Development of effective propagation techniques for Elabatu (*Solanum melongena* var. insanum)

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Abstract

A series of pot experiments were conducted at the Faculty of Agriculture, University of Ruhuna to develop effective seed and vegetative propagation techniques for the preservation and multiplication of Elabatu (*Solanum melongena* var. insanum), identified as an endangered plant.
For seed propagation studies, different levels of Nitric acid (HNO_3) (i.e. 20%, 30%, 40%) and Gibberellic Acid (GA) (i.e. 50 µmol, 100 µmol, 150 µmol and 200 µmol) combined with two soaking periods, 12 and 24 hours, on seed germination were tested. For the vegetative propagation, effects of maturity of cuttings (i.e. soft wood, semi-hard wood and hard-wood) and different frequencies of watering, on shoot length, root length and dry matter yield of plants were studied.
Percentage germination of seeds treated with HNO_3 increased significantly irrespective of dipping periods, compared to the control treatment. The highest rate was recorded for the treatment with 30% of HNO_3 dipping. Germination % of seeds was considerably reduced with increasing period of soaking time from 12 to 24 hrs. Seed germination also decreased with increasing period of storage and the highest germination was recorded for fresh seeds, soon after extraction. Among different levels of GA treatments, significantly higher seed germination was recorded at 100 µmol.
Results revealed that with maturity of cuttings, growth parameters (i.e. number of leaves, shoot height, root length and dry matter yield of plants) tend to decrease. Also numbers of leaves, shoot height, root length and dry matter yield of plants decreased significantly with increasing frequency of watering.
From the results, it can be concluded that seeds treated with 20% HNO_3 and 20 µmol GA enhanced seed germination, while germination was low under normal conditions. Among different types of cuttings (i.e. soft- wood, semi-hard wood and hard-wood), soft-wood cuttings were more suitable for vegetative propagation than other types.

Introduction

In Sri Lanka about 550 flowering plants have been identified as medicinal plants, of which several species have been identified as endangered plants. From biodiversity viewpoint there is a felt need for conservation of all endangered plant species, for future use. "Elabatu" (*Solanum melongena* var. insanum/*Solanum insanum*) is one such species, identified as an endangered plant. Elabatu belongs to the family *Solanaceae* and similar to the eggplant. Elabatu is found in the plains of India and neighbouring countries growing under semi-wild state around villages. *S. insanum* occurs in wild or semi-wild state with high prickliness and small (2.5cm diam.) oval or spherical, often white, inedible fruits; *S. melongena* on the other hand, is the cultivated form, often not prickly and has large coloured edible fruits of variously shapes ((Roxburg 1832, Prain 1903, Duthie 1911 and Gamble 1921). The interrelationship and the taxonomic status of *S. insanum* are still not fully understood. Hepper (1987) in his enumeration of *Solanaceae* from Sri Lanka mentioned the polymorphism prevalent in *S. insanum* populations. Neither of the authors, however, provided significant morphological features of the Elabatu. Elabatu, which is grown in many parts of Sri Lanka, shows variable characters and true Elabatu plant populations are dwindling from its natural habitats. On the other hand, most people describe Elabatu as "Thalanabatu". *S. melongena and S. insanum* are highly diverse species although no distinction has been made taxonomically (Hepper 1987).
Elabatu is a highly cross-pollinated plant and does not produce true to type plants. Due to the dwindling populations of elabatu from its natural habitat, it would be necessary to select and identify pure Elabatu plants. Once this process is completed, it would be necessary to develop an effective vegetative propagation technique for rapid multiplication and produce a large population of pure Elabatu plants. Further multiplication could be made by inducing self-pollination on this population and through seed propagation.
Seed germination of elabatu is very low, may be due to dormancy of seeds and there is hardly any literature available on possible seed treatments to break the seed dormancy of elabatu. But there are

several recommended treatments for breaking the seed dormancy of some other crops. For example, Tomer *et al.* (1997) standardized several dormancy breaking treatments such as predrying (400C), hot water treatment (800C) for 24 hrs. for tree seeds), scarification with sand paper, conc. H_2SO_4 treatment for 60 and 120 seconds, KNO3 (0.02%), Ethanol (5.50 ppm), GA3 (300-500 ppm), HNO3 (0.3 N), prechilling, low moisture, pre washing 30-45 minutes in running water, stratification, alternating wetting and drying and soaking in water etc. were applied. The dormancy breaking treatments were applied as per type of dormancy, kind of species/crops/trees etc. The same types of treatments have been recommended by ISTA (1985), ASOA; Seed Testing Manual (Chalam *et al.* 1967), Puri and Khosla 1993 and Verma *et al.* 1990.

