PRESENTATION OF THE ADVANTA SUNFLOWER RFLP LINKAGE MAP FOR PUBLIC RESEARCH

S.T. BERRY[1], A.J. LEON[2], R. PEERBOLTE[3], P. CHALLIS[1], C. LIVINI[1], R. JONES[1] and S. FEINGOLD[2]

[1] Jealott's Hill Research, Bracknell, Berkshire, RG42 6EY. UK.
[2] Balcarce Research Station, Ruta 226 Km 60.3, Casilla de Correo 30, 7620 Balcarce, ARGENTINA.
[3] Rilland Research Station, 4410 AA Rilland PO Box 1, THE NETHERLANDS.

INTRODUCTION

Cultivated sunflower (*Helianthus annuus* L.) is a diploid species ($2n=2x=34$) and is second only to soybean as an annual oilseed crop. Despite its economic importance, sunflower biotechnology is in its infancy when compared to other major crop plants. Sunflower is also disadvantaged in that the genetics of very few traits has been studied in any detail and as a consequence no classical genetic map exists for this species. However, in the last two years several genetic linkage maps have been published based on either RFLPs (Berry *et al.* 1995, 1996; Gentzbittel et al. 1995; Vick *et al.* 1996) or AFLPs (Peerbolte & Peleman, 1996). The relationship between the various linkage groups identified in the three published RFLPs maps is not known and up until now the probes have not been available to public institutions. The lack of DNA markers and a public map has obviously hindered academic research on sunflower. Therefore Advanta, which is the new seed company formed from the merger of ZENECA Seeds and Van der Have, are prepared to release a subset of 81 RFLP markers, evenly-spaced across the 17 linkage groups, to key academic groups to establish collaborative research projects.

MATERIALS AND METHODS

All experimental protocols and mapping procedures have been published by Berry *et al.* (1994, 1995 and 1996).

RESULTS & DISCUSSION

RFLP probes

Table 1 lists the 81 probes selected from the composite map published by Berry *et al.* (1996); however the probes have been re-coded and given the prefix ZVG. Only 3 of these probes are *Pst*I genomic clones (ZVG0009, ZVG0016 & ZVG0051), the rest are cDNA clones derived from mRNA isolated from etiolated seedlings. These probes were chosen from the 612 available on the basis of having:

a) simple and easy to score hybridisation patterns for fingerprinting germplasm (*i.e.* single locus probes)
b) a high polymorphic index (PI)
and c) providing good genome coverage

Table 1 also gives the PI's for 64 of probes and these values are based on the allele frequencies seen in 263 sunflower inbred lines from around the world. The mean PI is 0.544; however the values vary from 0.165 (ZVG0074) to 0.819(ZVG0019). This large range in PI is due to the main selection criteria being for simple hybridisation pattern in order that allelic variants may be scored unequivocally. The discriminatory power of these RFLP probes has all ready been demonstrated by their ability to clearly separate B and R lines (Berry *et al.* 1994). Commercial hybrid seed production is based on a cytoplasmic male sterility (CMS) system which has functionally divided sunflower germplasm into males (R lines) and females (A/B lines). Breeders generally confine their inbred line development to crosses made within these two groups, which has resulted in the production of two very broad heterotic groupings. In general, one would expect approximately 60% of RFLP markers to be polymorphic in a B x R cross.

Public RFLP linkage map

The 81 probes selected for release detect 81 loci which cover approximately 1200 cM of the sunflower genome and are on average 15 cM apart. The markers are arranged into 17 linkage groups (Figure 1), which probably correspond to the 17 haploid chromosomes of *H. annuus*. The map distances are taken from the composite map described by Berry *et al.* (1996) which was based on segregation data from nine different F_2 populations (Table 2) and constructed using JoinMap (Stam, 1993). The F_2 population HA89 x RHA271 was also segregating for restoration of the CMS cytoplasm and the position of the restorer gene *Rf1* is also shown in Figure 1. The linkage group numbers are completely arbitrary as no classical genetic map exists for sunflower; however they have been re-coded from the maps published by Berry *et al.* (1995 & 1996).

Table 1. A list of the 81 sunflower RFLP probes for public release with information on insert size and polymorphic index.

