Photoauto-, Photohetero- and Photomixotrophic *in vitro* Propagation of Papaya (*Carica papaya* L.) and Response of Seed and Seedlings to Light-emitting Diodes

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Abstract

The objective of this study was to assess whether papaya could be propagated under photoautotrophic conditions and what the response would be to light-emitting diodes (LEDs). Seeds of papaya (Carica papaya L. cv. 'Rainbow' and 'Sunrise Solo') that were soaked overnight and surface sterilized with 0.1% mercuric chloride solution + 2 drops of Tween-20 then with 80% ethanol, could germinate at 100% on Murashige and Skoog medium with 3% sucrose. Seeds were placed under different light conditions: positive control (100% white heat fluorescent lamps), negative control (darkness) and five combinations of LEDs with different red (R) and blue (B) ratios (100% R; 70% R + 30% B; 50% R + 50% B; 30% R + 70% B; 100% B) with a standard light intensity of 45 µmol/m^2/s for all treatments. Seed germination was high (95%-100%) independent of the treatment, but 100% R resulted in extremely long hypocotyls while and 100% B showed highly stunted seedlings. Control seedlings germinated in light were also placed under the same light conditions and stem and root growth were stimulated or stunted by R and B LEDs, respectively. Separately, one-month-old papaya plantlets were cultured photoautotrophically, i.e., in sucrose-free medium in the presence of 3000 ppm CO_2, either in non-aerated or aerated vessels. Even though leaf-drop was high, photoautotrophic in vitro propagation led to greater leaf production than control plantlets. Papaya could be propagated under photoautotrophic conditions and LEDs affected seedling growth differently.

Keywords : LED, light-emitting diode; MS, Murashige and Skoog.

1. Introduction

The most conventional way to propagate papaya (*Carica papaya* L.), a tropical fruit, is by seed (reviewed by Teixeira da Silva *et al*. 2007a). Papaya seed germination *ex vitro* is slow, erratic and incomplete (Chako and Singh 1966). Papaya seeds are orthodox (Chin and Roberts 1980; Hofman and Steiner 1989) as they retain high moisture content during maturation, they do not withstand desiccation, and they require high moisture content for germination. Many factors, including the type of substrate, environmental factors such as oxygen, water, temperature and light

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can affect seed germination (Hartmann *et al.* 2002).

The gelatinous sarcotesta (aril, or outer seed coat which is formed from the outer integument) of *C. papaya* seeds can prevent germination due to the presence of several phenolic compounds (Tokuhisa *et al*. 2007), even though dormancy can also be observed in seeds from which the sarcotesta has been removed (Lange 1961; Yahiro 1979). Removal of the sarcotesta improves germination (Perez *et al.* 1980; Sangakkara 1995). Pre-soaking papaya seeds in water for 24 h can also promote germination (Riley 1981).

Light-emitting diodes (LEDs) are an artificial light source that have been used for inducing organogenesis or for promoting or enhancing growth of plantlets *in vitro* and *ex vitro* in several horticultural commodities. In some cases, plant growth responses changed under different blue (B) to red (R) LED ratios. For example, Huan and Tanaka (2004) indicated that callus proliferation of *Cymbidium* Twilight Moon 'Day Light' was best under 75% R + 25% B compared to 100% R, 100% B, 50% R + 50% B, and 75% B + 25% R treatments. At various R:B ratios, Nhut *et al*. (2003) proved that strawberry plantlets grew better under the ratio of 70% R + 30% B. This is the first study on the effect of LEDs on seed germination. Recently published data on papaya also shows that seed germination, which could be optimized to 100% under *in vitro* conditions, is affected by the constitution of the LED R+B ratio (Giang *et al*. 2011).

