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ESTABLISHMENT OF THE HONEY CROP (*STEVIA REBAUDIANA*) IN HOT SEMI ARID CLIMATE

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ABSTRACT

Stevia rebaudiana is native plant of tropical climate of Brazil and Paraguay. Its leaves produce a sweetener- stevioside which is 150-300 times sweeter than cane sugar. An attempt is made to grow this plant in hot semi arid climate of Lahore region of Pakistan through tissue culture technique. A completely randomized design was used for the experiment with three replicates. The data was analyzed by applying one way ANOVA and the treatments' means were compared for significance by Duncan's New Multiple Range (DMR) test at 0.05% P. MS media supplemented with different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l), kinetin (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and combination of BAP and kinetin were used and BAP 1 mg/l proved to be the better micropropagating medium for *Stevia rebaudiana* as compared to other media. Whilst rooting of micro shoots of the Stevia gave the best response in MS+ IBA 0.4 mg/l. then these mericlones were hardened in green house in different soil mixtures among which the best mixture was sand, clay and vermicompost in the ratio of 1:1:1 with 85% survival rate. These semi hardened plants were then shifted to larger pots and open field of hot semi arid climate of Lahore and developed successfully.

Key words: Stevia, BAP, honey crop, sweetener, stevioside.

INTRODUCTION

With increasing awareness of the common man about obesity, diabetes and heart diseases, people are becoming more and more conscious about the use of sucrose in their daily meals. As a result, demand of non caloric sweetener is increasing day by day (Pinhiero and Oliviera, 2005).

Non caloric sweetener are of two types i.e., natural sweeteners and artificial sweeteners. However disadvantages overweigh the advantages of these sweeteners as artificial sweeteners like aspartame can cause cancers. Similarly patients of phenylketonuria cannot use aspartame in their diet due to the formation of phenylalanine during its metabolism (Butchko *et al*., 2001). Saccharin is considered to be associated with bladder cancer (Pearson, 2001). Cyclamate has a major metabolite, cyclohexylamine, which causes testicular atrophy and at high doses, it has unwanted cardiovascular effects (Bopp and Price, 2001). In such circumstances *Stevia rebaudiana* may be considered as the best alternative of sugar cane as it produces stevioside which is 150-300 times sweeter than sucrose and non caloric in nature without any harmful effects (Dacome *et al.,* 2005).

Stevioside can be used in baking at temperatures where other artificial sweeteners show browning. It is stable on wide range of temperatures and pH (Brandle and Rosa, 1992). It has longer shelf life as it acts against food deteriorating bacteria e.g., E. coli. It also shows anticancer, anti-hyperglycemic and anti-hypertension activity etc (Jeppeson *et al*., 2000; Kedik *et al*., 2003).

Stevia rebaudiana is a plant native to the tropical climate of Brazil and Paraguay where average annual rainfall is 1500-1800 mm and that of temperature tolerance range is -6 to 43˚C. But it also shows better results in fertile fields when grown as a crop (Oddone, 1999).

Present work was carried out to establish this important crop in Pakistan. As this plant, with very poor seed germination rate, is not native to Pakistan, so tissue culture technique was used to establish the crop here in hot semi-arid climate of Lahore, Pakistan, where annual rain fall is less than 500 mm and average temperature is greater than 20˚C (Khan and Jamil, 2010).

MATERIALS AND METHODS

Shoot tips of *Stevia rebaudiana* plants were taken from the green house of Lahore College for Women University, Lahore, Pakistan, to be used as explants for the tissue culture purposes. These explants were first washed with the running tap water and treated with household detergent to remove the dust particulates. Then explants were washed with tap water to remove the detergent. The explants were then treated with 20% sodium hypochlorite solution for 15 minutes. In next step, explants were washed with autoclaved water to remove the traces of the sodium hypochlorite. After completing the sterilization process, the explants were inoculated in different MS media formulations for micropropagation. At the end, plants were shifted to different mixtures of soil, sand and manures, to check the percentage survival

rate. Fully grown plants were then shifted to the field. The experiment was planned under completely randomized design (CRD) with six treatments with five replication.

