Research Article

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Comparison of biology of tea mosquito bug, *Helopeltis bradyi* Waterhouse (Hemiptera: Miridae) on different phenological stages of cocoa (*Theobroma cacao* L.)

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Abstract

Among the insect pests recorded on cocoa, tea mosquito bug, *Helopeltis* spp. is a major pest. Three species of tea mosquito bug *viz., Helopeltis antonii, H. bradyi* and *H. theivora* causes damage to cocoa. Among them, *H. bradyi* is a predominant species feeding on cocoa. Different phenological stages of cocoa *viz*., tender shoots, cherelles and pods were compared for fecundity, nymphal development, survival and adult longevity of *H. bradyi.* The fecundity, nymphal emergence and egg hatchability was highest in cocoa pods and lowest in cherelles. Highest numbers of eggs were recorded in tender shoot (110.5 numbers) followed by pods (107.5). Significantly lower number of eggs were recorded in cherelles (103.3 numbers). The total number of nymphs emerged and egg hatchability were significantly highest in tender shoots. The nymphs developed much earlier on pods than on tender shoots and cherelles. The total nymphal developmental period of *H. bradyi* was higher on tender shoot (224.5 h) followed by cherelles (218.3 h) and was lowest on pods (202.7 h). The adult survival was the highest (98.6%) when fed on cocoa pods compared to that of cherelles. Both females (31.5 days) and males (29.8 days) lived longest on pods than on cherelles. Whereas, in tender shoots both the male and female recorded significantly the lowest adult longevity of 21.3 days and 23.2 days, respectively. Tender shoots were found to be an inferior food source for adults than cherelles and pods. It is revealed that the availability of pods is critical for the fecundity, development, survival and longevity of *H. bradyi*.

Keywords: Cocoa, fecundity, developmental period, *H. bradyi,* longevity, tea mosquito bug

Introduction

Cocoa, (*Theobroma cacao* L.) is a small understory tree prevalent in the low land rain forests of the Amazon basin (Wood and Lass, 1985). This crop was domesticated in pre-Columbian times by the Olmec and Maya civilizations for using the seeds of cocoa to produce beverages for royalty and religious ceremonies and as currency (Coe and Coe, 1996; Motamayor *et al*., 2002; Emch, 2003). Now, cocoa is grown throughout the humid tropics, often in agro-forestry ecosystems with other crops. Many insect pests are found to attack cocoa and among them tea mosquito bug, *Helopeltis* spp. (Hemiptera: Miridae), is the predominant one which is a serious pests of cocoa worldwide (Entwistle, 1972). In India, cocoa is attacked by three species of tea mosquito bug *viz*., *Helopeltis antonii* Signoret, *Helopeltis bradyi* Waterhouse and *Helopeltis theivora* Waterhouse (Stonedahl, 1991; Sundararaju, 1996). Among the tea mosquito bugs present in India, *H. bradyi* is the most predominant species attacking cocoa (CPCRI, 2013). *H. bradyi* is a polyphagous sap sucking insect pest. Nymphs and adults suck the sap from tender shoots, cherelles and pods of the cocoa plant by inserting its stylet. While feeding, salivary secretions are injected into the feeding site which leads to appearance of necrotic spots on the tender shoots (Fig. 1). Toxic saliva injected into the tender shoots cause die back (Fig. 2). The damage on flower buds leads to premature shedding of flowers. Feeding punctures

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Fig. 1. Appearance of spots and necrosis on tender shoots due to tea mosquito bug feeding

Fig. 2. Die back of tender shoot due to tea mosquito bug feeding

Fig. 3. Cocoa pods with tea mosquito bug feeding lesions

Fig. 4. Severely infested cocoa pods with cankering, roughening on surface

causes appearance of depressed oily spots on cocoa cherelles and pods known as lesions (Fig. 3) (Mariau, 1999). On well matured cocoa pods, the bug feeding sites are marked by black spots, sometimes it attract secondary infection by microbes. High number of feeding punctures on tender shoots or cherelles may cause deformation during growth or premature death and shedding of cherelles and pods. These infections on pods and cherelles result in cankering and roughening on surface (Fig. 4). The cocoa pods are attacked by tea mosquito bugs at all the stages of development. Yield loss due to tea mosquito bug damage could be as high as 75 per cent in cocoa when attacked by the mirids (Padi, 1997; CPCRI, 1993). Estimated crop loss due to *Helopeltis* spp. are variable and it depends on cultural practices, methods of control, locality, type of weather, the plant and insect species involved.

