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Isotopic Tracing of Phosphorus Uptake in Oil Palm Seedlings Leaf Axil Using ^{32}P Labelled

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***Abstract*— Pot experiments have been carried out to investigate the distribution pattern of Phosphorus in oil palm seedlings axillary axes using ^{32}P radioisotope technique. The experiment was conducted in the greenhouse at the Center for Application of Isotope and Radiation – National Nuclear Energy Agency, Jakarta, Indonesia. $KH_2{}^{32}PO_4$ solution with the activity of 5 mCi was injected into five points viz through the lowest, middle, upper leaf axil, at the base of the trunk and the soil 5 cm from the trunk. The method used was randomized complete block design with two replications.The ^{32}P uptake is expressed in counted per minute (cpm) counted by Liquid Scintillation Counter then transformed to disintegration per minute (dpm). The results showed that at the injection point in the lowest leaf axil, the distribution of ^{32}P was more towards the crown before heading towards the roots. In the middle of the leaf axil, the distribution of ^{32}P was recorded higher, nutrients were actively distributed to the top of the plant while the roots were very low. The dynamics of ^{32}P at the upper axil injection point, the highest ^{32}P uptake at the start of the injection was seen in the lowest leaf axil. The ^{32}P distribution then leads to the stem, roots and finally to the canopy. At the injection point in the soil 5 cm from the trunk, the counted value shows ^{32}P distributed to the top of the plant, while at the base of the trunk, ^{32}P is distributed to the farthest injection point, the plant canopy. From this study, it can be concluded that ^{32}P radioisotopes can describe the distribution of nutrients in plant parts, wherever radioisotope is applied. Phosphorus can be applied through any leaf axil and can be distributed well to the canopy of plants.**

***Keywords*— distribution of phosphorus; leaf axil; oil palm; radioisotop ^{32}P**

I. INTRODUCTION

Fertilization of oil palm in the world, including Indonesia, has so far been almost entirely carried out through roots systems [1]. According to Ref. [2], the application of oil palm fertilization through rooting is less effective during the growth period, this is due to the dose, time and composition of nutrients absorbed are highly dependent on local land conditions. The effectiveness and efficiency of fertilizing oil palm through the land are relatively low. It is only around 30%.

Under certain conditions the fertilizer run into evaporation, leaching, erosion and fixation [3]. On tidal, swamp, sandy and peatland, fertilization response is very low because of nutrient fixations. Likewise, in barren soil such as a land former coal mine will require special efforts as stated by Ref. [4]. Nutrient competition between oil palm and weeds also needs serious attention [5]. Thus, a more efficient fertilizer application is needed [6]. The application of fertilizer through leaves and air is mostly done to overcome the inefficiency of fertilizing oil palm through the ground but the results are not yet satisfactory. On the matter of fulfilling the micronutrients of oil palm fertilizer is given through the leaf axil, for example, the microelement such as Boron [7]. Many applications have been carried out by the leaf axil so far in addition to Boron fertilization, also for efforts to control coconut leaf disease [8]. However, for macro nutrients such as phosphorus, the information available is very limited. Phosphorus is a macro nutrient for all types of plants including oil palm, but its availability in the soil is relatively difficult because it is highly dependent on soil pH so that it becomes a serious limiting factor for crop production [9]. By the reason of the nature of phosphorus, a more efficient P fertilizer application is needed which is quickly absorbed without depleting in the soil.

To ensure that phosphorus can be applied through the leaf axil, it is necessary to prove it using a ^{32}P tracking technique that is added to the fertilizer. Radioactive tracers have long been used extensively to study nutrient translocation from soil to plant tissue [10, 11]. It was also reported by Safuan *et al*. [1] that tracer techniques are widely used to measure P uptake in phosphate rock studies and provide a more reliable availability index than conventional methods and they correlate better with measurements of chemical extraction.

P nutrient tracer technique using ^{32}P isotope can be done directly or indirectly [11, 12]. The direct method is isotope as the tracer is mixed into fertilizer material or other P sources. The amount of tracer that is absorbed by plants is measured directly. In the indirect method of labeling with isotopes is done not on fertilizer but other P sources such as soil. This is done if the available fertilizer sources cannot be labeled, for example, natural phosphate. [13, 22].

