

DOMESTICATION GENES AND STRESS ADAPTATION GENES IN THE GENUS VIGNA FOR SUSTAINABLE AGRICULTURE UNDER STRESS ENVIRONMENTS

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His expertise is in the leguminous plants belonging to the genus *Vigna*. He worked at the Tropical Agriculture Research Center (TARC, presently called JIRCAS), Japan, Chai Nat Field Crops Research Center, Thailand, and at National Institute of Agrobiological Sciences (NIAS), Japan.

Currently, he is the Genetic Resources Coordinator of the Genebank Project in Japan, organized by Genetic Resources Center, NARO.



ABSTRACT

I propose a novel breeding strategy “Neo-Domestication” for sustainable food production especially under stress environments. It is estimated that the world food production should be doubled before 2050. In the past 50 years, world food production had increased 2.5 – 3 times owing to the yield increase of a few major crops, which is called “Green Revolution”. However, this system depended largely on irrigation (water resource), industrial fertilizers and pesticides (both produced by fossil energy) and resource consuming high yielding varieties. Therefore, it is far from sustainable and causes water resource deficit, soil salinization, environmental pollution, etc.

Present agriculture has used 11% of the world land. One approach to increase crop production is to use lands that are currently not suitable for crop production. These include lands with high salinity, high acidity, and high alkalinity, those that are drought prone and those that have water-logged environment. Even among the lands presently used for crop production, about 70% are categorized as problem soils. To achieve stable crop production on various problem soils, new crops that can grow well on these lands are needed. Hence, I propose “Neo-Domestication” of edible wild species already adapted to problem soils. Leguminous plants have an advantage in sustainable agriculture because of their ability of symbiotic N fixation.

As the causative change of the domestication gene is generally a “Loss-of-Function” type, “Neo-Domestication” could be achieved by mutation breeding. Screening process can be facilitated by gene screening using NGS-TILLING, in case a target gene sequence is known. Since 10 wild species had been domesticated to become crops in the genus *Vigna*, diverse domestication genes could be identified from *Vigna* crops and be used for NGS-TILLING during “Neo-Domestication”. On the other hand, more than 100 wild *Vigna* species had evolved by adaptation to diverse stressful environments including problem soils. Hence, wild *Vigna* plants are considered as rich sources of stress adaptation genes. In addition to “Neo-Domestication”, these domestication genes and stress adaptation genes could also be used for the breeding of other crops.

KEYWORDS

Genetic Resources, Evolution, Domestication, Adaptation, Symbiosis

REFERENCES

- Sakai, H., K. Naito, E. Ogiso-Tanaka, Y. Takahashi, K. Iseki, C. Muto, K. Satou, K. Teruya, A. Shiroma, M. Shimoji, T. Hirano, T. Itoh, A. Kaga, and N. Tomooka, 2015: Scientific Reports 5, 16780.
- Sakai, H., K. Naito, Y. Takahashi, T. Sato, T. Yamamoto, I. Muto, T. Itoh, and N. Tomooka, 2015: Plant and Cell Physiology, 57, 1-3.
- Tomooka, N., K. Naito, A. Kaga, H. Sakai, T. Isemura E. Ogiso-Tanaka, K. Iseki, and Y. Takahashi, 2014: Plant Genetic Resources: Characterization and Utilization, 12 (S1): S168-S171.
- Tomooka N., T. Isemura, K. Naito, A. Kaga, and D. Vaughan, 2014: *Vigna* species. In Singh, M., I.S. Bisht, and M. Dutta, eds., Broadening the Genetic Base of Grain Legumes. Springer, New Delhi, Heidelberg, New York, Dordrecht, London, Chapter 9, 175-208.

Chair Nakashima

The next speaker is Dr. Norihiro Tomooka. He is Genetic Resources Coordinator in the Genetic Resources Center, NARO, Japan. He has studied on *Vigna* germplasm for a long time, and the title of his presentation is 'Domestication genes and stress adaptation genes in the genus *Vigna* for sustainable agriculture under stress environments.' Dr. Tomooka, please start the presentation.

Dr. Norihiro Tomooka

Thank you very much Mr. Chairman. My name is Tomooka. I am belonging to the Genetic Resources Center, NARO. Today, I want to talk four topics. At first, I want to explain about *Vigna*, then domestication genes and stress adaptation genes, then finally I want to propose some idea of neo-domestication.

This slide shows *Vigna* and *Phaseolus* are very closely related genus, and in case of *Phaseolus*, its distribution is restricted to America. But in case of *Vigna*, this is *Vigna*, it can be found in Africa (cowpea group), in Asia (adzuki bean group) and also in America. If you look at the evolution of *Vigna*, the prototype of *Vigna* is believed to be in Africa. Then, Eurasian *Vigna* appeared and then Asian *Vigna* and then finally *Phaseolus* and American *Vigna* evolved.

The flower morphology becomes gradually complicated from very simple floral morphology of African *Vigna*. It took about 5 million years to attain this evolution.

In this slide, I want to show some *Vigna* crops. This is adzuki bean, mung bean, cowpea, black gram, and this is tuber cowpea, this is bambara groundnut and moth bean and rice bean. These are the main *Vigna* crops.

From now, I want to talk something about domestication genes. During the process of domestication, the organ size usually becomes bigger from wild, it becomes non-shattering and the seeds become non-dormant and sometimes the architecture of the plant changed. These are the main morphological changes or physiological changes during the domestication.

