plant, Algae, and Environment
Vol. 10, Issue 3, Sept. 2026

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Regional Records of *Tamarix* Species (Tamariacaceae) from Kerman City (Kerman, Iran)

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Received: 2026-05-18 Accepted: 2026-07-20

Abstract

The genus *Tamarix* (Tamaricaceae) is one of the important genera of medicinal plants, comprising approximately 70–104 species worldwide, of which about 35 species have been reported in Iran. In the present study, field botany, morphology, taxonomy, scanning electron microscopy (SEM), and biochemical analyses of the leaves and flowers of *Tamarix* species in Kerman City were conducted. Six species, including *T. kotschyi*, *T. androssowii*, *T. szowitsiana*, *T. tetragyna*, *T. karakalensis*, and *T. ramosissima*, were recorded. *T. androssowii* and *T. szowitsiana* showed a similar phylogenetic relationship, whereas *T. tetragyna* and *T. karakalensis* appeared in a distinct clade. SEM images provided evidence of salt glands and crystals of NaCl and $CaSO_4 \cdot 2H_2O$ in the leaves. These features indicate the migration of ions, including Na^+, Cl^-, K^+, Ca^{2+}, and SO_4^{2-}, from groundwater, surface water, soils, and sediments, followed by salt excretion through salt glands and the formation of salt crystals in the leaves. These findings were consistent with the high concentrations of Na^+, K^+, and SO_4^{2-} detected in the leaves and substrate soils. Concentrations of trace elements, particularly potentially toxic elements, were close to background levels and were comparable to published global data for *Tamarix* species, indicating no evidence of excessive accumulation of these elements in the studied plants. Furthermore, phytochemical extracts of *Tamarix* species may have beneficial applications in the treatment of type 2 diabetes, bacterial and fungal diseases, cancer, Alzheimer's disease, liver fibrosis, rheumatoid arthritis, and urinary stones.

Keywords: *Tamarix* species, Salt-tolerant, Salt crystals, Medicinal uses, Iran

Introduction

The genus *Tamarix* is becoming an important medicinal plant that distributed across in Europe, Asia, Africa, America, Oceania, and other regions of the globe with about 70 to 104 species (Fig. 1) (Li et al. ,2024; Terrones and Juan, 2023); Villar et al., 2019). *Tamarix* species are considered as salt-tolerant, halophytes, phreatophytes, and evergreen plants that mostly grow in arid, semi-arid, and hot regions along riversides, salty marshes and saline sediments,

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Doi: 10.48308/pae.2026.244960.1145

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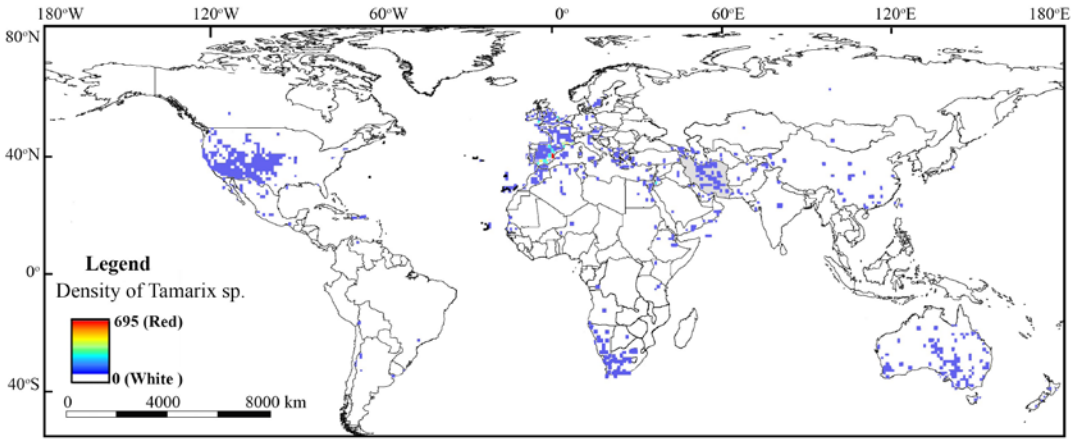


Fig. 1. Global distribution of *Tamarix* species with a considerable density in Iran (adopted from Mayonde et al., 2015)

where salinities range from 0.01% to more than 1%, NaCl. According to plant taxonomy (Tracy and Robbins 2009; Adams 2021; Villar et al. 2023), the genus Tamarisk is classified as: Tracheophytes, Angiospermae, Eudicots class, Magnoliopsida order, Caryophyllales family, Tamaricaceae genus, Tamarisk species.

Field and physical characteristics of the genus *Tamarix* display a shrubby plant ranging from 1-18 m height with rhombic, oval, needle-shaped leaves (1-2 mm) that contain white salt deposits on their surface due to evapor-transpiration and that the camels are the only livestock reported to feed (Adams, 2021). The red to purple and whitish-purple flowers are short with five stamens that are arranged in a 5 to 10 cm spike. The branches and twigs of *Tamarix* species are slender and delicate, with smooth, reddish-brown bark that becomes grayish-brown and furrowed as the plant mature. The wood of the *Tamarix* stem is hard and brittle, with the outer part being white and the inner part is reddish in color (Morisette et al., 2006).

The oldest reported fossils attributed to Tamarisk species, including *Tamarix minoa, Tamarix africana*, *Tamarix gallica*, were identified in the rock units of the western Mediterranean region dating to approximately 16 million years ago (Miocene) and from deposits dating from 2.58 million to 10,000 years ago (Pleistocene) (Terrones and Juan, 2023). Valuable studies in Iran reported (Abolhasni et al. 2020; Arianmanesh et al. 2015; Veisi et al. 2023; Sheidai et al. 2019) about 35 *Tamarix* species. In this study, a distribution map of the Tamarisk species is presented (Fig. 2), in all provinces under different climatic conditions, where the most abundant species are *Tamarix gallica, Tamarix aphylla*, and *Tamarix ramosissima*. Traditionally, *Gaz Angabin* of Khansar (Isfahan Province) and Gaz of Shahdad or Tamarix manna (Kerman Province) have been used in the preparation of nougat or Gaz sweet. Notably, in Persian culture, “Gaz” refers to *Tamarix.*

The term *manna* (*al-mann* in Arabic, Chapter Albaqarah, verse 57) refers to the exudate produced by the Tamarisk tree following insect feedingby *Euscelis decoratus*

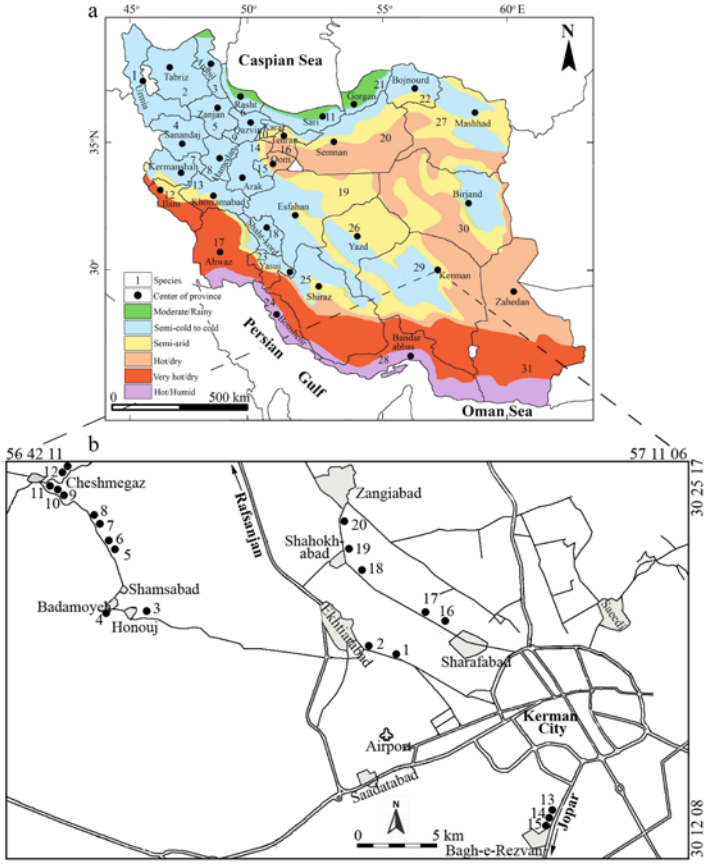


Fig. 2. Botanical and climatological map of *Tamarix* species in Iran. (a) distribution of *Tamarix* species in Iran, (b) sampling position of *Tamarix* species in Kerman city (Modified after Sheidai et al., 2019; Veisi et al., 2023; Abolhasani et al., 2020; Arianmanesh et al., 2015; Bahramsoltani et al., 2020; Mayonde et al., 2015, 1.*T. ramosissima Ledeb, T. octandra* , 2. *T. articulata, T.kotschyi,* 3. *T. austromongolica, T. ramosissima Ledeb,* 4. *T. ramosissima, Ledeb,* 5. *T. ramosissima Ledeb,* 6. *T. ramosissima Ledeb,* 7. *T. ramosissimma Ledeb,* 8. *T. ramosissima Ledeb,* 9. *T. habitatas,* 10. *T. ramosissima Ledeb,* 11. *T. aphylla, T. humboldtiana,* 12. *T. ramosissima Ledeb,* 13. *T. ramosissima Ledeb,T. aphylla,* 14. *T. habitatas,* 15. *T. kotschyi,* 16. *T. tetragyna,* 17. *T. passerinoides,* 18.*T. ramosissima Ledeb,* 19. *T. ramosissima Ledeb, T. aphylla,* 20. *T. ramosissima Ledeb, T. meyri,* 21. *T. arceuthoides,* 22. *T. ramosissima Ledeb, T. aphylla,* 23. *T. mascatensis,* 24. *T. aphylla, T. Jordanis,* 25. *T. tetrandra, T. mascatensis,* 26. *T. rosea,* 27. *T. ramosissima Ledeb,* 28. *T. tetrandra, T. humboldtiana,* 29. *T. kermanensis, T. karakalensis, T. ramosissima,* 30. *T. aphylla, T. hispida,* 31. *T. aphylla, T. humboldtiana.*

(Rahmani, 2004). Sheidai et al. (2019) reported various *Tamarix* species as primarily used as ornamental trees, windbreaks, and soil erosion control; however, more recent studies (Duan et al. 2022; Li et al. 2024; Aftabi 2024) show additional application

of *Tamarix* species in medicine, pharmacy, and environmental management (Figure 3). Comprehensive studies regarding Tamarisk species and their uses remain limited in Kerman city, although Baum (1967) only introduced *Tamarix kermanensis* as one of the important species. The main objectives of this study were to present a global distribution map of *Tamarix* species and their distribution in Iran, document regional records of *Tamarix* species in Kerman City, provide SEM images of the studied *Tamarix* species, characterize the macro- and microelement composition of *Tamarix* leaves, and discuss the major medicinal applications of *Tamarix* species and their potential environmental impacts.

Material and Methods

To identify Tamarisk species in the vicinity of the Kerman City, 24 leaf and flower samples were collected along Kerman-Jopar, Kerman-Cheshmehgaz, and Kerman-Zangiabad roads (Figure 2 and Table 1). The samples were dried for one month described by Mahdavi Maymand and Mirtadjdini (2011), Qaiser (1981), and Maden (2004) and were subsequently submitted to the Herbarium Laboratory of the Biology Department at Shahid Bahonar University of Kerman for identification. The*Tamarix* specieswere identified by Dr. Mirtadjadini and assigned Herbarium accession numbers ranging from 4700 to 4716.

Li et al. (2024) and Nisar et al. (2022) suggested that, for phytochemical, pharmacological, and medicinal applications of *Tamarix* species, the leaves, flowers, fruits, and other plant parts are dried in a shaded area, powdered and subsequently followed by dissolution and extraction.

To investigate the presence of salt glands and salt crystals in Tamarisk species, cleaned and dried leaves were analyzed using scanning electron microscopy (SEM) with a resolution of 3.5 nm and an acceler-

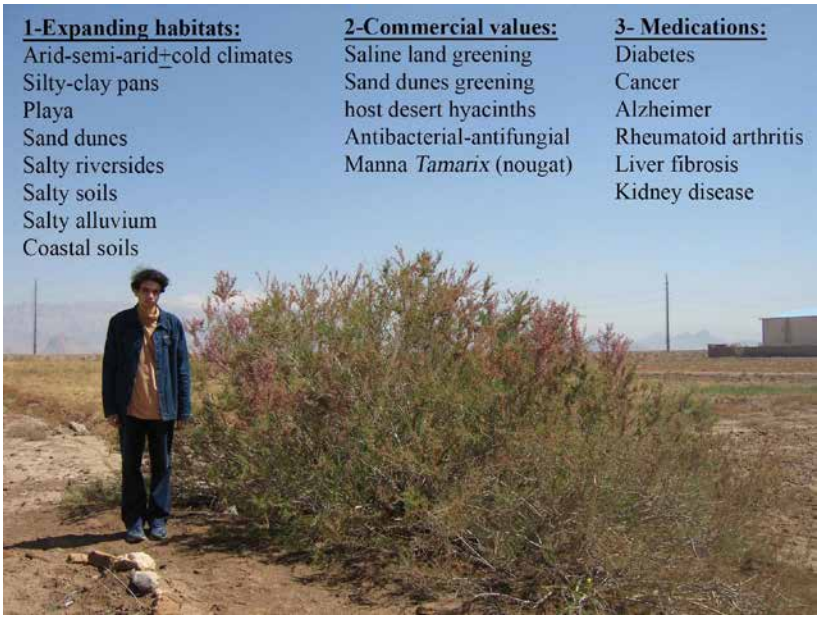


Fig. 3. Environments, commercial values and medicinal uses of the genus *Tamarix* (adopted from Duan et al., 2022; Li et al., 2024; Aftabi, 2024)

ating voltage 30,000 eV. The analyses were performed using an EDEX detector coupled to a Camscan MV 2300 instrument in the Department of Materials and Metallurgy Engineering at Shahid Bahonar University of Kerman, Iran. To integrate the phylogenetic associations of the *Tamarix* species, the morphological data were analyzed using SPSS software, and a dendrogram was constructed using Ward's linkage method with rescaled distance cluster combine. For the determination of biochemical determination of macro and microelements, the leaves were washed with distilled water, dried, powdered, and ashed, followed by dissolution in a mixture of HNO_3 and HCl (Atapour and Taheri, 2017). The concentrations of the elements were measured using inductively coupled plasma emission spectroscopy method (ICP-OES) (Varian-Agilent 700 Series) at the Applied Research Laboratory of Karaj branch of Geological Survey of Iran in Tehran. The accuracy, assessed against the certified reference materials (American National Standard 2001) ranged from 93.84 to 99.81%, respectively. The precision of the measurements was less than 10% RSD.

Table 1. Geographical position and herbarium numbers of *Tamarix* species in Kerman City

Sample No.	Herbarium No.	X	Y	Z	*Tamarix* Speice
1	4700	30°18'37.10"N	56°57'42.42"E	1749	*Tamarix cf androssowii*
2	4701	30°18'37.12"N	56°57'42.48"E	1749	*Tamarix szowitsiana*
3	4702	30°20'2.10"N	56°46'50.99"E	2184	*Tamarix szowitsiana*
4	4703	30°20'33.09"N	56°46'6.60"E	2274	*Tamarix szowitsiana*
5	4704	30°22'37.75"N	56°45'57.36"E	2202	*Tamarix szowitsiana*
6	4705	30°23'37.75"N	56°45'19.29"E	2126	*Tamarix kotschyi*
7	4706	30°23'37.54"N	56°45'19.29"E	2125	*Tamarix kotschyi*
8	4707	30°23'52.10"N	56°44'58.87"E	2125	*Tamarix kotschyi*
9	4708	30°23'49.64"N	56°45'12.80"E	2120	*Tamarix kotschyi*
10	4709	30°24'57.69"N	56°42'33.87"E	2167	*Tamarix kotschyi*
11	4709	30°24'57.56"N	56°42'33.89"E	2167	*Tamarix kotschyi*
12	4710	30°24'42.50"N	56°43'24.43"E	2161	*Tamarix kotschyi*
13	4711	30°12'32.80"N	57° 3'26.35"E	1768	*Tamarix cf androssowii*
14	4712	30°12'32.80"N	57° 3'26.33"E	1768	*Tamarix androssowii*
15	4713	30°12'32.56"N	57° 3'26.26"E	1768	*Tamarix Karakalensis*
16	4714	30°20'9.35"N	56°58'37.31"E	1749	*Tamarix androssowii*
17	4714	30°20'12.22"N	56°58'32.50"E	1749	*Tamarix androssowii*
18	4714	30°20'11.21"N	56°58'33.03"E	1749	*Tamarix androssowii*
19	4715	30°23'29.42"N	56°55'9.59"E	1737	*Tamarix androssowii*
20	4716	30°23'15.16"N	56°55'11.80"E	1737	*Tamarix tetragyna*

Results

Morphological studies and Tamarisk taxonomy

Morphological studies of 24 *Tamarix* samples revealed record several noteworthy species recorded in the Kerman City. The morphological characteristics of these species are presented in Tables 1 and 2, and Figure 4, and are described as below:

Tamarix androssowii Litv., Sched. Herb. Fl. Ross. 5: 41 (1905)

Tamarix androssowii (Fig. 4a, d) grows as a small tree (2- 3 m in height) with smooth, glabrous bark that is reddish-brown to brown. It ocuuresin sandy dunes, saline playas, and along riverbanks. The green to reddish-green vegetative branches reach nearly to 1 m in length and arise from the central branches of the plant, However, the youngest branches are attached to the main branches at nearly right angles. The green leaves growing on the main branches are 1-2 mm long and 0.5 mm wide, are amplexicaul form, whereas the leaves of younger lateral branches are green and egg-shaped with a layered margin and acute tips. The purple to purplish flowers bloom in May. Based on herbarium data (Fig. 4a, d, Table 2) and a comparison the morphological characteristics of the speciemens (Table 2) with published data (Baum 1967; Qaiser 1981; Sheidai et al. 2019; Arainmanesh et al. 2016), this tamarisk species resembles *T. androssowii*, which is reported for the first time in the Kerman City.

Tamarix kotschyi Bunge, Tent. Gen. Tamar. 30 (1852)

Tamarix kotschyi is a medium-sized species that predominantly grows along riversides, around springs and on spring sediments, reachinga height of 1- 2 min Cheshmehgaz Village (Fig. 2). Morphologically, the plant is characterized by greyish-brown to purplish-brown skin and sessile, green leaves measuring 1-2 mm in length long and 1 mm in width withtranslucent margins. The younger leaves may be amplexicaul and the purplish to light-purplish flowers bloom in May. Based on the herbarium data and comparized with published data (Fig. 4b, Table 2), the plant is is in consistent with *Tamarix* kotschyi and is reported here as a new record for the Kerman City.

Tamarix szowitsiana Bunge, Tent. Gen. Tamar.: 26 (1852).

Tamarix szowitsiana grows to 1-3 m in height and occurs in sandy, saline soils and along riversides. It is characterized by greyish-brown bark, on which sessile, ovate to egg-shaped leaves measuring 1-2 mm in lengt and approximately 0.5 mm in width. The purplish to light-purple flowers bloom in May. Based on herbarium data, morphological characteristics, and global distribution information (Tables 1 and 2, Fig. 4c), the specimens examined in this study are consistent with *Tamarix szowitsiana*, whichis reported here as a new record for Kerman City.

Tamarix karakalensis Freyn, Bull. Herb. Boissier, sér. 2, 3: 1060 (1903).

This *Tamarix* reaches a height of 1-3 m has reddish-brown bark and grows in sandy hills, sandy soils and river banks. The plant produces few bright-purplish to white flowers in April. The green, sessile leaves of 1-2 mm long and 0.75 mm wide, with a narrow base. Based on the herbarium data (Fig. 4e), morphological characteristics (Table 2), and

Table 2. Biometric and morphological aspects of *Tamarix* species studied and recorded in Kerman City (SABUKH = Shahid Bahonar University of Kerman Herbarium)

Taxa	Height	Leaves	Flowers	Disk	Racemes (length and width)	Sepal (shape and width)	Petal (shape and width)	Bract/ pedicel length	Herbarium number
T. kotschyi (7)	1-2m (2m)	Amplexicaul	4-merous	Synlo to parasynlophic	1-2 cm, 2-4 mm (1-3 cm, 3-4 mm)	Ovate, 0.7- 1mm (0.75- 1 m)	Elliptic, 1.5- 2mm (2-2.25 mm)	1-1.5	Mirtadjadini- Aftabi (SBUKH 4705-4710)
T.androssowii (7)	2-3 m (2-5 m)	Sessile	4-merous	Synlo to parasynlophic	1.2-3.5 cm, 3-4 mm (1.5 – 4 cm,3-5 mm)	Ovate to trullate, 0.7-1mm (1mm)	Obovate –elliptic, 1.5- 2 mm (2-2.25 mm)	1-1.25	Mirtadjadini-Aftabi (SBUKH 4700, 4711-4715
T.szowitsiana (4)	1-3 m (3-7m)	Sessile-elliptic	4-merous	Synlo to parasynlophic	1.7-4 cm, 6- 8 mm (2-4 cm, 6 mm)	Ovate-dentriculate, 1.2 mm (1-1.25 mm)	Elliptic- obovate, 1.5- 2mm (2.25-2.5 mm)	1-1.3	Mirtadjadini-Aftabi (SBUKH 4701-4704)
T. Tetragyna (1)	1-3 m (2-5 m)	Sessile-Ovate Sessile-ovate	4-merous to 4 and 5-merous	Paralophic	4-9 cm, 5-9 mm (5-15 cm, 8-10 mm)	Ovate-dentriculate, 1.5-2 mm (2 mm)	Elliptic-obovate, 2-5 mm (3.5- 5 mm)	1.5-2	Mirtadjadini-Aftabi (SABUKH 4716)
T.karakalensis (1)	1-3 m (2-3m)	Sessile-ovate	5-merous	Hololophic	0.7- 4 cm, 2-4 mm (0.5- 4 cm, 3-4 mm)	Trullate-ovate, 0.4-0.7 mm (0.5-0.75 mm)	Elliptic-obovate, 1-1.3 mm (1.25-1.5 mm)	1.5-2.5	Mirtadjadini-Aftabi SBUKH 4713
T.ramosissima (3)	1-3 m (1-6 m)	Sessile-ovate	5-merous	Hololophic	1.4-6.5 cm, 3-4 mm (1.5- 7 cm, 3-4 mm)	Trullate-ovate 0.5- 1 mm (0.5-1mm)	Elliptic- obovate, 1-1.7 mm (1-1.75 mm)	2.5-3	No enough data on leaves and flowers

The data in parenthesis represent numbers of samples and morphologic global data (bold data)

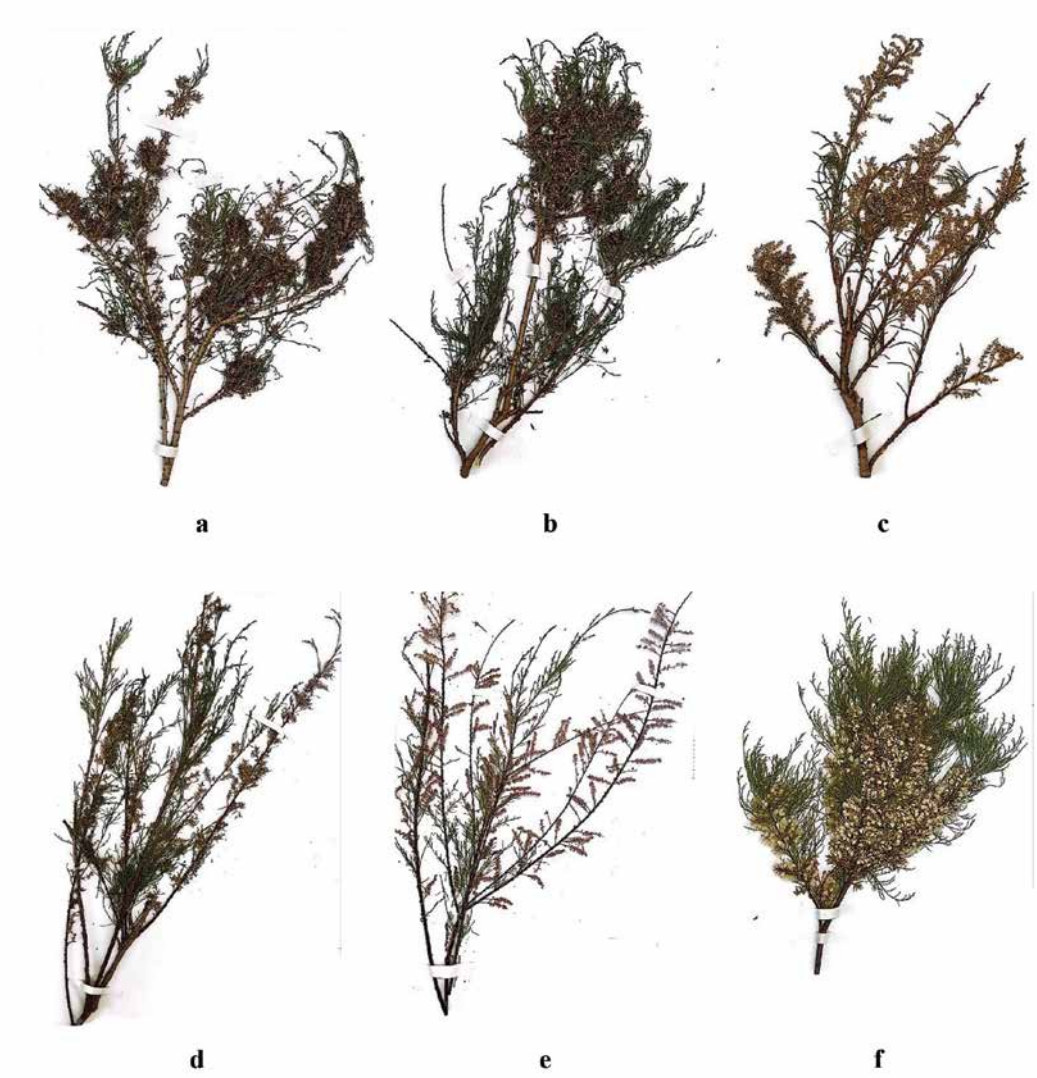


Fig. 4. Herbarium images of *Tamarix* species in Kerman City (a) *T. androssowii*, (b) *T. szowitssiana*, (c) *T. kotschyi*, (d) *T. androsswii*, (e) *T. karakalensis*, (f) *T.tetragyna*

comparison with global data, this plant species resembles *Tamarix karakalensis* resembles *Tamarix ramosissima*.

Tamarix tetragyna Ehrenb., Linnaea 2: 257 (1827).

This species grows to a height of 1-3 mand occurs in saline soils. The plants display smooth purplish to dark brown bark, on which narrow-based, sessile and egg-shaped leaves of 1-5 mm long and 1mm wide grow. The purplish to white flowers bloom in May. Based on herbarium data (Fig. 4f, No. 4716), morphological characteristics (Table 2), and global data, this species resembles *Tamarix tetragyna* and is reported for the first time in the Kerman city.

Tamarix ramosissima Ledeb., Fl. Altaic. 1: 424 (1829).

Tamarix ramosissima grows to a height 1-3 m and is also known as a highly branched salt cedar with lateral growth extending 2-3 m, whicht may exceed its height. It occurs in sandy hills, along small riversides, on sand dunes, and in saline soils. The plant is distinguished by reddish-brown bark and overlapping, blue-green, sessile -ovate, and

egg-shaped leaves measuring 2-3 mm in length and 1- 2 mm in width. The branch tips may bear few hairs and the purplish to white flowers bloom in April. In Based on bract-to-pedicel ratio (2.5-3, Table 2) and inflorescence characteristics, *Tamarix ramosissima* shows similarities to *Tamarix karakalensis*.

The phylogenetic dendrogram was constructed (Fig. 5) based on morphological characters, including the bark color, leaf and flower characteristics, inflorescence features, raceme length and width, sepal shape and length, petal shape and length, and bract-to-pedicelratio . Accordingly, the dendrogram indicates a considerable similarity between *Tamarix androssowii* and *Tamarix szowitsiana* in Clade I. *Tamarix Kotschyi* clustered separately in Clade II, whereas *Tamarix tetragyna* and *Tamarix karakalensis* are grouped in Clade III.

SEM analysis data

Some *Tamarix* species have been reported to exhibit salt glands and salt crystals as very small, dot-like features on the leaf surface. To investigate these features, *Tamarix* leaves were examined under SEM. As shown in Fig. 6, limited evidence of salt glands and crystals of sodium chloride (NaCl) and calcium sulfate dihydrate ($CaSO_4$, $2H_2O$) was observed in the different species examined. These salt glands are concentrated on the leaf surface and may have be associated with intense evaporation in arid and semi-arid regions as well as the migration of dissolved Na^+, K^+ and Ca^{2+} ions by surface waters, ground waters and moist in saline soils (Duan et al., 2022).

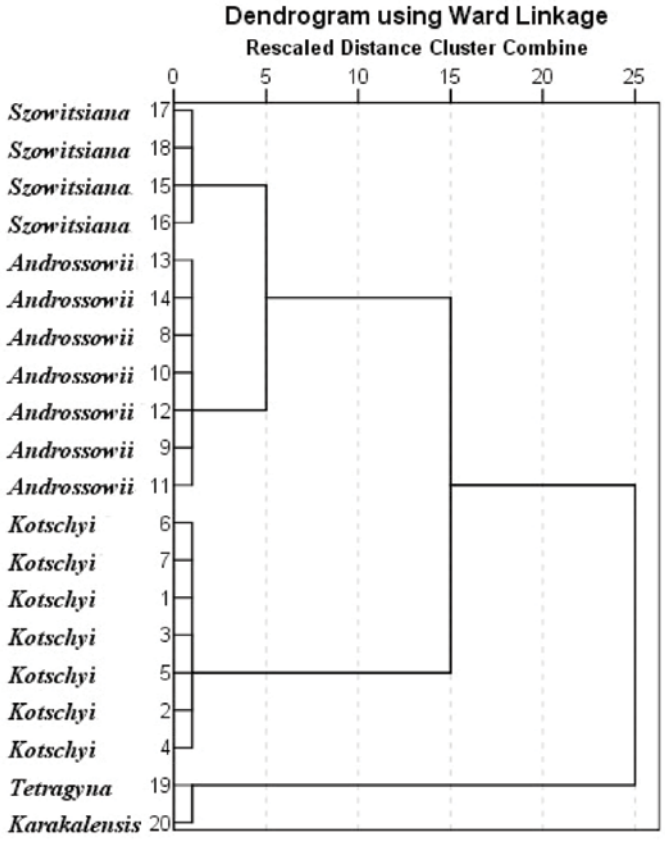


Fig. 5. Morphological dendrogram of *Tamarix* species in Kerman City

Biochemistry of macro and microelements in leaves

The concentrations of macro and microelements in *Tamarix* leaves are presented in Table 3 and compared with specific published data from various *Tamarix* species (Table 3). The results showed relatively high concentrations of sodium (759.7 µg), potassium (180.1 µg), and sulfur (500 µg) may be associated with the presence of salt glands and salt crystals. These findings are consistent with the SEM observations shown in Fig. 6. Based on Table 3, the highest concentrations of Na, K, and S were recorded in *Tamarix ramosissima* and in the substrate soils and sediments associated with the habitats of the studied *Tamarix* species. The metal bioaccumulation factor (MBAF) of the elements was calculated as the ratio of the concentration of elements in *Tamarix* leaves to its concentration in the substrate soil or sediment (Pachura et al., 2015). (MBAF values were classified as follows: (1) <0.01, no enrichment; (2) 0.01–0.1, weak enrichment; (3) 0.1–1, moderate enrichment; and (4) >1, high enrichment (Table 3). Table 3 shows that Na, P, and, S exhibit weak to moderate enrichment, whereas other elements do not show significant enrichmen in Tamarix leaves. On the other hand, the concentrations of toxic and heavy elements, including arsenic, lead, zinc, iron, manganese, titanium, molybdenum, and vanadiumare within the tolerable ranges reported for other *Tamarix* species worldwide and show no evidence of toxic effects on the studied *Tamarix* species.

Discussion

Discussion and possible medicinal applications and threats

The genus *Tamarix* (Tamaricaceae) comprises more than 70 species of the salt-tolerant, halophytes/phreatophytes plants that occur mainly in the arid, semi-arid and to a lesser extent, in cold regions of Northern Hemisphere. These species are reported to be useful as ornamental plants, windbreaks and afforestation of sand dunes and more importantly as medications for some diseases (Li et al., 2024; Aftabi, 2024; Maldonado, 2016). Paleo-geographically, the first generation of *Tamarix* probably appeared in the geological time of Eocene (54-36 million years ago) in the Mediterranean area under warm and humid climatic conditions (Zhang et al., 2018). In the geological time of Miocene (25.2-5.2 million years ago), due to the regression of the Tethys ocean the genus *Tamarix* expanded into the central Asia, China and probably western Asia under arid and cool climates (Zhang et al., 2018).

The global distribution of different *Tamarix* species in the world as well as their distributionin Iran (Figs. 1- 2) shows that these plants occur mostly in arid and semi-arid climates. Yet, some *Tamarix* species have been reported to occur in cold climates in the Northern Hemisphere, including Iran (Lehenhoff et al., 2011; Bean and Dudley, 2023; Sheidai et al., 2019). Lehenhoff et al. (2011) suggested that *Tamarix* seeds are more resistant to cold and temperate conditions, remaining viable at temperature -5 to 14 °C for up to 6 months, than under warm and hot conditions at 25-35°C for three months. Therefore, some *Tamarix* species may be capable of growing under cold climate conditions, provided that suitable soil

Table 3. Mean concentrations (ug/g) and metal bioaccumulation factors (MBACF) of macro-microelements in *Tamarix* species of Kerman city compared to *Tamarix s*pecies in Africa (Nedjimi, 2023; Soltan et al., 2004). The concentrations of all elements in substrate soils and sediments are in ug/g

Taxa	As	Al	Fe	K	Na	Mg	P	S	Mn
T. kotschyi (3)	0.01	4.1	2.86	112.6	169	56	26.6	100.15	0.422
MBACF	0.0004	0.0001	0.0001	0.006	0.009	0.004	0.046	0.200	0.0007
T. Androssowii (3)	0.01	5.01	3.24	166	166	74	20.7	293.8	0.358
MBACF	0.0004	$7.62*10^{-5}$	$7.77*10^{-5}$	0.008	0.008	0.006	0.036	0.588	0.0006
T. szowitsiana (3)	0.01	6	4.11	137.7	568.4	111.5	13.76	34.17	1.28
MBACF	0.0004	$9.13*10^{-5}$	$9.86*10^{-5}$	0.007	0.029	0.009	0.024	0.068	0.002
T. karakalensis (1)	0.01	5.72	3.62	170.45	43.3	118.2	10.94	29.76	1.41
MBACF	0.0004	$8.70*^{10-5}$	$8.68*10^{-5}$	0.009	0.002	0.009	0.020	0.060	0.002
T. tetragyna (1)	0.01	3.67	2.75	71.85	253.8	52.65	25.6	116.25	1.257
MBACF	0.0004	$5.58*^{10-5}$	$6.6*10^{-5}$	0.004	0.013	0.004	0.044	0.232	0.002
T. ramosissima (1)	0.01	3.71	2.36	180.1	759.7	97	13.3	148.43	1.93
MBACF	0.0004	$5.64*^{10-5}$	$5.66*10^{-5}$	0.009	0.039	0.007	0.023	0.297	0.003
T. gallica (Algeria)	0.29	---	458	6484	11082	---	---	---	---
T. nilotica (Egypt)	---	---	772	17888	16934	3158	---	---	79
Average in substrate soils and sediments	27	65716	41698	19798	19284	12698	571	500	596

Taxa	Ti	Cu	Mo	Pb	Zn	V	Sr	Ba	Li
T. kotschyi (3)	0.0964	0.115	0.002	0.007	0.367	0.004	1.36	0.045	0.2
MBACF	0.00001	0.003	0.0010	0.0003	0.004	0.00001	0.002	0.00001	0.005
T. Androssowii (3)	0.131	0.071	0.001	0.01	0.3	0.005	1.16	0.02	0.2
MBACF	$4.5*10^{-5}$	0.002	0.0005	0.0004	0.004	$5.38*10^{-5}$	0.002	$5.96*10^{-7}$	0.005
T. szowitsiana (3)	0.002	0.0001	0.024	0.005	0.2	0.006	0.8	0.05	0.04
MBACF	$6.87*10^{-7}$	$2.7*10^{-6}$	0.012	0.0002	0.0025	$6.45*10^{-5}$	0.001	$1.49*10^{-6}$	0.001
T. karakalensis (1)	0.14	0.11	0.001	0.006	0.24	0.005	0.9	0.05	0.07
MBACF	$4.81*10^{-5}$	0.003	0.0005	0.0002	0.003	$5.38*10^{-5}$	0.002	$1.49*10^{-6}$	0.002
T.tetragyna (1)	0.09	0.12	0.001	0.006	0.43	0.003	0.58	0.2	0.2
MBACF	$3.09*10^{-5}$	0.003	0.0005	0.0003	0.005	$3.23*10^{-5}$	0.001	$5.96*10^{-6}$	0.005
T. ramosissima (1)	0.09	0.11	0.005	0.009	0.03	0.003	0.4	0.03	0.04
MBACF	$3.09*10^{-5}$	0.003	0.0025	0.0004	0.0004	$3.23*10^{-5}$	0.0007	$8.94*10^{-7}$	0.001
T. gallica (Algeria)	---	---	---	---	20.5	---	---	8.8	---
T. nilotica (Egypt)	---	2.12	---	0.4	35	---	---	---	---
Average in substrate soils and sediments	2910	37	2	21	80	93	555	33540	40

and water salinity conditions are present. The distribution of 35 *Tamarix* species in Iran and Kerman City (Fig. 2a, b) highlights their potential as medicinal plant sources.

