

**Impact of nutrient biofortification as a crop nutrient
management strategy against insect pest infestation in
potato (*Solanum tuberosum* L.)**

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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

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

General Introduction

Potato (*Solanum tuberosum*) is one of the world's most important food crops ranking fourth after wheat, maize, and rice in terms of total production (FAO 2022). Globally, potato production has increased by almost 14 %, and the average yield by 16.6 % in the period from 2010 to 2020 (FAO 2022). The production trends show a strong shift towards developing countries of Asia, Africa, and Latin America where potatoes make a significant contribution to the food supply (Lutaladio and Castaldi 2009; Devaux et al. 2021). Africa alone registered a 50 % increase in potato production and a 20 % average increase in yield during the same time period. This underpins the importance of the crop in reducing food insecurity in developing countries of the world.

The yield and quality of potatoes, like many other crops, are largely dependent on the availability of nutrients in addition to light and water. Potatoes require relatively high amounts of potassium (K), nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S) (Hopkins et al. 2020). But, the N, P, and K contents of potato crop are typically higher than that of Ca, Mg and S, and thus the former are regarded as primary nutrients, whereas the latter are regarded as secondary nutrients (Hopkins et al. 2020). N, P, and K are the three fertilizer elements for potatoes that are most frequently used, and this is done at high concentrations (Hopkins et al. 2020). The efficiency with which these nutrients provided by fertilizers are absorbed and utilized has a significant impact on the biomass and properties of potatoes (Ray et al. 2013; Hachiya et al. 2021). Ca and Mg nutrients are not frequently included in the agronomic management of potatoes, despite the fact that they can further limit crop yield and quality, particularly during critical plant developmental stages (Cakmak and Yazici 2010; Palta 2010). Therefore, it is crucial to maintain a supply of these nutrients both on a local and global scale in order to improve potato yield and quality.

Pests and diseases have a significant impact on potato yield and quality, resulting in up to 30 % economic losses in global potato production (Oerke et al. 1994). Several pests, including aphids, potato psyllids, leafhoppers, and the Colorado potato beetle, are known to be economically significant in potato production due to the damage they cause and/or the pathogens they spread to the crop. Among the diseases affecting potatoes, potato virus Y (PVY), potato leafroll virus (PLRV), late blight, soft rot, black leg, bacterial wilt, and cyst nematodes are known to be of economic importance (Adolf et al. 2020). The PVY, a disease spread by aphids, can result in up to 70 % yield losses if not controlled (Nolte et al. 2004). Chemical, biological, and cultural control methods are commonly used by potato farmers to combat aphid infestations. The chemical pest control method involves the use of chemical insecticides with different modes of action such as a repellent and

those that interfere with nervous system and metabolic processes of the insect (Dewar and Denholm 2017). The use of insecticides has remained the major control strategy for suppressing or eliminating aphid populations, though it has always failed to prevent the transmission of viruses such as PVY (Martin et al. 2004; Dedryver et al. 2010). For viruses like PVY, the transmission occurs quickly during superficial stylet punctures before the insecticide becomes effective, and in some cases, the insecticide can increase aphid activity, speeding up virus spread (Budnik et al. 1996; Dedryver et al. 2010). Also, the ongoing development of resistance in novel aphid strains to the majority of pesticides currently in use, as well as the associated human health and environmental risks, has made insecticide use less appealing (Zhang et al. 2018; Kroschel et al. 2020). Biological pest control methods mostly involve the use of natural enemies such as parasitoids, predators, and entomopathogenic fungal species to control the pest populations (Hance et al. 2017). The main limitation of this approach is that it takes longer to suppress pest populations than most pesticides because the parasitized organisms may take several days to die; additionally, natural enemies require time to establish an economic level of pest suppression, thus, making it difficult to apply on a large scale (Bale et al. 2008). Cultural control methods include selection and breeding for pest resistant cultivars and agronomic practices such as intercropping and fertilizer management amongst others (Chang et al. 2017). Agronomic practices are always affected by environmental factors such as fluctuations in temperature (Skendžić et al. 2021). Breeding for resistant cultivars is costly, take longtime, and potato viruses can quickly overcome host-mediated resistance (Yi and Gray 2020). However, plant nutrition mediated host resistance turns to avert such a phenomenon (Huber and Haneklaus 2007). Introduction of integrated pest management (IPM) strategies on potato production can result in a more reliable system for large scale production that is cheap, easy, and practical (Wenninger et al. 2020).

Nutrient supply is often used to explain growth and yield of crop including potato, in relation to each nutrient's role in metabolic processes. Also, plant nutritional status (deficient to toxic) significantly impact on the morphology, anatomy, and chemical composition of a plant which can either increase or decrease pest resistance (Huber et al. 2012). Aphids, in particular, are renowned for being extremely sensitive to changes in plant nutrition, and a change in their preferred food source would have an impact on their behavior (Lees 1966; White 1972; Banerjee and Raychaudhuri 1987). N nutrition, depending on plant species, affect soluble N content, amino acid concentration, repellents or toxins synthesis, trichome density, and leaf thickness which may affect aphid behaviors (Jinwen et al. 2009; Bilkova et al. 2016; Huber et al. 2012). P nutrition affects the defense gene expression, accumulation of lignin and tannins, and production of phenols and terpenes that serve as either barriers or are deleterious to aphids (Khan et al. 2016; Shah 2017). K deficiency impairs sugar

translocation and protein synthesis which increase concentration of sugars and amino acids in the plant foliage and accelerate aphid feeding (Huber et al. 2012). Calcium ions (Ca^{2+}) plays a key role as second messenger to plant's invasion by pathogens (Huber et al. 2012). Calcium nutrition also maintains the integrity of cell membranes and strengthens cell walls, making plants more resistant to pathogens (Palta 2010). Szwarc et al. (2021) found a negative correlation between Ca content and resistance to cabbage aphid (*Brevicoryne brassicae* L.) and cabbage root fly (*Delia radicum* L.) in rapeseed plants. High Mg supply increases free amino acid concentrations in plants, whereas low Mg nutrition affects photosynthesis and photosynthates transportation and causes accumulation of soluble sugars (Ruan et al. 2011; Tränkner et al. 2018). Few studies have worked on Mg nutrition effects on insect pest. Facknath and Lallijee (2005) found no effect of Mg nutrition on serpentine leafminer, *Liriomyza trifolii* Burgess, supplied either alone or in combination with NPK nutrition in potato plants. There haven't been any studies on the effects of Mg nutrition on aphids. Plant nutrition affects pests and thus can be used for pest control.

Several studies have documented typical nutrient deficiency and toxicity symptoms in potato production systems. Nitrogen deficiencies causes stunted growth, yellowing in lower leaves (chlorosis), senescence, and early tuber initiation and bulking in potato plants (Westermann 1993). On the other hand, excessive N uptake favors vegetative growth over tuber growth, delays tuber initiation and skin development, reduces specific gravity and skin set, and makes tubers more susceptible to brown center and hollow heart disease (Westermann 1993; Hopkins et al. 2020). Phosphorus deficiencies in potato plants are known to cause stunted growth, poor root development, darker green leaves, and sometimes purple colored leaves (Uchida 2000; Hopkins et al. 2020). Phosphorus toxicity, on the other hand, causes reduction in growth, leaf necrosis and abscission in plants (Takagi et al. 2020). Inadequate K supplies in potato plants are known to cause stunted growth, decreased photosynthesis, chlorosis and necrosis of older leaves, leaf crinkling, reduced leaf size, and black or dark green colored leaves, whereas toxic supplies cause reduction in root growth, leaf necrosis, increased tuber water content and reduced specific gravity (Hopkins et al. 2020). Calcium deficiencies are reported to cause shoot tip necrosis, axillary shoot growth, chlorotic brown spots on leaves in foliage, and increased bacterial soft rot, internal brown spot, brown center hollow heart in potato tubers (Busse et al. 2008). Currently, little is known on the effects of excess Ca supply and their toxic levels in potato production systems. When Mg is in short supply, the processes of photosynthesis and photo-assimilate partitioning are halted, resulting in interveinal chlorosis in older leaves of the plant (Hermans et al. 2004). With excessive Mg supplies there is currently no evidence for a direct Mg toxicity in plants (Gerendas and Fuhrs 2013). However, interaction of Mg^{2+} with imbalanced supply of other cations such as K^+ , NH_4^+ , and Ca^{2+} may impair some physiological processes in

plants (White et al. 2012; Peng et al. 2020). These can be key limiting factors for sustainability of potato production in the face of almost 60% of the global cultivated soils having nutrient deficiency and toxicity (Schjoerring et al. 2019). And, consequently impact on the health of humans and herbivorous animals including insect pests that consume them (Agren et al. 2012).

Several studies attempted to analyze the effect of essential elements on crop yields as well as the agronomic efficiency of fertilizer utilization under various conditions. For the purpose of maximizing tuber yield. Walworth and Muniz (1993) provided a thorough review of studies on various potato cultivars in the field. They also identified the whole potato leaf N, P, K, Ca, and Mg contents from deficient to toxic levels at flowering stage. However, in field experiments, the availability of the essential elements can be influenced by various factors such as the complex soil chemical, physical and biological properties and their interactions with environmental factors such as temperature, radiation, and water supply (Warring and Cobb 1992; Butler et al. 2012; White et al. 2012; Niu et al. 2015; Liu et al. 2020). Moreover, the application of one fertilizer element can influence the uptake of another element and the vice versa (Ishizuka and Tanaka 1960; Robson and Pitman 1983; Jakobsen 1993; Aulakh and Malhi 2005; Xiao et al. 2022). In addition, these varying conditions interact with other biotic factors such as pests and diseases which may further affect the crop yield and quality levels (Bala et al. 2018; Atkinson and Urwin 2012).

Tissue culture-based *in vitro* research has recently gained popularity as a tool for breeding and testing potato resistance to biotic and abiotic stresses (Brown and Thorpe 1995; Gopal et al. 2008; Bridgen et al. 2018). The technique allows the elimination of viruses and other diseases in several crops including potato through meristem culturing in combination with thermotherapy and chemotherapy (Naik and Buckseth 2018; Bhat and Rao 2020). Thus, using disease free tissue culture plants would allow for a more precise investigation into the effect of abiotic and biotic factors on crop's biochemical and morphological attributes. Murashige and Skoog (MS) medium, a plant growth medium developed based on the nutrient composition of tobacco leaves to propagate tobacco pith, has been widely used in tissue culture (Murashige and Skoog 1962). Terrer and Tomas (2001) optimized peach almond tissue culture propagation by adjusting the nutrient composition of MS medium in relation to the macronutrient concentrations in peach-almond leaves at maximum growth and the macronutrient content of standard MS medium. A similar approach was used with success in Gerbera and Cymbidium plants (Gonçalves et al. 2005). This is also useful for modifying MS medium to produce potato plants with varying levels of nutrition status. Reviews of previous studies on N, P, and K on insect pest populations in natural field, agricultural field, and greenhouse conditions show unclear and conflicting results

(Warring and Cobb 1992; Butler et al. 2012). Positive and negative responses were recorded for N, P, and K, with majority being positive for N and negative for P and K (Butler et al. 2012). The authors attributed the inconsistencies to differences in environmental conditions within and among greenhouse, natural, and agricultural fields. Such inconsistencies may be resolved by studies in a controlled environment where only plant nutrition can be varied.

Varying responses of potato plants to changes in MS medium in efforts to optimize growth and micro-tuber production have previously been reported. Reducing the N concentration of the normal MS medium was reported to improve the performance potato plants (Zarrabeitia et al. 1997). Contrastingly, Sarkar and Naik (1998) found improved growth in potato with the reduction of the N concentration. Work on K showed large sized potato micro-tubers with increased K supply in MS medium (Naik and Sarkar 1998). Radouani and Lauer (2015) simultaneously increased N, P, and K contents in the MS medium and reported improved potato growth. However, these studies did not define the insufficient or excessive nutrient supplies. Conversely, the manipulation of the nutrient composition in the MS medium can be used to artificially produce potato plants with insufficient and excessive nutrition statuses mimicking field conditions (Groll et al. 2002; Ward et al. 2008; Ohmagari et al. 2020).

Several studies have been conducted to understand on how plants confer resistance against aphids using host settling choice tests, reproduction assays as well as feeding behaviors using electrical penetration graphs (EPG) (Tjallingii and Esch 1993; Smith and Chuang 2014; Leybourne and Aradottir 2022). Aphids feed on plant phloem sap as their primary source of nutrition, which has high ratios of sugars to amino acids, non-essential to essential amino acids and K^+ to Na^+ and incredibly low lipid levels (Douglas 2003). The primary nutrients for aphids are amino acids, and variations in their composition and concentration have a significant impact on aphid performance (Cao et al. 2018; Gruhn et al. 2021). Amino acids are used for protein synthesis, as key components for neurotransmitters and neurohormones, and as a constituent of aphid haemolymph (Douglas et al. 2017). Sugars, on the other hand, are an important source of carbon for aphids, and their concentrations in the phloem sap affects the feeding rate, with lower rates observed at higher sucrose concentrations (Douglas et al. 2006). However, the chemical composition of the sap varies between plant species and is influenced by environmental factors (Gruhn et al. 2021). Aphids must therefore find a suitable host plant that can provide them with the nutrition they require for fitness. According to Powell et al. (2006), aphids exhibit six sequential behaviors in the process of selecting a suitable host plant. Aphids initially use visual (plant-reflected wavelengths) and olfactory (volatiles) cues to locate a plant, regardless of whether it is a suitable host plant or

a non-host (Nottingham et al. 1991). When aphids come in contact with a plant, plant surface cues such as epicuticular waxes and trichomes exudates influence their behavior before initiating brief (< 1 min) stylet probes into the epidermis and ingesting sap to assess its quality (Powell et al. 1999; 2004). Later, aphids penetrate and inject watery saliva through their stylets into the mesophyll and parenchyma tissues, and then into the phloem sieve element, where they uptake the sap (Prado and Tjallingii 1994). According to Prado and Tjallingii (1994), saliva prevents plants from sealing the sieve elements, allowing for sustained sap ingestion. And, host plant acceptance is presented by longer and sustained phloem ingestion, which may last from 10 min to several hours. Plant resistance can thus occur at any point during the aphid suitable host selection process. While aphid fitness under no choice was evaluated using reproduction assays (Hosseini et al. 2010; Gómez-Trejo et al. 2020), the influence of volatiles and plant morphological characteristics on a plant's susceptibility was primarily evaluated using host settling tests (Nowak and Komor 2010; Bala et al. 2018; Ahmed et al. 2019). The presence of resistance at the leaf surface as well as in various layers of plant tissues was assessed using the EPG (Escudero-Martinez et al. 2020). When taken as a whole, these studies offered perceptive insights into aphid behavioral changes in relation to plant resistance with aphids showing increased host preference and reproduction, as well as sustained feeding, owing to phloem sap quality and the absence of resistance factors, especially at the phloem sieve element (Leybourne and Aradottir 2022; Twayana et al. 2022). To better understand how plant nutrition works, such studies can also be done in potato tissue culture. Currently, there haven't been many aphid behavioral studies done in potato tissue culture. Few studies examined the host plant selection patterns and PVY transmission efficiency of various aphid species and the findings were consistent with those from studies conducted in agricultural fields (Boquel et al. 2012; 2014). These findings suggest a possibility that studying the impact of plant nutrition on aphids using potato tissue culture may yield useful information for field potato research.

Therefore, the aims of this study were to: (1) establish a model nutrition control system in potato tissue culture and its influence on plant elemental composition (2) determine the influence of plant nutrition status on aphid host settling preference, reproduction, and feeding behaviors in potato plants *in vitro*. The information gathered provided new insights on how plant nutrition influence aphid behaviors particularly in potato plant. It further provides guidance for further studies on nutrient management under field or greenhouse to combat aphid infestation in potatoes. Better understanding of the individual nutrient and their interactive effect on aphid feeding behaviors may lead to improved fertilization and pest management strategies in potato production systems. This will reduce the costs and environmental risks associated with the use of chemical insecticides.

CHAPTER 1

A Model Nutrition Control System in Potato Tissue Culture and its Influence on Plant Elemental Composition

1.1 Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most important crops, providing major contributions to human nutrition, livestock feed, employment, and income (Devaux et al. 2020). Potato requires a variety of essential nutrients for growth and development, and intensive fertilizer applications remain the main viable option for improving yield and quality. Insufficient or excessive soil nutrient contents are known to influence plant nutrient levels and affect the sensitivity of potatoes to abiotic stresses such as heat and drought as well as biotic stresses such as diseases and insect pests (Hopkins et al. 2020). For instance, Ca status in the potato root zone is known to regulate abiotic stresses including heat and frost (Palta 2010). Increases in leaf N content have been linked to an increased host suitability of the potato plants to leaf miners, whereas increases in P and K were detrimental to the insects (Facknath and Lalljee 2005). Butler et al. (2012) reported an overall positive response of insect pests to increased fertilization but noted that findings are still conflicting. The results of field studies can vary because nutrient availability is influenced by a variety of factors, including the soil's complex chemical, physical, and biological properties, as well as their interactions with environmental factors such as temperature, radiation, and water supply. Therefore, research in a controlled environment may reveal clear interactions between plant nutrient status and biotic as well as abiotic stresses.

The tissue culture technique has been widely used for rapid large-scale multiplications of plants, including potatoes, in an aseptic and controlled environment. Murashige and Skoog (MS) medium, a plant growth medium developed based on the nutrient composition of tobacco leaves to propagate tobacco pith, has been widely used for tissue culture (Murashige and Skoog 1962). Manipulation of the nutrient composition in the MS medium could be used to produce potato plants with insufficient and excessive nutrition status of a particular element. Several studies have attempted to adjust MS medium nutrient concentrations for the particular purpose of increasing either plantlet growth or microtuber yield. Radouani and Lauer (2015) simultaneously increased N, P, and K contents from the standard 60 mM, 1.25 mM, and 20 mM, respectively, in MS medium, which resulted in an elevated number of microtubers, microtuber weight, and stem and root

weights. The purpose of this study was to increase microtuber yield, and the study did not measure the nutrient contents in the plants. Nguyen et al. (2021) assessed potato growth under insufficient N levels of 0 to 7.5 and 60 mM and reported the highest shoot biomass at 7.5 mM. Upadhyaya et al. (2016) assessed the growth of potato cultivar Desiree at Ca levels of 0 to 12 mM and reported the highest growth and tuber yield at 9 mM Ca. These studies found changes in the morphology of the plant by adjusting the nutrient concentrations. However, there are very few studies evaluating the nutrient contents that could alter visible plant growth or invisible plant functions.

Walworth and Muniz (1993) provided a comprehensive review of several field-grown potato cultivars with insufficient and excessive whole potato leaf N, P, K, Ca, and Mg contents at flowering stage, and their relationship to yield. Geary et al. (2015) established deficient, adequate, and excess N nutrient status in potato plants under hydroponic conditions by evaluating N content. No studies like this have been done for potato tissue culture, but the nutrient status has been successfully determined in tissue culture studies of other crops, including peach almonds, flowers, and legume trees (Terrer and Tomas 2001; Bouman and Tiekstra 2005; Gonçalves et al. 2005). The adoption of this approach may thus aid in growing potato plants with insufficient or excessive nutrient content in a tissue culture system.

Varying the rate of application of one element is known to affect the plant uptake of other elements. Major macronutrients have been found to interact with each other; i.e., N is known to interact with P, K, Ca, and Mg in many different plant species (René et al. 2017). These interactions could either be antagonistic or synergistic either in the soil or within the plant in a complex manner. For example, both competitive and synergistic uptake of multiple nutrients from the soil can occur among the elements (Kumar et al. 2021). In addition, root growth can be enhanced by some nutrients, which can accelerate the uptake of other nutrients (Fageria et al. 2014). These intricate interactions occur in both soil and plants, affecting nutrient composition, yield, and quality of crops. Currently, there is a scarcity of information on nutrient interactions in potato plants in vitro, and the tissue culture system with varying nutrient concentrations in the MS media could be an effective way to study this, under a controlled environment.

In this study, a model tissue culture nutrient control system was designed based on the whole potato leaf's nutrient contents in the field, and the effects of essential nutrient supply on potato growth and development were systematically analyzed. The objectives of the study were to (1) establish nutrient concentration levels in the media to produce potato plants with insufficient and excessive nutrient content and (2) evaluate the

nutritional interactions driven by variations in nutrient element concentrations in the media. This data could be valuable in investigations into the effects of nutrients on biotic and abiotic stresses on potato production.

