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SOME OBSERVATIONS ON SEEDS AND SEEDLINGS OF JOJOBA [*SIMMONDSIA CHINENSIS* (LINK) C.K. SCHNEIDER (FAMILY: SIMMONDSIACEAE)] – A NATIVE OF SONORAN DESERT – CULTIVATED IN PAKISTAN

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ABSTRACT

Seeds of Jojoba (*Simmondsia chinensis* (Link.) C.E. Schneider, collected from its plantation raised by Jojoba Research Station, Bahawalpur, Pakistan, were germinated in garden loamy soil and some of the seed and seedling characteristics were studied. The seeds of Jojoba are chocolate (mahogeny) brown in colour, ovate in shape and non-endospermic provided with a dorsal longitudinal raphe. Testa is thin but hard. A dense creamy-white mat of unicellular trichomes is present over the surface of the seed. The weight of seed averaged to 895.32 ± 11.62 mg varying from 551 to 1155 mg (CV: 12.98%). The seed weight tended to follow normal distribution. Jojoba seedlings appear to resemble the *Chisocheton* subtype of Vogel (1980). According to the Garwood (1996) scheme Jojoba seedling may be classed as "Crypto-cotylar hypogeal Reserve type". The embryo is straight. Cotyledons are massive, shortly petiolate succulent, food-laden, creamy white in colour and semi-ellipsoidal in shape. The outer surface of cotyledons is provided with longitudinally running grooves. In one-month old seedlings, shoot was 10.75 ± 1.05 cm and root 12.8 ± 1.8cm long and there were 9.75 ± 1.18 leaves per seedling. There existed variation with respect to leaf shape. The leaves of basal nodes were obtuse apexed, emarginate or round. The leaves of upper nodes were ovate or oblong or elliptic to lanceolate or oblanceolate in shape with acute apex. Venation appeared to be of brachidodromous type as evident by the grooves on the lamina. Stem and leaves densely pubescent. The trichomes of shoot were of two types – a) multicellular uniseriate trichomes of 2-9 (-11) and b) glandular multicellular uniseriate trichomes with apical cell globose. Some trichomes showed meander like cell wall. The shoot of seedling was thickly encrusted with cuticle and wax. The stomata of anomocytic type were the common one. The inner surface of cotyledons had tetracytic stomata. Some stomata with common subsidiary or no subsidiary also existed on stem and leaf. Leaves of Jojoba are amphistomatic. The occurrence of pericytic stoma besides anomocytic one was extremely rare feature on dorsal foliar surface. The average number of stomata was higher on the lower surface (81.91 ± 1.387 per mm^2) than on the dorsal surface (55.98 ± 1.452 per mm^2). The stomata were sunken. The size of the stomatal complex (stoma + epicuticular dome) was comparable on the two foliar surfaces. In all, seven fungal species (4 genera) were found to associate with Jojoba seeds and seedlings - *Rhizopus stolonifer, Aspergillus niger, A. flavus, A. terreus, A. fumigatus, Fusarium solani* and *Absidia* sp. *Absidia sp.* only associated with seeds in internalized state.

Key Words: Jojoba (*Simmondsia chinensis*), Seeds and seedlings, Leaf architecture and ornamentation, seed and Seedling mycoflora.

INTRODUCTION

Jojoba (*Simmondsia chinensis* (Link.) Schneider is a native to the desert of Southern Arizona, Sonora and Baja California and has been experimentally and commercially cultivated in several areas of the World due to its economic value (NAS, 1984; NRC, 1985; Khan and Abourashed, 2010). It is known by several names, Jojoba, hohoba, buck nut, goat nut and pig nut etc. It was introduced in Pakistan in mid 1980s (Bashir *et al.,* 2009). Jojoba Research Station, Bahawalpur (JRSB), Pakistan has been cultivating this plant since 1987-88. Hassanein *et al.* (2012) studied the germination of Jojoba under various conditions. Inoti *et al.* (2015) studied seed size and storage effects on germination and performance of young Jojoba seedlings in semi-arid areas of Kenya. Ashour *et al.* (2013) have studied Jojoba comprehensively from pharmacognostic view-point. *Glat et al. (198)* have described foliar stomata of American Jojoba and Gülz and Hangst (1983) had described the chemistry and morphology of epicuticular wax on various organs of dry Jojoba sample. Jojoba plants obtained from seeds have showed a high degree of variability in most characteristics including yield because it is a dioecious and obligate cross-pollinated long-lived perennial species (Gentry, 1958). The present paper describes the characteristics of Jojoba seedlings from the seed crop obtained from its plantation raised by JRSB, Pakistan.

MATERIALS AND METHODS

The seeds of *Simmondsia chinensis* were provided by Dr. Lal Hussain Akhtar, Guar Botanist, Agricultural Research Station, Bahawalpur, Pakistan. The seeds were recently collected from the plantation of Jojoba raised by Jojoba Research Station, Bahawalpur, Pakistan in 1987-88. The seeds were germinated in May 2015, without any dormancy-breaking treatment, in pots filled with garden loam soil maintained at 75% water holding capacity. Maximum germination was 80% achieved within a week. The seedlings were irrigated daily. The seedlings were studied for their morphological characters including leaf architecture and ornamentation i.e. trichome and stomatal types. Seedlings type was described according to Vogel (1980) and Garwood (1996). Hickey (1973) and LAWG (1999) were followed for description of leaf architecture. Leaf epidermal impressions were made with clear nail

polish (Wang *et al.*, 2006). Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. This nomenclature does not recognize actinocytic and stephanocytic stomata and categorize them as anomocytic type. As a basic criterion, all the cells abutting the guard cells were considered distinct by Prabhakar (2004) from the other epidermal cells by virtue of their position (i.e. abutting nature to the guard cells) hence he prefers to call them subsidiaries. To obtain epidermal peel, the leaves were placed in a test tube containing 70% lactic acid and 30% ammonia and boiled to soften leaves. Epidermis was removed and observed under light microscope (Shaheen *et al.*, 2010). To remove epicuticular waxes and elucidate the surface structure of epidermis to facilitate stomatal identification, the leaf was treated with hexane following Gülz and Hangst (1983). The nail polish imprint of hexane-treated leaves was obtained as described earlier. To determine the mycoflora the seeds (obtained from JRSB) and root, stem and leaf of 60-day old Jojoba seedlings were divided in two sets. One set was directly plated without any surface sterilization while other plated after surface sterilization with 1 % Sodium hypochlorite. Five pieces of each of the plant part were transferred to PDA poured plates (autoclaved) having antibiotics penicillin and streptomycin @ 2mg/litre. There were three replicates of each one. All plates with or without surface sterilization were incubated at room temperature for a week and percent colonization were determined as Colonization = Number of infected pieces / Total number of pieces expressed as percentage.

Mycoflora was identified using standard mycological literature (Domsch *et al.*, 1980; Ellis, 1971; Nelson *et al.*, 1983; Raper *et al.*, 1965; Gilman, 1950; Mycobank, 2013). The data was analyzed statistically (Zar, 2010).

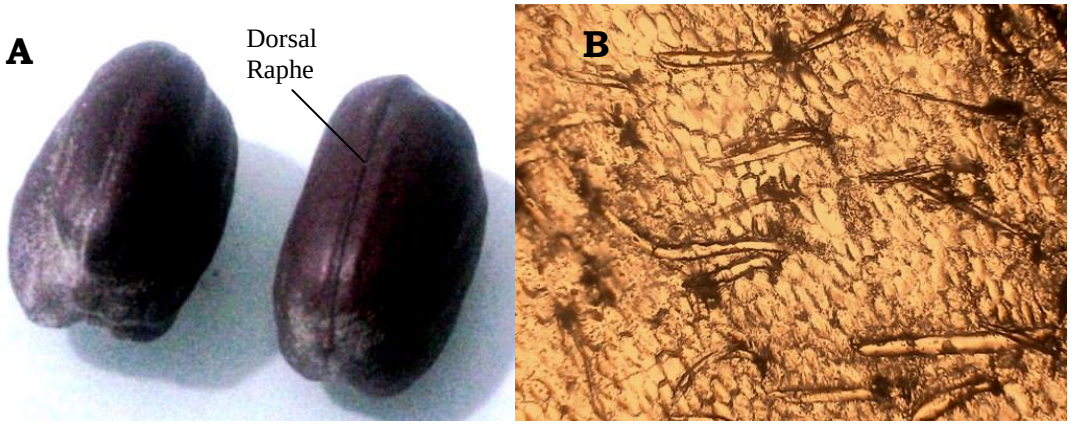

Fig. 1.The normal seeds of Jojoba (A) – the whitish mat over the surface are the trichomes. B, seed surface with scattered unicellular trichomes. The seed surface is papillate.

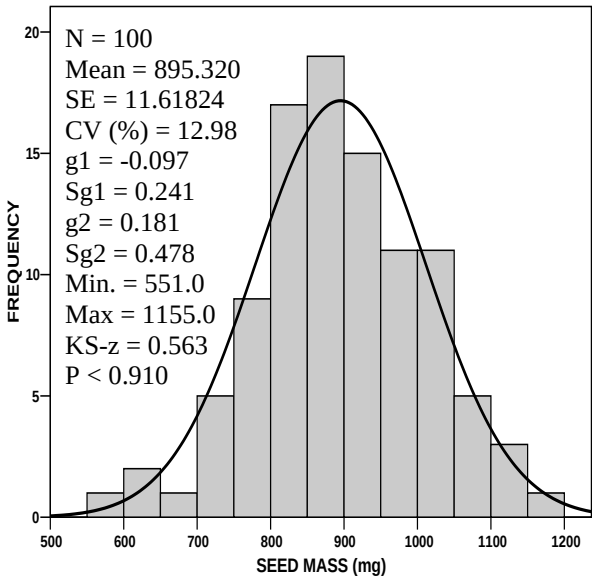

Fig. 2. Frequency distribution of biomass of air-dried seeds of Jojoba. g1, skewness; g2, kurtosis. Sg1, St Error of skewness and Sg2, St. Error of kurtosis.

RESULTS AND DISCUSSION

Seed :The seeds of Jojoba are chocolate (mahogeny) brown in colour, resembling peanut or acorn, ovate in shape and non-endospermic provided with a dorsal longitudinal raphe which is a characteristic feature of Family Simmondsiaceae. The seeds are rich in oil. The seed surface bears papillae (Fig. 1B) (cf. Gūlz and Hangst (1983). The seed has a typical anatropous ovule. Hilum is near micropyle. Testa is thin but hard. A dense creamy-white mat of unicellular trichomes is present over the surface of the seed (Fig. 1 A and B). The weight of seed averaged to 895.32 ± 11.62 mg varying from 551 to 1155 mg (CV: 12.98%). The seed weight tended to follow normal distribution (Fig 2) as evident from insignificant magnitude of KS-z (0.4563, $p < 0.910$).

