UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF RED-TIP OF PINEAPPLE LEAVES WITH NUTRIENT DEFICIENCY

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By

VIJIANDRAN A/L JUVA RAJAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

August 2007

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LIST OF FIGURES

DEDICATION

This thesis is dedicated to :-

Both **my parents** Mr. and Mrs. Juva Rajah-Punithavathy the souls responsible for what I am today

My wife Christina Vijiandran who had shared all the wonderful and troubled moments of my life since the very first day we met

To the contributors and potential contributors of any kind of knowledge that is beneficial to mankind and is as well in harmony with nature

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Associate Professor Ahmad Husni Mohd. Hanif, PhD,

Faculty: Agriculture

The red-tip of pineapple leaves which affected the Gandul variety was sighted in the early 1990's in Peninsula Plantations, Simpang Renggam, Johore where it has been found that about 10% or more of its leaves starting from the tip had turned red. It was speculated that it was due to sulphur deficiency as a result of the change of nitrogen carriers from ammonium sulphate to urea. This study was carried out with three main objectives: (i) to investigate whether the red-tip problem is related to nutrient deficiency through Missing Element Experimental Technique and thus identify the nutrients that are involved, (ii) to investigate the effect of ammonium sulphate and urea as the nitrogen carriers on the red-tip of pineapple leaves and also the plant performance in terms of vegetative growth and fruit yield at field conditions and (iii) to examine and compare the cell structural

differences between the normal (green) and infected (red-tip) part of the pineapple leaf from cv. Gandul

The Missing Element experiment was carried out in the glasshouse to achieve the first objective while the second objective was achieved through a field experiment in Simpang Renggam, Johore. Further to the above experiments, the red-tip area and the green area of the leaves were also viewed under a Confocal and Transmission Electron Microscope to look for any differences between them at the cellular level which covered the third objective of the study.

Visual observations of the plants from the first experiment could not reproduce the red-tip as found in Simpang Renggam for all treatments including plants treated without the input of sulphur though there was some occurrence of the red-tip in these plants at 150 days after planting (DAP). The deficiency symptom of other eliminated nutrients was mostly similar to that reported in literature and there were no similarities seen compared with the red-tip phenomenon. Vegetative growth variables studied from the destructive sampling carried out at 180 and 300 days after planting showed significant differences between treatments only at the later stage of plant growth, the second stage of sampling. The first experiment indicated that sulphur deficiency was not the primary cause for the occurrence of the red-tip phenomenon in the pineapple leaves.

Plants grown in the field from both treatments, i.e. plants fertilised with ammonium sulphate as nitrogen fertiliser (Treatment 1, T1) and plants fertilised with urea as nitrogen

fertiliser (Treatment 2, T2) did not show any significant differences between them in terms of vegetative growth, plant nutrient uptake, soil nutrient concentrations and yield. There were also no significant differences between T1 and T2 in the red-tip percentage at all sampling periods except one but further soil and plant nutrient analysis did not show any significant differences. Besides that, the uptake trend of the macro and micro nutrients was also not significant for both treatments and the levels had indicated healthy growth for both treatments. The sulphur and other nutrient concentrations for plant and soil were well above the critical point for deficiency to occur and the continuous occurrence of red-tip in plants treated with input of ammonium sulphate gave a doubt whether sulphur is the main cause of the phenomenon. The insignificant yield between the two treatments had further increased the doubt. The second experiment had further strengthened that the deficiency of sulphur is not the cause of the red-tip in the pineapple leaves.

With the above findings, observations carried out using the Confocal Microscope of both the green and red-tip area of the pineapple leaves revealed the presence of some blockages in the phloem cells of the red-tip area which was not found in the green area. Further magnification of this area using the Transmission Electron Microscope revealed presence of some globular structures in the sieve element cells of the red-tip area similar to the infection of Mycoplasma Like Organisms (MLO). The presence of these structures had most likely blocked the movement of nutrients through sieve element cells thus causing the disintegration of chlorophyll to anthocyanin causing the red-tip of pineapple leaves. However further trials are needed to confirm this speculation.

The study had given an understanding that the red-tip phenomenon is not caused by the deficiency of sulphur or any other plant nutrients but due to the presence of the globular structures in leave cells which may be MLO.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ASSOCIATION OF RED-TIP OF PINEAPPLE LEAVES WITH NUTRIENT DEFICIENCY

Oleh

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Fenomena hujung merah daun nanas yang telah menjangkiti varieti Gandul telah ditemui pada awal 1990an di Peninsula Plantations, Simpang Renggam, Johor dimana 10% atau lebih daripada daunya telah berubah menjadi merah bermula dari hujungnya. Spekulasi yang dijangka adalah kekurangan sulfur disebabkan oleh perubahan pembawa nitrogen dari ammonium sulfat kepada urea. Kajian ini telah dijalankan dengan tiga objektif utama iaitu (i) untuk mengkaji sama ada masalah hujung merah daun berkaitan dengan kekurangan nutrien melalui Teknik Eksperimen Pelupusan Nutrien dan seterusnya mengenalpasti nutrien yang mempengaruhinya, (ii) untuk mengkaji kesan ammonium sulfat and urea sebagai pembawa nitrogen pada hujung merah daun nanas serta prestasi tumbuhan dari segi pertumbuhan vegetatif serta hasil dalam keadaan lapangan dan (iii)

untuk mengkaji serta membandingkan perubahan struktur sel diantara bahagian biasa (hijau) dan yang dijangkiti (hujung merah) daun nanas cv. Gandul.

