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Morphological, cytological, physiological and genetic studies of *Bassia indica* (Amaranthaceae)

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ABSTRACT. *Bassia indica* is a natural herb of medical and economic importance with a worldwide distribution, including in various regions of Egypt. It is primarily used for healing and is also considered a green fodder and can be used to remediate salty soils. We investigated morphological and germination parameters as well as the karyotype and genetic variation of *B. indica* by DNA-RAPD. Three samples of this species were collected from various localities in Egypt¸ namely the Northern Coast, the Delta region and Upper Egypt, which each represent different ecosystems. The morphological analysis, which included several traits, showed no significant difference between the localities. However, germination varied among the different populations from the different localities. The chromosomes of this species were found to be diploid, being 2n=18 at all localities. Genetic distances based on DNA-RAPD ranged from 0.13 to 0.31 in the samples from the different localities. A dendrogram based on these distances showed close similarity between the *B. indica* populations collected from the Delta region and Upper Egypt, indicating that they are closely related to each other, while both these populations are quite distant from those from the Northern Coast. This study provides useful information for the classification, chromosomal identification, and germination of *B. indica* in these regions that have distinct soils and climate.

Key words: *Bassia indica*; Cytology; Morphology; RAPD-PCR; DNA Fingerprinting; Weed

INTRODUCTION

Bassia indica [Syn.: *Kochia indica*] is an annual herb found in India, Egypt (Delta and Upper Egypt, Great Southwestern Desert, Nile Delta, North-Coastal Egypt), Saudi Arabia (Aseer region, Makkah region, Nejd Desert), and other countries. *B. indica* is one of the species of the *Bassia* genus that is considered to have potential as green fodder on a global scale (Inam-ur et al., 2011; Hashem et al., 2016). It grows in saline soil and provides a dense cover for the soil surface, and thus aids soil conservation and management (Shaltout and El-Beheiry, 2000; Hand, 2003); these traits have also been used for the detection of microbial habitats in certain locations (Hashem et al., 2015). It is adapted to abiotic stress and has been used in the repair of desert ecosystems, in salt phytoremediation and for livestock grazing on land affected by salinity (Hashem et al., 2016). Shaltout and El-Beheiry (2000), Shaltout and Galal (2006) and El-Sheikh et al. (2012) focused on *B. indica*, investigating the plant's distribution in different habitats in Egypt, namely in the Delta region, the Northern Coast, and Lake Burullus in the Mediterranean region, respectively. People have used the *Bassia* plant for nutrition, house construction, clothes manufacture, medication, cosmetics, ceremonial use, and also in magic (Elsharkawy et al., 2018)

Bassia is used in folkloric medicine to treat many disorders, including renal and rheumatic diseases. An extract of its leaves is useful as an anti-inflammatory and analgesic (Shaker et al., 2013). Microbial studies by Bouaziz et al., (2009) have illustrated the antimicrobial activity of *B. indica* on *Pseudomonas aeruginosa* and *Aspergillus niger*. Furthermore, *B. indica* has a high content of sugars, lipids, and proteins, making it suitable as a safe fodder for animals, such as cattle, and as a natural organic fertilizer (Nafea, 2017).

Chromosomal karyotyping is progressively utilized in scientific plant classification and can offer information essential to clarifying the source, speciation, and phylogenetic relationship of plants (Halil et al., 2013). The RAPD technique is a simple, reliable, efficient, and economical means of cultivar identification and diversity analysis (Qari, 2017; Hammad and Qari, 2010). The DNA-RAPD technique has been successfully employed in the estimation of genetic diversity, including of *Ricinus communis* (Gajera et al., 2010), *Jatropha curcas* (Zhang et al., 2011) and *Aloe vera* (Nejatzadeh-Barandozi et al., 2012).

We examined morphological and germination differences between populations of *B. indica*. To this end, a chromosomal study was conducted and the genetic variance between the different populations of *B. indica* from different localities was examined.

MATERIAL AND METHODS

Plant materials

Samples from three different populations of *B. indica* were collected during the summer of 2018 from three different localities in Egypt: Location 1 "Loc.1" (Mariout, Alexandria governorate, Northern Coast region), Location 2 "Loc.2" (Mansoura governorate, Delta region) and Location 3 "Loc.3" (Assiut governorate, Upper Egypt region).

Morphological parameters

The morphological measurements of 10 individuals from the *B. indica* population in each locality were investigated. These parameters included stem diameter, internode length, leaf length, leaf width, seed length and width.

