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Full Length Research Paper

In vitro response from cotyledon and hypocotyls explants in tomato by inducing 6-benzylaminopurine

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Differential response of tomato was evaluated by hypocotyls and cotyledon explants on 6-benzylaminopurine (BAP) containing MS media. Among the four levels (0, 1, 2 and 4 mg/l) of BAP employed in Murashige and Skoog (MS) media, 2.0 mg/l BAP was found superior in growth traits (number of shoots/explants and shoot length) and cultivars. No significant difference was noticed between cotyledon and hypocotyls explants on media having 2.0 mg/l BAP. In the same treatment, shoot length was significantly different between cotyledon and hypocotyls derived explants. No adventitious shoots was noted in the control and higher concentration of BAP containing media but the explants turned into callus on media having 4.0 mg/l BAP.

Key words: Cotyledon, hypocotyls, in vitro, tomato, 6-benzylaminopurine.

INTRODUCTION

Tomato belongs to the family Solanaceae by its nature of a perennial plant but is commercially cultivated as an annual crop. Tomato (Lycopersicon esculentum. Mill) is regarded as the 2nd most important vegetable crop in the world after potato (Bhatia et al., 2004a; Foolad, 2004). The tomato crop is very versatile and is grown either for fresh market or processing. Tomato fruits are eaten fresh and used in processing more productions like pastes and ketchup. Tomato is rich in vitamins A and C and fiber, and is also cholesterol free (Block et al., 1992; Gerster, 1997; Rao and Agarwal, 2000). Tissue culture is an important tool of biotechnology, which can be used to improve productivity of crop via rapid availability of superior planting stock (Bhatia and Ashwath, 2004). In vitro regeneration of cultivated tomato has been a subject of research because of the commercial value of the crop and its amenability for further improvement via genetic manipulation (Evans, 1989).

The success in tomato regeneration response has been found to depend largely on genotype, explants, and plant growth regulator used in culture medium (Bhatia et al., 2004a). Plant growth regulators affect morphogenic tomato cultures (Branca et al., 1994). For tomato regeneration, a wide variety of plant growth regulators have been used with varying concentrations. Many cytokinin and auxin combinations could induce shoot proliferation in tomato from different source of explants. Jatoi et al. (2001) found that 6-benzylaminopurine (BAP) + indole-3-acetic acid (IAA) are the best for callus induction from shoot tips and also found that kinetin (Kin)+IAA are the best for regeneration shoots from callus. Gubis et al. (2004) studied the effect of different growth regulators and plant regeneration of tomato explants, they found that the best regeneration medium was the Murashige and Skoog (MS) medium supplemented with 1 mg/l of zeatin and 0.1 mg/l IAA and 100% frequency of regeneration was observed when hypoctyl explants were used. Chaudhry et al. (2004) found that callus formation from hypocotyl is best when gotten from leaf discs in 2 mg/l IAA + 2 mg/l BAP or 2 mg/l α–naphthalene acetic acid (NAA)+ 4 mg Kin and the maximum percentage of shoot formation on the MS medium supplemented with 2 mg/l IAA+5 mg/l BAP or 2 mg/l +4 mg/l Kin. They reported that hypocotyl is the best explant source for callus formation and regeneration.

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Abbreviations: BAP, 6-Benzylaminopurine; IAA, indole-3-acetic acid; Kin, kinetin; MS, Murashige and Skoog.

Table 1. Nature of response initiated by BAP in two types of tomato explants.

Cultivars	Explants	Response			
		0	1 mg/l BAP	2 mg/l BAP	4 mg/l BAP
Pearl	Hypocotyls	Roots	Shoots	Callus+ shoots	Callus
	Cotyledon	No response	Shoots	Callus +shoots	Callus
Beril	Hypocotyls	Roots	Shoots	Callus+ shoots	Callus
	Cotyledon	No response	Shoots	Callus+ shoots	Callus

