Journal of Applied Research on Medicinal and Aromatic Plants xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Morphological, phytochemical and genetic diversity of Ziziphus spina – Christi (L) Des. in South and Southeastern of Iran

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ARTICLE INFO

Keywords:
Ziziphus spina-christi
Christ's thorn
Germplasm variability
Saponin
Quercetin
DNA-sequencing

ABSTRACT

Morphological and genetic variations of the Iranian Christ's-thorn (Ziziphus spina-christi (L.) Desf., Rhamnaceae) give us a knowing perspective to investigate and select elite germplasms for breeding and conservation purposes. In this study, Christ's-thorn organs and parts of 101 genotypes were sampled from 12 habitats in the South of Iran. In total, 32 qualitative and quantitative characteristics were measured. Analysis of variance was carried out based on completely randomized design and this revealed significant differences for most variables, indicating a large-scale diversity among the genotypes. For instance, fruit weight ranged 0.68–7.7 g, fruit length ranged 10.68–27.45 mm, seed length 7.22–17.8 mm, fruit pulp 2.2–11.4 mm and leaf length 16–65.6 mm. The genotypes were clustered into three main groups. 5′ Partial 18s rDNA from 9 regions were cloned by specific primers. The partial sequence of 18s rDNA gene in seedling of studied Iranian wild types Christ's-thorns were shown to be identical with the *Ziziphus Spina-Christi* 18sr¬DNA gene. Flavonoid quercetin and saponin content were measured for 9 dominant populations. Maximum saponin content was related to the Shahdad sample (large leaves and fruits with least thorn density) with 2.6 μg ml^{-1}. Maximum flavonoid quercetin content (3.66 mg g^{-1}) was related to the Jahrom sample (large leaves and fruits with least thorn density).

1. Introduction

Ziziphus spina-Christi is commonly known as Christ's Thorn Jujube. "Konar" is a deciduous tree of the tropical and subtropical regions and is now widespread in North Africa, Southern Europe, the Mediterranean, Australia, sub-tropical America and in the south, east and Middle Eastern areas of Asia (Asgarpanah and Haghighat, 2012). *Christ's Thorn* Jujube belongs to the Rhamnaceae family of Rosales order that contains about 60 genera and more than 850 species. It has been used in folk medicine as a demulcent, depurative, anodyne and as an emollient for stomach pain, toothache astringents, a body wash (Ghafoor et al., 2012), liver and urinary problems, obesity, fever, diarrhoea, diabetes, digestive disorders, lethargy, insomnia and skin infections. Their seeds were also seen as a potential source of antimicrobial compounds (Bukar et al., 2014). A number of Cyclopeptide and Isoquinolin alkaloids, Flavonoids, Terpenoids and their Glycosides were found in most Ziziphus species (Asgarpanah and Haghighat, 2012). The fruit flavonoids of Christ's thorn and *Z. jujuba* (L.) also help to promote general good health while it is also said that the leaves have properties that contribute to the lowering of blood pressure. In addition it has been shown that the leave extract has antimicrobial, antinociceptive and antidiabetic properties (Adzu and Haruna, 2007; Avize et al., 2010; Niamat et al., 2012). The antidiabetic effect of the leaf extract is due to the existence of Saponin and Polyphenols. The low plant cover and food deficiency in the semi-arid and arid Savannah zones of Africa and Asia means there is a strong case for Christ's thorn Jujube playing a part in any food security programme. This is because its fruits have a very high energy value, the seeds are rich in protein while the leaves are rich in calcium, iron and magnesium and can also be used as animal feed. It is a cross pollinated plant that has a high range of genetic variability in its morphological and physiological traits. Because the Christ's thorn plant is dormant in the summer months and therefore requires very little water, it can tolerate very dry conditions. It can grow up to an altitude of 2000 m and also grow in poor and salinity soils (about 6.18 ds/m EC) due to its deep and strong roots. Thus it can be grown in areas that are not suitable for many other plants. The Christ 's thorn plant grows in most regions in the South of Iran such as the provinces of Khuzestan, Bushehr, Fars, Kerman, Sistan, Hormozgan and Lorestan. It is a multipurpose tree and is known locally as the "Konar". This very popular fruit is found in local markets and its area under cultivation is increasing. Because the native Konar plant has drought and salt tolerance genes, it can play an important part in

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http://dx.doi.org/10.1016/j.jarmap.2017.06.006
Received 27 September 2016; Received in revised form 29 April 2017; Accepted 26 June 2017

drought and salinity stress breeding programs. Its high nutritional value and extensive growth regions mean it must be protected from the risk of genetic eradication.

The phenolic compounds, especially flavonoids, are reported to have multiple biological effects such as antioxidants activity (Gagnon and Holdway, 2000). Molecular technology is considered a reliable alternative tool for the identification of plant species (Savolainen et al., 2000), A DNA barcode is a universally accepted short DNA sequence that allows for the prompt and unambiguous identification of species (Savolainen et al., 2005). DNA barcoding is the latest move towards the generation of universal standards (Kane and Cronk, 2008). More recently, the nuclear ribosomal internal transcribed spacer (ITS) has also been suggested as an efficient barcoding locus for complex plant groups (Hollingsworth et al., 2011). The final objective of our research is to analyse the morphological, enzymatic and genetic diversity of 101 Konar genotypes from 12 regions of South and South East of Iran.

