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Table of Content: Volume 12 Number 6 June 2018

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Full Length Research Paper

Biological effect of gamma irradiation on vegetative propagation of *Coffea arabica* L

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Coffee is the second most valuable commodity exported by developing countries, and supports the livelihood of more than 75 million people. There are limitations to preference for established cultivars due to the autogamous nature of the crop thereby making improvements via conventional breeding of many years to produce a new cultivar difficult. Mutation breeding can overcome these obstacles. One of the first steps in mutation breeding is to determine radio-sensitivity so that optimal irradiation treatments can be determined. Three cultivars: Kents, Mundo novo and Geisha were sourced from the coffee germplasm collection at the Mambilla Plateau substation of the institute, Taraba State, Nigeria. The biological effect of the physical was studied in the selected cultivars of *Coffea arabica* after the rooted seedlings were treated with various doses of Gamma rays: 0, 5, 10, 15, 20 and 25 Gy. Success takes percentage and seedling vigour were used as measures of radio-sensitivity at 4 weeks after settings (WAS), 8, 12, and 16 WAS. The results showed that genotype of the cultivars and dosage of irradiation significantly influenced response to irradiation treatments ($p < 0.05$). The effect of the irradiation on treated cultivars was inversely proportional to the emergence of the success takes, plant height, root length, number of roots and number of leaves. The optimal mutation treatment (LD_{50}) of M_1V_1 cuttings was in the range of 12 Gy in all the treated *Arabica* varieties an indication to buttress the narrow genetic base with reference to the similarity of their evolutionary trends. This work provides data on dose treatments for mutation induction in coffee, which may be exploited for coffee improvement.

Key words: Cultivars, autogamous, mutation, irradiation and genotypes.

INTRODUCTION

Coffee is known to be one of the most important beverages in the world. It has a current estimated value of US$10 billion and is one of the most traded commodities second in value only to oil and a huge contributor of foreign exchange earnings for developing countries (Labouisse et al., 2008). It has become an important contributor to cash income for many smallholders, who produce most of the world's beans

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with the annual output which now reached almost nine million tonnes (one million tonnes more than a decade ago) and the gross value production of green coffee now exceeds US$16 billion (Pietro, 2015). The crop belongs to the genus *Coffea* in the family of Rubiaceae with over 100 species; *arabica* coffee is the only tetraploid species ($2n$ = $4x$ = 44), the remaining species being diploid with ($2n$ = $2x$ = 22) chromosomes, (Davis et al., 2006). The species which are most cultivated world-wide are *Coffea arabica* (*Arabica* coffee) and *Coffea canephora* (Robusta coffee) responsible for 70 and 30% of production, respectively. Coffee is mainly grown in tropical and subtropical regions and is an important cash crop in more than 60 countries in South and Central America, Asia, and Africa with an acreage of over 11 million ha (Waller et al., 2007). The African coffee producing countries takes advantages of diverse agro ecological conditions with genetic diversity (Kawuma, 2015). The name "coffee" was derived from the forbidden poetic Arabic word for wine 'Quahweh'. It is called Café (French), caffee (Italian), Kaffee (German), Koffie (Dutch), coffee (English) and *Coffea* (Latin) (Smith, 1985). *Arabica* coffee has high quality and the more valuable of the two cultivated species and is the subject of this work.

Coffee is one of the fourteen exportable commodities cultivated in Nigeria, after the introduction in 1890's. It is a source of revenue to the country contributing over US$13.5 million to Nigeria economy in 2013. Change in climate has been a great threat towards food production. This is already affecting agriculture, with unevenly effects distributed across the world which would probably increase the risk of food insecurity for some of the poor (Porter, 2014).

With a worldwide decline in plant genetic resources, an increasing demand in plant production, and a public aversion in pesticide use, new methods of crop diversity are required to address agricultural challenges. Such methods include the use of plant breeding, genetic engineering and induced mutations. Coffee crop is becoming susceptible to more virulent diseases such as coffee leaf rust that is responsible for average reduction of 31 and 16% during the epidemics year (Cristancho et al., 2012).

