BIOLOGIA PLANTARUM 52 (3): 517-520, 2008

BRIEF COMMUNICATION

In vitro propagation of *Capsicum chinense* Jacq.

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Abstract

An efficient micropropagation protocol was established for *Capsicum chinense* Jacq. cv. Umorok, a pungent chilli cultivar. Shoot-tip explants were cultured on Murashige and Skoog (MS) medium containing cytokinins (22.2 - 88.8 μM 6-benzylaminopurine, BAP, 23.2 - 93.0 μM kinetin, Kin, or 22.8 - 91.2 μM zeatin, Z) alone or in combination with 5.7 μM indole-3-acetic acid (IAA). Maximum number of shoots were induced on medium containing 91.2 μM Z or 31.1 μM BAP with 4.7 μM Kin. The separated shoots rooted and elongated on medium containing 2.5 or 4.9 μM indole-3-butyric acid (IBA). Axillary shoots were induced from *in vitro* raised plantlets by decapitating them. The axillary shoot-tip explants were used for further multiple shoot buds induction. A maximum of about 150 plantlets were obtained from a single seedling. Hardened and acclimatized plantlets were successfully established in the soil.

Additional key words: axillary shoot induction, chillies, decapitation, multiple shoot induction.

Capsicum chinense Jacq. cv. Umorok is an indigenous chilli cultivar. Although, the tissue culture aspects of the genus *Capsicum* is well-studied (for recent information see *e.g.* Christopher and Rajam 1996, Hyde and Phillips 1996, Ramirez-Malagon and Ochoa-Alejo 1996, Anilkumar and Nair 2004, Khan *et al.* 2006, Peddaboina *et al.* 2006), several of these report a strong genotype dependence (Christopher and Rajam 1996, Hyde and Phillips 1996). Proliferation of multiple shoot buds from shoot-tip explants by the release of axillary buds were reported in limited cases (Christopher and Rajam 1994, Anilkumar and Nair 2004). Moreover, chilli pepper tissue culture is mostly confined to the more common species, *Capsicum annuum* L. and there has been few reports for the *in vitro* regeneration of *Capsicum frutescens* L. (Subhash and Christopher 1988, Wang *et al.* 1991, Reddy *et al.* 2002) and no report for *Capsicum chinense* Jacq. Therefore, keeping in mind the problems associated with conventional propagation and the need to develop *in vitro* regeneration protocols for the specific cultivars, we report an efficient protocol for *in vitro* clonal mass multiplication of this elite chilli cultivar by inducing multiple shoots on shoot-tip explants and further multiplication by using axillary shoots induced on regenerated plantlets by decapitating them. Such induction of axillary shoots proliferation by decapitation presents an efficient technique for mass multiplication of this elite cultivar.

Seeds extracted from mature fruits were washed in running tap water and treated with 0.1 % *Dhanustin-50* (fungicide) for 10 - 15 min followed by rinsing three times with distilled water. Thereafter, the seeds were surface-sterilized by 0.1 % $HgCl_2$ solution for 5 min and rinsed five times with sterile distilled water. Seeds were then sown in 250 cm^3 conical flasks containing sterile filter paper soaked in distilled water and incubated in the dark for 7 - 10 d at 25 ± 2 °C. After germination, the seeds were inoculated on basal Murashige and Skoog (MS) medium with 3 % (m/v) sucrose and 0.8 % (m/v) agar, adjusted to pH 5.8 by 1 M NaOH and 1 M HCl before adding agar. The media were autoclaved at 121 ºC for 20 min before dispensing into the culture vessels. Shoot apices (1 - 1.5 cm) were trimmed from 4-week-old seedlings and inoculated on shoot bud induction medium consisting of MS basal medium supplemented with cytokinins (22.2 - 88.8 μM 6-benzylaminopurine, BAP, 23.2 - 93.0 μM kinetin, Kin or 22.8 - 91.2 μM zeatin, Z) alone or combinations of cytokinins (22.2 - 44.4 μM BAP with 4.7 μM Kin) or 8.88 - 44.4 μM BAP in combination with 5.7 μM indole-3-acetic acid (IAA). The number of

Received 12 July 2006, *accepted* 17 January 2007.

Abbreviations: BAP - 6-benzylaminopurine; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; Kin - kinetin; MS medium - Murashige and Skoog medium; NAA - α-naphthalene acetic acid; Z - zeatin.

Acknowledgement: One of the authors (K.S.) is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi for the award of a Senior Research Fellowship (*vide* No. 9/476(26)/2K3-EMR-1).

