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Original scientific article

GENE FLOW AND GENETIC DIVERSITY IN *Consolida* (Ranunculaceae) USING SEQUENCE RELATED AMPLIFIED POLYMORPHISM

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The genus *Consolida* (DC.) Gray (Ranuculaceae) belongs to tribe Delphinieae. It comprises approximately 52 species, including the members of the genus *Aconitella* Spach. Iraq is one of the richest countries for the genus in South-West Asia.The genetic diversity was assessed through Sequence-related amplified polymorphism. To uncover genetic diversity and species characteristics in *Consolida* species, were studied through a molecular data. Seventy individuals related to five *Consolida* were collected in 5 provinces. A total of 75 (Number of total loci) (NTL) DNA bands were produced through polymerase chain reaction amplifications (PCR) amplification of five *Consolida* species. These bands were produced with the combinations of 5 selective primers. Present results showed that sequence-related amplified polymorphism have the potential to identify and decipher genetic affinity in *Consolida* species. Current results have implications in biodiversity and conservation programs. Besides this, present results could pave the way for selecting suitable ecotypes for forage and pasture purposes in Iraq.

Key words: Sequence-related amplified polymorphism, Gene Flow; Genetic Diversity, *Consolida*

INTRODUCTION

Sequence-related amplified polymorphism (SRAP) is PCR –based marker system. It is one of the efficient and simple marker systems to study gene mapping and gene tagging in plant

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species (LI and QUIROS, 2001), and SRAP are potential markers to assess plant systematics and genetic diversity studies (ROBARTS and WOLFE, 2014). Previously, WU *et al.* (2010) assessed genetic diversity and population structure in *Pogostemon cablin* with the aid of SRAP markers. SRAP markers were successfully implemented in Lamiaceae family to study natural populations and variations within the family (LI and QUIROS, 2001).

The genus *Consolida* S.F. Gray was considered as a separate genus based on one species (*C. regalis*) by GRAY (1821), who worked on British flora. But some researchers considered *Consolida* as a section of *Delphinium* (BOISSIER, 1867). Unlike the others based on annual life form, single spured petal, single follicle compared to 3 or 5 sessile follicles of *Delphinium* recognized *Consolida* as a separate genus (ERTUGRUL *et al.*, 2010). KEMULARIA-NATHADES (1939) recognized a new genus *Aconitopsis* from species of *Consolida* based on peculiar formation of the petal, upper sepal, and spur. The name *Aconitopsis* was later rejected by SOJAK (1969) and being replaced by *Aconitella* because of nomenclature priority. Some researchers have studied these genera taxonomically (IRANSHAHR *et al.* 1992; CONSTANTINIDIS *et al.* 2001). *Consolida* has about 40 species, of which 19 have been recorded from Iran. *Aconitella* with ca. 10 species (3 species in Iran) and 31 species of *Delphinium* (species in Iran) are centred in Irano-Turanian and Mediterranean phytogeographic regions (HASANZADEH *et al.*, 2017). Some biosystematic studies have carried out in various field such as chromosomal studies (HONG, 1986) chemical studies (AITZETMULLER *et al.*, 1999), palynological studies (JABBOUR and RENNER, 2011) and phylogenetic investigations by using DNA sequence data (YOSEFZADEH *et al.*, 2012). In the recent molecular studies (JABBOUR and RENNER, 2001; 2012) it was showed that *Consolida* and *Aconitella* form a clade embeded in *Delphinium* and also *Aconitella* is embedded within *Consolida*. The JABBOUR and RENNER (2011) results showed that *Consolida* diverged from *Delphinium* relatives at least in the early of middle Miocene.

Contrary to the vast majority of *Aconitum* and *Delphinium* species, *Consolida* always has an annual life cycle. Other unique *Consolida* features are the single spurred petal, probably resulting from merging of the two upper petals of *Delphinium* (SCHRODINGER, Cited By Tamura 1966), and the single follicle compared to 3 or 5 sessile follicles of *Delphinium* and 3 to several follicles of *Aconitum.*

The present study investigated the molecular variation of five species in Iraq. Objectives of the study were; a) to estimate genetic diversity; b) to evaluate population relationships using WARD approaches. Current results have implications in breeding and conservation programs.

MATERIALS AND METHODS

Plants collection

Seventy (70) individuals were sampled. Five *Consolida* species in Halabja, Sulaimanieh, Kalar, Chamchamal and Basreh Provinces of Iraq were selected and sampled during July-August 2018-2020 (Table 1). Five to twelve samples from each population belonging to five different species were selected based on other eco-geographic characteristics. Samples were stored at - 20°C till further use. Detailed information about locations of samples and geographical distribution of species are mentioned (Table 1).

