Development of a protocol for *in-vitro* propagation of black pepper (*Piper nigrum* L.) local selections

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Introduction

Black pepper (Piper nigrum L.) belongs to family Piperaceae and it is one of the most economically important spice crops in the world (Srinivasan, 2007; Mathew et al., 2001). Unavailability of sufficient mother plant stock in the field, obtaining basal runners for propagation and less success and multiplication rate of the high yielding local pepper cultivars are the major problems faced by the farmers who cultivate. Being in vitro propagation a promising option, this study was focused to develop a suitable protocol for in vitro propagation of black pepper local selections.

Methodology

This research was carried out at Central Research Station, Department of Export Agriculture, Matale. Four experiments were conducted during the research period. Experiment one was conducted to find out the suitable surface sterilization method for the sterilization of black pepper shoot tips. Selecting of appropriate media for the culture establishment of black pepper shoot tips were carried out in second experiment using 1/3Murashige and Skoog (MS) medium and 1/2 Woody Plant Medium (WPM).Experiment three was conducted to find out suitable combination of auxin and cytokinin for the shoot multiplication of black pepper local selections. In fourth experiment, priority was given for the selection of best media and hormonal combination for the callus initiation of TG7 black pepper local selection. Full and half strength MS media were used as the culture media and two different concentration levels of kinetin and NAA were used as the growth regulators. Complete Randomized Design (CRD) was used as the experimental design. ANOVA was used to analyze the statistical difference of parametric data and non-parametric data were subjected for logarithmic transformation. SAS statistical software was used to analyze the data and mean separation was performed using Least Significant Difference (LSD).

Results and Discussion

As the results summarized in Table 1, sterilization using 10%- 20% Clorox for five to ten minutes (T1 to T5) showed higher percentages of bacterial contamination (40 to 80 %). Lower percentages of fungal contamination was observed in T4 to T8 within the period of three to five days (3% to 7%).The highest survival percentage (66.6 %) was reported in T8, 0.04 % HgCl2 for five minutes. Similarly, the lowest percentages of bacterial and fungal contamination were observed in T8. The highest percentage of phenolic browning (80%) was shown in T6 and lowest percentage of phenolic browning (10.0%) was observed in T2, i.e. 10% Clorox for 10 minutes within four to seven days.

Table 2 : Percentages of survival, bacterial and fungal contamination and phenolic browning at different surface sterilization methods

Treatment	Survival %	Bacterial %	Fungal %	Browning %
T1- 10% Clorox for 5 minutes	15.0	50.0	20.0	15.0
T2- 10% Clorox for 10 minutes	0	80.0	10.0	10.0
T3- 15% Clorox for 5 minutes	15.0	40.0	15.0	30.0
T4- 15% Clorox for 10 minutes	35.0	40.0	5.0	30.0
T5- 20% Clorox for 5 minutes	10.0	50.0	5.0	35.0
T6- 20% Clorox for 10 minutes	15.0	0	5.0	80.0
T7- 0.1% $HgCl_2$ for 1 minute	38.5	26.9	7.6	26.9
T8- 0.04% $HgCl_2$ for 5 minutes	66.6	18.18	3.01	12.12

In experiment two, there was a significant difference between two media (1/3 Murashige and Skoogmedium and 1/2 Woody Plant Medium) for the shoot development of black pepper local selections. As shown in table 2, 1/2 Woody Plant Medium showed the highest mean survival (0.4892) three weeks after culturing.

Table 3: Mean survival rate of Black Pepper shoot tips in 1/3Murashige and Skoog (MS) medium and 1/2 Woody Plant Medium (WPM)

Medium	Mean of Survival
1/3 MS	0.16250 [b]
1/2 WPM	0.48920 [a]
LSD	0.0782

Means with same letters along the columns are not significantly different at probability level of 0.05

Table 4: Mean number of shoots, number of leaves and shoot length of shoot tip cultures affected by various growth hormones

Treatment			Mean shoot number	Mean leaves number	Mean shoot length
BA(mg/L)	NAA(mg/L)	Kinetin(mg/L)			
T1-3.0	0	0	2.0 [a]	4.4 [a]	0.1 [c]
T2-3.0	0.5	0.1	1.4 [c]	4.5 [a]	0.1 [c]
T3-3.0	0.5	0.2	1.5 [bc]	3.9 [a]	0.1 [c]
T4-3.0	1.0	0.1	2.3 [a]	4.6 [a]	0.3 [b]
T5-3.0	1.0	0.2	1.9 [ab]	4.4 [a]	0.4 [a]
	LSD		0.434	0.8446	0.125

Means with same letters are not significantly different at probability level of 0.05

According to the Table 3, higher number of shoots were observed in T4, followed by T1and T5.Higest number of leaves were observed in T4.There was a significant difference in shoot length between treatments. The highest shoot length was observed in T5. With considering number of shoots and shoot length, Woody Plant Medium with 3 mg/L BA, 1.0 mg/L NAA and 0.2 mg/L Kinetin was the best hormonal combination for shoot multiplication o f black pepper local selections(T5).

As the table 4 shows, there was no significant difference between two media (Full strength MS and half strength MS) for the callus formation.

Table 5. The status of callus formation on full and half strength MS media

Media	Mean Callus formation
Full strength MS medium	0.54 [a]
Half strength MS medium	0.49 [a]
LSD	0.07
CV%	18.7

Means with same letters are not significantly different at probability level of 0.05

As Table 5 indicates, there was a significant difference between the T2, T4 and T5 for the callus formation. The highest callus development (0.74 score) was observed in T4.

Table 6.Callus development in different kinetin and NAA level fourweeks after culture

Treatment No	Treatment		Mean score	Color
	NAA (mg/L)	Kinetin (mg/L)		
T1	0.5	1.0	0.69 [ab]	Whitish brown
T2	0.5	1.5	0.55 [c]	Whitish brown
T3	1.0	1.0	0.59 [bc]	White
T4	1.0	1.5	0.74 [a]	White
T5	0	0	0.0 [d]	No
LSD			0.11	
CV%			18.71	

Within the column means with same letters are not significant at probability level of 0.05

Conclusions

In development of a protocol for *in vitro* propagation of black pepper (*Piper nigrum* L.) local selections, following methods for surface sterilization, culture establishment, shoot multiplication and callus induction are established.The best surface sterilization method for the shoot tips of black pepper local selections is 0.04 % Mercuric Chloride for five minutes. Half of WPM supplemented with 3.0 mg/L of BA and 1.0 mg/L of kinetin is the best media for the culture establishment using shoot tips of black pepper local selections. The best hormonal combination for the shoot multiplication of black pepper local selections is WPM with 3.0 mg/L of BA, 1.0 mg/Lof NAA and 0.2 mg/L of Kinetin. Either full or half strength MS medium supplemented with 1mg/L of NAA and 1.5 mg/L of Kinetin is better to use for callus induction from leaves of black pepper local selections.

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