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Performance prediction and validation of three way cross and double cross hybrids for fruit yield in chilli (*Capsicum annuum* L.)

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Abstract

Predicting the performance of multi-parent hybrids even before their synthesis may conserve time and resources incurred during hybridization and evaluation. Five diverse chilli parental lines were crossed in half diallel mating design to produce 10 single crosses. Three-way cross and double cross hybrids were generated from single cross hybrids and evaluated for 4 quantitative characters viz., fruits plant⁻¹, fruit length, average green fruit weight and green fruit yield plant⁻¹. Their *per se* performance in terms of yield components were validated by two models, proposed by Jenkins, viz., mean of non-parental single crosses and mean of all possible single crosses among the parents involved in that cross. There was a perfect agreement between the observed and expected values in both prediction methods as per theory for all the traits under consideration, except for green fruit yield plant⁻¹ suggesting that epistatic interaction might be involved in inheritance of this trait.

Keywords: *Capsicum annuum*, double cross hybrids, half diallel, performance prediction, three-way

Introduction

Pepper (*Capsicum* spp., Solanaceae), is an important vegetable crop mostly used as a spice, condiment, vegetable and source of vitamin A and C throughout the world. Amongst the 5 cultivated species of genus *Capsicum*, *C. annuum* is the most widely cultivated species in India owing to its pungent (hot pepper) and non-pungent (sweet pepper syn. capsicum, bell pepper) fruits (Reddy *et al.*, 2014) [9]. Worldwide, India is the largest producer of chilli accounting for 1.1 m t annually, followed by China with a production of around 0.4 m t (Patel *et al.*, 2015) [10]. Single cross hybrids are most commonly cultivated types in chilli. When it is difficult to obtain the favourable combination of traits in pure lines derived from the cross of two parents then it would be advantageous to attempt multi-parent crosses. If the F₂s from a single cross is compared with multi-parent crosses, greater heterogeneity is observed in the populations derived from that of multiple parents. Moreover, the population derived from multiple parents will have more alleles at a locus as compared to biparental population. For instance, four alleles and three alleles can be harnessed from a population of double crosses and three-way crosses, respectively. This composite breeding technique is a means of creating and preserving genetic variation in an exploitable form with a good potential of selecting high yielding genotypes.

Single cross hybrids are considered most desirable because breeding and seed production is much easier in such hybrids than in multi-parent hybrids (Khan *et al.*, 2015) [8]. Multi-parent hybrids have wide genetic base, however as the number of parental lines increases the number of three way cross and double cross hybrids increases to a great extent. As a result, the synthesis and evaluation of a large number of multi-parent hybrids is labour-intensive and uneconomical. Therefore, it would be of great advantage if the performance of these hybrids is predicted well before. This possibly would eliminate the laborious testing of single crosses and breeders can proceed directly from testing of inbred-variety crosses to the testing of double crosses (Jenkins, 1934) [7]. Performance prediction is based on the performances of single crosses and their combinations in hybrids. Doxtator *et al.* (1936) [4] suggested that the performance of double cross hybrids can be predicted by appropriate use of data from single cross hybrids. Anderson (1938) [1] and Hayes *et al.* (1943) [6] reported good agreement of predicted and observed results when they used non-parental single cross means to predict double cross performance.

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This method has subsequently been used by maize breeders to reduce the number of double crosses to be tested to a manageable number. However, Carballoa (1957) while comparing the observed double cross mean yields and predicted means, obtained from non-parental single crosses, found a low correlation ($r = 0.12$) in 568 double crosses grown during a 6-year period. Hence, we are trying to validate it in chilli crop. So, out of the four methods proposed by Jenkins two methods were used to find if observed performances of these double cross and three-way cross hybrids are on par with the predicted performances.