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multiple shoot buds and the percentage of explantsforming multiple shoot buds were counted after four weeks. The shoot buds were then separated and transferred to rooting media consisting of MS basal medium fortified with different concentrations of auxins, 2.9 and 5.7μM IAA, 2.5 and 4.9 μM indole-3-butyric acid (IBA) or 2.7 and 5.4 μM α-naphthalene acetic acid (NAA). After four weeks of culture, data on percentage of rooting, length of regenerated shoots and the number of leaves formed were recorded.

Four-week-old rooted plantlets having 5 - 9 leaves were decapitated for inducing axillary shoot development by cutting the tips with a sterile blade. Axillary shoots developing in the axils of leaves of the decapitated plantlets were used for further multiple shoot bud induction by culturing on medium containing cytokinins (68.4 or 91.2 μM Z, and 66.6 or 88.8 μM BAP) alone or in combination (22.2 or 31.1 μM BAP with 4.7 μM Kin) and the number of shoot buds were counted after four weeks. The shoot buds formed were cut and rooted in MS basal medium fortified with 4.9 μM IBA. All cultures were maintained at temperature of 25 ± 2 °C and 16-h photoperiod with irradiance of 30 μmol m^{-2} s^{-1} (cool white fluorescent tubes). Four week-old rooted plantlets were carefully removed from the flasks and gently washed under running tap water to remove adhering pieces of gelled medium. These were then transplanted to perforated plastic cups containing pre-sterilized sand:soil (5:1) and maintained in a 50 % shaded net-house for hardening. The plants were initially covered with clear polyethylene bags having a few holes on it and were frequently watered to keep high humidity. After 10 d, the humidity was gradually decreased by increasing the size of holes in the polyethylene bags. The plants hardened here within 15 d and these were indicated by the emergence of new apical leaves. The polythene bags were finally removed and the plants were transferred to bigger earthen pots containing soil or to the field condition.

Data on shoot bud induction from shoot-tip explants, elongation of rooted shoots and the multiple shoot induction from axillary shoot explants were analyzed using one-way analysis of variance (*ANOVA*) and the means were evaluated at the $P < 0.01$ level of significance using Duncan's multiple range test. Each treatment for multiple shoot bud induction had six replicates while the treatment for rooting and axillary shoot culture had ten replicates. All the experiments were repeated thrice.

The regeneration systems of *Capsicum* reported so far had shown the critical effect of cytokinin, cytokinin-cytokinin or cytokinin-auxin ratio in regeneration from various explants (Gunay and Rao 1978, Phillips and Hubstenberger 1985, Agrawal *et al.* 1989, Arroyo and Revilla 1991, Ezura *et al.* 1993, Christopher and Rajam 1994, Szasz *et al.* 1995, Ramirez-Malagon and Ochoa-Alejo 1996, Anilkumar and Nair 2004). Therefore, we studied the morphogenetic response of shoot-tip explants of *Capsicum chinense* Jacq. cv. Umorok cultured on MS medium supplemented with various concentrations of cytokinins (BAP, Kin or Z) and combinations of BAP with Kin or IAA. After 2 - 3 weeks of culture, multiple shoot buds developed from the shoot-tip explants (Fig. 1*A*). The maximum proliferation of shoot buds (up to 7) occurred on medium containing 91.2 μM Z, or 31.1 μM BAP with 4.7 μM Kin (Table 1). Among the three cytokinins tested, Z proved to be the most effective for multiple shoot bud induction followed by BAP and Kin. The effectiveness of 4.6 μM Z alone (Gunay and Rao 1978) or in combination with 0.6 μM IAA (Arroyo and Revilla 1991) in pepper tissue cultures have been reported. However, in the present study, the frequency of shoot buds formed was low (1 - 2) at 4.6 μM Z and it increased with increasing concentration of Z. Christopher and Rajam (1994) reported that very high concentrations of BAP (66.6 μM for *Capsicum praetermissum* and 88.8 μM for *Capsicum annuum* L. cv. G4) were necessary for maximal shoot proliferation from shoot-tip explants. Our results also show a similar finding with maximal proliferation at 66.6 μM BAP with decrease in the frequency of shoot buds beyond 66.6 μM BAP. Low concentrations of BAP (8.8 - 22.2 μM) alone or in combination with 0.6 - 11.4 μM IAA was found to be effective for shoot bud induction from various explants in chilli tissue cultures (Gunay and Rao 1978, Agrawal *et al.* 1989, Husain *et al.* 1999, Arroyo and Revilla 1991). However, in the present study, when BAP was used in combination with 5.7 μM IAA the number of shoot buds increased with the increase in BAP concentration beyond 22.2 μM. When BAP was used in combination with Kin, maximum shoot bud proliferation from the shoot-tip explants occurred in MS medium fortified with 31.1 μM BAP and 4.7 μM Kin. Anilkumar and Nair (2004) also obtained similar results in *Capsicum annuum*.

