J. Indian bot. Soc. e-ISSN:2455-7218, ISSN:0019 - 4468 Vol. 98 (1 & 2) 2019: 44-51

EPICUTICULAR WAX IN *ASPARAGUS:* INTERSPECIFIC AND INTRASPECIFIC VARIATIONS

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Date of online publication: 31st March 2019
DOI: 10.5958/2455-7218.2019.00005.6

Genus *Asparagus* (Asparagaceae), is a woody, branched, spiny shrub with medicinally important fascicular roots containing steroidal saponins and sapogenins. In the present study, twenty five accessions of *Asparagus* collected from different regions of India, have been examined.. The micromorphological characters of epicuticular waxes were worked out to elucidate the intra and interspecific variability. The data on quantitative characters exhibited significant intra and interspecific variability, Photomicrographs were obtained through scanning electron microscopy. One way ANOVA depicted significant differences among the genotypes.

Key words: *Asparagus*, Micromorphological, Epicuticular Waxes

Plant epidermis is covered with cuticle which is made up of cutin and wax. Wax is an essential structural element exhibitive with considerable ultrastructural chemical diversity with immense functional and ecological importance. Epicuticular wax of plants plays an important role in transportation, water conservation and defense. The leaf surface properties and composition of wax vary with environmental condition as well as with the age of the plant (Herbin and Robins 1969).

Chemically, waxes are esters of long-chain acids and long-chain primary alcohols, composed of a mixture of different chemical compounds (Barthlott & Wollenweber 198l, Bianchi 1995, Martin & Juniper 1970, Tulloch 1976 and Walton, 1990). Plant epicuticular waxes have been of interest to botanists for more than a century. Several early publications (Sprengel 1793, Darwin 1886, Kerner von Marilaun 1913) described the macroscopic details and the functions of epicuticular waxes. Much of the work focused on the fine structure of the cuticle is reported by Lee and Priestley (1924), Jurasky (1934) and Fritz (1935). The first comprehensive study of epicuticular waxes was done by De Bary (1871) on about 60 angiosperms and he divided them into four categories: viz, layers or crusts, rodlets, granules and heaped coverings. Amelunxen *et al.* (1967) revised De Bary's classification. This revised classification was adopted by several other authors (Holloway, 1970; Napp-Zinn 1973, Wilkinson 1979). The most recent classification of epicuticular waxes was given by Jeffree (1986), who proposed fourteen 'wax morphological types' based on the results obtained from the investigations of about 480 taxa (including about 300 *Eucalyptus* species) of angiosperms. Juniper & Bradley (1958) studied epicuticular waxes by transmission electron microscopy (TEM), but only a limited number of species could be studied. Later the scanning electron microscope made the study quicker and easier. Frolich & Barthlott (1988) refined the classification of Jeffery and described for the first time 12 different wax types in monocotyledons. Also, they considered the orientation of wax crystalloids.

Keeping the significance of epicuticular waxes in view, an attempt has been made to study them in genus *Asparagus* and accessions of *Asparagus racemosus*.

MATERIAL AND METHODS

Twenty five accessions of *Asparagus* spp. (**Table 1**) were collected from different geographical zones of the country and

Received on December 22, 2018 www.indianbotsoc.org Accepted on January 07, 2019

J. Indian bot. Soc.97 (1) 2019 :45

grown in well maintained pots in the Department of Botany, Lucknow University. The taxonomic identities of the different species of *Asparagus* were determined with the help of herbarium at BSI (Botanical Survey of India, Dehradun). For the SEM analysis the pieces of cladodes were washed in distilled water , then fixed in 2.5% gluteraldehyde for 48 hours. All samples were cleared using 7.5 pH NaOH buffer solution, samples were washed in distilled water, then dehydrated by passing them through 30%, 50%, 70%, 90% and 100% reagent grade ethanol, and rinsed again in 100% ethanol. These samples were fixed to the specimen holder of SEM-JEOL, Japan JSM – 6490LV and the photomicrographs were observed under a scanning electron microscope.

