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Comparative foliar studies in saline sand and fresh water soil - grown *Trigonella foenum - graecum* Linn. plants

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Abstract: Vegetable markets in Mumbai sell "methi" at two stages of growth, the full grown vegetative to flowering stage and another at two cotyledonary-leaves stage. The latter are commonly grown in the sandy beaches of Mumbai whereas the former are the regular soil-grown ones. The beach grown *Trigonella foenum-graecum* L. plants are watered with saline beach- well waters whereas the soil-grown ones are watered with regular fresh water. The research work focused on studying the impact of this difference in growing conditions on many growth parameters, viz., morphology, anatomy and biochemical analyses of the sand and soil-grown plants in addition to sand, soil and water analyses. Current paper deals with the impact of these different growing conditions on some foliar parameters viz., anatomy, stomatal index, palisade ratio and venation patterns, in the two types of *Trigonella foenum-graecum* L. plants.

Key words: Anatomy of methi plant; sand and soil environment; stomatal index; palisade ratio and venation pattern

Introduction

Along some of Mumbai's beaches, notably the Seven Bungalows beach in the north-western suburb of Andheri, *Trigonella foenum-graecum* L. plants (locally called "methi") are cultivated regularly, round the year, in the saline sands of the beach. The week – old plants are harvested at the two-cotyledonary-leaf stage, tied into bunches and sold in the local markets as a leafy vegetable. The regular soil grown *Trigonella foenum - graecum* plants are a popular and widely consumed leafy vegetable. *Trigonella foenum-graecum* L. is a native of southern Europe and Asia. It is an economic plant grown the world over, especially in parts of Central and South Eastern Europe, Western Asia, Indian subcontinent and North Africa. It is cultivated both for its culinary and medicinal uses. Seeds are used as a diuretic, carminative and tonic. In addition, the seeds are also significant as astringent, demulcent, emollient and aphrodisiac (Nadkarni, 1976). It was decided to undertake a detailed comparative scientific study of these two-habitat based *T. foenum-graecum* plants *viz.,* sand and soil-grown. Present paper focuses on the comparative study of the leaves with respect to their anatomy, stomatal index, palisade ratio and venation patterns.

Materials and Methods

Cultivation of Test plants: Soil and Sand: the two types of *Trigonella foenum-graecum* plants were cultivated in Patkar - Varde college campus from November to March. For cultivation of soil grown plants, red soil and cow dung manure purchased from the local nursery, were mixed in the ratio of 3:1 and filled into a large number of 12-inch diameter and 16-inch long plastic bags. These bags were in turn lowered into slightly bigger black plastic bags to prevent algal growth.

For replicating the cultivation of beach sand-grown plants, sufficient sand from the cultivation plots ie actual site of methi farms from the beach at Seven Bungalows, Andheri West, was transported to the college campus where the study was undertaken. It was filled into many, clean, plastic bags of 12 inches' diameter and then lowered into black plastic bags.

The soil-grown plants were watered with regular municipal fresh water. But those grown in sand were given the beach-water collected from the 7-15 feet deep wells dug on the beach by the commercial cultivators.

Although *Trigonella foenum- graecum* plants are available in the vegetable markets during most parts of the year, healthy growth of the test plants, was observed in seeds sown from end October onwards. Test plants were cultivated between November and March.

Hand cut sections: Samples of leaves at different stages of growth were sectioned by hand, stained in 1 % aqueous Safranin and light green stains, mounted in glycerine and observed under the microscope. The observations reported in the present paper, are of samples of mature leaves collected at pre-flowering stage.

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Microtome sections: Samples of leaves were collected from the sand-grown and soil-grown plants at different stages of growth and fixed in normal FAA. for 24 hours. The materials were processed according to the usual method suggested by Johansen (1940) i.e., washing, dehydration, clearing, infiltration, using emerc paraffin wax, embedding, block making and taking microtome sections of 10 μ thickness. Wax ribbons containing serial sections were passed through xylol-alcohol-xylol series and stained with 1% Safranin followed by 1% fast green stain and mounted in Canada balsam. The observations reported here are of mature Samples of leaves collected at pre-flowering stage.