It has beensuggested that (Sheidai et al., 2019; Arianmanesh et al., 2015; Ijbari et al., 2014; Li et al., 2024) the best morphological features for distinguishing *Tamarix* species are primarily related to four-and-five-merous floral, the shape of the disk, and the shape of sepal and petals, although these features may show some degree of overlap among species (Bredenkamp and Phepho 2008). In this regard, this study presents some region-

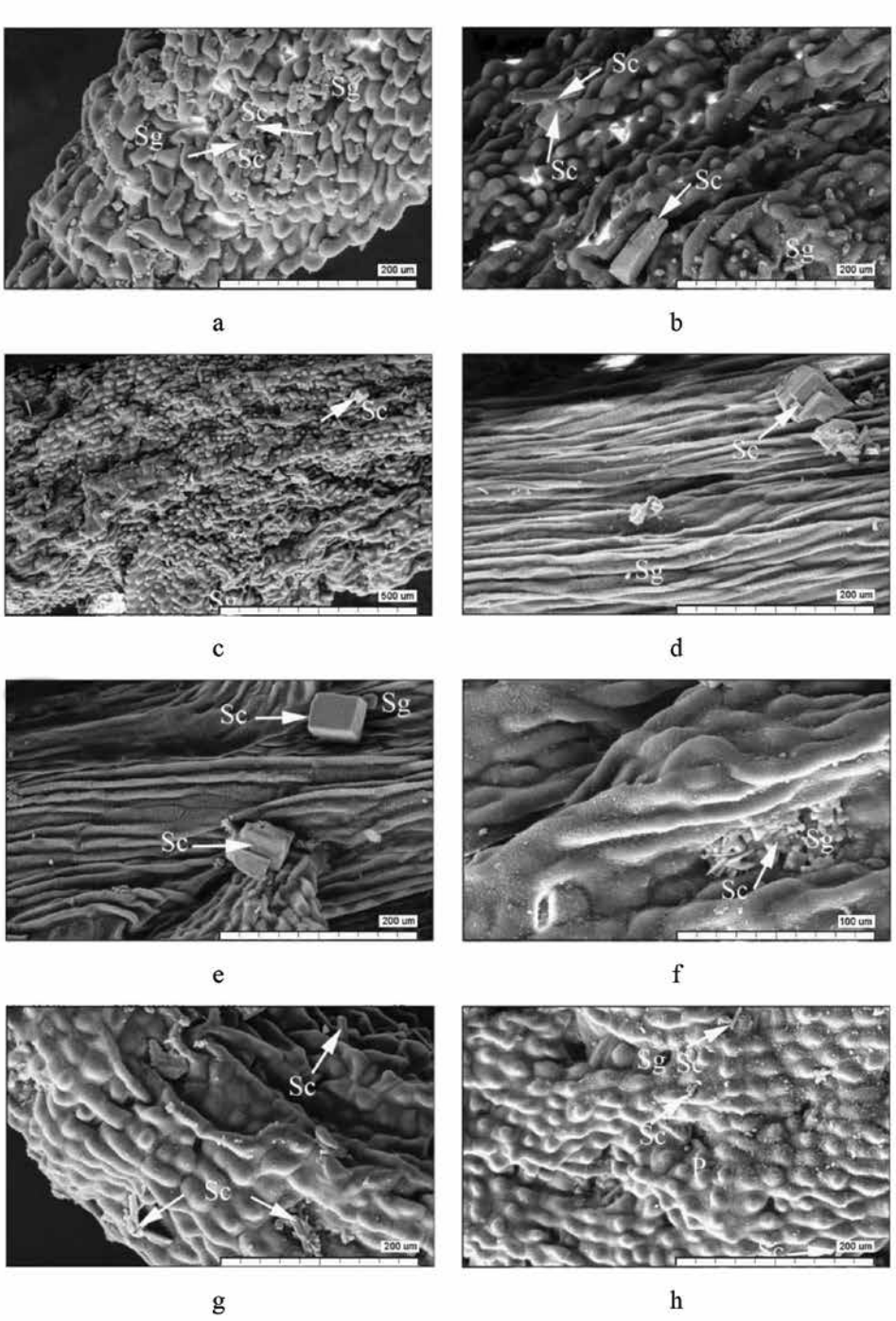


Fig. 6. SEM images of *Tamarix* species from Kerman City: (a) *T. androssowii*, (b) *T. szowitsiana*, (c) *T. kotschyi*, (d, e) *T. androssowii*, (f) *T. karakalensis*, (g) *T. tetragyna*, and (h) *T. tetragyna*. Sg, Sc, and P represent salt glands, salt crystals, and papillae, respectively. Salt crystals in (d) and (e) consist of NaCl, whereas those in the other SEM images consist of calcium sulfate ($CaSO_4 \cdot 2H_2O$).

al records of *Tamarix* species, as described in Tables 1 and 2, together with herbarium data (Fig. 4) and SEM images (Fig. 6). Morphologically, *Tamarix szowitsiana* and *Tamarix androssowii* show the greatest similarity in the dendrogram (Fig. 5). This similarity was also reported by Sheidai et al. (2019) and may be associated with their four--mer-

ous floral arrangements, sessile leaves, and similar disc morphology. The similarity of these two species with *Tamarix kotschyi* may be attributed primarily to their similar four-merous inflorescence and disk morphology. However, the amplexicaul nature of the leaves in *Tamarix kotschyi* represents a major morphological difference from other species. Some data (Shagholi et al. 2020; Arianmanesh et al. 2016) suggest that all three species could belong to the *Oligadenia* section. In the third branch of the cluster diagram (Fig. 5), *Tamarix karakalensis* and *Tamarix tetragyna* show morphological similarity, which may be associated with their five-merous inflorescences, a feature that not observed in the three previously mentioned species. The closet morphological similarity between *Tamarix karakalensis* and *Tamarix tetragyna* may be associated with a paralophic disk in *Tamarix tetragyna* and a hololophic disk in *Tamarix karakalensis*.

Table 2 shows that the bract-to-pedicel length ratio in *Tamarix karakalensis* is more similar to that of *Tamarix ramosissima*. This affinity was also reported by Baum (1967). It is noteworthy that *Tamarix ramosissima* is one of the most abundant species of *Tamarix* in Iran (Fig. 3). This species is mostly distinguished by its reddish to brownish bark, expanding branches, a high bract-to-pedicel length ratio (2.5-3) and five-merous inflorescence.

SEM images of dried leaves from the studied *Tamarix* species (Fig. 6) indicate the presence of papillate salt glands, sodium salt crystals and calcium sulfate crystals in different species. Sodium chloride crystals can be identified by their cubic crystalline structure, whereas calcium sulfate crystals are charecterized by their monoclinic prismatic crystals. The presence of these crystals suggests that surface waters, groundwaters, and soils associated with the habitats of the studied*Tamarisk* species contain relatively high concentrations of Na, Cl, Ca and S ions (Table 3). The migration of Na, K and S by groundwater and surface water to the leaves led may contribute to salt accumulation and crystallization on the leaf surface through evaporation and saturation. These findings are consistentwith those findings reported bySookbirsingh et al. (2010), Zhang et al. (2018), and Duan et al. (2022), who documented the occurrence of sodium chloride, potassium chloride, and calcium sulfate crystals on the leaves of the *Tamarix* species.

No additional data are currently available on the concentrations of macro-and microelements in the leaves of the *Tamarisk* species. Element concentrations exceeding natural background levels, especially those of potentially toxic elements, may adversely affect the growth and flowering of *Tamarix* species. The high values of toxic elements may seriously contaminate the *Tamarix* extracts and pose a potential risk to their phytochemical, pharmacological, and medicinal applications.

The biochemical data presented Table 3 show that the highest concentrations of Na (759.7 μg), K (180.1 μg), and S (148.43 μg) were recorded in the leaves of *T. ramosissima*, even though the concentrations of these elements were lower than the substrate soils. Among all the elements listed in in Table 3, Na, K, S, and P exhibited low to moderate

metal bioaccumulation factors, reflecting the presence of salt glands and crystals of sodium chloride and calcium sulfate in SEM images (Fig. 6) consistent with previous studies (Sookbirsingh et al., 2011; Duan et al. ,2022). Fortunately, the other elements detected in this study (Table 3) occured at very low concentrations (Table 3) that were close to or below the tolerance limits reported for other *Tamarisk* species worldwide Therfore, these elements are unlikely to pose a toxicity risk in the phytochemical extracts used for medicinal applications.

Recent studies (Alshehri et al., 2022, Nisar et al., 2022, Duan et al., 2022, Li et al., 2024, Aftabi et al., 2024) on medicinal plants have highlighted that *Tamarix* species are as potentially valuable sources for treatments of several diseases. In this context, diabetes mellitus is a metabolic disorder for which effective treatments are available, however, some synthetic α-glucosidase inhibitors may be associated with adverse effects, including hepatotoxicity. To addressthis issue, methalonic and ethalonic leaf extracts of *Tamarix aphylla, Tamarix nilotica*, *Tamarix dioica, Tamarix gallica, Tamarix articulate,Tamarix stricta* and other *Tamarix* species have demonstrated considerable antidiabetic potential. These extracts inhibit α-glucosidase and α-amylase activities, thereby potentially reducing carbohydrates digestion and helping to regulate blood sugar levels.

Most bacterial-fungal infections are treated with antibiotics, some of which may may cause allergies. However, Li et al. (2024) and Duan et al. (2022) suggested that the extracts of several *Tamarix* species, particularly *Tamarix gallica*, *Tamarix aphylla*, *Tamarix africana*, *Tamarix boveana*, *Tamarix ramosissima*, *Tamarix dioica*, *Tamarix arabica*, *Tamarix tetragyna*, *Tamarix karakalensis*, *Tamarix balansae*, and *Tamarix nilotica* can neutralize the harmful effects of bacteria and fungi.

Although effective treatments are available to manage some symptoms of Alzheimer's disease, no cure is currently available, and vitamin E and C can moderate the severity of the disease. Moreover, the antioxidant properties of extracts from *Tamarix gallica*, *Tamarix aphylla*, *Tamarix africana*, *Tamarix hispida*, *Tamarix ramosissima*, *Tamarix tetragyna*, *Tamarix karakalensis* and other *Tamarix* species may help mitigate oxidative damage and provide neuroprotective effects (Li et al. 2024; Duan et al. 2022).

Interestingly, syringic acid ($C_9H_{10}O_5$) is an antioxidant phenolic benzoic acid extracts from *Tamarix dioica*, *Tamarix hispida*, and *Tamarix ramosissima that* may prevent the progression of benign and malignant tumors and cancer cells. Methyl ferulate ($C_{11}H_{12}O_4$) is another antioxidant compound identified in the extracts of the various *Tamarix* species.that has been reported to exhibit cytotoxic activity against malignant cells associated with colorectal cancer (Duan et al. 2022; Li et al. 2024).

Rheumatoid arthritis is an autoimmune disease involving genetic and environmental factors. The compound Ramosissimin derived from *Tamarix ramosissima* extracts, may have potential for the treatment of this disease (Duan et al. 2022).

Liver fibrosis is a chronic liver disease that may initially be asymptomaticsbut can progressively lesions eventually develop into

liver cancer. pPhenolic and methanolic extracts from various *Tamarix* species have been reported to exhibit potential protective effects against liver fibrosis and liver cancer (Li et al. 2024, Duan et al. 2022).

Some kidney stones are composed primarily of calcium oxalate. Extract of *Tamarix hispida* and *Tamarix ramosissima* have been reported to exhibit potential anti-urolithiatic activity and may help inhibit the formation or growth of calcium oxalate and other urinary stones (Duan et al. 2022, Li et al. 2024). Despite the numerous reported medicinal applications of the *Tamarix* species, several disadvantages and potential threats have also been documented. Some fungal pathogenes, particularly species of *Botryosphaeria*, may cause branch dieback in different *Tamarix* species (Guerreio Madonado, et al. 2016). In addition, different *Tamarix* species excrete salts through their leaves and produce salt glands, and salt crystals, thereby increasing soil and sediment salinity and potentially inhibiting the growth of other plant species. (Ohrtman, 2009). Increased salinity in soils and sediments may also reduce interparticle bonding within soil-clay systems, thereby increasing soil susceptibility to soil erosion.

Conclusion

This paper highlights that the *Tamarix* species around the Kerman City mainly grow on alkali-tolerant salty sand dunes and soils. Morphological data indicated that the dominant occuring *Tamarix* species in this study area are *Tamarix kotschyi*, *Tamarix androssowii and Tamarix szowitsiana,* followed by the less frequently recorded *Tamarix ramosissima*, *Tamarix tetragyna*, and *Tamarix karakalensis*. Morphologically, *Tamarix androssowii* shows a greatest similarity to *Tamariix szowitsiana*, whereas *Tamarix karakalensis* shows relatively greater similarity to *Tamarix ramosissima* and *Tamarix tetragyna*. More importantly, SEM images displayed salt glands and crystals morphologically consistent with sodium chloride (NaCl) and calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), suggesting that the *Tamarix* species occur in alkaline and salinesoil environment. The bioaccumulation factors in *Tamarix* leaves and the concentrations of potentially toxic elements were within the ranges reported as tolerable for other *Tamarix* species. Therefore, these elements may not pose a significant environmental risks for using *Tamarix* extracts for medicinal applications. Further fresearch is warranted to investigate the molecular phylogeny and chemotaxonomy of *Tamarix* species and to develop a comprehensive atlas of *Tamarix* species. The main environmental threats associated with *Tamarix* species include fungi pathogens that cause branch dieback and the accumulation of salts in the substrate soils, which may increase the soil salinity and potentially inhibit the growth of neighboring plants species.

Acknowledgement

We sincerely thank Dr. M. Mirtadzadini for his professional identification of the *Tamarix* species and insightful suggestions during the study at Department of Biology, Shahid Bahonar University of Kerman, Iran. We greatly appreciate the Cooperation of Geological Survey of Iran in conducting precise and accurate analyses of the *Tam-*

arix leaves and soils. We also thank Dr. S. Kamali for performing SEM analyses at the Zand-Rahimi Research Center at Shahid Bahonar University of Kerman.

Author contributions

This paper was contributed by Hossein Aftabi and Habibeh Atapour, who collected the fieldwork samples, prepared the samples, managed the main text, and performed the data analysis.

Funding

The authors declare no funds and grants during the preparation of this manuscript.

Declarations

Conflict of interest

The authors declare that no competing financial interests or personal relationships that could have appeared to influence of the research reported in this paper.

Human and animal right

This manuscript does not contain any relationships with human participants or animals.

References

Abolhasani, F., Kharazian, N. and Jalilian, N., 2020. Floristic studies, life forms and chorology of plants in Kouh-payeh area, Isfahan province, Iran. Caspian Journal of Environmental Sciences, 19, pp. 59–73.

Adams, A., 2021. Treating invasive Tamarix as an intern at San Andres National Wildlife Refuge. *Geographical Bulletin*, 62, pp. 101–103.

Aftabi, H., 2024. The current progress on the antidiabetic, antibacterial, anticancer and anti-Alzheimer properties of the genus Tamarix: A review. In: *Proceedings of the 8th International Congress on Biomedicine (ICB2024)*, Tehran, Iran.

Alshehri, S.A., Wahab, S., Abullais, S.S., Das, G., Hani, U., Ahmad, W., Amir, M., Ahmad, A., Kandasamy, G. and Vasudevan, R., 2021. Pharmacological efficacy of Tamarix aphylla: a comprehensive review. *Plants*, *11*(1), p.118. https://doi.org/10.3390/plants11010118.

Arianmanesh, R., Mehregan, I., Assadi, M. and Nejadsattari, T., 2016. Comparative morphology of the genus Tamarix (Tamaricaceae) in Iran. International Letters of Natural Sciences, 60, pp. 1–12, https://doi.org/10.18052/www.scipress.com/ILNS.60.1.

Arianmanesh, R., Mehregan, I., Nejadsattari, T. and Assadi, M., 2015. Molecular phylogeny of Tamarix species from Iran based on ITS sequence data. *Pelagia Research Library*, 5, pp. 44–50.

Atapour, H. and Taheri, M., 2017. Exploration and environmental significance of heavy elements in plants and soils in urban areas of Kerman city. *Iranian Journal of Mining Engineering*, 12(35), pp. 105–126. [In Persian with English abstract].

Bahramsoltani, R., Kalkhorani, M., Zaidi, S.M.A. et al., 2020. The genus Tamarix: Traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology, 240, p. 112245. https://doi.org/ 10.1016/j.jep.2019.112245

Baum, B.R., 1967. A new species of Tam-

arix from southern Iran. Oesterreichische Botanische Zeitschrift, 114, pp. 374–382. DOI:

Bean, D.W. and Dudley, T.L., 2003. Saltcedars (Tamarix spp.): History and ecology in North America. In: Winston, R.L. (ed.) *Biological control of weeds in North America*. Milwaukee, WI: NAISMA.

Bredenkamp, C. and Phepho, N., 2008. *Hybridization of Tamarix usneoides and Tamarix ramosissima*. Unpublished data, South African Botanical Institute.

Duan, Q., Zhu, Z., Wang, B. and Chen, M., 2022. Recent progress on the salt tolerance mechanisms and application of tamarisk. *International Journal of Molecular Sciences*, *23*(6), p.3325. https://doi.org/10.3390/ijms23063325.

Ijbari, H., Vaezi, J., Ejtehadi, H. and Behroozian, M., 2024. Genetic diversity and population genetic structure of *Tamarix dubia* (Tamaricaceae) from Iran. *Plant Algae Environment*, 8(2), pp. 1462–1476. https://doi.org/10.48308/jpr.2024.237130.1089

Lehnhoff, E.A., Menalled, F.D. and Rew, L.I., 2011. Tamarix establishment in its most northern range. *Invasive Plant Science and Management*, 4, pp. 58–65, https://doi.org/10.1111.avsc.12011

Li, F., Xie, W., Ding, X., Xu, K. and Fu, X., 2024. Phytochemical and pharmacological properties of the genus Tamarix: a comprehensive review: F. Li et al. *Archives of Pharmacal Research*, *47*(5), pp.410-441. https://doi.org/10.1007/s12272-024-01498-x

Maden, K., 2004. Plant collection and herbarium techniques. *Our Nature*, 2, pp. 53–57.

Mahdavi Maymand, Z. and Mirtadjadini, M., 2011. Collection and identification of some plant species from Kerman province for herbarium of herbal drugs. *Journal of Herbal Drugs*, 1, pp. 1–24. [In Persian].

Maldonado, G.N., Lopez, M.J. and Caudullo, G., 2016. Tamarix in Europe: Distribution, habitat and threats. In: *European Atlas of Forest Tree Species*. Luxembourg: Publications Office of the European Union, published online at https://w3id.org/mtv/FISE-Comm/v01/e011f06.

Mayonde, S.G., Cron, G.V., Gaskin, J.F. and Byrne, M.J., 2015. Evidence of Tamarix hybrids in South Africa, as inferred by nuclear ITS and plastid trnS–trnG DNA sequences. *South African Journal of Botany*, *96*, pp.122-131. https://doi.org/10.1016/j.sajb.2014.10.011.

Morisette, J.T., Jarnevich, C.S. and Ullah, A., 2006. A Tamarix habitat suitability map for the continental United States. *Frontiers in Ecology and the Environment*, 4, pp. 11–17, https://doi.org/10.1890/1540.9295

Nedjimi, B., 2023. Analytical chemistry of some trace elements in Tamarix gallica L. using INAA. *Radiochimica Acta*, 111, pp. 633–639, https://doi.org/10.1515/ract-2022-0074.

Nisar, J., Shah, S.M.A. and Ayaz, S., 2022. In vitro comparative evaluation of Tamarix gallica extracts for antioxidant and antidiabetic activity. *Experimental Biology and Medicine*, 248, pp. 253–262, https://doi.org/10.1177/15353702221139208

Ohrtman, M., 2009. *Quantifying soil and*

groundwater chemistry in areas invaded by Tamarix species along the Middle Rio Grande, New Mexico. PhD Thesis, University of Denver, USA.

Pachura, P., Ociepa-Kubicka, A. and Skowron-Grabowska, B., 2015. Assessment of the availability of heavy metals to plants based on translocation index and bioaccumulation factor. *Desalination and Water Treatment*, https://doi.org/10.1080/19443994.2015.1017330.

Qaiser, M., 1981. The genus Tamarix (Tamaricaceae) in Pakistan. *Pakistan Journal of Botany*, 13, pp. 107–158.

Rahmani, G.H., 2004. Manna of Tamarix (gaz-e-Shahdad). *Iranian Journal of Medicinal and Aromatic Plants Research*, 19, pp. 403–410.

Shagholi, T., Keshavarzi, M. and Sheidai, M., 2020. Pollen morphology of some Tamarix species of Iran. *Acta Botanica Hungarica*, 62, pp. 151–161, https://doi.org/10.1556/034.62.2020.1-2.9.

Sheidai, M., Shagholi, T., Keshavarzi, M., Koohdar, F., Ijbari, H. 2019. Species delimitation and interspecific gene flow in *Tamarix*. *Hacquetia*, 18, pp. 313–322, https://doi.org/10.2478/hacq.2019/0001.

Soltan, M.E., Moalla, S.M.N., Rashed, M.N. and Fawzy, E.M., 2004. Assessment of metals in soil extracts and their uptake and movement within *Tamarix nilotica* at Lake Nasser Banks, Egypt. *Chemistry and Ecology*, *20*(2), pp.137-154. https://doi.org/10.1080/02757540410001664585.

Sookbirsingh, R., Castillo, K., Gill, T.E. and Chianelli, R.R., 2010. Salt separation processes in the saltcedar Tamarix ramosissima (Ledeb.). *Communications in soil science and plant analysis*, *41*(10), pp.1271-1281.https://doi.org/10.1080/00103621003734281.

Terrones, A. and Juan, A., 2023. First dated phylogenetic reconstruction of the genus *Tamarix*. *Botanical Journal of the Linnean Society*, 203, pp. 209–226, https://doi.org/10.1093/botlinnean/boad025.

Tracy, J.L. and Robbins, T.O., 2009. Taxonomic revision and biogeography of the Tamarix-feeding Diorhabda elongata species group. *Zootaxa*, 2101, pp. 1–52, https://doi.org/10.11646/zootaxa.2101.1.1.

Veisi, M., Sheidai, M. and Koohdar, F., 2023. Anatomical study of some Tamarix species in Iran. *Acta Botanica Hungarica*, 65, pp. 413–420. https://doi.org/10.1556/034.65.2023.3-4.11.

Villar, J.L., Alonso, M.Á., Crespo, M.B. and Martínez-Azorín, M., 2023. Nomenclatural type identification of names in north African Tamarix (Tamaricaceae). *Plants*, *12*(23), p.3969. https://doi.org/10.3390/plants12233969.

Zhang, J.W., Rozario, D., Duan, S.M. et al., 2018. Epidermal characters of Tamarix from Northwest China and taxonomic implications. *Journal of Palaeogeography*, 7, pp. 179–196, https://doi.org/10.1016/j.jop.2018.01.003.

Genetic Diversity and Trait Association Analysis in the Economically Important Fiber-Producing Species Calotropis procera (Aiton) W.T. Aiton

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Received: 2026-06-09 Accepted: 2026-08-10

Abstract

Calotropis procera (Aiton) W.T.Aiton is an evergreen, perennial shrub of the family Apocynaceae which has been utilized in traditional medicinal systems in North Africa, the Middle East Asia, as well as Souteast Asia. We can also cite this plant importance as a fodder, and used for fuel purposes, timber and fiber production, phytoremediation, and synthesis of nanoparticles. From the economic point of view, this species is in demand for its pharmacological applications. Beside the above said applications, *C. procera* has also been used as an ornamental species. We have limited geographical distribution of this species in Iran and almost no report is available on genetic diversity, gene flow and populations' genetic structure of this precious plant in the country. We used SCoT molecular markers to conduct a population genetic study of *C. procera* and to generate novel data on the associations of genetic regions with geographical, landscape as well as important morphological characters that are related to fiber production. DCA analysis showed that SCoT loci are appropriate molecular markers for population genetic studies in *C. procera* bevause these loci are scattered throughout the genome. Genetic diversity analyses revealed some private SCoT bands or loci in the geographical populations studied. A moderate level of genetic polymorphism was observed in these populations and DAPC revealed frequent and extensive gene flow among the studied populations, probably due to cross-pollination in these plants. Association studies identified several SCoT loci significantly associated with geographical and landscape variables. We also identified some genetic regions, which are closely associated with morphological characteristics related to fiber production, as well as loci associated with fiber color.

Keywords: *Calotropis procera* (Aiton) W.T. Aiton, CCA, Gene flow, LFMM, RDA, SCoT

Introduction

Calotropis procera (Aiton) W.T. Aiton, also known as the apple of Sodom, is an economically important plant species whose native range extends from Macaronesia

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and tropical Africa to Indo-China (Kaur et al., 2021). This evergreen perennial shrub belongs to the family Apocynaceae and has long been utilized in traditional medicinal systems of North Africa, the Middle East, and Southeast Asia. The species contains several biologically active compounds and has been investigated for various pharmacological properties. However, the species is also recognized as a toxic plant due to the presence of cardiac glycosides and other secondary metabolites. In addition to its reported medicinal uses, *C. procera* has attracted attention because of its applications in fiber production, phytoremediation, nanoparticle synthesis, fuel production, and as an ornamental plant.

C. procera is an evergreen shrub or small tree that usually reaches a height of 2.5–6 m,and possesses a deep taproot system. Young stems are grayish-green, while mature stems develop fissured bark. The species is characterized by large opposite succulent leaves covered with a waxy layer, abundant milky latex, and attractive white-to-purple flowers arranged in umbellate inflorescences. The fruits are inflated follicles containing numerous seeds bearing silky hairs that facilitate long-distance dispersal by wind (Kaur et al., 2021). *C. procera* is mainly distributed in the southern and southeastern parts in Iran, particularly along the Persian Gulf and Oman Sea Coasts. Natural populations have been reported from Hormozgan, Bushehr, Khuzestan, Kerman, and Sistan and Baluchestan provinces, where the species occurs in arid and semi-arid habitats, coastal plains, sandy soils, and disturbed areas (Mozaffarian, 1996; Assadi et al., 2008; Kaur et al., 2021).

In addition to its ecological and economic importance, *C. procera* has considerable ethnobotanical significance across several regions, particularly in arid and semi-arid areas of the Middle East and South Asia, including Iran. Ethnobotanical surveys have reported the traditional use of different parts of this species in folk medicine for the treatment of skin diseases, inflammation, rheumatism, as well as for topical treatment of various ailments, despite its known toxicity (Ghisalberti, 2004; Khan et al., 2015). In southern Iran, local communities have also been documented to use this plant in traditional practices, reflecting its long-standing role in local knowledge systems. However, due to the presence of potent cardiac glycosides, the application of this species requires caution and is generally limited to traditional, non-standardized applications (Kaur et al., 2021).

C. procera is well developed to arid and semi-arid climates, and can tolerate very low annual rainfall (150 mm) and dry seasons lasting up to 10 months. Mean annual monthly temperatures where *C. procera* is found are generally in the range 20-30°C. *C. procera* is not frost tolerant and can grow in a wide variety of soil types, including alkaline and saline soils, though it prefers well-drained sandy soils. It occures across a braod altitudinal range, from exposed coastal sites to medium elevations up to 1300 m (Kaur et al., 2021).

Due to morpho-physiological adaptations and ability to tolerate various abiotic stresses, *C. procera* has naturalized beyond its introduced range and is considered

an invasive weed in several parts of the world, particularly in pasture, overgrazed rangelands, and poorly managed hay fields. This taxon can compete with desirable pasture species and form dense thickets that interfere with livestock management, particularly mustering activities. Moreover, *C. procera* is known to contain *Calotropis* which is a cardiac poison, and for this, the plant is a main suspect for death of sheep and goats in West Africa (El-Badwi et al., 1998). This species is complicated to control and eradicate, mainly because its seeds are wind-dispersed and its plants are fire resistant. In addition to wind pollination, the carpenter bees *Xylocopa pubescens* and *Xylocopa fenestrata* visit this weed and serve as the main pollinators, while, the workers of *Apis florea* act as its minor pollinators (Ali and Ali, 1989). *C. procera* produces flowers and fruits throughout the year (Little et al., 1974), and each plants may produce hundreds to thousands of seeds annually. The seeds exhibited a high germination rate (89%), with germination occurring within 7-64 days after sowing in a potting mix (Francis, 2002). These characteristics facilitate the expansion of this species within its habitat place and further invasion of new areas.

C. procera is highly similar to the closely related species *C. gigantea*, and the two species are often confused. However, *C. gigantea* grows taller (4.5 m and above) and has smaller leaves borne on thicker leaf-bearing stems up, which can reach to 6 cm in diameter, compared with approximately 1 cm in *C. procera* (Crothers and Newbound, 1998).

The flowers of *C. gigantea* are more open and have petals with a uniform mauve-pink coloration. In Iran, *C. procera* is restricted to southern part of the country, where it occurs in a limited number of geographically distinct populations. Given the ecological and economic importance and invasive characteristics of *C. procera*, as well as its limited distribution in Iran, we conducted a population genetic study of this species using SCoT (Start Codon Targeted) molecular markers. SCoT markers have previously been reported as an appropriate molecular marker for population genetic studies of this species (Muriira et al., 2018). These markers prefentially target candidate functional genes and are based on short conserved sequences surrounding the start codons of plant genes. Consequently, SCoT markers offer several advantages, including no requirement for prior genomic information, high reproducibility, and high throughput (Zhang et al., 2019).

Identifying putatively adaptive genetic variation is a key objective in landscape genetic studies. Single nucleotide polymorphisms (SNPs), which represent single base-pair variations in the genome, along with other molecular markers such as SSRs and SCoTs, are widely used to detect genetically differentiated loci (outlier loci) that may be subjected to natural selection. These approaches also enable the assessment of associations between genetic variation and environmental variables while accounting for neutral evolutionary processes, including population genetic structure and demographic history (Zhang et al., 2019; Garot et al., 2021). In this study, we focused on SCoT molecular

markers to investigate genetic diversity and their associations with morphological, geographical, and environmental variables in *C. procera*. Multivariate analytical approaches, including RDA (redundancy analysis), DAPC (discriminant analysis of principal components), and LFMM (latent factor mixed models), were applied to identify potential patterns of genetic structure and association (Collins and Jombart, 2015; Zhang et al., 2019).

Material and methods

Plant sample studies

In total, 80 plants from 12 geographical populations were studied for both morphological and molecular investigations (Table 1). Plant specimens were identified based on morphological characteristics and available taxonomic references by Mr. Mohammadreza Kordasti. Voucher specimens of all studied populations were deposited in the Herbarium of Shahid Beheshti University (HSBU), Tehran, Iran. The corresponding herbarium accession numbers are provided in Table 1.

Morphological studies

Twelve morphological characters related to leaf, stem, fruit, and seed morphology were evaluated in the studied populations. These characters were selected based on their taxonomic significance, observed variability in *C. procera*, and their potential relevance to fiber production and seed dispersal, as determined by previous morphological studies of the species (Kaur et al., 2021; Khadivi and Mirheidari, 2025). These characters included: leaf base (funnel or heart), leaf length-to-width ratio (L/W), leafstalk (visible or gone), leaf thickness (thin or thick), internode size, peduncle size, plant height, follicle size, seed color (light brown or dark brown), seed size, popup color (shiny white or creamy), and popup seed size.

Morphological observations and measurements were performed on all 80 sampled individuals representing the 12 geographical populations. The subsequent analyses were restricted to mature and

Table 1. *Calotropis procera* populations and their locality

No	POP	Habit	soil type	Humidity	Temperature	Voucher number (Herbarium: HSBU)
1	Systan, Pashmak	Shurbs	Sandy- Clay	50%	40-28	HSBU-2022101
2	Boshehr, Ab pakhsh	Tree	Sandy- Clay	25%	50-38	HSBU-2022102
3	Kerman, Kahnoj	Shurbs	Sandy- Clay	31%	45-30	HSBU-2022103
4	Hormozgan, Bandar jask	Shurbs	Sandy	70%	37-33	HSBU-2022104
5	Khozestan, Dezfol	Shurbs	Sandy	18%	45-23	HSBU-2022105
6	Hormozgan, Minab	Shurbs	Sandy	40%	45-30	HSBU-2022106
7	Kerman, Bam	Tree	Sandy- Clay	33%	44-24	HSBU-2022107
8	Khozestan, Shoahtar	Shurbs	Sandy	15%	50-28	HSBU-2022108
9	Fars, Girkarzin	Shurbs	Sandy- Clay	33%	44-24	HSBU-2022109
10	Boshehr, Samal	Tree	Sandy- Clay	20%	50-35	HSBU-2022101
11	Hormozgan, Goarzonak	Shurbs	Sandy	40%	43-30	HSBU-2022110
12	Sistan, Chabahar	Shurbs	Sandy- Clay	65%	38-35	HSBU-2022112

healthy individuals. Qualitative and quantitative characters were recorded using a stereomicroscope and graph paper following standard botanical procedures. The obtained data were subsequently used for statistical analyses of morphological variation among populations.

Molecular studies

For SCoT molecular analyses, fresh leaf samples were collected and immediately dried in silica gel powder. Genomic DNA was extracted using the CTAB-activated charcoal protocol described by Sheidai et al. (2019). The quality of the extracted DNA was assessed by electrophoresis on a 0.8% agarose gel.

A preliminary screening was carried out using several SCoT primers developed by Collard and Mackill (2009). Based on amplification quality, band sharpness, reproducibility, and the degree of polymorphism obtained, three primers (SCoT1: CAACAATGGCTACCACCA, SCoT2: CAACAATGGCTACCACCC, and SCoT41: CAATGGCTACCACTGACA) were selected for further analyses. These primers produced clear, reproducible, and polymorphic banding patterns suitable for genetic diversity and association studies in *C. procera*.

PCR amplifications were performed using a ready-mix master mix (Parts Tools Co., Iran). Each reaction contained 20 ng of genomic DNA, 1 U of Taq DNA polymerase, and other reaction components in a final volume of 25 µl.

The amplification reactions were conducted in a Bio-Rad thermocycler (USA) under the following conditions; an initial denaturation step of 4 min at 95°C, followed by 45 cycles of 1 min at 94°C, 1 min at the primer-specific annealing temperature (49°C for SCoT1, 56°C for SCoT2, and 53°C for SCoT41), and 1 min at 72°C. A final extension step of 7 min at 72°C was performed.

The amplification products were separated on 2% agarose gel and stained with PowerLoad (PowerLoad, Kosar Co., Iran). Fragment sizes were estimated using a 100 bp DNA ladder (Fermentas, Germany).

Data analysis

Morphological analyses

Standardized data (mean= 0, variance= 1) were used for multivariate statistical analyses. Morphological data analyses were performed in PAST software version 4.03 (Hammer et al., 2001). A heat-map based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was constructed to study morphological divergence among individuals and their relationships with geographical and landscape features.

All statistical analyses were performed using individual-based morphological data, wheras population-level summaries were used exclusively for descriptive purposes and were excluded in multivariate analyses.

A Mantel test was conducted to examine whether morphological distance is associated with geographical distance among the studied populations (Mantel, 1967; Sokal and Rohlf, 1995). Pearson correlation coefficients were also calculated among variables.

Genetic diversity analyses

The SCoT bands obtained were treated as binary characters (Podani, 2000) and used for further analyses. Detrended correspondence

analysis (DCA) was performed to evaluate the distribution pattern of SCoT bands obtained for population genetic analysis (Hill and Gauch, 1980). Similarly, discriminant power of bands was determined by the DAPC method, which is appropriate for binary data (Jompard et al., 2010), as well as Neis' Gst value as determined in the POPGENE program. Genetic diversity parameters, including the number of effective alleles (Ne), Shannon index (I), Nei's genetic diversity (He), unbiased He (UHe), and percentage of polymorphism (P%), were determined in the studied populations. Genetic differentiation of the populations was studied by two different approaches. First, we used UPGMA (Unweighted Paired Group using average) cluster analysis to show if the replicates of each population are separated from the others. AMOVA (Analysis of molecular variance) was then performed using GeneAlex software to partition molecular variation among and within populations. Neis' genetic distance was also being determined among the studied populations, and populations grouping was subsequently performed based on DAPC analysis as well as heat map construction based on the Jaccard similarity index in R package 4.1. DAPC analysis was used to identify discriminating SCoT loci that differentiate the studied populations of *C. procera*, and an assignment test was performed to assess gene flow among the studied plants.

Association studies

Association analysis was performed using multiple approaches, from Chi-square and Fisher's exact test to DAPC and Bayesian-based LFMM (Collins and Jombbart, 2015; Frichot et al., 2013), combined to study associations between genetic data and both morphological and landscape data. LFMM algorithms correct for unobserved confounders, improving power and controlling false discoveries, with LFMM_ Ridge and LFMM_Lasso functions based on regularized least-squares solutions. The R package LFMM fits latent factor mixed models for genome-wide (GW), genome-environment (GE), and epigenome-wide (EW) association studies. Due to complex interactions between genetic structure and environmental variables, LFMM analysis used PCA followed by Bonferroni and false discovery rate (FDR) corrections to avoid type I and II errors, in R 4.1. DAPC accuracy was assessed by cross-validation in R 4.1. CCA and RDA were used to identify the contribution of environmental variables to population genetic structure. CCA, the canonical form of correspondence analysis (CA), is a direct gradient analysis where explanatory variables intervene in the CA solution, representing only explainable correspondence. It suits unimodal response variables, preserves chi-squared (χ^2) distances, and identifies response variables maximally related to linear combinations of explanatory variables, their importance determined by correlation to the final ordination (Greenacre, 2007). RDA, used in landscape genomics, identifies how spatial and environmental factors structure genetic variation among populations, modeling linear relationships among environment predictors and genomic variation. Integrating ordination and multiple regression, it

represents data matrix variation in reduced dimensions via multiple regressions through *principal components analysis (Legendre and Legendre, 2012; Segovia et al., 2020).*

Dispersal versus vicariance

Reconstruction of ancestral states (RASP) analysis was performed to investigate dispersal versus vicariance of SCoT loci in a population geographical context as implemented in RASP software. Statistical Dispersal–Vicariance Analysis (S-DIVA), as implemented in RASP software, was used to infer the historical biogeographic relationships among the populations based on a likelihood-based approach. (Yu et al., 2015).

Results

Morphological variation and population relationships

UPGMA dendrogram (Fig. 1) based on morphological data revealed that the studied populations have a high degree of morphological similarity which formed different clusters at approximately 0.15 Euclidean distance. However, some of the studied populations show a higher degree of within-population similarity as the plant samples from these populations were closely grouped. This holds true for populations 9 and 10. The other populations show an admixture of plant samples. PCA analysis of morphological features, revealed that morphological similarity among populations 9 and 10 was mainly due to leaf thickness, while similarity of populations 1, 4, and 6, is due to the size of internode.

Figure 2 illustrates clustering patterns based on combined datasets, showing morphological similarity and admixture among populations, with weak and non-significant association with geographical and environmental variables. Heatmap constructed from combined morphological, geographical, and landscape data revealed that morphological characters such as internode size, plant height, and leaf length-to-width ratio showed similar patterns in grouping of the studied populations. Similar patterns were also observed for peduncle size, temperature, leaf thickness, leaf base, and leaf stalk (Fig. 2). However, other geographical and landscape variables showed weak and non-significant

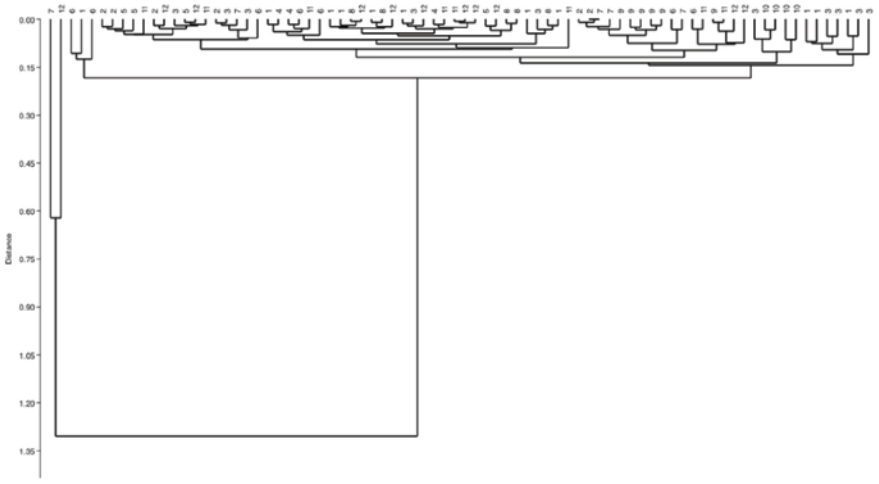


Fig. 1. UPGMA dendrogram of *Calotropis procera* populations based on morphological characteristics

associations with morphological grouping, indicating a generally mixed pattern of relationships. These findings were further supported by the non-significant Mantel test between morphological and ecogeographical distances among populations (R = 0.038, p = 0.33).