OBSERVATIONS

Wax was found on the cladode surface in almost all collected accessions though its distribution varied. In SEM studies

Table 1: Qualitative and quantitative characters of epicuticular wax in different accessions in *Asparagus*

Characters / Plant	Species name	Ocurrence	Type	Length of wax (µm)
A1	*A.racemosus*	++	Entire plate	1.3±0.023
A2	*A.racemosus*	++	Entire plate	0.89±0.025
A3	*A.racemosus*	+++	Irregular plate	0.70±0.045
A4	*A.racemosus*	++	Irregular plate	0.92±0.05
A5	*A.racemosus*	+	Irregular plate	0.87±0.025
A6	*A.racemosus*	++	Entire plate	0.99±0.054
A7	*A.racemosus*	+	Entire plate	0.85±0.025
A8	*A.racemosus*	++	Entire plate	0.81±0.054
A9	*A.filicinus*	+++	Irregular plate	0.42±0.017
A10	*A. densiflorus*	+	Entire plate	0.472±0.014
A11	*A.densiflorus*	+	Entire plate	0.496±0.021
A12	*A. setaceous*	+	Crust	0.474±0.345
A13	*A. lohaghat curillus*	+++	Irregular plate	0.36±0.024
A14	*A.adscendense*	+	Entire plate	0.65±0.045
A15	*A.racemosus* (Nepali)	+++	Irregular plate	0.64±0.036
A16	*A.racemosus*	+	Irregular plate	0.87±0.025
A17	*A.racemosus*	+	Parallel plate	0.76±0.014
A18	*A.racemosus*	++	Parallel plate	0.46±0.023
A19	*A.racemosus*	+	Rosettes	0.59±0.045
A20	*A.racemosus*	++	Irregular plate	0.41±0.054
A21	*A.racemosus*	+	Entire plate	0.88±0.023
A22	*A.acerosus*	+++	Parallel plate	2.8±0.021
A23	*A.racemosus*	+	Entire plate	0.90±0.023
A24	*A.racemosus*	+	Rosettes	0.91±0.056
A25	*A.racemosus*	++	Entire plate	0.97±0.084

variation in shape, size and arrangement was found. In *Asparagus* majorly crust and crystalloid type of waxes are found. Crusts are massive wax coverings or the product of the erosion of crystalloids. Crystalloids are characteristic in certain taxa. They may appear in the form of platelets and plate. These platelets may have entire margins with irregular and parallel orientation. The distribution can be dense, organized or restricted (Table 1).

Entire plate: Entire plates are observed in A1, A5, A6, A17, A18, A21, A23, and A25 (crystalloids with entire margin and regular shape), and maximum length observed was in A1 (1.3 µm), minimum in A6 (0.46 µm) protruding perpendicularly from the surface (**Fig. 1** 1, 5, 6; **Fig. 2** 1, 5, 6: **Fig. 4** 1, 2).

Irregular plate: Irregular plates are observed in A7, A8, A9, A11, A12, A13 and A20 (crystalloids with irregular margin) and the maximum length was observed in A7 (0.85 µm) while minimum in A13 (0.36 µm) (**Fig. 3** 1, 2, 3; **Fig. 4** 1).

