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GRAFT-TRANSMISSIBLE VIRUS RESISTANCE IN TOBACCO (*Nicotiana benthamiana*)

Dang Minh Tam, Neena Mitter

ABSTRACT

RNA silencing, which is triggered by double stranded (ds)RNA in conserved mechanism in eukaryotes, involved in defense against viruses and transposons. The dsRNA is processed into 21-25 nt small interfering (si)RNAs by the activity of an RNase III like enzyme called Dicer. In the present investigation we studied the graft transmission of RNA silencing based resistance against Cucumber mosaic virus (CMV). Transgenic tobacco lines carrying ***dsRNA hairpin constructs*** *targeting the* ***2b gene*** *of CMV were used as rootstocks and scions. Micrografting of selected lines was done at seedling stage using CMV immune transgenic lines (2bihp19 and 2bihp44) as rootstocks and susceptible transgenic lines (2bihp1, 2bihp15, 2bihp24 and 2bihp47) as scions. Transmission of resistance was recorded only in case of 2bihp1 as scions grafted on 2bihp19 or 2bihp44. In all other grafts the scions remained susceptible after grafting; and in all other susceptible parent lines we could not detect any siRNAs (21nt -24nt). Therefore, the presence or absence of siRNA may not be an indicator of virus resistance. Silencing signal generated by immune rootstock is transmitted across the graft junction in 2bihp1 grafting plants, and is able to confer resistance in the susceptible scion. The level of 21-24nt small interfering (si)RNA probably play a role in systemic signal spread through the plant.*

Key words: *Cucumber mosaic virus* (CMV), dsRNA hairpin constructs, 2b gene, Micrografting, *Graft-transmissible* virus resistance

INTRODUCTION

The RNA silencing pathways that exist in plants, animals and fungi have similar genetic requirements and biochemical features. A key conserved feature is that it is triggered by double-stranded RNA (dsRNA) processed into 21-25nt small interfering (si)RNAs by the activity of an RNase III like enzyme called Dicer. The siRNA is then incorporated into a RNA-induced silencing complex (RISC), so ensuring that it specifically degrades any RNA sharing sequence similarity with the inducing dsRNA (Braden M. Roth *et al.*, 2004).

RNA silencing can provide high-level virus resistance by specific targeting of viral sequences in transgenic plants. Viruses in turn encode potent suppressors of RNA silencing leading to breakdown of resistance. In plants, RNA silencing can be induced by highly transcribed sense transgenes (Que *et al.*, 1997 and Vaucheret *et al.*, 1997) or by transgenes coding for dsRNA (Waterhouse *et al*., 1998).

Silencing capable of spreading locally and systemically could be triggered by large sense, antisense, and dsRNAs as well as double stranded siRNAs (Ulrich Klahre *et al*., 2002). In plant, spreading of silencing can be short or long distance transmission between cells. Mechanism of long distance gene silencing from rootstocks to scions by micro grafting in *Arabidopsis* has investigated and shown that RNA interference (RNAi) and chromatin silencing components were involved in graft-transmissible gene silencing in scions (Brosnan *et al*, 2007) .

The mobile RNA silencing signal can travel and induce silencing in distant plant parts. The short-range movement of RNA silencing was studied in *Nicotiana benthamiana* by direct injection of *Agrobacterium* into the leaf (agroinfiltration). The result was transient gene expression by spreading of local silencing from 10 to 15 cells away the initial site of induction (Himber *et al*., 2003).

Transgenic tobacco plants were generated carrying dsRNA constructs targeting 2b and Coat protein gene of *Cucumber mosaic virus* (CMV). The independent transgenic lines were checked by PCR for the presence of the transgene. The positive transgenes were then challenged with CMV. In case of both the genes more than 75% plants showed resistance to CMV. Grafting experiments were then conducted to check the transmission of resistance across a graft junction from a resistant rootstock to a wild type non – transgenic scion. It was observed that the wild type scion remained susceptible even after grafting. The aims of the present investigation were to study the transfer of resistance across graft junction if transgenic susceptible lines were used as scion, and study any change in the level of siRNAs in the susceptible scions after grafting.

OBJECTIVES

- Micrografting of tobacco lines carrying the 2b gene as hairpin construct with susceptible lines as scions and immune lines as rootstocks.
- siRNA analysis of the grafted scions as well as parent lines before challenging them with CMV.
- Test the resistant status of the scions after grafting by subjecting them to challenge with CMV.