Genetic diversity analyses

DCA plot (Fig. 3), revealed that SCot molecular markers are scattered along the plot and therefore are distributed throughout the genome and are suitable for population genetic studies. We obtained 39 distinct and stable SCoT loci for population genetic analyses.

Genetic diversity parameters determined in

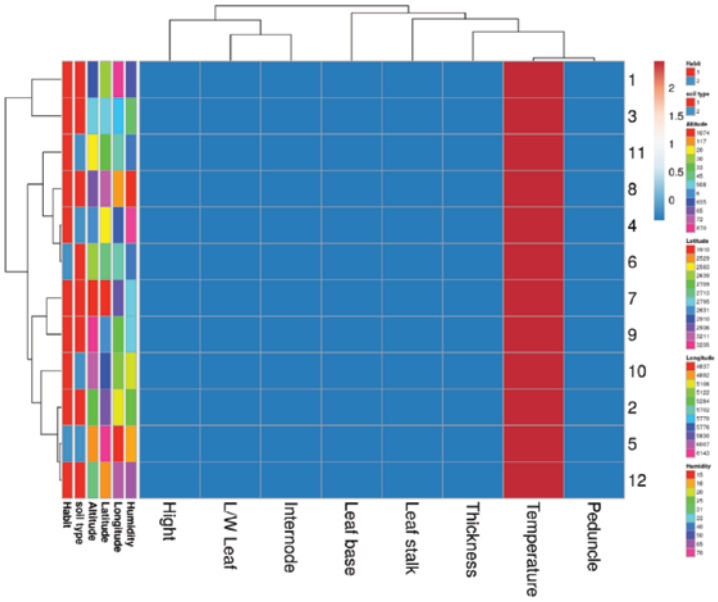


Fig. 2. Heatmap of morphological, geographical, and landscape variables in studied *Calotropis procera* populations.

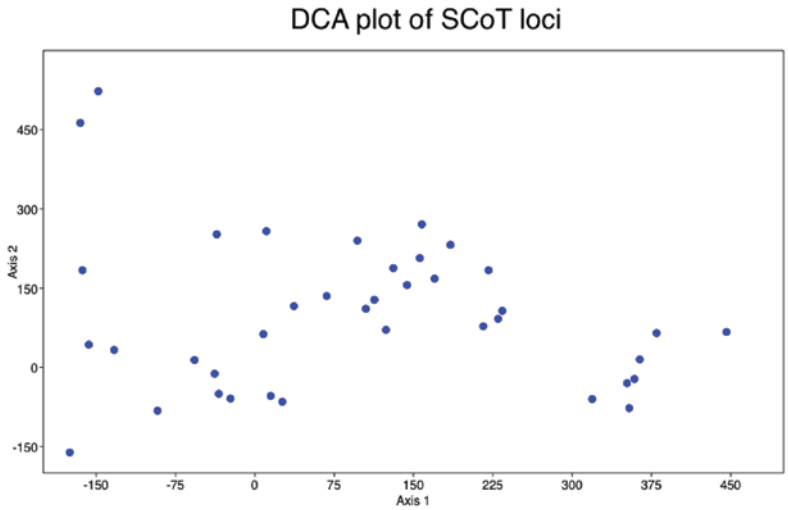


Fig. 3. DCA plot of SCoT loci obtained in *Calotropis procera* populations

the studied populations are provided in Table 2. Polymorphism percentages varied from 25% in population 4 to 64% in population 9. The moderate genetic polymorphism observed in these populations may be due to cross pollination behavior of this species. The mean gene diversity (He), varied from 0.09 to 0.191 in the same populations. This low gene diversity may be related to high magnitude of gene flow among geographical populations, as will be covered in coming paragraphs. AMOVA-paired revealed significant genetic difference (P< 0.01), in most of the studied populations, which shows inspire of frequent gene flow among populations, they still have a mean genetic distinctness which differentiate them from each other. This is further supported by overall AMOVA result which revealed that 70% of genetic variance is due to within population genetic variability and 30% due to among population genetic difference.

Nei genetic distance obtained among populations ranged from 0.02 to 0.22 only (Table 3). This is due to high level of genetic similarity in the studied populations (> 0.87). We obtained a significant correlation between genetic distance and geographical distance of the studied populations (r = 0.15, p. < 0.01), by Mantel test.

Genetic grouping and gene flow analysis

The mean Gst value of SCoT loci versus number of migration (Nm value), obtained by pop gene, produced Gst = 0.3688, and Nm = 0.85, which show a high magnitude of gene flow for SCoT loci. This is further

Table 2. Genetic diversity parameters in *Calotropis procera* populations

Pop	Na	Ne	I	He	uHe	%P
Pop1	1.333	1.368	0.339	0.223	0.233	66.67%
Pop2	1.103	1.342	0.283	0.192	0.209	51.28%
Pop3	1.179	1.310	0.277	0.184	0.194	53.85%
Pop4	0.795	1.150	0.138	0.091	0.110	25.64%
Pop5	0.897	1.248	0.227	0.150	0.171	43.59%
Pop6	1.154	1.372	0.312	0.212	0.228	56.41%
Pop7	0.744	1.257	0.208	0.143	0.159	35.90%
Pop8	1.051	1.279	0.255	0.167	0.186	51.28%
Pop9	1.282	1.316	0.296	0.191	0.209	64.10%
Pop10	0.513	1.155	0.130	0.088	0.101	23.08%
Pop11	0.718	1.185	0.173	0.113	0.120	35.90%
Pop12	1.128	1.300	0.266	0.175	0.184	56.41%

a. N: No of plants studied, b. Na: No. of alleles, c. Ne: Effective No. of alleles, d. He: Gene diversity, e. UHe: Unbiased gene diversity, f. %P: Polymorphism percentage

Nei genetic distance obtained among populations ranged from 0.02 to 0.22 only (Table 3). This is due to high level of genetic similarity in the studied populations (> 0.87). We obtained a significant correlation between genetic distance and geographical distance of the studied populations (r = 0.15, p. < 0.01), by Mantel test.

Table 3. Nei genetic distance among *Calotropis procera* populations (populations 1-12 are according to Table 1)

Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10	Pop11	Pop12	
0.000												Pop1
0.106	0.000											Pop2
0.102	0.163	0.000										Pop3
0.223	0.222	0.285	0.000									Pop4
0.066	0.140	0.147	0.310	0.000								Pop5
0.069	0.107	0.105	0.259	0.146	0.000							Pop6
0.055	0.154	0.197	0.367	0.086	0.131	0.000						Pop7
0.068	0.115	0.188	0.332	0.066	0.079	0.088	0.000					Pop8
0.035	0.132	0.108	0.335	0.060	0.085	0.069	0.046	0.000				Pop9
0.107	0.147	0.184	0.296	0.068	0.167	0.135	0.144	0.123	0.000			Pop10
0.029	0.145	0.141	0.270	0.046	0.110	0.063	0.079	0.038	0.069	0.000		Pop11
0.044	0.150	0.154	0.303	0.045	0.143	0.061	0.087	0.057	0.051	0.037	0.000	Pop12

supported by DAPC assignment analysis. Genetic grouping of the studied populations (Figure 4.I), differentiated theme in three main groups. Assignment test of plants by DAPC placed them in the three main genetic groups and also revealed a high level of gene flow and admixture among the studied populations (Figure 4.II).

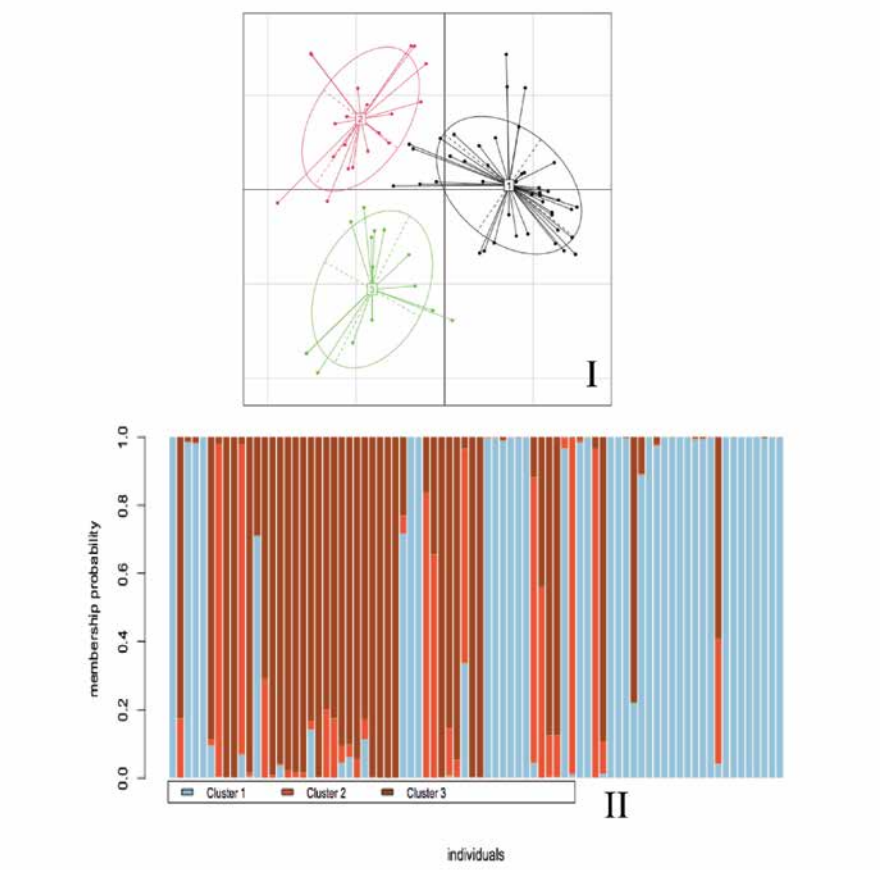


Fig. 4. I. DAPC plot of populations genetic groups in *Calotropis procera* plants studied, II. Assignment and admixture plot of *Calotropis procera* plants in three main genetic groups (clusters)

Dispersal versus vicariance

RASP analysis revealed that some of the populations were less involved in migration and gene flow as most of their plants were grouped together in an almost distinct clade. For example, populations 2, 3, 10 and 12 (Figure 5), show less degree of vicariance compared to the other studied populations. The reason for this is not known to us and it may be due to either presence of more severe ecological and environmental barriers they encounter, or shortage of frequent pollinating visitors in their geographical area.

Association studies

SCoT loci and geographical variables

Canonical correspondence analysis and RDA plots produced similar results and both produced significant association ($p < 0.01$) between SCoT loci and geographical variables. Here we provide only RDA plot to be brief (Figure 6). Some of the SCoT loci were closely associated with geographical variables. For example, SCoT loci. 24 and 31 are close to both longitude and altitude, while SCoT loci 2, 4, and 6, are closely associated to humidity. Loci 21 and 22

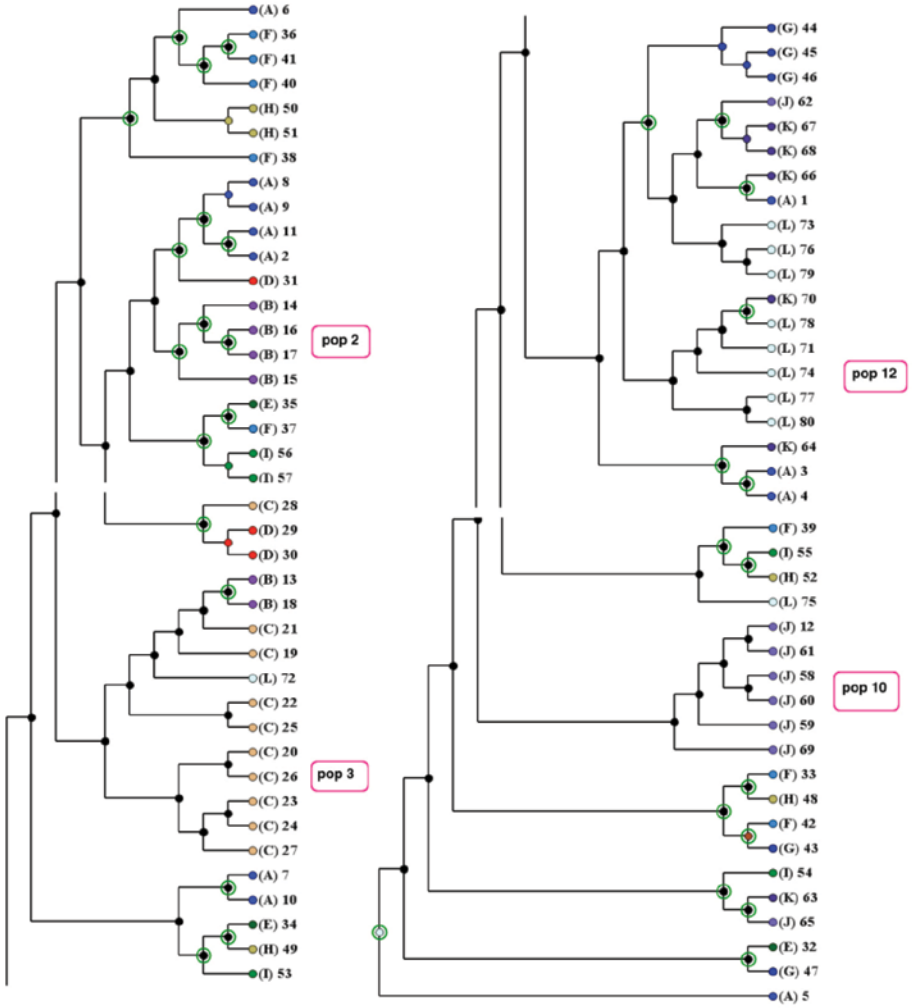


Fig. 5. RASP phylogenetic tree of *Calotropis procera* populations constructed by S-DIVA approach

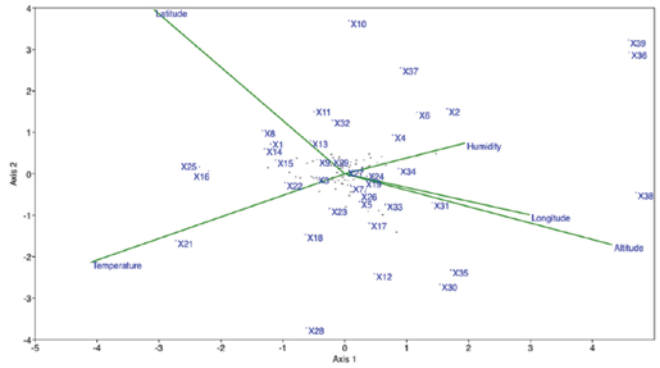


Fig. 6. RDA plot of SCoT loci against geographical variables showing close association between some of the loci and variables studied.

showed close association with temperature.

SCoT loci and soil type

Chi square analysis followed by Fishers' exact test produced significant association between SCoT loci and soil type (X-squared = 7.1, df = 2, p-value = 0.02) (Table 1). Similarly, DAPC analysis cross-validation (Figure 7-a), indicated that the model of association produced is efficient and identified three SCoT loci number 1, 3, 10, and 30, are significantly associated with the soil type habitat of plants. Ridge and Lasso models of LFMM analysis of the same data (Figure 7-b), followed by Bonferroni correction and false discovery rate test, also identified SCoT loci. No. 3, and 10, as well as 11 and 15, are significantly associated with the soil type.

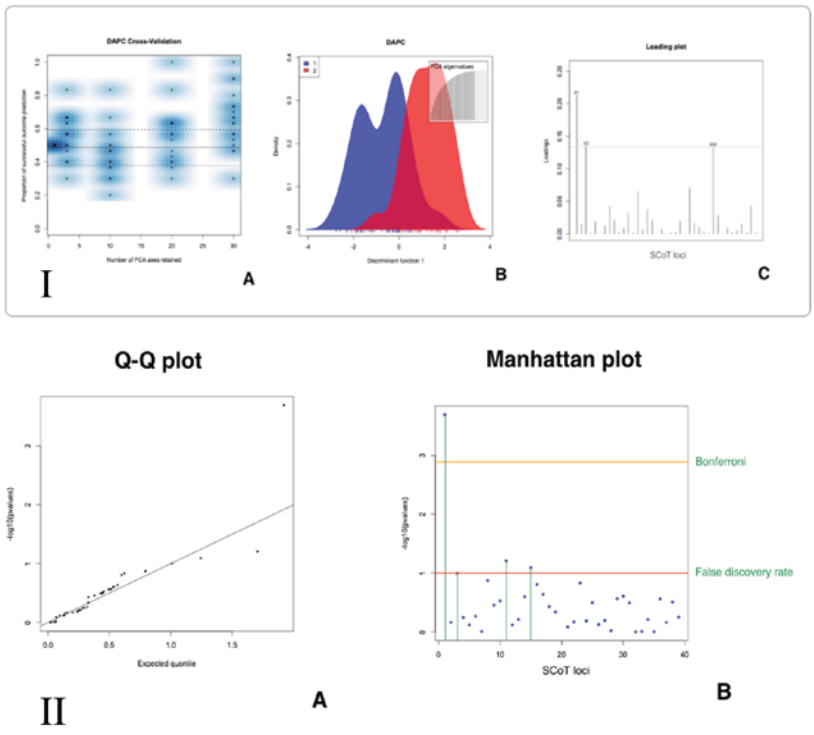


Fig. 7. I. DAPC association analyses of SCoT loci and the soil type, II. Q-Q plot and Manhattan plot of LFMM analysis showing significantly associated SCoT loci with the soil type.

SCoT loci and habit

Plants studied had two types of growth forms (Table 1). Although Fishers' exact test did not produce significant result, both DAPC and LFMM analyses showed significant association between some of the SCoT loci No. 7, 9, 11, 14, 15, 16, and 24, and habit form of the studied plants.

Association studies between SCoT loci and morphological characters

Some of morphological characters in *C. procera* are associated with fibber production. These are the size of internode, peduncle, seed size, and seed pod (follicle) which when dehisced releases seeds with silky white popups. Moreover, detailed observation of silky papas showed two types of colors, 1- shiny white and 2-creamy, which may be of economic importance. Significant association between genetic regions and these important morphological characteristics are of importance in future selection and breeding of this precious fiber producing plant species. Different methods used showed significant association between SCoT loci and significant morphological characters assumed to be related to fiber production. Details of SCoT loci associated with these characteristics are summarized and presented in Table 4.

Similar analysis for fiber color also produced significant association between SCoT loci and this interesting characteristics. As shown in Table 4, several significant associations were identified between SCoT loci and seed-related traits. Two SCoT loci (No. 2 and 26) were significantly associated with seed cob traits, whereas loci No. 14 and 23 showed significant associations with seed pod size. These characters are directly related to the fiber production and few SCoT loci are associated with them which can

Table 4. Association results of SCoT loci with morphological characters, geographical and landscape variables

Subject	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30	X31	X32	X33
Leaf base					+										+				+	+	+				+			+		+	+	+
Leaf width/length ratio					+	+	+																						+	+			
Leaf stalk	+		+	+	+	+	+			+				+	+		+			+	+	+	+		+			+	+	+			
Leaf thickness	+					+	+		+		+				+											+							
Internode	+								+									+	+	+	+	+			+					+			
Peduncle				+	+	+								+										+									
Plant height			+		+		+		+		+	+			+		+					+					+	+		+	+		
Fulicle size (seed pode)													+								+												
Seed color			+	+								+	+	+	+								+				+						
Seed popups size		+																					+										
popups color			+	+	+				+			+	+	+	+	+							+				+			+			
Soil type	+		+							+	+				+											+						
Altitude																	+									+							
Latitude	+							+	+				+													+							
Longitude																	+	+			+						+				+		
Humidity		+				+																		+									
Temperature			+																+	+													

be characterized and sequenced for future breeding and selection purposes. Some other interesting associations are between SCoT loci No. 18 and size of leaf stalk, SCoT loci No. 20 and 21 are related to the size of internode. These characters are also related to the size of plant and fiber production via more seed production. SCoT loci associated with the soil type and temperature, would also be important as they may contribute to the ecological and landscape tolerance for this plant species.

Discussion

Genetic and morphological diversity

This study reports morphological and genetic variability of *C. procera* in different localities of Iran. The studied populations showed both morphological and genetic admixture due to extensive gene flow, probably caused by cross-pollination. SCoT loci were efficient molecular markers for population genetic studies and germplasm evaluation of *C. procera*.

A moderate level of genetic polymorphism was obtained. Genetic diversity in *C. procera* has been analyzed using SSR and ISSR markers (Majeed et al. 2020; Navabpour et al., 2021), both showing moderate polymorphism (mean PIC = 0.575; Na = 3.625, Ho = 0.58 for SSR; ~36% for ISSRs), agreeing with our results. A significant correlation was found between genetic and geographical distance ($r = 0.15$, $p < 0.01$) by Mantel test, consistent with Majeed et al. (2020) ($r = 0.147$, $P = 0.010$).

AMOVA also showed significant genetic differences among populations. Genetically diverse populations can serve as an important resource for germplasm collection in breeding programs (Majeed et al., 2020; Navabpour et al., 2021). Ecological grouping based on combined genetic and environmental data revealed that populations tend to cluster partly according to geographical origin and environmental conditions. Although gene flow resulted in considerable genetic admixture, certain populations showed partial structuring reflecting adaptation to local conditions such as temperature, humidity, and habitat type. The weak but significant correlation between genetic and geographic distances supports isolation by distance in shaping population structure.

Overall, while *C. procera* populations in Iran are not strictly ecologically segregated, environmental heterogeneity may still contribute to subtle local differentiation. Both neutral processes (e.g., gene flow) and selective forces associated with habitat variability likely act simultaneously on the genetic structure of this species.

Association studies

This study identified genetic regions by SCoT loci significantly correlated with geographical and landscape variables as well as with important morphological traits related to fiber production, including fiber color in *C. procera*. These are novel findings. Different approaches used to identify genetic loci associated with geographical and ecological variables were all in agreement, improving understanding of associated SNPs or genetic regions and giving insights into contemporary evolutionary processes (Segovia et al., 2020).

Heterogeneous environmental conditions

bring about changes in genetic diversity, resulting in local adaptations (Zhang et al., 2019; Garot et al. 2021, Segovia et al. 2020). Studies on the genetic basis of local adaptation and identification of adaptive genetic loci or SNPs can improve knowledge of the genetic mechanism of local adaptation and species diversification within a genus (Zhang et al. 2019). *C. procera* is emerging as a new, yet undomesticated, fiber resource comparable to cotton and kapok (Majeed et al., 2020). Therefore, screening efficient genotypes from wild populations and identifying genetic regions associated with fiber production are important in the pre-domestication process. Desired genotypes can then be improved through conventional breeding. Identification of important genes is an important task in genetic studies of *C. procera*. For example, Ramadan et al. (2014) identified the Δ-pyrroline-5-carboxylate synthase (P5CS) gene, which synthesizes proline, a compatible solute that accumulates widely in plants to tolerate unfavorable conditions. Similarly, Shokry et al. (2014) detected a Usp-like gene in *C. procera*. The universal stress protein (USP) domain provides organisms with the ability to respond to environmental stresses. Mutwakil et al. (2017) studied *C. procera* response to drought and osmotic stress using polyethylene glycol and NaCl, with transcriptomic and metabolomics measurements across a five-day time-course, identifying a dynamic transcriptional response including changes in inositol signaling, stress response genes, and cytokinins, along with sharp increases of myo-inositol and elevated amino acid metabolites at later times.

Based on evaluated morphological traits related to fiber production, certain populations exhibited superior performance in key economically important characteristics such as internode length, plant height, and fiber-related seed and leaf traits. These populations may be considered potential candidate genetic resources for future breeding and domestication of *C. procera*. However, given the observed genetic admixture and absence of strong population-level structuring, these superior traits should be interpreted cautiously and further validated through multi-environmental and controlled breeding experiments before formal selection of elite genotypes.

In conclusion, we provided novel findings on genetic structure, gene flow as well as association of genetic regions to the geographical, land scape and important morphological characters related to fiber production in *C. procera*.

Funding

None

Conflicts of interest:

All authors have no conflict of interest

Availability of data and material

All data are available from the corresponding author on reasonable request.

Code availability

NONE

References

Ali, T. and Ali, S.I. (1989) 'Pollination biology of *Calotropis procera* subsp. *hamiltonii* (Asclepiadaceae)', *Phyton (Horn)*, 29(2), pp. 175–188.

Assadi, M., Maassoumi, A.A., Khatamsaz, M. and Mozaffarian, V. (eds.) (2008) *Flora of Iran*. Tehran: Research Institute of Forests and Rangelands.

Collins, C. and Jombart, T. (2015) *A tutorial for discriminant analysis of principal components (DAPC) using adegenet*. London: Imperial College London.

Crothers, M. and Newbound, S. (1998) *Rubber Bush (Calotropis procera)*. Northern Territory of Australia, Australia.

El-Badwi, S.M.A., Adam, S.E.I., Shigidi, M.T. and Hapke, H.J. (1998) 'Studies on laticiferous plants: toxic effects in goats on *Calotropis procera* latex given by different routes of administration', *Deutsche Tierärztliche Wochenschrift*, 105(11), pp. 425–427.

Francis, J.K. (2002) '*Calotropis procera* (Ait.) Ait.', in Francis, J.K. (ed.) *Wildland shrubs of the United States and its territories: thamnic descriptions*. General Technical Report IITF-WB-1. U.S. Department of Agriculture, Forest Service.

Frichot, E., Schoville, S.D., Bouchard, G. and François, O. (2013) 'Testing for associations between loci and environmental gradients using latent factor mixed models', *Molecular Biology and Evolution*, 30(7), pp. 1687–1699. https://doi.org/10.1093/molbev/mst063.

Garot, E., Dussert, S., Domergue, F., Joët, T., Fock-Bastide, I., Combes, M.C. and Lashermes, P. (2021) 'Multi-approach analysis reveals local adaptation in a widespread forest tree of Reunion Island', *Plant and Cell Physiology*, 62(2), pp. 280–292. https://doi.org/10.1093/pcp/pcaa160.

Ghisalberti, E.L. (2004) 'The medicinal plant *Calotropis procera*', *Fitoterapia*, 75(4), pp. 310–337.

Greenacre, M. (2007) *Correspondence Analysis in Practice*. 2nd edn. Boca Raton, FL: Chapman & Hall/CRC.

Hammer, Ø., Harper, D.A.T. and Ryan, P.D. (2001) 'PAST: paleontological statistics software package for education and data analysis', *Palaeontologia Electronica*, 4(1), pp. 1–9.

Hill, M.O. and Gauch, H.G. (1980) 'Detrended correspondence analysis: an improved ordination technique', *Vegetatio*, 42, pp. 47–58. https://doi.org/10.1007/BF00048870.

Jombart, T., Devillard, S. and Balloux, F. (2010) 'Discriminant analysis of principal components: a new method for the analysis of genetically structured populations', *BMC Genetics*, 11, 94. https://doi.org/10.1186/1471-2156-11-94.

Kaur, A., Batish, D.R., Kaur, S. and Chauhan, B.S. (2021) 'An overview of the characteristics and potential of *Calotropis procera* from botanical, ecological, and economic perspectives', *Frontiers in Plant Science*, 12, 690806. https://doi.org/10.3389/fpls.2021.690806.

Khadivi, A., Mirheidari, F., Tunç, Y., Saeidifar, A., Moradi, Y., Rabiei, V. and Razavi, F. (2026) 'Morphological characterization of *Calotropis procera* (Aiton) W.T. Aiton, a neglected medicinal plant', *PLOS ONE*, 21(2), e0341425. https://doi.org/10.1371/journal.pone.0341425.

Khan, M.A. et al. (2015) 'Ethnobotanical uses of medicinal plants in arid regions',

Journal of Ethnopharmacology, 173, pp. 1–15.

Legendre, P. and Legendre, L. (2012) *Numerical Ecology*. 3rd edn. Amsterdam: Elsevier.

Little, E.L. Jr, Woodbury, R.O. and Wadsworth, F.H. (1974) *Trees of Puerto Rico and the Virgin Islands*. Washington, DC: U.S. Department of Agriculture, Forest Service.

Majeed, A., Goel, B., Mishra, V., Kohli, R. and Bhardwaj, P. (2020) 'Elucidation of genetic diversity base in *Calotropis procera* – a potentially emerging new fibre resource', *Plant Genetic Resources*, 18(3), pp. 159–167. https://doi.org/10.1017/S1479262120000167.

Mantel, N. (1967) 'The detection of disease clustering and a generalized regression approach', *Cancer Research*, 27(2), pp. 209–220.

Mozaffarian, V. (1996) *A Dictionary of Iranian Plant Names*. Tehran: Farhang Moaser Publishers.

Muriira, N.G., Muchugi, A., Yu, A., Xu, J. and Liu, A. (2018) 'Genetic diversity analysis reveals genetic differentiation and strong population structure in *Calotropis* plants', *Scientific Reports*, 8, 7832. https://doi.org/10.1038/s41598-018-26275-x.

Mutwakil, M.Z., Hajrah, N.H., Atef, A. et al. (2017) 'Transcriptomic and metabolic responses of *Calotropis procera* to salt and drought stress', *BMC Plant Biology*, 17, 231. https://doi.org/10.1186/s12870-017-1155-7.

Navabpour, S., Yamchi, A. and Golcheshmeh, S. (2021) 'Assessment of genetic diversity between different accessions of *Calotropis procera* with ISSR molecular markers', *Plant Genetic Resources*, 8(1), pp. 17–28. https://doi.org/10.52547/pgr.8.1.2.

Podani, J. (2000) *Introduction to the Exploration of Multivariate Data*. Leiden: Backhuys Publishers.

Ramadan, A.M. and Hassanein, S.E. (2014) 'Characterization of P5CS gene in *Calotropis procera* plant from the de novo assembled transcriptome contigs', *Comptes Rendus Biologies*, 337(12), pp. 683–690. https://doi.org/10.1016/j.crvi.2014.09.002

Segovia, N.I., González-Wevar, C.A. and Haye, P.A. (2020) 'Signatures of local adaptation in the spatial genetic structure of the ascidian *Pyura chilensis*', *Scientific Reports*, 10, 14098. https://doi.org/10.1038/s41598-020-70798-1.

Sheidai, M., Shagholi, T., Keshavarzi, M., Koohdar, F. and Ijbari, H. (2019) 'Species delimitation and inter-specific gene flow in *Tamarix* L.', *Hacquetia*, 18(2), pp. 1–10. https://doi.org/10.2478/hacq-2019-0001.

Shokry, A.M., Al-Karim, S., Ramadan, A. et al. (2014) 'Detection of a Usp-like gene in *Calotropis procera* plant from genome contigs', *Comptes Rendus Biologies*, 337(2), pp. 86–94. https://doi.org/10.1016/j.crvi.2013.12.008.

Sokal, R.R. and Rohlf, F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. 3rd edn. New York: W.H. Freeman.

Yu, Y., Harris, A.J., Blair, C. and He, X. (2015) 'RASP (Reconstruct Ancestral

State in Phylogenies): a tool for historical biogeography', *Molecular Phylogenetics and Evolution*, 87, pp. 46–49. https://doi.org/10.1016/j.ympev.2015.03.008.

Zhang, X.X., Liu, B.G., Li, Y., Liu, Y., He, Y.X., Qian, Z.H. and Li, J.X. (2019) 'Landscape genetics reveals that adaptive genetic divergence in *Pinus bungeana* is driven by environmental variables relating to ecological habitats', *BMC Evolutionary Biology*, 19, 160. https://doi.org/10.1186/s12862-019-1489-x.

Effects of Zinc on Some Physiological and Biochemical Parameters of Dunaliella salina Algae

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Received: 2026-06-17 Accepted: 2026-08-25

Abstract

Recently, the application of algae as food and medicinal sources has gained significant attention. Heavy metals are recognized as major environmental pollutants because of their inherent toxicity. Their non-biodegradability and environmental persistence to natural degradation can result in prolonged exposure, posing serious threats to ecosystems and the health of living organisms. Algae have recently attracted considerable interest as valuable sources of food and bioactive compounds. Zinc (Zn) is an essential micronutrient in algal cells, contributing to enzymatic activity, photosynthetic processes, and overall cellular metabolism. This study evaluated the effects of different Zn concentrations (0.03, 0.06, 0.09, 0.25, and 0.5 µg/mL) on the physiological and biochemical characteristics of *Dunaliella salina* using a completely randomized design with three replications. The results showed that 0.03 µg/mL Zn significantly enhanced photochemical efficiency chlorophyll a, chlorophyll b , protein content, soluble sugars, and catalase activity by 50.76%, 154.5%, 88.88%, 13.86%, 89.47%, 71.42%, respectively, compared with the control. In contrast, the highest increase in carotenoid accumulation (92.85% compared to the control) was observed at 0.5 µg/mL Zn. Proline, total amino acids, ascorbate peroxidase, and polyphenol oxidase increased progressively with increasing Zn concentration, reaching their maximum values at 0.5 µg/mL and exceeding the control values by 68.18%, 88.88%, 88.46%, and 90.38%, respectively. Zn also affected carbohydrate metabolism in a biphasic manner, causing an initial increase followed by a decline in soluble sugar levels. Overall, the findings indicate that Zn concentration modulates photosynthetic performance, antioxidant defense, and metabolic activity in *D. salina*, while the species exhibits considerable sensitivity to changes in Zn availability. These characteristics highlight the potential of *D. salina* as a bio indicator for monitoring heavy-metal contamination in aquatic environments. These adaptive responses may also have potential significance for biotechnological applications, including the production of valuable secondary metabolites or the use of algae in heavy metal bioremediation strategies.

Keywords: *Dunaliella salina*, Zn strees, Antioxidant defense, Heavy metal bioremediation

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Doi: 10.48308/pae.2026.244255.1142

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Introduction

Dunaliella salina is a unicellular alga belonging to the family Dunaliellaceae (Salehipour-Bavarsad, et al., 2022). It was first identified along the Mediterranean coast of France and described by Teodoresco in 1905 (Riahi, 2002). *Dunaliella salina* is a green, halophilic, pear-shaped, biflagellated alga that lacks a cell wall and possesses a simple life cycle, enabling it to grow in a wide range of environmental conditions. This alga is capable of producing valuable pigments, particularly β-carotene (Whittaker, 1978). One of the most remarkable characteristics of *Dunaliella salina* is its ability to adapt to stressful environmental conditions, including extremely high salinity, elevated temperatures, nitrogen deficiency, and intense light exposure. This adaptability contributes to its broad distribution in natural habitats and diverse saline environments such as salt lakes and marshes (Massyuk, 1996). Zn (Zn), an essential micronutrient, plays numerous critical roles in the physiological and biochemical processes of living organisms. It is involved in the structure and function of more than 300 proteins, including key enzymes in metabolic pathways, and acts as a cofactor or structural component in various cellular processes, such as protein synthesis, carbohydrate metabolism, and cell membrane stabilization (Broadley et al., 2007).

In addition, Zn is involved in the regulation of gene expression and the maintenance of DNA stability, and may indirectly influence the photosynthesis process. Photosynthesis, the essential process by which light energy is converted into chemical energy, depends on the activity of several enzymes in which Zn may play either a catalytic or structural role. Zn also contributes to carbon fixation and may enhance photosynthetic efficiency in algae through its role in enzymes such as carbonic anhydrase (Palomares et al., 2004). Under environmental stress conditions, Zn functions as an antioxidant and helps reduce damage caused by reactive oxygen species (ROS). This element can enhance cellular tolerance to environmental stress by promoting the production of non-enzymatic antioxidants and modulating the activity of antioxidant enzymes (Pilon & Várkonyi, 2017). At low concentrations, such as 0.03- 0.09 µg/mL, Zn may improve photosynthetic efficiency, increase the activity of metal-dependent enzymes, and enhance cellular tolerance to environmental stress. These stimulatory effects are generally attributed to meeting cellular physiological Zn requirements and activating metabolic pathways associated with growth and survival (Kabata-Pendias, 2011; Nagajyoti et al., 2010). On the other hand, increasing Zn concentrations beyond the optimal range can exert inhibitory or toxic effects on the algal cell. At higher concentrations, particularly at or above 0.5 µg/mL, Zn may interfere with the uptake and balance of other essential elements, such as iron, magnesium, and copper, thereby disrupting cellular ionic homeostasis (Broadley et al.,2007; Xiong, 2006). Furthermore, excessive accumulation of Zn can promote the generation of reactive oxygen species (ROS) and induce oxidative stress, a condition in which the balance between the ROS production and antioxidant scavenging is disturbed, resulting in increased damage

to lipids, proteins, and nucleic acids. Consequently, photosynthetic activity may decline, cell growth is inhibited, and, in severe cases, cell death may occur (Cakmak, 2000). Determining the concentration at which Zn begins to exert toxic effects on algal growth, photosynthesis, and biochemical parameters is essential for evaluating the health of aquatic ecosystems and establishing permissible pollutant limits (Wang, 2002). Understanding how Zn is absorbed, metabolized, and released by algae can also provide insights into the bioavailability of this element to other organisms within the food chain, as well as its broader biogeochemical cycling in aquatic ecosystems (Sunda & Huntsman, 1995). In some cases, optimal Zn concentrations may enhance the production of valuable secondary metabolites in algae, such as carotenoids, which have significant for biotechnological applications (Rathur et al., 2015). In *Dunaliella salina*, a microalga known for its remarkable adaptability to harsh environmental conditions, responses to Zn may depend on its concentration, exposure time, and culture conditions. Therefore, investigating the effects of different Zn concentrations within the range of 0 -0.5 µg/mL is particularly important for determining the optimal balance between growth stimulation and the onset of Zn toxicity. Such studies can contribute to a better understanding of the mechanisms underlying heavy metal tolerance, photosynthetic regulation, and antioxidant responses in this alga.

Material and methods

Preparation of algae

Dunaliella salina, isolated from Lake Urmia (West Azerbaijan Province, Iran), was used as the experimental microalga in this study. Zn was applied at concentrations ranging from 0.03 to 0.5 µg/mL. The selected concentration range was determined through preliminary range-finding experiments designed to identify both growth-promoting concentrations and the highest non-lethal exposure level. All experimental procedures were conducted in 2023 at the Bioinstrumentation Laboratory, Faculty of Science, University of Mohaghegh Ardabili.

Preparation of Johnson's culture medium

To prepare Johnson's culture medium, 75.87 g of NaCl, 8.9 g of $MgCl_2 \cdot 6H_2O$, 0.53 g of $CaCl_2 \cdot 2H_2O$, 2.3 g of Na_2SO_4, and 0.85 g of K_2SO_4 were dissolved in distilled water and diluted to a final volume of 1 L. The medium was then sterilized by autoclaving at 120°C and 1 atm for 20 minutes. During preparation of the culture medium, 25.3 g of Tris base was added to adjust the pH to 7.5. Because trace elements are heat sensitive, 5 g of each of KNO_3, KH_2PO_4, and trace elements were added to the basal culture medium after it had cooled.