Table 1. Effect of growth regulators on multiple shoot bud induction from shoot-tip explants of *Capsicum chinense* Jacq. cv. Umorok. Means ± SE, $n = 6$. Means followed by the same letters are not significantly different at $P < 0.01$.

BAP [μM]	Kin [μM]	Z [μM]	IAA [μM]	Response [%]	Shoot number
-	23.3	-	-	33.3	1.3 ± 0.19e
-	69.8	-	-	83.3	2.0 ± 0.23de
-	93.0	-	-	100.0	3.3 ± 0.19bcd
-	-	22.8	-	66.6	2.2 ± 0.44cde
-	-	68.4	-	100.0	3.8 ± 0.28b
-	-	91.2	-	100.0	6.2 ± 0.28a
22.2	-	-	-	83.3	2.3 ± 0.38bcde
66.6	-	-	-	10.0	3.8 ± 0.44b
88.8	-	-	-	83.3	2.7 ± 0.45bcde
8.8	-	-	5.7	100.0	2.3 ± 0.19bcde
22.2	-	-	5.7	100.0	3.2 ± 0.28bcd
44.4	-	-	5.7	100.0	3.7 ± 0.45bc
22.2	4.7	-		83.3	2.7 ± 0.30bcde
31.1	4.7	-		100.0	5.5 ± 0.31a
44.4	4.7	-		83.3	2.5 ± 0.46bcde

