

INTRODUCTION

1.1 Introduction

Precision agriculture (PA) involves the integration of advanced technology and data management to enhance the efficiency and accuracy of farming practices. The primary objective of incorporating these technologies is to reduce production costs while enabling farmers to make more informed decisions through accurate predictions of future occurrences. One critical application of PA is in the early sex determination of dioecious crops, such as *Cannabis sativa* L. and *Phoenix dactylifera* L. (date palm), where female plants have higher agriculturally valuable.

Cannabis sativa L., an annual dioecious plant from the Cannabaceae family, is cultivated primarily for its medicinal and industrial uses. Male plants produce pollen necessary for reproduction, while unfertilized female plants produce flowers rich in cannabinoids compounds with significant medicinal value. For optimal cannabinoid yield, cannabis farmers aim for only unfertilized female flowers. However, the plant's sex is typically identifiable only 4–8 weeks after planting, which can lead to inefficiencies in resource use. Cannabis utilizes an XY/XX sex determination system (Mandolino et al., 2002), though alternative mechanisms such as an X-to-autosome balance system have also been proposed (Ming et al., 2007, 2011; Punja & Holmes, 2020; Razumova et al., 2016). Previous studies have focused on the development of male-associated DNA markers, such as the male-associated DNA from *Cannabis sativa* (MADC2) region, which provides a reliable genomic signature for male plants (Mandolino et al., 1999, 2002). Implementing molecular techniques to identify such markers in local cannabis cultivars, particularly in Thailand, can enable early selection of female seedlings.

Similarly, *Phoenix dactylifera* L., commonly known as date palm, is a dioecious fruit tree in the Arecaceae family, native to the desert regions of the Middle East. In recent years, it has adapted well to the climate in Thailand, producing high yields and

yields and gaining popularity in for fresh and dried forms due to its sweet taste and nutritional value. Only female date palm trees bear fruit, but determining the plant's sex typically takes 3–7 years, depending on the cultivar (Chao et al., 2007; Wellmann et al., 2007). Date palm propagation methods include seed propagation, offshoot division, and tissue culture. While seed propagation is cost-effective and suitable for large-scale planting, it presents a 50:50 chance of producing male or female plants. This uncertainty leads to high investment risks related to planting, fertilization, irrigation, and maintenance before the plant's sex is known. Although tissue-cultured plants offer a more predictable alternative, they are expensive and may involve varieties not well-suited to local growing conditions Thailand (NSTDA, 2022).

To address these challenges, this research aims to develop a rapid, cost-effective, and user-friendly molecular tool for early sex determination in dioecious crops such as cannabis and date palm. The study focuses on applying Loop-mediated Isothermal Amplification (LAMP), a technique that amplifies DNA at 60–70 °C and can increase target genetic material up to a billion times within an hour (Notomi et al., 2000). The reaction's results can be interpreted visually through a color change, using phenol red as an indicator, which shifts from red/orange to yellow upon successful amplification (Quan-Ying et al., 2023). With its high specificity, sensitivity, simplicity, and affordability, the LAMP-based test kit is suitable for field applications, empowering farmers to make wisely planting decisions without reliance on costly equipment or specialized expertise. The success of this research will significantly reduce production costs, minimize risk, and promote sustainable agricultural practices for Thai farmers and beyond.

1.2 Research Objectives

- 1) To develop simple DNA extraction method for cannabis and date palm leaves
- 2) To develop method for cannabis sex determination
- 3) To develop male date palm specific genetic markers for LAMP reaction
- 4) To develop a suitable reagent for LAMP reactions
- 5) To create a simple date palm sex determination test (kit) for field use

1.3 Scope of Research

DNA extraction methods for both cannabis and date palm were developed to obtain high-quality genetic material for downstream analysis. A PCR-based method was established for sex determination in cannabis. For date palm, the Loop-mediated Isothermal Amplification (LAMP) technique was employed to identify the sex of known samples. Various components of the LAMP reaction, as well as optimal conditions including the lyophilization of reagent components—were optimized. Furthermore, a simple, field-deployable test kit for early sex determination in date palm was developed to support practical applications in agricultural settings.