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HYBRIDIZATION IN *SIDA OVATA* COMPLEX: AN EVIDENCE FROM RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) PROFILE STUDIES.

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Abstract

In the present studies seed molecular analysis based on random amplified polymorphic DNA profile is proved more supportive method to determine the hybridization between *Sida ovata* Forssk. and *S. taigii* Bhandari. Hybrids display both additive and specific profiles and degree of polymorphism varies from 42-100% with different primers.

Key words: Hybridization, Seeds, RAPD, *Sida ovata* Forssk., *S. taigii* Bhandari.

Introduction

The phenomenon of natural hybridization i.e., the crossing between two distinct taxa is universal but rare, however the study of hybridization is important for the evolutionary process in either positive or negative means (Stebbins 1980, Genovart 2009, Liao *et al.,* 2015; Niemann *et al.,* 2019). *Sida ovata* complex belongs to the family Malvaceae and comprises *Sida ovata* Forssk., and *S. taigii* Bhandari and their intermediate with white, yellow and offwhite flowers respectively (Dawar *et al.,* 1996). Hybridization between both the species has been earlier reported from Pakistan by providing the evidences from breeding studies, morphology, cytology and chemistry (Dawar *et al.,* 1994, 1996). A recent attempt has also been made to confirm the hybridization between *S. ovata* complex by utilizing seed micromorphology and seed protein analysis (Abid *et al.,* 2014). In modern approach various molecular studies have been successfully applied to confirm and solving various problems associated with hybridization. One of the techniques is Random amplified polymorphic DNA (RAPD) technique which is simplest and rapid to analyze the genetic diversity and polymorphism in the populations (Alzate-Marin *et al.,* 1996, Ramirez-Rodriguez *et al.,* 2011). Previously RAPD technique has also been used to solve the phylogenetic relationships of various *Mentha* species and their hybrids (Fenwick & Ward 2001, Shasany *et al.,* 2002). Similarly, the application of RAPD technique on *Primula* (Mizuhiro *et al.,* 2001) and *Papaver* (Shoyama *et al.,* 1998) confirmed the coinheritance between the hybrids and parental species. Presently, seed RAPD profile studies are carried out to determine the hybridization between *S. ovata* and *S. tiagii* from Pakistan.

Materials and Methods

Plant material: Fresh seed were collected from field within the vicinity of Karachi, Pakistan. Specimens were deposited in Karachi University Herbarium (KUH).

DNA extraction: Genomic DNA was isolated from seed samples of each genotype using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle 1990). The concentration of extracted DNA in each sample was checked using Nano Photometer ™ P-360 (Implen, Germany) and the quality of the DNA was checked by horizontal agarose gel electrophoresis (Cleaver Scientific HU10, UK). The extracted DNA was stored at -20°C till further use.

RAPD analysis: In the present study total 45 random primers were used, 24 from Gene Link and 21 from Operon. The amplification was carried out using all primers. For Gene Link, primer PCR amplification was performed in 10 µl reaction volume, including 1X PCR buffer, 3mM $MgCl_2$, 0.33mM dNTPs, 0.6µM primer, 0.25 unit Taq polymerase, 50 ng DNA and appropriate amount of PCR grade water. The thermal cycler conditions were set as: initial denaturation of double stranded DNA at 95°C for 7 min, followed by 45 cycles of 94°C for 1 minute, 50°C for 1 min, 72°C for 1 min. the final extension was done at 72°C for 7 min. For operon primer, DNA amplification was also carried out in 10 µl reaction volume with 1 X PCR buffer, 4mM $MgCl_2$, 0.6mM dNTPs, 0.6µM primer, 1unit *Taq* polymerase, 50ng genomic DNA and appropriate volume of PCR grade water. The thermal cycler was programmed as follows: initial denaturation of DNA at 94°C for 1 minute, 45 cycles of denaturation of DNA at 94°C for 1 min, annealing of primer at 36°C for 1 min, primer extension at 72°C for 2 min and final extension at 72°C for 10 min. The amplified products were observed through agarose gel electrophoresis (Cleaver Scientific HU10, UK) on 1.5% agarose gel (Serva, Germany) and visualized on Gel Documentation System (UVI Tech, UK).