Table: three way cross hybrids, double cross hybrids show

Three-way cross hybrids	Abbreviation used	Double Cross Hybrids	Abbreviation used
(Gouribidanur × PBC 483) × Tiwari	TWCH 1	(CCA 9907-9611 × Byadagikaddi) × (Tiwari × PBC 483)	DCH 1
CCA 9907-9611 × (Tiwari × PBC 483)	TWCH 2	(CCA 9907-9611 × Byadagikaddi) × (Gouribidanur × PBC 483)	DCH 2
Byadagikaddi × (Tiwari × PBC 483)	TWCH 3	(CCA 9907-9611 × Byadagikaddi) × (Gouribidanur × Tiwari)	DCH 3
(CCA 9907-9611 × Byadagikaddi) × Gouribidanur	TWCH 4	(Gouribidanur × PBC 483) × (Tiwari × Byadagikaddi)	DCH 4
(Gouribidanur × PBC 483) × CCA 9907-9611	TWCH 5	(Byadagikaddi × Gouribidanur) × (Tiwari × PBC 483)	DCH 5
(Tiwari × Byadagikaddi) × Gouribidanur	TWCH 6	(Byadagikaddi × Gouribidanur) × (CCA 9907-9611 × PBC 483)	DCH 6
(Tiwari × PBC 483) × Gouribidanur	TWCH 7	(Byadagikaddi × Gouribidanur) × (CCA 9907-9611 × Tiwari)	DCH 7
Byadagikaddi × (CCA 9907-9611 × Tiwari)	TWCH 8	(CCA 9907-9611 × Gouribidanur) × (Tiwari × PBC 483)	DCH 8
(Byadagikaddi × PBC 483) × Gouribidanur	TWCH 9	(CCA 9907-9611 × Gouribidanur) × (Byadagikaddi × PBC 483)	DCH 9
(Gouribidanur × PBC 483) × Byadagikaddi	TWCH 10	(CCA 9907-9611 × Gouribidanur) × (Tiwari × Byadagikaddi)	DCH 10
(CCA 9907-9611 × PBC 483) × Tiwari	TWCH 11	(CCA 9907-9611 × PBC 483) × (Tiwari × Byadagikaddi)	DCH 11
Tiwari × (CCA 9907-9611 × Gouribidanur)	TWCH 12	(Gouribidanur × Tiwari) × (CCA 9907-9611 × PBC 483)	DCH 12
(Byadagikaddi × PBC 483) × Tiwari	TWCH 13	(CCA 9907-9611 × Tiwari) × (Gouribidanur × PBC 483)	DCH 13
(Tiwari × Byadagikaddi) × PBC 483	TWCH 14	(Gouribidanur × Tiwari) × (Byadagikaddi × PBC 483)	DCH 14
(CCA 9907-9611 × Gouribidanur) × PBC 483	TWCH 15	(CCA 9907-9611 × Tiwari) × (Byadagikaddi × PBC 483)	DCH 15
(CCA 9907-9611 × Gouribidanur) × Byadagikaddi	TWCH 16		
(Gouribidanur × Tiwari) × PBC 483	TWCH 17		
(Byadagikaddi × Gouribidanur) × Tiwari	TWCH 18		
(CCA 9907-9611 × PBC 483) × Byadagikaddi	TWCH 19		
(Gouribidanur × Tiwari) × CCA 9907-9611	TWCH 20		
Gouribidanur × (CCA 9907-9611 × Tiwari)	TWCH 21		
(Byadagikaddi × Gouribidanur) × CCA 9907-9611	TWCH 22		
(CCA 9907-9611 × Byadagikaddi) × PBC 483	TWCH 23		
(Byadagikaddi × PBC 483) × CCA 9907-9611	TWCH 24		
(Gouribidanur × Tiwari) × Byadagikaddi	TWCH 25		
(CCA 9907-9611 × Byadagikaddi) × Tiwari	TWCH 26		
(Tiwari × Byadagikaddi) × CCA 9907-9611	TWCH 27		
(CCA 9907-9611 × PBC 483) × Gouribidanur	TWCH 28		
(CCA 9907-9611 × Tiwari) × PBC 483	TWCH 29		
(Byadagikaddi × Gouribidanur) × PBC 483	TWCH 30		

