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A simple plant regeneration-ability assay in a range of *Lycopersicon* species

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Abstract

Plant growth regulator-dependent (PGR-dependent) *in vitro* shoot organogenesis has been extensively studied in tomato (*Lycopersicon esculentum*), whereas PGR-independent adventitious shoot organogenesis received marginal attention in *L. esculentum* and no consideration at all in other *Lycopersicon* species. In the present study, induction of PGR-independent adventitious shoots was by decapitation of the apex and removal of preexisting shoot meristems of the seedling, and seedling culture on a medium with no PGR supplements. The existence of PGR-independent regeneration-ability was verified in *L. esculentum* genotypes (high pigment photomorphogenic mutants and wild-type counterparts) and was uncover amongst *L. cheesmanii*, *L. chilense*, *L. chmielewskii*, *L. hirsutum*, *L. parviflorum*, *L. peruvianum* and *L. pimpinellifolium*. Compared to species other than *L. esculentum*, high pigment photomorphogenic mutants displayed the weakest PGR-independent regeneration-ability. Our results imply that decapitated seedlings cultured on a medium without PGRs can serve as a convenient assay system for genotypic variation in self-controlled, PGR-independent, shoot regeneration-ability in a wide range of *Lycopersicon* species. Using transverse thin slices of the hypocotyl placed onto a medium supplemented with 0.2 μM zeatin reboside and 0.04 μM IAA, we assessed PGR-mediated shoot regeneration in *L. esculentum* genotypes. In a given genotype, more plants per seedling were established by PGR-mediated than by PGR-independent regeneration. However, with both modes of organogenesis, only a fraction of shoot buds eventually grew into normal plants, while others developed into abnormal regenerants having no stem. Percentage of stem-deficiency, in a given genotype, was higher in PGR-treated cultures, which indicates that PGRs amplify the formation frequency of imperfect adventitious apical shoot meristems. Unlike *L. esculentum*, adventitious shoot buds of other *Lycopersicon* species, induced by wounding seedlings that were not treated with PGRs, rarely formed regenerants lacking a stem.

Abbreviations: IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; MES – 2-(*N*-morpholino)ethanesulfonic acid; PGR – plant growth regulator; RIM – root induction medium; SIM – shoot induction medium; ZR – zeatin riboside

Introduction

Tomato seedlings grown in culture generate a new replacement shoot following decapitation of the original shoot apex. Seedlings display this ability whether they still carry one cotyledon after decapitation or when they are decapitated below the cotyledonary node. Since induction of a new apical meristem at the wound as well as the following elongation of a shoot are controlled by factors (like growth substances) produced in sufficient amounts by adjacent tissues and remote organs, formation of an adventitious replacement shoot occurs in decapitated seedlings even if planted onto a medium without PGRs (Pozueta-Romero et al., 2001, and Figure 1 in the present work). Segments of the hypocotyl also generate adventitious shoots *in vitro*, however, they need PGR supplements to the medium (Bhatia et al., 2004). In the following we refer to shoot regeneration in the decapitated seedling grown on a medium without PGRs, or shoot regeneration by hypocotyl-derived tissue segments explanted onto a medium with PGRs, as PGR-independent or PGR-dependent regeneration, respectively.

Considerable variability in response to PGRs among *L. esculentum* genotypes and *Lycopersicon* species has been detected in numerous investigations during the last decades (Bhatia et al., 2004), and it is now generally recognized that it may be necessary to retune culture conditions for uninvestigated genotypes. PGR-independent shoot regeneration in tomato culture received little attention and was communicated once only (Pozueta-Romero et al., 2001). The three cultivars included in the latter study displayed wound-induced regeneration response, indicating thereby that time-consuming adaptation of plant regeneration protocol per genotype (for example, for the purpose of genetic transformation, see