MATERIALS AND METHODS

Plant materials:

In this experiment of the project, we started with the 2bihp transgenic tobacco lines resources. T1 seeds of the transgenic lines carrying hairpin construct 2bihp were provided:

2bihp CMV immune lines:
- 2bihp (19) (single copy)
- 2bihp (6) (two copies)
- 2bihp (44) (multiple copies)

2bihp CMV susceptible lines:
- 2bihp (1) (single copy)
- 2bihp (24) (two copies)
- 2bihp (15) (multiple copy)
- 2bihp (47) (single copy)

W38 was used as non-transgenic plant control.

The experiment treatment:

Table 1: The plants were grafted randomly with 8 grafting plants per each plate, 3 replications. A total of 360 grafting were done at the seedling stage.

No.	Treatments	Plates for grafting	Plants for grafting/plate
1	2bihp (1) / 2bihp (6)	3	8
2	2bihp (15) / 2bihp (6)	3	8
3	2bihp (24) / 2bihp (6)	3	8
4	2bihp (47) / 2bihp (6)	3	8
5	2bihp (1) / 2bihp (19)	3	8
6	2bihp (15) / 2bihp (19)	3	8
7	2bihp (24) / 2bihp (19)	3	8
8	2bihp (47) / 2bihp (19)	3	8
9	2bihp (1) / 2bihp (44)	3	8
10	2bihp (15) / 2bihp (44)	3	8
11	2bihp (24) / 2bihp (44)	3	8
12	2bihp (47) / 2bihp (44)	3	8
13	WT38 / 2bihp (6)	3	8
14	WT38 / 2bihp (19)	3	8
15	WT38 / 2bihp (44)	3	8
Total		45 plates	360 plants

Primers used for 2b gene:

- CMV 2b XhoI F
5'- **CCC TCG AGA TGT ATG TAA TTG AAC GTA GGT GCA**- 3'

- CMV 2b SalI R
5'- **CCC GTC GAC TCA AAA GCA CCT TCC GCC CA** -3'

2b primers give about 330bp band

Virus challenging and buffer:

- *Cucumber Mosaic Virus* (CMV-isolate 207)
- Buffer used to inoculate: 10mM phosphate, buffer pH=7.4 + 0.1% sodium sulfide.

Methods:

- Growing the seeds of the selected lines in Petri dishes on minimal media.
- Micro-grafting 2bihp plants: 2bihp susceptible lines and W38 as scions (shoots) and 2bihp immune lines as rootstocks.
- Transfer all the viable grafted plants to soil in the glasshouse condition.
- DNA extraction (by mini CTAB method **)** from each of the grafted plant moved to the seedling tray to confirm presence of transgene by PCR using 2b specific primers. The plants which tested positive for the transgene were moved into individual pots and maintained in the glasshouse for further analysis. Leaf material from each plant was collected for RNA extraction before challenging them with CMV. Total RNA was extracted from plant tissues by TRIzol method. Run the gel to check for total RNAs and then small RNAs were extracted from total RNAs.
- Test for the resistance status of the parent lines as well as grafted scions by challenging with virus *Cucumber mosaic virus* (CMV-207) in the glasshouse.
- RNA assay to check immune plants expression through the Northern Blot analysis of small RNAs.

RESULTS AND DISCUSSIONS

Micro-grafting result:

Tobacco plants at seedling stage were used for micrografting. Transgenic tobacco lines carrying intron spliced hairpin construct targeting the 2b gene of CMV were used as rootstocks and scions. The susceptible scions (2bihp 1, 2bihp 15, 2bihp 24, 2bihp 47) as well as wild type non transgenic W38 was grafted on transgenic immune rootstocks (2bihp 19 and 2bihp 44). For each combination, 24 grafts were made. Only grafts which formed a junction and remained viable were transferred into seedling trays in the glass house. The results of successful grafted plants are shown in Table 2.

Table 2: Number of grafted plants were transferred to the tray in glass-house.

No.	Treatments	Number of grafted plants were transferred to the tray in glass-house
1	2bihp (1) / 2bihp (19)	7
2	2bihp (15) / 2bihp (19)	4
3	2bihp (24) / 2bihp (19)	5
4	2bihp (47) / 2bihp (19)	9
5	2bihp (1) / 2bihp (44)	7
6	2bihp (15) / 2bihp (44)	9
7	2bihp (24) / 2bihp (44)	10
8	2bihp (47) / 2bihp (44)	4
9	WT38 / 2bihp (6)	5
10	WT38 / 2bihp (19)	10
11	WT38 / 2bihp (44)	11
Total		81

Thus out of a total of 360 grafts made, only 81 lines survived and were transferred to soil in seedling trays for further propagation.