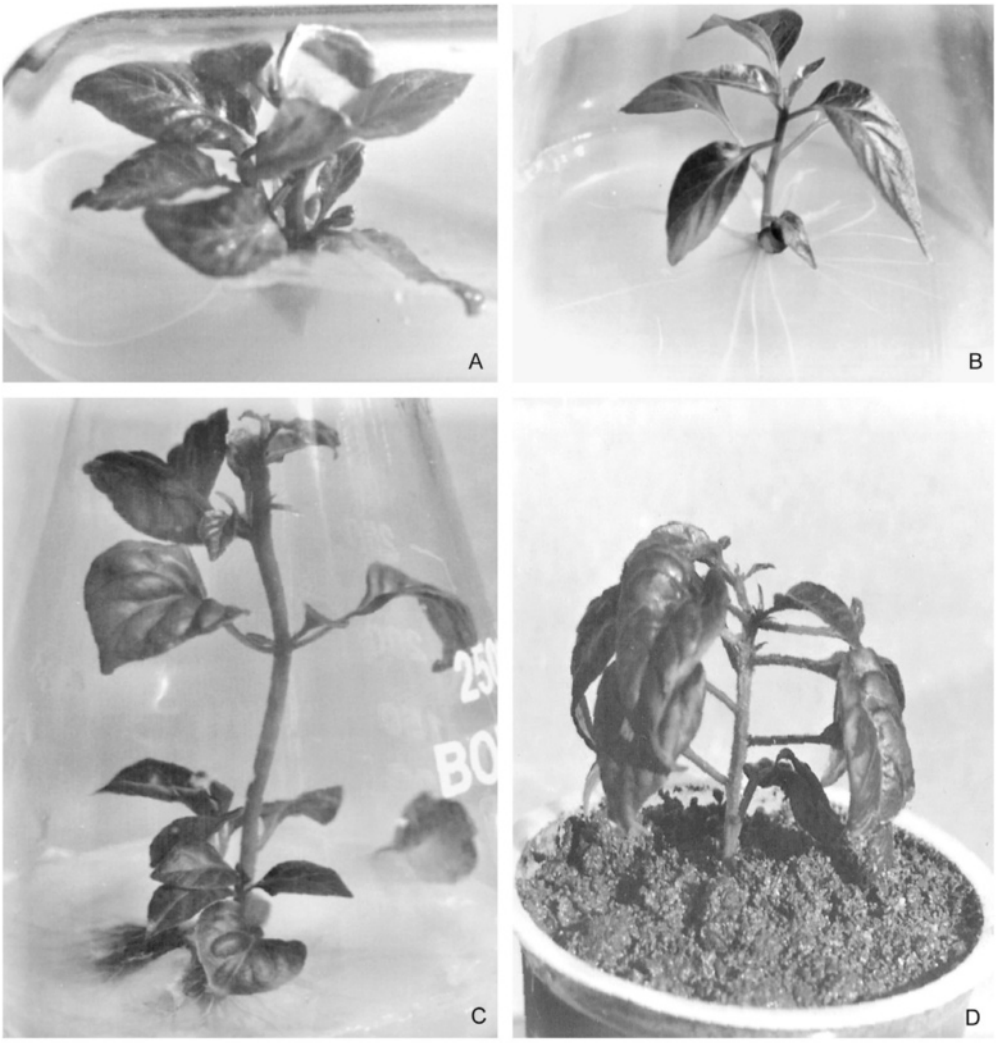

Fig. 1. *In vitro* propagation of *Capsicum chinense* Jacq. cv. Umorok. *A* - induction of multiple shoots from shoot-tip explant, *B* - rooted plantlet, *C* - induction of axillary shoots by decapitation, *D* - transplanted plantlet.

Table 2. Effect of auxins on rooting and elongation of shoot buds derived from shoot-tip explants of *Capsicum chinense* Jacq. Means ± SE, $n = 10$. Means followed by the same letters are not significantly different at $P < 0.01$.

IAA [μM]	IBA [μM]	NAA [μM]	Rooting [%]	Shoot length [cm]	Leaf number
2.9	-	-	70	1.9 ± 0.36b	5.7 ± 0.35bc
5.7	-	-	90	1.9 ± 0.30b	6.3 ± 0.51ab
-	2.7	-	100	4.1 ± 0.44a	6.0 ± 0.42bc
-	4.9	-	100	3.8 ± 0.36a	7.6 ± 0.25a
-	-	2.7	100	0.4 ± 0.11c	3.6 ± 0.29d
-	-	5.4	100	0.4 ± 0.07c	2.5 ± 0.21d

Table 3. Effect of growth regulators on multiple shoot bud induction from axillary shoot explants of *Capsicum chinense* Jacq. Means ± SE, $n = 10$. Means followed by the same letters are not significantly different at $P < 0.01$.

BAP [μM]	Kin [μM]	Z [μM]	Response [%]	Shoot number
-	-	68.4	100	2.9 ± 0.22b
-	-	91.2	100	5.2 ± 0.38a
66.6	-	-	100	4.3 ± 0.35a
88.8	-	-	80	2.6 ± 0.35b
22.2	4.7	-	80	2.4 ± 0.25b
31.1	4.7	-	100	4.8 ± 0.31a

Rhizogenesis followed by elongation of the shoot buds (Fig. 1*B*) occurred on rooting medium medium containing 2.5 or 4.9 μM IBA (Table 2). Agrawal *et al.* (1989) also reported the effectiveness of IBA on rooting of *in vitro* regenerated chilli pepper plantlets. On NAA containing medium, the roots produced were thick and

short with fine root hairs while on medium containing IAA or IBA, the roots were thin and long with branches and root hairs. A maximum of about 6 - 8 leaves developed on plantlets rooted in medium containing 5.7 μM IAA or 4.9 μM IBA (Table 2).

After four weeks of culture in the rooting medium, the elongated plantlets were decapitated and its effect on the growth of axillary shoots was studied. The decapitated plantlets showed the development of 2 - 5 axillary shoots within two weeks of culture (Fig. 1*C*). Earlier, we reported similar induction of axillary shoots by decapitation of *in vitro* regenerated plantlets in two cultivars of *Capsicum annuum* L. has been reported (Sanatombi and Sharma 2006). This system differs from that reported by Ramirez-Malagon and Ochoa-Alejo (1996). In that system the buds formed after 10 - 14 d of culture on MS medium without growth regulators from the wounded apical zone of hypocotyl tissues.

On culturing the axillary shoot-tips on bud induction medium, these proliferated to produce multiple shoot buds (3 - 6) on medium supplemented with 91.2 μM Z, 66.6 μM BAP or 31.1 μM BAP and 4.7 μM Kin (Table 3). These shoot buds also showed rooting and elongation in media containing 4.9 μM IBA. These rooted plantlets were decapitated again and used for further induction of axillary shoots or they were transplanted after hardening and acclimatization. The regenerated plants showed 90 % survival during hardening and acclimatization and there were no observable variations between the parent plants and *in vitro* raised plants. The transplanted plantlets established well in pots (Fig. 1*D*).

Thus, the present system presents a novel protocol for rapid micropropagation of this elite chilli cultivar by inducing multiple shoots (up to 7) from the shoot-tip explant of a seedling followed by *in vitro* induction of axillary shoots (up to 5 per plantlet) from the regenerated plantlets and further induction of multiple shoot buds from the axillary shoot explants.

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