STUDIES ON MACRO VEGETATIVE PROPAGATION OF BLACK PEPPER *(PIPER NIGRUM* LINN.) USING VINE CUTTINGS: EFFECTS OF PLANT AND CULTURAL FACTORS ON SPROUTING, ROOTING AND SEEDLING DEVELOPMENT

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ABSTRACT

The type of cutting with or without leaves, rooting media, the environment under which propagation is carried out and fungicide treatment of cuttings, influence the successful vegetative multiplication of planting materials of black pepper (Piper nigrum Linn.) from vines. All the softwood cuttings died within one week after setting them whilst more of the hardwood cuttings sprouted and survived producing more vegetative growth than the semi-hardwood cuttings. Rooting media did not significantly affect the production of sprouts but survival rate of developed seedlings was higher in top soil and the mixture of topsoil and river sand than cuttings set in river sand alone. Dipping cuttings in fungicides, retaining leaves on cuttings and propagating them under transparent polythene sheet or bags to raise the humidity in the rooting environment resulted in the production of more sprouts and enhanced seedling survival and development. Dry weights of seedlings were affected by the various treatments. Environment and rooting media each had significant interaction with nodal positions.

Keywords: *Vegetative Propagation, Nodal Positions, Rooting Media, Leaf Retention, Fungicides, Sprouting Black Pepper.*

INTRODUCTION

Black pepper *(Piper nigrum* Linn.) is one of the oldest and most important of all spices. It has been a foreign exchange earner for countries in the Far East for over 2,000 years [1]. The crop has recently caught the attention of several developing countries in the tropics including Ghana. In 1991, the Ghana Export Promotion Council (GEPC) and the Plant Genetic Resources Centre (PGRC) at Bunso introduced the crop to farmers for its large scale cultivation which was supported and financed by the United Nations Development Programme (UNDP). Following the inception of the programme, the demand for black pepper planting materials has become an ever-increasing one. Although black pepper can be propagated by seeds, plants from seeds show considerable variability and may even be unisexual. Commercial plantings therefore use vine cuttings which are also associated with problems in the preparation and establishment of planting materials.

Along the vine of black pepper, visible differences exist between nodal regions from the apex to the base, which may be associated with differences in internal and external factors such as anatomy and stem size respectively. These differences may influence the rooting ability of cuttings prepared from different positions on a particular vine [2]. The rooting of cuttings of many plant species may also be greatly influenced by the kind of rooting medium used, not only in the percentage of cuttings rooted but in the quantity and quality of root system formed [3]. Several workers have emphasized the need to obtain a near saturation around rooting cuttings to enhance sprouting and rooting [4, 5, 6, 7]. Fungicide treatment of cuttings may prevent or reduce pathogenic infection so that many potential planting materials may be available for planting. With leaf retention on cuttings, Bouillenne and Bouillenne - Walrand [8] showed that cotyledons, leaves and buds possess root forming substances that stimulate rooting of cuttings. Went [9] indicated that specific factors other than auxins were manufactured in the leaves and were necessary for root formation.

Two experiments were conducted to determine the influence of nodal positions set in different rooting media and environments and the effects

of nodal positions with or without leaves, polythene covering and fungicide application on sprouting, rooting and seedling development of black pepper.

MATERIALS AND METHODS

Experiment 1

A 3 x 3 x 2 factorial experiment involving nodal positions, rooting media and different environments was carried out under a lathhouse (a structure which provides half to two-thirds roofing cover to protect container grown nursery stock from the sun and has open sides to allow enough ventillation) at the Department of Horticulture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, for 12 weeks. Two node cuttings of softwood, semi-hardwood and hardwood were prepared from orthotropic black pepper vines of an imported variety 'Balancotta' collected from the field genebank of PGRC, Bunso. The cuttings, each carrying dormant buds were defoliated and set in three rooting media namely, sterilized top soil of Akroso series (a sandy loam soil belonging to the Asuansi - Kumasi Soil Association which is deep and well drained with good to moderately good water holding capacity), river sand and a 1:1 (v/v) mixture of top soil and river sand. There were five cuttings per treatment which were carried in 12.5cm clay pots containing the various rooting media. The cuttings were watered and half of them were individually enclosed in transparent polythene bags each measuring 39.5 cm long and 24.5 cm wide while the other half were exposed. Periodic watering of the exposed treatments was done when the soils in the pots dried. For the enclosed treatments, when the relative humidity dropped below 80%, the transparent polythene bags were removed, watered and the polythene bags fitted back. The treatments were assigned to experimental units using Completely Randomised Design and replicated three times.