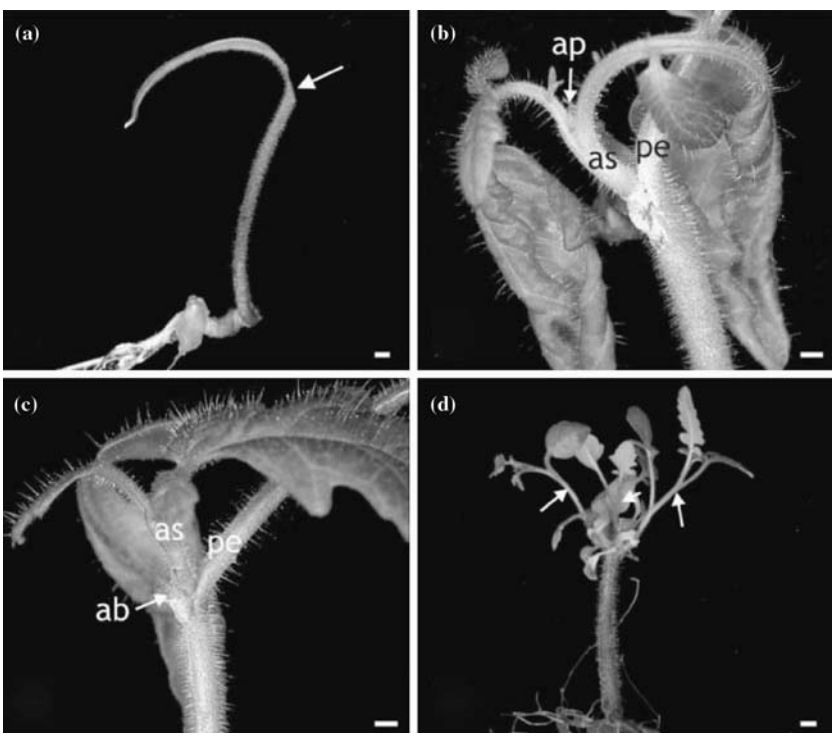

Figure 1. Autonomous adventitious shoot regeneration in decapitated seedlings of *Lycopersicon* spp. incubated on a medium without plant growth regulators. (*a*) Decapitated, 7 days old, tomato seedling. Arrow points at the cut made in the hypocotyl to remove preformed shoot meristems. (*b*) Regeneration of a single adventitious shoot (as) 3 weeks after decapitation (*L. esculentum*). The petiole (pt) of the remaining cotyledon and the shoot apex (ap) are denoted. (*c*) Regeneration of a dominant adventitious shoot (as) and suppressed incipient adventitious buds (ab) 3 weeks after decapitation (*L. esculentum*). The petiole (pe) of the remaining cotyledon is denoted. (*d*) Multiple shoots formation (arrows) on the hypocotyl, 4 weeks after decapitation below the cotyledonary node (*L. peruvianum*). Bars indicate 2, 1, 1 and 1.5 mm in (*a*), (*b*), (*c*) and (*d*), respectively.

Pozueta-Romero et al., 2001) would be unnecessary for genotypes carrying PGR-independent regeneration-ability. As of yet, however, we do not know how widespread PGR-independent shoot regeneration-ability appears among a broader range of tomato genotypes.

Abnormal morphogenesis *in vitro* is quite common and various aberrations have been described in the literature. A most critical type of anomaly is the formation of regenerants lacking a stem: this generally is an irreparable defect that arrests normal shoot development, it blocks plant establishment and limits what we term as "plant regeneration-ability". Studies with "shootless" mutants, mutants unable to form a shoot stem, revealed that complete absence or imperfect differentiation of the apical shoot meristem in zygotic embryos leads to lack of a stem (Keddie et al., 1998; Mayer et al., 1998; Satoh et al., 1999; Pilu et al., 2002). Moreover, somatic embryos with no apical shoot meristems or with a partially differentiated shoot meristem that eventually develop to regenerants with no stem have been discovered also in genotypes not carrying a "shootless" mutation (Nickle and Yeung, 1993; Padmanabhan et al., 1998; Steinitz et al., 2003). Notably, however, reports demonstrating stem-deficiency as an impediment to plant establishment via organogenesis are not available.

One objective of the present study was to uncover PGR-independent regeneration-ability in tomato species other than *L. esculentum*, and to examine to what extent wound-induced adventitious shoot regeneration response is genotype-dependent. A second objective was to examine whether stem deficiency could appear during organogenesis of adventitious shoots or not.

