Taxonomic significance of seed sculpture and pollen ecto-mycoflora at the infraspecific level: *Brassica tournefortii* case study

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A B S T R A C T

Several populations of *Brassica tournefortii* (Brassicaceae) occurring in Egypt are investigated from the micromorphological point of view (seed ornamentations). The species is known to show a notable phenotypic plasticity and five morphotypes was identified in the past. Furthermore, a soil analysis as well as a study of the fungal species from anthers were carried out. The aim of the study is to verify the taxonomic value of the morphotypes of *B. tournefortii* and their ecologic relationship with soil variables. The results obtained demonstrated that the five morphological forms can be distinguished based on the seed sculpture. The Canonical Correspondence Analysis (CCA) exhibited a clear correlation between the soil variables and the identified forms. Six species of fungi were detected from the ecto-anthers in the Forms (F2-F4), while F1 was lacking the fungal species. The study revealed that the morphological plasticity of studied *B. tournefortii* depends on ecological factors.

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1. Introduction

The genus *Brassica* L. (Brassicaceae Burnett) includes annual herbs which have high economic relevance in agriculture (see e.g., Amer et al., 2019a,b). *Brassica tournefortii* Gouan. is one out of the five *Brassica* species recorded in Egypt (Boulos, 1995). This species is distributed from the Mediterranean basin and the Middle East to India (Abdelhameed et al., 2020) and it is recorded as alien in southwest U.S.A., N- and C-Mexico, and Australia (America, n.d.; Australia, n.d.; Minnich and Sanders, 2000; VanTassel et al., 2014). *Brassica tournefortii* is characterized as having a high molecular and morphological (macro- and micro-) variability, and five forms were identified in Egypt (see Abdelhameed et al., 2020).

Starting from Heywood (1971) the use of SEM in the study of the seed coat was considered in Brassicaceae taxonomy (see e.g., Brisson and Peterson, 1977, 1976; Mulligan and Bailey, 1976; Stork, 1980). Biologists gave attention to the morphology and development of Brassicaceae seeds, also studied exo- and endomorphic characters (Beeckman et al., 2000; Berggren, 1962; Corner and Corner, 1976; Jonsell, 1986; Koch et al., 2003; Koul et al., 2000; Murley, 1951; Musil, 1948; Zou et al., 2001).

Testa topographic pattern during seed development was used to distinguish the cultivated forms of Brassicaceae (Zeng et al., 2004). In Egypt, Fayed & El Naggar (1996; 1988) studied seed coat sculpture of species of tribes Brassicaceae DC. and Lepidieae DC. Tantawy et al. (2004) investigated the seed exomorphic of 30 species including *B. tournefortii* and seed testa was used as a taxonomic criterion by El Naggar (2005). The latter author studied the seed characters of 93 Egyptian taxa. *B. tournefortii* was investigated among other species by El-Habashy et al. (2013) and Kasem et al. (2011).

Ecological factors play an important role to clarify the issues related to the spatial distribution and morphological variation, so contributing to understanding several taxonomic problems (see Sharma, 2009). Chauhan et al. (2006) and Bangle et al. (2008) studied the environmental limits (light, temperature, and salt concentration) affecting the germination of seeds of *Brassica tournefortii*. While the relation between soil features and *B. tournefortii* distribution in Egypt was studied by El-Gawad (2014).

The endophytic fungi can play a basic role in the plant life, and those reported in many of the wild *Brassica* members were summarized by Card et al. (2015) who stated that these fungi improve...
and promote plant growth, increase yield, reduce disease symptoms caused by plant pathogens, reduce insect herbivory, remove the contaminant from the soil, improve plant performance under extreme temperature and water availability conditions, solubilize phosphate and contribute assimilable nitrogen to their hosts.

On the other hand, the ecto-mycoflora can serve for other issues, the study of ecto-mycoflora was carried out on pollen and leaf surface of three species of Zygophyllum (El Naggar and Abdel-Hafez, 2007) and on native Brassicaceae species including B. tournefortii (El Naggar et al., 1993). Also, Abdel-Hafez and El Naggar (2006; 2001); El Naggar and Abdel Hafez (2003) studied the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records for the correlation among fungal biodiversity with the leaf and pollen morphology of native and medicinal species. No earlier records.

The aim of this work is to study 1) the relationship between the morphology of Brassica tournefortii in comparison with soil ecological factors and the ecto-mycoflora and 2) the value of seed sculpture in the taxonomy of this species.

2. Material and methods

2.1. Morphology

12 Plants of Brassica tournefortii from seven Egyptian populations were collected and examined during the spring of the years 2017 and 2018 (Table 1). 74 Macro- and micromorphological characters were investigated following the consideration by Abdelhameed et al. (2020) (See Annex). Seed sculpture was scanned using a scanning electron microscope (SEM) in electron microscope unit at Beni-Suef University.

