Universidad Autónoma de Sinaloa
Colegio de Ciencias Agropecuarias
Facultad de Agricultura del Valle del Fuerte
Doctorado en Ciencias Agropecuarias

TESIS:

INFLUENCIA DE *Bacillus subtilis* Y SUMINISTRO DE FÓSFORO EN CRECIMIENTO, RENDIMIENTO Y ABSORCIÓN NUTRIMENTAL DEL CULTIVO DE FRIJOL

Que para obtener el grado de
Doctor en Ciencias Agropecuarias

PRESENTA:

M. Sc. Jesús del Rosario Ruelas Islas

DIRECTOR DE TESIS:

Dr. Gabriel A. Lugo García

CODIRECTOR DE TESIS:

Dr. Fidel Núñez Ramírez

Juan José Ríos, Ahome, Sinaloa, México, 2020

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DOCTOR EN CIENCIAS AGROPECUARIAS

CONSEJO PARTICULAR

DIRECTOR

DR. GABRIEL A. LUGO GARCÍA

CODIRECTOR

DR. FIDEL NÚÑEZ RAMÍREZ

ASESOR

DR. JORGE FLORES VELÁZQUEZ

ASESOR

DR. FEDERICO VILLARREAL GUERRERO

JUAN JOSÉ RÍOS, AHOME, SINALOA, MÉXICO, 2020

UNIVERSIDAD AUTÓNOMA DE SINALOA
COLEGIO DE CIENCIAS AGROPECUARIAS

FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA
FACULTAD DE AGRONOMÍA CULIACÁN
FACULTAD DE AGRONOMÍA VALLE DEL FUERTE
FACULTAD DE CIENCIAS DEL MAR
FACULTAD DE AGRONOMÍA VALLE DEL CARRIZO

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Jesús del Rosario Ruelas Islas

CORREO ELECTRÓNICO: chuyitaruelas@favf.mx
CURP: RUIJ781209MSLLSS06

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“if I have seen further,

It has been by standing on the shoulders of giants”

Sir Isacc Newton

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I really feel totally blessed with everyone around for supporting me in this important achievement.

"Success is the power of willing, no matter how hard it can be"

"Excellence is not a gift,
but a skill that takes practice,
We do not act rightly
Because we are excellent
In fact we achieve excellence
By acting rightly**"**

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RESUMEN

El cultivo de frijol es uno de los mas importantes en el mundo como fuente principal de proteínas para la problacion consumidora. Recientemente, la productividad de este cultivo es afectada por factores de manejo y otros ocasionados por el clima. A pesar de esto, la información disponible sobre los requerimientos para el buen crecimiento y desarrollo, absorción nutrimental y partición de recursos es todavia limitada en algunas regiones de nuestro país. Dicha información es necesaria para predecir e identificar etapas criticas de desarrollo y con ello desarrollar un programa de manejo de fertilización fosfatada mas eficiente.

En esta investigación se establecieron experimentos de campo para determinar la respuesta del cultivo a diferentes dosis de fertilización fosfatada (0, 25, 50, 100 kg ha^{-1} como P_2O_5) y el rol de *Bacillus subitilis* Q11 en: 1) la dinámica de crecimiento y rendimiento del cultivo, 2) acumulación y distribución de biomasa y, 3) absorción nutrimental a través del tiempo. El experimento se diseñó en parcelas divididas en bloques completes al azar con tres repeticiones.

De acuerdo a los datos obtenidos, las dosis de fósforo influyeron positivamente en los parámetros de altura, cobertura foliar e índice de crecimiento, especialmente las dosis de 50 y 100 kg ha^{-1}. En general, se registró una altura promedio de 62 cm, cobertura foliar de 81 cm e índice de crecimiento de 75 cm, los cuales fueron maximizados al inicio del llenado de vaina (60 dds). Los componentes de rendimiento (peso seco de vainas, peso seco de grano y peso de 100 granos) se incrementaron para las dosis de 50 y 100 kg ha^{-1}. La misma tendencia se observó en el rendimiento final, donde la dosis media (50 kg ha^{-1}) expresó el máximo potencial productivo (3.2 Mg ha^{-1}) en comparación con el resto de las dosis de fósforo. El rendimiento promedio en los tratamientos inoculados y no inoculados fue de 2.9 Mg ha^{-1}. Tambien, se encontraron diferencias significativas en acumulación de biomasa bajo los tratamientos de fósforo e inoculación con *Bacillus subtilis* únicamente en etapas de llenado de vaina y madurez fisiológica. Al final del ciclo, la acumulación total de materia seca fue mayor en los tratamientos inoculados (6.143 Mg ha^{-1}) en comparación con los tratamientos no inoculados (4.854 Mg ha^{-1}). La acumulación promedio en ambos tratamientos fue de 5.498 Mg ha^{-1}. La dosis de 50 kg ha^{-1} produjo la mayor cantidad de biomasa (6.341 Mg ha^{-1}). Se observó que las estructuras vegetativas (hojas y tallos) fueron los órganos de mayor acumulación de MS. Al final del estudio, el promedio para todos los tratamientos en los órganos vegetativos representó aproximadamente el 56% de la MS total acumulada.

