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Full Length Research Paper

Effects of different fruit juices used as carbon source on cucumber seedling under *in-vitro* cultures

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In this study, the effect of various commercial fruit juices (used as plant carbon source) was assessed on cucumber (*Cucumis sativus* L.) cv. Liza at seedling stage under aseptic conditions. Seeds were germinated on ½MS medium (within 2-days) under dark conditions. They were sub-cultured on MS_0 (MS basal salts) medium and its derivatives [MS, supplemented with fruit juices in place of sucrose (3%) such as, MS_1 (orange), MS_2 (apple), MS_3 (red grapes) and MS_4 (strawberry)] for six weeks. Maximum seedling growth was observed in MS_1 and MS_3 cultures ($p > 0.05$). Significant increase in proline and reducing sugars was measured in MS_2 and MS_4 in comparison to MS_0 (control) culture. Abundance of chlorophyll contents (Chl *a*, *b* and *ab*) and total carotenoids including its precursor (lycopene) was also found in MS_1 medium. Both orange and grapes supplied cultures were most effective in relation to all growth related parameters.

Key words: *Cucumis sativus* L., seedling growth, proline contents, total carotenoids, lycopenes.

INTRODUCTION

Cucumber *(Cucumis sativus* L.) is a tender herbaceous annual vegetable vine crop used for its fresh fruit desserts. In addition to delicious taste, it is fairly abundant with calories and high medicinal values for human beings. This crop can serve as an active drug for secreting and promoting flow of urine as well as useful for both high and low blood pressures due to high contents of potassium (0.5 to 0.8 mg g^{-1}) in fruit (Kadans, 1979). In Pakistan, its total production is 6487 tons (Anon, 2006). According to these figures, production is quite low. Increase in cucumber production like that of any other crop, can be achieved either by bringing more area under cultivation or by adopting improved varieties and better cultural practices (Waseem et al., 2008). Because of narrow genetic basis, cross hybridization is very difficult between its closely related species (Den and Custers, 1990). Aseptic improvement against surface growing pathogens is possible. A reproducible tissue culture system of a plant either develop from seed or regeneration of complete plant that has to be improved for this crop (Gamborg et al., 1968).

Normally, plant growth requires carbohydrate as a source of free energy to run its all growth processes. Its continuous supply is essential because *in vitro* cultures have slow anabolic processes with limited light intensity, gas exchange rates and with high relative humidity. Sucrose is most widely used carbon source in plant tissue culture. It also acts as an osmotic agent to support the growth of cultured tissues (Alkhateeb, 2008)

Fruits are rich source of sugars and vitamins. These components may also be useful for plant tissue growth. Their use in nutrient medium not only enhances growth rate, but also triggers differentiation in cultured tissues (Kinnersley and Henderson, 1988: Siddique and Paswan, 1998; Waseem et al., 2008). Fruit juices also contain some growth inhibitors such as ethylene and ABA that could slow down growth rate but moderate the effects of

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Abbreviations: **MS**, Murashige and Skoog; **B5 Vit,** vitamins B 5; **Chl**, chlorophyll; **F Wt**, fresh weight; **D Wt**, dry weight; **NaOCl**, sodium hypochlride.

Table 1. Plant nutrient medium with composition of different juices used for the study of their effects on seedling growth in cucumber (*C. sativus* L.) cv., Liza (6-weeks culture).

Medium	Medium composition	Juice composition (1.0 g D wt)
MS_0	MS basal medium (control) (Murashige and Skoog (1962)	
½MS_0	Half concentration of MS basal salts (MS_0)	
MS_1	MS + 158 ml orange juice (contain 3% sugar)	0.3% vit A, 1.56% vit C, 1.5% riboflavin, 3.4% niacin, 1.9% Ca, 1.3% Fe, 3.4% vit B6, 1.5% folate, 3.2% P, 5.3% Mg, 3.1% pentathenate
MS_2	MS + 130 ml apple juice (contain 3% sugar)	12% sugars, 7% fructose, 2.7% sucrose, 2% glucose, 0.7% malic acid, 0.5% pectin, 0.5% starch, 0.01% polyphenols, 0.006 proteins, 0.0005% vitamins (mainly ascorbic acid), 0.02% ashes, 0.003% β-carotene.
MS_3	MS + 100 ml red-grapes juice (contain 3% sugar)	25% carbohydrates, 0.17% nitrate, 0.01% protein, 0.11% free amino acids, 0.002% humin, 0.6% Al, 0.007% B, 0.025% Ca, 0.01% Cl, 0.0003% Cu, 0.003% Fe, 0.025% Mg, 0.005% Mn, 0.25% K, 0.05% P, 0.001% Rb, 0.0002% Si, 0.02% Na, 0.003% SO_4.
MS_4	MS + 195 ml strawberry juice (contain 3% sugar)	0.4% protein, 0.15% fat, 12.5% carbohydrate, 0.2% fiber, 10.42% sugar, 1.1% Ca, 0.026% Fe, 1 Mg, 1.2% P, 0.4% Na, 10% K, 0.007% Zn, 0.005% Cu, 2.5% vit C, 0.006% thiamine, 0.003% riboflavin, 0.028% niacin, 0.006% vit B6, 8.78 vit A, 0.004% vit E,0.05% vit K.

