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Characterization of the complete chloroplast genome of alfalfa (*Medicago sativa*) (Leguminosae)

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1. Introduction

Alfalfa (*Medicago sativa*), a perennial, outcrossing and autotetraploid ($2n=4x=32$) species, is the third most valuable crop in the United States after corn and soybean (Bouton 2007; Zhang et al. 2005). It is the most widely cultivated forage legume throughout the world and has been planted for more than two thousand years in China (Bouton 2007; Zhang et al. 2005). Because it is highly productive, stress tolerant, and a valuable forage crop for livestock, alfalfa is referred to as “the king of forage crops” (Liu et al. 2013).

Chloroplasts, considered to be derived from cyanobacteria through endosymbiosis, are the photosynthetic organelles that provide essential energy for plants and algae (Gray 1989; Howe et al. 2003). This organelle contains chloroplast-specific components and regulates starch storage, sugar synthesis, the production of key cellular materials (several amino acids, vitamins, pigments, and lipids), and sulfur and nitrogen metabolic pathways (Martin et al. 2013). Furthermore, fatty acids are metabolized on the chloroplast membrane and synthesized in the chloroplast stroma or exported to the cytosol for phosphatidic acid (PA) synthesis, which is crucial for phospholipid formation in the endoplasmic reticulum (Joyard et al. 1998).

The availability of the complete chloroplast genome sequence should facilitate the chloroplast transformation technique (Bock 2001). There are several advantages of chloroplast genetic engineering over nuclear transformation, such as high copy number of transgenes. Through this genetic technique, polycistronic RNA giving rise to multiple transgenes may be inserted and expressed effectively in a single transformation event. In addition, chloroplast transformation is not associated with gene silencing and provides the benefit of transgene containment by maternal inheritance (Daniell et al. 2005). Legumes have experienced considerable numbers of chloroplast genomic rearrangements, are second only to grasses in their agricultural and economic value, and contain a great number of important species grown for food, wood, fodder, ornamentals, and raw materials for industry, and are also important for their ecologically vital role in biological nitrogen fixation (Lewis et al. 2005; Jansen et al. 2007). Therefore, it is necessary to the study of the chloroplast genome of alfalfa.

Gene mapping and genomic sequencing have demonstrated that chloroplast genome structure, gene order, and gene content are generally largely conserved among angiosperms (Palmer 1991; Raubeson and Jansen 2005). Most chloroplast genomes are characterized by a quadripartite structure, comprised of two copies of an inverted repeat (IRA and IRB), the large single copy (LSC), and the small single copy (SSC) region (Melodelima and Lobréaux 2013; Jansen et al.

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