Table 1. Voucher details of Consolida species in this study from Iraq

Taxa
C. hohenackeri (Boiss.) Grossh.
C. stocksiana Nevski
C. rugulosa Schrödinger
C. ambigua (L.) Ball & Heywood
C. orientalis (Gray) Schrödinger

Sequence-related amplified polymorphism method

Fresh leaves were used randomly from one to twelve plants. These were dried with silica gel powder. Genomic DNA was extracted while following previous protocol. SRAP assay was performed as described previously (LI and QUIROS, 2001). Five SRAP in different primer combinations were used (Table 2). A 25µl volume containing 10 mM of Tris-HCl buffer at pH 8; 50 mM of KCl; 1.5 mM of MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of single primer; 20 ng of genomic DNA and 3 U of Taq DNA polymerase (Bioron, Germany) were subjected to PCR reactions. The overall reaction volume consisted of 25 µl. This PCR reaction was carried out in Techne thermocycler (Germany). The following cycles and programs were observed. The initial denaturation step was performed for 5 minutes at 94°C. The initial denaturation step was followed by 40 cycles for 1 minute at 94°C; 1 minute at 52-57°C, and 2 minutes at 72°C. The reaction was completed by a final extension step of 7-10 min at 72°C. Staining was performed with the aid of ethidium bromide. DNA bands/fragments were compared against a 100 bp molecular size ladder (Fermentas, Germany).

Table 2. SRAP primers and other parameters. a: Number of total loci (NTL); b: Number of polymorphic loci (NPL); c: Polymorphic ratio(P %); d: Polymorphic information content (PIC); e: Resolving power (Rp)

Primer name	NTL[a]	NPL[b]	P[c]	PIC[d]	RP[e]
Em1-Me1	14	11	92.31%	0.34	33.77
Em2-Me2	10	10	100.00%	0.36	39.70
Em1-Me4	15	13	94.4%	0.33	40.40
Em3-Me1	20	15	79.00%	0.30	36.54
Em4-Me1	17	17	100.00%	0.24	34.21
Mean	15	14	89.00%	0.31	38.52
Total	75	66			159.85

Data Analyses

Molecular analyses

Sequence-related amplified polymorphism (SRAP) bands were recorded. Presence and absence of bands were scored present (1) and absent (0), respectively. Total loci (NTL) and the number of polymorphism loci (NPL) for each primer were calculated. Furthermore, the polymorphic ratio was assessed based on NPL/NTL values. Polymorphism information content was calculated as previously suggested by ROLDAN-RUIZ *et al.* (2000). Resolving power for individual marker system was calculated as: $Rp = \Sigma Ib$. Ib (band informativeness) was estimated while following equation: proposed as: Ib= 1 - [2 x (0.5-p)]. In the equation, p indicates the presence of bands (PREVOST and WILKINSON, 1999). Pairwise genetic similarity between species was evaluated to reveal genetic affinity between species (JACCARD, 1908). Unbiased expected heterozygosity and Shannon information index were calculated in GenAlEx 6.4 software (PEAKALL and SMOUSE, 2006). Gene flow was conducted in POPGENE software, version 1.32 (YEH *et al.*, 1999). Analysis of molecular variance test was conducted in GenAlEx (PEAKALL and SMOUSE, 2006). Mantet test was performed with 5000 permutations in PAST, version 2.17 (HAMMER *et al.*, 2012).

RESULTS

Species identification and genetic diversity

Five (5) suitable primer combinations (PCs), out of 10 PCs were screened in this research. Figure 1 illustrates the banding pattern of Em2-Me2, Em1-Me4, Em4-Me1, Em3-Me1 and Em1-Me1 primer by the SRAP marker profile. Sixty-six (66) amplified polymorphic bands (number of polymorphic loci) were produced. These bands (fragments) had different range i.e. 100bp to 3000 bp. Maximum and minimum numbers of polymorphic bands were 17 for Em4-Me1 and 10 Em2-Me2, respectively. Each primer produced 14 polymorphic bands on average. The PIC ranged from 0.24 (Em4-Me1) to 0.36 (Em2-Me2) for the 5 SRAP primers, with an average of 0.31 per primer. RP of the primers ranged from 33.77 (Em1-Me1) to 40.40 (Em1-Me4) with an average of 38.52 per primer (Figure 1, Table 2). The calculated genetic parameters of *Consolida* species are shown. The unbiased heterozygosity (H) varied between 0.21 (*C. ambigua*) and 0.32 (*C. rugulosa*) with a mean of 0.26. Shannon's information index (I) was maximum in *C. rugulosa* (0.43), where as we recorded minimum Shannon's information index in *C. ambigua* (0.23). The observed number of alleles (*N*a) ranged from 0.119 in *C. ambigua* to 1.147 in *C. stocksiana*. The significant number of alleles (*N*e) ranged from 1.095 (*C. orientalis*) to 1.240 (*C. rugulosa*).

