International Journal of Theoretical and Applied Research (IJTAR)
ISSN: 2812-5878

Homepage: https://ijtar.journals.ekb.eg

Original article

Genetic and biotechnological studies on *Euphorbia peplus* L. accessions growing in different habitats in Egypt

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ARTICLE INFO

Received 09/12/2022
Revised 26/03/2023
Accepted 30/03/2023

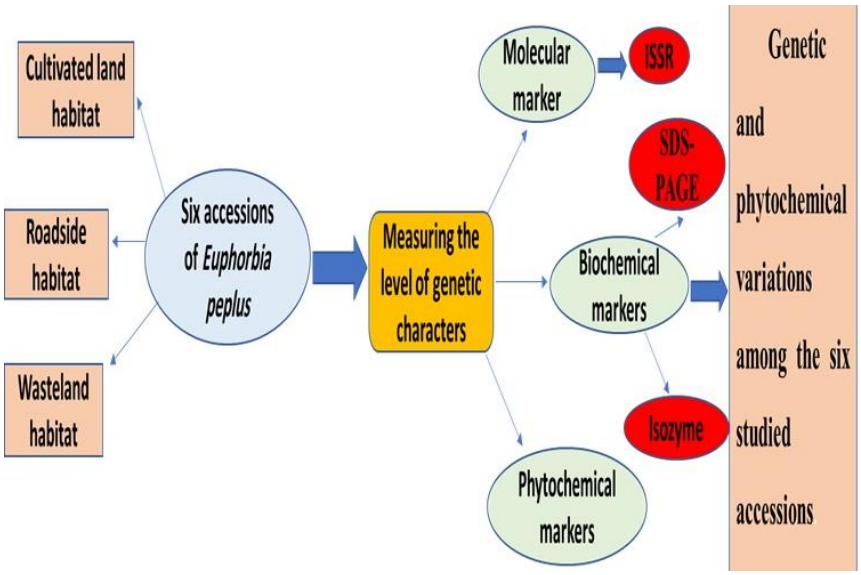
Keywords

ISSR
SDS-PAGE
Isozymes
Euphorbia peplus
Phytochemical components

ABSTRACT

Euphorbia peplus (Family Euphorbiaceae) is a medicinal annual weed found in many habitats in Egypt. The main goal of this study was to assess the diversity of *E. peplus* accessions growing in Egypt and to measure the levels of genetic characters of six selected accessions collected from different habitats in Egypt, by using Inter-Simple Sequence Repeat (ISSR), SDS-PAGE, and isozymes. Extracting, measuring, and identifying the bioactive compounds were also taken in our consideration. A total of 45 plant species were recorded as associates with *E. peplus* in various habitats. Poaceae and Asteraceae were the most common plant families. Therophytes were the most abundant life forms. Cosmopolitan species were the most common floristic category. *Cynodon dactylon*, *E. peplus*, and *Sisymbrium irio* had the highest importance values, of 58.3, 47.6, and 46.8, respectively. A total of six primers were evaluated for ISSR–PCR analysis, and the polymorphism percentages varied from 20–66.66%. Primer HB-9 produced the maximum number of polymorphic products (four bands), while primer HB-12 produced the lowest number of polymorphic products (one band). SDS-PAGE analysis revealed some variations among the studied accessions in the banding pattern and 50 % polymorphism. Two isozymes (peroxidase and polyphenol oxidase) were used to distinguish among the six accessions of *E. peplus*. The obtained data showed genetic and phytochemical compounds content variation among the six studied samples.

Graphical abstract



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DOI: 10.21608/IJTAR.2023.178173.1014

1. Introduction

By preserving biodiversity and conserving the environment, the plant community plays a critical role in long-term management [1] Biological diversity means the variability among living organisms from all sources including, inter alia terrestrial, marine and other aquatic ecosystems including diversity within species, between species, and habitat ecosystems [2]**.** Local adaptation refers to variations in allele frequencies that result in phenotypic differentiation in a population, whereas phenotypic plasticity refers to the variety of phenotypes that a single genotype may display as a function of its environment [3]. Variation within populations is predicted by changes in environmental circumstances, and genetic diversity is critical for the survival of plant populations in dynamic settings [4]. Several environmental variables can influence the genetic diversity of a population within a community [5]. Climate variables such as precipitation and temperature have an impact on plant genetic diversity [6].

Table1. Shows the differences between cultivated land, roadside and wasteland habitat soils

Cultivated land	Roadsides	Wastelands
Cultivated land which is the most suitable habitat as compared to other habitats means that the land has been emptied of its environmental vegetation and is presently planted with a crop. Cultivated lands have many services for the ecosystem not only food for direct human consumption but also feed and fodder for animals, fibers, biofuels, medicines, pharmaceutical products, dyes, chemicals and other raw materials. [7] showed that percentages of clay, Cl-, water-holding capacity, porosity, total N, CaCO3, HCO3- as well as a concentration of Ca+2 and organic carbon are the most critical edaphic factors controlling the distribution and abundance of weed vegetation in the cultivated land habitat. -Cultivated land soils were characterized by high amounts of silt and clay and low amount of sand and high amounts of soil porosity, water holding capacity, calcium carbonates and HCO_3^- in one group of the study [8].	Roadsides are located in the zone between the road surface and the adjacent landscape. Roadsides are stressful areas for biological organisms due to high concentrations of heavy metals, salt, organic molecules, and nutrients. Roadsides play an important role in the control of soil erosion and slope stabilization**.** Roadsides display a variety of physical and chemical changes as a result of road operation and traffic. Roadsides soils are characterized by a high concentration of CaCO3, SO4-2 and N according to [9].	Wastelands are urban habitats that are defined as neglected lands where plant species grow without human control**.** Importantly, urban wastelands can offer enormous ecological and social benefits, such as providing places for recreation, interactions with nature, and conservation of rare and threatened species**.** wasteland habitat is characterized by a wide variety of substrates; nutrient rich soil/clay, graded brick rubble, compacted rubble, compacted ballast and raw brick. -Wastelands soils had the highest number of soil variables with significant differences organic matter, Mg^{+2}, Cl^-, electrical conductivity, HCO^-_3, Ca^{+2}, SO_4^{-2} and nitrogen [10] .

Molecular markers are important tools for the breeding of plants as well as for gene mapping, genetic diversity analysis, and taxonomic and evolutionary studies [11]. These markers are divided into two groups: biochemical markers and DNA markers [12]. Some are used in breeding because of their codominant nature, and some are used in genetic diversity studies because of their high polymorphism [13]. Molecular markers of DNA are the most abundant and easiest molecular markers and can be used in any organism [14]. Inter-Simple Sequence Repeat (ISSR) and random-amplified polymorphic DNA (RAPD) are two DNA profiling approaches that have been effectively utilized to determine genetic diversity. These are dominant markers that employ anonymous areas to study genomic diversity using universal primers [15].

ISSR markers are highly polymorphic and may be used to examine genetic diversity and phylogeny. ISSR is a quick, easy, and efficient approach that yields amplified products ranging from 200–2000 bp in length. Because longer primers allow for higher annealing temperatures, the process is exceedingly repeatable [16]. According to [17] the ISSR technique is one of the simplest PCR-based marker techniques. It is a commonly used technique that comprises the amplification of a DNA fragment located between two amplifiable distances matching microsatellite duplication regions concerned with opposite directions. ISSR has been successfully employed to assess genetic diversity at the inter- and intra-specific level in a variety of medicinal plant species [18].

Biochemical markers are visible by electrophoresis and have many advantages, low cost, easy analysis and higher producibility. Biochemical markers, proteins and isozymes are considered very active in cultivar identification [19] . The assumption of homology can be more exact compared to other genomic DNA markers.