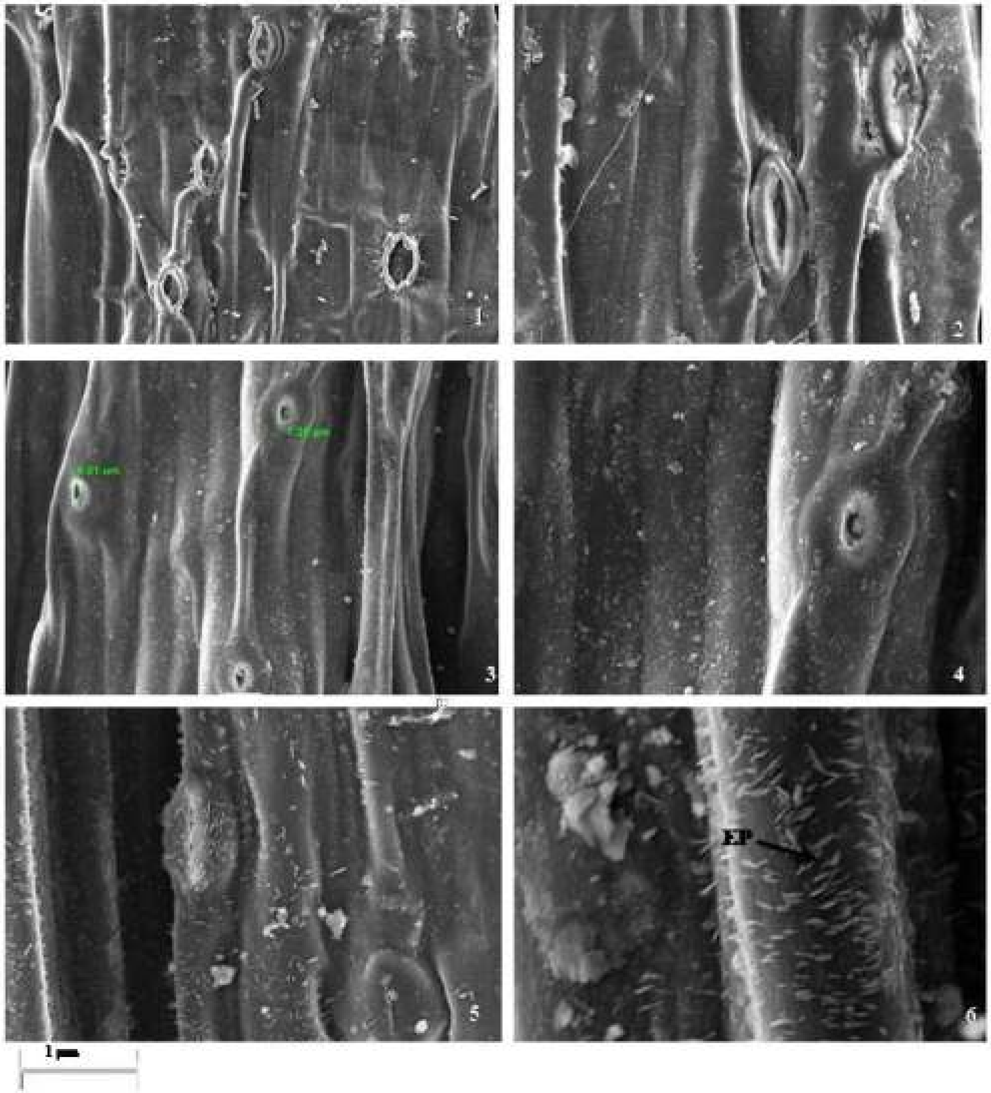


Figure. 1: Epicuticular wax on the surface of cladode . **1,** entire plate; **2, 3, 4,** crust; **5 , 6,** entire plate; **1, 2,** *Asparagus densiflorus*; **3, 4,** *Asparagus setaceous*; **5, 6,** *Asparagus curillus.* EP- entire plate

