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AUTO-TAXONOMY OF *BRASSICA TOURNEFORTII* GOUAN. (BRASSICACEAE) IN EGYPT

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Abstract

Brassica tournefortii Gouan. (family Brassicaceae) is one of the five species in the Egyptian flora. Its populations showed notable morpho-plasticity with taxonomic debates, which were not yet resolved. The current study was carried out to assess the species morpho-plasticity and its molecular identity based on ISSR. The study was applied to 27 herbarium and fresh populations, representing all the species distribution ranges in Egypt. The taxonomic revision included 70 morphological characters, revealed five distinct Forms (1-5), radical leaf, and fruit provided the major distinguishable traits among the studied 70 morphological characters based on them the morphologic key is provided to delimit these forms. The pollen grain features using SEM are a pioneer at the infra-specific level, two shapes observed the subprolate (Forms 1& 3) and prolate (Forms 2, 4 & 5). Furthermore, the exine micro-features possess taxonomic value at the infra-specific level. The cluster analysis based on ISSR data revealed two clusters congruent to those developed by morphological and pollen traits. The ISSR results indicated that the species morpho-plasticity is genetically controlled. The study highlights the importance of the multidisciplinary approach to assess the taxonomic identity at the infra-specific level, for the auto-taxonomy of morpho-plastic species.

Introduction

Brassicaceae (Cruciferae) is a monophyletic family, distributed in all continents with high diversity in Irano-Turanian, Mediterranean, and west N. American regions (Taiyan *et al.*, 2001). It includes 3977 species under 351 genera and 52 tribes (The Plant List, 2013).

Genus *Brassica* L. considered one of the most economically important genera of the tribe Brassiceae, with highly diverse morphology and wide-ranging utility, represented by 80 accepted species worldwide (The Plant List, 2013; Amer *et al.*, 2019a). Genus *Brassica* L. in Egypt includes five species namely: *B. rapa* L., *B. tournefortii* Gouan., *B. nigra* (L.) Koch, *B. deserti* Danin & Hedge, and *B. juncea* (L.) Czernj. & Coss. (Amer *et al.*, 2019a)**;** the first three species are widespread in Egypt, while the others are rare (Boulos, 1995, 1999, 2009).

The ecological range of *B. tournefortii* extends from the Mediterranean basin and much of the Middle East (including Egypt) to western India (Aldhebiani and Howladar, 2013). It was recorded as invasive species outside its ecological range it began to spread quickly throughout the southwest USA, northern & central Mexico, and Australia (Minnich and Sanders, 2000; VanTassel *et al.*, 2014). Various studies were carried out to detect its phenological, ecological impact, and management (Marushia, 2009; Marushia *et al*., 2010; Marushia *et al.*, 2012; Berry *et al.*, 2014; Abd El-Gawad, 2014; Winkler *et al.*, 2018).

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In Egypt, *B. tournefortii* distributed in all the phytogeographic regions except the Sinai Peninsula (Boulos, 1995), as a dominant weed in the newly reclaimed land (Abd El-Gawad, 2014). It possesses rapid phenological diversity compared to its relatives (Marushia *et al*., 2010). Worldwide, this infra-specific diversity induced taxonomic debates among four identified varieties (var. *dasycarpa* O.E. Schulz, var. *leiocarpa* Maire. & Weiller var. *recurvata* Bornm. and var. *sisymbroides* Fisch. ex DC.) and one forma (f. *dentata* O.E. Schulz), now all of them are grouped as species synonym (The Plant List, 2013). This taxonomic uncertainty also extended to Egypt where two varieties *i.e. B. tournefortii* var. *dentata* O.E. Schulz with simple dentate leaves, and var. *recurvate* Bornm. in which the fruit recurved on the stem. Later, both varieties were treated as *B. tournefortii* synonyms (Boulos, 2009, 1999).

Though Brassicaceae is a stenopalynous at the family level (Al-Shehbaz *et al*., 2006), the pollen characters are useful for assessing phenetic relationships and resolving taxonomic problems at the family, generic, and specific level (Anchev and Deneva, 1997; Carlo and Paula, 2004). Recently, SEM was applied to study the pollen grains at the infra-specific level in both of *Brassica nigra* biotypes (Amer *et al.*, 2019a), and *Capsella bursa-pastoris* genotypes (Amer *et al*., 2019b) in Egypt.

ISSR markers can differentiate the closely related species, at the interspecies level and assess the genetic relationships (Zietkiewicz *et al.*, 1994; Amer *et al.*, 2014). Brassicaceae was subjected to several molecular studies for generic delimitations and an understanding of its phylogenetic relationships (Warwick *et al.*, 2010). Liu and Wang (2006), applied the ISSR markers to prove the genomic evolution of *Brassica* allopolyploids in 35 genotypes of *Brassica* sp.

This work was carried out to assess the taxonomic identity of the *B. tournefortii* at the infra-specific level, through a multidisciplinary approach, including macro-morphological, SEM-micro-morphological traits and the retrieved data confirmed using ISSR markers.

Materials and Methods

Plant materials

Twenty-seven plant samples and their seeds were collected from different populations of *Brassica tournefortii* distributed in various localities in Egypt during the spring of 2017 and 2018. The herbarium specimens preserved at Cairo University Herbarium (CAI), Assiut University Herbarium (ASTU), and Beni-Suef University Herbarium (BNSU) were also studied. Fresh flowering and fruiting specimens of ten representative individuals were preserved in FAA (50 ml ethyl alcohol, 10 ml formaldehyde, 5 ml glacial acetic acid, and 35 ml distilled water) for further study.

Macro-morphological characters

Macro-morphological characters of the fresh and herbarium specimens, including stem, leaves, inflorescences, flowers, fruits, and seeds were investigated.

Micro-morphological characters

Fresh anthers were collected from the floral buds of the representative populations, cultivated in the experimental garden of Beni-Suef University and investigated pollen morphology using Scanning Electron Microscope (SEM) according to Punt *et al.* (1994).

