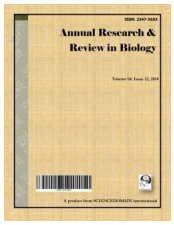

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Influences of Explant Type on *in vitro* Regeneration of Malaysian Chilli (*Capsicum annuum* L.) var CB 4

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Authors' contributions

This work was carried out in collaboration between both authors. Author ZA designed the study, wrote the protocol, performed the treatment and interpreted the data. Author ZZ managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Regeneration system *via* organogenesis has been developed for Malaysian chilli (*Capsicum annuum* L) var. CB4. Hypocotyls, cotyledons and petioles from *In vitro* germinated seedlings were cultured 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. Hypocotyls and petioles were found to be the best explants, based on its ability to produce shoot buds compared to cotyledons. Shoot buds were elongated on MS medium containing 3 mg/l BAP, 1 mg/l IAA, 15 g/L DJ, 2 mg/L GA3, 10 mg/l AgNO3 and 15 g/L DJ nutrient. Rooting was induced on MS basal containing IAA.

Keywords: Capsicum annuum; chilli; plant regeneration; tissue culture; explants.

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1. INTRODUCTION

Capsicum annuum L. belongs to the family Solanaceae and is an important vegetable and spice crop around the world. Fruits of this plant have been used worldwide as ingredients in a wide variety of dishes and also as salads, pickles, paprika, chilli powder, curry powder, and pepper sauces. Chilli fruits come with diversity of forms, colors, shapes, flavors, pungency and aromas. Classical breeding programs for chilli 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 lag behind compared to most other vegetable crops, mainly due to its recalcitrance to regeneration [1]. 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 [2]. Various explants have been tried such as shoot tip [3], rooted hypocotyl [4], leaf, stem, hypocotyl, cotyledon, root, shoot tip and embryo [5].

Though various reports on organogenesis in *C. annuum* varieties are available [2], 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 [6,7,8]. Several of these reports suggest a strong influence of source of explants on the regeneration process. However, inconsistency of these protocols with different genotypes and low reproducibilities among laboratories had meant that efficiency *In vitro* regeneration from seedling explants has remained a challenge. In the view of the above facts, the present research was designed to develop a protocol for Malaysian chilli var CB 4 by studying the effects of different source of explants on regeneration of Malaysian chilli.

2. MATERIALS AND METHODS

2.1 Explant Preparation

Seeds of *Capsicum annuum* L. var. CB4 were obtained from Universiti Kebangsaan Malaysia (UKM), Bangi. They were sterilized with 20% Clorox® for 20 min and rinsed three times with sterile distilled water. Sterilized seeds were germinated on MS basal medium [9] supplemented with 30 g/L sucrose, 2.8 g gelrite, pH adjusted to 5.8 before autoclaving at 121℃ and 1.2–1.3 kg/cm2 pressure for 20 min. Seeds were germinated in growth chamber at a temperature of 26°± 1℃ and 16 h photoperiod.

Cotyledons, petioles and hypocotyls were excised aseptically from 10-14-day-old seedlings and cultured on induction medium established in our previous study [10]. The induction medium consists of MS basal, 5 mg/L BAP, 1 mg/L IAA and 25 g/L DJ nutrient (chilli seedling extract) to induce 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 GA3, 10 mg/L AgNO3 and 15 g/L DJ nutrient) [10].

Elongated shoots were excised and transferred on to rooting medium consisting of full strength MS medium supplemented with 0.5 mg/L IAA [10]. Plantlets with well developed shoot and root systems were transferred to earthen pots containing garden soil and organic manure (1:1). Observation were made at regular intervals and tabulated. For each treatment, 25 replicates were used and all experiments were conducted thrice. Data were subjected to analysis of variance (ANOVA)

2.2 DJ Nutrients Preparation

DJ nutrients was prepared by grounding 25 g (fresh weight) of chilli seedlings with liquid nitrogen. 15 ml distilled water was added to the seedling powder [10]. The slurry was then transferred to a 50-ml centrifuge tube and centrifuged at 8,000 rpm for 3 min. Distilled 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℃.