Materials and methods

Plant material

Seeds of *L. esculentum* Mill. used were: MP1, a line with a superior regeneration and transformation response (Barg et al., 1997); open-pollinated cv. Moneymaker, MM +/+, and lines nearly isogenic and homozygous for the *hp-2* and *hp-2^j* mutations, open-pollinated cv. Manapal, Manapal +/+, and a line isogenic and homozygous for the *hp-2^{dg}* mutation, open pollinated processing lines n474 and n935, both homozygous for the *hp-2^{dg}* mutation. The homozygous photomorphogenic *high pigment* mutants (*hp-1*/*hp-1*, *hp-2*/*hp-2*, *hp-2^j*/*hp-2^j*) and *dark green* (*hp-2^{dg}*/*hp-2^{dg}*) have been introgressed into several commercial tomato cultivars currently marketed as Lycopene Rich Tomato. Seeds of these genotypes were from lots used in our current genetic research programs (Levin et al., 2003; Lieberman et al., 2004; Bino et al., 2005). Seeds of other tomato species such as *L. cheesmanii* Riley (accession LA 1447), *L. chilense* Dun (accession LA1932), *L. chmielewskii* Rick, Kes., Fob. & Holle (accession LA1028), *L. hirsutum* Dun (accession LA1777), *L. parviflorum* Rick, Kesicki, Fobes & Holle (accession LA0247), *L. pennellii* (Corr.) (accession LA0716), *L. peruvianum* (L.) Mill. (accession LA0111) and *L. pimpinellifolium* (Jusl.) Mill (accession LA1589), were obtained from The C. M. Rick Tomato Genetics Resources at UC Davis, Davis, California, U.S.A.

Culture conditions for shoot regeneration and plant establishment

Seeds were surface disinfected in 1% sodium hypochlorite solution containing a few drops of Tween-20 for 20 min, rinsed with sterile distilled water, and germinated in the dark for 2 d at 25 ± 1 °C, in Petri plates (90×20 mm), on filter paper wetted with sterile distilled water. Seedlings were transferred to the light and grown on a Murashige and Skoog (1962) (MS) basal medium (Duchefa, The Netherlands) supplemented with 60 mM sucrose, pH 5.8, in Magenta 7 boxes covered with a 1.5 cm diameter filter-vented lid. Explants for PGR-mediated regeneration and decapitated seedlings were prepared from 7 d old seedlings

Autonomous shoot regeneration was initiated in seedlings by excision of one cotyledon and the hypocotyl apex including the apical shoot meristem and the axillary meristem of the second cotyledon (Figure 1a). After decapitation, seedlings were placed in 100×25 mm glass test tubes, one seedling per test tube, with 10 ml medium, and covered with Kimble-25 caps.

Tissues derived from cotyledons and hypocotyls were used as explants for experiments on PGR-mediated regeneration. Cotyledons, excised at the proximal petiole edge, and 1 mm wide transverse strips of tissue prepared from the

proximal region of the cotyledons, were incubated with the adaxial side on SIM. Transverse hypocotyl discs, 0.5–1.0 mm thick, were sliced from a 10 mm hypocotyl segment cut immediately below the cotyledonary node. Discs were placed with their basal cut on SIM. Explants were cultured in Petri plates (90×20 mm) with 25 ml medium, and were subcultured to freshly prepared medium every 2 weeks.

SIM for cotyledon- and hypocotyl-derived explants consisted of MS basal medium, supplemented with Gamborg B5 vitamins (Gamborg et al., 1968) (Duchefa, The Netherlands), 110 mM maltose, 0.2 μM ZR, 0.04 μM IAA, 1.17 mM MES, 3 gl^{-1} Phytagel, pH 5.2. Decapitated seedlings were grown on the same medium but without ZR and IAA. RIM consisted of half strength MS basal medium fortified by 1.0 mM $MgCl_2$, 1.5 mM $CaCl_2$, full Gamborg B5 vitamins, 60 mM sucrose, 1 μM IBA, 3 gl^{-1} Phytagel, pH 5.8. Maltose, sucrose, IAA, IBA, ZR, MES and Phytagel were obtained from Sigma Co., St. Louis, MO, USA. Shoots were rooted in Magenta 7 boxes containing 30 ml RIM. The pH of all media was adjusted after addition of Phytagel, before autoclaving at 120 kPa for 20 min at 121 °C. Filter-sterilized vitamins and PGRs were added to the cooling autoclaved media. All cultures were exposed to 10 μmol m^{-2} s^{-1} fluorescent white light (Osram 36 W10), 16-h photoperiod, at 25 ± 1 °C.

Rooting percentage was scored 1 month after planting shoots on RIM. Rooted regenerants lacking a visible stem were transplanted to a peat: vermiculite (1:1, v:v) soil mixture, in styrofoam transplant trays, and were grown in a growth room at 25 ± 2 °C, 16-h photoperiod, 30 μmol m^{-2} s^{-1} fluorescent white light. Regenerants were irrigated periodically with tap water, and were grown for another month to verify whether or not they could grow a stem.