Explants of shoot apical meristem were inoculated in solid MS media (Murashige and Skoog, 1962) supplemented with cytokinins i.e., BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l), Kin (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and combinations of BAP 1 with Kin 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. The pH of media was set at 5.4-5.7 for all experiments and then media containers were solidified with phytagel at concentration of 1.5 g/l. Each time when medium was formulated, it was sterilized by autoclaving at 121°C and 15 lbs/Inch^2 pressure for 20 minutes. Cultures of *Stevia rebaudiana* were maintained in culture room under controlled conditions with fluorescent light having 2200 lux light intensity, temperature range of 22°C ± 2 with 16 hour light and 8 hour dark period in every 24 hour cycle. The data were recorded for 1) days for multiple shoot formation, 2) number of shoots per explant, 3) shoot length and 4) number of nodes per cm of the shoot length of the plant.

Then these *in vitro* grown micro shoots were transferred to the rooting medium (MS+ different concentrations of Auxins, NAA, IBA and IAA in concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l. In the next step, rooted *in vitro* plants or mericlones were transferred into pots containing different soil combinations for hardening. Potted plants were brought out from green house into open sun light after four weeks of hardening and finally seventy plants were shifted into the field for further growth and ten out of them were used to record the growth parameters.

The data thus generated were analyzed through one way analysis of variance (ANOVA) and the treatments' means were compared for significance by Duncan's New Multiple Range (DMR) test at 0.05% P using SPSS computer software. (Duncan, 1955)

RESULTS AND DISCUSSION

Shoot tips of *Stevia rebaudiana* were inoculated on different concentrations of cytokinins (i.e., BAP, Kinetin and their combinations) for the purpose of micropropagation. Table 1 depicts that the best results were obtained in case of MS+ BAP 1mg/l on the basis of days to shoot initiation (7.92±0.03) shoot length (8.48±0.17 cm) and number of nodes (6.74±0.32). Number of nodes is an important parameter studied to evaluate the efficiency of micropropagation method for *Stevia rebaudiana* as it determines the number of leaves per cm of the plant length. In turn leaves are the factory site for stevioside synthesis (Megejii *et al*., 2003). Maximum number of shoots was obtained from MS+ BAP 3mg/l. As MS+ BAP 1mg/l gave the best results, it was used with different concentrations of Kinetin (Table 2), in which somehow better results were shown by MS+ BAP 1mg/l +kin 1mg/l with least number of days to show initiation of multiple shoots from the explant (14.0±0.13), MS+ BAP 1mg/l+ Kin 2 mg/l with maximum number of shoots per explant (22.4±1.00) and MS+ BAP 1mg/l+ Kin 0.5 mg/l with maximum shoot length per explant (6.54±0.13 cm) and maximum number of nodes per cm of the plant (5.52±0.05). But as a whole, result was not better than MS+ BAP 1mg/l.

Hwang, (2006) used combination of IAA 2mg/l and Kin 0.5mg/l for optimizing micropropagation of *Stevia rebaudiana*. Ibrahim *et al*., (2008) obtained maximum number of shoots in MS+ BAP 2mg/l. They showed that kinetin gave best results at concentrations of 10mg/l but in present study kinetin showed response at quite lower concentration i.e., 2 mg/l. Similarly Debnath, (2008) concluded that *Stevia rebaudiana* explants gave maximum number of shoots with MS+ BAP 2 mg/l+ IAA 1.13 mg/l. Hossain *et al*., (2008) also concluded that MS+ BAP 1 mg/l as the best medium for the micropropagation of *Stevia rebaudiana* on the basis of days to shoot initiation, shoot length, number of multiple shoots and number of microcuttings per culture. Sairkar *et al.,* (2009), found better micropropagation method of *Stevia rebaudiana* with MS+ BAP 3.5 mg/l.

Cytokinins in fact determine the regeneration response in the explants (Schmulling, 2004) and enhance the number of meristematic cells as well as the cell division rate. Cytokinins are also involved in the function of certain enzymes, so having a vital role in the metabolism of plants (Ali *et al*., 2007). So Cytokinins promote plant growth by inducing active cell growth. These cytokinins bind to the high affinity binding proteins (Histidine kinases) which reside in the plasma membrane and thus form a signal transduction cascade which convey signal to the nucleus by two pathways (Schmulling, 2004). One of which is through histidine aspartame phospho/dephosphorylation or by guanine nucleotide binding and subsequent hydrolysis, thus causing transcription of specific genes which are involved in the synthesis of specific proteins (Cyclins) which are responsible for G1/S transition in cell cycle (Ding *et al*., 2009) .

Microshoots developed from the experiment were inoculated in the different rooting media containing different concentrations of NAA, IBA and IAA. Table 2 depicts the results of rooting experiment that explant took least days (6.14±0.27) to initiate roots in MS+ IBA 0.4 mg/l. This medium also produced maximum number of roots per explant (4.54±0.14) with maximum root length (10.9±0.24 cm).

