Screening and genetic analysis for root-knot nematode resistance in oats

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Backgrounds

In southern part of Japan, Kyushu, there are sustainable crop rotation systems of sweet potatoes and oats. Total 60,000 hectares of oats were grown for green-manures in last year.

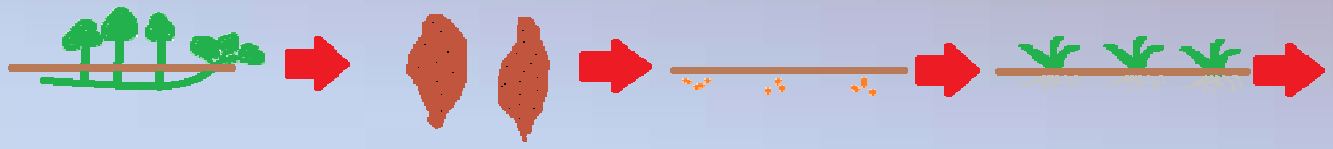
Agriculturally important species include the southern root-knot nematode, (*Meloidogyne incognita*), which is a major pest of sweet potatoes. The root-knot nematodes infected susceptible oats are the leading cause of yield loss of sweet potatoes.

We have, however, only one resistant variety 'Tachiibuki' derived from Argentina variety. It is needed to find new resistant genotypes of oats.

Rotation of Oat and Sweet-potato

1. Use of Mid-heading Oat Variety（Sowing in Autumn）

April to May → August to November → November → Vegetivly growing → Next Spring



Plowing as Green-manure

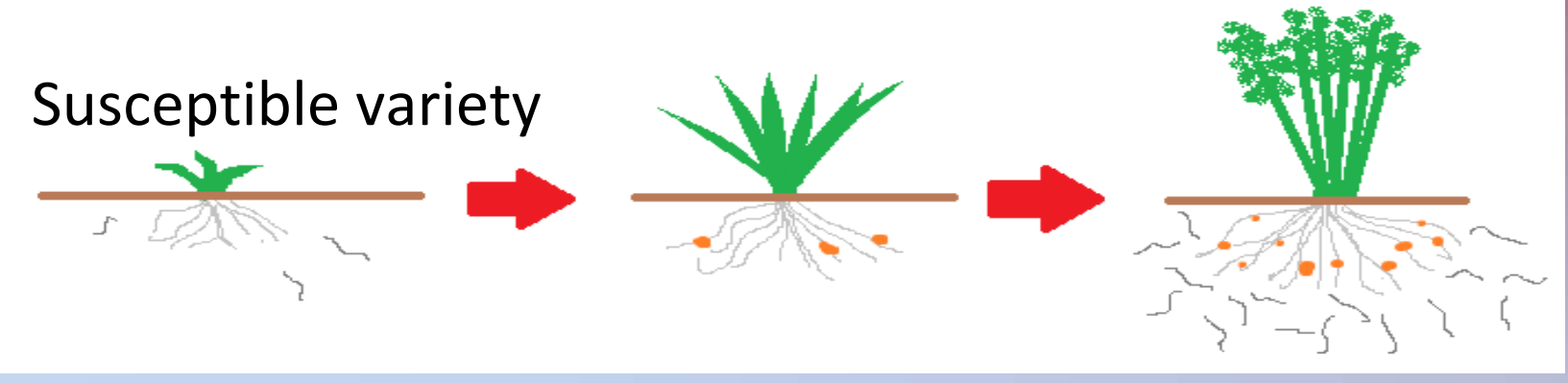
2. Use of Early-heading Oat Variety（Sowing in Summer)

April to May → August to September → September → Heading at the end of the year → December

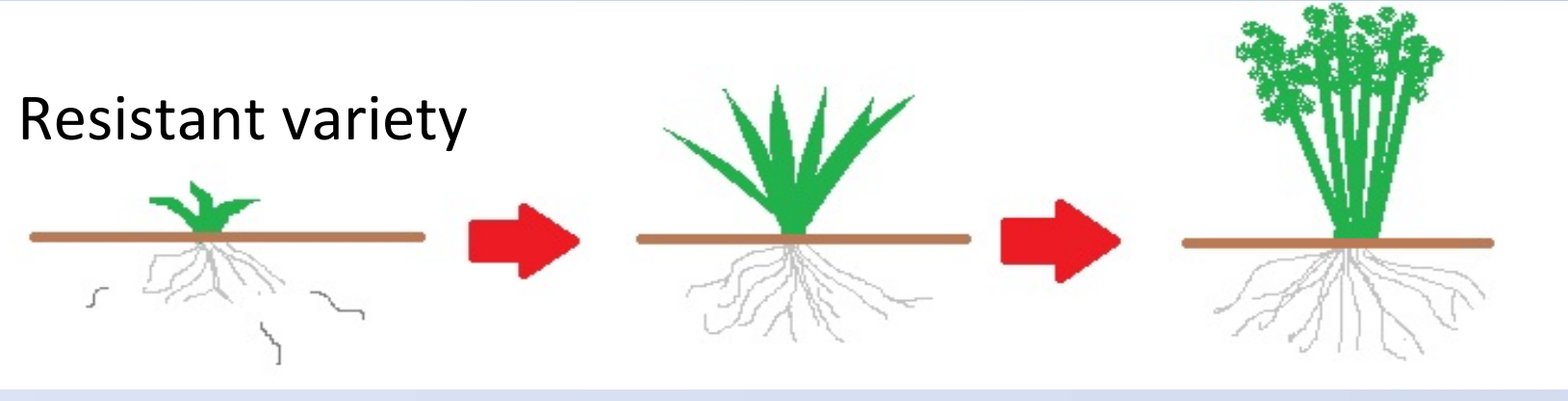
Harvesting Hay

There are serious damages of sweet-potatoes with the infection of root-knot nematodes.

