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Determination grafting techniques and compatible grafts between *piper* species - a case study in Vietnam

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Abstract

Black pepper (*Piper nigrum* L.) is one of the main crops in Vietnam. Currently, Vietnam is biggest black pepper producer globally and the crop has brought millions of US dollars to Vietnam economy. However, in recent years pepper plantation has been dying off thousands of hectares due to *phytophthora capsici*, a soil borne pathogen. Using resistant *piper* species as rootstock is considered an alternative option to control *phytophthora capsici*. This study was carried out to investigate suitable grafting methods and compatible grafts. Two way factor experiment consisting of six treatments and 3 replications was conducted. *Piper colubrinum*, *piper betle* large leaves and *piper betle* small leaves were used as rootstocks materials. They are being grafted by *piper nigrum* as scions in two grafting techniques: Splice graft and Cleft graft. Growth parameters such as successful rate (%), scion height (cm), scion diameter (mm), number of leaves and scion dry biomass (g/graft) were recorded after 30 days, 45 days, 60 days, 75 days and 90 days of grafting. The results showed that Cleft graft was better than Splice graft. *Piper nigrum* grafted on *piper colubrinum* by Cleft technique showed highest growth parameter as well as dry weight.

Keywords: *Piper nigrum*, *piper colubrinum*, *piper betle*, grafting

Introduction

Black pepper (*piper nigrum*) is one of main crops in Vietnam. Annually, the crop generates million of US dollars to Vietnam economy. In crop year 2019, it is estimated that Vietnam will export around 270,000 tons and becomes the biggest pepper producer in the world ^[1]. At the household level, the crop provides livelihoods for million people in the Central Highlands and South-eastern regions, Vietnam ^[2].

However, in recent years farming has been facing many challenges. A significant drop in pepper price resulted in low incomes to farmers, profits cannot cover production costs. Moreover, soil borne diseases such as Quick wilt disease and foot rot caused by *Phytophthora capsici* has killed thousands of hectares of pepper plantation ^[3].

Phytophthora capsici control by chemical fungicides seems to be less effectiveness and increase production costs as well as resulting in concerns about food safety. Therefore, new approaches and strategies are developed to control the fungi as well as enhance crop growth and productivity ^[3].

Many research works have concluded *piper colubrinum* as *phytophthora capsici* and *Meloidogyne incognita* resistance plant while *piper betle* is considered a *phytophthora capsici* tolerance plants ^[4]. These plants can be used as rootstock materials in grafting, a potential technique to control *phytophthora capsici* in black pepper plantation.

However, which grafting techniques show the best compatibility and initial growth have not been fully understood. The objective of this study is to investigate suitable grafting techniques and compatible grafts between *piper* species.

Materials and methods

Root stock preparation: Root stock cuttings of *Piper colubrinum*, *Piper betle* large leaves and *Piper betle* small leaves are harvested from free diseased gardens. Then, they are planted in the pots (14 cm in diameter and 25 cm in length) which are filled with potting mixtures consisting of top soil, cow dung, coir pit and river sand (ratio of 1:1:1:1 in volume).

The pots are placed in greenhouse and watered every two days. After 4 months of planting, cuttings are 4 to 5 leaves, and around 45 cm in length, they are ready for the experiment.

Scion preparations: semi-hardwood shoots of black pepper (*Piper nigrum*) are used as scions. Two nodes of shoots are collected from free disease gardens, then they are kept in moist condition.

Experiment design

A two factor experiment is used for this study. Factor A (root stocks): *Piper colubrinum*, *Piper betle* large leave, *Piper betle* small leaves. Factor B (grafting techniques): Splice graft and Cleft graft.

There are six treatments (Table 1), 30 pots for each treatment x 6 treatments x 3 replications = 540 pots.

Table 1: Details of six treatments for the experiment

Treatments	Root stock	Scion	Grafting techniques
Treatment 1	<i>Piper colubrinum</i>	<i>Piper nigrum</i>	Splice graft
Treatment 2	<i>Piper colubrinum</i>	<i>Piper nigrum</i>	Cleft graft
Treatment 3	<i>Piper betle</i> large leaves	<i>Piper nigrum</i>	Splice graft
Treatment 4	<i>Piper betle</i> large leaves	<i>Piper nigrum</i>	Cleft graft
Treatment 5	<i>Piper betle</i> small leaves	<i>Piper nigrum</i>	Splice graft
Treatment 6	<i>Piper betle</i> small leaves	<i>Piper nigrum</i>	Cleft graft

Data collection

Success rate (%): record all pots, 15 days interval after grafting.