2.2. Soil analysis

Three soil samples (depth = 20 cm) were collected from each locality for each forms, identified according to Abdelhameed et al. (2020). Soil samples were air-dried at 40 °C; sieved using a 2 mm sieve to remove gravel and debris, then packed and kept for physical and chemical analysis. Three soil replicates/locality, fifteen soil variables (physical and chemical) were investigated according to Carter and Gregorich (2007), as follows: soil particle size (Piper, 1950), soil reaction (pH), and electric conductivity (EC) were carried out according to Chapman and Pratt (1961). Anions and cations (Chloride by titration with AgNO₃, Bicarbonates by titration with HCl, Sodium, and potassium by flame photometer, Calcium, and magnesium by titration with versenat (EDTA), Sulphates were calculated by the difference between anions and cations were carried out in saturated soil paste extract according to Jackson (1967).

2.3. Pollen mycoflora

2.3.1. Sample collection and storage for mycoflora

Twenty flowers of three forms of Brassica tournefortii were collected in triplicates from the experimental garden in Beni-Suef University, each one placed in a sterile plastic bag and kept at 2–5 °C till fungal isolation and identification.

2.3.2. Isolation and identification of mycoflora

The anther ecto-mycoflora was determined using the anther-plate method (Naggar and Sallam, 2009). Six plates/Forms were used, four anthers/plate were placed on potato dextrose agar (PDA) medium supplemented with chloramphenicol (0.5 mg/ml medium) as a bacteriostatic agent (Wang et al., 2016). Then Petri dishes were incubated for 7–10 days at 25 °C for 3 days and periodically checked for purity. Pure fungal isolates were counted and identified based on macro- and microscopic morphological characteristics according to Moubasher (1993).

2.4. Data analysis

The cluster analysis (UPGMA method) was performed using the program SPSS version 23, whereas the Canonical Correspondence Analysis (CCA) was made using Past software version 3.26.

3. Results

3.1. Morphological identity of the studied forms

According to Abdelhameed et al. (2020), we identified all the five forms highlighted by these authors (F1-F5). Form no. 3 is characterized by pinnatipartite-pinnatisect basal leaves, with rhombic-triangular terminal lobe, whereas the other four forms display pinnatisect basal leaves. Basal leaves of Form no. 2 do not show clear midrib and lateral pseudo-alternate lobes, while in Forms nos. 1, 4, and 5 midrib and lateral opposite-alternate lobes are clear. Form no. 5 can be identified by terminal lobe cut to midrib (not winged), whereas Forms nos. 1 and 4 can be distinguished by the terminal lobe which is mostly lanceolate in Form no. 1 and ovate in Form no. 4 (see Fig. 1, Annex).

Morphological correlation between the studied forms

The results of cluster analysis, (UPGMA; Fig. 2) reveal that the three significative groups can be considered, i.e, Group (1) (corresponding to Form no. 1), Group (2) (corresponding to Form no. 3), and Group (3) (corresponding to Form nos. 2, 4, and 5). Note that Forms nos. 4 and 5 showed a high affinity.

3.2. Seed features

Seeds of the identified Forms are globose with visible hilum, red – light brown, lusterless, with glabrous texture. Seed sizes are 1.2 0–1.45 × 1.25–1.5 mm in general. The smallest size recorded in Form 2 (1.20–1.25 × 1.25–1.30 mm) and Form 5 (1.20–1.30 × 1.25–1.35 mm), whereas Form1 (1.30–1.40 × 1.35–1.45 mm) and Form 4 (1.30–1.45 × 1.30–1.5 mm) had the largest size. Form 3 shows the medium size (1.25–1.40 × 1.30–1.45 mm).

Seed sculpture using SEM

The SEM showed three types of seed sculpture: papillate in Forms 3, 4, and 5, and reticulate in Form 1, and reticulate-foveolate in Form 2 as shown in Fig. 3. The terminology of Murley (1951) was adopted to describe the seed coat sculpture
in addition to some extra terms from (Bojnansky and Fargašová, 2007) (see Table 2).

A key to the forms according to seed size and sculpture follows:

I. Seed coat reticulate, size (1.30–1.40 \times 1.35–1.45 \text{ mm})
   Form 1
II. Seed coat reticulate-foveolate, size (1.20–1.25 \times 1.25 – 1.30 \text{ mm}) Form 2
III. Seed coat papillate
   a) Seed size (1.25–1.40 \times 1.30–1.45 \text{ mm}) Form 3
   b) seed size (1.30–1.45 \times 1.30–1.5 \text{ mm}) Form 4
   c) seed size (1.20–1.30 \times 1.25–1.35 \text{ mm}) Form 5

3.3. Soil analysis

The results obtained by the soil analyses (see Table 3), showed that the percentage of soil fraction are relevant, particularly concerning the coarse sand which ranges from 31.0 to 38.0 for, respectively, Forms 4 and 3, while silt from 12 (Forms 3 and 4) to 20.8 (Form 4) and the clay from 2.3 (Form 2) to 4.0 (Forms 3 and 4). So, the soil type supports all these forms is a sandy-loam soil.

