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Research Article

Single marker analysis in sunflower (*Helianthus annuus* L.)

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Abstract

In sunflower marker-trait association were studied for nine yield and yield component traits using 30 SSR markers with a set of 21 CMS lines of sunflower genotypes. Association of mean performance of hybrids and *gca* effects of parents with corresponding marker score were assessed single marker analysis by using simple linear regression. A total of 16 markers gave significant association with at least one of the nine traits studied. Most of the markers were found to be related to more than one trait. The markers ORS388 and ORS852 were found to be associated with three traits each. Some of the markers were found related to only one trait. It was also observed that the same marker ORS811 was found related to days to 50% flowering, days to maturity, plant height, volume weight per 100 ml, oil content as well as seed yield. This indicates that the same gene is controlling the expression of these characters. Moreover, phenotypically these characters have more association with each other. Hence these markers may be useful for marker assisted breeding programme.

Key words: Sunflower, Single marker analysis, SSR markers, marker assisted breeding.

Introduction

In the past 20 years, the major effort in breeding has changed from traditional phenotypic-pedigree based selection systems to molecular genetics with emphasis on quantitative trait loci (QTL) identification and marker assisted selection (MAS) in sunflower. MAS, which uses DNA markers to select optimal genotypes, is an excellent tool for selecting beneficial genetic traits that are difficult to measure, that exhibit low heritability and/or are expressed late in development (Davies et al., 2006; Wilde et al., 2007; Ender et al., 2008), as well as for assessing the genetic potential. However, results of MAS/QTL have been modest (Kearsey and Farquhar, 1998; Collard and Mackill, 2008; Hospital, 2009). This may be because (i) in linkage based QTL analyses, non-availability of mapping populations, (ii) the absence of tight linkage between marker and QTLs (Thomas, 2003), (iii) the existence of QTL × environment interactions (Bouchez et al., 2002). In order to overcome these limitations, molecular marker-trait association identifications have been conducted through the regression technique (Wright and Mowers, 1994, Pradeep et al., 2007, Srivastava et al., 2007) and increasingly adopted in many plants (Maureira-Butler et al., 2007). In the present study, association of mean performance of hybrids and *gca* effects of parents with corresponding marker score were assessed single marker analysis by using simple linear regression.

Material and methods

In this present investigation, 55 hybrids derived from crossing between 55 CMS lines and one restorer by top cross fashion in sunflower (*Helianthus annuus* L.) were studied. They were raised in a randomized block design with two replication in the oil seeds farm, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University; Coimbatore during *kharif* 2009. In each replication, each entry was raised in two rows of 4.5m length adopting a spacing of 60 cm between the rows and 30 cm between the plants within each row. Normal agronomic practices were followed under irrigated condition. From these, a subset of 21 CMS lines (Table 1.) and their hybrids were subjected to single marker analysis. The data were recorded on five randomly selected plants of

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each entry of each replication for nine yield and yield contributing traits viz, days to 50% flowering, days to maturity, plant height, head diameter, 100-seed weight, volume weight per 100 ml, oil content, seed yield and oil yield.

DNA extraction and marker generation

Leaves were harvested from 21 CMS lines in the field conditions, freeze-dried and ground to powder. DNA extraction was performed according to the cetyl-trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). The extracted DNA content was measured using DNA standards in agarose gel (0.8 % w/v). A total of 30 SSRs (Table 2.) were utilized to find out the polymorphism between parents. The number of alleles generated by the primer varies from 1 to 3. On average, each locus revealed approximately two alleles. The PIC value ranges from 0.04 to 0.89 for 30 SSR primers studied. The PIC values of the 16 polymorphic loci reveal a high level of polymorphism (>0.5). The higher PIC value indicated the informativeness of the primer. Among the primers used in the study five primers as *viz*., ORS671 (0.89), ORS546 (0.78), ORS1088 (0.77), ORS378 (0.76) and ORS484 (0.76) exhibited the high PIC value.

The PCR reaction contained 20 ng DNA, 1X reaction buffer, 1.5 mM Mgcl$_2$, 0.2 mM of each of dNTP, 0.5 uM of each forward and reverse primer, 0.3 IU Taq DNA polymerase. DNA amplification was performed in a Veriti® 96-Well Fast Thermal Cycler (Applied Biosystems Inc., Foster city, CA) with 10 uL reaction volume. DNA samples were denatured initially at 94 °C for 3 min, then subjected to the following 20 cycles: 94 °C for 30 s, 63 °C for 30 s with a decrement of 0.5 °C per cycle, and 70 °C for 1 min. This was followed by another 20 cycles of 94 °C for 15 s, 55 °C for 30 s, and 70°C for 1 min. A 10 min extension was performed at 72 °C as the last step. Amplified products were analyzed using 1.5 % agarose gel. Electrophoresis was performed at 120 volts DC for 2.5 hrs in a submarine electrophoresis system (Maxi sub XL). After electrophoresis, remove the gel from the tank and view the gel under UV illumination and photograph using gel documentation system.

