Estimation of Combining Ability for Oil and Yield-related Traits in Sunflower (*Helianthus annuus* L.) by Using Line × Tester Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sunflower is an important oilseed crop cultivated worldwide because of its importance for oil and food. Sunflower is well suited to our local cropping pattern and contains much edible oil The research was conducted at the University of Agriculture Faisalabad, Pakistan to evaluate the combining ability of morphological traits of sunflower. Four lines were used as female and three testers (male) were crossed to make twelve hybrids by using a line × tester mating design. In the next season, the crosses and their parents were evaluated according to their performance for yield and its related traits. The data was taken based on ten morphological and physiological traits. The

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data of parents and their crosses were evaluated whether the data was significant or non-significant, by performing an analysis of variance test. A54 and A69 both lines and tester A53 are good general combiners and significant SCA effects for most traits observed in their combinations. Plant height and 1000-achene weight have significant SCA effects. Lines contain a very significant role for eight traits, i.e., flower initiation (20.57%), 50% flowering (16.65%), 100% flowering (22.19%), plant height (14.36%), leaf area (79.97%), head diameter (49.87%), seed yield (97.95%) and 1000 achene weight (18.58%) as compared to these testers that showed meager contributions for all traits that revealed minor or no parental effect for all parameters.

1. INTRODUCTION

Humans depend on edible oil in their daily lives to suit their various demands. It is employed in producing paints, plastics, lubricants, varnishes, cosmetics, soaps, detergents, pharmaceuticals, margarine, apparel dyes, and cooking. Sunflower is a member of an enormous angiosperm family, "Asteraceae." It is a diploid plant with 2n-34 chromosomes. Its genome size is 2871-3189 mbp. Sunflowers are annual by nature and grow twice a year. Helianthus contains 65 different species [1]. It consists of a hairy stem and broad leaves with a fibrous tap root system [2]. Its head is more extensive and has many small flowers that grow into seeds [3]. From the evidence of prehistoric times, the primary origin of the sunflower was Mexico around 3000 BC. It is an important oilseed crop cultivated worldwide because of its importance for oil and food. Its seeds are delightful and enriched with nutrient ingredients for different dishes. It is consumed directly, or the oil is extracted from it and used in cooking and many other purposes. Its oil is rich in nutrients, but most people do not know its nutritious importance. It contains 35-50% oil contents, and approximately 18.7 percent of national oil production is obtained from it [4].

It has broad utilization in food, medicines, makeup sectors, and biodiesel production [5]. One hundred grams of seeds are thought to own 20.78 g proteins, 50 g fats, 3.02g ash, 20g carbohydrates, and 8.6 g fiber, entirely 2445kj of energy. In addition, it has a decent supply of choline (55.1 mg) and adequacy (35.4 mg) [6]. Some elements of sunflowers, such as selenium have also been studied for their ability to scale back the incidence of bound forms of cancer. Moreover, it could lower blood cholesterol levels. Sunflower meal or cake (a superb result separated by Helianthus handled seeds) accounts for 36 percent of mass composition and protein level starting from 45-50% [7]. It is an excellent source of water-soluble B complex vitamins, including nicotinic acid and riboflavin, as well as nutrients like nitrogen, calcium, and potassium which are crucial for soil fertility.

Sunflower with 18-27.5 percent essential protein is bifurcated in monounsaturated and polyunsaturated fatty acids such as linoleic acid (21.08-80.13%). oleic acid (9.88-18.15%), and linolenic acid (0.11-19.96%). Saturated fatty acids (SFA), such as palmitic acid (4.76-9.60%) and stearic acid (0.23-7%), are in short supply [8]. It is a good source of vitamins like A, D, E, and K. Its oil has a nutritious quality and oxidative stability, which is why it is considered a good source for cooking food [9].

Sunflower is a nontraditional crop with a unique status with varieties and it is capable in producing significant oil yield compared to other crops. Sunflower is well suited to our local cropping pattern and contains much edible oil. The major challenge is to produce a short-duration crop; the life cycle of this crop is about 75 to 120 days. It has a high heterosis efficiency and combining ability. The ability of parental lines to mix during hybridization for the sake of a desirable gene is referred to as combining ability. Correlation is a strong association between two or more traits. If the relation between two traits is positive then by improving one trait, another trait will also be improved simultaneously [10].

