Molecular genetic studies on some natural medical plants

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The present study has been carried out in the Genetics and Cytology Unit, Desert Research Center and Genetics Lab, Faculty of Agriculture, Ain Shams University. The present work aimed to study the following: 1-Detection of biochemical and molecular genetic markers for two medicinal plants (Hyoscyamus muticus L. and Calotropis procera (Ait.) Ait f.) using biochemical and molecular genetic fingerprints.2-Studying genetic variation of these medicinal plants at the biochemical and molecular genetic level. The biochemical markers (protein and isozymes) and the molecular markers (RAPD and ISSR) were used to differentiate and study the relationship and genetic variations among two medicinal plants; henbane and Ushaar. Henbane' (Hyoscyamus muticus L.) was collected from three different locations (Wadi Sudr, El-Maghara and Shalateen). Ushaar (Calotropis procera (Ait.) Ait. f.) also was collected from three different locations (El-Maghara, Shalateen and Sant Kathrin).1. The obtained results from protein electrophoresis (SDS-PAGE) in henbane (Hyoscyamus muticus L.): Three henbane ecotypes were fingerprinted by SDS-PAGE of protein. They exhibited a maximum number of 13 bands which were not necessarily present in the three ecotypes in a range of 68.05 to 230.40 KDa. Results revealed that henbane ecotype from Wadi Sudr has two positive bands at the molecularweights; 202.03 and 112.17 KDa which may used as markers for this genotype. The results showed that the henbane ecotype from El-Maghara has two positive bands at the molecular weights; 190.57 and 97.00 KDa as a marker bands for this ecotype. Concerning henbane ecotype from Shalateen, it was found that four marker bands at the molecular weights; 22 .41, 163.33, 82.06 and 76.86 KDa. On the other hand, the band number 8 at the molecular weight 171.65 appeared in the henbane ecotype from both of El-Maghara and Shalateen regions. Four common bands at the molecular weights 127.55, 95.08, 88.37 and 68.05 KDa, respectively in the henbane genotype from the three regions. 2. The obtained results three isozyme systems in henbane (Hyoscyamus muticus L.):1.A total of four peroxidase bands were obtained. Results revealed that two positive bands were characterized in the henbane ecotype from Wadi Sudr as a marker bands at Rf 0.487 and 0.633, respectively. Only one positives pecific band was characterized in the henbane ecotype from both of El-Maghara and Shalateen regions at Rf 0.165. 2.The bands of poly phenyl oxidas were clearly revealed, but polymorphism was detected only in one band at the Rf 0.449 in the henbane ecotype from Shalateen and El-Maghara regions.3.The banding patterns of Alcohol Dehydrogenase isozyme revealed a total of four bands are scored for the studied plant. The results showed that there were two Characteristic positive bands (at Rf of 0.339 and 0.888) in the henbane genotypes from and El-Maghara and Shalateen regions. Depending on the combination between the banding patterns of protein SDS-PAGE and isozyme data when using NTsyspc2 software, the similarity indices (Table 8) were 53.30 % between the henbane ecotype from Wadi Sudr and El Maghara regions, followed by 26.70 % between the henbane ecotype from Shalateen and El-Maghara. The lowest similarity indices were detected between the henbane ecotype from Shalateen and Wadi Sudr (20.00 %). The dendrogram (Figure 7) was revealed that only one cluster (including the henbane ecotype from Wadi Sudr and El-Maghara regions) while the henbane ecotype from Shalateen detected alone 3. The obtained results from protein electrophoresis (SDS- PAGE) in Ushaar (Calotropis procera (Ait.) Ait. f.): The total number of bands is 12.50 % of them are polymorphic bands with molecular weights ranging

