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FACTORS AFFECTING AND ANATOMICAL ASPECTS OF *IN VITRO* SHOOT REGENERATION OF FLAX (*Linum usitatissimum* L.)

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ABSTRACT

Direct shoot regeneration of two flax cultivars; Blanka (a fiber cultivar) and Giza 8 (a dual purpose cultivar) was conducted *in vitro* using different explants from the seedling (hypocotyl, cotyledon and root). Factors affecting as well as stages and capacity of direct shoot regeneration and anatomical features of *in vitro* induced shoots comparing with *in vivo* shoots were studied. The most remarkable outcomes proved that the used culture medium is the key factor that affecting the shoot regeneration when combined with any other factor (explant type, explant age and genotype). Hypocotyl explant proved to elevate shoot regeneration percentages that varied between 33 to 95%. Three days aged explant bear out the highest percentage of shoot regeneration as compared with explant aged 7 days. The most medium was MS basel medium supplemented with 0.25 mg/l NAA + 0.50 mg/l BA. Inductive flax cv. Blanka showed a higher shoot regeneration percentage as compared with the other genotype flax cv. Giza8. Bud formation was initiated on the surface of the flax seedling hypcotyl as small projection, this projection showed a heavy wrinkled and waved sculpture, later it developed into normal shoot bud with mersitematic dome. The anatomical features of *in vivo* and *in vitro* stems and leaves were inversely distinctive as the *in vitro* stems were wider and maintained thicker epidermal cells as well as thicker cortex and pith whereas having lesser amount of supportive and vascular tissues. Comparing with *in vivo* leaves, the *in vitro* leaves were thiner with poor differentiated epidermal, mesophyll and vascular tissues. The *in vitro* leaves showed bigger and denser stomata area in both leaf surfaces.

Key words: *adventitious bud - Linum usitatissimum -shoot regeneration*

Abbreviations: BA: Benzyl adenine, 2, 4-D: Dichlorophenoxy acetic acid , NAA: Naphthalene acetic acid

1. INTRODUCTION

Flax (*Linum usitatissimum* L.) is one of the most earliest domestic and cultivated plant species. It is an important source of natural fibers for textile industries and oil for the production of paints, varnishes, inks, linoleum and pharamaceaticals. (Green and Marshall, 1984, and Belonogova and Raldugina, 2006). Moreover it has the potential of meeting edible oil and protein deficiency (Green, 1986). Flax improvement has not been developed at the same rate as in other crops. Biotechnology and genetic engineering can lead to speed up flax breeding and providing incorporation of valuable and desirable traits as salinity tolerance (Mc Hughen and Schwartz, 1984) and resistance to *Fusarium oxysporum* (Rutkowska *et al.*, 2003). Tissue culture of flax has been carried out for more than 30 years. *In vitro* flax regeneration through direct organogenesis is considered as the most valuable method as having higher regeneration percentage and lower number of somaclonal variants in comparison with regeneration through embryogenesis (Cristina *et al.*,1997; and Dedicova *et al.*, 2000) or indirect organogenesis (Bretagne *et al.*, 1994). For shoot regeneration, various flax explants were used; hypocotyl (Lane, 1979, Mustafa and Murat, 2002 and Blinstrubiene *et al.*, 2004), Cotyledon (Belonogova and Raldugina, 2006), root (Cristina *et al.*, 1997), anther (Burbulis *et al.*, 2007), ovary (Bartosova , 2006), protoplast (Ling and Binding, 2006), however , hypocotyl segments proved to be highly efficient (Friedt, 1990). Other factors as genotype, age of donor plant, medium composition, and stress also affected the regeneration process. Blinstrubiene *et al.* (2004) reported that flax shoot regeneration largely depends on the genotype, while (Cristina, *et al.*, 1997), stated a genotype- independent procedure. Although flax shown to be responsive *in vitro* to a wide range of growth regulators including thidiazuron (Bretagne *et al.*, 1994), naphthalene acetic acid and benzyl adenine are the most commonly used for direct shoot

regeneration. Flax hypocotyl segments from 5 and 7 day old seedlings were cultivated *in vitro* for direct regeneration by Dedicova *et al.* (2000) and Mustafa and Murat (2002).

Direct regeneration of adventitious shoot meristems formed directly on explants *in vitro* is often initiated by cell division beginning in the epidermal and subepidermal layers. The initial cell division results in a mass of cells forming new meristematic centers (meristemoides), later becoming a shoot apical meristem with meristematic dome and leaf primordia (shoot bud) and finally developing into normal shoot. Regeneration in this manner is widespread occurring in monocots (*e.g. Allium sativum* L., Mahammad *et al.*, 1999), dicots (*e.g. Aloysia polystachya* L., Brudyni *et al.*, 2006) and conifers (*e.g.* spruce, Saravatiz *et al.*, 1993) .On flax, only two reports were published ; Keiko *et al.* (1997) on decapitated seedlings and Dedicova *et al.*(2000) on hypocotyl explant .

With respect to the anatomical characteristics of the *in vitro* plants in comparison with *in vivo* plants, it is well known that the special conditions during the *in vitro* culture cause morpho-physiological disorders of plantlets that result in poor survival during acclimatization (Hazarika, 2005) and prevent the successful use of tissue culture technique in crop improvement through genetic engineering . The leaves that develop *in vitro* generally lack well developed epicuticular waxes and have thin cuticle (Sutter and Langhans, 1982), they have increased number of malfunctioning small size stomata (Brainerd and Fuchigami,1981 ; Marin *et al.*,1988 and Johanson *et al.*,1992) , poor development of the photosynthetic apparatus (Prece and Sutter,1991) with no or few rows of palisade cells (Rosna and Noorma, 2008) with lower chloroplast number, poorly developed thylakoid staking , and low amount of chlorophyll and disorganized grana (Majada *et al.*, 2002) . Moreover, leaves and stems of *in vitro* shoots are characterized by relatively poor developed supportive and vascular tissues (Hazarika, 2005). As far as the authors are aware, the anatomical characteristics of flax plantlets grown *in vitro* had not been studied.

