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Comparison of Morphological and Physiological Traits of Chili Pepper Capsicum Annuum l. Plants Grown from Seeds and Somaclons drom Salt Stress Medium

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Abstract

Somaclonal variations are a useful source of plants with desired traits. The current experiment was conducted to assess the effect of tissue culture under salt stress on the induction of morphological, physiological and biochemical changes in the regenerated chili pepper plants in comparison to seeds derived plants. Seed derived plants and plants regenerated from salt stress calli were grown in the greenhouse until maturity. Morphological analysis of the plants showed that tissue culture derived plants had useful variations. Tissue culture plants were significantly higher in the branches number and stem diameter which reached (150.1% and 63.4 *%) respectively. Also they surpassed the seed derived plants in the yield parameters which included: number of fruits, total fresh and dry fruit yield with increments of 34.6%, 13.9% and 17.8% respectively. Moreover, HPLC analysis of some biogenic compounds showed that the fruits of the tissue culture plants were rich in Ascorbic acid in an increment of 44.8% compared with the seed derived plants. Although there were no significant difference between the two plant groups in the capsaicin content, the amount of capsaicin in the total dry fruits of the tissue culture plants were higher than that of the seed grown plants which were (58.226 and 50.213 mg /dry fruits of one plant) respectively in an increment of 15.95%. Analysis of the Pungency showed that the plants of both groups are considered of high Pungency degree.*

Keywords: *Ascorbic acid, Capsaicin, fruits, salt stress, HPLC, Somaclonal variations*

1. Introduction

The developments of biotechnology and tissue culture have been used in the breeding programs to improve many plant species. Plant regeneration and the selection under biotic and abiotic stress, micropropagations and secondary metabolisms production are some of the applications of the technology (D.C.W. Brown and T.A.Thorpe, 1995; R. Ramachandra and G.A. Ravishankar 2002). Although the regenerated plants are clones and they should be identical, many researchers reported variations in micropropagated plants (A. B. Nwauzoma and E. T. Jaja 2013; M. A. Ramos Leal *et al*.1996; S. J. Khan *et al*. 2004; A. R. Leva *et al*., 2012). These variations refer to as somaclonal variations (P. J. Larkin and W.R.Scowcroft, 1981) which are very well documented in the regenerated plants of many crop species such as wheat (Z. N. Hashim and W. F. Campbell, 1990), Potato (S. A. Bannaceur *et al*. 1991) strawberry (M.K. Biswas, *et al*., 2009) rice (R. Joshi *et al*., 2011) and tomato (A.N. El-Banna, *et al*. 2015). Regeneration in chili pepper *Capsicum annuum* has been achieved from the culture of different tissues and organs, such as apical buds (L. Koleva *et al*., 2001) cotyledons (A. Joshy and S.L. Kothari 2007) hypocotils (E. A. J. El Kaaby *et al*., 2015; L. Gunai and P. S. Rao, 1978) protoplast (A. H. Prakash *et al* 1997) and stem segments (A. Hossain *et al*., 2003). Moreover, variability in many morphological and physiological characteristics has been reported in pepper regenerants (A. Hossain *et al*., 2003; A. Anu *et al*., 2004; L. K. Gudeva and S. S. Veselinovska, 2011). Regardless of the source and the mechanisms in which they occur, such variations are considered as a novel source for crop improvement.

Morphological traits like pigment production, biochemical characters like nicotine synthesis and chromosome number and structure were reported by several researchers (D. A. Evans *et al*. 1984; S. M. Jain, 2001). It has been reported that salt stress induced epigenetic variations as well as genetic in regenerated plants (L. Zhong *et al*., 2009; A. R. Leva, 2012). Therefore the objective of this study is to assess the effect of tissue culture under salt stress on morphological and biochemical traits of local chili pepper in comparison to seeds derived plants.