growth enhancers (auxins and cytokinins) in *in vitro* culture (Abeles, 1973; Mattoo and Suttle, 1991; Pua and Chi, 1993). Silver nitrate ($AgNO_3$) and cobaltous ions greatly improve plant regeneration efficiency in both moncots and dicots (Lau and Yang, 1976; Giridhar et al., 2003).

Plant development is compromised on various growth stages and seed germination, and its emergence stages are more sensitive. The aim of this study was to characterize the growth promoting activity of various juices on cucumber seedling under *in-vitro* by replacing sugar of nutrient medium.

MATERIALS AND METHODS

Healthy seeds of cucumber (*C. sativus* L.) cv., Liza were selected and washed thoroughly with running tap-water. They were disinfected for surface growing microbes with 30% Robin Bleach® (5 % NaOCl). After 20 min, seeds were rinsed for 3 times with sterilized dH_2O.

Sterilized seeds were soaked in sterilized dH_2O for 1 h, then cultured on ½MS_0 [MS (Murashige and Skoog, 1962) basal salts with B 5 vit complexes and 3% sugar] medium for 2 days in the dark. Germinated seeds were sub-cultured on five different plant nutrient medium and incubated under light conditions (Table 1). Almost 6-weeks old seedlings were removed from medium and cleaned from agar by washing with tap water. Their biomass was measured in order to weigh their fresh weight (F Wt), while their dry weight (D Wt) was measured by drying at 72°C for 3-days.

Chlorophyll contents and total carotenoids were analysed from fresh leaves by grinding in 80% acetone (Arnon, 1949; Nagata and Yamashita, 1992). Carbohydrates were extracted from plant dry materials according to Ciha and Brun (1978) by homogenization in 10 ml sugars extraction buffer (glacial acetic acid: methanol: water, 1:4:5, v/v/v). They were quantified following Dubois et al. (1956) method. Reducing sugars were also determined by following Miller (1959) and total proteins according to Bradford (1976) methods. Nitrate contents were determined by using Morris and Riley (1963) method. Ascorbate (Shalata and Neumann, 2001), proline (Bates et al., 1973) and phenolic (Ozyigit et al., 2007) contents were also determined.

The significance of the collected values from each culture (with 5 replicates) was calculated using COSTAT computer package (CoHort software, Berkeley, USA).

RESULTS AND DISCUSSION

An experiment was performed by culturing germinated seeds (2-days old) of cucumber (*C. saticus* L.) cv., Liza on MS cultures supplemented with various fruit juices (Table 1). Different fruit juices were added in the MS