A series of preliminary experiment was conducted using all seed treatments mentioned above but here we discuss a few effective seed treatments only.

The main objective of this study is to develop "effective propagation techniques for Elabatu" for large scale multiplication and cultivation as a medicinal plant.

Materials and methods

A series of pot experiments on seed and vegetative propagation were carried out at the Faculty of Agriculture, University of Ruhuna, Kamburupitiya during the period - April 2000 - August 2001. All experiments were set up following the Complete Randomized Design with three replications.

Seed propagation

Two separate experiments were conducted on seed propagation. Seeds of Elabatu were collected from mature fruits and washed thoroughly for about 30 minutes. Fresh seeds were dried in the shade for a week and stored in a dry, cool place until the commencement of experiments.

In these experiments, effect of different concentrations of Nitric Acid (Experiment 1) and Gibberellic Acid (Experiment 2) on seed germination was tested. Seeds, stored for different periods (fresh seeds, 2, 4, 8, 12, 16 and 20 weeks of storage) (Experiment 3) were used for the experiments. All seeds were allowed to soak for 12 hours and 24 hours in tap water. Soaked seeds were treated with either different concentrations of Nitric Acid (i.e. 20%, 30%, and 40%) for 1 and 2 minutes (Experiment 1) and or different concentrations of Gibberellic Acid (i.e. 50µmol, 100µmol, 150µmol and 200µmol) (Experiment 2). Seeds in all treatments were placed on petry dishes lined with wet filter papers at the bottom and covered with lids. Each replicate had 100 seeds. Seeds were allowed to germinate for a period of six weeks. The filter papers were kept moist continuously by adding small quantity of tap water and kept in a laboratory.

Vegetative propagation

Few elabatu plants were identified in a natural forest at Anuradhapura, North-Central Province of Sri Lanka and cuttings taken from these plants were multiplied vegetatively. After getting enough planting materials as mother plants vegetatively, vegetative propagation studies were started. Three types of cuttings (i.e. hard wood, semi-hard wood and soft-wood) were used for the experiment. A well matured, gray coloured pencil thickness cuttings were taken as hard-wood cuttings. The green coloured middle part of the stem was used as semi-hard wood cuttings and top most part of the stem taken as soft-wood cuttings. The length of the cuttings was about 15-20cm. All leaves were removed from cuttings and dipped in a commercially available hormonal solution of Clonex (i.e. IBA) for about 2 minutes. Treated cuttings were planted in polybags, filled with potting mixture of 1:1:1 - top soil, sand and compost. Just after planting, plants were watered and watering was continued daily for about 10 days and thereafter watering was done according to assigned treatments (i.e. daily, once in 2 days, once in 3 days and once in 4 days). Number of leaves, plant height, root length and dry weights of plants were recorded 8 weeks after planting.

Results and Discussion

Seed Propagation

Seed germination increased significantly in response to HNO3 treatment irrespective of dipping periods of 1 and 2 minutes, when compared to the control treatment. The highest germination rate was recorded at 30% level. Chalam *et al.* 1967 reported that seed dormancy of cereals and oil seeds can be removed by

HNO_3 (0.3N). Even though the trend was similar, germination was reduced considerably when soaking period increased from 12 hrs. to 24 hrs (Figure 1).