Jojoba seed weight in literature is found to vary geographically and with the clones studied. Osman and Abohassan (2013) reported performance of nine clones of Jojoba in Hadaal Sham, Atyotamah and Hail deserts of Saudi Arabia. The individual seed weight of Jojoba clones averaged to 0.87 (0.70 – 1.21) and 0.89g (058 -1.25) at Hadaal sham in 2003-2004 and 2004-2005, respectively. This parameter averaged to 0.82g (0.56 -1.06) and 0.80g (0.57 – 1.11) for these years at Atyotamah. The seed size was, however, smaller at Hail (0.57g (0.49 – 0.65) and 0.58g (0.45 – 0.67) in 2003-2004 and 2004-2005, respectively. Thagana *et al.* (2007) reported weight of 100 seeds of Jojoba, when grown in Kenya to be 71.96g (0.72g per seed) for germplasm Janca and 74.42g (0.744g per seed) for germplasm KJIL (Kenya Jojoba Industries Limited). One hundred seeds of Jojoba weighed 61-157.8g (Hassanein *et al.*, 2012) i.e. 0.60g to 1.58g per seed. Mean and range of seed weight of air-dried individual seeds procured from 9-year old populations at the University of California to be higher for selected population [1.15 ± 0.024g (0.75 – 1.69)] than unselected population [0.95 ± 0.037g (0.51 – 1.64)], respectively (Naqvi *et al.* (1990). Desai (2004) reported that 100-seed weight of Jojoba varies from 40 to 80g (1600 seeds / kg). Inoti *et al* (2015a) described seed size in Jojoba – large seeds around 1047mg, medium sized seeds around 697mg and smaller seeds to be around 333mg. Larger seeds have larger cotyledons and therefore, large nutrition base. The larger seeds are reported to show significantly higher germination (78%), than that of medium seeds (33%) and smaller seeds (37%).

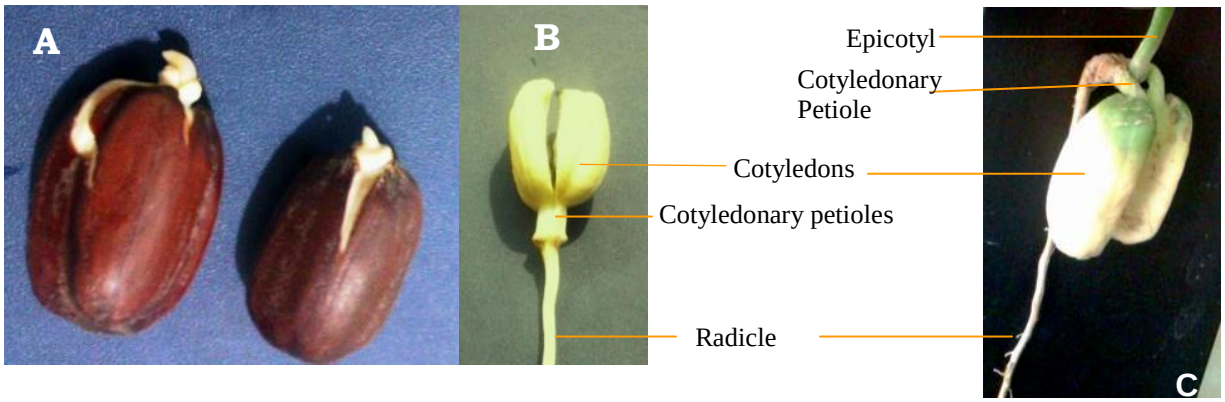

Fig. 3. Jojoba seeds imbibed in petri plates. Protrusion of radicle and hypocotylar sheath (A), early seedling type (B) and C) Arrangement of the embryo components in a seedling.

Seedling type and Seedling growth

"Seedling" is the final stage of the regenerative process of a plant from a seed. The germination of seeds in this species was around 80% when healthy seeds were sown in garden soil. On germination testa splits due to swelling of the cotyledons on imbibition (Fig. 3A). It is apparent with the emergence of the radicle (Fig. 3a) from one pole of the seed. On 5th day of sowing, the germinated seeds showed plumule (epicotyl) as green body emergent from the hypocotylar sheath (Fig. 4A, B and C) which is present in the region of cotyledonary node in a depression between the cotyledonary petioles (Rust *et al.*, 1977). Hassanein *et al.* (2012) have reported that plumule commences emerging after 4 days of sowing. The embryo is straight (Fig.3C). Cotyledons are massive, shortly petiolate, succulent, food-laden, creamy white in colour and semi-ellipsoidal in shape. A cotyledon is semi-elliptic in outline in cross-section with slightly concave to flat inner surface. Light-exposed surface of the cotyledons becomes green in colour (Fig. 4 and 5). Cotyledons remain underground (hypogeal germination). Cotyledons are oppositely placed and turn together lateral to the epicotylar stem (Fig. 3C). Cotyledons are non-spreading. They are pushed apart due to swelling of the cotyledonary node. Through this gap between the cotyledons the epicotyl grows (Fig. 4A, B and 5). The outer surface of cotyledons is provided with longitudinally running grooves (Fig. 7). The patterning of grooves over cotyledons varies from cotyledon to cotyledon. Cotyledons generally exhausted in all seedlings by 88th day of emergence. In one-month old seedling, shoot was 10.75 ± 1.05 cm and root 12.8 ± 1.8cm long and there were 9.75 ± 1.18 leaves per seedling. Total leaf area per seedling (1-month old) averaged to 3872.23 ± 323.41 mm^2. The

study of foliage morphology by Inoti *et al.* (2015b) had showed that single leaf area of male seedlings (4.4 cm^2) was significantly higher as compared to the females (3.2 cm^2).

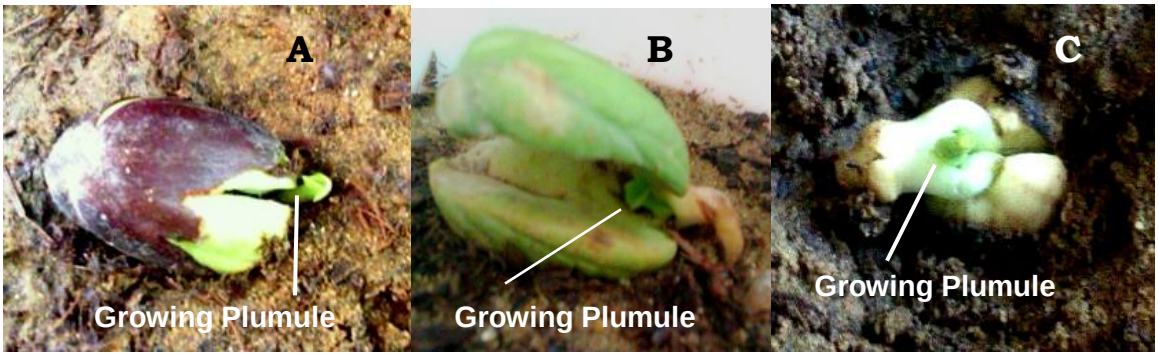

Fig. 4. Growth and emergence of epicotyl of Jojoba when sown in different positions in superficial soil layer or just above the soil; Fifth day of sowing. A, sown laterally, raphe above and seed semi-embedded; B, seed sown superficially, raphe below in contact with the soil; C, seed sown vertically, micropylar end above and seed embedded.

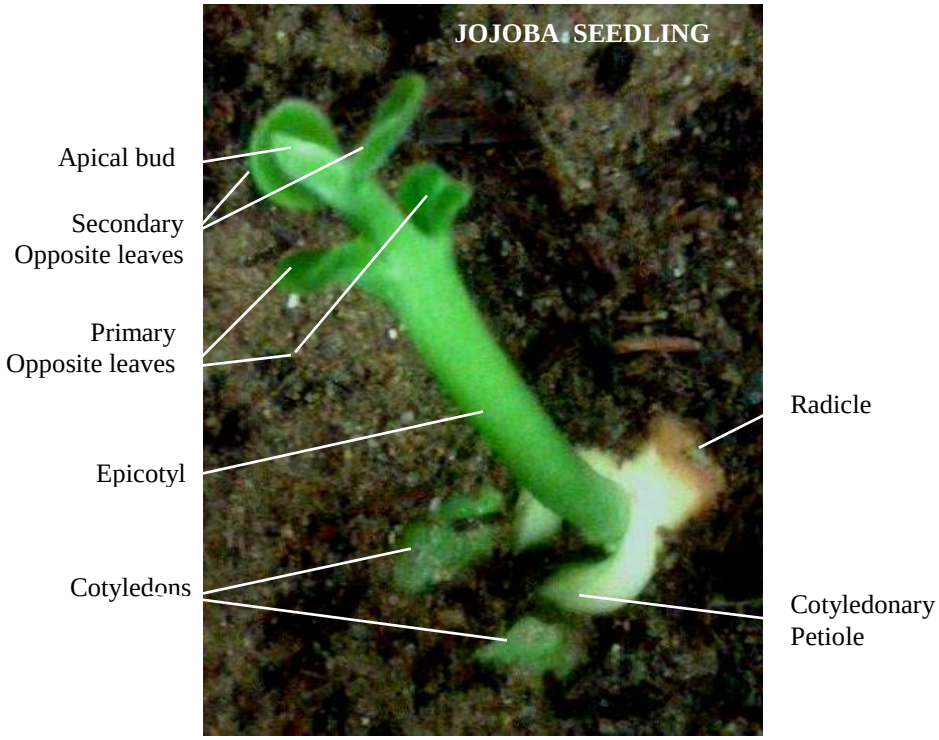

Fig. 5. Seedling of *Simondsia chinensis* after 6 days of emergence of epicotyl. Cotyledons are known not to come out of the soil. Cotyledons are visible due to shallow sowing of seed. Note that on exposure to light cotyledons turns green. The primary leaves are emarginate and the secondary leaves are round in shape. Epicotyl is c 2.3 cm long.

Vogel (1980) have made distinction of seedling types based on combinations of characters which express the mode of development of the seedling taking into consideration the fruit wall, testa, endosperm, hypocotyl, cotyledons, seedling axis and leaves. The position of the cotyledons in relation to the seedling axis and the persistence of envelopments are more fundamental in this classification. On the basis of characters of Jojoba seedlings, it appears to generally resemble the *Chisocheton* subtype (Vogel, 1980; Fig. 119, p. 335) of *Endertia* type (Vogel, 1980, pp. 69-70). *Chisocheton pentandrus* (Meliaceae), *Whifordiodendron myrianthum* (Papilionaceae), *Eugenia* (Myrtaceae) and *Lucuma* (Sapotaceae) are some examples to this subtype. The Garwood (1996) scheme of seedling types is based on the characters of cotyledonary position (epigeal or hypogeal), exposition (cryptocotylar or phanerocotylar) and texture (fleshy or foliaceous) during germination. According to this scheme Jojoba seedling may be classed as "Crypto-cotylar hypogeal Reserve type".