The aim of this research was to: 1- elucidate the effect of genotype, explant type, explant age and culture medium on the *in vitro* direct shoot regeneration of flax and 2- study the anatomical aspects of the *in vitro* shoots in comparison with *the in vivo* plants.

2. MATERIALS AND METHODS

Seeds of two flax cultivars namely. Blanka (fiber type) and Giza 8 (dual purpose type) were surface sterilized by 70% ethanol for 2 min followed by immersing in 80% Clorox for 20 min and then rinsed four times each of 5 min in sterile distilled water. Seeds were germinated on 0.7% agar full strength MS medium (Murshige and Skoog ,1962) supplemented with 3% sucrose and without growth regulators. After 3 and 7 days from germination the hypocotyl, cotyledon, root explants were placed on shoot regeneration media consisted of MS basal medium enriched with 3% sucrose and 0.2% gelrite. For induction of direct shoot regeneration various combinations of growth regulators were added 0.25 mg/l NAA + 0.5 mg/l BA, 2.0mg/l NAA+2.0mg/l Kin, 0.25 mg/l 2,4-D+2.0mg/l Kin, 0.25 mg/l 2,4-D+0.50 mg/l BA and the medium pH was adjusted to 5.7 – 5.8. The culture medium was sterilized by autoclaving at 121°C for 20 min. and incubated under controlled conditions of temperature 24 ± 2°C with 16 hours photoperiod under cool white fluorescent light. Intensity of illumination was 3000 Lux. At the end of the culture (5 weeks later) shoot regeneration percentage was estimated for scanning electron microscopy. Samples were fixed in 4% glutaraldehyde in 0.1 phosphate buffer (pH7.2) overnight at 4°C and then dehydrated through graded ethanol series and critical-point dried in liquid carbon dioxide. Specimens were mounted on stubs, sputter coated with gold, and examined with a scanning electron microscope (model JSM-T220; Jeol Ltd.). For the anatomical studies semi thin sections were prepared through cutting at 1mμ using the ultramicrotome model EM-UC6. Sections were stained with Toludine blue mounted and examined by light microscope.

3. RESULTS AND DISCUSSION

3.1. Factors affecting direct regeneration

Shoot regeneration capacity was influenced by many factors; *i.e.*, explant type, explant age, genotype and medium composition. The statiscal analysis showed that the interactions between these factors significantly affected the average percentages of shoot regeneration. So, the optimal combinations that produced high regeneration percentage, regardless the effects of the other factors, will be thrash out in this study.

The used culture medium was the key factor that affecting the shoot regeneration when combined with any other factor (explant type, explant age and genotype). The over all shoot regeneration percentages regardless the effects of

other factors were, 17,20, 49 and 54% for medium supplemented with 2,4-D (0. 25 mg/l) + BA (0.5 mg/l)+BA (0.5 mg/l), respectively (Fig. 1, A). The interaction between the used medium and the explant type showed different response. It is clear that, in both flax cultivars the hypocotyl was the best explant for the induction of direct shoot regeneration followed by cotyledon and root explant. Data illustrated in (Fig. 1,B) show that, the average shoot regeneration percentages due to explant type, calculated regardless of the other factors under study, were 82, 54 and 9% for the three studied explant hypocotyl, cotyledon and root, respectively. Moreover, hypocotyl explant combined with the four tested growth regulator combinations proved to induce high shoot regeneration percentages that varied between 33 to 95%. The highest shoot regeneration percentage 95% was recorded with MS medium supplemented with NAA (0.25 mg/l) + BA (0.5 mg/l). While the lowest recorded percentage 0.0% was noted with root explant incubated with the medium supplemented with NAA (2.0 mg/l) +Kin (2.0 mg/l). However, cotyledon explants gave intermediate direct regeneration percentages.

The shoot regeneration percentages as affected by explant age; 3 or 7 days combined with medium composition are shown in (Fig 1, C). Generally, 3 days aged explant proved to maintain the highest shoot regeneration as compared with explant aged 7 days. The average recorded shoot regeneration percentages using 3 days aged explant, regardless the other factors under study, were 72% compared with 36% for 7 days aged explant, (Fig 1, C). The interaction between the explant age and the used medium showed that, within each medium, shoot regenaration percentages were not greatly affected by the explant age. Where, the average percentage differences between explants aged 3 and 7 days within each used medium, ranged from 1% in case of NAA (0.25 mg/l) + BA (0.5 mg/l) to 16% in case of NAA (0.25 mg/l) + BA (0.5 mg/l). In addition, the highest recorded percentages were regarded for explants, cultured on MS medium supplemented with NAA (0.25 mg/l) + BA (0.5 mg/l), were 53 and 54% for explant aged 3 and 7 days, respectively. While, the lowest recorded percentages were 17 and 10% for explants aged 3 and 7 days and cultured on MS medium supplemented with 2, 4-D (0.25 mg/l) + Kin (2.0mg/l).

Regarding the genotype effects on direct regeneration, the two used genotypes were different regarding shoot regeneration capacities. Generally, the over all shoot regeneration percentage of Blanka regardless of the other mg/l), 2,4-D (0.25 mg/l) + Kin (2.0 mg/l), NAA (2.0mg/l) + Kin (2.0 mg/l) and NAA (0.25 investigated factors was 39% comparing with 33%. For the other genotype Giza 8. The interaction between genotype and the used medium was differed significantly. Blank showed 64% which was the highest shoot regeneration percentage with the medium supplemented with NAA (0.25 mg/l)+BA (0.5 mg/l). While it showed 10% shoot regeneration percentage with the medium supplemented with 2, 4 -D (0.25 mg/l) +Kin (2.0 mg/l). The same trend with relatively lower percentages was obtained with the other genotype Giza 8.