Algal cultivation and treatment

Sterilized 250-mL Erlenmeyer flasks were each filled with 100 mL of the prepared culture medium (1.5 M) and inoculated with algal cells. The remaining volume in the flasks provided sufficient headspace for gas exchange during algal growth. The flasks were then incubated for two weeks at 15°C in a germinator under 16 h light/8 h dark photoperiod. During the incubation period, the cultures were manually agitated every two days to prevent cell sedimentation. The experimental treatments included

a control group and cultures exposed to Zn concentrations of 0.03, 0.06, 0.09, 0.25, and 0.5 µg/mL. For each treatment, the required Zn concentrations were added to the culture medium as Zn sulfate. Subsequently, algal cells obtained from the initial cultivation step were exposed to the respective Zn concentrations and maintained under the same conditions in the germinator for an additional 14 days. The cultures were manually agitated every two days throughout the treatment period.

Measurement of physiological parameters

Optical density(OD)

Before and after the Zn treatments, 3 ml of each algal culture sample was transferred to a cuvette, and its absorbance was measured at 680 nm using a spectrophotometer. The absorbance value indicates the amount of light absorbed by the sample at the specified wavelength, with higher absorbance values generally indicating greater optical density and, under appropriate measurement conditions, a higher cell concentration.

Chlorophyll fluorescence

Chlorophyll fluorescence of the algal samples was measured using a PAM (Pulse-Amplitude Modulation) fluorometer. Prior to measurement, the samples were dark-adapted for 30 min to ensure that all photosynthetic reaction centers were in the open state. Basal fluorescence (F_0) was then measured under dim light conditions, followed by the application of a saturating light pulse to record maximum fluorescence (F_m). The maximum quantum efficiency of photosystem II (F_v/F_m) was calculated using the following equation: $F_v/F_m = (F_m - F_0) / F_m$ All measurements were performed at room temperature using three biological replicates. For data normalization, when necessary, cell concentration was determined based on light absorbance at a specific wavelength or dry biomass weight (Maxwell & Johnson, 200 0).

Chlorophyll a, b and Carotenoid levels

First, 0.5 g of a fresh algal sample was ground in a porcelain mortar. The resulting extract was then brought to a final volume of 20 mL using 90% acetone and centrifuged at 6000 rpm for 20 min. The supernatant obtained after centrifugation was transferred to a cuvette, and the absorbance values of chlorophyll a, chlorophyll b, and carotenoids were measured at wavelengths of 663, 645, and 470 nm, respectively, using a spectrophotometer. chlorophyll content was expressed as mg g^{-1} fresh weight, whereas carotenoid content was expressed as µg g^{-1} fresh weight using the following equations. In these equations, V represents the final volume of the extract, and W represents the fresh weight of the sample (Lichtenthaler, 1987)

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V / (1000 \times W)$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times V / (1000 \times W)$$

$$\text{Carotenoids} = [1000 \times A_{470} - 3.27 \times (\text{Chl a}) - 104 \times (\text{Chl b})] / 227$$

Proline content determination

To determine proline content, 0.5 g of frozen algal sample was ground, and 5 mL of 3% sulfosalicylic acid was added. The mixture was immediately transferred to microtubes and kept on ice. Samples were then centri-

fuged using a refrigerated centrifuge for 15 min at 15,000 rpm at 4°C. Next, 1 mL of the supernatant was transferred to a new tube, and 1 mL of acetic acid and 1 mL of ninhydrin were added. The mixture was incubated in a 100°C water bath for 1 h, followed by cooling in an ice bath. Then, 2 mL of toluene was added to each sample and mixed for 20 s using a vortex mixer. After mixing, two phases were formed, and the absorbance of the upper toluene phase was measured using a spectrophotometer (PG Instruments, Model T80 +, UK) at 520 nm (Bates et al.,1973).

Measuring biochemical parameters

Protein content measurement

For protein extraction, 0.1 g of fresh algal biomass was thoroughly ground in liquid nitrogen, and 6 mL of 0.01 M phosphate buffer was added. The resulting protein extract was transferred to microtubes. Samples were then centrifuged at 4 °C for 15 min at 13,000 rpm using a refrigerated centrifuge. After centrifugation, the supernatant (upper phase) was transferred to new microtubes, stored at −80 °C, and used to determine protein concentration and the activities of various enzymes. For protein quantification, 0.5 mL of the prepared protein extract was mixed with 2 mL of Bradford reagent. The absorbance of the resulting mixtures was measured at 595 nm using a spectrophotometer (Bradford, 1976).

soluble sugar determination

For soluble sugar determination, 0.1 g of fresh algal tissue was mixed with 2.5 mL of 80% ethanol. The mixture was then placed in a water bath at 90°C for 60 minutes to extract soluble carbohydrates. Following extraction, the mixture was filtered through filter paper, and the residual alcohol was allowed to evaporate. The resulting precipitate was dissolved in 2.5 mL of distilled water, and 0.2 mL of the extract was transferred to a tube. Next, 5 mL of anthrone reagent was added to each tube, and the contents were mixed thoroughly. The tubes were then incubated in a water bath at 90°C for 17 minutes. After cooling, the absorbance of the samples was measured at 625 nm using a spectrophotometer (Dubois et al., 1956).

Amino acids analysis

To determine amino acid content, 1 g of fresh algal tissue was homogenized in 10 mL of 0.05 M phosphate buffer. The mixture was then centrifuged at 3000 rpm for 20 mn. After centrifugation, the supernatant was collected, and ninhydrin reagent was added at a supernatant-to-reagent ratio of 1:5. The solution was incubated in a water bath at 100°C for 7 minutes. Following incubation and cooling, the absorbance of the samples was measured at 570 nm using a spectrophotometer, following the method described by Moore and Stein (1963).

Catalase enzyme activity measurement

For the determination of catalase activity, the reaction mixture was prepared by combining 2.8 mL of 0.05 M phosphate buffer, 0.1 mL of 3% hydrogen peroxide, and 0.1 mL of enzyme extract. The absorbance of this mixture was measured every 10 seconds using a spectrophotometer at a wavelength of 290 nm (Aebi, 1984).

Ascorbate peroxidase enzyme activity analysis

Ascorbate peroxidase activity was determined by preparing the reaction mixture in an ice bath. Specifically, 2.0 mL of 0.05 M phosphate

buffer was mixed with 0.2 mL of 3% hydrogen peroxide and 0.2 mL of 5 mM ascorbic acid. Immediately after mixing, 0.1 mL of enzyme extract was added, and the initial absorbance was measured spectrophotometrically at 290 nm (Nakano & Asada, 1981).

polyphenol oxidase activity determination

To measure polyphenol oxidase activity, 0.2 mL of pyrogallol was added to 2.5 mL of 0.2 M phosphate buffer in a test tube. The mixture was placed in a water bath until the temperature of the tubes reached 40°C. Subsequently, 0.2 mL of the previously prepared enzyme extract was added, and the change in absorbance was monitored spectrophotometrically at 430 nm (Mayer & Harel, 1979).

Statistical analysis

The experiment was conducted using a completely randomized design (CRD) with three replications. All statistical analyses were performed using SPSS software. Differences among treatments were evaluated using one-way analysis of variance (ANOVA), and mean comparisons were performed using Duncan's multiple range test at a significance level of $p \leq 0.05$.

Results

Physiological parameters

The results of the statistical analysis showed that Zn treatment significantly affected physiological parameters ($p \leq 0.05$). Exposure to 0.03 µg/mL Zn resulted in a 35.51% increase in light absorbance relative to the control group. Under Zn treatment, F_0 and F_m increased in a concentration-dependent manner, reaching 40.82% and 91.84% above the control values, respectively, at 0.5 µg/mL of Zn. m. The highest F_v/Fm value was also recorded at 0.03 µg/mL Zn, showing a 50.76% increase compared with the control. At a Zn concentration of 0.03 µg/mL, chlorophyll a and chlorophyll b contents increased by 154.5% and 88.88%, respectively, relative to the control group. In contrast, carotenoid content showed the greatest increase, reaching 92.85% above the control at 0.5 µg/mL Zn. Proline content also increased in a concentration-dependent manner under Zn treatment, with the highest value observed at 0.5 µg/mL Zn, representing a 68.18% increase relative to the control (Table 1).

Table 1. Mean values regarding to effects of different Zn content of medium on physiological parameters of *Dunaliella salina*

Zn content (µg ml^{-1})	Control	0.03	0.06	0.09	0.25	0.5
Physiological parameters						
OD (680)	0.01^{f}± 1.07	0.06^{a} ±1.45	0.03^{b} ±1.40	0.04^{c} ±1.36	0.02^{d} ±1.28	0.01^{e} ±1.15
F_0	0.33^{f} ±25.33	0.97^{e} ±29.72	1.1^{d} ±30.52	1.3^{c}±31.12	1.47^{b}± 33.67	1.45^{a} ±35.67
F_m	0.57^{f} 20.12	0.35^{e} ±24.64	0.29^{d} ±26.53	0.31^{c}±28.71	0.45^{b}±32.67	0.33^{a} ±38.60
F_V/F_m	0.01^{f} ±0.065	0.07^{a} ±0.098	0.03^{b} ±0.093	0.02^{c} ±0.082	0.06^{d}±0.074	0.05^{e}±0.070
Chlorophyll a (mg g^{-1} FW)	0.006^{f}±0.11	0.011^{a}±0.28	0.005^{b}±0.26	0.003^{c}±0.24	0.002^{d}±0.19	0.004^{e}±0.13
Chlorophyll b (mg g^{-1} FW)	0.003^{f}±0.09	0.005^{a}±0.17	0.002^{b}±0.15	0.001^{c}±0.14	0.003^{d}±0.12	0.002^{e}±0.10
Carotenoids (µg g^{-1} FW)	0.002^{f}±0.14	0.004^{e}±0.16	0.001^{d}±0.19	0.003^{c}±0.21	0.002^{b}±0.25	0.001^{a}±0.27
Proline (µm g^{-1} FW)	0.98^{f} ±14.52	1.1^{e}±15.82	1.3^{d} ±16.64	1.5^{c} ±17.72	1.7^{b} ± 20.84	1.9^{a} ±24.42

In each row, values followed by the same letters are not significantly different according to Duncan's multiple range test at a significance level of $p \leq 0.05$ (n = 3)

Biochemical parameters

Data analysis showed that protein content increased under Zn treatment; however, the changes were not concentration-dependent. Protein content increased by 13.86% at 0.03 μg/ml compared with the control group, followed by a slight decrease at higher Zn concentrations, although the values remained higher than the control group. Soluble sugar content initially increased and subsequently decreased under Zn treatment, indicating a non-concentration-dependent response. The highest soluble sugar content was recorded at 0.03 μg/mL Zn, showing an 89.47% increase compared with the control. Total amino acid content increased in a concentration-dependent manner under Zn treatment, reaching its maximum value at 0.5 μg/mL Zn, with an 88.88% increase relative to the control. The results further demonstrated that catalase activity was significantly affected by Zn treatment ($p \leq 0.05$), although the changes were not concentration-dependent. The highest catalase activity, representing a 71.42% increase compared with the control, was observed at 0.03 μg/mL Zn. In contrast, the activities of ascorbate peroxidase and polyphenol oxidase increased in a concentration-dependent manner, reaching maximum increases of 88.46% and 90.38%, respectively, at 0.5 μg/mL Zn relative to the control (Table 2).

Discussion

Zinc (Zn) is an essential micronutrient for the growth and metabolism of microalgae and plays important roles enzyme structure and activity, photosynthetic processes, and antioxidant defense systems (Elleuch et al., 2021). Therefore, the physiological and biochemical responses observed in *Dunaliella salina* under different Zn concentrations should be interpreted from two distinct perspectives: the nutritional and stimulatory effects at low concentrations and the stress-inducing or toxic effects at higher concentrations. The significant increase in F_v/F_m observed in Zn-treated cultures, particularly at 0.03 μg/mL, suggests that this concentration provided favorable conditions

Table 2. Mean values regarding to effects of different Zn content of medium on biochemical parameters of *Dunaliella salina* . In each row, values followed by the same letters are not significantly different according to Duncan's multiple range test at $p \leq 0.05$ (n = 3)

Treatments ($\mu g\ ml^{-1}$)	Control	0.03	0.06	0.09	0.25	0.5
Biochemical parameters						
Total protein ($ng\ ml^{-1}$)	0.06^{e}± 1.01	0.06^{a} ±1.15	0.05^{b} ±1.14	0.05^{b} ± 1.14	0.07^{c} ±1.06	0.06^{d} ±1.04
Soluble sugar ($mg\ g^{-1}$ FW)	0.007^{f}±0.19	0.004^{a}±0.36	0.004^{b}±0.32	0.002^{c}±0.30	0.003^{d} ± 0.28	0.005^{e} ±0.21
Free amino acid ($mg\ g^{-1}$ FW)	0.001^{e}±0.009	0.001^{d}±0.011	0.002^{c}±0.012	0.004^{c}±0.012	0.003^{b}± 0.015	0.005^{a} ±0.017
Catalase activity (μmol. min−1 mg−1)	0.003e±0.007	0.005a ±0.012	0.006b ±0.011	0.002b±0.011	0.002c ±0.010	0.001d ±0.008
Ascorbate peroxidase activity ($\mu mol.\ min^{-1}\ mg^{-1}$	0.002^{f}±0.052	0.001^{e}±0.059	0.001^{d}±0.061	0.002^{c}±0.063	0.003^{b} ± 0.072	0.006^{a} ±0.098
Polyphenol oxidase activity ($\mu mol.\ min^{-1}\ mg^{-1}$)	0.001f±0.52	0.002e ± 0.60	0.003d±0.61	0.005c±0.62	0.004b ± 0.78	0.007a ±0.99

for Photosystem II (PSII) performance and enhanced photosynthetic efficiency. Since Zn is involved in the activity of several enzymes associated with photosynthesis and electron transport, this response may reflect the significant nutritional role of Zn rather than a direct stress response (Wongsnansilp et al., 2018). In contrast, the increase in F_0 observed in some treatments may indicate alterations in PSII function and, at higher Zn concentrations, may be associated with the onset of photoinhibition and photosynthetic disturbances. The increase in chlorophyll content under Zn supplementation, particularly at 0.03 μg/mL, may be attributed increased pigment biosynthesis, improved activity of chlorophyll-synthesizing enzymes, and enhanced pigment stability (Alirahimi et al., 2022). Therefore, the elevated chlorophyll levels at this concentration may reflect the essential nutritional role of Zn. Conversely, the relative decline in chlorophyll content at 0.5 μg/mL may indicate the onset of inhibitory or toxic effects associated with excessive Zn accumulation and potential impairment to photosynthetic structures (Elleuch et al., 2021). The increase in soluble sugar content, particularly at 0.03 μg/mL Zn, may be associated with improved photosynthetic performance and enhanced carbon fixation. Although soluble sugars are known to function as osmoprotectants under stress conditions, their accumulation at low Zn concentrations may instead reflect enhanced carbon metabolism and energy storage rather than a direct stress response (Elleuch et al., 2021).

Similarly, the increase in total protein content at 0.03 μg/mL supports the potential role of Zn in promoting protein synthesis and metabolic activity. As a cofactor for numerous enzymes and a regulator of gene expression, Zn may promote protein production under optimal nutritional conditions. In contrast, the reduction in total protein content at 0.5 μg/mL may be associated with impaired protein synthesis or increased protein degradation under Zn-induced stress (Folgar et al., 2009). Regarding free amino acids, their accumulation at higher Zn concentrations, particularly at 0.5 μg/mL, may be associated with the synthesis of protective metabolites and the regulation of osmotic balance. This interpretation is consistent with the marked increase in proline observed at the same Zn concentration. Proline is widely recognized as a compatible osmolyte and antioxidant-related metabolite that accumulates under oxidative and metal-induced stress conditions (Hamed et al., 2017). Therefore, the substantial increase in proline and total amino acids at higher concentrations may indicate the activation of cellular defense mechanisms in response to Zn-induced stress, whereas the responses observed at lower Zn concentrations may primarily reflect the nutritional effects of Zn, (Sui et al., 2021). The pattern of antioxidant enzyme activities further supports this distinction. The increase in catalase (CAT) activity at 0.03 μg/mL may reflects enhanced cellular antioxidant capacity associated with adequate Zn availability. This response does not necessarily indicate severe stress but may instead suggests at enhanced capacity of the cells to detoxify reactive oxygen species (ROS) (Gao et al., 2024).

In contrast, the elevated ascorbate peroxi-

dase (APX) activity observed at 0.5 μg/mL may indicate enhanced antioxidant responses associated with increased ROS generation and a greater requirement for hydrogen peroxide detoxification under Zn-induced stress conditions (Roy, 2021). Likewise, the increase in polyphenol oxidase (PPO) activity at 0.5 μg/mL may be attributed to the activation of defense pathways involved in phenolic metabolism and oxidative stress mitigation. The higher PPO activity at elevated Zn concentrations is consistent with its proposed role in cellular defense against environmental stress and oxidative damage (Mishra & Jha, 2011). Overall, the results demonstrate that the response of *Dunaliella salina* to Zn varies with concentration and depends on the physiological and biochemical parameter examined. The low Zn concentration of 0.03 μg/mL enhanced photosynthetic performance, chlorophyll accumulation, soluble sugar content, total protein content, and selected antioxidant responses, suggesting a predominantly nutritional and stimulatory role of Zn in cellular metabolism. In contrast, the higher concentration of 0.5 μg/mL was associated with increased levels of proline, free amino acids, APX, and PPO, together with relative reductions in some metabolic parameters, suggesting the activation of stress-related defense responses and the possible onset of Zn toxicity. Therefore, not all observed responses should be interpreted as stress-related effects; rather, a distinction should be made between the beneficial effects of adequate Zn nutrition and the potentially detrimental effects associated with excessive Zn accumulation.

Conclusion

Zn supplementation induced concentration-dependent changes in several physiological and biochemical parameters in *Dunaliella salina*, reflecting both its essential nutritional role and its potential adverse effects at elevated concentrations. The lowest Zn concentration (0.03 μg/mL) enhanced photosynthetic efficiency, chlorophyll content, soluble sugar content, protein accumulation, and catalase activity, suggesting improved metabolic performance and antioxidant responses. These responses suggest a predominantly beneficial and stimulatory effect of Zn rather than a pronounced stress response. In contrast, the highest Zn concentration (0.5 μg/mL) increased proline, total free amino acid content, APX activity, and PPO activity while reducing protein content, suggesting the activation of stress-related protective responses. The control treatment generally exhibited lower levels of several metabolic and antioxidant parameters, highlighting the importance of Zn for optimal cellular function. Overall, *Dunaliella salina* exhibited considerable metabolic flexibility and modulation of antioxidants in response to Zn availability. These findings emphasize the importance of optimizing Zn concentrations to support algal physiological performance and potentially enhance applications in metabolite production and bioremediation.

Author contribution statement

S. M. R. conceived and designed the experiments. M. Kh. prepared the initial draft of the manuscript. Data analysis was performed by S. M. R., L. B., S. M. M., A. Gh., and M. Kh. All authors read and approved the final

version of the manuscript.

References

Aebi, H. (1984) 'Catalase in vitro', Methods in Enzymology, 105, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Alirahimi, N., Kordenaeej, A., Rezaei, A. and Talei, D. (2022) 'Changes in growth, beta-carotene production, traits, and protein banding patterns in *Dunaliella salina* microalgae in the presence of colchicine', *Journal of Marine Science and Technology*, 20(4), pp. 86–94. https://doi.org/10.22113/jmst.2016.38962.

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973) 'Rapid determination of free proline for water-stress studies', Plant and Soil, 39(1), pp. 205–207. https://doi.org/10.1007/BF00018060.

Bradford, M.M. (1976) 'A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding', *Analytical Biochemistry*, 72(1–2), pp. 248–254. https://doi.org/10.1006/abio.1976.9999.

Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I. and Lux, A. (2007) 'Zn in plants', *New Phytologist*, 173(4), pp. 677–702. https://doi.org/10.1111/j.1469-8137.2007.01996.x.

Cakmak, I. (2000) 'Possible roles of Zn in protecting plant cells from damage by reactive oxygen species', *New Phytologist*, 146(2), pp. 185–205. https://doi.org/10.1046/j.1469-8137.2000.00630.x.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) 'Colorimetric method for determination of sugars and related substances', *Analytical Chemistry*, 28(3), pp. 350–356. https://doi.org/10.1021/ac60111a017.

Elleuch, J., Hmani, R., Drira, M., Michaud, P., Fendri, I. and Abdelkafi, S. (2021) 'Zinc biosorption by *Dunaliella* sp. AL-1: Mechanism and effects on cell metabolism', *Science of the Total Environment*, 773, 145024. https://doi.org/10.1016/j.scitotenv.2021.145024.

Folgar, S., Torres, E., Pérez-Rama, M., Cid, A., Herrero, C. and Abalde, J. (2009) '*Dunaliella salina* as a marine microalga highly tolerant to, but a poor remover of, cadmium', *Journal of Hazardous Materials*, 165(1–3), pp. 486–493. https://doi.org/10.1016/j.jhazmat.2008.10.004.

Gao, M., Ling, N., Tian, H., Guo, C. and Wang, Q. (2024) 'Toxicity, physiological response, and biosorption mechanism of *Dunaliella salina* to copper, lead, and cadmium', *Frontiers in Microbiology*, 15, 1374275. https://doi.org/10.3389/fmicb.2024.1374275.

Hamed, S.M., Zinta, G., Klöck, G., Asard, H., Selim, S. and AbdElgawad, H. (2017) 'Zn-induced differential oxidative stress and antioxidant responses in *Chlorella sorokiniana* and *Scenedesmus acuminatus*', *Ecotoxicology and Environmental Safety*, 140, pp. 256–263. https://doi.org/10.1016/j.ecoenv.2017.02.039.

Kabata-Pendias, A. (2011) *Trace Elements in Soils and Plants*. 4th edn. Boca Raton, FL: CRC Press.

Lichtenthaler, H.K. (1987) 'Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes', *Methods in Enzymology*, 148, pp. 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

Massyuk, N.P. (1996) 'Mass culture of the carotene-bearing alga *Dunaliella salina* Teod.', *Ukrainian Botanical Journal*, 23, pp. 12–19.

Maxwell, K. and Johnson, G.N. (2000) 'Chlorophyll fluorescence—A practical guide', *Journal of Experimental Botany*, 51(345), pp. 659–668. https://doi.org/10.1093/jex-

bot/51.345.659.

Mayer, A.M. and Harel, E. (1979) 'Polyphenol oxidases in plants', *Phytochemistry*, 18(2), pp. 193–215. https://doi.org/10.1016/0031-9422(79)80057-6.

Mishra, A. and Jha, B. (2011) 'Antioxidant response of the microalga *Dunaliella salina* under salt stress', *Botanica Marina*, 54(2), pp. 195–199. https://doi.org/10.1515/BOT.2011.027.

Moore, S. and Stein, W.H. (1963) 'Methods for amino acid analysis', *Analytical Chemistry*, 35(13), pp. 1965–1970. https://doi.org/10.1021/ac60205a045.

Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M. (2010) 'Heavy metals, occurrence and toxicity for plants: A review', *Environmental Chemistry Letters*, 8, pp. 199–216. https://doi.org/10.1007/s10311-010-0297-8.

Nakano, Y. and Asada, K. (1981) 'Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts', *Plant and Cell Physiology*, 22(5), pp. 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.

Palomares, A.J., Rubio, F. and Florencio, F.J. (2004) 'Role of carbonic anhydrase in inorganic carbon uptake by phytoplankton', *Biochemical Journal*, 382(2), pp. 353–361. https://doi.org/10.1042/BJ20040484.

Pilon, M. and Várkonyi, A. (2017) 'Plant metal homeostasis—Challenges and opportunities', *Current Opinion in Plant Biology*, 38, pp. 151–159. https://doi.org/10.1016/j.pbi.2017.05.004.

Rathore, D., Singh, A.K. and Pandey, A. (2015) 'Effect of Zn on growth and β-carotene production in *Dunaliella salina*', *Journal of Environmental Biology*, 36(1), pp. 141–147.

Riahi, H. (2002) *Algology*. 2nd edn. Tehran: Alzahra University Press.

Roy, U.K., Nielsen, B.V. and Milledge, J.J. (2021) 'Tuning *Dunaliella tertiolecta* for enhanced antioxidant production by modification of culture conditions', *Marine Biotechnology*, 23(3), pp. 482–500. https://doi.org/10.1007/s10126-021-10039-7.

Salehipour-Bavarsad, F., Riahi, H., Hejazi, M.A. and Shariatmadari, Z. (2022) 'Screening terrestrial and aquatic strains of *Dunaliella* from the eco-taxonomical perspective: A comparative study on fatty acid compositions as habitat indicators', *Journal of Applied Phycology*, 34(1), pp. 461–474. https://doi.org/10.1007/s10811-021-02645-3.

Sui, Y., Mazzucchi, L., Acharya, P., Xu, Y., Morgan, G. and Harvey, P.J. (2021) 'A comparison of β-carotene, phytoene, and amino acid production in *Dunaliella salina* DF15 (CCAP 19/41) and *Dunaliella salina* CCAP 19/30 under different light wavelengths', *Foods*, 10(11), 2824. https://doi.org/10.3390/foods10112824.

Sunda, W.G. and Huntsman, S.A. (1995) 'Effect of free metal ion concentration on the survival of the marine diatom *Thalassiosira weissflogii*', *Limnology and Oceanography*, 40(3), pp. 512–517. https://doi.org/10.4319/lo.1995.40.3.0512.

Wang, W.-X. (2002) 'Interactions of trace metals and different marine food chains', *Marine Ecology Progress Series*, 243, pp. 295–309. https://doi.org/10.3354/meps243295.

Whittaker, R.H. and Margulis, L. (1978) 'Protist classification and the kingdoms of organisms', *BioSystems*, 10(1–2), pp. 3–18. https://doi.org/10.1016/0303-2647(78)90006-3.

Wongsnansilp, T., Juntawong, N. and Wu, Z. (2018) 'Effects of phosphorus on the growth and chlorophyll fluorescence of a *Dunaliella salina* strain isolated from saline soil under nitrate limitation', *Journal of Biological Research-Bollettino della Società Italiana di Biologia Sperimentale*, 91(1). https://doi.

org/10.4081/jbr.2018.7541.

Xiong, Z.T. (2006) ‘Bioaccumulation and physiological effects of excess Zn in plants’, *Reviews of Environmental Contamination and Toxicology*, 186, pp. 67–113. https://doi.org/10.1007/0-387-32883-2-3.

Exogenous Salicylic Acid Reverses Water Splitting and Electron Transport Activity of Leaves and Triggers the Transition to Flowering in *Phlomis tuberosa* Under Salt Stress

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Received: 2026-07-02 Accepted: 2026-08-29

Abstract

To investigate the protective role of salicylic acid (SA) in alleviating salt-induced damage in *Phlomis tuberosa* plants. Fifty-day-old seedlings were exposed to salt stress by irrigating 40 mM sodium chloride solution. Salt stress significantly inhibited plant growth by diminishing shoot and root dry weights, plant height, and relative water content (RWC). This growth inhibition was primarily associated with excessive Na^+ accumulation in plant shoots. However, root-application of SA at 0.1 mM mitigated the toxic effects of salt-induced damage by inhibiting Na^+ translocation to leaves, resulting in significant improvements in growth-related attributes, including dry mass. Salt stress markedly affected the chlorophyll *a* fluorescence transient (OJIP) curve, as indicated by an increase in the O-J phase coupled with reduced maximum quantum yield (F_v/F_m) and oxygen-evolving complex efficiency of PSII (F_v/F_o). Conversely, SA supplementation reversed these effects by improving JIP-test parameters, water- splitting acitivity, and electron transport within PSII. Furthermore, pretreatment with salicylic acid under salt stress conditions markedly enhanced the activities of antioxidative enzymes compared with those in the salinity alone treatment. Notably, SA enhanced flowering in *P. tuberosa* regardless of stress, and the positive correlation between nitric oxide (NO) levels and flower number suggested a role for NO in salicylic acidmediated floral transition. Overall, by enhancing carotenoids levels, reducing reactive oxygen species (ROS) accumulation, preserving thylakoid membrane integrity, improving the balance and functionality between PSI and PSII and optimizing energy fluxes; SA alleviated the detrimental effects of salt stress on photosynthesis and mitigated stress-induced damage.

Keywords: Ionic homeostasis, OJIP transient, Oxidative stress, *Phlomis tuberosa*, Photosynthesis, Salicylic acid, Salinity

Introduction

Among the abiotic stresses, salinity is a significant stressor that severely restricts plant growth and development, thereby reducing crop yield (Munns and Gilliham, 2015; Liu et al., 2024). Under saline con-

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Doi: 10.48308/pae.2026.245180.1149

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ditions, the excessive uptake of Na^+ and Cl^- reduces the K^+/Na^+ ratio in plant tissues and induces osmotic stress, leading to ionic toxicity and nutritional imbalances in plants (Isayenkov and Maathuis, 2019; Mukhopadhyay et al., 2021; Hryvusevich et al., 2021). One of the common consequences of this ionic toxicity and nutritional imbalance is the overproduction of reactive oxygen species (ROS), which causes damage to cellular membranes, stimulates tissue necrosis, and decreases plant vigor (Hasanuzzaman et al., 2021; İbrahimova et al., 2021). Under these conditions, excessive ROS accumulation reduces light-capturing efficiency by accelerating chlorophyll (Chl) degradation, impair membrane integrity and photosynthesis-related proteins (Miller et al., 2010), compromises the structural and functional integrity of photosystem II (PSII), and inhibit photochemical activity and electron transport chains (ETC), and consequently, ultimately reducing photosynthetic capacity and crop yield (Raihan et al., 2025).

To mitigate the excessive production of ROS and minimize oxidative damage to the photosynthetic apparatus, plants have broadly evolved several adaptive strategies, including the maintenance of osmotic balance, regulation of mineral homeostasis, activation of enzymatic and non-enzymatic antioxidants systems, (e.g., carotenoids, glutathione, ascorbic acid and phenolic compounds), synthesis of phytohormones, generation of nitric oxide, and accumulation of compatible solutes (Isayenkov and Maathuis, 2019; Ramasamy and Mahawar, 2023). Since these endogenous mechanisms provide only partial protection against the detrimental effects of salt stress, considerable progress has been made in recent years toward alleviating salt-induced toxicity and enhancing plant tolerance to salinity through the exogenous application of protective compounds, including proline (Koc et al., 2024), nitric oxide (Wang et al., 2024), jasmonic acid (Ali et al., 2022), salicylic acid (SA) (Kumar et al., 2024), and melatonin (Kaya et al., 2022). Among these protective compounds, exogenous SA is known to play an important role in modulating the antioxidant defense responses and mitigating the negative effects of ROS, thereby enhancing plants tolerance to challenging adverse environmental conditions, including heavy metal toxicity (Maghsoudi et al., 2020), water deficit (Kareem et al., 2019), salinity (Yadav et al., 2020), and heat stress (Arif et al., 2020).

SA supplementation at appropriate concentrations has been reported to enhance plant growth and improve stress tolerance to salt stress in various plant species (Hayat et al., 2010; Jayakannan et al., 2015; Barwal et al., 2024), including wheat (Hafeez et al., 2024), soybean (Farhangi-Abriz and Ghassemi-Golezani, 2018), and *Egletes viscosa* (Batista et al., 2019). Exogenous SA enhances plant tolerance to salt stress by increasing the activities of antioxidant enzymes, including catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), glutathione S-transferase, glutathione reductase, and glutathione peroxidase (Ahanger et al., 2020; Barwal et al., 2024; Liu et al., 2024). It may also promote the biosynthesis or accumulation of key plant growth hormones, such as auxins, gibberellins, and abscisic acid (Sharma

et al., 2020; Kashif et al., 2024), increasing the concentration of antioxidants, including proline, glycine betaine, flavonoids, and alkaloids (Alizadeh et al., 2024; Barwal et al., 2023) and improving Na^+ and K^+ balance (Jayakannan et al., 2015; Batista et al., 2019; Kumar et al., 2024).

Furthermore, under salinity stress, exogenous SA enhances photosynthesis by stabilizing Chl (Lotfi et al., 2020; Islam et al., 2023), improving stomatal conductance, and protecting PSII efficiency from ROS damage (Ma et al., 2017; Raihan et al., 2025). These mechanisms help plants for increasing the salt tolerance.

Given that the involvement of salicylic acid (SA) in floral development has not been fully characterized, the present study examined how exogenous SA and NaCl influence the onset of flowering in *P. tuberosa*. We aimed to clarify whether SA contributes to the acceleration of flowering under both optimal and saline conditions. Earlier reports have suggested that SA may modulate floral induction even in the absence of environmental stress (Martínez et al., 2004; Wada and Takeno 2013). Additional findings indicate that SA plays an important role in the regulation of flowering initiation in certain plant species. For instance, stressassociated increases in SA levels have been reported to promote early flowering in *Arabidopsis* and *Lemna paucicostata*, partly through suppression of the floral repressor *FLOWERING LOCUS C* (*FLC*) (Wada et al., 2010). Furthermore, nitric oxide (NO) is known to interact with redoxregulated signaling networks and has been proposed to influence the expression or activity of key flowering regulators (Hou et al., 2025). SAinduced NO generation may therefore contribute to activating molecular mechanisms underlying the floral transition. Accordingly, the present work investigated the potential involvement of NO in SAmediated control of flowering in *Phlomis tuberosa*.

Phlomis tuberosa belongs to the *Lamiaceae* family and is cultivated for its medicinal value. *P. tuberosa* is capable of surviving in extreme environmental conditions, including highaltitude and arid or semiarid habitats characterized by intense ultraviolet radiation and recurrent droughts can constrain its growth and yield (Ghasemifar et al., 2022). Despite its documented tolerance to various environmental stresses, the effects of salinity on *P. tuberosa* growth and productivity remain poorly investigated. To the best of our knowledge, no studies have investigated whether exogenous SA enhances salt stress tolerance in *P. tuberosa* . Hence, the mechanisms by which this growth regulator may enhance salt-stress tolerance in *P. tuberosa* are still not fully understood. The current study aimed to determine whether application mitigates salinity stress by improving photochemical performance. To address this question, we investigated in some detail the biochemical mechanisms by which SA affects the plant growth, PSII functioning, ionic homeostasis, and antioxidative system in salt-treated *P. tuberosa* plants. Unlike previous studies, particular attention was given to fluorescence-based parameters to elucidate how SA modulates PSII photochemical performance and contributes to the mitigation of salt stress in medicinal *P. tuberosa*.

The findings of this study are expected to provide valuable ecological and economic strategies for enhancing plant resilience in saline environments.

Materials and methods

Plant material and treatments

Seeds of *Phlomis tuberosa* were colleted from Mishoudagh, near the city of Marand, 65 km south of Tabriz (45°38' E, 38°22' N) at an elevation of 1800 m, East Azarbaijan Province, northwestern Iran . Healthy and uniform seeds were surface-sterilized with a 5% sodium hypochlorite (NaOCl) solution for 5 min and then rinsed thoroughly with distilled water. Ten-day-old seedlings were transferred to Hoagland nutrient solution (Johnson et al., 1957) and were pre-cultured for 34 days before the treatments were initiated. The plants were grown in a nutrient solution containing: 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4$, 50 µM H_3BO_3, 2 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM H_2MoO_4 and 0.02 mM $FeSO_4$-EDTA. Salicylic acid (SA) was supplied through the nutrient medium at a final concentration of 0.1 mM with the pH adjusted to 5.6–5.8, and the entire solution was replaced every 7 days. The concentration and mode of SA application method of SA were determined through a preliminary dose–response experiment. *P. tuberosa* seedlings were treated withconcentrations of SA, including 0.05, 0.1, 0.5, 1, 5, and 10 mM, applied either as a foliar spray or to the root zone. Based on growth responses, 0.1 mM SA applied to the root zone was selected for subsequent experimentsbecause it produced a significantly great increase in seedling growth than foliar application or the other concentrations tested. At 49 days after germination, 40 mM NaCl were added to the nutrient solution described above. The salt concentration was selected based on the effect of different NaCl concentrations, including 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM on seedling growth in a preliminary study. The seedling growth was reduced by approximately 50% compared with the control and this concentration was therefore selected as a semi-lethal concentration of salinity for further experiments (data not shown), at 40 mM. Plants were maintained in a controlled with temperatures of 30-35/18-22 ºC, respectively, under a 16 h light/8 h dark photoperiod. , The relative humidity maintained at 40-45 % and a daily photon flux density of approximately 400-450 µmol m^{-2} s^{-1}.

Determination of plant biomass and leaf relative water content

Fully expanded and mature leaves were collected for the measurement of physiological and biochemical parameters. Shoots and roots were carefully harvested to determine their dry weight (DW) and subsequently dried at 70 ºC for 48 h . For subsequent analyses, the samples were immediately immersed in liquid nitrogen and maintained under these conditions until analysis. Relative water content (RWC) and percentage (%) of yield loss were determined according to the the method described by Habibi (2020).

Determination of proline

Proline was determined according to the method described by Bates et al., (1973). Leaf samples from each group were homog-

enized in 3% (w/v) sulphosalicylic acid at 4 °C, and the homogenates were centrifuged at 3,000 × g for 20 min. Approximately 2 ml of the extract was transferred to a test tube, followed by an addition of 2 ml of glacial acetic acid and ninhydrin reagent. The tubes were cooled on crushed ice, and the absorbance of the resulting red-color complex was measured at 520 nm using a UV-visible spectrophotometer, with toluene serving as the blank. A standard curve was prepared using proline (Sigma-Aldrich) as the reference standard.

Determination of Na and K contents

All samples were dry-ashed at 550 °C for 8 h, and the resulting dry residues were mixed with 0.5 M HCl and brought to a final volume with double-distilled water. Following acid digestion, the samples were transferred to a hot plate maintained at 120 °C and heated for an additional 1 h. The digested samples were subsequently diluted to a final volume of 50 mL with double-distilled water to prior to analysis. Then, The concentrations of Na and K were subsequently determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, INTEGRA XL2, GBC; Australia).

Determination of total chlorophylls and carotenoids

The concentrations of chlorophyll *a*, chlorophyll *b*, and total carotenoids in leaf tissues were determined following pigment extraction in chilled acetone. The Samples were incubated in darkness at 4 °C for 24 h to ensure complete extraction of the pigments, following the procedure described by Lichtenthaler and Wellburn (1985).

Chlorophyll a fluorescence measurement

Chlorophyll a fluorescence parameters were measured daily between 09:00 and 11:00 husing a Pocket-PEA chlorophyll fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., UK). Prior to measurement, the leaves were dark-adapted for at least 30 min. The JIP-test analysis was used to quantify a range of phenomenological and biophysical parameters associated with the functions of photosystem II (PSII) and photosystem I (PSI). Analysis of the polyphasic chlorophyll fluorescence rise (OJIP curve) provided insights into photosynthetic electron fluxes (Strasser et al., 2004; Kumar et al., 2020). The JIP-test is based on the polyphasic rise in fast chlorophyll a florescence and is widely used for investigating the correlation between light dependent reactions and chlorophyll a florescence ChlF. The O–J phase of the fluorescence rise reflects the closure of afraction of the PSII reaction centers associated with the reduction of the primary electron acceptor QA. The J–I phase corresponds primarily to the reduction of the plastoquinone (PQ) pool, whereas the I–P phase is typically associated with the reduction of electron acceptors, on the PSI acceptors on the PSI acceptor side, including $NADP^+$. Specific parameters reflecting functional and structural alterations in PSII were calculated according to protocols (Kalaji et al., 2011), as ascribed in the subsequent sections.