Concerning the PH (see Table 3) it is moderately alkaline, ranging from 7.84 (Forms 1, 4, and 5) to 7.99 (Forms 3 and 4).

The forms sustained in mesophytic habitat with low salinity where the EC ranges from 1.26 (Form 1, 4 and 5) to 5.14 (Form 4).

Fig. 1. Basal leaves of different forms, A: Form 1, B: Form 2, C: Form 3, D: Form 4, D: Form 5.

Fig. 2. Cluster analysis of the studied Forms based on the morphometric data (Annex).

Fig. 3. Seed sculpture of the identified B. tournefortii Forms; A: reticulate in Form 1; B: reticulate-foveolate in Form 2; C, D&E: papillate in Forms 3, 4, and 5.
The detected ten chemical parameters for the studied *Brassica tournefortii* forms showed, however, no significant variation except for Form 4 which showed the highest Na⁺ and Cl⁻ (20.1 & 24.8 mEq/L; respectively; Table 3).

The Canonical Correspondence Analysis (CCA) applied to the studied physical and chemical soil variables, reveals that the occurrence of Form 1 is mainly affected by coarse sand, fine sand, and soil saturation percent, whereas Form 3 is affected by soil pH and clay. On the other hand, the distribution of Forms 2 and 5 is mainly affected by Mg²⁺, SO₄²⁻ and Ca²⁺, while HCO₃⁻, K⁺, and electric conductivity affects the distribution of Form 4. The successive decrease in Eigenvalues of the four CCA axes (0.46174, 0.385, 0.16339, and 0.11144, respectively) illustrated in Table 4, suggests a well-structured data set. The inter-set correlation resulted from CCA analysis showed Axis 1 is positively correlated with pH, SP, coarse sand, fine sand, and clay, and negatively correlated with EC, Ca²⁺, Mg²⁺, Na⁺, K⁺, HCO₃⁻, Cl⁻, SO₄²⁻, and silt. Soil saturation percent (SP) has the highest positive value (0.70703), while sulphates have the highest negative value (~0.490932), thus axis 1 can be interpreted as SP-sulphate gradient. Axis 2 is positively correlated with pH, SP, Mg²⁺. Coarse sand, and fine sand, and negatively correlated with EC, Ca²⁺, Na⁺, K⁺, HCO₃⁻, Cl⁻, SO₄²⁻, and clay. The highest positive value is for fine sand (0.647164) and the highest negative value for HCO₃⁻ (0.704459), thus axis 2 can be interpreted as fine sand-organic matter gradient.

### Table 2
Adopted technical terms for description of seed coat sculpture (Modified from Murley, 1951 and Bojnansky and Fargašová, 2007).

| Term                | Explanation in detail                                                                 |
|---------------------|---------------------------------------------------------------------------------------|
| Reticulate          | Having a raised network of narrow and sharply angled lines frequently presenting a geometric appearance, each area outlined by a reticulum being an interspace |
| Foveolate           | Marbled with small shallow pits                                                       |
| Reticulate-foveolate| A type intermediate between reticulate and Foveolate types                            |
| Papillate           | With minute, rounded, nipple-like projections                                           |