Results and Discussion

The presence of intermediate populations create a

great confusion to confirm the taxonomic entities and these intermediate populations point out the existence of natural hybridization. Presently molecular studies based on RAPD techniques are applied to confirm the hybridization between *Sida ovata* and *S. taigii.* For the RAPD profile analysis various primers viz., GL DecamerA-06 (I), GL DecamerA-08 (II), OPA-09 (III), OPA-10 (IV), OPC-05 (V), OPC-07 (VI), OPH-02 (VII), OPL-04 (VIII) and OPL-15 (IX) (Table 1) were used to analyze the polymorphism within *Sida ovata* complex. While, the degree of polymorphism may effectively predict the coinheritance between the parental species and their hybrid (Khanuja *et al.,* 2000, Patra *et al.,* 2001, Shasany *et al.,* 2002, Akhare *et al.,* 2008). The percentage of polymorphism varied with the different primers (Table 2; Figs. 1-3) such as, high percentage of polymorphism (70-100%) was observed in primers II, III, IV, V, VII and VIII. While primers I, VI and IX showed comparatively low percentage of polymorphism i.e., 42-50%, with the exception of primer VII which exhibited the exclusive bands at highest zone i.e., 3000 bp. In the present study most of the studied primers produced bands between 300-2500 bp zones. However, there are three categories present based on the presence or absence of bands in parents and hybrid, i.e., category A represents common bands between hybrid and parents, category B comprised common band of both the parents but was not found in hybrid, category C represented exclusive bands of hybrid and parents. Regarding to the degree of affinities of hybrid with their parents, *S. tiagii* was more closer, as at one side by applying primer-I, hybrid (H1 and H2) and *S. taigii* showed common bands at highest zone (2000 bp). However, with the addition of primer II these taxa also exhibited common bands at 300 bp zone and these bands were found to be specific for *S. taigii* and hybrid but were not found in *S. ovata* so these bands could also be effectively used to distinguish both the parental species. Present findings could also be well supported by the previous findings of Abid *et al*., (2014) where similar protein bands were observed at 150 kDa zone in *S. taigii* and hybrid. While, on the other hand, hybrid individuals also resembled to both the putative parents due to common band between 400-1500 bp zones which was also supported by sharing the glabrous or pubescent seed strophiole from both of the parental species (Abid *et al.,* 2014). Our studies also strengthen the opinion that hybrid display additive or specific profiles (Harborne 1975, Ali & Qaiser 1980, Dawar *et al.,* 1996, Abid *et al*., 2014). Exclusiveness of hybrid was also evident by abnormal behavior of chromosomes, gross morphology (Dawar *et al*., 1996), reticulate, ruminately foveate, foveolate or apressedly glochidiate seed surface patterns and specific protein profile banding (Abid *et al*., 2014). Similarly, RAPD profile by applying primers II, III, IV also proved to be significant to point out the specific nature of hybrid as well. Thus, present studies based on RAPD profile clearly proved the existence of hybridization between *Sida ovata* Forssk. and *S. taigii* Bhandari in Pakistan.

Table 1. List of RAPD primers used in the present study.

S. No.	Primer	Sequences
I	GL DecamerA-06	5' GGTCCCTGAC 3'
II	GL DecamerA-08	5' GTGACGTAGG 3'
III	OPA-09	5' GGGTAACGCC 3'
IV	OPA-10	5' GTGATCGCAG 3'
V	OPC-05	5' GATGACCGCC 3'
VI	OPC-07	5' GTCCCGACGA 3'
VII	OPH-02	5' TCGGACGTGA 3'
VIII	OPL-04	5' GACTGCACAC 3'
IX	OPL-15	5' AAGAGAGGGG 3'

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Table 2. Polymorphic/ monomorphic bands and their percentages.