Different scientists, who worked on rooting of *Stevia* plants, used different concentrations of Auxins, like Rafiq *et al.,* (2007) used different concentrations of NAA and IBA but concluded that MS+ NAA 0.5 mg/l to be the right medium for rooting of the plant . But Ahmed

et al., (2007) reported that IAA at concentration of 0.1 mg gave maximum number of roots per explant. Hossain, (2008) reported that NAA 1.5 mg/l with full strength MS was better for rooting.

Auxin control of multiple activities is due to its control over cell division, cell elongation and cell differentiation (Davies, 2004). Auxins are taken up by the cells through pH trapping or by influx carrier proteins (Delberre *et al*., 1998). Auxin signal is perceived right in the heart of the nucleus (George *et al*., 2006). Auxins control the transcription of some genes in the nucleus which in turn control the cell wall elongation (Dharmasiri *et al*., 2005).

Table 3 shows the hardening experiment of tissue cultured grown *Stevia rebaudiana* mericlones in green house level. All the plants which survived were successfully transferred to the large pots and field on canal bank near Multan Road, Lahore, Pakistan. Experimental data showed that a mixture of sand clay and vermicompost in ratio of 1:1:1 proved to be better with 85% survival rate and afterwards giving better shoot length values after two and four weeks (5.20±0.05 cm) and (15.40±0.24 cm) respectively.

Sairkar *et al.,* (2009), Patel and Shah, (2009), Ahmed *et al.,* (2007), Rafiq *et al.,*(2007), Macchia *et al*., (2003) reported only 78%, 63%, 70%, 83%, 80% survival rate for *Stevia rebaudiana* plantlets respectively. Hwang, (2006) was successful to get 98.6% survival rate of *Stevia* plants in Korean climate in soil.

Addition of compost in mixture improves the drainage while maintaining the water holding capacity of the soil and interrupts the aggregation of the clay particles so that the soil has a more granular structure. It helps maintain the soil's porosity so that air and water can move freely through the soil and makes the sticky soil more friable or workable. It reduces the bulk density of the soil helping it resist compaction and also helps the roots to penetrate in the soil.

The results obtained in the present study could be highly significant. This efficient and reliable plant regeneration system via micropropagation system can be exploited for improvement in yield and productivity through genetic transformation and other cellular techniques. The establishment of mericlones in semi arid environment also is a step forward for establishment of this exotic plant in Pakistan.

Table 1: Effect of different cytokinins on shooting of *Stevia rebaudiana*

Hormone	Treatment (mg/l)	Days to shoot initiation	Number of shoots per explant	Shoot length (cm)	Number of nodes per branch
MS+BAP	0.5	10.4^{e}±0.46	7.92^{e}±0.51	4.16^{de}±0.09	2.34^{ef}±0.19
	1.0	7.92^{f}±0.03	15.0^{d}±0.30	8.48^{a}±0.17	6.74^{a}±0.32
	1,5	10.1^{e}±0.21	21.2^{c}±0.66	6.30^{c}±0.25	4.50^{b}±0.15
	2.0	10.0^{e}±0.06	26.2^{b}±0.33	6.00^{c}±0.21	3.54^{cd}±0.16
	2.5	12.6^{d}±0.36	27.4^{b}±0.67	4.24^{de}±0.11	3.04^{de}±0.07
	3.0	14.8^{c}±0.47	35.4^{a}±0.61	3.00^{e}±0.17	1.88^{fg}±0.13
MS+Kinetin	0.5	16.9^{a}±0.29	1.14^{h}±0.07	1.16^{g}±0.061	1.30^{g}±0.10
	1.0	14.8^{c}±0.33	3.68^{g}±0.27	2.10^{g}±0.08	1.54^{g}±0.05
	1,5	13.4^{cd}±0.61	3.36^{g}±0.17	4.58^{d}±0.32	2.26^{efg}±0.13
	2.0	9.16^{e}±0.19	4.18^{g}±0.09	7.06^{b}±0.11	4.08^{bc}±0.09
	2.5	12.6^{d}±0.78	6.34^{f}±0.14	2.28^{f}±0.09	3.62^{bc}±0.16
	3.0	15.7^{ab}±0.17	6.68^{ef}±0.61	1.02^{h}±0.09	2.40^{ef}±0.14

No. of test tubes cultured = 10, each value is mean of five replicate with standard error (mean ± S. E). Means within a column not sharing a common superscript differ significantly (P<0.05) according to Duncan's multiple range test.