2. Materials and methods

The samples were collected from their natural origins between January and April 2015. Sampling regions included: Chabahar, Shahdad, Jiroft, Kahnuj, Roudan, Minab, Bandar Abbas, Qeshm Island, Larestan, Jahrom, Bushehr and Kangan. In total 101 Genotypes were collected from these 12 regions. All regional information includes: latitude, longitude, altitude and the rain and temperature annual average rates are registered (Table 1). Information was also recorded to describe bough colour, soil type, fruit flavour and shape and the thorn density score on the field. All of the samples were stored separately in 4 °C on dry ice for other laboratory observations.

2.1. Sampling for enzymes and phytochemicals

In each region, 20 Christ's thorn trees were randomly selected and the leaf samples from all selected trees were core collected. Four replications from each core collection were used to measure chlorophyll (Chl) a and b, Carotenoid, catalase (CAT), Peroxidase (POD), Ascorbic peroxidase(APX), polyphenol oxidase (PPO), saponin, and quercetin contents of Iranian *Ziziphus spina Christi*.

2.2. Laboratory observations

In total 32 quantitative and qualitative variables were measured (Table 2). Fruit and seed length and width (mm) was measured by caliper and length/width rate were calculated. A ruler was used to measure thorn length, leaf characters, fruit pulp thickness and petiole length. All characters related to the weight were measured with a digital balance with + 0.001 g sensitively. Seed and fruit weight was determined by weighting sample of 10 seeds or fruits and average weight was calculated (Thanna et al., 2011). A SPAD chlorophyll meter (SPAD-502; Konica Minolta, Osaka, Japan) apparatus was used for measuring the chlorophyll content of leaves. Five leaves were chosen and spade numbers were recorded for three areas of each leaf to generate an average measurement.

2.3. Determination of total phenolic content

One gram of pulverized fruit pulp was dissolved in 10 ml of 50% Ethanol. The solvent was centrifuged for 15 min, 1 ml of supernatant was taken and mixed with 2.5 ml folin-ciocalteu reagent (diluted tenfold) and 2 ml of 7.5% sodium bicarbonate. As blank, 1 ml of 50% ethanol, 2.5 ml dilute phenol reagent and 2 ml of 7.5% sodium bicarbonate mixed. Absorbance values were measured 30 min of reaction at 750 nm using a UV–vis spectrophotometer read set (CECIL, CE2501, 2000 series model) (Diana et al., 2007).Gallic acid was used as a standard compound and the total phenols were expressed as mg g^{-1} gallic acid equivalent using the standard.

Standard curve equation: $Y = 0.0029x + 0.0424$. $R2 = 0.999$ (1)

Where Y is absorbance at 750 nm and X is total phenolic content in the extracts. All determinations were carried out in triplicate. Folin-ciocaltue's reagent was purchased from Sigma-Aldrich Co., Germany. All other chemicals and reagents used had analytical grade.

2.4. Determination of quercetin- flavonoid content by HPLC method

Christ's thorn leaf samples were obtained from 9 native regions (Shahdad, Chabahar, Bandar Abbas, Jahrom, Bushehr, Larestan, Minab, Jiroft and Gheshm Island). Methanolic extract was prepared by the maceration method, using 100 g of dried plant leaves powder and 500 ml of absolute methanol (99% methanol from Merck Co., Germany) for 72 h. Extracts were kept at 4 °C until used. Dry extract (34 mg) was refluxed in 8 ml HPLC grade methanol and incubated for 1 h in 75 °C water bath. Then the extract was filtered and transferred quantitatively to a 100-ml measuring flask. After dilution to volume with methanol, the solution was passed through 0.45 µm syringe filter and 20 µl of the sample injected into a luna C18 column. Column temperature was 25 °C with elution mode of mobile phase composed of degassed mixture of acetonitrile and phosphoric acid aqueous solution. 100 ppm quercetin was used as standard. The wavelength for absorption of quercetin was 370 nm. All determinations were carried out in triplicate. Chemical components: quercetin from Roth Co., Germany, HPLC grade methanol, and acetonitrile were purchased from Roth Co., Germany.

2.5. Determination of total Saponin by Anis aldehyde reagent

Christ's thorn,Konar, leave dried methanolic extract (500 mg) was refluxed in 10 ml 2 N HCL and 10 ml ethanol and hydrolyzed in 90 °C water bath and extracted twice with 80 ml diethyl ether. The saponin

Table 1
Geographic distribution and continental information of collected genotypes.

Region	Local	Sample no	Latitude	Longitude	Altitude	Rain	Temperature
Hormozgan	Bandar Abbas	10	E56.27	N27.18	15	200	26.5
Hormozgan	Minab	10	E57.4	N27.9	40	227	33.5
Hormozgan	Roudan	7	E57.12	N27.25	200	250	28
Hormozgan	Qeshm	10	E56.16	N26.57	8	125.2	26
Sistan	Chabahar	8	E60.64	N25.29	11	200	26
Kerman	Shahdad	9	E57.71	N30.42	430	15	23.7
Kerman	Jiroft	10	E56.55	N28.15	690	182	23.5
Kerman	Kahnug	5	E57.75	N28.05	505	188	26
Fars	Laresten	10	E54.28	N27.68	806	203	23
Fars	Jahrom	10	E53.20	N28.24	700	200	23
Bushehr	Bushehr	10	E50.83	N28.95	18	220	25
Bushehr	Kangan	2	E57.28	N25.48	5	146	30

Rain and temperature describe as annual average rate.