Isozyme markers can properly differentiate many levels of taxa, accessions, and individuals [20] individuals Isozyme markers were the first to be utilized in molecular differentiation between humans. These markers are commonly employed to gain information on levels of genetic diversity in natural populations [21] [22] and [23]. Protein electrophoresis has been effectively used to study the inheritance of different pla nts for use in crop breeding programs and in their germplasm [24]. [25] studied leaf protein profiles to

estimate genetic diversity and illuminate the taxonomic affinity among six species of *Jatropha.*

Family Euphorbiaceae consists of 340 genera and over 8000 species [26]; [27] and [28]. *Euphorbia* is the fourth-largest genus of flowering plants [29],[30] reported that the genus *Euphorbia* includes 2000 cosmopolitan species, found in tropical, sub-tropical and warm temperate regions. *Euphorbia peplus* is an annual herb commonly found in various habitats in Egypt, and is a widespread weed of cultivated ground, orchards, and roadsides. It has been recorded in many phytogeographical regions in Egypt, including the Mediterranean, Nile, Oasis of the Western Desert, deserts, and Sinai [31]. In Australia, it is known as radium weed, while in the United Kingdom, it is known as petty spurge [32].

Euphorbia peplus extract has activity against human melanoma [33] and is used as a purgative and to treat cancers, warts, waxy growths, corns, asthma, catarrh, stomach, liver, uterus, diarrhea, dysentery, low blood pressure [33], skin cancers [34] and [32], skin diseases, migraine, and intestinal parasites [35]. Lipase enzyme was extracted from *E.* peplus latex and demonstrated good stability in the presence of organic solvents and suggested its industrial utility [36]. In addition, it has anticancer, cytotoxic, and antimicrobial activities, curative properties for warts, and insecticidal properties [37]. It plays an important role in breast cancer treatment [38] and recently, Jatrophane diterpenes was isolated from the seeds of *E.* peplus and were used for the lysosomal-autophagy pathway [39].

Various groups of large molecules, such as flavonoids, terpenoids phenolic acids and steroids act as a plant protection system [40]. It is also a collector for various phytohormones which protect the plant against any stress. They are used in a variety of chemical-pharmaceutical products, including medicines, agrochemicals, tastes, fragrances, colours, biopesticides, and food additives [41]. Biotic and abiotic stress are considered the largest problems that face decreasing the quality and quantity of crops [42]. Environmental factors influence on the types and contents of active substances produced by plants [43]. The production and accumulation of secondary metabolites are very complex and are influenced by numerous factors including internal developmental genetic materials that translate into genes and enzymes as well as, external environment factors (light, temperature, water, salinity etc.) and biotic factors [44]. Many studies provide that environmental factors have an effect on the manufacture of secondary metabolites [45], [46] and [47] .

The main objective of the present study was to investigate the ecological parameter, genetic characters, biochemical (SDS-PAGE and isozymes) and phytochemical markers for six accessions of *E. peplus* collected from different habitats in Egypt.

2. Materials and Methods

2.1. Vegetation analysis

The field trips were carried out during the spring and summer seasons of 2019 and 2020. A total of 23 sites from three different habitats (cultivated lands, roadsides, and wastelands) were studied. Four ($5 \times 5 = 25$ m^2) quadrants were investigated at each site, and the species present in each quadrant were listed. The sites were selected randomly from six governorates: Cairo, Ash Sharqia, Al-Gharbia, Alexandria, Al-Dakahlia, and El-Beheira. The floristic composition of various sites was recorded. All plant species found within each stand were listed after complete identification according to [48] [30][31][49][50][51]. Life-form categories were identified following [52]. Floristic categories were recognized after [53]. Species density, frequency, and abundance were recorded according to [54] and [55] to calculate the relative density, relative frequency, and relative abundance of each species, and these values were totaled to estimate its important value (IV) according to [56] The mean and standard error of species important value was calculated using SigmaPlot version 12.5.

2.2. Plant material and the study area

E. peplus plants were collected in March (2019) during the winter season from three different habitats in Egypt (wasteland, roadside, and cultivated land). The collected plant accessions were identified according to [48] and [30][31][49][50][51].

For the molecular biology analyses, only six of the twenty-three accessions (two accessions from each habitat) were selected, and these were numbered from 1 to 6. The site code number, name of the collection sites, habitats, and Global Positioning System coordinates are given in Table 2 and Figure1.

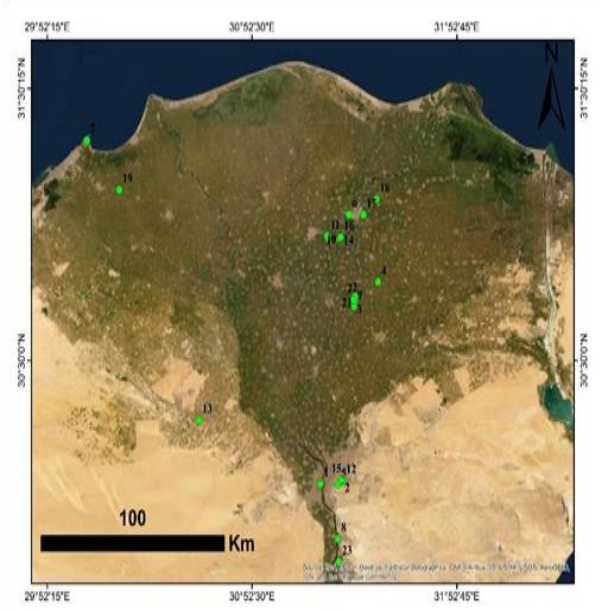


Fig. 1 Map of studied sites.

Table2. Site number, collection sites, habitats, and GPS position of *E. peplus*

Site no.	Collection sites	Habitats	Latitude (N)	Longitude (E)
1	Cairo	Wasteland 1	30° 2' 42.16"	31° 12' 39.64"
2	Cairo	Wasteland 2	30° 3' 34.46"	31° 18' 51.29"
3	Ash Sharqia	Roadside 1	30° 43' 13.79"	31° 22' 24.36"
4	Ash Sharqia	Roadside 2	30° 47' 25.33"	31° 29' 32.78"
5	Cairo	Cultivated land	30° 2' 37.28"	31° 17' 49.81"
6	Al-Dakahlia	Cultivated land	31° 2' 18.60"	31° 21' 1.04"
7	Alexandria	Cultivated land	31°18' 46.9"	30° 03' 56.23"
8	Cairo	Cultivated land	29° 50' 31.75"	31° 17' 47.25"
9	Ash Sharqia	Cultivated land	30° 42' 3.14"	31° 22' 28.81"
10	Al-Dakahlia	Wasteland	30° 57' 4.49"	31° 18' 14.56"
11	Al-Gharbia	Wasteland	30° 57' 33.74"	31° 14' 37.21"
12	Cairo	Wasteland	30° 3' 26.62"	31° 19' 17.88"
13	Cairo - Alexandria Desert Road	Roadside	30° 16' 45.22"	30° 36' 54.6"
14	Al-Dakahlia	roadside	30° 57' 47.0154"	31° 18' 44.5176"
15	Al-Dakahlia	roadside	30o 3 -35.50"	31o 18 -51.28"
16	Al-Dakahlia	Cultivated land	30° 57' 21.7"	31° 18' 36.8"
17	Al-Dakahlia	Cultivated land	31° 2' 17.7"	31° 25' 17.5"
18	Al-Dakahlia	Cultivated land	31° 5' 41.4"	31° 29' 9"
19	El-Beheira	Cultivated land	31º 7- 49.57"	30º 13- 26.49"
20	Ash Sharqia	Cultivated land	30º 43- 17.78"	31º22-41.53"
21	Ash Sharqia	Cultivated land	30º 44- 16.72"	31º 22- 48.25"
22	Ash Sharqia	Cultivated land	30º 43- 33.21"	31º22-21.49"
23	Al-Giza	Cultivated land	29 º45- 28.069"	31º 18- 1.39"

2.3. Soil analysis

At each site, four soil samples were collected and mixed well to form a representative homogeneous sample of the site. A total of 19 soil factors were measured in each sample. Soil texture was determined by soil sieves according to [57], and pH, total dissolved salts (TDS), and electrical conductivity (EC) were determined using a digital portable pH, TDS, and EC meter (Adwa), respectively. Calcium, magnesium, and chlorides were determined by the titration method according to [58]. Sodium and potassium were determined after [58] using a flame photometer at 589 and 767 nm wavelengths, respectively. [59] titration technique was used to determine carbonates and bicarbonates. The turbidimetric method was used to determine sulfates according to [60]. Phosphorus was determined after [60]. The titration method was used to determine organic carbon [57]. Calcium carbonate was determined after [58] by the titration method. Water content and saturation percentage were determined after [59] and [61], respectively. Available nitrogen was determined after [59].