Stomatal index: Epidermal peelings from the abaxial and adaxial surfaces of different leaves were stained in aqueous Safranin and mounted in Glycerine. The number of stomata and epidermal cells were counted in different fields of vision. Stomatal index was calculated by substituting the mean value in the following formula:

$$SI = (S / S+E) X 100$$

SI: Stomatal index; S: No. of stomata; E: No. of epidermal cells

Palisade ratio: Samples of leaves were fixed in formaldehyde, acetic acid and alcohol mixture for 24 hours followed by potassium hydroxide treatment for 2-3 days. Samples were washed in water and mounted in dilute Glycerine. Tracing was done with the help of camera lucida (drawing prism) for study of epidermal cells and palisade cells and ultimately palisade ratio was calculated.

Venation patterns: Samples of leaves were treated with 5% Potassium Hydroxide solution for 2 days, stained with 2% safranin and mounted in glycerin. The slides were studied under the compound microscope and the details of venation patterns were described based on Hickey's classification. For the description of tracheoid structure reference of Mohan and Inamdar (1984) was used. (1984).

Results

T.S. of leaves: Prophylls and trifoliate leaves of both the sand-grown and soil-grown plants were of dorsiventral type with single layered epidermis. Cuticle was indistinguishable. Stomata were present on both the epidermis, though more on the abaxial side. Bi-cellular, long epidermal hair were present on the abaxial surface of the leaves, more along the midrib and primary veins. Cells of the mesophyll tissue contained abundant chloroplasts. Palisade in both the sand-grown and soil-grown plants was made up of two-three layers of compactly arranged, elongated, capsule shaped cells. Spongy tissue in all the leaves studied was composed of irregular to bluntly lobed parenchyma cells, leaving ample air spaces. The midrib was composed of single vascular bundle. Phloem was composed of sieve tubes, companion cells and parenchyma. Xylem was made up of rows of bluntly angular vessels interspersed with parenchyma. Fibres were absent.

Cotyledonary leaves of both the sand-grown and soil-grown plants revealed four-five layered palisade tissue under the upper epidermis followed by the spongy tissue. Half to more than half the thickness of the leaf was occupied by the multilayered palisade tissue. Upper and lower epidermis was single layered, cuticularized and showed the presence of stomata. Bicellular hair, as seen in prophylls and trifoliate leaves, were absent in the cotyledonary leaves. Vascular bundles were smaller in size but otherwise similar to those of the trifoliate leaves. The palisade cells were much shorter than those of trifoliate leaves or prophylls. The spongy tissue was composed of absolutely spherical to very slightly oval shaped cells. Both the palisade and spongy cells were rich in chloroplasts. Chloroplasts in all the leaves were very properly arranged in a layer next to the membrane.

Epidermal cells in WM: Shapes of the epidermal cells in whole mounts of leaf peelings showed some differences between the sand-grown and soil-grown plants. Margins of upper epidermal cells of prophylls of soil-grown plants were comparatively straighter with very little waviness whereas the corresponding margins from the sand-grown plants were wavier. Trifoliate leaves of sand-grown and soil-grown plants, too gave similar results. Margins of lower epidermal cells of trifoliate leaves of both sand-grown and soil-grown plants appeared equally wavy in whole mounts.

Types of Stomata: Leaves of both the sand and soil-grown plants showed two types of stomata occurring simultaneously – anomocytic and anisocytic.

Stomatal Index: On the whole, the stomatal indices of upper and lower epidermis of different leaves of soil and sand-grown plants were not very different from each other. Nevertheless, the minute differences did show a clear pattern. (Table 1 and 2):

Table 1: Stomatal Index of prophylls:

Epidermis	Sand-grown			Soil-grown			%D
	Min	Avg	Max	Min	Avg	Max	
Adaxial	19.3	20.7	21.6	15.6	19.4	25.0	6.7
Abaxial	17.6	21.11	25.0	18.4	22.7	25.0	-7.0
%D1		1.94			14.53		

% D: Percent difference between the average readings of the leaves of sand-grown and soil-grown plants
% D1: percent difference between the average readings of the abaxial and adaxial epidermis

Table 2: Stomatal Index of trifoliate leaves:

Epidermis	Sand-grown			Soil-grown			%D
	Min	Avg	Max	Min	Avg	Max	
Adaxial	18.1	22.28	27.2	17.4	21.71	28	2.62
Abaxial	18.1	22.51	30.4	18.7	23.15	29.1	-2.76
%D1		1.02			6.22		

