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ORIGINAL ARTICLE

Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227 m height

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Abstract Altitudinal gradient-defined specific environmental conditions could lead to genetics and chemical variations among individuals of the same species. By using RAPD, ISSR, GC–MS and HPLC analysis, the genetic and chemical diversity of *Ziziphus spina-christi* plants at various altitudinal gradient namely; Abha (2227.86 m), Dala Valley (1424 m), Rakhma Valley (1000 m), Raheb Valley (505 m) and Al-Marbh (147 m) were estimated. RAPD markers revealed that the highest similarity value (40.22%) was between Raheb Valley and Al-Marbh while the lowest similarity (10.08%) was between Abha and Raheb Valley. Based on ISSR markers the highest similarity value (61.54%) was also between Raheb Valley and Al-Marbh, while the lowest similarity (26.84%) was between Abha and Rakhma Valley. GC–MS results showed the presence of various phytochemical constituents in each population. The dendrogram based on chemical compounds separated the *Z. spina-christi* grown at the highest elevations (Abha) from the populations in lower elevations. HPLC analysis showed that the leaves of *Z. spina-christi* plant contain considerable amount of vitamins including B_1, B_{12}, B_2 and folic acid. In conclusion, there is a close relation between altitudinal gradients, genetic diversity and chemical constituents of the leaves of *Z. spina-christi* plants.

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1. Introduction

Ziziphus spina-christi (L.) Desf. (family, Rhamnaceae), locally known as Sidr, or Nebeq is highly respected by people throughout the Middle East. There are about 50 species distributed in the tropical Asia, Africa and America and in the temperate regions of both hemispheres [1,2]. It was considered

Abbreviations: RAPD, random amplified polymorphic DNA; ISSR, inter-simple sequence repeats markers; GC–MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography

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as one of the few native tree species of Saudi Arabia that is still growing comparatively along with many newly introduced invasive weeds species [3]. It has the ability to grow in drought conditions and to adapt to the different environmental conditions in the Kingdom of Saudi Arabia. In traditional medicine *Ziziphus spina-christi* (Sidr) is a well known source of healthy food and energy. Recently, to analyze and estimate genetic diversity in plant species, a series of molecular markers techniques have been developed, for example, the randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR). The randomly amplified polymorphic DNA (RAPD) is potentially simple, rapid, reliable, effective, no prior knowledge of DNA sequence information is required and considered as one of the most popular DNA-based approaches [4,5]. The ISSR technique is more reproducible than the RAPD technique, since ISSR markers are designed from di- or trinucleotide repeat motifs with a 5′ or 3′ anchoring sequence of one to three nucleotides [6], producing a high degree of polymorphism to distinguish between individuals genetically related, without prior sequence information [7]. ISSR and RAPID techniques were successfully applied to study genetic diversity in *Phoenix dactylifera* L. cultivars [8], *Astragalus oniciformi* populations [9], *Jatropha* species [10] and in many else. Because of the only published results on genetic diversity of *Ziziphus* spp. was on ber of *Z. mauritiana* (Lam.) by Singh et al. [11] and Obeed et al. [12], who used the AFLP technique to study their genetic diversity. Therefore, the aim of our study is the molecular characterization of *Z. spina-christi* using randomly amplified polymorphic DNA (RAPD) technique and inter simple sequence repeat (ISSR) and the impact of altitudinal gradient on the plant genetic diversity.