3. RESULTS AND DISCUSSION

Explants obtained from 10-14 days old seedling of *Capsicum annuum* L. var. CB4 were cultured on the induction medium. Buds were produced directly from the explants within 1 week of culturing on MS basal media supplemented with 5 mg/l BAP, 1 mg/l IAA, 25 g/L DJ nutrient and 30% sucrose. Results showed that cotyledons produced the highest buds (78.3%) compared to hypocotyls (74.2%) and petioles (70.7%) (Fig. 1).

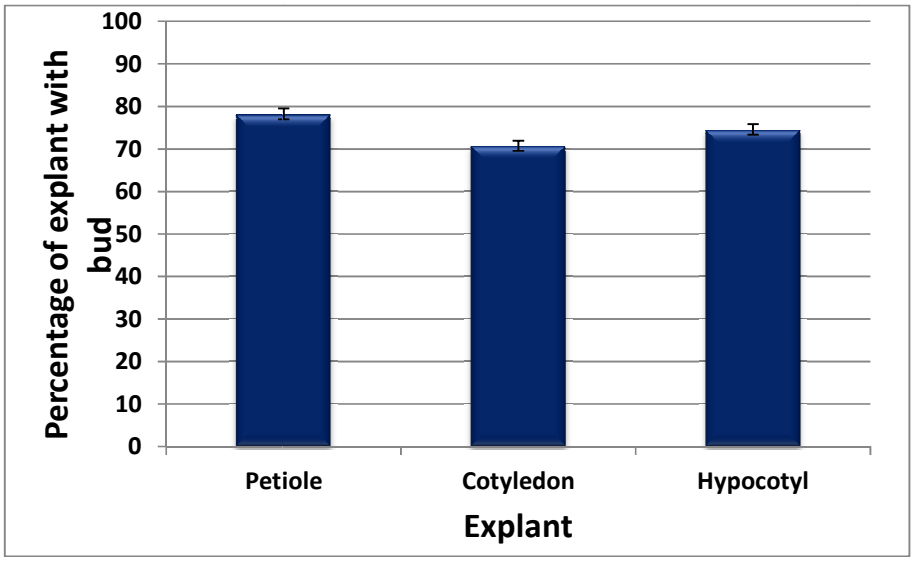

Fig. 1. Percentage of buds produced by explants on the induction medium

However, buds produced by cotyledons were ill-defined that look like crown as shown in and Fig. 2a. Hypocotyls produced 98% leafy structure buds (Fig. 2b) and buds initiated from petioles were combination of both (76.4% leafy structures) (Fig. 2c).

Crown structures (Fig. 3a and b) produced by cotyledons are actually the ill defined buds and these structures failed to develop and leafy buds produced by hypocotyl and cotyledon frequently developed into leafy structures (rosette) (Fig. 3c) or normal shoots (Fig. 3d and e).

The formation of rosettes or blind leaf structures without shoot elongation has been observed in *Capsicum* and it was reported as the major problem in establishing tissue culture regeneration [11,12]. This phenomenon is common in *Capsicum* [2] and it might be associated with fasciated and degenerated meristems [13]. Meanwhile, Ochoa-Alejo & Ramırez-Malagon [2] suggested a problem in auxin perception and/or signal transduction which lead to malformation meristems.

3.1 Shoot Elongation and *In vitro* Rooting

The shoots were transferred to a medium containing 3 mg/L BAP, 1 mg/L IAA, 2 mg/L GA3, 10 mg/L AgNO3 and 15 g/L DJ nutrient [8]. Ill-defined buds did not elongate and resulted in a rosette of shoots. The shoot-like structure developed either into normal shoots or abnormal shoots (rosette). The normal shoots elongated further within four weeks of subculture. Earlier study by Phillips & Hyde [14] showed silver nitrate promote shoot regeneration in *Capsicum annuum*. Silver nitrate has also been used to promote shoot in *Vigna unguiculata* [15] and *Manihot esculenta* [16]