from 68.05 to 230.40 KDa. The maximum number of bands was 10 which found in the Ushaar ecotype from Sant Kathrin. The minimum number of bands was 8 which found in the Ushaar ecotype from El-Maghara and Shalateen regions. Results revealed that Ushaar ecotype from Shalateen has two positive bands at the molecular weights; 230.40 and 175.86 KDa. Moreover, the obtained results showed that the Ushaar ecotype from Sant Kathrin has two positive bands at the molecular weights; 168.33 and 148.68 KDa as a marker bands for this ecotype. On the other hand, the bands number 10 and 11 with the molecular weights of 82.06 and 76.86 KDa were found in the Ushaar ecotype from Shalateen region. The protein SDS-PAGE data showed that the genetic similarity indices ranged from 00.00 % to 66.70 % (Table 12). The closet relationship was detected between the Ushaar ecotypes from El-Maghara and Sant Kathrin (66.70 %). On the other hand, the farthest relationship of similarity matrices was detected between the Ushaar ecotypes from Shalateen and Sant Kathrin (00.00 %), followed by El-Maghara and Shalateen (0.333 %). As indicated by the Figure (2) the dendrogram based on the similarity matrices of protein SDS-PAGE banding patterns separated the three Ushaar genotypes into one cluster (including El-Maghara and Sant Kathrin ecotypes) and Shalateen was separate.4.The obtained results from three isozyme systems in Ushaar (Calotropis procera (Ait.) Ait. f.): 1.Concerning peroxidase, a total of four bands are scored for the Ushaar genotypes. Results revealed that two positive bands were characterized in the Ushaar ecotypes from El-Maghara and Sant Kathrin as a marker bands at Rf 0.165 and 0.633, respectively. 2.For poly phenyl Oxidase isozyme, a total of four bands are scored for the studied genotypes of Ushaar. The results showed that there were two characteristic positive bands (atRf of 0.449 and 0.832) in the Ushaar ecotypes from El-Maghara. 3.In case of Alcohol Dehydrogenase (ADH) isozyme, a total of four bands are scored for the studied genotypes of Ushaar. Alcohol Dehydrogenase isozyme showed polymorphism in a percentage of 75%. The results revealed that the positive band at the Rf 0.198 is detected in the Ushaar ecotype from El-Maghara and Sant Kathrin. The band at the Rf. 0.225 is detected in both ecotypes from Shalateen and El Maghara. Moreover the band (at the Rf. 0.888) is detected in the Ushaar ecotype from Sant Kathrin was separate. Depending on the combination between the banding patterns of protein SDS-PAGE and isozyme data when using NTsyspc2 software, the similarity indices were 46.20 % between the Ushaar ecotype from El Maghara and Sant Kathrin regions, followed by 30.08 % between the Ushaar ecotype from El-Maghara and Shalateen. The lowest similarity indices were detected between the Ushaar ecotypes from Shalateen and Sant Kathrin (23.10 %). The dendrogram (Figure 17) revealed that only one cluster (including the Ushaar ecotype from El Maghara and Sant Kathrin regions) while the Ushaar ecotype from Shalateen was detected alone.5.The obtained results from randomly amplified polymerphic DNA (RAPD-PCR) in Egyptian henbane : The banding patterns of RAPD-PCR fragments using the seven arbitrary primers with the three henbane ecotypes showed 57 amplified fragments; 30 of them were polymorphic (52.63 %). Results revealed the presence of 21 positive RAPD molecular markers. 6.The obtained results from inter simple sequence repeats USSR) in henbane (Hyoscyamus muticus L.): The banding patterns of ISSR-PCR fragments using the five specific primers with the three henbane ecotypes revealed 41 amplified fragments; 15 of them were polymorphic (36.59 %).ISSR-PCR data revealed 13 positive and 2 negati e molecular markers for the three henbane genotypes. Depe ding on the combination between the banding patterns of RA D-PCR and ISSR-PCR data through using NTsyspc2 software, e similarity indices was 68.90 % between the henbane ecotyp from Wadi Sudr and El Maghara regions, followed by 22.20 % between the henbane genotype from Shalateen and El Maghara The lowest similarity indices were detected between the hen s ane ecotype from Shalateen and Wadi Sudr (8.90 %). 7.The obtained results from randomly amplified polymorphic DNA (RAPD-PCR) in Ushaa (Calotropis procera (Ait.) Ait. f.): The banding patterns of RAPD-PCR fragmen s using the seven arbitrary primers with the three Ushaar geno , l es showed 50 amplified fragments; 24 of them were polymo s hic (48.00 %). The resulted was revealed that presence of 18 positive RAPD molecular markers. RAPD-PCR amplificat on revealed different degrees of polymorphisms between e henbane ecotypes.8.The obtained results from inter simple sequ nce repeats (ISSR) in Ushaar (Calotropis procera (Ait.) • it. f.):The banding patterns of ISSR-PCR fragments sing the five specific primers with the three Ushaar ecotypes revealed 41 amplified fragments; 12 of them were

polymorphic (29.27%). ISSR-PCR data revealed 4 positive molecular ma kers for the three Ushaar ecotypes. Depending on the combination between the banding patterns of RAPD-PCR and ISSR-PCR s ata through using NTsyspc2 software, the similarity indices w re 44.40 % between the Ushaar ecotype from El Maghara and Sant Kathrin regions, followed by 33.30 % between the Ushaar genotype from Shalateen and El Maghara. The lowest similarity indices were detected between the Ushaar genotype from Shalateen and Sant Kathrin (22.20 %). The Dendrogram revealed that only one cluster (including the Ushaar ecotype from El Maghara and Sant Kathrin regions) while the Ushaar ecotype from Shalateen was detected alone.