Scion height (cm), scion diameter (mm), number of leaves: all survival grafts are recorded, 15 days interval after grafting.

Scion dry weight (g): 30 grafts per treatment (180 grafts in total) are randomly chosen. Then, scions are careful separated. Finally, scions are dried at 105°C during 10 hours until unchanged weight. Finally, they are measured by a balance.

Data analysis

Anova two factors analysis is used to identify differences between means and interactions between two factors.

Results

Success rate of grafts

Success rate of grafts after 30 days, 45 days, 60 days, 75 days and 90 days of grafting were showed at table 2. In general, there were significant differences between root stock materials and grafting techniques. *Piper nigrum* grafted in *Piper colubrinum* showed highest rate of success compare to *Piper betle* large leaves and *Piper betle* small leaves. In terms of grafting techniques, Cleft gave higher success rate than Splice Graft.

The data also showed that *Piper nigrum* grafted in *piper colubrinum* used Cleft method show highest rate of success.

The successful rate of grafts decreased following by days after grafting. The highest rate could be found after 30 days, these figures drop after 90 days of grafting.

Scion dry weight

Cleft graft showed highest dry weight in comparison to Splice graft. Dry weight measured in grafts used *Piper colubrinum* as rootstocks gave highest dry weight following by *Piper betle* large leaves and *Piper betle* small leaves.

Scion height

After 30 days and 45 days of grafting, *Piper nigrum* grafted on *Piper colubrinum* showed highest height growth, following by *Piper betle* large leaves and *Piper betle* small leaves. However, the period from 60 days to 90 days after being grafted the scion height of *Piper colubrinum* and *Piper betle* large leaves were similar compared to *Piper betle* small leaves.

Regarding to grafting techniques, Cleft method gave better scion height growth than Splice method.

Scion diameter

Scion diameter is one of growth characteristics to identify how fast growth in different grafts. Overall, from 30 days to 75 days after grafting, scion diameter of *Piper nigrum* grafted on *Piper colubrinum* was biggest, following by *Piper betle* large leaves and *Piper betle* small leaves. On the other hand, at 90 days of grafting there was no significant difference. Splice and Cleft techniques did not give different growth in scion diameter.

Number of leaves

In terms of grafting techniques, young plants grafted by Cleft technique produced more leaves than Splice technique (not significant difference at $\alpha = 0.05$). *Piper nigrum* being grafted on *Piper colubrinum* and *Piper betle* large leaves showed better leave growth than *Piper betle* small leaves.

Table 2: Success rates after grafting

	Root stocks (A)	Grafting techniques (B)		Mean (A)
		Splice graft	Cleft graft	
After 30 days of grafting	<i>Piper colubrinum</i>	86,66 ^c	97,00 ^a	91,83 ^a
	<i>Piper betle</i> large leave	85,33 ^c	90,33 ^b	87,83 ^b
	<i>Piper betle</i> small leave	81,33 ^d	87,00 ^c	84,16 ^c
	Mean (B)	84,44 ^b	91,44 ^a	
	CV(%) = 1,13; F _A [*] ; F _B [*] ; F _{A*B} [*]			
After 45 days of grafting	<i>Piper colubrinum</i>	82,66	93,33	88,00 ^a
	<i>Piper betle</i> large leave	81,66	87,00	84,33 ^b
	<i>Piper betle</i> small leave	77,66	84,33	81,00 ^c
	Mean (B)	80,66 ^b	88,22 ^a	