Molecular investigation

Genomic DNA was collected and purified from juvenile leaf samples of the taxonomically identified five *B. tournefortii* Forms (1-5) grown in GBSU using DNeasy plant mini kit (Qiagen). Five complimentary ISSR primers 14A (5`-CTCTCTCTCTCTCTCTTG-3`), 44B (5`-CTCTCTC-

TCTCTCTCTGC-3`), HB-9 (5`-GTGTGTGTGTGTGG-3`), HB-12 (5`-CACCACCACGC3`), and HB-15 (5`-GTGGTGGTGGC-3`) were used to perform amplification of genomic DNA according to Murray and Thompson (1980) and Williams *et al.* (1990).

Data analysis

The retrieved data from the morphology, pollen grains, and ISSR marker studies were subjected to statistical analysis. For cluster analysis dendrograms were generated by the similarity matrices using "R" software for windows version 3.5.1. Genetic Similarity coefficient (GS) among the studied Forms was carried out by the Dice coefficient formula (Sneath and Socal, 1973):

Dice formula: GSij = $2a/(2a + b + c)$

Where *GSij* represents the measure of genetic similarity between Forms "*i*" and "*j*", "*a*" is the number of characters shared by Forms "*i*" and "*j*", "*b*" is the number of characters present in "Form *i*" and absent in "Form *j*", while "*c*" is the number of characters present in "Form *j*" and absent in "Form *i*".

Results and Discussion

Species morphology:

Annual erect and spreading herb, up to 80 (-95) cm, stem more or less branched from the base. Leaves and stem densely covered with stiff retrorse white hairs. Radicle (basal) leaves rosette-forming, 4-25 (-40) × 2-6 (-12) cm, shortly to broadly petiolate, pinnatipartite-pinnatisect with 2-15 pairs of patent or slightly recurved oblong-obovate lateral lobes with serrate-dentate margin, obtuse-acute apex, terminal lobe lanceolate winged to broadly ovate and sometimes rhombic with obtuse-acute apex. Cauline (upper) leaves sessile-short petiolate, linear lanceolate-pinnatisect, entire-denticulate margin. The inflorescence is subtended by sessile petiolate bracts, 4-6 × 0.5 cm, linear-lanceolate, entire-dentate margin, and acute apex. Inflorescence corymbose 10-20 flowered, flowers yellow, green calyx or somewhat violet 3-5 × 1-2 mm; petal 5-10 × 1-3 mm, linear-oblong, obtuse, long-clawed, often tinged violet at the throat, sometimes white. Ovary c. 15 ovulate. Fruit elongated siliqua arranged in a lax raceme; fruit distinguished into three regions: fruit pedicel, fruiting part, beak; fruit pedicel erect, spreading 1-4 cm., the beak of 1-2 × 0.2-0.3 cm, beak non- 2-seeded; valve with a more or less distinct midrib. Seed shiny brown-red light brown, mucilaginous, and 1-2 × 1.2-1.5 mm in diameter.

Infra-specific diversity

Morphological diversity

Morphological diversity among the studied 27 *B. tournefortii* populations distinguished them into five forms by using 70 macro-morphological characters that were presented in Table 1 and Figs. 2–5. Macro-morphological characters were grouped the studied populations into five distinct forms based on the following key:

1	Radicle leaves pinnatipartite-pinnatisect, terminal lobe rhombic-triangular	**Form 3**
2	Radicle leaves pinnatisect, terminal lobe not so.	
a	Leaf with no clear midrib, lateral lobes pseudo-alternate lobes	**Form 2**
b	Leaf with clear midrib, lateral lobes opposite-alternate	
●	Terminal lobe cut to midrib (not winged)	**Form 5**
●	Terminal lobe not cut to midrib (winged)	
✓	Terminal lobe mostly lanceolate, fruit pedicel up to 1.5 cm	**Form 1**
✓	Terminal lobe ovate, fruit pedicel up to 2.5 cm	**Form 4**

Table 1. Macro-morphological characters of the five forms of *Brassica tournefortii* Gouan.

Character	Form 1	Form 2	Form 3	Form 4	Form 5
Stem:					
1. Basal branching	1-2 axes	3 axes	1-3 axes	1-3 axes	1-3 axes
2. Upper branching	Slightly	Slightly-much	Slightly	Slightly	Slightly
3. Hair density	Dense allover	Dense allover	Dense allover	Dense on basal part	Dense on basal part
4. Height cm	Up to 40	Up to 95	Up to 60	Up to 80	Up to 80
Radicle leaves:					
5. Leaf base	Petiolate	Petiolate	Petiolate	Petiolate	Petiolate
6. Shape -Mid rib	Pinnatisect	Pinnatisect	Pinnatipartite-sect	Pinnatisect	Pinnatisect
7. clarity	Clear	Not	Clear	Clear	Clear
8. Margin	Serrate – dentate	Serrate-dentate	Serrate	Serrate-dentate	Serrate-dentate
9. Apex	Acute	Obtuse-acute	Obtuse -acute	Obtuse-acute	Obtuse-acute
10. Hairs density	Moderate	Dense	Moderate	Moderate- dense	Dense
11. Terminal lobe	Lanceolate-ovate	Ovate-lanceolate	Rhombic-Triangular	Ovate winged	Ovate
12. Lateral lobes	Triangular	Elliptic-triangular	Rhombic	Elliptical-Oblong	Elliptical-Oblong
13. Lateral lobes 14. arrangement	Pseudo-opposite-opposite	Pseudo-alternate	Opposite	Alternate	Opposite-pseudo-opposite
15. Petiole L cm	Up to 5	Up to 5	Up to 2	Up to 5	Up to 3
16. Lamina L×W cm	15 × 5	40 × 5	7 × 2	30 × 5	25 × 5
17. Terminal lobe L×W cm	5 × 3	7 × 8	5 × 4	10 × 5	5 × 5
18. Lateral lobe pairs	2–5	5–15	2–5	2–10	2–10
Cauline leaves:					
19. Leaf base	Petiolate	Petiolate	Sessile-petiolate	Petiolate	Petiolate
20. Shape	Linear lanceolate	Pinnatisect	Pinnatisect -lanceolate	Pinnatisect-lanceolate	Pinnatisect-lanceolate
21. Margin	Serrate	Dentate – serrate	Entire-serrate	Serrate	Serrate
22. Apex	Acute	Acute	Acute	Obtuse-acute	Acute
23. Hairs	Dense	Dense	Dense	Dense	Dense

(Contd.)

