PHYSIOLOGICAL AND GENETICAL VARIABILITY AMONG SOME PLANTS UNDER DIFFERENT SALINITY CONDITIONS IN KUWAIT

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The Salt tolerant plant species (Halophytes) represent about 4% of the flora. Some of the wild species proved to be potential donors for salt-tolerance genes that can be transferred to their respective domesticated species by classical breeding or biotechnology. Exotic halophytes constitute an untapped genetic resource that can be used for developing crops for saline conditions (Jaradat, 1999). There was a tendency to perform researches in relatively extreme environments to find out plant responses to intense stresses. Understanding of the physiological, biochemical and genetically processes that regulate the whole plant alarm responses which are defensive and/or adaptive to the stimulus is greatly enhanced (Stewart and Larcher, 1980). Exposure of plants to biotic and abiotic stresses led to some changes in stress physiology and biochemistry. For instance, salinity and drought which are major abiotic stresses severely causes alterations of protein synthesis or degradation which is one of the fundamental metabolic processes that may influence salinity and drought tolerance (Chandler and Robertson, 2004).

The presence of high concentrations of salt (NaCl) in the soil solution induces a wide range of physiological and biochemical perturbations at the whole plant level. It has been demonstrated in several glycophyte and halophyte species such as *Mesembryanthemum crystallinum* (Vera-Estrella *et al*., 2009).

The *Chenopodiaceae* and *Nitrariaceae* are a family of flowering plants, also the family's main range is in the arid and semi-arid regions. Moreover most species are halophytes characteristic of saline soils and coastal habitats. Although widely recognized in most plant classifications. *Salsola baryosma (*Schult.), *Tragnum nudatum (*Delile) and *Nitraria retusa* (Forssk.) are halophytes wild plants and uses as medicines, agri-horticulture and as animal forage in desert and semidesert (Angiosperm Phylogeny Group, 2009). Changes in C and N decreased biosynthesis of chlorophyll and inefficiency of photosynthesis, (Christenhusz and Byng, 2016). Moreover, salt stress has also been found responsible for an increased respiration rate, ion toxicity (Sudhir and Murthy, 2004).

DNA barcoding is a molecular tool that uses a short locus from a standardized genome position to provide fast and accurate species identification

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Web Site (*www.esg.net.eg*)

(http://www.barcoding.si.edu). This technique is helpful in taxonomic, ecological, and evolutionary studies. In addition, it can be used in more applied fields (e.g. conservation, forensic science, and the food industry) and can enable the accurate identification of cryptic species (Lahaye *et. al*., 2008; Ragupathy *et. al*., 2009). The term "DNA barcode" is used here to refer to a DNA sequence-based identification system that may be constructed of one locus or several loci used together as a complementary unit (Kress and Erickson, 2007).

Therefore, the present work was intended to investigate the physiological and genetically responses of three plants *Salsola baryosma*, *Tragnum nudatum* and *Nitraria retusa* to their different environment in Kuwait state.

MATERIALS AND METHODS

Firstly, the concerned three halophytic plant species were identified and authenticated in January 2016. Three plant species were collected from three different sites in Kuwait state at (Keran, Montaza El-Watania and Shalehate El-Watania) to represent the variations within and between plant species encountered in the study area. The collected wild plants under study are as follows:

- ***Salsola baryosma*** (Romy and Schult.) Dandy Family *Chenopodiaceae.* A stout much-branched shrub to about 1 m high of saline and waste sandy places. The plant is very salty. The plants perennial herbs; very rare shrubs, or tree. Most of plants are halophytes (salt tolerant). They grow on saline soil. Uses ash Medicines Angiosperm Phylogeny Group (2009) (Fig. 1).

- ***Tragnum nudatum*** (Delile) genus *Traganum*, Family *Chenopodiaceae.* A straggling bush to 60 cm high of rocky waste places and desert pasturage of the Region. The foliage is readily browsed by camels but less so by other stock. It is collected in March. They bloom and bear fruit with twigs succulent Leaves needle. It is a much green and White branched bush growing to a maximum height of about 1 m. (Fig. 2). Uses Agri-horticulture: fodder Products: fuel and lighting Angiosperm Phylogeny Group (2009).

- ***Nitraria retusa*** (Forssk.) Asch. Family *Nitrariaceae*. The plant is a salt-tolerant and drought-resistant shrub or a much-branched bush growing to a maximum height of about 2.5 m .It plays an important role in the stabilization of sand dunes. It has tiny, white to green, fragrant flowers and small edible red fruit. It typically grows in salt marshes and semi-arid saline areas of deserts and it can help in the stabilization of loose soils, (Fig. 3). Many species are important as animal forage in desert and semidesert. It is one of a number of salt-tolerant plants that are being investigated as potential fodder crops for livestock. (Mold, 2012; (Christenhusz and Byng, 2016).

Soil analyses

Also, three associated soil profiles were dug wide open to the depths where the greatest amount of plant roots occurred. Accordingly three soil samples were collected and then air dried, crushed, sieved through 2 mm sieve and subjected to analysis.

- Particle size distribution of the fine earth was carried out by the dry sieving, (Gee and Bauder 1986), due to the coarse-textured nature of soils.

- Determined of the soluble constituents in the soil paste using the method described by Black (1986).

- Total salinity (ECe) in the soil saturation extract was determined conductimetrically (Zeng *et al.*, 2002).

- Cationic and anionic compositions of the soil extract were accomplished following the methods described by Stefania *et al.* (2006).

Determination of nutritive elements and heavy metals

- The digested samples were analyzed for macronutrients (N, P, K, Ca, and Mg), micronutrients (Fe, Mn, Cu, Zn, B, Mo and Ni) and heavy metals according to the methods of Cottenie *et al.* (1982) and Willson *et al.* (1997).
- Determination of total Nitrogen was carried out by kjeldahel method (Sàez-Plaza *et al.*, 2013).
- Phosphorous was determines by the colormetric method and measured spectrophotometrically.
- Potassium was determined flame photometrically (Meditsiiniline, 2012).
- Calcium, Magnesium, micronutrients and heavy metals were determined by inductivity coupled plasma (ICP).

Determination of biochemical components

Determination of moisture content, ash content, carbohydrates, protein, lipids and fiber in both leaves and stems of plant studies was carried out following the methods described herein :-

- Moisture content was measured according to AOAC (2006).
- Ash content was done by AOAC (2006).
- Total carbohydrate content was estimated as the method described by Chaplin and Kennedy (1994).
- Total lipids were measured according to AOAC (2006).
- Crude fiber was determined as done by AOAC (2006).
- Total protein was determined according to AOAC (2006).
- Total amino acids determination was performed according to method of analysis AOAC (2006), using an amino acid analyzer (Biochrom 30).

Molecular identification DNA extraction

Dried plant leaf samples from three plants were used. DNA extraction was carried out using SIGMA® Plant High Molecular DNA extraction KIT®, Plant tissue was disrupted by grinding in liquid nitrogen and DNA was released with detergent and chaotropic agents.

PCR and sequencing

The primer pairs of (rbcLaF 1,5) (5’-ATG TC CCA CAA ACA GAG ACT AAA GC-3’) and rbcLaR (5’-GTA AAA TCA AGT CCA CCR CG-3’) were used to amplify rbcL region, (White *et al*., 1990). Molecular identification, assignment of taxa and phylogenetic analysis.

For such, the BLASTn tool were used to determine the candidates for a supported phylogenetic analysis using several methods. To identify the evolutive position and study phylogenetic relationships of the three plants under study, the aligned sequences were analyzed by maximum likelihood (ML) analysis implemented in MEGA7 (Tamura *et al*., 2013).

RESULTS AND DISCUSSION

Soil of the study area

The obtained results of soil analysis (Table 1) revealed that the soil texture is sandy. The chemical composition of the examined soils, (Table 2) shows that soil reaction is neutral to alkaline where pH values range from 6.99 up to 8.91. EC fluctuates in narrow range, where it ranges from 3.11 to 6.85 dsm-1, however these values differ from one site to the other. The highest salinity level is detected in lying lands of site 1 whereas the lowest salinity characterizes the soil of site 3.