Public Code	Vector	mw (bp)	Linkage Group	Polymorphic Index		Public Code	Vector	mw (bp)	Linkage Group	Polymorphic Index
ZVG0001	pBluescript	850	1			ZVG0042	pBluescript	780	9	0.649
ZVG0002	pBluescript	1450	1	0.716		ZVG0043	pBluescript	1590	10	0.669
ZVG0003	pBluescript	590	1	0.587		ZVG0044	pBluescript	1020	10	
ZVG0004	pBluescript	1350	1	0.595		ZVG0045	pBluescript	620	10	0.654
ZVG0005	pBluescript	2290	2	0.5		ZVG0046	pBluescript	930	10	
ZVG0006	pBluescript	1290	2			ZVG0047	pBluescript	490	10	0.637
ZVG0007	pBluescript	1740	2	0.61		ZVG0048	pBluescript	2570	10	0.676
ZVG0008	pBluescript	2460	2	0.416		ZVG0049	pBluescript	1290	11	0.68
ZVG0009	pUC13	630	3	0.403		ZVG0050	pBluescript	440	11	
ZVG0010	pBluescript	930	3	0.476		ZVG0051	pUC13	780	11	0.582
ZVG0011	pBluescript	1020	3			ZVG0052	pBluescript	2140	11	0.687
ZVG0012	pBluescript	1820	3	0.61		ZVG0053	pBluescript	1180	11	0.769
ZVG0013	pBluescript	1660	3	0.722		ZVG0054	pBluescript	520	12	0.59
ZVG0014	pBluescript	2290	4	0.665		ZVG0055	pBluescript	2690	12	0.455
ZVG0015	pBluescript	1550	4	0.666		ZVG0056	pBluescript	960	12	
ZVG0016	pUC13	1910	4	0.528		ZVG0057	pBluescript	790	12	0.324
ZVG0017	pBluescript	760	4	0.744		ZVG0058	pBluescript	280	13	0.677
ZVG0018	pBluescript	890	4	0.373		ZVG0059	pBluescript	1620	13	
ZVG0019	pUC13	660	5	0.819		ZVG0060	pBluescript	2510	13	0.399
ZVG0020	pBluescript	1550	5			ZVG0061	pBluescript	620	13	0.738
ZVG0021	pBluescript	440	5			ZVG0062	pBluescript	2570	14	
ZVG0022	pUC18	810	5	0.666		ZVG0063	pBluescript	1780	14	0.489
ZVG0023	pBluescript	3020	5	0.608		ZVG0064	pBluescript	960	14	0.396
ZVG0024	pBluescript	1510	5			ZVG0065	pBluescript	870	14	0.52
ZVG0025	pBluescript	710	6	0.411		ZVG0066	pBluescript	580	15	0.643
ZVG0026	pBluescript	760	6	0.276		ZVG0067	pBluescript	420	15	0.243
ZVG0027	pBluescript	1050	6			ZVG0068	pBluescript	1290	15	0.517
ZVG0028	pBluescript	1740	6	0.458		ZVG0069	pBluescript	3800	15	0.443
ZVG0029	pBluescript	420	7	0.552		ZVG0070	pBluescript	890	15	0.554
ZVG0030	pBluescript	2140	7	0.497		ZVG0071	pBluescript	1050	16	
ZVG0031	pBluescript	1040	7	0.473		ZVG0072	pBluescript	960	16	
ZVG0032	pBluescript	870	7	0.602		ZVG0073	pBluescript	710	16	
ZVG0033	pBluescript	250	8	0.756		ZVG0074	pBluescript	2400	16	0.165
ZVG0034	pBluescript	2290	8	0.471		ZVG0075	pBluescript	1780	16	0.603
ZVG0035	pBluescript	590	8	0.62		ZVG0076	pBluescript	530	17	0.485
ZVG0036	pBluescript	960	8	0.379		ZVG0077	pBluescript	1150	17	0.684
ZVG0037	pBluescript	2400	8	0.454		ZVG0078	pBluescript	1000	17	0.419
ZVG0038	pBluescript	890	9			ZVG0079	pBluescript	1260	17	0.453
ZVG0039	pBluescript	3400	9			ZVG0080	pBluescript	1350	17	0.658
ZVG0040	pBluescript	790	9	0.516		ZVG0081	pBluescript	2160	17	0.662
ZVG0041	pBluescript	1900	9	0.522						

Table 2. The number of individuals and RFLP loci mapped in each of the nine F_2 populations used to construct the sunflower composite map published by Berry *et al.* (1996).