Data scoring and data analysis

Clear and unambiguous bands were scored for their presence or absence with the score 1 indicating their presence and 0 indicating their absence. The data matrix of binary codes thus obtained was subjected to further analysis. Phenotypic value of hybrids mean and *gca* effects of parents were subject to associate with corresponding marker score for its significance by using simple regression in SPSS software (version. 16).

Results and Discussion

Phenotype analysis

The phenotypic variation observed among 21 CMS lines of sunflower is summarized in the Table 3. The traits 50% flowering, days to maturity, plant height, volume weight per 100 ml and oil content were showing low coefficient of variation whereas head diameter and100-seed weight showed a medium coefficient of variation. All other traits had high coefficient of variation. The non-significant values of skewness and kurtosis also indicated that the traits have normal distribution. Hence these data were subjected into single marker analysis.

Simple linear regression was calculated for each of the phenotypic traits with all the marker classes. The potential relationship between the marker and trait was established considering the significance of the regression coefficient. It was found that a single marker was related with many traits and a single trait related to many markers. The marker which is having a strongest relationship can be judged from its adjusted R^2 value which will give the overall percentage of variability of that particular trait that the marker can explain. Single marker analysis for mean value of hybrids and *gca* effects of parents for various traits is presented in Table. 4.

The trait, days to 50% flowering was showing relationship with three SSR markers for both hybrids mean and *gca* effects of parents. Among the three SSR markers, the marker ORS388_214 will best ascertain the relation as they can account for almost 46 per cent variability of the trait. Four markers ORS628_385, ORS852_500, ORS811_100 and ORS677_406 were found to be related with the days to maturity for both mean value of hybrids and *gca* effects of parents. For plant height, four SSR markers were found to be linked of which ORS811_100 having highest adjusted R^2 value (0.46). Both hybrids mean and *gca* effects of parents, five markers were found to be associated with the 100-seed weight of which the marker ORS337_180 having highest R^2 value (0.39). The volume weight per 100 ml was having the highest number of markers i.e. seven markers associated with it. Out of the seven markers, ORS578_257 was having higher adjusted R^2 value

(0.31). Three markers ORS388_204, ORS578_257 and ORS811_175 were found to be associated with oil content for both hybrids mean and *gca* of parent and among the markers studied as a whole, the adjusted R^2 value of the marker ORS811_1175 was high (0.49) than other marker, so that can be used for further studies. Four markers were found to be relevant in the case of seed yield and two SSR markers were found related to the oil yield for both hybrids mean and *gca* effects of parents.

Most of the markers were found to be related to more than one trait. The markers ORS388 and ORS852 were found to be associated with three traits each. Many markers were found related to only one trait. It was also seen that the same marker ORS811 was found related to days to 50% flowering, days to maturity, plant height, volume weight per 100 ml, oil content as well as seed yield. This indicates that the same gene is controlling the expression of these characters. Moreover, phenotypically these characters have more association with each other. Hence these markers may be useful for yield improvement programme.

Molecular markers linked with QTL/major genes for traits of interest are being routinely developed in several crops using materials derived from planned crosses such as $F_{2,}$ RIL, back cross inbreds and DH populations. However, non-availability of mapping populations and substantial time needed to develop such populations are sometimes major limitations in the identification of molecular markers for specific traits. Another limitation is the absence of tight linkage between marker and traits observed in these studies. Also, it is difficult to eliminate false positives with available methods. Therefore, markers identified during the present study need to be subjected to validation and/or functional analysis of respective traits, which is beyond the scope of the present study. Sun *et al.* (2003) highlight that this approach could have advantages over the use of mapping populations as the markers are more likely to be applicable to a large number of breeding programmes. However, we believe that at least some of the markers identified during the present study would be validated and used for MAS involving sunflower breeding programme.

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Table.1. List of sunflower male sterile lines (CMS) and their cytoplasm source

S.No.	CMS lines	Source of cytoplasm
1	10A	PET1
2	17A	PET 1
3	62A	PET 1
4	115A	PEF
5	137A	PET 1
6	138A	PET 1
7	148A	PET 1
8	207DSA	PET 1
9	234A	PET 1
10	300A	PET 1
11	302A	PET 1
12	336A	PET 1
13	607A	PET 1
14	852A	PET 1
15	ARG2	ARG
16	ARG6	ARG
17	DEB1	DEB
18	FMS400A	FMS
19	MUT2	MUT
20	NDSMS1A	PET 1
21	86A3P5	PET 1

Table 2. Number of alleles and polymorphism information content (PIC) of the sunflower microsatellite (SSR) primers

