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大豆모자이크바이러스에 의한 結莢節位別 褐斑粒의 形成과 바이러스의 種子傳染

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The Relation of Soybean Seedcoat Mottling with Podding Location and Seed Transmissibility in Soybean Mosaic Virus

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ABSTRACT

Association of SMV infection in soybean plants with seedcoat mottling and with seed transmission of SMV was examined. Seedcoat mottling was increased positively in proportion to the SMV severity in variety Clark. No correlation was found between the incidence of mottled seed and the seed's position on the plant. Susceptible variety with virus symptoms gave rise to mottled seeds but only a small percentage of these mottled seeds could transmit the virus. SMV could be transmitted even by nonmottled seeds harvested from SMV infected plants in similar rate with mottled seed. It seemed that the amount of mottled seed could be used as an indicator of the amounts of SMV infection in a seed production field.

INTRODUCTION

Soybean mosaic virus(SMV) is a devastating disease in the large part of soybean growing areas. The symptom of soybean mosaic was first described by Clington[6] and reported in 1967 as being in Korea.[11] Significant yield losses,[4,8,12,14] reduction in seed quality,[12,13] decreased oil contents[3] and reduced nodulation[15] have been reported to result from the virus disease. It has been noted that seedcoat mottling is associated with the virus disease infection and the virus is transmitted through the mottled seeds.[7] Before the relationship was demonstrated,[5,7,12] earlier investigators recognized that seedcoat mottling was influenced by hereditary traits and only environmental factors.[13] Genetic control of mottling has been reported by Cooper,[2] who found the variety Merit to be immune to mottling due to a single gene, lm, which exhibited partial to complete dominance in an environment that favored expression of mottling. Recently, embryo infection in mature seed was doccumented to be an important factor affecting the seed transmissibility of SMV.[1]

Since the distribution of infected seeds by man is an obvious first step in the epidemiological pattern of the virus and that SMV occurs everywhere soybeans are grown is apparently because of seed transmission, many investigators exerted the efforts to determine the effect of seedcoat mottling to virus transmission. However, the relationship between seedcoat mottling and virus transmission is inconsistent and can be dependent upon environ-

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ment, cultivar and genotypes.[12,14] Therefore, the present study is to determine the association of SMV infection in plants with the incidence of mottled seeds, effect of seed position and pod location, and presence of infectious virus in seed of domestic soybeans.

MATERIALS AND METHODS

Twenty five plants of soybean variety Clark were grown in the field and inoculated with SMV at the second trifoliate leaf stage by conventional rubbing method. SMV severity of the plants was arbitrarily rated 1 to 5 grades based on mosaic symptom at 4-5th trifoliate leaf stage. After harvesting, number of mottled seeds and pods containing the mottled seeds were measured individually.

To determine seed transmission of SMV through the mottled seeds, SMV infectivity was identified by double agar immunodiffusion test with hypocotyl of the germinated seeds.[9] Hypocotyl of soybean was extracted by grinding in 1.5% sodium dodecyl sulfate with a motar and pestle. Agar medium containing 0.8% noble agar, 0.5% sodium dodecyl sulfate and 1% sodium azide in 0.05M Tris-HCl was prepared and then, cut wells by pressing the gel cutter in the gar. With a pasteur pipette, Soy MV-antiserum which was courtiously provided from D.E. Percifull, Plorida, U.S.A. was added to the central well and hypocotyl extracts as antigen to the peripheral wells. After incubating the dishes in a moist chamber at 24°C for 24-72 hours, reaction was observed on the fluorescent lamp.

For the growth-on test, mottled or nonmottled seeds were planted in the field and their seed transmission percentage were examined at the basis of mosaic symptom appearance at 3-4 trifoliate leaf stage.

RESULTS AND DISCUSSION

Frequency distribution of seedcoat mottling was determined in relation to infection grade of the plants (Fig. 1). Soybean plants inoculated by rubbing method produced distinct mosaic symptoms, puckering leaves with different grades in disease severity. The infected plants produced mottled seeds but all pods that was harvested from SMV-infected plants did not contain mottled seeds even in the plants that was rated as the highest severe grade. The mottled seeds (r=0.796) and the pods containing mottled seeds(r=0.879) were increased positively in proportion to the infection grade with significant correlation.