The cationic composition of soils is generally dominated by Na^+ which is the major cation, followed by Ca^{++} and Mg^{++} while K^+ is the least abundant cation. For more information about the cationic composition of the soil extract, the obtained data reveal that Na^+ constitutes 19.00-48.40 me/l with the highest and lowest contents in soils of the topographically low land of (3) and (1), respectively. Soluble Ca^{++} content ranges from as low as 7.34 me/l to as high as 15.3 me/l. In general, the highest contents of soluble Ca^{++} are characteristic of the site 1, whereas the lowest Ca^{++} content in site 3, while in the soils of moderately elevated land of site 2. Soluble Mg^{++} content ranges within a narrow limit between 3.75 and 8.20 me/l. Soluble K+ content fluctuates between 0.99 and 1.32 me/l, being at its highest content in the moderately elevated land of site 1, while its lowest content characterizes the low land of site 3.

The anionic composition of the soil extract reveals that Cl^- is the most predominant anion followed by SO_4^{--} while HCO_3^- is the least abundant anion .These results dictate that most soluble salts are in Cl^- and SO_4^{--} forms with a lesser contribution of HCO_3^-.It is also evident that the presence of HCO_3^- salts has exerted certain modifications of soil reaction (pH) towards light alkaline reaction. This is clearly evidenced from certain rise of pH values up to 7.55 that corresponds to the increase of HCO_3^- up to 4.35 me/l. Needless to mention that most salts in the forms of chlorides and sulfates are neutral.

Total content of micronutrients in soils

Table (3) shows the total contents of Fe, Mn, Cu, Zn, Ni, B and Mo in soils of the studied localities which are considered the main resource of supplying plants with such elements (metals and non-metals). An account on the distribution of these elements in soils of the concerned three sites is given hereafter.

Iron: The result in Table (3) showed that the total Fe content ranges widely from 422.0 to 1440.34 ppm. The highest content is found in the elevated land of (site 2) while the lowest is detected in the moderately elevated land of (site 1). It is also apparent that the lower content of Fe among the studied of (site 3) whose Fe content doesn't exceed 422.0 ppm. In general, the total Fe content of soils indicates a poor supply with Fe; this is due to the impoverishment of sandstone and granite which are the parent sediments of soils.

Manganese: Table (3) showed that total Mn content varies from 229.13 to 430.15 ppm. The highest content is detected in the (site 2) whereas the lowest content characterizes the land of (site 1). In fact, these results clarify the association of Fe and Mn since the highest content of both is found in site 2.

Copper: Table (3) showed that total Cu content varies within a very narrow limit between 2.90 and 7.31 ppm .The highest content characterizes the elevated land of site 3, while the lowest is that of the soil in site 2. In general, total Cu content is very low in soils of all the studied sites.

Zinc: Data in table (3) showed that total Zn ranges from 45.1 to 72.42 ppm. The highest content is recorded in the soils of (site 2) whereas the lowest is the land of (site 1). The close similarity between Zn contents in both low and moderately elevated lands of site 3 is clearly noticed.

Nickel: Total Ni content is also quite low, being in the range of 4.29 and 7.33 ppm. The highest content is strictly confined to the elevated lands of site 1 followed by the low lying lands of site 2. In contrast, the lowest content characterizes of site 3.

Molybdenum: Total Mo content in soils is generally very low, being in the range of 1.08 to 2.99 ppm. The highest content is found in the land of site 2, whereas the lowest is found in the land of site 1.

Boron: Total B content ranges from 13.31 to 23.84 ppm (Table 3). The highest content is associated with soils of the elevated lands of (site 3), whereas the lowest is the land of site 1.

From the foregoing results, one can conclude that the site 2 attained the highest levels of Fe, Mn, Cu, Zn, Ni, Mo and B. This clarifies some associations such as Fe and Mn, Cu and Zn, and Mo and Ni. In general, the total contents of the essential micronutrients suggest that the studied soils of different sites will be devoid of almost all essential elements, and plants grown under these conditions will suffer from the deficiency of essential micronutrients. In addition, the formation of soils in the studied three sites was inherited originally from sandstone and granite, i.e., soils derived from sandy matrix is poor in micronutrients and consequently infertile.

Total content of heavy metals in soils

This part is mainly concerned with heavy metals to assess the magnitude of soil pollution in the study area. In this regard, seven polluting elements were determined; Al, Cr, Sr, V, Pb, Cd, and Co. An account on the total content of each of the studied elements (Table 3) is given as follows:

Aluminum: Total Al content in soils varies within a somewhat narrow limit, being between 7331.0 and 12602.2 ppm. The highest content is found in the elevated land of site 3, whereas the lowest content is the land of site 1.

Chromium: Total Cr varies between 29.99 and 42.21 ppm. The highest content is detected in the elevated land of (site 3) whereas the lowest is the land of site 1. In general, the topographically low lands of the three sites under consideration attain the higher levels of Cr whereas elevated lands attain lower levels of Cr.

Strontium: Total content of Sr varies within a wider range, from as low as 11.59 to as high as 29.88. The highest content characterizes the site 2, while the lowest is found in the land of site 3. A common pattern of Sr abundance is strictly confined to the topographically low lands in the three sites under study. Noteworthy to mention that, the highest level of Sr is about three times its lowest content.

Vanadium: Total content of V varies from one site to another, being at its highest contents in the land of site 2 as well as the moderately elevated lands of site 1. In short, V content ranges from 29.13 and 46.85 ppm.

Lead: Data presented in Table (3) show that the total Pb content is of traceable and unique amounts, not exceeding 0.003 ppm. This content surely dictates that the soils of the studied sites are non-polluted with Pb.

Cadmium: Total Cd content has the same levels, < 0.0004 ppm. regardless of the studied three sites . Again, this level of Cd suggests that the soils of the studied three sites are non-polluted at all.

Cobalt: Total Co content is quite low, being in the range of 1.55 to 4.77 ppm. The highest content is recorded in site 2, whereas the lowest content is that of the low land of site 1.

Commenting on the above mentioned results, one can conclude that two associations of heavy metals are observed where the highest and lowest contents of Al and Cr are clearly associated since the moderately elevated land of site 3, which the plant grown was *Nitraria retusa* .On the other hand, the highest Sr and V contents are also associated and remarkably detected in site 2, which the plant grown was *Tragnum nudatum*. Moreover, Cd and

Pb constitute minor unified amounts that are considered far below the contaminant level.

Elemental composition of plants stduies leaves and stems

Over 95% of dry weight of plants is made up of carbon, hydrogen and oxygen taken from air and water. The remaining 5% of the plant dry weight is adsorbed from the soil where roots adsorb these elements from their surroundings. Among these elements, only 14 elements are necessary for plant growth; these 14 elements along with C, H and O are referred to as 17 essential inorganic nutrients, or elements. Within this number of essential elements, some are needed in larger amounts (macronutrients). Whereas the rest of elements are needed in lesser amounts (micronutrients). Note to mention that the macronutrients absorbed from the air are C, O and H while other macro and micronutrients are adsorbed from the soil.

In brief, the essential elements for plant growth are C, O, H, N, P, K, Ca, Mg and S as macronutrients while the micronutrients are Fe, B, Zn, Mn, Cu, Mo, Ni and Cl. This doesn't exclude that some trace elements such as Si and Co are beneficial for certain plants but not essential for all plants. Almost all elements are used in a variety of ways, such as catalysts for enzymatic reaction (either as part of the enzyme structure or as regulators of activators), as regulators of the movement of water in or out of the cell and maintenance of turgor pressure, as regulators of membrane permeability, as structural components of the cell of electron receptors or as buffers maintaining the pH within cells. In this regard, two thirds of chemical elements occur naturally in plants, of which some are used metabolically by particular species, but others with no function are accumulated because they are present in the soil from which the plant extracts water and ions (Bonner and Galston, 1952).

The obtained results in this study of the elemental composition of leaves and stems of the three plants studies are presented in Tables (4 and 5).The data include the macronutrients, micronutrients as well as heavy metals content in both leaves and stems. An account on each of the chemically studied elements is given hereafter.

