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Effect of Paclobutrazol Treatment on Some Leaf Physiological and Biochemical Characteristics of Rejuvenated Coffee (*Coffea arabica* L.) Trees

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ABSTRACT

Rejuvenation pruning has been proven effective in bringing back vigor and productivity of old coffee trees. The effectiveness of the present rejuvenation techniques however could be probably enhanced by the application of tree growth regulators with known reinvigorating or tree health enhancing effect such as Paclobutrazol but has not been tried so far. The present study aimed to evaluate some physiological and biochemical responses of the rejuvenated coffee trees to timing and level of PBZ application prior to cutting.

Plants applied with PBZ 2 months prior to pruning had higher leaf internal CO_2 concentration compared to those applied 1 month prior to pruning. Net photosynthesis, transpiration rate, PAR/LAI, chlorophyll content, and leaf N, P, K, Ca and Mg contents did not differ with time of PBZ treatment. Application of 0.5-1.0 g a.i. PBZ per meter canopy span significantly increased transpiration rate, internal CO_2 concentration and chlorophyll content but did not affect the rate of photosynthesis, PAR/LAI, leaf N, P, K, Ca and Mg contents.

Keywords: rejuvenation pruning, Paclobutrazol, growth retardant, physiological and biochemical responses.

INTRODUCTION

Paclobutrazol (PBZ) [2RS, 3RS)-1-4 (-chlorophenyl)-4,4-dimethyl-2-1, 2, 4-triazol-1-yl-penten-3-ol], a member of the triazole group with both fungicidal and plant growth regulatory effects is known to have reinvigorating effect on plants by influencing or altering crop physiology. Among the reported effects of PBZ application on plant physiological processes include increased rate of photosynthesis (Sankhla *et al.* 1986; Zhou and Xi, 1993; Berova and Zlatev, 2000; Dalziel and Lawrence, 1984),increased stomatal conductance (Qi *et al.* 2006), delayed onset of leaf senescence (Proietti *et al.* 1999) and improve water relations due to reduced transpiration rate and reduced leaf surface area (Fletcher and Nath, 1984).

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On the other hand, increased leaf tissue chlorophyll content (Sopher *et al*. 1999; Berova and Zlatev, 2000; Sebastian *et al*. 2002), increased soluble protein contents in leaves (Wang *et al.* 1985; Sopher *et al*. 1999), increased leaf mineral contents (Yelenosky *et al*. 1995; Yeshitela, 2004) and decreased shoot GA contents (Rademacher, 1991; Protacio, 2000)were among the reported plant biochemical responses to PBZ application.

In coffee, rejuvenation pruning was developed as a cultural management strategy to bring back old rundown trees to acceptable production level, with yield improvement as the ultimate benefit (Canell, 1983; Cabangbang, 1988; CEMARRDEC, 1990; Jativa, 1990; Netsere *et al.* 2006). An effective rejuvenation system should promote faster development and production of new shoot system which is required for early bearing. The different aspects of the rejuvenation process including specific cutting method (Netsere *et al.* 2006, Pugoy, 1991), fertilizer application (Pugoy, 1991; Bacasno, 2002),mulching (Bacasno, 2002) and shading (http://abstracts.aspb.org/pb2003/public/P72/1437) had been already studied. In the Philippines, rejuvenation techniques for old coffee trees had been recommended (Cabangbang, 1990). Common among the different rejuvenation technologies include specific pruning height, rate of fertilizer application, sprout selection and training technique. Application of tree growth regulators with known reinvigorating or reconditioning effect to enhance tree health like Paclobutrazol could probably further improve the effectiveness of the existing rejuvenation technologies.

To our knowledge, there has been no study determining the effects of pre-rejuvenation application of PBZ on the performance of rejuvenated coffee trees. Specifically, the study aimed to evaluate some physiological and biochemical responses of the rejuvenated coffee trees to timing and level of PBZ application prior to cutting.

MATERIALS AND METHODS

The Experimental Trees

Thirty (30) year old non-productive coffee trees planted at the Coffee Project of the Department of Horticulture, College of Agriculture and Food Science, Visayas State University, Visca, Baybay City, Leyte, were used. The trees were planted at 3 m x 3 m using triangular planting system and were under 30-35% shade provided by Thailand acacia (*Acasiaspectabilis* A. Cunn. ex Benth) trees. The trees had an average height of 6.60 m, base girth circumference of 65.52 cm and canopy span of 4.20 meters. The trees had multiple vertical stems due to non-removal of water sprouts. The lateral branches found mostly at upper part of the verticals were overcrowded, had already overlapped with lateral branches of the neighboring trees, and had only very few fruits mostly located near the tip of the branches. Trees with more or less the same stand (height and spread) were selected as experimental samples.