The average performance of an inbred line in a crossing series is called general combining ability (GCA). The specific combining ability (SCA) refers to a line's average performance in a particular cross. Sindagi et al. [11] argued that GCA significantly influences yield factor determination. According to the combining ability study of multiple characters, superior SCA values are related to dominant gene action, whereas superior GCA values reveal the presence of additive gene action which affects these features. When both GCA and SCA are non-significant, the plant characters are controlled by the epistasis gene effect [12].

The objectives of this study were to determine was to assess inbred lines based on their general and specific combining ability and to use these lines to develop hybrids with the maximum oil content, achene yield, and yield-related components. The line × tester analysis is a well-known method for assessing many inbreds GCA and SCA impacts. Line × tester analysis is a version of the top cross approach that evaluates lines based on their offspring. A line or variety with a broad genetic base noted for its strong combining ability is known as a tester [13].

2. MATERIALS AND METHODS

The research was conducted at the experimental sunflower area of the Department of Plant Breeding and Genetics (PBG) at Raja Wala Research Station, University of Agriculture Faisalabad, Pakistan. The germplasm for research was collected from the Department of PBG, UAF. Four lines of sunflower. i.e., A51, A52, A54, and A69, were grown along with three testers, i.e., A53, A68, and A70, in the field in February. Twelve crosses were made by using the Line × Tester approach technique. The seeds of parents and crosses were harvested separately. The sowing of parents and hybrids were done under an RCB design with three replications to minimize the variation in results. The flowers were selected for selfing purposes and covered with paper bags before the onset of anthesis to avoid cross-pollination. Selected a suitable head of flowers used as a female plant and tagged it. Emasculation was done late afternoon. Removed the immature whorls from the center, and emasculation was done by removing the anthers with the help of forceps. This process continued for 5 to 6 days. Selected the flowers used as a male plant and covered them with a paper bag before the onset of flowering. Pollen was sprinkled on emasculated flowers with the help of a camel hairbrush. After pollination, flowers were covered again with the respective paper bags to avoid adulterating foreign pollen. Sunflowers were harvested when the seeds were appropriately matured and later dried individually to remove the moisture content. When the seeds were dried at each end, seeds were packed.

2.1 Data Recording

For data collection, four parents and F1 hybrid plants were tagged randomly. Data on the following pre and post-harvest traits were collected for flower initiation (FI), days taken to 50% flowering (D50%F), days taken to 100% flowering (D100%F), plant height (PH), number of leaves (NOL), leaf area (LA) from three leaves of each plant, head diameter (HD), Achene yield per head (AY/H), 1000-achene weight (1000-AW), protein content (PC), and oil content (OC). Random five plants were selected from each replication for evaluation.

2.2 Statistical and Biometrical Analysis

Steel et al. [14] studied all traits to estimate the analysis of variance and to estimate the data for GCA and SCA. The effect of lines × testers, and the variances of these values were calculated [15] for parents and their hybrids through line tester mating design under RCB design.

3. RESULTS AND DISCUSSION

Four lines and three testers (pollen parent) were used to make twelve sunflower hybrids using the line × tester mating design. Variances, general combining ability (GCA), and specific combining ability (SCA) for different yields and their related traits were evaluated statistically. General and specific combining abilities are primary factors for plant breeders in sunflower breeding strategies. GCA depicts the genetic potential of parental lines, and SCA is accountable for parental interactions. Collected data of different traits analyzed through the analysis of variance. Among crosses, most genotypes showed significant results for observed traits.

3.1 Evaluation of Variability

Significant differences were observed between parental materials (Lines and Testers) for all traits shown in Table 1. In the lines, non-significant results for PH, NOL, and PC were observed. For testers, non-significant results were observed in some traits, i.e., FI, D100%F, PH, HD, and AY/P. Inbred lines showed significant results for all traits. The line × tester result was significant for FI, D50% F, D100% F, PH, NOL, HA, 1000-AW, PC, and OC while the interaction was non-significant for LA and, AY/P. All genotypes revealed significant results for all morphological traits. Hybrids portrayed highly significant (P<0.01) results for all traits except the PH, NOL, and HD. Parents and crosses were shown highly significant (P<0.01) results for all morphological traits except for head diameter, which showed non-significant results. Significant variations in yield and its related traits were also

observed by Zia et al. [16], Din et al. [17], Gejli et al. [18], Hlandi et al. [19] and Imran et al. [20] narrated significant variations among lines, testers and their crosses for HD, OC, AY/P, LA and 1000-AW.

