Evaluating effects of Antioxidant Turmeric and Ascorbic acid On Callus Forage Cactus (opuntia Ficus-indica) In vitro Condition

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Abstract: Micrpropagation is wildly used for multiplication of plants which the conventional methods can not propagate them desirably. However, some problems exist during *in vitro* propagation of these plants. Browning of explants due to phenolic compounds oxidation during micropropagation is considered as one of the barriers to achieve propagation through tissue culture technique. To obviate this problem, the application of two different antioxidants including ascorbic acid and Turmeric, each at concentrations of 0.1, 0.5 and 1% alone and in combination with each other, was studied on callus cultures of *O. ficus-india*. Application of the antioxidants affected the callus size, callus weight and shoot length significantly. The highest callus size was obtained in media containing both Turmeric and ascorbic acid at concentrations of 1% and 0.1%, respectively. While, the maximum callus weight was achieved at media including 1% Turmeric, the highest shoot length was observed in treatment containing ascorbic acid with the concentration of 0.5%.
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Introduction

Cactus forage includes about 130 genera and 1500 species (Roughley and Whiting, 1973). *Opuntia ficus-india* as one of important cactus crops is grown in arid and semiarid parts of the world. *O. ficus-india*, also known as prickly pear, is of CAM plants with water use efficiency (WUE) of 4-10 mMol Co_2 fixed per mol H_2O lost. Comparing to C3 and C4 plant with water use efficiency of 1-1.5 and 2-3 mMol Co_2 fixed per mol H_2O lost, respectively, prickly pear benefits of higher WUE (Fawzy and Thomasand, 2002). The ability of this crop to accumulate noticeable amount of water in tissues, confers it a higher tolerance against drought and better propagation.*O. ficus* is not planted just as forage, though for edible fruits and cladods.

Like other CAM plants, prickly pear has generally a slow growing rate that sometimes inhibits its reproductive capacities. Basically, *O. ficus* is propagated through conventional methods like seed germination or rooting of cladods. However, these common methods accompany main problems like: genetic segregation, long juvenile phase and low propagation rate. Therefore, micropropagation techniques can be used as an alternative method to overcome the difficulties of conventional production methods. Beyond all the advantages that the micropropagation techniques offer, they also might have some minor problems. Browning of explants due to the secretion of phenolic compounds can be named of these problems. Usually, browning of tissues happens following the poly phenol oxidase activity which exists in plastid of plant cells. The enzyme reacts with present phenolic compounds and causes browning (Khalafalla et al, 2007 ; Kiani Feriz et al, 2005).

Browning happens for most of plant explants after 3-5 days passing initial establishment which results in prevention of further explants growth. The browning problem often occurs in plant tissues containing high amounts of tannin or other hydroxyl phenols. Also, decayed agar, inappropriate autoclave, sever acidic or alkaline pH of culture medium can cause browning of explants(Hung, 2002; Simpson Mark and, 1990). Kiani feriz et al., (2005) showed that reduction in nutrition elements of culture medium embrace reduction of browning of *Olea europaea* L. explants. Commonly, excised explants are exposed to antioxidants for decreasing of browning(Ehsanpour And amini, 2001; Pulla Reddy and Lokesh).

To reduce browning during *in vitro* culture, application of different antioxidants like ascorbic acid, citric acid, cysteine, hydrochloride, 1, 4- di tiotritol, glutathione and mercaptoethanol in medium culture has been attempted(Bagheri et al, 2004). In this study, the effect of two different antioxidants

including ascorbic acid and Turmeric to inhibit browning of *in vitro* cultures of *Opuntia ficus-indica* has been examined.

Materials and Methods

Cladods of *Opuntia ficus-indica* were used as explants for callus induction in this study. The explants were surface sterilized and were cultured on MS medium containing BAP (1 mg/l) in combination with NAA (1 mg/l), sucrose (30 g/l) and agar (7 g/l) for callus induction. To evaluate antioxidant effects of Turmeric and ascorbic acid on callus cultures f *O. ficus-indica*, each of these components were added at concentrations of 0.1, 0.5 and 1% to callus induction medium separately and in combination with each other. As ascorbic acid is heat sensitive, it was filter-sterilized using 0.2µm filter and added to the culture medium under aseptic conditions. Each treatment had 4 replications and each replication contained 4 explants. The pH of all treatments was adjusted to 5.7.

The cultures were maintained at 25±1˚c under 16 h light conditions following 8h dark in a growth chamber. After one month, the callus size, callus weight and length of emerged shoots were measured. The experiment was analyzed based on factorial completely randomized design using MSTAT-C software. The means were separated by the Duncan's multiple range tests at P≤5%.

Results and Discussion

According to analysis of data, Turmeric and ascorbic acid had a significant effect on callus size, callus weight and length of emerged shoots (Table1).

Table1: Analysis of Variance for traits studied in vitro

S.O.V	df	Mean of Square		
		Callus size	Callus weight	Shoot length
ascorbic acid	2	**0.071	*4.707	**0.604
Turmeric	2	**0.555	**9.515	*0.101
Turmeric * ascorbic acid	4	**0.167	^{ns} 1.531	^{ns} 0.242
Error	27	0.005	1.403	0.121

*and ** Significantly at p < 0.05 and < 0.01, respectively; ns = non-significant.

The interaction effect of Turmeric and ascorbic acid was also significant on callus weight. The highest callus size of 1.115 mm was obtained from treatments containing both Turmeric and ascorbic acid at concentrations of 1% and 0.1%, respectively (Table2).