La tasa de crecimiento del cultivo se incrementó en función del tiempo y alcanzó el máximo de 181 kg ha^{-1} dia^{-1} en la etapa de llenado de vaina (75 DDS), luego declinó drásticamente hasta madurez fisiológica con 75 kg ha^{-1} dia^{-1} en los tratamientos inoculados con *Bacillus subtilis* Q11. Así mismo, se pudo apreciar que las dosis de 50 y 100 kg de P mostraron una tasa de crecimiento mayor en comparación con dosis menores.

La tasa de crecimiento en los tratamientos sin inocular fue de 146 kg ha^{-1} dia^{-1} en la misma etapa y se redujo a 60 kg ha^{-1} dia^{-1} al final del ciclo.
El periodo de máxima absorción y acumulación de nutrientes en los diferentes tejidos coincidió con el periodo de máxima acumulación de biomasa (llenado de vaina). La absorción total de macronutrientes fue en el orden de K>N>Ca>Mg>P con 284, 269, 221, 70 y 19 kg ha^{1}, mientras que la tasa de absorción en la etapa de máxima demanda fue de 5, 3.8, 3.8, 1.3 y 0.34 kg ha^{-1} dia^{-1}.
La absorción total de micronutrientes fue en el orden de Fe>Mn>Zn>B>Cu con 16, 4.3, 2.8, 1.2 y 0.540 kg ha^{-1} y su tasa de absorción en la misma etapa fue de 0.144, 0.066, 0.032, 0.019 y 0.01 kg ha^{-1} dia^{-1} respectivamente. Independientemente de los tratamientos, la distribución de nutrientes canalizada 100% a órganos vegetativos hasta la etapa de floración, se dividió en etapas posteriores y asignó cerca del 30% a los órganos reproductivos y el 70% a órganos vegetativos.

Palabras clave: Materia seca, absorción de nutrientes, partición, frijol común.

ABSTRACT

Actually, the information regarding requirements for a good growth and development, dry matter accumulation, nutrient uptake and partitioning is scarce in crops such as common bean. Such information is the basis to predict and indentify critical stages of growth with the purpose of developing a more efficient fertilization program.

Series of field experiments were performed to determine the response of the crop to different P fertilizer rates and the inoculation with *Bacillus subitilis* Q11 in growth dynamics, yield potential, gross production and partitioning of dry matter and nutrient uptake as a function of time.

Three investigations are presented in this document. In the first investigation, it was found that growth and development occurred consistently according to previous reports on the same crop. According to data, P rates positively influenced parameters of plant height, canopy closure and growth index. Overall, the average height recorded was 62 cm, canopy closure of 81 cm and growth index of 75 cm which were maximized at the beginning of pod filling (60 DAP) for all treatments.

Yield components increased for rates 50 and 100 kg ha^{-1}. Medium rate (50 kg ha^{-1}) had the better yield (3.2 Mg ha^{-1}) as contrast with the rest of P rates. Average yield was of 2.9 Mg ha^{-1} for inoculated and non-inoculated treatments.

In addition, significant differences were found in dry matter accumulation under P rates and inoculation with *Bacillus subtilis* only in pod filling and physiological maturity. At the end of the season, total dry matter accumulation was higher on inoculated treatments (6.143 Mg ha^{-1}) with respect to non-inoculated treatments (4.854 Mg ha^{-1}).

The average accumulation was that of 5.498 Mg ha^{-1} for both treatments. Rate of 50 kg ha^{-1} produced more biomass (6.341 Mg ha^{-1}) as compared to the rest of the rates.

It was observed that vegetative structures (leaves and stems) showed to be the strongest organs for dry matter accumulation. At the end of the trial, the average for all treatments in vegetative organs represented approximately 56% of total DM accumulated.

Crop growth rate (CGR) increased as a function of time reaching a peak growth at pod filling stage with 181 kg ha^{-1} day^{-1}, to then dramatically decline at physiological maturity with 75 kg ha^{-1} day^{-1} for inoculated treatments. Meanwhile, rates of 50 and 100 kg of P showed a higher growth rate as compared to low P rates. CGR in non-inoculated treatments was that of 146 kg ha^{-1} day^{-1} in the same stage, decreasing its rate at the end of the season with 60 kg ha^{-1} day^{-1}.