Photoautotrophic (i.e., using CO_2 as the primary carbon source to derive energy) micropropagation – vegetative propagation *in vitro* or under aseptic conditions – can reduce production costs and automate the micropropagation process by minimizing microbial contamination and by increasing photosynthetic rate, growth and rooting *in vitro* and survival percentage *ex vitro* (Norikane et al. 2010; Xiao et al. 2010). Tanaka (1992) and Hahn and Paek (2001) showed that photoautotrophic culture, unlike heterotrophic (exogenous organic carbon source required) growth, resulted in improved growth parameters such as larger and more vigorous uniform chrysanthemum plantlets. Teixeira da Silva et al. (2007a) showed that photoautotrophic conditions led to higher callus and protocorm-like body (PLB) fresh and dry weight, number of PLBs, and more robust hybrid *Cymbidium* plants. The Vitron (gas-permeable vessel created by Otsuka, Tokushima, Japan), was successful in the photoautotrophic micropropagation of *Spathipyllum* (Teixeira da Silva et al. 2006).

To date, no study has yet examined the response of papaya to photoautotrophic culture *in vitro*, this being the first such data set. The use of CO_2-enrichment to improve papaya growth *in vitro* has practical value. This experiment further explored the response of papaya seeds of two popular export cultivars to a wide range of LED R+B ratios to assess the basic biological response to different light spectra. The ability to manipulate seed germination based on spectral quality has practical applications for germination of tropical fruit germplasm.

2. Materials and methods

2.1 Chemicals and reagents

All plant growth regulators (PGRs) were purchased from Sigma-Aldrich (St. Louis, USA) and were of tissue culture grade. All other chemicals and reagents were of the highest analytical grade available and were purchased from Wako or Nacalai Tesque (Osaka, Japan), unless specified otherwise.

2.2 Seed surface sterilization and germination in vitro

The seed surface sterilization and germination protocol followed the Giang *et al*. (2011) protocol, with some modifications, as explained next. Several fruits of two commercially available hybrid

papaya (*Carica papaya* L. cv. 'Rainbow' and 'Sunrise Solo') cultivars were purchased from a local supermarket with guaranteed import quality and with no (or few) apparent surface infection or markings. Seeds were removed from the fruit when ripe and were left to soak for 48 h. Seeds were washed in running tap water to remove as much of the fruit as possible and the sarcotesta surrounding to seeds. A floatation test was performed to determine seed viability and only seeds that sunk were used while seeds that floated as well as any seed with split testas were discarded as these were not considered to be viable or were considered to be damaged and thus negatively affect seed germination. Seeds were gently scraped against the inside of a conventional kitchen sieve to remove all the remaining arils/sarcotestas. After dabbing between commercially-available kitchen paper towels to remove excess water, naked seeds were surface sterilized by placing them in a solution of 0.1% mercuric chloride ($HgCl_2$) + 2-3 drops of Tween-20 for 5 min, rinsed 3 times in sterilized distilled water (SDW) then sprayed with 80% ethanol – sufficient to cover the seeds but not soaked – for 1 min. Seeds were once again rinsed 3 times in SDW. Surface-sterilized seeds were placed on full-strength (macro- and micronutrients) Murashige and Skoog (1962) (MS) medium containing 3% sucrose and 2 g/L gellan gum (Gelrite®, Merck, USA). Medium was adjusted to pH 5.8 with 1 N NaOH or HCl prior to autoclaving at 100 KPa for 21 min. Seeds were slightly embedded into the medium, 5 per Petri dish, which were sealed with Parafilm® and incubated at 25°C under a 16-h photoperiod with a light intensity of 45 µmol/m^2/s provided by plant growth fluorescent lamps (Plant Lux, Toshiba Co., Japan) or LEDs, as explained next.

2.3 The LED irradiation source

R and B LEDs were arranged on the ceiling of "LED PACKS", 25 cm × 31 cm × 25 cm deep, originally described in Tanaka et al. (1998). Each LED PACK contained 176 LED bulbs (5 mm in diameter, Sharp Electric Ltd., Tokyo, Japan) and the R+B LED ratio was adjusted based on a uniform division of R+B LED bulbs, for example, 88 R : 88 B, evenly interspersed, for the 50% R : 50% B treatment. LED PACKS were placed in culture rooms in which CO_2 concentration was maintained at 3000 ppm.

2.4 Seed germination in response to LEDs

Seeds from each cultivar (50 seeds for each cultivar and for each treatment, arranged as 5 batches of 10 seed per Petri dish), were cultured under one of three light sources: L1: plant growth fluorescent lamps or heat fluorescent lamps (HFL; positive control). L2: complete darkness (negative control); L3: five LED B:R ratios: (100% R; 70% R : 30% B; 50% R : 50% B; 30% R : 70% B; 100% B). Germination (defined in this study as the clear formation of a hypocotyls and an epicotyl) percentage was calculated after 10 days.