Experimental design and statistical analysis

For PGR-mediated organogenesis, 7 transverse hypocotyl discs from each of two seedlings were placed in a Petri plate. The identity of explants and cultures derived from each seedling was traced throughout the experiment. Five plates were inoculated from each *L. esculentum* genotype in an experiment. For regeneration from decapitated seedlings, 15 seedlings from every *L. esculentum* genotype were prepared in an experiment. Due to limited seed availability of all other *Lycopersicon* species (Table 3), we used 5 seedlings per species per experiment. Experiments were repeated three times on different dates, and the data, representing the mean value of these replicates, were subjected to statistical analysis. Comparisons of means were made with the least significance test at the 5% level of probability.

Results

Plant growth regulator-independent shoot regeneration

Seedlings of all species, decapitated as illustrated in Figure 1a, formed a new shoot that was ready for excision and transplantation to RIM within 2 weeks of wounding. In *L. esculentum*, usually a single shoot emerged (Figure 1b). In some instances the formation of a shoot was accompanied by differentiation of incipient buds that did not grow further until the dominant shoot was removed (Figure 1c). Following excision of the dominant shoot, the decapitated seedling was placed on fresh growth medium, and one of the incipient buds developed to become the succeeding leader shoot. The appearance and development of the second and the following shoots was generally slower. We seldom encountered two shoots elongating at the same time in *L. esculentum*. Shoot formation ceased about 10 weeks from first decapitation.

Shoots and shoot-like buds planted on RIM generally rooted within 2 weeks. Rooting percentage was, however, scored after 30 d growth on RIM. At this point not all rooted shoot-like leafy structures had an obvious normal stem. Structures having no stem are termed here "rooted regenerants with no stem" (see Figure 2b and c). Rooted regenerants were transplanted to the soil and grown for another month in a growth room to verify that there was absolutely no recovery of a visible stem.

Results of organogenesis and plant establishment from decapitated seedlings of *L. esculentum* genotypes cultured on a medium without PGRs are presented in Table 1. These genotypes can be grouped into three classes. The first class includes MP1, MM +/+, Manapal +/+,

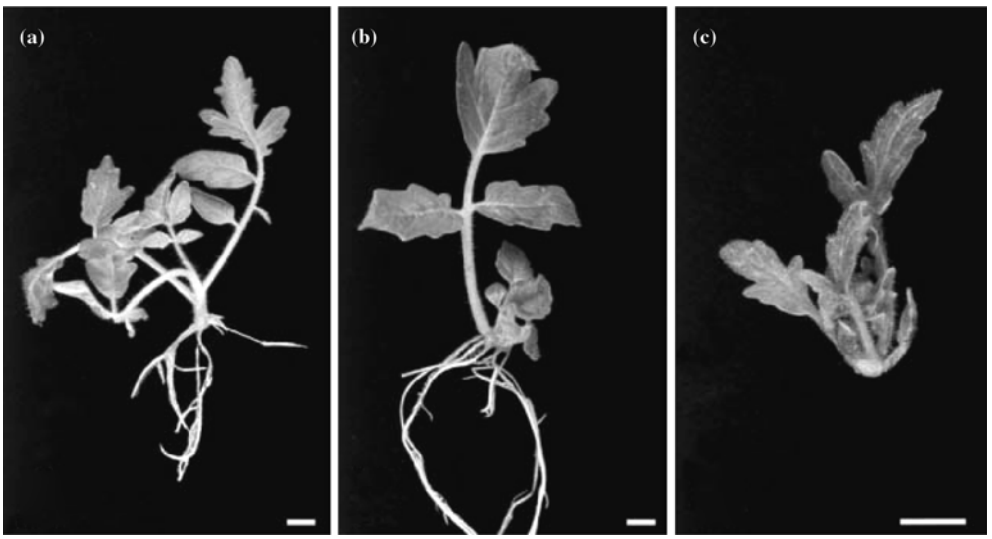

Figure 2. Normal and abnormal organogenesis in *L. esculentum* documented after 1 month growth on rooting medium. (*a*) A rooted plant. (*b*) A rooted regenerant with no stem. (*c*) A regenerant with no stem that failed to root. Bars indicate 5 mm.

n474 *hp-2^{dg}*/*hp-2^{dg}* and n935 *hp-2^{dg}*/*hp-2^{dg}*; some seedlings of these types excelled and produced four or five shoots, but on average two or three shoots per seedling were formed. A second class includes Moneymaker *hp-2^{j}*/*hp-2^{j}* and Manapal *hp-2^{dg}*/*hp-2^{dg}*; some seedlings in these genotypes did not regenerate any shoot, and yielded on average less than 1 shoot per seedling. Finally, an intermediate response class (MM *hp-2*/*hp-2*), with 1 or 2 shoots per seedling.

