GENETIC CHARACTERISATION OF *SOLANUM NIGRUM* AND RELATED SPECIES USING SIMPLE SEQUENCE REPEATS

RWIGI SUSAN WAGIO B.Ed (Science)
Reg No: I56/CE/22421/2010
Signature.....Date.30/7/2014
Department of Biochemistry and Biotechnology.

A Research Proposal Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Biotechnology) in the School of Pure and Applied Sciences of Kenyatta University

Supervisors

Dr. Steven Runo
Department of Biochemistry and Biotechnology.
Kenyatta University.
Signature_____ Date 30/7/2014

Dr. Alice Muchugi
Department of Biochemistry and Biotechnology.
Kenyatta University.
Signature_____ Date 30/7/2014.

Abstract

Solanum nigrum (commonly referred to as black nightshade) and related species are world-wide weeds on arable land, gardens, rubbish pits, soil rich in nitrogen in moderately light and warm situation which occur from sea to montare levels. Human consumption of leaves and fruits as food is wide spread, particularly in Africa and South East Asia. Nightshades have also been used in the field of medicine in the manufacture of analgesics, ointments and vasodilators. In Kenya there are three distinct varieties of black nightshade, grown in high, medium and low altitude areas. Broad leafed nightshades are found throughout Africa. There are several wild species but the most popular species is *Solanum scabrum*. A wild variety *Solanum villosum* has also been identified in a number of regions in Kenya. *Solanum nigrum* and related varieties can be grown in wide range of soil types but do not tolerate drought. Yields range from 20-40 tons per hectare under good management. Many of the species are poisonous giving varying degree of toxicity which may be mildly irritable to causing fatalities with small quantities. In Kenya these species are found extensively around Mt Kenya, Mau forest, Kisii highlands and Kakamega. Farming is not extensive and only few farmers produce the crop. The limited information available on importance and basic aspects of this neglected and underutilized family, such as black nightshade, hinders the development of these species as well as their sustainable conservation. Exisisting knowledge on the genetic potential of these plants are limited and the information regarding the genomic organization of *Solanum nigrum* complex species found in Kenya is scarce. However molecular markers could help us gain a better understanding and the information could improve the genetic analysis to facilitate future breeding programmes. This work targets the use of SSR molecular markers in determining the genetic diversity of both cultivated and wild varieties of black nightshades in selected parts of Kenya. Leaf samples will be collected from five regions and morphological analysis done on leaf shape, colour of the fruits, nature of leaf surface and type of leaf margin. Samples will then be dried in snap-top bag containing silica gel in readiness for DNA extraction using the CTAB protocol. A set of 7 SSR primers as used by Angeline van Biljon et al, 2010 will be used for PCR amplification of the DNA. Final DNA concentration will be confirmed by electrophoresis in 2% agarose gel run at 120v for 40min in a TAE (tris acetate ethlydiquine tetra acetic acid) buffer and visualized with ethidium bromide staining under UV light. Bands in the SSR profiles of each individual plant will be scored as present or absent (1 or 0, respectively) and subjected to sequential clustering algorithm using the Unweighted Pairgroup method with Arithmetic Means (UPGMA).The lengths will be analysed in an automated ABI^{R}310 sequencer (Applied Biosystem) that use gene mapper ^{TM}software. Morphological data will be subjected to analysis of variance (ANOVA) by the GLM procedure of the Statistical Analysis System and correlation coefficient to determine the degree of diversity. The relationships within populations of cultivated and wild types will be generated using phylogenetic analysis package GenAlex 6.3.and presented in a dendogram. Findings from this study will be useful in the improvement of wild black nightshade germplasm to develop suitable plants using Marker Assisted Selection