2.5 Photoauto-, photohetero- and photomixotrophic *in vitro* propagation

The experimental designs, as employed by Teixeira da Silva et al. (2006, 2007b) for *Spathiphyllum* and hybrid *Cymbidium*, were used. Twenty-five shoots (~5 cm in length with a developed epicotyl and hypocotyl) derived from control light seed germination treatment (i.e., under HFL) were transferred to an OTP® film culture vessel, the Vitron® (Fig. 1A). Each shoot was embedded in a 25-hole rockwool multiblock (Grodan® RW Multiblock™, AO 18/30, Grodiana A/S, Denmark; Fig. 1A), prior to which 200 mL Hyponex® (N:P:K = 6.5:6:19; 3 g/L; Hyponex, Osaka, Japan) (without agar) was evenly distributed, and placed at the same temperature and light conditions as described above. CO_2 gas was supplied at a constant (24 h) super-elevated concentration (3000 ppm). In addition, a total of 20 shoots (4-5 cm in length) were transferred to 80 mL of gellan gum (2 g/L)-

solidified Hyponex® medium (5 shoots/bottle) with 3% (w/v) sucrose in a glass bottle (75 mm wide × 130 mm tall) containing a Milliseal®, i.e., photoheterotrophic conditions, with or without CO_2 enrichment, i.e., photoautotrophic conditions, or with both sucrose (3%) and CO_2 enrichment (3000 ppm) (i.e. photomixotrophic conditions) and placed at the same temperature and light conditions as described above.

2.6 Morphogenic and photosynthetic analysis

Plantlet growth was quantified by the number of new leaves and roots, and leaf : root ratio. Chlorophyll content in the third leaf (counting downward from the top) of the plantlets was measured as the SPAD value by a chlorophyll meter (SPAD-502, Minolta, Japan).

2.7 Flow cytometry

Nuclei were isolated from 0.25 cm^3 of seedling leaves or callus derived from any treatment by chopping in a few drops of nucleic acid extraction buffer (Partec Cystain UV Precise P, Germany), digesting on ice for 5 min. The nuclear suspension was then filtered through a 30 μm mesh size nylon filter (CellTrics®) and five times of Partec Buffer A (2 μg/ml 4,6-diamidino-2-phenyl-indole (DAPI), 2 mM $MgCl_2$, 10 mM Tris, 50 mM sodium citrate, 1% PVP K-30, 0.1% Triton-X, pH 7.5; Mishiba and Mii 2000) was added at room temperature for 5 min. Thereafter, nuclear fluorescence was measured using a Partec® Ploidy Analyser. Three samples were measured, and relative fluorescence intensity of the nuclei was analyzed when the coefficient of variation was <3%. A minimum of 5000 nuclei were counted for each sample. The internal standard was barley as defined in Teixeira da Silva *et al*. (2005).

2.8 Statistical analyses

Seeds were observed daily and seed germination was quantified as number of days from plating. Experiments were organized according to a randomized complete block design (RCBD). Data was subjected to analysis of variance (ANOVA) with mean separation ($P \leq 0.05$) by Duncan's multiple range test (DMRT) using IRRISTAT version 3.0.

3. Results

A previous protocol (Giang et al. 2011) was used to sterilize the seeds of both papaya cultivars. In both cultivars, 100% or nearly 100% germination was possible. In that study, as well as in this study, shoot length was drastically stunted under blue LEDs and highly elongated under red LEDs (compare C vs D in Fig. 1) with the stunting increasing as the ratio of blue LEDs increased (Table 1). Aeration using the Vitron was more effective than aeration using a Milliseal® in producing more leaves and roots and in stimulating greater chlorophyll content (Table 2). Under heterotrophic (control), photomixotrophic or photoautotrophic conditions, plantlets tended to allocate more carbon to aerial sinks, i.e., leaves, as assessed by a shoot: root ratio > 1 (Table 2), although a weight-based measurement would be more accurate. Even though CO_2-enrichment increased shoot and root number, aeration was a stronger factor (Table 2). The R:B ratio of LEDs significantly influenced the *in vitro* growth response of papaya plantlets of both cultivars (Table 3). The use of LEDs tended to decrease photosynthetic capacity (i.e., the ability to form chlorophyll or SPAD value) even though, in the case of 100% red LEDs, slightly more leaves were produced relative to the control lighting conditions (Table 3). Red LED strongly stimulated the formation of shoots and a high shoot : root ratio while 100% blue LEDs stimulated root formation more (or alternatively, stunted shoot formation more) (Table 3). Although roots also became more stunted, this did not affect plantlet survival (data not shown). Callus, which tends to form at the base of shoots, demonstrates endopolyploidy, while the shoots and roots