Primers	Number of Polymorphic bands	Number of Monomorphic bands	Total number of bands	Percentage of polymorphic bands (%)
GL Decamer A-06	04	04	08	50%
GL Decamer A-08	07	03	10	70%
OPA-09	10	01	11	90%
OPA-10	09	01	10	90%
OPC-05	7	02	09	78%
OPC-07	06	06	12	50%
OPH-02	15	0	15	100%
OPL-04	16	02	18	89%
OPL-15	05	07	12	42%

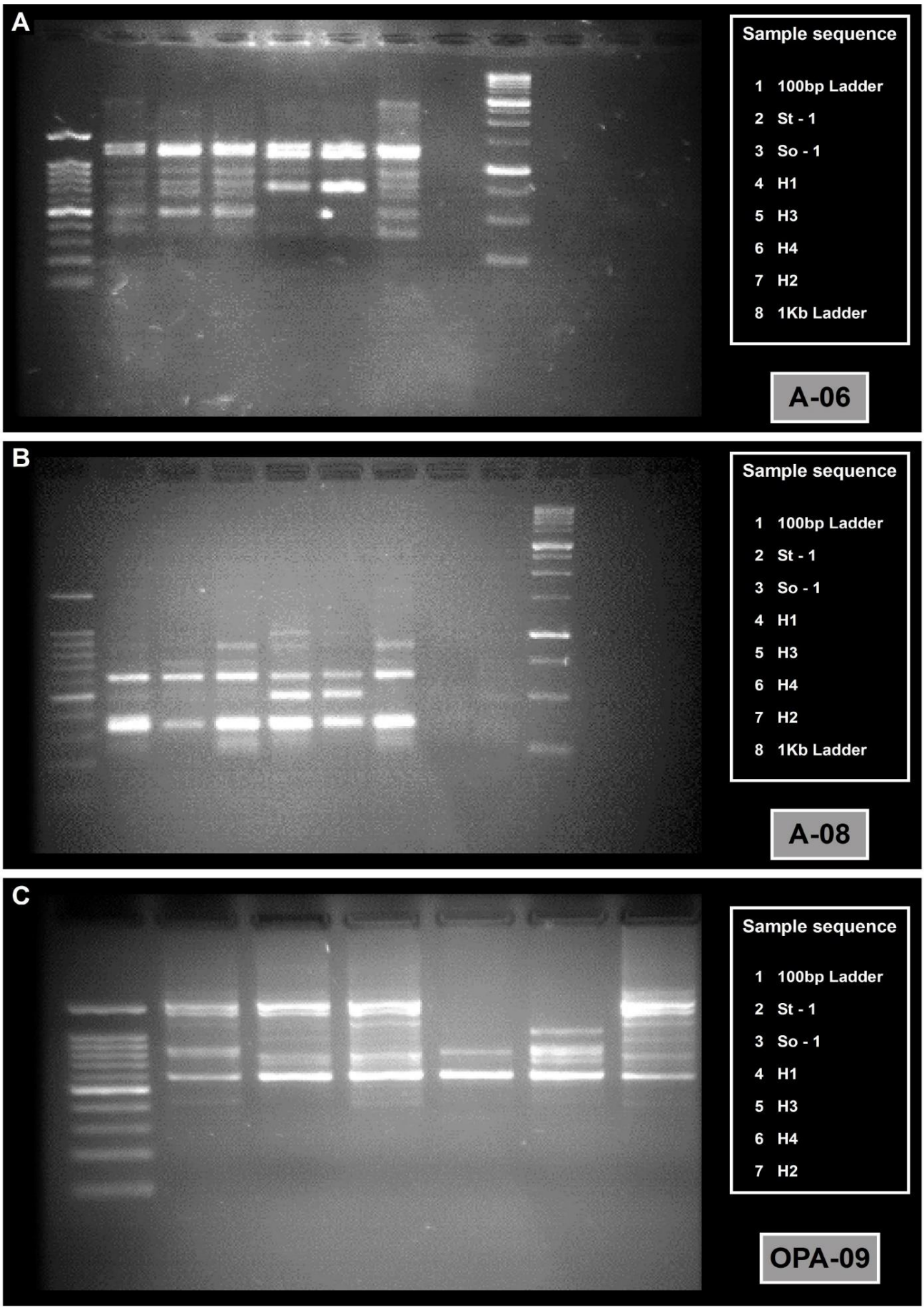

Fig. 1. A-C, RAPD analysis of *Sida ovata* complex (S.t.=*Sida* tiagii; S.o= *Sida ovata*; H1-4= Hybrid).