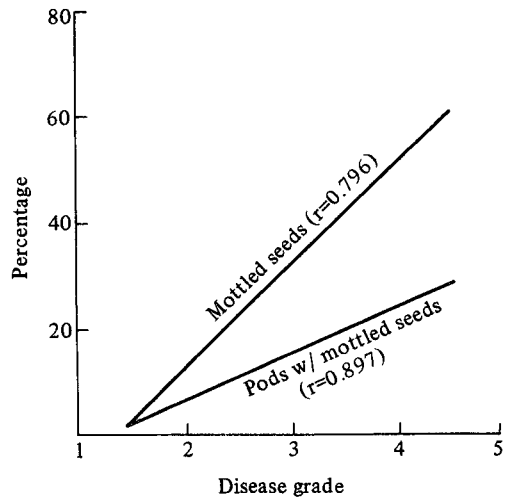

Fig. 1. Frequency distribution of seed coat mottling in SMV infected plants of soybean variety Clark.

To determine the effects of pod location and seed position to production of seedcoat mottling, seeds were harvested from each plants at maturity and examined for mottling in each node of the plants. As shown in Table 1, significant difference was found in the percentage of pods containing mottled seeds on different nodes of the plants. There was a slight tendency to be increased with topward position of nodes, but no significant correlation (r=0.34) between order of nodes and number of pods containing mottled seeds was recognized. It was found that in every case, some pods contained mottled seeds but some did not contain the mottled seeds on the same node of the infected plant. The pods containing mottled seed was averaged 21.7% in the plant.

Table 1. Distribution of the pods containing mottled seeds on each node of soybean plants inoculated with SMV at the second trifoliate leaf stage.

Plant node[a)]	Plant w/ PMS (%)[b)]	Ave. number of pod w/		PMS (%)
		Mottled seed	Nonmottled seed	
1	46.6	0.9	4.0	18.5
2	53.3	0.4	4.4	16.5
3	73.3	1.4	4.7	22.3
4	53.3	1.0	5.5	14.7
5	46.6	1.7	6.1	21.9
6	80.0	14.	6.5	17.2
7	66.7	1.8	6.4	22.2
8	66.7	0.9	3.6	19.4
9	75.0	1.3	4.3	22.5
10	66.7	3.3	4.7	41.5
Ave.	62.8	1.4	5.0	21.7
r[c)]	–	–	–	0.34NS

a) Order of nodes from the bottom of the soybean plants.
b) Plants with the pods containing mottled seeds among 25 soybean plants inoculated.
c) Correlation coefficient between the order of nodes and number of pods containing mottled seeds.

For seed base, difference in the percentage of mottled seed taken from different node was found, but no pattern was discerned (Table 2). Some seeds were mottled but some were not even in the same pod of the infected plants. The mottled seed was averaged 48.5% in the plants. This agrees with the conclusion[1)] that is no relationship between TRSV transmission and the position of seed in the pod, or of pod location on the soybean plants.

Table 2. Distribution of the mottled seeds in the pods with mottled seeds produced from soybean plants inoculated with SMV at the second trifoliate leaf stage.

Node[a)]	Ave. number of		Mottled seed percentage
	Mottled seed	Nonmottled seed	
1	0.8	1.0	45.7
2	0.9	0.8	54.5
3	1.3	1.6	45.6
4	1.1	1.0	51.2
5	1.6	2.1	44.4
6	1.7	1.4	54.7
7	2.0	2.9	40.9
8	1.0	1.0	50.0
9	0.8	1.0	42.9
10	5.5	4.5	55.0
Ave.	1.6	1.7	48.5
r[b)]	–	–	0.31 NS

a) Order of nodes from the bottom of the soybean plants.
b) Correlation coefficient between the order of nodes and the number of mottled seeds.

To determine whether seed transmission of SMV is associated with seedcoat mottling, presence of virus in soybean seed was detected by double agar immunodiffusion test with hypocotyl of germinated seeds. Most of susceptible varieties with virus symptom gave rise to mottled seeds but a small percentage of these mottled seeds contained virus in the seeds. The percentage of Bukhae seeds containing detectable virus was 33.3% at germinated seeds, and the corresponding values for Woodworth and Clark were 4.2% respectively (Table 3). The percentage of nonmottled seeds containing SMV was almost same as those of mottled seeds. It disagrees with the reports showing greater SMV transmission from mottled seed than from nonmottled seeds.[3,9)] The discrepancy may have occurred either because occasional field plants escaped infection and produced nonmottled seeds, or because of environmental differences during seed drying.

