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MARKER TRAIT ASSOCIATION IN BACKCROSS POPULATION OF INTERSPECIFIC CROSS (*JATROPHA CURCAS X JATROPHA INTEGERRIMA*)

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Abstract- In Jatropha marker-trait association were studied for twenty eight yield and yield component traits using 21 SSR markers and 22 ISSR markers with a set of 97 genotypes of BC_4F_1 population. Association of mean performance with corresponding marker score were assessed single marker analysis by using simple linear regression. Among this, 16 SSR and 18 ISSR primer pairs were identified as putatively linked to at least one of the investigated trait. The number of associated marker varies of associated marker varies from 6 (oil yield per plant) to one (number of female flower per inflorescence, number of seeds per fruit, fruit aspect ratio and oil content). The adjusted R^2 for the regression equation varies from 3.1 to 11.6 %. Traits namely canopy cover between rows (ISSR 856), capsule weight per plant (ISSR 817) and seed yield per plant (ISSR 817) recorded above 10 % R^2 value. 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- Jatropha, SSR markers, ISSR markers, marker assisted breeding.

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Introduction

Jatropha curcas is becoming one of the world's key crops for biodiesel production. Oil containing a high amount of unsaturated fatty acid can find an application as biodiesel feed stock. To make the production of jatropha profitable and sustainable, genetic improvement of oil yield demanded. However, oil traits cannot be evaluated until the seeds are harvested and analyzed in laboratory, and detailed selective breeding has not been carried out. Meanwhile molecular breeding in jatropha has limited due to lack of molecular bases of economically important traits such as seed yield, seed oil traits, biotic or abiotic stress resistance. 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 [1,2,3], as well as for assessing the genetic potential. To study the genetic nature of a trait, phenotypic data and genotypic data from molecular markers can, by detecting associations between markers and traits, help determine the number and nature of a gene/quantitative trait locus (QTL) controlling a trait. Single marker analysis is one of a series of quantitative trait locus (QTL) analysis techniques that can detect associations between molecular markers and traits of interest to plant breeders, such as disease resistance, increased yield, and improved fruit quality.

Material and Methods

In the present investigation, 97 backcross progenies derived from crossing between *Jatropha curcas L* x *Jatropha integerrima* L and one check TNMC7 were studied. They were raised in a randomized block design with three replication in the millet farm, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University; Coimbatore during September 2010. Normal agronomic practices were followed under irrigated condition. The data were recorded for 28 yield and yield contributing traits *viz*., plant height, collar diameter, leaf length, leaf width, leaf length width ratio, canopy cover in rows, canopy cover between rows, canopy volume, number of branches per plant, days to flowering, number of male flower per inflorescence, number of female flower per inflorescence, male to female flowers ratio, number of bunches per branch, number of fruits per bunch, number of seeds per fruit, fruit length, fruit width, fruit aspect ratio, seed length, seed width, seed aspect ratio, capsule weight per plant, hundred seed weight, shelling percent, oil content, seed yield per plant and oil yield per plant. Leaf samples of 179 BC_4F_1 progenies and parents were collected and DNA was extracted as per Doyle and Doyle (1987) [4] and stored at OSMAS Lab, Department of Oilseeds, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. From these, 97 individuals were subjected to determine the association of 21 SSR and 22 ISSR marker [Table.-1] to the respective phenotype. The markers were subjected to single factor regression analysis using the marker as independent and the respective phenotype as dependent. The results of the single market analysis for various traits along with the significance and adjusted R^2 values are presented in Table 36. In all the cases, more than one marker was related with a trait and in many cases a single marker was related to more than one trait.

DNA Extraction and Marker Generation

Leaves were harvested from 97 BC_4F_1 progenies 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). *Jatropha curcas* and *Jatropha integerrima* were chosen as parents for parental polymorphism study using 23 SSR primers and 100 ISSR primers. Among the primers studied, 21 SSR primers and 22 ISSR primers were polymorphic between parents. 21 SSR and 22 ISSR polymorphic markers were used for profiling of 97 BC_4F_1 progenies