Dynamics of root-knot nematode in soil

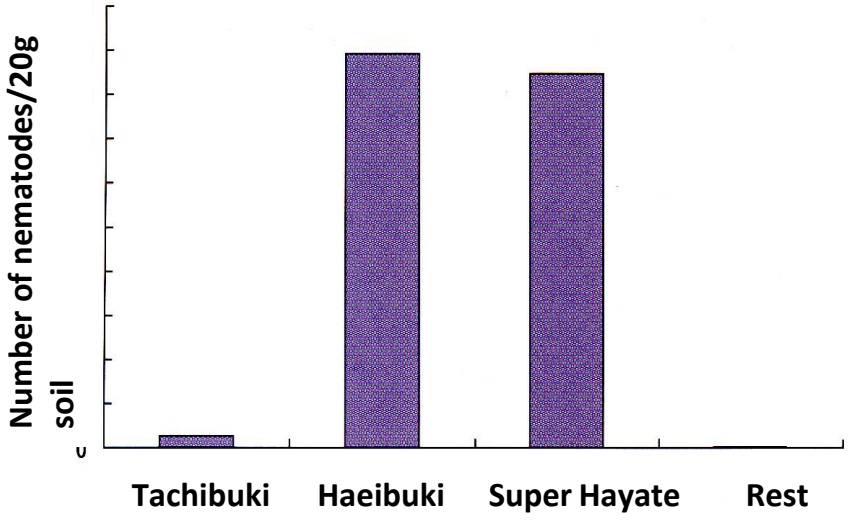


Infection of nematodes Production of egg-capsules Propagation of nematodes



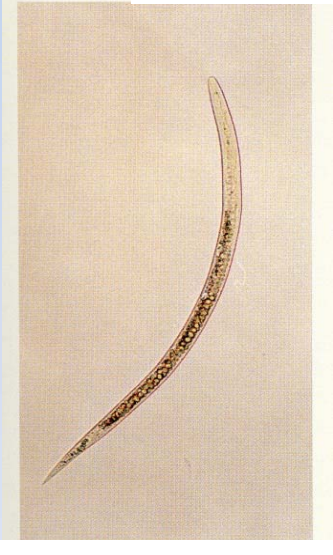
Infection of nematodes Restriction of egg-capsules Restriction of nematodes

Resistant variety ‘Tachiibuki’in summer.

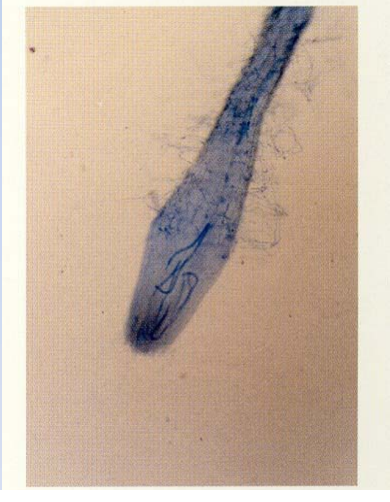


Density of nematodes in soil after growing of three oat varieties.

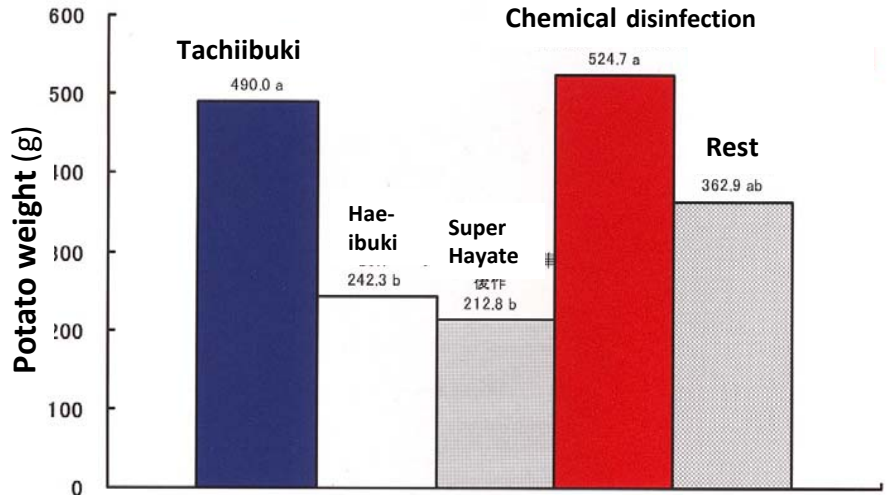
Imago of root-knot nematode laying eggs.



Larva of root-knot nematode.



Larva penetrating into root-tip.



Comparison of potato yield among oat varieties or Chemicals.

Sweet-potatoes after resistant oat variety

Sweet-potatoes after susceptible oat variety

Research Objectives

1. Screening oat accessions derived from Mediterranean countries for finding new root-knot nematode resistant genotypes.
2. Analyzing the mode of inheritance for the root-knot nematode resistance by examining the F2 hybrids between resistant 'Tachiibuki' and susceptible 'Super Hayate'.