Arabica coffee improvement is a serious concern for plant breeders as the coffee industry is geared towards the processing of specific and preferred cultivars, e.g. from Columbia, Indonesia, India, Kenya and Brazil with the fact of being a tree crop with a long generation time of 3 to 4 years from sowing a seed to the next generation, required an alternative breeding technique. The *Arabica* species does not have adequate genetic diversity from which breeding of varieties suitable to face the problems and constraints that result from climate change (Schilling, 2015), therefore, mutation breeding can provide useful mutant directly in preferred elite germplasm and thus reduce the time in developing new cultivars. Coffee has been a neglected crop with respect to mutation breeding and the methods need to be developed and optimized (Dada et al., 2014). This will be a first step to determine the most effective irradiation treatment for which radio-sensitivity testing is required

Radio-sensitivity is a relative measure that gives an indication of the effects of irradiation treatments on the irradiated objects (Morishita et al*.,* 2013). In many radio-biological reactions, the effect of a given dose depends on the intensity of irradiation and/or the manner in which the total dose is fractioned. Physical mutagen (Gamma and X rays) are known to influence plant growth and development by inducing cytological, genetical, biochemical and physiological changes in nuclei, cells and tissues where damaging effects are proportional to the dose or concentration received (Mba et al., 2010). This test is recommended to determine the best treatment in relative to the seeds, budwoods and explant. Here, we target bud wood of three *Arabica* cultivars for study as the cuttings is the propagating material that capture true to type traits of the selected varieties.

C. arabica is suffering from a limited variability for breeding as a result of a narrow genetic base due to inbred nature of the species (Carvalho, 1988). Mutation breeding is capable of inducing variability in the crop within a shorter time frame compared to conventional breeding which may take up to 30 years in perennial species such as coffee. It is capable of increasing biodiversity for desired traits in crop plants and hence accelerates the breeding of varieties with higher yield, yield stability, nutrition and improved resistance to environmental stress such as disease, drought and salinity (FAO/IAEA, 2014). About 3,200 mutant cultivars in over 220 crop species are registered in FAO/IAEA database (https://mvd.iaea.org), with little work recorded on tree breeding. Although mutation breeding techniques have been applied successfully in a wide range for crops, there has been little application to coffee. Conventional breeding methods are inadequate for coffee due to its narrow genetic base and long generation time. Hence, mutation breeding is attractive as a means of coffee improvement. Gamma and X rays are physical mutagens classed as ionizing electromagnetic radiation, and are capable of causing biological injuries in higher plants through interactions with genetic materials (Forster and Shu, 2012). These mutagens are commonly used in plant mutation breeding.

The effect of gamma ray is reported on selected cultivars of coffee using data on cuttings takes and vigor after treatment to determine radio-sensitivity. This is an important step in developing effective mutagenic treatments for mutation breeding in coffee.

MATERIALS AND METHODS

Three cultivars of *C. arabica* were selected for study: Kents, Mundo-novo and Geisha. They were selected because they are commercially cultivated by farmers in diverse *Arabica* growing

Figure 1. *Coffea arabica* irradiated cuttings (M_1V_1) germinated in the nursery.

areas of Taraba, Plateau and Cross River. These three cultivars were sourced from the Cocoa Research Institute of Nigeria (CRIN), Mambilla, substation in Taraba State of Nigeria.

Fresh harvested berries were soaked in water for 12 h, and de pulped. The seeds from the de pulped berries were air dried for 24 h at the Plant Breeding Section, CRIN, Nigeria. Good beans with no infection or injury to the embryo were sorted from the population in a seed room. Seeds with parchment were transported to the FAO/IAEA Plant Breeding and Genetics Laboratory (PBGL), Austria and re-packaged on arrival for short term storage (2 days) before transferring the seeds to a desiccator for 2 weeks and stored in the desiccator for two weeks prior to irradiation to equilibrate seed moisture content. The seeds were kept in vacuum desiccators over glycerol (60% v/v) and left at room temperature for 4 to 7 days in order to reduce and standardize seed moisture content to 12 to 14% prior to irradiation treatments. Seeds with parchment were soaked for five days in warm tap water (40°C) for imbibition. Batches of 100 seeds with parchment were sown in soil-filled trays in a greenhouse at a sowing depth of 60 mm. Nursery soil was prepared with 2 parts peat moss, 4 parts Seibersdorf soil and 1 part sand. This was complemented with 27 g of Urea and 15 g of TSP per 40 kg mixture soil. Plastic seed trays (600 × 400 × 80 mm) were filled with prepared soil in a greenhouse at 27°C with a 12 h photoperiod. Trays were watered every two days to avoid over wetting and overwatering. The germinated seedlings were uprooted for irradiation at 8 months after sowing with standard Gamma Cell 220 with ^{60}Co isotope source (Atomic Energy of Canada Limited, Ottawa, Canada) at 7.8 Gy/min from a Cobalt-60 source. The seedlings were package and shipped to Plant Breeding Department of CRIN for vegetative propagation. Each nodal cutting were treated with Indole Butyric Acid (IBA) as rooting hormone at 8000 ppm before sowing on top soil, watered and kept in a humid environment under air tight polythene sheet (CRIN, 2005)