Table 2: Effect of different combinations of cytokinins on shooting of *Stevia rebaudiana*

Hormone	treatment (mg/l)	Days to shoot initiation	Number of shoots per explant	Shoot length Cm	Number of nodes per branch
	1.0+0.5	17.8^{bc}±0.18	10.8^{cd}±0.95	6.54^{a}±0.13	5.52^{a}±0.05
	1.0+1.0	14.0^{e}±0.13	12.1^{c}±0.63	6.24^{a}±0.12	4.26^{b}±0.73
MS+	**1.0+1.5**	15.6^{d}±0.36	18.2^{b}±0.33	4.48^{b}±0.14	3.48^{c}±0.26
BAP + Kin	**1.0+2.0**	16.8^{cd}±0.20	22.4^{a}±1.00	3.30^{c}±0.04	2.84^{cd}±0.19
	1.0+2.5	18.7^{b}±0.40	15.8^{b}±1.18	3.40^{c}±0.09	2.46^{d}±0.21
	1.0+3.0	20.6^{a}±0.73	8.40^{d}±1.43	2.36^{d}±0.07	0.98^{e}±0.25

No. of test tubes cultured = 10, each value is mean of five replicate with standard error (mean ± S. E). Means within a column not sharing a common superscript differ significantly (P<0.05) according to Duncan's multiple range test

Table 3: Effect of different auxins on rooting of *Stevia rebaudiana*

Hormone	Treatment (mg/l)	Days to root initiation	Number of roots per explants	Root length Cm
MS+NAA	**0.2**	15.5^{de}±0.16	2.14^{e}±0.06	1.10^{i}±0.29
	0.4	14.4^{e}±0.45	2.64^{cd}±0.16	6.20^{d}±0.64
	0.6	14.0^{e}±0.28	2.94^{cd}±0.04	9.10^{b}±0.36
	0.8	13.8^{e}±0.41	3.24^{bc}±0.04	10.5^{a}±0.42
	1	17.5^{c}±0.32	3.72^{b}±0.05	6.38^{d}±0.20
MS+IBA	**0.2**	15.2^{de}±0.05	3.36^{b}±0.13	8.72^{b}±0.23
	0.4	6.14^{g}±0.27	4.54^{a}±0.14	10.9^{a}±0.24
	0.6	10.9^{f}±0.84	4.36^{a}±0.15	9.18^{b}±0.09
	0.8	7.72^{g}±0.20	3.90^{ab}±0.05	8.26^{bc}±0.11
	1	9.38^{f}±0.60	4.12^{ab}±0.09	7.24^{c}±0.18
MS+IAA	**0.2**	23.2^{a}±0.67	1.94^{e}±0.05	2.06^{hi}±0.06
	0.4	21.4^{b}±1.09	2.18^{e}±0.10	2.56^{gh}±0.18
	0.6	18.0^{c}±0.49	2.32^{de}±0.18	3.14^{g}±0.08
	0.8	16.7^{cd}±0.32	3.24^{bc}±0.17	4.16^{f}±0.07
	1	20.0^{b}±0.48	3.08^{d}±0.24	4.34^{e}±0.22

No. of test tubes cultured = 10, each value is mean of five replicate with standard error (mean ± S. E). Means within a column not sharing a common superscript differ significantly (P<0.05) according to Duncan's multiple range test

Table 4: Effect of different combinations of soil and manure on hardening of *Stevia rebaudiana*

Sr. #	Treatments				percentage	Shoot length (cm)	
Mixtures	sand	clay	vermi-compost	Leaf manure	survival	After 4 weeks	After 8 weeks
1	1	0	0	0	30.67%	6.03^{f}±0.03	06.20^{j}±0.05
2	0	1	0	0	30.11%	7.07^{e}±0.05	07.40^{i}±0.05
3	0	0	1	0	49.93%	8.30^{cd}±0.12	13.20^{f}±0.12
4	0	0	0	1	44.67%	8.00^{d}±0.04	14.30^{e}±0.07
5	1	1	0	0	50.17%	8.17^{d}±0.07	08.87^{h}±0.06
6	1	0	1	0	53.00%	8.43^{bcd}±0.05	14.20^{e}±0.12
7	1	0	0	1	60.00%	9.00^{abc}±0.05	15.80^{d}±0.13
8	0	1	1	0	76.10%	9.00^{abc}±0.07	19.57^{b}±0.24
9	0	0	1	1	34.97%	6.43^{ef}±0.24	11.03^{g}±0.03
10	1	1	1	0	85.00%	9.80^{a}±0.05	23.40^{a}±0.24
11	0	1	1	1	63.80%	9.20^{ab}±0.05	15.96^{d}±0.07
12	1	1	1	1	80.00%	8.37^{bcd}±0.11	18.20^{c}±0.12