S.No.	Primer	No. of alleles	PIC value
1	ORS309	2	0.54
2	ORS337	2	0.65
3	ORS358	1	0.62
4	ORS371	2	0.07
5	ORS378	2	**0.76**
6	ORS388	2	0.23
7	ORS 407	2	0.58
8	ORS 484	3	**0.76**
9	ORS 546	2	**0.78**
10	ORS 552	3	0.32
11	ORS 561	2	0.16
12	ORS 578	2	0.71
13	ORS 628	2	0.47
14	ORS 671	2	**0.89**
15	ORS 677	2	0.59
16	ORS 767	1	0.42
17	ORS 780	2	0.54
18	ORS 807	3	0.68
19	ORS 811	2	0.69
20	ORS 852	2	0.50
21	ORS 930	2	0.62
22	ORS 938	1	0.27
23	ORS 959	3	0.61
24	ORS 996	2	0.04
25	ORS 1024	2	0.18
26	ORS 1068	2	0.50
27	ORS 1088	1	**0.77**
28	ORS 1159	3	0.61
29	ORS 1220	1	0.67
30	ORS 1245	2	0.43
	Total	60	15.66
	Mean	**2**	**0.52**

Table 3. Variability for different characters among 21 CMS lines

Characters	Range	Mean	CV (%)	Skewness	Kurtosis
50% flowering	8.00	55.21	4.25	0.65	-0.32
Days to maturity	4.00	86.86	1.33	0.20	-0.82
Plant height (cm)	48.39	169.47	8.03	0.12	-0.47
Head diameter (cm)	5.40	13.15	10.36	-1.00	1.20
100 seed weight (g)	1.83	3.91	14.21	0.32	-0.94
Volume weight per 100 ml (g)	10.05	41.50	6.94	0.20	-0.78
Oil content (%)	6.35	36.33	5.27	0.12	-0.97
Seed yield per plant (g)	30.80	41.32	21.93	0.35	-0.81
Seed yield (Kg/ha)	1537.00	1782.14	22.40	-0.03	-0.48
Oil yield per plant (g)	12.06	15.05	23.18	0.45	-0.54
Oil yield (Kg/ha)	591.32	650.92	23.55	-0.02	-0.35

Table 4. Single marker analysis for mean and *gca* effects of various traits.

Characters	Markers_bp	Mean value		General combining ability	
		Probability value	Adjusted R^2 value	Probability value	Adjusted R^2 value
50% flowering	ORS388_204	0	0.34	0	0.35
	ORS388_214	0	0.46	0	0.46
	ORS996_250	0.04	0.16	-	-
	0RS996_300	0.04	0.16	0.04	0.16
	ORS811_175	0.02	0.33	0.02	0.33
Days to maturity	ORS628_385	0.03	0.19	0.03	0.19
	ORS852_500	0.01	0.27	0.01	0.27
	ORS811_100	0.02	0.34	0.02	0.34
	ORS677_406	0.01	0.42	0.01	0.42
Plant height (cm)	ORS552_221	0.02	0.26	0.02	0.26
	ORS552_233	0.02	0.2	0.02	0.2
	ORS484_429	0.03	0.27	0.03	0.27
	ORS811_100	0.01	0.46	0.01	0.46
100_seed weight (g)	ORS1088_300	0.01	0.28	0.01	0.28
	ORS388_204	0.01	0.25	0.01	0.25
	ORS388_214	0.02	0.21	0.02	0.21
	ORS552_221	0.01	0.26	0.01	0.26
	ORS930_490	0.04	0.21	0.04	0.21
	ORS930_500	0.04	0.21	0.04	0.21
	ORS337_180	0.01	0.39	0.01	0.39

Table 4. Contd..

Characters	**Markers_bp**	**Mean value**		**General combining ability**	
		Probability value	**Adjusted R^2 value**	**Probability value**	**Adjusted R^2 value**
Volume weight (g)	ORS1068_380	0.03	0.23	-	-
	ORS1068_400	0.03	0.23	0.03	0.23
	ORS371_300	0.01	0.24	0.01	0.24
	ORS552_221	0.04	0.17	0.04	0.17
	ORS552_233	0.04	0.17	0.04	0.17
	ORS767_362	0.03	0.18	0.03	0.18
	ORS578_257	0.03	0.31	0.03	0.31
	ORS780_310	0.03	0.3	0.03	0.3
	ORS811_175	0.05	0.25	-	-
Oil content (%)	ORS388_204	0.05	0.14	0.05	0.14
	ORS578_257	0.04	0.26	0.04	0.26
	ORS811_175	0.01	0.49	0.01	0.49
Seed yield (kg/ha)	ORS337_200	0.04	0.28	0.04	0.28
	ORS484_451	0.04	0.23	0.04	0.23
	ORS780_300	0.04	0.24	0.04	0.24
	ORS811_100	0.04	0.28	0.04	0.28
Oil yield (kg/ha)	ORS337_200	0.04	0.28	0.04	0.28
	ORS780_300	0.05	0.23	0.05	0.23