### Table 3
Features of soil variables (physical & chemical), as mean values and range (between brackets).

| Soil variables | Form 1 M ± SE (Range) | Form 2 M ± SE (Range) | Form 3 M ± SE (Range) | Form 4 M ± SE (Range) | Form 5 M ± SE (Range) |
|----------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Physical       |                        |                        |                        |                        |                        |
| Coarse sand (%) | 36.0 ± 0.57 (35–37)    | 35.5 ± 0.28 (35–36)    | 36.5 ± 0.86 (35–38)    | 34.5 ± 2.02 (31–38)    | 36.5 ± 0.28 (36–37)    |
| Fine sand (%)   | 45.5 ± 1.44 (43–48)     | 47.0 ± 0.57 (46–48)    | 47.0 ± 0.57 (46–48)    | 45.0 ± 1.15 (43–47)    | 44.5 ± 0.86 (43–46)    |
| Silts (%)       | 17.55 ± 0.55 (16.6–18.5)| 16.6 ± 1.09 (14.7–18.5)| 15.25 ± 1.87 (12–18.5)| 16.4 ± 2.54 (12–20.8) | 15.65 ± 0.55 (14.7–16.6) |
| Clay (%)        | 3.45 ± 0.03 (3.4–3.5)   | 2.9 ± 0.34 (3.5–4)     | 3.75 ± 0.14 (3.5–4)    | 3.15 ± 0.49 (2.3–4)    | 3.35 ± 0.03 (3.3–4)    |
| Textural class  | Sandy loam              | Sandy loam             | Sandy loam             | Sandy loam             | Sandy loam             |
| Chemical        |                        |                        |                        |                        |                        |
| pH (1:2.5)      | 7.89 ± 0.031 (7.84–7.95)| 7.91 ± 0.02 (7.88–7.95)| 7.97 ± 0.011 (7.95–7.99)| 7.91 ± 0.04 (7.84–7.99)| 7.88 ± 0.02 (7.84–7.93)|
| EC (ds/m)       | 1.36 ± 0.05 (1.26–1.46)| 2.71 ± 0.72 (1.46–3.97)| 1.39 ± 0.037 (1.33–1.46)| 3.20 ± 1.12 (1.26–5.14)| 3.02 ± 1.12 (1.26–4.24)|
| % Soil saturation (SP) | 34.5 ± 1.44 (32–37) | 31.0 ± 3.46 (25–37) | 34.5 ± 1.44 (32–37) | 29.5 ± 1.44 (27–32) | 28.3 ± 2.02 (25–32) |
| Ca²⁺ (mEq/L)   | 3.25 ± 0.14 (3–3.5)    | 6.7 ± 1.85 (25–37)     | 3.95 ± 1.01 (3.5–4.4)  | 6.45 ± 1.99 (3–3.9)    | 4.75 ± 0.26 (3–6.5)    |
| Mg²⁺(mEq/L)    | 3.75 ± 0.14 (1.5–2)    | 5.5 ± 2.02 (2–9)       | 2.05 ± 0.02 (2–21)    | 5.25 ± 2.16 (1.5–9)    | 1.75 ± 1.29 (1.5–6)    |
| Na⁺ (mEq/L)    | 6.85 ± 0.37 (6.2–7.5)  | 12.8 ± 3.06 (7.5–18.1) | 6.45 ± 0.61 (5.4–7.5)  | 20.1 ± 8.48 (5.4–34.8) | 8.35 ± 1.24 (6.2–10.5) |
| K⁺ (mEq/L)     | 1.1 ± 0.06 (1–1.2)     | 1.5 ± 0.29 (1–2)       | 1.0 ± 0.00 (1–2)      | 1.55 ± 0.32 (1–1.1)    | 1.1 ± 0.06 (1–1.2)     |
| HCO₃⁻ (mEq/L)  | 0.4 ± 0.06 (0.3–0.5)   | 0.4 ± 0.11 (0.2–0.6)   | 0.4 ± 0.06 (0.3–0.5)  | 0.7 ± 0.29 (0.2–1.2)   | 0.5 ± 0.03 (0.5–0.6)   |
| Cl⁻ (mEq/L)    | 8.8 ± 0.46 (8–9.6)     | 13.5 ± 2.25 (9.6–17.4) | 9.4 ± 0.11 (9.2–9.6)  | 24.8 ± 9.6 (8–41.6)    | 12.7 ± 2.71 (8–17.4)   |
| SO₄²⁻ (mEq/L)  | 3.75 ± 0.14 (3.5–4)    | 5.1 ± 0.63 (4–6.2)     | 3.65 ± 0.20 (3.3–4)   | 5.25 ± 1.12 (3.3–7.2)  | 4.75 ± 0.72 (3–5.6)    |

#### 3.4. Anther’s mycoflora

Anthers ecto-mycoflora was investigated in the identified forms of *Brassica tournefortii*, and the overall results revealed the presence of 22 colonies representing six species and three genera (Table 6, Fig. 5). Form 1 showed no emerging fungi with all the inoculated anthers. The six identified species are: *Alternaria alternata* (Fr.) Keissl, *Cladosporium oxysporum* Berk & Curtis, *C. tenuesisms* Cooke, *Penicillium chrysogenum* Thom, *P. citrinum* Thom., and *P. raistrickii* Smith (Table 5 & Fig. 5). The highest colonization frequency (58.33%) showed in Form 2, and the lowest (4.16%) in Form 3 which showed the presence of *Cladosporium tenuesisms* Cooke only. And similar species were detected in Form 4, in addition to *Cladosporium oxysporum* Berk & Curtis (Table 6).
4. Discussion

4.1. Taxonomic impression of the morphometric characters

Brassicaceae is highly homoplasious in its morphological characters, accordingly the taxonomic significance of the morphological characters plays a limited role at the generic and family levels in resolving the phylogenetic affinities (Al-Shehbaz et al., 2006). Despite this, the notable phenoplasticity (Table 1 & Fig. 2) of the studied Brassica tournefortii populations revealed delimiting the infra-specific taxa into five forms (1, 2, 3, 4, and 5; Fig. 3) depending mainly on the shape, midrib-clarity, and the arrangement of the leaf segments. Our data was congruent to that reported by Abdelhameed et al. (2020). The character of the basal leaf is significant in delimiting the infra–taxa [for example, it was applied by Amer et al. (2019b) to classify the phenoplasticity in the Capsella bursa-pastoris (L.) Medik. in Egypt into morphotypes, these results were verified later as genetically distinct biotypes by molecular approach by Amer et al. (2020). Linear-terete, erect, and spreading fruit was the common character in the studied populations, congruent with Gabr (2018). The var. recurvata characterized by recurved fruit (Tackholm, 1974) not traced during this study, similar data were reported in Egypt by Abdelhameed et al. (2020). The fruit traits were delimited the studied forms (Annex); this result is supported by Amer et al. (2019a) in delimiting the Brassica nigra varieties.