Macronutrients

Nitrogen: Data depicted in Table (4) revealed that total nitrogen content of leaves ranges from 1.17 to 1.25% with an average of 1.12%. The highest content is associated with *Nitraria retusa* grown in (site 3) .With regard to nitrogen content in the stems, Table (5) shows that total nitrogen content varies within a very narrow limit (0.63 to 0.73%) with an average of 0.69%. The highest content is found in plants *Salsola baryosma*, *Tragnum nudatum which* grown in site 1 and site 2. While the low elevated was in *Nitraria retusa which* grown in site 3.

Phosphorous: Phosphorous is an essential nutrient and plays an important role as a structural and regulatory element in plant growth and development (Sacala *et. al.*, 2008). Adequate P nutrition may minimize and ameliorate negative effects of water stress (Shobhra *et. al.*, 2004).

Data in Tables (4 and 5) include the phosphorous content of leaves and stems of *Nitraria retusa* grown in the (site3). These data clarify that phosphorous content is generally higher in leaves relative to stems, except in *Tragnum nudatum* grown in the site 2.

For convenience, phosphorous content in leaves varies from 0.29 to 1.09% with an average of 0.69%. The highest content (1.01%) is found in leaves of plants grown in the low lying bed of *Nitraria retusa* grown in the site 3, whereas the lowest P level is (0.29%) that found in plant leaves of *Tragnum nudatum* grown in the (site 2).On the other hand, P content in stems ranges within a narrow limit between 0.41 and 0.56% with an average of 0.52%. The highest content is found in the stems of plants grown in moderately elevated of *Nitraria retusa* grown in the site 3, while the lowest P content is that of plants grown on the low of *Salsola baryosma* grown in the site1.

Potassium: Potassium is an important nutrient and plays an essential role in water relation, osmotic adjustment, stomatal movement and finally plant resistance to drought. Decrease in K^+ concentration was reported in many plant species under water deficient conditions, mainly due to membrane damage and disruption in ion homeostasis and K^+ deficient plant has lower resistance to water stress (Lisar *et. al.*, 2012).

Tables (4 and 5) postulate the total potassium content in both leaves and stems of three plants under study grown in the concerned three sites of study. The tables show that potassium content is apparently higher in leaves compared to stems. To suffices, K^+ content in leaves varies from 1.31 to 1.88% with an average of 1.48%. The highest content corresponds to leaves of plants *Nitraria retusa* grown in the site 3, while the lowest content is that of leaves of plants *Tragnum nudatum* grown in site 2. With respect to K^+ content in stems, it ranges from 0.94 to 1.56% with an average of 1.32%. The highest K^+ content is found in the low of plants *Tragnum nudatum* grown (site 2).

Commenting on the above mentioned data of macronutrients, one can conclude that N, P and K contents are generally higher in leaves relative to stems regardless of *Nitraria retusa* grown in site 3. Moreover, macronutrient elements could be arranged according to their content in either leaves or stems in the descending order; K, N and P.

Calcium: Tables (4 and 5) show that Ca content in leaves is higher than that of stems, in plants *Salsola baryosma*, *Tragnum nudatum* which grown in sites 1 and 2, while Ca in stems is higher than that of leaves of *Tragnum nudatum* and *Nitraria retusa* which grown in sites 2 and 3. This may be due to local condition of this site which accelerates Ca accumulation in an extraordinary case in leaves. For convenience, Ca content in leaves ranges from 0.11- 0.12% while in stems it ranges from 0.03 to 0.06%. The highest content of Ca in leaves is recorded in plants *Salsola baryosma*, *Tragnum nudatum* grown different sites through the two sites (1 and 2). Whereas the lowest content is found in plants *Nitraria retusa* grown on the site 3.

With regard to Ca content in stems, the highest content is recorded in plants *Nitraria retusa* grown in the site 3, whereas the lowest content is found in plants *Salsola baryosma*, grown in the site 1. It is also remarkable that the variations encountered in the Ca content of leaves are within a narrow range whereas the range is wider in stems.

Magnesium: Like Ca, Mg constitutes higher levels in leaves relative to stems. To suffices, Mg content in leaves ranges from 0.01 to 0.02%.The highest content is recorded in the leaves of plants *Tragnum nudatum* grown in the site 2, while the all other sites share in the same Mg percentage (0.01%). With respect to Mg content in stems, data show that Mg content ranges from 0.004 to 0.01%. The highest content corresponds to stems of plants *Salsola baryosma*, grown in site 1, while the lowest content is estimated in the stems of plants *Tragnum nudatum* and *Nitraria retusa* grown in the two sites 2 and 3.

Micronutrients

Iron: Tables (4 and 5) indicate that the mean total Fe content in the leaves of *Salsola baryosma* is considerably high relative to stems, being about three times. For convenience, the total Fe content in leaves ranges from 8.00 to 9.45 ppm with an average content of 9.11 ppm. The highest Fe content (9.45) is found in leaves of plants *Tragnum nudatum* grown in site 2, while the lowest one (8.00) is assigned to plants *Nitraria retusa* grown in site 3.

On the other hand, Fe content of stems varies within a somewhat narrow limit, being in the range of 3.39-3.88 ppm with an average of 4.27 ppm. The highest content (3.88) is associated with plants *Nitraria retusa* grown in site 3, whereas the lowest content (3.39) is found in plants *Salsola baryosma* and *Tragnum nudatum* grown in (sites 1 and 2).Worth mentioning to note that the variations in iron content within leaves is wider than that of stems.

Manganese: Like Fe, Mn content is appreciably higher in leaves relative to stems, being about 1.42 to 3 times, Tables (4 and 5). In fact, Mn content in leaves ranges from 1.42 to 2.05 ppm with an average of 1.80 ppm. The highest content is recorded in plants *Salsola baryosma* grown in site 1, whereas the lowest is confined to plants *Nitraria retusa* grown in site 3. With regard to Mn content in stems, it ranges within a narrow limit between 0.33 and 0.49 ppm. The lowest content is recorded in plants *Salsola baryosma* grown in the site 1, whereas the highest contents are found in plants *Tragnum nudatum* and *Nitraria retusa* grown in sites 2 and 3.

Copper: Data presented in Tables (4 and 5) dictate that total Cu content in leaves is considerably lower than its content in stems. In most cases, Cu content in stem is two times that in leaves except in plants *Salsola baryosma* grown in site 1, where Cu content in total, leaves total equals that of stems. In details, the total Cu content in leaves ranges from 0.24 to 0.68 ppm with an average of 0.28 ppm. In contrast, total Cu in stems varies widely from 0.26 to 1.07 ppm with an average of 0.83 ppm.

Zinc: Tables (4 and 5) present the total zinc content in both leaves and stems of three studied plants. The tables show that Zn content in leaves varies from 0.59 to 0.70 ppm with an average of 0.63 ppm. The highest content characterizes the low of *Salsola baryosma* grown in site 1, whereas the lowest with plants *Tragnum nudatum* grown in site 2. With regard to Zn content in stems, it seems evident that Zn content is somewhat higher in stems relative to that of leaves, being in the range of 0.67 to 0.99 with an average of 0.80 ppm. The highest content is recorded in the *Nitraria retusa* grown in (site 3), while the lowest is confined to the *Tragnum nudatum* grown in site 2. It is quite evident that Zn contents in both leaves and stems range within very narrow limits. Moreover, Zn content is in all stems cases more than Cu content in leaves.

Boron: Data in Tables (4 and 5) include the B content in both leaves and stems of three studied plants . These data indicate that B content in leaves is quite high, being in the range of 3.19 to 5.51 ppm with an average of 5.00 ppm. The highest content (5.51 ppm) is recorded in leaves of plants *Nitraria retusa* grown in site 3, whereas the lowest (3.19 ppm) is that of plants *Salsola baryosma* grown in site 1. Considering the B content in the stems, it is apparent that its content is far below that of the leaves, being in the range of 0.21 to 0.44 ppm with an average of 0.39. In other words, B content of leaves is more than stems.

Molybdenum: Data in Tables (4 and 5) reveal that Mo content in leaves ranges from 0.02 to 0.04 ppm.with an average of 0.03 ppm. The highest content is recorded in plant *Salsola baryosma* and *Nitraria retusa* samples representing sites 1 and 3, whereas, the least content is recorded in plants *Tragnum nudatum* grown in site 2.