F_o: Minimum fluorescence, when all PSII reaction centers (RC) are open.

F_m: Maximum fluorescence, when all PSII reaction centers are closed.

F_v: Variable fluorescence.

F_v/F_m: Maximum quantum yield of PSII.

F_v/F_o: Efficiency of the oxygen-evolving complex on the donor side of the PSII.

F_m/F_o: Maximum fluorescence normalized by minimum fluorescence.

V_j: Relative variable fluorescence at 2 ms (J-step), that refers to the number of closed RCs relative to the total number of RCs.

V_i: Relative variable fluorescence at 30 ms (I-step), that reflects the ability of PSI and its acceptors to oxidize reduced plastoquinone.

N: Turnover number: number of QA reduction events

S_m: Normalized total complementary area above the OJIP transient (reflecting multiple-turnover QA reduction events) or total electron carriers per RC.

ABS/RC: Absorption flux per RC; that reflects the proportion between chlorophyll *a* molecule amounts in fluorescence-emitting antenna complexes and the active reaction centers.

TR_o/RC: Trapped energy flux per RC.

ET_o/RC: Electron transport flux per RC.

DI_o/RC: Dissipated energy flux per RC.

ABS/CS: Absorption flux per CS; represents the amount of photon energy absorbed by the antenna associated with active and inactive reaction centres of PSII.

TR_o/CS_m: Trapped energy flux per CS.

ET_o/CS_m: Electron transport flux per CS.

DI_o/CS_m: Dissipated energy flux per CS.

RC/C_m: Amount of active PSII RCs per CS.

$ABS/CS_m = \approx F_o$: Absorbed photon flux per excited PSII cross section at time zero.

φP_o: Maximum quantum yield of primary photochemistry*;* that indicates the probability of trapping the energy of absorbed photons by PSII reaction centers.

φE_o: Quantum yield for the reduction of end acceptors of PSI per photon absorbed.

ψE_o: Probability (at time 0) that trapped exciton moves an electron into the electron transport chain beyond QA.

PI_{abs}: The performance index.

Assay of antioxidative enzymes and related metabolites

Enzyme activity was assayed in leaves harvested at midday. The activities of superoxidase dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and ascorbate peroxidase (APX; EC 1.11.1.11) were determined according to the methods described by Habibi (2020). SOD activity was determined using the nitro blue tetrazolium (NBT) photoreduction method described by Giannopolitis and Ries (1977), using a reaction mixture containing 0.1 mM EDTA, 50 mM Na_2CO_3 pH 10.2, 13 mM methionine, 63 μM nitroblue tetrazolium chloride (NBT), 13 μM riboflavin. CAT activity was determined following a protocol adapted from Simon et al. (1974) using the H_2O_2 as substrate. CAT activity was determined from the decrease in absorbance at 240 nm, which reflects the decomposition of hydrogen peroxide (H_2O_2). APX activity was assayed by monitoring the oxidation of ascorbate using a modified reaction mixture containing 50 mM phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 mM ascorbic acid, and 50 μg BSA, as detailed by Boominathan and Doran (2002). The rate of lipid peroxidation was estimated based on the concentration of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid (TBA, Sigma) at 532 nm. MDA concentra-

tion was quantified using a standard curve prepared with 1,1,3,3-tetraethoxypropane (Sigma-Aldrich). H_2O_2 content was determined according to the method of Velikova et al. (2000) using a standard curve of H_2O_2 (Sigma-Aldrich).

Determination of NO concentration

Nitric oxide(NO) concentration was determined using the modified method described by Wu et al. (2016). Fresh leaf sample of a known weight were homogenized in ice-cold 50 mM acetic acid buffer (pH 3.6) containing 4% Zn diacetate. Absorbance was measured at 540 nm, and NO concentration was calculated using a standard curve prepared with known concentrations of $NaNO_2$.

Statistical analysis

The experiments were conducted using a completely randomized design using four independent replicates. Data were analyzed using one-way analysis of variance (ANOVA) in Sigma Stat version 3.5. Treatment means were compared using Tukey's honestly significant differences among treatments (HSD). test at a significance level of $P< 0.05$. Data are presented as means±-standard deviation (SD) in all Figures and Tables, with different letters indicate statistically significant differences among treatments. Spearman rankorder correlation analysis was performed using SigmaStat version 3.5 to measure the relationship between the number of flowers and NO levels, based on 15 independent replicates. Chlorophyll florescence data were assessed using the PEA Plus software, version 1.10.

Results

The root application of SA improved the growth parameters under salt stress

Measurement of the growth parameters in *P. tuberosa* plants under salt stress showed that salinity significantly reduced shoot fresh and dry weight, root dry weight, and plants height (Table 1). Exogenous application of SA improved significant morphological parameters , including shoot fresh and dry weights . However, SA application did not significantly affect root dry weight and plant height parameters in salt-stressed plants..

Plants treated with NaCl exhibited a significant reduction in relative water content (RWC) compared with the control group (Table 2). In contrast, plants treated with both NaCl and SA maintained RWC levels similar to those of the control plants, highlighting the positive role of SA in maintaining plant water status under salt stress.

Table 1. Effect of SA on fresh and dry weight and tap root and stem length of *P. tuberosa* under NaCl stress. Values are mean ± SD (n = 4); different letters indicate significant differences ($p < 0.05$, Tukey's test)

Treatment	Shoot FW	Root FW	Shoot DW	Root DW	Plants height	Root length
Control	5.10±0.32 [a]	3.44±0.22 [a]	0.85±0.10 [a]	0.62±0.10 [a]	23.2±2.32 [a]	6.53±0.73 [b]
NaCl	3.42±0.30 [b]	3.24±0.33 [a]	0.58±0.13 [b]	0.41±0.08 [b]	17.5±.1.89 [b]	6.10±0.08 [b]
SA	5.27±0.28 [a]	3.54±0.37 [a]	0.89±0.12 [a]	0.66±0.09 [a]	24.1±3.02 [a]	8.75±1.02 [a]
SA+NaCl	4.92±0.24 [a]	3.09±0.26 [a]	0.84±0.11[a]	0.42±0.07 [b]	18.3±1.26 [b]	7.99±0.07 [a]

Furthermore, salt stress increased proline content compared with the control treatment. Salt-treatment significantly increased Na^+ concentrations both in shoots and roots compared with the control plants. However, the application of SA to the root zone under salt stress reduced shoot Na^+ concentration by 32%. (Table 2). Under salinity stress, shoot K^+ concentration decreased compared with the control. However, SA application under NaCl stress treatment alleviated this reduction, improving shoot K by 27% relative to NaCl-stressed plants (Table 2). However, root K was not significantly influenced by SA application in stressed plants.

SA mitigated the adverse effects salt stress on the PSII function

While after 15 days of salt stress, *P. tuberosa* plants showed a significant decrease in chlorophyll *a* contents, plants exposed to only salt stress could maintain chlorophyll *b* and carotenoids contents at relatively high levels (Fig. 1). However, the decreases in chlorophyll *a* contents in the leaves of stressed plants were significantly mitigated by SA application. Carotenoids contents were not significantly affected by exposure to salinity alone, however, the application of SA raised this parameter in plants under salt stress. Although we detected a clear decrease in the maximum quantum yield (F_v/F_m) of leaves after 15 days of exposure to salt stress (Fig. 2), the application of SA mitigated this reduction, improving F_v/F_m relative to stressed plants. Exposure to salinity alone caused a marked decline in the oxygen-evolving complex efficiency of PSII (F_v/F_o) and the performance index of photosystems (PI_{abs}), however, the application of SA raised these parameters in plants under salt stress.

According to the fast chlorophyll *a* fluorescence (OJIP) kinetics, fluorescence dropped sharply after 15 days of salt stress, indicating reductions in the I-P phases compared to control plants. However, the O-J phase showed a marked increase in fluorescence intensity relative to control plants (Fig. 3). In contrast, SA application under NaCl stress effectively suppressed this O-J phase of the fluorescence rise.

A sharp increase in V_j (relative variable fluorescence at J step) was also detected in leaves of salt-stressed *P. tuberosa* plants(-Fig. 4). In addition, exposure salinity alone resulted in significant increase in several specific energy fluxes, including absorption flux per reaction center (ABS/RC), trapped energy flux per reaction center (TR_o/RC)

Table 2. Effect of SA on RWC, proline, and element content in *P. tuberosa* under NaCl stress. Values are mean ± SD (n = 4); different letters indicate significant differences ($p < 0.05$, Tukey's test)

Treatment	RWC (%)	Proline	Shoot Na	Root Na	Shoot K	Root K
Control	86.7±5.5 ^{a}	10.2±1.2 ^{c}	15.3±2.0 ^{b}	25.3±2.7 ^{b}	74.3±6.8 ^{a}	65.9±6.2 ^{ab}
NaCl	70.3±4.1 ^{b}	16.3±1.7 ^{b}	25.6±2.3 ^{a}	38.4±3.5 ^{a}	40.6±6.5 ^{c}	52.3±4.7 ^{b}
SA	87.2±5.8 ^{a}	11.0±1.4 ^{c}	14.6±1.5 ^{b}	24.7±3.6 ^{b}	72.6±4.6 ^{a}	69.2±4.9 ^{a}
SA+NaCl	83.2±3.9 ^{ab}	19.5±1.3 ^{a}	17.3±1.9 ^{b}	35.8±5.2 ^{a}	55.6±5.1 ^{b}	60.1±9.5 ^{ab}

and electron transport flux per reaction center (ET_o/RC) compared with the control (Fig. 4). These changes reveal substantial deviations from the control values, as reflected by the greater distances from the control profile in the spider plot (Fig. 4). Conversely, the phenomenological energy flux model identified PSII as a critical site of affected by salt stress . This stress resulted in decreases in several phenomenological energy fluxes, including absorption flux per cross-section (ABS/CS_m), trapped energy flux per cross-section (TR_o/CS_m), electron transport flux per cross-section (ET_o/CS_m), and the reduction flux beyond PSI (RE_o/CS_m) under salt stress compared with the control. These changes in JIP-test parameters represented a considerable impact on the structural and functional integrity of the photosynthetic apparatus under salt stress. In addition, salt stress markedly affected the quantum yield of primary photochemistry F_v/F_m (φP_o), the quantum yield for electron transport (φE_o), () and the efficiency/probability that an elec-

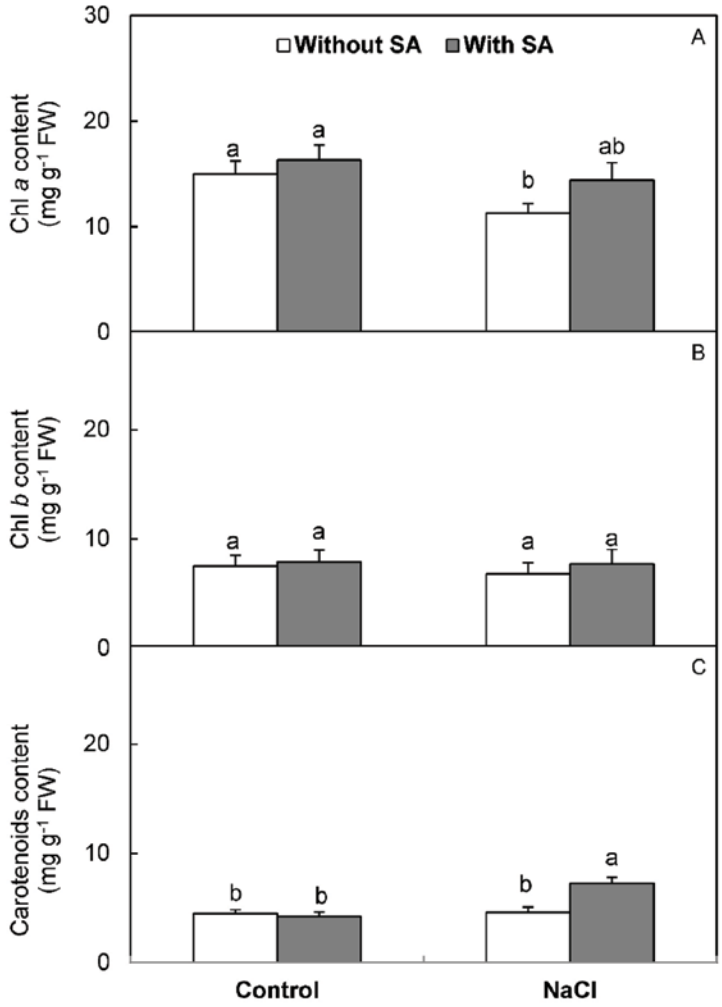


Fig. 1. Effect of SA on chlorophyll *a*, chlorophyll *b*, and carotenoid contents in *P. tuberosa* leaves under NaCl stress. Values are mean ± SD (n = 4). Different letters indicate significant differences ($p < 0.05$, Tukey's test).

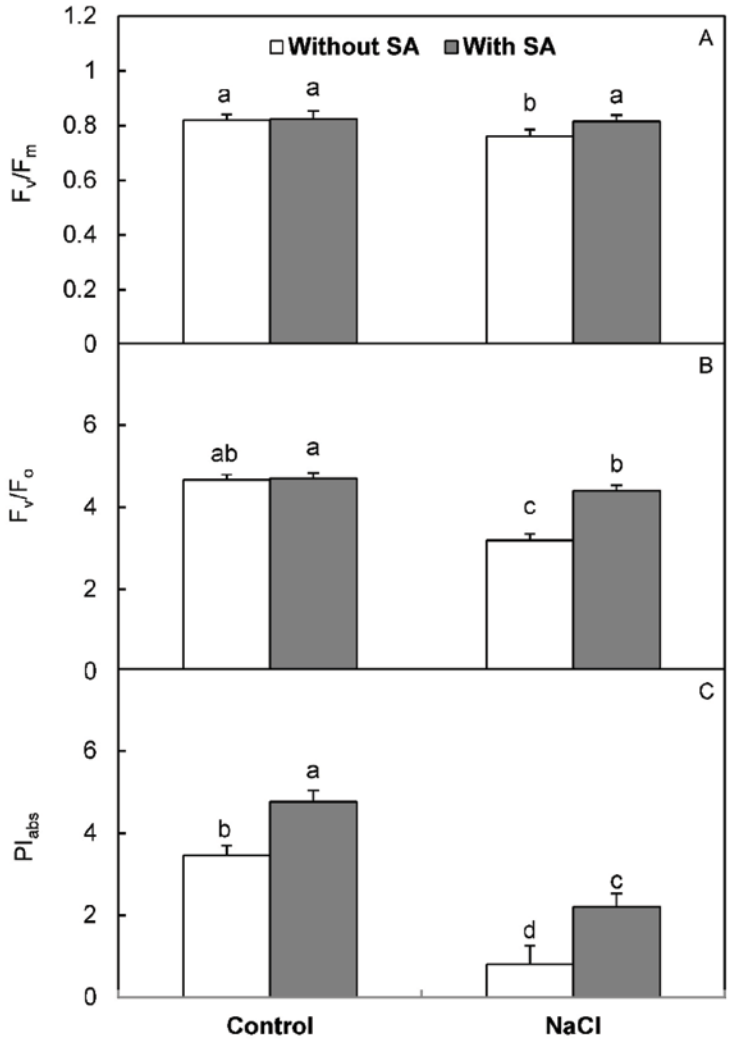


Fig. 2. Effect of SA on Fv/Fm, Fv/Fo, and PIabs in *P. tuberosa* leaves under NaCl stress. Values are mean ± SD (n = 4). Different letters indicate significant differences ($p < 0.05$, Tukey's test).

Table 3. Effect of SA on Chl *a*, Chl *b*, Car (Carotenoids), F_v/F_m, F_v/F_o and PI_{abs} in *P. tuberosa* under NaCl stress. Values are mean ± SD (n = 4); different letters indicate significant differences ($p < 0.05$, Tukey's test)

Treatment	Chl *a*	Chl *b*	Car	F_v/F_m	F_v/F_o	PI_{abs}
Control	15.1±1.23 ^{a}	7.6±0.99 ^{a}	4.51±0.36 ^{b}	0.82±0.02 ^{b}	4.6±0.12 ^{a}	3.46±0.23 ^{ab}
NaCl	11.3±0.89 ^{b}	6.8±1.05^{a}	4.62±0.47 ^{b}	0.76±0.025 ^{a}	3.2±0.15 ^{c}	0.81±0.45 ^{b}
SA	16.3±1.42 ^{a}	7.9±1.10 ^{a}	4.23±0.45 ^{b}	0.83±0.03 ^{b}	4.7±0.14 ^{a}	4.76±0.28 ^{a}
SA+NaCl	14.4±1.65 ^{ab}	7.71±1.35 ^{a}	7.25±0.55^{a}	0.81±0.024 ^{a}	4.4±0.13 ^{b}	2.22±0.33 ^{ab}

tron is transferred beyond Q^A (ψE_0)) (Fig. 4). However, SA s pretreatmentsignificantly alleviated these stress-induced changes, as indicated by reductions in F_o, ABS/RC, TR_o/RC and ET_o/RC, compared with plants exposed to salt stress alone. Thus, SA application under salt stress effectively modulated these parameters toward values closer to control. In addition, ABS/CS_m, TR_o/CS_m, ET_o/CS_m and RE_o/CS_m were significantly improved in plants treated with both NaCl and SA, highlighting the positive role of SA in maintaining photosystem function under salt stress.

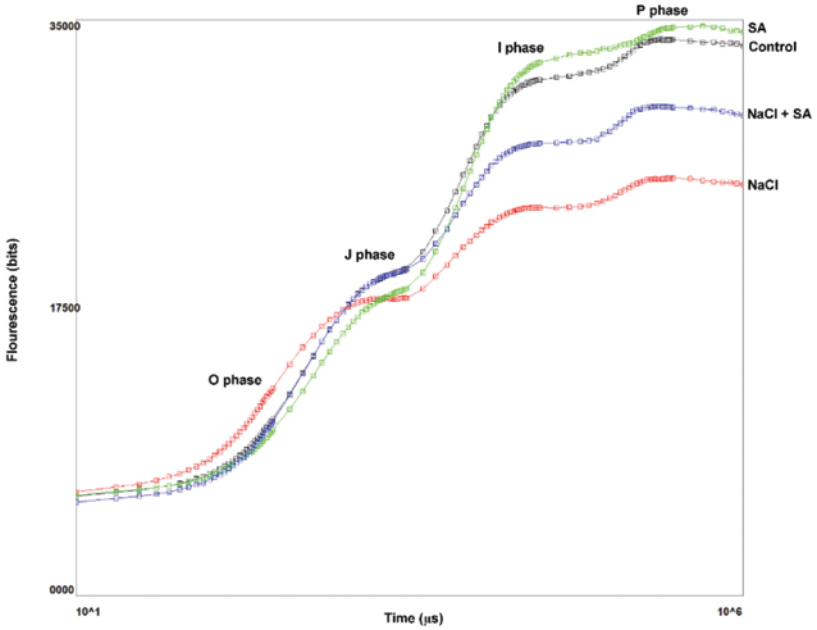


Fig. 3. Effect of SA application on the fast chlorophyll a fluorescence transient kinetics of *P. tuberosa* under NaCl stress

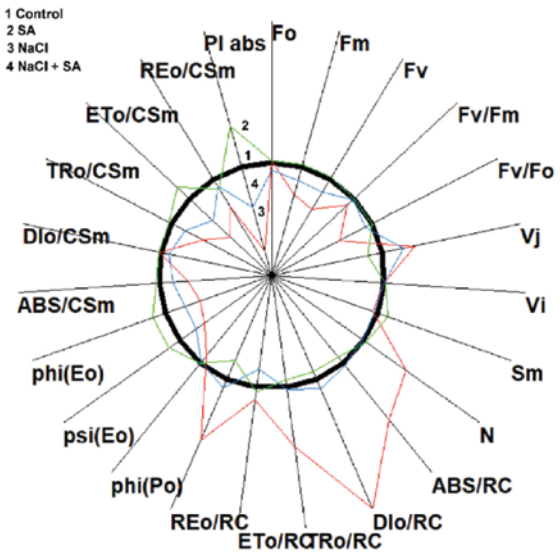


Fig. 4. Radar plots showing various technical fluorescence parameters of *P. tuberosa* plants affected by SA under salt stress.

SA at 0.1 mM was an alleviant for the oxidative stress effects caused by salinity

Significant changes in the activities of antioxidant enzymes, including APX and CAT, were observed under salinity alone treatment (Fig. 5).

Furthermore, the exogenous application of SA under salt stress conditions markedly enhanced the activities of APX, SOD, and CAT compared with those in the salinity alone treatment. Interestingly, plants treated with SA and salinity combination exhibited significantly higher in both NO and H_2O_2 content than the control (Fig. 6). Under salt stress, MDA content increased compared with the control, however, this increases in the leaves of salt-stressed *P. tuberosa* plants was significantly prevented by SA application at 0.1 mM.

SA application induces flowering of P. tu-

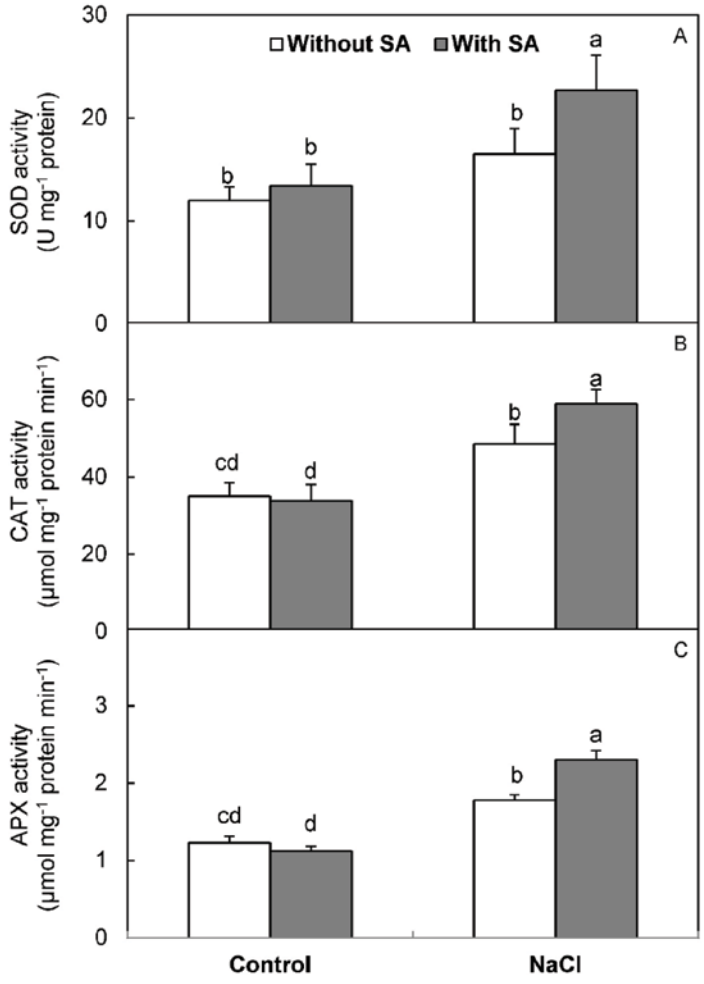


Fig. 5. Effect of SA on SOD, CAT, and APX activities in *P. tuberosa* leaves under NaCl stress. Bars with the same letter are not significantly different ($p < 0.05$, Tukey's test). Values are mean ± SD (n = 4).

berosa under both stress and non-stress conditions

Although the number of flowers was not affected by salt stress, salicylic acid (SA) treatment significantly increased flower number under both saltstress and nonstress conditions. Our results indicated that while exogenous SA application under control conditions did not significantly alter endogenous NO levels, it led to a pronounced accumulation of NO under salinity stress, suggesting that the SA-mediated enhancement of the NO signal is triggered under stressful conditions. In addition, a positive correlation was observed between flower number and nitric oxide (NO) levels in *P. tuberosa* plants subjected to SA alone ($r = 0.52$, $p < 0.05$) or exposed to SA and NaCl combination ($r = 0.86$, $p < 0.01$) (Fig. 7).

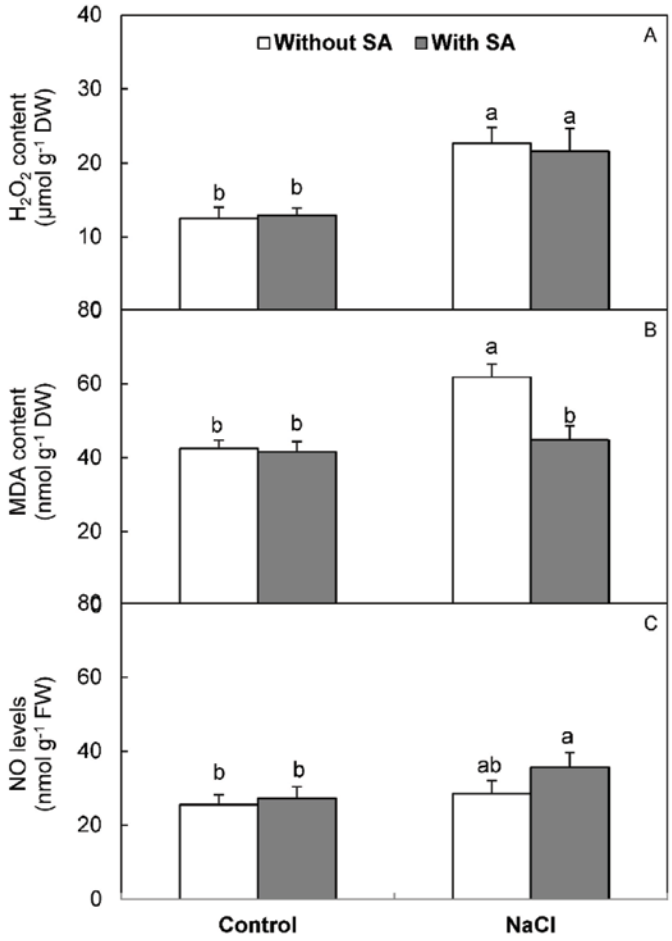


Fig. 6. Effect of SA application on the content of hydrogen peroxide (H_2O_2), maloendialdehyde (MDA), and nitric oxide (NO) in *P. tuberosa* leaves under NaCl-stressed conditions. Bars indicated with the same letter are not significantly different ($p < 0.05$, Tukey test). Values are the mean ± SD (n = 4).

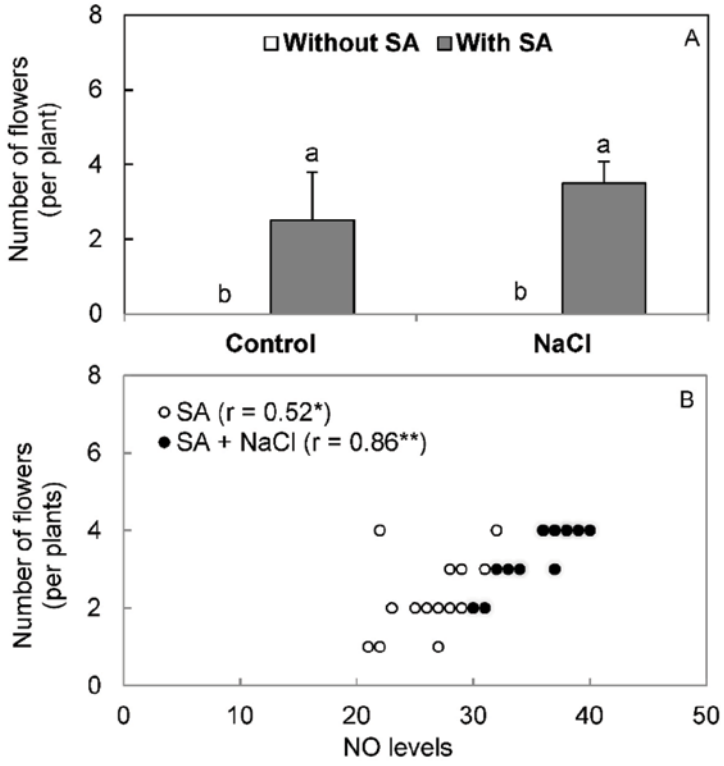


Fig. 7. Effect of SA application on the number of flowers per plant in *P. tuberosa* under NaCl-stressed conditions (A). Correlation between the number of flowers and NO levels (B) recorded in *P. tuberosa* plants exposed to SA or SA + NaCl. **p* < 0.05; ***p* < 0.01; n.s., not significant.

Discussion

Salinity induces hyperosmotic and hyperionic conditions, which lead to excessive ROS production, ultimately resulting in stunted plant growth and development (Chaves et al., 2009). The present study revealed that salt stress significantly inhibited plant growth, which is primarily related to excessive ion accumulation in plant cells coupled with decreased uptake of essential nutrients and water, which together restrict cell division and development (Acosta-Motos et al., 2015). Similar reductions in biomass and shorter shoots under salt stress have been shown in various plant species, including maize (Raza et al., 2019), barley (Akhter et al., 2023), wheat (Genc et al., 2019), and black gram (Ahmad et al., 2024). In this study, the results revealed that under salt-stressed conditions, the K^+ content decreased, and the Na^+ content increased in the *P. tuberosa* leaves. Increased Na^+can also interfere with the uptake and homeostasis of other nutrients (Wakeel et al., 2011; Guo et al., 2019) and cause ionic imbalance (Roshdy et al., 2021), which can may disrupt plasma membrane integrity in this species. SA alleviated salt stress by reducing excessive Na^+

translocation to leaves through the transpiration stream, thereby enhancing plant growth (X et al., 2025). Furthermore, the application of SA in *P. tuberosa* plants was capable of increasing shoot K^+ content, and lowering Na^+/K^+ ratio, which maintained the integrity of the cell membranes from oxidative damage (El-Hawary et al., 2023). Additionally, a significant improvement in proline content was detected in leaves of salt-stressed *P. tuberosa* plants, however, exogenous SA application under NaCl stress further increased proline. Similar results were reported by Liu et al., (2016), who showed that SA application further increased the contents of proline in the leaves of NaCl-treated *Nitraria tangutorum* seedlings. Furthermore, our results demonstrated that SA significantly improves water status under salt stress, as evidenced by maintaining the leaf RWC. This observed increase in leaf RWC may be attributed to SA's role in enhancing water use efficiency and protecting cellular hydration. In conclusion, the current study's findings were consistent with previous reports on *Egletes viscosa* (Batista et al., 2019) and *Olea europeae* (Methenni et al., 2018) in which SA application significantly alleviated the detrimental effects of salt stress on plant growth. In the present study, chlorophyll *a* concentration was markedly decreased in salt-stressed *P. tuberosa* plants, highlighting that salt stress may have impaired chlorophyll biosynthesis of leaf pigments and accelerated their degradation. In fact, salt stress damages chloroplasts and reduce the chlorophyll content, thereby impeding the photosynthesis capacity (Chen et al., 2021; Khalvandi et al., 2021). Additionally, This reduction in leaf pigment contents may be associated with the Na^+ accumulation, K^+depletion , and subsequent lipid peroxidation within leaf cells when exposed to salinity (Taïbi et al., 2016; Nieves-Cordones et al., 2019). In our research, carotenoids concentrations were substantially greater in plants treated with NaCl+SA than in those treated with NaCl alone. This increase suggests that SA application stimulates carotenoids biosynthesis and reduces carotenoids degradation under salt stress, possibly because of diminished oxidative stress (Farhangi-Abriz and Ghassemi-Golezani 2018). This increased carotenoids content may contribute to maintaining PSII efficiency under salt stress (Habibi 2021) and may play a major role in photoprotection (Zhang et al., 2025). In this study, we also employed OJIP transients, which provides indicators of photosynthetic electron transport rate and the efficacy of light energy capture in the PSII, to evaluate PSII performance in plants treated with salt stress and SA treatment. Findings revealed that salt stress led to a more rapid elevation in fluorescence during the O−J phase, suggesting impaired re-oxidation of QA and its increased accumulation, which may reflect decreased efficiency of electron transport beyond QA (Kalaji et al., 2016). In addition, salt stress significantly increased F_o consistent with previous findings reported by Che et al., (2022) and Li et al., (2020). These finding showed that the increase in F_o may be associated with stress-induced inhibition of PSII reaction centers, which can restrict electron transfer from QA to QB and reduce the efficacy of energy trapping within PSII. The quantum yield of primary photochem-

istry F_v/F_m, a key chlorophyll fluorescence parameter, was lower in salt-stressed *P. tuberosa* plants, indicating decreased photosynthetic capacity. This reduction may have lead to greater susceptibility to oxidative stress (Mohagheghian et al., 2025). The F_v/F_o parameter reflects the performance of the water-splitting complex on the donor side of PSII (Schreiber et al., 1995). In this study, the decline in the F_v/F_o ratio under salt stress indicated an inhibition of osmotic water uptake(Fricke and Peters 2002) and disruption of the electron transport and photophosphorylation processes (Pereira et al., 2000). In addition, the decreased PI_{abs} in the salt stress treatment group revealed a reduced efficiency in the number of functional reaction centers and the ability to channel and convey electrons to the electron transport chain (Kalaji et al., 2011). SA supplementation diminished ABS/RC and improved φP_o and φE_o in the salt-stressed *P. tuberosa* plants, highlighting more efficient electron transfer from antennae to RCs (Li et al., 2022). Additionally, salt stress significantly increased Vj and N levels as compared with the control , reflecting increased electron transport through QA reduction and enhanced energy fluxes associated with chlorophyll fluorescence transients. These changes likely indicated the plant's physiological adjustments to cope with stress and meet the elevated energy demands (Akhter et al., 2021). SA supplementation notably reduced these parameters, suggesting that SA suplementation helped stabilizing electron transfer processes and restoring balance to chlorophyll fluorescence transients (Li et al., 2022). Reductions in the phenomenological energy flux parameters, including ABS/CS_m, TR_o/CS_m, ET_o/CS_m, DI_o/CS_m and RE_o/CS_m revealed that PSII was strongly affected by salt stress compared with the control. These reductions may be associated with the conversion of active PSII reaction centers into inactive centers, thereby impairing energy trapping and electron transport within PSII (Chen et al., 2021). Similar results were reported in tomato leaves by Chen et al., (2021) demonstrating the universal impact of salt stress on photosynthetic machinery. Therefore, these findings highlighted the protective role of SA in improving photosynthesis and sustaining productivity under saline conditions.

In addition to the non-enzymatic antioxidant system, the enzymatic antioxidant system including SOD, CAT and APX, is another defense strategy in plants against stress by scavenging excessive ROS (Mahawar and Shekhawat 2019; Mahawar et al., 2024). SOD is typically recognized as the primary antioxidant enzyme that converts superoxide radicals into H_2O_2, a compound subsequently neutralized into H_2O and O_2 by CAT and APX (Khalvandi et al., 2021). APX is a pivotal enzyme within the AsA-GSH cycle, performing the detoxification of H_2O_2 to H_2O by using AsA as an electron donor (Leng et al., 2021). Our research showed increased APX and CAT activities in *P. tuberosa* plants under NaCl stress, corroborating studies on various plant species, including tomato (Faizan et al., 2021), hybrid P*ennisetum* (Li et al., 2020), rice (Koc et al., 2024) and wheat (Khalvandi et al., 2021) and saffron (*Crocus sativus* L.) (Niknam et al., 2025). Our research also revealed that

the activities of SOD, APX and CAT were markedly enhanced upon SA supplementation. This positive effect of SA on the activities of antioxidative enzymes was similar to that observed in salt-stressed Chinese cabbage (Kaya et al., 2020) and wheat (El-Hawary et al., 2023) plants. Under salt stress treatment, increased H_2O_2 and MDA levels exhibited over-accumulation of ROS, which induced significant oxidative damage to cellular biomolecules (Foyer et al., 2020; Liu et al., 2024). The significant increase in ROS accumulation in the leaves of the stressed *P. tuberosa* plants, which was consistent with the decrease in F_v/F_m, highlighting that the reduction of photochemical activity closely attributed to the marked variations in oxidative status. Similarly, were previously demonstrated by. This finding also aligns with previous reports of Azzabi et al., (2012), which the relationships between oxidative status and a decrease in F_v/F_m was observed. In contrast, when the *P. tuberosa* seedings was primed with SA, MDA levels decreased in NaCl-treated plants. These results revealed that SA can play a protective role in diminishing ROS accumulation and diminishing membrane oxidation damage. Similar reduction in MDA values have been observed in tobacco seedlings subjected to SA treatment under salt stress (Song et al., 2025) further corroborating our findings. Interestingly, in this study, endogenous NO content of SA-treated plants exhibited a significant increase which was only detected under salinity stress, revealing that SA affects NO accumulation under stress conditions. Regarding to relationships of SA and NO, several studies have highlighted that SA acts upstream of NO to enhance antioxidant biosynthesis and mitigate oxidative damage caused by environmental stresses, including salt stress in cotton seedlings (Dong et al., 2015), drought stress in wheat (Kolupaev et al., 2018), and heat stress in *Lablab purpureus* L (Rai et al., 2020). However, to determine the synergistic effects of NO and SA in boosting antioxidant defense mechanisms and mitigating NaCl stress damages, will require to be further analysed.

Exogenous SA promoted flowering in *P. tuberosa* under both saline and non-saline conditions, indicating that its effect on floral transition was not strictly stress-dependent. This observation was consistent with earlier reports showing that SA can regulate flowering under both normal and stress conditions (Cleland et al., 1982; Martínez et al., 2004; Wada and Takeno 2013). The positive correlation between flower number and endogenous NO further suggested a potential involvement of NO in SA-related flowering regulation, although the present data support an associative rather than definitive mechanistic role (Hou et al., 2025).

Conclusion

The present study showed that salt stress significantly inhibited plant growth by reducing shoot dry biomass, which was primarily attributed to excessive Na^+ accumulation in plant cells coupled with reduced uptake of K^+ and water. However, SA application reversed the toxic effects of salt-induced damages by inhibiting excessive Na^+ translocation to leaves, resulting in a significant increase in growth-related attributes. The significant increase in ROS

accumulation in the leaves of the stressed *P. tuberosa* plants, which was consistent with the decrease in F_v/F_m, highlighting that the reduction of photochemical activity closely attributed to the marked variations in oxidative status. In contrast, when the *P. tuberosa* plants was primed with SA, MDA levels decreased in NaCl-treated plants. Our findings suggested a potential synergistic or interactive role of NO in SA-mediated antioxidant defense mechanisms and mitigating NaCl stress damages. SA application promoted flowering in *P. tuberosa* irrespective of salinity. Moreover, the positive correlation between NO levels and flower number suggested that NO may function as a signaling component in SAmediated regulation of the floral transition. Our results revealed that SA application can play a protective role in reducing ROS accumulation and diminishing membrane oxidation damage resulting in significant photosynthetic improvements.

