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Short communication

Breeding cycle of fifth generation inbred of cocoa and performance analysis of progenies over generations

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ABSTRACT

Cocoa (*Theobroma cacao* L), the only source of chocolate has a highly complex genetic structure. Kerala Agricultural University (KAU) started inbreeding programme since 1987 with the objective of producing homozygous inbreds for use as parents for hybridization programme. Inbred crosses between two distinct genotypes are expected to produce exceptionally high vigour in F_1 generation. Use of homozygous inbreds as parents eliminates the problem of high variability among the progenies when heterozygous parents are produced. World's first reported fifth inbreeding generation (S_5) of GII 7.4 accession was field established at KAU during 2006. Seedlings established in the field in successive generation were very less because of high level of abnormalities expressed in the nursery. But when performance of field established inbreds over generations was analyzed, there was not much significant inbreeding depression when compared to preceding generation. This might be due to the presence of characters controlled by additive genes, only vigorous inbreds were field established and ancestral parents of GII 7.4 may be adapted to self pollination. The results of some former studies indicated that different genotypes of cocoa responded in different way to inbreeding depression. Hence, non-expression of inbreeding depression by a single genotype may not rule out the possibility of using cocoa inbreds in commercial hybrid production. More over hybrids produced from two diverse inbreds will have an added advantage of combination of only superior alleles from both genetically divergent parents.

Key words: Cocoa, inbreeding depression, inbreds, homozygosity, hybrid vigour.

Cocoa (*Theobroma cacao* L.) is the only source of chocolate and is the one of most luxurious indulgent foods in existence. It is a perennial diploid (2n = 20) with highly complex genetic structure. Kerala Agricultural University started cocoa breeding since 1987 and divergent heterozygous parents were used for the production of hybrids, which exhibited a high amount of variability among them. Moreover superiority of each hybrid seedlings produced by crossing heterozygous parents cannot be assured. Heterozygous parents may lead to the expression of inferior alleles in their hybrid progenies, which was originally masked in heterozygous condition. The only method to overcome this situation is to produce inbreds and then cross between two genetically distinct inbreds. After studying the positive results achieved by using different inbred lines of ICS (Imperial College Selection) clones from Trinidad, Soria (12) suggested taking up inbreeding in cocoa for production of uniform F_1 hybrids.

By the year, 2006 our centre succeeded in producing first ever fifth inbred generation of cocoa reported in the world. 968 inbreds of various genotypes belonging to different generations were field established and details are furnished in Table 1. This paper explains breeding cycle of S_5 (fifth selfed generation) inbred of GII 7.4, and performance of inbred progenies of this genotype over generations. The seedling raised from pods were collected from farmers field and field established during 1980 was selected for the study. This self compatible genotype was selfed during 1987. The method of assisted pollination was followed as described by Mallika *et al.* (5). One hundred flowers were selfed. Seedlings from these pods were raised in the nursery for the production of selfed first generation (S_1 generation). 10 inbreds survived were planted during 1989. To advance the selfed generation assisted self pollination of these plants were taken up during 1991 and out of 10 S_1 nine turned out to be self incompatible (SIC). Subsequently, selfed generation two (S_2) inbreds of GII 7.4 (1 number) was field established during the year 1992. In 1994, selfing of S_2 was carried out and luckily it turned out to be self-compatible by setting pods. Seeds from these pods were raised in the nursery for the production of next selfed generation S_3. 16 seedlings of S_3 generation were field established during 1995 and when they started flowering during 1998 selfing programme was taken up. Out of these 16 S_3 only three seedlings set pod on selfing and 7 S_4 (selfed generation four) plants were planted on during 1999. In 2004, S_4 generation plants were selfed and from three SC ones only one S5 (selfed

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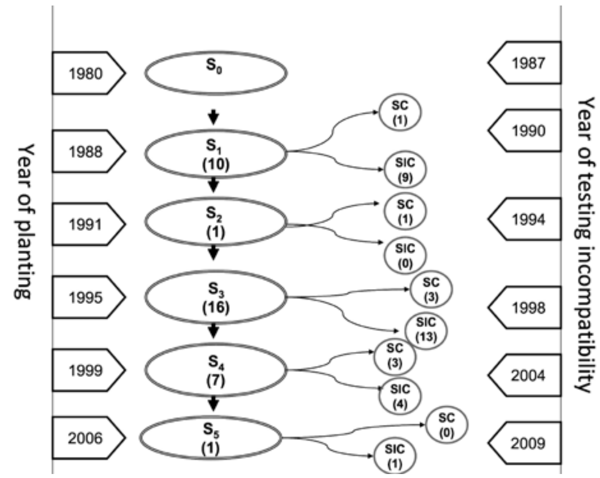
Table 1. Details of cocoa inbreds which were field established.