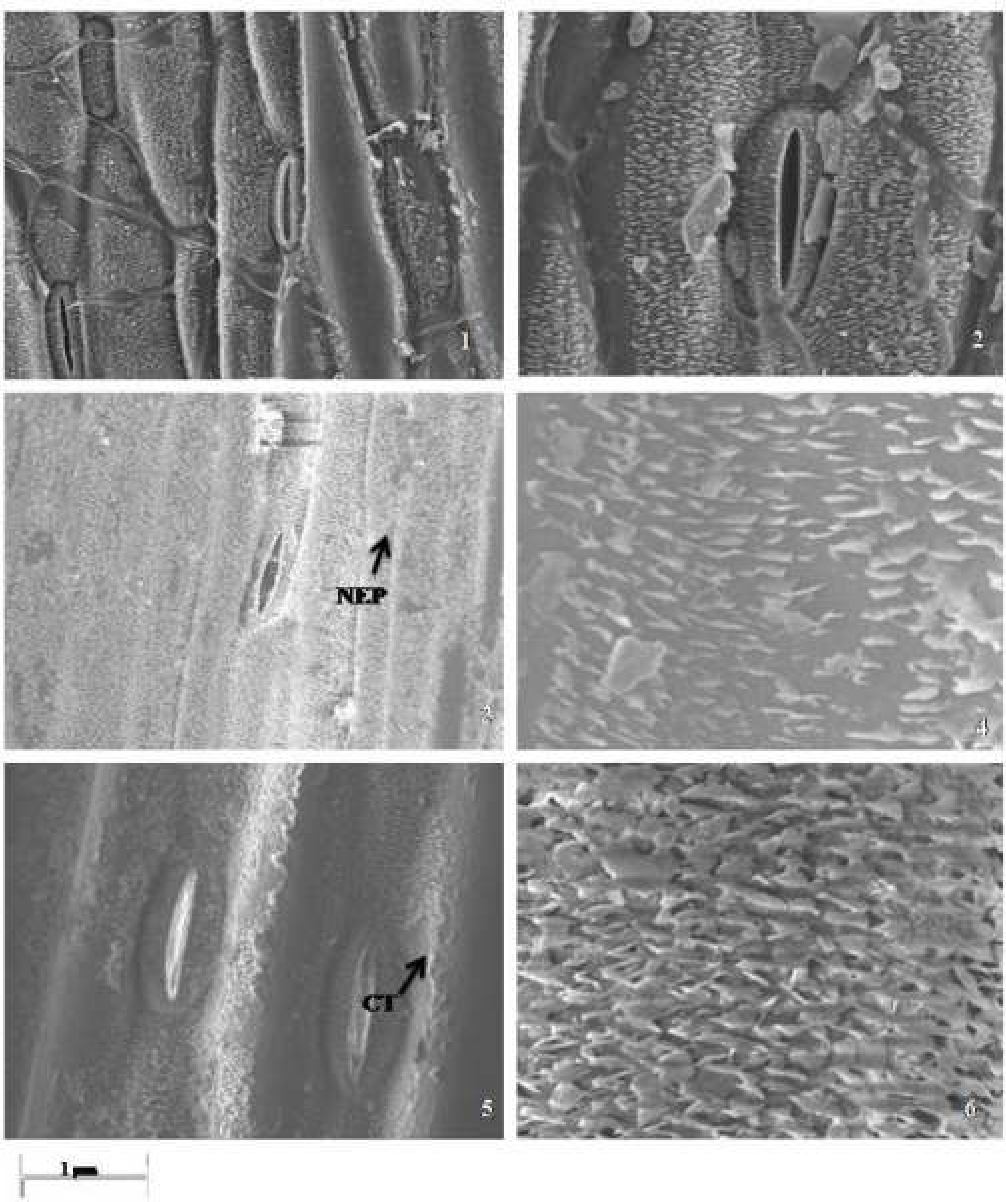


Figure 2: Epicuticular wax on the surface of cladode. **1,2,3,** irregular plate; **4,5**, non entire plate and crust; **6,** non entire plate. 1, 2, *Asparagus adscandense*; **3, 4,** *Asparagus racemosus* var., nepali ; **5, 6,** *Asparagus racemosus*. NEP- non entire plate, CT- crust

Crust: Crusts are continuous coverings or surface sculpturing observed in A2, A3, A4 and A16. The maximum length of crusts was observed in A4 (0.92 µm) and minimum in A22 (0.47 µm) (**Fig. 1** 2, 3, 4**; Fig. 2** 2, 3, 4**; Fig. 3** d**; Fig. 4** 2, 4, 6).

Parallel plate: Parallel rows of longitudinally aligned platelets were found in A10, A15, and A19 showing either entire or non-entire margins. Their length was maximum in A15 (0.64 µm) and minimum in A10 (0.47 µm) (**Fig. 4** 3, 5).

Rosettes: Rosettes are non-entire platelet and rodlet like structures spread over surface. The maximum length of rosette was found in A24 (0.91 µm) while minimum in A19 (0.59 µm) (**Fig. 3** 5, 6; **Fig. 4** 4).