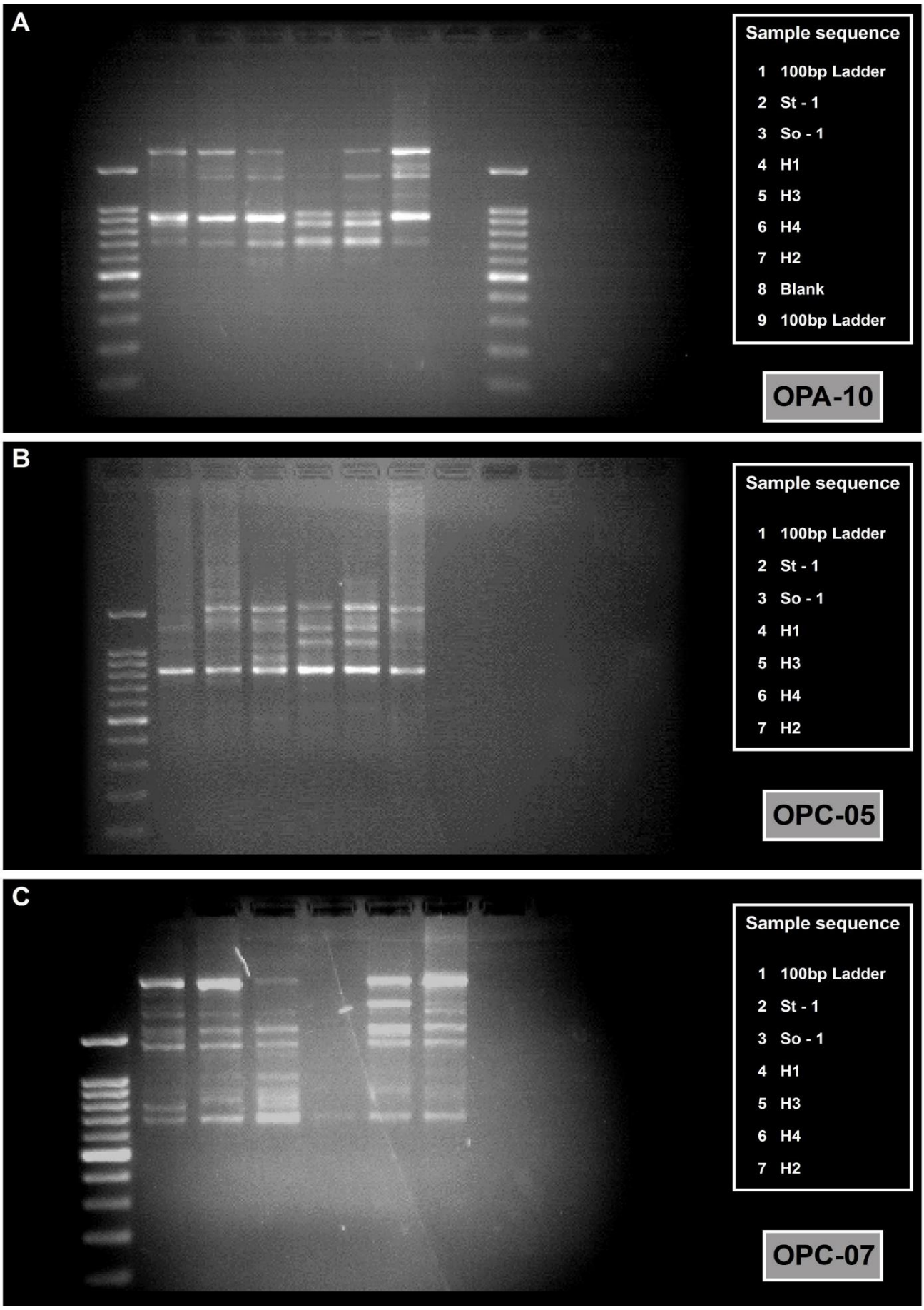

Fig. 2. A-C, RAPD analysis of *Sida ovate* complex (S.t.= *Sida tiagii;* S.o.= *Sida ovate;* H1-4= Hybrid).