1.2 Materials and Methods

1.2.1 Plant material and growth conditions

The potato, *Solanum tuberosum*, cv. Irish Cobbler, was used to generate plant materials in the entire experiment, utilizing a tissue culture approach. Disease-free stock plantlets were multiplied and maintained from aseptically excised 1 cm long leafless single-node sections in cup-closed 60 mL transparent glass tubes containing 10 mL of standard MS medium, which contained MS inorganic salts, Fe-EDTA, H vitamins, 30 g L⁻¹ of sucrose, and 7 g L⁻¹ of Bacto Agar (Murashige and Skoog 1962). The MS inorganic salts contained 0.83 mg L⁻¹ of potassium iodide (KI), 6.2 mg L⁻¹ of boric acid (H₃BO₃), 8.6 mg L⁻¹ of zinc sulfate heptahydrate (ZnSO₄·7H₂O), 24.1 mg L⁻¹ of manganese (II) sulfate pentahydrate (MnSO₄·5H₂O), 0.17 g L⁻¹ of potassium dihydrogen phosphate (KH₂PO₄), 1.65 g L⁻¹ of ammonium nitrate (NH₄NO₃), 1.9 g L⁻¹ of potassium nitrate (KNO₃), 0.44 g L⁻¹ of calcium chloride dihydrate (CaCl₂·2H₂O), 0.37 g L⁻¹ of magnesium sulfate heptahydrate (MgSO₄·7H₂O), 0.25 mg L⁻¹ of disodium molybdate (VI) dehydrate (Na₂MoO₄·2H₂O), 0.025 mg L⁻¹ of copper (II) sulfate pentahydrate (CuSO₄·5H₂O), and 0.025 mg L⁻¹ of cobalt (II) chloride hexahydrate (CoCl₂·6H₂O). The Fe-EDTA was composed of 46.60 mg L⁻¹ of disodium ethylenediaminetetraacetate dihydrate (C₁₀H₁₄O₈N₂Na₂·2H₂O) and 34.75 mg L⁻¹ of iron (II) sulfate heptahydrate (FeSO₄·7H₂O). The H vitamins were composed of 100 mg L⁻¹ of myo-inositol, 2 mg L⁻¹ of glycine, 0.5 mg L⁻¹ of thiamine hydrochloride (vitamin B1 hydrochloride), 0.5 mg L⁻¹ of pyridoxine hydrochloride (vitamin B6 hydrochloride), 5 mg L⁻¹ of nicotinic acid, 0.05 mg L⁻¹ of biotin, and 0.5 mg L⁻¹ of folic acid. Subsequently, the multiplied disease-free plantlets were used to generate plantlets with insufficient and excessive N, P, K, Ca, and Mg contents, and five replicates were randomly selected for subsequent assessments. About 1 cm of leafless single-node sections were aseptically excised and inserted into cup-closed 60 mL transparent glass tubes containing 30 mL of MS media of different individual amounts of N, P, K, Ca, and Mg (Table 1-1). Then, the cup was opened when the shoots reached the mouth of the tube to allow the plantlets to develop 5 to 6 leaves outside the tube before further assessments. To prevent contamination from the outside and also moisture loss, sterilized glass beads (ca. 1 mm in diameter) and activated carbon were added to the media surface to a depth of about 0.7 mm and 0.5 mm, respectively. The

approximate entire plant growth period was 5 to 6 weeks depending on the individual nutrient and supply amounts. All the plantlets were grown in a controlled room with the temperature maintained at 20 ± 2 °C with 16 h of light and 8 h of darkness throughout the entire experimental period. All the media had their pH adjusted to 5.6 and were autoclaved at 121 °C for 20 min before use.

Table 1-1. Amount of salts that were varied in MS media for propagation of potato plantlets with insufficient and high N, P, K, Ca, and Mg nutrient status for plant nutrient effect bioassays.

	Concentration (mM)	NH ₄ NO ₃	KNO ₃	KH ₂ PO ₄	KCl	Ca(NO ₃) ₂ ·4H ₂ O	CaCl ₂ ·2H ₂ O	MgCl ₂ ·6H ₂ O	MgSO ₄ ·7H ₂ O	KI
		(g l ⁻¹)								
Standard ^a		1.65	1.90	0.17			0.440		0.37	0.00083
N	20	0.775		0.17	1.39		0.440		0.37	0.00083
	70	1.925	1.90	0.17			0.440		0.37	0.00083
P	0.4	1.65	1.90	0.058	0.062		0.440		0.37	0.00083
	3.5	1.74	1.67	0.479			0.440		0.37	0.00083
K	13.5	1.65		0.17	0.921	0.0312	0.421		0.37	0.00083
	30	1.65	1.90	0.17	0.752		0.440		0.37	0.00083
Ca	1.0	1.65	1.90	0.17			0.147		0.37	0.00083
	10.0	1.65	1.90	0.17			1.467		0.37	0.00083
Mg	1.5	1.65	1.90	0.17			0.440		0.37	0.00083
	9.0	1.65	1.90	0.17			0.440	1.52	0.37	0.00083

^a as in standard MS medium, NH₄NO₃: ammonium nitrate, KNO₃: potassium nitrate, KH₂PO₄: potassium dihydrogen phosphate, KCl: potassium chloride, Ca(NO₃)₂·4H₂O: calcium nitrate tetrahydrate, CaCl₂·2H₂O: calcium chloride dihydrate, MgCl₂·6H₂O: magnesium chloride hexahydrate.

1.2.2 Calculations of MS media N, P, K, Ca, and Mg concentrations

The N, P, K, Ca, and Mg concentrations in the MS media were manipulated to various concentrations using the insufficient and excessive N, P, K, Ca, and Mg contents provided by Walworth and Muniz (1993) for the whole leaf of field-grown potatoes at flowering stage and formulas provided by Terrer and Tomas (2001). The excessive amount of each nutrient in the new media was calculated using Formula (1)

$$Em = B \times \frac{\sum M}{\sum El} \quad (1)$$

where Em is the amount (mg L⁻¹) of the excessive nutrient in the new media; B is the excessive content (%) of each nutrient in the potato whole leaf defined by Walworth and Muniz (1993); $\sum M$ is the total sum of N, P, K, Ca, and Mg (mg L⁻¹) in the standard MS medium; and $\sum El$ is the total sum of the excessive content of N, P, K, Ca, and Mg in potato whole leaf. The deficient amounts (mg L⁻¹) of each nutrient were calculated using Formula (2)

$$Dm = Em \times \frac{\sum Dl}{\sum El} \quad (2)$$

where Dm is the deficient amount (mg L⁻¹) of the nutrient in the new media; Em is the calculated excessive amount (mg L⁻¹) of each nutrient in the new media (Formula (1)); $\sum Dl$ is the total sum of deficient N, P, K, Ca, and Mg (mg L⁻¹) in potato whole leaf defined by Walworth and Muniz (1993); and $\sum El$ is the total sum

of the excessive content of N, P, K, Ca, and Mg in potato whole leaf Walworth and Muniz (1993). Further adjustments were made to the calculated nutrient concentrations when potato plantlets exhibited severe deficiency or toxicity symptoms to ensure asymptomatic plantlets can be produced. In the MS media, only the MS inorganic salts were changed according to the formulas above, while other nutrients, H vitamins, Fe-EDTA, sucrose, and Bacto Agar were kept the same as in the standard MS medium (Table 1).

1.2.3 Plant growth assessments

In order to understand the effects of the manipulated nutrient supply on potato growth *in vitro*, the fresh and dry weights of aerial biomass (stem plus leaves), hereafter referred to as fresh and dry shoot biomass, were measured on plantlets with 5 to 6 fully grown leaves outside the growth glass tubes. Five plantlets of uniform growth were randomly selected for each nutrient type and concentration for measurements of fresh and dry biomass weight using a digital scale. The fresh weights were measured immediately after sample collection, and the dry weights were determined after oven drying at 60 °C for 4 days. The plant's morphological characteristics were visually observed to identify severe nutrient deficiency or toxicity symptoms. In this study, dry shoot biomass and morphological symptoms were used to define “deficient”, “insufficient”, “optimum”, “excessive”, and “toxic” levels. Deficient level was concluded when the visible symptoms of nutritional deficiency were observed. Insufficient level correlated with a significantly low dry shoot biomass. The optimum level was within the range of maximum biomass. Excessive level was concluded when, despite an increase in nutrient concentration, no additional increase in biomass was observed. Toxic level was when visible symptoms of nutrient toxicity were observed.

1.2.4 Plant nutrient composition analyses

The nutrient composition was determined in the shoots of 5 individual plantlets as 5 replicates per each treatment after oven drying and fine grinding. Total nitrogen was measured using a dry combustion method by a CHN automated elemental analyzer (Vario EL III, Elementar Analysensysteme, Hanau, Germany). To determine shoot P, K, Ca, and Mg, approximately 0.01 g of fine ground sample was ashed using a muffle furnace followed by dissolution of the ash with 1 mol L⁻¹ hydrochloric acid (HCl) according to Miller (1998). The digest was measured using an inductively coupled plasma atomic emission spectrometer (ICPE-9820, Shimadzu Corporation, Kyoto, Japan). Five replicates (potato plantlets) were analyzed for each treatment.

These analytical machines were part of the Obihiro University of Agriculture and Veterinary Medicine Common Equipment. All measurements were calculated on a dry matter basis. The nutrient uptake was calculated by multiplying the shoot nutrient content by the dry shoot biomass.

1.2.5 Statistical analyses

All statistical analyses on plant growth parameters and nutrient composition were performed using JASP statistical software version 0.15 (JASP Team 2021). Data on plant growth and nutrient composition were subjected to one-way analysis of variance followed by Tukey's honestly significant difference to separate the means at $p < 0.05$. To check whether changing one nutrient causes a change in the uptake of other nutrients, the Bayesian Pearson's correlations were tested on measured shoot nutrient uptake. The Bayes factor (BF_{10}) values were used to categorize the strength of evidence as follows: >100 : extreme, 30–100: very strong, 10–30: strong, 3–10: moderate, 1–3: anecdotal, and 1 or <1 : none (Nuzzo 2017).

1.3 Results and discussion

1.3.1 Definition of N nutrient status

From the dry shoot biomass and morphological symptoms, 16 mM N was defined as deficient, 20 to 40 mM as insufficient, 60 to 80 mM as optimum, and 100 mM as toxic. The maximum shoot dry biomass was observed between 60 and 80 mM, and the dry shoot biomass was significantly reduced in N supplies below 40 mM (Figure 1-1 A). Deficiency symptoms including chlorosis and retarded root growth were observed at 16 mM (Figure 1-2 A). Nguyen et al. (2021) reported increased dry shoot biomass with a decreased N supply of 7.5 mM compared to 60 mM, and no chlorotic symptoms were observed in potato cultivar Iwa. Schum et al. (2017) reported decreased shoot biomass in 13 out of 17 potato cultivars with an N supply of 7.5 mM compared to 60 mM, and five of the cultivars showed heavy chlorosis. These results affirm that potato N response is cultivar-dependent and a cultivar-specific experiment needs to be run to determine the N levels for a deficient to toxic range.

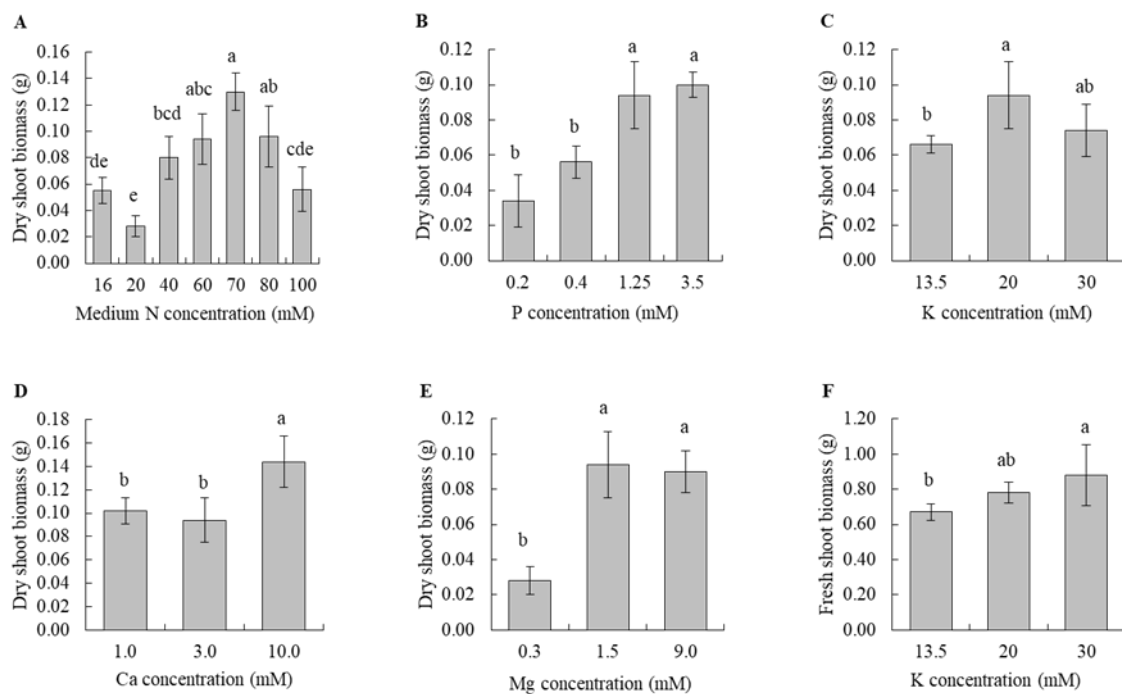


Figure. 1-1 Effect of varying nutrient supplies in MS media on potato plant dry shoot biomass weight: (A) nitrogen; N, (B) phosphorus; P, (C) potassium; K, (D) calcium; Ca, (E) Magnesium; Mg, and (F) K on fresh shoot biomass weight. Error bars on bar graphs represent standard deviations. Means separated according to Tukey's HSD at $p < 0.05$.

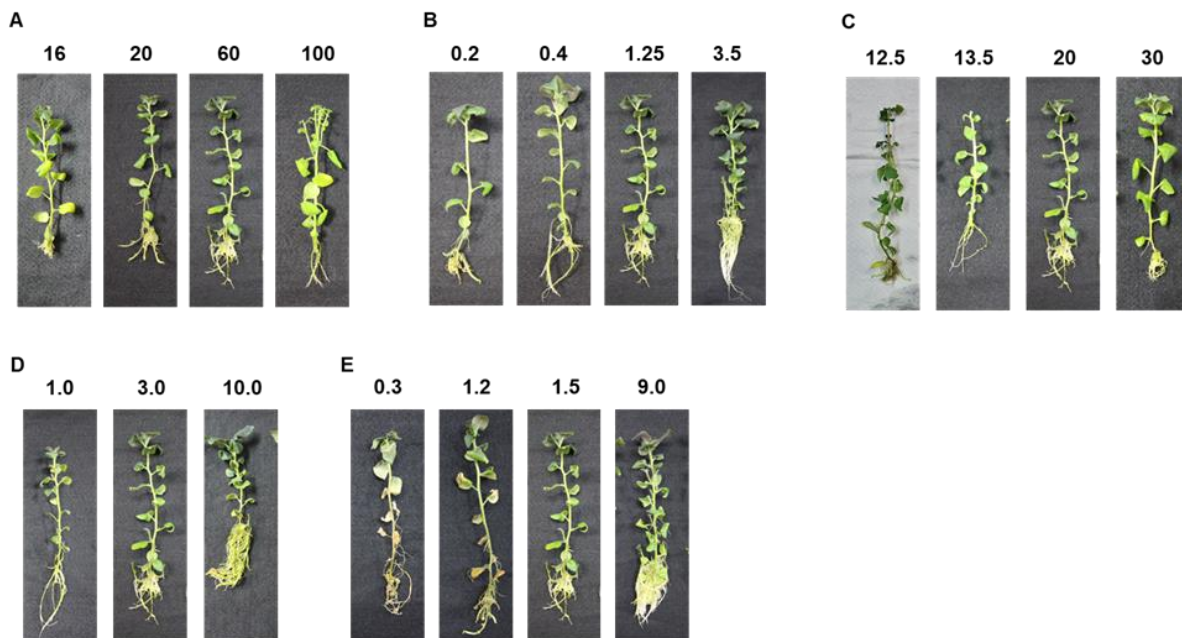


Figure. 1-2 Photographs showing the morphological characteristics of potato plantlets supplied with varying concentrations (mM) of (A) nitrogen (N: 16-20 = insufficient, 60 = standard, 100 = toxic), (B) phosphorus (P: 0.2 – 0.4 = insufficient, 1.25 = standard, 3.5 = excessive), (C) potassium (K: 12.5 – 13.5 = insufficient, 20 = standard, 30 = excessive), (D) calcium (Ca: 1.0 = insufficient, 3.0 = standard, 10.0 = excessive), and (E) magnesium (Mg: 0.3 – 1.2 = insufficient, 1.5 = standard)

In contrast, at 100 mM, significantly lower dry shoot biomass was observed, as were symptoms linked to toxicity, including many small leaves, heavy branching, early leaf drying, and minimal lateral root growth (Figure 2A). The N uptake also significantly decreased (Table 1-2), but shoot N content was significantly higher than that in all other N supply levels (Table 1-3). This poor growth might be caused by poor root growth. A previous study suggested that N concentrations in solution and in tissue, and thus toxicity level, greatly changed with the N forms and that the optimal range of N was higher with NO_3^- than with NH_4^+ nutrition (Cao and Tibbits 1998). This suggests that the N toxicity observed in the present study could be due to increased NH_4^+ ion concentration in the medium.

Table 1-2. Effect of changes in nutrient element concentrations in MS medium on nutrient uptake in shoots of potato plants *in vitro*

Medium element concentration															
Element	(mM)	N		P		K		Ca		Mg					
		(mg plantlet ⁻¹)													
N	20	1.90	± 0.40 c	0.26	± 0.06 b	0.73	± 0.17 c	0.26	± 0.04 c	0.10	± 0.02 b				
	40	4.36	± 0.71 b	0.51	± 0.13 a	3.61	± 0.39 a	0.44	± 0.06 b	0.19	± 0.03 a				
	60 ^a	6.03	± 1.15 ab	0.59	± 0.13 a	3.96	± 1.40 a	0.43	± 0.07 b	0.19	± 0.06 a				
	70	7.39	± 0.62 a	0.45	± 0.03 ab	3.02	± 0.28 ab	0.58	± 0.07 a	0.20	± 0.02 a				
	80	7.50	± 1.89 a	0.66	± 0.12 a	2.53	± 1.08 ab	0.30	± 0.07 c	0.15	± 0.05 ab				
	100	4.98	± 1.43 b	0.44	± 0.15 ab	1.77	± 0.38 bc	0.21	± 0.06 c	0.10	± 0.03 b				
P	0.2	2.22	± 0.85 c	0.14	± 0.04 c	1.14	± 0.52 b	0.14	± 0.07 c	0.06	± 0.02 c				
	0.4	3.75	± 0.44 b	0.24	± 0.05 c	1.83	± 0.29 b	0.24	± 0.04 c	0.10	± 0.01 c				
	1.25 ^a	6.03	± 1.15 a	0.59	± 0.13 b	3.96	± 1.40 a	0.43	± 0.07 b	0.19	± 0.06 b				
	3.5	7.36	± 0.59 a	1.35	± 0.17 a	4.71	± 0.42 a	0.62	± 0.05 a	0.25	± 0.04 a				
K	13.5	4.18	± 0.46 b	0.48	± 0.14 a	2.53	± 0.73 a	0.34	± 0.07 a	0.15	± 0.02 a				
	20 ^a	6.03	± 1.15 a	0.59	± 0.13 a	3.96	± 1.40 a	0.43	± 0.07 a	0.19	± 0.06 a				
	30	4.33	± 0.89 b	0.57	± 0.08 a	3.94	± 0.91 a	0.32	± 0.08 a	0.15	± 0.03 a				
Ca	1.0	6.09	± 0.57 b	0.52	± 0.03 a	2.07	± 0.42 b	0.20	± 0.02 b	0.15	± 0.01 b				
	3.0 ^a	6.03	± 1.15 b	0.59	± 0.13 a	3.96	± 1.40 a	0.43	± 0.07 b	0.19	± 0.06 ab				
	10.0	8.61	± 1.17 a	0.63	± 0.07 a	4.57	± 0.49 a	1.56	± 0.29 a	0.25	± 0.04 a				
Mg	0.3	1.60	± 0.30 c	0.33	± 0.14 b	0.40	± 0.15 b	0.28	± 0.09 b	0.06	± 0.02 c				
	1.5 ^a	6.03	± 1.15 b	0.59	± 0.13 a	3.96	± 1.40 a	0.43	± 0.07 a	0.19	± 0.06 b				
	9.0	8.04	± 1.22 a	0.59	± 0.07 a	3.50	± 0.14 a	0.33	± 0.04 ab	0.81	± 0.11 a				

^aStandard as in MS medium [6]. n = 5. Means are presented with ± standard deviations. Means separated according to Tukey's HSD at p < 0.05.

When we compared the interaction of N with other elements, we observed a significant decrease in shoot Ca and Mg uptakes in the toxic N range (Table 1-2), in line with Roosta and Schojoerring (2007)'s report of poor growth of cucumber with toxic levels of NH_4^+ levels and associated with reduced tissue Ca and Mg contents. Additionally, changes in shoot N uptake had a significant positive interaction with the changes in

P, K, Ca, and Mg uptakes (Table 1-4). Similar findings were reported for N and K in *Actinidia arguta* grown under tissue culture and for N, Ca, and Mg in cucumber grown under hydroponic conditions (Roosta and Schojoerring 2007; Arteta et al. 2022). Therefore, N supply studies in potato plants would require careful consideration of balance with other elements because the reduced uptake of these nutrients can occur before any apparent symptoms.

Table 1-3. Effect of changes in nutrient element concentrations in MS medium on nutrient content in shoots of potato plants *in vitro*.