Physiological parameters (seed germination)

A germination test was conducted on thermogradient bars that provided a temperature range from 5°C to 44°C rotationally with 3°C as interval temperature. For each temperature, about 100 seeds were germinated on wet filter paper (Wittman # 1) soaked with water and kept in a chamber held at the respective temperature in the dark. The papers were replaced every two days. After seven days, the germination percentage was calculated by dividing the number of healthy seedlings by the total number of seeds in the test and multiplying this by 100 (Fei-Yian et al., 2016).

Cytological studies

Seeds from the different *B. indica* populations were germinated for the chromosome count according to Caperta et al. (2006). The root tips of 10 individuals from each population were collected in 0.05% colchicine with cooling for 3 h. Subsequently, the root tips were fixed in Carnoy's solution (3 ethanol: 1 glacial acetic acid) overnight at a cool temperature. They were hydrolyzed in 1N HCl for 15 min at 60°C, then washed with distilled H_2O three times. Finally, they were stained with Feulgen stain for 30 minutes. To achieve a good resolution of the stained chromosomes, drops of 45% acetic acid were added to the squashed roots to remove the excess stain, according to Sharma and Sharma (1999). The stained chromosomes were examined under a Jena Lab electric microscope (magnification power 100x).

Molecular markers

DNA isolation and RAPD assay

The modified CTAB protocol from Doyle and Doyle (1990) was applied to isolate DNA from small leaves of the three B. indica populations. Samples consisting 0.5 g of whole leaves from random individuals were ground to a fine powder using liquid nitrogen in a chilled mortar. This was followed by the addition of 1 ml preheated CTAB buffer (CTAB 2%, 1% PVP, 2 M NaCl, 100 mM Tris HCl- pH 8, 20 mM EDTA). The slurry was then transferred to an Eppendorf tube and incubated at 60°C for 30 min. After incubation, 1 ml of chloroform: isoamyl alcohol (CHCl3: IAA, 24:1) was added and mixed carefully for 10 min. The content was centrifuged at 10,000 RPM for 10 min at 4°C. The upper phase (about 700 µl) was collected into a new Eppendorf tube, with the addition of RNase (10 µg/ml), and incubated at 37°C for 30 min. To inactivate RNase A, 1 ml CHCl3: IAA (24:1) was added and the content was centrifuged at 10,000 RPM for 10 min at 4°C. The upper phase was transferred again into a new tube and 150 µl of 3 M potassium acetate (pH 5.2)

was added. To precipitate the DNA, two volumes of chilled absolute ethanol were used, and the tubes were kept at -20°C for 1 h. The tubes were recentrifuged at 10,000 RPM for 10 min at 4°C. The supernatant was discarded, and the pellet was rinsed with 70% ethanol, air dried, and dissolved in 150 µl of TE buffer.

The PCR was conducted in a 25 µl reaction volume containing 50 ng DNA, 0.5 units of Taq DNA polymerase, 12.5 µl of Bioline Master mix, and 50 pmol of 10-mar primers (Table 1). The final volume was made up with sterile distilled H_2O. The amplifications were conducted in a Biometra thermocycler. The PCR amplification conditions for RAPD consisted of an initial step of denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min.

The amplified DNA was loaded onto 1.2% agarose gel in a 1x TBE buffer containing 5 µl of ethidium bromide (10 mg/ml) and photographed using a gel documentation system (Vilber LOURMAT, Germany).

Table 1. List of primers used for the RAPD analysis of *Bassia Indica*; all had 60% GC content.

Primer Code	Nucleotide sequence
OPAB-11	5′-GTGCGCAATG-3’
OPAQ-04	5′-GACGGCTATC-3’
OPAQ-15	5′-TGCGATGCGA-3’
OPBB-19	5′-TTGCGGACAG-3’

Statistical data analysis

The morphological description analysis was conducted through calculation of the mean, standard deviation, multivariate analysis (ANOVA) test, and Duncan analysis using SPSS21 software. The data analysis of the molecular markers for the RAPD-PCR was performed by band scoring of the amplified fragments and phylogenetic cluster analysis using the BioRAD Quantity One software. A dendrogram was constructed on the basis of the similarity matrix data from the complete linkage cluster analysis.