Data was collected on mean number of sprouted cuttings 8 weeks after setting, total number of surviving seedlings, total number of developed leaves and roots at harvest at 12 weeks after setting. Measurements were also made of seedling height, average root length and dry weights of the various vegetative parts. Data was analysed statistically using analysis of variance (ANOVA) and differences between means were separated using Duncan's Multiple Range Test (DMRT) at 5% level. The square root transformation was performed on all count data prior to analysis of variance. The HM 34 H & T meter was used to record relative humidity and temperature at 0900h, 1200h and 1500h GMT during the experimental period and the mean figures are presented in Table 1.

Table 1: Means of temperature and relative humidity taken during period of experiment (10th May to 2nd August, 1994) using HM 34 H & T meter.

Time of day (GMT)	Outside environment		Inside polythene cover	
	Temp. (°C)	Relative Humidity (%)	Temp.(°C)	Relative Humidity (%)
0900h	22.5	73.1	22.9	87.7
1200h	30.8	48.1	33.4	94.2
1500h	29.9	39.3	33.1	82.4
Mean	27.7	53.5	29.8	88.1

Table 2 shows the chemical nutrient analysis of media used in the experiment and the determinations were based on standard procedures adopted by the Association of Official Analytical Chemists (AOAC) [10].

Table 2: Chemical nutrient analysis of media used in the experiments based on standard procedures adopted by the AOAC (1984).

Media	N (%)	P_2O_5 (%)	K_2O (%)	Na (%)	Ca (mg/100g)	Mg (mg/100g)
Top soil	0.18	0.020	0.020	0.009	102	26
River sand	0.03	0.005	0.004	0.006	12	6

Experiment 2

A factorial experiment involving nodal positions, polythene covering, leaf retention and fungicide application as the treatments was set up for 12 weeks. The place of the experiment, source and vine type of planting material, soil media used, watering schedule, statistical analyses of data recorded were as described under Experiment 1.

For each nodal position, one half the number had leaves retained whilst the other half had their leaves removed. Half the cuttings were totally

immersed in 0.1% suspension of 1:1 (w/w) mixture of 'Bavistin' and 'Kocide 101,' a systemic and contact fungicide respectively for 15 minutes while the other half were untreated. The treated cuttings were air dried for 25 to 30 minutes before setting them and untreated ones in 12.5cm clay pots. The pots were filled with 1:1 (v/v) mixture of sterilized topsoil and river sand. Pots that were to receive fungicide treated cuttings were earlier drenched with 0.3% suspension of the fungicide mixture according to Maag [11] before the cuttings were inserted in the medium after which judicious watering was done. One half of the cuttings were covered with 0.003 mil transparent polythene sheet over split bamboo loops, 28cm high from the ground, making sure that excess sheets were tucked in the ground using stones to prevent any vapour from the soil.

Organic carbon was determined by the standard Walkley and Black wet oxidation method [12] and nitrogen by macro Kjeldahl's method [13]. Carbon: Nitrogen (C:N) ratios were the quotients of values of organic carbon divided by the respective values of nitrogen of the various nodal positions.

RESULTS

In both experiments, all the softwood cuttings died within a week after setting them in the various media. Statistical analysis was therefore performed on data collected on only the semi-hardwood and hardwood cuttings.

Experiment 1

Table 3 shows data on the main effects of nodal positions, rooting media and environment on some vegetative growth parameters.

The number and percentage of cuttings that sprouted were significantly higher ($P < 0.05$) in hardwood than in the semi-hardwood cuttings. The number of surviving seedlings, developed leaves and roots were all higher in the hardwood cuttings. Seedlings developed from the hardwood cuttings were taller and had longer roots than the semi-hardwood cuttings.

Table 3: Main effects of nodal positions, rooting media and environment on some vegetative growth parameters.

Parameters	Number of sprout from five cuttings	Number of surviving seedlings from five cuttings	Mean seedling height (cm)	Number of leaves per cutting	Number of roots developed per cuting	Average root length (cm)
Nodal position						
Semi-hardwood	2.0b* (80)	1.5b	6.3b	2.4b	1.8b	2.0b
Hardwood	2.4a (100)	1.7a	7.9a	2.8a	2.1a	2.4a
Rooting medium						
River sand	1.8a (65)	1.2b	2.0c	1.6b	1.4c	1.7c
Top soil	1.8a (65)	1.5a	7.7a	2.5a	1.9a	2.3a
Mixture	1.8a (65)	1.4a	4.6b	2.1a	1.7b	1.8b
Environment						
Polythene covered	1.8a (65)	1.5a	6.2a	2.3a	1.9a	2.2a
Exposed	1.7b (38)	1.3b	3.3b	1.8b	1.5a	1.7b