Analysis of Molecular Variance results in significant genetic difference (p = 0.01) among *Consolida* species. The majority of genetic variation occurred among species. AMOVA findings revealed that 71% of the total variation was between species and comparatively less genetic variation was recorded at the species level. Genetic difference between *Consolida* species was highlighted by genetic statistics (Nei's G_{ST}), as evident by significant p values i.e. Nei's G_{ST} (0.38, p = 0.01) and D_est values (0.148, p = 0.01). The constructed dendrogram highlighted two major clusters (Fig 2). Group A consisted of 1 species *C. hohenackeri*.Two sub-clusters were in the B group: four species of *C. stocksiana, C. rugulosa, C. orientalis and C. ambigua.*

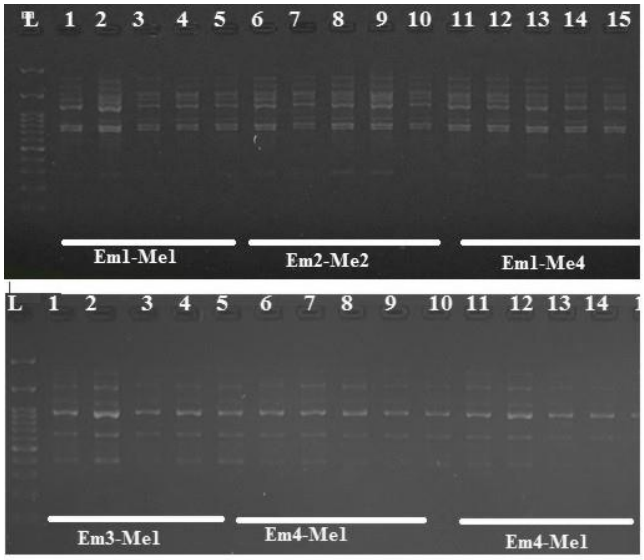

Fig 1. Electrophoresis gel of studied *Consolida* species from DNA fragments produced by SRAP profile; 1,6,11: *C. hohenackeri* ; 2, 7,12: *C. stocksiana*; 3,8, 13: *C. rugulosa*; 4, 9, 14: *C. ambigua*;; 5, 10, 15: *C. orientalis* , L = Ladder 100 bp

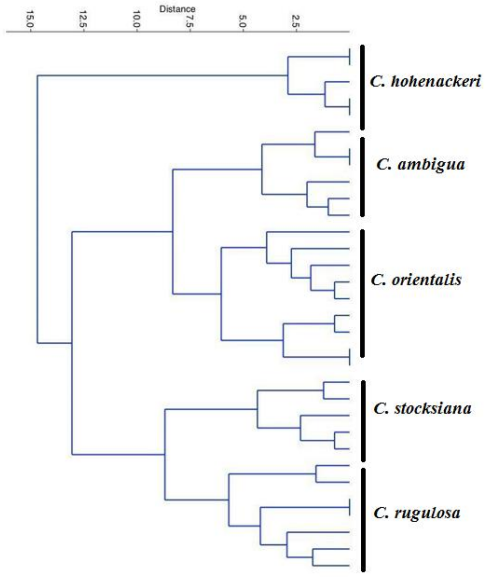

Fig 2. WARD tree of Sequence-related amplified polymorphism data in the studied *Consolida* species

We detected strong correlation between geographical and genetic distances (r = 0.45, p=0.0002) and gene flow ($N_{m)}$ score of 0.287 was reported among species. Detailed information about genetic distances and genetic identity (Nei's) are described. The findings suggested that there was the highest degree of genetic similarity (0.88) between *C. stocksiana* and *C. rugulosa*. On the contrary to this, *C. hohenackeri* and *C. stocksiana* (0.70) had lowest genetic resemblance.

DISCUSSION

In the present study, we used molecular (SRAP) data to evaluate species relationships in *Consolida*. SRAP molecular markers detected clear genetic difference among species. These results indicate that SRAP have potentials to study plant systematics and taxonomy in *Consolida* members.