do not (Fig. 2), independent of treatment (Table 4). Endopolyploidy was not induced by any treatment related to photoautotrophic culture *in vitro*, aeration, or altered R:B LED ratio, although blue LEDs stimulated a very low level of endopolyploidy (Table 4).

Table1. Response of two papaya (*Carica papaya* L.) cultivars to light vs dark conditions, including to 5 different combinations of red:blue LED ratios.

	Rainbow		**Sunrise Solo**	
Light treatment	**Germination %**	**Shoot length (mm)**	**Germination %**	**Shoot length (mm)**
L1	100 a	26 c	100 a	36 c
L2	98 a	61 bc	100 a	49 b
L3				
100% R	100 a	103 a	96 a	86 a
70% R + 30% B	100 a	74 b	100 a	61 b
50% R + 50% B	96 a	68 bc	100 a	53 b
30% R + 70% B	100 a	54 c	100 a	47 b
100% B	98 a	6 d	100 a	8 d

L1: plant growth fluorescent lamps or heat fluorescent lamps (HFL; positive control). L2: complete darkness (negative control); L3: five LED blue (B) : red (R) ratios. n = 5 × 10 (total = 50) for each treatment. Different letters within a column and for each cultivar indicate significant differences ($P < 0.05$) using DMRT.

Table 2. Subsequent organogenesis (formation of leaves and roots) of seed-derived papaya (*Carica papaya* L.) shoots (n = 25) derived from L1 in Table 1 under heterotrophic (control) [A], photomixotrophic [B] or photoautotrophic [C] conditions on a Hyponex® basal medium (with or without 3% sucrose and with or without aeration).

Treatment	Cultivar	№ Leaves	Leaf:Root*[1]	№ Roots	SPAD*[2]
Glass bottle (+3% sucrose) ($-CO_2$)[A]	Rainbow	4.5 c	1.10 b	4.1 b	36.2 b
	Sunrise Solo	5.1 c	1.42 ab	3.6 bc	38.1 b
Glass bottle (+3% sucrose) (Milliseal®-CO_2)[A]	Rainbow	4.7 c	1.21 ab	3.9 b	39.6 b
	Sunrise Solo	5.2 c	1.58 a	3.3 c	41.2 ab
Glass bottle (+3% sucrose) (Milliseal®+CO_2)[B]	Rainbow	6.8 b	1.28 ab	5.3 ab	44.8 a
	Sunrise Solo	7.2 b	1.53 a	4.7 ab	47.2 a
Vitron (-3% sucrose ($+CO_2$)[C]	Rainbow	9.8 a	1.58 a	6.2 a	42.6 ab
	Sunrise Solo	8.6 a	1.48 a	5.8 a	44.3 a

Data presented as means; different letters within a column, and within a single treatment and for a single parameter, indicate significant differences across cultivars at P<0.05 according to Duncan's multiple range test. CO_2 concentration = 3000 ppm. *[1] = measurement of carbon partitioning; *[2] = measurement of chlorophyll content on the 3rd youngest leaf.