Table 1 Contd.

24. Terminal lobe	–	Triangular – lanceolate	Lanceolate winged - lanceolate	Lanceolate winged –ovate	Lanceolate winged
25. Lateral lobes	–	Elliptical oblong-triangular	Triangular	Elliptical oblong	Elliptical oblong
26. Position of lateral lobes	–	Opposite	Opposite	Opposite-alternate	Opposite-alternate
27. Petiole L cm	Up to 3	Up to 2	Up to 1	Up to 3	Up to 1
28. Lamina L×W cm	6 × 1	16 × 4	5 × 1	19 × 5	7 × 1
29. Terminal lobe L×W cm	–	5 × 4	3 × 1	7 × 3	2 × 1
30. No. lateral pairs	–	3–7	1–3	1–4	1–5
Inflorescence bract:					
31. Leaf base	Sessile-petiolate	Sessile-petiolate	Sessile	Sessile-petiolate	Sessile-petiolate
32. Shape	Linear lanceolate-lanceolate	Linear lanceolate -pinnatisect	Linear lanceolate-lanceolate	Linear lanceolate	Linear lanceolate
33. Margin	Serrate	Dentate -serrate	Entire-serrate	Entire-serrate	Entire-serrate
34. Apex	Acute	Acute	Acute	Acute	Acute
35. Hairs density	Moderate	Moderate	Moderate	Moderate	Moderate
36. Petiole L. cm	Up to 0.5	Up to 1.5	-	Up to 2.5	Up to 0.5
37. Lamina L×W cm	4 × 0.5	5 × 0.5	4 × 0.5	6 × 0.5	3.5 × 0.5
Inflorescence:					
38. Number/Plant	Up to 40	Up to 70	Up to 25	Up to 135	Up to 85
39. Length cm	Up to 20	Up to 25	Up to 50	Up to 70	Up to 50
40. Number of Fruits	Up to 20	Up to 35	Up to 30	Up to 30	Up to 25
Fruit:					
41. Fruiting part L×W cm	3.5 × 0.1	4 × 0.1	4 × 0.3	5 × 0.3	5.5 × 0.2
42. Beak length cm	Up to 1.5	Up to 1.5	Up to 1.5	Up to 2	Up to 2
43. Pedicel L cm	Up to 1.5	Up to 2	Up to 1	Up to 2.5	Up to 2.5
44. Seeds/fruit	Up to 25	Up to 30	Up to 30	Up to 30	Up to 30
45. Seeds/beak	0-1	1-2	0-1	0-2	0-2
46. Valve veins	1-2	3-4	3	1-3	1-3

Table 1 Contd.

Flower:					
47. Pedicel surface	Hairy	Glabrous	Glabrous	Glabrous	Glabrous
48. Pedicel L cm	Up to 2	Up to 1.5	Up to 1	Up to 0.5	Up to 1.5
Sepal:					
49. L×W cm	Up to 0.5 × 0.1	Up to 0.5 × 0.2	Up to 0.3 × 0.1	Up to 0.3 × 0.1	Up to 0.5 × 0.2
50. Surface	Hairy	Glabrous	Glabrous	Glabrous	Glabrous
51. Base	Succate base	Succate base	Succate base	Succate base	Succate base
52. Patent	Erect- spreading	Erect-spreading	Erect-spreading	Erect-spreading	Erect-spreading
53. Persistence	Caduceus	Caduceus	Caduceus	Caduceus	Caduceus
54. Shape	Elliptical-oblong	Elliptical-oblong	Elliptical-oblong	Elliptical-oblong	Elliptical-oblong
55. Apex	Acute	Acute	Acute	Acute	Acute
56. Margin	Entire	Entire	Entire	Entire	Entire
Petal:					
57. L×W cm	1 × 0.2	0.5 × 0.2	0.5 × 0.1	0.5 × 0.2	0.5 × 0.2
58. Shape	Obovate	Obovate	Obovate	Obovate	Obovate
59. Apex	Rounded	Rounded	Rounded	Rounded	Rounded
60. Margin	Entire	Entire	Entire	Entire	Entire
61. Color	Yellow - creamy	Yellow	Yellow	Yellow	Yellow
Outer stamen (2):					
62. Filament L cm	Up to 0.3	Up to 0.2	Up to 0.1	Up to 0.2	Up to 0.3
63. Anther L×W cm	0.1 × 0.1	0.1 × 0.1	0.2 × 0.1	0.1 × 0.1	0.2 × 0.1
Inner stamen (4):					
64. Number	4	4	4	4	4
65. Filament L.cm	Up to 0.6	Up to 0.5	Up to 0.5	Up to 0.3	Up to 0.5
66. Anther L×W cm	0.1 × 0.1	0.2 × 0.1	0.2 × 0.1	0.1 × 0.1	0.2 × 0.1
67. Anther shape	Oblong	Oblong	Oblong	Oblong	Oblong
68. Ovary L cm	Up to 0.5	Up to 0.4	Up to 0.5	Up to 0.5	Up to 0.7
69. Style L cm	Up to 0.1	Up to 0.2	Up to 0.2	Up to 0.3	Up to 0.3
70. Stigma L cm	Up to 0.05	Up to 0.05	Up to 0.04	Up to 0.03	Up to 0.05
71. Style	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
72. Seed Size (mm)	1.30-1.39 × 1.35-1.45	1.19-1.25 × 1.24-1.27	1.23-1.37 × 1.28-1.42	1.28-1.42 × 1.3-1.5	1.22-1.34 × 1.22-1.33

Morphological similarity between the identified forms

The heat map analysis using 70 macro-morphological characters (as shown in Table 1), reveals the dendrogram (Fig. 6), in which the five studied forms grouped into two main clusters, one of them includes Forms 1 and 3, and the other comprises Forms 2, 4, and 5. The last cluster is divided into two sub-clusters, one of which combines Forms 4 and 5, while the other subgroup includes Form 2.