The period of maximum nutrient uptake and accumulation in tissues coincided with the period of maximum biomass accumulation. Macronutrient uptake was in the following order: K>N>Ca>Mg>P with 284, 269, 221, 70 and 19 kg ha^{-1} while nutrient flux in the stage of maximum demand was that of 5, 3.8, 3.8, 1.3 and 0.34 kg ha^{-1} day^{-1} in the same order of preference.

Micronutrient uptake was Fe>Mn>Zn>B>Cu with 16, 4.3, 2.8, 1.2 and 0.540 kg ha^{-1} whereas its uptake rate in the same stage was of 0.144, 0.066, 0.032, 0.019 and 0.01 kg ha^{-1} day^{-1} respectively. Finally, nutrient distribution in vegetative organs was of 100% up to flowering independently of treatments applied. In later stages, around 30% was distributed in reproductive organs and 70% in vegetative organs, acting these as the strongest sources in nutrient accumulation during growth and development.

Keywords: Dry matter, nutrient uptake, partition, common bean

CAPITULO 1

1.1. INTRODUCCIÓN

El frijol común *(Phaseolus vulgaris* L.) es una leguminosa muy importante para la nutrición humana a nivel mundial por su contenido de almidón, proteínas, fibra, vitaminas y calorías (Anderson *et al.,* 2004). América Latina es la región de mayor producción y consumo, con cerca del 45% de la producción mundial (Voysest, 2000). En México, el dato más reciente del ciclo 2018 registra una superficie de 83,994 hectáreas y una producción de 158,227 Mg ha^{-1} (SIAP, 2018). Se considera que el rendimiento del cultivo todavía permanece debajo de su rendimiento potencial debido a una serie de factores bióticos (enfermedades) y abióticos (altas temperaturas, fertilización inapropiada y/o déficit hídrico.

La nutrición de las plantas es un proceso fundamental para el crecimiento y potencial productivo, ya sea en el hábitat natural o en el agroecosistema. Sin embargo, la pérdida continua de fertilidad en suelos agrícolas y la baja disponibilidad de elementos presentes ha causado que el uso de fertilizantes sea cada vez alto, con el propósito de incrementar las metas de rendimiento actuales. Por otro lado, el uso irracional de productos químicos causa efectos adversos al suelo y a los cuerpos acuíferos (Zhang, 1996), es por ello que uno de los principales retos que enfrenta la agricultura actual es sostener y/o aumentar la productividad de los cultivos mediante la implementación de programas de fertilización más racionales y propuestas ecológicamente sustentables para promover la fertilidad natural del suelo (Jilani *et al.,* 2007; Yazdani *et al.,* 2009; Yasmin y Bano, 2011).

La baja disponibilidad de fósforo en la mayoría de los suelos lo ha convertido recientemente en el primer factor limitante del crecimiento vegetal y radical (Borch *et al.,* 1999; Kanako *et al.,* 2004), ya que además tiene funciones escenciales en la captación, almacenamiento y transferencia de energía.

El porcentaje de fósforo total en el suelo es aproximadamente de 0.04 a 0.15%, pero únicamente una pequeña fracción puede ser absorbida por las plantas (Lin, 1990). Cuando este nutriente es aplicado en fosfatos inorgánicos (fertilizantes) se transforma rápidamente en formas no disponibles (Ryan *et al.,* 2012) por su gran capacidad de adsorción (arcillas 2:1, 1:1, óxidos e hidróxidos de fierro y aluminio) y precipitación ($CaCO_3$, HCO_3) (Rengel y Marschner, 2005; Hidayatullah *et al.,* 2013; Iqbal *et al.,* 2015). Este material oxidado e hidratado como ortofosfato (H_2PO_4 HPO_4^{2-}) (Banerjee *et al*., 2010) depende fuertemente del pH del suelo y, al igual que el nitrógeno, está sujeto a procesos de mineralización e inmovilización, por lo que su liberación dependerá de la concentración del mismo y de los requerimientos de la población microbiana (Asadi *et al.,* 2005; Remans *et al.,* 2008). Como consecuencia, es recomendable inocular la rizósfera con rizobacterias (PGPR), denominados biofertilizantes (Compant *et al.,* 2010), los cuales favorecen la solubilización de nutrientes y promueven el crecimiento vegetal (Patel *et al.,* 2010, García-López y Delgado*,* 2016).