This slide shows the domestication in Asia. In Asia, there are about 24 or 25 species of *Vigna* distributed, and from these 24-25 species, moth bean has been domesticated and adzuki bean domesticated, black gram domesticated, and mung bean domesticated, creole bean domesticated, and rice bean domesticated. Among these domesticated crops, I selected four crops for the comparison of the domestication genes. First, I analyzed domestication genes of adzuki bean and the results were published in these journals, then rice bean domestication genes analyzed and mung bean and black gram domestication genes were analyzed and published.

This is one result of the comparison of the pod shattering genes. This is the linkage map of each crop. Four pod shattering genes were detected. Adzuki bean gene on linkage group 7, this one, was our target for the gene cloning. We named it TIP gene. This slide shows the effect of TIP gene. The wild adzuki bean shattered when it matured and the cultivated one not shattered, but when we introduced TIP gene into cultivated adzuki bean, its pod became shattered.

Then, we tried to identify the reason of the shattering and we found that the lignification may be the cause of the pod shattering. The wild adzuki bean has a very thick lignin layer inside the pod wall, but cultivated adzuki bean has very thin lignin layer. The near isogenic line of cultivated adzuki bean with wild TIP gene, lignin layer – the red part shows the lignin layer - became very thick.

We tried to clone the TIP gene, and we found this thymine insertion is responsible for the loss of lignin accumulation inside the pod wall.

In case of soybean, there is still a problem of the pod shattering, especially under the dry conditions. Soybean pod shattering genes were recently reported by two different scientists, one is Shat 1-5 gene and the other is Pdh 1 gene, but the TIP gene of the adzuki bean is different from these two genes. So, we tried to introduce TIP gene into soybean.

This is mutant tip homo soybean pod, and this is mutant hetero soybean pod, and pod shattering was compared under the experimental condition (40°C 16hrs treatment). This mutant tip homo showed complete non-shattering phenotype, while mutant hetero showed 80% pod shattering.

This is the second example of the domestication gene, seed size increase gene. In this case, there are many, many genes detected. In total, 16 seed size genes were detected from four crops. This gene on the linkage group 2 was commonly found from these four crops. In this case, we have targeted this black gram seed size increase gene for the gene cloning.

This slide shows the effect of MOG (multiple organ gigantism) gene of black gram. When the MOG gene was destroyed, the plant became big and the leaf also became big and the seed also became big, but the cell size was not affected by this mutation.

We have identified the responsible gene, this is the MOG gene and when the 8 base deletion occurred, plant organs became bigger. In this case also, we tried to test this mutation gene effect using soybean. In this case, we used the RNAi method to lose the function of MOG gene and the plant became bigger. The seed size also became bigger. So, these two examples showed the domestication gene of adzuki bean or black gram can be applied to other crops, in our case to soybean.

From now, I want to talk about some stress adaptation genes in *Vigna*. There are many kinds of environmental stresses in the world. Total Japanese crop land is only 4 million hectares but the stress area in the world is big. Crops were suffered from these stresses. For each stress, we found stress adapted *Vigna*; *Vigna marina*, *Vigna exilis*, *Vigna vexillata*, *Vigna trilobata* *Vigna mungo*, and *Vigna stipulacea*. *Vigna marina*, it can tolerate up to 400 mM NaCl and the symbiotic rhizobium strains can tolerate up to 800 mM NaCl. So, we tried to identify the responsible genes of the saline tolerance by crossing and we found one major QTL responsible for this salt tolerance which can explain 50% of phenotypic variations. So, from Riverside habitat to Marine Beach habitat adaptation, one mutation has revealed to have very big contribution.

Then, I will talk about neo-domestication. Usually, if we found stress tolerant wild species, we cross it with stress susceptible crop and try to develop more resistant crop. The new idea (neo-domestication) is trying to domesticate stress tolerant wild species and develop new crop. It is called neo-domestication. Important thing is that the phenotypic changes during domestication were caused by loss of function type mutations. So, we can use mutagen to accelerate mutation and select the domesticated phenotype, a traditional mutation breeding methodology. And we tried to use *Vigna stipulacea* as an example of neo-domestication. We used EMS chemical treatment and grew the plants and we found new non pod shattering mutant and seed color mutants and at the same time, these seed color mutants showed loss of dormancy. So, these genes can be called domestication genes. But the process of the mutation breeding is very time-consuming and labor-consuming. If we know the gene sequence, we can use the Next Generation Sequencer screening (NGS TILLING), not by the phenotypic screening.

This is the final slide - my recommendations. We must learn more from diverse domestication events is the first recommendation. The second one is we must learn more from diverse adaption of wild plants. And it is better to include nitrogen fixation system of the wild plants for the sustainable crop production research. This is all. Thank you very much.

Chair Nakashima

Thank you for the interesting presentation. Dr. Tomooka explained about varieties of *Vigna* and he showed some domestication genes and adaptation genes. He also recommended neo-domestication for the sustainable culture and stress environments.

Now, the paper is open for discussion. Anybody has any questions or comments? No comments or questions? So, I have a question. You have succeeded to isolate some QTLs such as TIP and MOG. Can you find function of the genes: the protein function?

Dr. Norihiko Tomooka

Not yet clarified.

Chair Nakashima

Not yet. I see.

Dr. Norihiko Tomooka

It's all isolated but not yet tested of the function.

Chair Nakashima

Thank you. Are there any questions or comments? Okay, please.

Male Questioner

You mentioned that MOG gene can increase the seed size and plant size of soybean, but this gene does not have an effect on seed number or the final yield?

Dr. Norihiko Tomooka

Yes. The seed size becomes double, but the number of seeds per plant is about half. So, the single plant basis the yield may be the same.

Chair Nakashima

Okay. So, it's time. Thank you, Dr. Tomooka.

Dr. Norihiko Tomooka

Thank you very much.