From the above mentioned results it is clear that shoot regeneration capacity was influenced by the used culture media. This was early reported by Tejklova (1992) and Cointry *et al.* (1993). They reported that flax direct shoot regeneration explants were cultured *in vitro* on MS medium supplemented with 0.01, 0.1, 1.00 or 3.0 mg/l IAA, NAA or 2, 4-D alone or in combination with the same concentrations of [BA]. The optimal combination of growth substances for bud regeneration on shoot segments was 1.0 mg/l BA.

The type of the explant also, affects the shoot regeneration capacity. This was early reported by Netherland *et al.* (1988) and Zhan *et al.* (1989). They mentioned that hypocotyl explant seemed to be the best explant source for bud regeneration, followed by roots. Moreover, the present outcomes revealed that explant age and the They mentioned that hypocotyl explant seemed to be the best explant source for bud regeneration, followed by roots. Moreover, the present outcomes revealed that explant age and the used genotype affect shoot regeneration. This is confirmed by Cointry *et al.* (1993), Dedicova *et al.* (2000) and Blinstrubiene *et al.* (2004).

3.2. Shoot bud regeneration on hypocotyl explant

The adventitious bud formation was traced using electron microscopy. It is evident that bud formation was initiated without distinct orientation on the surface of the flax seedling hypocotyl as small projection from the hypocotyls surface. This projection showed a heavy wrinkled and waved waxy sculpture (Fig2, a). From the top view of the developed bud a minute differentiated leaf primordia could be seen (Fig 2, b). The adventitious bud elongated and formed the central shoot axis (Fig 2, c). Finally, the adventitious bud showed a typical dicotyledonous shoot tip flanked by leaf pirmodia (Fig 2, d). The developmental histological study of adventitious shoot bud formation on decapitated flax seedling was reported by Marchuk and Raju (1978) and Keiko

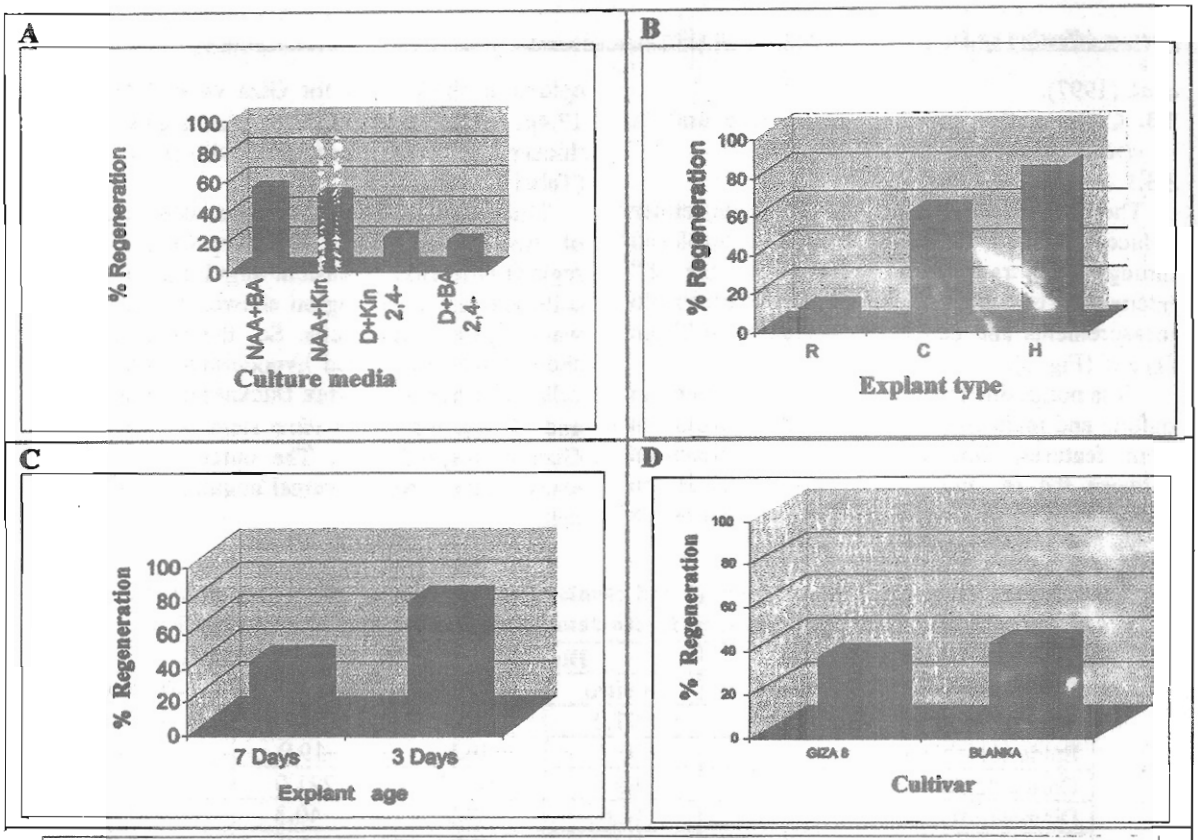

Fig. (1): The effects of the culture media, explant type, explant age and cultiv on direct shoot regeneration percentages of flax.