Consequently, numerous studies on plant regeneration from a wide range of tissues and organs of wild and cultivated tomato germplasm have been conducted (Cassells, 1979; Zapata et al., 1981). Mass propagation of tomato has been attempted through the use of various types of explants viz. cotyledon, hypocotyl, pedicel, peduncle, leaf, stem sections and inflorescence for organogenesis. On the other hand, Sheeja et al. (2004) found that the presence of 2 mg/l BAP and 0.5 mg/l kinetin produced maximum shootlets and they reported that the use of young hypocotyls explants enhanced plantlet regeneration and length plantlets and maximum root length was observed in the presence of 2 mg/l BAP+ 1 mg/l IAA. Half strength of MS was found to be the best rooting medium. Jabeen et al. (2005) found that the regeneration capacity was strongly influenced by the cultivar and explant type. The explant types of shoot tip were found to be the best explant source for direct shoot formation (80% shoot primordial were regenerated) while hypocotyl was found to be the best explant source for shoot formation through callogenesis (64.5% shoot primordial were regenerated).

Capote-Rodriguez et al. (2000) found that the best results for shoot regeneration from leaf tissue were obtained with MS media supplemented with 0.175 mg NAA and 1.5 mg BA/l. Venkatachalam et al. (2000) in his study found that BAP was more suitable compared to kinetin for maximum shoot bud differentiation as well as multiple shoot induction. BAP was the most effective plant growth regulator in this study, indicating cytokine specificity for shoot bud regeneration and multiple shoot induction in these tissues. The experiment was conducted to find out the effectiveness of BAP on shooting morphogenesis in two hybrid tomato cultivars.

MATERIALS AND METHODS

Seeds sterilization culture

Seeds of two F1 tomato hybrid cultivars, Pearl and Beril, were obtained from the local market in Malaysia from Sin Seng Huat Seeds Sdn. BHD and Rijk Zwaan companies respectively. Tomato seeds were surface sterilized with 8% clorox (sodium hypochlorite) for 10 min and rinsed four times with autoclaved distilled water and cultured in test tubes in MS (Murashige and skoog, 1962) basal medium. The cultures were incubated in the dark for three days and were transferred to 16/8 light/dark cycle and illuminated under white fluorescent light (15.8 $\mu molm^{-2}s^{-1}$) and 25±2ºC.

Explants isolate and culture

Two weeks after germination, cotyledons and hypocotyls explants were separated by aseptic manipulations. Explants of hypocotyls and cotyledons (1 cm) were isolated and cultured in MS (Murashige and skoog, 1962) medium supplemented with 30 g sucrose at different concentrations of BAP (0, 1, 2 and 4 mg/l). The pH of the medium was adjusted to 5.7 and solidified by 7 g/l agar. One explant was cultured in every test tube and test tubes were placed in slope condition. The cultures were incubated in normal growth room conditions (16/8 light/dark regime) having the same light intensity and temperature as above for six weeks. Data were collected, and evaluated in terms of shoots number and length.

Design and statistics analysis

The factorial experiment (2 × 2 × 4) was arranged in a completely randomized design (CRD) with four replications. Each replication consisting of three samples and data were analyzed using analysis of variance (ANOVA) and LSD was used $p = 0.05$ for comparison between treatment means.

RESULT AND DISCUSSION

The cytokinin BAP promotes cell division, shoot multiplication and auxiliary bud formation (Sutter, 1996). Combination of phytohormones (cytokines and auxins) has been reported to determine the course of morphogenesis such as shoot organogenesis in tomato cultivars. In this study, BAP alone induced shoot organogenesis without auxins comparison with other studies that used a combination of hormones in tomato cultivars such as Chaudhry et al. (2004), Duzyaman et al. (1994), El-Bakry, (2002) and Sheeja et al. (2004). The morphogenetic response of tested explants differed depending on the concentrations of BAP in the medium (Table 1). Differential response was noticed in cultivars and explants under the levels of BAP in the media. The medium supplemented with 2 mg/l BAP was most effective in the induction of adventitious shoots for both the hypocotyls and cotyledons. The formation of roots from hypocotyls explants were found at the control treatment, whereas the cotyledons did not show any response. The media supplemented with 4 mg/l BAP resulted to callus in both the explants, while hypocotyls showed most response than cotyledons explants in both cultivars (Figure 1). Bhatia et al. (2004b) indicated the formation of callus from cotyledons explants under the effect of BAP and Zeatin and that BAP