Pollen grains micro-morphological characters

Scanning electron microscopic investigation showed that pollen grains of morphologically identified Forms (1-5) are tricolpate reticulate hetero-brochate (irregular lumen shape and size) exine, the five forms belonging to two pollen shapes; subprolate in Forms 1 and 3 (P/E= 1.17 and 1.32 µm; respectively) and prolate in Forms 2, 4 and 5 where (P/E= 1.34, 1.66 & 1.57; respectively), as outlined in (Table 2 and Fig. 7). These forms are grouped into two clusters congruent to the morphological cluster; the most significant characters are the muri width and the pollen shape (Table 2).

Fig. 1. Distribution map of the studied *B. tournefortii* populations based on collection and herbaria specimens.

Table 2. Pollen features of the five identified forms of *Brassica tournefortii* (mean value within the brackets).

Form number	Polar view (µm)	Equatorial view (µm)	P/E	Muri (W) (µm)	Lumen (L× W) µm	Pollen shape
Form 1	12.339-14.699 (13.67)	9.954-10.931 (10.32)	1.32	0.275-0.421 (0.35)	(0.474-0.906) × (0.288-0.620) (0.68 × 0.50)	Subprolate
Form 2	13.653-14.888 (14.36)	9.818-11.591 (10.75)	1.34	0.311-0.528 (0.41)	(0.541-0.913) × (0.413-0.726) (0.77 × 0.53)	Prolate
Form 3	13.370-19.141 (17.27)	11.064-16.641 (14.76)	1.17	0.209-0.480 (0.33)	(0.638-1.347) × (0.525-0.754) (0.88 × 0.63)	Subprolate
Form 4	12.630-19.994 (17.98)	8.996-15.303 (10.81)	1.66	0.360-0.664 (0.49)	(0.375-0.564) × (0.315-0.482) (0.47 × 0.38)	Prolate
Form 5	16.184-18.414 (17.24)	10.577-11.324 (10.97)	1.57	0.305-0.494 (0.39)	(0.541-0.913) × (0.413-0.726) (1.03 × 0.78)	Prolate

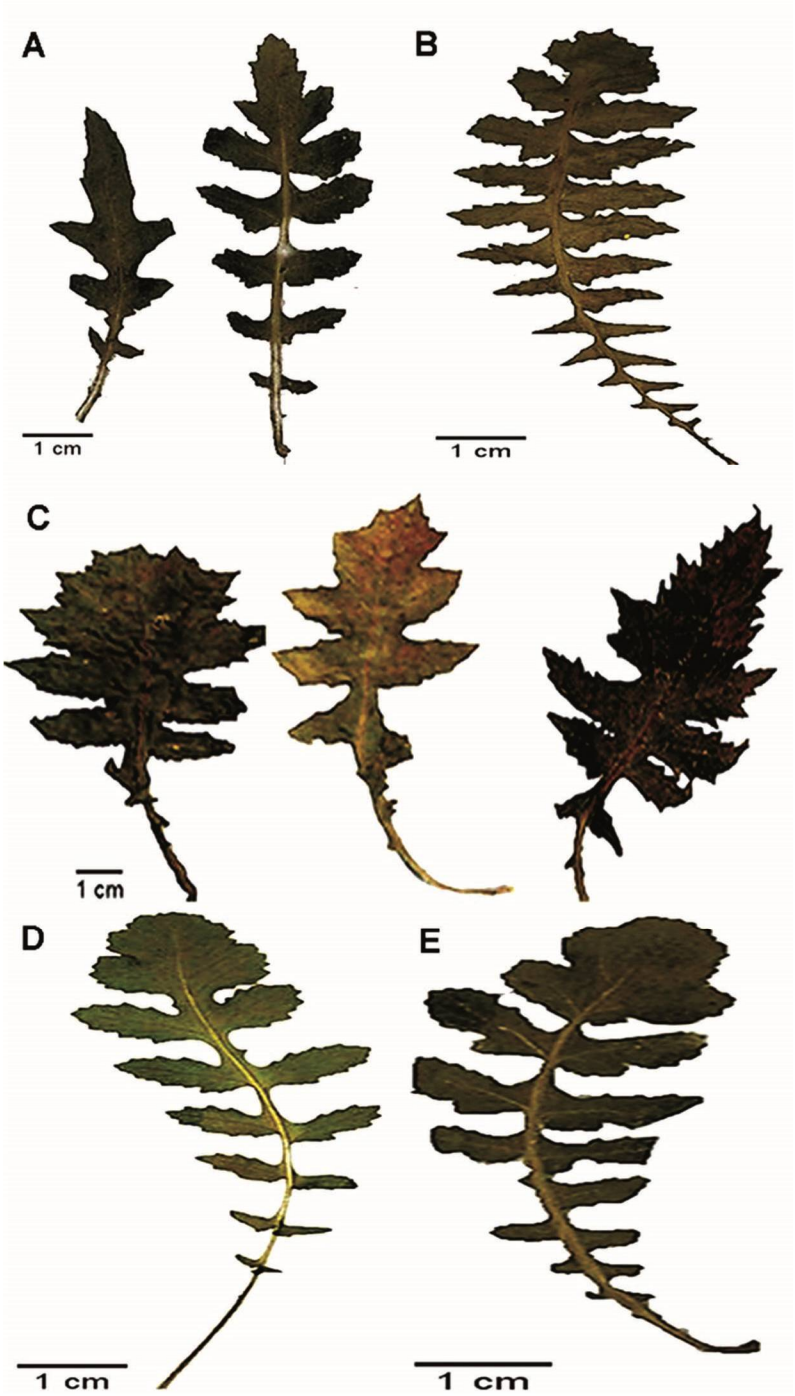

Fig. 2. Morphologic diversity of radical leaves between the five identified *B. tournefortii* forms; A: Form 1, B: Form 2, C: Form 3, D: Form 4 and E: Form 5.

