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Identification of the most organogenic-responsive variety of tomato using the variety x medium interaction

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Abstract

The in vitro culture of tomatoes was initiated with the goal of creating an edible malaria vaccine which could be successfully expressed in tomatoes. The identification of the best culture medium and of the most suitable tomato variety was performed by conducting variety x media interaction study. Six tomato varieties were used in order to carry out the tissue culture experiment, namely Roma, Brandy, Pink Girl, Pineapple, Rutgers and Summers. The cotyledon explants used for this experiment were obtained from 7-10 days-old seedlings placed on six different culture media. All the media contained MS salts with different concentrations of Zeatin and auxin. These are: MS+1Z, MS+1Z+.05IAA, MS+1Z+.01NAA, MS+2Z, MS+2Z+.05IAA, MS+2Z+.01NAA. Cultured cotyledons were grown for a period of 8 weeks, data being recorded by counting the number of plants regenerated from each explants and also by scoring shoot initiation response percentages. Data were analyzed using Statistical program SPSS and the mean differences were compared by Duncan's multiple range test. The culture medium which recorded the highest number of regenerants was MS + 1Z + .05IAA, this medium performed significantly better than the MS+ 1Z+ .01NAA medium. Brandy and Roma tomato varieties have produced significantly higher number of regenerants, although these varieties did not perform better against all media.

Keywords: Tomato, Auxins, Cytokinins, Medium, ANOVA.

Introduction

Tomato (*Lycopersicon esculentum*) is among the most important vegetable crops amenable to plant tissue culture and genetic transformation processes. The genetic transformation of tomatoes and the in vitro regeneration have been successfully used for genetic improvement in order to trigger higher resistance to different diseases and insects [1, 2]. The tomato varieties are popular and can be grown in all continents, their fruits being mostly consumed raw as salad. It's consumption is being correlated with beneficial effects against diseases such as: some types of cardiovascular diseases and cancer [3]. Being commonly eaten raw as salad, tomato is a perfect candidate for the delivery of oral vaccines. To date, tomatoes are widely used to produce vaccine antigens, successful transformation being reported by several researchers [4, 5, 6, 7, 8, 9].

Cotyledons, hypocotyls, stems, petioles, leaves, anthers, inflorescences and apical meristems have been the tomato explants used so far to induce callus formation and regeneration from different explants [10, 11, 12]. According to the literature, the best medium for tomato tissue culture is the one containing a cytokinin (Zeatin), the most used concentration of Zeatin being 1mg/l [1, 10, 13, 14, 15, 16, 17, 18, 19]. The most popular auxin used in tomato tissue culture and tomato transformation is IAA, 0.05 mg/l [11, 14, 16,

17, 20, 21]. We optimized the tissue culture to obtain a large number of plants using cotyledon explants due to their higher capacity for shoot regeneration [1, 5, 22, 23].

The main goal of our research was to identify the best variety and the best medium for the in vitro regeneration of six tomato varieties with the aim of transforming tomato cultivar with malaria antigen gene. Malaria genes that are used to transform the tomato plants are PfCP-2.9 and PfCSP-RC. These genes were selected after a detailed research on different malaria vaccine candidates [24].

Materials and methods

Six tomato varieties were used in order to carry out the tissue culture experiment, namely Roma, Brandy, Pink Girl, Pineapple, Rutgers and Summers. Approximately 300 tomato seeds from each variety were surface sterilized in a 50-ml tube in a few ml of 70% ethanol for one min. After removal of ethanol, 40 ml of 20% commercial bleach containing one drop of Tween-20 / 100 ml of solution was added and seeds were surfaced sterilized for 15 minutes. After three washings with sterile double distilled water, seeds were transferred to Petri dishes containing sterile autoclaved semi-solid basal Murashige and Skoog (MS) medium with 2% Dixie Crystal sugar. Ten seeds were placed in each plate and incubated in an environmental control growth chamber (25ºC, 16 h photoperiod, light intensity of 30-40 µEm-2s-1).