Since there are alternative feeding sites within a stand of cocoa, it is often assumed that food is not a limiting factor, but the quality of nutrients derived from each part of the plant may affect the growth and development of the tea mosquito bugs. In South India, the peak pod availability is twice a year (April-May and October-November). So, there will be relative pod scarcity during certain months of the year, which can affect the biology and

population dynamics of the tea mosquito bugs and it is a significant limiting factor for the population increase (Azhar, 1986). The present investigation was conducted to compare the influence of different phenological stages of cocoa *viz.,* tender shoots, cherelles and pods on the fecundity, egg hatchability, nymphal development, survival and adult longevity of *H. bradyi.*

Materials and methods

Mass culturing of *H. bradyi*

Mature gravid females of *H. bradyi* were collected from cocoa plantations at ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka ($12^0$15'N latitude and 75^o25'E longitude) and were allowed to lay eggs on three month old potted cocoa seedlings in the laboratory. Each shoots containing eggs was labeled with date of oviposition until hatching. The seedlings with eggs were maintained in the laboratory avoiding direct impact from rainfall. Five to seven days after oviposition, the shoots of potted seedlings containing eggs were cut off from the potted seedlings and placed in the nymphal rearing cages as described below. Four glass vials (5 mL capacity each) were fixed on a small aluminium stand using an adhesive (Araldite) which consists of an aluminum plate (size: 2.5 cm width x 25.5 cm length and 18 gauge) and a handle of 15 cm height fixed at the centre of aluminum plate. One tender shoot was kept erect inside each vial, filled with water and mouth of the vial was closed with wet absorbent cotton. The stand with four tender shoots was placed inside the nymphal rearing cage (Aluminum cage size: 15x15x20 cm and thickness: 15 gauge).

In the nymphal rearing cage, two side windows were provided with black coloured muslin cloth sleeve in order to facilitate the removal of the adults after final moulting (Sundararaju and John, 1992). The other two side windows were covered with transparent polyester film (147 micron thickness) for better viewing. Fifteen to twenty nymphs were transferred in each cage and the top of the rearing cage was covered with wet muslin cloth. On the second day another aluminum stand along with four fresh tender shoots of cocoa was placed adjacent to the already existing aluminum stand without transferring/disturbing the nymphs feeding on the shoots kept on the previous day. This process was repeated every day. On every third day, the tender shoots kept on the first day along with aluminum stand were removed after examining the shoots for the absence of nymphs and this process was repeated daily till the adult emergence. Temperature and relative humidity in the laboratory were monitored throughout the experiment.

Fecundity and egg hatchability of *H. bradyi*

Muslin sleeve cages (30 cm long × 30 cm diameter) were used to confine the adult bugs after pairing adult bugs on tender flushing shoots, one month old cherelles and two months old cocoa pods. Male and female tea mosquito bug were released within 24 h after emergence in to muslin cloth sleeve cage at the rate of one pair of male and female in each cage. Each cage constituted a replication. Both ends of the sleeve cage were securely tied to prevent the bugs from escaping. The shoots were kept in their natural position by tying the upper part of the sleeve cage to a stake and lower part to the twigs. The sleeve cages were labeled with oviposition date and precautions were taken to prevent the disturbance from ants. The presence of a pair of fine respiratory filaments projecting from the surface of the plant tissue was the indication of the presence of eggs embedded in the cocoa tissues. Muslin sleeve cloth cage was left as such until the nymphs emerged. After 7 to 10 days, the tender shoots, cherelles and pods were daily observed for the emergence of nymphs. The newly emerged nymphs were counted and removed. The egg hatchability was calculated using the following formula:

$$\text{Egg hatchability} = \frac{\text{Total number of nymphs emerged}}{\text{Total number of eggs laid}} \times 100$$

The number of viable eggs was determined by the number of first instar nymphs that were emerged from eggs (Awang *et al.,* 1988).

Nymphal developmental period and survival rate of *H. bradyi*

The study was carried out on fresh (3 to 5 days old) tender shoots, one month old cherelles and two month old developing pods. Using camel hair brush,

Fig. 5. Muslin cloth sleeve cage enclosed on the cocoa pods

20 newly emerged nymphs (0 to 12 h old) were transferred onto tender shoots, cherelles and pods (serving as the food sources) and enclosed with muslin sleeve cages (30 cm long × 30 cm diameter) (Fig. 5). Each insect constituted a replication. The nymphs were observed at 24 hrs intervals to determine the moulting status, as indicated by the presence of moulted skins. The duration of different nymphal instars was recorded in hours. The nymphs survived to subsequent instars on tender shoots, cherelles and pods were recorded.

