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Use of Chelating Agents to Modify Cacao Roots for Enhanced Nutrient Absorption in Phosphorus Deprived Media

Ian Marc Cabugsa* **and Travis Idol**
*Corresponding author email id: imgcabugsa@addu.edu.ph

Abstract **– Nutrient deficiency is one of the prominent causes for limited production in cacao farms in the Philippines. To increase nutrient absorption of cacao in fertilizer-starved farms, root modification was studied. Chelating agents were the root modifiers introduced in cacao seedlings. Several 3 and 6-month old cacao seedlings were grown in one of the greenhouses at the Magoo site of University of Hawaii, Manoa. The 98 best grown seedlings from each group were selected for further studies. Samples were transplanted to a media with 50:50 mixture of vermiculite and oxisol soils. Chelating agents; glycine (G), oxalic acid (OA), citric acid (CA), trisodium citrate (Na3), 2, 3-dimercapto-1-propanesulfonic acid (DPS) and meso-2, 3-dimercaptosuccinic acid (MDS) were added to groups. The growing seedlings were watered only with phosphorus-deprived Hoagland solution to mimic macronutrient deficiency. Morphological developments were routinely measured. After 10 weeks, the plants were harvested. Micro and macronutrients levels in the leaves were also analyzed. The combined results of morphological and nutrient uptake indicate better development for plants treated with chelating agents such as oxalic acid (OA). Plants treated with DPS, however, performed poorer than the control. Further studies would be needed to evaluate its efficacy especially during the cacao fruit bearing phase, since this study focused only on the developing stage.**

Keywords **– Cacao, Chelating Agents, Micronutrients and Phosphorus-Deficient soils.**

I. Introduction

Agricultural plants yield more produce when its nutrition is adequate [1]. Nutrients can be sourced from the soil itself or by addition of fertilizers which can be organic or inorganic. Well-nourished plants do not only yield more but are also resilient to pest and deceases. [2]. Cacao, like all other plants, is not an exemption to this nutritional needs and effects.

The underfertilization of soils in Eurasia, Africa and some parts in Latin America is causing soil mining, whereas overfertilization is observed in North America, Western Europe and China [3]. Farming in Asia is very challenging as farmers would want to harvest more despite minimal efforts in fertilization. This is coupled with the ubiquitous presences of pest and disease. All these conditions combine for a decreased annual harvest. Nevertheless, farmers still need to generate produce for their livelihood.

Phosphorus is one of the major nutrients that plants, like cacao, need in its development [4, 5, 6]. Phosphorus deficiency will lead to stunted growth of cacao and ultimately affecting its development [7]. The under-fertilization problems in Asia, particularly Philippines, has made farming a challenging tasks. With cacao becoming popular in the southern part of the Philippines, improved techniques must be placed to address the continued underfertilization. Plants with better genotypes that thrive well in nutrient deficient soils are explored [8]. But ultimately, it is the root-soil interaction that must be studied in order to provide better understanding on the mechanisms on fertilization thus answer underfertilization problems.

Roots are the main channel for nutrient absorption [9]. The plasticity of the roots produce modifications for the plants to survive and adapt to its environment [10]. Ideally, the roots of the plants should be well developed to have increased nutrient uptake [11]. Deficiency of P in the soil elicits modifications in the roots to better adsorb the needed nutrient [12]. However, not all plants develop the same responses to P deficiency [13]. The modifications are affected by numerous factors including plant type, amount of water, nutrients, microorganisms, type of media and a lot more.

Chelating agents are one of the factors that can modify the structure and functionality of the roots [14]. The effects of chelating agents can vary from plant to plant and concentration. Some of the notable effects is the increased uptake of minerals from the soil [15]. Since plants have different responses to different chelating agents, this study was devised to investigate the effects of various chelating agents on the nutrient uptake of cacao in a phosphorus deficient environment during its developing months. This investigation seeks to unravel if induced root modifications will make plants absorb more nutrients ideal for an Asian setting.

II. Materials and Methods

To test the effect of chelating agents to the growing cacao seedlings, 49 3-month old and 6-month old seedlings were selected from a pool of readied cacao inside the greenhouse of UH in Magoon facility. The seedlings where transplanted to another container where a mixture of 50:50 vermiculite and oxisol served as the media. The 49 seedlings per age group were divided into 7 subgroups each with 7 seedlings.

