Influence of Genotype Source on the *In Vitro* Regeneration Ability of Malaysian Chilli Varieties

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Abstract

Effect of variety on regeneration of Malaysian chilli explants was studied using two varieties MC11 and CB4. Variety CB4 showed the best response while MC11 was the least responsive. Explants were inoculated on medium comprising of Murashige and Skoog's basal medium supplemented with 5 mg/L BAP, 1 mg/L IAA and 25 g/L DJ nutrient. In vitro regeneration from chilli explants were achieved by direct organogenesis. Shoot buds were transfered on MS medium containing 3 mg/L BAP, 1 mg/L IAA, 2 mg/L $GA_{3,}$ 10 mg/L $AgNO_3$ and15 g/L DJ nutrient to elongate. The elongated shoots were rooted on medium containing with 0.5 mg/L IAA.

Keywords: Capsicum annuum, chilli, plant regeneration, organogenesis

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Introduction

Capsicum annuum L. belongs to the family Solanaceae and is an important vegetable and spice crop around the world. Chilli fruits come with diversity of forms, colors, shapes, flavors, pungency and aromas has been used worldwide as ingredients of a wide variety of dishes and also as salads, pickles, paprika, chili powder, curry powder, and pepper sauces.

Classical breeding programs for pepper cultivation have been well established. However productivity of this plant is threatened by its susceptibility to fungal and viral pathogens. Plant tissue culture techniques can be used to increase the speed and/or efficiency of the breeding

process, to improve the accessibility of existing germplasm, and to create new variation for crop improvement.

However, tissue culture techniques in chilli still lag behind most other vegetable crops, mainly due to its recalcitrance to regeneration (Liu et al. 1990). *Capsicum* sp. was found to be highly recalcitrant to regenerate compared to other solanaceae members. The most common approach for tissue culture regeneration of this plant has been through organogenesis and a number of protocols have been published using a range of explants and different medium combinations (Ochoa-Alejo & Ramirez-Malagon 2001). Various explants have been tried such as shoot tip (Christopher & Rajam 1994), rooted hypocotyl (Valera-Montero & Alejo 1992), leaf, stem, hypocotyl, cotyledon, root, shoot tip and embryo (Agrawal et al. 1989). However, these procedures failed or had to be modified when they were used to regenerate plants from other chilli varieties in other laboratories. Researchers around the world faced the same problem in regenerating capsicum; formation of ill defined buds or shoot like structures either resisting elongation or producing rosettes of distorted leaves which generally do not produce normal shoots (Steinitz et al. 1999, Ochoa-Alejo & Ramirez-Malagon 2001). Several of these reports suggest that the chilli regeneration process is strongly influenced by genotype (Ramirez-Malagon & Ochoa-Alejo 1996). In the view of the above facts, the present research was designed to evaluate the effect of variety on regeneration of Malaysian chilli explants.

Materials and Methods

Seeds of *Capsicum annuum* L.var CB4 and MC11 were obtained from Universiti Kebangsaan Malaysia (UKM), Bangi and Malaysian Research and Development Institute (MARDI). They were sterilized with 20% Clorox® for 20 min and rinsed three times with sterile distilled water. Sterilized seeds were germinated on MS (Murashige & Skoog 1962) basal medium supplemented with 30 g/L sucrose, 2.8 g gelrite, pH adjusted to 5.8 before autoclaving at 121°C and 1.2–1.3 kg/cm2 pressure for 20 min. Seeds were germinated in growth chamber at a temperature of 26° ± 1°C and 16 h photoperiod.

Cotyledons and hypocotyls of *C. annum* var. MC11 and CB4 were excised aseptically from 10- to 14-day-old seedlings and cultured on MS medium containing 5 mg/L BAP, 1 mg/L IAA and 25 g/L DJ nutrient (chilli seedling extract) to induce shoot bud. Shoots induced were excised and placed on shoot elongation medium (MS medium containing 3 mg/L BAP, 1 mg/L IAA, 2 mg/L GA_3, 10 mg/L $AgNO_3$ and 15 g/L DJ nutrient).

Elongated shoots were excised and transferred on to rooting medium consisting of full strength MS medium supplemented with 0.5 mg/L IAA. Plantlets with well developed shoot and root systems were transferred to earthen pots containing garden soil and organic manure (1:1).

DJ nutrients

DJ nutrients nutrient was prepared by grounding 25 g (fresh weight) of chilli seedlings with liquid nitrogen. 15 ml water was added to the seedling powder. The slurry was then transferred to a 50-ml centrifuge tube containing and then centrifuged at 8,000 rpm for 3 min. water was added to a final volume of 25 ml (final concentration: 1 g fresh weight/ml). The solution was filtered through a 0.22 mm filter paper and stored at 4^0C.