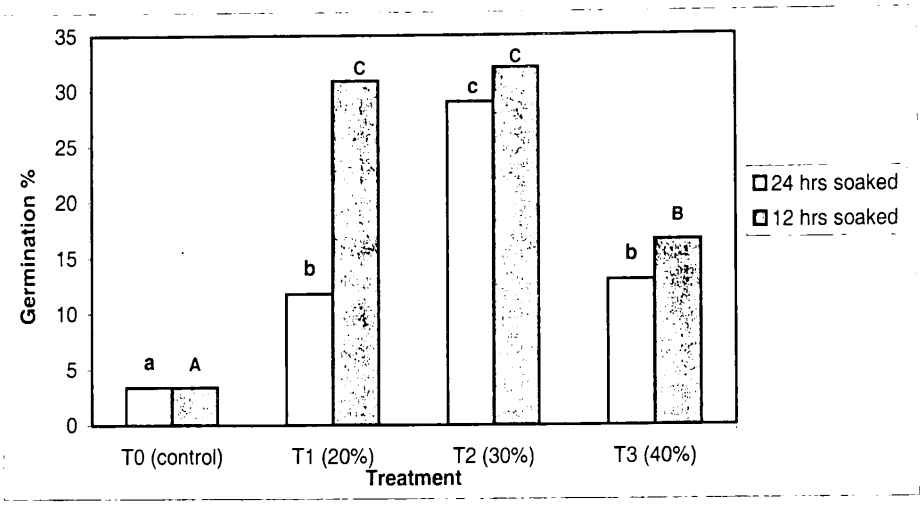

Figure 1. Effect of different concentrations of HNO_3 acid and different soaking periods on seed germination

Means with the same letter on the bar are not significantly different at P≤ 0.05.

The highest germination was recorded when fresh seeds were used just after the extraction, irrespective of the soaking period (12 and 24 hrs.) over all other treatments. Seed germination decreased with increasing period of storage and it was almost negligible 4-5 months after extraction of seeds (Figure 2). The viability of seeds decreased with increasing period of storage and that may be the reason for decreasing germination when seeds are stored for a long period of time.

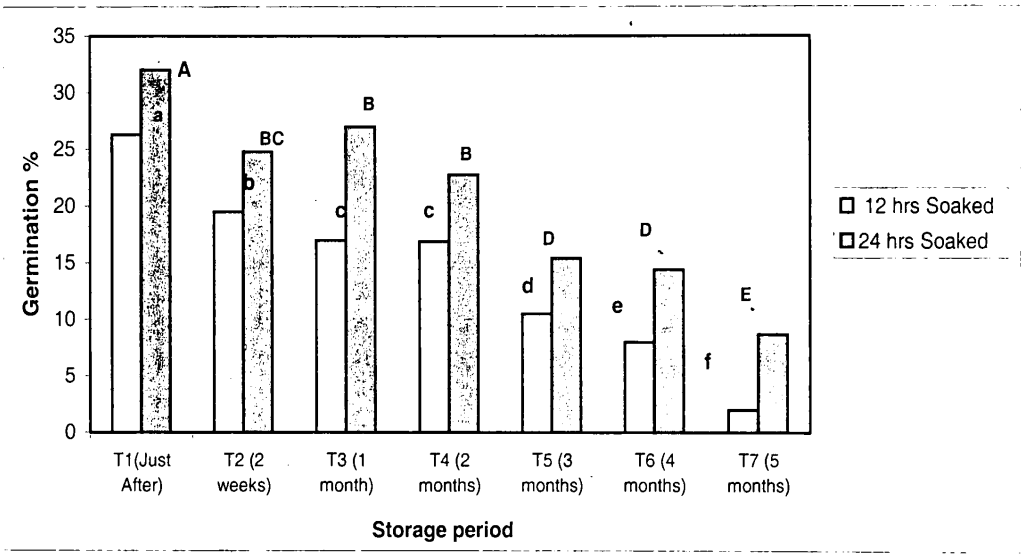

Figure 2. Effect of different storage and soaking time on seed germination

Means with the same letter on the bar are not significantly different at P ≤ 0.05.

Since seed germination decreased with increasing periods of soaking from 12 hrs. to 24 hrs., and highest germination was recorded in fresh seeds, we used only fresh seeds, just after extracted and soaked only for 12 hrs. Seed germination was significantly higher in all treatments when compared to the control. Even though seed germination between 100 µmol and 200 µmol of GA was not significantly different, highest germination was recorded at 100 µmol level (Figure 3). Chalam *et al.* (1967) and Puri and Khosla (1993) reported similar results in cereal seeds and oil seeds that dormancy can be broken by applying Gibberellic Acid, 300-500 ppm.