Element	Medium element concentration	N		P		K		Ca		Mg						
	(mM)															
(g kg ⁻¹)																
N	20	64.4	± 3.4	c	9.8	± 2.6	a	27.7	± 8.2	b	9.7	± 1.9	a	3.7	± 0.8	a
	40	55.0	± 5.3	d	6.6	± 1.7	b	46.0	± 5.9	a	5.5	± 0.8	b	2.3	± 0.2	b
	60 ^a	64.4	± 3.4	c	7.0	± 0.9	ab	45.6	± 4.8	a	4.3	± 0.5	bc	2.1	± 0.1	bc
	70	57.0	± 3.5	cd	3.5	± 0.4	c	23.4	± 3.2	b	4.4	± 0.6	bc	1.5	± 0.2	c
	80	78.0	± 4.1	b	6.9	± 0.9	b	25.6	± 4.4	b	3.1	± 0.3	c	1.5	± 0.1	c
	100	89.2	± 3.4	a	8.0	± 1.3	ab	32.5	± 5.3	b	3.6	± 0.1	c	1.7	± 0.1	bc
P	0.2	66.6	± 5.8	ab	4.3	± 1.4	c	33.3	± 2.8	b	4.0	± 0.3	b	1.8	± 0.2	bc
	0.4	67.4	± 4.7	ab	4.4	± 1.2	c	32.7	± 2.8	b	4.2	± 0.3	b	1.7	± 0.2	c
	1.25 ^a	64.4	± 3.4	b	7.0	± 0.9	b	45.6	± 4.8	a	4.3	± 0.5	b	2.1	± 0.1	b
	3.5	73.6	± 2.5	a	13.4	± 1.2	a	47.4	± 6.3	a	6.3	± 0.5	a	2.5	± 0.3	a
K	13.5	63.2	± 3.0	ab	7.3	± 1.7	a	38.0	± 8.9	b	5.2	± 0.8	a	2.3	± 0.2	a
	20.0 ^a	64.4	± 3.4	a	7.0	± 0.9	a	45.6	± 4.8	ab	4.3	± 0.5	a	2.1	± 0.1	a
	30.0	58.6	± 2.6	b	7.9	± 1.2	a	53.7	± 8.6	a	4.4	± 0.3	a	2.1	± 0.1	a
Ca	1.0	59.8	± 2.6	a	5.2	± 0.5	b	20.2	± 3.2	c	1.9	± 0.1	c	1.5	± 0.1	c
	3.0 ^a	64.4	± 3.4	a	7.0	± 0.9	a	45.6	± 4.8	a	4.3	± 0.5	b	2.1	± 0.1	a
	10.0	60.0	± 2.5	a	4.4	± 0.6	b	32.2	± 5.1	b	10.8	± 1.0	a	1.7	± 0.2	b
Mg	0.3	47.2	± 6.4	b	11.7	± 2.0	a	14.2	± 3.2	b	9.8	± 0.7	a	2.1	± 0.2	b
	1.5	64.4	± 3.4	a	7.0	± 0.9	b	45.6	± 4.8	a	4.3	± 0.5	b	2.1	± 0.1	b
	9.0	66.6	± 6.1	a	6.6	± 0.6	b	39.4	± 4.4	a	3.6	± 0.3	b	9.0	± 0.7	a

^aStandard as in MS medium [6]. n = 5. Means are presented with ± standard deviations. Means separated according to Tukey's HSD at p < 0.05.

Table 1-4. Bayesian Pearson's correlations among N, P, K, Ca, and Mg uptake in shoot of potato plants as affected by individual nutrient concentrations in MS medium in vitro.

Change in shoot nutrient	Shoot nutrient uptake	n	Pearson's r	p value	BF ₁₀	Lower 95% CI	Upper 95% CI
N	P	30	0.723	< 0.001 ^{†††}	7231.98 ^{***}	0.461	0.850
	K	30	0.554	0.001 ^{††}	56.03 ^{**}	0.225	0.747
	Ca	30	0.520	0.003 ^{††}	28.12 [*]	0.183	0.724
	Mg	30	0.541	0.002 ^{††}	42.89 ^{**}	0.209	0.738
P	N	20	0.870	< .001 ^{†††}	54778.27 ^{***}	0.647	0.944
	K	20	0.825	< .001 ^{†††}	5984.41 ^{***}	0.552	0.922
	Ca	20	0.935	< .001 ^{†††}	11040000.00 ^{***}	0.805	0.974
	Mg	20	0.908	< .001 ^{†††}	727016.73 ^{***}	0.735	0.961
K	N	15	0.382	0.160	1.43	0.031	0.713
	P	15	0.856	< .001 ^{†††}	1152.63 ^{***}	0.537	0.947
	Ca	15	0.384	0.157	1.45	0.032	0.715
	Mg	15	0.363	0.183	1.29	0.029	0.704
Ca	N	15	0.881	< 0.001 ^{†††}	3138.88 ^{***}	0.599	0.957
	P	15	0.399	0.070	1.58	0.034	0.722
	K	15	0.629	0.006 ^{††}	11.24 [*]	0.152	0.839
	Mg	15	0.743	< 0.001 ^{†††}	62.26 ^{**}	0.310	0.895
Mg	N	15	0.812	< 0.001 ^{†††}	291.83 ^{***}	0.440	0.927
	P	15	0.533	0.020 [†]	4.23	0.079	0.791
	K	15	0.523	0.023 [†]	3.87	0.073	0.786
	Ca	15	0.060	0.415	0.38	0.009	0.554

[†] $\alpha = 5\%$, ^{††} $\alpha = 1\%$, ^{†††} $\alpha = 0.1\%$. *BF₁₀ > 10, **BF₁₀ > 30, ***BF₁₀ > 100, CI: Confidence interval.

1.3.2 Definition of P nutrient status

Based on the dry shoot biomass and morphological symptoms, 0.2 mM P was defined as deficient, 0.4 mM as insufficient, 1.25 mM as optimum, and 3.5 mM as excessive. At 0.4 mM, a relatively normal growth, stronger stems and leaves, and improved rooting were observed. The relatively normal growth could be due to the slight increases in nutrient uptake (Table 1-2) caused by an improved root system. However, the dry shoot biomass was significantly reduced at 0.4 mM and was significantly different from 1.25 mM P (Figure 1-1 B). The maximum dry shoot biomass was observed at a concentration of 3.5 mM, which was not significantly different from 1.25 mM. This was the first in vitro study to report the effect of varied P alone and the evaluation of P content in potato plants. Most in vitro studies that have assessed the effect of varied P supply on potato plants were performed in combination with changes in other nutrients (Sarkar et al. 2004; Radouani et al. 2015). In this study, P deficiency symptoms such as weaker stems and darker leaves, as well as fewer and smaller roots, were observed in 0.2 mM N supply (Figure 1-2 B). Similar symptoms were observed by Barben et al. (2010) in potato plants grown in P-deficient nutrient solution under hydroponic

conditions. The P deficiency was reported to impair both the synthesis and translocation of sugars, which negatively impacts plant growth.

Although the dry shoot biomass did not significantly increase, the P uptake at 3.5 mM significantly increased compared to 1.25 mM, indicating an excessive P uptake (Table 1-2). This excessive P uptake was more favorable for root growth than for shoot growth (Figure 1-2 B). In a greenhouse study, a similar trend in shoot growth has been reported with a decline in the P use efficiency in shoot growth with high P applications (Fernandes and Sorrato 2012). In addition, the lack of shoot growth could be related to the plant allocating more sugars to the roots, which has been reported in potato plants grown under deficient P (Kavka et al. 2021). In this study, the same phenomenon also occurred at excessive P supply, as evident from the improved root growth (Figure 1-2 B), suggesting that it occurs not only at deficient but also at excessive P. This correlated with a significant increase in Mg content from 1.25 to 3.5 mM P (Table 1-3). Mg is known to improve root growth in potato plants (Koch et al. 2020). In the field, high P fertilizer applications increase the below-ground biomass (Rosen and Bierman 2008), reflected in the present study by improved root growth. However, previous studies reported P toxicity in potato cultivar Russet Burbank at an excessive P supply of 1024 μ M in a hydroponic solution (Barben et al. 2010). This suggests that further increases in P supply above 3.5 mM may produce symptomatic potato plants. In addition, the effect of Mg changes may need careful consideration in P fertilization field studies.

Increases in P supply also increased the uptake of N, K, and Ca in potato shoots (Table 1-2). The shoot Ca and Mg uptake increased similarly to the P uptake, while for N and K there was no significant difference between 1.25 and 3.5 mM P supply. The changes in shoot P uptake had a significant positive correlation with the changes in N, K, Ca, and Mg uptake (Table 1-4). Studies on several other crops have reported a positive relationship between P and N (Broadley et al. 2004), and K (Abd Lateef et al. 2021), as well as both positive and negative relationships between P and Ca (Gonçalves et al. 2005). Broadley et al. (2004) attributed the P and N relationship to their involvement in photosynthesis and protein formation, and that P can be taken up and translocated with K, Ca, and Mg to different parts of the plant.

1.3.3 Definition of K nutrient status

Based on the dry shoot biomass and the morphological symptoms, 12.5 mM K was deficient, 13.5 mM was insufficient, 20 mM was optimum, and 30 mM was excessive. The 12.5 mM concentration was not included

in further analyses due to poor growth and early defoliation of the plant. The plantlets showed K deficiency symptoms such as lighter green and early yellowing of the lower leaves (Figure 1-2 C). In 13.5 mM, there were significant reductions in dry shoot biomass compared to 20 mM K (Figure 1-1 C). In fact, the insufficient supply of 13.5 mM K was a critical margin below which typical K deficiency symptoms were expressed (Figures 1-1 C and 1-2 C). Similar K deficiency symptoms were observed under K-deficient sand culture. The potato plants exhibited reduced CO₂ net assimilation and biomass production (Koch et al. 2019). This could be attributed to an impaired photosynthesis as well as photoassimilate translocation to newly growing tissues, which negatively impact plant growth (Tränkner et al. 2018). On the other hand, smaller biomass produced at an insufficient K supply of 13.5 mM could also be attributed to the reduction in osmoregulation and cell expansion in the plant (Gerardeaux et al. 2010). In contrast, at an excess K supply of 30 mM, no increase in dry shoot biomass but a slight increase in fresh shoot biomass was observed (Figure 1-1 C and F), suggesting excessive water uptake in potato plant shoots. This has previously been reported in field-grown potatoes (Allison et al. 2001) and several other crops (Grzebisz et al. 2013) where excessive K supply increased water uptake in the plants.

The shoot K uptake showed no significant increase with the increase in MS media K concentrations (Table 1-2). However, as the media K supply increased, the shoot K content increased significantly (Table 1-3), which corresponded to an increase in fresh shoot biomass, indicating excessive K content. At excessive K supply, the shoot K uptake did not change while the K content and fresh shoot biomass increased (Tables 2 and 3, Figure 1-1 C and F). This suggests that the increase in shoot K content creates an osmotic potential due to reduced sugar translocation to the roots resulting in higher water uptake than K uptake. In fact, the lack of sugar translocation to the roots is evident from the poor root growth at excessive K supply (Figure 1-2 C). At both insufficient and excessive K supply, the shoot N uptake decreased (Table 1-2), indicating that at a lower K supply range, K and N are synergic, while they are antagonistic at an excessive range. Similar findings have been reported in apple dwarf seedlings where N uptake was reduced at both deficient and excessive K supplies (Xu et al. 2020). Excessive K supply induces competition between K⁺ ions and NH₄⁺ ions during the uptake, whereas insufficient K supply hinders the assimilation and translocation of N as observed in *Arabidopsis* (Armengaud et al. 2009; ten Hoopen et al. 2010). Therefore, such interactions are apparent in the present study, and K supply would require careful consideration of N supply. Additionally, a significant positive correlation between changes in K and P uptake in potato shoots was observed (Table 1-4). This is the first report of such interaction.

1.3.4 Definition of Ca nutrient status

From the dry shoot biomass and morphological symptoms, the Ca supplies of 1.0 mM and 3.0 mM were insufficient, and 10.0 mM was optimal. Ozgen et al. (2011) observed Ca supply below 3.0 mM showing no effect on plant shoot biomass weight but further decreases below 1.0 mM producing symptomatic potato plants, characterized by increased axillary shoots. This phenomenon was attributed to the loss in apical dominance. In the present study, the reduction in Ca supply from 3.0 to 1.0 mM showed no effect on the shoot biomass, but it reduced root growth (Figures 1-1 D and 1-2 D). The poor root growth could be attributed to the damage in the meristematic regions of the roots due to inadequate Ca supply (Busse et al. 2008). A significant increase in the dry shoot biomass accompanied by enhanced plant vigor and root growth was observed at 10.0 mM (Figures 1-1 D and 1-2 D). Upadhyaya et al. (2016) reported enhanced plant growth, tuber number, and tuber yield at 9.0 mM Ca under in vitro conditions. On the higher end of Ca concentration, it is still unclear whether Ca can become toxic to potato plants. In fact, we observed that even at 30 mM there was no effect on plant growth when compared to 10 mM, and the plants were asymptomatic, suggesting excessive Ca supply.

While the shoot Ca uptake remained stable between 1.0 and 3.0 mM, it drastically increased at 10 mM Ca supply (Table 1-2) due to a large increase in shoot Ca content (Table 1-3) and better shoot growth. The shoot Ca uptake was associated with a steady increase in shoot N, K, and Mg uptake up to 10.0 mM Ca (Table 1-2), where improved root growth was observed (Figure 1-2 D). Moreover, the Ca uptake was correlated with N, K, and Mg uptake (Table 1-4). Therefore, future Ca supply studies must consider this synergistic effect with N, K, and Mg supply in order to achieve nutrient balance.

1.3.4 Definition of Mg nutrient status

From the dry shoot biomass and morphological symptoms, the supply of 0.3 to 1.2 mM Mg was deficient, 1.5 mM was optimum, and 9.0 mM was excessive. The maximum dry shoot biomass was observed at 1.5 mM, and the dry shoot biomass was significantly reduced at 0.3 mM (Figure 1-1 E). Mg deficiency symptoms such as necrotic lower leaves and fewer and shorter lateral roots were observed in 0.3 and 1.2 mM (Figure 1-2 E). The symptoms of Mg deficiency observed in this study were similar to those reported by Koch et al. (2020) in potato plants grown in a Mg-deficient nutrient solution. The authors attributed this to

an impaired Mg re-translocation from source to sink tissues, which resulted in the accumulation of sugars in the source organs and sugar starvation in the sink organs. The asymptomatic potato plants produced at 1.5 mM suggested a slight decrease in Mg supply below this level is thus critical. The supply of 9.0 mM decreased the shoot dry biomass slightly when compared to 1.5 mM (Figure 1-1 E), indicating an excess level of Mg, but greatly enhanced lateral root growth when compared to all other Mg levels (Figure 1-2 E). A similar observation has been reported in *Arabidopsis* with an increased Mg supply (Gruber et al. 2013).

The Mg uptake in the shoot increased in accordance with the dry matter up to a point where only the Mg uptake continued to increase, indicating luxury absorption (Tables 1-2 and 1-3). The synergistic increase and significant correlation between N and Mg were observed and appear to be caused by complementary functions within the plant (Table 1-4). Both Mg and N are involved in chlorophyll synthesis, and N assimilation and uptake require energy from Mg-ATP. Peng et al. (2020) observed an increase in the uptake of nitrate but not ammonium in soybean plants grown in high Mg nutrient solution. The shoot P and K uptake were found to be affected only when the Mg supply was deficient (Table 1-2). This could be due to the poor root growth since both P and K are taken up by diffusion which requires well-developed roots (Liebersbach et al. 2004; Santner et al. 2012). These nutrient changes suggest that the Mg supply must take into consideration N, P, and K supplies in order to achieve balanced plant nutrition.

1.4 Conclusion

The present study aimed at establishing a model nutrient control system in potato tissue culture by manipulation of the MS media nutrient concentrations. The N, P, K, Ca, and Mg uptake in the potato plants was consistent with the growth and morphological symptoms reported in the literature. Very few studies have considered correlations in element content in potato plants; our findings show that drastic interactions among the nutrient elements exist. Therefore, there is a need for careful consideration of these interactions when growing potato plants for experiments as well as in fertilizer management under field conditions. This approach offers a potential platform for the acquisition of precise data and conducting further research on biotic and abiotic stresses in potatoes, which can be adapted in field research. However, depending on the potato cultivar and nutrient interactions, additional adjustments may be required to maximize the effectiveness of this method. As part of integrated vector control management in potatoes, we plan to subject these potato plants with insufficient and excessive nutrition status to biotic stresses such as aphid feeding behaviors.

CHAPTER 2

Influence of Plant Nutrient Status on *Aphis gossypii* Host Settling Preference, Reproduction, and Feeding Behaviors in Potato (*Solanum tuberosum* L.) Plants

2.1 Introduction

Aphids are one of the notorious agricultural pests causing yield and economic losses in many crops including potatoes. In potatoes, yield losses of up to 5 % have been reported due to direct damage by aphid feeding on the plant's phloem sap and up to 70 % due to viral diseases vectored by aphids (Beemster and De Bokx 1987). Apart from the yield losses, viral diseases vectored by aphids negatively affect potato tuber quality and the certification of seed potato. Seed potato with excessive levels of potato virus Y are downgraded or rejected in some parts of the world resulting in significant economic losses in the seed potato producers (Fox et al. 2016). Despite the fact that it has failed to prevent non-persistently transmitted viruses in many cases, chemical insecticide application has remained the major and most effective control strategy for suppressing or eliminating aphid populations in agricultural fields for decades (Martin et al. 2004; Dedryver et al. 2010). Furthermore, chemical insecticide use has become less appealing due to the ongoing development of resistance in novel aphid strains to the majority of chemical insecticides currently in use, as well as global human health and environmental concerns (Zhang et al. 2018; Kroschel et al. 2020). As a result, safer and more effective control methods that aim to reduce agricultural chemical insecticide use while improving host plant resistance are urgently needed. Host resistance is a component of integrated pest management (IPM) and can be enhanced through cultural practices such as fertilizer management (Huber and Haneklaus 2007).

Aphids are a large group of insects in the superfamily Aphidoidea (order Hemiptera), highly specialized to feed from the phloem of the plants causing immense damage to crops. Based on their feeding nature, aphids are categorized into generalists and specialists (Gadhav et al. 2020). Out of 137 aphid species, 62 feed on plants from multiple families (polyphagous), 52 mostly on plants from multiple genera within the same plant family (oligophagous), and 23 species mostly feed on plant(s) from same genus and family (monophagous). Due to their feeding nature, aphids directly cause damage to the crops by (i) modifying the morphology of the plant (ii) cytological changes (iii) disruption of resource allocation, and (iv) perturbation of

photosynthesis. It has been reported that 10 to 20 % of aphid species cause plant morphological changes such as gall formation (Alvarez et al. 2013). These plant morphological changes can result in the reduction of water flow within the plant (Brown et al. 1991). The cytological changes caused by aphids include chlorosis and macroscopic cell death at the feeding site (Belefant-Miller et al. 1994). The disruption of resource allocation involves the aphid colonies redirecting large amounts of the plant resources to support their own growth and development (Sorensen 2009). These damages mostly occur on a suitable host plant as they increase in their densities.

Aphids locate a suitable host and avoid a non-host uses both visual and olfactory cues (Doring and Chittka 2007; Webster 2012). Once aphids land on a prospective host, they use the tactile and gustatory cues, such as monitoring surface features and short probes to sample host cell contents, to ascertain host's suitability (Muller and Riederer 2005; Powell et al. 2006). Plant emit a variety of different volatile compounds; which aphids use to identify their hosts. Nutritional cues are thought to be the most likely cues facilitating host selection in generalists, whereas plant secondary metabolites are thought to be the most important cues for host selection in specialists (Lankau 2007). Three major aphid feeding behavioral activities have been identified using a visualization technique known as Electronic Penetration Graph (EPG; Tjallingii and Esch 1993). Within the plant, the aphid uses its stylet to initiate several behavioral activities known as pathway, xylem, and phloem phases. During these stages, the aphid uses gustatory cues to monitor plant tissue conditions. The pathway phase involves intercellular stylet movements characterized by brief intracellular punctures. The brief intracellular punctures are identified as a sudden drop in the voltage when observed using EPG technique (Pettersson et al. 2017). This feeding behavior implies stylet penetration into the plasmalemma, resulting in a potential drop in the cell membrane and corresponding to a "pd waveform" in the EPG (Pettersson et al. 2017). The phloem and xylem phases involve the activities in the vascular bundle. The phloem phase is characterized by aphid salivation in the sieve elements and ingestion of the phloem sap and such behaviors are designated as "E1 and E2 waveforms", respectively. The xylem phase involves ingestion of the xylem fluids and this activity is designated as a "G waveform" (Pettersson et al. 2017). The xylem ingestion is reported as a feeding behavior that controls dehydration and water deficit due to the high concentration of solutes in the phloem sap and does not contribute to the aphid nutrition (Kuhlmann et al. 2013). A prolonged ingestion of the xylem indicates unsuitability of the host plant (Pettersson et al. 2017). Therefore, the understanding of changes in these aphid feeding behaviors can help in explaining the resistance locations in the plant.

As vectors, aphids are also conceivably the most successful vectors of plant viruses. This is the case due to their ability to (i) precisely deliver the virus particles (virions) using their needle like mouth part (stylet) into the plant cells, (ii) efficiently and abundantly reproduce within a short time period, (iii) access host plants across different families using several feeding modes, and (iv) adapt to adverse conditions and develop wings to allow aphids and viruses to spread over long distances. The transmission of viruses from plant to plant has been linked to the feeding behaviors. The brief intracellular stylet punctures have been linked with the acquisition of a non-persistent virus from the infected plants and inoculation on uninfected plants (Martin et al. 1997). Almost 75 % of the viruses vectored by aphids are transmitted non-persistently using the brief stylet penetrations (Nault 1997). However, longer periods of the stylet penetration beyond the epidermis cell layer of the plant tissues reduces the efficiency of transmission and aphids also lose their ability to transmit the virus within minutes upon removal of the stylet from the plant (Ng and Perry 2004). This also applies to the semi-persistent viruses with exception that the longer period of penetration increases their transmission efficiency. The acquisition of the persistent virus requires that aphids ingest the phloem sap whereas its inoculation requires longer periods of salivation in the sieve elements (Prado and Tjallingii 1994). Therefore, understanding these feeding behaviors is critical in the management of the aphid as vectors of viral diseases in potatoes.