These multi-parental hybrids were synthesised in a way that no parental line should be repeated in each TWC and DC hybrid. Five parental lines and ten SC hybrids were evaluated separately in a randomised complete block design (RCBD) with three replications. While, the TWC and DC hybrids being heterogeneous were evaluated in an unreplicated trial during *rabi* 2012. Forty days old seedlings of parents and their SC hybrids were planted in two rows each of 5 m length with a spacing of 0.75 m between rows and 0.45 m between plants within a row. Same aged seedlings of 30 TWC and 15 DC hybrids each were planted in the similar fashion evaluated in an unreplicated trial side by side. Observations for the number of fruits plant⁻¹, fruit length, average green fruit weight and green fruit yield plant⁻¹ were recorded on randomly selected five plants in each replication for single crosses whereas single plant observations were recorded on 30 randomly chosen plants in case of TWC and DC hybrids. Mean of single cross hybrids over replications were used to estimate the predicted means of TWC and DC hybrids, whereas mean of 30 randomly selected plants in each TWC and DC hybrid were considered as realised values.

The quantitative trait mean of each DC hybrids was predicted by two methods *viz.*, mean of non-parental SC hybrids involved in DC hybrids as parents (method 1) and mean of all

Materials and Methods

Five diverse genotypes of chilli namely PBC-483, Gouribidanur, Tiwari, Byadagi Kaddi and CCA 9907-9611 were used as parental lines and crossed in half diallel mating design to obtain ten single cross (SC) hybrids during *kharif* 2011. These ten single cross hybrids were, in turn, used to synthesise 15 double cross (DC) hybrids and 30 three-way crosses (TWC) hybrids during summer 2012. The multi-parental hybrids used were as under:

possible single cross hybrids involved in DC hybrids (method 2) [Jenkins, 1934] [7].

$$\text{Method 1: } (\overline{A \times B}) \times (\overline{C \times D}) = \frac{(A \times C) + (A \times D) + (B \times C) + (B \times D)}{4}$$

$$\text{Method 2: } (\overline{A \times B}) \times (\overline{C \times D}) = \frac{(A \times C) + (A \times D) + (B \times C) + (B \times D) + (A \times B) \times (C \times D)}{6}$$

The quantitative trait means of TWC hybrids was predicted as

$$\overline{(A \times B)} \times C = \frac{(A \times C) + (B \times C)}{2}$$

Where A, B, C and D are the parental lines

The agreement of predicted quantitative trait mean of each TWC and DC hybrids with those of respective realized means was tested using χ^2 test (Pearson, 1900) [11]. As TWC and DC hybrid means are not estimated with equal precision and are associated with different variances, therefore the weight defined as the reciprocal of trait variances of each TWC and DC hybrids was used as weights while calculating χ^2 test statistic.

$$\text{Calculated } \chi^2 = (O-E)^2 \times \text{Weight}$$

$$\text{Where Weight} = \frac{1}{\sigma_{TWC/DC}^2}$$

O and E refer to observed and expected values; TWC and DC refer to three way cross and double cross hybrids respectively. To standardise the χ^2 value corresponding weights were used instead of dividing it with expected value. If calculated chi square is more than table χ^2 value at $(n-1)$ *df*, it suggested the non-agreement of predicted and realized trait means.

Results and Discussion

The predicted performance was in perfect agreement with realised number of fruits plant⁻¹ in all the TWC hybrids. All the crosses, except two TWC hybrids, expressed mean fruit length which did not differ significantly from that of predicted mean for fruit length (Table 1). In case of average fruit weight, the TWC hybrids, except two hybrids, manifested non-significant differences between realised and predicted performances for average fruit weight. Differences between realised and predicted performances of 73% TWC hybrids were significant for green fruit yield plant⁻¹ (Table 1). There was a good agreement between realised and predicted performances of TWC hybrids for all studied, except green fruit yield plant⁻¹.