DNA extraction and PCR to check for transgenic plants:

The grafted scions were first tested for the presence of the transgene by PCR using 2b gene specific primers. Only the plants which were found to be positive for the transgene were then transferred to individual pots. The table of the PCR positive plants is given below (Table 3). The positive plants showed the presence of 330bp applicant which was absent in wild type W38 (Figures 1, 2, 3, 4 , 5 and 6).

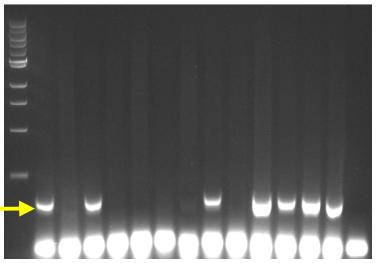

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Figure 1: DNA testing results from the grafted tobacco plants

Notes:

1. W38/2bihp(19) # 7
2. 2bihp(47)/2bihp(44) # 1
3. 2bihp(24)/2bihp(19) # 1
4. W38/2bihp(19) # 1
5. W38/2bihp(19) # 5
6. 2bihp(24)/2bihp(44) # 8
7. 2bihp(15)/2bihp(44) # 6
8. 2bihp(24)/2bihp(44) # 6
9. 2bihp (15)/2bihp(44) # 8
10. 2bihp (24)/2bihp(44) # 5
11. 2bihp(15)/2bihp(44) # 7
12. 2bihp(24)/2bihp(44) # 7

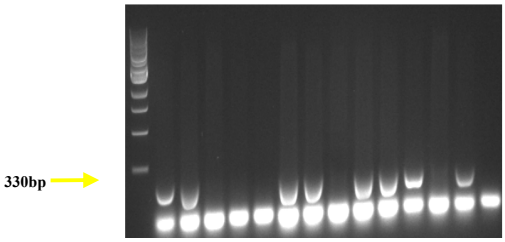

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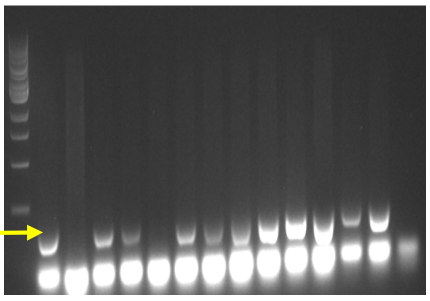
Figure 2: DNA testing results from the grafted tobacco plants

Notes:

13. 2bihp(15)/2bihp(44) # 4
14. W38/2bihp(19) # 4
15. W38/2bihp(19) # 3
16. W38/2bihp(19) # 6
17. 2bihp(15)/2bihp(44) # 1
18. 2bihp(24)/2bihp(44) # 2
19. W38
20. 2bihp(15)/2bihp(44) # 5
21. 2bihp(15)/2bihp(44) # 3
22. 2bihp(15)/2bihp(44) # 2
23. 2bihp(24)/2bihp(44) # 3
24. 2bihp(24)/2bihp(44) # 1



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Picture 3: DNA testing results from the grafted tobacco plants

Notes:

25. W38/2bihp(19) # 2
26. 2bihp(24)/2bihp(19) # 3
27. 2bihp(1)/2bihp(44) # 3
28. 2bihp(1)/2bihp(19) # 5
29. 2bihp(1)/2bihp(44) # 2
30. 2bihp(1)/2bihp(44) # 7
31. 2bihp(1)/2bihp(44) # 1
32. 2bihp(15)/2bihp(19) # 2
33. 2bihp(47)/2bihp(19) # 2
34. 2bihp(47)/2bihp(19) # 7
35. 2bihp(15)/2bihp(19) # 3
36. 2bihp(47)/2bihp(19) # 4

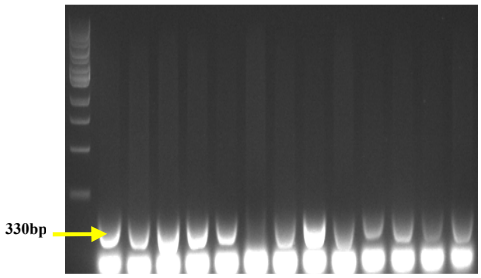

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Figure 4: DNA testing results from the grafted tobacco plants

Notes:

37. 2bihp(24)/2bihp(19) # 2
38. 2bihp(47)/2bihp(19) # 5
39. 2bihp24
40. 2bihp(47)/2bihp(19) # 1
41. W38/2bihp(19) # 10
42. 2bihp(1)/2bihp(44) # 6
43. 2bihp(47)/2bihp(44) # 2
44. 2bihp(1)/2bihp(19) # 4
45. 2bihp(24)/2bihp(19) # 4
46. 2bihp(1)/2bihp(19) # 6
47. 2bihp(1)/2bihp(44) # 5
48. 2bihp(1)/2bihp(44) # 4

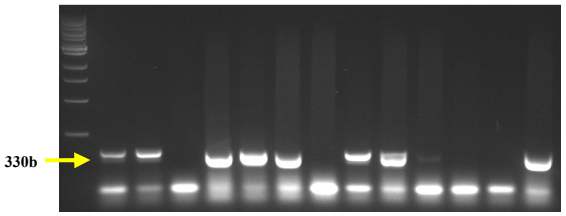

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Figure 5: DNA testing results from the grafted tobacco plants

Notes:
49. 2bihp(15)/2bihp(19) # 1
50. W38/2bihp(19) # 3
51. 2bihp(47)/2bihp(19) # 6
52. 2bihp(47)/2bihp(19) # 3
53. 2bihp(1)/2bihp(19) # 2
54. W38/2bihp(19) # 5
55. 2bihp(1)/2bihp(19) # 3
56. 2bihp(1)/2bihp(19) # 1
57. W38/2bihp(19) # 6
58. W38/2bihp(19) # 9
59. W38/2bihp(19) # 8
60. 2bihp(24)/2bihp(44) # 4

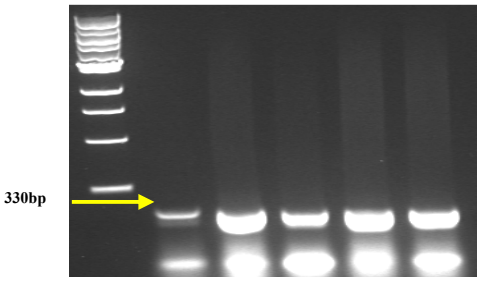

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Figure 6: DNA testing results from the tobacco parent plants

+: positive control
- : negative control

Notes:
61. 2bihp47
62. 2bihp15
63. 2bihp19
64. 2bihp1
Positive control: 2bihp44

Table 3: PCR results of positive plants from 1% agarose gel electrophoresis.

No	Grafting plants (scion / rootstock)	Ratio of positive plants
1	2bihp(24)/2bihp(44)	5/8
2	2bihp(15)/2bihp(44)	8/8
3	2bihp(47)/2bihp(44)	2/2
4	2bihp(1)/2bihp(44)	7/7
5	2bihp(24)/2bihp(19)	3/4
6	2bihp(15)/2bihp(19)	3/3
7	2bihp(47)/2bihp(19)	7/7
8	2bihp(1)/2bihp(19)	5/6
9	W38/2bihp(19)	Negative 100%
10	2bihp(1) # 1	Parents: positive 100%
11	2bihp(15) # 1	
12	2bihp(24) # 1	
13	2bihp(47) # 1	
14	2bihp(19) # 1	
15	W38	Negative

The PCR results of positive plants by 1% gel electrophoresis (Table 3) showed that 100% of parent plants were positive (transgenic). The non-transgenic plants (W38) and grafted plants of them (W38/2bihp19) were 100% of negative results. The results of micro-grafted plants (2bihp15/2bihp44, 2bihp47/2bihp44, 2bihp1/2bihp44, 2bihp15/2bihp19, 2bihp47/2bihp19) were positive (100%). However, 2bihp24/2bihp44, 2bihp24/2bihp19 and 2bihp1/2bihp19 were not completely positive in results. Their ratios were 5/8, 3/4 and 5/6 respectively.

From each of the transgenic grafted scion as well as W38 scions, leaf samples were collected for RNA extraction before challenging them with the virus. The grafted plants as well as parents were inoculated with CMV- isolate 207 in the glasshouse. Non- transformed W38 plants were challenged as positive control for virus replication.

The symptom expression after inoculating with Cucumber Mosaic Virus (CMV-isolate 207)

When the tobacco grafting plants were inoculated with CMV-207 in the glasshouse CMV symptoms

were observed after 10 days of challenging with the virus. The symptoms recorded were initial vein clearing followed by development of characteristic mosaic with dark green islands. The symptoms first appeared on young leaves. (Figure 7A and 7B). The classic mosaic was observed after 2 weeks of challenging (Figure 7C).