Regarding Mo content in stems, it varies within a narrow limit between 0.01 and 0.02 but the common content of Mo in most cases is 0.02 ppm. In general, the highest content is recorded for stems of plants *Salsola baryosma* and *Nitraria retusa* grown in the two sites 1 and 3, while the least content is reported for the plants *Tragnum nudatum* grown in site 2. In brief, the content of Mo in leaves is either equal to that of stems or being twice as much of it depending on the abiotic environment in which plants are grown.

Nickel: Data presented in Tables (4 and 5) dictate that Ni content of leaves is commonly 0.03 ppm except for plants *Nitraria retusa* grown in site 3. Likewise, Ni content in stems is commonly 0.03 ppm except for one cases in plants *Salsola baryosma* and *Tragnum nudatum* grown in two sites 1 and 2, where Ni content rises up to 0.03 and 0.04 ppm.

Based on the foregoing results, it is quite evident that the micronutrient elements in leaves and stems of three plants under study could be ranked according to their contents in two distinct descending orders; i) Fe, B, Mn, Zn, Cu and Ni (for leaves) and ii) Fe, Cu, Zn, Mn, B, Ni (for stems). Furthermore, it is also remarkable that Fe, Mn, B and Mo contents are considerably higher in leaves relative to stems and the converse is true for Cu and Zn where their contents in stems are somewhat higher than in leaves but Ni displayed a similar content in both leaves and stems.

Heavy metals

Data tabulated in Tables (4 and 5) present the contents of non-essential elements or heavy metals that were detected in leaves and stems of three plants under study. These data clarify that the plant attains considerable amounts of some heavy metals like Al, Sr and traceable amounts of other heavy metals. For convenience, Al content is high in leaves. Its content ranges between 12.08 and 15.92 ppm in leaves, while being in the range of 3.24 to 5.24 ppm in stems. In other words, Al content in leaves is about 1.5 times to more than 4 times that of stems. The presence of considerable amounts of such element in plant parts is mainly rendered to the nature of soil on which this plant is grown since it is mainly composed of aluminum-silicates together with Al components that stimulate the uptake of Al by grown plants.

Chromium content is present in traceable amounts that range from 0.03 to 0.06 ppm and 0.02 to 0.06 ppm in leaves and stems of three plants under study, respectively, being somewhat higher in leaves relative to stems. Likewise, Cobalt constitutes very traceable amounts ranging from 0.003 to 0.005 ppm and 0.002 to 0.003 ppm in leaves and stems, respectively.

Vanadium content is quite similar content in three plants in leaves in the 0.03 ppm and 0.01 to 0.02 ppm in stems, i.e., being somewhat higher in leaves relative to stems.

Strontium content is in common considerably high, being in the ranges of 5.73 to 6.34 ppm and 0.02 to 4.302 ppm in leaves and stems, respectively. Its content is remarkably higher in leaves relative to stems.

Cadmium content in leaves of three plants under study is of minute amounts (0.001 ppm), whereas Cd content in stems is slightly higher where its mean value is 0.01 to 0.02 ppm. Barium is also present in traceable and variable amounts, being between 0.50 to 0.95 ppm and 0.20 to 0.68 ppm in leaves and stems, respectively with a common higher content in leaves compared to stems.

Lead content varies from 0.01 to 0.04 ppm with no distinct pattern for leaves or stems. In other words, Pb content is apparently low in both leaves and stems.

Commenting on the above mentioned results one can conclude that the studied plants is not polluted by heavy metals, reflecting the safe environment in which three plants is grown. In other words, none of the determined elements in plant reaches the polluting range of these elements. This also confirms that the presence of an element in plant doesn't necessarily indicate its essentiality but may merely signalize the fact that this element is present in the medium surrounding the roots. In short, the accumulated and determined elements in three plants under studies reflect, in a way, the status of these elements in soils and surrounding environment that stimulates or contradicts their absorption and uptake by grown plants.

Data presented in Tables (4 and 5) dictate that Ni content of leaves is commonly 0.04 ppm except for plants *Nitraria retusa* grown in site 3. Likewise, Ni content in stems is commonly 0.03 ppm except for two cases in plants *Salsola baryosma*, *Tragnum nudatum* grown in two sites 1 and 2.

Based on the foregoing results, it is quite evident that the micronutrient elements in leaves and stems of three plants under study could be ranked according to their contents in two distinct descending orders; i) Fe, B, Mn, Zn, Cu and Ni (for leaves) and ii) Fe, Cu, Zn, Mn, B, Ni (for stems). Furthermore, it is also remarkable that Fe, Mn, B and Mo contents are considerably higher in leaves relative to stems and the converse is true for Cu and Zn where their contents in stems are somewhat higher than in leaves but Ni displayed a similar content in both leaves and stems.

Biochemical profile of three plants under study

Concerning the biochemical components of the aerial parts of three plants under study, Tables (6 and 7) present the contents of moisture, ash, total carbohydrates, total lipids, fiber and total protein, in the plants collected from the different three sites. A brief account on each of these components is given hereafter.

Moisture content

The moisture content in the aerial plant parts varies within narrow limit, being in the ranges of 6.48 to 6.67% and 2.07 to 5.45% in leaves and stems, respectively with a relatively higher content in leaves Tables (6 and 7). This is expected since the plant is grown in arid desert environment and sandy soils impoverished in water and nutritive elements with a common shortage of water essential for plant growth.

Ash content

Tables (6 and 7) show that ash content in leaves ranges from 10.50 to 14.14% while being in the range of 4.83 to 9.92 % in stems which means that the ash content of leaves is about 2 folds higher than that of stems. This confirms the previous findings that the elemental composition of leaves is more pronounced if compared to that of stems.

Total carbohydrates

Carbohydrates act as nutrient and signaling molecules, modulating the expression of a large number of genes (Osuna *et. al*., 2007) and they are also involved in the response to abiotic stresses. Tables (6 and 7) show that total carbohydrates content either in leaves or in stems varies within a very narrow limit. For instance, carbohydrates contents in leaves range from 53.3-58.40% while in stems the carbohydrates are appreciably lower and range from 39.10-44.12%. The minute variations express the prevailing aridity (hyper arid conditions) and open unique environments that contribute to photosynthesis in the same way and magnitude.

In this respect, Strogonove *et al.* (1970) and Kramer (1983) reported the accumulation of carbohydrates in plants as a response to salinity or drought, despite a significant decrease in net CO_2 assimilation rate. This is also confirmed by global gene expression studies which showed a reduction in the expression level of most genes encoding chloroplast enzymes involved in carbon fixation, while genes encoding cytoplasmic and vascular enzymes in the pathways leading to glucose, fructose and fructan production were upregulated under drought stress (Xue *et al.*, 2008), suggesting a coordination in the regulation of transcripts of key enzyme genes involved in carbon fixation and carbohydrate accumulation. Besides, maintenance of active life and green foliage during the long dry period may be explained by the fact that this species depends in its water supply on the water stored in its tissues by slight succulence, high latex content with high bound water for viscous cytoplasm for more drought resistance behavior.

Total lipids

Water stress can lead to a disturbance of the association between membrane lipids and proteins as well as enzymes activity and transport capacity of membranes. Lipid peroxidation is the well known effect of drought and many other environmental stresses via oxidative damage.

Data given in Tables (6 and 7) reveal that total lipids constitute 0.52 to 1.30% and 0.80 to 1.16% of leaves and stems, respectively. This means that the total lipids don’t change considerably neither in leaves nor in stems, i.e., lipids content are within the same range of magnitude irrespective of plant organs. Among the most familiar lipids, fatty acids and glycerolipids are probably concerned with the organization and function of cellular membrane. Though lipids are important membrane components, and changes in their composition may help to maintain membrane integrity and preserve cell compartmentation under water stress conditions (Gigon *et al.,* 2004), yet the content of lipids in *C. pendulus* is seemingly low. This behavior was explained by

Monteiro de paula *et al*. (1993) and Matos *et al.* (2001) who correlated the decrease in membrane lipid content under water stress to an inhibition of lipid biosynthesis and a stimulation of lipolytic and peroxidative activities.