The 7 groups were:
OA – Oxalic acid.
G – Glycine.
CA – Citric acid.
Na3 – Trisodium citrate.
DPS – 2, 3-dimercapto-1-propanesulfonic acid.
MDS – Meso-2, 3- Dimercaptosuccinic acid.

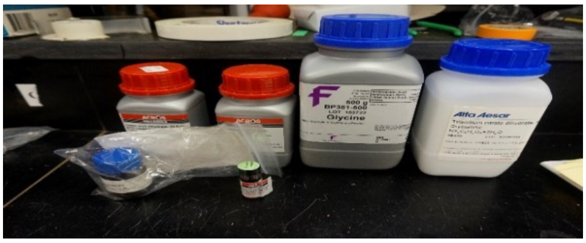
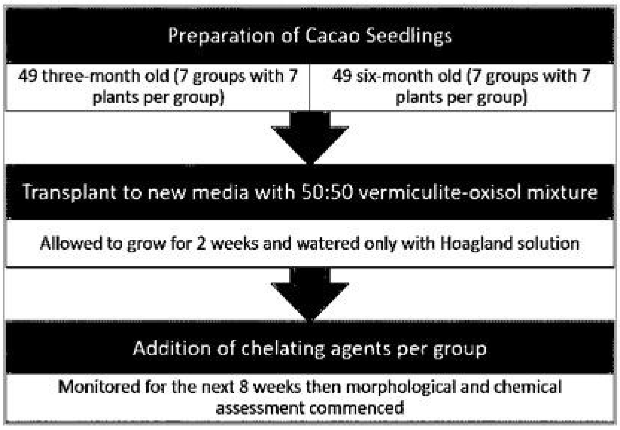


Fig. 1. Chelating agents used for this study.

The groups represent the type of organic chelating agent added to the seedlings. All reagents were purchased from Sigma-Aldrich. After the transplant, the seedlings were let to adjust to its current environment for 2 weeks and was watered only with Hoagland solution that is deprived of phosphorus. The Hoagland solution was prepared by adding 17.14 mL KNO_3, 27.15 mL $Ca(NO_3)_2$ and 6. 58 mL $MgSO_4$ solution in 10L water producing a mixture with 100, 67, 109, 16 and 21 ppm of N, K, Ca, Mg and S respectively. The saturated weight of the seedlings were monitored and kept for the entire duration of the experiment.

Two weeks after transplant, the first treatment of the seedlings with the 100 mL of 10 mM concentration for OA, G, CA and Na3, while the concentration of DPS and MDS were 0.01 mM and 0.10 mM respectively commenced. The latter two had lower concentrations due to economic constraints. The second treatment with chelators happened 4 weeks later. In the entire course of the experiment, the condition of the glasshouse like the light, humidity and temperature where constantly monitored using a data logger.

Fig. 2. Photo of the 6-month old cacao seedlings used in the study. The far left are the 3-month old seedlings.
Table 1. Schematic diagram of the procedure.



At the end of the experiment, the final height, weight and stem diameter of the plants were measured. The chemical properties of the leaves where analyzed by the Analytical laboratory of NREM in UH.