4.2. Seed sculpture and its taxonomic significance:

Seed coat structure and sculpture are conservative and stable characters, which have been used successfully in taxonomy and phylogeny of different taxa (El Naggar, 2005; Goda, 2018). The seed sculpture of Brassicaceae was applied to distinguish between Egyptian members of Brassicaceae including Brassica tournefortii (El Naggar, 1992). The current study used the SEM of the seed sculpture (Fig. 4) to assess the morphologically identified Brassica tournefortii Forms. The seed sculpture of the identified Forms distinguished them into three patterns: papillate (Form 3, 4 and 5), reticulate (Form 1), and reticulate-foveolate (Form 2). These sculpturing were already reported, for Egyptian specimens, by Kasem et al. (2011), Tantawy et al. (2004), and El Naggar (2005). Then, the morphological characters and seed sculpture showed its taxonomic potentialities at the infra-specific level of Brassica tournefortii.

Table 4
CCA analysis results showing the inter-set correlation of the soil variables together with eigenvalues of the studied Brassica tournefortii Forms.

| Eigenvalue | Axis 1 | Axis 2 | Axis 3 | Axis 4 |
|------------|--------|--------|--------|--------|
| pH         | 0.689104 | 0.144124 | 0.369861 | -0.296883 |
| EC         | -0.297309 | -0.325004 | -0.83592 | -0.0140878 |
| SP         | 0.70703 | 0.272903 | 0.8051582 | 0.395777 |
| Ca²⁺       | -0.303423 | -0.0412736 | -0.886014 | -0.335602 |
| Mg²⁺       | -0.415462 | -0.232908 | -0.798197 | -0.412512 |
| Na⁺        | -0.232708 | -0.473286 | -0.709608 | 0.143502 |
| K⁺         | -0.213401 | -0.413586 | -0.926408 | 0.126643 |
| HCO₃⁻       | -0.253556 | -0.704459 | 0.0529331 | 0.0420772 |
| Cl⁻        | -0.256726 | -0.52492 | -0.42353 | 0.0903005 |
| SO₄²⁻       | -0.490832 | -0.0732143 | -0.70948 | -0.13691 |
| Fine sand  | 0.165636 | 0.267724 | 0.601651 | -0.409942 |
| Clay       | 0.528133 | 0.647164 | -0.223969 | 0.020504 |
| Silt       | -0.155239 | -0.163519 | -0.354952 | 0.772959 |
| Coarse sand| 0.439864 | -0.253496 | 0.860021 | -0.00810499 |

Table 5
Isolated ecto-mycoflora species from the five Brassica tournefortii Forms, collected from Beni-Suef university experimental garden under the same conditions.

| Form  | Alternaria alternata (Fr.) Keissl | Cladosporium oxysporum Berk & Curtis | Cladosporium tenuissimum Cooke | Penicillium chrysogenum Thom | Penicillium citrinum Thom | Penicillium raistrickii Smith |
|-------|----------------------------------|-------------------------------------|-------------------------------|-----------------------------|--------------------------|-----------------------------|
| Fungi | 0                                | 1                                   | 1                             | 0                           | 0                         | 0                           |
| Species|                                  |                                      |                               |                             |                          |                             |
|       |                                  |                                      |                               |                             |                          |                             |

Table 6
Number of isolated colonies, Relative frequencies, and Colonization frequencies of the isolated fungal species from the studied Brassica tournefortii five Forms.

| Form Species          | Number of pure isolated colonies | Relative frequency (RF%) | Colonization frequency (CF%) |
|-----------------------|----------------------------------|--------------------------|------------------------------|
|                       | F1 | F2 | F3 | F4 | F5 | F1 | F2 | F3 | F4 | F5 | F1 | F2 | F3 | F4 | F5 |
| Alternaria alternata (Fr.) Keissl | -  | 3  | -  | -  | 1  | -  | 18.75 | -  | -  | 16.66 | -  | 16.66 |
| Cladosporium oxysporum Berk & Curtis | -  | 1  | -  | 1  | 1  | -  | 6.25  | -  | 50 | 16.66 | 50 | 16.66 |
| Cladosporium tenuissimum Cooke | -  | 8  | 1  | 1  | 2  | -  | 50    | 100 | 50 | 33.33 | -  | 33.33 |
| Penicillium chrysogenum Thom | -  | 1  | -  | -  | 2  | -  | 6.25  | -  | -  | 33.33 | -  | -  |
| Penicillium citrinum Thom | -  | 1  | -  | -  | -  | -  | 6.25  | -  | -  | -  | -  | -  |
| Penicillium raistrickii Smith | -  | 2  | -  | -  | -  | -  | 12.5  | -  | -  | -  | -  | -  |
| Total                 | 0  | 16 | 1  | 2  | 6  | -  | 58.33 | 4.16 | 8.33 | 20.83 | -  | -  |
4.3. Soil factors and spatial distribution of the identified forms:

The data obtained from the present study was congruent with that reported by El-Gawad (2014), who claimed that *Brassica tournefortii* expressed a highly significant correlation with sand, calcium, and magnesium. This species is susceptible to salinity (El-Gawad, 2014), the studied Forms can tolerate high sodium and pH (up to 34.8 mEq/L and 7.99; respectively, Table 3).