4. Discussion

Spectral quality can influence plant growth and development. LEDs are an interesting alternative light source for *in vitro* plant growth and propagation since they are small, have specific narrow-bandwidth wave length emissions, cool emitting surfaces and longevity and with positive effects on plant growth of several species. For example, Huan and Tanaka (2004) indicated that 100% red LEDs was the most effective for callus induction from protocorm-like body segments of *Cymbidium* Twilight Moon 'Day Light' while Nhut et al. (2003) showed that the best R: B LED ratios for plantlet growth *in vitro* of strawberry cv. 'Akihime' was 70% R + 30% B. *Spathiphyllum* 'Merry' plants grown under LED *in vitro* or in the greenhouse did not perform better than under regular HFL (Nhut et al. 2005). 50% R +50% B LEDs was the best ratio for the growth of upland cotton (*Gossypium hirsutum* L.) plantlets *in vitro* (Li *et al*. 2010). *Phalaenopsis* protocorm-like bodies showed increased endopolyploidy in

response to extreme spectral ranges (Park et al. 2010), indicating that changes to both cellular and genetic components takes place. This was also evident in the papaya cultures (Fig. 2). There was an increase in antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos irradiated by red light, indicating that cell damage may take place (Shohael et al. 2006). LEDs also profoundly affect photosynthetic attributes in chrysanthemum (Kim et al. 2004), which was also evidenced by the influence on SPAD value in papaya in this study. IN addition to photosynthetic attributes, LED red:blue ratio can affect biochemical attributes, such as sucrose, amino acid, starch and soluble sugar content, in cotton (Li et al. 2010). Extreme stem elongation (Fig. 1D) may be as a result of synergistic interactions between phytochrome and blue or red light receptors (Kim et al. 2004). Schuerger et al. (1997) claimed that leaf thinning, which was observed in this study in response to 100% red light (Fig. 1D) might be a result of a decrease in blue photons.

Photoautotrophic micropropagation has previously been shown to affect the shoot: root ratio and photosynthetic capacity of *Cymbidium* plantlets and aeration generally improved plantlet growth, as was also observed in this study for papaya (Teixeira da Silva et al. 2007b). *Limonium* plantlets grown in photoautotrophic and photomixotrophic conditions had superior growth characteristics (more leaves, greater leaf surface area, higher chlorophyll and sugar contents, higher photosynthetic rate, and higher percentage survival) than plantlets grown under heterotrophic conditions (Lian *et al*. 2002). Interestingly, maximum photosynthetic rate decreased in avocado leaves under elevated CO_2 concentrations, which the authors ascribed to a decrease in Rubisco activity (de la Viña *et al*. 1999). A similar decrease in net photosynthetic rate was observed in eucalyptus leaves following an increase (Khan *et al*. 2002). Photoautotrophic micropropagation is an important means to improve rooting of tree peony (Wang *et al*. 2012), a woody ornamental that exhibits, along with herbaceous peonies, poor rooting *in vitro* (Shen *et al*. 2012). The relevance of high light intensity together with high CO_2 level on *in vitro* rooting of difficult-to-root woody plants was confirmed in rain tree in which photoautotrophic conditions increased root number and biomass relative to photomixotrophic conditions (Mosaleeyanon et al. 2004). Moreover, improved rooting of plantlets *in vitro* can lead to better survival *ex vitro* (Yoon et al. 2009). In banana cultures, the higher the CO_2 level, the greater the accumulation of biomass (Navarro et al. 1994) while in tomato, a higher CO_2 level resulted in lower photosynthetic rate (Le et al. 2001). The use of ventilated vessels (vents made of 10-mm microporous polypropylene membrane with a 0.22 µm pore size) improved rooting in *Phillyrea latifolia* but negatively influenced shoot parameters such as shoot length and plantlet fresh weight (Lucchesini and Mensuali-Sodi 2004). Leaf area, chlorophyll concentration and net photosynthetic rate were greater in statice plants grown under photomixotrophic conditions than those under heterotrophic conditions (Xiao and Kozai 2006). Further, St. John's wort plants grown under photomixotrophic conditions performed more poorly than under photoautotrophic conditions (Couceiro et al. 2006). The effect of CO_2 enrichment on growth performance of tomato, chrysanthemum and petunia plants was not linear (Qu et al. 2009). For example, while shoot length of chrysanthemum was significantly improved, the number of leaves was significantly lower. Increased ventilation usually decreases the amount of accumulated ethylene in the culture vessel and thus decreases hyperhydricity (Lai et al. 2005), although, in our study, almost total leaf drop or senescence was observed when the Vitron was used (Fig. 1A), which might

reflect the difference in number of air exchanges between the Vitron and the Milliseal® (Zobayed et al. 1999). Using super-elevated CO_2 concentrations (10,000 ppm), *Oncidesa* plant growth could be performed when grown in te Vitron (Norikane et al. 2013).