III. Results and Discussion

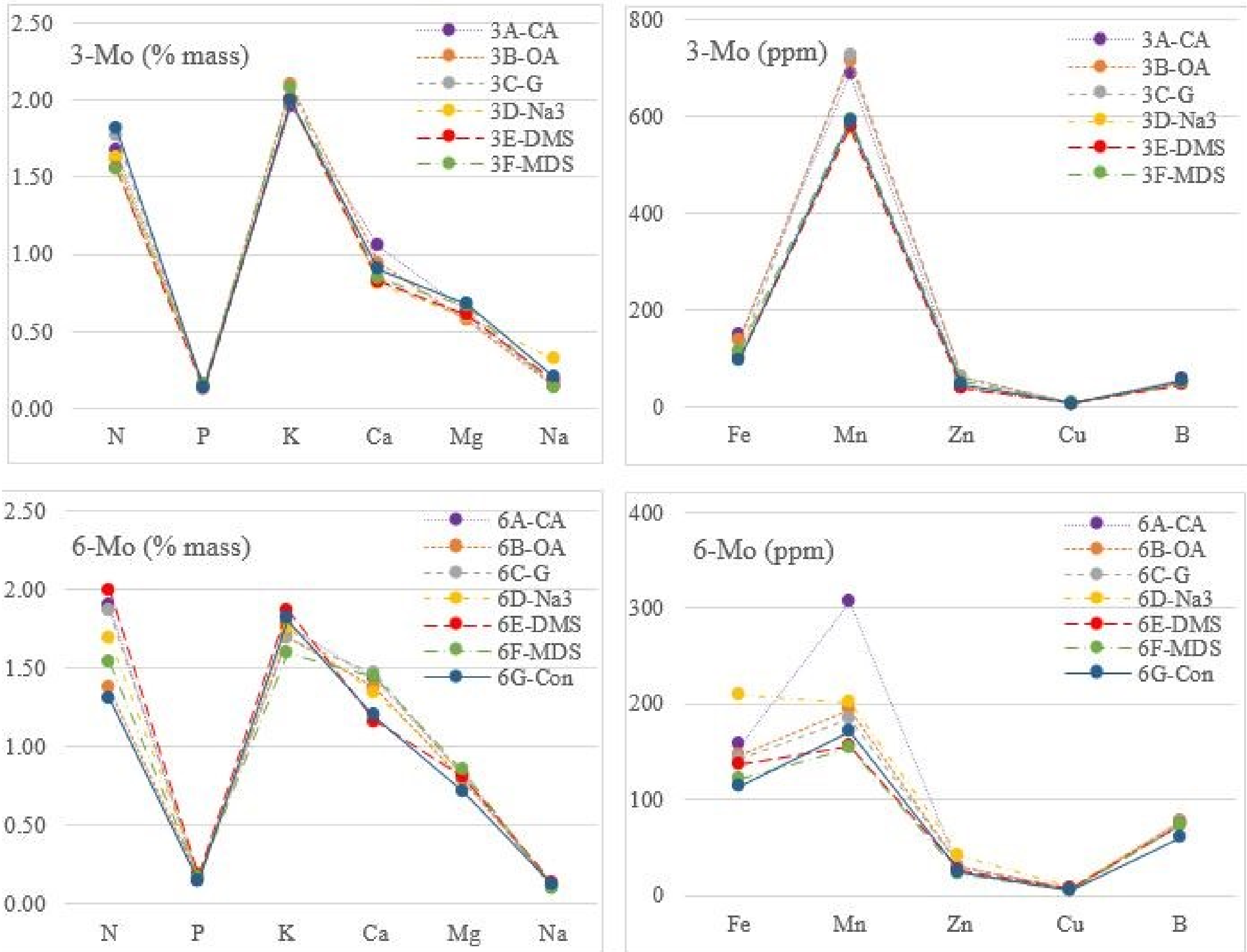
The morphological results of the experiment are shown in Table 2. The results of the 3 and 6 month old seedlings have minimal variance. The initially effect of citric acid (CA) was evident as it produce plants with better mass, height and stem diameter. Comparing the two results, calculating the statistical t-test indicate that the difference was insignificant (p = 0.9693) when compared to the control. The minimal changes produced was not overwhelming to produce substantial difference. The 6-month old seedlings produced very similar results to the 3-month olds, however, the best performance this time was taken by the oxalic acid (OA). Similarly, there was no significant difference (p = 0.3383) between the best result (OA) from the control.

Table 2. Average final dry weight in grams, diameter in centimeters and height in centimeters of the harvested cacao seedlings after 10 weeks of study.

3-Mo Old Plants	Chelating Agent	Dry Mass in g	Ave Diameter in cm	Height in cm	6-Mo Old Plants	Chelating Agent	Dry Mass in g	Ave Diameter in cm	Height in cm
3A	CA	8.72	0.816	35	6A	CA	26.88	1.190	61
3B	OA	7.72	0.835	30	6B	OA	31.83	1.162	72
3C	G	8.14	0.827	32	6C	G	29.06	1.163	64
3D	Na3	6.90	0.771	34	6D	Na3	28.18	1.210	65
3E	DPS	7.38	0.774	34	6E	DPS	21.53	1.056	63
3F	MDS	7.54	0.832	34	6F	MDS	30.93	1.241	64
3G	Control	8.64	0.787	33	6G	Control	27.13	1.205	66

The summary of the nutrient concentration analysis in the leaves of the cacao seedlings are shown in Graph 1. The results indicate minimal changes in the uptake of macro and micronutrients of the treated seedlings. The macronutrients are expressed in % mass while the micronutrients in ppm. For the 3 - month old seedlings, the uptake of the macronutrients did not essentially differ with the control, however, the results for the micronutrients show that some of the elements like iron and manganese were higher than the control.