Author contributions GH planned and designed the research, collected and analyzed the data and wrote the manuscript and revised the manuscript.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdullaev, F., Pirogova, P., Vodeneev, V. and Sherstneva, O. (2024) 'Chlorophyll fluorescence in wheat breeding for heat and drought tolerance', *Plants*, 13(19), p.2778. https://doi.org/10.3390/plants13192778.

Acosta-Motos, J.R., Ortuño, M.F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M.J. and Hernandez, J.A. (2015) 'Physiological and biochemical mechanisms of', *Eugenia myrtifolia* L. plants in response to NaCl stress. *Planta*, 242(4), pp.829–846. https://doi.org/10.1007/s00425-015-2330-0.

Ahanger, M.A., Aziz, U., Alsahli, A.A., AlYemeni, M.N. and Ahmad, P. (2020) 'Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis and ascorbate–glutathione cycle in salt-stressed', *Vigna angularis*. *Biomolecules*, 10(1), p.42. https://doi.org/10.3390/biom10010042.

Ahmad, W., Ijaz, M., Zafar-ul-Hye, M., Tahir, M., Khan, M.A. and Ahmad, I. (2024) 'Silicon-mediated improvement in drought and salinity stress tolerance of black gram (', *Vigna mungo* L.). *ACS Omega*, 9(35), pp.37231–37242. https://doi.org/10.1021/acsomega.4c05342.

Akhter, M.S., Hossain, M.A., Hasanuzzaman, M. and Fujita, M. (2023) 'Silicon supplement improves growth, yield and antioxidant defense system in barley under salt stress', *Journal of Soil Science and Plant Nutrition*, 23(2), pp.1694–1712. https://doi.org/10.1007/s42729-023-01234-5.

Akhter, S.M., Andersen, M.N. and Liu, F. (2021) 'Influence of salinity stress on photosystem II efficiency and electron

transport in barley genotypes probed by chlorophyll', *a* fluorescence. *Journal of King Saud University - Science*, 33(1), p.101239. https://doi.org/10.1016/j.jksus.2020.101239.

Ali, L., Asghar, H.N., Khan, M.Y., Saleem, M.F. and Hussain, S. (2022) 'Growth, photosynthesis and antioxidant enzyme modulation in broccoli under salinity stress', *South African Journal of Botany*, 148, pp.104–111. https://doi.org/10.1016/j.sajb.2022.04.010.

Alizadeh, M.M., Gerami, M., Majidian, P. and Ghorbani, H.R. (2024) 'Potential application of biochar and salicylic acid to alleviate salt stress in soybean', *Heliyon*, 10(4), p.e26677. https://doi.org/10.1016/j.heliyon.2024.e26677.

Arif, Y., Sami, F., Siddiqui, H., Bajguz, A. and Hayat, S. (2020) 'Salicylic acid in relation to other phytohormones: a study towards understanding its role in plant defense signaling under environmental challenges', *Environmental and Experimental Botany*, 175, p.104040. https://doi.org/10.1016/j.envexpbot.2020.104040.

Azzabi, G., Pinnola, A., Betterle, N., Bassi, R. and Alboresi, A. (2012) 'Enhancement of non-photochemical quenching as an adaptive strategy of the moss', *Physcomitrella patens* to cope with salt stress. *Plant and Cell Physiology*, 53(10), pp.1815–1825. https://doi.org/10.1093/pcp/pcs121.

Barwal, S.K., Singh, P., Kumar, A., Yadav, S. and Singh, V.P. (2024) 'Mechanistic insights of salicylic acid–mediated salt stress tolerance in maize seedlings', *Heliyon*, 10(16), p.e34486. https://doi.org/10.1016/j.heliyon.2024.e34486.

Bates, L.S., Waldren, R.P. and Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39(1), pp.205–207. https://doi.org/10.1007/BF00018060.

Batista, V.C.V., do Amaral, M.N., Riffel, A., Auler, P.A. and Moraes, D.M. (2019) 'Salicylic acid modulates primary and secondary metabolism to alleviate salt-induced photosynthesis impairment in', *Egletes viscosa*. *Environmental and Experimental Botany*, 167, p.103870. https://doi.org/10.1016/j.envexpbot.2019.103870.

Boominathan, R. and Doran, P.M. (2002) 'Ni-induced oxidative stress in roots of the Ni hyperaccumulator,', *Alyssum bertolonii*. *New Phytologist*, 156(2), pp.202–205. https://doi.org/10.1046/j.1469-8137.2002.00506.x.

Chaves, M.M., Flexas, J. and Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, 103(4), pp.551–560. https://doi.org/10.1093/aob/mcn125.

Che, Y., Xu, X., Wang, Y., Wang, H. and Zhao, H. (2022) 'Potassium application mitigates salt stress impacts by alleviating proton diffusion in thylakoids and maintaining photosynthetic efficiency', *Environmental and Experimental Botany*, 194, p.104708. https://doi.org/10.1016/j.envexpbot.2021.104708.

Chen, L., Zhao, Y., Xu, S., Zhang, Z., Xu, Y., Zhang, J. and Chong, K. (2021) 'Melatonin promotes seed germination

under salt stress by regulating ABA and GA balance in cotton (', *Gossypium hirsutum* L.). *Plant Physiology and Biochemistry*, 162, pp.506–516. https://doi.org/10.1016/j.plaphy.2021.03.016.

Chen, X., Wang, Y., Li, J., Jiang, A. and Cheng, Y. (2021) 'Ascorbic acid-induced photosynthetic adaptability to salt stress monitored by OJIP chlorophyll fluorescence transient analysis', *Frontiers in Plant Science*, 12, p.594400. https://doi.org/10.3389/fpls.2021.594400.

Cleland, C.F., Tanaka, O. and Feldman, L.J., 1982. Influence of plant growth substances and salicylic acid on flowering and growth in the Lemnaceae (duckweeds). *Aquatic Botany*, 13, pp.3–20. https://doi.org/10.1016/0304-3770(82)90036-190036-1).

Dong, Y.J., He, M.R., Wang, Z.L., Chen, W.F., Hou, J., Qiu, X.K. and Zhang, J.W. (2015) 'Interaction of nitric oxide and salicylic acid alleviates salt stress in cotton seedlings', *Journal of Soil Science and Plant Nutrition*, 15(2), pp.561–573. https://doi.org/10.4067/S0718-95162015005000041.

El-Hawary, M.M., Hashem, O.S.M. and Hasanuzzaman, M. (2023) 'Seed application with ascorbic acid and salicylic acid mitigates salt stress in wheat', *Agronomy*, 13(2), p.493. https://doi.org/10.3390/agronomy13020493.

Faizan, M., Faraz, A., Yusuf, M., Khan, S.T. and Hayat, S. (2021) 'Zinc oxide nanoparticles induce salt tolerance by enhancing photosynthetic performance and antioxidant system in tomato', *Plant Physiology and Biochemistry*, 161, pp.122–130. https://doi.org/10.1016/j.plaphy.2021.02.015.

Farhangi-Abriz, S. and Ghassemi-Golezani, K., 2018. How can salicylic acid and jasmonic acid mitigate salt toxicity in soybean plants? *Ecotoxicology and Environmental Safety*, 147, pp.1010–1016. https://doi.org/10.1016/j.ecoenv.2017.09.070.

Foyer, C.H., Kyndt, T. and Hancock, R.D. (2020) 'Vitamin C in plants: novel concepts, new perspectives, and potential applications', *Antioxidants & Redox Signaling*, 32(7), pp.463–485. https://doi.org/10.1089/ars.2019.7812.

Genc, Y., Oldach, K., Verbyla, A.P., Lott, G., Hassan, M., Tester, M., Wallwork, H. and McDonald, G.K. (2019) 'Sodium exclusion and salt tolerance in bread wheat', *Frontiers in Plant Science*, 10, p.1280. https://doi.org/10.3389/fpls.2019.01280.

Ghasemifar, E., Habibi, G. and Bakhshi-Khaniki, G. (2022) 'Pretreatment with salicylic acid and nitric oxide mitigated silver nanoparticles toxicity and enhanced their removal in medicinal', *Phlomis tuberosa* plants. *Acta Biologica Cracoviensia Series Botanica*, 64(2), pp.35–48. https://doi.org/10.24425/abcsb.2022.140302.

Guo, Q., Liu, L. and Barkla, B.J. (2019) 'Membrane lipid remodeling in response to salinity', *International Journal of Molecular Sciences*, 20(17), p.4264. https://doi.org/10.3390/ijms20174264.

Habibi, G. (2020) 'Comparison of CAM expression, photochemistry and antioxidant responses in', *Sedum album*

and *Portulaca oleracea* under combined stress. *Physiologia Plantarum*, 170(4), pp.550–568. https://doi.org/10.1111/ppl.13193.

Hafeez, M.B., Zahra, N., Khan, Z.A., Raza, A., Ahmad, S., Khan, A. and Farooq, M. (2024) 'Plant growth-promoting rhizobacteria improve photosynthetic performance and antioxidant defense system under salinity stress', *Plant Stress*, 12, p.100480. https://doi.org/10.1016/j.stress.2024.100480.

Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., Fujita, M. and Fotopoulos, V. (2021) 'Reactive oxygen species and antioxidant defense in plants under abiotic stress', *International Journal of Molecular Sciences*, 22(17), p.9326. https://doi.org/10.3390/ijms22179326.

Hasanuzzaman, M., Shabala, L., Brodribb, T.J., Zhou, M. and Shabala, S. (2017) 'Screening methods as proxies for drought tolerance in barley', *Functional Plant Biology*, 44(3), pp.253–266. https://doi.org/10.1071/FP16202.

Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. *Environmental and Experimental Botany*, 68(1), pp.14–25. https://doi.org/10.1016/j.envexpbot.2009.08.005.

He, Y., Zhu, Z., Yang, J., Ni, X. and Zhu, B., 2009. Grafting increases the salt tolerance of tomato by improving photosynthesis and antioxidant enzyme activities. *Environmental and Experimental Botany*, 66(2), pp.270–278. https://doi.org/10.1016/j.envexpbot.2009.02.005.

Hou, X., Shi, M., Zhang, Z., Yao, Y., Li, Y., Li, C., Yu, W., Wang, C. and Liao, W. (2025) 'DNA demethylation is involved in nitric oxide-induced flowering in tomato', *Journal of Integrative Agriculture*, 24, pp.1769–1785. https://doi.org/10.1016/j.jia.2024.03.076.

Hryvusevich, P., Nekrasov, V., Dunaeva, E., Baluška, F. and Shabala, S. (2021) 'Sodium influx and potassium efflux in sunflower roots under high salinity', *Frontiers in Plant Science*, 11, p.2181. https://doi.org/10.3389/fpls.2020.613936.

Isayenkov, S.V. and Maathuis, F.J.M. (2019) 'Plant salinity stress: many unanswered questions remain', *Frontiers in Plant Science*, 10, p.80. https://doi.org/10.3389/fpls.2019.00080.

Johnson, C.M., Stout, P.R., Broyer, T.C. and Carlton, A.B. (1957) 'Comparative chlorine requirements of different plant species', *Plant and Soil*, 8(4), pp.337–353. https://doi.org/10.1007/BF01398944.

Kalaji, H.M., Govindjee, Bosa, K., Kościelniak, J. and Żuk-Gołaszewska, K. (2011) 'Effects of salt stress on photosystem II efficiency and CO_2 assimilation of barley', *Environmental and Experimental Botany*, 73, pp.64–72. https://doi.org/10.1016/j.envexpbot.2010.10.009.

Kalaji, H.M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I.A., Cetner, M.D., Łukasik, I., Goltsev, V. and Ladle, R.J. (2016) 'Chlorophyll', *a* fluorescence as a tool to monitor physiological status of plants

under abiotic stress conditions. *Acta Physiologiae Plantarum*, 38, p.102. https://doi.org/10.1007/s11738-016-2113-y.

Kareem, F., Rihan, H. and Fuller, M.P. (2019) 'The effect of salicylic acid on drought tolerance in wheat (', *Triticum aestivum* L.). *Journal of Crop Science and Biotechnology*, 22(1), pp.37–45. https://doi.org/10.1007/s12892-018-0014-3.

Kashif, M.H., Hussain, S., Raza, A., Ahmad, S., Shahzad, B. and Farooq, M. (2024) 'Salicylic acid-mediated alleviation of salt stress in', *Asarum sieboldii* Miq. *Chemosphere*, 362, p.142604. https://doi.org/10.1016/j.chemosphere.2024.142604.

Kaya, C., Ashraf, M., Alyemeni, M.N., Corpas, F.J. and Ahmad, P. (2020) 'Nitric oxide-mediated enhancement of the ascorbate–glutathione cycle under arsenic toxicity in maize plants', *Journal of Hazardous Materials*, 399, p.123020. https://doi.org/10.1016/j.jhazmat.2020.123020.

Kaya, C., Ashraf, M., Corpas, F.J. and Ahmad, P. (2022) 'Melatonin and salicylic acid regulate nitrogen metabolism and detoxify arsenic toxicity in maize', *Environmental Pollution*, 297, p.118727. https://doi.org/10.1016/j.envpol.2021.118727.

Kaya, C., Ugurlar, F., Ashraf, M., Corpas, F.J. and Ahmad, P. (2020) 'Endogenous nitric oxide in salicylic acid-induced salinity tolerance of pepper plants', *Plant Physiology and Biochemistry*, 147, pp.10–20. https://doi.org/10.1016/j.plaphy.2019.11.038.

Kaya, C., Ugurlar, F., Ashraf, M., Corpas, F.J. and Ahmad, P. (2023) 'Synergistic effects of melatonin and salicylic acid in improving drought tolerance of tomato plants', *Scientia Horticulturae*, 321, p.112368. https://doi.org/10.1016/j.scienta.2023.112368.

Khalvandi, M., Siosemardeh, A., Roohi, E. and Keramati, S. (2021) 'Salicylic acid alleviates drought stress in winter wheat by regulating photosynthetic capacity and antioxidant defense', *Heliyon*, 7, p.e05908. https://doi.org/10.1016/j.heliyon.2021.e05908.

Koc, Y.E., Aycan, M. and Mitsui, T. (2024) 'Exogenous proline improves salinity tolerance by enhancing antioxidant defense and photosynthetic performance in rice', *Plant Physiology and Biochemistry*, 214, p.108914. https://doi.org/10.1016/j.plaphy.2024.108914.

Kolupaev, Y.E., Yastreb, T.O. and Obozny, A.I., 2018. Combined effect of salicylic acid and nitric oxide donor on antioxidant enzymes and heat resistance of wheat seedlings under drought stress. *Applied Biochemistry and Microbiology*, 54, pp.418–424. https://doi.org/10.1134/S0003683818040120.

Kumar, D., Singh, H., Raj, S. and Soni, V., 2020. Chlorophyll *a* fluorescence kinetics of mung bean (*Vigna radiata* L.) grown under artificial continuous light. *Biochemistry and Biophysics Reports*, 24, p.100813. https://doi.org/10.1016/j.bbrep.2020.100813.

Leng, X., Wang, P., Wang, C., Zhu, X. and Li, X. (2021) 'Genome-wide identification

and characterization of the ascorbate peroxidase gene family in', *Populus trichocarpa*. *Genes*, 12(3), p.334. https://doi.org/10.3390/genes12030334.

Li, G., Peng, X., Wei, L. and Kang, G. (2013) 'Salicylic acid increases antioxidant capacity in wheat leaves under salt stress by enhancing glutathione and ascorbate accumulation', *Gene*, 529(2), pp.321–325. https://doi.org/10.1016/j.gene.2013.08.023.

Li, L., Song, Y., Guan, C., Li, Y. and Chen, X. (2022) 'Silicon ameliorates salt stress by regulating photosynthesis and antioxidant metabolism in cotton', *Antioxidants*, 11(8), p.1520. https://doi.org/10.3390/antiox11081520.

Li, P., Zhu, Y., Song, X. and Song, F. (2020) 'Effects of salinity and drought stress on growth, photosynthesis and antioxidant metabolism of hybrid', *Pennisetum*. *Plant Physiology and Biochemistry*, 155, pp.93–104. https://doi.org/10.1016/j.plaphy.2020.07.003.

Li, X., Zhang, L., Ahammed, G.J., Li, Z., Wei, J., Shen, C., Yan, P., Zhang, L., Han, W. and Fu, J. (2019) 'Salicylic acid acts upstream of nitric oxide in regulating flavonoid biosynthesis and abiotic stress tolerance in tea plants', *Environmental and Experimental Botany*, 161, pp.367–374. https://doi.org/10.1016/j.envexpbot.2019.01.012.

Lichtenthaler, H. and Wellburn, A.R. (1985) 'Determination of total carotenoids and chlorophylls a and b of leaf in different solvents', *Biochemical Society Transactions*, 11, pp.591–592. https://doi.org/10.1042/bst0110591.

Liu, W., Zhang, Y., Yuan, X., Xuan, Y., Gao, Y. and Yan, Y. (2016) 'Exogenous salicylic acid improves salinity tolerance of', *Nitraria tangutorum* by enhancing antioxidant capacity. *Russian Journal of Plant Physiology*, 63(1), pp.132–142. https://doi.org/10.1134/S1021443716010101.

Liu, Y., Zhang, L., Liu, B., Wu, Y., Sun, Y. and Guo, S. (2024) 'Biochar improves soil properties and growth of', *Melissa officinalis* under salt stress. *Scientia Horticulturae*, 338, p.113704. https://doi.org/10.1016/j.scienta.2024.113704.

Maghsoudi, K., Arvin, M.J. and Ashraf, M., 2020. Mitigation of arsenic toxicity in wheat by salicylic acid. *Journal of Soil Science and Plant Nutrition*, 20, pp.577–588. https://doi.org/10.1007/s42729-019-00146-6.

Mahawar, L. and Shekhawat, G.S. (2019) 'Heme oxygenase 1 mediated mitigation of NaCl-induced oxidative stress in', *Eruca sativa*. *Physiology and Molecular Biology of Plants*, 25(4), pp.895–904. https://doi.org/10.1007/s12298-019-00672-1.

Mahawar, L., Sharma, P. and Shekhawat, G.S., 2024. Copper oxide and zinc oxide nanoparticles influence photosynthesis and antioxidant defense in *Raphanus sativus* under salinity stress. *Plant Physiology and Biochemistry*, 206, p.108281. https://doi.org/10.1016/j.plaphy.2023.108281.

Martínez, C., Pons, E., Prats, G. and León, J., 2004. Salicylic acid regulates flowering time and links defense responses and reproductive development. *The Plant*

Journal, 37(2), pp.209–217. https://doi.org/10.1046/j.1365-313X.2003.01980.x.

Methenni, K., Balti, R., Chehab, H., Ouled-Messaoud, M. and Trabelsi, N. (2018) 'Salicylic acid and calcium alleviate salinity effects on growth and physiological traits of olive', *Scientia Horticulturae*, 233, pp.349–358. https://doi.org/10.1016/j.scienta.2018.01.046.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S. and Mittler, R. (2010) 'Reactive oxygen species homeostasis and signalling during drought and salinity stresses', *Plant, Cell & Environment*, 33(4), pp.453–467. https://doi.org/10.1111/j.1365-3040.2009.02083.x.

Mukhopadhyay, R., Sarkar, B., Ghosh, S., Mondal, S., Tripathi, D.K., Pandey, S., Corpas, F.J., Khan, M.I. and Ahmad, A., 2021. Role of salicylic acid in enhancing plant tolerance to salt stress. *Plant Physiology and Biochemistry*, 164, pp.196–212. https://doi.org/10.1016/j.plaphy.2021.04.024.

Munns, R. and Gilliham, M. (2015) 'Salinity tolerance of crops – what is the cost?', *New Phytologist*, 208(3), pp.668–673. https://doi.org/10.1111/nph.13519.

Munns, R. and Tester, M. (2008) 'Mechanisms of salinity tolerance', *Annual Review of Plant Biology*, 59, pp.651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.

Nazar, R., Iqbal, N., Syeed, S. and Khan, N.A., 2011. Salicylic acid alleviates the adverse effects of salt stress by improving photosynthesis and antioxidant metabolism in mustard. *Plant Physiology and Biochemistry*, 49(2), pp.123–131. https://doi.org/10.1016/j.plaphy.2011.01.003.

Nikolaeva, M.K., Maevskaya, S.N., Shugaev, A.G. and Bukhov, N.G. (2010) 'Effect of drought on chlorophyll fluorescence parameters and antioxidant activities in wheat leaves', *Russian Journal of Plant Physiology*, 57(1), pp.87–95. https://doi.org/10.1134/S1021443710010130.

Noreen, S., Fatima, N., Akhtar, S., Hussain, K. and Ashraf, M. (2021) 'Regulation of photosynthetic efficiency, ion homeostasis, and antioxidant defense by salicylic acid in rice under salt stress', *Journal of Plant Growth Regulation*, 40(4), pp.1753–1767. https://doi.org/10.1007/s00344-020-10245-y.

Pandey, P., Srivastava, R.K. and R.S., D., 2024. Alleviatory role of ascorbic acid and proline on salt-induced oxidative stress in wheat seedlings. *Biologia Plantarum*, 68, pp.417–428. Available at: https://doi.org/10.32615/bp.2024.035.

Porcel, R. and Ruiz-Lozano, J.M. (2004) 'Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress', *Journal of Experimental Botany*, 55(403), pp.1743–1750. https://doi.org/10.1093/jxb/erh188.

Qiu, Z., Guo, J., Zhang, F., Sun, L. and Li, C. (2014) 'Improved salt tolerance through overexpression of a chloroplast-localized small heat shock protein in', *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, 80, pp.47–53. https://doi.org/10.1016/j.plaphy.2014.03.025.

Rady, M.M. and Hemida, K.A. (2016)

'Salicylic acid increases salt tolerance and improves antioxidant systems in maize', *Environmental Science and Pollution Research*, 23(8), pp.7013–7022. https://doi.org/10.1007/s11356-015-5910-7.

Rady, M.M., Taha, R.S., Mahdi, A.H., Metwally, I.H. and Salem, M.F. (2023) 'Ascorbic acid enhances salt tolerance in barley by boosting antioxidant defense and maintaining photosynthetic efficiency', *Agronomy*, 13(7), p.1765. https://doi.org/10.3390/agronomy13071765.

Rao, M., Ahmed, S., Younis, U., Raza, A., Mumtaz, S., Jatoi, F.A., Anwar, S., Alharby, H.F. and Bamagoos, A.A. (2024) 'Interactive effects of salicylic acid and zinc oxide nanoparticles on salinity tolerance in tomato', *Chemosphere*, 345, p.140567. https://doi.org/10.1016/j.chemosphere.2023.140567.

Rizwan, M., Ali, B., Qayyum, A., Ok, Y.S., Adrees, M., Ibrahim, M., Zia-ur-Rehman, M., Farooq, Q. and Abbas, F. (2016) 'Mechanisms of salinity tolerance in plants: a review', *Environmental Science and Pollution Research*, 23(9), pp.8697–8710. https://doi.org/10.1007/s11356-016-6470-9.

Sarker, U. and Oba, S. (2018) 'Salinity stress and red amaranth: oxidative stress response and antioxidant defense', *Scientia Horticulturae*, 239, pp.314–322. https://doi.org/10.1016/j.scienta.2018.05.058.

Sayfzadeh, S., Ahmadi, A. and Shikhi, S. (2024) 'Salicylic acid improves chlorophyll fluorescence, photosynthesis and antioxidant response of maize under salt stress', *Plant Stress*, 12, p.100512. https://doi.org/10.1016/j.stress.2024.100512.

Shahverdi, M.A., Omidi, H., Azizinezhad, R. and Taleei, A. (2017) 'Salicylic acid-mediated effects on photosynthesis, osmolytes and antioxidative enzymes in salt-stressed rapeseed', *Acta Physiologiae Plantarum*, 39, p.275. https://doi.org/10.1007/s11738-017-2565-y.

Sharma, A., Sidhu, G.P.S., Araniti, F., Bali, A.S., Shahzad, B., Tripathi, D.K., Brestic, M., Skalicky, M. and Landi, M. (2020) 'Exogenous application of salicylic acid improves salinity tolerance in plants: a meta-analysis', *Ecotoxicology and Environmental Safety*, 196, p.110537. https://doi.org/10.1016/j.ecoenv.2020.110537.

Shemi, R., Wang, R., Peng, Y., Liu, D., Meng, Y., Sun, Y., Li, K., Chen, S., Xiao, F. and Ma, X. (2020) 'Silencing of MAPK genes enhances salinity tolerance in rice seedlings via boosting ROS scavenging and osmotic adjustment', *International Journal of Molecular Sciences*, 21(9), p.3220. https://doi.org/10.3390/ijms21093220.

Simon, L.M., Fatrai, Z., Jonas, D.E. and Matkovics, B. (1974) 'Study of peroxide metabolism enzymes during the development of', *Phaseolus vulgaris*. *Biochemie und Physiologie der Pflanzen*, 166, pp.387–392.

Song, X., Chen, J., Xu, C., Cai, X., Song, W., Chang, A., Zhang, Y. and Luo, C. (2025) 'Physiological and molecular mechanisms of exogenous salicylic acid in enhancing salt tolerance in tobacco seedlings by regulating

antioxidant defence system and gene expression', *Frontiers in Plant Science*, 16, p.1545865. https://doi.org/10.3389/fpls.2025.1545865.

Strasser, R.J., Tsimilli-Michael, M. and Srivastava, A. (2004) 'Analysis of the chlorophyll', *a* fluorescence transient. In: Papageorgiou, G.C. and Govindjee, eds. *Chlorophyll a fluorescence: a signature of photosynthesis*. Dordrecht: Springer, pp.321–362.

Tavallali, V., Karimi, S., Rahemi, M., Ramezanian, A. and Eshghi, S. (2010) 'Role of salicylic acid and nitric oxide in salt tolerance of', *Salvia officinalis*. *Acta Physiologiae Plantarum*, 32, pp.263–270. https://doi.org/10.1007/s11738-009-0403-1.

Turkan, I. and Demiral, T., 2009. Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany*, 67(1), pp.2–9. https://doi.org/10.1016/j.envexpbot.2009.05.008.

Velikova, V., Yordanov, I. and Edreva, A. (2000) 'Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines', *Plant Science*, 151(1), pp.59–66.

Wada, K.C. and Takeno, K., 2013. Salicylic acid-mediated stress-induced flowering. In: Hayat, S., Ahmad, A. and Alyemeni, M.N., eds. *Salicylic acid: plant growth and development*. Dordrecht: Springer Netherlands, pp.163–182.

Wada, K.C., Yamada, M., Shiraya, T. and Takeno, K. (2010) 'Salicylic acid and the flowering gene FLOWERING LOCUS T homolog are involved in poor-nutrition-stress-induced flowering of', *Pharbitis nil*. *Journal of Plant Physiology*, 167(5), pp.447–452. https://doi.org/10.1016/j.jplph.2009.07.009.

Wang, X., Su, L., Wang, X., Zhang, Q. and Guo, Y. (2024) 'Melatonin improves salt tolerance in', *Brassica napus* by modulating photosynthesis and ROS homeostasis. *Plant Physiology and Biochemistry*, 214, p.108922. https://doi.org/10.1016/j.plaphy.2024.108922.

Wu, Q., Su, N., Zhang, X., Liu, Y., Cui, J. and Liang, Y. (2016) 'Hydrogen peroxide, nitric oxide and UV RESISTANCE LOCUS interact to mediate UV-B-induced anthocyanin biosynthesis in radish sprouts', *Scientific Reports*, 6(1), p.29164.

Xiao, X., Wang, Y., Qiu, Q., Zhang, M. and Ma, X. (2022) 'Salicylic acid enhances salt tolerance in potato by improving ion homeostasis and antioxidant defense', *Plant Physiology and Biochemistry*, 187, pp.140–151. https://doi.org/10.1016/j.plaphy.2022.08.020.

Xu, J., Tian, Y., Li, F., Li, P., Gao, Y. and Chen, W. (2016) 'Exogenous ascorbic acid improves photosynthesis and antioxidant capacity in tomato plants under salinity stress', *Biologia Plantarum*, 60(1), pp.181–188. https://doi.org/10.1007/s10535-015-0577-0.

Yadav, K., Singh, N. and Verma, P. (2021) 'Salicylic acid-induced modulation of photosynthesis and oxidative stress tolerance in', *Triticum aestivum* under salt stress. *Physiology and Molecular Biology of Plants*, 27(6), pp.1263–1275.

https://doi.org/10.1007/s12298-021-00987-0.

Yin, J., Jia, J., Lian, Z., Liang, X., Xin, G., Liu, W. and Li, T. (2019) 'Effects of salinity stress on chlorophyll fluorescence and photosynthetic performance in soybean', *Agronomy*, 9(12), p.655. https://doi.org/10.3390/agronomy9100655.

Zarei, M., Shabani, L., Sabzalian, M.R. and Fazeli, A. (2016) 'Salicylic acid regulates secondary metabolite production and antioxidant capacity in', *Stevia rebaudiana* under salinity stress. *Plant Physiology and Biochemistry*, 100, pp.67–75. https://doi.org/10.1016/j.plaphy.2016.01.015.

Zhang, J., Jia, W., Yang, J. and Ismail, A.M. (2006) 'Role of ABA in integrating plant responses to drought and salinity stresses', *Field Crops Research*, 97(1), pp.111–119. https://doi.org/10.1016/j.fcr.2005.08.018.

Zhang, L., Gao, M., Zhang, L., Li, B. and Han, M. (2024) 'Salicylic acid enhances salt tolerance in maize through improved photosynthesis and redox regulation', *Environmental and Experimental Botany*, 212, p.105555. https://doi.org/10.1016/j.envexpbot.2023.105555.

Building a Framework for Resilience Assessment and Monitoring: Integrating Human-Environment Interactions to Protect Anzali Wetland's Aquatic Ecosystems

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Received: 2026-07-12 Accepted: 2026-09-05

Abstract

Urban wetlands are critical socio-ecological systems that provide essential ecosystem services while facing increasing anthropogenic and environmental pressures. This study aims to develop a site-specific framework for assessing and monitoring the resilience of Anzali Wetland, a Ramsar Wetland of International Importance in northern Iran, by integrating ecological and social dimensions of resilience. A three-round Delphi process was conducted with 10 experts selected for their relevant expertise and direct experience with Anzali Wetland. Expert assessments were analyzed using descriptive statistics and Kendall's coefficient of concordance to examine the convergence of judgments across Delphi rounds. The results identified seven resilience factors spanning ecological and social dimensions. The overall resilience of the wetland was assessed at 2.7 on a five-point scale, with 50% of experts rating its resilience as weak or very weak. Water quality (mean = 4.9), biodiversity (mean = 4.7), and hydrological regime (mean = 4.6) were the three highest-priority factors, while human pressure was the highest-ranked social factor (mean = 4.3). Expert agreement increased across the Delphi rounds, with Kendall's W rising from 0.62 in Round 1 to 0.75 in Round 2 and 0.82 in Round 3 ($p = 0.001$). The prioritized factors were translated into an operational monitoring framework linking major threats with measurable indicators. These indicators included BOD, COD, nitrogen, and phosphorus concentrations for water pollution; annual sedimentation rate for hydrological and sediment-related pressures; and the abundance of indicator species for biodiversity changes. By linking expert-based prioritization of ecological and social resilience factors with measurable monitoring indicators, the framework provides a site-specific basis for repeated resilience assessment and adaptive management of Anzali Wetland. Its contribution lies in translating an integrated socio-ecological resilience assessment into an operational monitoring structure that can support evidence-based evaluation of management responses over time.

Keywords: Socio-ecological resilience, Anzali Wetland, Resilience assessment, Delphi method; Monitoring indicators, Adaptive management

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Doi: 10.48308/pae.2026.244306.1143

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Introduction

Wetlands are among the most ecologically and socio-economically significant ecosystems globally, providing water purification, flood control, carbon storage, and habitat provisioning (Gardner et al., 2015; Gardner & Finlayson, 2018), yet they remain highly vulnerable to climate change, pollution, and urban expansion. These pressures are best understood through the lens of resilience, defined as the capacity of a social-ecological system (SES) to absorb disturbance while retaining its core structure, functions, and identity (Folke, 2006; Walker et al., 2004; Ostrom, 2009). SES framework formalizes this perspective by conceptualizing the interdependence between ecological subsystems and the human communities. This interdependence is particularly pronounced in urban-adjacent wetlands, where anthropogenic activity and ecological function are tightly coupled (Alberti & Marzluff, 2004). Within this framework, resilience operates along two interlinked dimensions: ecological resilience, referring to a system's capacity to sustain biodiversity and ecosystem services under stress (Folke et al., 2010). Social resilience, refers to the economic, institutional, and adaptive capacities of dependent communities to absorb and recover from shocks (Cutter et al., 2008; Folke et al., 2010). A central concept in resilience research is that of critical thresholds, beyond which incremental disturbances can trigger abrupt and potentially irreversible changes in system function (Scheffer et al., 2012) underscoring the practical stakes of timely resilience assessment. In this context, ecosystem services refer to the benefits humans derive from ecosystems, spanning provisioning, regulating, cultural, and supporting functions (Assessment, 2005).

Researchers have shown the importance of governance of SES in building resilience across various dimensions. (Evans, 2011) investigated how ecologists integrated adaptive experiments into urban governance to promote resilience against climate change. (Gotham and Campanella 2011) described urban ecosystems as containing both resilient and vulnerable elements.

Anzali Wetland, covering approximately 20,000 hectares, is a vital habitat for various aquatic and terrestrial bird species, fish, and other aquatic life, playing an important role in maintaining regional biodiversity (Fathi et al., 2010). Yet, this valuable ecosystem is threatened by multiple factors, including pollution due to industrial and agricultural activities, inflow of urban wastewater, illegal fishing, climate change, drought, and reduced precipitation (Ghazban & Zare Khosh Eghbal, 2011). These threats can undermine the resilience of Anzali Wetland by reducing its capacity to maintain essential ecological functions, biodiversity, and ecosystem services. Local communities surrounding the wetland also depend on its resources for their livelihoods through activities such as agriculture, fishing, and tourism. (Salehi & Qolamdoust, 2013). Therefore, understanding the social resilience of these communities to the threats and challenges associated with wetland degradation is as importance as ecological resilience.

In Iran, several studies have been conducted on the resilience of Anzali Wetland. (Ebrahimi and Kordevani 2014) evaluated the

ecological resilience of Anzali Wetland to climate change and showed that the wetland is at serious risk of reduced water levels and altered precipitation patterns. They suggested that enhancing resilience requires integrated management actions, including pollution control and vegetation protection. (Khosravi et al., 2021) examined the impact of human activities on the resilience of Anzali Wetland and found that urban and industrial development in the wetland's catchment area has significantly affected its water quality and biodiversity. Their findings emphasized the importance of considering human-natural system interactions in wetland management.

Despite this body of research, a closer examination reveals a specific and identifiable broad gap in the literature on Anzali Wetland and socio-ecological resilience assessment. With respect to Anzali Wetland itself, prior studies remain predominantly single-dimensional. (Ebrahimi and Kordevani, 2014) assessed ecological resilience to climate-driven hydrological change, while (Khosravi et al., 2021) examined the impact of urban and industrial development on water quality and biodiversity. However, both studies largely considered ecological degradation in isolation from the social dynamics of dependent communities. Conversely, (Salehi and Qolamdoust 2013) addressed community dependence on wetland resources but do not integrate these findings into a structured, quantifiable resilience assessment. To date, no existing study on Anzali Wetland has combined a systematic expert-based evaluation of ecological and social resilience factors within a single integrated instrument, or translated such an assessment into an operational set of site-specific monitoring indicators.

This gap is not unique to Anzali Wetland; it reflects a broader limitation in existing resilience assessment frameworks applied to similar urban-adjacent socio-ecological wetland systems. Frameworks such as (Ostrom, 2009) general SES model and subsequent applications in comparable contexts (e.g., Mosleh et al., 2023), in the context of urban stormwater infrastructure are largely diagnostic and typologic. They identify the categories of variables relevant to resilience but do not, in their standard form, generate a site-specific, empirically ranked set of factors or link that ranking to a concrete, ongoing monitoring structure. As a result, even where such frameworks have been applied to comparable wetland systems elsewhere, they have tended to produce conceptual maps of resilience determinants rather than operational tools that management authorities can directly use.

These limitations highlight the need for an integrated, site-specific framework for Anzali Wetland. The wetland has experienced substantial ecological degradation, including an estimated 30% reduction in area over three decades (Rahimi et al., 2019), while the resilience of communities dependent on its resources has not been systematically assessed alongside ecological conditions. This study addresses this gap by combining expert-based prioritization of ecological and social resilience factors with a set of operational monitoring indicators tailored to the wetland.

The novelty of the proposed framework can

be clarified along four distinct dimensions, relative both to standard SES models and prior empirical work on Anzali Wetland specifically. First, with respect to standard SES models, SES framework, and its subsequent applications in wetland research are predominantly diagnostic and typological in nature (Ostrom, 2009). They specify which categories of variables, including resource systems, resource units, governance systems, users should be considered, but do not themselves generate a ranked, empirically weighted set of factors for a specific site or translate directly into measurable monitoring indicators. The present framework differs from this standard usage in that it operationalizing the SES logic through a structured, three-round Delphi process. This process empirically ranks ecological and social resilience factors based on expert-assigned importance (Table 2) and statistically verified consensus (Kendall's W = 0.62 to 0.82), rather than treating the SES categories as a fixed conceptual checklist. Thus, the framework applies the SES perspective as a site-specific and quantifiable assessment approach rather than solely as a classificatory scheme.

Previous empirical studies on Anzali Wetland e.g., (Ebrahimi & Kordevani, 2014; Khosravi et al., 2021; Salehi & Qolamdoust, 2013) have primarily examined ecological and social dimensions separately. They have not integrated these dimensions into a single assessment instrument or developed a structured monitoring framework. The present framework addresses to advance this body by integrating ecological and social resilience into a consensus-based assessment (Table 2) and linking the findings to a second-stage monitoring framework (Table 3; Figure 2). The framework's theoretical contribution lies in how it treats the relationship between expert and lay knowledge rather than assuming its relative importance. Specifically, community participation was ranked alongside ecological and other social factors according to expert judgments. Its ranking (Table 2, rank 7) provides insight into how the expert panel prioritized this factor relative to other resilience factors. (Table 2, rank 7). this approach provides a basis for examining the relationship between the theoretical importance attributed to participatory governance in the literature (e.g., Ostrom, 2009; Berkes et al., 2008) and its comparatively low prioritization by the expert panel itself. This bridging of expert and lay perspectives, and the empirical exposure of the gap between them, constitutes a distinct theoretical contribution beyond simply asserting that participation matters.

A further contribution of the framework is its practical application of the Delphi consensus to wetland monitoring. Previous studies on Anzali Wetland have generally concluded with general policy recommendations, such as pollution control and integrated management. This framework identified BOD, COD, nitrogen (N), and phosphorus (P) levels for water pollution; annual sedimentation rates for hydrological loss; and the abundance of indicator species for biodiversity loss (Table 3). These indicators are associated with an explicit assessment-to-monitoring pipeline (Figure 2). Furthermore, its management authorities can operationalize directly, rather than a static diagnostic report requiring

further translation into practice.