Seed transmission of SMV was verified by growing-on test as measuring infection of the seedlings arising from those seeds in field. Virus transmission was 41.5% and 32.6% with mottled

Table 3. Occurrence of soybean mosaic virus in mottled and non-mottled soybean seeds as determined by double agar immunodiffusion test and growing-on test.

Soybean variety		Serology		Growing-on test[a]	
		No. of seeds (infected/tested)	Infection (%)	Emergence (%)	Infected plant (%)
Bukhae #1,	M[b]	16/48	33.3	73.9	41.5
	N	14/48	29.2	79.5	32.6
Woodworth,	M	2/48	4.2	87.5	9.4
	N	2/48	4.2	89.4	6.7
Clark,	M	2/48	4.2	82.6	13.3
	N	0/48	0.0	85.7	6.5

a) Results of growing-on test based on appearance of symptoms in seedlings at 3-4 trifoliate leaf stage in field.

M; mottled seeds, N; non-mottled seeds

l nonmottled seeds of Bukhae, 9.4% and 6.7% Woodworth, 13.3% and 6.5% for Clark, respectively. The incidence of infection obtained by the growing-on test was slightly higher than that obtained by the double agar immunodiffusion test. This might be because either the procedure was not sufficiently sensitive to detect all virus in the infected seeds due to low titer, or the virus was further multiplied in the seedlings during the soybean growth.

From the result, it can be seen that the percentage of mottled seed was somewhat higher than that of others[7,12,13] and transmission of SMV through the seeds occurred at relatively low frequency. With this regard, evidence for variability in seedcoat mottling and SMV seed transmission in different soybean germplasm has been doccumented.[1,5,7,10] Incidence of seed transmission was higher when infection occurred prior to the onset of flowering and dependent upon infection of the embryo.[1] The virus in seed may be inactivated in some case during maturity and drying[7] and the inactivation could account for the lack of, and variation in rates of seed transmission in soybean.

As indicated in the result that a reasonable correlation was obtained between SMV severity of the plants and percentage of mottled seeds produced on these plants, the association of seedcoat mottling with SMV seed transmission suggests an attractive means for certification of seed for absence of virus. Although the correlation might not be absolute and SMV could be transmitted even by nonmottled seeds obtained from infected plants, it would be useful if the amounts of mottled seeds could be used as an indicator of the amounts of SMV infection in a seed production field. Removal of mottled seeds would reduce SMV seed transmission since the mottled seed occurs only on infected plants.

摘 要

콩모자이크 바이러스(SMV)에 의한 褐斑粒의 형성율과 SMV의 種子傳染率을 조사하기 위하여 대두 품종 Clark, Woodworth, 北海 1 號를 재료로 SMV 罹病程度와 褐斑粒率 結莢節位 및 褐斑粒形成등의 관계를 조사하고 그 갈반립을 통한 SMV의 종자전염율을 免疫二重擴散法과 幼苗感染率로 조사하였다.

1. 褐斑粒의 形成率은 植物體의 罹病程度와 正比例的인 相關($r = 0.796$)을 보였다.

2. 罹病植物體의 褐斑粒形成은 結莢節粒 및 莢中種實位置와 有意相關이 없었으며 ($r = 0.34$), 同一節位 및 莢內에서도 褐斑粒과 非褐斑粒이 함께 形成되었다.

3. 免疫二重擴散法으로 조사한 SMV 의 종자전염율은 4.2－33.3 %로 비교적 낮았으며 品種에 따라 현저한 차이가 있었다.

4. 幼苗에 나타난 SMV 症狀으로 조사한 種子傳染率은 免疫二重擴散法으로 검정한 종자감염율보다 약간 높게 나타났으며 이는 抗血淸의 力價 또는 Virus의 體內增殖과 관계가 있는 것으로 보였다.

5. 罹病植物의 褐斑粒은 SMV 종자전염율과 一致

하지는 않으나 SMV의 圃場罹病率 推定에 有用한 指標가 될 수 있으며 播種時 갈반립의 제거는 SMV 傳染源의 감소효과를 가져올 수 있을 것으로 보였다.

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