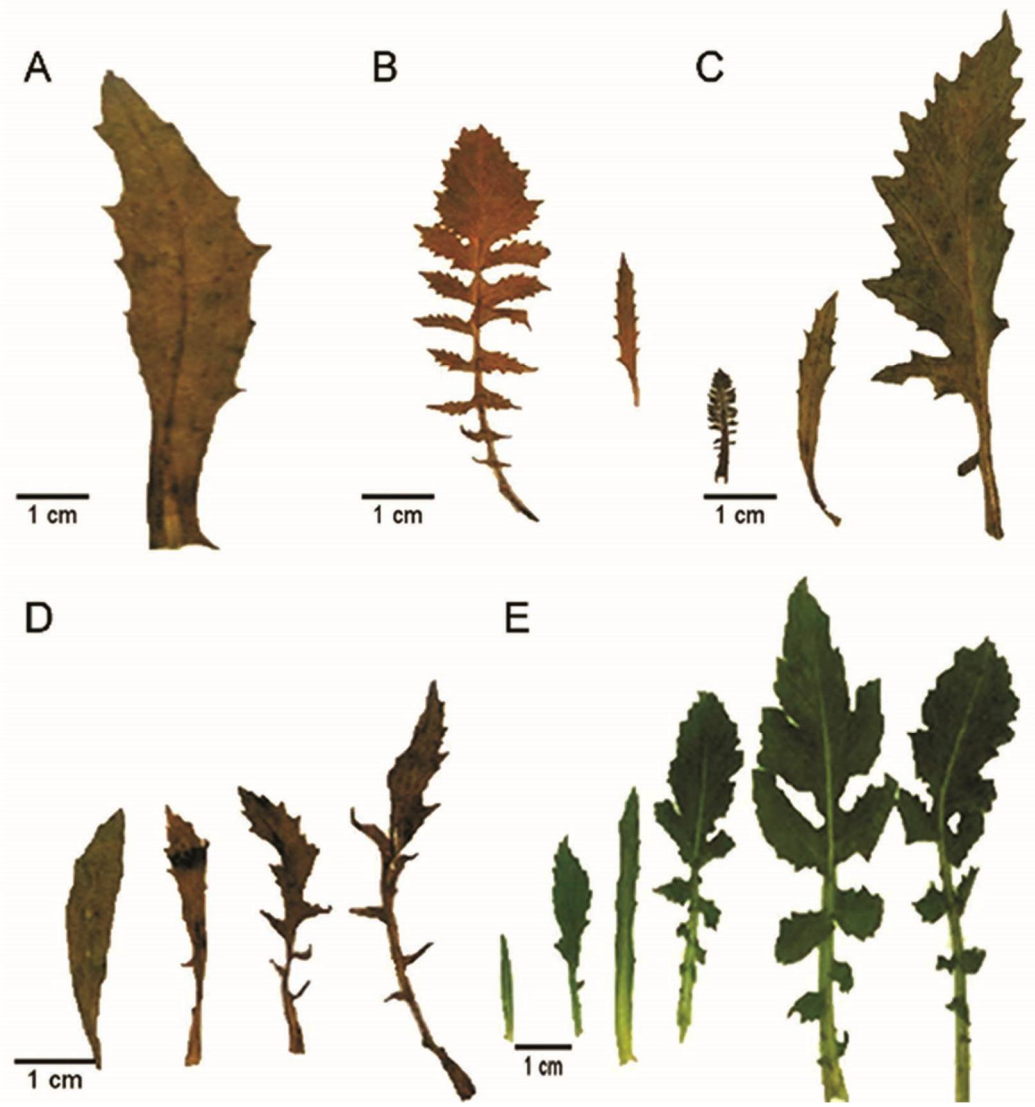

Fig. 3. Morphologic diversity of cauline leaves between the five identified *B. tournefortii* forms; A: Form 1, B: Form2, C: Form 3, D: Form 4 and E: Form 5.

Molecular analysis:

Results of infra-specific molecular investigation of the five Forms using ISSR primers to elucidate the molecular identity of Forms identified by macro-morphological taxonomic key based characters were depicted in Figs. 2-5. The ISSR primers reveal a total of 38 bands across the five Forms (1-5). Out of the amplified bands, 20 bands are polymorphic, reflecting an allelic diversity among the morphologically identified forms (Tables 3-4). We detected the highest polymorphism (77.77%), in Form 1 using primer HB-12, while primers HB-9 and HB-15 showed absence bands with Forms 1 and 5 and Forms 1 and 3; respectively.