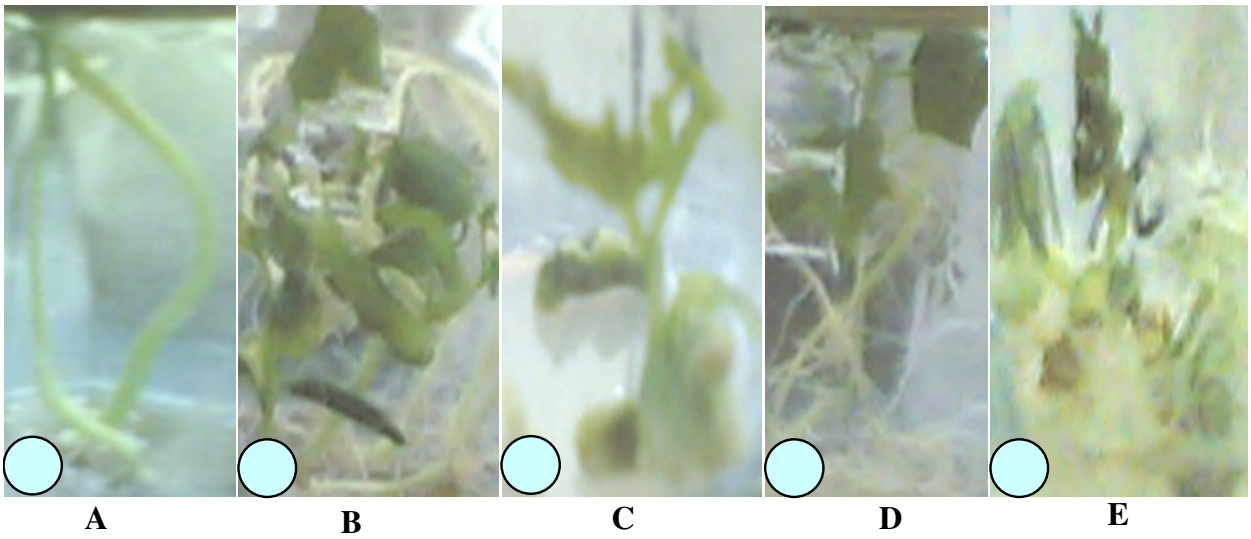

Figure 1. Development of seedlings of cucumber (*C. sativus* L.) cv., Liza on different plant nutrient basal medium [Murashige and Skoog, (1962) basal salts] supplied with different juices in place of carbon source under *in-vitro* condition (6 weeks culture). a: Growing seedling on simple MS0 medium; b: seedling on simple MS0 medium supplied with orange juice in place of 3% sugar; c: seedling on MS0 medium supplied with apple juice instead of 3% sugar; d: seedling growing on MS0 plant nutrient medium supplied with red-grapes juice in place of 3% sugar and e: seedlings growing on MS0 plant nutrient medium supplemented with strawberry juice in place of 3% sugar.

medium just to bring the level of sugar up to 3% (MS without sugar), which is required for plant growth under *in-vitro* conditions. These juices could also be a source of various nutrient substances, while this experiment was focused on sugar contents only. These cultures were maintained in light conditions for 6-weeks. Different responses of growing seedlings were observed from control to juice supplied nutrient cultures (Figures 1 and 2).

Significant change in growth was observed among the juice cultures. The plant biomass of the cucumber seedlings was increased ($p > 0.05$). The most effective plant growth cultures were MS_1 (orange) and MS_3 (grapes). Maximum plant height and root length was observed in MS_1 and MS_3 cultures. These two cultures were considered to enhance the vegetative plant growth of the seedlings significantly. Meanwhile, shoot dry weight was higher in MS_1 and root dry weight in MS_2 (apple), not in MS_3 (Figure 2).

The performance of the morphological characters was not affected adversely because of the application of fruit juices in MS medium than control (MS supplied with 3% sugar) cultures. Supplement of juices enhanced the growth rate of the cultured seedlings in the form of plant height, root length and plant biomass (Figures 1 and 2). Such accelerated growth rate seems to be triggered by the applications of hormones in the cultures (Kelly et al., 1971; Balestri et al., 1998; Reddy et al., 2000; Puchooa and Ramburn, 2004).

Chlorophyll contents were also affected significantly. Chlorophyll *a* (Chl *a*) was higher in MS_1 cultures but quantified almost the same in MS_0 and MS_4 (strawberry) cultures. Similarly, maximum Chl *b* was also observed in MS_1 cultures. Lycopenes were measured higher in MS_1 and MS_2 cultures, while total carotenoids were higher in MS_1 and MS_4 cultures. Biosynthesis of lycopene was substantially lower among the cultures because it is a precursor (acyclic) of carotenoids. Some external stresses on the growing plants cause an increase in total carotenoids (Sandmann, 2001; Marova et al., 2004). Meanwhile, the MS_1 culture represented the best biosynthesis of all plastids but each decreased differentially among other fruit juice supplied cultures.

Minimum plastids were observed in MS_0 and MS_2. It means that both were involved in the inhibition of their biosynthesis. Such inhibitions are the prime roles of aseptic culturing to stop or reduce the differentiation processes of the developing tissues. Total carotenoids are also the sign of growth inhibition due to certain applied stresses but its maximum concentration was observed in orange juice supplied cultures. There seedling growth rate was very efficient than other cultures. The influencing factors of the carotenoids are not clear.

Significant increase in total proteins, sugars and reducing sugar contents was observed in the seedlings cultured on MS_3 medium. Each decreased in MS_0 as well as in MS_2 cultures. Similarly, protein contents were higher in MS_4, while phenolics and nitrates in MS_1. Maximum ascorbate contents were measured in MS_4 cultures. Almost each parameter was performed differently in the presence of various fruit juices in the cultures (Figure 2).