Data on settings take were taken from 4, 8, 12 and 16 weeks after settings (WAS), while data for seedling vigour (plant height, root length, number of root and number of leaves) were taken 36 WAS before transplanting. Settings take and seedlings vigour was obtained on the three cultivars in three replicates.

The data obtained were analyzed by Microsoft Excel 2010 to determine the LD_{50} and LD_{30} (dosage required to reduce the population by 50 and 30%, respectively) of the cultivars. Cuttings morphological data were analyzed using statistical analysis system (SAS).

RESULTS AND DISCUSSION

Success takes commenced at exactly 4 WAS in the nursery with a visible protrusion from the nodal leaves; however, complete success takes occurred after 8 weeks of hardening process before transplanting 14 DAT (Figure 1). About 100% success takes were observed at 4, 8, 12 and 16 WAS at 0 and 5 Gy treated cuttings. The success takes at an irradiation of dose of 15 Gy was 55 and 45% at 4 and 16 WAS, respectively. A highest percentage of 40% die-back occurred at a treatment of 10 Gy in Figure 2.

Response of cultivars Kents, Mundo-novo and Geisha to gamma irradiation in terms radio-sensitivity based on success takes at 16 WAS for different dosage as represented in form of curves is as shown in Figure 3. The required dose to reduce the population by 50% (LD_{50}) was 12.8, 12.4 and 12.5 Gy for Mundo novo, Kents and Geisha, respectively as determined by extrapolation from the curve. Data obtained for the cuttings vigour at various treatments of 0, 5, 10, 15, 20 and 25 Gy were used to establish the effect of the treatments on observed vigour parameter understudied. From Table 1, there were no significance difference in the root length, number of roots and number of leaves at 0 and 5 Gy, however, plant height were more significantly enhanced at 5 Gy than the control at 0 Gy. Generally, the means value for the vigour reduces as the dosage increases which could be as a result of reduction in mitotic activity due to irradiation.

Mundo novo and Geisha genotype vigour were not significantly different, however, the number of roots in all the three genotypes were not significantly different at 5% (Table 2). The analysis of variance (ANOVA) analysis (Table 3) showed that variations were highly significant in the treatments in terms of applied dosages. It showed similar trends in the genotypes in exception of the number of roots (N.R = 0.46) that were not significant.

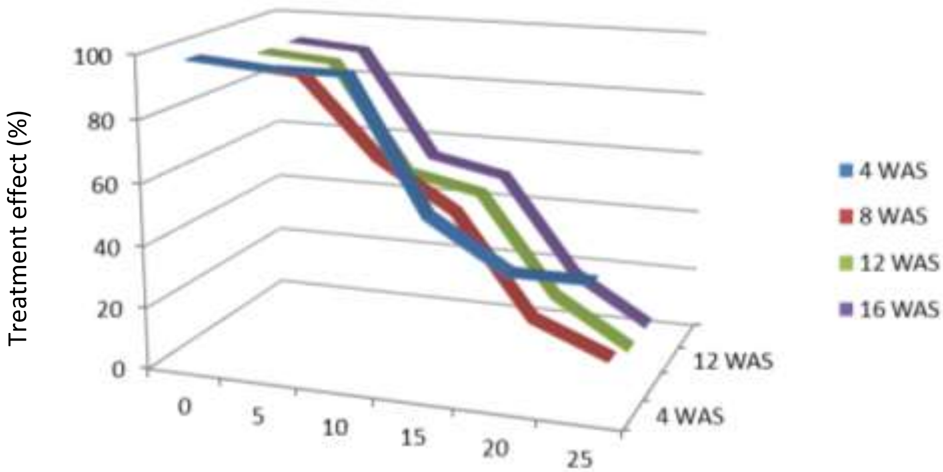

Figure 2. Curves to shows the effect of treatment on success takes at different period of after settings.