For the 6-month old seedlings, the concentration of the nutrients is much diverse than the 3-month old. There is now better uptake for some of the treated samples compare to the control. The increase in the uptake of nutrients might be attributed to the adjustment and adaptation of the seedlings in response to the earlier treatment with chelating agents. Plant have adaptive capability in response to changes in the environment [16]. The action of the chelating agents might have been responsible for the adaptation of the seedlings.



Graph 1. Summary of the macro and micronutrients concentration in the leaves of the cacao seedlings. The macronutrients are expressed in % mass while the micronutrients are in ppm.

The amount of P present in the leaves were very minimal (less than 0.50% of the total dry mass) in all the test subjects. The phenotypical result of P starvation is not obvious in the study as all the test subjects had minimal differences. Nevertheless, the plant will still be able to thrive in media with minimal presence of phosphorus. Though it cannot be denied that P is a very important macronutrient for plants. Phosphorus is mainly used in the energy functions from the generation of ATP, GTP, CTP and UTP. Furthermore, P is used in the DNA and RNA along with the numerous metabolic processes it support such as cytoplasmic streaming, membrane transport, photosynthesis, respiration and the biosynthesis of proteins, lipids and nucleic acids [3].

Remarkably, despite the fact that the plants were starved of P, the plants were still able to accumulate it from the inherent P from the media. As shown in Table 3. The soil media to which the seedlings were grown is inherently rich in P, K, Mg and Ca. With the exception of P, the concentration of the other metals in the soil is augmented with the addition of the Hoagland solution. The amount of P in the leaves of the cacao is similar to the study of [15] where they analyzed the effect of organic and inorganic fertilizer application to cacao.

Table 3. Initial Soil Physical Properties.

Parameter	Concentration
Soil pH	5.97 ± 0.1
Phosphorus (µg/g)	50.00 ± 4.0
Potassium (µg/g)	571.33 ± 8.1
Calcium (µg/g)	733.67 ± 23.5
Magnesium (µg/g)	539.67 ± 10.1
Nitrogen (g/g, %)	0.19 ± 0.1
Total Carbon (g/g, %)	2.07 ± 0.1

Generally, the uptake of micro and macro nutrients in all test subjects were similar except for Ca, Mn and Zn. Comparing the 3-month old with the 6-month old seedlings across all treatments, the uptake of calcium increased by around 50%. This macronutrient must have been needed more by the seedling as it develops. On the other hand, the uptake of both Mn and Zn decreased significantly (p <0.0001 for Mn, p = 0.0007 for Zn). Both micronutrients are noted to be vital in plant development. This must mean that younger cacao seedlings will need more Mn and Zn during its developing stage. This finding is useful in engineering the soil quality for growing young cacao

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seedlings. Furthermore, the uptake of Mn in CA chelated 6-month old cacao seedlings remarkably higher than the rest of the test subjects along with the control (>300 ppm while others are <200 ppm).

Table 4. Summary of rank points of the test groups. Ranking is from 1-7 with one as the highest. The groups with the lowest total (**T**) will be ranked (**R**) first and so on.