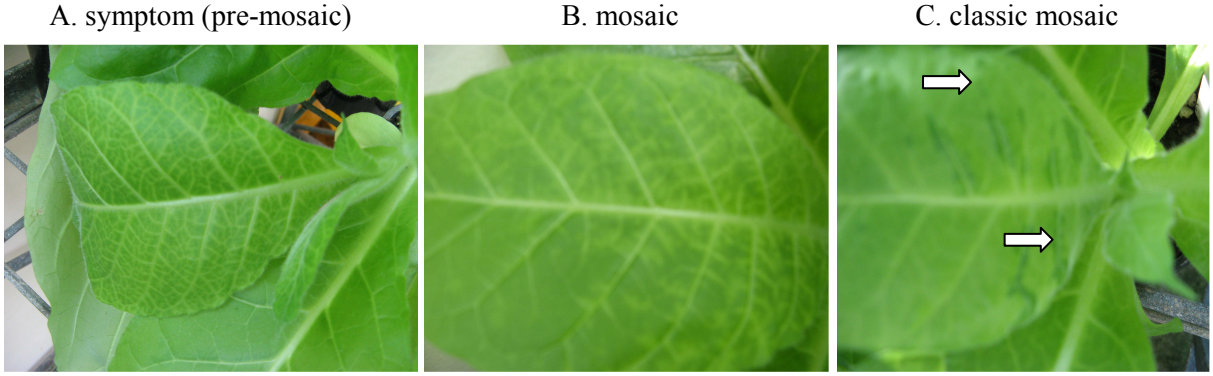

Figure 7: Phenotype of *Cucumber Mosaic Virus* (CMV-207) infection. A. symptom (pre-mosaic), B. mosaic, C. classic mosaic.

The symptom results were recorded after 10 days, 2 weeks, 4 weeks and 6 weeks of CMV-207 challenging. The transgenic parents have got both susceptible (2bihp1, 2bihp15, 2bihp24 and 2bihp47) and immune (2bihp19 and 2bihp44). The most interesting discovery was that all of the grafting plants with 2bihp1 (susceptible) as scion (shoot) resisted to CMV-207 and were normal (no symptom) in resistant phenotype; meanwhile the other scions, not 2bihp1 grafted, were recorded susceptible (Table 4)

Table 4: The resistant expression of 2bihp1 grafting lines (2bihp1/2bihp44 and 2bihp1/2bihp19) in comparing with their parents.

Grafting plants (scion / rootstock)	Expression	10 days after challenging with CMV	2 weeks after challenging with CMV	4 weeks after challenging with CMV	6 week after challenging with CMV
2bihp(1)/2bihp(44) # 1	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(44) # 2	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(44) # 3	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(44) # 4	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(44) # 5	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(44) # 6	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(44) # 7	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(19) # 1	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(19) # 2	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(19) # 3	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(19) # 4	-	normal (no symptom)	normal	normal	normal
2bihp(1)/2bihp(19) # 6	-	normal (no symptom)	normal	normal	normal
2bihp(1)	+	mosaic	classic mosaic	classic mosaic	classic mosaic
2bihp(19)	-	normal (no symptom)	normal	normal	normal
2bihp(44)	-	normal (no symptom)	normal	normal	normal

From the table, it is evident that transfer of resistance was observed only in case of 2bihp1 as scion (see the resistance on Figure 8). All other grafts showed CMV symptoms after 2 – 4 weeks post inoculation. Thus to understand the mechanisms, further siRNA analysis was done from selected plants. Leaf material for this had been collected earlier.

2bihp1 (susceptible) 2bihp1 / 2bihp19 (resistance) 2bihp1 / 2bihp44 (resistance)

Figure 8: Virus resistant expression of 2bihp1 grafting lines (immune) compared with 2bihp1 (susceptible) after 3 weeks of challenging with CMV-207.

Total RNA extraction and run a gel to check for the quantity of RNA

The RNA extraction from a leaf collected before inoculated with *Cucumber Mosaic Virus* (CMV-207) was checked for the quantity and quality of total RNA on the 1% agarose gel electrophoresis. The results showed the presence of both high and low molecular weight RNA (Figure 9).