Materials

1. **Common Oats（*Avena sativa L.*）**

Germ-plasms from Mediterranean countries

Osaka Pref. University 115

Nat. Agri. Center Kyushu-Okinawa 44

Resistant variety：'Tachiibuki' 1

Susceptible variety：'Haeibuki' 1

Total number of accessions: 162

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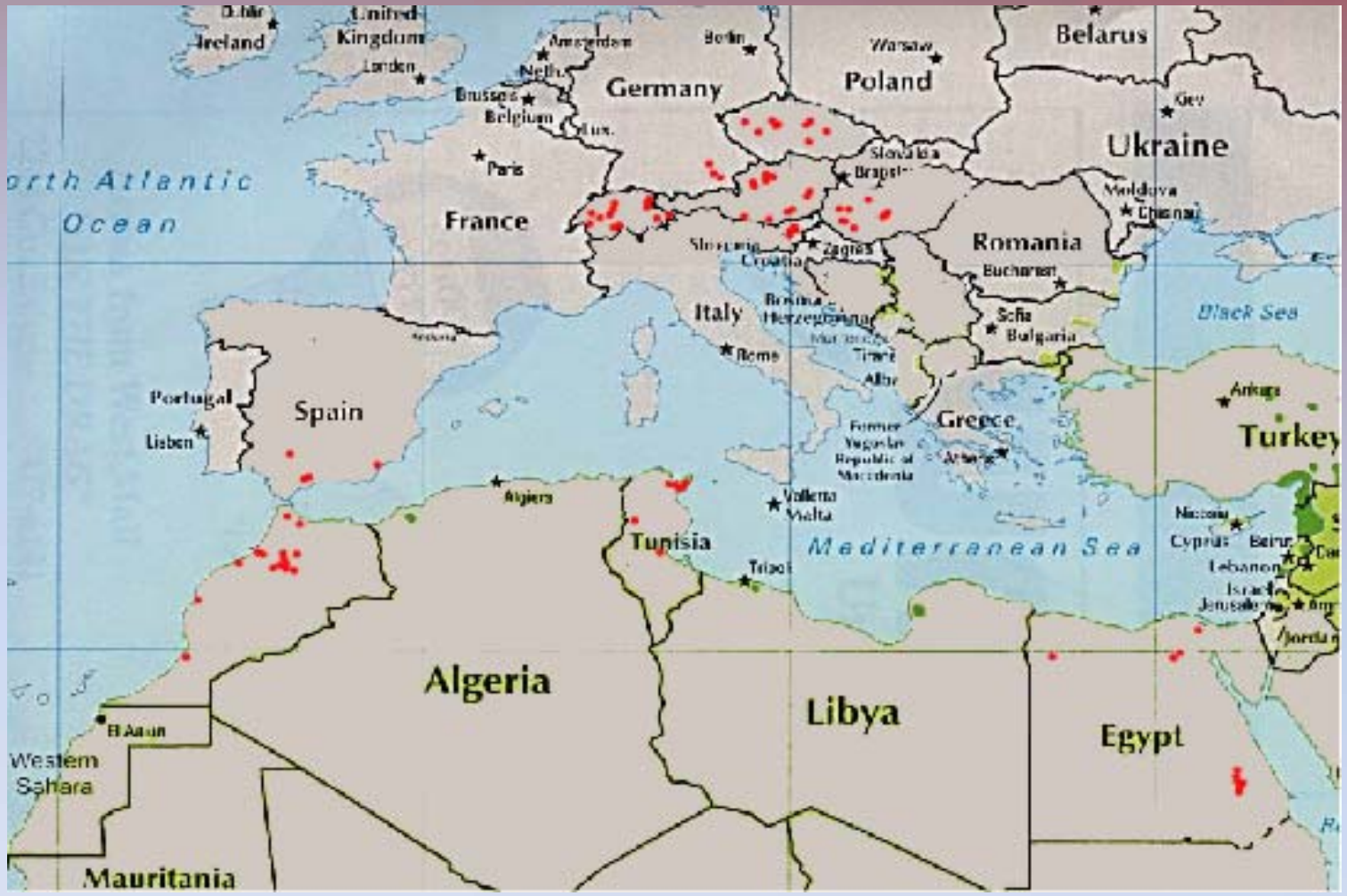
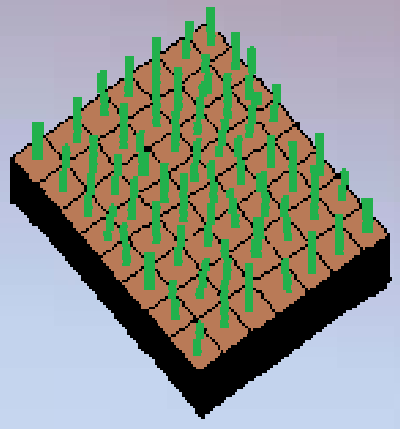
2. **Susceptible tomato variety：'Prize MR'** 1



Geographical distribution of the 115 cultivated oat (*A.sativa*) accessions collected from Mediterranean areas（GSEM95, 96 and 97）.