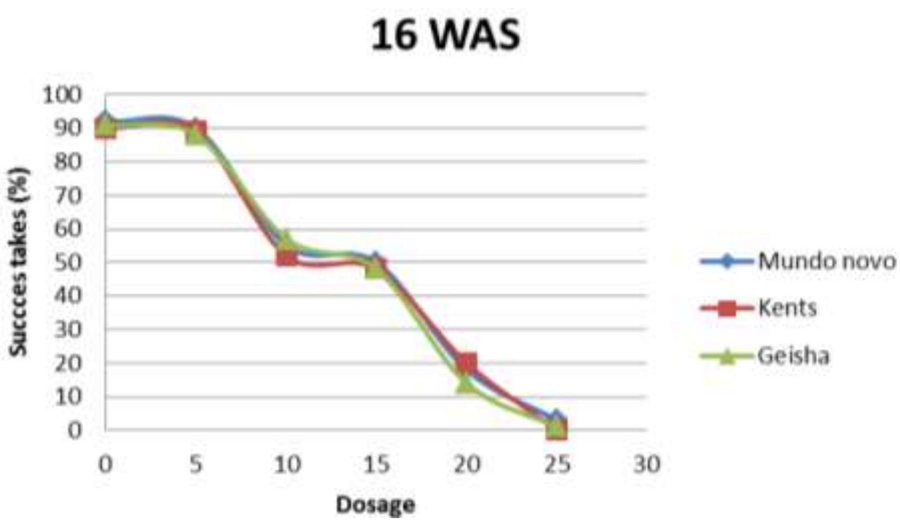

Figure 3. Response curves of cultivars Kents, Mundo-novo and Geisha to gamma irradiation: radio-sensitivity based on germination percentage plotted against dose.

Table 1. Mean separation of four characters in the treated dosage.

Treatment (Gy)	PH (cm)	RL (cm)	NR	NL
0	46.11^{b}	26.88^{a}	15.78^{a}	15.78^{a}
5	48.66^{a}	28.33^{a}	17.00^{a}	15.44^{a}
10	34.67^{c}	20.67^{b}	10.89^{b}	13.44^{a}
15	19.89^{d}	16.44^{c}	6.67^{c}	8.89^{a}
20	8.78^{e}	9.11^{d}	2.33^{d}	5.11^{d}
25	1.11^{f}	1.56^{e}	0.4^{e}	0.67^{e}

PH: Plant height; RL: root length; NR: number of roots; NL: number of leaves.

The interaction between the genotype and the treatments also produce a significant variation in all the vigours in exception of number of roots. There are positive interactions within the traits evaluated for vigour as the

Table 2. Mean separation of four characters of *Arabica* genotypes.

Genotype	PH (cm)	RL (cm)	NR	NL
Mundo novo	28.06[a]	19.17[a]	8.94[a]	10.83[a]
Kents	23.50[b]	13.17[b]	8.67[a]	8.00[b]
Geisha	28.06[a]	19.17[a]	8.94[a]	10.83[a]

PH: Plant height; RL: root length; NR: number of roots; NL: number of leaves.

Table 3. Analysis of variance of four characters of *Coffea arabica*

Source of variation	df	PH	RL	NR	NL
Genotypes (G)	2	124.52***	216***	0.46 NS	48.17***
Treatment (T)	5	3501.04***	773.06***	425.62***	336.71***
GXT	10	10.39***	11.15**	0.51NS	10.74***
Error	34	6.82	3.15	2.9	2.04
Cv		9.85	10.34	19.48	14.43

***Significant at P≤0.01 and p≤0.05. PH: Plant height; RL: root length; NR: number of roots; NL: number of leaves.

Table 4. Correlation coefficient between four characters evaluated in *Coffea arabica*.