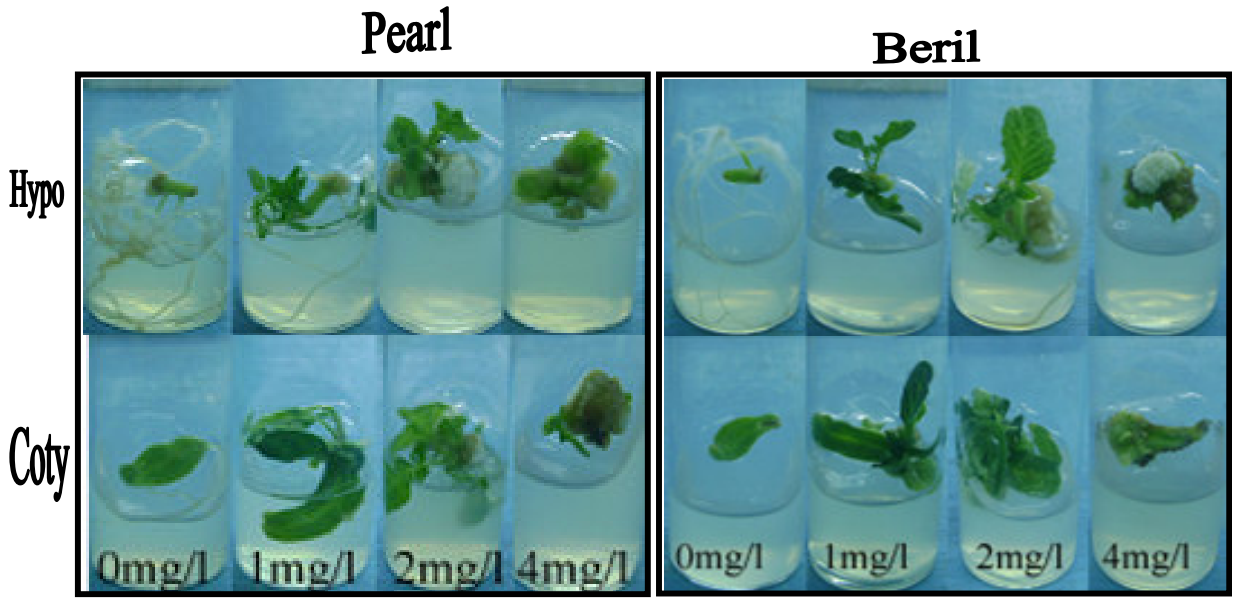

Figure 1. Formation of shoots appeared from hypocotyls and cotyledons explants in two tomato cultivars under different level of BAP.