3.2 General Combining Ability (GCA) Effects

GCA refers to the average performance of a parent across multiple hybrid combinations for a particular trait of interest. In sunflower breeding, GCA is used to evaluate the overall genetic potential of a parental line for yield, oil content, disease resistance, and other important traits. The general combining ability of a parental line is then calculated as the average performance of all the hybrids in which it was a parent, minus the average performance of all the other hybrids in the trial. In other words, GCA measures the inherent genetic potential of a parental line for a particular trait, regardless of the specific hybrid combinations. The difference between the performance of hybrids produced by a particular line and the average performance of hybrids produced by all the testers is an estimate of the GCA of that line for that trait [21,22]. The general combining ability for lines is presented in Table 2. Line A51 showed highly significant positive GCA with FI, D100% F, HD, and OC, while negative general combining ability was recorded with PH, LA, and AY/P. In the A52 line, a positive genotypic correlation was witnessed in PH, LA, and AY/P. At the same time, negative GCA was shown in FI, D100% F, and 1000-AW. Line A54 showed negative general combining ability with D50% F, NOL, LA, HA, AY/P, and 1000-AW. In A69, the highly significant positive general combining ability was recorded for traits like LA, AY/P, and 1000-AW. The sum of errors shows a positive genotypic correlation with all traits under study. Same results were reported by Mustafa et al. [23]. The general combining ability of testers is presented in Table 3. Tester A53 showed a negative general combining ability with NOL, LA, and PC. In tester A68, positive general combining ability is demonstrated with traits like PH, PC, and OC, while negative general combining ability is shown by 1000-AW. Tester A70 shows significant positive general combining ability with 1000-AW and LA, while negative general combining ability was shown with D50% F, PH, and OC. The sum of errors shows a significant positive general combining ability with all the traits under study. Similar findings were reported by Khan et al. [24].

3.3 Specific Combining Ability (SCA) Effects

SCA effects show non-additive gene activity. SCA effects should be used in conjunction with other factors, such as the respective parents' GCA, as SCA effects alone have little importance for parental choice in breeding programs [25]. When high-high and high-low general combiners were crossed, the resulting hybrids had significant SCA effects. Hybrids with a high SCA may be produced by combining dominant and recessive genes from weak and good combiners [26]. Table 4 shows the SCA effects for yield and related traits of hybrids. The hybrid A51× A53 showed significant positive specific combining ability effects with D50% F and D100% F. At the same time, negative specific combining ability was shown with PH, NOL, HD, 1000-AW, and OC. In hybrid A51 × A68, negative specific combining ability effects were shown with D50% F and 1000- AW while the positive effect was shown with NOL. In the A51 × A70 cross, significant positive specific ability effects were shown with PH. The hybrid A52 × A53 showed negative specific combining ability effects with FI and D100% F, while a positive effect was shown by PC. A52 × A68 hybrids showed highly significant positive specific combining ability effects with PH and 1000-AW while negative specific combining ability effects with NOL. A52 × A70 crosses showed negative specific combining ability effects with PH, 1000-AW, and PC while significant positive specific combining ability effects with FI and D100% F. In hybrid A54 × A53, negative specific combining ability effects showed with PH while positive specific combining ability effects showed NOL and 1000-AW. The A54 × A68 hybrid showed positive specific combining ability effects with 1000-AW, PC, and OC. In hybrid A54 × A70, the positive specific combining ability was showed by PH, while the negative effect of specific combining ability was with 1000-AW and OC. The A69 × A53 hybrid showed significant positive specific combining ability effects with FI, D100% F, HD, and 1000- AW and highly significant positive specific combining ability effects with PH. Hybrid A69 × A68 showed highly significant positive specific combining ability effects with FI and D100% F while negative specific combining ability effects with PH. The A69 × A70 hybrid showed positive specific combining ability effects with PC while significant negative specific combining ability effects with FI, D50% F, D100% F, PH, and 1000-AW.