Nutrients transport from the ground to the leaves is well known. The method used to monitor the passage of nutrients in plant tissues is by tracer, where nutrients are labeled isotopes which will be detected wherever these elements are located. Investigation of nutrient translocation in particular P using a ^{32}P tracer technique from the soil into the plant has been carried out [8],[9],[12]. However, a study regarding the P nutrient route from the leaf axil organ into the stem and other organs has not been done much. The novelty of this research was that there has been no study of the tracing of P translocation patterns from leaf axil to other organs in oil palm plants. In other plants translocation of P from the soils to the plants may have been reported [11-15] or from the leaves in a foliar fertilizer application. With the same analogy to foliar fertilizing, there was hope that monitoring of nutrient travel from the leaf axil to photosynthetic organs can be carried out.

This study aimed to investigate the pattern or route of P nutrient translocation that is applied through the oil palm leaf axil of one-year seedling using ^{32}P radioactive.

II. Material and Method

This research is a P fertilizer application with ^{32}P isotope labeled applied by 1 year old palm seedling axil.

A. Plant material and soil

Dura variety of oil palm seedlings were obtained from the experimental garden of INSTIPER Yogyakarta on Ungaran with 10 polybags. Growing media in the form of ultisol with a pH of 5.6 and NPK 330g / polybag fertilization have been carried out [16] for a year. Phosphorus fertilizer in the form of KH_2PO_4 labeled ^{32}P isotope with a specific activity of 5 mCi provided by Center for Application of Isotope and Radiation – National Nuclear Energy Agency, Jakarta, Indonesia.

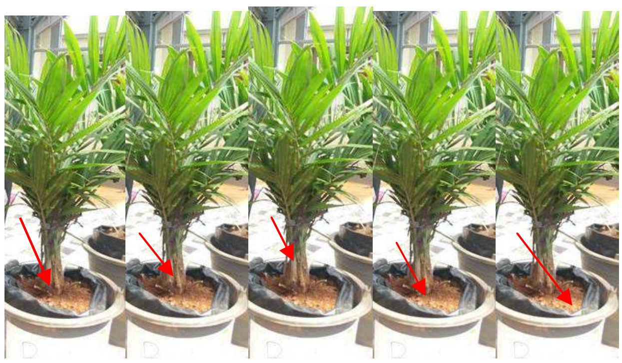
B. Research Method

This pot experiment used a randomized complete block design consisting of five injection point treatments of fertilizer labeled ^{32}P isotope, namely $KH_2{}^{32}PO_4$ with two replications. Each replication used one seedling [21]. Fertilization is done by injection through (1) lower, (2) middle, (3) upper leaf axil, (4). soil with a distance of 5 cm from the trunk, and (5) at the base of the trunk. The experiment was carried out in a greenhouse.

The indirect isotope method with A-value was applied in tracing techniques using ^{32}P in the form of carrier-free $KH_2{}^{32}PO_4$ solution [13]. That was, isotope as a tracer is combined into fertilizer material or other P sources then the amount of tracer absorbed by the plant is measured direct *^{32}P*. About A-value method, see Ref. [21] for detail explanation.

C. Aplication and Analysis

There were ten pot of one-year-old oil palm seedlings arranged in a greenhouse. Five plants took at replication 1 and Five more took as replication 2. Radioisotope ^{32}P was applied by injecting a 10 ml $KH_2{}^{32}PO_4$ solution into a leaf axil with a specific activity of 5 mCi or activity type 220 g / uCi (microCurrie). This means that there are 220 g of ^{32}P in each microCurrie radioactive activity of $KH_2{}^{32}PO_4$ solution [11]. The first seedling is injected with $KH_2{}^{32}PO_4$ solution at a lower point of leaf axil. The second to fifth plant seedlings were consecutively injected at the injection point in the middle and upper leaf axil, and in the soil at a 5cm distance from trunk and at the base of the stem or trunk. Each seedling was injected with fertilizer solution as much as 10 ml and repeated twice. The injection point of the solution labeled ^{32}P is illustrated in Figure 1.



1 2 3 4 5

1. Lowest leaf axil
2. Middle leaf axil
3. Upper leaf axil
4. Base of trunk
5. 5 cm from trunk

Fig. 1. The place of oil palm seedlings leaf axil as the injection point of $KH_2{}^{32}PO^4$ solution.

Five days after the treatment seedlings were dismantled to be counted of ^{32}P at each treatment. Plants are dismantled by unloading until to the roots and then separated between roots and canopy. Furthermore, the dismantled plants were smashed at 500°C for 5 hours, then cleaned using HCl 2N solution. ^{32}P activity was counted/chopped with a liquid scintillation counter (LSC) using Cherenkov calculation [11].