Root: Long tap root system.

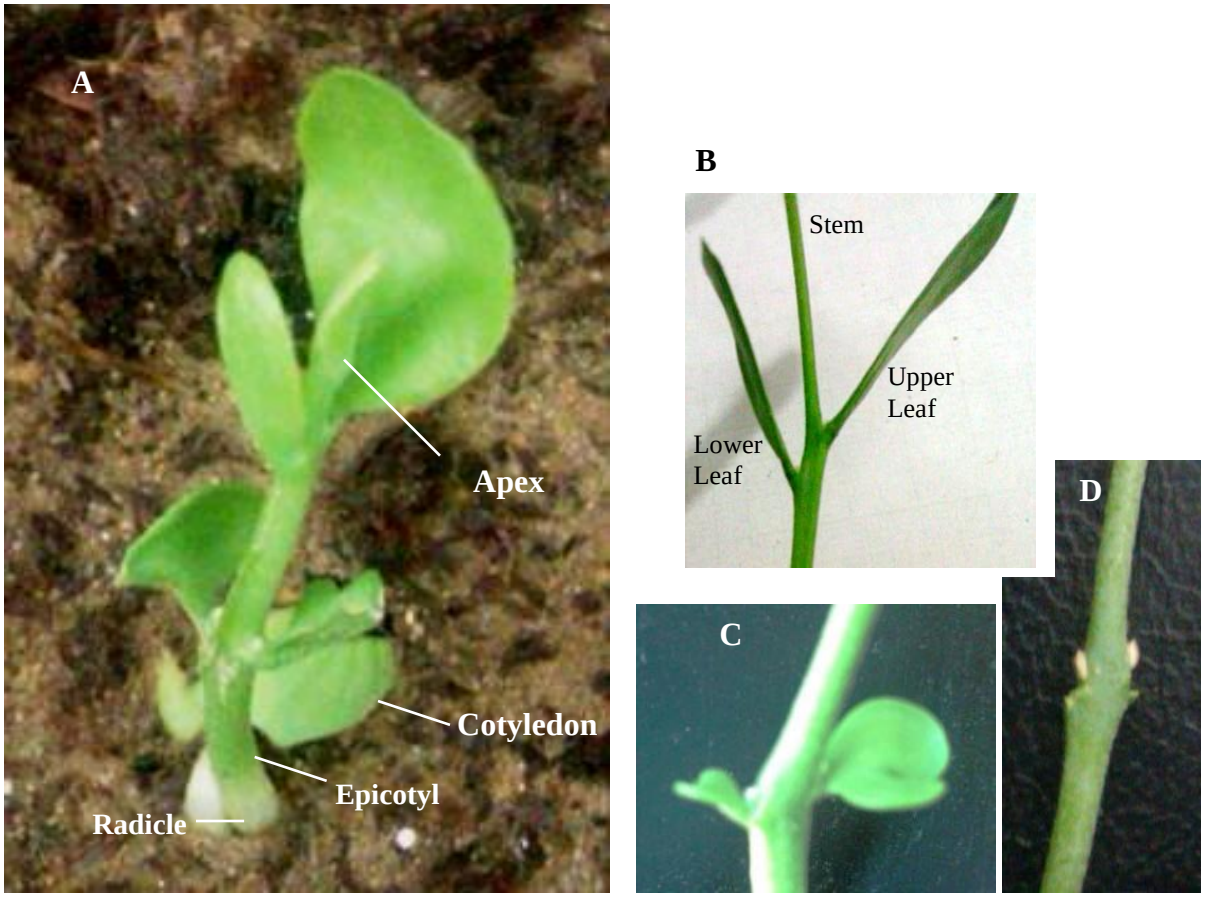

Fig. 6. A, Seedling after 8 days of emergence. At this age each seedling had four leaves and averaged to shoot length of 3.76 ± 0.25 cm; B, Leaves sometimes not exactly opposite; C, Primary unequal retuse leaves; D, stem of 1-month old seedling – leaves removed to show yellow brown buds. Stem's grayishness is due to trichomes.

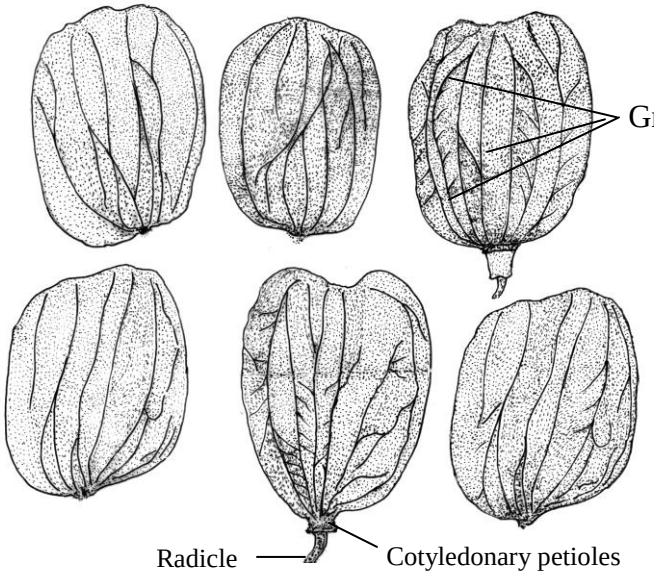

Fig. 7. Six different cotyledons of Jojoba showing surface grooves.

Fig. 8. Fleshy leaf.

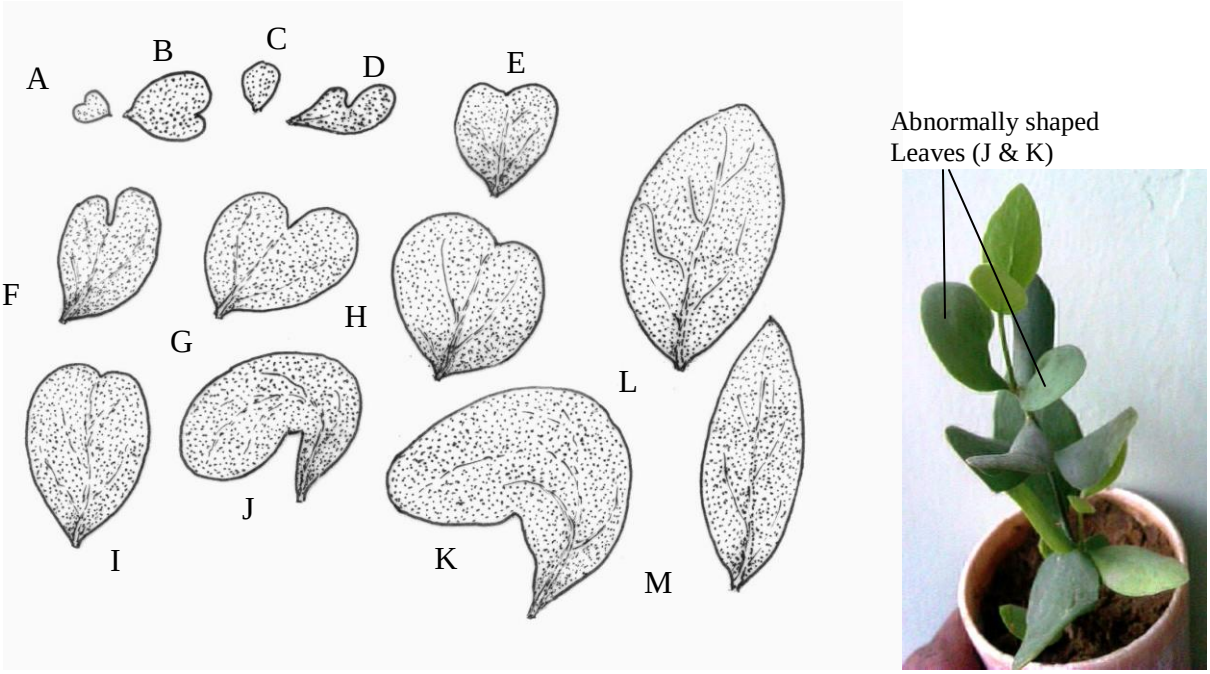

Fig. 9. Leaves of various shapes seen in Jojoba seedlings. Abnormal leaves (A-K) and normal leaves. (L and M).

Table 1. Leaf architecture of one month old Jojoba seedlings.

Parameter	N	Mean	SE	Min-Max	CV (%)
Petiole length (cm)	c 0.15 cm in mature leaves				
Lamina length (cm)	43	2.921	0.196	0.6-4.8	44.06
Lamina breadth (cm)	43	1.626	0.096	0.4-2.5	38.74
Aspect ratio * ◊	32	0.536	0.0113	0.444-0.70	11.89
Aspect ratio * ◊◊	11	1.01	0.0890	0.79-1.70	29.23
Leaf apex extension (La) (cm) - abnormal leaves	5	0.26	0.04	0.2-0.4	34.40
Leaf base extension (Lb) (cm)	Zero				
Apex angle (^{o}) – abnormal leaves (Obtuse apex)	11	113.45	5.903	68-130	17.26
Apex angle (^{o}) – Normal leaves (Acute apex)	28	79.93	1.084	70-90	7.17
Base angle (^{o}) – Abnormal leaves	11	85.91	5.98	60-120	23.12
Base angle (^{o}) –Normal leaves	28	82.96	1.33	70-95	8.49
Leaf area per leaf (mm^2)	43	352.25	33.986	18-886	63.26
Leaf area per seedling (mm^2)	4	3872.23	323.41	2028-4603	16.70

*, lamina width / lamina length - (after Lu *et al.*, 2012); ◊, acute apexed leaves; ◊◊, Obtuse apexed leaves;

Stem: Stem cylindrical, green when young and densely pubescent with multicellular trichomes of 2-9 (-11) and thickly encrusted with wax and cuticle.

Leaves: Leaves are long-lived, xerophytic, cauline exstipulate, shortly petiolate, entire, coriaceous in texture, astringent in taste and odourless. Young leaves are light green and adult leaves are gray dark green in colour. Thick Cuticular encrustation present over leaf surface. Epidermal wall thick. Epidermal hairs present. Leaves are generally opposite (at times not exactly opposite, Fig. 6B). Lamina length of leaves averaged to 2.92 ± 0.196 cm and breadth 1.63 ± 0.096 cm (Table 1). Two leaves of a pair of opposite leaves of a node were generally unequal in size (Fig. 10). The leaves were ovate or oblong or elliptic to lanceolate or oblanceolate in shape. Gray-green in colour. Double palisade. No spongy tissue. Venation obscure due to flashiness of leaf. It appeared, however, to be brachidodromous type as evident by the grooves on the lamina (Fig. 8). Leaves not spreading but oriented vertically upwards with an angle between two opposite leaves c ≤ 90^{o}. Leaf pairs decussate. Leaves on a stem pose a whirling effect when seen from the top (Fig. 9). The leaves were pubescent on blade and margins. On spray of water, they appeared to channelize water toward base of the seedling besides retaining droplets on the margins (Fig. 11A and B).

