UNIVERSITI TEKNOLOGI MARA

IDENTIFICATION OF SOMACLONAL VARIATION USING INTER SIMPLE SEQUENCE REPEATS (ISSR) MARKERS AND OPTIMIZATION OF TYPES OF FERTILIZERS IN ACCLIMATIZATION OF *IN VITRO* REGENERATED PINEAPPLE (*ANANAS COMOSUS* (L.) MERR. VAR. MD2).

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Dissertation submitted in partial fulfilment
of the requirements for the degree of
Master of Science
(Plant Biotechnology)

Faculty of Plantation and Agrotechnology

March 2022

ABSTRACT

Ananas comosus (L.) Merr. is propagated asexually from its crowns, slips, hapas, and suckers using the traditional method. In comparison to traditional propagation, the plant tissue culture approach is used to mass-produce clonal pineapple in a shorter time. However, the existence of variation in clonal seedlings var. MD2 prevented it to be planted in the field. Somaclonal variation was referred to the changes in the electrophoretic profile of accessions belonging to the same botanical variety. Hence, this research aims to identify the effect of different types of plant growth regulators on the formation of somaclonal variation and to identify the existence of somaclonal variation in traditional propagated suckers and *in vitro* plantlets. This research also aims at optimizing the types of fertilizers for the acclimatization of clonal pineapple *ex vitro*. The *in vitro* regeneration was carried out by adding different concentration of 6-Benzylaminopurine (BAP) ranging from 5.0 – 10.0 mg/l in the first stage treatments. Later, the plantlets produced was sub-cultured into a various concentration of Indole-3-Acetic Acid (IAA) ranging from 1.0 – 5.0 mg/l as the second stage of treatments. Inter simple sequence repeats (ISSR) markers were used to assess genetic variation. Genomic DNA samples was extracted from young leaves' traditional propagated suckers (one sample), six (6) samples from the young leaves from the plantlets produced in the first stage, and 30 samples were extracted from the young leaves of the plantlets that had been sub-cultured into second stage. A dendrogram showing genetic distance among pineapple was constructed based on polymorphic bands using the Darwin Software (version 6). For the acclimatization, the treatments were applied on secondary acclimatization with two different types of fertilizers served as treatments. The fertilizers used were compound fertilizers, NPK green with (15:15:15) ratio and controlled-release fertilizer AJIB CRF with NPKMg ratio (10:5:18:3). The planting media for clonal seedlings in secondary acclimatization consisted of topsoil, sand, and compost composition in a ratio of 2:1:2. As a result, 5.0 mg/l BAP produced the best result with the second highest for the mean of leaves number (6.07±0.23), the highest mean for leaf length (cm) and plantlet height (cm) with 3.29±0.10 and 5.57±0.07, respectively. In second stage treatments, 4.0 mg/l IAA produced the highest mean for roots length (cm) with 2.16±0.04 while 5.0 mg/l IAA produced the highest mean for root number with 7.67±0.33. Out of 10 primers screened only six (6) primers (Primer 3, Primer 4, Primer 5, Primer 6, Primer 7, and Primer 9) were amplified and showed 45.85% of polymorphism with 188 bands. A total of 410 DNA fragments amplified, varying in size from less than 3.0kb to 6.0kb. Based on the results from the dendrogram, two major clusters were formed for both stages of treatments when compared to the control treatments. The genetic distance ranging from less than 0.04 to 0.42. Meanwhile, the acclimatization method found that controlled-release fertilizer (AJIB CRF), NPKMg (10:5:18:3) showed about 90% of survival rate. The mean value for the number of leaves and leaves length (cm) in acclimatized clonal pineapple using Controlled-release fertilizer (AJIB CRF) were 5.48±0.96 and 6.00±1.04, respectively. Hence, the use of inter simple sequence repeats (ISSR) markers are suitable to detect somaclonal variation in pineapple. For acclimatization, the used of controlled-released fertilizer (AJIB CRF) optimized the growth performance of clonal pineapple in field.

ACKNOWLEDGEMENT

Firstly, I wish to thank God for giving me the opportunity to embark on my masters and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor Prof Ts Dr Asmah Awal and my co-supervisor Assoc Prof Ts Dr Shamsiah Abdullah.

My appreciation goes to the faculty and all the staffs who provided the facilities and assistance during the whole research. Special thanks to my colleagues and friends for helping me with this project.

Finally, this thesis is dedicated to my father, family, close friends, and myself. Lastly, for the loving memory of my very dear late mother for the vision and determination to educate me. This piece of victory is dedicated to all of you.

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CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Ananas comosus (L.) Merr. or also known as pineapple is a tropical fruit plant that belongs to the Bromeliaceae family and is planted widely as a vital commercial crop in the tropical and sub-tropical countries. Pineapple mature fruits contain sugar, bromelain (a protein-digesting enzyme), citric acid, malic acid, vitamin A, and vitamin B (Joy, 2010). United Nations Conference on Trade and Development (UNCTAD) in 2012 stated that over 20% of the world's production of tropical fruit is pineapple. The pineapple was grown in over 82 countries with estimated 849,840 hectares for the crop alone (Ndugwu, 2014). The global pineapple production in 2019 was estimated to be about 28.18 million metric tons (Jaji et al., 2018). The Food and Agriculture Organization of The United Nations (FAO) in 2013 reported that Costa Rica, Philippines, Brazil, Indonesia, and China are the top five fresh fruit producing countries. On the other hand, there are twelve (12) countries as the top importers of fresh pineapple are United States, France, Japan, Belgium, Italy, Germany, Canada, Spain, England, Korea, Netherlands, and Singapore (Hossain, 2016).

In Malaysia, pineapples are widely planted in Johor and Sarawak. In the year 2014, thousands of pineapples were planted on a total area of 16 101 hectares in Malaysia. The harvested area are 10 785 hectares with the total pineapple production 294 161 metric tonnes (Department of Statistic, 2015). The focus of this study is on the most commercialized pineapple var. MD2. In 2014, MD2's production in Malaysia was 31 317 metric tonnes. National Agrofood Policy had identified pineapple var. MD2 to be a key crop and as a part of Malaysia's 2011-2020 agenda, it was chosen as one of the country's new export goals for pineapple. MD2 cultivar exchanged around 80% of the world trade in pineapples (Ndugwu, 2014). The excellent fruit of MD2 comes with a high amount of juiciness, flavourful, and compromising of Vitamin C, potassium, calcium, crude fibre, and carbohydrates which are all major nutritional components (Hossain, 2016).