Set No.	No. of parents	Generation(s)	No. of plants	Year of planting
I	4	S1	28	1988
II	2	S2	21	1991
III	13	S1, S2	188	1992
IV	9	S1	137	1994
V	10	S1, S2, S3	146	1995
VI	1	S2	3	1997
VII	2	S3, S4	64	1999
IX	4	S1, S3	58	2001
X	5	S1	78	2002
XI	14	S1	71	2003
XII	6	S2, S3, S4	52	2004
XIII	1	S5	9	2006
XIV	2	S1, S2	46	2007
XV	6	S2, S4	56	2009
XVI	1	S2	11	2010

generation five) was successfully field established in 2006. Upon flowering this, S_5 inbred was continuously selfed during 2009 and 2010 and resulted in no pod set and it was concluded to be SIC and therefore further advancement of generation was not possible. Advantage of perennial crops over annual crops is that all generation inbreds can be maintained at the same time in the field for a long period. So at any time of research we can trace back to the parent of each generation. Taking this advantage in 2011- 2012 inbreeding of S_4 plants of GII 7.4 is again taken up in anticipating for getting a self compatible S_5, so that the generation can be advanced further. As a result three more S5 generation inbreds are field planted during 2013. Inbreeding cycle of GII 7.4 is schematically represented in Fig 1. Number of seedlings that could be field established in each generation are very small because of high level of abnormalities expressed in the nurseries as reported by Mallika *et al.* (6). The general tendency of cocoa population to exhibit more self compatible types (Nair, 8) was exhibited in inbreeding population also.

Growth and yield attributes (number of pods per tree, pod weight (g), pod length (cm), pod breadth (cm), husk thickness (mm), number of seeds, wet bean weight per pod (g), single dry bean weight, dry bean length (cm), dry bean breadth (cm), dry bean thickness (cm) were recorded in each generation. For each generation observations were taken after three years of planting (3 YAP) ie for S0 generation



SC = Self compatible SIC : Self incompatible

Fig. 1. Inbreeding cycle of GII 7.4

observation was taken during the year 1990, S1 during the year 1994, S2 in 1995, S3 in 1998, S4 in 2002 and S5 in 2009. From the recorded data pod value (g), pod index, efficiency index, conversion index and average dry bean yield per tree per year (g) were also computed using slandered formulas. Pod value (g) is actually dry bean weight obtained per pod (g) and computed with the formula, single dry bean weight (g) × number of beans per pod (Toxopeus and Jacob, 14). Pod index is the number of pods required to produce one kilogram of dried cocoa beans and the formula is 1000 (g) / pod value (g) (Morera *et al.*, 7). Efficiency index is the indication the pod weight required to produce one gram of dry bean and it is estimated as pod weight (g)/ pod value (g) (Jacob and Atanda, 3). Conversion index indicates the part of dry bean realized from a given part of wet bean, *i.e.* pod value (g) / wet bean weight per pod (g). Yield (g) is total dry bean weight per tree per year and can be estimated using the formula total number of pods × pod value (g). Observations on yield and yield attributes of each generation are presented in Table 2. Average yield (number of pods per tree per year) recorded after three years of planting (3YAP) showed that S_2 generation had a hike in the yield (50 pods) and all other generations were low yielding than parental generation. Similar result was observed in the case of the parameter, dry bean weight per tree per year (Fig. 2). Rubino and Wehner (11) also reported some yield improvement during the inbreeding process of pickling cucumber population, which is also a cross-pollinated crop. When average girth (cm) at three years after planting was compared among generations not much differences were observed between inbred populations and parental populations.

Table 2. Growth and yield attributes of GII 7.4 over inbreeding generations.