Results & Discussion

Explants obtained from 10-14 days old seedling of *Capsicum annuum* L. var CB4 and MC11 were cultured on induction medium supplemented with 5 mg/L BAP, 1 mg/L IAA and 25 g/L DJ nutrient and 30 % sucrose. Buds were produced directly from the hypocotyls and cotyledons within 1 week of culturing. After 4 weeks, result showed that CB4 was more responsive compared to MC11 in producing buds (Figure 1). Only 1% of MC11 explants produced buds compared to 74% of CB4 explants.

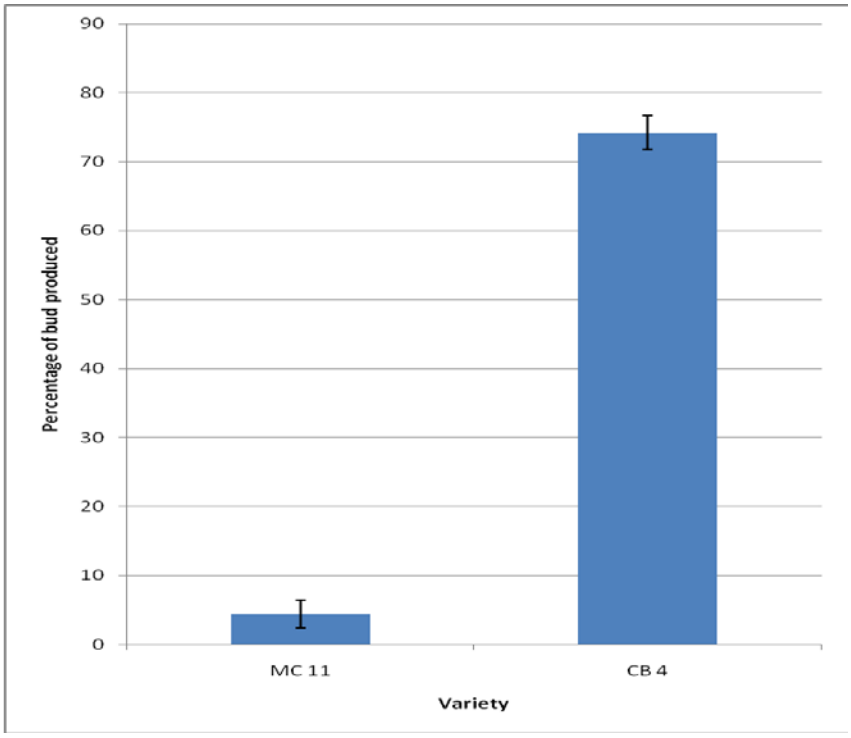

Figure 1: Effect of MC11 dan CB4 varieties on bud induction after one month cultivation.

Buds produced by MC11 was ill defined (Figure 2a). Meanwhile, CB4 produced combination of ill defined buds and shoot like structure buds (Figure 2b & 2c). The shoots were transferred to medium containing 3 mg/L BAP, 1 mg/L IAA, 2 mg/L GA_3, 10 mg/L $AgNO_3$ and 15 g/L DJ nutrient to elongated. Ill defined buds induced from MC11 and CB4 did not elongate and resulted in a rosette of shoots. The shoot like structure produced by CB4 frequently developed either into normal shoots or abnormal shoots (rosette). The normal shoots elongated further within four weeks of subculture.

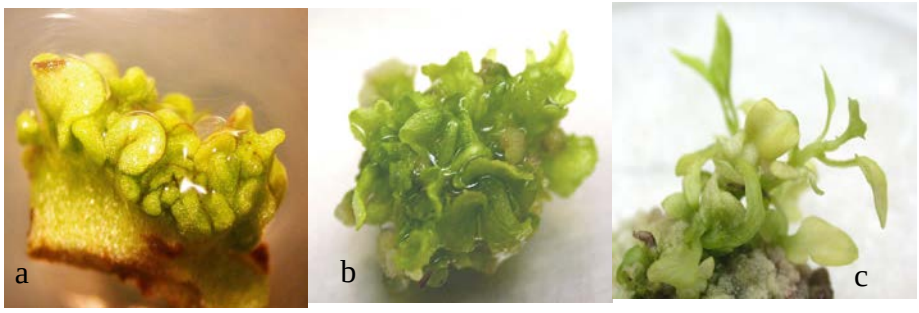

Figure 2: Type of shoot bud. (a) Ill defined buds, (b) shoot like structures and (c) normal shoots produced from explant of *Capsicum annum* cultured on induction medium.