No. of test tubes cultured = 10, each value is mean of five replicate with standard error (mean ± S. E). Means within a column not sharing a common superscript differ significantly (P<0.05) according to Duncan's multiple range test

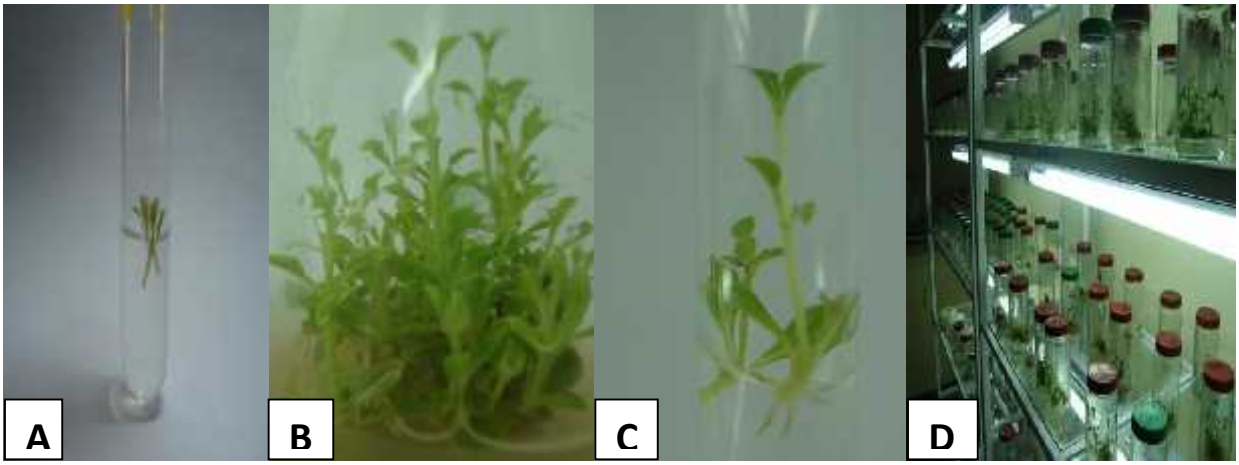

Fig A to H: Illustrating micropropagation and hardening stages of *Stevia rebaudiana*

A) Explant
B) Vigorous growth in MS+ BAP 1 mg/l
C) Rooting in MS+ IBA 0.4 mg/l
D) Rich cultures of *Stevia rebaudiana* in culture room
E) Hardening stage
F) Hardened plants in larger pots
G) Field grown Stevia Plants

REFERENCES

Ahmed, M. B., M. Salahin, R. Karim, M. A. Razvy, M. M. Hannan, R. Sultana, M. Hossain, and R. Islam (2007). An efficient method for *In Vitro* clonal propagation of newly introduced sweetener from plant *Stevia rebaudiana* Bertoni in Bangladesh. Amr. Euras. J. Scientific Res. 2(2): 121-125.

Ali, A., M. Asifa and N. Shagufta. (2007). An *in vitro* study on micropropagation of *Caladium bicolor*. Int. J. Agri. Biol., 9(5): 731-735.

Bopp, B. A. and P. Price. Cyclamate. (2001). In O'Brien Nabors, L. (Ed.). Alternative Sweeteners: Third Edition, Revised and Expanded. Marcel Dekker, New York, pp. 63-85.

Brandle, J. E. and N. Rosa (1992). Heritability for yield, leaf: stem ratio and Stevioside content estimated from a landrace cultivar of *Stevia rebaudiana*. Canadian J. Plant Sci. 72: 1263-1266.

Butchko, H. H., W. W. Stargel, C. P. Comer, D. A. Mayhew and S. E. Andress. Aspartame. (2001) In O'Brien Nabors, L. (Ed.). Alternative Sweeteners: Third Edition, Revised and Expanded Marcel Dekker, New York, pp. 41-61.

Dacome, A. S., C. D. S. Cleuza, E. M. D. Cecila, D. F. Jose, A. Juliana and C. D. C. Silvio (2005). Sweet diterpenic glycosides balance of a new cultivar of *Stevia rebaudiana* (Bert.) Bertoni: isolation and quantitative distribution by chromatographic, spectroscopic and electrophoretic methods. Process Biochem. 40: 3587-3594.