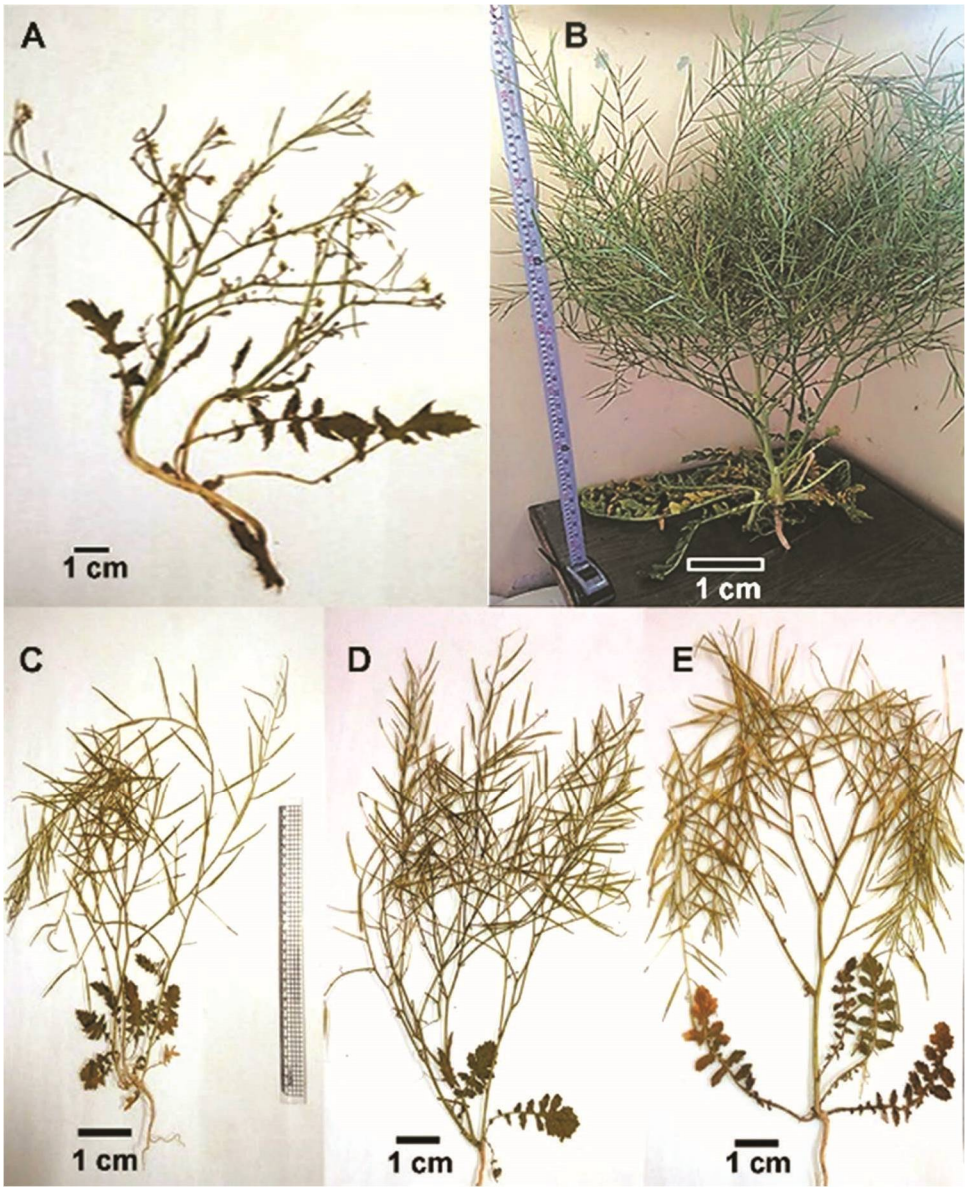

Fig. 4. Morphologic diversity of inflorescences between the five identified *B. tournefortii* forms; A: Form 1, B: Form2, C: Form 3, D: Form 4 and E: Form 5.

The genetic relationships among the studied five populations were constructed using Dice formula *(GSij = 2a/ (2a + b + c).* The highest similarity value (89%) were recorded between Forms 2 and 4. On the other hand, the lowest similarity value (75%) was observed between Forms 3 and 5 (Table 5). Cluster analysis of the DNA bands of the studied Forms (1-5) by UPGMA cluster analysis software produced three clusters; the first one includes Form 2, while the second cluster comprises both Forms 1 and 3, but the last cluster combines Forms 4 and 5 (Fig. 8).

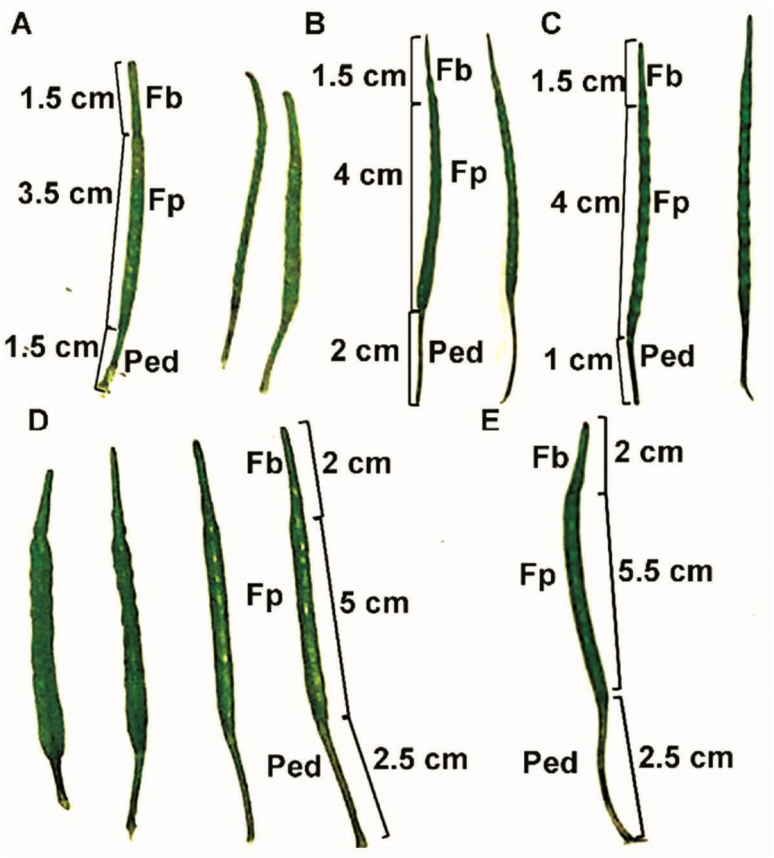

Fig. 5. Morphologic diversity of fruit between the five identified *B. tournefortii* forms; A: Form 1, B: Form2, C: Form 3, D: Form 4 & E: Form 5

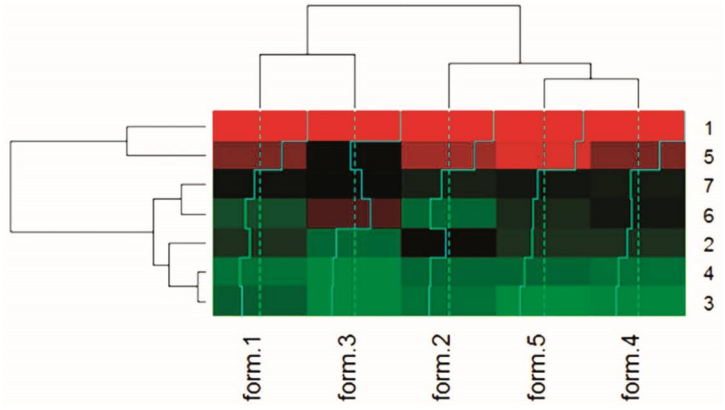

Fig. 6. Heat map with hierarchical clustering of studied five forms (X-axis) and the most seven affected morphological characters (Y-axis). Red indicates a high level of expression; green represents a low level of expression; increasing color intensity is directly proportional to the value of the studied character.

