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Abstract of Thesis/Dissertation

Applicant

Doctoral Program of Animal Science and Agriculture

Graduate School of Animal and Veterinary Sciences and Agriculture

Obihiro University of Agriculture and Veterinary Medicine

Student ID: 19180006

Name of Applicant: Chandiona MUNTHALI

Signature of Applicant:

Title : Impact of Nutrient Biofortification as a Crop Nutrient Management Control Strategy against Insect Pest Infestation in Potato *(Solanum* tuberosum L.)

（作物栄養管理における栄養強化が、ジャガイモ（*Solanum tuberosum* L.）での害虫の発生に与える影響）

Abstract

Low or excessive soil fertility as well as pests and diseases are a major constraint to potato production. The influence of each individual nutrient element on potato plant growth, nutrient uptake and interactions, and interaction with herbivorous insect pests under field studies remain ambiguous due to the influence of environmental variations. Creating an in vitro model plant with deficient or excessive nutrient content will provide a more controlled study and allow for a better understanding of how the concentration of one element can affect the uptake of other elements. Also, how the nutrient status of each element in the plant interact with insect pests can well be elucidated under a controlled environment.

Here, we first designed a tissue culture-based nutrition control system to systematically analyze the effects of essential nutrients on potato plants. Insufficient or excessive nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) contents were created by modifying the Murashige and Skoog (MS) medium. Deficient to toxic plant nutrient statuses were successfully defined by the evaluation of dry biomass and morphological symptoms. The N supplies of 20, 60-80, and 100 mM were defined as insufficient, optimum and toxic, respectively, whereas the P supplies of 0.4, 1.25, and 3.5

mM were defined as insufficient, optimum, and excessive, respectively, and K supplies of 13.5, 20, and 30 mM were defined as insufficient, optimum, and excessive, respectively. The Ca supplies of 1 and 10 mM were defined as insufficient and optimum, respectively, whereas Mg supplies of 1.5 and 9 mM were defined as optimum and excessive, respectively. The results showed that plant shoot growth, nutrient uptake and content, and nutrient interactions were all significantly impacted by the changes in the MS media nutrient concentrations. The increase in MS medium N supplies significantly increased shoot N uptake up to a toxic level where it drastically decreased. The changes in shoot N uptake had significant positive interactions with the changes in shoot P, K, Ca, and Mg uptakes. Increases in P supplies showed a steady increase in shoot P uptake up to a point where it became excessive. The changes in shoot P uptake induced a significant increase in shoot Mg content. The increase in MS medium K supplies showed slight increases shoot K uptake and this was associated with a significant decrease in shoot N uptake at both insufficient and excessive K supplies. The shoot Ca uptake remained stable between 1 and 3 mM of Ca supplies but drastically increased at 10 mM, and the changes in shoot Ca uptake was associated with significant steady increases in shoot N, K, and Mg uptakes. Increases in MS medium Mg supplies showed a steady increase in shoot Mg uptake with 9 mM having a huge uptake, and the changes in shoot Mg uptake had a significant positive interaction with shoot N uptake. Therefore, each nutrient would need to be carefully balanced with other elements because changes in their uptake can occur and have an unnoticed influence on the experimental results.

Second, we evaluated the influence of these variations of each individual nutrient element on aphid *Aphis gossypii* host settling preference, reproduction and feeding behaviors. To assess the aphid settling preference, dual host settling choice tests were conducted using intact potato tissue culture plants. The reproduction tests were conducted by infesting aphids to freely move on the whole plant and reproduce, and the number of nymphs were counted. The EPG analyses were conducted using a single aphid on each plant where the time, intensity and frequency of different feeding behaviors such as non-probing, intracellular stylet puncture, salivation into the sieve elements, and phloem sap ingestion. Our findings showed that host settling preference, reproduction, and feeding behaviors of aphid were all (except for Ca and Mg on reproduction) significantly influenced by the changes in the potato nutrition status. Increases in N nutrition potato plants significantly increased aphid host settling preference, but the number of nymphs were significantly reduced. The aphids spent

significantly shorter total non-probing time on potato plants with insufficient N nutrition compared to plants with optimum N nutrition. The insufficient N nutrition significantly increased the phloem sap feeding duration. Increase in P nutrition significantly increased aphid host settling preference with aphids showing more preference on excessive P potato plants compared to those with insufficient P nutrition. The number of nymphs significantly increased with the increase in P nutrition. The time to first intracellular stylet puncture, number of intracellular punctures, and total duration of intracellular puncture were all significantly shortened in potato plants with insufficient P nutrition when compared to potato plants with optimum and excessive P nutrition. Insufficient P nutrition significantly shortened the total duration of salivation in the sieve elements. The increase in potato plants' K nutrition significantly reduced aphid host settling preference when insufficient and excessive K plants were compared. The number of nymphs significantly increased with the increase in K nutrition up to optimum where it drastically decreased at excessive K nutrition. The total duration of aphid's phloem sap feeding was significantly shortened in potato plants supplied with excessive K nutrition compared to those with insufficient K nutrition. The aphids significantly preferred settling on potato plants with insufficient Ca than on those with optimum Ca nutrition and did not show any influence on number of nymphs. The aphid non-probing time was significantly shorter in potato plants with insufficient Ca compared to those with optimum Ca nutrition, whereas the time spent in phloem sap feeding was significantly longer in potato plants with insufficient Ca nutrition compared to those with optimum Ca nutrition. The aphids showed significantly higher preference for potato plants with excessive Mg nutrition than those with optimum Mg nutrition, but no significant change was observed on the number of nymphs. The time for aphid's first intracellular stylet puncture was significantly longer in potato plants with optimum Mg nutrition compared to those with excessive Mg nutrition. The duration for aphid salivation in the sieve elements was significantly longer in potato plants with optimum Mg nutrition compared to those with excessive Mg nutrition. These results showed that the influence of plant nutrition in potato plants against aphid feeding behavior is at leaf surface, epidermis or mesophyll, and phloem sieve elements cell layers. The influence on each layer vary with each nutrient.

Together, these tissue culture systems can be successfully used for further investigations of how each nutrient effects the efficiency of aphids to inoculate acquire viruses, and the morphological and molecular mechanisms associated with each nutrient in vitro.