During seedlings growth, accumulation of proline and reducing sugars has also been considered as a sign for abiotic stresses on the growing plants or tissues in

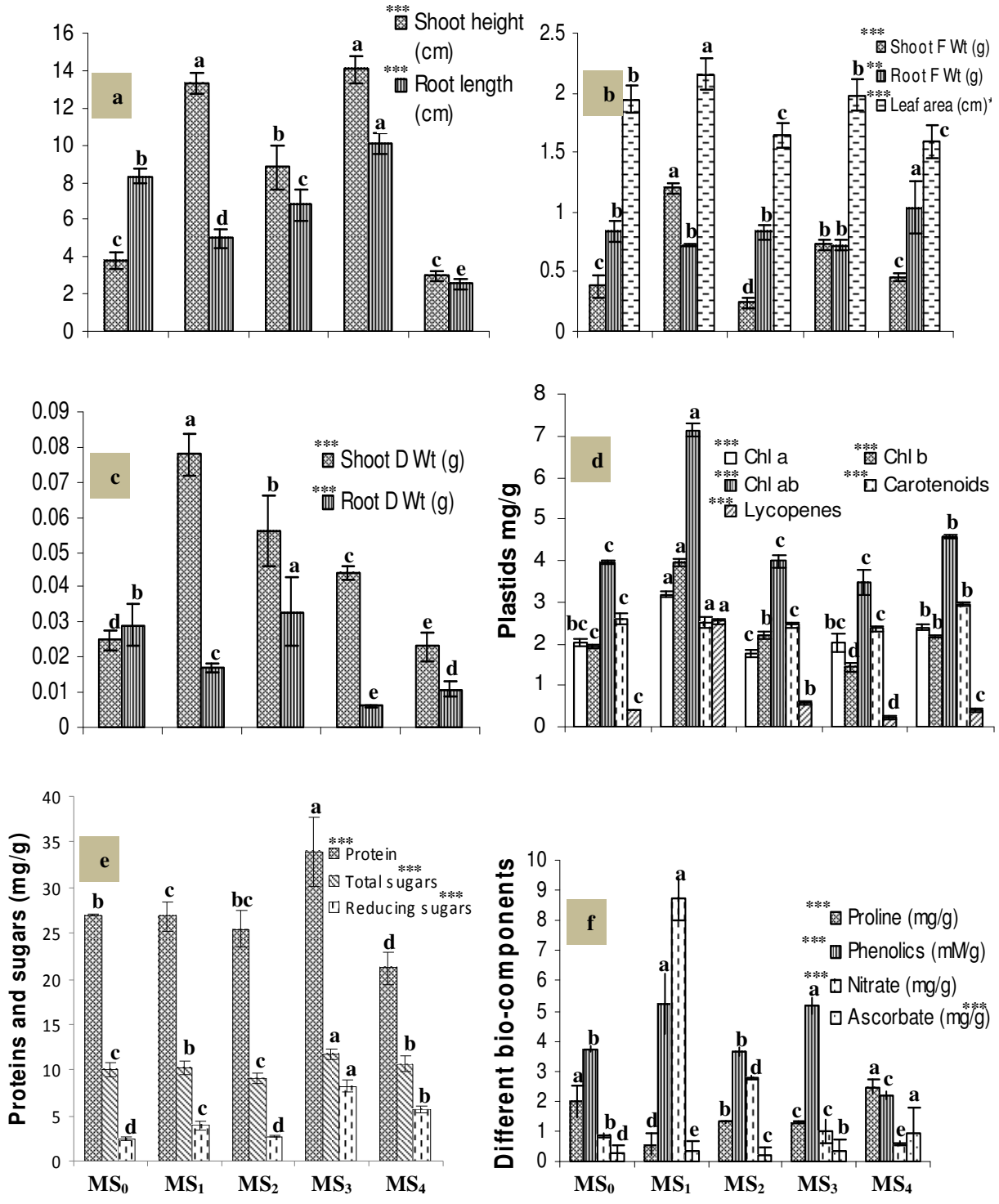

Figure 2. Effect of commercially available fruit juice on plant growth related morphological and bio-chemical parameters of the 6 weeks old seedling culture of cucumber (*C. sativus* L.) cv., Liza under *in-vitro* conditions. Bars with letters shown at different growth parameter are significantly different at $p<0.05$ by using the Duncan's multiple range (DMR) test.

medium. Both of these contents were higher in MS_1 and MS_3 cultures. It means that orange and grape juices were involved in the enhancement of seedling growth andapplication of somewhat stresses.

In this study, the applications of different fruit juices performed a significant role in seedling growth. Each growth character has shown beneficial increment than control cultures. Some cultures supplied with apple and

strawberry juices were not performed as good as orange and grape. This behavior of growth may be because of difference in juice compositions. Some juice substances are growth supportive, while others may not trigger some other mode of growth. Meanwhile, there is more need to perform such experiments for the purpose of identifying any specific mode of growth that is under control of a specific juice component. Orange and grape juices are best nutrient sources for cucumber seedling growth. They may also have the ability to perform the best *in-vitro* plant mass production or in-direct plant regeneration from a single plant cell or its tissue.

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