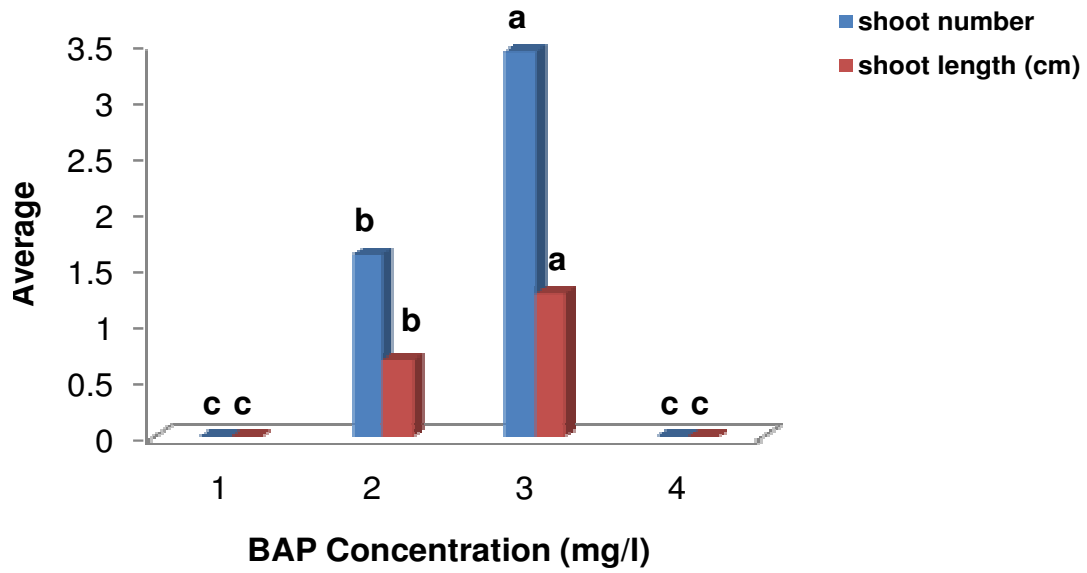

Figure 2. Effect of BAP levels on shoots number and shoots length average in tomato cultivars. Columns that have same alphabetical latter in each group is not significantly different, using revised LSD Test at p = 0.05

induced a higher amount of callus.

Medium supplemented 2 mg BAP was the best in the number of shoot and shoots length in both cultivars and explants (Figure 2). The number of shoots were maximum (3.43) on media having 2 mg/l BAP followed by 1mg/l BAP containing media in both explants tested and the shoots length were 1.28, 0.687cm in medium supplemented with 2 and 1 mg/l, respectively (Figure 2). No regeneration was observed in the control and 4 mg/l BAP supplement. The results agree with Ntui et al. (2009) and Sheeja et al. (2004) who found that the highest shoot induction frequency was obtained on the medium containing 2 mg/l BAP. The results indicated a significant difference between cultivars in shoots number and shoots length, the cultivar pearl was the best in both traits (Figure 3), and agrees with the results reported by Bhatia et al. (2005) and El-Bakry (2002). The responses of explant for shoots regeneration were different. Chaudhry

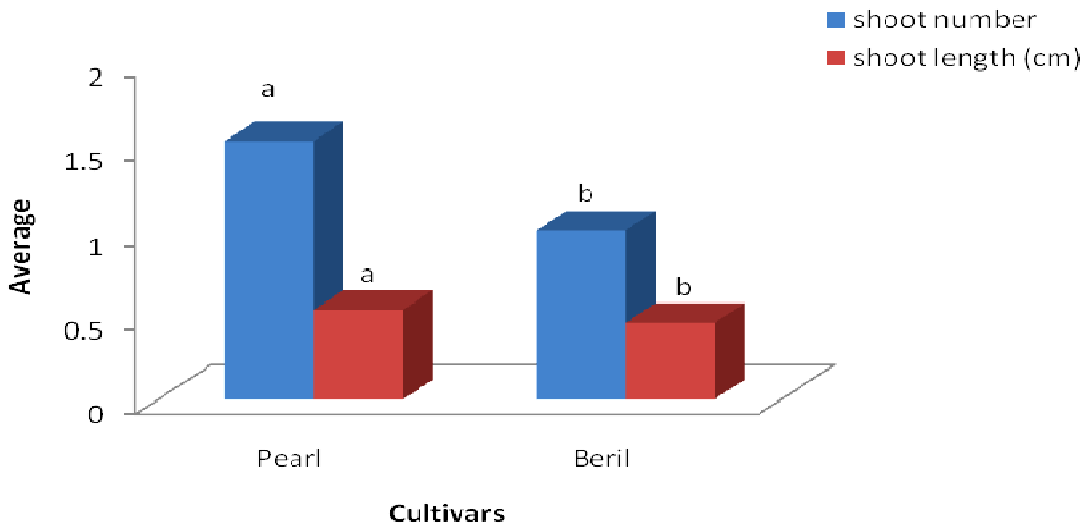

Figure 3. Effect of tomato cultivars on shoots number and shoots length average under different levels BAP. Columns that have same alphabetical letter in each group is not significantly different, using revised LSD test at p = 0.05