Experimental Design and Treatments

The experiment was laid out in factorial randomized complete block design (RCBD) with three replications, each replicate with 3 sample trees. The time of Paclobutrazol application (T_1- 1 month before cutting and T_2- 2 months before cutting) was assigned as Factor A and the three levels of PBZ application namely: L1 – 0 (water, control), L_2–0.5g.a.i.PBZ /meter span of canopy or 2 ml Cultar/meter canopy span and L_3 - 1.0g.a.i.PBZ/meter span of canopy or 4 ml Cultar/meter canopy span as Factor B.

For L_2 and L_3 trees, a total of 8 and 16 ml Cultar per tree, respectively, was applied because their average canopy span was 4 m.

Paclobutrazol Application

Commercial grade PBZ with 25% active ingredient (a.i.) (Trade name 'Cultar 25 SC', Syngenta UK Ltd.) was used. The required amount of Cultar was dissolved in one gallon water and was applied following the collar drench method (Figure 1). Furrows were made around the tree 30 cm away from the base and the PBZ solution was applied evenly along the furrows. For the untreated control, one gallon plain tap water was applied per plant.

Figure1. PBZ formulation and application by collar drenching technique

Rejuvenation Pruning

The procedures in heading back or rejuvenation pruning recommended by Cabangbang (1990) was followed. The main vertical stem was cut following a slanting cut one foot from the ground using sharp pruning saw. The cut surface was allowed to dry for 5 days and then was painted with coal tar (Figure 2).

Maintenance of the Experimental Trees

The rejuvenated plants were applied with 250 g complete fertilizer (14-14-14) per plant one month after pruning following the holing method (six 5-cm deep holes around the stump at a distance of 50 cm).The base of experimental plants were ring weeded and mulched with 5 cm thick rice hull. The areas in between rows were regularly underbrushed at three

months interval. When the sprouts were about 10 cm tall, preliminary sprout selection was done by removing the weak sprouts and retaining the 3 most vigorous sprouts per stump. When the retained sprouts were about 30 cm tall, the 2 weakest sprouts were removed retaining the most vigorous sprout per stump. Three shoots (1per stump) were allowed to grow per tree for 10 months (Figure 3). Subsequent shoots that emerged were regularly removed.

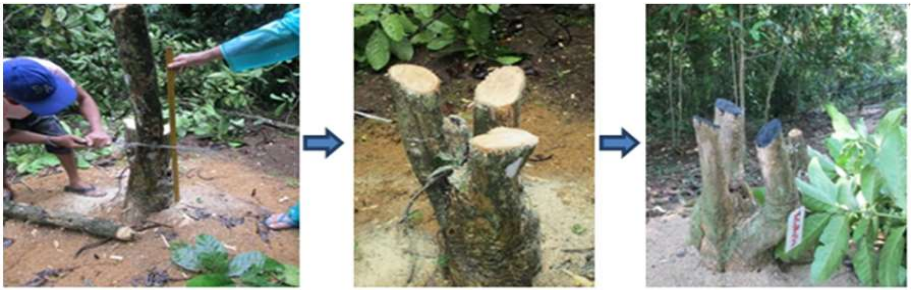

Figure 2. The rejuvenation pruning technique followed in rejuvenating old coffee trees

Figure 3. The selection of sprouts to establish plants with 3 shoots (1 shoot per stump)

Measurement of Parameters

Using the 4th pair from the youngest open leaf of the second pair of lateral branches from the base as index leaf, photosynthetic rate, transpiration rate and internal CO_2concentration were monitored using a Portable Photosynthesis System LI-6400 XT (LI-COR Biosciences Inc. 4421 Superior St. Lincoln, Nebraska 68504)while PAR/LAI using Digital Plant Canopy Imager CI-110 (CID Bio Science 1554 NE 3rdAve. Camas, WA USA) after 8 and 10 months from pruning. Leaf chlorophyll content was quantified using Minolta Chlorophyll Meter SPAD-502 (Minolta Co. Ltd. Japan). The leaves used in chlorophyll analysis were collected in the morning. The leaf samples were placed in properly labeled paper bag and brought to the Crop Physiology Laboratory of the Department of Horticulture, cleaned of dust and then oven-dried at 60-65°C for 5 days using forced draft oven. The dried samples were ground using Willey mill, placed in properly labeled paper bags and were submitted to the Central

Analytical Laboratory of the Philippine Rootcrop Research and Training Center for determination of N, P, K, Mg and Ca contents.

Statistical Analysis

Data were analyzed by performing analysis of variance (ANOVA) and treatment means were compared by Least Significant Difference (LSD) test at 5% level of significance using the STAR, version 2.0.1 2014 Biometrics and Breeding Informatics, PBGB Division International Rice Research Institute, Los Banos, Laguna.

RESULTS AND DISCUSSION

Leaf Physiological Responses to PBZ

Time of PBZ application significantly influenced internal CO_2 concentration 10 months after cutting but not the transpiration rate, net photosynthesis and PAR/LAI 8 and 10 months after cutting (Table 1). Regardless of level of PBZ application, leaves of plants applied with PBZ 2 months before cutting had significantly higher internal CO_2 concentration than leaves from plants applied with PBZ 1 month before cutting. The high internal CO_2 concentration in leaves among plants treated with PBZ 2 months before cutting over those applied with PBZ 1 month before cutting could be probably attributed to the effect of PBZ in improving plant water relations resulting from production of more fine roots (Ramos and Acedo, 2014) thereby increasing water absorption. Improved water relations also improved opening of stomata which promote gas exchange that raised leaf internal CO_2 concentration.