RESULTS AND DISCUSSION

Morphological parameters

The morphological description of the different populations of *B. indica* collected from the different habitats in this study is presented in Table 2. The morphological parameters were estimated for about 10 individuals from each population. There was no significant difference between the individuals from the different populations. This implies that habitat variation does not affect these morphological characteristics. Shaltout and El-Beheiry (1997) investigated the standing aboveground phyto-mass and nutrient status of this plant in the Nile Delta region, focusing on locations different from this study but also including the Northern Coast and Upper Egypt regions. Shaltout and El-Beheiry (2000) described the plant as erect, growing up to 2 meters in height and adapted for seed dispersal.

They also noted that it is common in arid zones across the globe. It has a compact pyramidal habit with much branching near the ground, forming dense thickets.

Table 2. Morphological parameters of the three different *assia. indica* populations collected from different localities (mean ± SD of the 10 individuals).

Location	Stem diameter (mm)	Internode length (mm)	Leaf length (mm)	Leaf width (mm)	Seed length (mm)	Seed width (mm)
1	3.15±1.11	21.78±2.40	10.48±2.26	1.48±0.88	0.67±0.69	0.75±0.03
2	3.18±1.54	22.68±2.42	9.03±1.49	1.53±0.18	1.18±0.52	0.80±0.09
3	3.06±0.90	15.20±2.47	8.86±1.99	1.50±0.46	1.15±0.38	0.79±0.02

Location 1 (Northern Coast), Location 2 (Delta region), Location 3 (Upper Egypt)

Seed germination

A seed germination bioassay was applied to the seeds of *B. indica* collected from the different localities. The seeds were germinated at temperatures ranging from 5 to 44°C, as shown in Table 3. The outcomes indicate that there are differences and variation in the response of the germinated seeds according to different habitats. These results imply that although the different habitats might not affect the morphological characteristics, they could have an effect on the physiological response; therefore, they could also affect the genes as all physiological processes are reflections of gene expression. The variation in the germination percentages between different temperatures, e.g. 29 and 32°C, may be attributed to a number of factors, such as the viability of the tested seeds, the geographical distribution, and the nature of the soil at each location.

Table 3. Comparison of the germination percentages (G%) along a temperature gradient from 5°C to 44°C for *Bassia indica* for different localities in Egypt.

Temperature °C	5	8	11	14	17	20	23	26	29	32	35	38	41	44
Location	0	10	18	60	70	90	85	60	23	8	40	38	17	0
Location 2	0	0	85	100	100	100	100	100	100	90	100	42	80	0
Location 3	0	0	0	60	70	90	100	100	100	60	80	70	70	10

Location 1 (Northern Coast), Location 2 (Delta region) Location 3 (Upper Egypt)

Cytological studies

For all the investigated *B. indica* populations collected from the different localities, the basic diploid chromosome count was 2n = 18. The cytologies are illustrated in Figure 1. There are no previous records for chromosome counts for *B. indica*. However, Aguilera et al. (2014) mention the chromosome counts of other *Bassia* species (*B. prostrata* 2n = 36, *B. dasyphylla* 2n = 18 and *B. scoparia* 2n = 18), and Lee et al. (2005) found that three different species of the *Bassia* plant collected from the far eastern region have diploid, tetraploid and hexaploid chromosome contents.

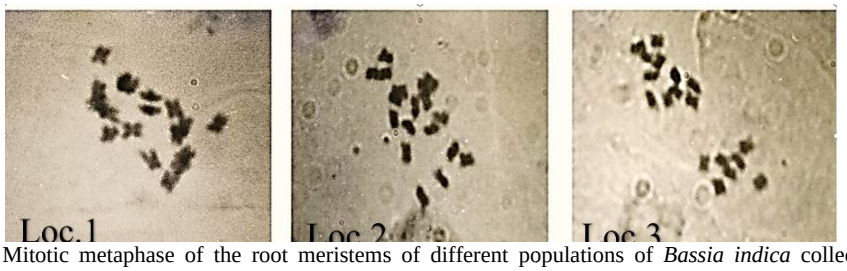

Figure 1. Mitotic metaphase of the root meristems of different populations of *Bassia indica* collected from different localities (2n = 18). Loc.1 = Location 1 (Mariout, Alexandria governorate); Loc.2 = Location 2 (Mansoura governorate) and Loc.3 = Location 3 (Assiut governorate).

Molecular markers

Molecular studies of *B. indica* are very rarely conducted, and there is virtually no information on this species. In our study, the results based on RAPD explain that a total number of 26 bands result from the four different RAPD primers. These contain a total of nine polymorphic bands. Each primer has a polymorphism percentage range from 0 (OPAQ-15) to 50% (OPBB-19) and the total average polymorphism percentage is 40.96% (Table 4). The gel electrophoresis images are shown in Figure 2. The genetic distance is shown in Table 5, indicating that there is a small genetic difference, ranging from 0.13 to 0.31. This slight variation could be due to the differences in both the geographic distribution and climatic conditions.