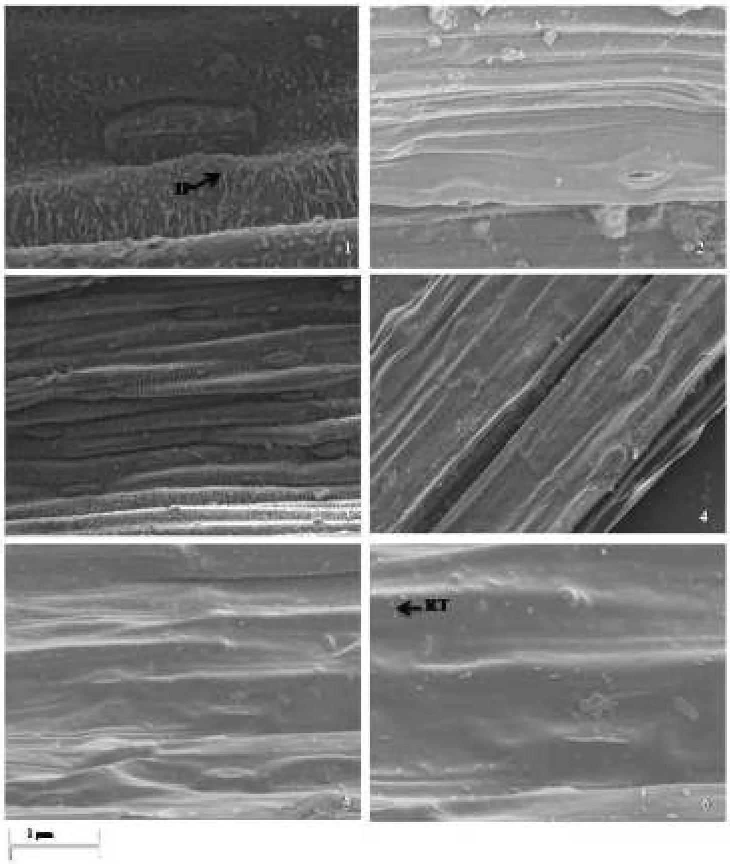


Figure. 3: Epicuticular wax on the surface of cladode of *Asparagus racemosus.* **1, 2, 3,** irregular plate; **4,** crust; e, f, rosettes RT- rodlets: IP- irregular plate

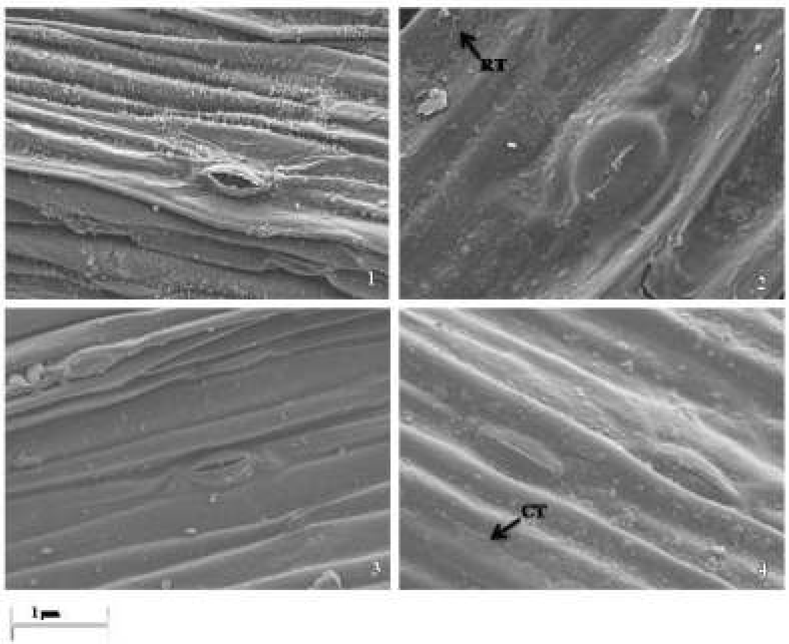
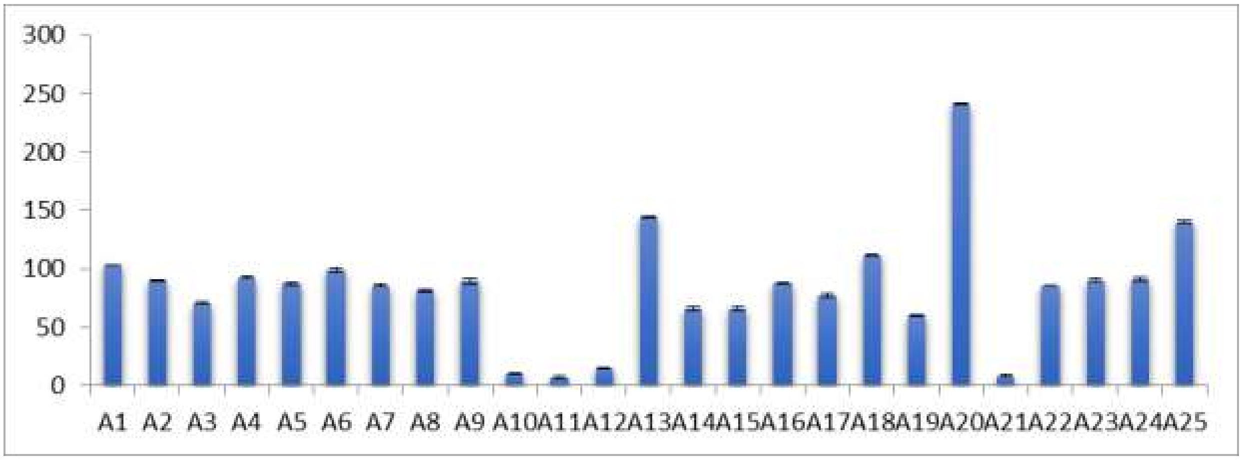