		CV(%) = 2,15; F _A [*] ; F _B [*] ; F _{A*B} ^{ns}		
After 60 days of grafting	<i>Piper colubrinum</i>	79,66	88,00	83,83 ^a
	<i>Piper betle</i> large leave	78,33	83,00	80,66 ^b
	<i>Piper betle</i> small leave	76,00	79,00	77,50 ^c
	Mean (B)	78,00 ^b	83,33 ^a	
		CV(%) = 2,41; F _A [*] ; F _B [*] ; F _{A*B} ^{ns}		
After 75 days of grafting	<i>Piper colubrinum</i>	78,00	84,66	81,33 ^a
	<i>Piper betle</i> large leave	75,66	80,66	78,16 ^b
	<i>Piper betle</i> small leave	72,66	76,33	74,50 ^c
	Mean (B)	75,44 ^b	80,55 ^a	
		CV(%) = 1,67; F _A [*] ; F _B [*] ; F _{A*B} ^{ns}		
After 90 days of grafting	<i>Piper colubrinum</i>	75,66 ^c	81,66 ^a	78,66 ^a
	<i>Piper betle</i> large leave	73,33 ^d	78,00 ^b	76,66 ^b
	<i>Piper betle</i> small leave	70,00 ^c	72,66 ^d	71,33 ^c
	Mean (B)	73,00 ^b	77,44 ^a	
		CV(%) = 1,32; F _A [*] ; F _B [*] ; F _{A*B} [*]		

Different letters showed differences in means at P_{0.05}; ns = not significant differences

Table 3: Scion height after grafting

Days after grafting	Root stocks (A)	Grafting techniques (B)		Mean (A)
		Splice graft	Cleft graft	
After 30 days of grafting	<i>Piper colubrinum</i>	4,73 ^{bc}	7,70 ^a	6,21 ^a
	<i>Piper betle</i> large leave	5,03 ^b	3,30 ^{bcd}	4,16 ^b
	<i>Piper betle</i> small leave	2,26 ^d	3,16 ^{cd}	2,71 ^c
	Mean (B)	4,01	4,72	
		CV(%) = 20,96; F _A [*] ; F _B ^{ns} ; F _{A*B} [*]		
After 45 days of grafting	<i>Piper colubrinum</i>	9,03	10,66	9,85 ^a
	<i>Piper betle</i> large leave	7,8	6,03	6,91 ^b
	<i>Piper betle</i> small leave	3,70	5,26	4,48 ^c
	Mean (B)	6,84	7,32	
		CV(%) = 20,29; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}		
After 60 days of grafting	<i>Piper colubrinum</i>	12,86 ^{bc}	16,36 ^a	14,61 ^a
	<i>Piper betle</i> large leave	15,23 ^{ab}	10,33 ^{cd}	12,78 ^a
	<i>Piper betle</i> small leave	5,73 ^c	9,43 ^d	7,58 ^b
	Mean (B)	11,27	12,04	
		CV(%) = 14,47; F _A [*] ; F _B ^{ns} ; F _{A*B} [*]		
After 75 days of grafting	<i>Piper colubrinum</i>	18,36 ^{bc}	22,33 ^a	20,35 ^a
	<i>Piper betle</i> large leave	21,66 ^{ab}	17,36 ^c	19,51 ^a
	<i>Piper betle</i> small leave	10,83 ^d	17,7 ^c	14,26 ^b
	Mean (B)	16,95 ^b	19,13 ^a	
		CV(%) = 11,28; F _A [*] ; F _B [*] ; F _{A*B} [*]		
After 90 days of grafting	<i>Piper colubrinum</i>	26,70 ^b	27,33 ^b	27,01 ^a
	<i>Piper betle</i> large leave	30,73 ^a	24,73 ^{bc}	27,73 ^a
	<i>Piper betle</i> small leave	21,73 ^c	24,73 ^{bc}	23,23 ^b
	Mean (B)	26,38	25,60	
		CV(%) = 6,98; F _A [*] ; F _B ^{ns} ; F _{A*B} [*]		

Different letters showed differences in means at P_{0.05}; ns = not significant differences

Table 4: Scion diameter

Days after grafting	Root stocks (A)	Grafting techniques (B)		Mean (A)
		Splice graft	Cleft graft	
After 30 days of grafting	<i>Piper colubrinum</i>	2,30	2,26	2,28 ^a
	<i>Piper betle</i> large leave	2,10	1,83	1,96 ^{ab}
	<i>Piper betle</i> small leave	2,00	1,80	1,90 ^b
	Mean (B)	2,13	1,96	
		CV(%) = 12,04; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}		
After 45 days of grafting	<i>Piper colubrinum</i>	2,83	2,90	2,86 ^a
	<i>Piper betle</i> large leave	2,73	2,66	2,70 ^{ab}
	<i>Piper betle</i> small leave	2,46	2,60	2,53 ^b
	Mean (B)	2,67	2,72	
		CV(%) = 7,05; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}		
After 60 days of grafting	<i>Piper colubrinum</i>	3,03	3,10	3,06 ^a
	<i>Piper betle</i> large leave	2,83	2,90	2,86 ^b
	<i>Piper betle</i> small leave	2,60	2,70	2,65 ^c
	Mean (B)	2,82	2,90	
		CV(%) = 4,63; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}		
After 75 days of grafting	<i>Piper colubrinum</i>	3,16	3,30	3,23 ^a
	<i>Piper betle</i> large leave	3,03	3,13	3,08 ^b
	<i>Piper betle</i> small leave	2,86	2,90	2,83 ^c
	Mean (B)	3,02	3,11	
		CV(%) = 3,20; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}		