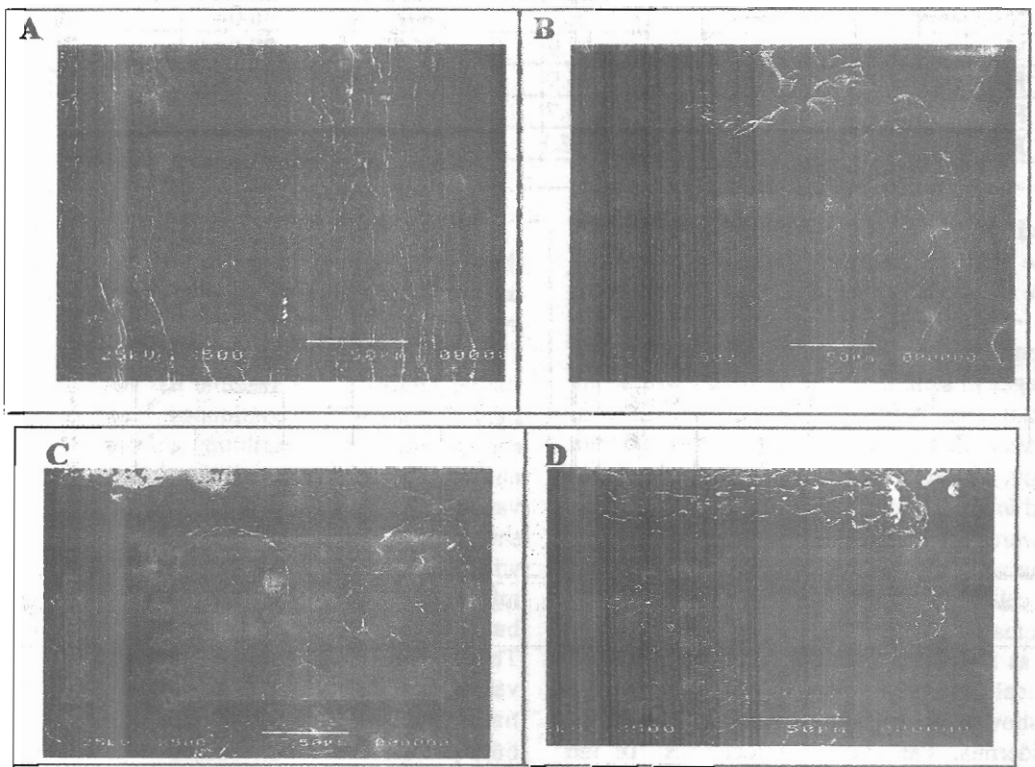

Fig. (2): Developmental stages of adventitious shoot bud formation on flax hypocotyl as seen by electron microscope.

et al. (1997).

3.3. Comparative anatomy of *in vitro* and *in vivo* developed shoots

3.3.1. Stem anatomical structure

The anatomical structure of the stems, either induced *in vitro* or grown *in vivo* was explored through the transverse sections in the 2nd internode of the stem. Full microscopic measurements and counts are presented in Table (1) and (Fig. 3).

It is noticeable that the stem layout is round in outline and maintained the typical dicotyledonous stem features. One of the major differences between the *in vivo* and *in vitro* stems is the average stem diameter. Generally, *in vivo* stems exhibited narrower stem diameter as compared with the *in vitro* stems. As well, stem internal structure of the two genotypes under investigation Balnka and Giza 8 showed different measurements and counts. The average increased percentages in stem diameter of the *in vitro* stems were 22.0 and 14.0% for Blanka and Giza 8, respectively. However, the average stem diameters of flax cv. Blanka scored, 816 and 997µ for the *in vivo* and *in vitro* stems. While, in the case of Giza 8 they were 907 and 1034µ. The stem epidermis is characterized by uniform thin walled barrel shaped cells. It is evident that the epidermis of *in vivo* stems is covered by relatively thick waxy cuticle as compared with the *in vitro* stems. The anatomical features of both *in vitro* and *in vivo* stems showed no trichomes on both surfaces of the epidermis. The average thicknesses of the epidermis as shown in the transverse sections of the Blanka were 20.7 and 16.8µ for *in vitro* and *in vivo* stems, respectively. The corresponding epidermis thicknesses for Giza were 8 19.9 and 17.4µ. indcating that , *in vitro* plant stems showed thicker epidermis as compared with *in vivo* stem, (Tabel 1) and Fig 3).

The cortex of the *in vivo* stems was composed of two main regions; the hypodermis (outer region) with firm consistent angular collenchyma cells and the inner region showed typically thin walled parenchyma cells. So, the *in vivo* stems showed well developed hypodermis collenchyma cells. The average cortex thicknesses were 198.5 and 221.0µ for the *in vitro* stem of Blanka and Giza 8, respectively . The outer cortex region showed five rows of normal angular collenchyma cells.

Table (1) : Average measurements (µ) and counts of certain microscopical features in transverse sections of the *in vitro* and *in vivo* stems of two flax cultivars Blanka and Giza 8 .

Cultivars	Blanka		Giza 8	
Characters	*In vitro*	*In vivo*	*In vitro*	*In vivo*
Stem diameter	997.0	816.0	1034.0	907.0
Epidermis thickness	20.7	16.8	19.9	17.4
Cortex thickness	198.5	164.2	221.0	183.2
Diameter of cortical cells	46.6	38.2	40.3	36.1
Number of cortical layers	5.0	7.0	5.0	8.0
Vascular Bundles dimensions				
Length	88.9	109.8	90.1	103.2
Width	57.1	60.5	60.5	66.8
Number of xylem rows/ bundle	5.0	8.0	6.0	7.0
Vessel diameter	27.9	36.2	28.6	33.6
Phloem thickness	44.9	58.2	48.4	51.6
Pith cell diameter	73.7	63.0	76.2	60.8
Pith diameter	532.1	441.8	496.7	462.3

Furthermore, the cortex of the *in vitro* stems showed relatively large parenchyma cells as compared with that of *in vivo* stems, where the average size of cortex parenchyma cells gained 17.2 and 12.6% increase in diameter, for the two studied cultivars. The vascular tissues forming the stem stele showed discontinuous ring of fascicular and inter-fascicular cambium, differentiated to a number of different sized diffuse collateral vascular bundles. Reversely, the *in vitro* stems showed reduced vascular bundles as compared with those of the *in vivo* stems. This reduction reflected on both length and width of the vascular bundles and all tissues shared in their structure. The average dimensions (length - width) of the vascular bundles of the *in vivo* stems as shown in transverse section were 109.8, 60.5 and 103.2 , 66.8 µ for Blanka and Giza 8, respectively. The corresponding dimensions of the *in vitro* stems were 88.9, 57.1 and 90.1, 60.5µ, for the two flax

cultivars in the same order. The reduction in vascular bundle length was mainly due to the reduction occurred in both xylem vessels diameter and number of differentiated vessels rows in the stem radial direction (Fig 3, a&c).