Table2: The interaction effect of Turmeric and ascorbic acid on callus size

Turmeric	Ascorbic acid		
	Concentrations of 0.1%	Concentrations of 0.5%	Concentrations of 1%
concentrations of 0.1%	0.405 d	0.632 c	0.65 c
concentrations of 0.5%	0.345 d	0.615 c	0.6475 c
concentrations of 1%	1.115 a	0.660 c	0.9875 b

Similar letters in each column indicate non significant difference at 5% probability level

However, media including either Turmeric or ascorbic acid at concentrations of 1% caused the maximum callus weight of 1.115 and 0.66 mg, respectively (Table3).

Table3: The interaction effect of Turmeric and ascorbic acid on callus weight

	Ascorbic acid	Turmeric
concentrations of 0/1%	0.405 b	0.6325 b
concentrations of 0/5%	0.345 c	0.615 b
concentrations of 1%	1.115 a	0.66 a

For shoot development, comparing the effect of Turmeric and ascorbic acid showed that ascorbic acid could induce shoots with higher length. According to data shown in table 4, media containing ascorbic acid with the concentration of 0.5% induced the highest shoot length (1.698 cm) and media with 0.1% ascorbic acid caused the lowest shoot length (0.405 cm). However, the highest shoot length achieved in media containing 1% Turmeric was 1.116 cm.

Table4: The interaction effect of Turmeric and ascorbic acid on shoot length

Turmeric	Ascorbic acid		
	Concentrations of 0/1%	Concentrations of 0/5%	Concentrations of 1%
concentrations of 0/1%	0.7803 b	1.603	0.8261
concentrations of 0/5%	1.698 a	0.9163 abc	0.7071
concentrations of 1%	1.117 abc	1.425	0.7075

Numbers with the same letters in each column indicate non significant difference at 5% probability level.

Antioxidants like Turmeric and ascorbic acid are applied in the *in vitro* cultures due to their capacity to reduce phenolic compounds in the medium (Elmore et al, 1990). Ascorbic acid as one of the important metabolites along with other antioxidants in plants protects the plant against adverse conditions. Also, ascorbate plays a role as cofactor to activate some kind of enzymes like hydrolyses and Violaxanthin de-epoxidase. (Vahdatpour et al, 2009;Elmore et al, 2004). Another type of antioxidant that affects the phenol production of *in vitro* plants is Turmeric. The antioxidant characteristic of Turmeric has ascribed to curcumin. Krishna and Riavo (1995) reported that cucurmin and its derivates keep the antioxidant activities of enzymes like superoxide dismutase, catalyze, glutathione peroxidase, high so that the lipid peroxidation will decrease and subsequently the production of free radicals is controlled (Govindarajan, 1980; Tian-ming et al, 2004). Elmore *et al*., (2004) used Turmeric and ascorbic acid in plant cell and tissue cultures to control production of phenolic compounds. They observed that applying Turmeric at concentration of 0.1% in culture medium caused the highest callus growth. They also showed that the highest callus weight was produced while using Turmeric at concentration of 1%. The result of present study is in accordance with Elmore's findings. Vahdatpour et al. (2008) also utilized Turmeric as an antioxidant in the callus culture media of *Ulmas pavrifolia* Jasc to reduce browning. They compared the effect of Turmeric, ascorbic acid and active charcoal on callus weight and found that both Turmeric and ascorbic acid at the concentration of 0.1% and 1%, respectively, induced the highest callus weight Vahdatpour et al, 2009). Hung et al., (2002) also reported similar results.

Findings in the current research indicate that using Turmeric as an antioxidant in culture media of *Opuntia ficus-indica* at appropriate concentration can play a significant role in rectifying the problem of browning.

Figure 1 - Comparison between treatments was performed after 4 weeks.
a) callus in non-treated(control) b) callus on medium containing 5% of ascorbic acid c) callus in medium containing Turmeric1%.

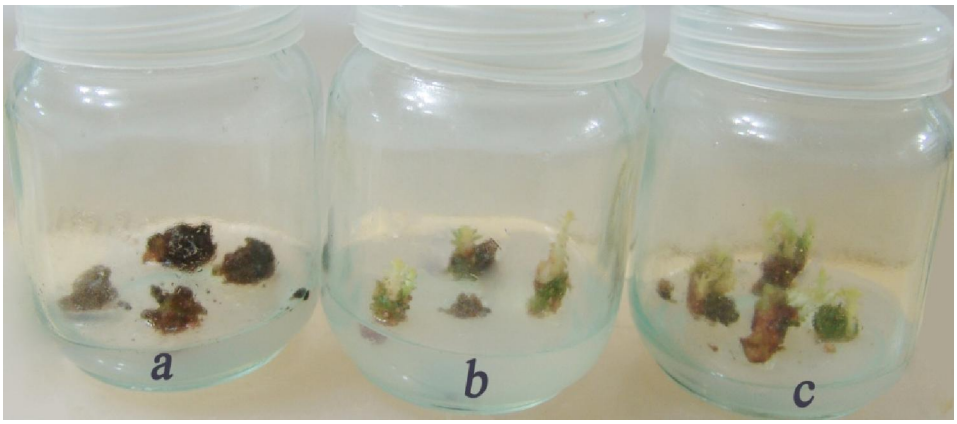

Figure 2 - Comparison of the growth and propagation of forage cactus
a) control b) ascorbic acid concentrations of 0.5% c) the concentration of Turmeric 1%

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