The cotyledons from 7-10 days old seedlings were used as explants in this experiment. All six media used in this experiment contained basal salts of MS containing 3% sucrose with different growth regulators (Zeatin alone and with IAA or NAA). The media used were: 1Z (1 mg Zeatin / l), 1Z+0.05 IAA (1 mg Zeatin + 0.05 mg IAA /l), 1Z+0.01NAA (1 mg Zeatin + 0.01 mg NAA /l), 2Z (2 mg Zeatin / l), 2Z+0.05 IAA (2 mg Zeatin + 0.05 mg IAA /l), and 2Z+0.01NAA (1 mg Zeatin + 0.01 mg NAA). The pH of all media were adjusted to 5.7 before autoclaving for 15 min at 121^{o}C and 15 lb pressure. Six media and six tomato varieties in all possible (6 x 6 = 36) combinations were used for the tissue culture experiments. For each of the 36 combinations, 5 plates were cultured with 10 explants in each plate. The plates containing cotyledon explants were incubated in an environmental control growth chamber (25ºC, 16 h photoperiod, light intensity of 30-40 µEm-2s-1). Experimental design used was 6 x 6 factorial completely randomized design (CRD). The same experiment was repeated using the same media and the same varieties (36 combinations) under the same environmental condition.

For shoot elongation, the explants were transferred to fresh media after two weeks interval. Number of regenerated shoots was counted after 6-8 weeks. Elongated shoots were rooted in MS media without growth regulator. Rooted shoots were transferred to GA7 Magenta box containing autoclaved greenhouse soil. Plants were gradually acclimatized to ambient temperature and then transferred to 20 cm pots containing peat moss/vermiculite (1:1) mixture and maintained in a greenhouse at 25–28º C.

Results and discussion

Eight weeks after initial culturing of explants, numbers of shoot/explant data were collected from each of the 36 combination factorial design experiment. The data collected on shoot initiation response of explants are percentages. In most instances, percentage data are not normally distributed therefore cannot be analyzed using ANOVA. However, when percentage data is transformed using a variety of methods such as log10, those data show normal distribution therefore ANOVA is appropriate. When ANOVAs of both transformed and non transformed data show similar results, results of non transformed data can be presented without reservation.

The shoot initiation response data from the first experiment was analyzed using Univariate Analysis (analysis of variance) of SPSS Statistical Software Package. After log10 transformation of the shoot initiation response data, ANOVA was performed and the result is shown in Table 1. Highly significant variation was observed among the six tomato varieties tested in this investigation. Similarly, significant variation was observed among the six media and Variety x Media interaction.

Table 1. ANOVA showing the effect of Variety and Media interaction (%)

Source	Sum of squares	Df	Mean square	F	Sig.
Variety	12.833	5	2.567	8.894	0.000
Media	4.015	5	0.803	2.783	0.019
Variety x Media	12.650	25	0.506	1.753	0.021
Error	43.350	150	0.289		

Significance level alpha = 0.05

Significant F value suggests that at least one of the mean is significantly different from other means but does not provide pair wise mean comparison information. To find that information in a most efficient and fast way, Duncan's multiple range test (DMRT) analysis was conducted on variety and media. Results are shown in Tables 2 and 3. These two tables also included standard error of means and 95% Confidence Interval data for each mean. Table 2 shows the grouping of the six varieties into three significantly different subsets, Brandy and Roma being the highest producers and Pineapple and Rutgers being the lowest producers while Pink and Summers being the intermediate types. Table 3, on the other hand, does not show a clear cut grouping of media means into subsets. The results indicate that 1Z+0.05IAA is significantly better medium that 1Z or 1Z+0.05NAA medium.

Table 2. Variety means comparison using Duncan's Multiple Range Test (DMRT) analysis

Variety	N	Mean	S.E.	95 % Confidence interval	
				Lower	Upper
Brandy	30	1.181-a	0.088	1.006	1.356
Pineapple	30	0.340-c	0.085	0.173	0.507
Pink Girl	30	0.674-b	0.088	0.501	0.847
Roma	30	1.008-a	0.089	0.832	1.184
Rutgers	30	0.396-c	0.079	0.24	0.553
Summers	30	0.653-b	0.084	0.488	0.819

Table 3. Medium means comparison using DMRT analysis

Media	N	Mean	S.E.	95 % Confidence interval	
				Lower	Upper
1Z	30	0.600-b	0.084	0.433	0.766
1Z + 0.01NAA	30	0.579-b	0.084	0.413	0.744
1Z + 0.05IAA	30	0.907-a	0.085	0.738	1.075
2Z	30	0.717-a	0.089	0.54	0.893
2Z + 0.01NAA	30	0.709-a	0.084	0.544	0.875
2Z + 0.05IAA	30	0.742-a	0.087	0.571	0.913

Although we identified the best varieties and best medium for tomato shoot production among the varieties and media tested in this investigation, the significant variety x medium interaction indicates that best variety Brandy or Roma does not perform best against all

media. Similarly, the best medium 1Z+0.05IAA does not perform best for all varieties (Fig. 1). This significant interaction will allow us to identify the best specific variety and medium combination for clonal propagation or transformation study.