The formation of rosettes or blind leaf structures without shoot elongation has been observed in *Capsicum* and reported as the major problem in establishing tissue culture regeneration (Szasz et al. 1995; Franck-Duchenne et al. 1998). This phenomenon is common in *Capsicum* (Ochoa-Alejo & Ramırez-Malagon 2001) and may be associated with fasciated and degenerative meristems (Mezghani et al. 2007). Meanwhile, Ochoa-Alejo & Ramirez-Malagon, (2001) suggesting a problem in auxin perception and/or signal transduction leading to malformed meristem.

This result indicated that buds induction ability are greatly influenced by the genotype and are in agreement with those reported in Ezura et al. (1993). They observed varietal differences in the percentage of explants forming buds and in the percentage of explants with elongated shoots when working with 14 bell pepper cultivars. Meanwhile Binzel et al. (1996) worked with two hot pepper varieties, also concluded that cultivars used will affect the explant ability to form bud and regenerate. Dabauza & Pena (2001) reported that bud induction mainly depended on explant type and variety. Szász et al. (1995) analysed shoot regeneration ability of 17 bell pepper genotypes using intact cotyledons with petioles and rooted hypocotyls from 12 to

14-day-old seedlings with different concentrations of IAA and BA. They observed different responsiveness of the 17 pepper varieties and hybrids used depending on culture medium and explant type. Thus, the strong influence of the chilli variety makes it necessary to optimize regeneration protocols for specific variety.

The elongated shoots, 2 cm and more in length were excised and placed on the MS medium consisting of 0.5 mg/L IAA. Root initiation occurred directly from the cut ends of micro-shoots after 2 weeks of culture. Rooted plantlets were removed from agar, washed thoroughly and placed in a mixture of sterilized vermiculite and sterilized soil (1:1), before being acclimatized in greenhouse.

Although the protocol has limited applications in mass propagation *in vitro*, it can be utilized for genetic transformation studies of chilli. Further studies on increasing the percentage of shoot elongation could be useful in Malaysian chilli.

Conclusion

In conclusion we have demonstrated that shoot formation primarily depends on the variety used. CB4 was more responsive compare to MC11 in producing shoot buds and we have established a promising protocol for regeneration of *Capsicum annuum* var CB4.

Abbreviation

BAP - 6-benzylaminopurine
MS - Murashige and Skoog basal medium (1962)
IAA - Indole acetic acid
$AgNO_3$ - Argentum nitrate

References

1. Binzel M.L., Sankhla N., Joshi S. & Sankhla D. 1996. *In vitro* regeneration in chile pepper (*Capsicum annuum* L.) from ‘halfseed explants’. *Plant Growth Regul*. 20: 287–293.
2. Ezura H., Nishimiya S. & Kasumi M. 1993. Efficient regeneration of plants independent of exogenous growth regulators in bell pepper (*Capsicum annuum* L.). *Plant Cell Rep*. 12: 676–680.

3. Franck-Duchenne, M., Wang, Y., Ben Tahar, S. & Beachy, R.N. 1998. *In vitro* stem elongation of sweet pepper in media containing 24-epi-brassinolide. *Plant Cell Tiss. Organ Cult.* 53: 79-84.

4. Kumar, V., Gururaj H. B,. Prasad, B.C,N., Giridhar, P. & Ravishankar, G.A. 2005. Direct shoot organogenesis on shoot apex from seedling explants of *Capsicum annuum* L. *Sc. Hort.* 106: 237–246.

5. Li, D., Zhao. K., Xie, B., Zhang, B. & Luo K. 2003. Establishment of highly efficient transformation system for pepper (*Capsicum annuum* L.). *Plant Cell Rep.* 21: 785-788.

6. Mezghani, N., Jemmali, A., Elloumi, N., Gargouri-Bouzid, R. & Kintzios, S. 2007. Morpho-histological study on shoot bud regeneration in cotyledon cultures of pepper (*Capsicum annuum*). *Biologia* 62(6): 704-710

7. Murashige, T. & Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497

8. Ochoa-Alejo, N. & Ramırez-Malagon, R. 2001. *In vitro* chili pepper biotechnology. *In Vitro Cell. Dev. Biol. Plant* 37:701–729.

9. Ramirez-Malagon, R. & Ochoa-Alejo, N. 1996. An improved and reliable chili pepper (*Capsicum annuum* L.) plant regeneration method. *Plant Cell Rep*. 16: 226-231.

10. Steinitz, B., Wolf, D., Matzevitch, J.T. & Zelcer, A. 1999. Regeneration *in vitro* and genetic transformation of pepper (*Capsicum* spp.). The current state of art. *Capsicum* and eggplant. *Newsletter* 18: 9-15.

11. Szasz, A., Nervo, G. & Faari, M. 1995. Screening for *in vitro* shoot-forming capacity of seedling explants in bell pepper (*Capsicum annuum* L.) genotypes and efficient regeneration using thidiazuron. *Plant Cell Rep*. 14: 666-669.