Aphid host settling preference and fecundity are known to be greatly influenced by the quality of phloem sap (Banerjee and Raychaudhuri 1987). Phloem sap is mainly composed of high ratios of sugars to amino acids, non-essential to essential amino acids and K^+ to Na^+ , and extremely low lipid levels. Amino acids are the primary source of N for aphid which is used for protein synthesis, as a key component for neurotransmitters and neurohormones, and as a constituent of aphid haemolymph (Douglas et al. 2017; Cao et al. 2018; Gruhn et al. 2021). Wu et al. (2020) observed significant reductions in aphid weight and fecundity with a 50 % reduction in dietary amino acid supply, indicative of high sensitivity of aphids to amino acid supply. Sugars are an important source of carbon for aphids, and act as phagostimulants where decreasing feeding rates are observed at higher sucrose concentrations (Douglas et al. 2017). The quality of phloem sap varies between plant species and is influenced by environmental factors such as nutrient supply to the plant (Gruhn et al. 2021). As a result, aphids are compelled to finding a suitable host that can supply them with the required nutrition to increase their fecundity.

Plants as hosts express resistance to aphid's invasion. The resistance is classified into three categories (i) antixenosis, (ii) antibiosis, and (iii) tolerance. The antixenosis resistance is where the plant is able to drive

away aphids within a few hours after some superficial probes. The mechanisms behind this are said to be due to color of the foliage, palatability as influenced by the secondary metabolites, waxiness of the leaf surface, difficulties in reaching the phloem, local necrosis on the site of stylet insertion, and the presence of trichomes (van Emden 2017). The antibiosis resistance is the ability of the host plant to reduce or stop the growth and or development of the aphid. It is mostly measured as reduced survival, growth and fecundity, and extended development time. The mechanisms for antibiosis include presence of glandular trichomes that secrete distasteful polyphenols that also disable the aphid mouthpart, toxins produced after disruption of plant cell wall, nutritional factors such as amounts of amino acids, and extrinsic factors (van Emden 2017). Tolerance is when a plant provides better yield than another plant yielding poorly at the same aphid burden, both in numbers and duration. The mechanisms include compensation through enhanced plant growth and absence of symptoms. At molecular level, plants respond to aphid feeding by both general plant defense mechanisms and specific resistance gene-aphid interaction (Zust and Agrawal 2016). Using a general response, plants produce phytohormones which include jasmonic acid, ethylene, and salicylic acid (Smith and Boyko 2007). This response is known to have a negative influence on the aphid performance. Plant secondary metabolites are also known to be part of the plant's defense against aphids but their efficacy is mostly dependent on the ingestion. Plants contain protease inhibitors, which are thought to interfere with protein digestion in the aphid gut, resulting in decreased aphid growth and fecundity (Nalam et al. 2018). Occlusion of phloem sieve elements is also one of plants' defense mechanisms for keeping sap-sucking insects away from their valuable nutrients, phloem sap. Two well-known mechanisms for preventing sap loss from sieve elements are callose deposition and the use of proteins to seal the pores of sieve elements (Eschrich 1975). Callose deposition is a slow process that can take up to 10 minutes or more, whereas proteins are used to quickly (within seconds) plug the sieve pores and prevent sap loss (Knoblauch et al. 2001; Xie et al. 2011). Both mechanisms are repairable if the sieve is not severely damaged (Furch et al. 2010). These sealing processes are said to be aphid species specific (Walker and Medina-Ortega 2012). Plants recognize the presence of herbivorous insects by using herbivore-associated molecular patterns (HAMPs), which activate the defense system known as effector-triggered immunity (ETI) when the insect effectors are recognized (Nalam et al. 2018).

Plant defense mechanisms require a well-balanced plant nutrition to be optimized because nutrient supply affects plant physiology and biochemistry, which are linked to resistance. Excessive or inappropriate fertilizer application are thus reported to lower pest resistance (Bala et al. 2018). Sagar et al. (2014) found increased populations of leafhoppers in *Bt* cotton supplied with high N fertilizer doses. Jansson and Ekbon

(2002) reported increased reproduction of *Macrosiphum euphorbae* on petunia plants with high P fertilizer dose. Ahmed et al. (2019) observed increased settling preference of *M. persicae* on cabbages with high N content and volatile emission. Higher reproduction of aphids was also reported in plants with high N content (Hosseini et al. 2010). Walter and DiFonzo (2007) observed higher aphid densities in K-deficient soybean compared to K-sufficient soybean plants. Ai et al. (2011) reported a negative interactive effect of N and K nutrition on aphid population in *Bt* cotton. However, recent comprehensive reviews of several studies done under natural, greenhouse, and field conditions have revealed contradictory findings (Butler et al. 2021). In the case of N nutrition, 114 of the 166 studies found a positive effect, while 44 found a negative effect on insect pest performance. Fourteen of the 21 reported studies on P nutrition showed a negative response, while 7 studies showed a positive response. In contrast, 7 of 10 studies on K nutrition found a negative response and 3 found a positive response. Environmental factors, as well as the presence of natural enemies, were held responsible for the differences in insect pest response (Butler et al. 2021). As a result, more research is required to understand the effects of plant nutrition on insect pest infestation, especially in a controlled environment where only plant nutrition can be easily changed.

In this study, the effect of N, P, K, Ca, and Mg nutrition on aphid behaviors were evaluated on potato plants under *in vitro* conditions. The objectives of the study were to (1) establish the nutrient effect on host settling preference (2) evaluate the effect of individual plant nutrient on aphid reproduction, and (3) evaluate the effect of individual nutrient on aphid feeding behavior in potato plants. Understanding individual nutrients and how they interact with aphid behavior will help improve fertilization and pest management in potato production systems. As a result, the costs and environmental risks associated with current chemical insecticide could be reduced.

2.2 Materials and Methods

2.2.1 Plant material and growth conditions

The potato, *Solanum tuberosum*, cv. Irish Cobbler, was used to generate plant materials in the entire experiment, utilizing a tissue culture approach as described in Chapter 1. Plants for this experiment were grown in media tailored to each nutrient element, as defined in Chapter 1. For N nutrition, 20 and 60 mM were "insufficient" and "optimum," respectively; for P nutrition, 0.4, 1.25, and 3.5 mM were "insufficient",

"optimum", and "excessive", respectively; for K nutrition, 13.5, 20, and 30 mM were "insufficient", "optimum", and "excessive", respectively; for Ca nutrition, 1.0 to 3.0 mM and 10.0 mM were "insufficient" and "optimum", respectively, and; for Mg nutrition, 1.5 and 9.0 mM were "optimum" and "excessive", respectively. "Insufficient" was defined as a supply level that showed significant lower dry biomass while "optimum" was when the plants showed maximum dry biomass weight. On the other hand, "excessive" was defined as when additional nutrient supplies did not increase dry biomass weight. For some elements we couldn't get the excessive levels that were distinct enough, therefore we adopted the optimum range. Plants were chosen at random from tissue culture generated plants for each nutrient and level and subjected to subsequent aphid experiments. To replenish the tissue culture plants, new plants were propagated using the same protocols as before, and this was done throughout the experimental period.

2.2.2 Aphid rearing

In this study, the *Aphis gossypii* (Figure 2-1 A) was used as a test insect reared on potato plants. First, potato plantlets were propagated in the tissue culture following the protocol as described in Chapter 1, using a standard MS medium (Murashige and Skoog 1962). After 21 days, the plantlets were later transplanted into plastic pots containing vermiculite soils and covered with transplanted plastic cups for seven days to acclimatize them to the ex vitro environment (Figure 2-1 B).

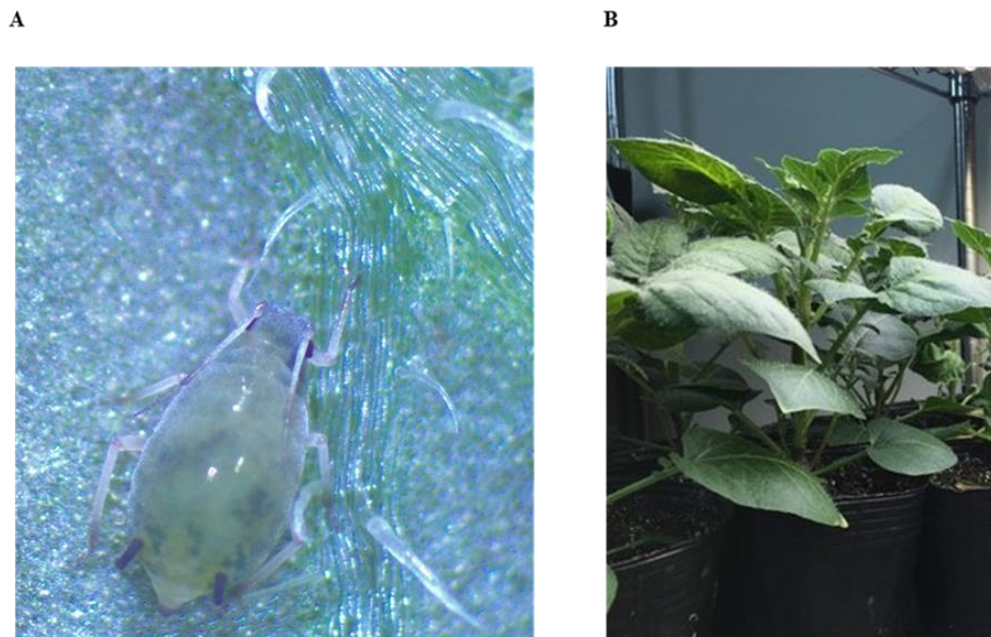


Figure 2-1 (A) *Aphis gossypii* feeding on potato leaf. **(B)** potted potato (*Solanum tuberosum* L) for rearing *A. gossypii*.

The plants were left to grow under room temperature for almost 2 weeks until they were ready for aphid rearing. Later, the plants were put in cages with the dimensions of 24.5 cm in height, 24 cm in width and 24.5 cm in depth. The aphids were released onto caged potted potato plants and left to freely reproduce in an incubator (MIR-254, SANYO) with temperature set at 24 ± 2 °C, light duration of 16 hours, and 8 hours of darkness. When the potato plants began to wilt, they were replaced with new potted potato plants. Throughout the experiment, the potato plants were watered twice a week.

2.2.3 Host settling choice tests

A dual settling choice experiment was carried out to determine the effect of individual nutrition status on *A. gossypii* preference in potato plants. A 5 cm x 1.5 cm petri dish was used as an arena, and a 150 ml tube with one end closed was used as a holder for the aphids (Figure 2-2).

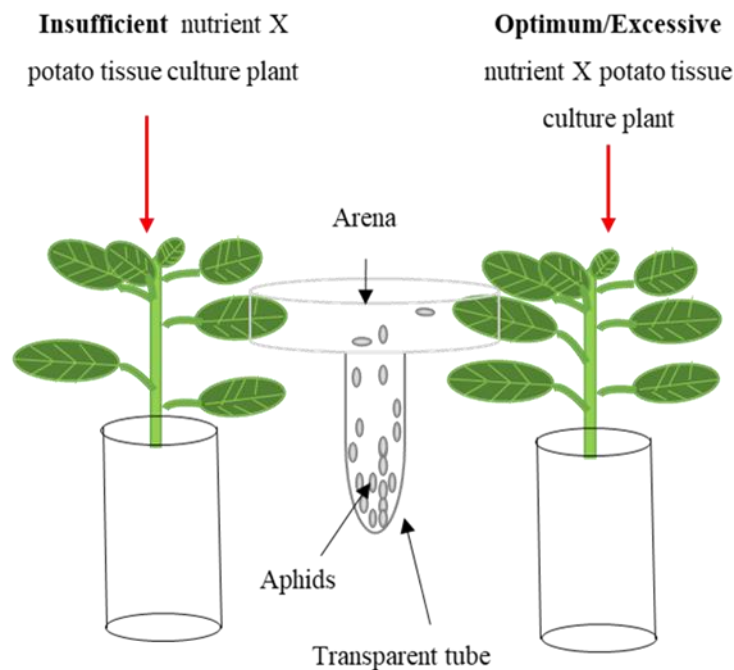


Figure 2-2 An illustration of the dual aphid settling preference test

The open end of the 150 ml tube was inserted into a fitting hole drilled in the arena's center. A slit-sized cut was made equidistant from the center of the arena on either edge of the arena, across the diameter of the petri dish. A petiole of the experimental potato plantlet was inserted into each slit, followed by a release of 30

adult apterae aphids in the holder tube. The experiment was carried out in a growth chamber at 24 ± 2 °C, 70% relative humidity, 16 hours of light, and 8 hours of darkness. The number of aphids on the entire plant was counted and recorded after 24 hours. Ten independent replicates were performed for each test.

2.2.4 Aphid reproduction experiments

The reproduction capacity of *A. gossypii* was assessed in this experiment by counting the number of nymphs on tissue culture potato plants with varying nutrition (section 2.2.1). Ten adult apterae aphids were placed on an individual tissue culture potato plantlet to move and reproduce freely (Figure 2-3). A transparent polypropylene tube (25 cm long and 9 cm in diameter) was used to cover the plants, with one end (upper side) closed with gauze mesh. To prevent aphids from escaping, a transparent plastic cup containing distilled water was placed beneath the tube. After that, the assay was placed in an incubator with the temperature and relative humidity set to 24 ± 2 °C and 70 %, respectively. Light and darkness were set to last 16 and 8 hours, respectively. The aphids were allowed to reproduce for 7 days. After 7 days, the number of nymphs was counted and recorded. Each nutrient level was subjected to 10 independent repetitions.



Figure 2-3 Potato tissue culture plants in an incubator infested with 10 adults of *Aphis gossypii* apterae to freely move and reproduce.

2.2.5 Aphid feeding behavior experiments

The *A. gossypii* feeding behaviors were monitored using an EPG recording system (Giga-8d EPG systems: The Netherlands) for a duration of 6 hours under room temperature, and constant light supply (Figure 2-4).

The EPG recording system is a device that can record and analyze the time, frequency, and onset time of various behaviors in detail (Figure 2-4 C), such as intracellular stylet punctures or potential drop (pd; Figure 2-4 B1), salivation into sieve elements (E1; Figure 2-4 B2), and phloem sap feeding (E2; Figure 2-4 B3).

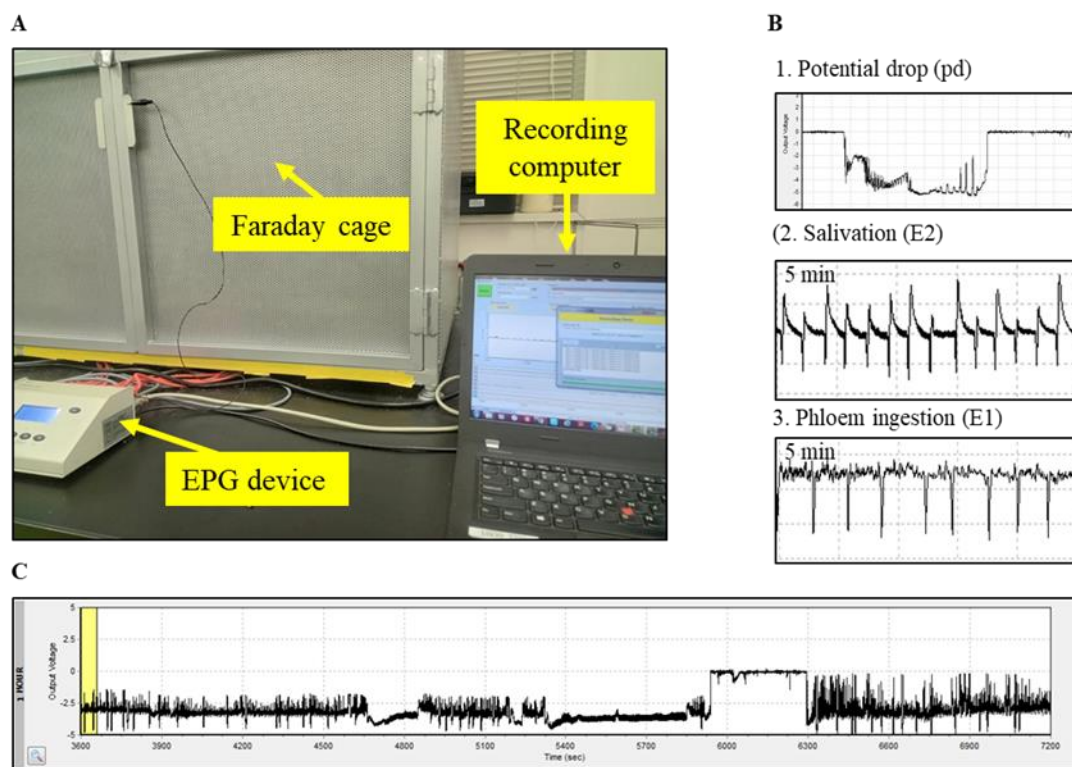


Figure 2-4 (A) An overview of an EPG setup. **(B)** Some important waveforms for aphid feeding behaviors. **(C)** An overview of feeding behavior waveforms over a period of 1 hour.

Eight plants, varying in nutrient status were put in a faraday cage with the dimensions of 0.64 m long, 1.04 m wide, and 0.69 m in depth. On each plant, one apterae aphid, which was first starved for 1 hour, was put on the adaxial side of the leaf while attached to a gold wire electrode on its dorsum using water-based silver glue. The other end of this gold wire was connected to a copper wire which was used to connect to the EPG amplifier input. Another copper electrode from the output voltage of the EPG device was inserted in the plant growth medium while avoiding contact with the plant. The recordings on feeding behaviors were started immediately after all the aphids were set up. For each treatment, 7 independent repetitions were conducted using fresh plants and aphids. The recordings of the aphid behavioral signals on all eight plants were done at the same time using an EPG data acquisition software installed in a computer connected to the EPG Giga-8d device. Later, the waveforms identified using an EPG signal analysis software and data analysed using a Microsoft Excel Workbook (Sarria et al. 2009).

2.2.5 Statistical analyses

All statistical analyses on dual settling choice tests, reproduction, and EPG data were done in Microsoft Excel version 2019. Data on dual settling choice test was treated as binominal, therefore the data were arcsine transformed before conducting statistical analyses. All comparisons between two treatments of the same nutrient element were done using *t*-test at $p < 0.05$.

2.3 Results and discussion

2.3.1 Influence of N nutrition on aphid host choice, reproduction, and feeding behaviors

2.3.1.1 Aphid host settling preference

Nitrogen nutrition influenced the *A. gossypii* host settling preference (Figure 2-5). The majority of the aphids significantly preferred ($p < 0.001$) settling on potato plants with optimum N nutrition compared to insufficient N nutrition (71 % versus 29 %). This suggests that resistance factors related to surface resistance were increased in potato plants with insufficient N and decreased in plants with adequate N. The difference in N content between these two nutrient statuses was not statistically different but uptake was almost 4 times higher in the optimum N (Chapter 1, Tables 1-2 and 1-3). Higher N uptake was associated with higher dry biomass, which can affect the amount of volatiles emitted by the plant and may attract aphids (Agelopoulos et al. 2000). Ahmed et al. (2019) observed an increased *Myzus persicae* settling preference on high N cabbage cultivars which were associated with higher total volatile organic compounds emission. In another study, Ahmed et al. (2022) found that *M. persicae* preferred cabbage plants that had higher concentration of amino acids and lower levels of indole glucosinolates in their leaves than those with lower concentrations. Nowak and Komor (2010) reported higher preference of *Uroleucon tanacetii* (Mordvilko) and *Macrosiphoniella tanacetaria* (Kaltenbach) on tansy plants with high N than those with insufficient N. The authors attributed this mainly to increased concentration of amino acids in the phloem sap and not plant surface factors. In contrast, Jakobs and Müller (2019) showed that olfactory cues did not influence the preference of *M. tanacetaria* on tansy plants but did so for the *U. tanacetii*, when subjected to only olfactory cues. When both aphid species were subjected to visual, olfactory, and plant surface cues, they both were affected by visual and surface cues. High N fertilization is also known to inhibit the formation of lignin and waxy cuticle and

synthesis of secondary metabolites which can ease the aphid probing (Gomez-Trejo et al. 2020). Though not measured in the present study, these studies suggest that the total volume of volatiles and phloem sap nutrition quality may explain the aphid settling preference in the present study, but olfactory cues may be species specific. Therefore, future studies need to consider verifying possible changes in total volatile and phloem sap nutrition quality with varying N nutrition in potato plants in reference to higher aphid settling preference on high N potato plants.

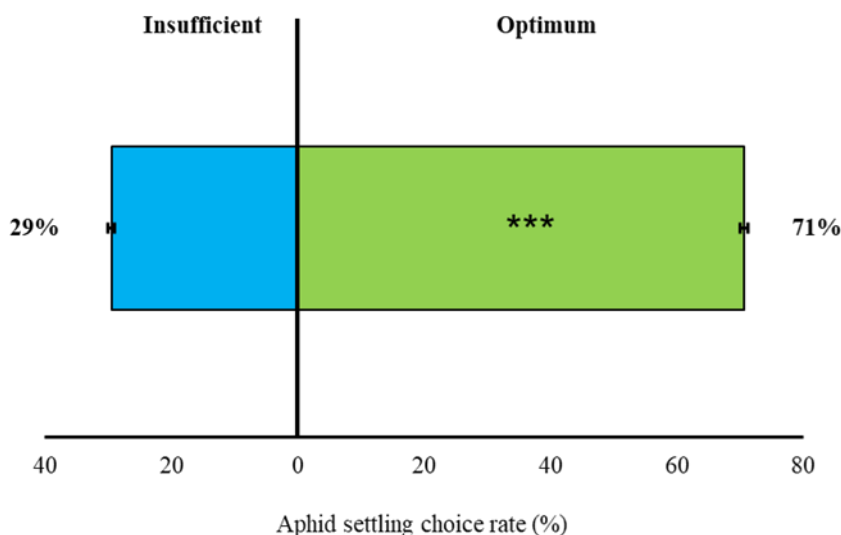


Figure 2-5 Influence of plant N nutrition on *Aphis gossypii* host settling preference in potato plants after 24 hours. Insufficient = MS medium containing 20 mM of N and Optimum = MS medium containing standard 60 mM of N. Mean comparison between two treatments according to *t*-test at $p < 0.05$. *** $p < 0.001$. Means presented with \pm standard error.