Resistant test for root-knot nematodes

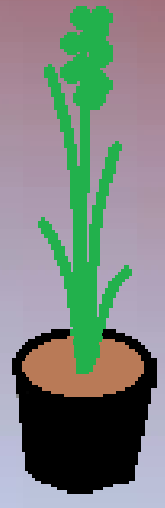


3 weeks seedlings at multi-pots

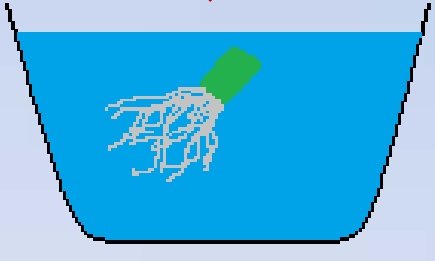
Transferring to each small pots

Five days later, inoculating 700 larvae

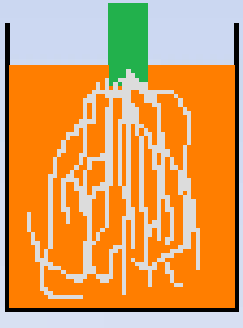
Incubating at 25℃ in glass house



50 days later



Washing roots with water



Staining egg capsules with Phroxin B

Washing with water

Counting number of egg capsules in roots

Resistant test for root-knot nematodes

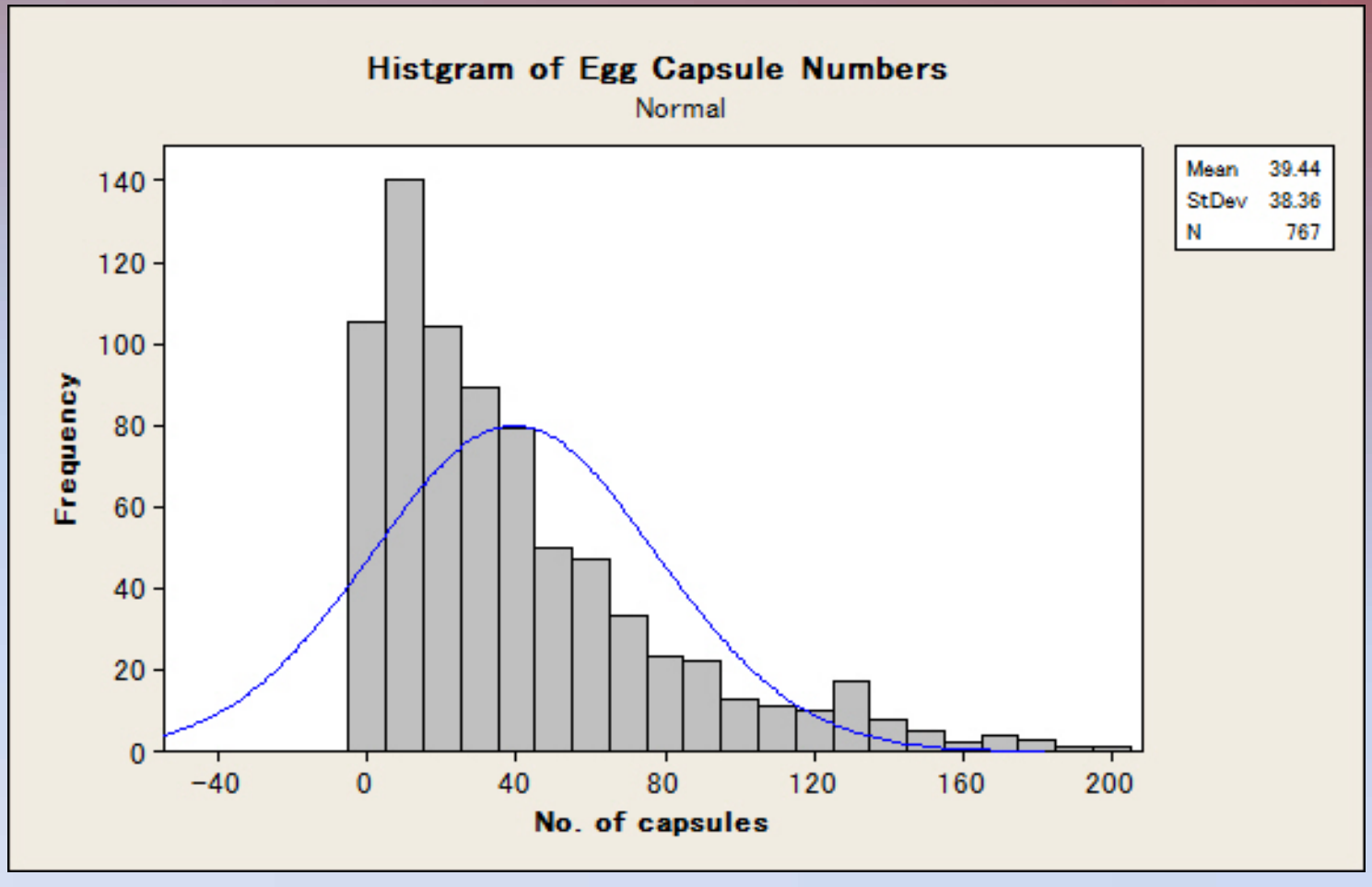
Three-week-seedlings of oats

Fifty days after inoculation

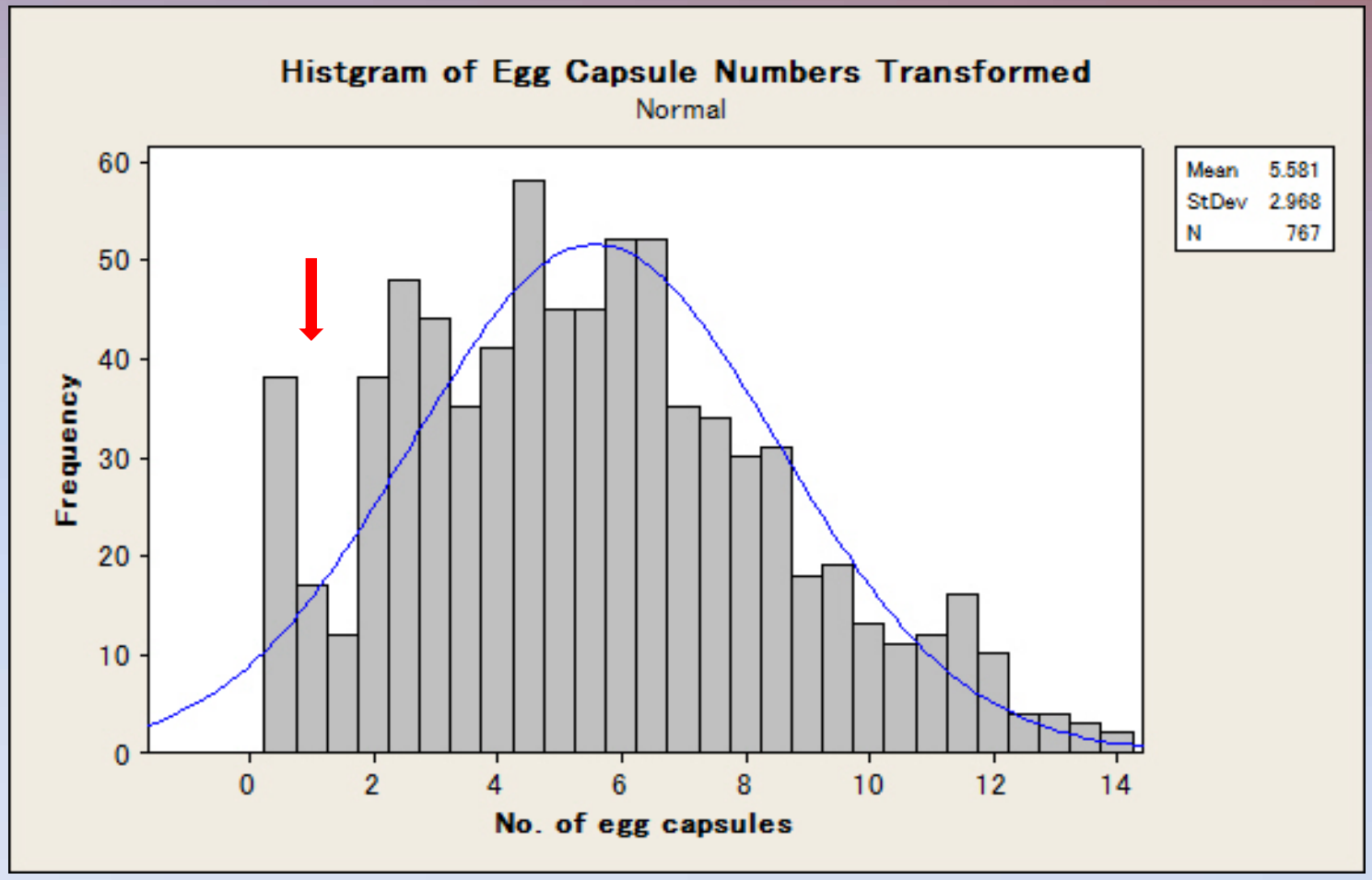
Washing the roots with tap-

Staining egg capsules

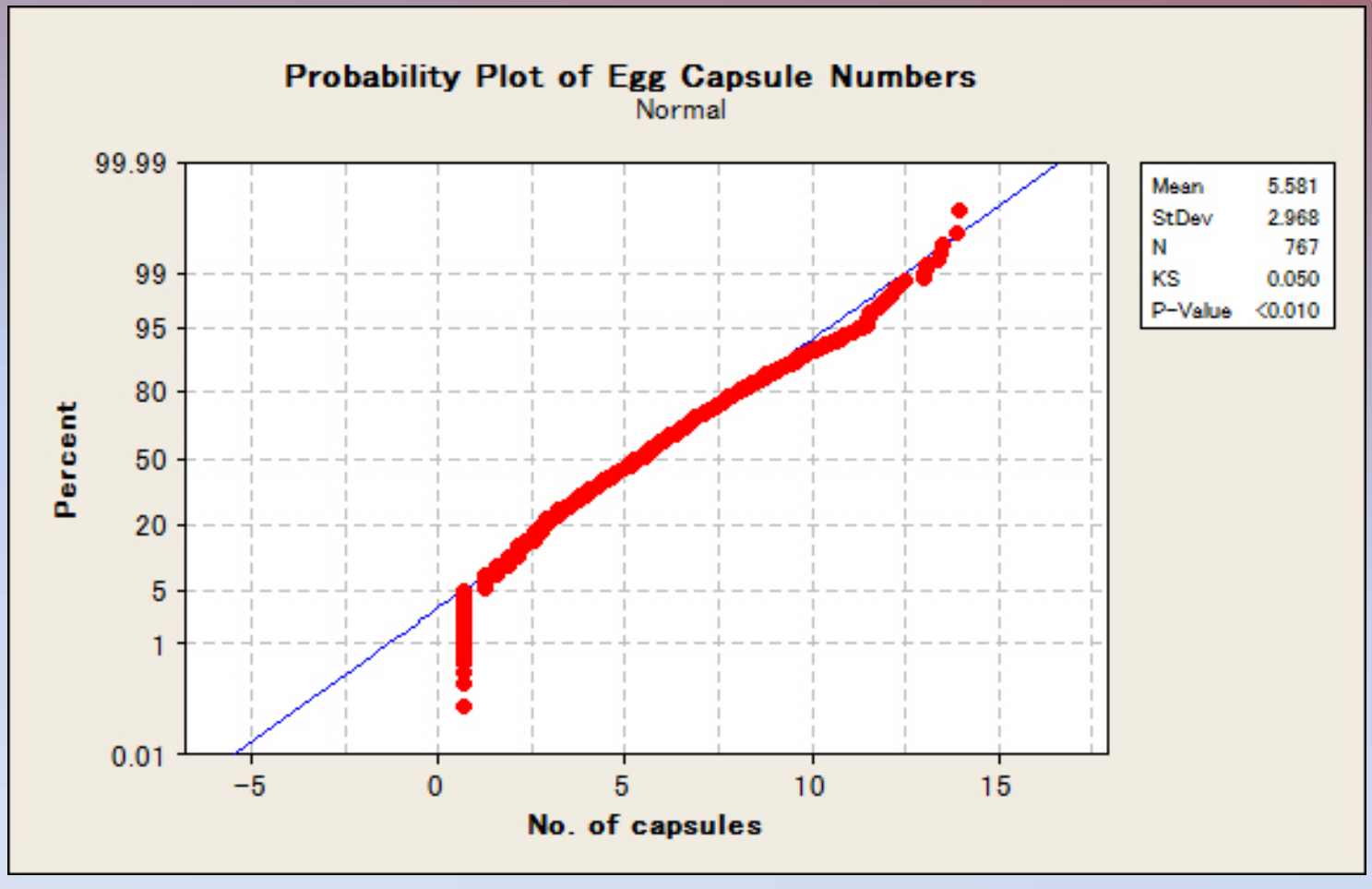
Egg capsules stained with Phroxin B (L:tomato, R:oats). Yellow circles indicate the egg capsules.