The vascular bundles of the *in vitro* stems had compressed phloem with minute amounts of sieve elements mixed with phloem fibers which are quite undifferentiated as it showed scattered clusters of fiber cells mixed with extensive amounts of parenchyma cells. The average thicknesses of the phloem of the *in vivo* stems of . Blanka and Giza 8 were 58.2µ and 51.6µ , and 44.9µ and 48.4µ for the *in vitro* respectively. Xylem vessels exhibited variable size as the average vessel diameters of the *in vitro* stems. The *in vitro* stems showed wider pith as compared were 27.9 and 28.6µ for the two studied flax cvs. Blanka and Giza 8, respectively. These values were reduced by 25.0 and 15.1% as compared with the vessels diam eters of the *in vivo* stem of both cultivars. The average pith diameter increased by 20.0 and 7% for Blanka and Giza 8, respectively. The pith cells of the *in vitro* stem increased in diameter by 11.6 and 25.0% as compared with the *in vivo* stems (Table 1).

The above mentioned results proved that the anatomical structure of the *in vivo* and *in vitro* stems are inversely distinctive. As, the *in vitro* stems were wider and maintained thicker basic epidermal cells as well as thick cortex and pith. This enlargement was mainly due to the increase in average cell size. In addition, the *in vitro* flax stems characterized by absence of supporting collenchyma and poor development of both sclernchyma tissues and vascular bundles. These results agree with those mentioned before by Sabh (1998) in clover Abd El-Zaher (2008) in jackfruit and Fauguel *et al.*, (2008) in sun flower. Moreover, Hazarika (2005) reported that the stems

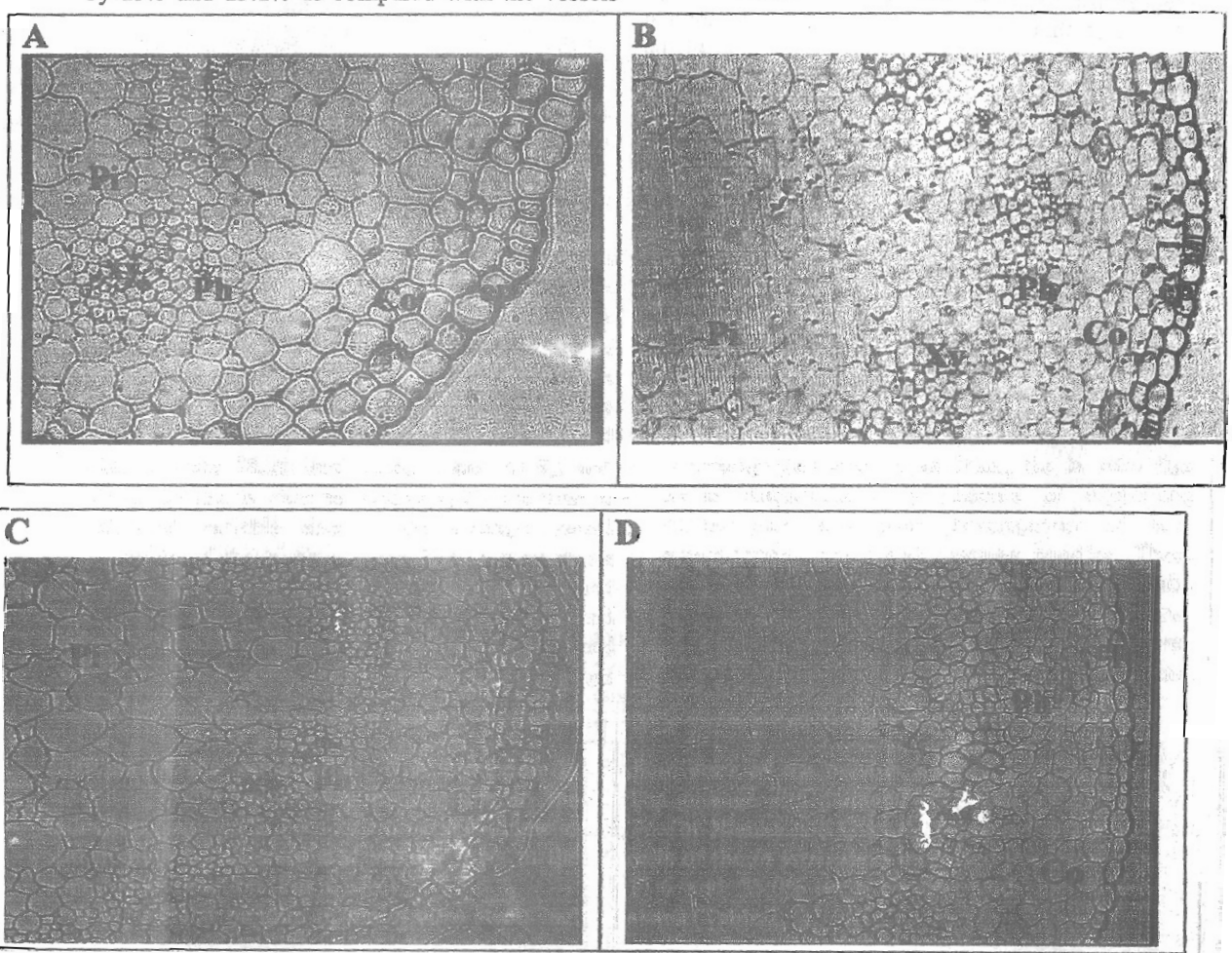

Fig. (3): Light micrographs of transverse sections of flax stems; flax cv. Blanka *in vivo* (A) and *in vitro* (B), flax cv. Giza 8 *in vivo* (C) and *in vitro* (D).
Details; Co: cortex, Ep: epidermis, Ph: phloem, Pi: pith, Xy: xylem.

of red raspberry plantlets grown *in vitro* were most slender and had considerably less collenchyma and sclerenchyma supportive tissues than plants grown in the field.