Additionally, we found that the insufficient N plants had significantly higher foliar Ca and Mg contents while K content was significantly lower when compared to the optimum N plants (Chapter 1, Table 1-3). These nutrient interactions may also confer some indirect influence on the aphid preference; for example, decreases in K can increase preference due to increased free amino acid content, whereas increases in Ca can decrease preference due to increased plant toughness (Zehnder and Hunter 2009). Thus, how plant N nutrition and interactions with other nutrients may alter each cue and consequently influence on aphid settling preference requires further investigations.

2.3.1.2 Aphid reproduction

The N nutrition in potato plants influenced the *A. gossypii* reproduction capacity. Significantly higher numbers of nymphs were observed in potato plants with insufficient N nutrition compared to those with high N nutrition (Figure 2-6).

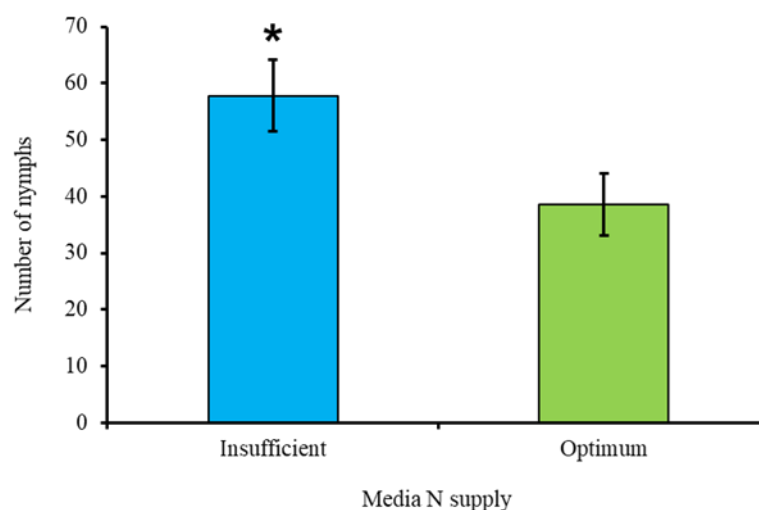


Figure 2-6 Influence of plant N nutrition on number of *Aphis gossypii* nymphs in potato plants after 7 days. Insufficient = MS medium containing 20 mM of N and Optimum = MS medium containing standard 60 mM of N. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$. Means presented with \pm standard error.

This is contrary to most previous reports under hydroponics and greenhouse where higher aphid reproduction capacities were observed only on higher N plants (Petitt et al. 1994; Hosseini et al. 2010). However, the present findings agree with the observations by Zehnder and Hunter (2009) in *A. nerii* where its performance was decreased on milk weed plants with high foliar N content. The variations in the results could be attributed to additional supplies of K, Ca, and Mg compared to the present study where only N supply was varied while other nutrients were kept constant. These additional nutrient supplies might have confounding effects on aphid reproduction; for example, increase in K and Ca may reduce aphid reproduction. In the present study, insufficient N supply was associated with significant decreases in foliar K and increases in Ca and Mg contents (Chapter 1, Table 1-3). These nutrient interactions were not reported in previous studies. Hu et al. (2017) observed a 40 to 63 % increase in free amino acids under K deficiency despite a reduction in plant N content, because free amino acids are a dominant N source for aphids (Douglas 1993). Wu et al. (2020) reported a reduction in *M. persicae* weight and fecundity with a 50 % reduction in dietary amino acid supply.

High Ca content results in strong plant cell walls, which may confer toughness against insect pest attack, but its impact on phloem sap quality is unknown (Palta 2020). Whereas high Mg content may result in high sugar contents in the phloem sap, which can increase aphid performance (Mewis et al. 2012; Koch et al. 2020). Therefore, the present study suggest that insufficient N supply may have indirectly influenced *A. gossypii* reproduction by causing K deficiency and increasing Mg content. Therefore, a careful consideration of these interactions between N and K, Ca, and Mg is needed when assessing plant N nutrition on insect pest reproduction.

2.3.1.3 Aphid feeding behavior

From the EPG analyses, the N nutrition influenced potato plant resistance to the *A. gossypii* at leaf surface, epidermis and mesophyll layers and sieve elements (Figure 2-7 and 2-8). This is the first study to demonstrate the influence of N nutrition on aphid feeding behaviors in potato plants using EPG. Significantly ($p < 0.05$) shorter aphid non-probing time was observed on insufficient N nutrition plants compared to optimum N nutrition plants (Figure 2-7 A).

This suggests the absence of resistance structures on the leaf surfaces of N-insufficient plants. Leaf surface structures and olfactory stimuli such as leaf color, leaf toughness, and trichomes provide the first line defense against herbivorous insect pests (van Helden and Tjallingii 1993). There were no visible N deficiency symptoms in the N-insufficient plants, in the present study (Chapter 1, Figure 1-2 A), ruling out the possibility that leaf color influenced aphid feeding behaviors. The higher Ca content found in N-deficient plants (Chapter 1, Table 1-3), could possibly confer leaf toughness, but this did not result in delayed aphid probing. The shorter aphid non-probing time also suggests absence of the trichome effect. Contrastingly, thicker leaf surfaces have been reported under insufficient N nutrition compared to higher N nutrition in rice plants (Jinwen et al. 2009). Also, Bilkova et al. (2016) observed increased trichome density in leaves of *Artemisia annua* under low N supply. Thicker leaf epidermis and the presence of trichomes increased resistance to aphid *Brevicoryne brassicae* in oilseed rape, and this was attributed to their restrictive effect on the aphid movement (Hao et al. 2020). Similarly, Nalam et al. (2021) observed that higher density and longer trichomes prolonged the non-probing time for *A. glycines* on soybean plants. Trichomes can thus impart physical resistance to insect pests by interfering with their movement, feeding, and releasing toxic secondary metabolites exudates (Levin 1973; Norris and Kogan 1980). The surface resistance observed in the present study corroborates well with the lower aphid settling preference on potato plants with insufficient N nutrition

over those with optimum N nutrition (Figure 2-5). This suggests that other factors may have had a stronger influence on aphid non-probing time than surface structures. In fact, plants with insufficient N were lower in K and higher in Mg content, which can increase free amino acids and sugars in phloem sap, respectively, and thus increase aphid performance (Mewis et al. 2012; Koch et al. 2020). Although these changes were not evaluated in this study, future research may look into assessing these changes with N fertilization to gain more insight into aphid surface resistance in potatoes.

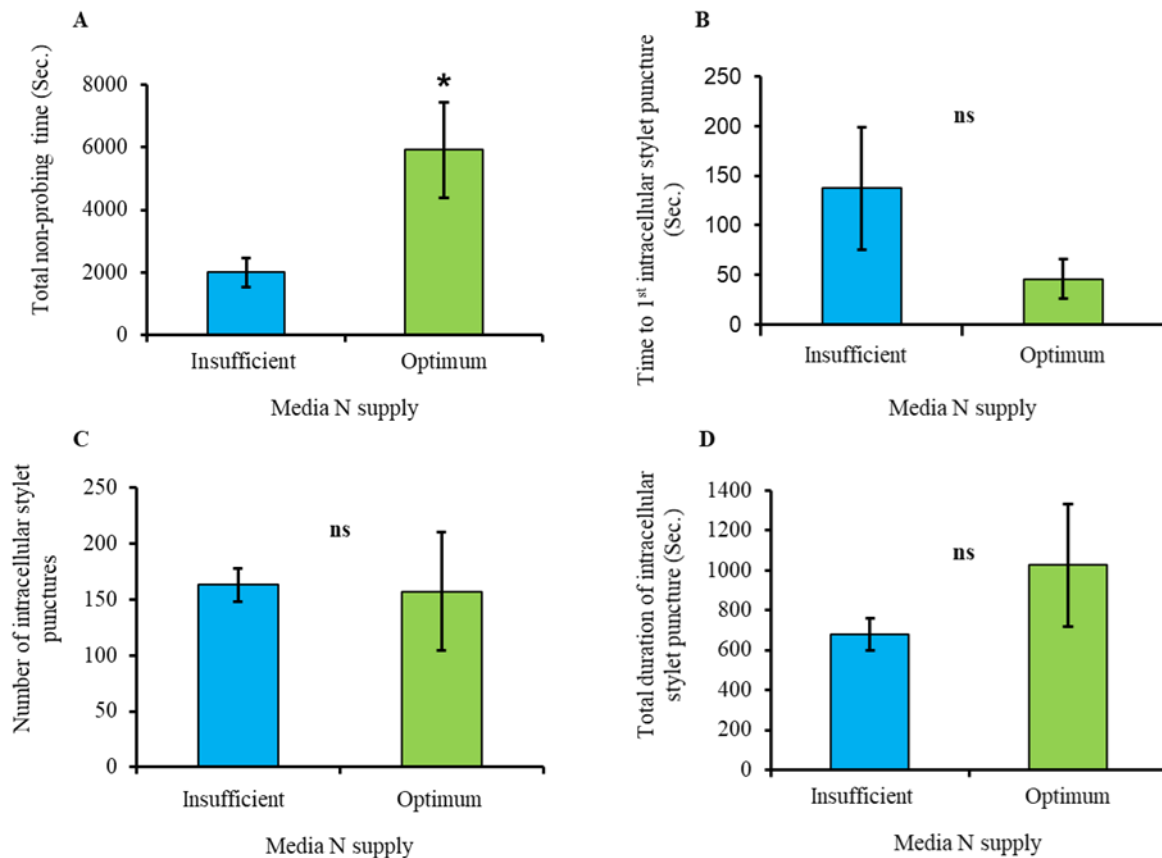


Figure 2-7 Influence of plant N nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. **(A)** non- probing (np), **(B)** time to first potential drop (pd), **(C)** number of potential drops (n_pd), and **(D)** total duration for potential drops (s_pd). Insufficient = MS medium containing 20 mM of N and Optimum = MS medium containing standard 60 mM of N. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, ns = non significant. Means presented with \pm standard error.

The aphid phloem ingestion was significantly ($p < 0.05$) increased in the insufficient N nutrition plants compared to optimum N nutrition plants (Figure 2-8 C). This is the first report on the effect of insufficient N nutrition on aphid feeding behaviors in potato plants. Prolonged phloem ingestion is important for the

aphids to successfully acquire nutrients for their fitness (Kuhlmann et al. 2013). This result corroborates well with the higher aphid populations observed in the plant with insufficient N nutrition (Figure 2-6). This phenomenon could be well explained by changes in the phloem sap quality as a result of low K content. Though not evaluated in this study, other studies have reported increases in free amino acids of up to 63 % due to K deficiency under low N nutrition in tea crops (Hu et al. 2017). Ma et al. (2019) observed enhanced survivorship and reproduction in *A. gossypii* fed artificial diets without cotton and cucumber secondary metabolites compared to when fed on diets which contained the secondary metabolites from cotton and cucumber. This also suggests a possible differential effect of secondary metabolites between insufficient and optimal N, with the former resulting in better phloem sap quality than the latter. Further research to quantify these changes is required in light of these scenarios. Though not significant at 5 % level, the duration of aphid salivation into the phloem sieve elements was almost 3 times longer on potato plants with insufficient N nutrition compared to the duration in optimum N nutrition (Figure 2-8 B).

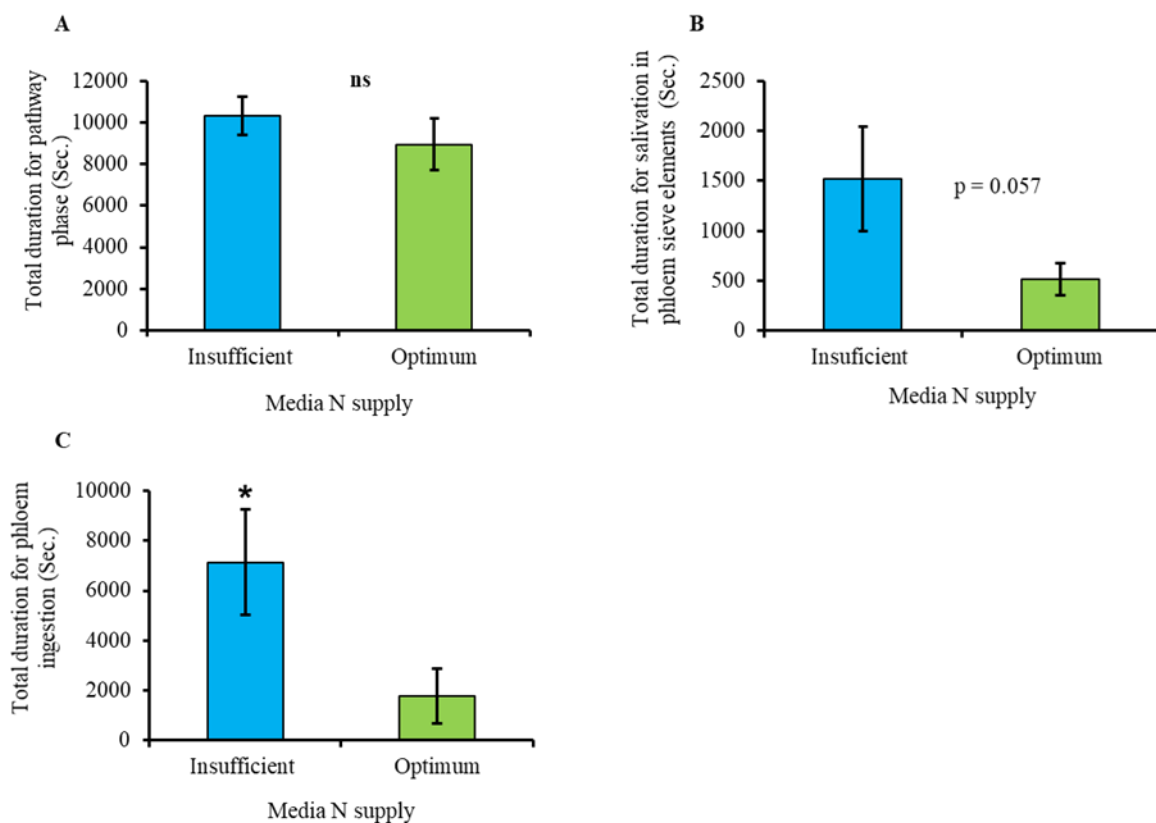


Figure 2-8 Influence of plant N nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. **(A)** total time spent in pathway phase (s_C), **(B)** total time spent salivating in phloem sieve elements (s_E1), and **(C)** total time spent feeding on phloem (s_E2). Insufficient = MS medium containing 20 mM of N and Optimum = MS medium containing standard 60 mM of N. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, ns = non significant. Means presented with \pm standard error.

Medina-Ortega and Walker (2013) reported increased aphid salivation as a defense mechanism against sieve element occlusion, a plant response to damage on sieve element for the protection of its photo-assimilate-rich phloem sap. This suggests that sieve element plugging was enhanced in the potato plants with insufficient N nutrition than in potato plants with optimum N nutrition. However, the increased phloem ingestion observed in the insufficient N nutrition suggests that aphids were able to overcome the occlusion effect. In addition, the reduced salivation and phloem ingestion in the optimum N nutrition suggests some mechanisms that would require further investigations. Together, a moderation of N nutrition while avoiding N deficiencies may improve the ability of the plant to resist against aphid infestations.

2.3.2 Influence of P nutrition on aphid host choice, reproduction and feeding behavior

2.3.2.1 Aphid host settling preference

The P nutrition positively influenced the *A. gossypii* host settling preference (Figure 2-9). The aphids showed a significant ($p < 0.001$) settling preference on potato plants with excessive P nutrition compared to those with insufficient P nutrition (Figure 2-9 A).

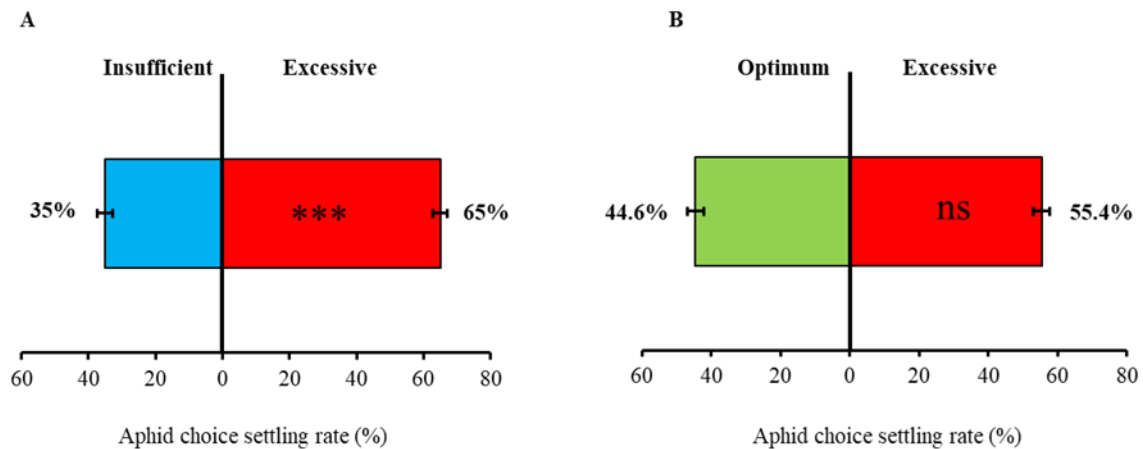


Figure 2-9 Influence of plant P nutrition on *Aphis gossypii* host settling preference in potato plants after 24 hours; (A) dual host settling preference between insufficient P and excessive P nutrient statuses and (B) dual host settling preference between optimum P and excessive P nutrient statuses. Insufficient = MS medium containing 0.4 mM of P, Optimum = MS medium containing standard 1.25 mM of P, and Excessive = MS medium containing 3.5 mM of P. Mean comparison between two treatments according to *t*-test at $p < 0.05$. *** $p < 0.001$ and ns = non significant. Means presented with \pm standard error.

The present findings agree with Bala et al. (2018) who observed higher insect pest infestations with higher P levels. Similar findings have been reported by Shah (2017) where higher P levels were linked to an increase in insect pest incidence. Khan et al. (2016) reported that inorganic P deficiencies induce jasmonic acid pathway which improves plant defense against insect pests. Jasmonic acid pathway modulates the biosynthesis of secondary metabolites that can either be toxic or repellent to insect pests (Wang et al. 2019). These results suggest induced plant resistance to aphids rather than presence of leaf surface inhibitors reduced aphid preference on P insufficient plants. Contrastingly, the acceptability of excessive P nutrition plants suggests that excessive P fertilization may accelerate aphid infestations in potato production systems.

2.3.2.2 Aphid reproduction

The *Aphis gossypii* reproduction capacity increased with the increase in potato plant P nutrition (Figure 2-10). The number of nymphs were significantly higher in potato plants with excessive P nutrition compared to plants with insufficient ($p < 0.01$) and optimum ($p < 0.05$) P nutrition.

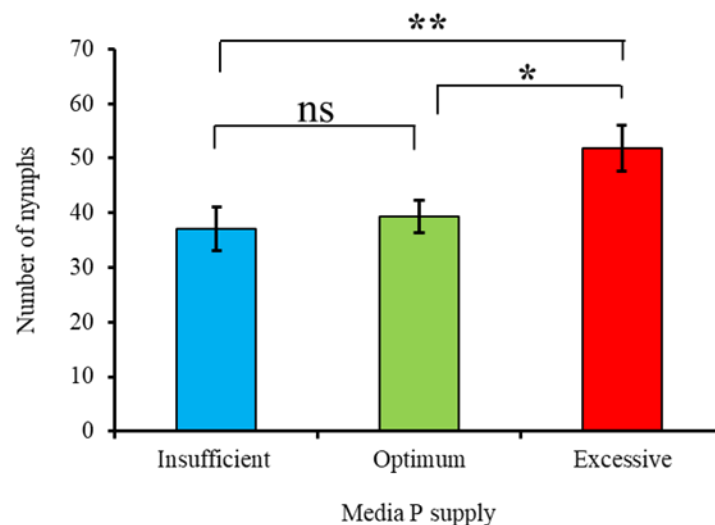


Figure 2-10 Influence of plant P nutrition on number of *Aphis gossypii* nymphs in potato plants after 7 days. Insufficient = MS medium containing 0.4 mM of P, Optimum = MS medium containing standard 1.25 mM of P, and Excessive = MS medium containing 3.5 mM of P. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, *** $p < 0.001$, and ns = non significant. Means presented with \pm standard error.

Jansson and Ekbom (2002) reported that the number of nymphs in aphid *Macrosiphum euphorbiae* increased with high P nutrition on petunia plant. Several other previous studies have reported both increases and

decreases in insect pests when P levels are high (Gómez-Trejo et al. 2021). According to Chang et al. (2008), P deficiency reduces the concentration of amino acids in plants. The P insufficiency, in present study, was also associated with significant decrease in shoot K uptake (Chapter 1, Table 1-2) which is also known to increase the amino acids. In view of these two opposing phenomena, the amino acid could not well explain our findings. The possible mechanism could be higher secondary metabolites in the insufficient P plant as previously described (Section 2.3.2.1). The present findings suggest that excessive P fertilizer may increase aphid infestation, however, the actual mechanisms for the higher reproduction are still unknown, hence requires further studies.