Sample	Chelator	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	M	D	H	T	R
3A	**CA**	3.0	3.0	7.0	1.0	3.0	5.0	1.0	3.0	7.0	3.5	1.0	1.0	4.0	1.0	43.5	**1**
3B	**OA**	5.0	3.0	1.0	2.0	7.0	6.5	2.0	2.0	1.0	2.0	5.0	4.0	1.0	7.0	48.5	**3**
3C	**G**	2.0	5.5	5.0	5.5	6.0	4.0	5.0	1.0	2.0	6.0	6.0	3.0	3.0	6.0	60.0	**5**
3D	**Na3**	4.0	3.0	5.0	7.0	5.0	1.0	4.0	7.0	5.0	3.5	4.0	7.0	7.0	3.0	65.5	**6**
3E	**DPS**	6.5	5.5	3.0	5.5	4.0	3.0	6.0	6.0	6.0	5.0	7.0	6.0	6.0	3.0	72.5	**7**
3F	**MDS**	6.5	1.0	2.0	4.0	2.0	6.5	3.0	4.0	3.0	1.0	3.0	5.0	2.0	3.0	46.0	**2**
3G	**control**	1.0	7.0	5.0	3.0	1.0	2.0	7.0	5.0	4.0	7.0	2.0	2.0	5.0	5.0	56.0	**4**
6A	**CA**	2.0	7.0	3.0	3.0	4.0	5.5	2.0	1.0	5.0	3.0	2.0	6.0	4.0	7.0	54.5	**3**
6B	**OA**	6.0	4.0	5.0	4.0	6.0	3.0	3.0	3.0	3.0	4.0	1.0	1.0	6.0	1.0	50.0	**2**
6C	**G**	3.0	4.0	6.0	1.0	4.0	7.0	4.0	4.0	2.0	5.0	3.0	3.0	5.0	4.5	55.5	**4**
6D	**Na3**	4.0	1.5	4.0	5.0	2.0	3.0	1.0	2.0	1.0	1.0	6.0	4.0	2.0	3.0	39.5	**1**
6E	**DPS**	1.0	1.5	1.0	7.0	4.0	1.0	5.0	6.0	4.0	2.0	4.0	7.0	7.0	6.0	56.5	**5**
6F	**MDS**	5.0	4.0	7.0	2.0	1.0	5.5	6.0	7.0	6.0	6.0	5.0	2.0	1.0	4.5	62.0	**6**
6G	**control**	7.0	6.0	2.0	6.0	7.0	3.0	7.0	5.0	7.0	7.0	7.0	5.0	3.0	2.0	74.0	**7**

Legend: M – Total dry Mass, **D** – 1 cm above ground Diameter, **H** – total Height from ground to tip.

Ranking the mineral uptake and morphological development of the seedlings by the different chelating agents from 1 to 7 where 1 being the highest and 7 being the lowest, Table 4 summarizes the points gathered by the respective groups. The result for the 3-month old seedlings indicate citric acid (CA) was the best chelating agent followed by meso-2, 3- Dimer - captosuccinic acid (MDS) then the oxalic acid (OA) while dimercapto-1-propanesulfonic acid (DPS) was the least with the control in the middle. For the 6-month old seedlings the best chelating agent was the trisodium citrate (Na3) followed by oxalic acid (OA) and the citric acid (CA) and the least performing was the control.

The changes in the adsorption of nutrients must have been influenced by the root architecture. Studies on root development show high correlation between nutrient uptake and root area [17]. Unfortunately, the observed root architecture in the study is not concise to produce a substantial generalization. It is deemed important to increase the sample population to arrive at a consensus.

The use of chelating agents in cacao seedlings produced plants that are able to develop and uptake nutrients better. In the case for the 6-month old seedlings, though Na3 treated cacao ranked first, the researcher would personally choose the second rank over the first. This is due to the better overall morphological development of the plant compared to the first. Na3 ranked first indeed but it was due to the number of nutrients absorbed, regardless of nutrients, it failed to translate the accumulated nutrients to plant development. On the other hand, OA treated seedlings developed better as indicated by the most dry mass and height.

IV. Conclusion

The use of chelating agents to cacao seedlings increases its rate of morphological development and nutrient uptake. Oxalic acid and citric acid chelating agents produce best results in the study. More analysis and variation of this study are needed to produce a more comprehensive result especially until the plants bare fruits which is the most important part for cacao farming.

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AUTHORS PROFILE'

Ian Marc G. Cabugsa is a faculty of the Chemistry Department of Ateneo de Davao University, Davao City Philippines and is currently specializing in agricultural chemistry. The principal author is a recipient of Norman Borlaug cacao Fellowship with funding from the USDA and in partnership with World Cacao Organization when he conducted this research.

Travis Idol Ph.D. is a faculty of the Natural Resources and Environmental Management of University of Hawaii in Manoa.