Given the critical importance of Anzali Wetland and the communities that depended on it, this study seeks to answer three main questions: What is the current state of ecological and social resilience in Anzali Wetland? What factors influence the ecological and social resilience? What strategies can enhance the ecological and social resilience of Anzali Wetland, and how can their effectiveness be improved?

Materials and method

Study area and environmental setting

The Anzali Wetland complex (approximately 139 km^2) is located adjacent to the port city of Bandar Anzali in Gilan Province, northern Iran, along the southern shores of the Caspian Sea. This wetland is one of the closest wetlands to the city of Ramsar, the birthplace of the Ramsar Convention, and was registered in the Ramsar List of Wetlands of International importance on June 23, 1975 (Stroud et al., 2022). The Anzali Wetland is a large marsh fed by freshwater from numerous rivers and separated from the Caspian Sea by a sandy shoreline, featuring extensive reed beds and abundant floating vegetation. This permanent wetland is surrounded by seasonal marshes, agricultural fields (mainly rice), and fish farming ponds, and is of international importance for bird breeding and wintering (Aghsaei et al., 2020).

The biodiversity of the Anzali Wetland is remarkable. The wetland is home to more than 100 bird species, 50 fish species, 230 plant species, and various mammals, reptiles, and amphibians. Specific animal species in this wetland include Gray Pelican, Mallard, Swan, White Stork, and fish species such as Pike-perch, Carp, and Pike. In terms of vegetation. The dominant species includes reed, cattail, and bulrush, which play important roles in the wetland's ecology (Amini et al., 2022).

However, the Anzali Wetland has faced serious challenges during recent decades. Water pollution from urban and industrial wastewater, excessive sedimentation, the invasion of exotic species such as water hyacinth, and climate change are among the most important threats to this valuable ecosystem. Studies have shown that over the past 30 years, the area of the Anzali Wetland has decreased by approximately 30 % (Rahimi et al., 2019).

Studies have shown that the Anzali Wetland ecosystem faces serious challenges. Increased sedimentation, water pollution, and declining biodiversity are among the issues that have affected the wetland's resilience (Jafari, 2009). Furthermore, changes in precipitation patterns and increasing temperatures due to climate change have affected the wetland's hydrological conditions (Khazaei et al., 2019). Research on the Anzali Wetland, has also shown that cooperation among various stakeholders, including local communities, government organizations, and researchers, can improve management and increase the ecosystem's resilience (Ebrahimi & Kordevani, 2014).

Finally, the resilience of social-ecological systems is a dynamic and continuous process. Continuous changes in environmental, social, and economic conditions require ongoing review and updates to our under-

standing of these systems' resilience. For the Anzali Wetland, this means the need for continuous monitoring of the wetland's ecological and socio-ecological status, evaluation of management action effectiveness, and adaptation of strategies based on new findings.

Data Collection

Delphi method

This research uses the Delphi method to collect and analyze expert opinions on the resilience of Anzali Wetland. The Delphi method is a structured and iterative process for gathering and converging expert opinions through a series of questionnaires with controlled feedback (Hsu & Sandford, 2007). This method originally introduced by (Linstone & Turoff, 1975), facilitates confidential group communication and collaboration. It is particularly effective for gathering insights from experts on complex issues where empirical data may be insufficient. The method aims to build a collective understanding of these issues and to reach consensus on viable solutions (Hasson et al., 2000; Linstone & Turoff, 1975). According to (Fletcher & Marchildon ,2014), the main objective of the Delphi method is to gather informed opinions on topics that are largely undefined, context-specific, or focused on future developments. This method has gained traction as a valuable technique in social and policy research, especially for decision-making in areas such as environmental governance, including forest management, public transportation, and energy transitions (Caglayan et al., 2021; Filyushkina et al., 2018; Hirschhorn, 2019; Makkonen et al., 2016; Revez et al., 2020; Waldron et al., 2020). In Indonesia, the Delphi method has been applied to identify interventions for addressing complex forest and land governance challenges (Toumbourou, 2020).

Process and participants

Data collection was conducted in identification, exploration, and consolidation. Experts were purposively selected using a set of formal inclusion criteria. These criteria were designed to ensure both depth of expertise and breadth of perspectives. Eligible candidates were required to have at least eight years of professional or research experience in wetland ecology, hydrology, natural resource management, or socio-ecological systems. They also needed demonstrated



Fig. 1. Study area location- Anzali Wetland (Source: Google Maps, 2024)

experience with Anzali Wetland, through research, management responsibilities, or field-based work within its catchment area. In addition, candidates were required to hold an advanced academic degree (M.Sc. or Ph.D.) or have equivalent professional standing in a relevant discipline. Beyond these baseline criteria, the expert panel was deliberately balanced across institutional sectors to avoid the dominance of any single perspective. the final panel included four representatives from academic institutions, three from governmental environmental and water-management agencies, two from non-governmental organizations involved in wetland conservation, and a local institution with direct experience in local community engagement. This cross-sectoral structure was intended to capture both technical and scientific expertise and applied management experience. This structure is consistent with recommendations in the Delphi literature for panels addressing complex social-ecological problems (Hsu & Sandford, 2007). A summary of the anonymized panel composition is presented in Table 1. .

The panel size of ten experts was determined based on established guidance in the Delphi literature rather than an arbitrary threshold. (Skulmoski et al. ,2007) and (Okoli and Pawlowski, 2004) note that Delphi panels composed of highly specialized, purposively selected expertise typically require fewer members than survey-based research. This is because validity of the method depends on the depth and relevance of expert knowledge rather than on statistical generalizability. A panel of 10–15 members is widely considered adequate when participants are selected based on their expertise and direct familiarity with the specific system under study (Hsu & Sandford, 2007). In this study, the panel was deliberately kept homogeneous in its focus on Anzali Wetland. While, remaining heterogeneous through the six relevant disciplines, including wetland ecology, water resource management, rural sociology, environmental economics, crisis management, and environmental sociology and across institutional sectors. This sectors allowed the

Table 1. Anonymized composition of the expert panel (n = 10)

Expert ID	Sector	Field of Expertise	Years of Experience	Degree
P1	Academia	Wetland Ecology	14	Ph.D.
P2	Governmental	Water Resource Management	18	Ph.D.
P3	NGO	Environmental Sociology	10	M.Sc.
P4	Academia	Rural Sociology	12	Ph.D.
P5	Governmental	Crisis Management	9	M.Sc.
P6	Local institution	Environmental Economics	8	M.Sc.
P7	Academia	Wetland Ecology	20	Ph.D.
P8	NGO	Wetland Conservation / Community Engagement	11	M.Sc.
P9	Governmental	Hydrology/Water Resource Management	15	Ph.D.
P10	Local institution	Rural Sociology / Local Governance	13	Ph.D.

Source: Authors

panel to reach a point of information richness. Additional experts within this narrow geographic and thematic scope were judged unlikely to introduce substantially new categories, given the convergence already observed by Round 3. This pattern is consistent with the concept of theoretical saturation in expert-based qualitative inquiry, where consensus stability across successive rounds serves as an empirical indicator that the panel size was sufficient for the scope of the study.

This assessment is further supported by the study's own data: Kendall's coefficient of concordance increased from 0.62 in Round 1 to 0.75 in Round 2 and 0.82 in Round 3, indicating that expert opinions converged toward a stable consensus well within the three-round process. This pattern suggest that the panel capture the relevant range of expertise for this specific, geographically bounded site. The process of implementing the Delphi method in this research is as follows.

Phase I: Anzali wetland resilience system Identification

The Identification phase aimed to gather information about the state of Anzali Wetland resilience system. We conducted biweekly meetings to discuss and refine our activities and methods. For the first Delphi Round interview, we developed a concise set of open-ended questions informed by an initial literature review. After collecting some basic background information with three introductory questions, the Phase 1 interview included five specific questions. At this stage, an open-ended questionnaire with general questions regarding the current state of resilience of Anzali Wetland, the main challenges, and opportunities for improvement was administered to the experts. The goal was to explore how participants from different disciplines perceived the resilience of Anzali Wetland, the factors influencing the social resilience of communities dependent on the wetland, their perceptions of its current condition, potential strategies for enhancing SES resilience, and actions needed to strengthen community resilience. The responses were examined using qualitative content analysis. The qualitative content analysis of Round 1 responses was conducted at the level of the individual statement or idea unit, that is, each distinct proposition or claim within an expert's response, whether expressed as a full sentence or as a clause within a longer sentence, was treated as a separate unit of analysis. This unit of analysis was chosen because expert responses frequently combined multiple distinct ideas within a single sentence, and coding at the sentence level alone risked conflating conceptually separate observations. The coding process was carried out by a single member of the research team, consistent with common practice in small-panel Delphi studies where the same researcher who conducts the interviews is best positioned to interpret contextual nuances in participants' responses (Hasson et al., 2000). To safeguard analytical consistency in the absence of a second independent coder, an intra-coder reliability check was performed. A random subsample of approximately 20 % of the Round 1 transcripts was re-coded by the same researcher after two-week interval without reference to the original coding decisions. The two sets

of codes were then compared, and the level of agreement between the original and re-coded labels was used as an indicator of coding stability. Any discrepancies identified through this process were reviewed and resolved by refining the coding definitions before the scheme was finalized and applied consistently across the full dataset. Following open coding, axial coding was used to group related open codes into broader sub-categories based on shared conceptual meaning. These sub-categories were subsequently consolidated into the final resilience factors reported in Table 2. To illustrate this analytical pathway, consider the following coding path, which traces a raw expert statement through to a final resilience factor.

Raw statement (illustrative excerpt) → Open code → Sub-category → Final factor

" Untreated wastewater from the surrounding cities keeps flowing into the wetland, and it's clearly affecting the water" → Open code: "untreated wastewater inflow" → Sub-category: "urban and industrial pollution loading" → Final factor: Water Quality

This same pathway was applied consistently across all Round 1 transcripts: idea units were first assigned open, descriptive codes; conceptually related open codes were then grouped into sub-categories through axial coding; and sub-categories were finally consolidated into the seven resilience factors presented in Table 2, spanning both the ecological and social domains. This structured coding pathway underlies the questionnaire items subsequently distributed to experts in the Round 2 (Explore) phase. Based on this analysis, a list of factors influencing the resilience of Anzali Wetland was compiled, including factors affecting ecological resilience and social resilience.

Phase II: Explore

At this stage, a questionnaire was designed based on the results of the first round. The Round 2 questionnaire items were derived directly from the categories and sub-categories identified through the open and axial coding of Round 1 responses, ensuring content validity by grounding each item in expert-generated language rather than researcher-imposed categories. Prior to distribution, the questionnaire was reviewed internally for clarity and item redundancy, and minor wording adjustments were made to ensure that each factor was phrased as a single, unambiguous evaluative statement appropriate for scoring on a 5-point Likert scale. Experts were asked to evaluate the importance of each identified factor on a 5-point Likert scale, ranging from 1 (not important) to 5 (very important). The responses were analyzed, and the mean, median, and standard deviation for each factor were calculated. Kendall's coefficient of concordance was used to assess the level of agreement among the experts.

Phase III: Consensus consolidation

At this stage, the results of the second round, along with a new questionnaire, were sent to the experts. They were asked to reassess their evaluations based on the collective feedback. Experts were given the opportunity to adjust their opinions if they wished or to maintain their previous opinions. After receiving the responses from the third round, the final analysis was conducted. The mean, median, and standard deviation for each factor and solution were calculated.

The Kendall's coefficient of concordance was also recalculated to determine the final level of agreement among the experts. To analyze the data obtained from the Delphi process, descriptive and analytical statistical methods were employed. SPSS version 26 was used to perform the statistical analyses.

Results

The results obtained from the Delphi process can be presented in several main sections, including the current state of the resilience of Anzali Wetland and the main factors and indicators influencing its resilience. According to the experts' assessment, Anzali Wetland is currently facing serious challenges regarding its resilience. The mean score of overall resilience of the wetland (on a scale of 1 to 5) was 2.7, indicating a below-average and declining status. This assessment was based on a combination of ecological and socio-economic indicators. In the ecological dimension, experts pointed to declines in water quality and biodiversity, as well as changes in the hydrological regime of the wetland.

Specifically, increased pollution from urban and industrial wastewater, a decline in the population of native species, and changes in water-flow patterns were identified as major indicators of reduced ecological resilience. In the socio-economic dimension, experts reported declining incomes among communities dependent on the wetland and increased migration from surrounding villages, which may have reduced local participation in wetland management. These factors indicate reduced in social resilience in the region. 50% of the experts assessed the resilience of the Anzali Wetland as weak or very weak. Only 10% of the experts rated the wetland's resilience as good, whereas none rated it at the highest level of the assessment scale.

Indicators identified to assess resilience of Anzali Wetland

The factors influencing the resilience of Anzali Wetland were divided into two categories, Ecological Resilience and Social Resilience. The identified factors primarily comprised ecological aspects, including water quality, biodiversity, hydrological regime, human pressure, climate change effect, integrated management, and of local community participation. Based on the experts' assessments of the importance of these indicators, the first three ranked highest within ecological domain. However, factors within the social domain were received lower rankings than those within the ecological domain (Table 2). Further details of interview findings are presented in Table 2.

In the first round, there was moderate disagreement among experts (n = 10) regarding the relative importance of the factors (score range: 2-5, Kendall's coefficient of concordance= 0.62). In the second round, the level of disagreement decreased (score range: 3-5, Kendall's coefficient of concordance:0.75), and by the third round, it had decreased further (score range: 4-5, Kendall's coefficient of concordance:0.82). A Kendall's coefficient of concordance of 0.82 being close to one, indicates a high level of agreement among the experts. Higher values of the coefficient indicate greater agreement among the experts. The Delphi process facilitated the collection of diverse expert perspectives and supported rthe development of

consensus regarding the main factors influencing the resilience of Anzali Wetland. It also enabled the identification of potential strategies to improving the resilience of this ecosystem.

After identifying the indicators, experts assessed the current status of the wetland by gathering comprehensive data on both ecological and social factors. This evaluation involves looking at various ecological indicators, such as the vegetation health, water quality, and species diversity, which provided insights into the overall condition of the ecosystem. Simultaneously, social indicators are considered, examining how local communities interact with and depend on the wetland, as well as their awareness of and involvement in conservation efforts. Once the current status was established, experts identified specific threats to the wetland, including pollution, habitat destruction, or the impact of climate change. Understanding these threats was crucial for developing effective solutions. Based on a comprehensive analysis, the experts then proposed potential strategies to address these issues. These included restoration measures, such as replanting native species or improving water management practices, as well as policy recommendations aimed at regulating harmful activities. Additional strategies are presented in Table 3. A critical aspect of this process is establishing a monitoring framework that allows for the ongoing assessment of the proposed strategies. By tracking relevant indicators over time, experts can determine whether their interventions are effective or if adjustments are needed. At this stage, monitoring indicators were also initially identified. Overall, this holistic approach combines scientific evaluation with community engagement to support the sustainable management of the wetland.

Analytical interpretation of the ranking pat-

Table 2. Ranking factors affecting the social-ecological resilience of Anzali Wetland after three rounds of Delphi

Factor	Domain	Mean	STD	Rank	Details
Water quality	Ecological	4.9	0.16	1	Pollution from urban and industrial wastewater, agricultural runoff, and sedimentation has significantly harmed water quality, leading to diminished biodiversity, altered aquatic communities, and reduced ecosystem services.
Biodiversity	Ecological	4.7	0.24	2	Experts note that a decrease in the diversity of native plant and animal species undermines the ecosystem's ability to cope with environmental changes, highlighting the need to preserve genetic diversity in existing populations.
Hydrological regime	Ecological	4.6	0.26	3	Experts highlight that changes in water flow patterns, precipitation, evaporation, and Caspian Sea levels can impact resilience, stressing the need to maintain a natural hydrological regime to support the wetland's ecological functions.
Human Pressure	Social	4.3	0.27	4	Human pressures include urban and industrial development in the watershed, increased agricultural activities, overfishing, and uncontrolled tourism. Experts noted that these activities can lead to habitat destruction, pollution, and overexploitation of the wetland's resources.
Climate change	Ecological	4.2	0.39	5	Experts noted that rising temperatures, changes in precipitation patterns, increased occurrence of droughts and floods, and rising sea levels can affect the wetland's resilience. They emphasized that the effects of climate change may be exacerbated by other stressors, creating complex challenges for wetland management.
Integrated management	Social	4.0	0.41	6	Experts believe that the absence of a comprehensive and integrated management approach can reduce the resilience of the Anzali Wetland. They highlighted the importance of coordination among various organizations, the integration of scientific and local knowledge, and considering the entire watershed in decision-making to enhance resilience.
Participation of local communities	Social	3.9	0.44	7	Experts emphasized that active involvement of local communities in the management and conservation of the wetland can enhance the overall resilience of the system. They noted that local knowledge, a sense of ownership, and community responsibility can significantly contribute to the preservation and restoration of the wetland.

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The ranking pattern in Table 2 provides information not only about factor priority but also about the degree of agreement among experts. The three top-ranked factors-water quality, biodiversity, and hydrological regime-cluster together not only in their mean scores (4.6–4.9) but also in the narrow range of their standard deviations (0.16–0.26), indicating related high degree of agreement among experts regarding their importance and close conceptual relationship. This is not a coincidence of ranking; rather, it reflects an underlying ecological sequence evident in the factor descriptions themselves in Table 2. Pollution and sedimentation degrade water quality, which in turn contributed to the biodiversity loss described by the experts. While, both are influenced by hydrological disruptions reduced flow, altered precipitation, sedimentation, captured by the third-ranked factor. Taken together, these three top ranked factors describe a connected sequence of ecological pressures rather than three separate problems. This finding has implications for management, suggesting that interventions targeting water quality may also contribute to improved biodiversity outcomes, rather than relying solely on biodiversity-focused interventions. The patterns change noticeably for factors in the social domain. Integrated management and local communities' participation, ranked sixth and seventh, respectively. These factors showed the highest standard deviations in Table 2 (0.41 and 0.44), approximately twice those of the top-ranked ecological factors. This difference in agreement is itself a noteworthy finding. While the expert panel showed strong agreement on the biophysical drivers of wetland decline, agreement was considerably weaker regarding the relative importance of governance and participation, even after three rounds of Delphi feedback designed to reduce disagreement. Human pressure, ranked fourth, occupies an instruc-

Table 3. Threats, solutions, and monitoring indicators mentioned by experts for SES resilience enhancement

Threats	Details	Solution	Monitoring indicator
Water pollution from urban and industrial wastewater	Experts noted that the inflow of untreated wastewater leads to increased nutrient levels (eutrophication), reduced dissolved oxygen, and contamination with heavy metals and chemicals, all of which can have detrimental effects on the wetland's biological communities.	Improvement of wastewater treatment systems in cities and industries around the wetland.	BOD, COD, N, P
Hydrology flow loss, Sedimentation and reduction of wetland depth	It is caused by soil erosion in the watershed and the inflow of sediments into the wetland. This process leads to decreased wetland depth, changes in hydrology and morphology, and reduced water retention capacity, all of which can diminish the ecosystem's resilience.	Restoration of vegetation cover along the wetland and control of soil erosion. Execution of dredging projects and sediment management.	Rate of annual sedimentation
Loss of biodiversity due to habitat destruction	Experts noted that habitat destruction and fragmentation, particularly in the fringe areas of the wetland, have led to a decrease in biodiversity. This includes the loss of native plant species, a decline in aquatic bird populations, and a reduction in fish diversity, all of which play crucial roles in the functioning of the wetland ecosystem.	Establishment of protected areas to preserve biodiversity	Diversity and abundance of indicator species (such as aquatic birds and native fish).
Land use change, particularly the conversion of natural areas to agricultural and residential lands	These changes lead to increased soil erosion, alterations in runoff patterns, and an increase in non-point source pollution, all of which can diminish the resilience of the wetland.	Formulation and implementation of an integrated management plan.	Percentage of wetland vegetation cover
Increased pressure from tourism without proper management	Experts noted that the rise in visitor numbers without adequate infrastructure can lead to habitat destruction, pollution, and disruption of wildlife activities.	Development of sustainable tourism and ecotourism.	The level of local community participation
Climate change and the increasing frequency of droughts	Experts noted that these changes can lead to alterations in the wetland's hydrological regime, increased temperatures and evaporation, and shifts in species composition, all of which can impact the resilience of the ecosystem.	Implementation of educational and participatory programs for local communities	Water level and its seasonal variations

tive midpoint between these two groups. Although human pressure, is classified as a social factor, its fourth-place ranking positions it between the two clusters It encompasses overfishing, unmanaged tourism, agricultural expansion into wetland margins describes human behavior with direct ecological consequences. This illustrates that the boundary between the ecological and social categories used in this study is itself somewhat porous in practice.

This structure carries a direct implication for how the overall resilience score of 2.7 out of 5 should be interpreted and acted upon. Because the highest-priority factors also show the strongest expert-consensus and are concentrated in the biophysical domain. Te results suggest that near-term interventions with the clearest expert backing wastewater treatment upgrades, sediment control, hydrological restoration have relatively strong expert support. These interventions may therefore be implemented without first resolving the comparatively unsettled question of how participatory governance should be structured. At the same time, the lower ranking and greater disagreement surrounding community participation should not be mistaken for evidence that it matters less. they indicate that expert consensus on this factor remains limited. To keep this issue visible over time. the monitoring framework proposed in Table 3 includes the level of local community participation as an explicit, ongoing indicator. This approach allows participations to be monitored consciously over time rather than assessed only once, reducing the risk that it will be less attention as more immediately measurable ecological interventions are underway.

Discussion

The results of this study show that Anzali Wetland is currently facing serious challenges in terms of resilience and that could be a situation that may also characterize for numerous wetlands within the urbanized watershed. Similar challenges have been reported for aquatic ecosystems worldwide (Folke et al., 2010), particularly, in relation to the factors influencing their resilience. However, water quality received the highest mean score in this study (4.8 out of 5), indicating the severity of water-related challenges in Anzali Wetland. Similarly, (Jamshidi Zanjani & Saeedi, 2014) reported unfavorable conditions in Anzali wetland based on several ecological indicators. Water quality, biodiversity and hydrological regime were identified as the most important factors affecting wetland resilience. This finding is consistent with global studies emphasizing the importance of these factors in the resilience of wetland ecosystems. The consistency between the present study and earlier assessments also suggests that key ecological challenges in Anzali Wetland have remained over time and continue to require sustained the management attention.

The proposed solutions for improving the resilience of Anzali Wetland encompass several approached, including technical measure to improve treatment systems, ecological measures to restore vegetation, and social measures to educate and engage communities). This comprehensive approach is aligned with the principles of resilience of coupled human–natural systems proposed

by (Berkes et al. ,2000). However, implementing of these solutions in practice may present challenges that require further investigation. The general framework from the result of the results of this paper (Figure 2). Such a resilience assessment and monitoring can support the development of strategies to enhance the ecosystem resilience, s particularly in aquatic ecosystems such as wetlands (Figure 2). Understanding Knowing the main function of an ecosystem and the potential threats plays an essential role for assessing and strengthening its resilience. After identifying the current condition, we need to develop indicators. The proposed indicators for assessing resilience include a combination of ecological and socio-economic indicators. This comprehensive, in line with the resilience assessment presented by (Folke et al., 2010), can help capture the dynamics of the complex system of Anzali wetland. Nonetheless, identifying indicators have to be done by monitoring and communicating the results with local communities and stakeholders. This integrated monitoring can help identify practical solutions and adaptive planning.

The role of local community participation in the proposed strategies highlights the importance of human–environment interactions for ecosystem resilience. This finding is consistent with studies on the role of participatory management in enhancing the resilience of social–ecological systems (Ostrom, 2009). Although the literature generally emphasizes the role of civil society and local participation in strengthening social and ecological resilience, community participation received the lowest ranking among the seven factors assessed in this study (mean =3.9; rank=7). This finding suggests that the expert panel assigned greater priority to ecological factors than to community participation. However, the relatively low ranking of com-

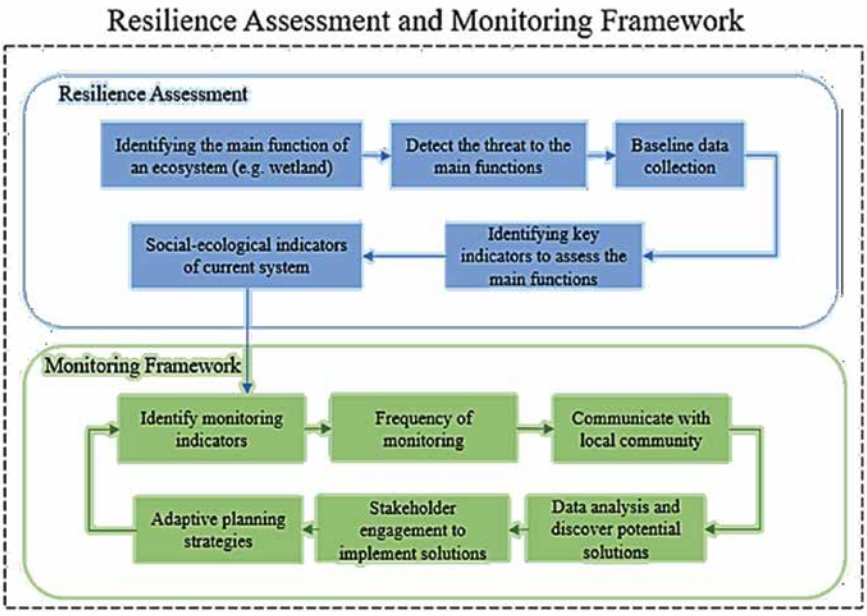


Fig. 2. Resilience assessment and monitoring framework for aquatic ecosystem

Source: Authors

munity participation highlights the need to consider this factor more explicitly in future planning and resilience strategies. This issue warrants greater attention because several ecological pressures, including agricultural water use and changes in wetland depth, are closely connected to local practices, awareness, and participation (Vera-Herrera et al., 2022; Zhao et al., 2021). The finding may also indicate a gap between the perspectives of academic experts and governmental and non-governmental organizations and the requirements of local communities and the wetland environment. Similar tensions have been reported in other environmental governance contexts (Basu et al., 2024; Zebardast et al., 2021).

The comparatively low ranking of local community participation (mean = 3.9; rank 7 and its highest standard deviation observed among all factor in this study (0.44) provide the empirical anchor for the following interpretation. The numerical pattern indicates that the panel reached stronger agreements on the main ecological drivers of wetland decline namely water quality, biodiversity, and hydrological regime, each of with showed a standard deviation below 0.27. In contrast, agreement on the governance and participatory dimensions of resilience remained weaker. despite three full rounds of structured feedback specifically designed to reduce disagreement.

This gap between technical and participatory consensus can be interpreted through broader critical perspectives on knowledge and power in environmental governance. These perspectives are applied here explicitly as interpretive lenses rather than as conclusions demonstrated by the study, since the Delphi design was not considered to test these theoretical propositions. (Gordon and Foucault ,1980) concept of power-knowledge relations offers one such interpretive lens: according to this context, the panel's strongly agreement on measurable technical indicators than on participatory ones raises the possibility that is perceived technical expertise as more "legitimate" than community-based knowledge in environmental decision-making. This reading is consistent with (Li's, 2007) observation that development experts often favor technical solutions over socio-cultural considerations. A similar pattern can be observed in the present study, where technical factors were both higher-ranked and associated with lower variance, while participatory factors were lower-ranked and associated with higher variance. (Spivak's, 2023) notion of epistemic violence and (Bourdieu, 1991) related concept of symbolic violence extend this interpretation by providing a theoretical basis for understanding how experiential and local knowledge may be structurally devalued relative to technical expertise in environmental governance. Dean (2009) and Agrawal (2005) provide complementary perspectives on governmental rationality, while Dryzek, (2022) demonstrated that technocratic discourse can obstruct environmental democracy and Swyngedouw (2011) similarly examines the "depoliticization" of environmental issues. Together, these perspectives suggest that the disagreement documented in this study regarding community participation may reflect a broader structural pattern in how socio-ecological governance values

and prioritizes technical and participatory forms of knowledge.

However, the present study does not directly test any of these frameworks. The collected data cannot determine which framework is more appropriate or confirm which specific mechanism of exclusion or symbolic devaluation operate in the management of Anzali Wetland. A more immediate and methodologically grounded explanation is also available and should be weighed alongside these theoretical interpretations. The Delphi method employed in this study aggregates the judgment of technically and scientifically trained experts. Consequently, the resulting rankings may reflect the perspective of these experts more strongly than those local residents, whose direct evaluation of the same factors might allocate greater weight to community participation. Distinguishing between a structural, governance-level explanation and this narrower methodological one would require research that directly engages local community members. We identify it as a clear and necessary priority for future work building on the present framework. Regardless of which interpretations proves correct, present data support anarrower, practical implication with the greatest confidence. Drawing on Ostrom (2009), participatory mechanisms warrant deliberate strengthening in future governance strategies. Given the disconnect, this study identifies between the theoretical weight typically assigned to community participation in the wetland-resilience literature and its comparatively low, less-consensual ranking by this study's own expert panel.

This study has several limitations and the expert panel was relatively small (n = 10). Although the panel size was appropriate for the specialized Delphi design and the defined study area, the findings may not be generalizable beyond Anzali Wetland. A larger or more geographically diverse panel might have identified additional resilience factors or assigned different weights resilience to the assessed factors. The assessment was based primarily on expert judgment rather than longitudinal field data. Expert assessment is useful for complex and data-limited socio-ecological systems, but it cannot replace direct measurements of ecological and social conditions over time. The proposed monitoring indicators (Table 3) have not yet been field-validated. Their sensitivity, feasibility, and cost-effectiveness therefore remain to be evaluated. Future research should pilot the framework in collaboration with local management authorities and compare its indicators with independently observed ecological and social outcomes.

Conclusions

Anzali Wetland is facing significant resilience challenges that mirror what is happening to urbanized aquatic ecosystems more broadly around the world. The factors identified in this study as most consequential water quality, biodiversity, and hydrological regime echo findings from previous research. (Folke et al, 2010) reinforcing that what is happening in Anzali is part of a larger, familiar pattern rather than an isolated case. The technical, ecological, and social strategies proposed here align with established principles of resilience in coupled human-natural systems, though translating any

of them from expert consensus into effective practice may present challenges that call for further exploration. The resilience assessment and monitoring framework developed in this study provides a systematic basis for identifying ecosystem functions, potential threats, and relevant indicators. These indicators bring together ecological and socio-economic dimensions of the wetland's condition.

Local community participation remains an important component of wetland resilience. Yet one of this study's more striking findings is precisely how little that importance was reflected in practice. While civil society's role was expected to weigh heavily, the results reveal a real disconnect between how significant experts believe local participation to be and how much weight they actually assigned to it. This gap deserves serious attention in future planning, since local actions bear directly on the wetland's health. It also points toward a broader and more difficult question: whether technocratic approaches to Anzali Wetland's management may be quietly overshadowing local knowledge. We raise this possibility as an interpretation this the study's findings make plausible, rather than one they directly prove. Confirming it would require research that engages local communities on their own terms. Future assessments should therefore consider both scientific and local knowledge and include community members directly in evaluating resilience priorities.

The resilience and monitoring framework proposed for Anzali Wetland offers a structured basis for understanding how ecological and socio-economic factors interact to shape the resilience of aquatic ecosystems more broadly. The proposed framework provides a structured basis for linking resilience assessment with ongoing monitoring. It integrates ecological and socio-economic factors and connects identified threats with measurable indicators and potential management responses. However, the framework should be regarded as a starting point rather than a fully validated management tool, field testing and longitudinal monitoring are required to assess the performance of the proposed indicators. They are also needed to determine whether the framework can be adapted to other wetland systems.

References

Aghsaei, H., Mobarghaee Dinan, N., Moridi, A., Asadolahi, Z., Delavar, M., Fohrer, N. and Wagner, P.D. (2020) 'Effects of dynamic land use/land cover change on water resources and sediment yield in the Anzali wetland catchment, Gilan, Iran', *Science of the Total Environment*, 712, 136449. https://doi.org/10.1016/j.scitotenv.2019.136449

Agrawal, A. (2005) *Environmentality: Technologies of Government and the Making of Subjects*. Durham, NC: Duke University Press. https://doi.org/10.1215/9780822386421

Alberti, M. and Marzluff, J.M. (2004) 'Ecological resilience in urban ecosystems: Linking urban patterns to human and ecological functions', *Urban Ecosystems*, 7, pp. 241–265. https://doi.org/10.1023/B:UECO.0000044038.90173.c6

Amini, J., Malekmohammadi, B. and Jafari, H.R. (2022) *Developing a wetland*

management framework based on an ecosystem approach (Case study: Anzali International Wetland).

Assessment, M.E. (2005) *Ecosystems and Human Well-being: Wetlands and Water Synthesis*. Washington, DC: World Resources Institute.

Basu, S., Nagendra, H., Verburg, P. and Plieninger, T. (2024) 'Perceptions of ecosystem services and knowledge of sustainable development goals around community and private wetlands users in a rapidly growing city', *Landscape and Urban Planning*, 244, 104989. https://doi.org/10.1016/j.landurbplan.2023.104989

Berkes, F., Colding, J. and Folke, C. (2008) *Navigating Social-Ecological Systems: Building Resilience for Complexity and Change*. Cambridge: Cambridge University Press.

Berkes, F., Folke, C. and Colding, J. (2000) *Linking Social and Ecological Systems: Management Practices and Social Mechanisms for Building Resilience*. Cambridge: Cambridge University Press.

Bourdieu, P. (1991) *Language and Symbolic Power*. Cambridge: Polity Press.

Caglayan, İ., Yeşil, A., Kabak, Ö. and Bettinger, P. (2021) 'A decision making approach for assignment of ecosystem services to forest management units: A case study in northwest Turkey', *Ecological Indicators*, 121, 107056. https://doi.org/10.1016/j.ecolind.2020.107056

Cutter, S.L., Barnes, L., Berry, M., Burton, C., Evans, E., Tate, E. and Webb, J. (2008) 'A place-based model for understanding community resilience to natural disasters', *Global Environmental Change*, 18(4), pp. 598–606. https://doi.org/10.1016/j.gloenvcha.2008.07.013

Dean, M.M. (2009) *Governmentality: Power and Rule in Modern Society*. London: SAGE.

Dryzek, J.S. (2022) *The Politics of the Earth: Environmental Discourses*. 4th edn. Oxford: Oxford University Press.

Ebrahimi, H. and Kordevani, P. (2014) *Study of climate change in Anzali International Wetland using Mann-Kendall method*.

Evans, J.P. (2011) 'Resilience, ecology and adaptation in the experimental city', *Transactions of the Institute of British Geographers*, 36(2), pp. 223–237. https://doi.org/10.1111/j.1475-5661.2010.00420.x

Fathi, F., Azari Dehkordi, F. and Bagherzadeh Karimi, M. (2010) *Determining the protective buffer zone of Anzali wetland complex based on ecological indices*.

Filyushkina, A., Strange, N., Löf, M., Ezebilo, E.E. and Boman, M. (2018) 'Applying the Delphi method to assess impacts of forest management on biodiversity and habitat preservation', *Forest Ecology and Management*, 409, pp. 179–189. https://doi.org/10.1016/j.foreco.2017.10.022

Fletcher, A.J. and Marchildon, G.P. (2014) 'Using the Delphi method for qualitative, participatory action research in health leadership', *International Journal of Qualitative Methods*, 13(1), pp. 1–18. https://doi.org/10.1177/160940691401300101

Folke, C. (2006) 'Resilience: The emergence of a perspective for social–ecological systems analyses', *Global Environmental*

Change, 16(3), pp. 253–267. https://doi.org/10.1016/j.gloenvcha.2006.04.002

Folke, C., Carpenter, S.R., Walker, B., Scheffer, M., Chapin, T. and Rockström, J. (2010) 'Resilience thinking: Integrating resilience, adaptability and transformability', *Ecology and Society*, 15(4), 20. https://doi.org/10.5751/ES-03610-150420

Gardner, R.C., Barchiesi, S., Beltrame, C., Finlayson, C.M., Galewski, T., Harrison, I., Paganini, M., Perennou, C., Pritchard, D., Rosenqvist, A. and Walpole, M. (2015) *State of the World's Wetlands and Their Services to People: A Compilation of Recent Analyses*. Ramsar Briefing Note No. 7. Gland, Switzerland: Ramsar Convention Secretariat. https://doi.org/10.2139/ssrn.2589447

Gardner, R.C. and Finlayson, C.M. (2018) *Global Wetland Outlook: State of the World's Wetlands and Their Services to People*. Gland, Switzerland: Secretariat of the Convention on Wetlands. https://doi.org/10.69556/GWO-2018-eng

Ghazban, F. and Zare Khosh Eghbal, M. (2011) *Investigating the origin of heavy metal contamination in the sediments of Anzali Wetland (Northern Iran)* [Conference paper or document]. Civilica. Available at: https://civilica.com/doc/1578123

Gordon, C. and Foucault, M. (1980) *Power/Knowledge: Selected Interviews and Other Writings, 1972–1977*. New York: Pantheon Books.

Gotham, K.F. and Campanella, R. (2011) 'Coupled vulnerability and resilience: The dynamics of cross-scale interactions in post-Katrina New Orleans', *Ecology and Society*, 16(3), 12. https://doi.org/10.5751/ES-04292-160312

Hasson, F., Keeney, S. and McKenna, H. (2000) 'Research guidelines for the Delphi survey technique', *Journal of Advanced Nursing*, 32(4), pp. 1008–1015. https://doi.org/10.1046/j.1365-2648.2000.01567.x

Hirschhorn, F. (2019) 'Reflections on the application of the Delphi method: Lessons from a case in public transport research', *International Journal of Social Research Methodology*, 22(3), pp. 309–322. https://doi.org/10.1080/13645579.2018.1543841

Hsu, C.-C. and Sandford, B.A. (2007) 'The Delphi technique: Making sense of consensus', *Practical Assessment, Research, and Evaluation*, 12(10), pp. 1–8. https://doi.org/10.7275/pdz9-th90

Jafari, N. (2009) 'Ecological integrity of wetland, their functions and sustainable use', *Journal of Ecology and the Natural Environment*, 1(3), pp. 45–54. https://doi.org/10.5897/JENE.9000042

Jamshidi Zanjani, A. and Saeedi, M. (2014) 'Metals pollution assessment of surface sediment in Anzali Wetland and their quality zonation', *Journal of Environmental Studies*, 39(4), pp. 157–170. https://doi.org/10.22059/jes.2014.36469

Khazaei, B., Khatami, S., Alemohammad, S.H., Rashidi, L., Wu, C., Madani, K., Kalantari, Z., Destouni, G. and Aghakouchak, A. (2019) 'Climatic or regionally induced by humans? Tracing hydro-climatic and land-use changes

to better understand the Lake Urmia tragedy', *Journal of Hydrology*, 569, pp. 203–217. https://doi.org/10.1016/j.jhydrol.2018.12.004

Khosravi, Y., Attaran, L. and Bahramifar, N. (2021) 'Assessment of heavy metal pollution in water and estimation of potential risk caused by metals on the life of Anzali Wetland in 2017', *Journal of Environmental Health Engineering*, 8(4), pp. 412–424. https://doi.org/10.52547/jehe.8.4.412

Li, T.M. (2007) *The Will to Improve: Governmentality, Development, and the Practice of Politics*. Durham, NC: Duke University Press.