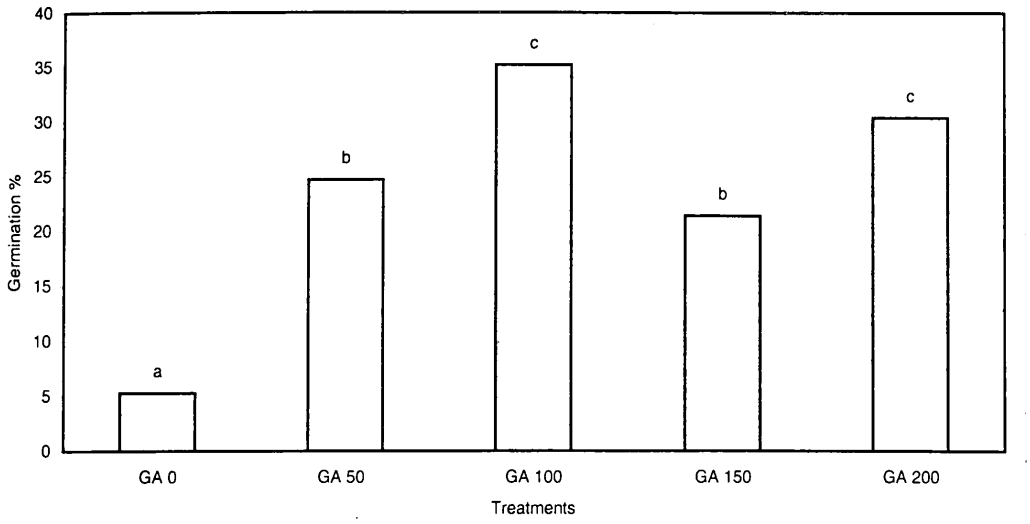

Figure 3. Effect of different concentrations of Gibberalic acid on seed germination
Means with the same letter on the bar are not significantly different at $P \leq 0.05$.

Vegetative propagation

Results revealed that soft-wood cuttings are more suitable for vegetative propagation than other two types. In regard to the dry matter yield, it could be argued that hard wood cuttings gave the highest dry matter yield mainly because of higher initial weight of hard-wood cuttings at planting. Otherwise there was no significant difference in other growth parameters (i.e. number of leaves, shoot height and root length etc.) in response to stage of maturity of cuttings (Figure 4).

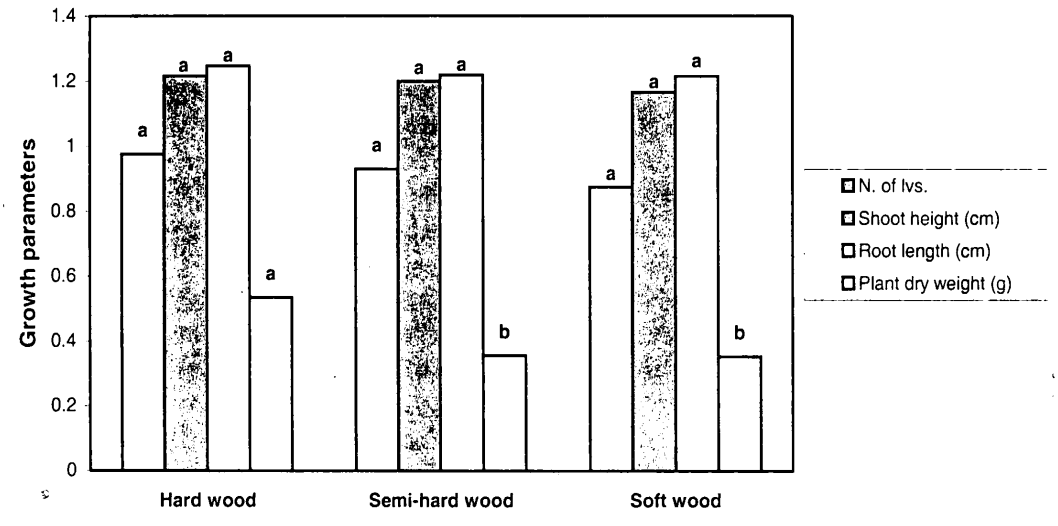

Figure 4. Effect of maturity of cuttings on growth parameters of Elabatu

Number of leaves, shoot height, root length and dry matter yields of plants significantly decreased by increasing the frequency of watering. The highest values of all the above parameters were obtained from cuttings watered daily, followed by once in 2 days, once in 3 days and once in 4 days (Figure 5).