Adult longevity of *H. bradyi*

The adult longevity of tea mosquito bugs was determined by releasing 20 numbers of adult in to the muslin sleeve cages (30 cm long × 30 cm diameter) enclosed with tender shoots, one month old cherelles, and two month old developing pods. Each insect constituted a replication. Both ends of the sleeve cage were securely tied to prevent the escape of nymphs. The shoots were kept in their natural position by tying the upper part of the sleeve cage to a stake and lower part to the twigs. Precautions were taken to prevent the attack of ants. The adults were observed for their longevity until death. Monthly rainfall, maximum and minimum temperature and relative humidity in the cocoa plantation at ICAR-CPCRI, Regional station, Vittal were monitored throughout the experiment. Each experiment was carried out in completely randomized block design, the data were statistically analyzed and mean were compared with least significant difference using AGRES package.

Results and discussion

Fecundity and egg hatchability of *H. bradyi*

The results from Table 1 revealed that *H. bradyi* preferred tender shoot for egg laying then on pods and cherelles. On tender shoots, highest numbers of eggs were recorded (110.5 number) followed by 107.5 number in pods, however, both were statistically on par with each other. The lowest number of eggs was recorded in cherelles (103.3 numbers). The incubation period was high (12.8 days) in cherelles followed by 11.2 days in pods and 8.8 days in tender shoots and the treatments significantly differed from each other. Total number of nymphs emerged were significantly high in tender shoots (85.2 numbers) followed by in pods (60.8 numbers) and in cherelles (50.5 numbers). The egg hatchability was also significantly high in tender shoots (77.1%) followed by pods (56.5%) and cherelles (48.9%).

Table 1. Fecundity, incubation period and egg hatchability of *H. bradyi* on different parts of cocoa

Cocoa plant parts	Number of eggs laid per female	Incubation period (days)	Total no. of nymphs emerged	Per cent egg hatchability
Tender shoot	110.5±5.1	8.8±2.1	85.2±5.6	77.1±17.0
Cherelle	103.3±5.9	12.8±2.1	50.5±5.8	48.9±11.3
Pod	107.5±6.6	11.2±1.9	60.8±6.6	56.5±12.9
CD (p=0.05)	3.7	1.2	3.9	3.9
CV%	5.5	17.5	9.3	10.1
SE	1.9	0.6	1.9	1.2
	**	**	**	**

** Highly significant

Table 2. Nymphal developmental period of *H. bradyi* on different parts of cocoa

Cocoa plant parts	Mean nymphal developmental period (h) (Mean±SD)					
	1st instar	2nd instar	3rd instar	4th instar	5th instar	Total
Tender shoot	45.4±5.7	32.5±3.7	37.4±5.6	39.7±3.8	69.5±5.5	224.5±5.9 (9.4)
Cherelle	43.5±5.9	33.4±1.8	35.7±5.6	37.5±5.4	68.3±4.4	218.3±5.9 (9.1)
Pod	42.7±6.6	35.5±5.2	29.2±6.6	31.5±5.6	63.8±5.9	202.7±5.2 (8.4)
CD (p=0.05)	3.9	2.4	3.8	4.2	3.4	3.6
CV%	13.9	11.4	17.4	13.8	7.9	2.6
SE	1.9	1.2	1.9	1.6	1.7	1.8
	NS	*	**	**	**	**

NS-Not significant; * Significant; ** Highly significant; Figures in parentheses indicate duration in days

Nymphal developmental period of *H. bradyi*

The nymphal developmental period on cocoa parts revealed that nymphs from first to fifth instar developed faster when fed on cocoa pods than on cherelles and tender shoots (Table 2). The duration from egg to first instar nymphs on pods was 42.7 hours, while that on the cherelles and on tender shoots it was 43.5 and 45.4 hours, respectively. However, the treatments did not significantly differ each other. The nymphal duration from first to second instar on pods was 35.5 hours, while that on cherelles and tender shoots it was 33.4 and 32.5 hours, respectively. The treatments were on par with each other. From 5th instar to adulthood, duration of development was 63.8 hours on pods and 68.3 and 69.5 hours on cherelles and tender shoots, respectively. Similarly, the total developmental period was significantly faster when *H. bradyi* fed on cocoa pods, it was 202.7 hours and it were 218.3 hours in cherelles and 224.5 hours in tender shoots. The developmental time from first to second instar was 2.7 hours longer on the tender shoot than on pods. The significant difference in the development rate occurred during the third to fourth instars, where it took 8.3 hours more on tender shoot than on pods. The total nymphal developmental period was about 21.8 and 15.6 hours longer on tender shoot and cherelles, respectively, than on pod (Table 2).