Establishment of plants was not achieved in all instances, as can be seen from the discrepancy between number of buds harvested per decapitated seedling and the number of plants established per seedling among *L. esculentum* genotypes (Table 1). Two difficulties in organogenesis were noticed:

– Some leafy buds excised from the hypocotyl top and transplanted onto RIM did not have a stem. Stem-less regenerants appeared among all genotypes.
– With the exception of Manapal +/+ that rooted 100%, various percentages of stems of all other genotypes did not root.

PGR-independent shoot regeneration in decapitated seedlings of other *Lycopersicon* species was assessed similarly (Table 2). Occasionally thin

Table 1. Organogenesis and plant regeneration from decapitated seedlings of various tomato (*Lycopersicon esculentum*) genotypes

Genotype	Buds harvested per seedling[z]	Rooted plants per seedling[z]	Rooted regenerants with no stem[z] [%]	Non-rooted shoots[z] [%]
MP1	3.6 ± 0.4^{a}	2.7 ± 0.40^{a}	17 ± 6^{a}	8 ± 4^{a}
MM[y] +/+	3.4 ± 0.4^{a}	2.4 ± 0.36^{a}	22 ± 9^{a}	19 ± 6^{a}
MM[y] *hp-2*/*hp-2*	2.4 ± 0.5^{ab}	1.6 ± 0.34^{b}	23 ± 9^{a}	7 ± 5^{a}
MM[y] *hp-2^{j}*/*hp-2^{2j}*	1.9 ± 0.5^{b}	0.54 ± 0.22^{c}	32 ± 9^{a}	29 ± 12^{a}
Manapal +/+	3.0 ± 0.4^{a}	2.4 ± 0.34^{a}	15 ± 4^{a}	0^{b}
Manapal *hp-2^{dg}*/*hp2^{dg}*	1.6 ± 0.5^{b}	0.45 ± 0.25^{c}	32 ± 9^{a}	14 ± 6^{a}
n474 *hp-2^{dg}*/*hp-2^{dg}*	3.4 ± 0.3^{a}	2.0 ± 0.37^{a}	26 ± 7^{a}	12 ± 6^{a}
n935 *hp-2^{dg}*/*hp-2^{dg}*	3.8 ± 0.3^{a}	2.58 ± 0.31^{a}	17 ± 6^{a}	9 ± 4^{a}

[z]Values represent means ± SE. In a column, different superscript letters indicate statistically significant difference between means ($p < 0.05$).
[y]cv. Moneymaker.
Decapitated seedlings were cultured on a medium without plant growth regulators. Adventitious shoots and buds were harvested over a period of about 10 weeks. Rooting was scored after 4 weeks growth on RIM. Definition of rooted regenerants as lacking a stem was done after 1 month growth in soil.

Table 2. Plant regeneration in *Lycopersicon* species via shoot organogenesis on decapitated seedlings

Species	Accession or line	Plants per seedling[z]
L. esculentum	MP1	1.6 ± 0.34^{b}
L. esculentum	Moneymaker +/+	1.9 ± 0.31^{b}
L. cheesmanii	LA1447	3.4 ± 0.95^{a}
L. chilense	LA1932	3.1 ± 0.80^{a}
L. chmielewskii	LA1028	4.0 ± 1.34^{a}
L. hirsutum	LA1777	4.1 ± 1.14^{a}
L. parviflorum	LA0247	3.3 ± 0.84^{a}
L. peruvianum	LA0111	3.5 ± 0.97^{a}
L. pimpinellifolium	LA1589	4.6 ± 0.98^{a}

[z]Values represent means ± SE. In a column, different superscript letters indicate statistically significant difference between means ($p < 0.05$).
Plants were established from shoots harvested for 4 weeks from first seedling decapitation.

hypocotyls of seedlings broke during repeated shoot harvests and could not be replanted. Unlike the first experiment, in which termination of shoot harvest was dictated by cessation of bud regeneration, in the species *L. chilense*, *L. chmielewskii*, *L. hirsutum* and *L. peruvianum* we did not see a similar decline in adventitious shoot production. We therefore decided to determined regeneration performances for all species by counting number of buds harvested during a period of 4 weeks following first seedling decapitation.