Hoy en día, la biotecnología aplicada a la agricultura ha contribuido fuertemente al desarrollo de numerosas variedades con alta resistencia a enfermedades, mayor tolerancia a estrés hídrico y salinidad, así como un mejor valor nutricional (Berg, 2009). Desafortunadamente, la interacción benéfica microorganismo-planta es generalmente ignorada en las estrategias de mejoramiento genético, a pesar de que estos microorganismos constituyen una parte fundamental en las funciones de las plantas y el suelo (Morrissey *et al.*, 2004).

De manera general, existen dos posibilidades para influir en el potencial antagónico y/o promotor de crecimiento vegetal: 1) la aplicación de enmiendas orgánicas e inorgánicas (Conn y Lazarovits 2000) y, 2) la aplicación de microorganismos nativos o no nativos en la zona radical, ya sea como aislados o como inoculantes microbianos (Compant *et al.*, 2005; Weller, 2007). Sin embargo, es ampliamente recomendable utilizar inoculantes o aislados nativos de la región porque esa característica aumenta sus posibilidades de adaptación y multiplicación (López-Valenzuela *et al.,* 2015).

En el caso contrario, la funcionalidad de los microorganimos puede ser altamente variable, principalmente por las condiciones del medio edáfico y climático (Hungría *et al.,* 2010).

Actualmente existen cepas comerciales de *Bacillus subtilis* que han probado ser buenos promotores de crecimiento y agentes de bio-control (Yuan *et al.,* 2012) y otros han facilitado la solubilización de compuestos fosfatados (Orhan *et al.,* 2006). Además, se ha comprobado que las bacterias del suelo son capaces de transformar el fósforo y otros nutrientes en formas mineralizadas previniendo la adsorción y/o fijación. Cabe mencionar que todavía existe información ambigua acerca de las formas químicas en las que algunos nutrientes son movilizados y cómo su solubilización se relaciona con las propiedades del suelo (Khan *et al.,* 2014).

Finalmente, Algunos informes señalan que el uso de inoculantes biológicos es una estrategia viable en zonas de mayor producción de cultivos para disminuir la aplicación de fertilizantes y reducir los costos de producción (Compant *et al.,* 2010; Nadeem *et al.,* 2013; Lugtenberg y Kamilova, 2009; Parmar y Dufresne, 2011; Trivedi *et al.,* 2012).

De la misma manera, Reyes *et al*. (2008) consideran que estos inoculantes son un componente del manejo integrado de la nutrición vegetal. Al aplicarse a las semillas, superficies de las plantas o al suelo, los microorganismos inoculados colonizan la rizósfera o el interior del huésped y producen efectos benéficos para el crecimiento y sanidad de la planta.

Con el propósito de desarrollar una técnica eficiente de fertilización fosfatada y determinar la influencia de *Bacillus subtilis* Q11 en la solubilización de nutrientes, se necesita generar información en términos de máxima acumulación de materia seca y nutrientes asociadas al rendimiento optimo del cultivo, tasa de acumulación de materia seca y nutrientes durante todo el ciclo productivo, etapas de desarrollo que coincidan con la máxima acumulación de biomasa, nutrientes y distribución de asimilados en órganos vegetativos y reproductivos.

Actualmente, es limitada la información sobre desarrollo del cultivo de frijol en relación a producción de biomasa y partición de la misma asi como requerimientos nutrimentales y concentración de nutrientes en tejidos. Por consiguiente, es fundamental desarrollar una mejor comprensión de la agronomía del cultivo de frijol que permita la identificación de etapas criticas de desarrollo vinculadas a la acumulación de materia seca y nutrientes asi como el proceso de distribución de los mismos en los distintos órganos de la planta.

Los objetivos de la presente investigación fueron:

1. Determinar la respuesta del cultivo a la aplicación de fósforo y *Bacillus subtilis* en parámetros de crecimiento, rendimiento y sus componentes.
2. Determinar la acumulación y distribución de materia seca en función del tiempo.
3. Determinar la concentración, absorción, distribución y flujo nutrimental en función del tiempo.

1.2. REVISIÓN DE LITERATURA

1.2.1. Fósforo (P) en el suelo.

El suelo es un sistema dinámico y abierto donde ocurren numerosas interacciones físicas, químicas, biológicas y mineralógicas que controlan la disponibilidad de nutrientes. El fósforo (P) no se encuentra en el suelo tan abundantemente como el nitrógeno o potasio. El P total en la superficie varía desde 0.005 a 0.15% (Havlin *et al.,* 2005), distribuído en fracciones lábiles (fácilmente disponible) no lábiles (no disponibles), minerales secundarios y materia orgánica (Hansen *et al.*, 2004; Turner *et al.*, 2006). Por consiguiente, el entendimiento de las relaciones e interacciones de las distintas formas de P en el suelo y los factores que influyen en la su disponibilidad es esencial para el manejo eficiente de la fertilización fosfatada.