The field data revealed the co-distribution of the five identified Forms (F1-F5), along their geographical range in Egypt. It distributed mainly along in the Western desert and Mediterranean strip dominating the newly reclaimed land; this is confirmed by Boulos (2009) and El-Gawad (2014).

4.4. Pollen ecto-mycoflora:

To the best of our knowledge, this is the first study dealing with the associated ecto-mycoflora to flower anthers at the infra-specific level. However, earlier studies on leaf and/or anthers ecto-mycoflora of wild species in the Arab region were carried out to the species level (Abdel-Hafez and El Naggar, 2006, 2001; El-Naggar et al., 1993; El Naggar and Abdel-Hafez, 2007; El Naggar and Abdel Hafez, 2003). The studied forms revealed the presence of six fungal species (Table 6). *Alternaria alternata* (Fr.) Keissl detected in Forms nos. 2 and 5, is a common saprophyte on leaf surface of several plant species wild species (*Adonis dentata*, *Erodium laciniatum*, *Malva parviflora*, *Matthiola longipetala* and *Papaver dubium*) from Burg El Arab, Egypt (El Naggar and Abdel Hafez, 2003). It was also, detected as endophytic fungi in *Brassica napus* (Card et al., 2015). Relevant fungal species (*Alternaria alternata* (Fr.) Keissl and *Cladosporium herbarum* (Pers.) Link) isolated from leaves of the Egyptian wild species namely: *Solanum elaeagnifolium*, *Pluchea dioscoridis*, *Haplophyllum tuberculatum* and *Cleome amblyocarpa* in addition to *Penicillium chrysogenum* and *Penicillium citrinum* was also isolated from the latter species and *Pluchea dioscoridis* (El Naggar and Abdel Hafez, 2003). Among the identified fungi *Cladosporium tenuissimum*
Cooke is the common species in the studied Forms (it traced in 4 out of 5 forms), *Cladosporium* sp. was isolated from anthers of *Withania somnifera* (Naggar and Sallam, 2009) and from *Sorbus domestica* (Card et al. 2015). The dominance of *Cladosporium* sp. over *Penicillium* and *Alternaria*, is not the case in other studies among them Card et al. (2015) on Sorbus domestica, where *Penicillium* sp. was the dominant species with high frequency. Lack of fungal species in Form 1 needs future work for clarification, however, this data supporting the morphological data where Form 1 separated in a distinctive cluster; confirming its uniqueness.

5. Conclusions

The current study revealed that the studied *Brassica tournefortii* that showed a notable phenotypic plasticity can be distinguished based on the seed sculpture, also the CCA exhibit a clear correlation between soil variables and the identified forms.

| Character | Form 1 | Form 2 | Form 3 | Form 4 | Form 5 |
|-----------|--------|--------|--------|--------|--------|
| **Stem:** |        |        |        |        |        |
| 1. Basal branching (<3 = 1 & up to three = 0) | 1 | 0 | 0 | 0 | 0 |
| 2. Upper branching (slightly branched = 1 & not so = 0) | 1 | 0 | 1 | 1 | 1 |
| 3. Hair density (dense allover = 1 & dense at base = 0) | 1 | 1 | 1 | 0 | 0 |
| 4. Height cm (up to 40 = 1 & >40 = 0) | 1 | 0 | 0 | 0 | 0 |
| **Basal leaves:** |        |        |        |        |        |
| 5. Leaf base (petiolate = 1 & sessile = 0) | 1 | 1 | 1 | 1 | 1 |
| 6. Leaf shape | 1 | 1 | 0 | 1 | 1 |
| 7. (pinnatissect = 1 & not so = 0) | 1 | 1 | 0 | 1 | 1 |
| 8. Margin (serrate-dentate = 1 & others = 0) | 0 | 1 | 1 | 1 | 1 |
| 9. Apex (obtuse acute = 1 & acute = 0) | 0 | 1 | 0 | 0 | 1 |
| 10. Hairs density (dense = 1 & not so = 0) | 0 | 1 | 0 | 1 | 1 |
| 11. Terminal lobe (ovate = 1 & not so = 0) | 0 | 1 | 0 | 1 | 1 |
| 12. Lateral lobes (triangular = 1 & not so = 0) | 1 | 1 | 0 | 0 | 0 |
| 13. Lateral lobes arrangement (clear opposite = 1 & not so = 0) | 0 | 0 | 1 | 0 | 0 |
| 14. Petiole L cm (up to 5 = 1 & <5 = 0) | 1 | 1 | 0 | 1 | 0 |
| 15. Lamina L cm (equal or < 15 = 1 & >15 = 0) | 1 | 0 | 1 | 0 | 0 |
| 16. Lamina W cm (equal 5 = 1 & >5 = 0) | 1 | 1 | 0 | 1 | 1 |
| 17. Terminal lobe L cm (equal 5 = 1 & >5 = 0) | 1 | 0 | 1 | 0 | 1 |
| 18. Terminal lobe W cm (equal or < 5 = 1 & >5 = 0) | 1 | 0 | 1 | 1 | 1 |
| 19. Number of lateral lobe pairs (equal or < 5 = 1 & >5 = 0) | 1 | 0 | 1 | 0 | 0 |