Survival of *H. bradyi*

The survival of first instar nymphs was highest in tender shoots (98.3%) followed by pods (95.3%) and cherelles (94.5%). The treatments were not significantly different from each other (Table 3). In the second, third and fourth instar nymphs the survival was significantly high in pods followed by cherelles. The tender shoots was least preferred by nymphs for survival. The mean nymphal survival was significantly higher (98.8%) when fed on pods, compared to cherelles (95.4%) and tender shoots (92.5%). This study showed that the cocoa pods are the best food source for survival of nymphs than cherelles and tender shoots.

Table 3. Mean percentage survival of *H. bradyi* on different parts of cocoa

Cocoa plant parts	Survival (%) (Mean±SD)					
	1st instar	2nd instar	3rd instar	4th instar	5th instar	Mean survival
Tender shoot	98.3±5.1	94.8±5.4	88.5±4.7	86.5±5.7	94.8±14.5	92.5±1.4
Cherelle	94.5±6.9	98.5±7.4	94.8±5.9	90.5±6.9	98.5±5.8	95.4±2.1
Pod	95.3±6.6	100.0±4.0	100.0±6.6	98.5±5.6	100.0±6.6	98.8±0.2
CD (p=0.05)	3.7	3.9	3.9	3.9	6.2	0.2
CV%	6.1	6.2	6.4	6.6	10.9	1.3
SE	1.9	1.9	1.9	1.9	3.1	0.5
	NS	*	**	**	**	**

NS-Not significant * Significant ** Highly significant

Adult longevity of *H. bradyi*

A significant difference in the longevity of male and female mosquitoes of *H. bradyi* was observed in this study. The females lived significantly longer period than the males on all the cocoa plant parts tested. In both sexes, those fed on cocoa pods shows highly significant adult longevity than those fed on tender shoots. The male and female recorded significantly lower adult longevity of 21.3 days and 23.2 days on tender shoots, respectively (Table 4).

Table 4. Adult longevity of *H. bradyi* on different parts of cocoa

Cocoa plant parts	Adult longevity (days) (Mean±SD)	
	Male	Female
Tender shoot	21.3±5.1	23.2±5.7
Cherelle	25.5±5.9	27.5±5.5
Pod	29.8±6.6	31.5±5.8
CD (p=0.05)	3.7	3.9
CV%	23.1	22.2
SE	1.9	1.9
	**	**

** Highly Significant

The mean number of egg laid by *H. bradyi* was significantly higher on tender shoots then on the pods and cherelles. This significant difference in fecundity might be attributed to nutritional value of tender shoots which might be favored for egg laying. Pillai *et al*. (1976) investigated the preferred site of egg laying of *Helopeltis*, which mostly depended on the host plant. The *H. theivora* prefers cocoa pods for egg laying (Miller, 1941) but rarely lays egg on young shoots. But on tea, the same species preferred new shoots, petioles and midribs of leaves (Das, 1984) for egg laying. The first and foremost site of oviposition of *H. antonii* was on the young shoots, inflorescence stalks and developing nuts of cashew, but will sometimes prefer the petioles and ventral midribs of leaves (Ambika and Abraham, 1979). The incubation period of the egg depends on locality and season, but it is generally in the range of 6 to 11 days (Stonedahl, 1991). Longer durations were observed occasionally for *H. theivora* in North East India (20 to 27 days) and 13 to 16 days for winter populations of *H. bradyi* (Das, 1984). *H. schoutedeni* exhibited a mean incubation period of 8.2 days. The difference observed could be attributed to climatic factors and rearing conditions like temperature and relative humidity of the location (Betrem, 1953).