3.3.2. Leaf anatomical structure

The anatomical structure of the leaves, either induced *in vitro* or grown *in vivo* was explored through the transverse section in the 2nd leaf on the stem. Full microscopic measurements and counts are presented in (Table 2) and (Fig. 4). Generally, *in vitro* leaf was thinner as compared with the *in vivo* leaves as their thickness recorded 330 and 405μ ,respectively for Blanka, and 317 and 355μ. for Giza 8. Thus, the average reductions in leaf thickness of the *in vitro* leaves were 11.0 and 9.0% for Blanka and Giza 8, respectively.

The upper and lower epidermis of both *in vivo* and *in vitro* leaves of Blanka are characterized by uniform thin walled barrel shaped cells. While,leaf epidermis of the Giza 8 *in vitro* plants showed irregular thin walled cells. Moreover, the cuticle of both adaxial and abaxial side of the leaf of the *in vivo* plants was thicker as compared with the *in vitro* plants. The average thicknesses of the epidermis as shown in the transverse sections of Blanka were 35.5 and 37.5 μ for the *in vitro* and *in vivo* leaves, respectively. The corresponding epidermis thickness for flax cv. Giza 8 were 22.5 and 30.5μ, (Table 2 and Fig. 4). It is evident that the mesophyll of the *in vivo* leaf showed well developed spongy mesophyll parenchyma cells with considerable amount of chloroplasts as compared with those of *in vitro*. Moreover, the mesophyll of *in vitro* leaf contained more loosely parenchyma cells with appreciably wide intercellular spaces . Little number of chloroplasts was observed in the *in vitro* spongy parenchyma which gave the leaves pale green color and negatively reflected on photosynthetic activity. The average mesophyll thicknesses were 285.0 and 225.0μ for the *in vitro* leaves of Blanka and Giza 8, respectively. The increase in leaf thickness found in the *in vivo* leaves was due to the increased number of spongy parenchyma cells rather than the enlargement in spongy cell size. On the contrary, the spongy parenchyma cells of *in vitro* leaves showed 17.2 and 12.6% increase in diameter than corresponding *in vivo* leaves of flax cvs. Blanka and Giza 8 , respectively. These values were reduced by 19.0 and 12.0% as compared with vessel diameters of the *in vitro* leaves for the two cultivars in the same order.

Regarding the midrib, it is obvious that, the *in vivo* leaf showed well differentiated midrib bundle. On reverse, the *in vitro* leaves showed reduced vascular bundles as compared with was *in vivo*. This reduction was reflected on the thickness of the midrib bundle and all tissues shared in their structure. The average thicknesses of the vascular bundles of the *in vivo* leaves were 175.0 and 152.0 μ for Blanka and Giza 8, respectively. The corresponding thicknesses recorded for the *in vitro* leaves were 123.0 and 112μ for the two flax cultivars in the same order, Table (2) and Fig. (4). The phloem of the *in vitro* midrib was characterized by minute amounts of sieve elements mixed with extensive amounts of parenchyma cells. The phloem of *in vivo* leaves were 29.0 and 16.0% more than those of the *in vitro* leaves for flax cvs. Blanka and Giza 8, respectively. The xylem of the *in vitro* plants was also remarkably reduced as compared with the *in vivo* xylem. The average xylem thicknesses were 144.0 and 124.8μ for the *in vivo* as compared with 92.0 and 85.0μ for *in vitro* leaves. In addition, the average vessel diameters of the *in vivo* midrib were 29.1 and 27.7μ for the two studied flax cultivars Blanka and Giza 8, respectively. These

Table (2): Average measurements (μ) and counts of certain microscopical features in transverse sections of the leaves of the two flax cultivars Blanka and Giza8 grown *in vitro* and *in vivo*.

Cultivars / Characters	Blanka		Giza 8	
	In vitro	*In vivo*	*In vitro*	*In vivo*
Leaf thickness	330.0	405.0	317.0	355.0
Upper epidermis thickness	35.5	37.5	22.5	30.5
Lower epidermis thickness	198.5	164.2	221.0	183.2
Thickness of mesophyll	285.0	300.0	225.0	375.0
Diameter of mesophyll cells	25.7	20.2	20.1	17.8
Mid rib bundle thickness	123.0	175.0	112.0	152.0
Xylem thickness	92.0	144.0	85.0	124.8
Number of xylem rows	4.0	6.0	4.0	6.0
Vessel diameter	23.7	29.1	24.4	27.7
Phloem thickness	58.2	44.9	51.6	48.4