% D: Percent difference between the average readings of the leaves of sand-grown and soil-grown plants
% D1: percent difference between the average readings of the abaxial and adaxial epidermis

i). Average stomatal index values for the abaxial leaf surfaces (of both prophylls and compound leaves) of sand-grown plants were lower than those of soil-grown plants. Whereas the similar values for those of adaxial leaf surfaces (of both prophylls and compound leaves) were higher than those of soil-grown plants.

ii). Average stomatal index values for the abaxial surfaces of all the leaves, in both the soil-grown plants and sand-grown plants were higher than those of their corresponding adaxial surface values.

iii). Percent difference between the average stomatal index values of abaxial and adaxial surfaces of sand-grown prophylls and compound leaves was much less (1.942% and 1.022% respectively) as compared to the soil-grown prophylls and trifoliate compound leaves (14.537% and 6.22% respectively).

Palisade Ratio: Observations made on the palisade ratio values of prophylls and compound trifoliate leaves of sand-grown and soil-grown plants were as follows: (Table 3):

Table 3: Palisade ratio of prophylls and trifoliate leaves:

Epidermis	Sand-grown			Soil-grown			%D
	Min	Avg	Max	Min	Avg	Max	
Prophylls	2.22	2.74	3.0	2.38	2.73	2.91	0.36
Trifoliate leaves	3.33	3.77	4.0	3.0	3.09	3.28	22.0
%D1		27.32			11.65		

i). Average palisade ratio values for prophylls and compound leaves were higher in sand-grown plants than those of soil-grown plants.

ii). Percent difference between the average palisade ratio values of sand-grown and soil-grown prophylls was negligible (0.36%) as compared to the corresponding values of trifoliate leaves (22%).

iii). Average palisade ratio values for prophylls, in both the soil-grown plants and sand-grown plants were lower than the corresponding values for trifoliate leaves.

iv). the percent difference between the average palisade ratio values of sand-grown prophylls and compound leaves was much higher (27.32%) as compared to the corresponding values of soil-grown plants (11.65%).

Discussion

Cultivation Season: Results from current project indicated that the best period for cultivation of *Trigonella foenum- graecum* plants was from end October onwards. Parallel findings were reported by the following researchers:

Bhatti (1988) experimented with fenugreek to evaluate the effect of sowing date and concluded that 7th November was the best time. Similarly, Baswana and Pandita (1989) inferred that maximum yield of fenugreek was when it was started on 5th October followed by 15th September. Thus, proving October November to be the ideal period for sowing *Trigonella foenum- graecum.* L. Some margin can be allowed on the basis of geographical position of the place of research and thus the environmental conditions.

Leaf anatomy: Anatomical studies of prophylls and trifoliate compound leaves revealed that there was hardly any difference between the leaves of sand-grown and soil-grown plants.

Epidermal cells in WM: the margin of lower epidermal cells of prophylls and trifoliate leaves of soil-grown plants was too wavy as compared to those from upper epidermis. But in case of sand-grown plants this difference in extent of waviness was much reduced.

Stomatal Index: While stomatal number varies considerably with the age of the leaf, stomatal index is highly constant for a given species. (Salisbury, 1927).

Observations made during the current project (Tables 1 and 2) revealed that as a result of salinity the stomatal index of the adaxial surfaces of different leaves increased and that of abaxial surfaces decreased, thereby drastically decreasing the percent difference values – more so in prophylls than in the trifoliate compound leaves.

De Villiers, *et al.,* (1996) have reported an increase in stomatal index values (abaxial or adaxial surfaces not specified) of *Atriplex semibaccata* R. Br. in response to increasing salinities; whereas others have reported a decrease in stomatal index values with an increase in salinity stress viz., Pratima Kadam *et al.,* (2010) in *Crotolaria retusa, C. verrucosa, C. juncea,* Pares J. *et al.,* (2008) in *Carica papaya* and Bray Shirley (2002) in the second trifoliate leaves of *Phaseolus vulgaris*. Mariana Andrea Reginato *et al.,* (2013), Qiu D. L. *et al.,* (2007) and Mansoor Hameed *et al.,* (2013) have reported decrease in stomatal density values in *Prosopis strombulifera*, *Kandelia candel* L. and *Cynodondactylon* L. respectively. On the contrary, Curtis Peter S. *et al.,* (1987) and Ilknur Solmaz *et al.,* (2011) have reported an increase in stomatal density values of *Hibiscus cannabinus* and dihaploid melon lines and their hybrids respectively, under salt stress conditions. Stomatal frequency values have been observed to have increased due to salinity in 42% of *Phaseolus vulgaris* L. plants (Miroslava Kaymakanova *et al.,* 2009) and some cotton cultivars (Jafri Ali Zafar *et al.,* 1995).