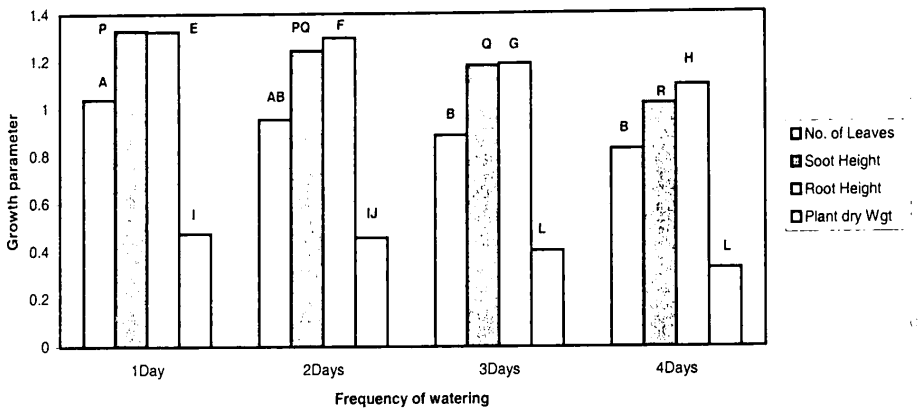

Figure 5. Effect of different frequencies of watering on growth parameters of Elabatu

Means with the same letter on the bar are not significantly different at $P \leq 0.05$.

Conclusions

Fresh seeds, immediately after extraction, treated with 30% HNO3 and 200 µmol GA could be recommended as treatments induced higher percentage of germination. All cuttings (soft, semi-hard and hard-wood) types could be recommended for vegetative propagation, but soft-wood cuttings were better than other types. The higher growth performance can be obtained by watering plants daily.

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Dissolution of Eppawala phosphate rock in low country Ultisols as influenced by rate and method of application

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Abstract

The phosphorus (P) fertiliser requirement of tea is low (below 15 kg ha^{-1} yr^{-1}), despite high P fixing capacity of Ultisols in tea lands. Locally available, low cost, but sparingly soluble Eppawala Rock Phosphate (EPR) has been recommended as a source of P fertiliser for tea in Sri Lanka. However, no information is available on the changes of EPR in tea soils, under field conditions. The objective of this research was to study the effects of P rates *and method of application on EPR dissolution in an acid tea soil.*

The soil belongs to the Rhodustult (Red Yellow Podzolic soil – older Sri Lankan classification system) collected from a long–term phosphate fertiliser field trial started in 1993 located at field No.7, lower division of Walahanduwa Estate, in Galle. The trial comprised of annual application of P fertiliser treatments involving six rates i.e. 20, 40, 60, 80, 100 and 120 kg P_2O_5 $ha^{-1}yr^{-1}$ and a control. P fertiliser was applied in the form of EPR in all treatments using two methods i.e. broadcasting on the soil surface and incorporation to a depth of 15-20 cm. Nitrogen and potassium fertilisers were applied at the rate equivalent of 240 kg N $ha^{-1}yr^{-1}$ and 120 kg K_2O $ha^{-1}yr^{-1}$ as Urea and Muriate of Potash, respectively. Treatments were arranged in a RCBD with three replicates.

Results showed that dissolution of EPR in soil was higher when applied to the soil surface by broadcasting than incorporation. In both methods, dissolved P was greater at lower rates of P application (20 kg P_2O_5) than higher rates. More than 50% of P in EPR was dissolved when broadcast, while release of P was relatively lower when incorporated. Therefore, surface application of EPR recommended for the tea lands is more preferred than that of incorporation.

