the rice breeding program is also aimed at improving complex traits such as yield and grain quality. These complex traits are determined by the action and interactions of many genes spread across the entire genome.

DArT technology offers a means of representing the entire genome of individual breeding lines, allowing detailed comparison of progeny with parents. DArT analysis shows, at a large number of specific regions within the genome, the parent from which DNA at that specific location is derived.

Assessing genetic similarity

The initial phase of the project involved development of a DArT reference panel, constructed using approximately 50 varieties and breeding lines commonly used in the breeding program at Yanco, as well as genetically diverse germplasm

from other species of rice (*Oryza glaberrima* and *O. rufipogon*) used in other international research programs. A total of 243 varieties were used in the development of the final DArT reference panel. This is an array of 6144 DNA fragments, each of which showed variation among the 243 reference varieties.

Second, a complete set of Yanco varieties and breeding lines were genotyped using this reference panel, providing an index of the genetic similarity and relationships between cultivars.

Third, a series of segregating lines were genotyped using the array after detailed phenotyping for seedling vigour under controlled-environment conditions. Seedling vigour was measured using a range of traits including seed size, the time taken to emerge from the soil, the rate of shoot elongation immediately following emergence, and the leaf area and dry matter produced after approximately three weeks of growth. There was a total of 61 DNA fragments (DArT markers) which varied among the parents and the high and low vigour lines.

Using DArT to select for seedling vigour

An example of the DArT score table is shown in Figure 1.

The parental lines are shown in the two left-most columns, the nine high-vigour lines are shown in the next nine



columns, while the nine low-vigour lines are on the right-hand side of the table. For example, in the first row of the table, most of the high-vigour lines have the DNA fragment or marker for vigour from the WAB 450-I-B-P-160 parent, indicated by the pink box marked '0'; while in the low-vigour lines it is mostly from Quest, indicated by the green box marked '1'.

Further analysis is necessary to associate phenotypic (physical) performance with the constellation of DArT markers necessary for high vigour. Three markers were strongly associated with seedling vigour, this raises the option of sequencing the fragment to determine the genes involved.

The subsequent rows in the table are for each DNA fragment which varied among the lines selected for high and low seedling vigour. Many of these were only weakly associated with differences in seedling vigour and represent other genetic differences among the lines.

Project outcomes

This project has resulted in the development of a DArT reference panel which can be used to represent the genome of future breeding lines and introduced varieties. It has already been used to highlight the genetic similarity between an *Oryza sativa*/*O. glaberrima* inter-specific cross and *O. sativa japonica* sub-species.

Analysis of Yanco varieties and breeding lines showed that

each variety has a unique pattern of DArT markers, and the technology is therefore a means of "fingerprinting" NSW varieties providing useful additional information for Plant Breeder's Rights protection.

DArT analysis of the lines developed from the *Oryza sativa*/*Oryza glaberrima* inter-specific cross is unlikely to lead to markers for seedling vigour traits as the population was already subject to strong selection for adaptation to the NSW environment, semi-dwarf stature and agronomic acceptability. This reduced the population size significantly and hence the opportunity to confirm relationships between DArT markers and seedling vigour traits.

In summary, DArT analysis provides a powerful tool for measuring genetic diversity among lines and cultivars at the molecular level. It will prove useful for existing and future crosses and backcrosses between NSW cultivars and other species such as *Oryza rufipogon* to determine the extent to which sections of DNA from *O. rufipogon* genome can be inserted (introgressed) into NSW cultivars, to give rise to new and useful genetic variation. It will continue to be used for specific crosses and populations, with careful attention to the population structure and the extent of selection imposed.

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WAB450-I-B-P-160-HBS1	QuestS1	YSB04_18:55	YSB04_19:146	YSB04_18:59	YSB04_19:219	YSB04_19:287	YSB04_19:139	YSB04_19:249	YSB04_20:273	YSB04_18:122	YSB04_19:156	YSB04_18:35	YSB04_19:91	YSB04_18:165	YSB04_19:281	YSB04_19:287	YSB04_18:136	YSB04_19:167	YSB04_19:279
0	1	0	0	0	1	0	1	0	0	0	1	1	1	1	0	1	0	1	0
1	0	1	1	1	1	0	1	1	1	0	0	0	1	0	1	0	0	0	1
1	0	0	1	0	1	1	0	1	1	1	0	0	0	0	1	0	1	0	1
1	0	0	1	0	1	1	0	1	1	1	0	0	0	0	1	0	1	0	1
1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	1	1
0	1	0	0	0	0	0	1	0	1	1	1	1	1	1	0	1	1	1	0
0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0
0	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1	1
0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	1	1	0	1	1	0		0	1	0	1	1	1	1	1		1	1	1
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0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0
0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0
0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0
1	0	0	1	0	1	1	0	1	1	1	0	0	0	1	0	1	0	1	1
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0
0	1	0	0	1	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0
1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	1	1
1	0	1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	1	0
0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0
0	1	0	0	1	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0
1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	1	1
1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	1	1
0	1	0	0	0	1	0	1	0	1	0	1	1	1	1	0	1	0	1	0
0	1	0	0	1	0	0	1	0	1	0	1	1	1	1	0	1	0	1	0
1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	1	1

Figure 1 Score table for 21 of the 61 DArT markers (DNA fragments) which varied among the parents and nine high and nine low vigour progeny from the cross. Parents are shown in the first two columns on the left, followed by nine high-vigour progeny, and nine low-vigour progeny on the right. Each row of coloured boxes represents DNA fragments which varied among the parents and high and low vigour lines. Thus for each DArT marker, the table indicates from which of the parents the DNA segment was derived. For example, in the fifth row of the table, all the high vigour lines have DNA derived from Quest, while only three of the low-vigour lines have Quest DNA at this location.