Figure. 4: Epicuticular wax on the surface of cladode . **1.** Parallel plate; **2,** crust and rosettes; **3,** rosettes; d, crust. a- c *A. racemosus*; **4,** *A. acerosus* RT- Rodlets; CT- crust



Graph 1: Length of epicuticular wax in different accessions of *Asparagus*

DISCUSSION

Leaf wax is an essential structural element of leaf surface, and the morphology is of functional and ecological importance vis-à-vis interaction between plants and their environment. The dense arrangement of waxes on leaf surfaces provides a protective barrier to the plant. Study of type of wax, its size and distribution pattern in the accessions of *Asparagus* revealed different types viz., crusts and crystalloids (rosettes, entire plate, parallel plate and irregular plate). The majority of wax type was crystalloid, present in most of the plants. Barthlott & Frolich, (1983) reported that parallel plates are characteristic of liliflorous families within the monocotyledons. The dominant components of crystalloid wax that appeared as platelets are primary alcohols (Barthlott 1997 et al., and Jeffree, 1986). In *Asparagus* the arrangement of wax has inter and intraspecific significance (**Fig. 1, 2, 3 and 4**). In few accessions the dense arrangement of wax was spread on the whole epidermal surface (**Fig. 2** 3-6; **Fig. 3** 1-6) while in A7, A8, A19, A20 in the form of patches (**Fig. 1** 1-6; **Fig. 3** 2- 6; **Fig. 4** 1-4). Some patterns are restricted to the coastal region only as in A8 and A19. In A7, A8, A9, A17, A22 parallel platelets are arranged around stomatal complex resembling electromagnetic field lines.

The leaf wax pattern were similar in A1, A5, A6, A7, A17, A18, A21, A23 and A25 (entire plate) while irregular pattern observed in A7, A8, A5, A9, A11, A12 and A13. Entire plate and irregular pattern are the most dominant leaf wax patterns. The species of *Asparagus* could be distinguished on the basis of distinct wax patterns where crust appears in (A2, A3, A4, A16, A22, A12), parallel plate in (A10, A15, A19) and rosette type arrangement in two accessions of *Asparagus* (A19 and A24). Epicuticular wax is found across the whole surface of cladode except guard cells. The length of wax plates among different accessions (Graph 1) showed significant difference statistically (ANOVA).

CONCLUSION

The wax morphological data of *Asparagus* revealed intraspecific and interspecific variability in type, size and its distribution pattern. The different types of waxes in *Asparagus* species as a whole can be considered as a biomarker in taxonomic identification.

The micromorphology of epicuticular waxes are modified by changes in plant growth conditions such as temperature, relative humidity, soil moisture, and irradiance as well as by wind. The variable structure of epicuticular wax affects the interactions at the organism environment interface. Presence of distinct types of wax patterning could aid in species identification as crust, parallel plates and rosettes were restricted to *Asparagus setaceous*, *Asparagus acerosus* and two accessions of *Asparagsus racemosus* respectively. It appears that the expression of specific type of wax is controlled by genetic constitution and rarely influenced by environmental factors.

Authors would like to express their gratefulness to Prof. Seshu Lavania, for her able guidance and encouragement during the present work. This work was supported by the University Grant Commission (U.G.C.), New Delhi in the form of Rajiv Gandhi National Fellowship. Our sincere thanks are due to USIC Lab of Baba Saheb Bhimrao Ambedkar University for the help in Scanning Electron Microscopy.

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