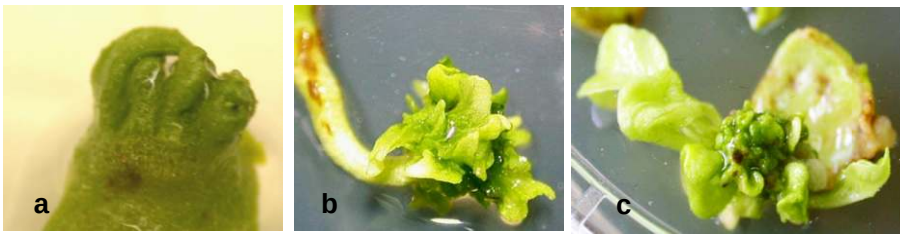

Fig. 2. Shoot type produced by explants on the induction medium. (a) Cotyledon produced ill-defined buds, (b) hypocotyl produced leafy shoots and (c) petiole produced a combination of leafy shoots and ill-defined buds

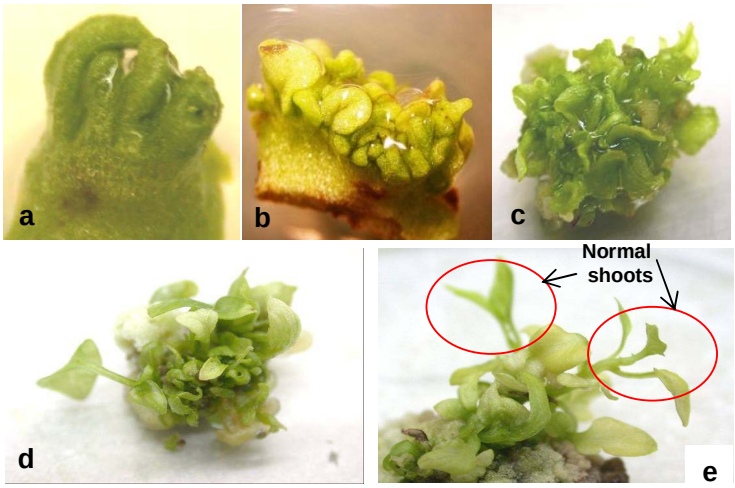

Fig. 3. Types of buds. (a and b) Ill defined buds, (c) shoot-like structures (rossette) and (d & e) normal shoots produced from the explant of *Capsicum annum* cultured on the induction medium

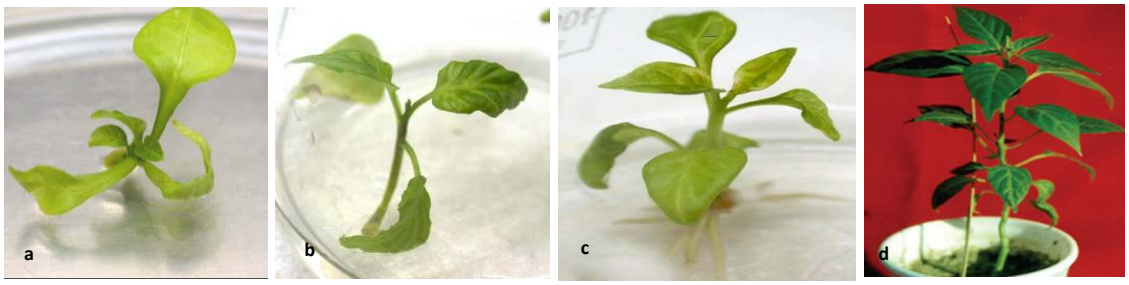

Fig. 4. (a and b) Elongation of normal shoots on elongating medium. (c) Elongation of *In vitro* roots. (d) Rooted plantlet in soil

The elongated shoots, 2 cm and more in length were excised and placed on the MS medium consisting of 0.5 mg/L IAA [10]. Root initiation occurred directly from the cut ends of micro-shoots after 2 weeks of culture.

Results indicated that the regeneration of CB 4 was highly influenced by the source of explants. The best response was obtained from hypocotyls and petioles. These buds proliferated further into numerous shoots when they were sub-cultured on elongating medium. In an earlier study by Jayashankar [17] and Dabauza & Peña [18], showed that high frequency of buds could be induced in cotyledons, but with low frequency of shoot elongation. They also concluded that the percentage of explants producing buds and shoots depended on the explant type. 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. 155 plantlets were transplanted into earthen pot with 85% survival.