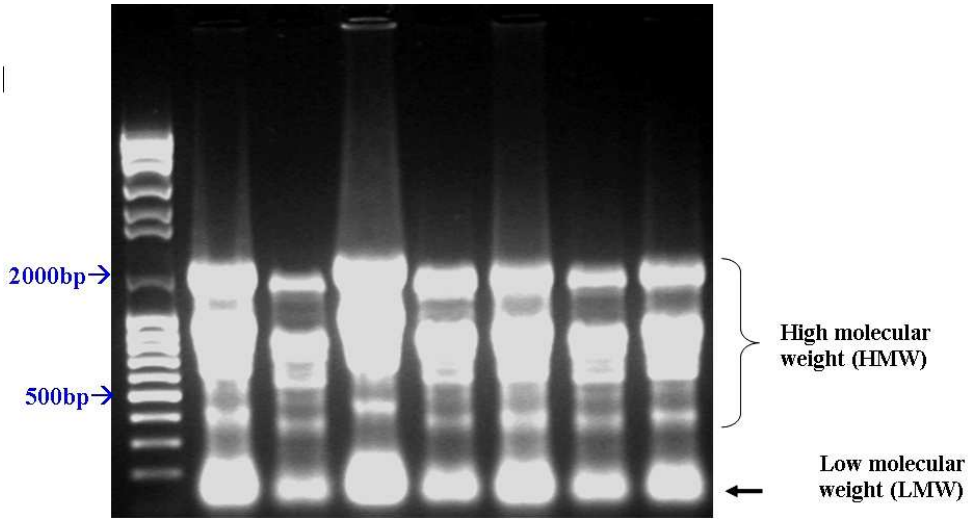

Figure 9: Total RNA extraction for good quantity of RNA on 1% agarose gel.

Small RNA extraction and small interfering (si)RNA assay

Low molecular weight RNA was precipitated from the total RNA and once again checked on Agarose gel for quality and quantity. The results are shown on Figure 10.

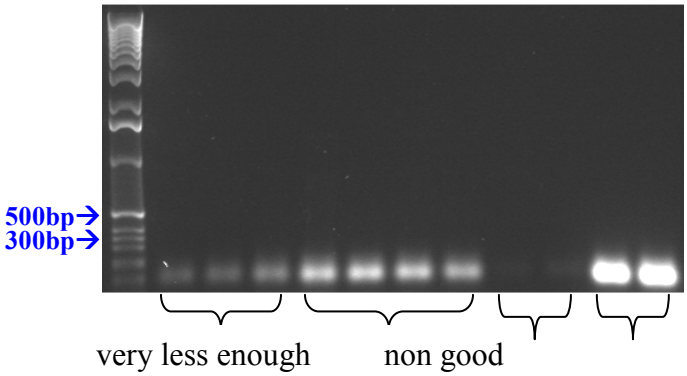

Figure 10: Small RNA quantity results from the 1% gel electrophoresis

Low molecular weight (LMW) RNA was then used to detect siRNA by polyacrylamide gel electrophoresis in Northern Blots.

Small interfering (si) RNA assay – Northern Blots

Equal quantity of LMW RNAs was loaded in 17% polyacrylamide gel electrophoresis and transferred to nylon membranes. Autoradiography revealed the presence of 21-24 nucleotide siRNAs. The immune lines (2bihp19 and 2bihp44) and the grafted plants of these with 2bihp1(susceptible lines) as scions, which showed resistance to CMV after grafting, showed small interfering RNAs (siRNAs) from 21 to 24 nucleotides. The susceptible parent lines (2bihp1) also had siRNAs at low level. After grafting (2bihp1/2bihp19 and 2bihp1/2bihp44), the 2bihp1 scions showed the presence of both 21nt and 24nt siRNAs at high level (Figure 11).