Eksperimen untuk Pelupusan Nutrien telah dijalankan di Rumah Kaca, untuk memenuhi objektif pertama, manakala objektif kedua kajian ini dicapai melalui satu ekperimen di lapangan di Simpang Renggam, Johor. Tambahan kepada kedua-dua eksperimen tersebut, bahagian hujung merah serta bahagian hijau daun nanas telah diperhatikan di bawah Mikroskop 'Confocal' dan Transmisi Elektron untuk melihat perbezaan diantara kedua-dua bahagian tersebut diperingkat selular untuk memenuhi objektif ketiga.

Pemerhatian visual pokok-pokok yang ditanam untuk eksperimen pertama, tidak boleh menghasilkan hujung merah seperti yang diperhatikan di Simpang Renggam terutamanya pokok-pokok yang dirawat tanpa input sulfur meskipun terdapat sedikit pembentukan hujung merah pada pokok-pokok tersebut iaitu 150 hari selepas penanaman. Simptom kekurangan bagi nutrien lain yang diperhatikan telah menunjukan pemerhatian yang sama seperti dalam literatur dan tidak mempunyai persamaan dengan fenomena hujung merah. Parameter-parameter pertumbuhan vegatatif yang dikaji daripada persampelan pemusnahan yang dijalankan pada 180 dan 300 hari selepas penanaman hanya menunjukan perbezaan rawatan yang bererti pada peringkat lewat pertumbuhan tanaman, iaitu pada peringkat persampelan yang kedua. Eksperimen pertama menunjukkan kekurangan sulfur bukan punca utama yang membawa kepada fenomena hujung merah didalam daun nanas.

Pokok-pokok yang ditanam dilapangan daripada kedua-dua rawatan iaitu pokok-pokok yang dibaja dengan ammonium sulfat sebagai baja nitrogen (Rawatan 1, T1) dan pokok-pokok yang dibaja dengan urea sebagai baja nitrogen (Rawatan 2, T2) tidak menunjukkan sebarang perbezaan bererti diantara mereka dari segi pemerhatian pertumbuhan vegetatif, pengambilan nutrien oleh pokok, kepekatan nutrien di dalam tanah serta hasil pengeluaran. Tiada sebarang perbezaan bererti diantara rawatan T1 dan T2 dalam peratus hujung merah yang pada semua tempoh persamplen kecuali satu dimana pemerhatian lanjutan dengan analisis tanah dan nutrien tanaman yang tidak menunjukkan perbezaan yang beerti. Selain itu, tren pengambilan nutrient makro dan mikro juga tidak menunjukkan sebarang perbezaan yang bererti diantara kedua-dua rawatan serta menunjukkan pertumbuhan pokok yang sihat. Tahap kepekatan sulfur dan nutrien lain didalam pokok serta tanah telah melebihi tahap kritikal untuk membolehkan masalah kekurangan nutrien berlaku serta kewujudan hujung merah berterusan pada pokok-pokok yang diberi input ammonium sulfat telah mewujudkan suatu kesangsian sama ada sulfur adalah penyebab utama fenomena tersebut. Hasil pengeluaran yang tidak bererti bagi kedua-dua rawatan tersebut turut menambah kesangsian ini. Eksperimen kedua telah memperkuatkan pendapat bahawa kekurangan sulfur bukan merupakan punca kejadian fenomena hujung merah pada daun nanas.

Pemerhatian dengan Mikroskop ‘Confocal’ untuk kedua-dua bahagian hujung hijau dan merah daun nanas, telah menunjukkan kehadiran sedikit sumbatan di sel-sel floem pada bahagian hujung merah daun dimana sumbatan sebegini tidak wujud dibahagian hijau daun. Pembesaran lanjut bahagian ini dengan menggunakan Mikroskop Transmisi

Elektron pula menunjukkan kehadiran sedikit struktur berbentuk globular di sel-sel elemen penapis pada bahagian hujung merah yang mempunyai persamaan seperti jangkitan Organisma Seperti Mikoplasma (MLO). Kewujudan struktur sebegini diramalkan telah menghalang perjalanan nutrien melalui sel elemen penapis yang seterusnya membawa kepada perubahan klorofil kepada antosianin yang seterusnya menyebabkan hujung merah daun nanas. Walaubagaimanapun kajian yang lebih lanjut diperlukan untuk mengesahkan spekulasi ini.