All *Lycopersicon* species included in this experiment generated shoots at a faster pace than *L. esculentum*, and thus more plants per decapitated seedling could be established following 4 weeks repeated shoots harvest (Table 2). Species like *L. chilense*, *L. chmielewskii*, *L. hirsutum* and *L. peruvianum* differed distinctly from *L. esculentum* in the production of 2 or 3 shoots which elongated concomitantly (e.g. Figure 1d), indicating that suppression of shoot elongation by a dominant leader shoot was weaker or absent. Unlike *L. esculentum*, adventitious shoots of all other *Lycopersicon* species very rarely formed regenerants lacking a stem, or shoots that failed to root, during the first 4 weeks from decapitation. No data on these defects are presented in Table 2 because of their negligible incidence.

PGR-mediated shoot regeneration

Barg et al. (1997) demonstrated that the *L. esculentum* line MP1 possesses a superior regeneration and transformation response, and therefore we chose MP1 as a reference genotype to which the performance of the photomorphogenic mutants and other tomato genotypes was compared. Based on preliminary experimentation (data not shown) we defined culture conditions for PGR-mediated shoot organogenesis and plant establishment suitable for the *L. esculentum* genotypes of the present study. We found that transverse thin hypocotyl slices placed on SIM responded with a profuse bud induction (Figure 3). The optimum slice thickness was 0.5–1.0 mm; thinner slices decayed or did not form buds, while thicker slices gave a smaller number

Table 3. Organogenesis and plant regeneration from thin transverse hypocotyl slices of tomato (*Lycopersicon esculentum*) genotypes

Genotype	Buds harvested per seedling[z]	Rooted plants per seedling[z]	Rooted regenerants with no stem[z] [%]
MP1	25.8 ± 6.0^{a}	12.1 ± 3.1^{a}	46.5 ± 14.0^{b}
MM[y] +/+	15.0 ± 7.4^{ab}	5.3 ± 2.4^{b}	33.0 ± 9^{b}
MM[y] *hp-2*/*hp-2*	10.1 ± 4.5^{b}	2.3 ± 1.3^{bc}	37.2 ± 9^{b}
MM[y] *hp-2*^{j}/*hp-2*^{j}	17.3 ± 6.2^{ab}	1.2 ± 0.5^{c}	85.1 ± 9^{a}
Manapal +/+	17.0 ± 7.4^{ab}	4.8 ± 1.5^{b}	47.1 ± 17^{b}
Manapal hp-2^{dg}/*hp-2*^{dg}	18.2 ± 7.1^{ab}	5.4 ± 1.7^{b}	56.0 ± 9^{b}
n474 hp-2^{dg}/*hp-2*^{dg}	7.5 ± 1.8^{c}	1.6 ± 0.9^{c}	62.8 ± 7^{b}
n935 hp-2^{dg}/*hp-2*^{dg}	16.9 ± 5.9^{ab}	6.7 ± 1.3^{b}	57.6 ± 16^{b}

[z]Values represent means ± SE. In a column, different superscript letters indicate statistically significant difference between means ($p < 0.05$).
[y]cv. Moneymaker.
Adventitious shoots and buds were harvested over a period of 10 weeks. Rooting was scored after 4 weeks growth on RIM. Definition of rooted regenerants as lacking a stem was done after 1 month growth in the soil.

of explants per hypocotyl. Consistent with the report by El-Bakry (2002), 110 mM maltose was observed to be the most beneficial sugar and concentration compared to equimolar concentrations of sucrose or glucose. Further, thin transverse hypocotyl discs displayed a markedly higher bud formation response than transverse thin cotyledon slices or proximal cotyledon cut edges (see Figure 3a). Consequently, thin transverse hypocotyl discs were the preferred explant type chosen to investigate PGR-mediated organogenesis in *L. esculentum* genotypes (Table 3).

Incipient buds were visible on the circumference of thin hypocotyl slices after 12 d culture initiation on SIM (Figure 3). Expanded discs were divided into two or three sectors by 3 weeks, and first buds were harvested for rooting 6 weeks after culture initiation. Repeated bud harvest was terminated by secession of new organ production, after 3–4 months (depending on genotype) culture on SIM. Shoots and shoot-like buds were excised from the source tissue and transferred to RIM. Rooted regenerants with no stem were planted into soil and scored as described in the previous