Palisade Ratio: is the average number of palisade cells beneath one epidermal cell using four contiguous epidermal cells for the count (Wallis and Dewar, 1933). This ratio has been shown to be sufficiently constant for a genus. The current work revealed that average palisade ratio values for prophylls and compound leaves were higher in sand-grown plants than those of soil-grown plants. But the difference in the values in sand and soil-grown prophylls was negligible as compared to the trifoliate leaves. This indicates that salinity has an

enhancing effect on the palisade ratio of compound trifoliate leaves but negligible effect in case of prophylls.

Another observation made was that the palisade ratio values exhibited much more variation in the leaves of sand-grown plants than the soil-grown ones indicating that perhaps due to salinity, the palisade cells in the leaves of sand- grown plants were not so homogenous and uniform.

Shirley Bray, *et al.,* (2002) studied the impact of salinity on the second trifoliate leaves of *Phaseolus vulgaris* L. and reported that it had no effect on the palisade density; whereas Hussein M.M. *et al.,* (2012) have observed an increase in the palisade tissue of *Jatropha curcas* grown under salinity stress. Magdalena Gapińska *et al.,* (2014) have observed changed cell shape (shrunk and deformed) simultaneously with increased volume of intercellular spaces when *Lycopersicon esculentum*) cv. Perkoz seedlings were subjected to increasing sodium chloride salinities. This perhaps explains the wide variation in palisade ratio values noted in the leaves of saline sand-grown plants.

Venation patterns in compound leaves of sand-grown plants: Venation pattern-study revealed that in compound leaves of sand-grown plants the angle of divergence of secondary veins was acute narrow as compared to acute moderate in soil-grown leaves. Though rare, a secondary branch in the distal half of leaf showed second order ramification which was totally absent in the soil-grown ones. According to the angle of origin of tertiary branches from the secondary on the exmedial and admedial sides, there were only AA and AR combinations whereas soil –grown ones showed AA (acute, acute), AR (acute, right), RR (right, right), and AO (acute, obtuse) combinations. All the areoles were of medium size (0.3-1 mm) but the soil-grown leaves had a mixture of medium (1-0.3 mm) and few large (2-1 mm). Tracheoids on the marginal ultimate veins were fewer as compared to those of soil-grown leaves. Loops were absent in salinity affected sand-grown leaves.

Venation patterns in prophylls of sand-grown plants: Based on their angle of origin from secondary's, the tertiaries showed RR combinations in addition to AA, AR and AO combinations also seen in soil-grown ones. The marginal ultimate veins showed fewer tracheoids as compared to those of soil-grown ones. Few isolated vein endings, isolated tracheoids and extension cells were seen in the prophylls as against their complete absence in those of soil-grown ones. Distal areoles were comparatively smaller and proximal ones larger and incomplete as against no clear pattern of distribution in the prophylls of soil-grown plants.

Conclusion

Saline sand-grown conditions did not affect the leaf anatomy and stomata of prophylls and trifoliate leaves. Margin of lower epidermal cells of prophylls and trifoliate leaves of soil-grown plants was too wavy as compared to those from upper epidermis. But in case of sand-grown plants this difference in extent of waviness was much reduced.

Average stomatal index values for the abaxial surfaces of all the leaves, in both the soil and sand-grown plants were higher than those of their corresponding adaxial surface values. But sand – grown conditions decreased the percent difference between the average stomatal index values of abaxial and adaxial surfaces of prophylls and compound leaves. Saline sand-grown conditions increased the average palisade ratio values of trifoliate leaves but decreased the stomatal index values on the abaxial sur- faces of both prophylls and trifoliate leaves.

The greater variation in the palisade ratio values in the leaves of sand-grown plants than in those of soil-grown ones, indicates that perhaps due to salinity, the palisade cells in leaves of sand- grown plants were not so homogenous and uniform, may be shrunk and deformed with increased volume of intercellular spaces.

Leaves of saline sand-grown plants exhibited fewer tracheoids, medium sized areoles, and absence of loops.

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