Predicted number of fruits plant⁻¹ of all DC hybrids, except (Gouribidanur × Tiwari) × (Byadagikaddi × PBC 483), was in accordance with that of realised number of fruits plant⁻¹ in both methods (Table 2). All DC hybrids, except (Byadagikaddi × Gouribidanur) × (Tiwari × PBC 483), exhibited fruit length which did not differ significantly from the predicted length in both methods. Two DC hybrids expressed significant deviation from realised average fruit weight in both prediction methods (Table 3). In both the prediction methods, nearly 13 out of 15 DC hybrids

manifested significant difference between realised and predicted green fruit yield plant⁻¹.

Significance of differences between predicted and realized performance of TWC and DC hybrids indicate involvement of epistasis (Chahal and Gosal, 2002) [3] for the inheritance of green fruit yield plant⁻¹. However, good agreement between realised and predicted performances of TWC and DC hybrids for all other traits is an indication of adequacy of additive-dominance model in the inheritance of these traits. Sujiprahati *et al.* (2003) [12] observed a good agreement between actual and predicted yields in maize at two locations. The rationale of high predictive power of the method (that involve mean of non-parental single crosses as a predictor) is that for any individual locus, the DC hybrid {(A × B) × (C × D)} includes only those genotypes which are produced in AC, AD, BC and BD single-crosses. Thus, the magnitude of additive and dominant effects expressed in DC hybrids would be the same as that of non-parental SC hybrids. These two populations *i.e.*, 'double crosses' and group of non-parental single crosses however, may be different with respect to a few specific combinations of genes at different loci which is of course inconsequential so long as genes at different loci are independent in action, *i.e.*, epistasis is absent (Hallauer and Miranda, 1988) [5]. However, genotype × environmental interactions and plot error may affect the accuracy of prediction formula to a greater extent than the epistatic bias unless adequate numbers of replications and environments are used to obtain the single cross or three-way cross means. Further, triple test cross involving contrasting parents can be used as a tool to confirm the involvement of epistatic gene action in governing fruit yield plant⁻¹.

Table 1: Estimates of realised and predicted mean performances of three-way cross hybrids for number of fruits plant⁻¹, fruit length, Average green fruit weight and Green fruit yield plant⁻¹ in chilli