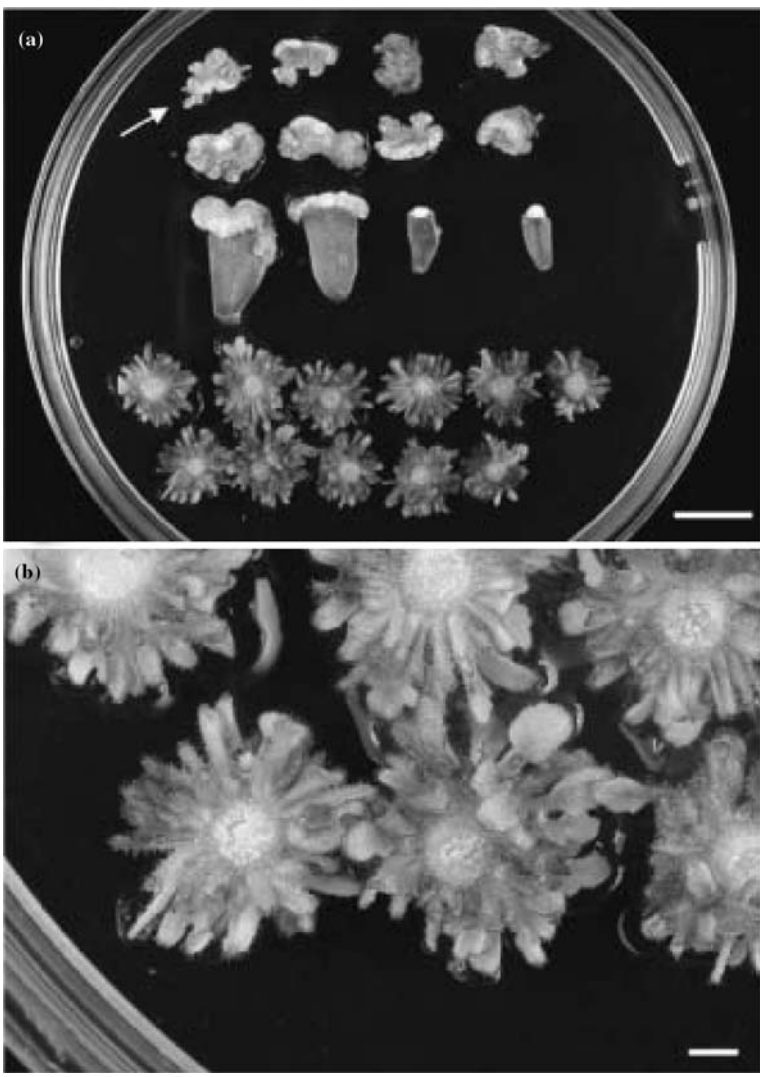

Figure 3. Shoot bud induction by PGRs in L. esculentum. (*a*) Cotyledon-derived (3 upper rows) and thin transverse hypocotyl slice explants (2 lower rows) after 3 weeks culture on SIM. Tow upper rows were transverse strips of tissue prepared from the proximal region of the cotyledons. In the third row, the distal halves of cotyledons. Notice scarce bud induction in cotyledon-derived tissue (arrow) and profuse bud development in hypocotyl slices. (*b*) Bud formation in thin transverse hypocotyl slices at a higher magnification. Bars indicate 15 and 6 mm in (*a*) and (*b*), respectively.

experiments. Table 3 summarizes organogenesis and plant establishment in *L. esculentum* genotypes using thin transverse hypocotyl slices incubated on a PGRs-supplemented SIM.

The reference line MP1 exceeded all other genotypes in bud regeneration and in number of plants established per seedling. Mutants *hp-2*/*hp-2*, *hp-2^{j}*/*hp-2^{j}* in MM background and *hp-2^{dg}*/*hp-2^{dg}* in the n474 background displayed the poorest performance, yielding 1–2 plants per seedling only. Invariably, in all genotypes 33–85% of buds harvested produced regenerants lacking a stem. For mutants such as *hp-2^{j}*/*hp-2^{j}* in MM background and *hp-2^{dg}*/*hp-2^{dg}* in n474 background, the gap between number of buds harvested and the markedly low number of plants eventually established was due to the high frequency of buds unable to form a stem. In these mutants, as well as in *hp-2^{dg}*/*hp-2^{dg}* in Manapal background and *hp-2^{dg}*/*hp-2^{dg}* in n935 background, this aberrancy was a major barrier to plant production. The difference between yield of buds and number of plants in +/+ and *hp-2*/*hp-2*, both in Moneymaker background, was due mainly to rooting difficulties.