2.4. Genomic analysis

2.4.1. Genomic DNA extraction for ISSR assay

Bulked samples of young leaves and shoots of *E. peplus* plants were collected randomly from the selected sites to represent the studied populations. The selected samples were separately cleaned, pressed, protected against insects and microorganisms, and then ground using liquid nitrogen to a fine powder. Next, 0.1 gram of the powder was transferred to an appropriately sized tube. The bulked DNA extraction of each selected sample was performed using a DNeasy Plant Mini Kit (QIAGEN).

2.4.2. Screening of PCR

Six selected primers were used (Table 3). The DNA amplifications were performed in an automated thermal cycle (Model Techno 512) programmed for one cycle at 94 °C for 4 min, followed by 45 cycles of 1 min at 94 °C for denaturation, 1 min at 57 °C for annealing, and 2 min at 72 °C extension. The reaction was finally stored at 72 °C for 10 min. The electrophoresis of the ISSR–PCR products was performed on 1.5% agarose gel in TAE buffer, visualized by staining with ethidium bromide, and transilluminated under short-wave UV light. The sizes of the generated ISSR fragments were estimated with a DNA size standard by using the Gel Analyzer 3 program for Gel Leader. PC-ORD software version 5 was used to examine the links between genotypes.

Table 3. Sequence of the ISSR selected primers

Code no.	Name	Sequence
1	14A	5` CTCTCTCTCTCTCTCTTG 3`
2	49A	5` CACACACACACAAG 3`
3	HB-9	5` GTGTGTGTG TGT GC 3`
4	HB-10	5` GAGAGAGAGAGA CC 3`
5	HB-12	5`CACCACCACGC 3`
6	HB-13	5´ GAGGAGGAGGC 3`

2.4.3. Protein electrophoresis

For protein extraction, 0.2 gram of each sample's leaves were ground to a fine powder using a mortar and pestle, and then homogenized in a clean Eppendorf tube with 1 M Tris-HCl buffer at pH 6.8 and left in the

refrigerator overnight before being centrifuged at 10,000 rpm for 10 min. The supernatant from each sample was kept frozen until electrophoretic analysis and then boiled for 5 min in a water bath before loading on the gel. SDS-polyacrylamide gel electrophoresis was performed on 12% acrylamide slab gels [62] as modified by [63]; [64] and [65].

2.4.4. Extraction of isozymes

Using a mortar and pestle, isozymes were extracted from the selected plant samples using 0.5 g fresh leaf samples in 1 ml extraction buffer (10 % glycerol). Next, the extract was placed into clean Eppendorf tubes and centrifuged for 5 min at 10,000 rpm. The supernatant was transferred to new, clean Eppendorf tubes and stored at −20 °C until electrophoretic analysis was performed.

2.4.5. Isozyme electrophoresis

Following [66] native-polyacrylamide gel electrophoresis (Native-PAGE) was performed to discover isozyme differences among the examined accessions using two isozyme systems (peroxidase and polyphenol oxidase). A Gel Doc VILBER LOURMAT system was used to photograph, scan, and analyze the gels. The relative mobility value (Rm) was calculated as Rm = (distance to which the isozyme band migrated / distance to which the marker dye migrated). The Biotechnology Laboratory of the Horticulture Research Institute performed the DNA genomic analysis, protein analysis, and isozyme analysis.

2.5. Phytochemical analysis

Whole plants of *E. peplus* were collected from six sites that are shown in Table 2 (sites from 1 to 6) and were dried at 50 °C in an oven and ground into fine powders. Dried powder material (10 g) of each sample was added to 100 ml of methanol (70 %) for the purpose of extraction. The preliminary phytochemical screening of methanolic extract (70 %) of *E. peplus* was applied for the estimation of tannins, sterols or terpenes, flavonoids, saponins, alkaloids and resins.

Tannins were determined according to the procedure described by [67] , terpenes and sterol according to Libermann-Burchard's test [68], flavonoids were determined according to [69] .saponins were determined as described by [69] and [67], alkaloids were attained using the techniques developed by [69] and resins were estimated according to [67].

3. Results

3.1. Vegetation analysis

A total of 45 taxa belonging to 20 plant families and 38 genera were recorded at the study sites. Poaceae and Asteraceae were the most common families, represented by 20 % (nine species) each. Chenopodiaceae was represented by 8.9 % (four species). Brassicaceae, Caryophyllaceae, Convolvulaceae, Cyperaceae, Euphorbiaceae and Fabaceae were represented by two species each (4.4%), while 11 families (Amaranthaceae, Asclepiadaceae, Loranthaceae, Malvaceae, Orobanchaceae, Oxalidaceae, Plantaginaceae, Primulacae, Portulacaceae, Solanaceae, and Urticaceae) were represented by a single species (Table 3 and Figure 2).

With regard to life span, the majority of the recorded species in this study were annuals, with 32 species (71.1 %). Perennials were represented by 13 species (28.9 % of the total species) (Table 4).

Six life forms were recorded in the study sites. Therophytes were the most abundant life form (32 species = 71%), followed by hemicryptophytes (6 species = 14 %), and geophytes (4 species = 9 %); phanerophytes, chamaephytes, and parasitic plants were represented by a single species each, and therophytes comprised 71 % of the total number of listed species (Table 4).

From the phytogeographical point of view, monoregionals were represented by three species (6.7 %). Biregional geoelements were represented by 8 species (17.8 %). Pluriregional geoelements were represented by 10 species (22.2 %). The remaining 24 species were distributed as follows: 17 cosmopolitan species (37.8 %), four paleotropical species (8.9 %), and three pantropical species (6.7 %).

The important value (IV) was calculated for the 45 species recorded at the 23 sampled sites where *E. peplus* occurred. The species with the highest IV at each site was considered the dominant species, while the species with the next highest IV was considered the co-dominant species. The cultivated land habitat contained 35 species dominated by *Cynodon dactylon*, which had the highest IV in this habitat (IV = 28.4). *E. peplus* recorded the second highest IV at 24.3, followed by *Malva parviflora* (IV = 21.8). The other species had IV ranging from 13.1 to 0.69. The roadside habitat comprised 22 species and was dominated by *Sisymbrium irio*, which had the highest IV in this habitat (IV = 46.8). *E. peplus* had the second highest IV at (45.6), followed by *Chenopodium murale* (IV = 35.1). The other species had IV ranging from 35.1 to 1.9. Finally, the wasteland habitat contained 16 species and was dominated by *C. dactylon*, which had the highest IV in this habitat (IV = 58.3). *E. peplus* recorded the second highest IV at 47.6, followed by *Sisymbrium irio* (IV = 40.7). The other species had IV ranging from 34.1 to 2.6 (Table 5).

3.2. Vegetation analysis

Table 6 shows descriptive statistics of the soil factors for the six selected sites, and Table 7 shows the mean values of soil factors for the three habitats. The mean values of soil content of EC, TDS, $Ca^{+2,}$ Mg^{+2}, Na^{+}, Cl^{-}, SO_4^{-2}, Nitrogen and the saturation percentages were highest in soil collected from the roadside habitat, followed in descending order by those in wasteland soil and in cultivated soil.