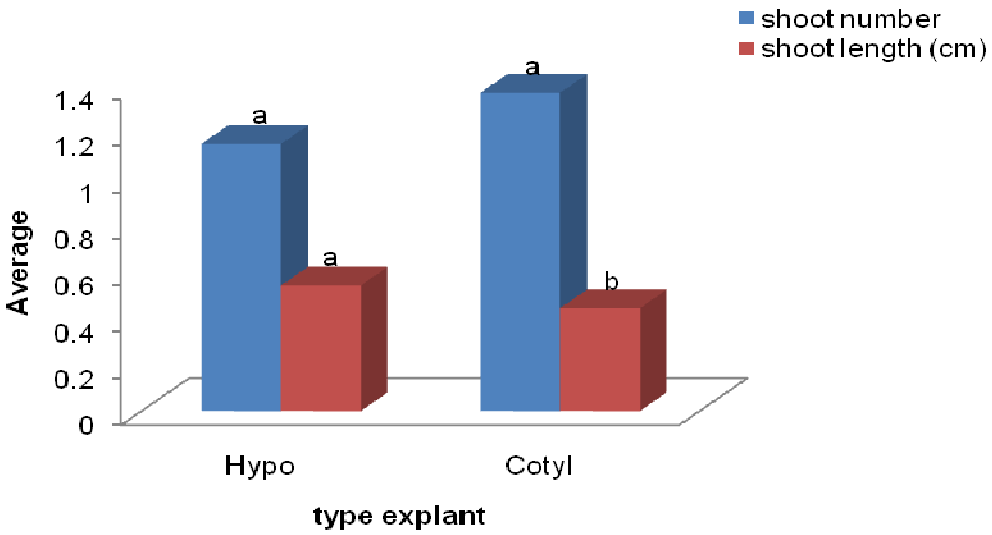

Figure 4. Effect of type explants on shoots number and shoots length average under different levels BAP. Columns that have same alphabetical latter in each group is not significantly different, using revised LSD Test at p = 0.05

et al. (2004), Sheeja et al. (2004) and Gubis et al. (2004) reported that hypocotyls explants were the best in shoots length and had an average number of shootlets. No significant differences were found between hypocotyls and cotyledon explants in shoots number at different concentrations of BAP treated media. In both coefficients of variation (CV), the numbers of shoots were 1.156 and 1.375, respectively (Figure 4).

Significantly, the hypocotyls explants were found to be superior to cotyledon in the shoots length, where the shoots length were 0.54 and 0.44 cm. Tables 2 and 3 indicated the interaction effects between cultivars, explants and BAP levels on shoots number and shoots length. The successive increase of BAP level was associated with increase of shoots number and shoots length in 1 and 2 mg/l BAP, whereas there was no response to shoots formation in 4 mg/l and control treatment. The medium supplemented with 2 mg/l BAP was the most effective in adventitious shoots formation and shoots length in both explants and cultivars. Shoot length was found significantly different between two explants in both CV's. The highest shoot length was obtained in media having 2.0 mg/l BAP

Table 2. Interaction effects of cultivars, explants and BAP concentration on shoots number.

Cultivars	Explants	Number of shoots/explants Mean±SE			
		0	1 mg/l BAP	2 mg/l BAP	4 mg/l BAP
Pearl	Hypocotyls	0	1.75±0.25	4±0.41	0
	Cotyledon	0	2±0.62	4.5±0.645	0
Beril	Hypocotyls	0	1.25±0.25	2.25±0.25	0
	Cotyledon	0	1.5±0.288	3±0.4	0

LSD (P = 0.05) = 0.3873.

Table 3. Interaction effects of cultivars, explants and BAP concentration on shoots length.

Cultivars	Explants	Shoots length (cm/explants, mean±SE)			
		0	1 mg/l BAP	2 mg/l BAP	4 mg/l BAP
Pearl	Hypocotyls	0	0.83±0.06	1.625±0.047	0
	Cotyledon	0	0.625±0.063	1.175±0.063	0
Beril	Hypocotyls	0	0.725±0.085	1.15±0.13	0
	Cotyledon	0	0.575±0.047	1.175±0.118	0

LSD (P = 0.05) = 0.0835.

and hypocotyls explants achieved maximum shoot length (1.625 cm) in pearl and the number of shoots was the highest (4.5) in the cotyledons explants in pearl CV (Table 3).

Conclusion

This study has shown that 2 mgl/ BAP is better for shoots induction in tomato cultivars and the tomato cultivars varied in response to shoots formation and shoots length. Also, higher concentrations of BAP (such as 4 mg/l) encouraged the formation of callus.

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