Crude fiber content

Tables (6 and 7) indicated that fiber content varies widely in leaves while being within a narrow limit in stems. To suffices, fiber content in leaves ranges from 10.3 to 13.3% while being much higher in stems as it ranges from 39.8 to 42.9%. This is expected due to the nature of the plants under study as a climber and adapted for hyper arid conditions.

Total protein

Data in Tables (6 and 7) showed that total protein content ranges from 13.91 to 14.15% in leaves and 5.33 to 7.96% in stems of the plants under study. These data clarify small variations in total protein content either in leaves or stems. In this regard Nour El-Din and Ahmed (2004) found that the increase in soil moisture stress may remarkably increase the assimilation and accumulation of nitrogenous compounds. Align with them, Campllans *et al.* (1999) showed evidence that protein residues may be altered during drought stress and some proteins are irreversibly damaged by the effect of drought stress and degraded by proteases. They further suggested that proteases mobilize amino acids from proteins to the synthesis of compatible osmolytes.

Total amino acids

Numerous studies have appraised the metabolic adjustments of nitrogen metabolism in plants subjected to water deficit and plant survival either during or after the period of stress. Among these studies, Stewart and Larcher (1980), Hanson and Hitz (1982) and Navari-Izzo *et al.* (1990) clarified that marked differences have been found in the amino acids pattern under stress conditions. Some other studies have delineated certain amino acids as medicators for drought resistance and defence.

Since the concerned plants *Salsola baryosma*, *Tragnum nudatum* and *Nitraria retusa* are actually grown in Kuwait state desert areas of arid to hyperarid climatic conditions with edaphic and abiotic factors controlling the prevailed environment, it is of interest to shed light on the protein amino acids in the aerial parts of such plant.

Figures (4 and 5) depict the protein amino acid composition in the aerial parts (leaves and stems) of three plants grown in the three sites .In this respect, 15 amino acids in a combined and free forms are determined. Examination of the obtained data reveals variations in amino acids concentrations in plants grown in different sites. Therefore, variations encountered in amino acids assortment and percentages in the aerial plant parts are postulated as follows.

Amino acids in leaves

Data presented in Fig. (4) shows clearly that the most predominant amino acids in leaves are aspartic (0.90 to 1.40%), glutamic (0.91 to 1.30%) and leucine (0.43 to 0.60%). In contrast, the least abundant amino acids are tyrosine (0.20 to 0.70%) and histidine (0.20 to 0.43%). Other amino acids are detected in concentrations that are in between those extremes. In general, the identified amino acids could be arranged according to their average concentrations in the descending order: aspartic>glutamic> leucine> alanine> valine> argenine > phenylalanine, lysine > isoleucine> proline> glycine>threonine> serine>histidine > tyrosine.

Though this pattern of protein amino acids in leaves of three plants under study is almost unique for plants grown in the three studied sites their concentrations showed marked changes according to location sites. This is clearly manifested by the highest concentrations of each aliphatic amino acid which are strictly confined to the leaves of plants *Salsola baryosma*, *Nitraria retusa* and *Tragnum nudatum* grown in the sites 1, 3 and 2, respectively. Whereas the concentrations of each cyclic amino acid are associated with plants *Tragnum nudatum*, *Salsola baryosma* and *Nitraria retusa* grown in sites 2, 1 and 3, respectively. Further examination of data dictates that the leaves concentrations of both glutamic and aspartic acids (acidic amino acids) which are the most abundant among identified amino acids are quite similar (Table 7).Within aliphatic amino acids; leucine is the most abundant followed by alanine, valine, isoleucine, glycine, threonine and serine in a descending order. Their concentrations range from 0.65 to 0.99%, 0.45 to 0.64%, 0.44 to 0.61%, 0.43 to 0.60%, 0.40 to 0.51%, 0.39 to 0.50% and 0.28 to o.45%, respectively. The highest content is strictly confined to plants *Nitraria retusa* grown in site 3, while the least concentrations are mostly associated with plants *Tragnum nudatum* grown in the site 2.

Within the basic amino acids, argenine and lysine predominate and have almost equal concentrations, 0.46-0.62% and 0.42-0.63%, respectively while histidine concentration (0.20-0.43%) is the least abundant in this group of amino acids.

The highest concentrations of basic amino acids characterize plants *Salsola baryosma* grows in site 1 and *Nitraria retusa* in site 3, whereas the lowest concentrations are found in plants *Tragnum nudatum* grown in site 2.

Within the cyclic amino acids, tyrosine is the most abundant together with proline while phenylalanine is the least abundant among this amino acids group. Their concentrations range from 0.39 to 0.70%, 0.34 to 0.62% and 0.20 to 0.41%, respectively. The highest concentrations are found in plants *Tragnum nudatum* grown in site 2, whereas the least concentrations are found in plants *Nitraria retusa* grown in site 3. The specific pattern of amino acids groups may indicate that the synthesis of protein types rich in acidic amino acids could be the key to survival of three plants species under the prevailing climatic (drought stress) or edaphic conditions. In other words, glutamic and aspartic acids have considerably high concentrations in drought stressed. While proline (cyclic amino acid) is known by its usefulness in adaptive response and helping plants to with stand stress effect accounted only for 0.34 to 0.62% (average 0.50%), i.e., for less than 8% of protein amino acids concentration already mentioned by Matysik *et al*. (2002) as esitical level.

With reference to Nanjo *et al*. (2009), one may state that *C. pendulus* is not able to produce considerable amounts of proline and therefore has a lower drought stress defense or tolerance. Since proline is of lower magnitude in this plant species, it is thought that its role in drought stress defense may be indirectly through its formation from glutamate or alternatively synthesized forming the glutamic acid semi aldehyde as a precursor of proline, Kenklies *et al.* (2006). Moreover, hydroxyproline is produced in the peptide-bound form proline by oxidation with ascorbic acid-dependent monooxygenase, Matysik *et al.* (2002). Despite the confirmed role of proline in drought defence, one cannot ignore the possible function of threonine (aliphatic) and argenine (basic) in drought defense (Stadtman, 2003).

Amino acids in stems

Figure (5) revealed that glutamic and aspartic acids are the most predominant among the 15 protein amino acids identified in the three plants under study where their concentrations range from 0.29 to 0.50% with an average of 0.27 to 49%, respectively. The highest and lowest concentrations of both amino acids are strictly confined to the stem of plants *Salsola baryosma* grown in site 1 and the stem of plants *Nitraria retusa* grown in site 3, respectively. In contrast, histidine and tyrosine are the least abundant amino acids in the stems of all plants grown in all sites. In this respect, histidine and tyrosine concentrations are in the ranges of 0.07-0.19% and 0.08-0.13% that averaged at 0.07 and 0.06% respectively. The higher concentrations of those amino acids are strictly associated with stems of plants *Salsola baryosma* grown in site 1, whereas the least concentrations are found in the stems of plants *Nitraria retusa* grown in site 3 and the stems of plants *Tragnum nudatum* grown in site 2.

For convenience, the combined amino acids in the stems of three plants could be arranged according to their concentrations in the descending order: glutamic > aspartic > leucine> valine> alanine> lysine> proline> phenylalanine> isoleucine> argenine > glycine> serine> threonine> histidine > tyrosine. Within the acidic amino acids, glutamic and aspartic acids have an almost similar concentration in the stems of plants grown in each sites irrespective of location. Within the aliphatic amino acids, it is apparent that leucine, valine and alanine are seemingly the most abundant followed by isoleucine, glycine, serine and threonine in a descending order regardless of locality. Within the basic amino acids, lysine is apparently the most abundant followed by argenine while histidine is the least abundant among this group of amino acids. Within the cyclic amino acids, proline and phenylalanine are the dominant amino acids while tyrosine is the least abundant.

The former sequence within each group of amino acids may suggest a specific and characteristic pattern of building units of protein in the stems of the three plants irrespective of locality in which plants are grown. This is surely manifested on the assortment and concentrations of these acid groups where variations in amino acids concentrations encountered in various localities of the three sites. In short, the specific pattern of amino acids in stems of the three plants under study may indicate that the synthesis of protein types rich in acidic amino acids (glutamic and aspartic) could be the key to survival of this plant species under the prevailing climatic (drought stress) and edaphic conditions. Noteworthy to mention that the concentrations of the identified amino acids in leaves far exceeds their concentrations in stems, being 2 to 7 folds in leaves relative to stems. This is expected since leaves are the most vital in synthesis of all plant constituents.