The rate of nymphal development depends on quality of the food source (Betrem, 1953; Awang *et al.*, 1988). In the present investigation, all the nymphal stages developed rapidly when fed on pods than on cherelles and tender shoots. The results of the present investigation were comparable with most of the reports on *H. theobromae* on cocoa, in which the nymphs that were fed on cocoa pods developed faster than those fed on tender shoots (Awang *et al.*, 1988). Faster rates of development were shown when *H. schoutedeni* and *H. antonii* were fed on fruits compared with feeding on tender shoots or on panicles (Dwomoh *et al*., 2008; Srikumar and Bhat, 2011). The development of *H. antonii* was faster on cocoa pods which could be probably due to the quality of food and nutrients found in the pods, which might be more juicy, succulent and may be more suitable to tea mosquito bugs compared to the tender shoots and panicles. The results suggested that the cocoa pods are the best food source for survival of nymphs than cherelles and tender shoots.

Adult longevity of tea mosquito bugs was reported to diverge with the source of the food (Betrem, 1953). Both male and females recorded their longest life span on cocoa pods while their shortest life spans were recorded on tender shoots. In Malaysia, the mean adult longevity of *H. theivora* on cocoa pod was 30 days (Tan, 1974). The same species recorded a mean longevity of only six days when raised on cocoa shoots (Awang *et al*., 1988). The significant differences in adult longevity probably due to nutritional effects and the tender shoot is not a suitable substrate for adult longevity (Tan, 1974; Betrem, 1953; Awang *et al*., 1988). The results clearly revealed that longevity of *H. bradyi* depend on availability of cocoa pods in the field during the fruiting season. The studies by Azhar (1986) have emphasized pod shortage as great limiting factor which influence the biology and population dynamics *H. bradyi*.

Conclusions

The present study revealed that, among the plant parts tested on cocoa, the pod was the best

(Rimberia *et al.*, 2006), late uni-nucleate or early bi-nucleate stages in *Brassica* had been documented as suitable candidates for successful anther culture (Fan *et al.,* 1988; Kott *et al.,* 1988; Pechan and Keller, 1988). Identifying visible and measurable traits like flower bud length linked with microspore developmental stage may fasten the process of choosing explant for anther culture (Srivastava and Chaturvedi, 2008). This report showed that 2 to 3 mm long flower buds (anthers containing uni-nucleate microspore stage) was found to be better than 4 to 6 mm long flower buds (anthers containing binucleate microspores stages) for callus induction.

Effect of flower bud sizes in callus induction

Overall, callus induction values suggested that 2 to 3 mm long flower bud was superior to 4 to 6 mm long flower buds. This result agrees with earlier report in neem *(Azadirachta indica)*, where 2 mm long flower bud (anthers containing early to late-uninucleate microspore stage) induced maximum callus/regeneration (Chaturvedi *et al.,* 2003).

Effect of genotypes in callus induction

With 2 to 3 mm long flower buds CCRP 1 and with 4 to 6 mm long flower buds, CCRP 1 and CCRP 2 genotypes were statistically similar and produced highest frequencies of callus production. CCRP 1, CCRP 3 and CCRP 5 genotypes performed well in CIM 4 medium with respect to 2 to 3 mm long flower bud with respect to pre-treatments. CCRP 1 produced more callus in CIM 1 medium with respect to 4 to 6 mm long flower buds. Accordingly, the results of this investigation confirm with previous reports which affirmed the existence of the effect of cocoa genotype on morphogenic response under *in vitro* conditions, physiological stage of plant materials, type of explant, the concentration and type of growth regulator used (Velasquez *et al.,* 2006).

Influence of pre-treatment of floral buds in callus induction

In order to induce pollen embryogenesis, temperature treatment is considered to be the most effective approach to compare with other pre-treatment methods. Pre-treatment of excised flower bud for certain period in cold conditions has been demonstrated to enhance the callus induction efficiency in anther culture (Mishra and Gowswami, 2014).