Table 1. F- Values from analysis of variance for yield and related traits

SOV	DF	FI	D50%F	D100%F	PH	NOL	LA	HD	AY/P	1000AW	PC	OC
Replications	2	5.10*	1.46	3.47*	3.82*	13.18**	15.25**	8.88**	2.82	5.44*	0.19	0.61
Genotypes	18	33.42**	18.98**	36.19**	10.53**	10.64**	84.29**	6.67**	7.62**	56.73**	27.16**	23.01**
Crosses	11	15.48**	6.17*	12.52**	4.94*	4.76*	20.32**	4.71*	8.37**	45.59**	8.04**	10.46**
Lines (C)	3	11.68**	3.77*	10.19**	2.60	3.37	59.60**	8.62**	30.05**	31.06**	2.09	4.99*
Testers (C)	2	1.78	5.53*	1.87	1.78	5.76*	17.58**	1.65	0.42	22.09**	17.36**	20.23**
Lines X Testers	6	21.95**	7.59**	17.24**	7.16**	5.13*	1.60	3.78*	0.17	60.70**	7.90**	9.93**
Parent	6	71.39**	40.96**	84.85**	8.09**	21.25**	94.13**	5.16*	7.13**	42.26**	55.06**	45.39**
Lines (P)	3	28.84**	8.09**	14.11**	10.89**	16.63**	91.14**	5.27*	6.43*	6.44*	13.49**	6.52*
Testers (P)	2	108.55**	62.22**	19.02**	1.36	14.07**	78.01**	4.21*	4.43*	3.84*	3.99*	1.31
Lines (P) Vs Testers (P)	1	124.72**	97.05**	428.75**	13.18**	49.45**	135.34**	6.71*	14.64**	226.57**	281.91**	250.17**
Crosses Vs Parents	1	2.87	27.92**	4.50*	86.58**	11.61**	728.88**	37.20**	2.33	266.01**	70.16**	26.84**

Significance at the P < 0.05 of the Probability
Significance at the P < 0.01 of the Probability

Table 2. General Combining Ability (GCA) effects of lines for various yield and related traits

Lines	FI	D50%F	D100%F	PH	NOL	LA	HD	AY/P	1000AW	PC	OC
A51	1.01 **	0.38	1.08 **	-1.49 *	0.21	-3.27 **	1.43 **	-4.59 **	0.17	0.29	1.81 **
A52	-0.99 **	0.43	-1.03 **	2.79 **	0.45	1.50 *	-0.59 ns	4.51 **	-1.53 *	-0.39	-0.82
A54	-0.32	-1.07 *	-0.31	-0.97	-1.15 **	-2.96 **	-0.98 *	-4.70 **	-2.46 **	-0.48 ns	-0.99
A69	0.29	0.26	0.25	-0.33	0.49	4.72 **	0.14	4.78 **	3.82 **	0.58 ns	-0.001
S.E	0.2737	0.4306	0.2794	0.5477	0.3714	0.5450	0.3943	1.1560	0.5496	0.4099	0.6024

Significance at the P < 0.05 of the Probability
Significance at the P < 0.01 of the Probability

Table 3. General Combining Ability (GCA) effects of testers for various yield and related traits

Lines	FI	D50%F	D100%F	PH	NOL	LA	HD	AY/P	1000AW	PC	OC
A51	0.17	0.63	0.13	-0.52	-1.00 **	-1.52 **	-0.21	-0.63	0.41	-1.43 **	-1.34 *
A52	0.17	0.21	0.25	1.56 **	0.34	-0.47	0.46	0.25	-2.20 **	1.06 **	2.58 **
A54	-0.33	-0.83 *	-0.38	-1.04 *	0.66	1.99 **	-0.25	0.38	1.79 **	0.37	-1.24 *
A69	0.2370	0.3729	0.2420	0.4743	0.3216	0.4720	0.3415	1.0012	0.4760	0.3550	0.5217
S.E	0.17	0.63	0.13	-0.52	-1.00 **	-1.52 **	-0.21	-0.63	0.41	-1.43 **	-1.34 *

Significance at the P < 0.05 of the Probability
Significance at the P < 0.01 of the Probability