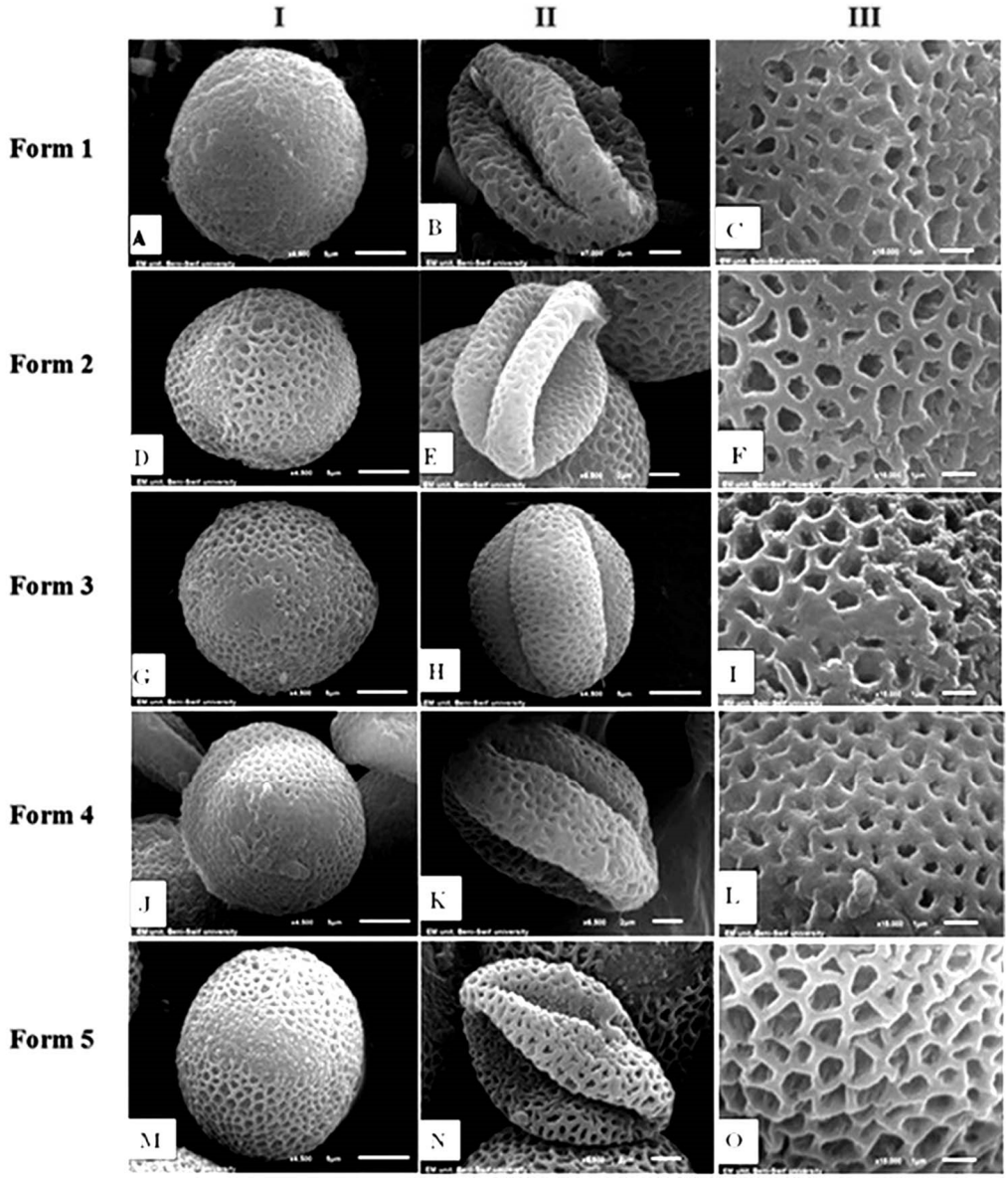

Fig.7. Scanning electron microscope micrographs (I: polar view, II: equatorial view, and III: magnified exine) of the *B. tournefortii* pollen grains of forms 1-5.

Brassicaceae is characterized by remarkable uniformity in the fundamentals of flower and fruit characters (El Naggar, 2000). Despite this, the studied 27 populations of *Brassica tournefortii* Gouan revealed notable phenoplasticity (Table 1 and Figs. 2–5). Congruent infra-specific diversity was also identified as biotypes by Amer *et al.* (2019b), in Egyptian populations of *Capsella bursa-pastoris.* The phenoplasticity of this species was reported earlier in America, which was grouped under five varieties (Maire, 1965).

Table 3. The similarity between the studied forms based on the macro-morphological and pollen characters.

	Form.1	Form.2	Form.3	Form.4	Form.5
Form.1	100				
Form.2	95.8	100			
Form.3	88.5	81.8	100		
Form.4	97.4	98.2	90.3	100	
Form.5	97.5	97.5	86.6	98.6	100

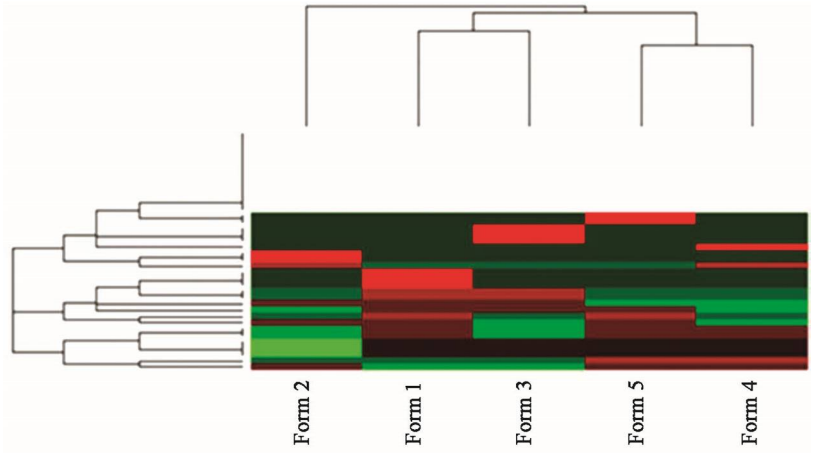

Fig. 8. Heat map with hierarchical clustering of studied five *B. tournefortii* Forms (1-5; X-axis) using ISSR markers, Y-axis representing the developed band. Red indicates a high level of expression; green represents a low level of expression; increasing color intensity is directly proportional to the value of the studied character.