Our analysis revealed that, the effects of pre-treatments were not consistent across flower bud sizes. With 2 to 3 mm long flower buds, fresh and cold pre-treatment showed better results than gamma irradiation for callus induction. Culturing anthers at uni-nucleate microspores taken from 4°C pre-treated flower buds showed highest frequency of callus (Raghavan, 1986; Stoehr and Zsuffa,1990; Khatun *et al.,* 2012). The material exposed to low temperatures for long periods near to a week, had a positive effect on the cocoa morphogenic response (Teixeira *et al.,* 2002). The flower buds 4 to 6 mm long revealed maximum callus induction frequency when used either as fresh or treated with gamma irradiation, compared to cold pre-treatment. Another way of pre-treatment has been done by exposing flower buds to gamma irradiation and was reported to enhance microspore embryogenesis in some plants (Sangwan and Sangwan, 1986; Macdonald *et al.*, 1988; Pechan and Keller 1989). The use of irradiation on flower buds would be possible for obtaining haploid cocoa plantlets (Falque *et al.,* 1992), as observed in the present study. Pre-treatment conditions had significant effect on callus induction and it depended on factors like clones/genotypes (Powell, 1988; Osolnik *et al.,* 1993; Khiabani *et al.,* 2008) and flower bud size with appropriate microspore stage (Pedroso and Pais, 1994; Sopory and Munshi, 1996; Chaturvedi *et al.,* 2003).

The results of interaction effect between pre-treatments and genotypes revealed that anthers from cold pre-treated CCRP 1 buds significantly showed higher callus induction compared to others with 2 to 3 long flower buds. With respect to longer flower buds (4 to 6 mm) CCRP 1 and CCRP 5 treated with gamma irradiation and fresh buds from CCRP 1 and CCRP 2 were best for callus induction. This finding conveys that, each genotype requires specific pre-treatment conditions.

Effect of media combinations

The medium combination CIM 4 with 2 to 3 mm long flower buds and CIM 1, CIM 4 medium with 4 to 6 mm long flower buds respectively, induced significantly greater callus induction frequencies

from anthers than other medium combinations. In all the genotypes,three pre-treatment conditions were on par but they all responded better in particular at CIM 4 medium for callus induction than other media combinations when 2 to 3 mm long flower buds were used. The callus formations was on par with fresh and gamma irradiated flower buds than cold pre-treatment in both CIM 1 medium and CIM 4 medium with 4 to 6 mm long flower buds.

Selecting types of callus and time of transferring calli to new media is very important factor to get the embryos/plantlets. In this study, CIM 1, CIM 3 and CIM 4 were appropriate medium for producing friable callus. Supplementation of DKW medium with 2,4-D, TDZ and glutamine enhanced the callus formation from anthers that were isolated from 2 to 3 mm and 4 to 6 mm long flower buds. Treatment with DKW medium induced embryogenic callus growth and development of somatic embryos (Li *et al.,* 1998; Maximova *et al.,* 2002). The excess presence of Ca^{2+}, S and Mg^{2+}ions in DKW medium favours for *in vitro* propagation of woody perennial species than MS medium. Appropriate auxin to cytokinin ratio is necessary for successful *in vitro* culture (Ramawat *et al.,* 2014). The auxin 2,4-D is widely used in anther culture systems (Zheng and Konzak, 1999). Similarly the cytokinine TDZ is widely used in woody plant tissue culture and enables micro-propagation of the most recalcitrant species (Huetteman and Preece, 1993). The addition of other compounds like casein, biotin, coconut water and glutamine has improved callus induction during androgenesis of few woody plants (Drira and Benbadis, 1975; Custodio *et al.,* 2005).

Regeneration

Embryogenic callus, somatic embryos and plants have been obtained in different cocoa genotypes and explants *viz.*, staminodes and petals (Ajijah *et al.,* 2016). Attempts were made to induce somatic embryo using various floral parts as explants, and successful report documented in staminode and stamen filament culture (Alemanno *et al.*, 1996). Santana *et al.* (2010) obtained rhizogenic callus from cocoa anthers of genotype Ocumare-61 and petals of genotype Chuao-2. The chromosomal analysis of roots derived from anther callus revealed haploid number of chromosome (2n=X=10), thus indicating the feasibility of haploid production through anther culture (Santana *et al.*, 2010). The genotypes under study showed recalcitrant behaviour to regeneration. The inability of callus to regenerate despite greening might be due to the very low regeneration ability of the genotypes under investigation. More cocoa genotypes have to be evaluated for identifying the contributing factors such as pre-treatment conditions and medium combinations for achieving better regeneration.

Conclusion

Genotype specific optimization of culture media composition and pre-treatment of flower buds is necessary to succeed in anther culture in woody crops. Results in the present study indicate that, each genotype requires specific pre-treatment conditions and media combination for optimum androgenic callus response. The optimized protocol for induction and regeneration of callus from immature anthers in this report provides a step ahead in the development of *in vitro* cocoa haploid production.

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