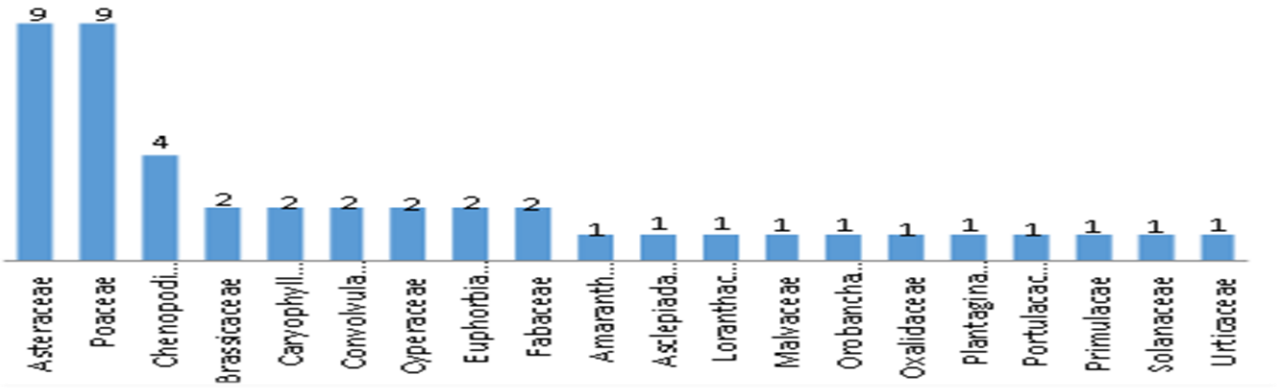


Fig. 2 Plant families according to the numbers of the species collected from the studied area.

Table 4. List of species recorded in different habitat of the study area. The species referred to their families, and life span; Annual=A and Perennial=P., life forms; Therophyte=T, Hemicryptophyte=H, Chamaephyte=C, Phanerophyte=Ph, Parasite=Par, Geophyte=G. Floristic categories; COSM=Cosmopolitan, ER–SR=Euro–Siberian, IR–TR= Irano–Turanian, ME=Mediterranean, PAL=Paleotropical, PAN=Pantropical, SA–SI=Saharo–Sindian, S–Z=Sudano–Zambesian

Species	Family	Life span	Life form	Floristic categories
Amaranthus caudatus L.	Amaranthaceae	A.	T.	COSM
Cynanchum acutum L.	Asclepiadaceae	P.	H.	ME+IR–TR+ER–SR
Bidens pilosa L.	Asteraceae	A.	T.	PAN
Cichorium endivia L.		A.	T.	ME+IR–TR
Conyza bonariensis (L.) Cronquist		A.	T.	ME
Launaea nudicaulis (L.) Hook. f.		P.	H.	IR–TR
Pluchea dioscoridis (L.) DC.		P.	Ph.	SA–SI+S–Z
Pulicaria undulata (L.) C.A.Mey.		P.	C.	SA– SI +S–Z
Senecio glaucus L.		A.	T.	IR–TR+SA– SI
Senecio vulgaris L.		A.	T.	ME+IR–TR+ER–SR
Sonchus oleraceus L.		A.	T.	COSM
Eruca sativa Mill.	Brassicaceae	A.	T.	ME+IR–TR+ER–SR+SA–SI
Sisymbrium irio L.		A.	T.	ME+IR–TR+ER–SR
Stellaria pallida (Dumort.) Murb.	Caryophyllaceae	A.	T.	ME+ER–SR
Vaccaria pyramidata Medik.		A.	T.	ME+IR–TR+ER–SR
Beta vulgaris L.	Chenopodiaceae	A.	T.	ME+IR–TR+ER–SR
Chenopodium album L.		A.	T.	COSM
Chenopodium murale L.		A.	T.	COSM
Kochia indica Wight.		A.	T.	IR–TR
Convolvulus arvensis L.	Convolvulaceae	P.	H.	PAL
Cressa cretica L.		P.	H.	PAL
Cyperus digitatus Roxb.	Cyperaceae	P.	G.	ME+IR–TR+ER–SR
Cyperus rotundus L.		P.	G.	PAN
Euphorbia helioscopia L.	Euphorbiaceae	A.	T.	COSM
Euphorbia peplus L.		A.	T.	COSM
Melilotus indicus (L.) All.	Fabaceae	A.	T.	PAL
Trifolium resupinatum L.		A.	T.	ME+IR–TR+ER–SR
Emex spinosa (L.) Campd.	Loranthaceae	A.	T.	ME+SA–SI
Malva parviflora L.	Malvaceae	A.	T.	ME+IR–TR
Orobanche cernua Loefl.	Orobanchaceae	P.	Par.	ME+IR–TR
Oxalis corniculata L.	Oxalidaceae	A.	T.	COSM
Plantago major L.	Plantaginaceae	P.	H.	COSM
Avena fatua L.	Poaceae	A.	T.	COSM
Avena sativa L.		A.	T.	COSM
Cynodon dactylon (L.) Pers.		P.	G.	PAN
Lolium perenne L.		P.	H.	COSM
Phragmites australis (Cav.) Trin. ex Steud.		P.	G.	PAL
Polypogon monspeliensis (L.) Desf.		A.	T.	COSM
Polypogon viridis (Gouan) Breistr.		A.	T.	ME+IR–TR+ER–SR
Setaria viridis (L.) P. Beauv.		A.	T.	COSM
Setaria verticillata (L.) P. Beauv.		A.	T.	COSM
Portulaca oleracea L.	Portulacaceae	A.	T.	COSM
Anagallis arvensis L.	Primulacae	A.	T.	COSM
Solanum nigrum L.	Solanaceae	A.	T.	COSM
Urtica urens L.	Urticaceae	A.	T.	ME+IR–TR+ER–SR

Table 5. Mean and standard error of species importance values in the different habitats

Species	Cultivated land habitat IV	Roadside habitat IV	Wasteland habitat IV
Amaranthus caudatus L.	-	-	2.56±2.56
Anagallis arvensis L.	10.56±2.54	-	-
Avena fatua L.	7.59±1.81	-	-
Avena sativa L.	0.86±0.86	-	-
Beta vulgaris L.	0.69±0.69	-	-
Bidens pilosa L.	7.26±2.39	-	-
Chenopodium album L.	3.63±2.49	-	-
Chenopodium murale L.	12.66±2.46	35.06±16.71	34.1±8.67
Cichorium endivia L.	4.74±1.55	2.08±2.08	2.56±2.56
Convolvulus arvensis L.	5.51±1.56	10.54±5.04	11.62±7.125
Conyza bonariensis (L.) Cronquist	1.33±0.93	-	-
Cressa cretica L.	-	1.94±1.94	-
Cynanchum acutum L.	-	9.82±7.63	4.18±4.18
Cynodon dactylon (L.) Pers.	28.42±5.06	34.4±18.44	58.28±20.311
Cyperus digitatus Roxb.	4.22±2.26	-	10.3±10.3
Cyperus rotundus L.	1.72±1.72	-	-
Emex spinosa (L.) Campd.	-	1.94±1.94	-
Eruca sativa Mill.	5.35±1.71	3.08±3.08	-
Euphorbia helioscopia L.	2.87±2.05	-	5.1±5.1
Euphorbia peplus L.	24.3±1.41	45.6±9.53	47.62±8.82
Kochia indica Wight.	-	1.94±1.94	-
Launaea nudicaulis (L.) Hook. f.	-	1.94±1.94	-
Lolium perenne L.	1.32±1.32	-	-
Malva parviflora L.	21.79±2.07	26.06±15.39	24.46±17.39
Melilotus indicus (L.) All.	4.15±2.28	3.5±3.5	-
Orobanche cernua Loefl.	0.71±0.71	-	-
Oxalis corniculata L.	5.44±3.058	-	-
Phragmites australis (Cav.) Trin. ex Steud.	2.29±1.21	-	5.1±5.1
Plantago major L.	0.95±0.95	9.98±6.93	5.22±5.22
Pluchea dioscoridis (L.) DC.	-	-	15.8±6.48
Polypogon monspeliensis (L.) Desf.	12.85±2.34	-	-
Polypogon viridis (Gouan) Breistr.	1.91±1.908	-	-
Portulaca oleracea L.	13.13±3.05	-	-
Pulicaria undulata (L.) C.A.Mey.	-	8.76±8.76	-
Senecio glaucus L.	3.34±2.26	13.68±8.41	-
Senecio vulgaris L.	-	6.78±6.78	-
Setaria verticillata (L.) P. Beauv.	-	4.22±4.22	-
Setaria viridis (L.) P. Beauv.	2.4±2.4	-	-
Sisymbrium irio L.	8.36±3.69	46.82±13.46	40.7±21.185
Solanum nigrum L.	3.4±2.45	4.58±4.58	11.18±6.89
Sonchus oleraceus L.	5.89±1.93	17.94±7.35	-
Stellaria pallida (Dumort.) Murb.	1.82±1.82	-	-
Trifolium resupinatum L.	1.13±1.13	-	-
Urtica urens L.	5.15±2.91	5.4±5.4	5.1±5.1
Vaccaria pyramidata Medik.	1.51±1.51	-	-