Frequency distribution of nematode egg capsules in 154 oat accessions derived from Mediterranean areas.



Frequency variables were transformed to fit normal distribution by using the formula, $\sqrt{X+0.5}$.


Probability Plot of Egg Capsule Numbers
Normal
Percent
99.99
99
95
80
50
20
5
1
0.01
-5
0
5
10
15
No. of capsules
Mean 5.581
StDev 2.968
N 767
KS 0.050
P-Value <0.010

Mean no. of egg capsules and 95% Cis in North Africa and Europe

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Country      N     Mean   StDev   ---+---------+---------+-------
--+------
Algeria     20    3.564   2.201           (-*-)
Austria    101    6.119   2.954                 (*)
Czech       43    2.942   1.583         (-*)
Egypt       45    6.124   1.206                 (*-)
France      25    5.773   2.569               (--*-)
Germany     70    5.816   2.646                (-*)
Hungary     45    2.930   2.138         (-*)
Morocco     92    7.575   3.650                     (*)
Norway      25    4.519   2.217             (-*-)
Resist.      5    0.707   0.000  (---*----)
Slovenia    43    5.582   1.639                (*-)
Soviet      50    6.895   1.617                  (-*)
Spain       30    5.943   4.075                (-*-)
Suscept.     5   12.726   0.658                           (---*--
--)
Sweden      35    4.904   1.705             (-*-)
Switzer.   103    5.308   2.748                (*)
Tunisia     30    4.905   2.673             (-*-)
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Results of the nematode resistance test

	No. of accessions tested		Candidate of Resistance
	Osaka Pref. Univ.	Nat. Agri. Center	
Hungary	10		
Czech	10		
Slovenia	10		
Switzer land	21		
Austria	21		
Germany	8	7	
Spain	5	1	**5-22-2**
France		5	
Morocco	15	2	**6-9-1**
Tunisia	6		
Egypt	9		
Algeria		3	**ALGERIBEE、 Algerian**
Russia		5	
Sweden		6	
Norway		3	
Italy		6	**Ava**
Holland		4	
Portugal		1	
Yugoslavia		1	
Total	115	44	5

Results

1.The frequency distribution of number of egg capsules (NEC) was fitted to normal distribution in almost all accessions, however, parts of them were discontinuance showing existence of major resistant genes.

2.The analysis of variance for NEC in the 20 countries showed highly significant differences among the countries from North Africa and Europe (Fs=15.09, P<0.001）.

3. Five new resistant lines were found in the accessions derived from Spain, Algeria, Morocco and Italy

Reciprocal crosses were made between resistant ‘Tachiibuki’ (P_1) and susceptible ‘Super Hayate’ (P_2) for examining the inheritance of root-knot nematode resistance in oats.