4. CONCLUSION

We have demonstrated that shoot formation in Malaysian chilli var CB4 depended on the types of explant used. We have established a promising protocol for the regeneration of *Capsicum annuum* var CB4.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Liu W, Parrott WA, Hildebrand, DF, Collins GB, Williams EG. *Agrobacterium* induced gall formation in bell pepper

(*Capsicum annuum* L.) and formation of shoot-like structures expressing introduced genes. Plant Cell Rep. 1990;9:360-364.
2. Ochoa-Alejo N, Ramırez-Malagon R. *In vitro* chilli pepper biotechnology. *In vitro* Cell. Dev. Biol. Plant. 2001;37:701–729.
3. Christopher T, Rajam MV. Effect of genotype, explant and medium on *In vitro* regeneration of red pepper. Plant Cell Tiss. Organ Cult. 1996;46:245-250.
4. Valera-Montero LL, Ochoa-Alejo NA. Novel approach for chili pepper (*Capsicum annuum* L.) plant regeneration: Shoot induction in rooted hypocotyls. Plant Science. 1992;84:215.
5. Agrawal S, Chandra N. Differentiation of multiple shoot buds and plantlets in cultured embryos of *Capsicum annuum* L. var. Mathania. Curr. Sci. 1983;52:645-646.
6. Franck-Duchenne M, Wang Y, Ben Tahar S, Beachy RN. *In vitro* stem elongation of sweet pepper in media containing 24-epi-brassinolide. Plant Cell Tiss. Organ Cult. 1998;53:79-84.
7. Steinitz B, Wolf D, Matzevitch JT, Zelcer A. Regeneration *In vitro* and genetic transformation of pepper (*Capsicum spp*.). The current state of art. *Capsicum* and eggplant. Newsletter. 1999;18:9-15.
8. Ramirez-Malagon R, Ochoa-Alejo N. An improved and reliable chilli pepper (*Capsicum annuum* L.) plant regeneration method. Plant Cell Rep. 1996;16:226-231.
9. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia. Plantarum. 1962;15:473–497.
10. Alizah Zainal. Functional study of cDNA CUKM2-CCS via antisense technology. Ph. D dissertation. Universiti Kebangsaan Malaysia, Bangi; 2009.
11. Szasz A, Nervo G, Faari M. 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. 1995;14: 666-669.
12. Ezura H, Nishimiya S, Kasumi M. Efficient regeneration of plants independent of exogenous growth regulators in bell pepper (*Capsicum annuum* L.). Plant Cell Rep. 1993;12:676–680.
13. Mezghani N, Jemmali A, Elloumi N, Gargouri-Bouzid R, Kintzios S. Morpho-histological study on shoot bud regeneration in cotyledon cultures of pepper (*Capsicum annuum*). Biologia. 2007;62(6):704-710.
14. Hyde C, Phillips GC. Silver nitrate promotes shoot development and plant regeneration of chilli pepper (*Capsicum annuum* L.) via organogenesis. *In Vitro* Cell Dev. Biol. Plant. 1996;32:72-80.
15. Brar MS, Moore MJ, Al-Khayri JM, Morelock TE, Anderson EJ. Ethylene inhibitors promote *In vitro* regeneration of cowpea (*Vigna unguiculata* L.). *In Vitro* Cell. Dev. Biol. Plant. 1999;35: 222–225.
16. Zhang P, Phansiri S, Puonti-Kaerlas J. Improvement of cassava shoot organogenesis by the use of silver nitrate *In vitro*. Plant Cell Tissue Organ Cult. 2001;67:47–54.
17. Jayashankar S. Comparison of different *In vitro* regeneration and genetic transformation strategies for chilli pepper (*Capsicum annuum*). Ph.D. dissertation, New Mexico State University, Las Cruces; 1998.
18. Dabauza M, Peña, L. High Efficiency organogenesis in sweet pepper (*Capsicum annuum* L.) tissues from different seedling explants. Plant Growth Regulation. 2001; 33:221-229.

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