* Means with similar letters within treatments are not significantly different. DMRT. $P < 0.05$

Rooting media did not significantly ($P > 0.05$) influence the number of cuttings that sprouted but cuttings set in top soil and in the mixture, had more ($P < 0.05$) surviving seedlings with more leaves. Seedlings from the top soil were taller, had more ($P < 0.05$) and longer roots than those set in the mixture which in turn performed better than cuttings set in river sand. Covering the cuttings with transparent polythene bags resulted in more ($P < 0.05$) sprouts and seedling survival than those exposed. Seedlings developed from the covered cuttings were taller, had more leaves and roots which were longer than those of the exposed cuttings.

The main effects of nodal positions, media and environment on dry weights of the vegetative parts (Table 4) followed a similar pattern to their effects on seedling height (Table 3).

Table 4: Main effects of nodal positions, rooting media and environment on total dry weights of vegetative parts and whole plant.

Parameters/ Treatments	Total dry weights (g)			
	Leaves	Stems	Roots	Whole plant
Nodal position				
Semi-hardwood	0.48b*	0.17b	0.29b	0.94b
Hardwood	0.66a	0.24a	0.45a	1.35a
Rooting medium				
River sand	0.15c	0.05c	0.12c	0.32c
Top soil	0.61a	0.22a	0.38a	1.21a
Mixture	0.39b	0.14b	0.25b	0.78b
Environment				
Polythene covered	0.48a	0.17a	0.31a	0.9b
Exposed	0.28b	0.10b	0.96a	0.5 b

* Means with similar letters within treatments are not significantly different, DMRT. $P < 0.05$.

Significant treatment interactions existed among some of the parameters. Covered hardwood cuttings resulted in more sprouts than both exposed hardwood and covered semi-hardwood cuttings which did not differ significantly ($P > 0.05$) from each other (Table 5).

Table 5: Interaction effect between nodal positions and environment on some vegetative growth parameters.

Parameters/ Interactions	Number of sprouts from five cuttings	Number of surviving seedlings from five cuttings	Mean seedling height (cm)	Number of leaves per cutting	Number of roots developed per cutting	Average root length (cm)
Semi-hardwood x Polythene covering	2.1b*(88)	1.9a	9.5a	3.2a	2.2a	2.4a
Semi-hardwood x Exposure	1.9c (72)	1.2c	3.1b	1.6b	1.3c	1.2b
Hardwood x Polythene covering	2.5a (100)	1.7ab	9.1a	2.8a	2.1ab	2.5a
Hardwood x Exposure	2.1b (88)	1.6b	6.8ab	2.7a	1.9b	2.4a

* Means with similar letters within treatments are not significantly different. DMRT, P > 0.05. Figures in parenthesis are percentage sprouts.

The number of surviving seedlings and their root number were also higher in both the covered hardwood and semi-hardwood cuttings whilst the exposed semi-hardwood cuttings recorded the lowest survival and root number. The exposed semi-hardwood cuttings produced significantly fewer leaves, shorter seedlings with shorter roots (Table 5) and low leaf, root, stem and total plant dry weights (Table 6) compared to the other treatment interactions which did not differ significantly from each other.

Table 6: Interaction effect between nodal positions and environment on total dry weights of vegetative parts and whole plant

Parameters/ Interactions	Total dry weights (g)			
	Leaves	Stems	Roots	Whole plant
Semi-hardwood x Polythene covering	0.74a*	0.26a	0.48a	1.48a
Semi-hardwood x Exposure	0.21b	0.08b	0.12b	1.48a
Hardwood x Polythene covering	0.69a	0.26a	0.44a	1.39a
Hardwood x Exposure	0.64a	0.22a	0.46a	1.32a

* Means with similar letters within treatments are not significantly different, DMRT, $P < 0.05$

Hardwood cuttings set in top soil produced more ($P < 0.05$) leaves and roots, taller seedlings with longer roots (Table 7) and higher dry weights (Table 8) than the other treatment interactions.

Table 7: Interaction effect between nodal positions and rooting media on some vegetative growth parameters.