The PCR reaction contained 20 ng DNA, 1X reaction buffer, 1.5 mM $MgCl_2$, 0.2

Table-1 *List of primers used*

S. No	SSR Name	Forward primer	SSR Name	Reverse primer
1	SSR-AG104-F	5'-GTGCGTTATGGGTTTATTTGGT-3'	SSR-AG104-R	5'-CTCCCGTAGCATTTGCATTTAT-3'
2	SSR-AG112-F	5'-AACCAAACCTTCCCCACTAAAT-3'	SSR-AG112-R	5'-CTAATGATGGAGCTTGCACAAA-3'
3	SSR-AG157-F	5'-TACTTCCTCCCTCTTCCCAAAC-3'	SSR-AG157-R	5'-CAATGCTCATGTTGCTCTTAGG-3'
4	SSR-AG220-F	5'-GCTCTCATTTCTAGTGAACGCA-3'	SSR-AG220-R	5'-ACACCATCTGGCTCAATTTTCT-3'
5	SSR-AG232-F	5'-TGCTGGTGGGCTTTTACTTACT-3'	SSR-AG232-R	5'-ACCTTTATCCTGTCTCCATCCA-3'
6	SSR-AG446-F	5'-CTTCAGTAGTTCGGTGCAAGC-3'	SSR-AG446-R	5'-ACTTATCCCTCTCCCGTTCCT-3'
7	SSR-AG517-F	5'-AGAGCCCATTGAGGACAAAA-3'	SSR-AG517-R	5'-GGCATTTGATGAGACCCAATA-3'
8	SSR-AG629-F	5'-CAGGGGAAAATGTAGAAGAGCA-3'	SSR-AG629-R	5'-CTAACTGCAACAGAAACGGTCA-3'
9	SSR-AG706-F	5'-GGTCTCGGCTTCTCTAGCAATA-3'	SSR-AG706-R	5'-CCGACTCCTACTCCTGCTTCTA-3'
10	SSR-AG818-F	5'-TCACCACCGTGACCATTATT-3'	SSR-AG818-R	5'-CCGTCACCAACACAGTAACCT-3'
11	SSR-AG848-F	5'-GAGCTTCTGTGGGCTTTATTGT-3'	SSR-AG848-R	5'-TTCTGCTCCTGTTGCTTTTGTA-3'
12	SSR-EST02-F	5'-AAGGGTGAATTTGTGCAGCTAT-3'	SSR-EST02-R	5'-CAACAATGGTAAACGAAGCAAG-3'
13	SSR-EST06-F	5'-TTTCAGTACATACTTTCTCACACACA-3'	SSR-EST06-R	5'-TGAGATCTCTGGTTAAGTTTCAGC-3'
14	SSR-EST10-F	5'-CCTGCCTGACTTCCAAAATAAC-3'	SSR-EST10-R	5'-TAGAGCTTCTGCTATTCCCAGC-3'
15	SSR-EST13-F	5'-ACCGAAAAGAAAATCCAGGAG-3'	SSR-EST13-R	5'-CGTGGTCTACATACGCCATTT-3'
16	JcSSR-613-F	5'-AGTGCCCAAATAGATTCCTCAA-3'	JcSSR-613-R	5'-GAAGGATGGGAAGTGGGAC-3'
17	JCT23-F	5'-ACAGGGACCAGACCCAGGAAAAGG-3'	JCT23-R	5'-AGGTCAGCAGAGGCGACGGT-3'
18	JCT36-F	5'TGTGGTGGAAAAGGTACATTGTAGGGA-3'	JCT36-R	5'-GGCAGAACCACTCACCTTTCGGG-3'
19	JCT51-F	5'-AGCATGTGGGTGTGGGTGTGC-3'	JCT51-R	5'-TGGCCCCAGTGTAGCTGGTGT-3'
20	JCT59-F	5'-GGTGACTCCTGAATGCTTGGACCT-3'	JCT59-R	5'-CCAGACCAACCCACTGAAATGGCA-3'
21	JCT86-F	5'-GCTGTTCAGCTTCCTCCATCTTCAG-3'	JCT86-R	5'-AGGCGGCTCTTGTGCAAGAAAGA-3'
22	JCT92-F	5'-AGCCAAAAGCCTACCAGTGCCA-3'	JCT92-R	5'-CTCTGCCCCCGCTTTCACCG-3'
23	JCT135-F	5'-TCACCCAGGAAAACAGTCCTTCACT-3'	JCT135-R	5'-TCGCCTATGGCAGGGATCCAGA-3'