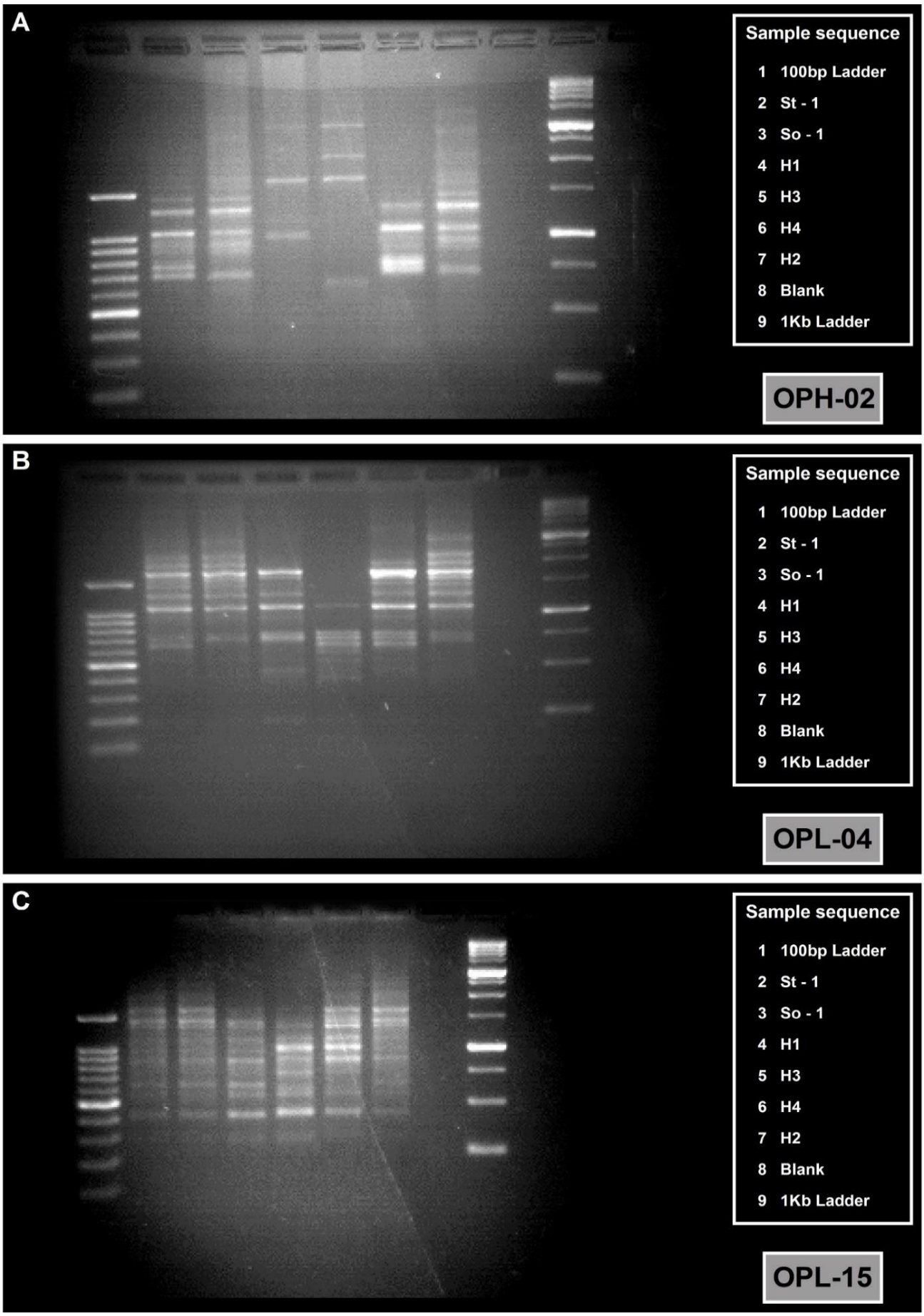

Fig. 3. A-C, RAPD analysis of *Sida ovata* complex (S.t.= *Sida tiagii;* S.o.= *Sida ovate;* H1-4= Hybrid).

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