Table 6. Descriptive statistics of soil factors for the six selected sites

Soil factors	Mean ± Standard deviation	Range (Maximum - Minimum)	Median	Standard error
Coars Sand (%)	27.23 ± 30.6	77.78 (79.9 - 2.1)	17.49	12.50
Fine Sand (%)	16.88 ± 7.3	17.26 (25.7 - 8.5)	16.69	3.00
Total Sand (%)	44.11 ± 26.4	69.39 (88.4 - 19)	41.44	10.80
Clay (%)	33.04 ± 15.8	45.69 (55.6 - 9.9)	32.78	6.47
Silt (%)	22.84 ± 18.7	55.02 (56.5 - 1.5)	19.86	7.64
EC (dS/m)	1.42 ± 0.67	1.57 (2.00- 0.43)	1.7	0.27
TDS (ppm)	906.5 ± 427.3	1005.74 (1278.06 - 272.32)	1059.8	174.43
Ca^{+2} (meq/l)	6.81 ± 3.3	7.61 (10.14 - 2.54)	8.3	1.34
Mg^{+2} (meq/l)	3.26 ± 2.01	5.10 (6.33 - 1.23)	2.7	0.84
Na^{+} (meq/l)	2.67 ± 0.95	2.53 (3.35 - 0.82)	3.0	0.39
K^{+2} (meq/l)	0.97 ± 0.97	2.61 (2.76 - 0.14)	0.5	0.39
$HCO3^{-}$ (meq/l)	0.61 ± 0.32	0.94 (1.13 - 0.19)	0.6	0.13
Cl^{-} (meq/l)	3.3 ± 1.37	3.89 (5.09 - 1.19)	3.6	0.56
P^{-2} (mg/l)	3.75 ± 2.01	5.6 (7.4 - 1.8)	3.6	0.82
$SO4^{-2}$ meq/l	9.82 ± 4.59	11.12 (14.48 - 3.36)	11.5	1.87
nitrogen (mg/kg)	191.33 ± 91.98	259 (371 - 112)	171.5	37.55
Saturation percentages (%)	51 ± 16.82	43 (71 - 28)	52.0	7.52
Water content (%)	6.18 ± 4.12	9.4 (10.1 - 0.7)	7.4	1.68
Organic carbon (%)	1.2 ± 0.29	0.84 (1.68 - 0.84)	1.2	0.12
Organic matter (%)	2.08 ± 0.51	1.5 (2.9 - 1.4)	2.1	0.21
$CO3^{-2}$	0	0	0	0.00
CaCO3 (%)	4.67 ± 1.38	3.2 (6.4 - 3.2)	4.8	0.56

The highest value of soil pH for the three habitats was represented 7.4 while the lowest value was 7.2.

Table 7. Soil factors comparison between the studied three habitats

Soil factors	Mean Wasteland	Mean Roadside	Mean Cultivated land
Coarse Sand (%)	17.49	22.43	41.77
Fine Sand (%)	25.19	12.96	12.50
Total Sand (%)	42.68	35.40	54.27
Clay (%)	37.82	44.66	16.66
Silt (%)	19.51	19.95	29.07
EC (dS/m)	1.38	1.73	1.13
TDS (ppm)	884.40	1110.07	724.96
Ca^{+2} (meq/l)	6.48	8.73	5.21
Mg^{+2} (meq/l)	3.26	4.12	2.39
Na^{+} (meq/l)	2.97	3.07	2.02
K^{+2} (meq/l)	0.55	0.91	1.45
HCO_3^{-} (meq/l)	0.57	0.61	0.66
Cl^{-} (meq/l)	3.69	3.56	2.63
P^{-2} (mg/l)	5.70	3.05	2.50
$SO4^{-2}$ (meq/l)	9.00	12.67	7.78
nitrogen (mg/kg)	147.00	255.50	171.50
Saturation percentages (%)	57.00	71.00	35.00
Water content (%)	7.60	9.65	1.30
Organic carbon (%)	1.44	1.08	1.08
Organic matter (%)	2.50	1.90	1.85
CO_3^{-2}	0.00	0.00	0.00
$CaCO_3$ (%)	4.80	4.40	4.80
Average for Sum total bands	23	24/22	20

The highest value of soil pH for the three habitats was represented 7.4 in the roadside (site 4), and 7.3 in the wasteland (sites 1 and 2) while the lowest value was *7*.2 in the cultivated land habitat (sites 5 and 6).

3.3. Investigation of markers product

The An effort was made to investigate any possible genetic variation among the six selected accessions of *E. peplus* from different habitats in Egypt (roadside, and cultivated land).

3.3.1. Euphorbia peplus ISSR loci

The number of amplified fragments with the selected primers ranged from 2–6 bands. The molecular weight of the generated fragments ranged from 130–1240 bp. Fourteen of the 29 total ISSR markers were polymorphic (4 unique and 10 polymorphic), as shown in Table 7. The unique markers included three Zero Unique Markers (ZUM) that were recorded by three primers (primer 14A at fragment 1240 bp, which were common to all the studied accessions except that collected from site 6**,** primer HB-9 at fragment 370 bp, which was common to all the studied samples except that collected from site 4, and primer HB-10 at fragment 365 bp, which was not recorded at site 4**.** Only one unique marker (OUM) was produced by primer HB-13 at fragment 500 bp, which was generated by primer HB-13 from site 3 only. The remaining 15 ISSR markers were monomorphic. All of the fragments can be considered genomic markers for *E. peplus* collected from the studied habitats (Table 8); as shown in Figures (3–8), the DNA amplification profiles using the six ISSR primers were obviously reproducible across gels based on the obtained results.

Table 9 shows that the primers were able to generate 48.27% polymorphism. The number of bands /primers ranged from two (HB-10) to six (49A and HB-9), as shown in Figs 2–7. The number of polymorphic bands/primers ranged from one (HB-10 and HB-12) to four (HB-9), with percentages ranging from 20 to 66.66% and allele size of 130–1240.

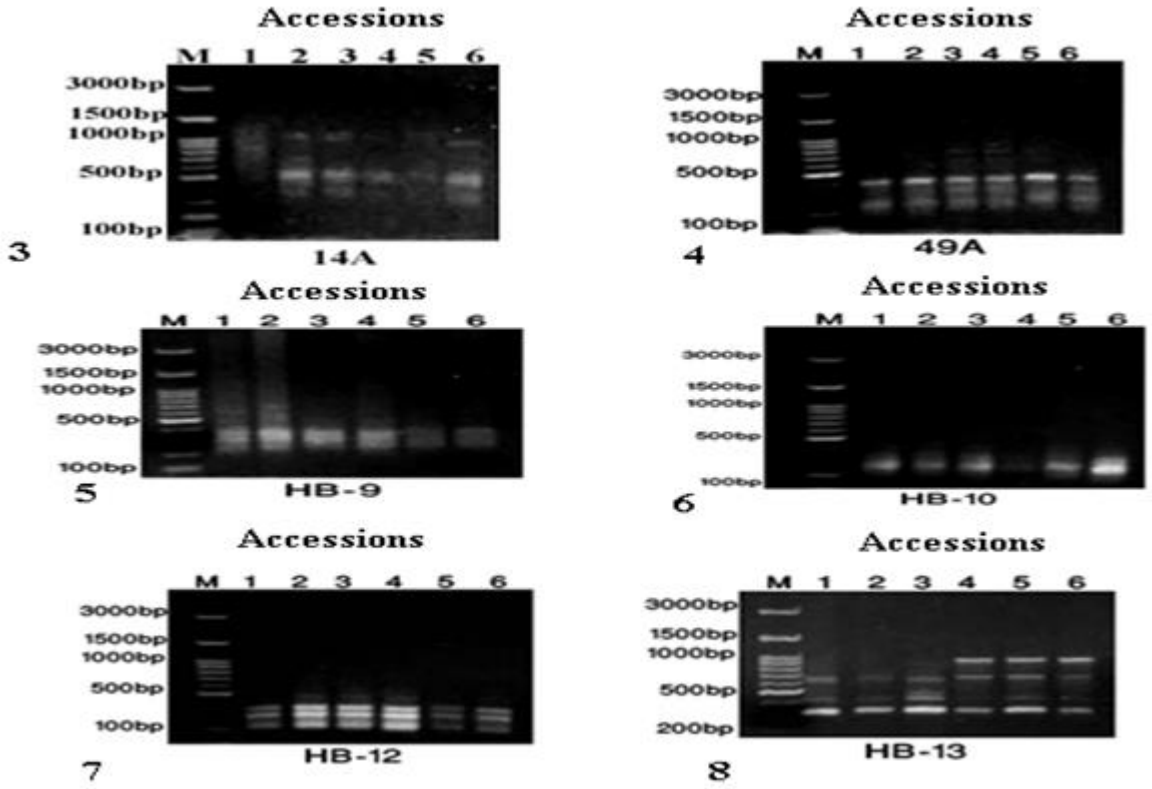
Table 8. Soil factors comparison between the studied three habitats