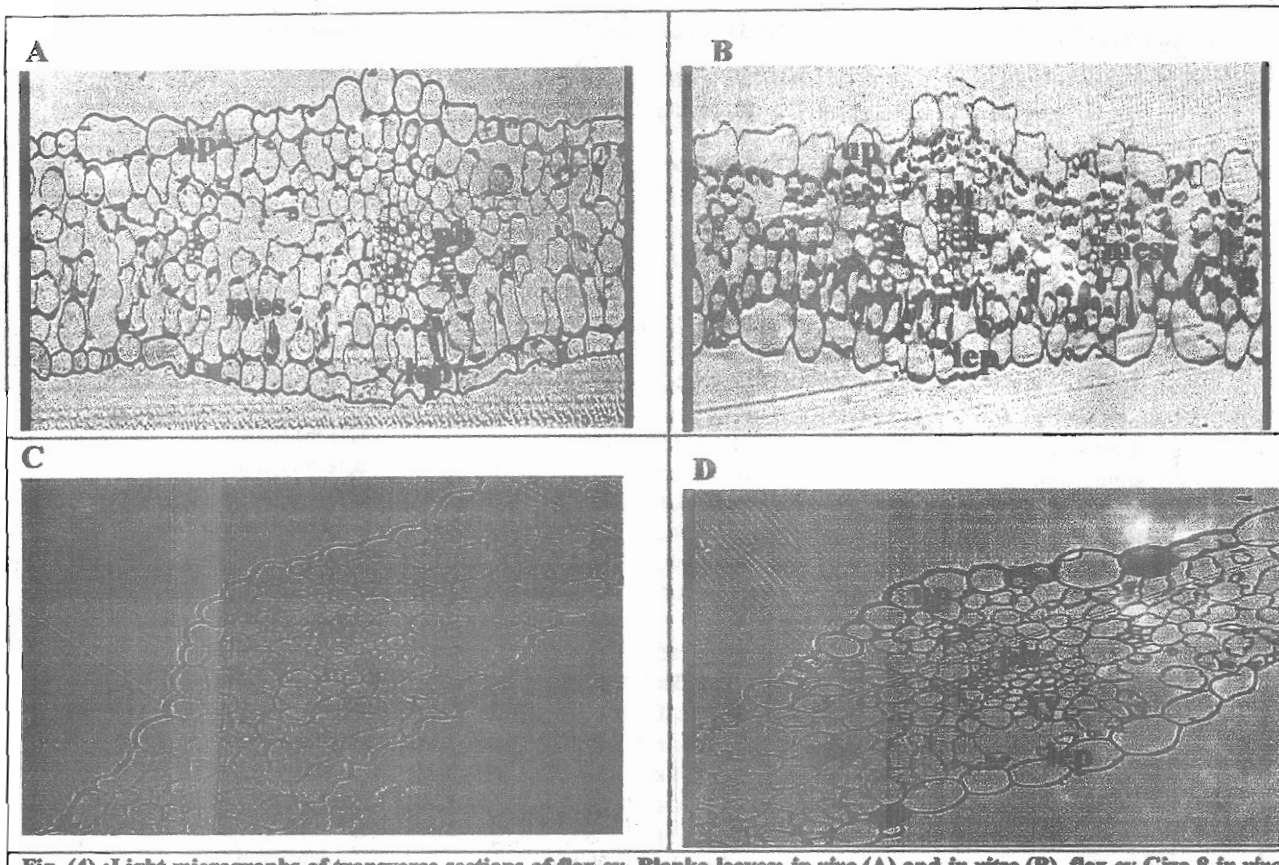

Fig. (4) :Light micrographs of transverse sections of flax cv. Blanka leaves; *in vivo* (A) and *in vitro* (B), flax cv Giza 8 *in vivo* (C) and *in vitro* (D).Details ; Lep: lower epidermis, Mes: mesophyll, Ph: phloem, Xy: xylem, Up: upper epidermis.

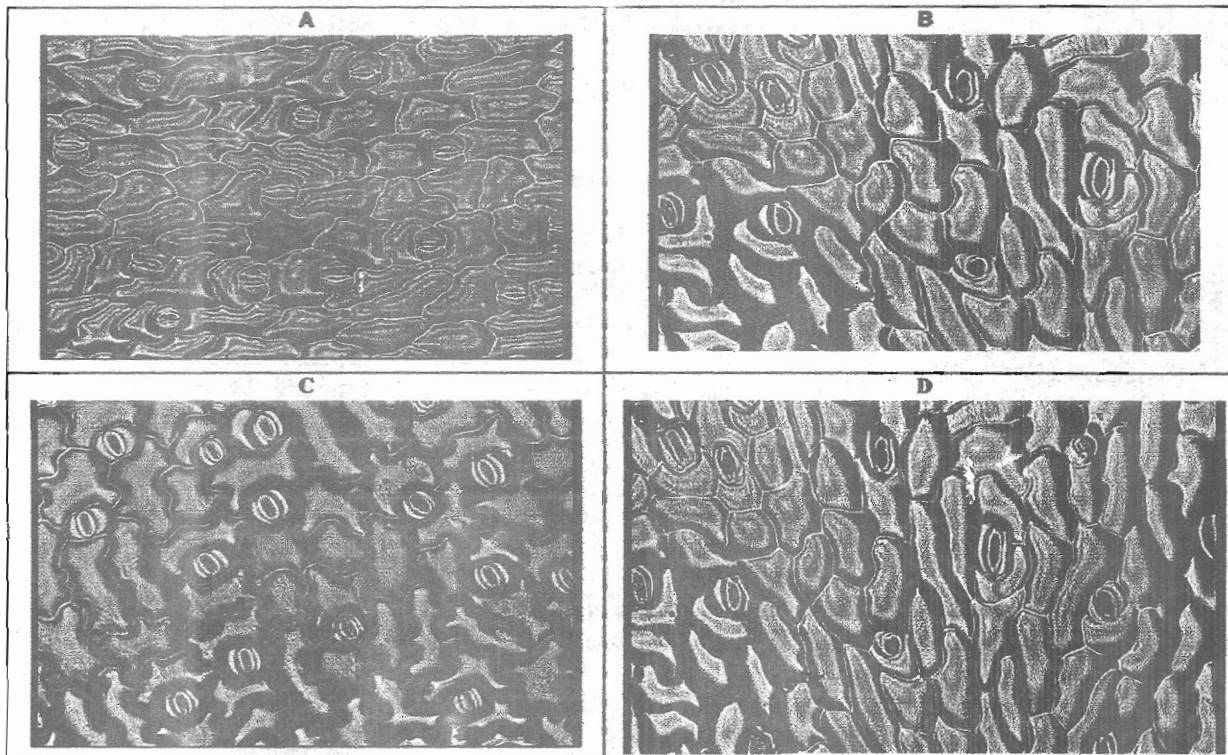

Fig. (5): Stomata of leaves surface of flax cv. Blanka; Lower surface *in vivo* (A), Lower surface *in vitro* (B), Upper surface *in vivo* (C), Upper surface *in vitro* (D).

values were reduced by 19.0 and 12.0% as compared with vessel diameters of the *in vitro* leaves for the two cultivars in the same order.