2.3.2.3 Aphid feeding behavior

The P nutrition influenced potato plant resistance to the *A. gossypii* at leaf surface, epidermis and mesophyll cell layer, and sieve elements, according to the EPG analyses (Figures 2-11 and 2-12).

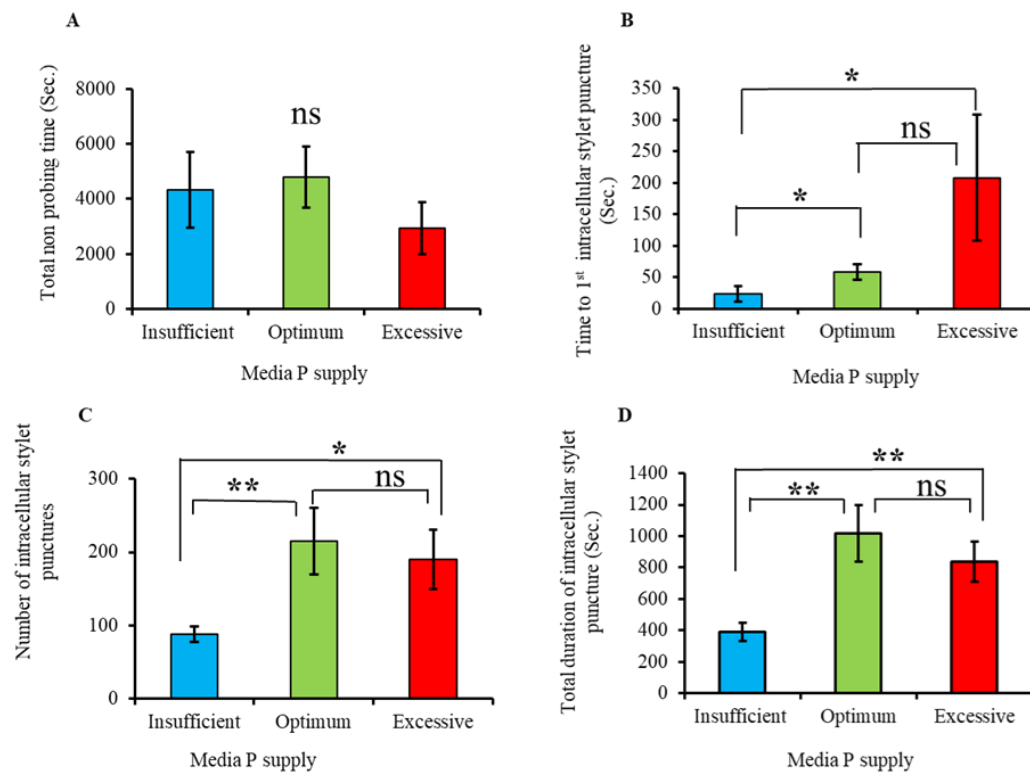


Figure 2-11 Influence of plant P nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. (A) non- probing (np), (B) time to first potential drop (pd), (C) number of potential drops (n_pd), and (D) total duration for potential drops (s_pd). Insufficient = MS medium containing 0.4 mM of P, Optimum = MS medium containing standard 1.25 mM of P, and Excessive = 3.5 mM of P. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, ** = $p < 0.01$, and ns = non significant. Means presented with \pm standard error.

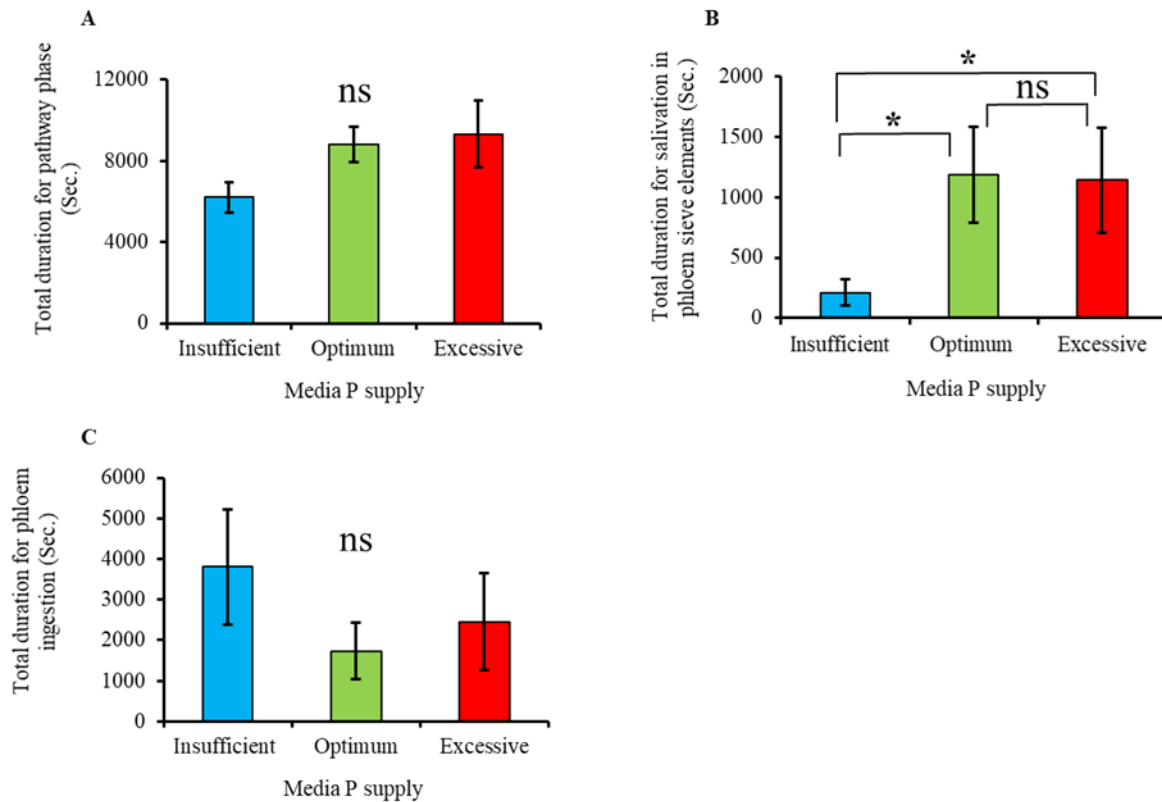


Figure 2-12 Influence of plant P nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. **(A)** total time spent in pathway phase (s_C), **(B)** total time spent salivating in phloem sieve elements (s_E1), and **(C)** total time spent feeding on phloem (s_E2). Insufficient = MS medium containing 0.4 mM of P, Optimum = MS medium containing standard 1.25 mM of P, and Excessive = 3.5 mM of P. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, ns = non significant. Means presented with \pm standard error.

This is the first study to report the aphid behavior under varying P nutrition in potato plants using EPG analyses. The time taken to first intracellular stylet puncture, number of intracellular stylet punctures, and duration of intracellular stylet punctures were all significantly shortened at insufficient P nutrition (Figure 2-11 B, C, and D).

The aphid salivation in the phloem sieve element was significantly ($p < 0.05$) reduced in the insufficient P nutrition when compared to both optimum and excessive P nutrition levels (Figure 2-12 B). This could possibly be due to better nutrition quality and accessibility in the P insufficient plants and reduced leaf structural inhibitors. Bala et al. (2018) observed that P nutrition play a key role in the synthesis of lignin and

tannins in leaf surfaces which can be feeding deterrents or toxic to aphids. Lignin is a phenolic heteropolymer that confers resistance by increasing the leaf toughness and decreasing the phloem sap nutritional quality (Johnson et al. 2009). Tannins are bitter polyphenols and deter the insects from feeding by reducing nutrient absorption efficiency and causing midgut lesions in insects (Barbehenn and Constabel 2011). However, the mechanism for the decreased salivation under insufficient P nutrition in potato plants remain unknown in the present study, necessitating further investigations.

2.3.3 Influence of K nutrition on aphid host choice, reproduction, and feeding behaviors

2.3.3.1 Aphid host settling preference

The host preference choice test showed that the *A. gossypii* host preference was influenced by K nutrition status in potato plants (Figure 2-13). The aphids significantly ($p < 0.01$) preferred settling on insufficient K nutrition over excessive K nutrition (Figure 2-13 A).

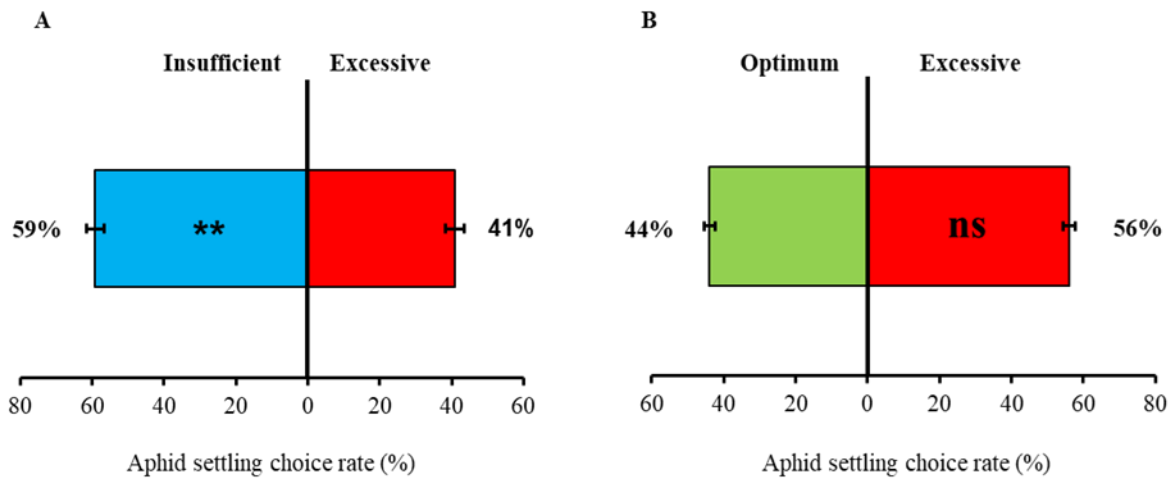


Figure 2-13 Influence of plant K nutrition on *Aphis gossypii* host settling preference in potato plants after 24 hours; (A) dual host settling preference between insufficient K and excessive K nutrient statuses and (B) dual host settling preference between optimum K and excessive K nutrient statuses. Insufficient = MS medium containing 13.5 mM of K, Optimum = MS medium containing standard 20 mM of K, and Excessive = MS medium containing 30 mM of K. Mean comparison between two treatments according to *t*-test at $p < 0.05$. ** = $p < 0.01$ and ns = non significant. Means presented with \pm standard error.

Potassium is involved in the activity of pyruvate kinase, an enzyme responsible for the incorporation of carbon into amino acids which consequently reduce the accumulation of free sugars and amino acids (Oria-Hernandez et al. 2005). Thus, in K deficiency, the activity of pyruvate kinase is hampered leading to accumulation of free amino acids. In addition, K is involved in the activity of starch synthase, and when K is deficient it results in accumulation of carbohydrates in the source organs. The accumulation of these metabolites is known to attract insects (Amtmann et al. 2008). Contrastingly, the increase in K nutrition reduces N, carbohydrate accumulation, and free amino acids which decreases the acceptability of the plant by insect pest as a suitable host (Rashid et al. 2016). Walter and DiFonzo (2007) observed higher aphid densities in K deficient soybean compared to non-deficiency soybean plant under field conditions, and attributed this to improved N nutrition under K deficiency. These mechanisms could explain the settling preference of aphid *A. gossypii* on K insufficient potato plants over excessive K nutrition, and the lack of significant preferential change between optimum and excessive K nutrition. However, further studies are needed to verify these mechanisms. The present findings suggest adequate K nutrition can reduce aphid infestation in potato production.

2.3.3.2 Aphid reproduction

The *A. gossypii* reproduction capacity was influenced by potato plant K nutrition (Figure 2-14). This is the first study to report the influence of K nutrition on reproduction of *A. gossypii* in potato plants. Reduction in *A. gossypii* population density at high K fertilization has previously been reported in cotton plants (Ai et al. 2010). Schlickmann-Tank et al. (2020) also reported decreased aphid populations in high K fertilized sorghum plants. The authors attributed this phenomenon to the reduced nutrition quality for aphids with increased K supply. These phenomena may also explain the significant reduction of number of nymphs at excessive K nutrition in the present study. As a result, adequate K nutrition can aid in the management of aphid population densities in potato production as part of the integrated pest management.

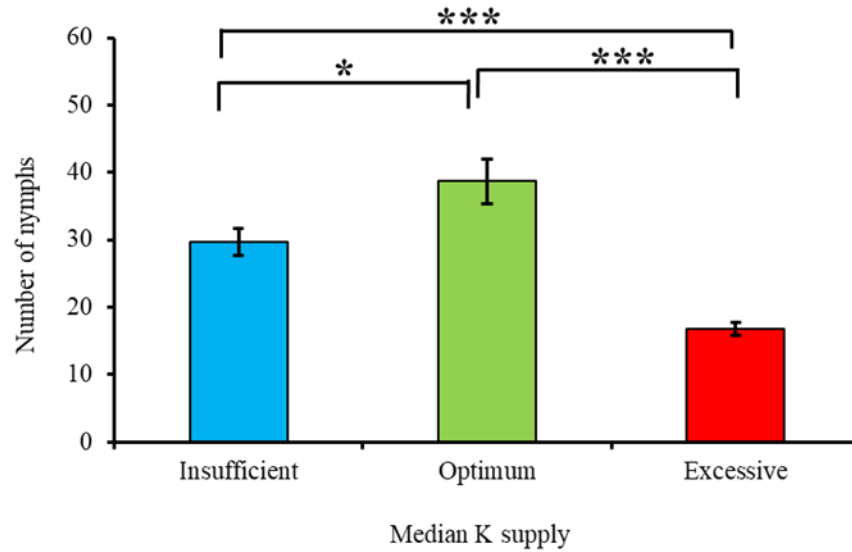


Figure 2-14 Influence of plant K nutrition on number of *Aphis gossypii* nymphs in potato plants after 7 days. Insufficient = MS medium containing 13.5 mM of K, Optimum = MS medium containing standard 20 mM of K, and Excessive = MS medium containing 30 mM of K. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$ and *** = $p < 0.001$. Means presented with \pm standard error.

2.3.3.3 Aphid feeding behavior

The EPG analyses showed that K nutrition influenced *A. gossypii* feeding behavior in potato plants phloem sieve elements (Figures 2-15 and 2-16). This is the first study to document the effect of K nutrition on *A. gossypii* behavior in potato plants using EPG analyses. The duration of aphid phloem ingestion was significantly decreased ($p < 0.05$) in potato plants with excessive K nutrition compared to aphids fed on insufficient K nutrition (Figure 2-16 C).

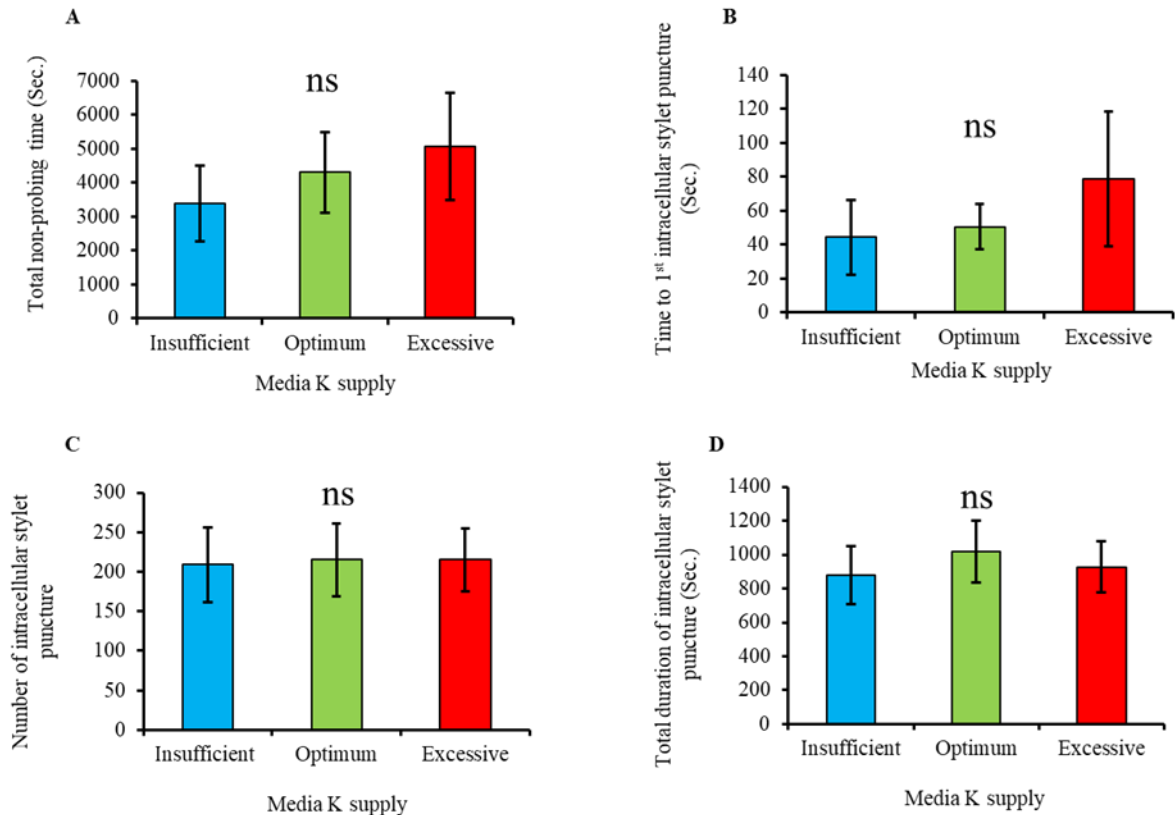


Figure 2-15 Influence of plant K nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. (A) non- probing (np), (B) time to first potential drop (pd), (C) number of potential drops (n_pd), and (D) total duration for potential drops (s_pd). Insufficient = MS medium containing 13.5 mM of P, Optimum = MS medium containing standard 20 mM of P, and Excessive = 30 mM of P. Mean comparison between two treatments according to *t*-test at $p < 0.05$. ns = non significant. Means presented with \pm standard error.

This behavior may explain the significantly lower numbers of nymphs observed in excessive K nutrition plants compared to K insufficient plants (Figure 2-14). Generally, the phloem feeding behavior was inversely associated with the K nutrition in the potato plants suggesting that its influence is also affected by other factors such as translocation of sugars and water relations in the plant. Potassium is involved in the enzymatic activities responsible for the synthesis of secondary metabolites from free sugars and amino acids (Facknath and Lalljee 2005). Also, it is involved in the translocation of amino acids to different parts of the plant (Ruiz and Romero 2002). These processes decrease the content of free amino acid in the phloem sap, depriving aphids of their dominant N source (Douglas 2003). Therefore, the influence of K nutrition on phloem resistance may be attributed to poor phloem sap quality. And this corroborates well with the significantly lower numbers of nymphs under excessive K supply. In view of the present findings, increases in K fertilization may play a key role in reducing aphid infestations in potato production systems.

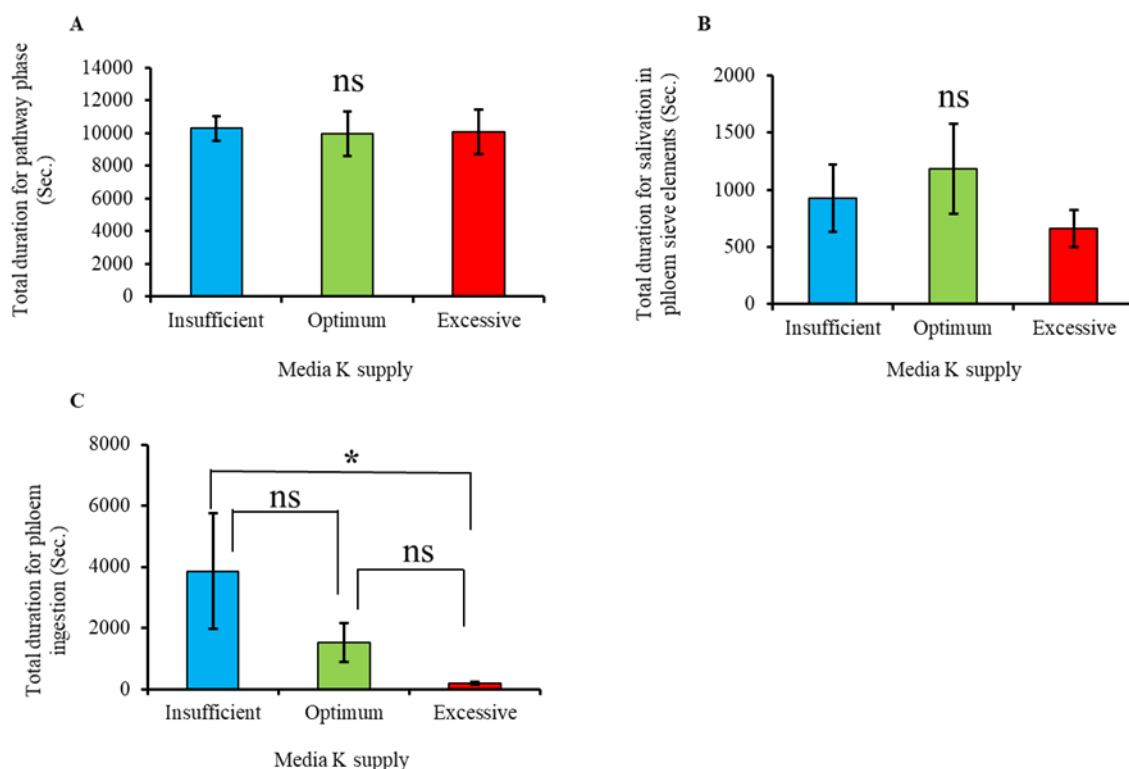


Figure 2-16 Influence of plant K nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. **(A)** total time spent in pathway phase (s_C), **(B)** total time spent salivating in phloem sieve elements (s_E1), and **(C)** total time spent feeding on phloem (s_E2). Insufficient = MS medium containing 13.5 mM of K, Optimum = MS medium containing standard 20 mM of K, and Excessive = 30 mM of K. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, ns = non significant. Means presented with \pm standard error.