Primer name	Band No.	Molecular weight (M.W)	Accessions						Band type
			1	**2**	**3**	**4**	**5**	**6**	
14A	1	1240	1	1	1	1	1	0	Unique
	2	975	1	0	0	0	0	1	Polymorphic
	3	720	1	1	0	0	0	0	Polymorphic
	4	530	1	1	1	1	1	1	Monomorphic
	5	365	1	1	1	1	1	1	Monomorphic
49A	1	830	0	0	1	1	0	0	Polymorphic
	2	675	0	0	1	1	0	0	Polymorphic
	3	415	1	1	1	1	1	1	Monomorphic
	4	340	0	0	1	1	1	1	Polymorphic
	5	260	1	1	1	1	1	1	Monomorphic
	6	220	1	1	1	1	1	1	Monomorphic
HB-9	1	715	1	1	0	0	0	0	Polymorphic
	2	600	1	1	0	0	0	0	Polymorphic
	3	480	1	1	1	1	0	0	Polymorphic
	4	370	1	1	1	0	1	1	Unique
	5	300	1	1	1	1	1	1	Monomorphic
	6	260	1	1	1	1	1	1	Monomorphic
HB-10	1	365	1	1	1	0	1	1	Unique
	2	230	1	1	1	1	1	1	Monomorphic
HB-12	1	510	0	1	1	1	0	0	Polymorphic
	2	465	1	1	1	1	1	1	Monomorphic
	3	300	1	1	1	1	1	1	Monomorphic
	4	270	1	1	1	1	1	1	Monomorphic
	5	130	1	1	1	1	1	1	Monomorphic
HB-13	1	970	0	0	0	1	1	1	Polymorphic
	2	680	1	1	1	1	1	1	Monomorphic
	3	500	0	0	1	0	0	0	Unique
	4	420	1	1	1	1	1	1	Monomorphic
	5	325	1	1	1	1	1	1	Monomorphic
Sum of total bands for each sample to six primers			**23**	**23**	**24**	**22**	**20**	**20**	

(1) means presence and (0) means absence of band.

The numbers 1-6 refer to the sites of the collected samples as shown in Table 1.

1Cairo (wasteland1),2 Cairo(wasteland2),3 Ash-Sharqia (roadside1),4 Ash-Sharqia(roadside 2),5 Cairo (cultivated land) and 6Al-Dkahahlia(cultivated land).



Figs. 3-8 ISSR fragments of *E. peplus* generated by 6 primers. The numbers 1-6 refer to the sites of the collected samples as shown in Table2.

Table 9. Scored number of bands, polymorphic bands, monomorphic bands, percentage polymorphism, and allele size range as revealed by ISSR analysis

Primers name	Total Bands	Monomorphic Bands	Polymorphic Bands	Polymorphic (%)	Allele size range base pair (bp)
14A	5	2	3	60	365-1240
49A	6	3	3	50	220-830
HB-9	6	2	4	66.66	260-715
HB-10	2	1	1	50	230-365
HB-12	5	4	1	20	130-510
HB-13	5	3	2	40	325-970
Total	29	15	14	48.27	
Average	4.83	2.5	2.33		

A dendrogram based on the investigated accessions classified ~~them~~ into two main groups. The first group was clearly separated into two subgroups: samples of roadside habitat (sites 3 and 4) and samples of cultivated land habitat (sites 5 and 6). On the other hand, the second group included sites 1 and site 2 (wasteland habitat), as shown in Figure 9.

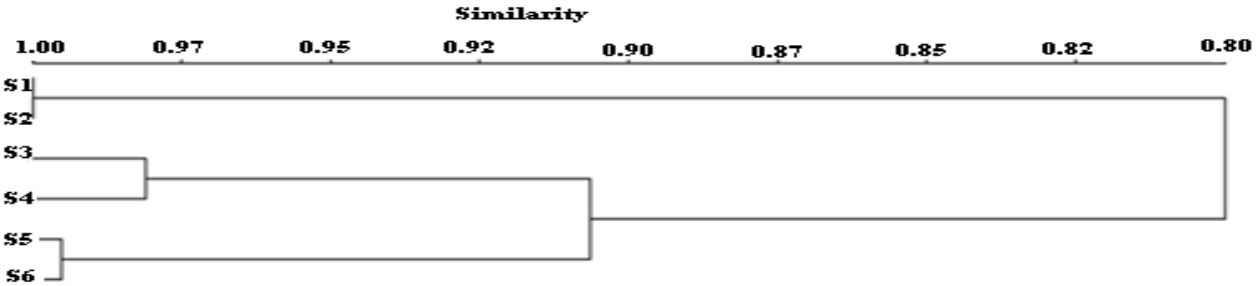


Fig. 9 Dendrogram using Sorensen distance measure and flexible beta as a group linkage method.

3.4. Biochemical analysis

3.4.1. SDS-PAGE

Crude protein from *E. peplus* leaves of the six selected accessions was analyzed by SDS-PAGE. The banding patterns of the studied sample, their molecular weight, the polymorphism percentage for each accession, polymorphic bands, and polymorphism percentage are shown in Table 9 and Fig. 10. The number of polypeptide bands varied from eight at site 3 (Ash Sharqia-roadside1) to 10 at site 1 (Cairo wasteland1), site 2 (Cairo wasteland2), site 5 (Cairo-cultivated land), and site 6 (Al-Dakahlia-cultivated land). The MW of the investigated polypeptides ranged from 10–330 KDa. The number of unique bands was two (ZUM at 98 KD_a and OUM at 58 KD_a) (Table10 and Figure 9).

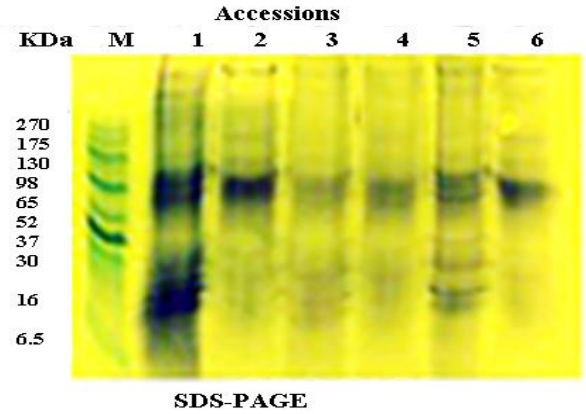


Fig. 10 The protein banding pattern of leaves accessions of *E. peplus*

Table 10. Data for SDS-PAGE gel image of leaves

Band No.	M.W. (KD_a)	Accessions 1	2	3	4	5	6
1	330	1	1	0	0	1	1
2	245	0	1	0	1	0	1
3	115	1	1	1	0	1	0
4	98	1	1	0	1	1	1
5	96	1	1	1	1	1	1
6	94	1	1	1	1	1	1
7	86	1	0	1	1	0	1
8	58	0	0	0	0	1	0
9	33	1	1	1	1	1	1
10	28	1	1	1	1	1	1
11	24	1	1	1	1	1	1
12	18	1	1	1	1	1	1
Total		10	10	8	9	10	10
Polymorphism for each population (%)		40	40	25	33.33	40	40
Total bands				12			
Polymorphic bands				6			
Polymorphism (%)				50			

(1) means presence and (0) means absence of band.