Key words: Eppawala Phosphate Rock, Ultisols, Tea, P dissolution, Broadcasting, Incorporation

Introduction

The Phosphorus (P) nutrition of tea plants has received less attention in the past than nitrogen (N) nutrition. The response to applied phosphate fertilisers has been far less marked than response to N mainly because most P studies have been conducted on fertile soils or soils in which P fertilisers have been applied before (Zoysa, 2000). However, it is well known that in most tea soils frequently present problems limit tea production due to conditions associated with high soil acidity. Therefore, P availability in acid soils has become a subject of wider agricultural interest.

Tea soils are highly acidic (pH in water < 5.5) and tea growing areas receive considerably high rainfall (> 2000 mm yr^{-1}). Phosphate Rocks (PR) when applied to these soils are expected to dissolve and supply adequate amounts of P to plants (Zoysa *et al.* 1998). Therefore, direct application of finely ground, locally available PR, may be an economically attractive alternative to the use of more expensive imported soluble P fertilizers.

In mid 1970s, a large PR deposit estimated to be over 40 million metric tones (Jayawardana 1976) was discovered in Eppawala (North Central Province). PR is now recommended as a P fertiliser for direct application to several perennial crops including mature tea in Sri Lanka (Dahanayake *et al.* 1995).

However, published information on the chemical reactions and transformations of applied P fertilisers in tea soils under field conditions is scanty. Therefore, research was undertaken to study the optimum rate and effective method of application of Eppawala Phosphate Rock (EPR) dissolution in an acid tea soil.

Materialas and Methods

Soil analysis

The soil used belongs to Red Yellow Podzolic great soil group (De Alwis and Panabokke 1972) classified as Hapludults according to the US soil taxonomy (Soil Survey Staff 1996). Soil samples were collected from a long-term phosphate fertiliser field trial located at field No.7, Lower Division,

Walahanduwa Estate at Galle. The trial was started in 1994 and the field is grown with tea clone TRI 2025. Important physical and chemical characteristics of soil at the site are given in Table 2.1

Table 2.1 Physico-chemical characteristics of soil.

Soil characteristics	Unit	Value
Sand	%	64
Silt	%	16
Clay	%	20
Soil pH	water	4.76
	$CaCl_2$	4.12
Organic C	%	0.5
Total N	%	0.21
Borax-P	$\mu g\ g^{-1}$ soil	9.98
Resin-P	$\mu g\ g^{-1}$ soil	2.79
Bray and Kurtz-P	$\mu g\ g^{-1}$ soil	1.94
Ex. K	$\mu g\ g^{-1}$ soil	83.3
Ex. Na	$\mu g\ g^{-1}$ soil	137.5
Ex. Ca	$\mu g\ g^{-1}$ soil	157
Ex. Mg	$\mu g\ g^{-1}$ soil	83.3
CEC	meq per 100 g soil	12.10

Treatment and experimental design

There were six rates of P including 20, 40, 60, 80, 100, 120 kg P_2O_5 ha^{-1} yr^{-1} and a control. Different P fertiliser treatments were applied using two methods viz. broadcasting on the soil surface and soil incorporation to a depth of 15-20 cm. Each treatment was replicated thrice and treatments were arranged in a randomized completely block design. Each experimental plot consists of 38 to 42 tea bushes. Fertiliser treatments were applied to the experimental plots four times a year, leaving a three month interval. Nitrogen (N) and potassium (K) were applied to the plots at the rate of 360 and 180 kg ha^{-1} yr^{-1}, respectively.

Soil sampling

Representative soil samples were drawn randomly from 0 -`20 cm depth after removing the surface litter of plots. Soils were air-dried and crushed with a rubber tipped pestle and sieved through 2 mm mesh.

Chemical analysis

The amount of EPR dissolution in soil was determined according to the method of Tambunan et al. (1993). Exchangeable cations were extracted by 1 M NH_4Cl buffered at pH 7.0 (Blackmore *et al.* 1987). Soil Organic C was determined by the method of Walkley and Black (1934). Soil pH was measured using deionized water (10 g soil : 25 cm^3 water) and 0.01 $CaCl_2$ (10g soil : 25 cm^3 0.01 $CaCl_2$) using a pH meter.