The majority of leaves were elliptical, ovate, oblong and acute apexed. There were few abnormally shaped leaves with retuse to emarginate apex (obtuse apex) (Fig. 9). Acute and obtuse apex leaves are reported in a ratio of

2.8: 1 (Khan *et al.*, 2015). Obtuse apexed leaves were generally located on the basal node (s) and acute apexed leaves were present on the higher nodes. There was significant variation in leaf shape (Fig. 9) as also pointed out by Janick and Paull (2008). Apex angle of abnormal (obtuse apexed) leaves was larger (113.45 ± 5.90^o) as compared to that of normal acute apex leaves (79.93 ± 1.08^o). The base angle of normal and abnormal leaves was more or less comparable (82.96 ± 1.33^o and 85.91 ± 5.98^o, respectively). The magnitude of the base angle of normal leaves was also comparable to the apex angle of the normal leaves (Table 1). This indicated to the consistency in shape of the normal leaves. The young acute apex leaves, however, had small mucro at the apex. In 30-day old seedling, the leaf maximally reached to 886 mm^2 in size. Leaf with an average area of 352.25 ± 33.99 mm^2 fell in the microphyllous category of leaf size (Raunkiaer, 1934), a characteristic common in arid land plants.

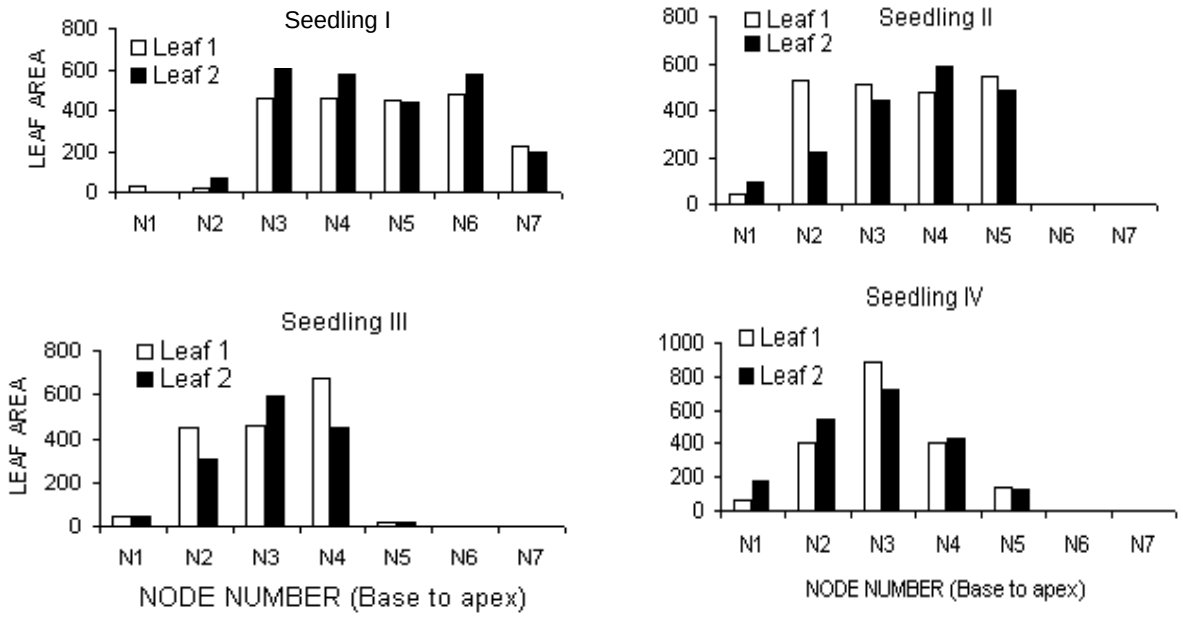

Fig. 10. Area of opposite leaves (mm^2) present on different nodes (from base to apex) of one month old seedlings of Jojoba.

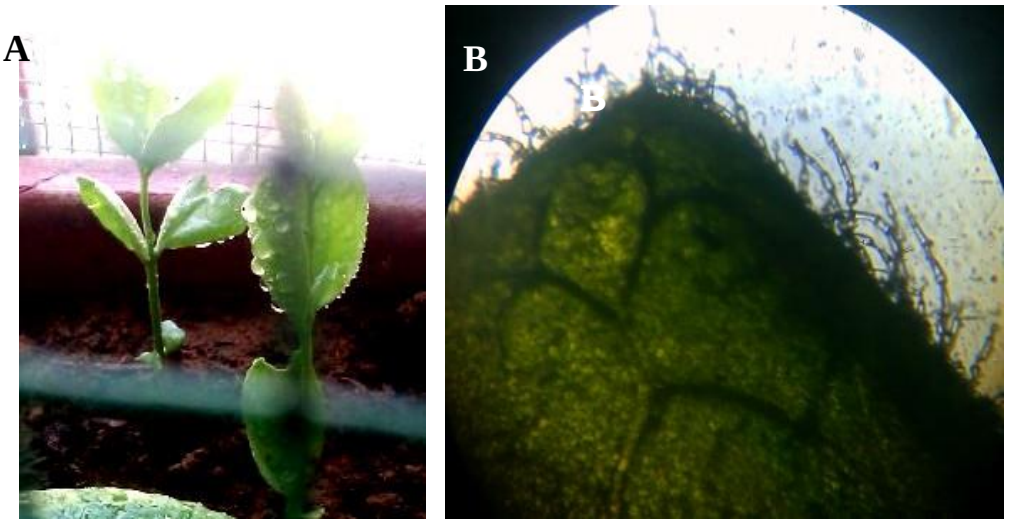

Fig.11. Leaf showing water droplets entangled in marginal trichomes (A) while sprayed with water and non-glandular trichomes on the margins and apex (B). Magnification: 10x10 X.

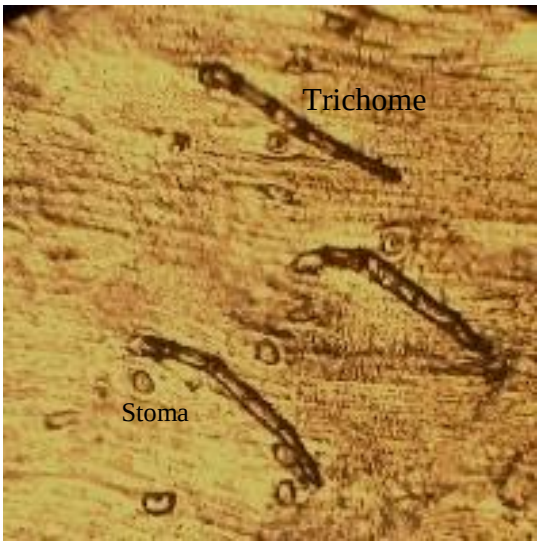

Fig. 12. Multicellular uniseriate non-glandular trichomes on the epicotylar stem surface of Jojoba seedlings. Epicuticular encrustation is prominent.

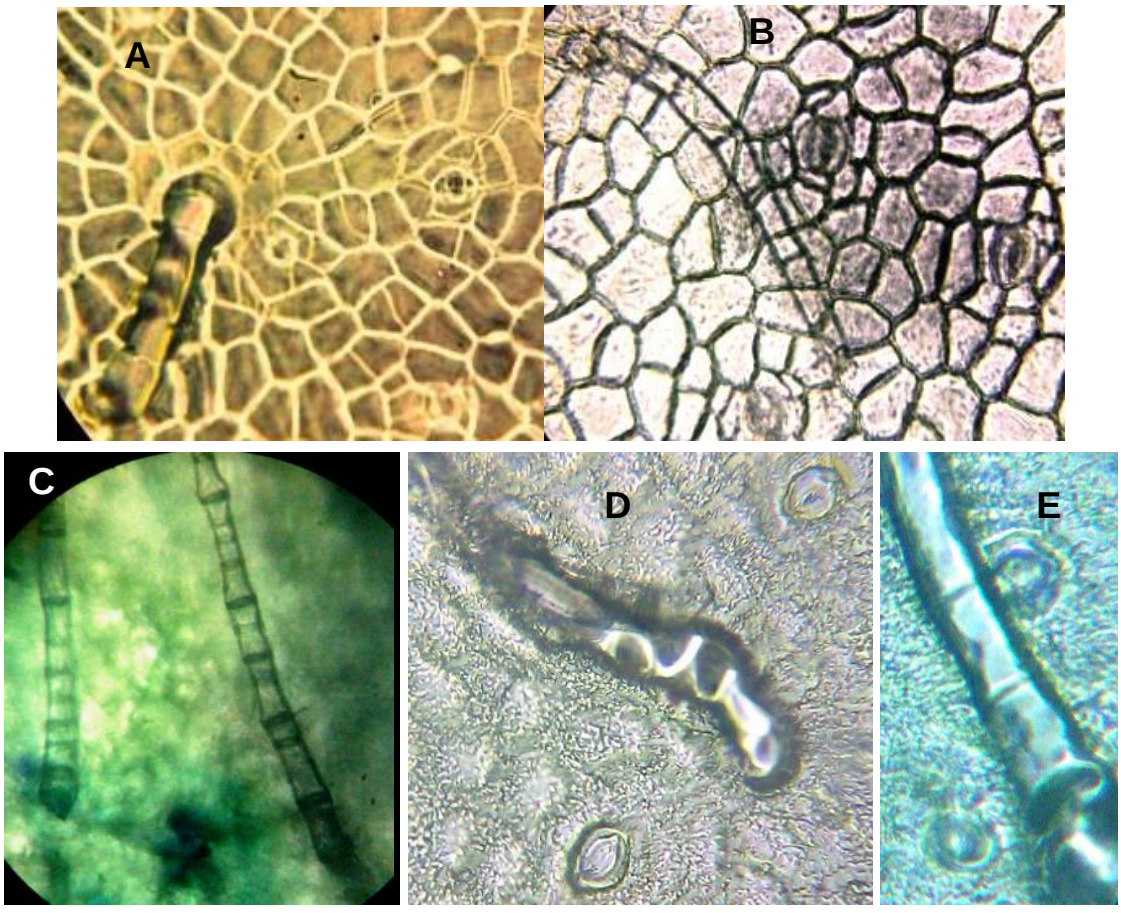

Fig.13. Multicellular trichomes on leaf of Jojoba. A, Glandular multicellular trichome on dorsal surface of leaf with few stomata (nail polish imprint). B, Non-glandular multicellular trichome on dorsal surface of adult leaf with few stomata (Epidermal peel of lactic acid + ammonia treated leaf). C, Two non-glandular trichome on ventral surface of young alive leaf; and D and E, trichome showing the meandering nature of the cell wall.