Three way cross hybrids	Number of fruits plant ⁻¹			Fruit length (cm)			Average green fruit weight (g)			Green fruit yield plant ⁻¹ (g)		
	Realised	Predicted	Chi square statistic	Realised	Predicted	Chi square statistic	Realised	Predicted	Chi square statistic	Realised	Predicted	Chi square statistic
TWCH 1	70.33	83.4	1.74	7.31	6.97	0.19	2.29	1.90	11.68	159.90	273.67	24.4*
TWCH 2	55.87	58.6	0.07	8.68	8.73	0.04	2.95	2.86	0.89	159.31	349.5	55.7**
TWCH 3	46.25	52.6	0.69	9.35	9.50	0.18	2.46	2.31	0.85	110.47	392.02	237.0**
TWCH 4	59.15	40.0	6.49	8.23	8.63	3.24	2.37	2.69	7.58	132.87	189.66	10.30
TWCH 5	59.30	40.5	7.63	8.83	8.95	0.58	2.80	2.76	0.08	162.41	196.12	3.30
TWCH 6	88.40	51.6	15.46	7.65	7.68	0.02	2.19	2.15	0.29	190.54	157.59	2.10
TWCH 7	79.28	66.4	1.55	9.64	6.67	75.63**	2.23	2.02	15.01	158.48	196.49	3.70
TWCH 8	47.69	50.5	0.06	10.2	10.13	0.02	2.56	2.40	0.94	122.19	296.55	25.8**
TWCH 9	43.69	47.2	0.20	7.81	7.95	0.23	1.96	2.41	12.26	83.74	214.09	54.3**
TWCH 10	42.41	38.2	0.13	7.87	9.20	20.51	2.02	2.47	18.73	80.49	271.00	71.5**
TWCH 11	76.75	89.9	1.87	7.67	7.70	0.01	2.54	2.54	0.00	168.22	459.11	231.0**
TWCH 12	70.34	77.5	1.42	7.92	7.13	14.20	2.28	2.41	1.55	177.67	325.43	84.9**
TWCH 13	72.25	78.5	0.48	9.12	8.55	3.00	2.29	2.12	2.74	179.07	412.29	105.2**
TWCH 14	65.40	69.9	0.26	9.97	8.48	20.99	2.74	2.21	12.97	176.56	387.08	93.5**
TWCH 15	65.29	47.6	11.59	9.42	8.27	52.65**	2.50	2.48	0.09	163.32	220.56	20.2
TWCH 16	45.64	36.1	1.49	8.93	9.83	5.19	2.10	2.56	26.39*	98.85	175.53	26.9*
TWCH 17	71.65	78.9	0.77	8.77	7.23	22.64	2.65	2.16	17.22	181.94	330.17	62.1**
TWCH 18	67.25	66.0	0.03	8.74	7.98	8.21	2.77	1.99	32.04**	166.91	278.61	25.9*
TWCH 19	42.00	41.9	0.00	10.12	10.07	0.01	3.06	2.49	14.59	104.28	271.34	106.6**
TWCH 20	60.94	65.9	0.44	8.61	8.08	2.80	2.57	2.95	6.75	145.01	357.49	86.1**
TWCH 21	74.75	59.3	3.43	6.3	7.35	25.61	2.14	2.31	1.66	143.11	172.05	2.70
TWCH 22	39.85	43.8	0.34	8.91	9.50	3.30	2.45	2.72	3.03	94.43	189.99	32.4**
TWCH 23	40.93	38.6	0.13	9.09	9.52	1.85	2.19	2.53	4.67	97.33	277.47	132.6**
TWCH 24	41.91	36.5	0.36	9.36	10.15	5.69	2.18	2.63	9.94	81.00	182.00	26.9*
TWCH 25	54.67	46.7	3.91	9.67	9.27	1.35	2.55	2.37	3.64	136.65	296.21	123.8**
TWCH 26	54.88	72.6	4.83	9.70	8.72	12.56	2.72	2.64	0.58	172.60	464.05	127.3**
TWCH 27	41.19	62.0	15.15	9.93	9.28	3.54	2.81	2.82	0.00	121.20	343.37	96.5**
TWCH 28	68.10	54.9	1.52	7.03	7.62	8.64	2.50	2.57	0.97	155.26	228.55	7.80
TWCH 29	71.31	64.5	0.35	8.12	8.57	4.79	2.78	2.35	7.17	189.12	297.74	9.70
TWCH 30	49.68	53.0	0.21	7.91	8.18	1.44	2.02	2.34	19.64	110.33	309.90	261.5**

*Significant @P=0.05, **Significant @P=0.01

Table 2: Estimates of realised and predicted mean performances of double cross hybrids for fruits plant⁻¹ and fruit length in chilli