Statistical analysis

The statistical analyses of data were performed using SAS programe (SAS 1987).

Results and Discussion

The concentration of undissolved-P recovered from P-dissolution was high in both methods of application for all treatments (Figure 1). The recovery of P increased with the rate of P applied to the soil. Repeated annual application of EPR for eight years resulted in an accumulation of considerable amount of EPR left in the soil undissolved. The accumulated EPR did not transformed in to other P-forms and remained as apatite P. It could be seen that the method of EPR application exerted a strong influence on the dissolution of EPR. When EPR was broadcast, the concentration of recovered EPR was lower, indicating that more EPR was dissolved. On the other hand, incorporation of EPR caused more recovery of EPR in the soil, indicating less dissolution in the soil.

Using results of undissolved P, the amount of dissolved-P (as P%) from applied EPR during eight years was estimated and results are shown in Figure 2. Dissolution of EPR was higher in all treatments when applied on the soil surface by broadcasting than incorporation to a depth of 15 - 20 cm. In both methods of application, amount of dissolved P being greater at lower levels of P (< 20 kg P ha^{-1} yr^{-1}) than at higher rates. More than 40% of applied P was dissolved in all the treatments. The possible reason is that at lower rates of EPR, there was proportionately higher acidity to enhance EPR dissolution and also more sinks for removal of dissolved products P, Ca, and F (Zoysa, 2000). Using glasshouse trials, Zoysa et al. (1999) reported that more than 50% of P from EPR was dissolved in the presence of tea plants in 10 months, at P rates below 20 kg P ha^{-1} in acid tea soils. Tambunan (1992) also observed in field trials on Ultisols of Indonesia under *Calopogonium*, the dissolution of NCPR (North Carolina Phosphate Rock) and MPR (Moroccan Phosphate Rock) added at the rate of 80 kg P ha^{-1} increased with the increase in contact period of PR and soil. For example, after 180, 360 and 545 days, dissolution of NCPR was 40, 82 and 98% respectively. EPR is a non-reactive phosphate rock and according to classification of PR based on their chemical reactivity (< 14% total P in EPR is soluble in 2% citric acid). Though EPR is sparingly soluble P fertiliser, high soil acidity (pH 4.5) and rainfall (> 2000 mm yr^{-1}) at the site enhanced dissolution of EPR considerably.

The amount of H^+ consumed for dissolving EPR in the soil was estimated using the amount of EPR dissolved and from the relationship that 2 moles of H^+ were consumed for every mole of P dissolved (%P in EPR/atomic weight of P/ 1/ 100/2 = 0.00933 μ mol H^+ μg^{-1} of EPR dissolved) (Table-1). Mineralogical analysis of EPR using XRD showed that EPR has no detectable amounts of free carbonates ($CaCO_3$ or $MgCO_3$) (Tazaki *et al.* 1987); therefore all acids consumed to be due to the reaction of acids with the apatite in EPR.

The H^+ consumption for the dissolution of EPR was lower for lower rates of P application in both methods of P fertilizer application. At higher rates of P application, H^+ consumption was higher. However, it was seen that less H^+ ions were required for the dissolution of EPR when incorporated than surface application. When EPR was incorporated to the soil, EPR gets concentrated as pockets or bands so that the dissolved products could not diffuse out of the reaction sites and accumulated around the particles. Therefore less acidity could penetrate in to the reaction site so that dissolution could not proceed. On the contrary, when EPR was applied to the soil surface, the dissolved products of EPR could move away from the reaction site along with percolating water and runoff etc. Hence more acidity could be expected for the dissolution of EPR with surface application.

Conclusion

The dissolution of EPR was higher when applied broadcast than incorporation to a depth of 15 – 30 cm. Therefore, deep placement of P in tea plantations cannot be considered as an agronomically feasible practice. In contrast, broadcasting of EPR is practically easy and economically viable due to the low cost of application.

Although EPR is considered as a non-reactive phosphate rock, more than 50% P in EPR was dissolved in the soil and the available acidity in soil was found to be sufficient for this purpose. The amounts of EPR dissolved were lower at higher rates and this could be due to the accumulation of dissolved products at the reaction site.

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