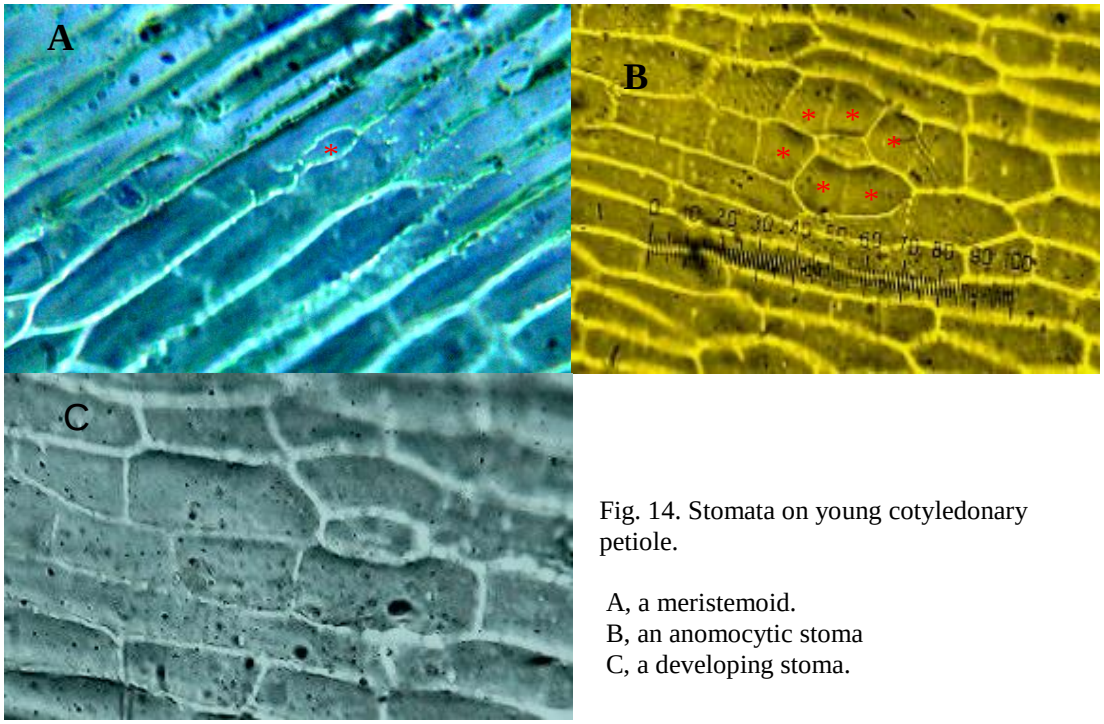

Fig. 14. Stomata on young cotyledonary petiole.

A, a meristemoid.
B, an anomocytic stoma
C, a developing stoma.

Shoot Ornamentation

Epicuticular encrustation: The surface of the seedling was heavily encrusted with thick cuticle and wax (Fig. 12, 13D & F, 16, 18, 19). In these figures the white thread like substance is presumably the wax deposited in intricate design as suggested by Glat *et al.* (1981). The waxy substance is considered to bring a counteracting effect to the loss of water during transpiration. The wax coating is also considered to serve as a mechanism to increase energy dissipation from the leaf surface in hot hours of the desert. Gūlz and Hangst (1983) have estimated that the amount of epicuticular waxes from Jojoba leaves is 0.34% D. wt. and 0.03% of seed coat on dry wt. basis. On area basis of leaf and seed coat, the proportion of epicuticular waxes was reported by Gülz and Hangst (1983) to be 0.079 mg waxes / cm^2 and 0.057 mg waxes / cm^2, respectively. According to their studies different organs were covered with similar amount of wax per surface area but due to differences in composition these wax coat differ in their morphological structure. The presence of epicuticular layer posed difficulty in identification of stomatal types due to difficulty in distinguishing subsidiary cells.

Trichomes: There are three types of simple trichomes appressed to the surface in Jojoba. Trichomes on seed surface were non-glandular unicellular (Fig. 1B) and have also been reported by Rust *et al.* (1977). On stem and leaves, two types of trichomes were seen- 1) four-celled glandular multicellular trichomes with globose apical cell (Fig. 13A) 2) Non-glandular uniseriate multicellular trichomes composed of 2-9 (-11) rectangular cells and present on stem and leaves (Fig. 12; 13B, C, D and E and 16A). We could also find meander like folded wall in some trichomes (Fig. 13D) as reported by Gülz and Hangst (1983). The presence of multicellular trichomes has been reported from petiole, floral stalk, dorsal and ventral surface of leaf and perianth, pericarp, and fruit stalk and unicellular trichomes on seed surface of Jojoba by Ashour *et al.* (2013).

Stomata: The main stomatal type in Jojoba is anomocytic type which may be seen on cotyledonary petioles (Fig. 14), stem (Fig. 16 and 17) and both surfaces of leaves (Fig. 18-24). Cotyledons had no stomata on their outer surface (Fig. 15A and C). The inner cotyledonary surface, however, had anisocytic and tetracytic type of stomata (Fig. 15B, D, E and F). On cotyledonary petioles and stem, besides anomocytic stomata, tetracytic stomata were also present (Fig. 14 and 16). There were several contiguous stomata scattered over the epicotylar stem oriented in various directions (Fig. 17). The dorsal as well as ventral surface of leaf showed anomocytic stomata when the leaves were treated with lactic acid + ammonia mixture (to obtain epidermal peel) or treated with hexane to remove epicuticular waxes (Fig. 20-22). On dorsal surface of a leaf a few contiguous and abnormal stomata with no subsidiary cell in between were visible (Fig. 21) besides pericytic stomata (Fig. 21C) as a rare feature also. There were some small clusters of stomata on the ventral leaf surface and in some instances two stomata sharing a common subsidiary and at some occasion anomocytic stomata were surrounded with two or more cycles of subsidiaries (Fig. 23 and 24).

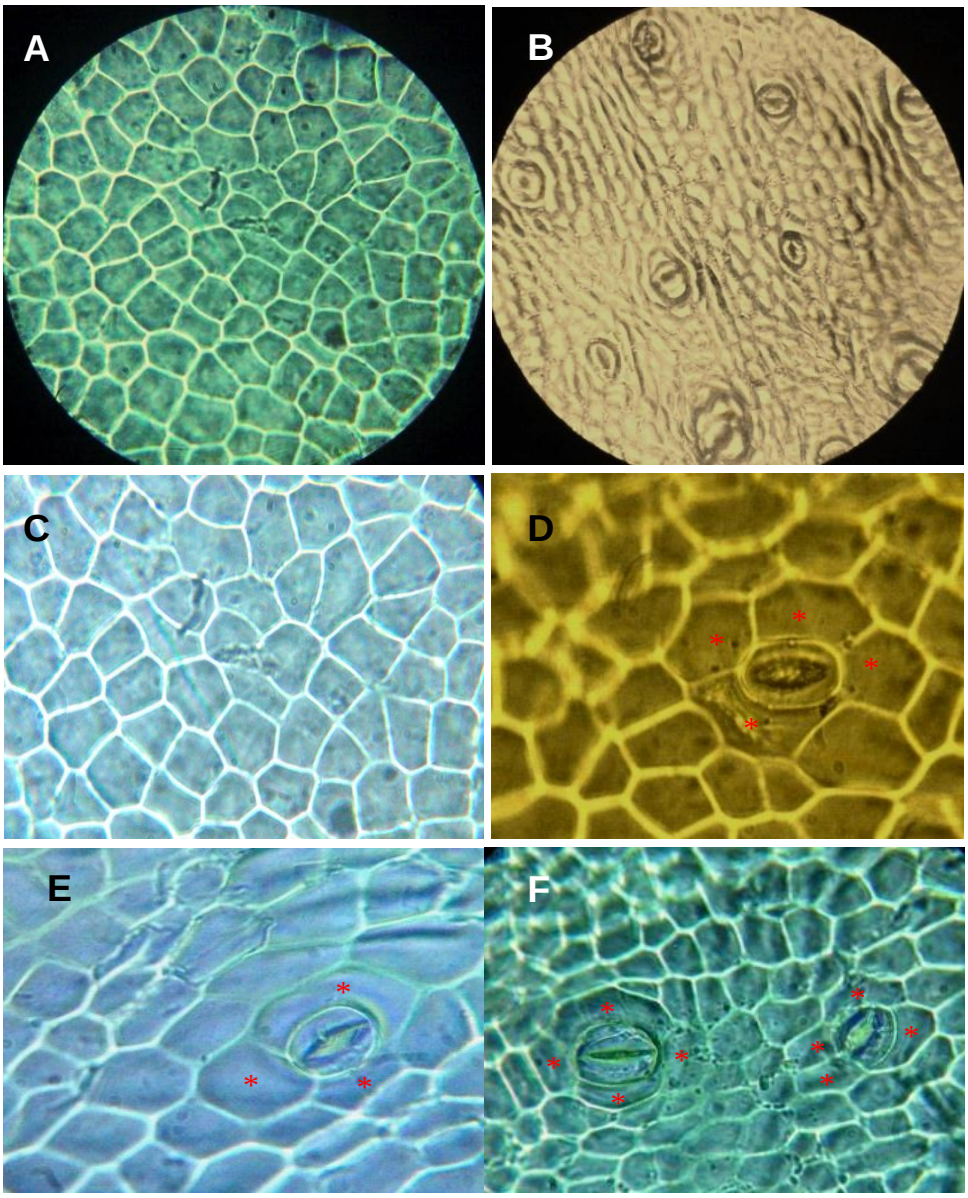

Fig. 15. Cotyledonary stomata. A and C, Outer surface with no stomata. Inner surface (B, D, E, and F) with anisocytic and tetracytic stomata.

Stomata are generally situated at the epidermal level or raised above the level of epidermal pavement. In Jojoba, however, each of the stomata was found to be sunken i.e. the guard cells were below the epidermal level. Fig. 25). Each stoma was protected by a collar of cuticular material called "epicuticular dome by Yermanos (1967).