Materials and Methods

The research was conducted at Genetic Engineering Department, Ministry of Science and Technology, during the years 2012-2015. Local chili pepper seeds were

obtained from a local supplier. The seeds were treated with 2mgl^{-1} of Gibrillic acid (GA_3) solution and germinated on filter paper in the test tubes and kept in the culture room at 25 ± 2Cº, 16 h light of 1000 Lux intensity and 8 h darkness. After two weeks of germination, the seedlings were transferred to plastic pots filled with peat moss and kept in the greenhouse conditions. Meanwhile plants regenerated from callus induced from shoot tip explants on MS medium of (9 dSm^{-1}) salt stress level (Z. N. Al Hattab, *et al*., 2015) were transplanted into the same pots and all the plants kept in the greenhouse until maturity. Five plants were selected randomly from each group and the following parameters were recorded: plant height (cm), number of branches, stem diameter (cm) dry weight of vegetative system (g) and yield component which included: number of fruits per plant, fruit weight (g)in addition to the total fresh and dry fruit yield (g/plant). Moreover, physiological parameters included Capsaicin, Chlorophyll, and Ascorbic acid (V.C) were assessed by HPLC. Capsaicin was extracted and estimating according to Z. A. A. Al Othman *et al*., (2011) method with slight modification and pungency degree was calculated by S. Tilahun *et al*., (2013) method using the following equation:

Pungency degree= Capsaicin concentration x 1.6 x 10^7

Furthermore Chlorophyll was extracted and analyzed following M. Roca and M. I. Mianguez-Mosquera, (2006) protocol and finally Ascorbic acid (V.C) extracted and estimated according to S.S.Mitic *et al*., (2011) with slight modification.

The experiments were conducted in Completely Randomized Design (C.R.D), the results were analyzed by Genestat software and the means were compared by L.S.D at p≥0.05.

Results

The results (Table 1, Figure 1) showed that pepper plants grown from seeds surpassed the tissue culture plants in plant height (56.4 cm) and fruit weight (5.03 g) compared with (26.9 cm) and (4.25 g) the increments were 109.6% and 18.3% respectively.

Figure 1 Regenerated plant (right) seed-derived plants (left)

However tissue culture plants were significantly higher in the branches number, fruits number, and stem diameter which were (8.33 branches, 127 fruits and 2.06 cm) compared with seed derived plants (3.33 branch, 94.33 fruits and 1.26 cm) respectively in a percentage reached to (150.1%, 34.6%, **63.4** %) respectively. Moreover, tissue culture plants significantly surpassed the conventional plants in shoot dry weight, total fresh and dry fruit yield which were (68.64 g, 540.90 g, 186.5 g) respectively compared with (57.55g, 474.60 g, 158.2 g) respectively in a percentage reached 19.27%, 13.9%, 17.8% respectively.

Table 1 Morphological, agronomic and yield characterization of *in vitro* and seed-derived plants

Traits	Plant origin		L.S.D (0.05)
	In vitro	seed-derived plants	
Plant height (cm)	26.9	56.4	8.6
Number of branches	8.33	3.33	2.62
Number of fruits	127	94.33	10.26
Stem diameter (cm)	2.06	1.26	0.35
Leaf area (Dc^2. Plant $^{-1}$)	54.79	48.08	3.82
Average fruit weight (g)	4.25	5.03	**0.55**
Shoot dry weight (g)	68.64	57.55	5.37
Total fresh fruit yield (g/plant)	**540.9**	474.6	49.71
Total dry fruit yield (g/plant)	**186.5**	**158.2**	17.12

Chlorophyll analysis (Table 2) showed that seed grown plants contain significantly higher amount of chlorophyll (42.5 µg/g fresh weights) than the tissue culture plants (31.4 µg/g fresh weight) in a percentage of 35.3%. While the tissue culture plants contain significantly higher amount of ascorbic acid than seed grown plants which were (84.9 and 58.6 µg/g fresh weights) respectively in an increment of 44.8%. Although there were no significant difference between the two plant groups in the capsaicin content, the amount of capsaicin in the total dry fruits of the tissue culture plants were higher than that of the seed grown plants which were (58.226 and 50.213 mg /dry fruits of one plant respectively in an increment of 15.95%. Analysis of the Pungency showed that the plants of both groups are considered of high Pungency degree.