Kajian ini telah memberi suatu pemahaman bahawa fenomena hujung merah tidak dipengaruhi oleh kekurangan sulfur atau nutrien-nutrien pokok lain tetapi disebabkan oleh kewujudan struktur globular didalam sel-sel daun yang berkemungkinan jangkitan MLO.

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to carry out our experiment in the estate and also for the supply of planting materials and media needed for the study. Their kind hospitality given to us during all our visits to the estate is also deeply appreciated.

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APPROVAL

I certify that an Examination Committee has met on 28 August 2007 to conduct the final examination of Vijiandran a/l Juva Rajah on his Master of Science thesis entitled "Association of Red-tip of Pineapple Leaves with Nutrient Deficiency" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the relevant degree.

Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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VIJIANDRAN JUVA RAJAH

Date: 12 December 2007

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CHAPTER I

INTRODUCTION

Pineapples *(Ananas comosus)*, originated from South America (Othman and Subhadrabanthu, 1995; Barthlomew and Malézieux, 1994), like many other crops in Malaysia, have been domesticated, improved and developed since pre-historic times, often with no records of agronomy (Othman and Subhadrabanthu, 1995). Originated from the Bromiliaceae family, the cultivation of pineapples in Malaysia is rather unique as it is cultivated largely on peat (Ahmed et al., 2002). In Malaysia, the crop is planted in an area of 6400 hectares (2003) for both 'Table' consumption and canning. Seventy eight percent of the total area is under pineapple estates while the balance belongs to small holders. Over the last few years, the production of canned pineapples in Malaysia has decreased. Malaysia was producing 189,700 tonnes of fresh fruit in 1991 at its peak and dropped to 73,500 tonnes (forecast) in 2003. Most of the pineapples were produced by the estates which accounted between 77 to 92 percent of total country production from 1990 to 2003. The decrease in the production is mainly due to the reduction of planting areas especially among the small holders where the area decreased by about 30% over the same period mentioned above (AGRIQUEST 2006).

Pineapple planted in Malaysia serves two main purposes, 'Table' consumption and canning. The Sarawak pineapple, which originated from the Cayenne variety and Morris from the Queen variety are commonly planted for 'Table' consumption while the Gandul and Masmerah from the Spanish variety for canning purposes (Selamat and Ramlah,

1993). The Gandul, which is largely planted in the Johore area, has been selected for its good yielding even though there are several shortcomings (Selamat and Abdul ,1996).

Over the years, numerous researches have been carried on the pineapple fruit and the plant itself with the aim of improving the yield thus increasing the income of farmers and making the industry profitable. In addition to that, various studies have been also been carried out to control diseases and pests infecting the plants such as mealy bug wilt (*Dysmicoccus brevipes*) the common pineapple pest. Recently, a new problem had arisen which has affected the gandul variety called red-tip of pineapple leaves.

The red-tip phenomenon was first sighted in Peninsula Plantations and speculations were that it was caused by nutrient deficiency. It is believed that this phenomenon had caused a reduction in yields which is an effect of nutrient deficiency which led to this study. This study was aimed to look into the red-tip of pineapple leaves in the nutrient point if view with three main objectives, (i) to investigate whether the Red-tip problem is related to nutrient deficiency through Missing Element Experimental Technique, (ii) to investigate the effect of both ammonium sulphate and urea as the nitrogen carriers on the red-tip of pineapple leaves and also plant performance in terms of vegetative growth and fruit yield under field conditions and (iii) To examine and compare the cell structural differences between the normal (green) and infected (red-tip) part of the pineapple leaf from cv. Gandul.

CHAPTER II

REVIEW OF LITERATURE

Background of The Pineapple Industry

Pineapple is one of the 45 genera and 2000 species available in the Bromeliaceae family (Nakasone and Paull, 1998). Originated from South America and has been well developed and now its world trade is dominated by the canned fruit. The world pineapple production fluctuated between 38 000 to 52 000 million standard cases (4,516 pineapples per standard case) from 1990 to 2002. Thailand and Philippines continued to lead the world in total canned pineapple export, accounting for 44.6% and 14.5% respectively (AGRIQUEST, 2006).

Pineapple has been the oldest agricultural export sector in Malaysia but unfortunately it never really took off and had always been a subsidiary crop for the country. The export of this product by Malaysia however decreased significantly from 6% in 1990 (41 300 standard cases) to 2.6% in 2003 which accounts for 38 170 standard cases (AGRIQUEST, 2006). The area under pineapple crop in Malaysia is decreasing steadily in recent years as the growers shift to more profitable and less intensive crops. But the decrease is all among the smallholdings because the nature of the industry makes the estate area more resistant to change, as most estate owners are also owners of the canning factories. Another reason for the drop in the area is clearly seen in the price of pineapple.