After 90 days of grafting	<i>Piper colubrinum</i>	3,46	3,53	3,50
	<i>Piper betle</i> large leave	3,56	3,43	3,50
	<i>Piper betle</i> small leave	3,23	3,26	3,25
	Mean (B)	3,42	3,41	
CV(%) = 6,60; F _A ^{ns} ; F _B ^{ns} ; F _{A*B} ^{ns}				

Different letters showed differences in means at P_{0.05}; ns = not significant differences

Table 5: Number of leaves

Days after grafting	Root stocks (A)	Grafting techniques (B)		Mean (A)
		Splice graft	Cleft graft	
After 45 days of grafting	<i>Piper colubrinum</i>	1,86	1,76	1,81 ^a
	<i>Piper betle</i> large leave	2,23	1,70	1,96 ^a
	<i>Piper betle</i> small leave	1,23	1,30	1,26 ^b
	Mean (B)	1,77	1,58	
CV(%) = 12,22; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}				
After 60 days of grafting	<i>Piper colubrinum</i>	2,16	2,00	2,08 ^a
	<i>Piper betle</i> large leave	2,63	2,53	2,58 ^b
	<i>Piper betle</i> small leave	1,63	1,63	1,63 ^c
	Mean (B)	2,14	2,05	
CV(%) = 8,95; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}				
After 75 days of grafting	<i>Piper colubrinum</i>	2,40	3,06	2,73 ^a
	<i>Piper betle</i> large leave	2,93	2,96	2,95 ^a
	<i>Piper betle</i> small leave	1,96	2,06	2,01 ^b
	Mean (B)	2,43 ^b	2,70 ^a	
CV(%) = 9,85; F _A [*] ; F _B [*] ; F _{A*B} ^{ns}				
After 90 days of grafting	<i>Piper colubrinum</i>	3,23	3,83	3,53 ^a
	<i>Piper betle</i> large leave	3,66	3,40	3,53 ^a
	<i>Piper betle</i> small leave	2,33	2,60	2,46 ^b
	Mean (B)	3,07	3,27	
CV(%) = 11,37; F _A [*] ; F _B ^{ns} ; F _{A*B} ^{ns}				

Different letters showed differences in means at P_{0.05}; ns = not significant differences

Table 6: Scion dry weight

Root stocks (A)	Grafting techniques (B)		Mean (A)
	Splice graft	Cleft graft	
<i>Piper colubrinum</i>	3,21	3,75	3,48 ^a
<i>Piper betle</i> large leave	2,45	3,60	3,03 ^b
<i>Piper betle</i> small leave	2,89	3,35	3,12 ^b
Mean (B)	2,85 ^a	3,57 ^b	

Different letters showed differences in means at P_{0.05}; ns = not significant differences

Discussions

Grafting in black pepper has been carried out by several studies. Albuquerque (1969) concluded that only *Piper colubrinum* showed highest success to 95 per cent. In this study the author also used folk method which is also known as Cleft graft [5].

Alternative grafting techniques have been used such as wedge, saddle, Splice, Modified Splice, Tongue, Double rootstock. The highest success rate was found in double rootstock methods while Wedge (Cleft) and Splice were only 62.00% and 60% [6]. The different success rate was explained by Vanaja, T., *et al.*, 2007. The author concluded that different months of grafting will result in different success rate. In addition, *Piper nigrum* varieties also affect grafting survivals [7].

Conclusion

Cleft graft was better than Splice graft. *Piper nigrum* grafted on *piper colubrinum* by Cleft technique showed highest growth parameter as well as dry weight.

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