Table 2 Physiological parameters of in vitro and seed-derived chilli pepper plants

L.S.D (0.05)	Plant origin		Traits
	seed-derived plants	In vitro	
9.95	42.5	31.4	Chlorophyll (µg)
7.63	58.6	84.9	Ascorbic acid(µg)
N. S.	312.2	317.4	Capsaicin (µg)
5.69	50.21	58.23	Capsaicin/plant dry weight mg/g
	49952	50784	Pungency(SHU)

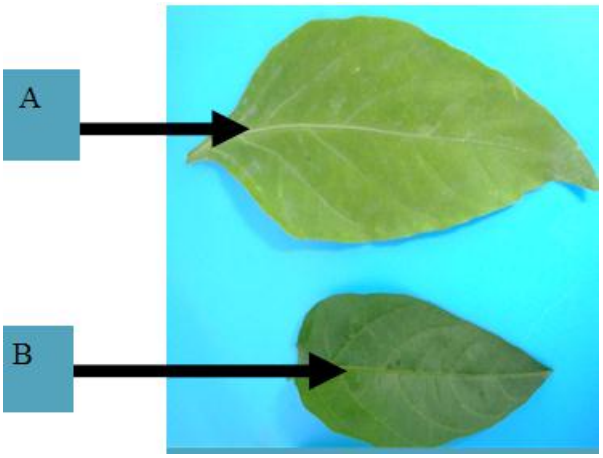

Figure 1 Pepper plant leaves A. *in vitro* B. seed-derived plants

Discussions

Tissue culture has been utilised in plant improvement and the selection of variants with desirable traits. Therefore researchers are interested in comparing the regenerants with the mother plants in the normal growth conditions. The results of the present experiment are in agreement with the findings of some published results in terms of the variation in some traits. Plant height of the regenerats was reduced while the branches and the number of the fruits were increased. The reduction in the number of fruits in plants grown from seeds causes an increment in the fruit weight. The main reason for the increase in the branches of the tissue culture plants could be due to the high concentration of cytokinines in the tissue which is accumulated in the plants from the culture medium.

Variations in the color and the shape of the leaves were detected in the tissue culture plants compared with the seed grown plants (Fig.1). Seed grown plants have small leaves with dark green color while tissue culture plans have large leaves with light green color. This result could be attributed to the Gibberellins accumulation from tissue culture medium (F. H. Al Khazaly *et al*., 2002). Reduction in the amount of chlorophyll was also detected in the tissue culture plants. This result is in agreement with what was reported recently by S. Grozeva and V. Todorova, (2015). The tissue culture plants in the current experiment were regenerated under salt stress condition which induced changes in the pigment-protein complexes of the thylakoid, PSII activity and thermoluminescence in chloroplasts (A. N. F. Misra, *et al*., 1998). Abiotic stress has an impact on the photosystem 2 reaction centers which is detected by chlorophyll changes in the plant (A. N. F. Misra, *et al*., 2001). Concerning the time of flowering, seed growing plants flowered before the tissue culture plants and gave fruits two weeks earlier. Tissue culture plants grown on medium supplemented with different growth regulators which enhanced plant branching and delayed the flowering. This result is in agreement with the results found by Ma.G. Valadez-Bustos *et al*., (2009). Capsaicin is produced in the placenta of the fruits which is responsible for the Pungency test of the chili pepper (V. Pandhair and S.S. Gosal. 2009). The increase of capsaicin in the tissue culture plants in the current study increased the pungency degree of the fruits. These changes could be attributed to the genetic changes in the regenerated plants. The degree Pungency varied in the fruits of the genus *Capsicum* depending on the genetic contain of the plant which control the production of capsaicin **(**R. Arora, *et al*., 2011). Moreover, high Ascorbic acid level in the regenerated plants could be responsible for the increments of capsaicin. It has been found that medium supplemented with L-ascorbic acid increases in the production of capsaicin to three folds (C. Veeresham *et al*., 1993).

Conclusions

The results of the current experiment indicated that tissue culture derived chili pepper plants grown in the greenhouse showed useful somaclonal variations. They surpassed the seed derived plants in the yield parameters which included number of fruits, total fresh and dry fruit yield. Moreover, the fruits of the tissue culture plants were rich in Ascorbic acid and Capsaicin with high Pungency (SHU).

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