Molecular identification

- ***Morphological identification***

Based on the morphological aspect, the three wild plant samples that were collected from different sites of Kuwait state protectorate have been identified as follows:

Sample no. (1) is *Salsola baryosma,* while sample no. (2) is *Tragnum nudatum* and sample no. (3) is *Nitraria retusa.*

- ***Molecular Identification and DNA Barcoding***

Using (rbcL), the phylogenetic analysis was performed using these samples together with GenBank accessions. Tree was rooted between samples that revealed two purified main clads. First clad possessed two samples no (1) *Salsola baryosma* and no (2) *Tragnum nudatum* per se and belong to a certain family. While the second clad included sample no. (3) is *Nitraria retusa*.

The (rbcL) phylogenetic analysis showed that, (sample no. 1 and sample no. 2) does belong to the order *sub family Salsoloideae*, and specifically to *family chenopodiaceae*. Sample no. (1) was supported to *Salsola vermiculata spp*. (bootstrap support of 96.2%). Sample no. (2) does belong to the family *chenopodiaceae* and specifically supported to *salsola ferganica spp* (bootstrap support of 94.7%) (Fig. 6). On the other hand, sample no. (3) was supported to *Nitraria retusa* (bootstrap support of 100%).

Species identification based on rbcl chloroplast region agrees with Kress *et al.* (2005) and Chase *et al.* (2005). *Salsola* is a genus of the subfamily *Salsoloideae* of the Amaranthaceae family (formaly known as chenopodiaceae). As the studied species (1 and 2) are newly recoded in the GenBank, an exact match wasn't found in the database. Meanwhile species 3, has found an exact match *Nitraria retusa* in the database that revealed correctly as a part of the genus *Nitraria* family *Nitrariaceae* rbcL region showed a better resolution among families and subfamilies. However, on the species and genus level it is not very recommended to resolve identification and taxonomic question for genera belong to the the *Amaranthaceae* family.

In conclusion, this study provides preliminary assessment data that will be useful for wider application of DNA barcoding in wild plants. With the current development of primers, we found that rbcL will be very useful for the barcoding of identification of plant species. However, further protocol development to enhance clean DNA extraction, PCR amplification strategies, including the development of new primers, and local authenticated databases could play important roles in efficient utilization of plant barcoding.

SUMMARY

The present study is devoted to physical, biochemical and genetical investigations of three halophytic plants *Salsola baryosma* (Romy and Schult), *Tragnum nudatum* (Delile), and *Nitraria retusa* (Forssk.) from Khiran area to their three different environment (site 1) in front of Al Wataniyah Chalet Resort, (site 2) Behind Al Wataniyah Chalet Resort and (site 3) 5 Km. away from Al Wataniyah Chalet Resort) in Kuwait state. Abiotic and biotic factors of the study area are defined through soils and plant studies. Aerial parts of three plants were collected from three different sites of Kuwiat state. The elemental composition of leaves and stems reveals the macronutrients, micronutrients and heavy metals contents and the plant is not polluted, reflecting the safe environment. With regard to the biochemical components of the aerial parts, the moisture content varies within narrow limits is higher in leaves of plants *Nitraria retusa*, *Tragnum nudatum* and *Salsola baryosma* which grown in sites (3, 2 and 1), also in the stems of plants which grown in sites (3, 1 and 2), respectively. Also, ash content is relatively higher in leaves relative to stems. Moreover, total carbohydrates and total protein are quite higher in leaves relative to stems *Nitraria retusa*, *Tragnum nudatum and Salsola baryosma* in plants which grown in sites (3, 2 and 1), respectively. Total lipids are almost the same for stems and leaves and crude fiber is much higher in total amino acids in both leaves and stems, the acidic ones (aspartic and glutamic) are the most predominant while the least abundant are the cyclic and basic (tyrosine and histidine). Physiological and biochemical researches have shown that salt tolerance depend on a range of adaptations embracing many aspects of plant physiology one of these includes the compartmentation of ions.

The (rbcL) species identification sequences reveled that *Salsola* is a genus of the subfamily *Salsoloideae* in the family (*Amaranthaceae*) *or family* (*Chenopodiaceae*) .That might be due to the in sufficient rbcL similar sequences of two plant species (*Salsola baryosma*, *Tragnum nudatum*) in the GenBank database, or the rbcL marker is the most suitable to be applied for the DNA barcoding for such families. However, the results revealed that, *Nitraria* is a genus of the family *Nitrariaceae* and *Nitraria retusa* is a spp.

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Table (1): Particle size distribution and textural class of the soil samples representative different sites in Kuwait state.

Sites	Particle size distribution (mm)%						*Texture class*
	2-1	1-0.5	0.5-0.25	0.25-0.125	0.125-0.063	<0.063	
1	43.05	25.87	14.32	8.40	5.56	3.91	Sandy
2	17.64	21.31	14.69	17.63	11.54	17.18	Sandy
3	35.53	22.97	16.49	9.99	8.99	6.07	Sandy

(1*) site in front of Al Wataniyah Chalet Resort .Which plant grown is *Salsola baryosma*
(2**) site Behind Al Wataniyah Chalet Resort. Which plant grown is *Traganum nudatum*
(3***) site 5 Km. away from Al Wataniyah Chalet Resort. Which plant grown is *Nitraria retusa*

Table (2): Chemical composition of the soil saturation extract of the different representative sites in Kuwait state.

Sites	PH	ECe ds/m	Soluble cations (me/1)				Soluble anions (me/1)		
			Na+	K+	Ca++	Mg++	HCO_3-	CL-	SO4--
1	8.91	6.85	48.10	1.32	15.3	3.75	3.92	40.61	17.45
2	7.40	5.47	36.22	1.01	10.22	8.20	3.11	29.40	20.00
3	6.99	3.11	19.42	0.99	7.34	4.11	3.12	12.22	7.21

** EC: Electric conductivity *** Me /l: mille equivalent / Liter
**** 1, 2, 3: See Table (1)

Table (3): Total content of micronutrients and heavy metals in the studied soil sample representing different sites in Kuwait State.

Plant Samples	Micronutrients							Heavy Metals						
	Fe	Mn	Cu	Zn	B	Mo	Ni	Al	Cr	Sr	V	Pb	Cd	Co
	Mg/l													
1	678.10	229.13	2.90	45.10	13.31	1.08	7.33	7331.0	29.99	20.12	29.13	0.003	0.0004	1.55
2	1440.34	430.15	4.82	72.42	16.37	2.99	7.23	8076.9	32.54	29.88	46.85	0.003	0.0004	4.77
3	422.70	293.87	7.31	55.30	23.84	1.99	4.29	12602.2	42.21	11.59	45.35	0.003	0.0004	3.92

••EC: Electric conductivity ••me/I: Millie equivalent Tr.: Below the detection level. *** 1 , 2 , 3 : See Table (1)

Table (4): Macronutrients, micronutrients and heavy metals composition of three plants under study in Kuwait State.

Plant Samples	Leaves																			
	Macronutrients %					Micronutrients (ppm)							Heavy Metals (ppm)							
	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B	Mo	Ni	Al	Cr	Co	V	Sr	Cd	Ba	Pb
1	1.17	0.35	1.41	0.12	0.01	9.91	2.05	0.24	0.70	3.19	0.04	0.03	15.92	0.06	0.004	0.03	6.34	0.001	0.84	0.01
2	1.18	0.29	1.31	0.12	0.02	9.45	1.61	0.23	0.59	4.44	0.02	0.03	13.33	0.06	0.005	0.03	5.73	0.001	0.50	0.04
3	1.25	1.01	1.88	0.11	0.01	8.00	1.42	0.68	0.68	5.51	0.04	0.04	12.08	0.03	0.003	0.03	6.25	0.001	0.95	0.02
Mean Value ±SD	1.21± 0.07	0.69± 0.37	1.48± 0.22	0.11± 0.01	0.01± 0.0	9.11± 2.28	1.80± 0.66	0.28± 0.05	0.63± 0.06	5.00±1 .33	0.03± 0.006	0.03± 0.006	13.4± 4.24	0.05± 0.02	0.004± 0.001	0.03± 0.01	5.73± 0.60	0.001± 0.00	0.66± 0.25	0.02± 0.01

*** 1, 2, 3: See Table (1)

Table (5): Macronutrients, micronutrients and heavy metals composition of three plants under study in Kuwait State.