2.3.4 Influence of Ca nutrition on aphid host choice, reproduction, and feeding behaviors

2.3.4.1 Aphid host settling preference

The dual host settling preference results showed that *A. gossypii* significantly preferred ($p < 0.05$) settling on potato plants with insufficient Ca nutrition over the optimal Ca nutrition plants (Figure 2-17). This finding suggests that potato plants with optimal Ca nutrition had more resistance factors than plants with insufficient Ca nutrition. These resistance factors are generally related to volatiles, visual cues, and plant leaf surface cues. During orientation in the early stages of host selection, volatiles attract or repel aphids (Pickett et al. 1992). Effah et al. (2020) reported a decrease in volatile organic compounds emission in *Calluna vulgaris* grown soils containing low Ca and K nutrients. These volatiles can also influence phloem sap quality as

feeding deterrents (Gebretsadik et al. 2022). According to Jakobs et al. (2019), aphids select a suitable host plant after recognizing the quality of the phloem sap. This suggests a possibility that the emitted volatiles in the insufficient Ca nutrition were lower than those in plants with optimal Ca nutrition, and that this significantly affected the aphid preference for the former plants. Since the plants were visually similar and visual cues are typically considered for winged aphids rather than wingless aphids, which were used in the current study, it is possible that the leaf color, did not play a significant role in the orientation of the aphids (Pettersson et al. 2017). Calcium play a critical role in enhancing cell wall strength through the formation of intra-molecular crosslinks between pectin molecules in plants (Thor 2019). When Ca is insufficient, cell walls become weak, and salts and organic compounds leak out making the plant susceptible to pest attack (Palta 2010). As a result of having sufficient Ca, the plant's surface toughness may have affected physical barriers to aphid mechanical probing, which may also have influenced aphids' attraction to potato plants with insufficient Ca nutrition.

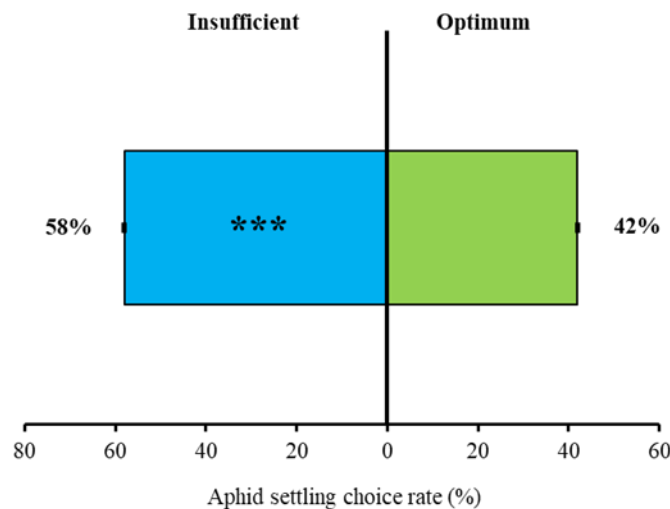


Figure 2-17 Influence of plant Ca nutrition on *Aphis gossypii* host settling preference in potato plants after 24 hours. Insufficient = MS medium containing 1.0 mM of Ca and Optimum = MS medium containing 10 mM of Ca. Mean comparison between two treatments according to *t*-test at $p < 0.05$. *** = $p < 0.001$ and ns = non significant. Means presented with \pm standard error.

In addition, in the current study, K content in potato plants with insufficient Ca nutrition was significantly lower than in those with optimal Ca nutrition (Chapter 1; Table 1-3). K is known to be important in the activities of pyruvate kinase and starch synthase, which are responsible of carbon incorporation into amino acids and glucose deposition into starch, respectively, processes that control the accumulation of free amino acids and free sugars (Oria-Hernandez et al. 2005). With insufficient K nutrition, these activities are halted,

which may result in the accumulation of free amino acids, which can increase the plant's acceptability as a host to aphids (Amtmann et al. 2008). Adequate Ca fertilization in potato production can thus be considered as one of plant nutrition management strategies to reduce potato plant susceptibility to aphid infestation. The mechanisms by which Ca influenced aphid settling preference were not clearly identified in the current study, necessitating further research.

2.3.4.2 Aphid reproduction

Calcium nutrition in potato plants had no effect on the reproduction capacity of *A. gossypii* (Figure 2-18). The quality and quantity of amino acids in phloem sap are known to influence *A. gossypii* reproduction (Banerjee and Raychaudhuri 1987). In the present study, the N uptake was significantly higher in optimal Ca plants than in insufficient Ca plants, and insufficient Ca plants had significantly lower K content than optimal Ca plants (Chapter 1; Table 1-2).

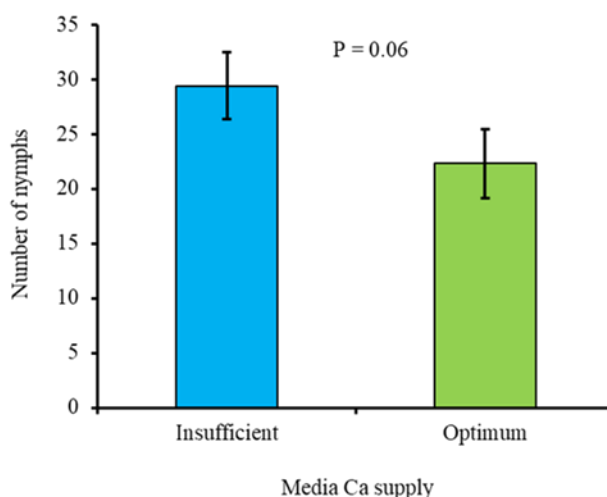


Figure 2-18 Influence of plant Ca nutrition on number of *Aphis gossypii* nymphs in potato plants after 7 days. Insufficient = MS medium containing 1.0 mM of Ca and Optimum = MS medium containing 10 mM of Ca. Mean comparison between two treatments according to *t*-test at $p < 0.05$. Means presented with \pm standard error.

Both higher N uptake and K content may influence N content in phloem sap. The lack of difference in aphid reproduction suggest that variation in Ca nutrition may not influence the N composition of phloem sap to the extent that it affects reproduction in *A. gossypii*. As a result, Ca fertilization as part of nutrient management control against aphids may necessitate additional interventions that inhibit aphid reproduction.

Further research may look into the addition of nutrients like K and reduction in N, which have shown interactions with Ca fertilization.

2.3.4.3 Aphid feeding behavior

Increases in potato plant Ca nutrition increased resistance to *A. gossypii* at the leaf surface and phloem sieve elements, as revealed by the EPG analyses (Figures 2-19 and 2-20). This is the first study to report this phenomenon in potato plants.

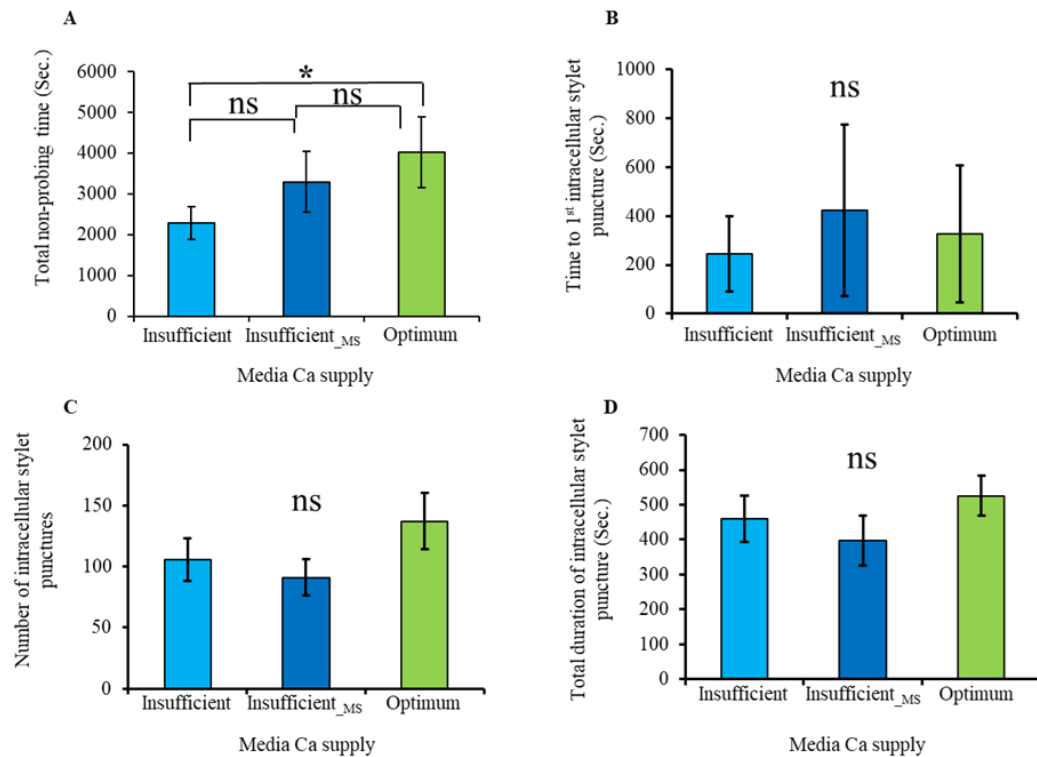


Figure 2-19 Influence of plant Ca nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. **(A)** non- probing (np), **(B)** time to first potential drop (pd), **(C)** number of potential drops (n_{pd}), and **(D)** total duration for potential drops (s_{pd}). Insufficient = MS medium containing 1.0 mM of Ca, Insufficient_{MS} = MS medium containing 3.0 mM of Ca, and Optimum = MS medium containing 10 mM of Ca. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$ and ns = non significant. Means presented with \pm standard error.

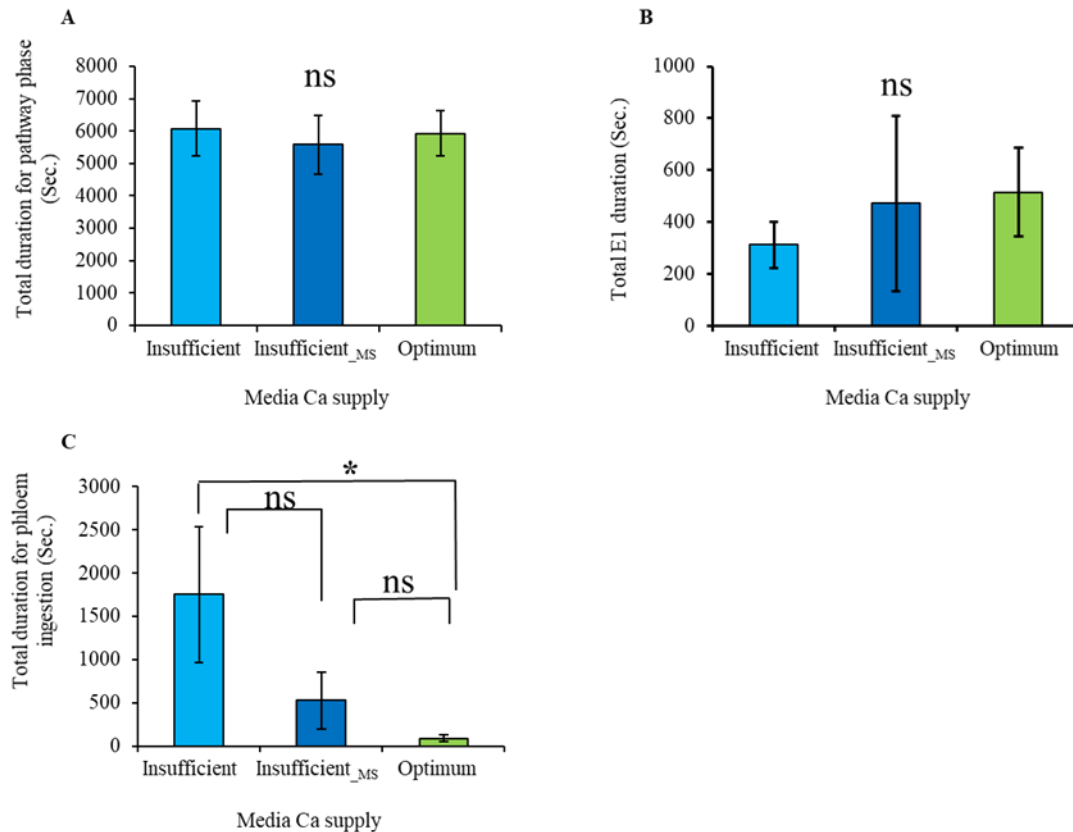


Figure 2-20 Influence of plant Ca nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. **(A)** total time spent in pathway phase (s_C), **(B)** total time spent salivating in phloem sieve elements (s_E1), and **(C)** total time spent feeding on phloem (s_E2). Insufficient = MS medium containing 1.0 mM of Ca, Insufficient_MS = MS medium containing standard 3.0 mM of Ca, and Optimum = MS medium containing 10 mM of Ca. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, ns = non significant. Means presented with \pm standard error.

When plants with optimal Ca nutrition were compared to those with insufficient Ca nutrition, the duration of non-probing for *A. gossypii* was significantly prolonged ($p < 0.05$) and time spent in phloem sap ingestion was significantly shortened ($p < 0.05$) (Figure 2-19 A and 2-20 C). These findings point to the presence of resistance factors associated with Ca's role in enhancing plant physical barriers and as a second messenger in plant defense against pathogen attack (Thor 2019). Ca nutrition strengthens plant physical barriers by forming cross-linkages of pectin in cell walls, and it also stabilizes cell membranes through interaction with phospholipids (Thor 2019). Sap sucking insects, such as aphids, are known to be affected by such physical strength on plant leaves (van Helden and Tjallingii 1993). Szwarc et al. (2021) observed a negative correlation between Ca content and resistance to cabbage aphid (*Brevicoryne brassicae* L.) in rapeseeds, which is consistent with the current findings. As a result, adequate Ca fertilization is critical for increasing resistance to aphid infestations in potato plants. Because the precise mechanisms underlying the resistance

are unknown, future research is required to identify the specific mechanisms at both the plant surface and the phloem sieve element levels.

2.3.5 Influence of Mg nutrition on aphid host choice, reproduction and feeding behaviors

2.3.5.1 Aphid host settling preference

The dual host settling preference test showed that *A. gossypii* significantly preferred ($p < 0.05$) settling on potato plants with excessive Mg nutrition over those with optimal Mg nutrition (Figure 2-21).

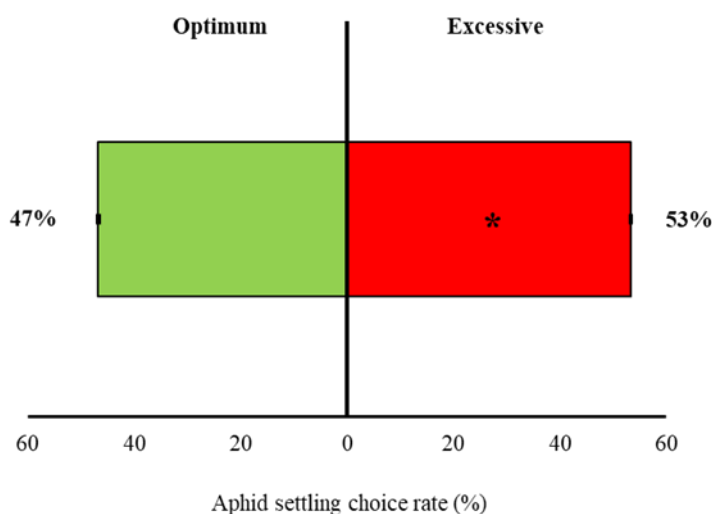


Figure 2-21 Influence of plant Mg nutrition on *Aphis gossypii* host settling preference in potato plants after 24 hours. Optimum = MS medium containing standard 1.5 mM of Mg and Excessive = MS medium containing 9.0 mM of Mg. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$. Means presented with \pm standard errors.

Mg is involved in a variety of metabolic processes in plants, including photosynthesis, protein synthesis, and the phloem loading of photosynthates and amino acids (Cakmak and Yazici 2010). Mg deficiency reduces phloem loading of sugars and amino acids, causing them to accumulate in the source leaves while reducing their content in phloem sap (Lalonde et al. 2004; Koch et al. 2020). In contrast, Ruan et al. (2012) found increases in the amounts of total sugars and amino acids in xylem saps and phloem exudates of tea plants given sufficient Mg. We can presume on a possible differential effect of increased Mg nutrition on phloem sap amino acid and sugar content from optimal to excessive Mg. Sugars act as phagostimulants, so high

sucrose content in aphid diets increases aphid feeding (Mittler and Meikle 1991). The composition and concentration of amino acid have a significant impact on aphid performance (Gruhn et al. 2021). Though amino acids and sugars were not quantified in the present study, our findings suggest that optimal Mg nutrition may have lower phloem loading, resulting in decreased sugar and amino acid content in the phloem sap, which reduced the acceptability of Mg optimal plants for *A. gossypii* settling. Pescod et al. (2007) found that the sugar content of phloem sap in potato plants has no effect on the settling of aphids *M. persicae* and *Aulacorthum solani* but it did for *Macrosiphon euphorbiae*. These findings point to the fact that aphid response to sugar content in phloem sap varies by species. Facknath and Lallijee (2005) found no effect of Mg nutrition on serpentine leafminer, *Liriomyza trifolii* in potato plants. The authors attributed this to lack of significant change in the plant Mg content after fertilization. In the present study, excessive Mg plants had significantly higher shoot Mg content (Chapter 1; Table 1-3). As a result, more research is needed to quantify the sugar and amino acid contents of potato plants under varying Mg nutrition and investigate their influence on different aphid species colonizing potato plants.

2.3.5.2 Aphid reproduction

The reproduction assay results showed that plant Mg nutrition did not affect *Aphis gossypii* reproduction capacity (Figure 2-22).

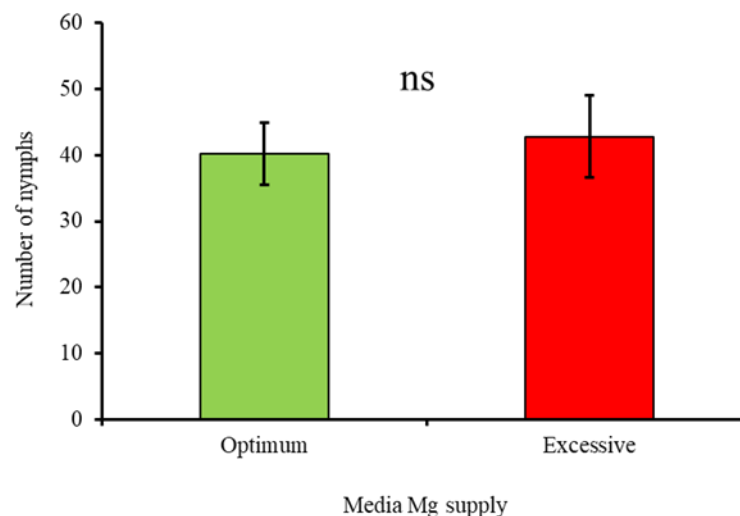


Figure 2-22 Influence of plant Mg nutrition on number of *Aphis gossypii* nymphs in potato plants after 7 days. Optimum = MS medium containing standard 1.5 mM of Mg and excessive = MS medium containing 9.0 mM of Mg. Mean comparison between two treatments according to *t*-test at $p < 0.05$. ns = non significant. Means presented with \pm standard error.

This agrees with the findings by Schlickmann-Tank et al. (2020) who found no effect of Mg nutrition on aphid *Melanaphis sacchari/sorghii* population increases in sorghum plants. The authors, however, reported positive correlation between aphid populations and sucrose and total soluble proteins in leaves. In addition, Mg nutrition was evaluated in combination with NPK other than alone. Schmidt-Jeffris (2021) reported reduced oviposition of European corn borer on *Bt* corn plants with increased Mg nutrition. Contrastingly, the application of Mg alone or in combination with N and P increased the populations of lice, *Eulecanium refulum* in oak trees (Huber et al. 2012). The authors attributed this to the fact that Mg nutrition induced K deficiency which increased soluble N concentration, a favorable condition for lice development. In the present study, excessive Mg induced higher N uptake which could be expected to increase the aphid production (Chapter 1; Table 1-2). Therefore, the effect of Mg nutrition on aphid reproduction remains unclear. Other factors other than changes in amino acid and sugar content in the phloem sap may have a major role affecting aphid reproduction, and thus requires further investigations.

2.3.5.3 Aphid feeding behavior

The EPG analyses showed that *A. gossypii* feeding behavior at plant leaf surface, as well as mesophyll and phloem sieve elements, was affected by potato Mg nutrition (Figures 2-23 and 2-24). This is the first time this phenomenon has been observed in potato plants. The aphid spent significantly more time ($p < 0.001$) before initiating the first intracellular stylet puncture in potato plants with optimal Mg nutrition than in potato plants with excessive Mg nutrition (Figure 2-23 B).