Significant effect of PBZ concentration was also obtained for transpiration rate and leaf internal CO_2 concentration (Table 1). Leaves of plants applied with 1.0g.a.i. PBZ/meter canopy span had significantly higher transpiration rate than those applied with 0.5g.a.i. PBZ/meter canopy span and the non-PBZ treated control sampled 8 months after cutting. After 10 months from cutting, the PBZ and the non-PBZ treated control had comparable leaf transpiration rate.

Furthermore, plants applied with 0.5 and 1.0 ga.i. PBZ per meter canopy span had higher leaf internal CO_2 concentrations than leaves of the non-PBZ treated plants. The improved plant water relations among PBZ-treated plants explained earlier may account for their higher leaf internal CO_2 concentrations over the non-PBZ-treated control plants.

PAR/LAI which is the measure of the fraction of absorbed photosynthetically active radiation (PAR) that the coffee plant canopy absorbed was not significantly affected by PBZ application. The coffee plant LAI was not also significantly altered by PBZ application (Ramos and Acedo, 2014).

Table 1. Effect of timing and level of PBZ application on some leaf physiological processes of the rejuvenated coffee trees

Treatments	Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)		Transpiration Rate (mmol H_2O $m^{-2}sec^{-1}$)		Internal CO_2 Concentration (µmol CO_2 $m^{-2}s^{-1}$)		PAR/LAI (µmolm^2s)
	Month After Cutting		Month After Cutting		Month After Cutting		10th Month
	8	10	8	10	8	10	
Time of PBZ Application							
1 month before cutting	13.53	16.03	6.89	7.05	155.92	183.91b	3.07
2 months before cutting	14.08	15.36	6.74	7.27	161.48	204.54a	2.48
PBZ Concentration							
0 (water, control)	13.34	15.18	6.39b	6.84	151.52	174.79b	3.06
0.5g.a.i. PBZ/m canopy span	13.50	15.28	6.25b	7.26	168.06	214.10a	2.56
1.0g.a.i. PBZ/m canopy span	14.57	16.62	7.79a	7.38	178.84	193.79ab	2.58
CV (%)	13.86	9.50	14.17	11.44	13.02	9.66	30.28

Mean separation within columns by LSD, 5%.

Leaf Biochemical Responses to PBZ

PBZ application (0.5 and 1.0 g a.i./m canopy span) significantly increased the leaf chlorophyll content 10 months after cutting (Table 2). These results corroborate earlier findings. Higher leaf chlorophyll contents resulting from PBZ application were found in maize seedlings (Sopher *et al*. 1999), tomato (Berova and Zlatev, 2000) and Dianthus (Sebastian *et al*. 2002). Berova and Zlatev (2000) and Sopher *et al.* (1999) attributed PBZ-induced increase in chlorophyll content to the ability of PBZ to increase the level of endogenous cytokinin which is known to stimulate chlorophyll biosynthesis and/or reduced chlorophyll catabolism. Chaney (2005) added that PBZ has enhancing effect on the production of phytol which is an essential part of the chlorophyll molecule.

PBZ had no marked influence on total N, P, K, Ca and Mg contents of the leaf after 10 months from pruning (Table 2). Earlier, lack of PBZ effect on leaf macronutrient status was also obtained in apple (Wieland and Wample, 1985) and mango (Leal *et al.* 2000; Yeshitela *et al.* 2004).

CONCLUSION

PBZ treatment significantly enhanced some physiological parameters of the coffee leaf evaluated in the study particularly the internal CO_2 concentration and transpiration rate but did not influenced net photosynthesis and PAR/LAI. The growth retardant treatment also significantly increased the leaf chlorophyll content but had no mark effect on the leaf total N, P, K, Ca and Mg contents.

Table 2. Effect of timing and level of PBZ application on chlorophyll and leaf nutrient contents of rejuvenated coffee plants 10 months from cutting

Treatments	Chlorophyll Content (SPAD reading)	Total N (%)	Total P (mg/kg)	Total K (g/kg)	Total Ca (g/kg)	Total Mg (g/kg)
Time of PBZ Application						
1 month before cutting	67.35	1.92	287.57	14.44	15.42	2.74
2 months before cutting	66.95	1.99	288.52	14.43	15.02	2.81
PBZ Concentration						
0 (water, control)	62.35b	2.00	287.74	14.27	15.28	2.80
0.5g.a.i. PBZ/m canopy span	67.75a	1.98	289.12	14.61	15.70	2.79
1.0g.a.i. PBZ/m canopy span	70.91a	1.97	287.26	14.43	14.67	2.75
CV (%)	4.87	8.56	3.70	12.86	10.24	6.46

Mean separation within columns by LSD, 5%.

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