Plant Samples	Stems																			
	Macronutrients %					Micronutrients (ppm)							Heavy Metals (ppm)							
	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B	Mo	Ni	Al	Cr	Co	V	Sr	Cd	Ba	Pb
1	0.73	0.41	1.65	0.03	0.01	3.39	0.33	0.26	0.75	0.21	0.02	0.04	3.24	0.06	0.002	0.01	2.28	0.02	0.20	0.02
2	0.73	0.49	0.94	0.05	0003	3.39	0.40	0.77	0.67	0.31	0.01	0.03	4.30	0.03	0.003	0.01	4.30	0.01	0.63	0.01
3	o.63	0.56	1.41	0.06	0.004	3.88	0.49	1.07	0.99	0.44	0.02	0.03	5.24	0.02	0.003	0.02	0.02	0.02	0.68	0.01
Mean Value ±SD	0.69± 0.047	0.52± 0.114	1.32± 0.25	0.05± 0.013	0.006± 0.004	4.27± 0.953	0.43± 0.062	0.83± 0.295	0.80± 0.119	0.39± 0.127	0.02± 0.006	0.04± 0.008	5.11± 1.204	0.05± 0.050	0.003± 0.001	0.02± 0.005	3.78± 0.860	0.02± 0.006	0.44± 0.19	0.07± 0. 14

*** 1 , 2 , 3 : See Table (1)

Table (6): The biochemical profile of the plants under studies (leaves) in different sites in Kuwiat stats.

Sites	Moisture Content (%)	Total Ash (%)	Total carbohy-drates (%)	Total Lipids (%)	Fiber content (%)	Total Protein (%)
1	6.48	14.14	53.30	0.52	13.3	14.15
2	6.71	11.30	55.10	0.91	13.1	14.12
3	6.67	10.50	58.40	1.30	10.3	13.91
Range	6.48 - 6.67	10.50 - 14.14	53.30 - 55.10	0.52 - 10.30	10.3 – 13.3	13.91 - 14.15
Mean value ±SD	6.42±0.45	12.42±1.71	55.15±3.55	0.87±0.19	12.02±2.25	13.12±0.48

*** 1, 2, 3: See Table (1)

Table (7): The biochemical profile of the plants under studies (stems) in different sites in Kuwait stats.

Sites	Moisture Content (%)	Total Ash (%)	Total carbohy-drates (%)	Total Lipids (%)	Fiber content (%)	Total Protein (%)
1	5.12	4.83	39.10	1.00	42.9	7.96
2	2.07	9.92	44.12	0.80	39.8	7.61
3	5.45	5.67	41.18	1.16	42.1	5.33
Range	1.07- 5.45	4.83 - 9.92	39.10 - 44.12	0.80 - 1.16	39.8 - 42.9	5.33 - 7.96
Mean value ±SD	4.82 ± 1.06	5.56±0.67	41.76±1.83	0.79 ± 0.284	40.2 ±1.771	6.83 ± 097

*** 1, 2, 3: See Table (1)

Fig. (1): *Salsola baryosma*.

Fig. (2): *Tragnum nudatum*

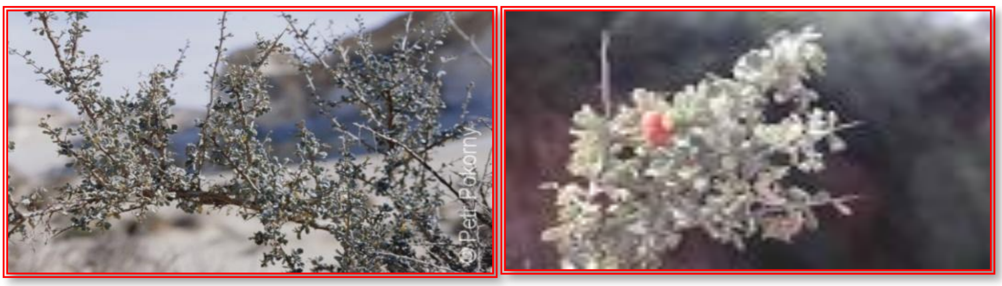

Fig. (3): *Nitraria retusa*

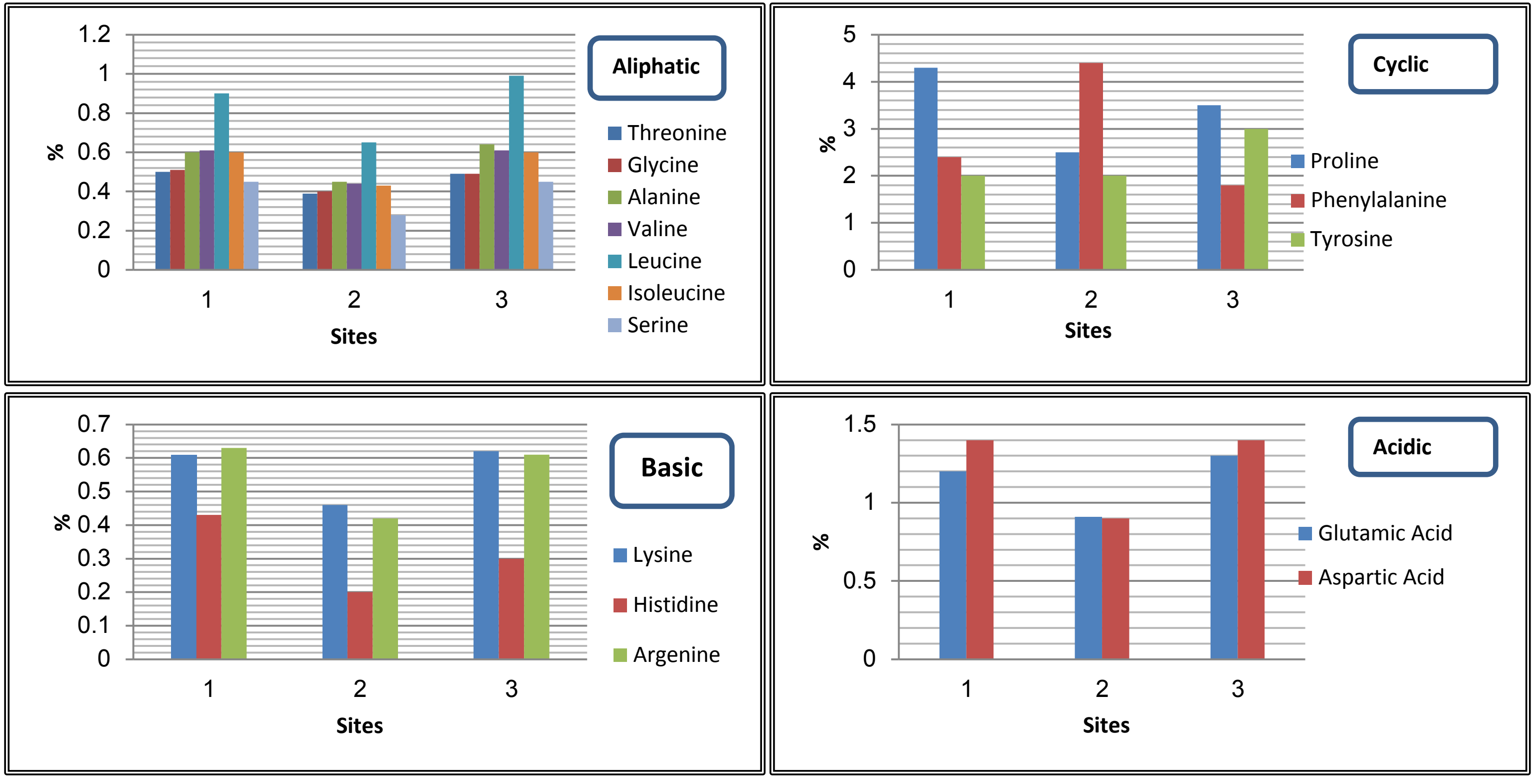

Fig. (4): Percentage of different amino acid in leaves of plant studies grown in the different sites. (1*, 2**, 3***) see Table (1).