Correlation	PH	RL	NR	NL
PH	1.0	0.96***	0.98***	0.92***
RL		1.0	0.92***	0.92***
NR			1.0	0.91***
NL				1.0

NR: number of roots; NL: number of leaves.

plant height was seen to be influenced by increase in root length, number of root and number of leave gave an indication that much more water and nutrient could be absorbed with an increase in root length and number of roots. So also the manufacturing of food through photosynthesis could be enhanced with an increase in the number of leaves. Correlation coefficient among seedling vigour characters showed that all the characters considered were positively correlated with each other in Table 4. An increase in the root length resulted in increase in plant height (0.96***), so also an increase in the number of roots (NR) and number of leaves (NL) gave an increase in plant height at (0.98***) and (0.92***), respectively. Generally, an increase in the number of roots gave an increase in number of leaves. The correlation in Table 4 shows the interaction of the traits in all the genotypes. There are positive interactions between the traits under consideration. The bar chart shows a stimulation effect at 5 Gy in all the vigour parameter considered in this study with a relative increase in value compared to the control at 0 Gy. The plant height, root length, number of root and number of leaves reduce as the treatment increases in exception of plant height at 5 Gy. The protrusion observed at 4 WAS signifies the commencement of success takes, however, there could be die-back after if the interaction between the clonal materials and the humid environment could not be sustained. Eight weeks of hardening process was to prepare the clonal materials for the condition on field as shown in Figure 1. At 4, 8, 12 and 16 WAS, the success takes recorded were not affected by the treatment at 0 and 5 Gy in all the varieties. Though, the success takes was high at 4 WAS at 10 Gy, the 40% of die back and subsequent takes of 55% at 15 Gy shows that an effective irradiation took place within 10 to 15 Gy of treated genotypes. The sensitivity of the genotype to the induction as represented in the curve (Figure 3) was taken at 16 WAS before transplantation. This shows that the optimum dosage to reduce the population to 50% (LD_{50}) were relatively the same in the three varieties, this could be as a result of similar evolutionary trends of the genotypes under consideration as reported by Schilling (2015). The descending nature of the curves shows that the success takes tends to 0 at an increase of the dosage.

The effect of treatments in terms of dosage on the vigour of the cuttings as shown in Figure 5 at 0, 5, 10, 15, 20 and 25 Gy showed the plant height recorded at 5 Gy were taller than the control at 0 Gy, though with no significant difference. The information available on the

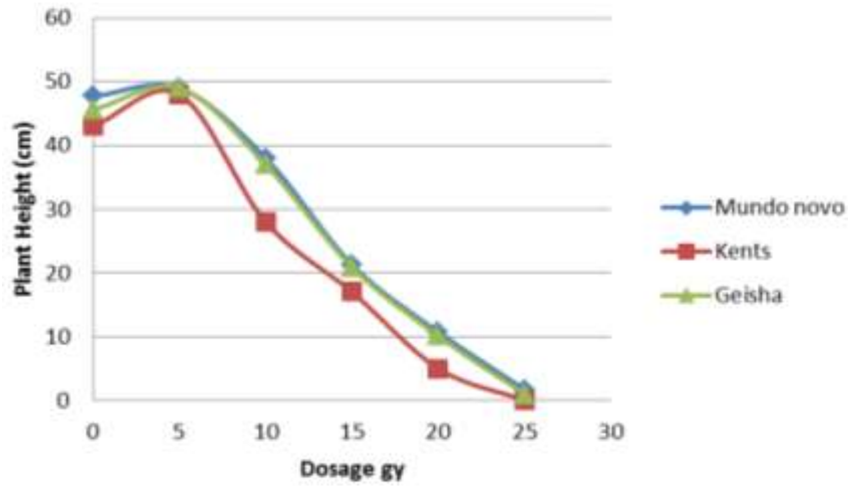

Figure 4. Curve to show the effect of dosage on plant height.