CRediT authorship contribution statement

Walaa Hassan: Conceptualization, Supervision, Data curation, Visualization, Writing - original draft. Asmaa Abdelhameed: Formal analysis, Investigation. Najla Al Shaye: . Wafaa Amer: .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Annex . Macro-morphological characters of the studied Forms of *Brassica tournefortii*

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| Character                                      | Form 1 | Form 2 | Form 3 | Form 4 | Form 5 |
|-----------------------------------------------|--------|--------|--------|--------|--------|
| Cauline leaves:                               |        |        |        |        |        |
| 20. Leaf base                                 | 1      | 1      | 0      | 1      | 1      |
| (petiolate = 1 & not so = 0)                  |        |        |        |        |        |
| 21. Shape                                     | 1      | 0      | 0      | 0      | 0      |
| (linear = 1 & others = 0)                     |        |        |        |        |        |
| 22. Margin                                    | 1      | 0      | 0      | 1      | 1      |
| (serrate = 1 & others = 0)                    |        |        |        |        |        |
| 23. Apex                                      | 1      | 1      | 1      | 0      | 1      |
| (acute = 1 & others = 0)                      |        |        |        |        |        |
| 24. Hairs                                     | 1      | 1      | 1      | 1      | 1      |
| (dense = 1 & sparse = 0)                      |        |        |        |        |        |
| 25. Terminal lobe                             | 0      | 0      | 1      | 1      | 1      |
| (lanceolate = 1 & others = 0)                 |        |        |        |        |        |
| 26. Presence of wings in terminal lobe        | 0      | 0      | 1      | 1      | 1      |
| (present = 1 & absent = 0)                    |        |        |        |        |        |
| 27. Shape of the lateral lobes                | 0      | 0      | 1      | 0      | 0      |
| (triangular = 1 & others 0)                   |        |        |        |        |        |
| 28. Arrangement of lateral lobes              | 0      | 1      | 1      | 0      | 0      |
| (clear opposite = 1 & others 0)               |        |        |        |        |        |
| 29. Petiole L cm                              | 0      | 1      | 1      | 0      | 1      |
| (up to 2 = 1 & >2 = 0)                        |        |        |        |        |        |
| 30. Lamina L cm                               | 1      | 0      | 1      | 0      | 1      |
| (up to 7 = 1 & >7 = 0)                        |        |        |        |        |        |
| 31. Lamina W cm                               | 1      | 0      | 1      | 0      | 1      |
| (up to 1 = 1 & >1 = 0)                        |        |        |        |        |        |
| 32. Terminal lobe L cm                        | 0      | 0      | 1      | 0      | 1      |
| (up to 3 = 1 & not so = 0)                    |        |        |        |        |        |
| 33. Terminal lobe W cm                        | 0      | 1      | 0      | 1      | 0      |
| (up to 4 = 1 & not so = 0)                    |        |        |        |        |        |
| 34. Number of lateral pairs                   | 0      | 0      | 1      | 0      | 0      |
| (up to 3 = 1 & not so = 0)                    |        |        |        |        |        |
| Inflorescence bract:                          |        |        |        |        |        |
| 35. Leaf base                                 | 0      | 0      | 1      | 0      | 0      |
| (clear sessile = 1 & not so = 0)               |        |        |        |        |        |
| 36. Shape                                     | 0      | 0      | 0      | 1      | 1      |
| (linear lanceolate = 1 & others = 0)          |        |        |        |        |        |
| 37. Margin                                    | 1      | 0      | 0      | 0      | 0      |
| (clear serrate = 1 & others = 0)              |        |        |        |        |        |
| 38. Apex                                      | 1      | 1      | 1      | 1      | 1      |
| (acute = 1 & others = 0)                      |        |        |        |        |        |
| 39. Hairs density                             | 1      | 1      | 1      | 1      | 1      |
| (moderate = 1 & dense = 0)                    |        |        |        |        |        |
| 40. Petiole L cm                              | 1      | 0      | 0      | 0      | 1      |
| (up to 0.5 = 1 & not so = 0)                   |        |        |        |        |        |
| 41. Lamina L cm                               | 0      | 1      | 0      | 1      | 0      |
| (>4 = 1 & equal or < 4 = 0)                    |        |        |        |        |        |
| Inflorescence:                                |        |        |        |        |        |
| 42. Number/Plant                              | 1      | 1      | 1      | 0      | 1      |
| (>100 = 1 & < 100 = 0)                        |        |        |        |        |        |
| 43. Length cm                                 | 0      | 0      | 1      | 1      | 1      |
| (up to & >50 = 1 & < 50 = 0)                   |        |        |        |        |        |
| 44. Number of Fruits/inflorescence cm         | 1      | 0      | 0      | 0      | 0      |
| (up to 20 = 1 & up to > 20 = 0)                |        |        |        |        |        |
| Fruit:                                        |        |        |        |        |        |
| 45. Fruiting part L cm                        | 0      | 0      | 0      | 1      | 1      |
| (>4 = 1 & equal or < 4 = 0)                    |        |        |        |        |        |
| 46. Beak length cm                            | 1      | 1      | 1      |        |        |
| (up to 1.5 = 1 & up to > 1.5 = 0)              |        |        |        |        |        |