3.4.2. Isozymes analysis

The banding profiles of leaf peroxidase and polyphenol oxidase (PPO) of the studied *E. peplus* were recorded and are illustrated in Tables 11 and 12 and Figures11 and 12. Generally, all the selected samples showed variations in the density of banding profiles for peroxidase and polyphenol oxidase confirming the polymorphism within and among the collection sites.

3.4.2.1. Peroxidase (px)

A total of three loci of peroxidase isozyme, which differed in their amount and relative mobility, were distinguished. Three loci (px1, px2, and px3) with relative mobilities of 0.1, 0.3, and 0.4, respectively, were recorded at moderate density in all accessions except site 2 and site 3, which showed low density; high density was not represented in this study (Table 11 and Figure11).

3.4.2.2. Polyphenol Oxidase (PPO)

Three loci of polyphenol oxidase isozyme, which differed in their amount and relative mobility, were distinguished. Locus PPO1, with a relative mobility of 0.1, was present at a high density at sites 1, 5, and 6, at moderate density in sites 2 and 4, and at a low density at site 3. Locus PPO2, with a relative mobility of 0.3, was present at sites 1, 3, 5, and 6 at a low density, and at site 4 at a moderate density. Site 2 showed high density. Finally, locus PPO1, with a relative mobility of 0.4, was found in site 2 and site 4 accessions at a moderate density, but other accessions were represented by low density, with none at high density (Table 12 and Figure. 12).

The results of the present study showed that this technique could be used as a biochemical marker, and the application of this technique in combination with SDS-PAGE and ISSR–PCR markers should aid in measuring genetic diversity according to habitat.

Table 11. Peroxidase groups and relative mobility of the 6 studied accessions of *E. peplus*

Peroxidase groups	Relative mobility	Accessions					
		1	2	3	4	5	6
Px1	0.1	1^{+}	1^{-}	1^{-}	1^{+}	1^{+}	1^{+}
Px2	0.3	1^{+}	1^{-}	1^{-}	1^{+}	1^{+}	1^{+}
Px3	0.4	1^{+}	1^{-}	1^{-}	1^{+}	1^{+}	1^{+}

(1^{+}) means moderate density and (1^{-}) means low density of band.

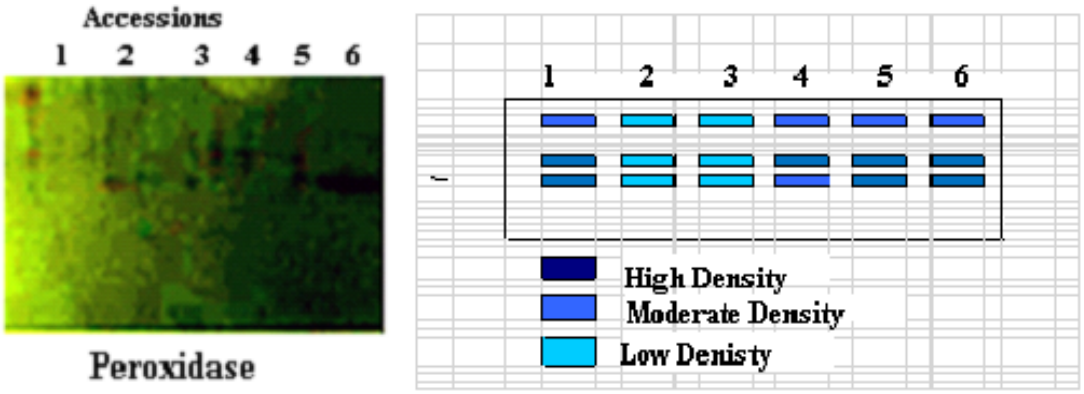


Fig. 11 Peroxidase banding pattern of leaves accessions of *E. peplus*

Table 12. Polyphenol Oxidase (PPO) groups and relative mobility of the 6 studied accessions of *E. peplus*

polyphenol Oxidase groups	Relative mobility	1	2	3	4	5	6
PPO1	0.1	1^{++}	1^{+}	1^{-}	1^{+}	1^{++}	1^{++}
PPO2	0.3	1^{-}	1^{++}	1^{-}	1^{+}	1^{-}	1^{-}
PPO3	0.4	1^{-}	1^{+}	1^{-}	1^{+}	1^{-}	1^{-}

(1^{++}) means high density, (1^{+}) means moderate density and (1^{-}) means low density of band.

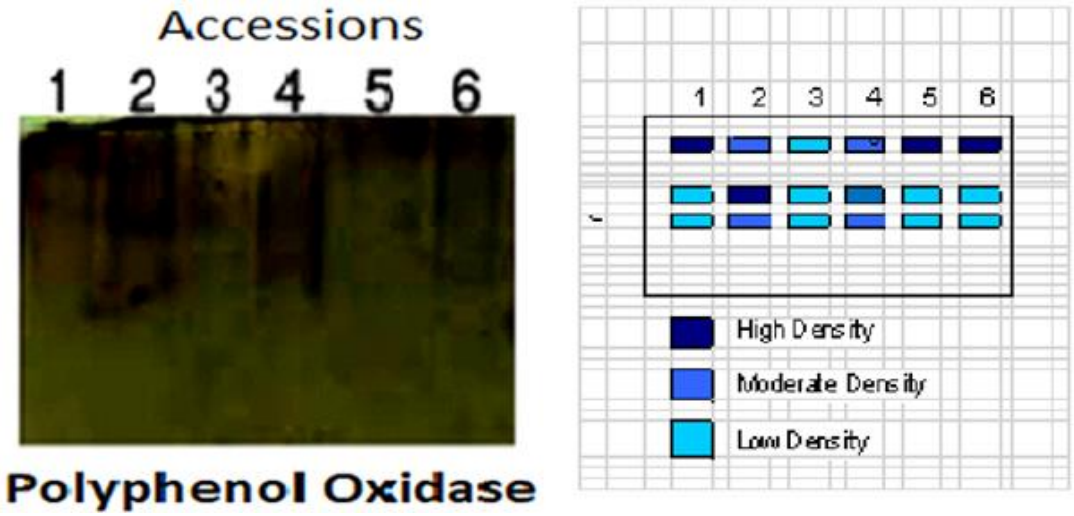


Fig. 10 The protein banding pattern of leaves accessions of *E. peplus*

3.5. Preliminary qualitative phytochemical screening of crude methanolic extract (70%)

The preliminary phytochemical screening of methanolic extract (70 %) of the six selected samples of *E. peplus* was tabulated and illustrated in Table 13 and showed that the samples contained tannins, sterols or terpenes, flavonoids, saponins, alkaloids and resins. Color density explains the amount of secondary metabolites that are produced under environmental stress. The high density of tannins was obtained from

samples collected from sites 1, 2, 3 and 4, site 6 showed moderate densities but the sample of site 5 showed low density. Flavonoids and alkaloids showed high density at samples of *E. peplus* collected from sites 2, 3 and 4, moderate density at site 1 but low density recorded at sites 5 and 6. In the investigated plant samples of sterols or terpenes showed high density at sites 1, 2,3 and 4 but moderate density at sites 5 and 6. Saponins showed high density at all sites except sites 5 and 6 were moderate density. The high density of resins was obtained in all tested samples except that were collected from site 5 showed moderate density.

Table 13. Polyphenol Oxidase (PPO) groups and relative mobility of the 6 studied accessions of *E. peplus*

Test	1	2	3	4	5	6
Tannins	+++	+++	+++	+++	+	++
Flavonoids	++	+++	+++	+++	+	+
Alkaloids	++	+++	+++	+++	+	+
Sterols or terpenes	+++	+++	+++	+++	++	++
Saponins	+++	+++	+++	+++	++	++
Resins	+++	+++	+++	+++	++	+++

+: lower color density, ++: moderate color density, +++: high color density. The numbers 1-6 refer to the sites of the collected samples as shown in Table 2.