S.No	ISSR Primer	Sequence	S.No	ISSR Primer	Sequence
1	ISSR 801	ATA TAT ATA TAT ATA TT	40	ISSR 840	GAG AGA GAG AGA GAG AYT
2	ISSR 802	ATA TAT ATA TAT ATA TG	41	ISSR 841	GAG AGA GAG AGA GAG AYC
3	ISSR 803	ATA TAT ATA TAT ATA TC	42	ISSR 842	GAG AGA GAG AGA GAG AYG
4	ISSR 804	TAT ATA TAT ATA TAT AA	43	ISSR 843	CTC TCT CTC TCT CTC TRA
5	ISSR 805	TAT ATA TAT ATA TAT AC	44	ISSR 844	CTC TCT CTC TCT CTC TRC
6	ISSR 806	TAT ATA TAT ATA TAT AG	45	ISSR 845	CTC TCT CTC TCT CTC TRG
7	ISSR 807	AGA GAG AGA GAG AGA GT	46	ISSR 846	CAC ACA CAC ACA CAC ART
8	ISSR 808	AGA GAG AGA GAG AGA GC	47	ISSR 847	CAC ACA CAC ACA CAC ARC
9	ISSR 809	AGA GAG AGA GAG AGA GG	48	ISSR 848	CAC ACA CAC ACA CAC ARG
10	ISSR 810	GAG AGA GAG AGA GAG AT	49	ISSR 849	GTG TGT GTG TGT GTG TYA
11	ISSR 811	GAG AGA GAG AGA GAG AC	50	ISSR 850	GTG TGT GTG TGT GTG TYC
12	ISSR 812	GAG AGA GAG AGA GAG AA	51	ISSR 851	GTG TGT GTG TGT GTG TYG
13	ISSR 813	CTC TCT CTC TCT CTC TT	52	ISSR 852	TCT CTC TCT CTC TCT CRA
14	ISSR 814	CTC TCT CTC TCT CTC TA	53	ISSR 853	TCT CTC TCT CTC TCT CRT
15	ISSR 815	CTC TCT CTC TCT CTC TG	54	ISSR 854	TCT CTC TCT CTC TCT CRG
16	ISSR 816	CAC ACA CAC ACA CAC AT	55	ISSR 855	ACA CAC ACA CAC ACA CYT
17	ISSR 817	CAC ACA CAC ACA CAC AA	56	ISSR 856	ACA CAC ACA CAC ACA CYA
18	ISSR 818	CAC ACA CAC ACA CAC AG	57	ISSR 857	ACA CAC ACA CAC ACA CYG
19	ISSR 819	GTG TGT GTG TGT GTG TA	58	ISSR 858	TGT GTG TGT GTG TGT GRT
20	ISSR 820	GTG TGT GTG TGT GTG TC	59	ISSR 859	TGT GTG TGT GTG TGT GRC

21	ISSR 821	GTG TGT GTG TGT GTG TT	**60**	ISSR 860	TGT GTG TGT GTG TGT GRA
22	ISSR 822	TCT CTC TCT CTC TCT CA	**61**	ISSR 861	ACC ACC ACC ACC ACC ACC
23	ISSR 823	TCT CTC TCT CTC TCT CC	**62**	ISSR 862	AGC AGC AGC AGC AGC AGC
24	ISSR 824	TCT CTC TCT CTC TCT CG	**63**	ISSR 863	AGT AGT AGT AGT AGT AGT
25	ISSR 825	ACA CAC ACA CAC ACA CT	**64**	ISSR 864	ATG ATG ATG ATG ATG ATG
26	ISSR 826	ACA CAC ACA CAC ACA CC	**65**	ISSR 865	CCG CCG CCG CCG CCG CCG