Stomatal Density: Stomatal density is important from water conservation view-point. It may range from 20 to 1000 stomata per mm^2 of leaf surface in plants (Meidner and Mansfield, 1968). Distribution of stomatal density on stem and dorsal and ventral foliar surfaces of Jojoba is presented in Fig. 26 and 27, respectively. Stomatal density in Jojoba was found to be comparatively low. On a young epicotylar stem, the stomatal density averaged to 49.05 ± 1.62 stomata per mm^2 ranging from 10 to 108. The distribution of stomatal density was asymmetrical and characterized with significant positive skewness and leptokurtosis (Fig. 26). Epicotylar stomatal density was more or less comparable to that on the dorsal foliar surface but substantially lesser than that of the ventral foliar surface (Fig. 26 and 27). On dorsal surface of leaf a sizeable proportion of 71.4 % was occupied by the stomatal density size classes ranging from 49 to 69 stomata per mm^2 whereas on ventral surface predominating classes, occupying a proportion of 86.7%, ranged from 69 to 98 stomata per mm^2. In both cases the distribution tended to be non-normal as indicated by the Kolmogorov-Smirnov test (Fig. 27). Jojoba leaves were amphistomatic with more stomata on lower surface. The average number of stomata on upper epidermis of leaves from the third node of one month old

seedlings (Fig. 27) in our studies (55.98 ± 1.452 per mm^2) was found to be somewhat lower than that reported on dorsal surface (79 per mm^2) of 10 Jojoba seedlings (age not known) by Glat *et al.* (1981). The average number of stomata on lower surface as per our studies (81.91 ± 1.387 per $mm^{2)}$ was, however comparable to that reported by Glat *et al.* (1981) for Jojoba (89 per mm^2) of the American desert. Glat *et al.* (1981) had reported significant variation in stomatal density amongst various clones of Jojoba. Salinity is also one such reason which has been reported to affect the stomatal density in Jojoba (Hassan and Ali, 2014). Our results of inequality of stomatal density on foliar surfaces are in agreement with Ashour *et al.* (2013) who also reported that stomata are more frequent on ventral foliar surface of Jojoba. Janick and Paull (2008) have, however, described the leaves as isolateral with equal number of stomata on dorsal and ventral surfaces. stomatal density is known to vary among individuals of a species, among leaves of a plant and even on different locations over the same leaf surface (Cole and Dobrenz, 1970).

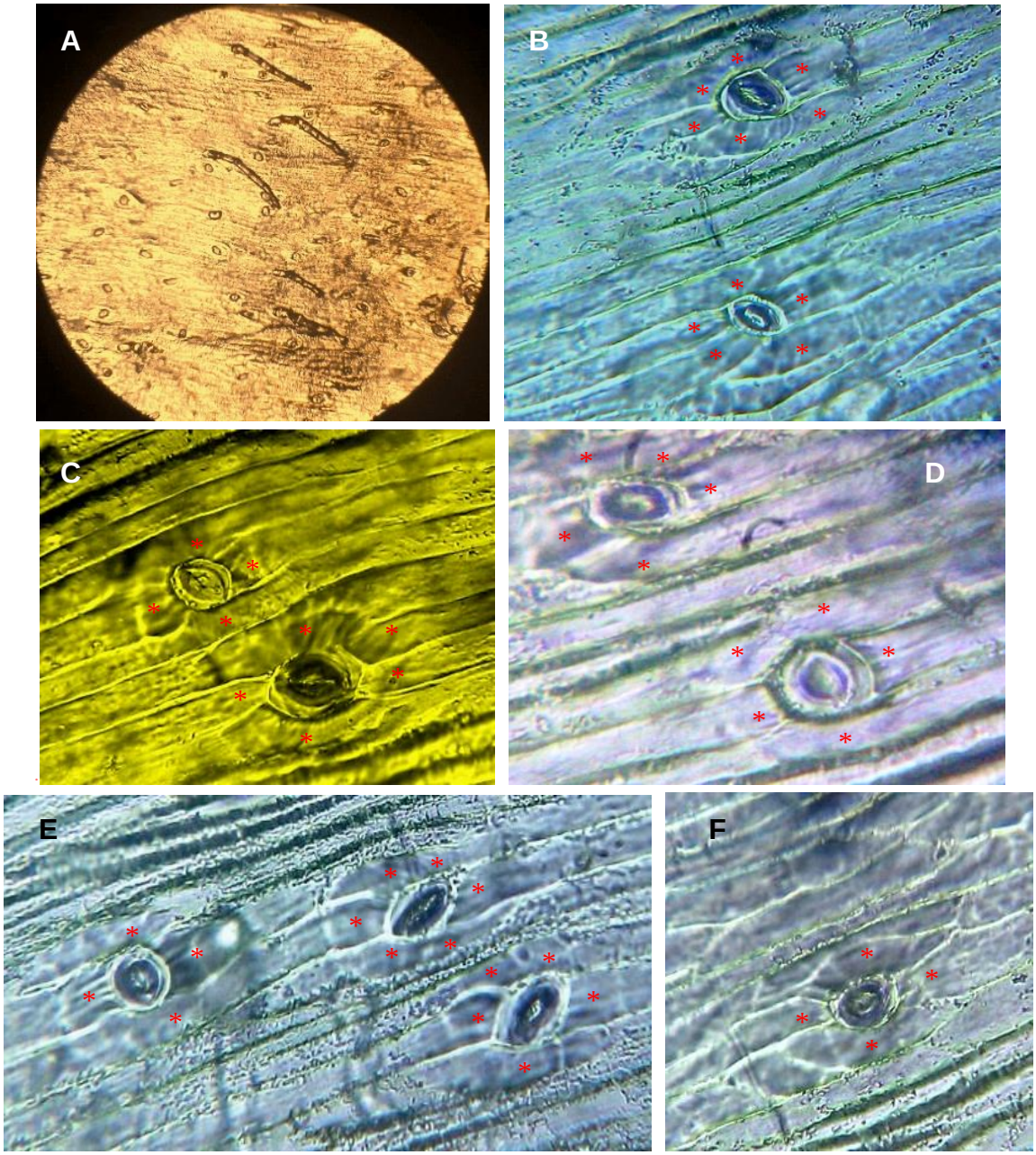

Fig. 16. Epicotylar stomata of Jojoba. A, surface view under low power showing stomata and multicellular trichomes (10 x 10 X). There are two types of stomata - tetracytic and anomocytic (B, C, D, E and F – Magnification: 45 x 10 X). Note that stomatal pores are oriented in different directions.

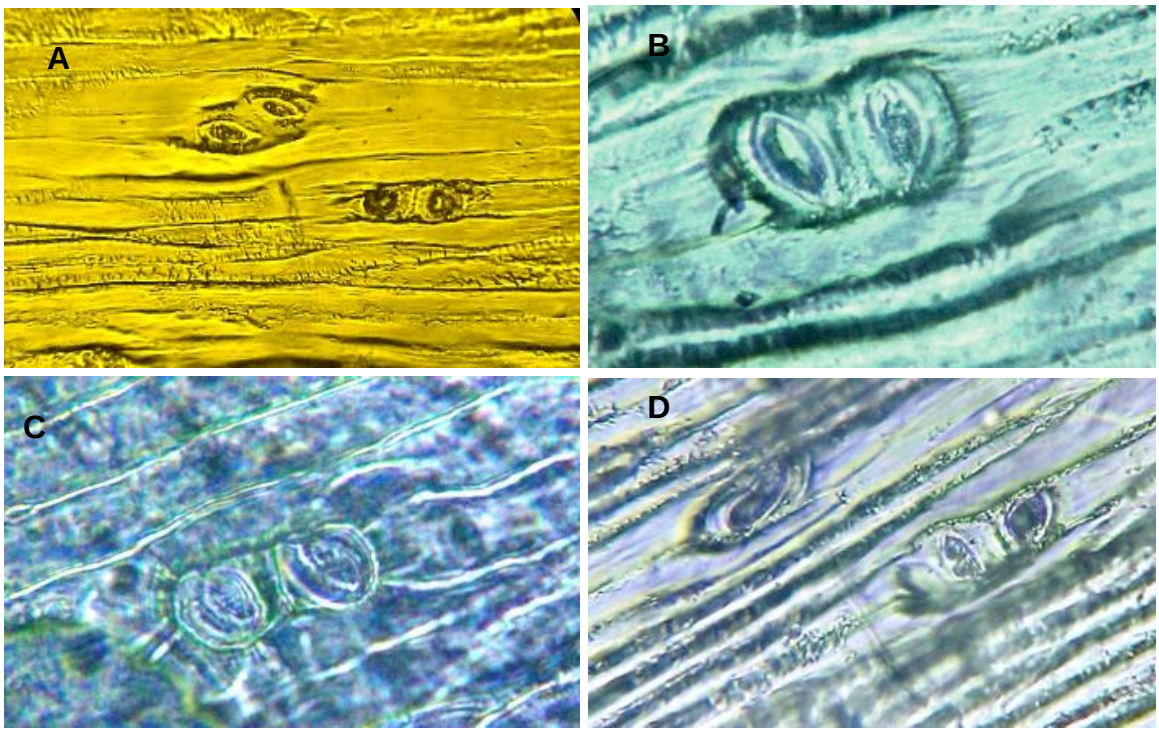

Fig. 17. Contiguous stomata (no subsidiary in between) on the surface of epicotyl of Jojoba seedlings. Such stomata were substantially frequent. The axes of stomatal pores of the two contiguous stomata may be parallel to each other or directionally discordant.

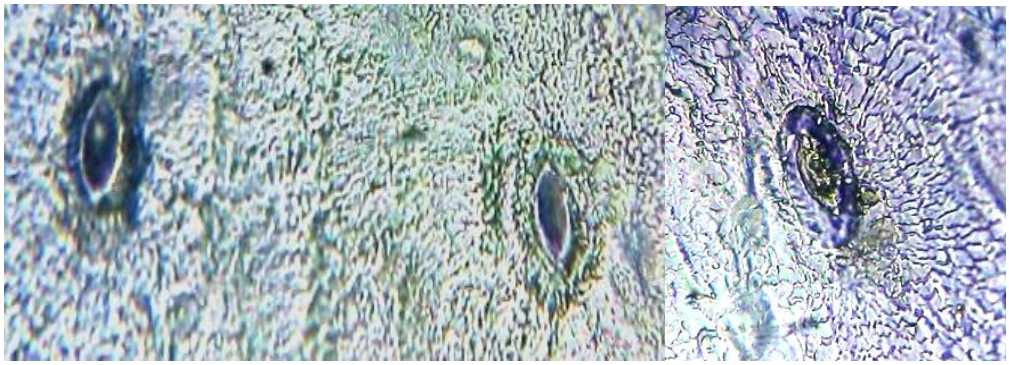

Fig. 18. Dorsal surface of adult leaf of Jojoba showing thick encrustation of cuticle and wax over the surface except the stomatal pores. The pore sunken with a doom around. (45 x 15 X and zoomed).