Table 3. Subsequent organogenesis (leaves and roots) of seed-derived papaya (*Carica papaya* L.) shoots (n = 25) derived from L3 in Table 1 under different LED ratios. All plants were grown under optimal photomixotrophic conditions, as defined by the results of Table 2, namely a CO_2 concentration of 3000 ppm, 3% sucrose and aeration with a Milliseal®.

		№ Leaves	Leaf:Root*[1]	№ Roots	SPAD*[2]
Treatment	**Cultivar**				
HFL	Rainbow	6.8 ab	1.28 de	5.3 a	44.8 a
	Sunrise Solo	7.2 ab	1.53 de	4.7 a	47.2 a
100% R	Rainbow	8.1 a	20.25 a	0.4 c	31.6 bc
	Sunrise Solo	7.9 a	13.17 b	0.6 c	29.3 bc
70% R + 30% B	Rainbow	6.1 b	8.71 c	0.7 c	36.0 b
	Sunrise Solo	6.2 b	8.86 c	0.7 c	34.6 b
50% R + 50% B	Rainbow	4.1 c	3.73 d	1.1 bc	36.4 b
	Sunrise Solo	3.8 c	2.71 d	1.4 bc	33.1 b
30% R + 70% B	Rainbow	3.6 c	1.29 de	2.8 b	39.4 ab
	Sunrise Solo	3.8 c	1.26 de	3.1 b	37.6 ab
100% B	Rainbow	3.1 c	0.56 e	5.5 a	26.4 c
	Sunrise Solo	3.6 c	0.78 e	4.6 a	28.3 c

Data presented as means; different letters within a column, and within a single treatment and for a single parameter, indicate significant differences across cultivars at $P<0.05$ according to Duncan's multiple range test. CO_2 concentration = 3000 ppm. *[1] = measurement of carbon partitioning; *[2] = measurement of chlorophyll content on the 3rd youngest leaf. HFL = plant growth fluorescent lamps or heat fluorescent lamps (control)

Table 4. Ploidy levels of shoots derived from all treatments in Table 2 and Table 3 of seed-derived papaya (*Carica papaya* L.) shoots. All measurements with a CV < 3% and a minimum of 5000 nuclei counted.

Treatment	Cultivar	Ploidy ratio (C: 2C: 4C: 8C)
Shoots	Rainbow	25: 74: 1: 0
	Sunrise Solo	22: 78: 0: 0
Roots	Rainbow	6: 94: 0: 0
	Sunrise Solo	8: 92: 0: 0
Callus	Rainbow	12: 78: 8: 2
	Sunrise Solo	16: 71: 10: 3
Glass bottle (+3% sucrose) (-CO_2)	Rainbow	15: 85: 0: 0
	Sunrise Solo	12: 88: 0: 0
Glass bottle (+3% sucrose) (Milliseal®-CO_2)	Rainbow	16: 84: 0: 0
	Sunrise Solo	14: 86: 0: 0
Glass bottle (+3% sucrose) (Milliseal®+CO_2)	Rainbow	12: 87: 1: 0
	Sunrise Solo	16: 84: 0: 0
Vitron (-3% sucrose (+CO_2)^C	Rainbow	11: 89: 0: 0
	Sunrise Solo	17: 83: 0: 0
HFL	Rainbow	14: 84: 2: 0
	Sunrise Solo	14: 86: 0: 0
100% R	Rainbow	21: 79: 0: 0
	Sunrise Solo	19: 81: 0: 0
70% R + 30% B	Rainbow	19: 81: 0: 0
	Sunrise Solo	17: 83: 0: 0
50% R + 50% B	Rainbow	12: 88: 0: 0
	Sunrise Solo	16: 84: 0: 0
30% R + 70% B	Rainbow	17: 82: 1: 0
	Sunrise Solo	12: 87: 1: 0

100% B	Rainbow	15: 83: 2: 0
	Sunrise Solo	16: 82: 2: 0

* = average of one organ from every treatment formed, pooled, then 3 independent readings made. ** = average of one shoot and root from that treatment (pooled separately for each cultivar), then 3 independent readings made.