27	ISSR 827	ACA CAC ACA CAC ACA CG	**66**	ISSR 866	CTC CTC CTC CTC CTC CTC
28	ISSR 828	TGT GTG TGT GTG TGT GA	**67**	ISSR 867	GGC GGC GGC GGC GGC GGC
29	ISSR 829	TGT GTG TGT GTG TGT GC	**68**	ISSR 868	GAA GAA GAA GAA GAA GAA
30	ISSR 830	TGT GTG TGT GTG TGT GG	**69**	ISSR 869	GTT GTT GTT GTT GTT GTT
31	ISSR 831	ATA TAT ATA TAT ATA TYA	**70**	ISSR 870	TGC TGC TGC TGC TGC TGC
32	ISSR 832	ATA TAT ATA TAT ATA TYC	**71**	ISSR 871	TAT TAT TAT TAT TAT TAT
33	ISSR 833	ATA TAT ATA TAT ATA TYG	**72**	ISSR 872	GAT AGA TAG ATA GAT A
34	ISSR 834	AGA GAG AGA GAG AGA GYT	**73**	ISSR 873	GAC AGA CAG ACA GAC A
35	ISSR 836	AGA GAG AGA GAG AGA GYA	**74**	ISSR 874	CCC TCC CTC CCT CCC T
36	ISSR 835	AGA GAG AGA GAG AGA GYC	**75**	ISSR 875	CTA GCT AGC TAG CTA G
37	ISSR 837	TAT ATA TAT ATA TAT ART	**76**	ISSR 876	GAT AGA TAG ACA GAC A
38	ISSR 838	TAT ATA TAT ATA TAT ARC	**77**	ISSR 877	TGC ATG CAT GCA TGC A
39	ISSR 839	TAT ATA TAT ATA TAT ARG	**78**	ISSR 878	GGA TGG ATG GAT GGA T
S.No	**ISSR Primer**	**Sequence**	**S.No**	**ISSR Primer**	**Sequence**
79	ISSR 879	CTT CAC TTC ACT TCA	**90**	ISSR 890	VHV GTG TGT GTG TGT GT
80	ISSR 880	GGA GAG GAG AGG AGA	**91**	ISSR 891	HVH TGT GTG TGT GTG TG
81	ISSR 881	GGG TGG GGT GGG GTG	**92**	ISSR 892	TAG ATC TGA TAT CTG AAT TCC C
82	ISSR 882	VBV ATA TAT ATA TAT AT	**93**	ISSR 893	NNN NNN NNN NNN NNN
83	ISSR 883	BVB TAT ATA TAT ATA TA	**94**	ISSR 894	TGG TAG CTC TTG ATC ANN NNN
84	ISSR 884	HBH AGA GAG AGA GAG AG	**95**	ISSR 895	AGA GTT GGT AGC TCT TGA TC
85	ISSR 885	BHB GAG AGA GAG AGA GA	**96**	ISSR 896	AGG TCG CGG CCG CNN NNN NAT G
86	ISSR 886	VDV CTC TCT CTC TCT CT	**97**	ISSR 897	CCG ACT CGA GNN NNN NAT GTG G
87	ISSR 887	DVD TCT CTC TCT CTC TC	**98**	ISSR 898	GAT CAA GCT TNN NNN NAT GTG G
88	ISSR 888	BDB CAC ACA CAC ACA CA	**99**	ISSR 899	CAT GGT GTT GGT CAT TGT TCC A
89	ISSR 889	DBD ACA CAC ACA CAC AC	**100**	ISSR 900	ACT TCC CCA CAG GTT AAC ACA