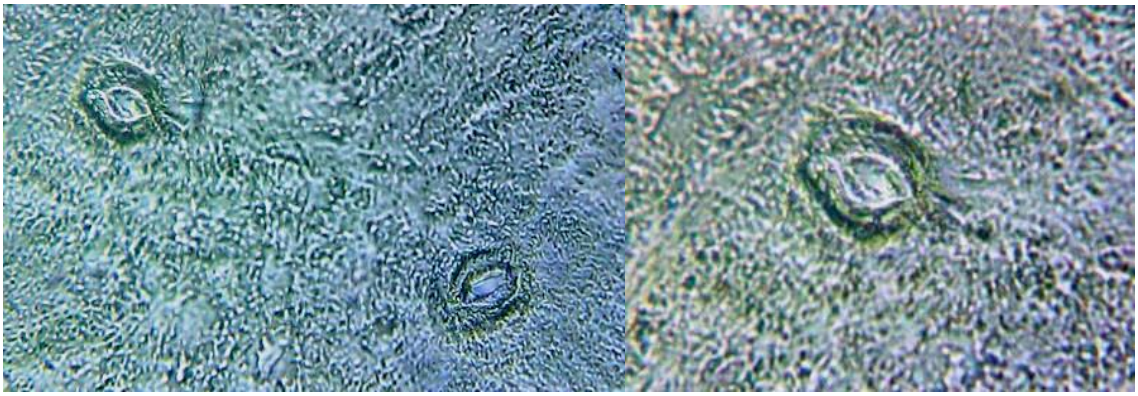

Fig. 19. Ventral surface of adult leaf of Jojoba showing thick encrustation of cuticle and wax over the surface except the stomatal pores. The pore sunken with a dome around. (45 x 10 X).

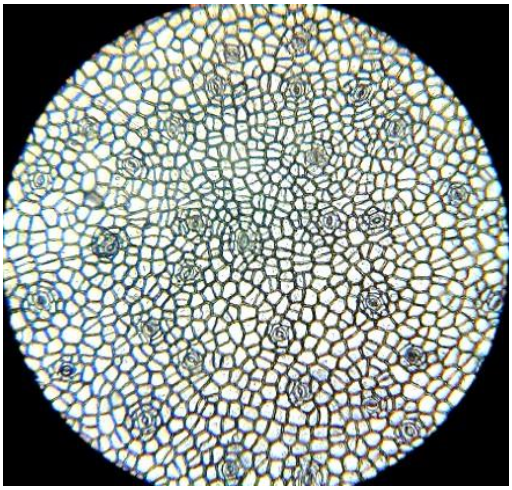

Fig.20. Surface view of dorsal epidermis peel obtained by treatment of leaf with lactic acid and ammonia. Cuticle, wax and trichomes removed. Stomata are scattered in the epidermal pavement with no or very small intercellular spaces between the cells. (Magnification 10 x 10 X).

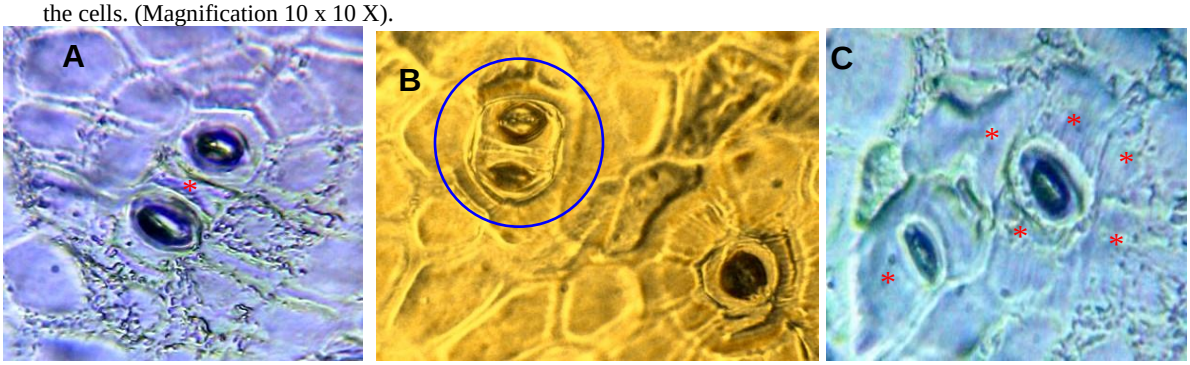

Fig. 21. Dorsal leaf surface showing two adjacent stomata with a common subsidiary (A); two stomata with no subsidiary in between and abutting together forming a compound stomatal structure (shown inside circle) (B). This compound structure was 32 x 15 µm in size – the upper stoma was 16 x 14.4 µm in size and lower one 19.2 x 11.2 µm in size. An unusual stomata surrounded by a subsidiary all around (pericytic stoma) is visible in C beside an anomocytic stomata. This is a rare feature.

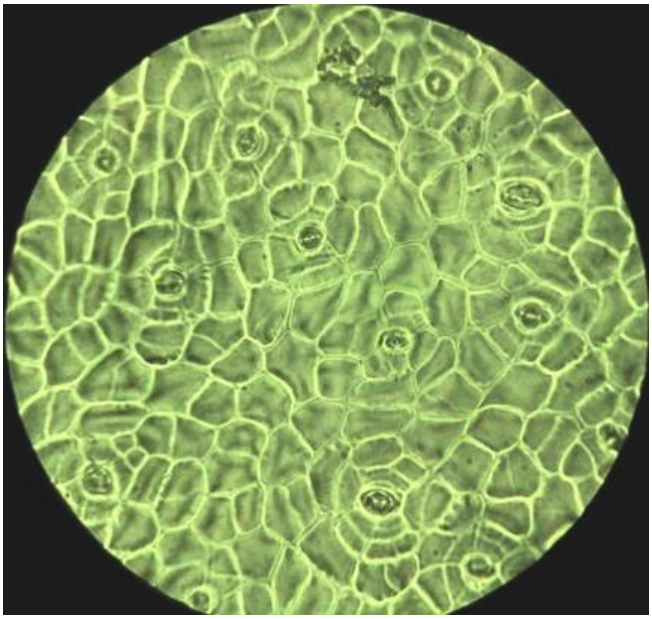

Fig. 22. Anomocytic stomata scattered over the ventral foliar surface of Jojoba. Intercellular cavities are very small or not at all. (Nail polish imprint of leaf treated with Hexane to remove epicuticular encrustation. Image seen under magnification of 45 x 10 X).

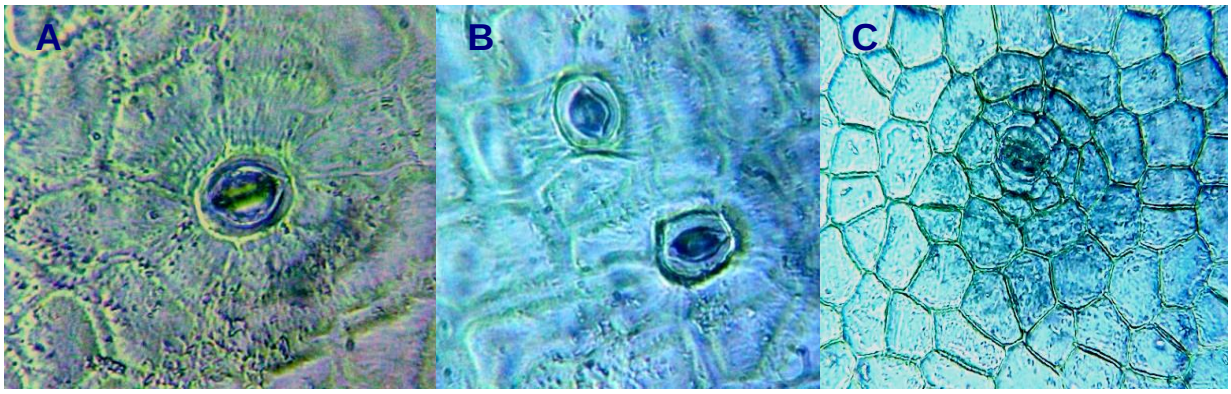

Fig. 23. Epidermal peel of ventral side of leaf Jojoba (Lactic acid + Ammonia treated leaf). A single anomocytic stoma; B, Two adjacent stomata and C, Single anomocytic stomata with three cycles of subsidiaries.

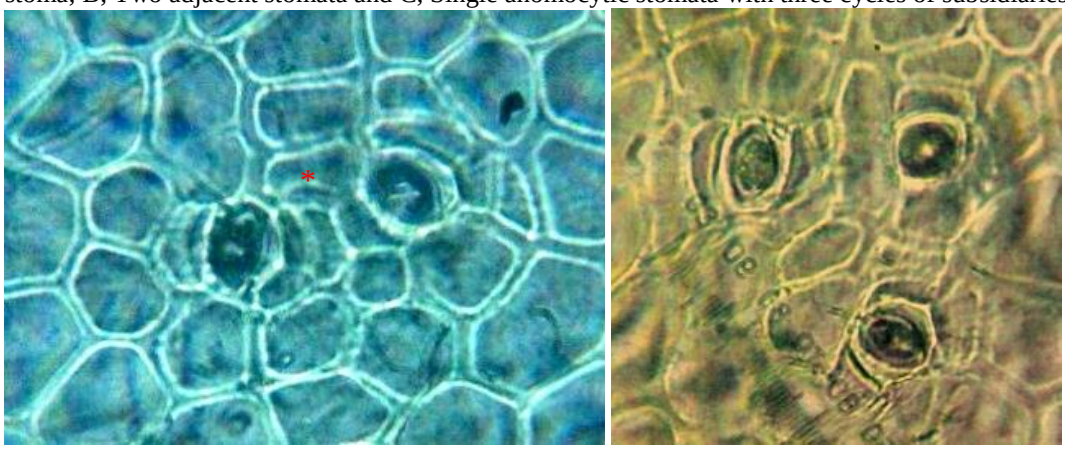

Fig. 24. Two adjacent stomata with common subsidiary (shown by an asterisk) and a cluster of three stomata. Ventral surface of a hexane treated leaf.

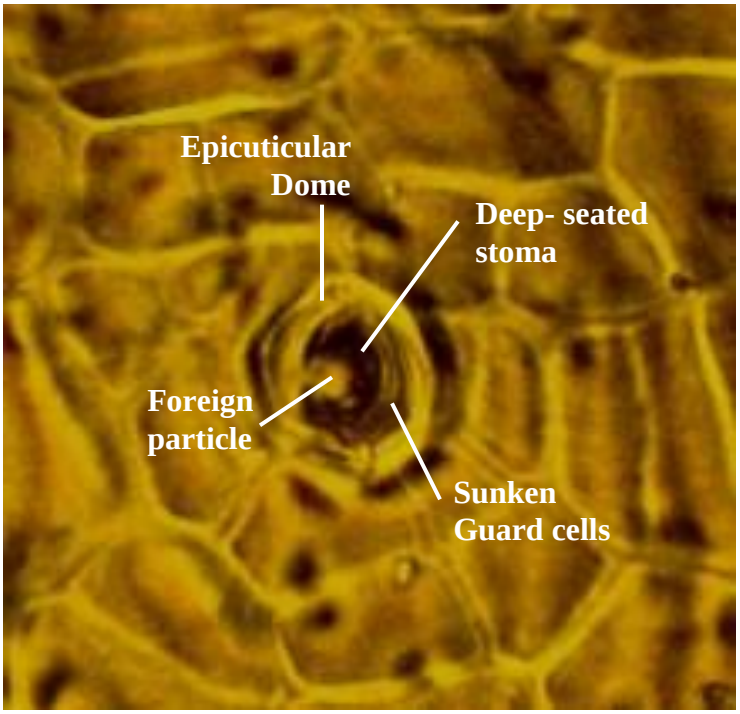

Fig. 25. Image showing the sunken nature of stomata in a hexane treated leaf. Epicuticular dome is prominently visible.

