**Doctoral Thesis**

# Nutritional and physiological studies on productivity and grain quality of a low-phytate soybean line

**Summary**

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Phytic acid, known as *myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate or phytate in its salt form, is present in seeds such as soybean, maize, wheat, and rice. It is a chelating agent for cations and performs the function of P storage, accounting for up to 80% of the total P in seeds. However, phytic acid is poorly digested by monogastric animals such as pigs, chickens, and humans. As agriculture production has intensified worldwide, there have been growing concerns about the sustainability of global P supplies. P is a crucial element of agricultural fertilizer and supplementation of inorganic P (Pi) is necessary to satisfy the nutritional requirements of livestock. It is a non-renewable, limited resource, and we are running out of it. Furthermore, dietary phytate chelates divalent cations, such as iron (Fe) and zinc (Zn), and this reduces the bioavailability and utilization of these essential nutrients. To solve these phytate-related problems, low phytic acid lines of cereals and legumes have been developed. Drought stress and low P levels are the primary abiotic constraints on crop productivity, with the capacity to severely reduce yields and challenge global food security. Drought stress at the reproductive stages, especially at the grain-filling stage, results in a substantial reduction in yield. This study investigated growth, productivity, and seed quality of a low-phytate soybean line compared to a normal-phytate soybean cultivar under drought stress and low P fertilization and evaluated the practicability of the low-phytate soybean line.

**1. Effects of phosphorus fertilization on the growth, mineral accumulation, seed yield and seed quality of a low-phytate soybean line**

In order to evaluate the effects of P fertilization on the growth, mineral accumulation, seed yield and seed quality of a low-phytate line compared to a normal-phytate cultivar (Akimaro) from western Japan; two levels of P fertilizers: 20 kg $P_2O_5$ $ha^{-1}$ (low P treatment) and 100 kg $P_2O_5$ $ha^{-1}$ (high P treatment) as single super phosphate were applied in a randomized complete block (RCB) design with four replications. The results revealed that P fertilization significantly increased growth, seed yield, yield components, and seed quality of experimental plants, and the low-phytate line had higher growth, seed yield, yield components, and seed quality than the normal-phytate cultivar under both low P and high P conditions. Minimal differences were observed in total P concentration of seed between the low-phytate line and the normal-phytate cultivar, while total P concentration was higher under the higher P application treatment. The effect of P fertilization on phytate P and inorganic P concentrations was significant in both low-phytate line and normal-phytate cultivar. Phytate P concentration in the normal-phytate cultivar was greater than in the low-phytate line under both P fertilization levels. The ratio of phytate P to total P was reduced with increased P fertilization in the low-phytate line; however, it was not greatly affected by the P application level in the normal-phytate cultivar. The lipid and protein concentrations of seeds in both the line and the cultivar were significantly higher in the higher P application level. The molar ratios of phytic acid to Zn, Fe, Mn, and Cu were lower in the higher P application level in both the line and the cultivar. The molar ratios of phytic acid to these minerals were lower in the low-phytate line than in the normal-phytate cultivar in both P application levels, which might increase the bioavailability of the minerals in the low-phytate line.

**2. Effects of phosphorus fertilization on growth, grain yield and quality of a low-phytate soybean line under drought stress condition**

To investigate the effects of P fertilization on growth, grain yield and quality of a low-phytate line under drought stress conditions compared to normal-phytate cultivar (Enrei) cultivated in western Japan; two irrigation levels, 85.5 ± 2.4% of the field capacity (control) and 35 ± 1.5%

of the field capacity (drought stress treatment), and two levels of P fertilizer, 20 kg $P_2O_5$ $ha^{-1}$ (low P treatment) and 100 kg $P_2O_5$ $ha^{-1}$ (high P treatment), were applied in a RCB design with four replications. Drought stress treatment was applied at the grain-filling stage for a total of fifteen days. The results of this study indicated that drought stress and low P treatment significantly reduced seed yield, yield components and seed quality in both the line and the cultivar; however, the reduction was less in the low-phytate line than in the normal-phytate cultivar. Drought stress treatment significantly reduced whole plant dry weight and relative water content in both the line and the cultivar. Phytic acid concentration was significantly lower in the low-phytate line than the normal-phytate cultivar under drought stress treatment. The molar ratio of phytic acid to minerals in low-phytate line and normal-phytate cultivar was significantly less under drought stress treatment compared to the control in both low P and high P treatments; however, the low-phytate line showed lower molar ratios of phytic acid to minerals compared to the normal-phytate cultivar.

**3. Effects of phosphorus fertilization on dry matter distribution and P translocation in a low-phytate soybean line**

The effect of low P fertilization on dry matter distribution and P translocation were investigated at the vegetative stage in a low-phytate line compared to the normal-phytate cultivar (Enrei). Two levels of P fertilizers, 25 kg $P_2O_5$ $ha^{-1}$ (low P treatment) and 100 kg $P_2O_5$ $ha^{-1}$ (high P treatment), were applied in RCB design with four replications. The results showed a significant difference in total dry matter production between low P and high P conditions in both the line and the cultivar, and the low-phytate line produced significantly higher total dry matter compared to the normal-phytate cultivar in both P treatments. Under low P treatment, the ratio of dry matter distribution between lower leaves and upper leaves was lower in the low-phytate line compared to the normal-phytate cultivar. Phosphorus concentration and its accumulation in different parts of the plant showed a significant difference between low P and high P treatments in both the line and the cultivar. The translocation of P from lower leaves to upper leaves in the low-phytate line was highly affected by low P treatment compared to that of the normal-phytate cultivar. The low-phytate line showed a higher tolerance to low P treatment and higher accumulation of P in the upper leaves than the normal-phytate cultivar. The low-phytate line had higher photosynthetic rate, stomatal conductance, and intercellular $CO_2$ concentration than the normal-phytate cultivar under low P treatment. This suggests that low-phytate line is more tolerant to low P conditions, and could produce significantly more dry matter overall, allocate more dry matter to the upper leaves, accumulate a greater amount of N and P, and adapt better to low P fertilization than the normal-phytate cultivar.

**Conclusion**

These results suggest that the low-phytate line achieved greater physiological performance in response to low P fertilization compared to the normal-phytate cultivar. The low-phytate line produced higher seed yield than the normal-phytate cultivar under low P conditions. The molar ratios of phytic acid to minerals in the low-phytate line was lower under both low P and high P conditions compared to the normal-phytate cultivar; thus, there was a greater bioavailability of microelements in the low-phytate line. Drought stress had a significant impact on physiological characteristics, seed yield, and seed quality of both the line and the cultivar; however, the low-phytate line had greater physiological performance and produced higher seed yields compared to the normal-phytate cultivar. The effect of P fertilization under drought stress treatment was similar between the low-phytate line and the normal-phytate cultivar. The low P fertilization level significantly reduced bioavailability of microelements, productivity, and nutritional quality of seeds compared to the high P fertilization level; however, this reduction was lower in the low-phytate line compared to the normal-phytate cultivar. Dry matter production and P accumulation in all parts of the plant, particularly the upper leaves, were significantly decreased by the low P treatment; however, dry matter production and P accumulation in the upper leaves of the low-phytate line were not significantly lower than those of the normal-phytate cultivar. It could be concluded that the low-phytate soybean line was not negatively affected by low P fertilization and displayed higher tolerance to drought-related physiological stress, as it achieved greater nutrient distribution and translocation from lower leaves to the upper leaves, produced higher seed yields, and had superior yield components, seed quality, and microelement bioavailability.