Egyptian forms vs identified varieties

The current study used 70 morphological characters (Table 1), reveals grouping of the studied 27 populations into five distinct Forms (1, 2, 3, 4 & 5; Figs 2–5). These forms mainly depend on the shape of radicle leaves with particular reference to terminal lobe and midrib clarity. Non, of the identified forms, was related to the earlier described varieties and forms (The Plant List, 2013) and those given by Täckholm (1974).

The identified forms showed notable diversity compared to the other relevant specimens described earlier in relative flora. The plant height of the studied five Forms (27 populations), recorded the highest stem length worldwide (95 cm in Form 2) that supported by EL-Habashy *et al.* (2013) and Boulos (1999). Also, it measures 70 cm in Asian specimens, given by Davis *et al.* (1965), from Turkey (Zohary, 1966), from Palestine and Iraq (Townsend and Guest, 1980). The morphological diversity extends to stem branching; the identified forms show sub-simple stem, basi-branched in Forms (2,3,4 & 5), this congruent with specimens of neighbouring areas. While, the samples from Arizona (USA), is simple much-branched above, with incredible size variation (Felger *et al.*, 2015). Here the results described for the first time, the pseudo-dichotomous branching in Form 1.

Table 4. Genetic polymorphism of the studied forms based on ISSR data.

Primer sequence	Form	No. of total amplified bands					No. of polymorphic bands Unique bands between bracts					% of polymorphism/primer				
		F1	F2	F3	F4	F5	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
14 A	$(CT)_8TG$	5	6	8	7	5	1 (0)	2 (0)	4 (0)	3 (0)	1 (0)	20	33.33	50	42.85	20
44 B	$(CT)_8GC$	6	7	5	8	8	2 (0)	3 (0)	1 (0)	4 (0)	4 (0)	33.33	42.85	20	50	50
HB-9	$(GT)_6GG$	3	6	5	4	3	0 (0)	3 (1)	2 (0)	1 (0)	0 (0)	0	50	40	25	0
HB-12	$(CAC)_3GC$	9	8	7	7	4	7 (0)	6 (0)	5 (0)	5 (0)	2 (0)	77.77	75	71.42	71.42	50
HB-15	$(GTG)_3GC$	5	6	5	6	6	0 (0)	1 (0)	0 (0)	1 (0)	1 (0)	0	16.66	0	16.66	16.66
Total	5	28	33	30	32	26	10 (0)	15 (1)	12 (0)	14 (0)	8 (0)	35.74	45.45	40	34.75	3.76

Radicle leaves are the most divergent morphological traits, distinguished the current Egyptian *B. tournefortii* forms, its length up to 40 cm (Fig. 2). Similar data reported earlier in Egypt (Boulos, 1999), and Palestine (Zohary, 1966). Felger *et al.* (2015) reported an extreme leaf length up to 80 cm in *B. tournefortii* invaded Arizona, USA.

Table 5. Similarity values between the studied *Brassica tournefortii* five forms based on ISSR data.

	F1	F2	F3	F4	F5
F1	100				
F2	78	100			
F3	83	82	100		
F4	80	89	84	100	
F5	85	78	75	86	100

Taxonomic significance of the morphological characters

Zohary (1966) reported the shape of the terminal lobe of radicle leaves that was demonstrated in Forms (1, 2, 4 & 5). While the lanceolate and more-less rhombic (Form 1 and Form 3; respectively), are new records to examined species. The simple leaf in var. *dentata* (Täckholm, 1974), absent in this revision, as the identified forms show pinnate-segments of diverse shape (Fig. 2), with dentate margins as reported earlier by Boissier (1867); Oliver and Thiselton-Dyer (1868); Zohary (1966); Boulos (1999); Minnich and Sanders (2000).

The fruit characters in the identified Forms (1-5) are linear-terete, erect, and spreading; this is congruent with Gabr (2018b), in Saudi Arabian specimens. While the recurved fruits of var. *recurvata* mentioned in Egypt (Täckholm, 1974), lacked in this revision. The fruit beak length, of studied Forms, was c. 2.0 cm that supports the earlier report of Felger *et al.* (2015).

The length of the fruit pedicel also shows distinct diversity in the identified forms. The number of seeds/fruits was 25-30 in recognised Forms. While the fruit beak contained 1-2 seeds/beak in Form 2; as reported by Zohary (1966) and Boulos (1999), while 0-2 seeded in Forms 4 and 5, as recorded by Gabr (2018b) in *B. tournefortii* of Saudi Arabia. On the other hand, Forms 1 and 3 showed novel seed/beak diversity with 0-1 seeded. The seed size in the studied forms was 1.19-1.42 × 1.22-1.5 mm, exceeded that recorded earlier (1.0-1.2 mm) by Tantawy *et al*. (2004) and Kasem *et al.* (2011).

Taxonomic significance of pollen characters:

The pollen grains of identified forms of *B. tournefortii* are were reticulate, tricolpate, heterobrochate (Fig. 7) as reported earlier in Egyptian and Saudi Arabian specimens by El-Naggar *et al*. (1993) and Gabr (2018a); respectively. The pollens of studied forms were grouped under subprolate and prolate shapes (Table 1). EL-Habashy *et al*. (2013) detected subprolate pollen in Egyptian specimens. El-Naggar *et al.* (1993), reported prolate-spheroidal (P/E <2) in Egypt and perprolate (P/E>2) in Saudi Arabia (Gabr, 2018a). Despite, the general similarity of pollen in the five identified forms, the micro-features (Table 2) signify the taxonomic value of pollen grains at the infra-specific level of *B. tournefortii.*

Molecular diversity

ISSR tool was carried out to assess the genetic polymorphism with and within the morphologically identified *B. tournefortii* Forms (1-5). The results revealed low genetic similarity

among the studied forms (Table 5). This genetic diversity is consistent with the results of Winkler *et al*. (2019), who claimed that the low genetic diversity of *B. tournefortii* is a result of self-fertilization. The retrieved molecular data from the ISSR tool reflect the presence of genotypic variation in the studied Forms (1-5), which is expressed as species morpho-plasticity.

This is the first report of the genetic features of *B. tournefortii* at the infra-specific level. This data will help the other taxonomists and ecologists, who dealing with the origin of the population diversity (phenoplasticity) in this autogamous species, which invaded the new world.

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