Genetic Materials

P1： Resistant ‘Tachiibuki’, 8 plants

P2： Susceptible ‘Super Hayate’, 8 plants

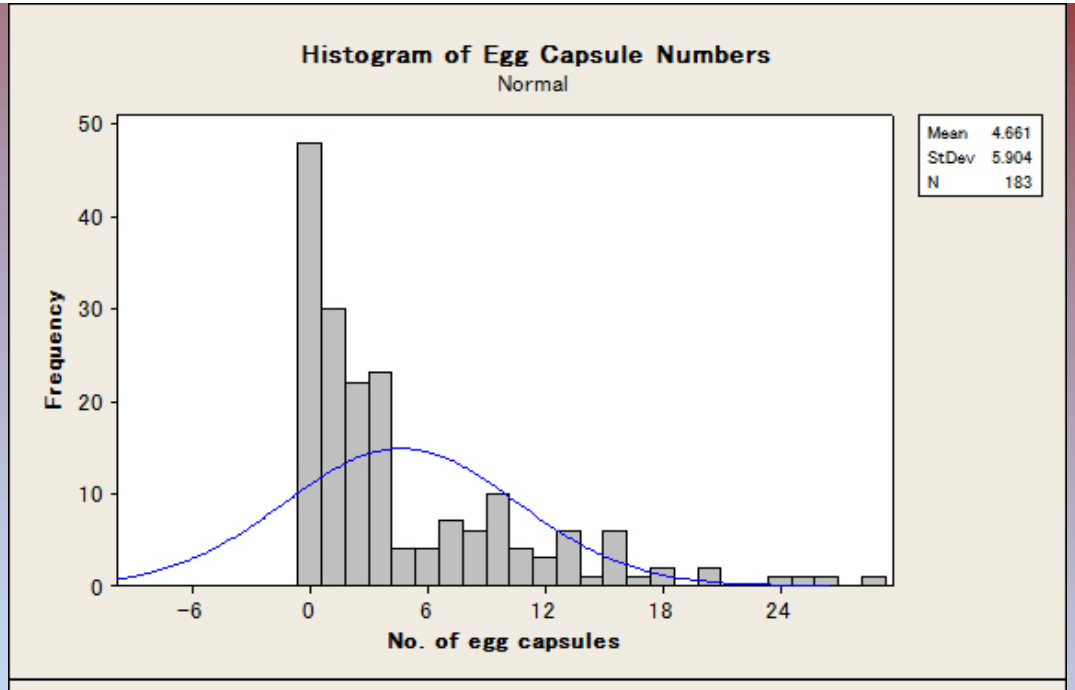
F1： Straight cross, P1 x P2, 4 plants

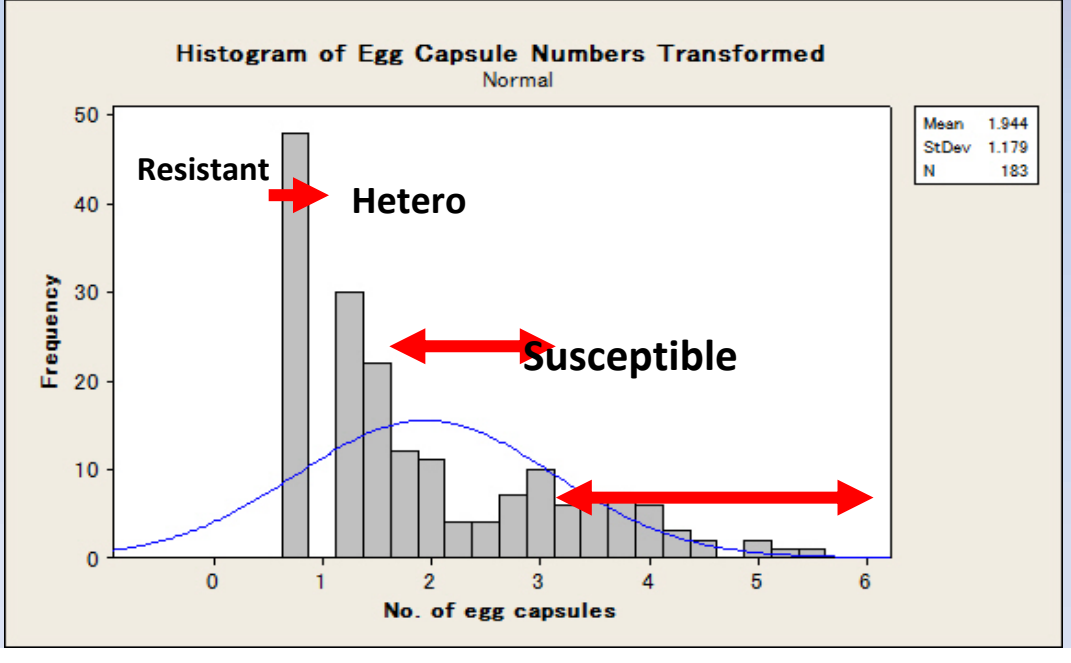
F1： Reciprocal cross, P2 x P1, 4 plants

F2： Straight cross, 183 plants

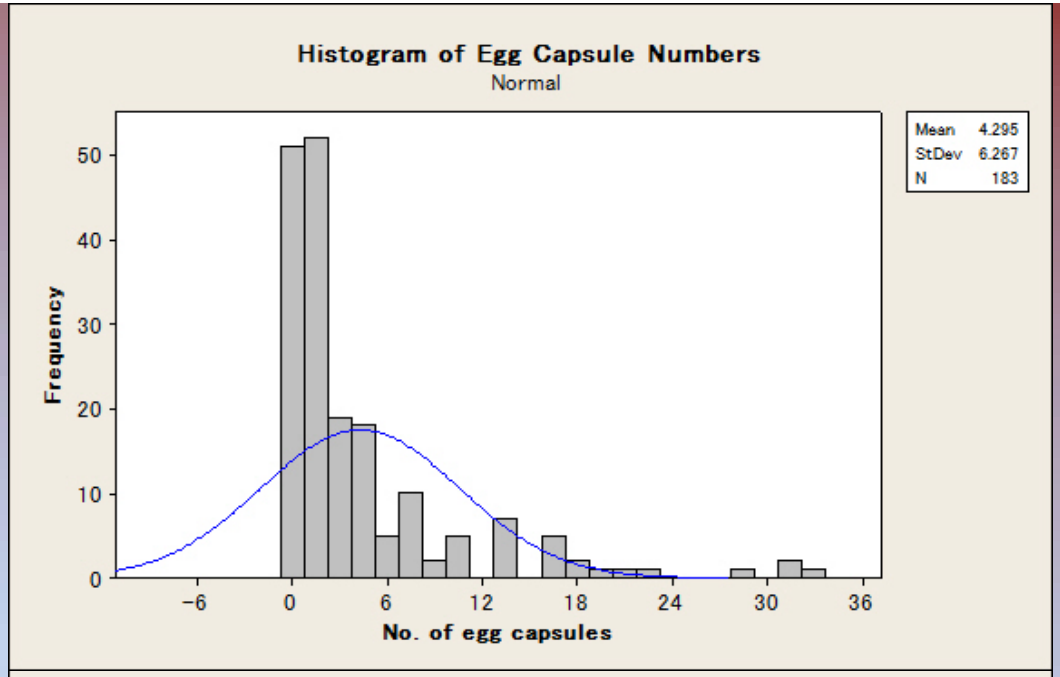
F2： Reciprocal cross, 183 plants

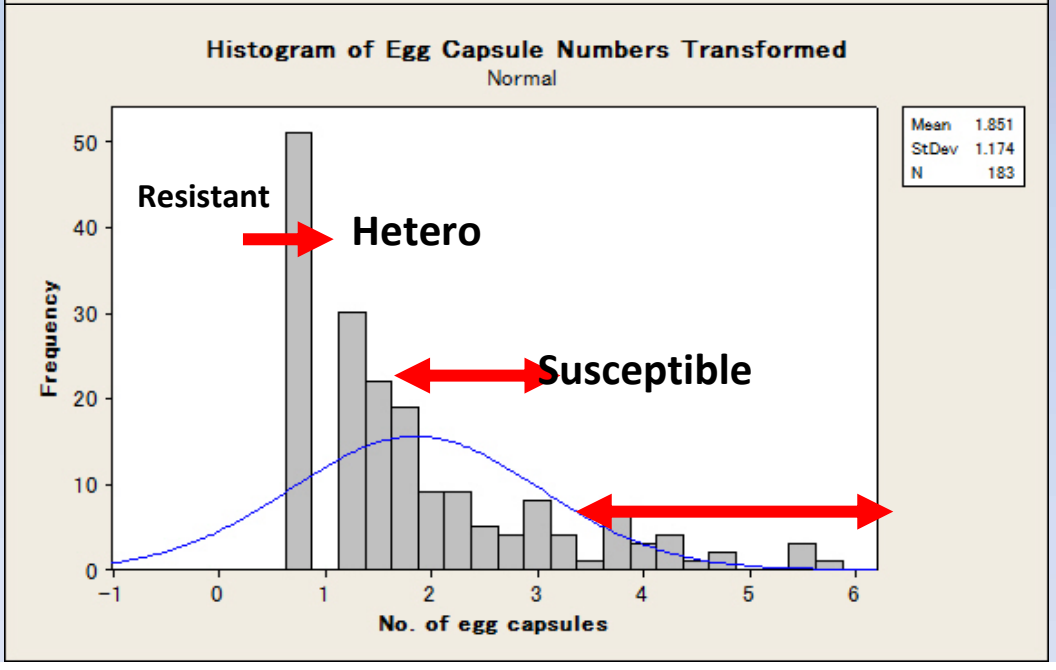
Frequency distribution of egg capsules in the F2 of straight cross.





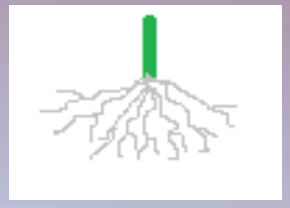
Frequency distribution of egg capsules in the F2 of reciprocal cross.





P1
Resistant 'Tachiibuki'

P2
Susceptible 'Super Hayate'



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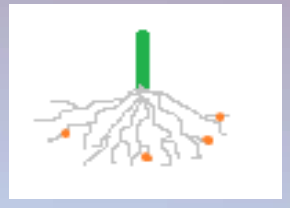
Egg capsules: 0

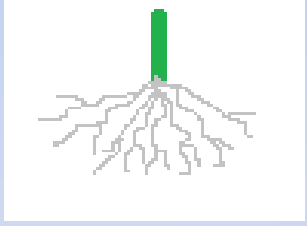
Egg capsules: 15-40

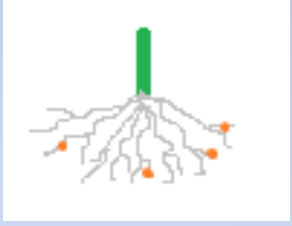
F1

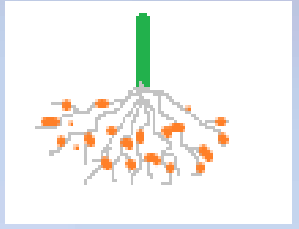


Egg capsules: 1-6

F2







Expected ratio = 1 : 2 : 1

Observed ratio

Straight Cross:	179 =	44	:	83	:	52	χ^2=1.659	P=0.436
Reciprocal Cross:	179 =	48	:	97	:	34	χ^2=3.447	P=0.178

Conclusions

1. **Five new resistant lines were found in the accessions derived from Spain, Algeria, Morocco and Italy.**
2. **The resistant genes for root-knot nematode were localized in the west Mediterranean countries.**
3. **The resistance of 'Tachiibuki' for root-knot nematode was controlled by a single incomplete gene.**

Further study

1. **AFLP and the *Mi* gene of tomato will be applied to balk segregant analysis by utilizing the F2 of 'Tachiibuki' x 'Super Hayate'、for finding the DNA markers linked with the root-knot nematode resistant genes.**
2. **Allelism test will be carried out among five resistant accessions for clarifying new resistant alleles and loci.**


Hello, oat workers.
I am a graduate student.
I like oats very much.


Thank you