D. Data collection

Data collected was the average of two replications. Data obtained from LSC (Liquid Scintillation Counter) was in units of cpm (counts per minute) which were then transformed into units of dpm (disintegration per minute) by dividing cpm by efficiency of LSC; dpm = cpm / efficiency [11]. The efficiency of LSC with Cherencov counting is 40% = 0.4. To determain the initial ^{32}P uptake by the plants, the dpm is transformed to dpm* by dividing the original dpm by the decay factor as shown by Annunziata cit. Citraresmini [11],[23].

E. Data Analysis

The collected data were analyzed with analysis of variance at a 5% confidence level. If there was a significant effect then is continued with the Duncan's multiple range test at 5% significance level to see the difference between treatments. The differences of the initialy and end caounted were analysed using the t test.

III. Result and Discussion

The results of the ^{32}P count at the five injection points of the solution were listed in Table1, while the dynamics of the counting were illustrated in Figure 2 to 6. Generally it can be seen at which point the solution was injected with ^{32}P count in the plant parts showing high at the beginning and decreasing at the end. This means that nutrients have been distributed from the injection site to other parts of the plant.

Figure 2 shows the ^{32}P dynamics data at the lower axil injection point. The highest ^{32}P uptake at the start of the injection was seen at the axil injection point 1. The position of the leaf axil 1 was the first axil near the stem. Therefore the second highest ^{32}P count is towards the trunk, and spreads upwards to the second axil which is higher (toward the canopy) than the axil 1. The ^{32}P distribution then went to the upper leaf axil, which is higher than the second one. Then the ^{32}P distribution also reached the root. When compared to ^{32}P counted data in the root section with the canopy section it was seen that the pattern of ^{32}P-distribution was more towards the canopy before heading towards the root. This condition occurs when ^{32}P is injected in the lower leaf axil.

Nutrient transportation in the phloem of the stem is caused by the compressive force of the root and osmotic potential gradient. Nutrient transportation to the bottom of the stem, due to gravity in the phloem. Oil palm is a monocotyledonous plant, including the palmae family. The location of the xylem and phloem are close and scattered, so that injection piercing of both and results ^{32}P is also spread up and down [18],[24].

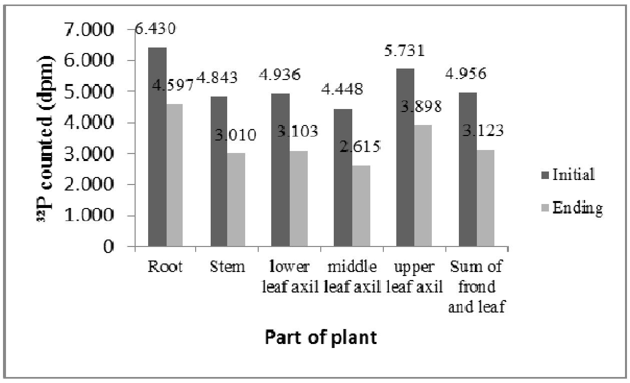


Fig. 3. The dynamics of ^{32}P counted in some parts of oil palm seedlings at the injection point of $KH_2{}^{32}PO_4$ solution in lthe middle leaf axil

At the middle axil injection point, the highest ^{32}P uptake was seen at the root, while the middle axil itself which was the injection point had the lowest ^{32}P counted value. This condition illustrateds that ^{32}P spreads rapidly after being injected at the middle axil point and accumulates a lot at the root, (Figure 3). When interpreted based on the principle of isotope labeling, high counted values indicate low isotope dilution rates [13, 23]. In the soil-plant relationship, the isotope dilucent factor in the soil and plant body is the presence of nutrients [9].

^{32}P's behavior in this condition shows a high ^{32}P dilution rate at the injection point in the middle leaf axil. This means that at the injection point there was a high nutrient content. It was suspected that the nutrient distribution is very good at the midpoint axil. The highest ^{32}P counted value at the root represents a low nutrient content in the root, because it was actively distributed to the top of the plant [11].