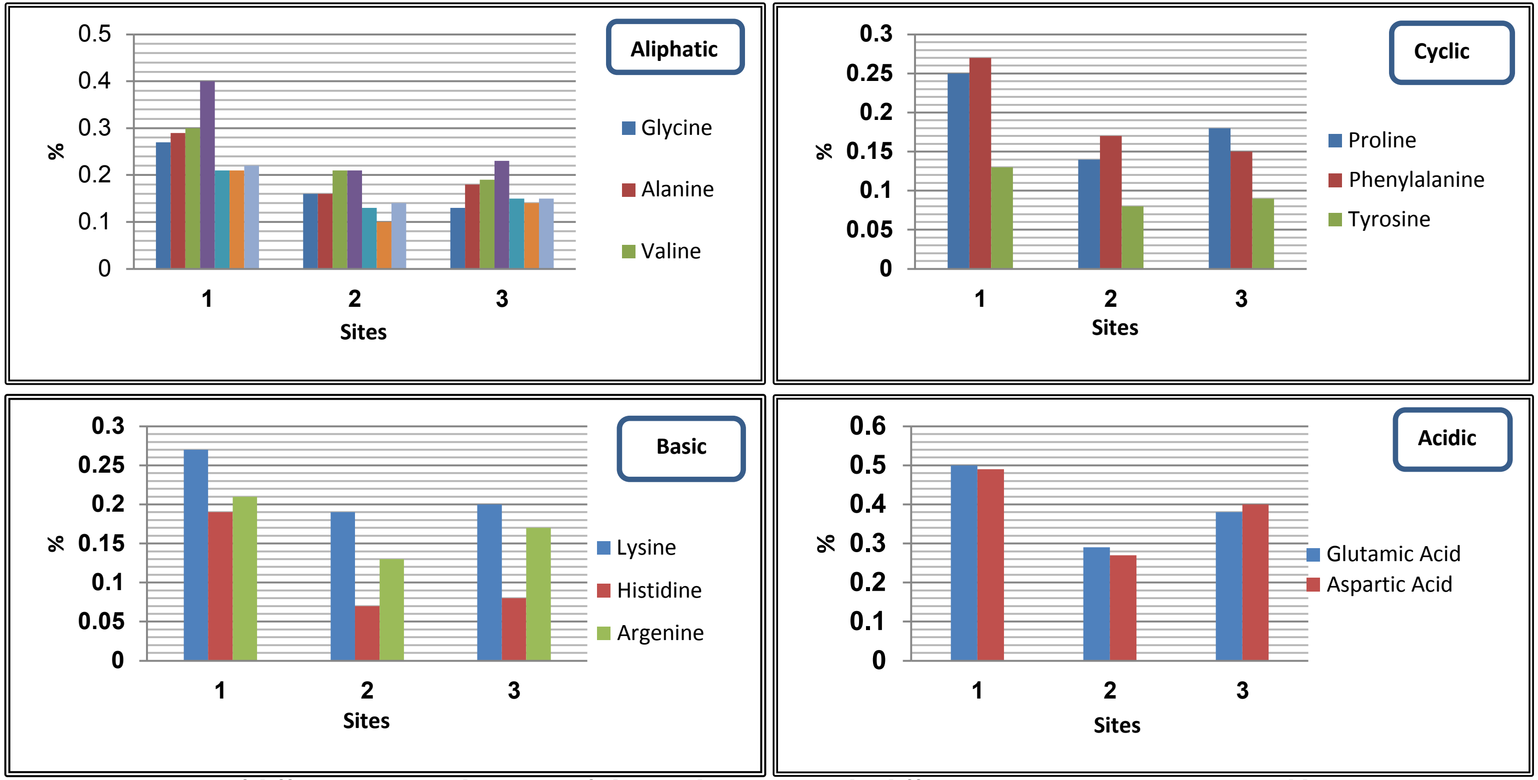

Fig. (5): Percentage of different amino acid in stems of plant studies grown in the different sites. (1*, 2**, 3***) see Table (1)

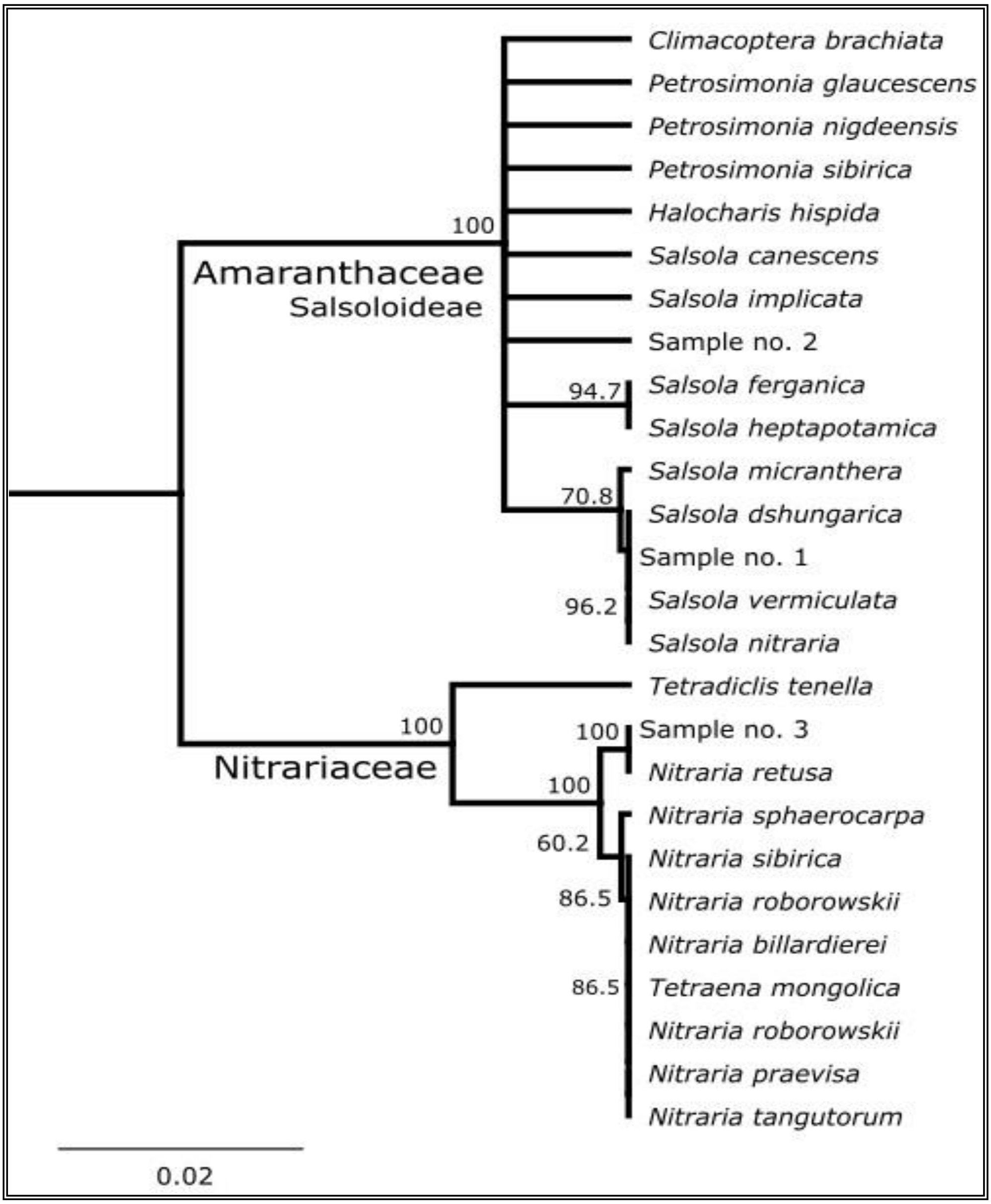

Fig. (6): rbcL based maximum likelihood tree for both collected three samples.
(1*, 2**, 3***) see Table (1).