Linstone, H.A. and Turoff, M. (1975) *The Delphi Method: Techniques and Applications*. Reading, MA: Addison-Wesley.

Makkonen, M., Hujala, T. and Uusivuori, J. (2016) 'Policy experts' propensity to change their opinion along Delphi rounds', *Technological Forecasting and Social Change*, 109, pp. 61–68. https://doi.org/10.1016/j.techfore.2016.05.020

Mosleh, L., Negahban-Azar, M. and Pavao-Zuckerman, M. (2023) 'Stormwater green infrastructure resilience assessment: A social-ecological framework for urban stormwater management', *Water*, 15(9), 1786. https://doi.org/10.3390/w15091786

Okoli, C. and Pawlowski, S.D. (2004) 'The Delphi method as a research tool: An example, design considerations and applications', *Information & Management*, 42(1), pp. 15–29. https://doi.org/10.1016/j.im.2003.11.002

Ostrom, E. (2009) 'A general framework for analyzing sustainability of social-ecological systems', *Science*, 325(5939), pp. 419–422. https://doi.org/10.1126/science.1172133

Rahimi, L., Malekmohammadi, B. and Yavari, A.R. (2019) 'The ecosystem services assessment of wetlands based on the classification of hydrological-ecological structures and functions (Case study: Shadegan Wetland)', *Geography and Environmental Sustainability*, 9(30), pp. 51–72. Available at: https://sid.ir/paper/221214/en

Revez, A., Dunphy, N., Harris, C., Mullally, G., Lennon, B. and Gaffney, C. (2020) 'Beyond forecasting: Using a modified Delphi method to build upon participatory action research in developing principles for a just and inclusive energy transition', *International Journal of Qualitative Methods*, 19, 1609406920903218. https://doi.org/10.1177/1609406920903218

Salehi, S. and Qolamdoust, A. (2013) 'Assessment of a model of collaborative management for wetlands from a community viewpoint (Case study: Anzali Wetlands)', *Advanced Environmental Sciences*, 11(1).

Scheffer, M., Carpenter, S.R., Lenton, T.M., Bascompte, J., Brock, W., Dakos, V., van de Koppel, J., van de Leemput, I.A., Levin, S.A., van Nes, E.H., Pascual, M. and Vandermeer, J. (2012) 'Anticipating critical transitions', *Science*, 338(6105), pp. 344–348. https://doi.org/10.1126/science.1225244

Skulmoski, G.J., Hartman, F.T. and Krahn, J. (2007) 'The Delphi method for graduate

research', *Journal of Information Technology Education: Research*, 6, pp. 1–21. https://doi.org/10.28945/199

Spivak, G.C. (2023) 'Can the subaltern speak?', in *Imperialism*, pp. 171–219. London: Routledge.

Stroud, D.A., Davidson, N.C., Finlayson, C.M. and Gardner, R.C. (2022) 'Development of the text of the Ramsar Convention: 1965–1971', *Marine and Freshwater Research*, 73(10), pp. 1107–1126. https://doi.org/10.1071/MF21312

Swyngedouw, E. (2011) 'Depoliticized environments: The end of nature, climate change and the post-political condition', *Royal Institute of Philosophy Supplements*, 69, pp. 253–274. https://doi.org/10.1017/S1358246111000300

Toumbourou, T. (2020) 'Using a Delphi approach to identify the most efficacious interventions to improve Indonesia's forest and land governance', *Land Use Policy*, 99, 102768. https://doi.org/10.1016/j.landusepol.2017.05.017

Vera-Herrera, L., Romo, S. and Soria, J. (2022) 'How agriculture, connectivity and water management can affect water quality of a Mediterranean coastal wetland', *Agronomy*, 12(2), 486. https://doi.org/10.3390/agronomy12020486

Waldron, K., Thiffault, N., Bujold, F., Ruel, J.-C., Lussier, J.-M. and Boucher, D. (2020) 'Ecological issues related to second-growth boreal forest management in eastern Quebec, Canada: Expert perspectives from a Delphi process', *Forest Ecology and Management*, 470–471, 118214. https://doi.org/10.1016/j.foreco.2020.118214

Walker, B., Holling, C.S., Carpenter, S.R. and Kinzig, A. (2004) 'Resilience, adaptability and transformability in social–ecological systems', *Ecology and Society*, 9(2).

Zebardast, L., Akbarpour, S., Jafari, H.R. and Bagherzadeh Karimi, M. (2021) 'Sustainable wetland management through bridging the communication gap between conservation projects and local communities', *Environment, Development and Sustainability*, 23(7), pp. 11098–11119. https://doi.org/10.1007/s10668-020-01082-5

Zhao, X., Zhang, Q., He, G., Zhang, L. and Lu, Y. (2021) 'Delineating pollution threat intensity from onshore industries to coastal wetlands in the Bohai Rim, the Yangtze River Delta, and the Pearl River Delta, China', *Journal of Cleaner Production*, 320, 128880. https://doi.org/10.1016/j.jclepro.2021.128880

Effects of Household Detergent Concentrations on Seed Germination and Early Seedling Growth of Alfalfa (*Medicago sativa* L.) and Cumin (*Cuminum cyminum* L.)

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Received: 2026-07-05 Accepted: 2026-09-07

Abstract

Household wastewater contains detergents that may adversely affect rivers and soil in large quantities. One of the compounds found in household wastewater is household detergents. This study aimed to determine the germination characteristics of alfalfa (*Medicago sativa* L.) and cumin (*Cuminum cyminum* L.) seeds under the influence of different concentrations of water contaminated with household detergent. A laboratory experiment was conducted to evaluate the effect of different concentrations of water contaminated with household detergent on the germination characteristics of alfalfa and cumin seeds, in a completely randomized design with three replications and six treatments. This concentrations including, zero, 0.002, 0.02, 0.2, 2, and 20 g/l of household detergent in water. Results showed that the difference between the highest and lowest concentrations of detergent in terms of seed germination was 90% in alfalfa and 30% in cumin. The highest seedling length (7.2 cm) and seed vigor (6.9 %cm) in alfalfa were obtained from the 0.02 g/l household detergent treatment. The highest seed germination percentage (56%), seedling length (3.4 cm), and seed vigor (2.05% cm) of cumin were obtained from the 0.2 g/l household detergent concentration. The result showed a 50% increase in germination, 3,300% increase in seedling length and 20,400% increase in seedling vigor compared to the 20 g/L household detergent concentration. Therefore, according to the results of this study, for cumin, irrigation water contaminated with household detergent up to 0.2 g/L can be used, and for alfalfa, up to 0.02 g/L. Higher concentrations are not recommended due to severe loss of seed vigor.

Keywords: Household detergents, Seed germination, Seedling growth, Alfalfa, Cumin

Introduction

Water dissolves and transports many environmental pollutants. Among these, household detergents are a serious threat to the environment. These detergents, which enter the environment through household wastewater, can cause water and soil pollution and affect plant and animal life. Their environmental effects include eutrophication and changes in temperature,

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Doi: 10.48308/pae.2026.246193.1153

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salinity, and pH of soil and water (Mousavi and Khodadoost, 2019). These detergents comprise potentially harmful compounds that can negatively affect the growth of living organisms, including plants (Vanitha et al., 2017; Valkó et al., 2020; Alfred et al., 2024; Cai, 2024; Lukács et al., 2025). Household detergents contain various compounds, each with specific effects and capable of causing environmental stresses such as salinity, drought, and heavy metal contamination (Heidari et al., 2017). The effect of detergents on seed germination was assessed in *Cucumis sativus* L., (Silva and Silva, 2023), *Amaranthus hybridus* L. and *Solanum lycopersicon* L. (Ehilen et al., 2017), monocotyledonous and dicotyledonous (Erhenhi, 2011), two horticultural crops (Gálvezal., 2019), habanero pepper (*Capsicum chinense* Jacq., Hernández-Baranda et al., 2023), maize (*Zea mays* L., Uzma et al., 2018), and wheat (Yosefi and Heidari, 2022). In a study examining the effect of water contaminated with household detergent on the germination of pinto bean (*Phaseolus vulgaris* L.) seeds. It was observed that the germination percentage was not affected by the contaminated water. However, detergent concentrations of 2 and 20 g/L resulted in the shortest radicle lengths (Heidari, 2015b). The effect of water contaminated with household detergent was evaluated on wheat (*Triticum aestivum* L.) seed germination. In this study, household detergent up to a concentration of 0.2 g/L did not affect seed germination characteristics. However, at a household detergent concentration of 2 g/L, germination percentage, shoot length, root length, and seed vigor decreased. While, at a household detergent concentration of 20 g/L, all seed germination characteristics were zero (Heidari, 2016). A study examining the effect of treated hospital wastewater on the germination characteristics of three vegetables species— coriander (*Coriandrum sativum* L.), dill (*Anethum graveolens* L.), and cress (*Lepidium sativum* L.)— found no difference between raw and treated wastewater in terms of germination characteristics. Higher wastewater concentration increased roots, stems, and leaves lengths in the plants studied (Gholami-Borujeni et al., 2019). In another study the effect of different concentrations of water contaminated was investigated with household detergent on the germination characteristics of bitter vetch (*Vicia ervilia* L.) seeds. It was observed that with increasing detergent concentration germination percentage, stem length, root length, and seed vigor was reduced. At a detergent concentration of 20 g/L, all seed germination characteristics were zero (Heidari, 2015c).

The effect of cadmium stress on the germination characteristics of some medicinal plants at with six concentrations of 5, 10, 15, 20, 25 and 30 mg/L were studied, along with a cadmium-free treatment. The results showed that low cadmium concentrations stimulated germination in some plants, whereas a concentration of 30 mg/L reduced root and shoot length, root and shoot dry weight, and seed vigor. Fenugreek (*Trigonella foenum-graecum* L.), black cumin (*Nigella sativa* L.), and dill were the most sensitive to cadmium (Spanany and Fallah, 2016). The

effect of different concentrations (0, 50, 100, 200, 400, and 800 mg/L) of lead and nickel on radish (*Raphanus sativus* L.) seed germination were investigated. At low concentrations of lead and nickel, radish germination increased. While, at concentration of 800 mg/L of lead and nickel, the germination percentage was reduced compared to the control (0 mg/L). The greatest radicle length was obtained at concentrations of 100 and 400 mg/L of lead and nickel (Aminifard and Bayat, 2019). The effect of different concentrations of lead nitrate (0, 0.25, 0.5, 1, and 2 mM) on lemon balm (*Melissa officinalis* L.) germination were also investigated. Increasing lead nitrate concentration reduced germination percentage, root and shoot dry weights, shoot and root length (Torabi et al., 2014).

The effects of different concentrations of heavy metals copper, zinc and silver were studied at concentrations of 0, 5, 10, 15 and 20 mg/L on the germination of Bermuda grass (*Cynodon dactylon* L.). It was observed that copper, zinc, and silver stimulated seed germination and root growth of Bermuda grass (Taghizadeh et al., 2013). In a study investigating the effects of irrigation with wastewater from a treatment plant on fenugreek, increasing wastewater concentration decreased the fresh and dry weight of aerial organs and roots. Heavy metals accumulated in the aerial organs and roots of the plant (Fazeli and Eyni, 2022). In another study the effect of water and soil contaminated with cadmium at different concentrations (0, 0.01, 0.05, 1, and 2 mg/L) was observed on corn (*Zea mays* L.) growth. It was found that the highest corn growth occurred at the lowest cadmium concentration. Increasing cadmium concentration reduced plant growth (Riahi-Farsani et al., 2021). In a study examining the effect of salinity stress treatments (0, 4, 8, and 12 dS/m) on the germination of giant reed (*Arundo donax* L.) seeds. It was observed that salinity reduced the germination percentage, germination rate, root and shoot length (Elahinejad et al., 2023). Salinity reduced the germination of *Arundo donax* L. seeds (Mahmoodi and Hosseini, 2018). As salinity increased to 300 mM, the root length of *Salsola orientalis* increased, however, concentrations higher than this value reduced root length (Panahi et al., 2015). Various studies have been conducted on the effect of wastewater on germination characteristics and early seedling growth. However, fewer studies have been conducted on the effects of household detergent on seed germination characteristics of crop plants. So far, no comprehensive study has been conducted on the effect of household detergents on seed germination characteristics and seedling growth in alfalfa and cumin. Therefore, the objectives of the research were to assess the tolerance of alfalfa and cumin to household detergent during the germination and the initial growth stage of seedlings. The study also aimed to determine the germination characteristics of alfalfa and cumin seeds at different concentrations of household detergent.

Materials and methods

A laboratory study was conducted at Razi University in 2015 using six household

detergent concentrations in water (0, 0.002, 0.02, 0.2, 2, and 20 g/l) on the germination characteristics of alfalfa (*Medicago sativa* L.) and cumin (*Cuminum cyminum* L.) seeds. The selection of household detergent concentrations was based on previous experiments (Heidari, 2015b; Heidari, 2016). These concentrations were selected solely for initial evaluation and to investigate the tolerance to household detergent and the extent of damage caused under laboratory conditions.

Laundry powder was used as the household detergent used**.** The main chemical composition of the detergent included sodium silicate, sodium carbonate, sodium sulfate, zeolite, sodium carboxy methyl cellulose, sodium tripolyphosphate, bleach and fragrance.

The study was conducted using a completely randomized design with three replications. Each Petri dish containing 10 seeds considered as an experimental unit. The seeds were placed at 25°C for one week under a 12-h light period. A filter paper was placed in each Petri dish, and 6 mL of a solution with a specific concentration was added to the filter paper. The Petri dishes for each replication were wrapped in plastic film to maintain ambient humidity and solution concentration throughout the experiment.

After one week, the germination characteristics of alfalfa and cumin seeds were measured. These characteristics included the germination percentage, seedling length, and vigor of alfalfa and cumin seeds. Germination percentage was measured by the ratio of germinated seeds to the total number of seeds in each petri dish. To measure seedling length, three seedlings were selected from each Petri dish. Seedling length was measured using a ruler with millimeter accuracy, from the tip of the longest root to the end of the stem. Seed vigor was measured using the method described by Heidari (Heidari, 2015a, Eq 1.):

Seed vigor (%cm) = Seed germination (%) × Seedling length (cm) (1)

After data collection and before data analysis, outliers were identified and removed using Minitab software, if necessary. Data normality was then assessed using Minitab software. First, an analysis of variance (ANOVA) was performed. When the result were significant, the means were compared using Duncan's test at the 5% probability level. SAS software was used for analysis of variance and mean comparison.

Results and Discussion

Effects of household detergent concentration on alfalfa germination, seedling length, and seed vigor

Analysis of variance showed that the experimental treatments had a significant effect on alfalfa seed germination percentage (Table 1). Comparison of the means indicated that the household detergent concentrations from 0 to 2 g/L resulted in the highest alfalfa seed germination percentages. (Table 2). At the concentration of 20 g/L, germination percentage was reduced to zero. The difference between the highest and lowest concentrations of detergent in terms of seed germination was 90% in alfalfa.

Analysis of variance showed that the experimental treatments had a significant effect on alfalfa seedling length (Table 1). Compari-

son of the means demonstrated that a concentration of 0.02 g/L resulted in the greatest alfalfa seedling length (7.2 cm). Whereas, concentrations of 2 and 20 g/L resulted in the lowest seedling length (1.2 and 0 cm). Concentrations of 0 and 0.002 g/L of detergent had lower seedling length than the concentration of 0.02 g/L of household detergent (Table 2).

ANOVA showed that the experimental treatments had a significant effect on alfalfa seed vigor (Table 1). Comparison of the mean germination characteristics demonstrated that a household detergent concentration of 0.02 g/L resulted in the highest alfalfa seed vigor (6.9 % cm). Concentrations of 2 and 20 g/L resulted in the lower seed vigor (0.9 and 0 %cm, respectively). Concentrations of 0 and 0.002 g/L of detergent also had lower seed vigor than the concentration of 0.02 g/L of household detergent (Table 2).

It is observed that detergent up to 0.002 g/L detergent increased seedling length and seed vigor of alfalfa and detergent is a growth stimulant at this concentration. A concentration of 20 g/L, which is the highest concentration studied, reduced germination percentage, seedling length, and seed vigor to zero. Among germination characteristics analyzed, seedling length and seed vigor were more sensitive to increasing detergent concentrations, than seed germination percentage. Germination percentage declined only at 20 g/L, whereas seedling length and seed vigor at 2 g/L were already comparable to those observed at 20 g/L. This is because germination is defined as the emergence of the shoot from the seed, a process that occurred in a shorter period than subsequent seedling growth. During the seedling growth stage, seeds are exposed not only to the stress during the germination stage but also to additional during subsequent growth. Therefore, longer exposure to stress may have contributed to reduction in the length of the seedling and the vigor of the alfalfa seed. The reason for the decrease in the length of the seedling and the vigor of the seed at concentrations of 0 and 0.002 g/l of detergent is probably that the detergent at a low concentration of 0.02 g/l acts like a disinfectant and prevents damage caused by pathogens such as fungi to the germination of the seed and subsequent growth, but at a high concentration (20 g/l of detergent) it causes various stresses such as salt stress, drought, and heavy elements, like a chemical.

Salts in household detergent can act as a seed priming agent and improve seed germination characteristics, which is consistent with the results of others (Tahmasebi et al., 2017). Salt priming resulted in the preservation of chlorophyll, reduced malondialdehyde accumulation, and decreased electrolyte leakage in mung bean (*Vigna radiate* L.), thereby enhancing salinity tolerance (Wang et al., 2026(. Salt priming increased total seed germination and leaf area under salinity stress conditions while reducing membrane permeability. This priming treatment and plant growth-promoting bacteria mitigated the adverse effects of salinity stress and could serve as a strategy to address salinity in the future (Dolu et al., 2025). The reduction in seed germination characteristics at a concentration of 20 g/L of detergent could be due to the negative effects of this substance

at high concentrations. Detergent contains salts such as sodium, which can be absorbed by seeds in large quantities and disrupt the physiological processes of seed germination (Heidari and Karimi, 2024). Detergent can also create osmotic stress, which has been reported to reduce seed germination characteristics with drought stress by other researchers (Heidari and Moradi, 2019).

Effects of household detergent concentration on cumin germination, seedling length, and seed vigor

Analysis of variance of the effect of contaminated water on the germination percentage of cumin seeds showed that the experimental treatments had a significant effect on the germination percentage of cumin seeds (Table 3). Comparison of the mean for the effect of different concentrations of water contaminated with household detergent on the germination percentage of cumin seeds showed that the concentration of 0.2 g/l of household detergent had the highest germination percentage of cumin seeds (56%), which resulted in a 50% increase in germination compared to the 20 g/l household detergent concentration. The lowest germination percentage of cumin seeds (6%) was related to the treatment of 20 g/l of household detergent. The

Table 1. Analysis of variance (mean square) of the effect of water contaminated with household detergent on the germination characteristics of alfalfa seeds

Sources of variation	Degree of freedom	Germination percentage	Seedling length	Seed vigor
Treatment	5	3408**	22.6**	17.3**
Error	12	138	0.8	0.8

** Significant at the 1% probability level.

Table 2. Mean comparison for the effect of different concentrations of water contaminated with household detergent on alfalfa seed characteristics

Treatments [a]	Germination (%)[b]	Seedling length (cm)	Seed vigor (%cm)
T1	90a	4.8bc	4.3bc
T2	76a	3.6c	2.7c
T3	76a	7.2a	6.9a
T4	86a	5.6ab	4.8b
T5	76a	1.2d	0.9d
T6	0b	0d	0d

a T1, T2, T3, T4, T5, and T6 represent household detergent concentrations of 0, 0.002, 0.02, 0.2, 2, and 20 g L^{-1} in water, respectively.
b Means within each column followed by the same letter are not significantly different at the 5% level according to Duncan's test.

concentration of 0 g/l of household detergent had a lower germination percentage than 0.2 g/l of household detergent (Table 4). The difference between the highest and lowest concentrations of detergent in terms of seed germination was 30% in cumin.

ANOVA showed that the experimental treatment had a significant effect of contaminated water on the length of cumin seedlings (Table 3). Comparison of the means indicated that household detergent concentration of 0.2 g/L of household detergent had the highest length of cumin seedlings (3.4 cm), which resulted in a 3,300% increase in seedling length compared to the 20 g/L household detergent concentration. The lowest length of cumin seedlings (0.1 cm) was related to the treatment of 20 g/L of household detergent. Concentrations of 0, 0.002, and 0.02 g/L of household detergent had shorter seedling lengths than 0.2 g/L of household detergent (Table 4).

Analysis of variance of the effect of contaminated water on cumin seed vigor showed that the experimental treatments had a significant effect on cumin seed vigor (Table 3). Comparison of the mean for the effect of different concentrations of

Table 3. Analysis of variance (mean square) of the effect of water contaminated with household detergent on the germination characteristics of cumin seeds

Sources of variation	Degree of freedom	Germination percentage	Seedling length	Seed vigor
Treatment	5	943**	3.6**	1.2**
Error	12	94	0.1	0.1

** Significant at the 1% probability level.

Table 4. Mean comparison for the effect of different concentrations of water contaminated with household detergent on cumin seed characteristics

Treatments [a]	Germination (%)[b]	Seedling length (cm)	Seed vigor (%cm)
T1	36b	1.4b	0.55b
T2	46ab	0.7c	0.32bc
T3	33b	1.6b	0.56b
T4	56a	3.4a	2.05a
T5	50ab	0.3cd	0.15c
T6	6c	0.1d	0.01c

a T1, T2, T3, T4, T5, and T6 are concentrations of 0, 0.002, 0.02, 0.2, 2 and 20 g/l of household detergent in water, respectively.

b Means within each column that have the same letter are not significantly different at the 5% level according to Duncan's test.

household detergent contaminated water on cumin seed vigor showed that the highest seed vigor (2.05 % cm) was related to 0.2 g/L household detergent, which resulted in a 20,400% increase in seedling vigor compared to the 20 g/L household detergent concentration. The lowest seed vigor (0.01 and 0.15 %cm) was associated with the treatments of 20 and 2 g/L household detergent. Concentrations of 0 to 0.02 g/L household detergent had lower seed vigor than 0.2 g/L household detergent (Table 4).

The enhancement in cumin seed germination percentage, seedling length and vigor at 0.2 g/L may be attributed to a stimulatory response to a low concentration of household detergent. Because the cumin used in this study was a native variety rather than modified variety, it may retain certain wild characteristics, including dormancy associated with the seed husk, and may therefore require environmental stimuli to initiate germination. At a low concentration, household detergent may have facilitated germination by removing the husk and external obstacles, due to the presence of certain salts and elements.

Based on the observed results, household detergent may play a dormancy breaker role. Probably, this effect may be associated with its detergent compounds that facilitate removal or weakening of seed coat, thereby enhancing the absorption of moisture and air. In addition, the salts present in household detergent may be absorbed by the seeds and potentially exert a priming-like effect, contributing to increasing the germination percentage and seed vigor. The potential of various compounds as seed priming has been proven in previous studies (Azarnia and Eisvand, 2013; Nouriani, 2018).

The results of the present study are consistent with those of previous studies (Heidari et al., 2017), which reported that high concentrations of household detergent contaminated- water reduced seed germination characteristics. Similarly, a study examining the effect of different concentrations of wastewater on wheat seed germination found wastewater that reduced seed germination percentage. However, at high wastewater concentrations, seed vigor, root length, and shoot length of the plant did not differ from the control (distilled water). These findings are in consistent with the results of the present study (Heidari and Moradi, 2019). Improvement of seed germination characteristics through the application of salts and elements has also been reported by other researchers (Hameed et al., 2025, Ellouzi et al., 2023, Hussain et al., 2022). At the highest household detergent concentration (20 g/l, germination percentage reduced significantly to 6% in cumin, while no germination was observed in alfalfa. Cumin seed vigor appeared to be more sensitive to household detergent than germination percentage. As there was no significant difference in seed vigor between the 2 and 20 g/L of household detergent. The reduction in germination characteristics at high household detergent concentrations may be associated with various stresses, such as drought, salinity, and heavy elements, which have been proven in previous studies (Barnawal et al., 2017; Armendariz et al., 2019; Heidari and Parhoon, 2022, Islam et al., 2023). A notable difference between, alfalfa and cumin in the pres-

ent study was their responses to the highest household detergent concentration (20 g/L). In cumin, seed germination characteristics were still observed at this concentration, with germination percentage, seedling length, and seed vigor values of 6%, 0.1 cm, and 0.01 % cm, respectively. In contrast, all germination characteristics were completely inhibited in alfalfa, with values of 0 %, 0 cm, and 0%cm for germination percentage, seedling length, and seed vigor, respectively. Another difference was that in cumin, seedling length and seed vigor were maximum at a higher concentration of household detergent compared to alfalfa. These differences could be attributed to genetic differences between the two plants, and differences the degree of plant breeding. For example, with respect to seed composition and environmental adaptation, cumin is a medical plant commonly cultivated in arid regions and is relatively tolerant of drought, potentially due to its secondary metabolites. In contrast, alfalfa is a water-demanding crop generally cultivated in water-rich regions.

Irrigation with household detergent-contaminated wastewater can contribute to surface water and groundwater pollution. The discharge of household wastewater containing detergent into rivers may also promote eutrophication. In this process, nutrients such as nitrogen and phosphorus in the wastewater can stimulates excessive growth of aquatic plants and algae. As these biomass accumulates and subsequently decomposes dissolved oxygen levels may decline, that adversely affected fish and other aquatic organisms. Ultimately, these changes decline water quality and disrupt aquatic ecosystem functioning (Akinnawo, 2023). The entry of household wastewater into agricultural soils can affect the growth of both crops and weeds. Previous research confirmed that detergents are able to remove the hard seed coats of some weed species, thereby stimulating germination. In addition, the nutrients present in these detergents can also provide an appropriate source of nutrients for weeds (Heidari and Parhoon, 2022; Lukács et al., 2025). Household detergents can also alter soil properties, including pH, organic matter content, and bulk density (Mohamed et al., 2018; Zhang and Dongdong, 2022). The detergent concentrations studied in this study were higher than those typically found in household wastewater. Previous research has indicated that detergents may stimulate seed germination and plant growth at low concentrations, whereas higher concentrations, can cause severe damage or plant mortality (Heidari et al., 2017).

Conclusion

Given that household detergent at certain concentrations reduced or prevented seed and seedling growth in alfalfa and cumin. Therefore, in areas where the use of such wastewater is unavoidable, irrigation with water free of household detergent contamination should be prioritized during sensitive stages, particularly seed germination. Based on the results of this study, detergent concentrations of up to 0.2 g/L for cumin and 0.02 g/L for alfalfa were associated with more favorable germination responses under the experimental conditions. Higher concentrations are not recommended because of their adverse effects on seed vigor.

Further studies should investigate the effects of other compounds present in wastewater on the germination characteristics of alfalfa and cumin.

References

Akinnawo, S.O. (2023) 'Eutrophication: Causes, consequences, physical, chemical and biological techniques for mitigation strategies', Environmental Challenges, 12, 100733. https://doi.org/10.1016/j.envc.2023.100733

Alfred, N.B., Ogaboh, A.M.I., Anietie, B.R. and Egwu, E.A. (2024) 'Effect of irrigation with household detergent on germination, activities of oxidative stress enzymes and chlorophyll content of pod maize', *Pakistan Journal of Agricultural Research*, 37(3), pp. 290–299. https://doi.org/10.17582/journal.pjar/2024/37.3.290.299

Aminifard, M.H. and Bayat, H. (2019) 'The effect of heavy metals nickel and lead on seed germination and radish seedling growth', *National Conference on Medicinal Plants and Sustainable Agriculture*, Hamadan, 23 January 2019.

Armendariz, A.L., Talano, M.A., Nicotra, M.F.O., Escudero, L., Breser, M.L., Porporatto, C. and Agostini, E. (2019) 'Impact of double inoculation with *Bradyrhizobium japonicum* E109 and *Azospirillum brasilense* Az39 on soybean plants grown under arsenic stress', *Plant Physiology and Biochemistry*, 138, pp. 26–35. https://doi.org/10.1016/j.plaphy.2019.02.018

Azarnia, M. and Eisvand, H.R. (2013) 'Seed quality improving by priming to increase crops growth and yield', *Research Achievements for Field and Horticulture Crops*, 2(4), pp. 277–287. https://doi.org/10.22092/rafhc.2013.100151

Barnawal, D., Bharti, N., Pandey, S.S., Pandey, A., Chanotiya, C.S. and Kalra, A. (2017) 'Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression', *Physiologia Plantarum*, 161(4), pp. 502–514. https://doi.org/10.1111/ppl.12614

Cai, X. and Ostroumov, S.A. (2022) 'Phytotoxicity of "Tide" detergent powder using *Lens culinaris* seeds as a bioassay', *Acta Scientific Microbiology*, 5(2), pp. 21–26. https://doi.org/10.31080/ASMI.2022.05.0998

Dolu, H., Killi, D., Bas, S. *et al.* (2025) 'Effectiveness of salt priming and plant growth-promoting bacteria in mitigating salt-induced photosynthetic damage in melon', *Photosynthesis Research*, 163(7). https://doi.org/10.1007/s11120-024-01128-z

Ehilen, O.E., Obadoni, B.O., Imade, F.N., Eseigbe, D. and Mensah, J.K. (2017) 'The effect of detergents on the germination and growth of *Amaranthus hybridus* L. and *Solanum lycopersicon* L.', *Nigerian Annals of Natural Sciences*, 16(1), pp. 100–108.

Elahinejad, M., Asadi, G. and Tavakol Afshari, R. (2023) 'The role of salinity and drought stress on the germination indices of giant reed (*Arundo donax* L.) in the next generation', *Iranian Journal of Weed Science*, 19(1), pp.

119–138. https://doi.org/10.22034/ijws.2024.363154.1438

Ellouzi, H., Zorrig, W., Amraoui, S., Oueslati, S., Abdelly, C., Rabhi, M., Siddique, K.H.M. and Hessini, K. (2023) 'Seed priming with salicylic acid alleviates salt stress toxicity in barley by suppressing ROS accumulation and improving antioxidant defense systems, compared to halo- and gibberellin priming', *Antioxidants*, 12(9), 1779. https://doi.org/10.3390/antiox12091779

Erhenhi, A.H. (2011) 'Effects of detergents and local soap "SODA" on germination of the seeds of monocots and dicots', *Nigerian Journal of Science and Environment*, 10, pp. 219–224.

Fazeli, F. and Eyni, H. (2022) 'Effect of urban wastewater treatment plant effluent irrigation on heavy metals uptake by fenugreek (*Trigonella foenum-graecum* L.)', *Journal of Plant Research*, 35(2), pp. 397–409.

Gálvez, A., López-Galindo, A. and Peña, A. (2019) 'Effect of different surfactants on germination and root elongation of two horticultural crops: implications for seed coating', *New Zealand Journal of Crop and Horticultural Science*, 47(2), pp. 83–98. https://doi.org/10.1080/01140671.2018.1538051

Gholami-Borujeni, F., Zahedi, A. and Sheikhi, M. (2019) 'Evaluation of hospital treated wastewater on seed germination and plant growth indices', *Journal of Health Research and Community*, 5(1), pp. 49–59.

Hameed, A., Hussain, S., Nisar, F., Rasheed, A. and Shah, S.Z. (2025) 'Seed priming as an effective technique for enhancing salinity tolerance in plants: mechanistic insights and prospects for saline agriculture with a special emphasis on halophytes', *Seeds*, 4(1), 14. https://doi.org/10.3390/seeds4010014

Heidari, H. (2015a) 'Effect of defoliation based on leaf position on maize yield, yield components and produced seed germination', *Bulgarian Journal of Agricultural Science*, 21(4), pp. 801–805.

Heidari, H. (2015b) 'Evaluating effect of laundry detergent on pinto bean seed germination', *The 1st International Congress on Earth, Space & Clean Energy*, Ardabil, p. 3. Available at: https://civilica.com/doc/456685

Heidari, H. (2015c) 'Study of the effect of polluted water on the germination of bitter vetch', *Second National Congress of Iranian New Technologies with the Aim of Achieving Sustainable Development*, Tehran. Available at: https://civilica.com/doc/399807

Heidari, H. (2016) 'Evaluation of germination characteristics of wheat seeds (Pishtaz variety) under the influence of polluted water', *Third Conference on New Findings in Environment and Agricultural Ecosystems*, Tehran. Available at: https://civilica.com/doc/586548

Heidari, H. and Moradi, S. (2019) 'Comparison of refined and non-refined wastewater effect on wheat seed germination and growth under drought', *Water SA*, 45(4). https://doi.org/10.17159/wsa/2019.v45.i4.7547

Heidari, H. and Parhoon, Z. (2022) 'Effect of water contaminated with hand washing

liquid on germination characteristics of wheat, flax, and lentil', *Tenside Surfactants Detergents*, 59(4), pp. 339–343. https://doi.org/10.1515/tsd-2021-2411

Heidari, H., Yosefi, M., Sasani, S. and Nosratti, I. (2017) 'Effect of contaminated water with laundry detergent on foxtail millet root and physiological traits', *Tenside Surfactants Detergents*, 54, pp. 118–124. https://doi.org/10.3139/113.110487

Heidari, H. and Karimi, S. (2024) 'Effect of contaminated water (handwashing detergent) on seed germination traits in wheat, mung bean, and chickpea', *Tenside Surfactants Detergents*, 61(2), pp. 189–193. https://doi.org/10.1515/tsd-2023-2578

Hernández-Baranda, Y., Echevarría-Machado, I., Rodríguez-Hernández, P. and Estrada-Medina, H. (2023) 'Effect of detergents on the germination and initial growth of habanero pepper (*Capsicum chinense* Jacq.) plants', *Revista Colombiana de Ciencias Hortícolas*, 17(3). https://doi.org/10.17584/rcch.2023v17i3.16577

Hussain, S., Shakil, A., Waheed, A., Guihua, L. and Ahmad, Y.N. (2022) 'Selenium seed priming enhanced the growth of salt-stressed *Brassica rapa* L. through improving plant nutrition and the antioxidant system', *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.1050359

Islam, M. and Sandhi, A. (2023) 'Heavy metal and drought stress in plants: the role of microbes—a review', *Gesunde Pflanzen*, 75, pp. 695–708. https://doi.org/10.1007/s10343-022-00762-8

Lukács, K., Kiss, R., Tóth, Á., Godó, L., Deák, B. and Valkó, O. (2025) 'Effects of laundry washing on germination of cloth-dispersed seeds depends on washing intensity not on detergent type', *Journal of Environmental Management*, 375, 124345. https://doi.org/10.1016/j.jenvman.2025.124345

Mahmoodi, S. and Hosseini, S.M. (2018) 'Effect of salt stress and temperature on germination of *Arundo donax* (L.) seeds', *APPlant*, 40, 123.

Mohamed, R.M., Al-Gheethi, A.A., Noramira, J. *et al.* (2018) 'Effect of detergents from laundry greywater on soil properties: a preliminary study', *Applied Water Science*, 8, 16. https://doi.org/10.1007/s13201-018-0664-3

Mousavi, S.A. and Khodadoost, F. (2019) 'Effects of detergents on natural ecosystems and wastewater treatment processes: a review', *Environmental Science and Pollution Research*, 26(26), pp. 26439–26448. https://doi.org/10.1007/s11356-019-05802-x

Nouriani, H. (2018) 'The effect of seed priming on germination components, biochemical changes and early growth of sesame seedlings (*Sesamum indicum*)', *Iranian Seed Research*, 5, pp. 43–58.

Panahi, F., Asareh, M.H., Jafari, M., Givar, A.R., Tavili, A., Arzani, H. and Ghorbani, M. (2015) 'The responses of *Salsola orientalis* to salt stress', *International Journal of Advanced Biological and Biomedical Research*, 3(2), pp. 163–171.

Riahi-Farsani, H., Ghobadinia, M., Noori Emamzadei, M., Danesh Shahraki, A.

and Motaghian, H. (2020) 'The effect of cadmium contamination water and soil on corn yield components', *Journal of Water and Soil Conservation*, 27(5), pp. 167–184. https://doi.org/10.22069/jwsc.2021.18319.3392

Silva, L.M. da and Silva, F.J. da (2023) 'Toxic effects promoted by a commercial detergent on the germination and initial development of cucumber seedlings (*Cucumis sativus* L.)', *Ecotoxicology and Environmental Contamination*, 18(2), pp. 3–9. https://doi.org/10.5132/eec.2023.02.02

Spanany, A. and Fallah, S. (2016) 'The effect of cadmium stress on seed germination characteristics of some medicinal plants under in vitro conditions', *Iranian Journal of Medicinal and Aromatic Plants Research*, 32(3), pp. 527–542. https://doi.org/10.22092/ijmapr.2016.106833

Taghizadeh, M., Solgi, M., Karimi, M. and Shahcheragi, T. (2013) 'Effects of some heavy metals application on seed germination and in vitro regeneration of bermudagrass', *Plant Productions Technology*, 5(1), pp. 95–108.

Tahmasebi, R., Sajedi, N.A. and Shoaei, S. (2017) 'Evaluation effect of different solutions and seed priming treatments on germination, agronomic and quality characteristics of red bean genotypes', *Iranian Journal of Pulses Research*, 8(1), pp. 60–72. https://doi.org/10.22067/ijpr.v8i1.26145

Torabi, A., Safipour Afshar, A. and Saeed Nematpour, F. (2014) 'Effect of heavy metal lead on seed germination and grain growth of the medicinal plant lemon balm', *First National Conference on Medicinal Plants, Traditional Medicine and Organic Agriculture*, Hamadan, 29 December 2014. Available at: https://civilica.com/doc/329976

Uzma, S., Khan, S., Murad, W., Taimur, N. and Azizullah, A. (2018) 'Phytotoxic effects of two commonly used laundry detergents on germination, growth, and biochemical characteristics of maize (*Zea mays* L.) seedlings', *Environmental Monitoring and Assessment*, 190, pp. 1–14. https://doi.org/10.1007/s10661-018-7031-6

Valkó, O., Lukács, K., Deák, B., Kiss, R., Miglécz, T., Tóth, K., Tóth, Á., Godó, L., Radócz, S., Sonkoly, J., Kelemen, A. and Tóthmérész, B. (2020) 'Laundry washing increases dispersal efficiency of cloth-dispersed propagules', *NeoBiota*, 61, pp. 1–16. https://doi.org/10.3897/neobiota.61.53730

Vanitha, S., Vighnesh, L. and Sreekar, V. (2017) 'A study on the effects of cleaning agents (household) on seed germination', *International Journal of Advanced Research in Science and Engineering*, 6, pp. 198–203.

Wang, Z.W., Liu, M., Sang, H.T. *et al.* (2026) 'Salt priming coordinates transcriptional and epigenetic states for enhanced salt tolerance in mung bean (*Vigna radiata*)', *Communications Biology*. https://doi.org/10.1038/s42003-026-10574-5

Yosefi, M. and Heidari, H. (2022) 'Evaluation of wheat tolerance during germination and early growth stages to detergent-contaminated water', *Tenside Surfactants Detergents*, 59(1), pp. 95–103. https://

doi.org/10.1515/tsd-2021-2380

Zhang, S. and Dongdong, L. (2022) ‘The effect of powder detergent water on shrinkage cracks of soil due to drying’, *Geofluids*, 2022, 3307135. https://doi.org/10.1155/2022/3307135