Population	No. of plants	No. of loci mapped
ZENB8xHA89	289	250
ZENB8xPAC2	70	334
ZENB8xZENR7	142	91
BSA52xRHA297	58	114
HA89xRHA271	94	118
ZENR7xRHA801	94	93
HA89xZENR9	115	38
ZENR1xZENR8	207	103
ZENB4xHA300	218	152

It is hoped that these 81 markers will provide a backbone for a public sunflower linkage map and that eventually segregation data will be available on the Internet through a sunflower web site. To this end, Advanta is currently collaborating with Steve Knapp at Oregan State University in order to produce a set of recombinant inbreds based on the cross RHA801 x RHA280. The 81 RFLP probes are available to public institutions for the purpose of collaborative academic research and can be requested via E-mail from:

Dr. Simon Berry	simon.s.t.berry@gbjha.zeneca.com
Dr. Rindert Peerbolte	peerbolte@vanderhave.com
Sr. Alberto Leon	zenleon@argenet.com.ar

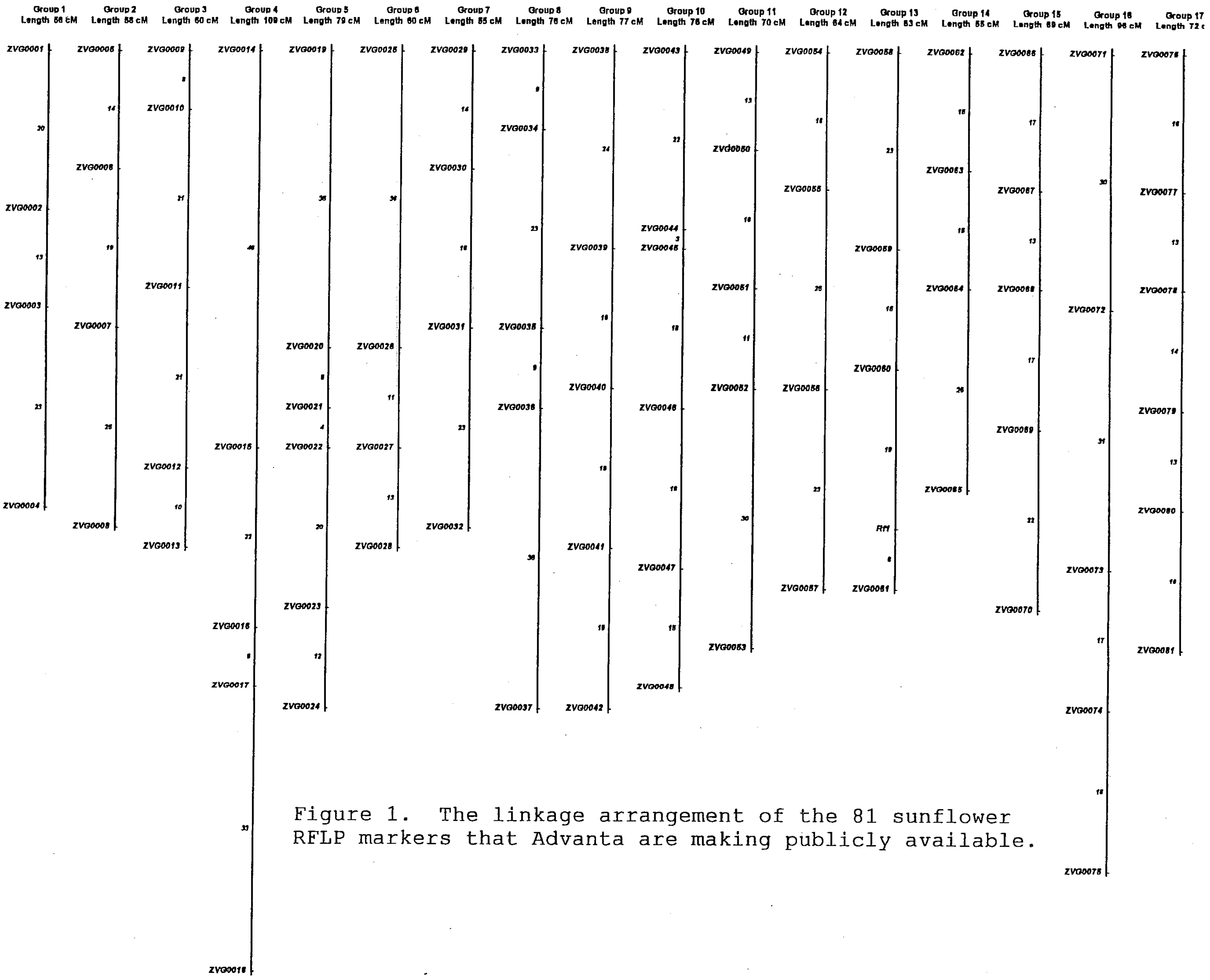

Figure 1. The linkage arrangement of the 81 sunflower RFLP markers that Advanta are making publicly available.

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