Table 4. Specific Combining Ability (SCA) effects for various yield and related traits

Hybrids	FI	D50%F	D100%F	PH	NOL	LA	HD	AY/P	1000AW	PC	OC
A51 × A53	1.28 *	1.38	1.21 *	-3.71 **	-1.92 **	0.31	-1.69 *	-0.74	-5.83 **	0.04	-2.48 *
A51 × A68	-0.89	-1.88 *	-0.75	1.57	2.27 **	0.16	0.50	0.78	-5.46 **	-1.01	-1.44
A51 × A70	-0.39	0.50	-0.46	2.14 *	-0.35	-0.47	1.19	-0.04	11.29 **	0.97	3.91 **
A52 × A53	-1.72 **	-1.51	-1.68 **	1.24	-0.52	-0.86	-0.40	-0.10	0.20	1.49 *	1.46
A52 × A68	-0.89	0.74	-0.97	3.93 **	0.24	0.93	0.39	-0.49	3.01 **	0.10	-2.54 *
A52 × A70	2.61 **	0.78	2.65 **	-5.17 **	0.29	-0.07	0.01	0.59	-3.21 **	-1.59 *	1.08
A54 × A53	-0.72	-1.35	-0.74	-4.30 **	1.74 *	0.06	0.56	0.77	3.39 **	-0.72	1.22
A54 × A68	0.28	0.57	0.31	-0.88	-1.26	0.58	-0.09	-0.61	2.23 *	1.99 *	2.53 *
A54 × A70	0.44	0.78	0.43	5.18 **	-0.48	-0.64	-0.47	-0.17	-5.62 **	-1.26	-3.75 **
A69 × A53	1.17 *	1.49	1.21 *	6.77 **	0.70	0.49	1.53 *	0.06	2.24 *	-0.81	-0.20
A69 × A68	1.50 **	0.57	1.42 **	-4.62 **	-1.24	-1.66	-0.81	0.31	0.22	-1.07	1.44
A69 × A70	-2.67 **	-2.06 *	-2.63 **	-2.15 *	0.54	1.18	-0.73	-0.38	-2.46 *	1.88 *	-1.24

Significance at the P < 0.05 of the Probability
Significance at the P < 0.01 of the Probability

Table 5. Estimation of Variance due to GCA, SCA, Additive, Dominance, Ratio of SCA to GCA and Degree of Dominance

Traits	Cov H.S Lines GCA (σ^2 GCA)	Cov H.S Testers GCA (σ^2GCA)	Cov H.S Ave (σ^2 D)	Cov H.S F.S (σ^2 A)	Var of GCA GCA (σ^2GCA)	Var of SCA GCA (σ^2GCA)	F=1 A (σ^2 A)	F=1 D (σ^2 D)	1000AW	PC	OC
FI	-0.6412	-0.9444	-0.1568	2.0382	-0.1568	3.8864	-0.3136	3.8864	-5.83 **	0.04	-2.48 *
D50%F	-0.5201	-0.2106	-0.0749	1.7674	-0.0749	2.5434	-0.1498	2.5434	-5.46 **	-1.01	-1.44
D100%F	-0.5502	-0.8997	-0.1429	2.0999	-0.1429	3.802	-0.2859	3.802	11.29 **	0.97	3.91 **
PH	-6.4537	-5.7058	-1.2204	15.8469	-1.2204	29.5016	-2.4407	29.5016	0.20	1.49 *	1.46
NOL	-0.3152	0.0845	-0.0254	2.1544	-0.0254	2.3484	-0.0508	2.3484	3.01 **	0.10	-2.54 *
LA	14.2584	2.9473	1.7871	17.8818	1.7871	0.2894	3.5742	0.2894	-3.21 **	-1.59 *	1.08
HD	0.632	-0.2087	0.0473	1.3535	0.0473	1.0155	0.0946	1.0155	3.39 **	-0.72	1.22
AY/P	28.6089	0.1775	3.0459	24.3195	3.0459	-3.5108	6.0918	-3.5108	2.23 *	1.99 *	2.53 *
1000GW	-7.3218	-7.1524	-1.4484	27.7017	-1.4484	44.0773	-2.8968	44.0773	-5.62 **	-1.26	-3.75 **
PC	-0.7407	0.9028	0.0065	2.9758	0.0065	2.5149	0.0131	2.5149	2.24 *	-0.81	-0.20
OC	-1.6264	2.5441	0.0672	10.4679	0.0672	8.7246	0.1345	8.7246	0.22	-1.07	1.44