Recalling that transverse slices were prepared only from the upper 1 cm of the entire hypocotyl, plant production per seedling by PGR-mediated shoot regeneration could potentially be greater than reflected by the data in Table 3. Nevertheless, most genotypes yielded more buds and plants per seedling with PGR-treated thin transverse hypocotyl slices, yet the differences in plant yields under the two modes of shoot induction were minor in the mutants *hp-2*/*hp-2* (Moneymaker background), *hp-2^{j}*/*hp-2^{j}* (Moneymaker background) and *hp-2^{dg}*/*hp-2^{dg}* (n474 background) (cf. Tables 2 and 3).

Discussion

Our study shows for the first time that PGRs-independent shoot regeneration is widespread among *Lycopersicon* species. PGRs-independent regeneration competence is certainly genotype-dependent, and *L. esculentum* genotypes display a comparative low capacity (Tables 1 and 2). Three distinct components contributed to a higher productivity of *L. chilense*, *L. chmielewskii*, *L. hirsutum* and *L. peruvianum* compared to *L. esculentum* genotypes: the differentiation and elongation of more than one shoot at a time, a more persistent shoot production capacity, and a considerably much lower incidence of stem-deficient shoot organogenesis.

Genotypic differences in organogenic competence among *Lycopersicon* species and genotypes have always been characterized by growing cultures under defined media composition including PGRs (e.g. Stommel and Sinden, 1991; Koornneef et al., 1993; Peres et al., 2001; de Faria et al., 2002; El-Bakry, 2002; Pratta et al., 2003; Bhatia et al., 2004, 2005). The results obtained, and consequently interpretations and conclusions drawn from such studies, inevitably depend on the PGR regime employed. Our work indicates that genotypic variation in regeneration-ability can be determined conveniently in a wide range of *Lycopersicon* species by growing decapitated seedlings on a medium containing no growth regulators. This system permits the assessment of genetic determinants controlling adventitious shoot meristem induction and shoot growth without the interference of exogenous PGRs.

Thin cell layer technology has been applied in tomato tissue culture for shoot (Compton and Veilleux, 1991) or root regeneration (Radin and Eisenback, 1991) both from flower peduncle, and for the formation *in vitro* of ripe fruits from explants of flower pedicels (KaurSawhney et al., 1996). Although the seedling is the plant development stage most often used for tomato regeneration and transformation studies, we are unaware of previous reports referring to the utilization of thin cell layers prepared from seedlings. Our work demonstrates that hypocotyl slices from young seedlings are preferable explants for profuse shoot bud induction over cotyledon-derived explants. The relatively large surface area of the small explant offers a large contact area with the incubation medium, and minimal transport distances for medium ingredients with low mobility or low transport rates in the tissue. This could explain the relatively higher regeneration efficiency of thin hypocotyl slices.

Morphological aberrations like leaf and stem deformations, or stem fasciation, are common among systems of PGR-dependent shoot (or somatic embryogenesis) regeneration. Amelioration or remedy of defects and recovery of normal growth patterns may be possible following transfer of cultures to a medium devoid of PGRs or after transplantation of plants to the soil. In contrast, as

we have experienced in this study, shoot-like regenerants with no stem are generally unable to correct the deficiency. In other words, this type of acute defect is a terminal one because the regenerant will not complete a life cycle.

Impaired *in vitro* organogenesis has been attributed to detrimental influences of PGRs, and a pivotal role for cytokinins specifically in the differentiation of normal or abnormal shoot meristems has been suggested (Toponyanon et al., 1999; Howell et al., 2003; Ramage and Williams, 2004). We show here for the first time that stem-deficiency appears also in cultures not treated with PGRs (Table 1). A high incidence of aberrant regenerants in *L. esculentum*, compared to a negligible incidence of stem deficiency among other *Lycopersicon* species, in cultures not exposed to PGRs, implies the contribution of a genetic component to the appearance of this defect. However, since the percentage of buds developing into regenerants without a stem is, in every *L. esculentum* genotype, higher in PGR-treated cultures (cf. Tables 1 and 3), it is evident that PGRs amplify the formation frequency of imperfect shoot meristems. Uncovering the innate and exogenous cues responsible for the stem-less regenerants is interesting from a point of view of control of meristem differentiation and function, and is essential for the improvement of plant production efficiency in tissue cultures of genotypes suffering from stem- and meristem-less disorders.

Another potential source of difficulties associated with shoot regeneration from hypocotyl explants is the relative high percentage of polyploid regenerant shoots, probably reflecting polyploidization in hypocotyl explant tissue (Bulk et al., 1990; Sigareva et al., 2004). It remains to be seen in the future whether changes in ploidy level occur at a lower frequency in PGR-independent regenerants.

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