Fig.1. Papaya 'Sunrise Solo' *in vitro*. (**A**) Photoautotrophic culture *in vitro* in a Vitron™ (i.e. whole vessel allows for air exchange) or using Milliseal® (allowing localized aeration; **B**) and 3000 µg/l CO_2. Manipulation of growth and size of seed-derived plantlets under 100% blue (**C**) or 100% red (**D**) light-emitting diodes. (**E**) Control plants growing under fluorescent lamps at 40 µmol/m^2/s. (**C-E**) Medium: Hyponex 3 g/l, 3% (w/v) sucrose in 1 L culture bottles. Figure reproduced from Teixeira da Silva JA, Rashid Z, Nhut DT, Sivakumar D, Gera A, Souza Jr. MT, Tennant PF (2007a) Papaya (*Carica papaya* L.) biology and biotechnology. *Tree and Forestry Science and Biotechnology* 1(1): 47-73, ©2007, with kind permission of Global Science Books (Ikenobe, Japan).

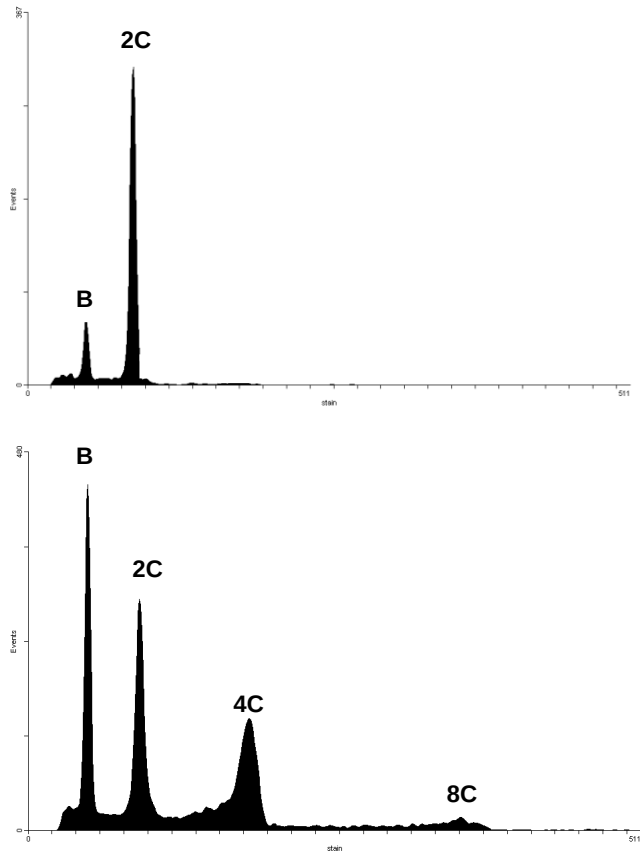

Fig .2. Ploidy changes found in *in vitro*-grown 'Sunrise Solo' papaya following DAPI staining. Upper histogram: control *in vitro* papaya leaves. Lower histogram: Callus, whether formed naturally at the base of shoots, or induced by 2,4-D, results in endopolyploidy, as high as 8C. B = control, barley (*Hordeum vulgare*). Figure reproduced from Teixeira da Silva JA, Rashid Z, Nhut DT, Sivakumar D, Gera A, Souza Jr. MT, Tennant PF (2007a) Papaya (*Carica papaya* L.) biology and biotechnology. *Tree and Forestry Science and Biotechnology* 1(1): 47-73, ©2007, with kind permission of Global Science Books (Ikenobe, Japan).

5. Conclusions

Papaya can be cultured successfully under photoautotrophic conditions provided that the culture vessel has suitable aeration or if, as in the case of this study, a gas-permeable vessel (the Vitron) is used. LEDs can be used to manipulate seed germination and plant architecture.

6. Acknowledgements and declarations of possible conflicts of interest

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small portion of the papaya data set (morphological results) was presented as a poster in Norway (Teixeira da Silva 2005). Figures 2 and 3 were previously published in Teixeira da Silva et al. (2007a), but none of the data set was included in that review.

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