Significance at the P < 0.05 of the Probability
Significance at the P < 0.01 of the Probability

3.4 Genetic Variance

Genetic variance refers to the variation in a trait that is due to genetic differences among individuals in a population. In sunflowers, several types of genetic variance can be important in breeding and genetic analysis, including Additive variance and Dominance variance. Additive variance is the component of genetic variance that is due to the additive effects of individual genes on the trait. Dominance variance is the component of genetic variance that is due to the interaction between different alleles at the same gene locus. Dominance variance is important because it can affect the expression of the trait in heterozygous individuals and can also influence the response to selection. In the case of FI, the GCA variance is less than SCA variance; dominance variance is greater than additive variance, and fraction variance is greater than the average variance. For D50% F, the GCA variance is less than the SCA variance, which means the dominance variance is greater than the additive variance, and the fraction variance is also greater than the average variance. Greater dominance variance, SCA variance, and fraction variance were also observed in D100% F, PH, NOL, HD, 1000-AW, PC, and OC. In contrast, greater additive, GCA, and fraction variance were observed in LA and AY/P. Overall, in all traits, SCA variance is greater than GCA variance, which means the involvement of recessive genes has more than dominant genes except for the LA and AY/P. These traits shown recessive gene involvement than dominant gene, and the findings were related to Patil et al. [27], Kang et al. [28], Jhondale et al. [29], Oshhundia et al. [30], Imran et al. [20], Qamar et al. [31], Azad et al. [32], Khalid et al. [33], Kulkarni and Supriya [34] and Rameeh and Andarkhor [35].

3.5 Proportional Involvement of Lines, Testers, and their Interactions to the Total Variance

A line × tester mating design was used to check the interaction of lines and testers. For this purpose, four lines and three testers were used under the line x-tester design. The overall variance for various parameters is shown in Table 6. The lines contain a very significant role for eight traits, i.e., FI (20.57%), D50% F (16.65%), D100% F (22.19%), PH (14.36%), LA (79.97%), HA (49.87%), AY/P (97.95%) and 1000-AW (18.58%) as compared to the tester's contribution for all these traits, i.e., FI (2.09%), D50% F (16.28%), D100% F (2.71%), PH

(6.57%), LA (15.73%), HA (6.37%), AY/P (0.91%) and 1000-AW (8.81%). At the same time, lines had shown a non-significant role for three traits, i.e., NOL (19.31%), PC (7.08%), and OC (13.02%), as compared to the tester's contribution for these three traits, i.e., NOL (21.97%), PC (39.27%), and OC (35.17%). Maternal and paternal collaboration (influence of line and testers) was great for all the traits except the LA (4.3%) and AY/P (1.13%).

Table 6. Proportional Involvement of Lines, Testers, and their Interactions to the Total Variance for Indicated Plant Characters of Sunflower

Traits	Lines	Testers	Lines × Testers
FI	20.57	2.09	77.34
50%F	16.65	16.28	67.07
100%F	22.19	2.71	75.09
PH	14.36	6.57	79.07
NOL	19.31	21.97	58.72
LA	79.97	15.73	4.3
HD	49.87	6.37	43.76
SY	97.95	0.91	1.13
1000GW	18.58	8.81	72.61
PC	7.08	39.27	53.65
OC	13.02	35.17	51.8

4. CONCLUSION

Line × tester analysis in sunflower revealed promising lines for hybrid development. A54 and A69 emerged as good general combiners, while specific combinations showed potential for targeted trait improvement, especially in plant height and achene weight. Lines played a significantly larger role than testers in influencing key yield and morphological traits, suggesting greater breeding potential within the female lines. Utilizing these findings, sunflower breeding programs can prioritize promising lines and exploit both general and specific combining abilities to create high-yielding hybrids with desirable agronomic characteristics.

in the study design, collection, analysis and interpretation of data; in the writing of the manuscript. If the study sponsors had no such involvement, the authors should so state.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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