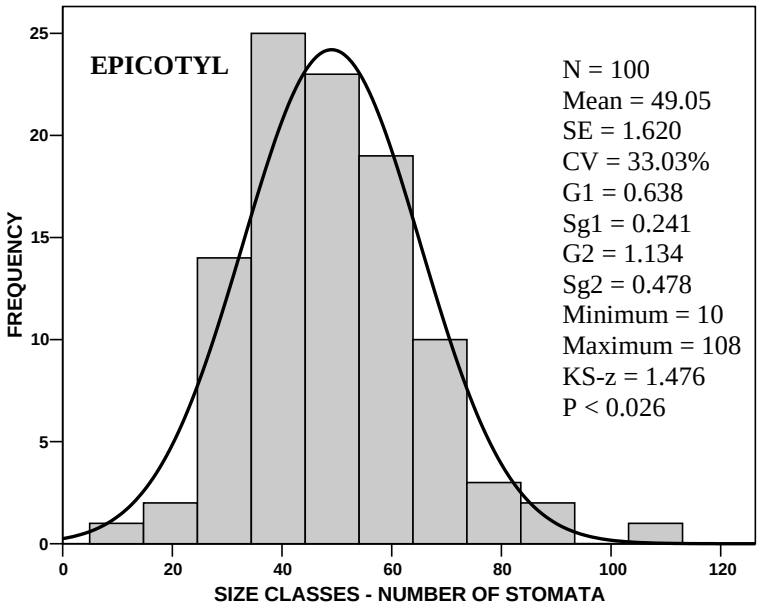

Fig. 26. Frequency distribution of number of stomata per mm^2 on surface of epicotylar stem of Jojoba seedling. g1 = skewness; g2 = kurtosis. Sg1 = St. Error of skewness and Sg2 = St. Error of kurtosis.

Table 2. Length and breadth (μm) of stomatal complex (stoma + epicuticular dome) on dorsal and ventral surfaces of Jojoba leaf (N =50).

Parameter	Dorsal Surface		Ventral Surface	
	Length	Breadth	Length	Breadth
Mean	26.94	19.07	25.98	20.83
SE	0.469	0.445	0.702	0.481
Min-Max	19.2-33.6	12.8-25.6	16.0-40.0	12.8-27.8
CV (%)	12.30	16.50	19.09	16.31

Table 3. Colonization (%) of the fungi on seed and seedlings of Jojoba.

Organ	*Rhizopus stolonifer*	*Aspergillus niger*	*A. terreus*	*A. flavus*	*A. fumigatus*	*Fusarium solani*	*Absidia* sp.
Seeds unsterilized	91.11 ± 3.51	86.67 ± 7.45	6.67 ± 3.33	0.0	0.0	37.78± 10.24	0.0
Seeds sterilized	33.33 ± 6.67	82.22 ± 8.46	26.67 ± 8.16	15.56 ± 10.42	10.33 ± 8.76	33.33± 11.06	8.89 ± 6.76
Roots Unsterilized	73.33 ±	6.67 ± 6.67	6.67 ± 6.67	6.67 ± 6.67	0.0	100 ± 0.0	0.0
Roots sterilized	40.00 ± 20.00	20.0 ± 11.55	13.33 ± 13.33	13.33 ± 13.33	6.67 ± 6.67	66.67± 33.33	0.0
Stem Unsterilized	86.67 ± 13.33	13.33 ± 13.33	0.0	0.0	20.0 ± 11.55	100 ± 0.0	0.0
Stem sterilized	86.66 ± 13.33	46.67± 6.67	13.33 ± 13.33	13.33 ± 13.33	6.67 ± 6.67	73.33 ± 13.33	0.0
Leaf Unsterilized	73.33 ± 6.67	33.33± 17.64	0.0	0.0	6.67 ± 6.67	60.0 ± 0.0	0.0
Leaf sterilized	93.33 ± 6.67	46.67 ± 24.04	0.0	0.0	0.0	80.0 ± 20.0	0.0

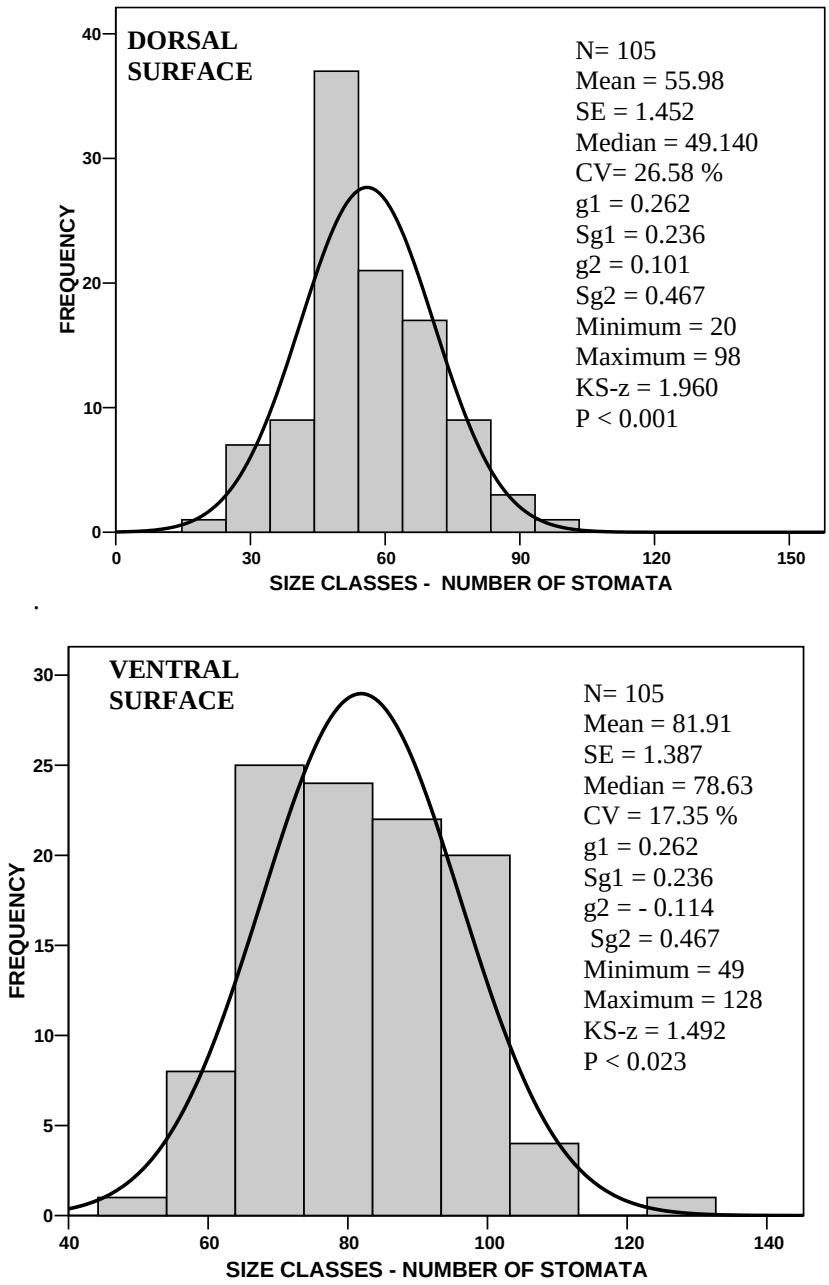

Fig. 27. Frequency distribution of number of stomata per mm^2 on upper (dorsal) and lower (ventral) surfaces of Jojoba leaf. g1 = skewness; g2 = kurtosis. Sg1 = St. Error of skewness and Sg2 = St. Error of kurtosis.

Size of stomatal complex: Size and ranges of the Stomatal complex (stoma + epicuticular dome) on dorsal and ventral leaf surfaces were more or less comparable (Table 2). The length of the stomatal complex averaged to 26.94 ± 0.409 µm on dorsal and 25.98 ± 0.702 µm on the ventral surface. The stomatal breadth averaged to 19.07 ± 0.445 µm on dorsal surface and 20.83 ± 0.481 µm on the ventral surface. The length and breadth of the stomatal complex varied by 12.3 and 16.5 % on the dorsal surface, respectively and by 19.09 and 16.31% on the ventral surface, respectively. Stomatal size of Jojoba appears to be roughly comparable with the guard cell size of *Prosopis cineraria* and *Crotalaria burhia* (29.39 ± 2.01 and 28.05 ± 6.93 µm in length), the two desert species of Thal and Cholistan, Pakistan (Bokhari and Dasti, 1991).

Jojoba seedlings, in present studies, were characterized with relatively longer roots, small leaf size, trichomes all over the plant body (even seed), thick cuticle and wax on stem and leaf surfaces and smaller and sunken stomata – the useful adaptations to withstand hot and dry conditions of the desert environment.

Seed and seedling Mycoflora: In all, seven fungal species (4 genera) were found to associate with Jojoba seeds and seedlings (Table3). *Rhizopus stolonifer, Aspergillus niger* and *Fusarium solani* were isolated from all parts of seedlings (root, stem and the leaf) and the seeds as well. These fungi were present on the surface and in internalized state. *Aspergillus terreus* and *A. flavus* were not found to associate with leaf. They were more frequent as seed and root borne fungi. *A. fumigatus* was another such fungus which was seed and root borne and also associated with stem. With leaf, it associated only externally. *Absidia sp.* only associated with seeds in internalized state. In all, seven species were seed borne (Table 3). Four of the seven species belonged to genus *Aspergillus*. These are called storage fungi and have been reported from lentil (Rahim *et al.*, 2010). Rahim and Dawar (2015) have reported the in hand *Aspergilli* along with *R. stolonifer* from Okra. Dawar *et al.* (2014) reported *Absidia* sp. and *Fusarium solani* from cumin seeds. All the species isolated here were reported to associate with deteriorating cellulosic materials (Dawar *et al.*, 2015).

Orhan (2012) have reported that mature Jojoba plants raised through cuttings were mainly infected by two species of *Fusarium* - *F. solani* and *F. oxysporium.* Other pathogens reported from Jojoba are, *Alternaria* spp., *Pythium* sp., *Phytophthora* spp., *Rhizoctonia* spp., *Cylindrocladium* spp., *Diplodia* spp., *Colletotrichum* spp., *Phoma* spp. and *Erwinia* spp. (Orhan, 2012). Lucero *et al.* (2005) have reported *Phytophthora nicotianae* infecting Jojoba in North-West Argentina. Sharma and Champawat (2000) have reported *Aspergillus flavus*, *A. nudilens*, *A. niger*, *Fusarium pallidoroseum* and *Rhizopus* sp. from Jojoba seeds

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