To investigate the chemicals in various plant groups gas chromatography mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) have been successfully applied. For examples, Alamri and Moustafa [13], identified chemical constituents by HPLC in ethanol extracts of fresh fruits of *Solanum incanum*, fresh leaves of *Ricinus communis* and *Allium ampeloprasum* var. *porrum*, found that they have varying amounts of phenols. Nadir et al. [14] found 78 unreported chemicals constituents from *Salvia santolinifolia* and this species was identified to be α-pinene chemotype. The essential oils of 4 species namely *Hypericum linarifolium*, *Hypericum perfoliatum*, *Hypericum humifusum* and *Hypericum pulchrum* from Portugal analyzed by GC and GC–MS found that alpha-pinene, beta-pinene and *n*-nonane separated the four species from each other [15]. Since, there are various disciplines associated with plant taxonomy to improve the identification, classification and systematic position of plant taxa, our study also aimed to use the distribution pattern of chemical compounds for the better understanding of the phylogenetic relationships between the five populations of *Z. spina-christi* collected from various elevations in Aseer region, KSA. In addition, we will establish chromatographic fingerprints to some vitamins present in the leaves by means of HPLC collected *Z. spina-christi* plants.

2. Materials and methods

2.1. Study area

The studied plant populations covered five sites of the Aseer region, KSA at various altitudinal gradients namely; Abha (latitude 18° 13′ 40″, longitude 42° 30′ 11″ – Alt.-2227.86 m), Dala Valley (latitude 18° 9′ 35″, longitude 42° 30′ 41″ – Alt.-1424 m), Rakhma Valley (Latitude 18° 4′ 7″, longitude 42° 30′ 21″ – Alt.-1000 m), Raheb Valley (Latitude 17° 56′ 13″ longitude 42° 28′ 7″ – Alt.-505 m) and Al marbh (Latitude 17° 45′ 32″, longitude 42° 19′ 51″ – Alt.-147 m). Contour Lines and spot elevations from the raster image (topographical map), are extracted then converted to digital vectors to calculate the elevation values using (Digital Elevation Model, DEM) (Fig. 1A and B). The topography of the study area is characterized by a series of semi arid undulating mountains, with max/min rainfall of 549.68 mm/133.47 mm with an average of 234.14 ± 114.99 mm per year, whereas the period between June and October has the bulk of rainfall with some deviations from year to year [16].

2.2. Plant material

From each site 500 g of leaves of *Z. spina-christi* plants reaching a height of 1.5 m was randomly sampled without any prior fertilization to the plants. The sampled leaves were immediately placed in a sealed plastic bag and about 3 g from each preserved in dry ice for the DNA extraction. A voucher specimen from each population was deposited in the Biology Department, Faculty of Science, King Khalid University.

2.3. DNA extraction from fresh leaves Z. spina-christi plants

The genomic DNA was extracted using 0.80 mg from fresh leaves of *Z. spina-christi* after grinding them to a fine powder using liquid nitrogen and commercial kit, (DNeasy plant mini kit) provided by QIAGEN-USA. All the steps were indicated in the Kit.

2.4. The quality and quantity extracted DNA

The DNA fragments were separated using 1% agarose gels after staining with ethidium bromide in a final concentration of 0.5 μg/ml. Agarose gels were run horizontally in 0.5× Tris–borate-EDTA (TBE) buffer and run for 60 min at 90 V. The DNA bands were visualized by UV transilluminator at 365 nm wavelengths and its conc. was estimated using a Thermo Scientific™ BioMate 3S UV–Visible at 260 nm [17].

2.5. RAPD-PCR and ISSR-PCR analysis of genomic DNA

Twenty-four biomarkers from RAPD and ISSR were used as shown in (Table 1). Standard PCRs buffer were performed using GoTaq Green Master Mix (2×). Each PCR reaction contained 1× GoTaq Green Master Mix, 23 *p*mol from each primer (either RAPD or ISSR), 25 ng DNA template and nuclease free water to obtain a final volume of 25 μl [18]. Cycling procedure was run using PTC 200 Peltier Thermal Cycler (MJ Research — USA) as follows: Initial denaturation at 94 °C for 5 min followed by 49 cycles of denaturation at 94 °C for 1 min, annealing temperature of 30 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 7 min. 17 μl of PCR amplified products were separated by electrophoresis in 1.4% agarose gels for about 120 min at 100 V. A negative control reaction in each PCR experiment was set up

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