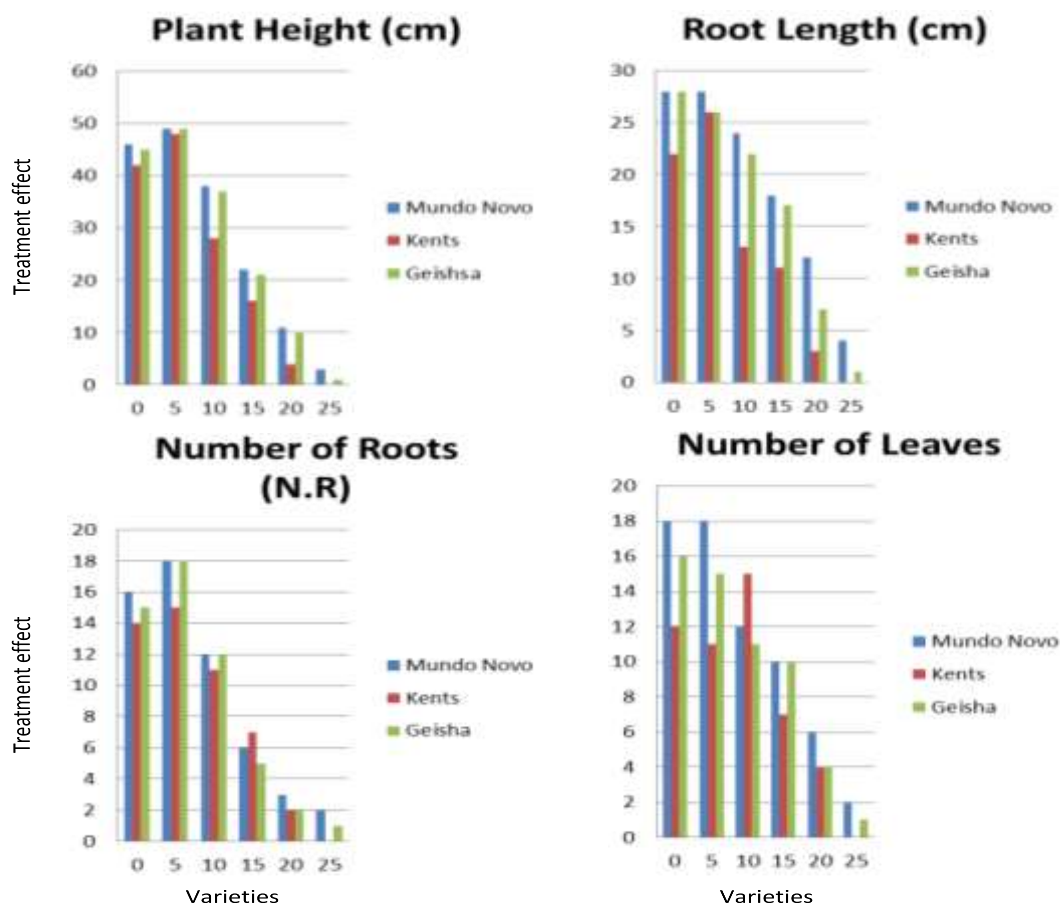

Figure 5. Chart to show the effect of treatment at different dosage on the *Arabica* varieties.

bar chart shows a stimulation effect at 5 Gy in all the vigour parameter considered in this study with a relative increase in value compared to the control at 0 Gy in exception to Kents variety where there were no success takes at 25 Gy of irradiation (Figure 4). The plant height, root length, number of root and number of leaves reduces as the treatment increases in exception of plant height at 5 Gy. The stimulation in the meristematic activities of the mitotic cell could be responsible for the occurrence. Generally, there was a decrease in the vigour as the treatment increases. The effect of the genotype on the cuttings vigour shows that the effect of Mundo novo and Geisha are the same in plant height, root length and number of leaves. A relationship to establish a narrow genetic base in *Arabica* coffee, however, numbers of roots in all the three genotypes were the same.

Conclusion

Results obtained from this work is a protocol for clonal mutation breeding whereby the cuttings and the vegetative propagation have shown different response to the dosage in terms of success takes and seedling vigour. The optimum dose (LD_{50}) for the success takes were in the range of 12 Gy in all three varieties as a result of similarity in the evolutionary trends of the varieties. A low dose of irradiation that stimulated the plant height in this work confirms an initial recommendation of increasing plant vigour (Chandrashekar et al., 2013). However, an increase in the dosage generally reduces the seedling vigour and eventually resulted in die back in all the irradiated cuttings. This work is a prerequisite for mutation breeding in coffee using vegetative propagules with the overall objective of improving the yield and quality of coffee plantations in spite of threat from change in climate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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