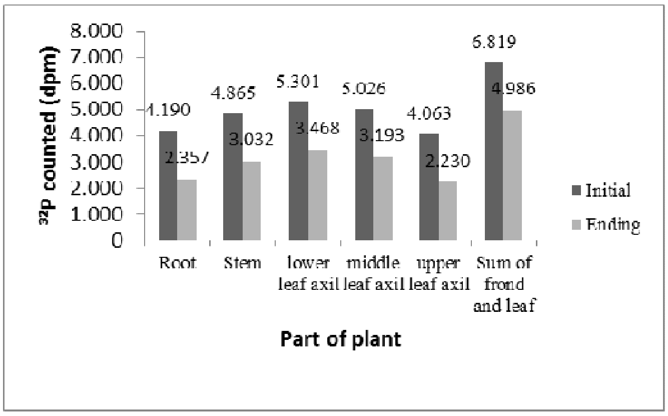


Fig. 2 .The dynamics of ^{32}P counted in some parts of oil palm seedlings at the injection point of $KH_2{}^{32}PO_4$ solution in lthe lowest leaf axil

TABLE I

COUNTED OF ^{32}P IN MANY PART OF PLANT AT FIVE INJECTION POINT OF $KH_2{}^{32}PO_4$ SOLUTION

Counted (dpm) at a part of	root		stem		Upper leaf axil		Middle leaf axil		Lowest leaf axil		Sum of frond and leaf	
Point of injection	Initial	Ending	Initial	Ending	Initial	Ending	Initial	Ending	Initial	Ending	Initial	Ending
Upper leaf axil	4.222a^{p}	2.389b^{q}	4.938d^{p}	3.105d^{q}	5.407f^{p}	3.574g^{q}	4.811i^{p}	2.978j^{q}	4.459l^{p}	2.626l^{q}	4.755n^{p}	2,922n^{q}
Middle leaf axil	6.430a^{p}	4.597a^{q}	4.843d^{p}	3.010d^{q}	4.936f^{p}	3.103g^{q}	4.448i^{p}	2.615j^{q}	5.731k^{p}	3.898k^{q}	4.956n^{p}	3,123n^{q}
Lowest leaf axil	4.190a^{p}	2.357b^{q}	4.865d^{p}	3.032d^{q}	5.301f^{p}	3.468g^{q}	5.026i^{p}	3.193i^{q}	4.063l^{p}	2.230l^{q}	6.819m^{p}	4,986m^{q}
At the soil 5 cm from the trunk	5.094a^{p}	3.261c^{q}	4.342d^{p}	2.509e^{q}	4.030f^{p}	2.197h^{q}	4.680i^{p}	2.847j^{q}	4.374l^{p}	2.541l^{q}	6.611m^{p}	4,778m^{q}
Base of trunk	5.024a^{p}	3.191c^{q}	4.618d^{p}	2.785e^{q}	6.622g^{p}	4.789f^{q}	4.921i^{p}	3.088i^{q}	4.908l^{p}	3.075l^{q}	4.284n^{p}	2,451o^{q}

Note: the mean number followed by the same subscript shows no difference in counts between injection points according to the DMRT level of 5%, while the same superscript shows no difference in counting at the initial and at the ending of the counted according to the t test at the 5% level

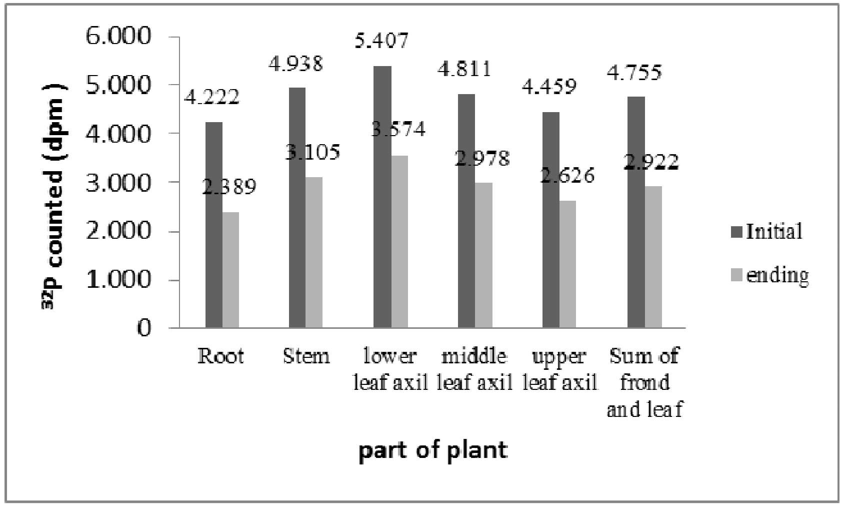


Fig.4. The dynamics of ^{32}P counted in some parts of oil palm seedlings at the injection point of $KH_2{}^{32}PO_4$ solution in lthe upper leaf axil

Data on the dynamics of ^{32}P at the upper of leaf axil injection point showed, the highest ^{32}P uptake at the start of the injection was seen at the lower injection point. The^{32}P distribution then leads to the stem, roots and finally to the upper leaf axil. However, when comparing the ^{32}P counts in he root, ^{32}P distribution was higher in the canopy (Figure 4).