(continued on next page)
### Character Form

| Character | Form 1 | Form 2 | Form 3 | Form 4 | Form 5 |
|-----------|--------|--------|--------|--------|--------|
| 47. Pedicel L cm | 1 | 0 | 1 | 0 | 0 |
| (up to 1.5 = 1 & up to > 1.5 = 0) | | | | | |
| 48. Number of seeds/fruit | 0 | 1 | 1 | 1 | 1 |
| (up to 30 = 1 & not so = 0) | | | | | |
| 49. Number of seeds in beak | 1 | 0 | 1 | 0 | 0 |
| (0–1 = 1 & 0–2 = 0) | | | | | |
| 50. Number of valve veins | 0 | 1 | 1 | 1 | 1 |
| (up to 1–3 = 1 & 1–2 = 0) | | | | | |
| **Flower:** | | | | | |
| 51. Pedicel surface | 1 | 0 | 0 | 0 | 0 |
| (hairy = 1 & glabrous = 0) | | | | | |
| 52. Pedicel L cm | 0 | 1 | 1 | 1 | 1 |
| (up to 1.5 = 1 & up to > 1.5 = 0) | | | | | |
| **Sepal:** | | | | | |
| 53. L cm | 1 | 1 | 0 | 0 | 1 |
| (up to 0.5 = 1 & up to 0.3 = 0) | | | | | |
| 54. Surface | 1 | 0 | 0 | 0 | 0 |
| (hairy = 1 & glabrous = 0) | | | | | |
| 55. Base shape | 1 | 1 | 1 | 1 | 1 |
| (sucate = 1 & not so = 0) | | | | | |
| 56. Patent | 1 | 1 | 1 | 1 | 1 |
| (erect – spreading = 1 & not so = 0) | | | | | |
| 57. Persistence | 1 | 1 | 1 | 1 | 1 |
| (caduceus = 1 & not so = 0) | | | | | |
| 58. Shape | 1 | 1 | 1 | 1 | 1 |
| (elliptical –oblong = 1 & not so = 0) | | | | | |
| 59. Apex | 1 | 1 | 1 | 1 | 1 |
| (acute = 1 & not so = 0) | | | | | |
| 60. Margin | 1 | 1 | 1 | 1 | 1 |
| (entire = 1 & not so = 0) | | | | | |
| **Petal:** | | | | | |
| 61. L × W cm | 0 | 1 | 0 | 1 | 1 |
| (0.5 × 0.2 = 1 & not so = 0) | | | | | |
| 62. Shape | 1 | 1 | 1 | 1 | 1 |
| (obovate = 1 & not so = 0) | | | | | |
| 63. Apex | 1 | 1 | 1 | 1 | 1 |
| (rounded = 1 & not so = 0) | | | | | |
| 64. Margin | 1 | 1 | 1 | 1 | 1 |
| (entire = 1 & not so = 0) | | | | | |
| 65. Color | 0 | 1 | 1 | 1 | 1 |
| (yellow = 1 & yellow-creamy = 0) | | | | | |
| **Outer stamen (2):** | | | | | |
| 66. Filament L cm | 1 | 0 | 0 | 0 | 1 |
| (up to 0.3 = 1 & < 0.3 = 0) | | | | | |
| 67. Anther L mm | 1 | 0 | 0 | 1 | 0 |
| (1.0 = 1 & 2.0 = 0) | | | | | |
| **Inner stamen (4):** | | | | | |
| 68. Number | 1 | 1 | 1 | 1 | 1 |
| (4 = 1 & not so = 0) | | | | | |
| 69. Filament L cm | 0 | 1 | 1 | 0 | 1 |
| (up to 0.5 = 1 & not so = 0) | | | | | |
| 70. Anther L mm | 1 | 0 | 0 | 1 | 0 |
| (1.0 = 1 & 2.0 = 0) | | | | | |
| 71. Anther shape | 1 | 1 | 1 | 1 | 1 |
| (oblong = 1 & not so = 0) | | | | | |
| 72. Ovary L cm | 1 | 0 | 1 | 1 | 0 |
| (up to 0.5 = 1 & not so = 0) | | | | | |
| 73. Style L mm | 0 | 0 | 0 | 1 | 1 |
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