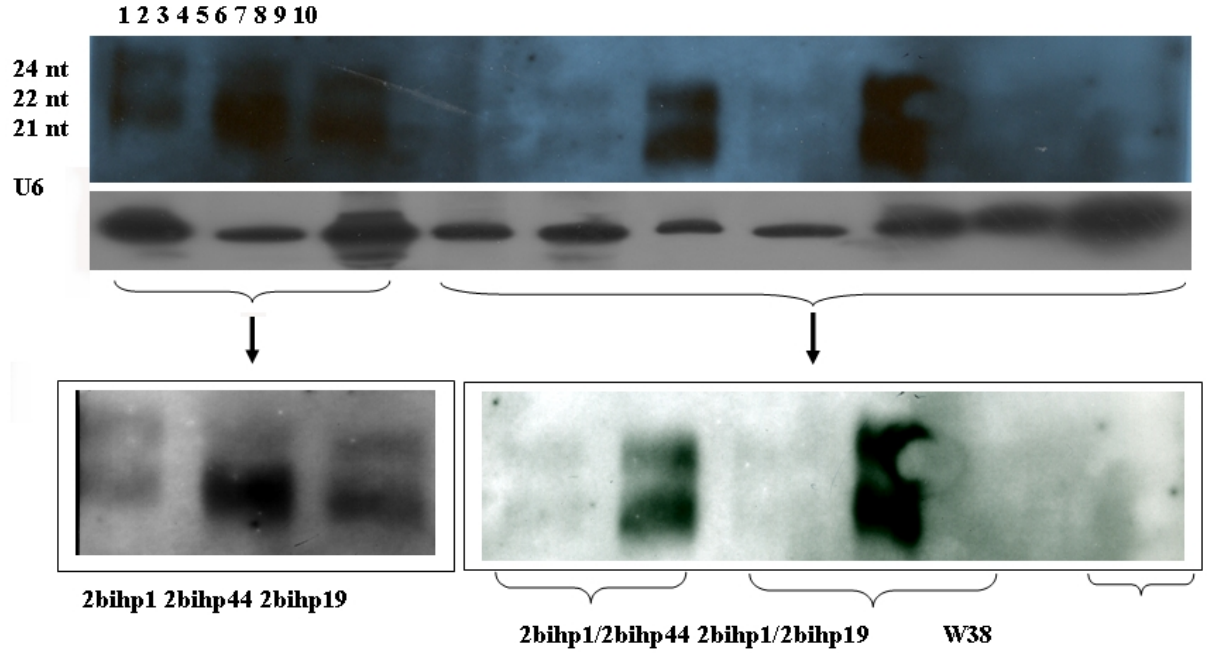

Figure 11: Northern blot analysis of siRNAs expression on 2bihp1, parents and grafting plants of 2bihp1 (2bihp1/2bihp44 and 2bihp1/2bihp19) and autoradiography showing equal loading analysis with U6 specific probe for the respective blots

The immune lines expressed on Figure 11 (2bihp44, 2bihp19 and the grafting 2bihp1/2bihp44 and 2bihp1/2bihp19), have got siRNA from 21 to 24 nucleotides. The level of 21 and 22 nucleotides was higher than that of 23 and 24 nucleotides clearly at 2bihp44 and 2bihp19. Meanwhile the susceptible lines (2bihp1) got 21-24 nucleotides and W38 did not form any siRNA or dsRNA. As a result, the virus resistance may not be indicated by the present or absence of siRNA. However, it related to the level of 21-22 nucleotides in this experiment. In addition, silencing signal generated by immune rootstock is transmitted across the graft junction in 2bihp1 grafting plants, and is able to confer resistance in the susceptible scion. Further, the presence of transgenes in the scions of 2bihp1 is required for the reception of the signal. Therefore, the transmission of silencing in terms of CMV resistance depends not only on the resistant rootstock but also on the susceptible scion (if there were the transgenes presented in the scion).

The 2bihp47, 2bihp24, 2bihp15 were susceptible and the scions remained susceptible as well after grfating. These lines did not show any siRNA. This means that either there were no dsRNA formation in these transgenic lines or the level of siRNAs was too low to be detected (Figure 12).

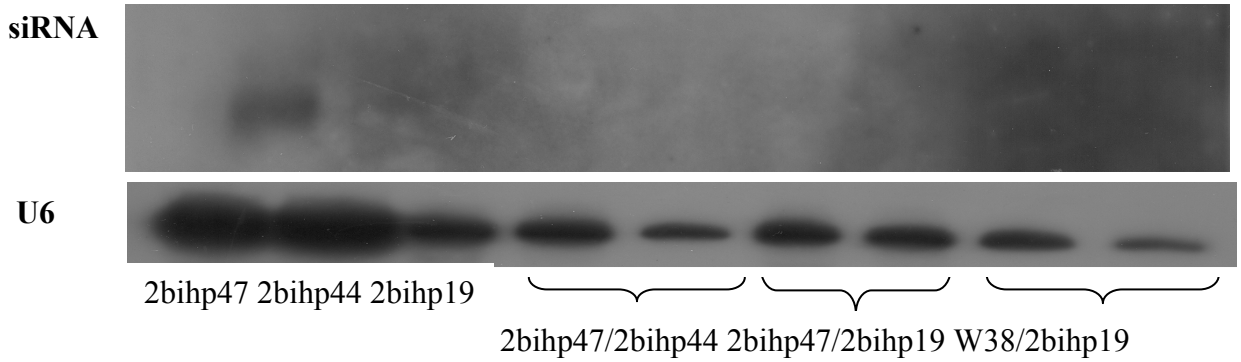

Figure 12: Northern blot analysis of siRNAs expression of susceptible grafted lines of 2bihp47 and W38, and their parents; and autoradiography showing equal loading analysis with U6 specific probe for the respective blots