Given the negative impact of biodiversity threats and overexploitation of *Consolida* plant species in Iran, it is necessary to conduct genetic diversity studies on *Consolida* species. Genetic diversity based studies pave our understanding to develop conservation strategies. Genetic diversity studies are conducted through appropriate selection of primers and indexes including Polymorphic information content (PIC) and marker index (MI) are important indexes to fathom genetic variation in species (SIVAPRAKASH *et al.*, 2004). Common logic suggests that different makers have different abilities to assess genetic diversity, and usually, genetic diversity is linked with polymorphism (SIVAPRAKASH *et al.,* 2004). In this research, we reported PIC values of SRAP primers from 0.24 to 0.36, with a mean value of 0.31. PIC values indeed show low and high genetic diversity among genotypes. Values are ranging from zero to 0.25 show low genetic diversity; in contrast to this, 0.25 to 0.50 highlight mid-level of genetic diversity. In addition to this, values higher than 0.5 are associated with high genetic diversity (TAMS *et al*., 2005). Present results highlighted the efficiency of SRAP markers to estimate genetic diversity in *Consolida* species. In our study, SRAP markers detected average percentage of polymorphism (89%). Current research results also described average PIC values of SRAP makers (0.31) and average RP (resolving power) values i.e. 38.52 of SRAP markers. These current reported values are higher than other reported markers on *Consolida* species (MARIA *et al*., 2007). In the recent study, low gene flow (N_m) was detected among *Consolida* species. The present study also depicted a significant correlation between genetic and geographical distances. Our findings revealed that isolation by distance (IBD) existed between *Consolida* species (Mantet test results). Several mechanisms, such as isolation, local adaptation, and genetic drift, shape the species or population differentiation (FRICHOT *et al*., 2013; DE KORT *et al.*, 2014). The magnitude of variability among *N*a, *N*e, *H*, and *I* indices demonstrated a high level of genetic diversity among *Consolida* species. Dendrogram and principal component analysis results showed clear difference among *Consolida* species. This shows the high utilization of the SRAP technique to identify *Consolida* species. Our results have implications for conservation and breeding programs. Furthermore, it may identify suitable ecotypes for forage and pasture.

CONCLUSIONS

The present study investigated the molecular variation of five species. Molecular analysis confirmed genetical difference between *Consolida* species. This was first attempt to assess genetic diversity through Sequence-related amplified polymorphism analysis in Iraq.

Current study reported two major clusters. These two major groups were separated on the basis of genetic. The genetic similarities between 5 species was estimated from 0.70 to 0.88. SRAP (Sequence-related amplified polymorphism**)** markers analysis, showed that *C. hohenackeri* and *C. stocksiana* had the lowest similarity. Current study also reported correlation between genetic and geographical distances. This clearly indicated isolation mechanism envloved in the ecology of *Consolida* species. Present results indicated the potential of sequence-related amplified polymorphism to assess genetic diversity and genetic affinitiy among *Consolida* species. Current results have implications in biodiversity and conservation programs. Besides this, present results could pave the way for selecting suitable ecotypes for forage and pasture purposes in Iraq.

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ISPITIVANJE PROTOKA GENA I GENETIČKOG DIVERZITETA KOD *Consolida* (Ranunculaceae) POMOĆU SRAP-a

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Izvod

Rod *Consolida* (DC.) Grei (Ranuculaceae) pripada plemenu Delphinieae. Obuhvata oko 52 vrste, uključujući članove roda *Aconitella* Spach. Irak je jedna od najbogatijih zemalja za ovaj rod u jugozapadnoj Aziji. Genetiči diverzitet je procenjen putem SRPA-a. Da bi se otkrio genetički diverzitet i karakteristike vrsta, vrste *Consolida* proučavane su na molekularnom nivou. Sedamdeset uzoraka povezanih sa pet *Consolida* prikupljeno je u 5 provincija. Ukupno 75 (Broj ukupnih lokusa) (NTL) DNK traka je proizvedeno putem PCR-a pet vrsta *Consolida*. Ove trake su proizvedene sa kombinacijama 5 selektivnih prajmera. Rezultati su pokazali da SRAP (pojačani polimorfizam povezan sa sekvencom) ima potencijal da identifikuje i dešifruje genetski afinitet kod vrsta *Consolida*. Dobijeni rezultati imaju implikacije na biodiverzitet i programe očuvanja. Pored toga, oni bi mogli otvoriti put za odabir odgovarajućih ekotipova za ishranu i ispašu u Iraku.

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