Davies, P. J. (2004). "The plant hormones: their nature, occurrence, and function," in Plant Hormones: Biosynthesis, Signal Transduction, Action, ed. P. J. Davies (Dordrecht: Kluwer Academic Publishers), 1–15

Debnath, M. (2008) Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. J. Medicinal Plant Res. 2(2): 45-51.

Delbarre, A., Muller, P. and Guern, J. 1998. Short-lived and phosphorylated proteins contribute to carrier-mediated efflux, but not to influx, of auxin in suspension-cultured tobacco cells. Plant Physiol. 116: 833–844.

Dharmasiri, N., Dharmasiri, S., and Estelle, M. (2005). The F-box protein TIR1 is an auxin receptor. Nature 435, 441–445.

Ding, J., N. Beifang, G. Wenfeng, W. Wen, W. Kun, L. Junqing, H. Guoping, H. Shanna, S. Wen, H. Tao, H. Lei, C. Guangwen, W. Mengchao, X. Weifen and W. Hongyang (2009). Cyclin D1 induction by Benzo[a]pyrene-7,8-diol-9,10-epoxide, via PI-3K/Akt/MAPKs and p70s6k-dependent pathway, promotes cell transformation and tumorigenesis. J. Biol. Chem., 284: 33311-33319.

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics 11:1–42.

Hossain, M. A., A. H. M. Shamim, T. A. J. Kabir and M. N. Hasan (2008). Micropropagation of *Stevia*. Inter. J. Sustainable Crop Prod. 3(4): 1-9.

Hwang, S. J. (2006). Rapid *In vitro* micropropagation and enhanced stevioside accumulation in *Stevia rebaudiana* Bert. J. Plant Biol. 49: 267-270.

Ibrahim, A. I., I. N. Mahmoud, R. M. Berlant and M. E. Mohammed (2008). Plant growth regulators affecting *In Vitro* cultivation of *Stevia rebaudiana*. Sug. Tech. 10(3): 254-259.

Jeppesen, P. B., S. Gregessen, C. R. Poulsen and K. Hermansen (2000). Stevioside acts directly on pancreatic β-cells to secrete insulin: Actions independent of cyclic adenosine. Metabolism 49(2): 208-214.

Kedik, S. A., S. V. Fedorov, N. A. Yanul, L. V. Prokhorova, E. V. Smirnova, and A. V. Panov (2003). Chromatographic determination of Stevioside in Raw Plant Material. *Pharmaceutical Chem. J.* 37(10), 19-22.

Khan, I. and M. Jamil (2010). Sugar crops and sugar policy of Pakistan. Sci. Vision 9(1-2), 1-8.

Macchia, M., S. Morini, G. Fiaschi and L. Andolfi (2003). *In Vitro* Propagation of *Stevia rebaudiana* Bertoni: Results with Different Genotypes. Agricoltura Mediterraneae 133(2): 117-123.

Megeji, N. W., J. K. Kumar, V. Singh, V. K. Kaul and P. S. Ahuja (2003). Introducing *Stevia rebaudiana,* A Natural Zero Caloric Sweetener. Curr. Sci. 88(5): 801-804.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Oddone, B. (1999). How to grow Stevia, Guarani Botanicals, Inc.: Pawcatuck, Connecticut. p. 1-30

Patel, R. M. and R. R. Shah (2009). Regeneration of Stevia plant through callus cultures. Ind. J. of pharm. Sci. 71: 46-50.

Pearson, R. L. (2001). Saccharin. In O'Brien Nabors, L. (Ed.). Alternative Sweetener s: Third Edition, Revised and Expanded. Marcel Dekker, New York, pp. 147-165.

Pinheiro, M. V. S., M. V. Oliveria (2005). The effect of different sweeteners in low calorie yogurts- a review. Int. Dairy Tech. 58(4): 193-199.

Rafiq, M., U. D. Muhammad, M. M. Sher, A. N. Habib and A. Q. Iqbal (2007). *In Vitro* Clonal Propagation and Biochemical Analysis of Field Established *Stevia rebaudiana* Bertoni. Pakistan J. Bot. 39(7): 2467-2474.

Sairkar, P., N. P. S. Chandravanshi and N. N. Mehrotra (2009). Mass Production of an Economically Important Plant *Stevia rebaudiana* using *In Vitro* Propagation Technique. J. Med. Res. 3(4): 266-270.

Schmulling, T. (2004). Cytokinin. In Encyclopedia of Biological Chemistry, 1-7.