Table 4. Polymorphic bands generated by the different RAPD primers for different *Bassia indica* populations in Egypt.

Treatments	Loc.1	Loc.2	Loc.3	Total bands	Polymorphic bands
OPAB-11	6	8	7	9	4
OPAQ-04	7	5	6	7	2
OPAQ-15	4	4	4	4	0
OPBB-19	5	5	4	6	3
Total no. of bands for all primers					26
Total no. of polymorphic bands for all primers					9

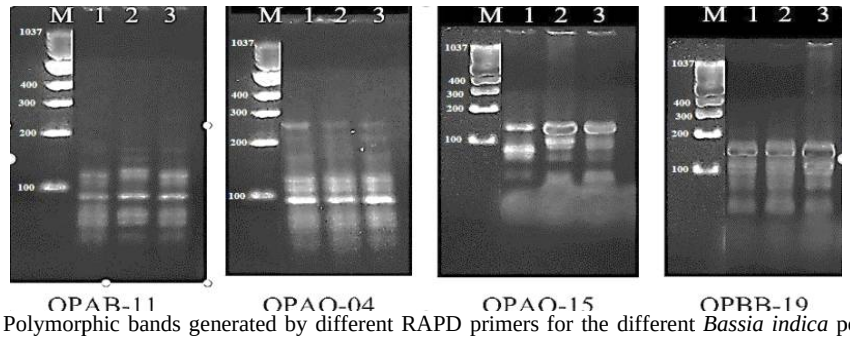

Figure 2. Polymorphic bands generated by different RAPD primers for the different *Bassia indica* populations (M: 1Kb ladder, 1: Loc.1, 2: Loc.2 and 3: Loc.3).

Table 5. Genetic distances based on RAPD analysis between the different *Bassia indica* populations from the different habitats.

	1	2	3
1	0.00	0.31	0.28
2	0.31	0.00	0.13
3	0.28	0.13	0.00

The dendrogram presented in Figure 3 shows a close similarity between the clusters of the *B. indica* populations collected from the Delta region Loc. 2 and Upper Egypt Loc. 3, indicating that these are closely related to each other. On the other hand, both these populations are quite distant from the population collected from the Northern Coast Loc. 1. These results can be explained based on the nature of the soil in these regions. Both the Delta region and Upper Egypt. 3 have inland, cultivated clay soils, while the Northern Coast of the Mediterranean tends to have sandy soil. Lee et al. (2005) used a RAPD molecular technique to differentiate among three different *B. indica* plants collected from the far eastern region. They also constructed a cluster analysis to determine the relationships among these species. Location 1 (Northern Coast), Location 2 (Delta Region), Location 3 (Upper Egypt).

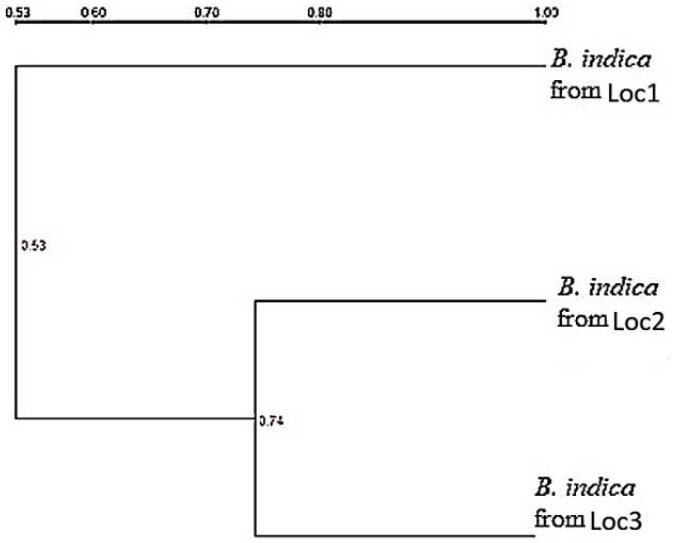

Figure 3. Dendrogram constructed according to the UPGMA cluster analysis of three different *B. indica* populations from different habitats.

CONCLUSIONS

We used morphological, physiological, cytological and molecular parameters to evaluate *B. indica* populations collected from different localities in Egypt. The results show a genetic variation and polymorphism percentage of 40.96% between these different populations.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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