This suggests the presence of resistance factors related to mechanical or olfactory cues located at the leaf surface, such as presence of repellent volatiles, leaf color, or toughness (van Helden and Tjallingii 1993). More research is needed to identify these structural leaf changes. These EPG results are consistent with the dual settling preference, in which aphids preferred settling on potato plants with excessive Mg nutrition over those with optimal Mg nutrition (Figure 2-21). Salivation in the phloem sieve elements was significantly prolonged ($p < 0.05$) in optimal Mg nutrition potato plants compared to excessive Mg nutrition potato plants (Figure 2-24 B). This behavior indicates that resistance factors are present at the mesophyll and phloem sieve elements in the optimal Mg nutrition potato plants. Prolonged aphid salivation indicates the aphid's response to plant defense suppression. Increased aphid salivation was reported by Medina-Ortega and Walker (2013) as a defense mechanism against sieve element occlusion, a plant response to sieve element damage for the

protection of its photo-assimilate-rich phloem sap. Kindler and Staples (1970) reported decreased resistance to spotted alfalfa aphid (*Therioaphis maculata* Buck ton) in alfalfa plants treated with excessive Mg nutrition, which is consistent with our current findings. Therefore, the present study suggests that adequate Mg nutrition in potato plants can play an important role in reducing aphid infestations.

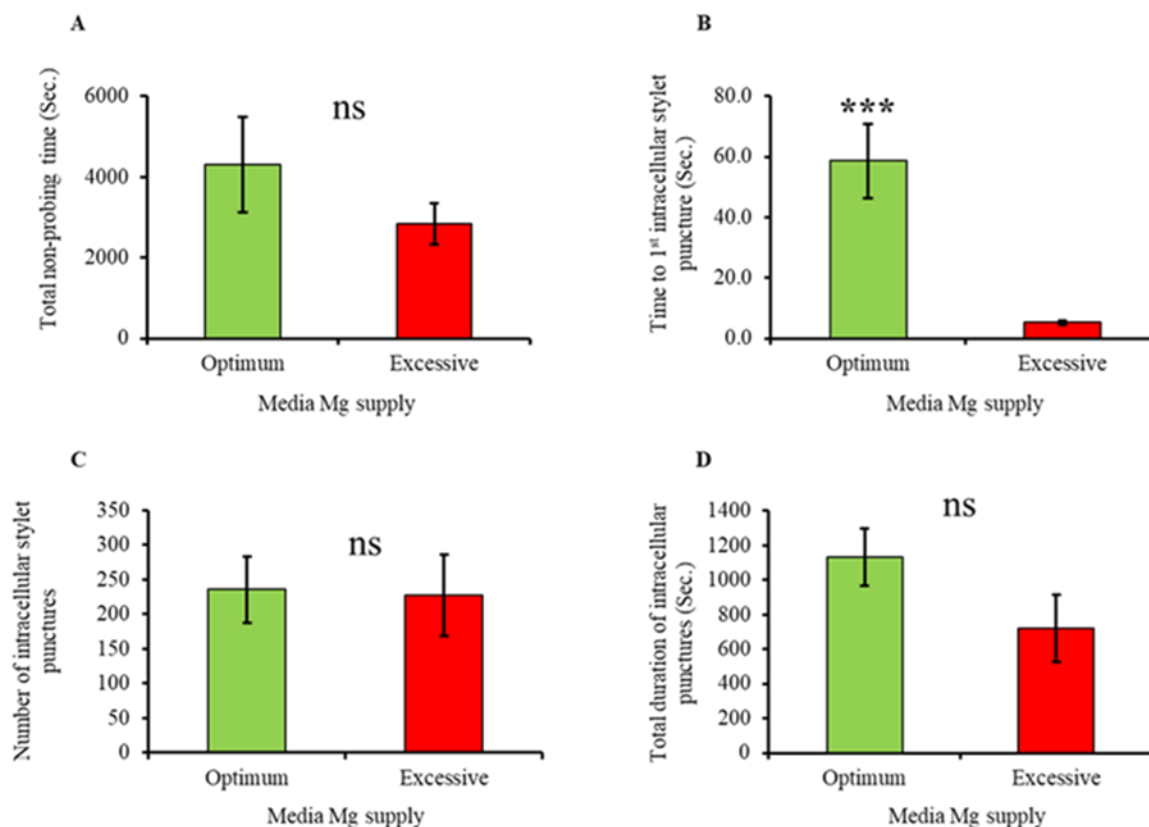


Figure 2-23 Influence of plant Mg nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. **(A)** non- probing (np), **(B)** time to first potential drop (pd), **(C)** number of potential drops (n_pd), and **(D)** total duration for potential drops (s_pd). Optimum = MS medium containing standard 1.5 mM of Mg and Excessive = MS medium containing 9.0 mM of Mg. Mean comparison between two treatments according to *t*-test at $p < 0.05$. *** = $p < 0.001$ and ns = non significant. Means presented with \pm standard error.

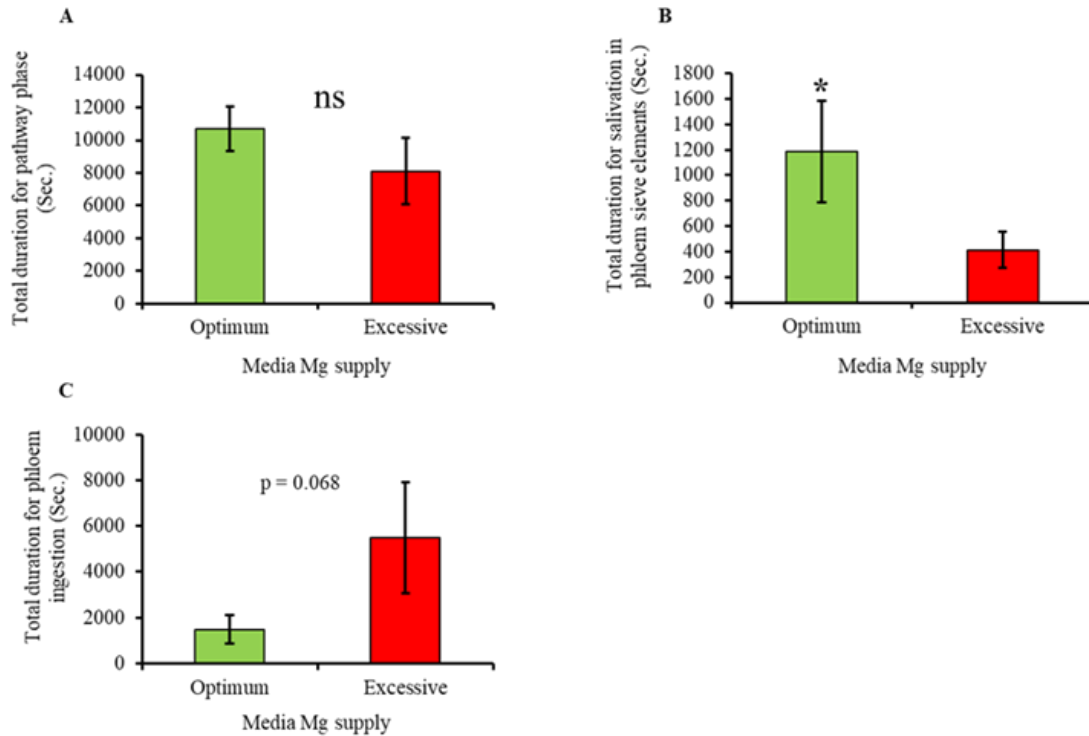


Figure 2-24 Influence of plant Mg nutrition on *Aphis gossypii* feeding behaviors as observed in Electric Penetration Graphs (EPG) in potato plants for a period of 6 hours. (A) total time spent in pathway phase (s_C), (B) total time spent salivating in phloem sieve elements (s_E1), and (C) total time spent feeding on phloem (s_E2). Insufficient = MS medium containing 1.0 mM of Ca, Insufficient_{MS} = MS medium containing standard 3.0 mM of Ca, and Optimum = MS medium containing 10 mM of Ca. Mean comparison between two treatments according to *t*-test at $p < 0.05$. * = $p < 0.05$, ns = non significant. Means presented with \pm standard error.

2.3.4 Conclusion

Despite several studies and contradictory findings on the effect of plant nutrition on insect pest behavior, it is clear that *A. gossypii* feeding behaviors are influenced by the plant's N, P, K, Ca, and Mg nutritional status. This is demonstrated by the fact that variation in each individual nutrient element affected aphid host settling preference, reproduction, and feeding behaviors at the leaf surface, epidermis, and sieve elements. High N, P, and Mg nutrition, as well as insufficient K and Ca nutrition, may increase host settling preference. High P and Mg nutrition, as well as insufficient N and K nutrition may increase reproduction. Increases in N, P, Ca, and Mg nutrition may confer leaf surface resistance, whereas increases in N, P, K, and Ca nutrition may confer sieve element resistance. The potential for interactive effects of nutrient elements on aphid performance was also emphasized. Furthermore, these behavioral changes can have a significant impact on the transmission of aphid-vectored viral diseases in potatoes. The current findings can help in the development of appropriate fertilizer regimes for potato production systems as part of cultural control

measures in integrated pest management to reduce aphid infestations. As a result, the costs and risks to human health and the environment associated with chemical insecticide applications may be reduced, while potato yield and quality could be improved or maintained. However, the precise molecular and morphological mechanism for each nutrient element remain unknown, necessitating further research.

General discussion

Integrated pest management (IPM) which employs chemical, biological and cultural pest control measures, is becoming increasingly popular as a safe, less costly, and practical pest control strategy (Bajwa and Kogan (2004). Cultural control as part of IPM strategy involves the use of agronomic practices such as pest resistance cultivars, as well as fertilizer management, to limit pest invasion and population growth in an agroecosystem (Bajwa and Kogan 2004; Chang et al. 2010). The control measure in this context focuses on increasing host resistance to pest as a primary defense strategy to prevent and reduce pest outbreaks. Enhanced host plant resistance is one of the most effective and safe pest control strategies, with pest and disease cultivar breeding receiving a lot of attention (Stout 2014). However, breeding programs have frequently proven to be expensive, time consuming and aphids can quickly overcome host-mediated resistance usually by development of virulent biotypes (Solomon-Blackburn and Baker 2001; Dogimont et al. 2010; Wenger et al. 2014). Even a resistant variety may have different resistance according to the nutritional status. The breakdown of plant genetic resistance to aphids, for instance, has been reported for *Nr* gene in lettuce plant against lettuce aphid as well as for *Rag* genes conferring resistance to *A. glycine* in soybeans (Dogimont et al. 2010). And virulent biotypes have been reported in several aphid species such as raspberry aphid (*Amphorophora agathonica*), woolly aphid (*Eriosoma lanigerum*), pea aphid (*A. pisum*), cabbage aphid (*Brevicoryne brassicae*), and greenbug aphid (*Schizaphis graminum*). The plant host resistance traits can be strengthened if the crop receives adequate and balanced nutrients and this can reduce the chances of the aphid developing virulent biotypes to easily overcome host resistance.

Plant nutrition can change the morphology and biochemistry of plants which can directly influence the behavior of aphids on the plant either as a host or a non-host (Huber et al. 2012). The plant morphological attributes that are changed include trichome density and its toxic exudates, cell wall thickness and lignification, and volatiles which provide the first line defense against insect pest attack (War et al. 2012). Therefore, management of fertilization regimes that enhance these morphological features can aid in keeping away aphids from the crop. Aphids, are also renowned for being extremely sensitive to changes in plant nutrition. A change in the composition of amino acid in the phloem sap affects the performance of aphids leading to high mortality and poor reproduction (Benerjee and Raychaudhuri 1987; Karley et al. 2002). Sugar composition of the phloem sap also, apart from being an important source of carbon for aphids, its concentrations affects the aphid feeding rate, with lower rates observed at higher sucrose concentrations (Douglas et al. 2006). As a result, manipulation

of phloem sugar and amino acid contents in plants through fertilizer management can be one way of creating unsuitable host plants for aphids, thus keeping them away from the crop. Previous research in the natural and agricultural fields, as well as in greenhouses, has yielded conflicting results on how plant nutrition affects herbivorous insect feeding behaviors (Warring and Cobb 1992; Butler et al. 2012). As a result, we developed a technique for more precise assays using tissue culture to better understand the effect of fertilization on plant growth and insect pest feeding behaviors.

The developed nutrient control system in potato tissue culture allowed for a better understanding of plant nutrient status, ranging from deficient to toxic levels. These nutrient status variations resulted in potato plant growth disorders that have been widely reported in previous studies under a variety of growth conditions ranging from tissue culture to field conditions (Cackmak and Yazici 2010; Palta 2010; Hopkins et al. 2020). The potato response to N supply was revealed to be cultivar dependent and that studies require cultivar specific experiments to obtain precise information. Apart from improving shoot biomass, P, Ca, and Mg enhanced root growth. Because root dry matter is known to be positively related to shoot dry matter and final tuber yield, this could be ideal for increasing potato tuber yield (Wishart et al. 2013). In the current study, both synergistic and antagonistic interactions between nutrients were observed, which was not previously reported in potato tissue culture studies (Naik and Sarkar 1998; Saik and Naik 1998; Randouani and Lauer 2015). There were synergistic interactions in uptake between: N and P, K, Ca, and Mg; P and N, K, Ca, and Mg; K and P; Ca and N, K, and Mg; and Mg and N. There were antagonistic interactions in uptake between toxic N and P, K, Ca, and Mg; excessive K and N; and deficient Mg, P, and K. These interactions were consistent with previous findings in greenhouse and field studies (Roosta and Schojoerring 2007; Fageria et al. 2014; Kumar et al. 2021; Arteta et al. 2022). Taking advantage of synergistic interactions while minimizing antagonistic interactions could be critical in improving nutrient use efficiency and developing appropriate fertilization regimes for improved host plant resistance to pest infestations.

We used the potato tissue culture system to learn more about the effects of insufficient and excessive nutrient supplies, with the hypothesis that these conditions would make a crop more vulnerable to insect pest infestation (Bala et al. 2018). In fact, aphid feeding behaviors were influenced by the nutritional status of the potato plants, which was consistent with previous observations in greenhouse and field conditions (Butler et al. 2012; Bala et al. 2018). This was the first study to document such phenomena *in vitro*. The lower aphid settling preference revealed the importance of plant nutritional status in repelling insect pests, which could be ideal not only for reducing pest pressure but also for lowering the chances of non-persistent virus transmission in plants. Non-

persistent virus transmission takes seconds to minutes for an aphid to acquire from an infected plant, while inoculation requires the aphid to land on the plant and initiate brief superficial stylet probes. As a result, changing these behaviors is critical for controlling persistently transmitted viral disease in crops. In contrast, persistent virus transmission necessitates a prolonged phloem feeding and salivation for several hours or a few days to acquire and inoculate the virus, respectively (Gadhav et al. 2020). As a result, the reduced phloem feeding and salivation observed as a result of plant nutrient status, combined with repellence, could play an important role in reducing the chances of aphids transmitting the persistent viruses. Despite the fact that repulsion can occur as a result of changes in plant morphological features such as trichomes and leaf surface toughness, as well as volatiles, the specific features for repulsion in the current study are still unknown. Reduced phloem feeding can have an effect on insect development and reproduction, lowering pest infestation pressure. The aphid's reduced reproduction capacity as a result of plant nutritional status was demonstrated in the current study. By removing large amounts of phloem sap, high pest densities can have an immediate impact on crop growth, resulting in growth disorders, wilting, and even plant death. High aphid densities can also result in the accumulation of sooty molds on the leaves due to honeydew accumulation. This can reduce photosynthesis and promote fungal diseases, which can cause more damage than phloem sap feeding alone (Sorensen 2009).

According to the findings of this study, plant nutrition strengthens plant resistance to aphids at three distinct levels: the leaf surface, mesophyll cells, and phloem sieve elements, which can reduce direct crop damage as well as viral disease transmission. Due to the study's limitations, the underlying morphological and chemical mechanisms on host resistance to aphid settling, reproduction, and feeding behaviors for each nutrient remain unknown. These mechanisms, as well as the possible effects of nutrient interactions, will need to be studied further in the future. It is clear that artificially manipulating plant nutrients in cultural insect pest control practices as part of IPM can help reduce aphid infestations in potato production systems.

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Abstract (要旨)

土壌栄養の不足や過剰、および病害虫による被害は、ジャガイモ生産の重要な制限因子となっている。ジャガイモの植物体の生長や、栄養の吸収およびその相互作用、植食性昆虫との相互作用に対する各種栄養元素の影響に対する理解は、環境変動の影響を受ける圃場試験では曖昧な状態である。そこで、栄養状態が不足または過剰なモデル植物を作出すれば、より制御された環境下での実験を可能にし、特定の元素濃度が他の元素の吸収にどのように影響を与えるか、また、植物体中の元素含量が害虫とどのように相互作用するかをより深く理解することができることが期待される。

本研究では、まず、ジャガイモに対する必須元素の影響を解析するために、組織培養ジャガイモの栄養含量制御系を確立した。ムラシゲ・スクーグ (MS) 培地を改良し、窒素 (N)、リン (P)、カリウム (K)、カルシウム (Ca)、マグネシウム (Mg) が欠乏または過剰の場位置を作成した。また、植物の栄養状態を、欠乏・不足・最適・過剰・有毒の5段階に分け、これらは植物体の乾燥重量および病徴の観察によって判断した。培地中のN濃度は、20mMで不足、60-80mMで最適、100mMで過剰となった。P濃度は、0.4mMで不足、1.25mMで最適、3.5mMで過剰となった。K濃度は、13.5mMで不足、20mMで最適、30mMで過剰となった。Ca濃度は、1mMで不足、10mMで最適となった。Mg濃度は、1.5mMで最適、9mMで過剰となった。これらの結果から、植物の生長および栄養吸収・含量、元素間の相互作用の全てが、MS培地の元素濃度の変化によって有意な影響を受けることが明らかとなった。MS培地中のN濃度の増加により、植物体中のN吸収量は有毒レベルにまで増加した。Nの吸収量の変化は、植物体中のP、K、Ca、Mgの吸収量を増加させた。MS培地中のP濃度の増加により、植物体中のP吸収量が過剰になるまで増加した。Pの吸収量の変化は、植物体中のMg吸収量を増加させた。MS培地中のK濃度の増加により、K吸収量はわずかに増加した。また、K濃度が不足および過剰の植物において、N吸収量は減少した。Ca濃度の吸収量は、MS培地中のCa濃度が1~3mMの濃度では一定であったが、10mMで急激に増加した。Caの吸収量の変化は、N、K、Mgの吸収量を増加させた。MS培地中のMg濃度の増加により、9mMで急激に吸収量が増加した。また、Mg吸収量の変化は、植物体中のN吸収量を増加させた。以上の結果から、特定の元素の吸収量の変化により、他の元素吸収量に影響を与えることが明らかとなった。したがって、意図しない元素吸収量の変化が起こる可能性を考慮し、他の元素とのバランスを取る必要がある。

次に、これらの各栄養元素の変動が、ワタアブラムシ (*Aphis gossypii*) の宿主嗜好性、繁殖、吸汁行動に与える影響を調査した。ワタアブラムシの宿主嗜好性は、元素含量の異なる植物を用いた、選択試験により評価した。繁殖試験は、元素含量の異なる植物にワタアブラムシを放飼し、自由に産子させ、若虫の個体数を計数した。吸汁行動は、EPG解析装置を用い、非吸汁、細胞内口針穿刺、師管への唾液分泌、師管液の摂取など異なる吸汁行動の時間、強度、頻度を測定した。その結果、ワタアブラムシの宿主嗜好性および繁殖、吸汁行動の全て（繁殖率におけるCaおよびMgの影響を除く）において、ジャガイモの栄養状態の変化が影響することが明らかとなった。ジャガイモ中のN含量が増加すると、ワタアブラムシの宿主嗜好性は上昇したが、産子数は減少した。また、N不足ジャガイモでは、最適の植物体に比べ、総非吸汁行動時間が短くなった。ジャガイモ中のP含量が増加すると、ワタアブラムシの宿主嗜好性は高くなった。また、三枝数は、P含量の増加に伴い増加した。P不足の植物体では、最適植物に比べ、初回口針穿刺時間および細胞穿刺回数、総細胞穿刺時間が増加し、総師管唾液分泌時間が減少した。K含量が不足するジャガイモは、過剰の植物に比べ、嗜好性が高まった。産子数は、最適レベルまでは増加するものの、過剰植物では劇的に減少した。K過剰植物では、K不足植物に比べ、総師管液摂取時間が減少した。Ca最適植物体に比べ、Ca不足ジャガイモでワタアブラムシの宿主嗜好性が高まったものの、産子数に影響は見られなかった。Ca不足植物体では、非吸汁行動時間が短かく、師管液摂取時間が長かった。Mg過剰ジャガイモは、最適レベルの植物体に比べ嗜好性が高まったが、産子数に影響は見られなかった。Mg過剰ジャガイモでは、初回口針穿刺までの時間および師管唾液分泌時間が短かった。これらの結果から、ジャガイモ中の元素含量は、ワタアブラムシの嗜好性および産子数、吸汁行動に対し影響を与え、その作用は各元素によって異なることが明らかとなった。

今後、これらの栄養状態を制御した組織培養ジャガイモを用いることで、各元素含量の変化がアブラムシによる植物ウイルス伝播効率にどのような影響を与えるか、また、その分子メカニズムに関して研究を展開していくことができるものと期待される。