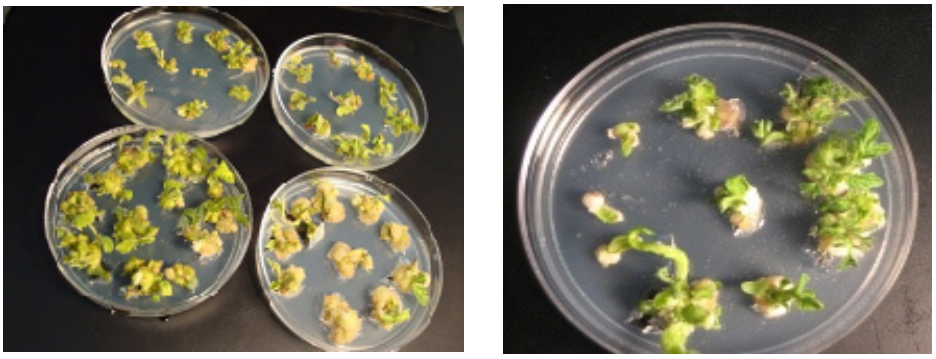

Figure 1. Explant response on different media

The effect of the different concentrations of Zeatin on shoot production

The effects of Zeatin concentration on explant response of shoot proliferation is shown in Table 4. When 1Z mg/l was compared to 2Z mg/l, three out of six varieties responded with higher shoots production and one variety did not show any difference. Similarly, when 1Z + 0.01NAA was compared with 2Z + 0.01NAA a very similar response was observed between the varieties. On the other hand, when 0.01 NAA was replaced with 0.05IAA, 1Z media was better than 2Z in five out of six varieties tested in this investigation. This information is very useful because Zeatin is one of the most expensive cytokinin growth regulator and therefore the growth regulator cost can be reduced to almost half. This is very encouraging information for the scientists of developing countries where research support is very limited.

Table 4. Variety x Media Average

MEDIA	ROMA	BRANDY	RUTGERS	SUMMERS	PINEAPPLE	PINK GIRL	AVERAGE
1Z	0.580	1.187	0.512	0.389	0.470	0.462	0.600
1Z + 0.01 NAA	0.620	1.243	0.387	0.606	0.017	0.600	0.579
1Z + 0.05 IAA	1.449	1.263	0.583	0.513	0.651	0.980	0.907
2Z	1.480	1.017	0.236	0.575	0.375	0.618	0.717
2Z + 0.01 NAA	0.760	1.617	0.293	0.717	0.150	0.720	0.710
2Z + 0.05 IAA	1.160	0.760	0.369	1.122	0.378	0.664	0.742
AVERAGE	1.008	1.181	0.397	0.654	0.340	0.674	

ANOVA of second experiment data was very similar to the first experiment (data not shown). Therefore we combined the results from both experiments and total number of shoots of Variety x Media interaction were calculated and presented in Table 5.

Table 5. Total Number of Shoots from Variety x Media Combinations

MEDIA	ROMA	BRANDY	RUTGERS	SUMMERS	PINEAPPLE	PINK GIRL	AVERAGE
1Z	37.11	89.03	41.21	44.95	58.70	43.38	51.47
1Z + 0.01 NAA	58.37	73.53	35.22	55.73	34.25	49.00	51.30
1Z + 0.05 IAA	96.80	112.96	58.11	46.02	73.12	57.14	72.50
2Z	89.76	92.27	21.70	53.21	42.17	31.52	53.27
2Z + 0.01 NAA	52.77	90.12	26.25	66.63	43.54	48.47	53.47
2Z + 0.05IAA	87.98	65.29	29.00	94.39	65.90	35.59	62.65
AVERAGE	69.16	87.20	34.53	59.37	53.03	45.09	

Combined results from both experiments shown in Table 5 are consistent with the results obtained in the first experiment. For example, medium 1Z + 0.05 IAA performed better for 5 out of 6 varieties. Similarly, the variety Brandy performed better in 5 out of 6 media tested.

Conclusion

In conclusion, based on the proposed methods and the experiments conducted so far, it was found that media containing 1Z mg/l + 0.05IAA mg/l would be the best media for tomato shoots regeneration, the result being consistent with prior scientific observations. Furthermore, it was established that a higher concentration level of Zeatin does not lead to an increase in the number of shoots obtained, which would help reduce future costs by purchasing a smaller quantity of Zeatin. For future transformation experiments only one medium will be used out of the six already tested in the tissue culture experiment. Similarly, since the Brandy variety performed the best, the future experiments will be focused on this variety.

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