4. Discussion

Vegetation analysis of the present results showed that 45 taxa belonging to 20 plant families and 38 genera were present at study sites representing three habitats (roadside, wasteland, and cultivated land), 32 of which were annuals. The increase in the number of annuals in this and other studies is due to the short life cycle of annuals and early flower production, which enables them to resist instability of the agro-ecosystem [70], as well as to complete their life cycle in adverse environmental conditions [71]. A feature of most weeds, especially annuals, is their ability to set seeds without the need for pollinator visits, either by autogamy (self-fertilization) or agamospermy [72]. Annuals also have more ecological, morphological, and genetic adaptability, as well as a larger reproductive potential when disturbed [73] and subjected to agricultural methods [74].

Poaceae and Asteraceae were the most common families present. These families are among the most common families in Mediterranean North African flora [75]. In addition, these plant families were the most frequent in many ecological studies dealing with the floristic composition of different regions in Egypt, such as [76], [77], [78], [79], [80] and [81] .

The increase in therophytes is due to many environmental factors, such as topographical variance and biological influences, in addition to the hot and dry climate [82].

The mean values of soil content of some soil factors EC, TDS, $Ca^{+2,}$ Mg^{+2}, Na^{+}, Cl^{-}, $SO_4^{-2,}$ and nitrogen were higher in soil collected from the roadside soil, followed by wasteland soil and cultivated soil, respectively. Roadsides are located in the zone between the road surface and the adjacent landscape. Roadside vegetation may also purify polluted runoff from roads. Roadsides are stressful areas for biological organisms due to high concentrations of heavy metals, salt, organic molecules, and nutrients [83]. Soils of urban wastelands are characterized by a wide variety of substrates; nutrient-rich, graded brick rubble, compacted rubble, compacted ballast, and raw brick [84] and [85].

The data confirmed genetic diversity among the six samples of *E. peplus* by using six selected ISSR primers based on di-, tri-, or tetranucleotide repeats. Because a species' evolutionary potential is dependent on its genetic variety, it is critical to acquire enough information about the amount of genetic diversity to aid in the development of conservation and sustainable utilization strategies [86] and [87] .

Using ISSR, [88] found high genetic polymorphism among 10 populations of *Euphorbia heterophylla* collected from 10 different soybean fields in Brazilian agroecosystems. [89] demonstrated genetic diversity among three different varieties of *Euphorbia milii* (*splendens*, *hislospii*, and *longifolia*) by using 30 different ISSR primers. [90] found genetic differences among 20 wild *Mallotus oblongifolius* populations in China.

The product of interactions among numerous evolutionary forces is the distribution of genetic diversity among populations. Selection, effective population size and the ability of the species to distribute pollen and seeds are all critical factors. Phenotype flexibility and significant genetic variation are common characteristics of plant species with a widespread distribution [91]. [92] showed genetic diversity among different species of the genus *Euphorbia*, such as *E. prostrata, E. peplus,* and *E. terracina*. In the present study, ISSR markers revealed high genetic diversity among the six accessions of *E. peplus* collected from the different habitats.

This study agrees with the results of [93] of which genetic diversity was found among 14 populations of

Silybum marianum collected from Egypt. The percentage of polymorphism determined by ISSR was about 80 % of the studied accessions and agrees with study [94] that showed genetic diversity between fifty accessions of *Poa pratensis* were collected from different habitats by using ISSR.

The results of the present study show qualitative and quantitative differences in leaf protein banding within and among samples of *E. peplus*. Such differences may be due to microheterogeneity according to the habitat and collection site, and other factors that were not investigated in the present study.

Many SDS-PAGE studies were performed on seed storage proteins to show genetic diversity, but in the current study, SDS-PAGE was carried out on leaf proteins such as [95] and [96].The results showed genetic diversity between the studied accessions and agreed with [26], which showed taxonomic affinity of the six species of *Jatropha* by using SDS-PAGE of leaf protein profiles, and [97], which revealed the genetic variation and relationship among 15 species of subtribe *Cassiinae* (Family Fabaceae) by SDS-PAGE on total leaf proteins.

Three isozymes were used by [98] for estimation of genetic diversity between some species of family Malvaceae. [22] used various isozymes for estimation of genetic variability, population structure, and relationships of *Lactuca* germplasm. Two isozymes (peroxidase and polyphenol oxidase) were used to distinguish among the six accession of *E. peplus*. Two isozymes are effective for differentiation among these accessions. This study agrees with that of [99], which used isozyme markers to show genetic variation among nine species of Euphorbiaceae and agree with the study [23] which showed that peroxidase banding patterns in cowpea landraces gave high polymorphism.

Higher values of the aforementioned soil factors (EC, TDS, $Ca^{+2,}$ Mg^{+2}, Na^{+}, Cl^{-}, SO_4^{-2} and nitrogen) are considered to be stress factors regarding plant biological processes and healthy growth, as they increase the osmotic pressure of the soil solution and decrease the availability of soil water to be absorbed by most mesophilic plants, such as *E. peplus*. All of these stressful factors in soil from the roadside and wasteland habitats, as compared with the soil of cultivated land, were associated with the variation in the number of bands recorded with DNA markers and biochemical markers. [100] reported that the soils properties are crucial for the plant's capacity to create primary and secondary metabolites so, varied soil conditions can cause important differences in primary and secondary metabolite synthesis and accumulation in plants of the same species. Plants respond to variations in the ecosystem by shifting their morphology, physiology, and by producing secondary metabolites involved in plant defense [101] and [102] .

Generally, the data obtained from phytochemical analysis on six samples of *E. peplus* that were collected from different sites in Egypt showed variation in the amount of tested phytochemical compounds. The samples collected from wasteland and roadside habitats contained higher amounts of some phytochemical compounds than that collected from cultivated lands habitat it may be due to the high stress that caused increasing soil content of EC, TDS, $Ca^{+2,}$ Mg^{+2}, Na, Cl^{-}, SO_4^{-2}, nitrogen and the saturation percentages in soil collected from the roadside habitat, followed in descending order by those in wasteland soil and in cultivated soil.

This result is in agreement with many previous studies [103] indicated that flavonoid concentration increases in *Oryza sativa* in response to high salinity. Saponins are defensive secondary metabolites that help plants in adapting to environmental challenges such as water availability, predators and difficult weather conditions [104]. [105] reported that alkaloid amounts increased when the plant was under several stress conditions. [106] that showed that soil properties have an effect on the contents but not the types of the bioactive compounds in *Brassica rupestris* which was collected from two different localities. [41] found that the highest alkaloid content, tannin and phenolic compounds concentration were recorded in *Deverra tortuosa* samples collected from sites where the soil was distinguished by high EC. [42] showed that the phytochemical compounds like saponins, flavonoids, tannins, phenols and terpenoids content were affected by different sites by using preliminary qualitative phytochemical screening test in some species.

This suggests that *E. peplus* plants grown under the unfavorable habitats of the roadside and wasteland may have acquired changes in amount of phytochemical compounds and variations in their genetic constituents to cope with those unfavorable conditions, as shown from the number of bands the plants produced as compared with plants grown under the favorable conditions of the cultivated land habitat.

5. Conclusion

The total number of plant species recorded in this study was 45, and the most common plant families were Poaceae and Asteraceae. The dominant life span consisted of annuals, whereas therophytes were the dominant life form. Cosmopolitan species were the most frequent floristic categories in this study. Molecular DNA marker ISSR combinations and biochemical markers (SDS-PAGE and isozymes) were effective at investigating the genetic variation among the six accessions of *E. peplus* collected from different habitats in Egypt and provided accurate results. The study sites varied in soil factors, which affected genetic variation between studied accessions. The results of the present study are essential for constructing effective and efficient strategies to discover genetic diversity among *E. peplus* accessions. The obtained polymorphism levels were sufficient to establish partial informative fingerprints with the six primers used. Phytochemical compounds like saponins, flavonoids, tannin, phenols and terpenoids content are affected by different sites as a result of high stress.

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