Parameters/ Interactions	Number of sprouts from five cuttings	Number of surviving seedlings from five cuttings	Mean seedling height (cm)	Number of leaves per cuttings	Number of roots per cutting	Average root length (cm)
Semi-hardwood x River sand	1.9a*(72)	1.3b	3.0d	1.7c	1.5c	1.4d
Semi-hardwood x Top soil	2.0a(80)	1.7a	10.5ab	2.9ab	2.1b	2.2b
Semi-hardwood x Mixture	2.0a(80)	1.6a	5.4cd	2.4bc	1.9bc	2.1b
Hardwood x River sand	2.3a(100)	1.3b	2.9d	2.0c	1.6c	2.5b
Hardwood x Top soil	2.4a(100)	2.0a	12.7a	3.5a	2.4a	2.8a
Hardwood x Mixture	2.3a(100)	1.7a	8.0bc	2.9ab	2.0b	1.9c

* Means with similar letters within treatments are not significantly different. DMRT, $P < 0.05$. Figures in parenthesis are percentage sprouts.

Table 8: Interaction effect between nodal positions and media on total dry weights of vegetative parts and whole plant.

Parameters/ Interactions	Total dry weights (g)			
	Leaves	Stems	Roots	Whole plant
Semi-hardwood x River sand	0.15e*	0.05e	0.13cd	0.33c
Semi-hardwood x Top soil	0.08ab	0.20ab	0.44b	1.53ab
Semi-hardwood x Mixture	0.48cd	0.17cd	0.33b	0.98cd
Hardwood x River sand	0.28de	0.09de	0.24bc	0.61de
Hardwood x Top soil	1.02a	0.38a	0.71a	2.11a
Hardwood x Mixture	0.68bc	0.25bc	0.41b	1.34bc

* Means with similar letters within treatments are not significantly different, DMRT, $P < 0.05$

The poorest treatment in the performance of the above parameters was from the semi-hardwood cuttings set in river sand.

Experiment 2

Nutritional analysis of the various nodal positions (Fig. 1) indicates that C:N ratio was very narrow in the softwood cuttings and widened in the semi-hardwood with the hardwood cuttings having the widest ratio. The results of the main effects of the treatments on the various parameters are

summarized in Tables 9 and 10.

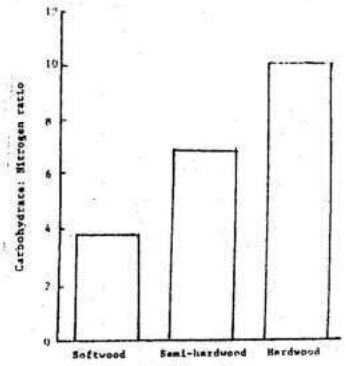

Fig. 1 Nutritional analysis of the various nodal positions indicating C:N ratio

Table 9: Main effects of nodal positions, polythene covering, fungicide application and leaf retention on some vegetative growth parameters.

Parameters/ Treatments	Number of sprouts from five cuttings	Number of surviving seedlings from five cuttings	Mean seedling height (cm)	Number of leaves per cutting	Number of roots developed per cuttings	Average root length (cm)
Nodal position						
Semi-hardwood	2.08b*(86)	1.37a	11.60a	1.86a	1.9a	2.2a
Hardwood	2.21a (98)	1.41a	11.80a	1.93a	1.9a	2.5a
Polythene						
Covered	2.14a(92)	1.46a	13.89a	2.13a	2.0a	2.6a
Exposed	2.06b(85)	1.02b	4.96b	1.25b	1.5b	1.6b
Fungicide						
Treated	2.55a(100)	1.37a	11.99a	1.89a	1.9a	2.3a
Untreated	2.21b (98)	1.11b	6.86b	1.49b	1.6b	1.6b
Leaves						
Undefoliated	2.56a (100)	1.83a	18.34a	2.65a	2.3a	2.5a
Defoliated	1.19b (28)	0.65b	0.52b	0.73b	1.0b	0.8b

* Means with similar letters within treatment groups are not significantly different, DMRT, $P < 0.05$. Figures in parenthesis are percentage sprouts.

Table 10: Main effects of nodal positions, polythene covering, fungicide application and leaf retention on dry weights of vegetative parts and whole plant.

Parameters/ Treatments	Total dry weights (g)			
	Leaves	Stems	Roots	Whole plant
Nodal position				
Semi-hardwood	0.64a*	0.31a	1.48a	2.43a
Hardwood	0.69a	0.34a	1.62a	2.65a
Polythene				
Covered	0.79a	0.39a	1.27a	2.45a
Exposed	0.29b	0.13b	0.26b	0.68b
Fungicide				
Treated	0.64a	0.33a	1.11a	2.08a
Untreated	0.44b	0.19b	0.42b	1.05b
Leaves				
Undefoliated	1.04a	0.51a	1.49a	3.04a
Defoliated	0.05b	0.02b	0.04b	0.11b

* Means with similar letters within treatment groups are not significantly different, DMRT, $P < 0.05$.