Double cross hybrids	Fruits plant ⁻¹					Fruit length (cm)				
	Realised	Predicted (Method 1)	Chi square statistic	Predicted (Method 2)	Chi square statistic	Realised	Predicted (Method 1)	Chi square statistic	Predicted (Method 2)	Chi square statistic
DCH 1	44.95	55.6	4.58	59.68	8.78	8.91	9.12	0.05	9.12	0.05
DCH 2	45.43	39.3	1.21	43.19	0.16	9.82	9.08	9.58	8.99	11.93
DCH 3	50.85	56.3	0.36	55.99	0.32	7.67	8.68	9.28	8.63	8.53
DCH 4	47.25	60.8	3.95	61.02	4.11	8.85	8.08	11.19	8.14	9.63
DCH 5	76.13	59.5	4.31	61.02	3.56	9.89	8.08	61.26**	8.14	57.55**
DCH 6	38.85	48.4	4.27	43.19	0.88	9.90	8.84	14.21	8.99	10.53
DCH 7	84.33	54.9	4.74	55.99	4.39	8.56	8.74	0.24	8.63	0.04
DCH 8	66.50	62.5	0.29	43.19	10.14	8.20	7.70	4.46	8.99	11.11
DCH 9	50.09	41.8	2.19	43.19	1.53	9.60	9.05	3.48	8.99	4.30
DCH 10	51.75	56.8	0.54	55.99	0.39	8.50	8.48	0.01	8.63	0.18
DCH 11	58.71	65.9	1.59	59.68	0.03	8.30	8.88	8.88	9.12	17.40
DCH 12	41.00	72.4	20.57	43.19	0.10	6.89	7.66	19.93	8.99	148.72**
DCH 13	45.28	61.9	3.30	43.19	0.05	7.54	7.96	0.58	8.99	6.99
DCH 14	14.00	62.8	51.58**	61.02	47.86**	7.57	8.25	2.59	8.14	1.81
DCH 15	52.23	57.5	0.83	59.68	1.65	9.01	9.35	3.09	9.12	0.30

*Significant @P= 0.05, **Significant @P=0.01

Table 3: Estimates of realised and predicted mean performances of double cross hybrids for Average green fruit weight, number of fruits plant⁻¹ and Green fruit yield plant⁻¹ in chilli

Double cross hybrids	Average green fruit weight (g)					Green fruit yield plant ⁻¹ (g)				
	Realised	Predicted (Method 1)	Chi square statistic	Predicted (Method 2)	Chi square statistic	Realised	Predicted (Method 1)	Chi square statistic	Predicted (Method 2)	Chi square statistic
DCH 1	2.58	2.59	0.00	2.49	0.68	130.02	370.8	238.6**	344.38	189.20**
DCH 2	2.60	2.61	0.01	2.55	0.13	117.77	233.6	87.0**	227.19	77.68**
DCH 3	2.26	2.66	6.37	2.50	2.25	126.84	326.9	118.8**	270.54	61.31**
DCH 4	2.34	2.18	0.87	2.20	0.63	128.09	272.3	69.1**	293.26	90.57**
DCH 5	2.58	2.17	25.22*	2.20	20.80	197.83	294.3	23.7*	293.26	23.18
DCH 6	2.97	2.53	10.88	2.55	9.75	115.03	249.9	70.0**	227.19	48.40**
DCH 7	2.56	2.35	1.76	2.50	0.15	216.90	234.3	0.20	270.54	2.10
DCH 8	2.92	2.44	8.47	2.55	5.00	171.47	273.0	19.1	227.19	5.76
DCH 9	2.77	2.52	4.58	2.55	3.34	141.17	198.0	10.4	227.19	23.80*
DCH 10	2.49	2.48	0.00	2.50	0.01	118.85	250.5	88.1**	270.54	117.01**
DCH 11	2.80	2.52	10.34	2.49	12.17	178.64	365.2	89.2**	344.38	70.39**
DCH 12	2.03	2.56	35.60**	2.55	35.26**	90.30	343.8	363.1**	227.19	105.84**
DCH 13	1.97	2.33	5.48	2.55	14.42	90.37	234.9	56.7**	227.19	50.81**
DCH 14	1.77	2.27	25.35*	2.20	19.46	80.13	313.2	433.9**	293.26	362.89**
DCH 15	1.79	2.37	61.25**	2.49	88.32**	99.97	297.1	509.4**	344.38	782.64**

*Significant @P= 0.05, **Significant @P=0.01

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