siRNAs were detected in the immune parents (2bihp19 and 2bihp44 as rootstocks) but absent in the susceptible parents (2bihp47, 2bihp15 and 2bihp24 as scions) and their grafted plants, except just only one line of the graft 2bihp15/2bihp19#1 (which has the immune phenotype). Non-transformed tobacco plants (W38), remained susceptible after grafting and had no siRNAs detected (Figure 12), indicated that the presence of transgene in the scion may be required for reception of the mobile silencing signal as the present navigation finding in 2bihp1(Figure 11).

The appearance of siRNA in 2bihp1 may be one of the factors that triggered the virus resistant mechanism on the grafting plants with a silencing signal which could be caused by themselves or moved from the immune rootstocks to susceptible scions. This result looks like the report of Ulrich Klahre *et al.* 2002: "siRNAs themselves or intermediates induced by siRNAs could comprise silencing signals and are generated in a self-amplifying fashion".

CONCLUSIONS

The identity of RNA silencing signals remains unknown. However, silencing signal generated by immune rootstock is transmitted across the graft junction and is able to confer resistance in the susceptible scion. The transmission of silencing in terms of CMV resistance depends not only on the resistant rootstock but also on the presence of dsRNA on susceptible scion.

The presence of transgene or the long dsRNA in the scion, which is the precursor of siRNAs is required for the reception of the mobile silencing signals. In addition, the level of 21-22nt small interfering (si)RNA probably play a role in systemic signal spread through the plant.

The molecular nature of the mobile signal also remains to be determined. The future aims will be to identify genes involved in transmission of long-distance gene silencing from the root in *Tobacco*, to further define genes involved in perception of the signal in the shoot and to determine the molecular nature of the mobile silencing signal.

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Nghiên cứu tính kháng virus của cây thuốc lá *(Nicotiana benthamiana)* theo kỹ thuật "Graft-Transmissible"

Phân tử RNA gây im lặng gen, từ phân tử dây kép (ds)RNA theo cơ chế bảo thủ của sinh vật eukaryotes, có trong cơ chế tự bảo vệ của cây chống lại các viruses và transposons. Chính dsRNA này được thực hiện ở kích thước phân tử 21-25 nt hình thành nên phân tử (si)RNAs nhờ hoạt động của enzyme RNase III mà người ta thường gọi là Dicer. Trong nghiên cứu này, chúng tôi đã thực hiện kỹ thuật "graft transmission" của một phân tử RNA silencing trên cơ sở tạo ra tính kháng virus gây bệnh khảm trên dưa chuột: *Cucumber mosaic virus* (CMV). Các dòng thuốc là được chuyển gen như vậy mang **dsRNA hairpin constructs** nhằm kiểm soát **2b gene** của CMV như là những vật liệu làm gốc ghép và mắt ghép. Kỹ thuật "Micrografting" các dòng đã chọn lọc được thực hiện trong giai đoạn cây con, trên các dòng miễn nhiễm với CMV do chuyển gen mà có (2bihp19 và 2bihp44) như gốc ghép; và các dòng chuyển gen nhiễm bệnh (dòng 2bihp1, 2bihp15, 2bihp24 và 2bihp47) làm mắt ghép. Sự kiện chuyển tính kháng được ghi nhận chỉ xảy ra trong trường hợp dòng 2bihp1 làm mắt ghép được tháp trên dòng 2bihp19 hoặc 2bihp44. Trong tất cả những gốc ghép khác, các dòng mắt ghép vẫn duy trì tính trạng nhiễm. Trong tất cả các dòng bố mẹ nhiễm bệnh, chúng tôi không phát hiện bất cứ phân tử siRNAs (21nt -24nt) nào cả. Do đó, sự có mặt hoặc vắng mặt của siRNA có thể không trở thành một chỉ dẫn về tính kháng bệnh virus này. Tín hiệu im lặng được phát sinh bởi gốc ghép miễn nhiễm được chuyển vào cây ghép ở dòng 2bihp1, và có thể lien quan đến tính kháng trong các mắt ghép bị nhiễm bệnh. Kích cỡ phân tử 21-24nt của (si)RNA can thiệp có thể đóng vai trò quan trọng trong hệ thống tín hiệu của cây.