Generation	Av. yield (No. of pods/tree/ year) 3YAP*	Av. dry bean yield/tree/year (kg) 3 YAP	Av. girth (cm) 3YAP	Pod wt. (g)	Pod length (cm)	Pod breadth (cm)	Husk thickness (mm)	No. of seeds	Wet bean wt. (g)	Dry bean wt. (g)	Dry bean length (mm)	Dry bean breadth (mm)	Dry bean thickness (mm)	Pod value (g)	Pod index	Efficiency index	Conversion index
S0	30.00	0.94	30.20	274.80	13.46	6.74	5.78	37.40	65.80	0.84	15.58	10.52	5.82	31.42	31.83	8.75	0.48
S1	21.30	0.56	29.00	248.40	12.64	7.04	8.34	38.80	66.26	0.68	16.67	10.48	6.17	26.38	37.90	9.41	0.98
S2	59.00	1.91	29.50	234.00	13.40	6.98	0.91	36.60	54.01	0.89	16.65	10.67	4.22	32.43	30.84	7.23	0.60
S3	15.80	0.59	29.10	246.00	13.46	6.98	0.87	46.00	72.55	0.82	16.47	10.24	3.28	37.54	26.64	6.55	0.52
S4	13.75	0.47	24.70	298.00	12.94	7.30	0.98	43.00	88.76	0.80	16.25	10.50	4.03	34.49	28.99	8.64	0.39
S5	15.40	0.60	27.00	308.00	13.16	7.10	1.05	39.80	70.06	0.98	17.83	11.28	4.44	39.08	25.59	7.88	0.56

*YAP = Year After Planting

The extent of inbreeding depression in the selfed generation was computed as per the formula given by Talebi *et al*. (13). Percentage of inbreeding depression of each generation on preceding generation was computed, it was observed that there is not much significant inbreeding depression except in the case of few traits (Tables 3 & 4). S_3 generation showed inferiority in maximum number of traits (7 traits) when compared to its preceding generation S_2. Generally most of the characters were at par or even superior when compared to preceding generation indicating little inbreeding depression. This is supported by the study of Luiz *et al*. (4). Similar reports were also recorded in a highly cross-pollinated family cucurbitaceous (Allard, 1; Nurgul and Rana, 9; Oviedo *et al*., 10). Non-significant role played by dominance and dominance x dominance form of epistasis may be the reason for lack of inbreeding depression. It was reported in INGENIC (2) that most of the economic traits of cocoa is controlled by additive genes.

The reason for non expression of inbreeding depression by GII 7.4 genotype can be attributed to additive gene control of most of the traits, survival of vigorous seedlings and adaptability of this genotype to self-pollination. In cocoa percentage of inbreeding depression varied among the genotypes selected (Mallika *et al*., 6). More over hybrids produced from two diverse inbreds will have an added advantage of combination of superior alleles from both genetically different parents.

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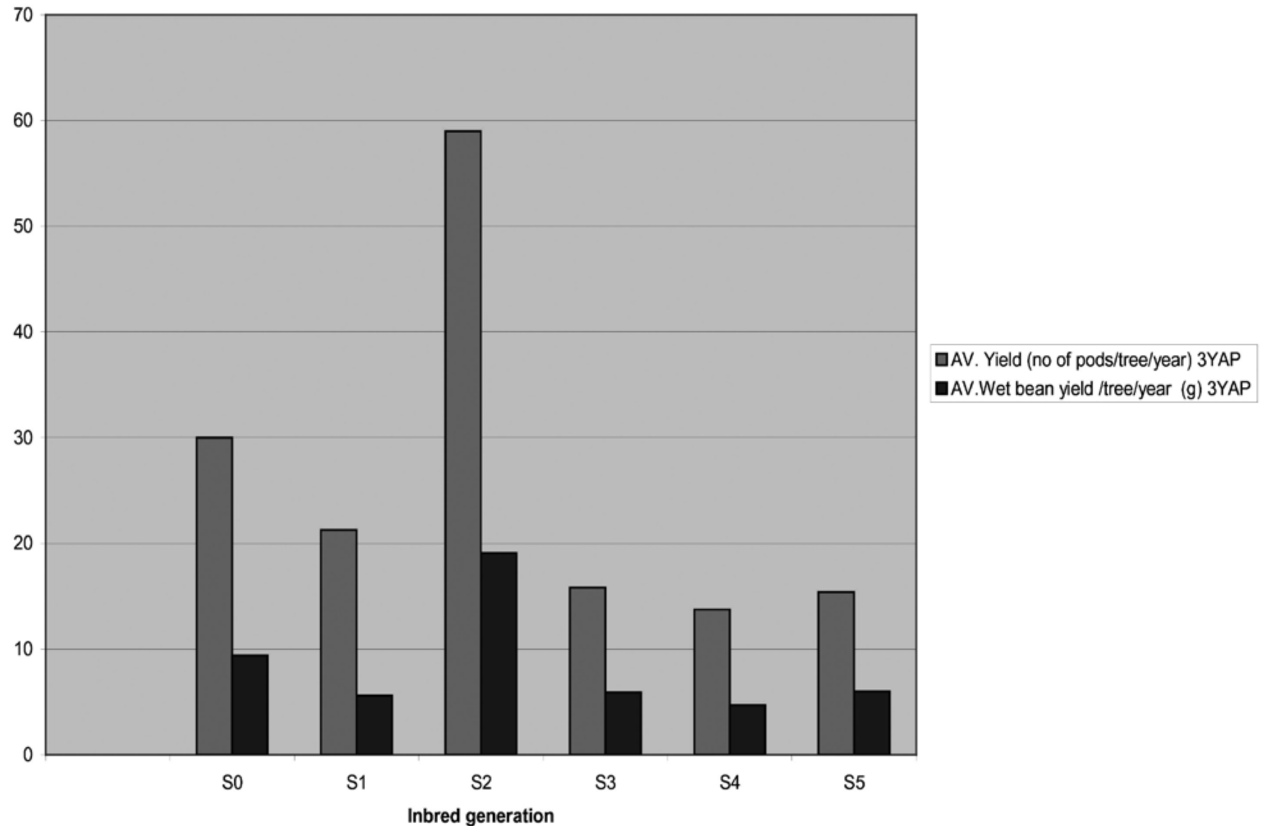