The above mentioned results indicate that the *in vitro* flax shoots achieved thin leaves with poor differentiated epidermal, mesophyll and vascular tissues. This was previously reviewed by Hazarika (2005) who reported that, the poor mesophyll differentiation and weak vasculature of the leaves formed the *in vitro* render the plants highly susceptible to transplantation shock. Leaves of the plants grown *in vitro* were thinner and had a characteristically poor developed palisade layer with significant amount of mesophyll air space compared to the *in vivo* plants. Both micropropagated cauliflower (Grout and Aston, 1977) and sweet gum (Wetzstein and Sommer, 1982) plantlets failed to develop a clearly defined palisade layer *in vitro*.

Stomata on both lower and upper leaf surfaces of *in vivo* and *in vitro* leaves of. Blanka was further investigated. It is obvious that flax leaf lamina exhibited anomotetracytic stomata arrangement according to the subsidiary cells shape (Fig. 5). Also, it is evident that stomata were numerous in the lower leaf surface as compared with the upper leaf surface. On the lower leaf surface the stomata density varied between the *in vivo* and *in vitro* plants. The *in vivo* plants showed approximately $100/mm^{2,}$ while the *in vitro* leaves showed $76/mm^2$. The reduction in stomata density of *in vitro* plants may be referred to the increase occurred in stomata size. As, the stomata size was increased by 44.0 and 18.0% for both stomata length and width, respectively. Stomata pore of *in vitro* plants was also increased in size comparing with the *in vivo* plants, (Fig 5). Same trend was obtained in the lower epidermis, where, the stomata density was greatly reduced in the *in vitro* plants. The average stomata density was $47/mm^2$ for *in vitro* leaves as compared with $82/mm^2$ for *in vivo* leaves.

The reduction in stomata density and size of *in vitro* plants was reported by many workers; among them Johansson *et al.* (1992), Radochova *et al.* (2000). On the contrary Hazariks (2005) reviewed that, there was no significant difference in stomatal frequency among *in vitro*, acclimatized and greenhouse-grown plants (Conner and Conner, 1984). But Zaid and Hughes (1995) reported that the stomatal frequency of greenhouse green leaves of date palm was significantly higher than the *in vitro* plantlets. It could be concluded from the comparative anatomy of *in vitro* and *in vivo* plants that, *in vitro* plants are very delicate owing to high humidity in the culture media, low light intensity and hetero or mixotrophic mode of nutrition. As a result, they lack the protective mechanisms like waxy cuticle, stomatal regulation, and poor development of photosynthetic tissues.

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العوامل المؤثرة والخصائص التشريحية لتجدد النموات الخضرية معمليا لنبات الكتان

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ملخص

اجريت تجربة على التجدد المباشر للنموات الخضرية معمليا لصنفين من الكتان : بلانكا (صنف للالياف) و جيزة ٨ (صنف ثنائى الغرض معمليا) باستعمال منفصلات مختلفة من البادرة (الجذور– السويقة السفلى – الفلقات) و تمت دراسة العوامل المؤثرة على كفاءة التجدد و مراحل العملية بالاضافة للخصائص التشريحية للنموات الخضرية الناتجة معمليا بالمقارنة مع مثيلتها خارج المعمل و اظهرت النتائج ان البيئة الغذائية المستعملة تمثل العامل الاساسى المؤثر على تخلق النموات الخضرية عند تفاعلها مع بقية العوامل : نوع المنفصل النباتى المستعمل – عمره – الطراز الوراثى للنبات الام و قد ظهر ان منفصلات السويقة الجنينية العليا قد اعطت اعلى نسبة للتجدد تراوحت من ٣٣–٩٥ % كما ثبت ان المنفصلات من البادرة في عمر ٣ ايام تفوقت على تلك من البادرة فى عمر ٧ ايام و كانت اكبر البيئات الغذائية تحفيزا على التجدد هى بيئة موراشيج و سكوج مضاف اليها ٢٥، ٠ ملجم من النفتالين حمض الخليك و ٥٠ ، ٠ ملجم من البنزيل ادينين لكل لتر من البيئة – واما بالنسبة لاستجابة الصنفين المستعملين فى الدراسة لعملية التجدد فقد تفوق الصنف بلانكا على الصنف جيزة ٨ . كما ظهر ان نشأة البرعم الخضرى تكون سطحية على أجزاء السويقة السفلى المزروعة على شكل بروزات صغيرة بها مسطح متعرج جدا و هذه البداءات تتطور لاحقا لتكون البرعم الخضرى الطبيعى بقبة مرستيمية و بداءات ورقية. واظهرت الدراسة اختلاف النموات الخضرية الناتجة داخل و خارج المعمل من الناحية التشريحية فالنموت الخضرية الناتجة معمليا لها سيقان اكبر قطرا و كذلك زاد فيها سمك كلا من البشره و القشرة و النخاع بينما احتوت على كميه اقل من الانسجة الوعائية و الدعامية . أما بالنسبة لاوراق النموات الخضرية داخل المعمل فقد كانت اقل سمكا مع ضعف تطور كلا من البشرة و النسيج المتوسط و الوعائى كما اظهرت الدراسة ان الثغور في أوراق النموات داخل المعمل كانت اكبر حجما و اكثر عددا على كل من سطحى الورقة.

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