Apart from total number of sprouts in which the hardwood cuttings produced more sprouts than the semi-hardwood cuttings, the nodal positions were not significantly different from each other in total number of surviving seedlings, number of leaves, roots produced, seedling height and average root length of shoots. Fungicide treatment resulted in the production of more sprouts ($P < 0.05$), higher survival of seedlings, an increased number of leaves and roots. Fungicide treated cuttings produced shoots which were taller with longer roots compared to the untreated seedlings. Retaining leaves on cuttings significantly ($P<0.05$) influenced the vegetative growth parameters. Undefoliated cuttings had significantly ($P < 0.05$) higher values in the parameters studied than the defoliated cuttings (Table 9).

Regarding the dry weights (Table 10), the main effects of polythene covering, fungicide application and leaf retention followed similar pattern to their performance in the total number of sprouts (Table 9). There were no significant ($P > 0.05$) differences between the nodal positions in the total dry weights of all the parameters studied (Table 10).

DISCUSSION

All the softwood cuttings died within a week in the nursery. Results showed C:N ratio to be narrow in the softwood cuttings (Fig. 1). Kraus and Kraybill [14] observed in the rooting of tomato cuttings that green succulent stems which were very low in carbohydrates but high in nitrogen all decayed without producing either roots or shoots. This might have accounted for the death of the seedlings. This is so because since the softwood cuttings contained large amounts of nitrogen in the tissues, it was possible for them to be damaged by heat as the mean temperature in the environment was hot (28.8°C). This is in support of Hartmann and Kester [7] who stated that leafy softwood or semi-hardwood stem cuttings are easily damaged by high temperatures.

The presence of leaves on the cuttings might have exerted some stimulating influence on root initiation as reported by Rappaport [15]. Carbohydrates, which are vital for root formation, might have been translocated from the leaves to the base of cuttings as reported by Evans [4]. Leaves left attached to the cuttings

served as source of auxins [16] and photosynthate, therefore normal plant life could go on to some extent. The overall effect was earlier rooting and subsequent survival of seedlings.

Covering cuttings with transparent polythene sheets or bags created a micro-climate and this might have helped reduce dehydration, breakdown of cell contents and also to re-activate the growth of dormant buds as reported by Hartmann and Kester [7]. The relatively higher temperature within the polythene sheet coupled with the higher humidity might have re-activated the biochemical processes that initiate sprouting and root formation as observed in rooting of chrysanthemums [17].

Apart from the number of sprouts which was higher in the hardwood cuttings, there were no significant ($P > 0.05$) differences between the nodal positions in the other parameters. Sax [18] reported that cuttings taken from various plants in the growth phase, often form new roots readily than those taken from adult growth phase. The stored photosynthate in the hardwood cuttings might have also encouraged more sprouting and rooting as experienced by Samish and Spiegel [19]. It is possible that in black pepper propagation, both semi-hardwood and hardwood cuttings play a significant role in sprouting and rooting. Fungicides play a very significant role in preventing or minimizing fungal infection of plants especially at the nursery. Bacteria and fungi are notorious for deterioration of plant products and wood [20]. Since some of the cuttings were treated with both contact and systemic fungicides, there was reduced pathogenic infection of the treated cuttings than the untreated ones resulting in enhanced seedling performance from the treated cuttings.

The type of rooting medium was not of significance as cuttings initially depended on accumulated photosynthate. As such, cuttings in river sand performed similarly to those set in top soil and the mixture. However, in the sustenance of the sprouted cuttings to develop into independent seedlings, the type of rooting medium used was important in that, the sprouted cuttings, having exhausted their accumulated photosynthate, depended on nutrients in the rooting medium to grow and develop. The top soil and the mixture were therefore better in sustaining the sprouted cuttings and the development of vegetative parts since the amount of plant nutrients (Table 2) vital for plant growth was higher in them than in the river sand.

CONCLUSION

Although all the softwood cuttings died probably due to its high nitrogen content since black pepper planting materials are scarce and are in high demand, possibilities of using plant growth regulators should be tried. It is clear from the present work that both undefoliated semi-hardwood and hardwood cuttings treated with fungicides and set under polythene cover will enhance rooting and sprouting of cuttings whilst black pepper seedling survival depends on high nutrient level medium.

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