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 30s, 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 progenies was subject to associate with corresponding marker score for its significance by using simple regression in SPSS software (version. 16).

Result and Discussion

Single Marker Analysis is used to detect the potential association between marker classes (presence or absence of band) and their respective phenotypic values. 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. Single marker analysis was performed for the 97 progenies of the BC_4F_1 populations of interspecific cross *Jatropha curcas* x *Jatropha integerrima* with 28 phenotypic traits and 43 markers using a simple regression approach was given in [Table-2].

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 explainable by marker. The adjusted R^2 value from 3.1 (SSR AG818 for number of female flower per inflorescence and fruit aspect ratio) to 11.6 (ISSR 817 for capsule weight per plant.

The marker ISSR 817 was found to be associated with seven traits *viz*., plant height, number of branches per plant, days to first flowering, and capsule weight per plant, hundred seed weight, seed yield per plant and oil yield per plant. It was also seen that the same marker was found to be related to the canopy cover in

Table-2. *Single marker analysis for SSR and ISSR primers linked to oil yield and yield components in the cross of J.curcas x J.integerrima*

Trait	Marker	Regression coefficient(b)		Adjusted R^2 (%)
Plant height	JCT92	-6.07	**	8.4
	ISSR817	9.30	*	5.4
Collar diameter	JCT59	1.51	*	4.2
	ISSR811	2.71	*	7.3
	ISSR835	2.20	*	5.6
Leaf length	SSRAG157	-0.20	*	3.8
	SSRAG232	0.20	*	4.9
	JCT92	-0.15	*	3.5
	ISSR860	0.22	*	6.1
Leaf width	JCT135	-0.16	*	4.5
Leaf length width ratio	-	-	-	-
Canopy cover in rows	ISSR856	-0.09	**	7.8
	ISSR899	0.06	*	5.0
Canopy cover between rows	JCT92	-0.05	*	4.3
	ISSR856	-0.09	**	11.6
	ISSR868	-0.05	*	4.8
Canopy volume	SSRAG232	0.15	*	4.5
	JCT92	-0.14	*	5.6
	ISSR856	-0.20	*	6.4
	ISSR899	0.16	*	6.0
Number of branches per plant	ISSR856	-0.57	*	4.5
	ISSR817	0.69	*	4.1
	ISSR899	0.42	*	4.6
Days to first flowering	JCT23	-3.43	*	5.8
	JCT135	-3.98	*	6.6
	ISSR868	4.90	**	5.5
Number of male flower per inflorescence	JCT135	7.46	*	5.9
	ISSR835	4.67	*	4.4
Number of female flower per inflorescence	SSRAG818	-0.63	*	3.1
Male female flower ratio	ISSR842	0.61	*	4.0
	ISSR848	-0.92	*	8.3
Number of bunches per branch	SSRAG157	-0.08	*	3.4
	JCT92	-0.07	*	4.1
	ISSR808	0.08	*	3.4
Number of fruits per bunch	SSRAG220	-0.51	*	3.8
	ISSR889	**-0.62**	*	**5.0**
Number of seeds per fruit	SSRAG220	-0.06	*	4.2
Fruit length	**EST13**	**-0.04**	*	**3.8**
	JCT86	0.04	*	4.1
	ISSR868	**0.04**	*	**5.5**
	ISSR888	0.05	*	4.8
	ISSR889	**0.04**	*	**3.8**
Fruit width	SSRAG232	-0.04	*	6.2
	SSRAG446	**0.02**	*	**5.7**
	ISSR808	-0.04	*	5.7
	ISSR856	**-0.04**	*	**6.9**
Fruit aspect ratio	SSRAG818	-0.03	*	3.1
Seed length	**ISSR835**	**0.03**	*	**5.9**
	ISSR856	-0.03	*	6.1

Seed width	**ISSR818**	**-0.02**	*	**3.9**
	ISSR886	-0.02	*	4.6
Seed aspect ratio	**SSRAG112**	**-0.04**	*	**4.9**
	SSRAG848	0.03	*	5.2
Capsule weight per plant	**SSRAG629**	**6.18**	*	**5.2**
	EST02	15.35	*	5.6
	EST10	**13.17**	*	**3.2**
	JCT92	-14.85	*	5.7
	ISSR817	**40.30**	**	**11.6**
Hundred seed weight	SSRAG220	-1.72	*	4.9
	ISSR817	**3.81**	*	**5.4**
	ISSR820	1.75	*	4.1
	ISSR891	**2.76**	*	**8.5**
Shelling per cent	-		-	-
Oil content	**ISSR891**	**1.49**	*	**8.5**
Seed yield per plant	SSRAG629	-9.70	*	6.0
	EST02	**8.75**	*	**5.1**
	JCT92	-8.55	*	5.0
	ISSR817	**23.30**	**	**10.4**
Oil yield per plant	SSRAG629	-2.62	*	3.1
	EST02	**1.30**	*	**5.6**
	EST10	3.09	*	4.2
	JCT92	**-2.91**	*	**4.8**
	ISSR817	8.00	**	9.8
	ISSR820	**2.79**	*	**4.0**

rows, canopy cover between rows and canopy volume (ISSR 856 and ISSR 899). The markers SSRAG 629, EST 02, JCT 92 and ISSR 817 [Fig-1,2] were had relation with capsule weight per plant, seed yield per plant and oil yield per plant. This indicates that the same genomic region may be controlling the expression of these characters. Moreover phenotypically these characters have more association with each other. Hence, these markers, which are found to be related to these economically important characters, may be useful for further studies. Traits namely canopy cover between rows (ISSR 856), capsule weight per plant (ISSR 817) and seed yield per plant (ISSR 817) recorded above 10 % R^2 value. These markers are of potential markers and could be used in marker assisted breeding programme.

Fig 1. Segregation pattern of SSR EST02 for BC_4F_1 progenies

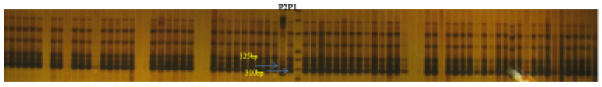
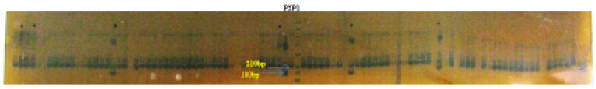


Fig 2. Segregation pattern of SSR JCT92 for BC_4F_1 progenies



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 F2, RIL, back cross inbreeds 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 are just a preliminary investigation to find out the potential relationship between the marker and the phenotypic traits. As we are dealing with the quantitative characters, it could not be concluded that these markers found related will be applicable universally. Sun et al (2003) [5] 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. For a valid conclusion to be drawn, the studies are to be repeated over locations, over seasons, on different set of populations and with a larger population. It is always better to go for the populations in which there is maximum variability so that we can identify the potential relationship between the trait and the marker.

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