Fig. 2. Distribution of average yield (No. of pods/tree/year) and av. wet bean yield/tree/year (g) at 3 YAP over generations.

Table 3. Inbreeding depression of GII 7.4 genotype over generations.

Character	S1	S2	S3	S4	S5
No. of pods/tree/year	29	-176.99	73.22	12.97	-12
Av. Girth (cm)	3.973	-1.724	1.36	15.12	-9.31
Pod wt. (g)	9.61	5.8	-5.13	-21.14	-3.36
Pod length (cm)	6.09	-6.01**	-0.45	3.86*	-1.7
Pod breadth (cm)	-4.45**	0.85	0	-4.58**	2.74**
Husk thickness (mm)	-44.29**	89.09**	4.4**	-12.64**	-7.14**
No. of seeds	-3.74	5.67	-25.68	6.52	7.44
Wet bean wt. (g)	-0.699	18.48	-34.31	-22.34	21.06
Dry bean wt. (g)	19.05**	-30.29**	7.9**	1.72**	-22.44**
Dry bean length (mm)	-6.996**	0.12**	1.08**	1.34	-9.72**
Dry bean breadth (mm)	0.38	-1.812	4.03	-2.54	-7.43
Dry bean thickness (mm)	-6.013	31.6	22.274	-22.87	-10.17
Pod value	16.017	-22.904	-15.76	8.13	-13.33
Pod index	-19.073	19.635	13.609	-8.84	11.76
Efficiency index	-7.625	23.348	9.187	-31.86	8.81
Conversion index	16.56	-50.75	13.83	24.95	-43.56
Av. wet bean yield /tree/year	40.44	-240.998	69.001	20.067	-26.79

*, **Significant at 5 & 1% levels

Table 4. Distribution of family mean in relation to preceding generation (p< 0.05).

Character	S1	S2	S3	S4	S5
Pod wt. (g)	On par	On par	On par	On par	On par
Pod length (cm)	On par	Superior	On par	Inferior	On par
Pod breadth (cm)	Superior	On par	On par	Superior	Inferior
Husk thickness (mm)	Superior	On par	On par	Superior	Inferior
No. of seeds	On par	On par	On par	On par	On par
Wet bean wt. (g)	On par	On par	On par	On par	On par
Dry wt./ bean (g)	Inferior	Superior	Inferior	Inferior	Superior
Dry bean length (mm)	Superior	Inferior	Inferior	On par	Superior
Dry bean breadth (mm)	Inferior	Superior	Inferior	Superior	Superior
Dry bean thickness (mm)	Superior	Inferior	Inferior	Superior	Superior
Pod value	Inferior	On par	On par	On par	On par
Pod index	Superior	On par	On par	On par	On par
Efficiency index	Superior	Inferior	Inferior	Superior	Inferior
Conversion index	Inferior	Superior	Inferior	Inferior	Superior

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