At the injection point of soil ^{32}P radioisotope was incubated into the soil at a distance of 5 cm from the stem (trunk). The counted number shows that the P-^{32} is distributed up to the injection point of the uppermost leaf axil located at the top of the plant (Figure 5). The low value of counted P-^{32} at the root indicated a high level of dilution by nutrient elements in the roots, stems, lower and middle axil. At the top injection point, it is assumed that nutrient elements have not reached this part, so that the dilution level of P-^{32} isotope is very low which results in high counted value in this section. According to Ref. [19], the dilution of the ^{32}P radioactive element is influenced by the P nutrient content in the part of the plant where the element is given. If there are a lot of nutrients in that part, there will be fast ^{32}P dilution so that this isotope is distributed quickly as well as marked by the low value of the counted in that place. Ref. [20] also mentioned that the status of P plants and leaf physiology influences the uptake and translocation of P nutrients in fertilization.

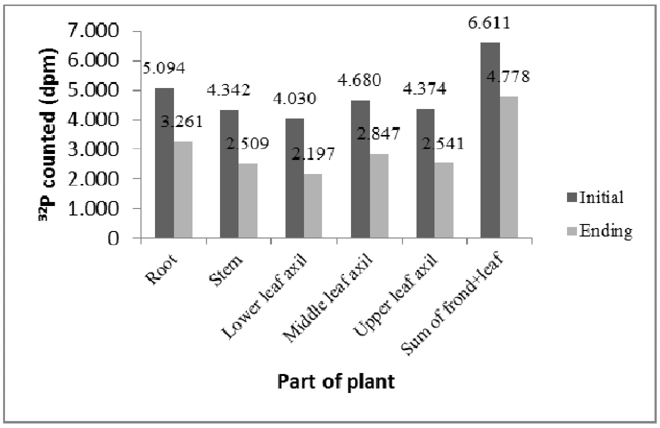


Fig. 5. The dynamics of ^{32}P counted in some parts of oil palm seedlings at the injection point of $KH_2{}^{32}PO_4$ solution at soil 5 cm distance from root zone

At the last treatment, radioisotope P-^{32} was incubated into the base of trunk or stem. The counted number showed that the radioisotope wass distributed to the farthest injection point, which was the upper leaf axil. The high number of counted at the lowest axil injection point and the low number of counted on the top axil indicate that nutrient distribution has taken place from the bottom to the top of the plant thereby diluting the P-^{32} radioisotope in plant parts (Figure 6).

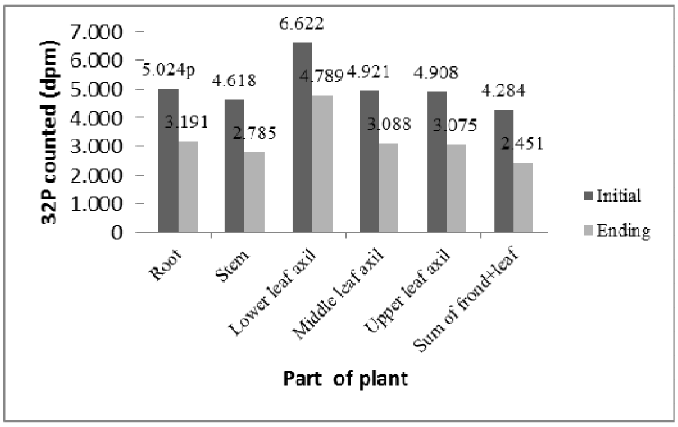


Fig. 6 . The dynamics of ^{32}P counted in some parts of oil palm seedlings at the injection point of $KH_2{}^{32}PO_4$ solution at the base of trunk

IV. Conclusion

It can be concluded that ^{32}P radioisotopes can trace the distribution of nutrients in oil palm plant parts, wherever radioisotope is given. Provision of radioisotopes through the leaf axil shows the most satisfying results, where most P nutrients are distributed towards the canopy of oil palm seedlings. Phosphorus nutrient can be given through any leaf axil and can be distributed well to the canopy of plants. ^{32}P nutrient translocation from the point of administration was influenced by the nutrient contents in the plants' organs and in the soils. This needs to be considered later in the fertilizer application of oil palm through the leaf axil.

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