1.2.2. Formas del fósforo en el suelo.

Fósforo en solución

La cantidad de $H_2PO_4^-$ y HPO_4^{2-} presente en la solución depende del pH. A pH de 7.2 existen aproximadamente cantidades iguales. Por debajo de umbral de pH predomina la forma de ortofostato primario ($H_2PO_4^-$) y por encima predomina la forma de ortofostato secundario (HPO_4^{2-}). La absorción por las plantas es mayor con $H_2PO_4^-$ (Havlin *et al.,* 2005).

Fósforo inorgánico

A medida que el P orgánico se mineraliza y transforma en P inorgánico, la parte que no es absorbida por las raíces de las plantas se distribuye a otros compartimentos del suelo. Una parte es inmovilizada por los microorganismos y se puede adsorber en superficies de minerales (P lábil).

Otra parte puede precipitarse en fosfatos de Ca insolubles en suelos alcalinos o como fosfatos de Fe/Al en suelos ácidos (Oelkers y Valsami-Jones, 2008).

Esta fracción (P no disponible) es alrededor del 35 al 70% del P total en el suelo (Harrison, 1987). Por ello es ampliamente recomendable la acidificación de la rizósfera, una estrategia eficaz para transformar el fósforo insoluble a formas altamente disponibles, especialmente en suelos neutros-alcalinos (Wang y Nancollas, 2008).

Fósforo orgánico (Po)

El P orgánico representa alrededor del 50% del fósforo total en el suelo (Havlin *et al.,* 2005). La mayoría de los componentes de P orgánico son ésteres de ácido ortofosfórico ($H_2PO_4^-$), tales como fosfatos de inositol, fosfolípidos y ácidos nucleicos (Turner *et al.*, 2002; Condron *et al.*, 2005). Los iones fosfato pueden ser liberados como formas de P organico mediante los procesos de producción de ácidos orgánicos y fosfatasas, así como de la mineralización por los microrganismos del suelo (Turner *et al.*, 2006).

1.2.3. Retención del fósforo en el suelo.

Existen muchas propiedades fisicoquímicas que influyen en la solubilidad de P y las reacciones de adsorción (fijación). En consecuencia, tales propiedades afectan la concentración de P en la solución y su disponibilidad para las plantas. Entre las propiedades más influyentes se encuentran las Arcillas: > adsorción en óxidos e hidróxidos de Fe y Al y arcillas 1:1, pH del suelo: > fuerte adsorción en presencia de Gibsita $Al(OH)_3$, Goetita (α-$Fe^{3+}O(OH)$, Hematita (Fe_2O_3) y precipitación con $CaCO_3$ en suelos calcáreos, temperatura: > adsorción en suelos de climas cálidos y materia orgánica presente en el suelo e inundación.

1.2.4. Solubilización de fósforo.

El P es uno de los nutrientes mayores que limitan actualmente la producción agrícola en muchas partes del mundo. Debido a ello se aplican grandes cantidades de fertilizantes fosfatados, sin superar las deficiencias de este elemento. La rápida transformación del P aumenta el interés de buscar estrategias que permitan aumentar su solubilidad.

Típicamente, la solubilización del P inorgánico es una consecuencia de la acción de ácidos orgánicos de bajo peso molecular, los cuales son sintetizados por algunas bacterias presentes en el suelo (Zaidi *et al.,* 2009). En sentido contrario, la mineralización del P orgánico sucede por la síntesis de una gran variedad de fosfatasas que catalizan el hidrolisis de los esteres fosfóricos (Glick, 2012), de tal manera que la solubilización y mineralización pueden coexistir en la misma cepa bacteriana (Tao *et al.,* 2008).

A pesar de que los microorganismos solubilizadores de nutrientes se encuentran comúnmente en la mayoría de los suelos, su establecimiento y su modo de acción son severamente afectados por factores ambientales, especialmente bajo condiciones de estrés (Ahemad y Khan, 2012a). En ese sentido, existen estudios que ponderan el efecto de fertilizantes orgánicos (compostas) en el comportamiento del P en el suelo, con una mayor solubilidad y absorción por las plantas.

1.2.5. Comportamiento del fertilizante fosfatado en el suelo.

La fertilización fosfatada y su método de aplicación siempre ha sido de suma importancia ya que puede quedar fuertemente fijado como fosfatos de hierro y aluminio en suelos ácidos y fosfatos de calcio en suelos alcalinos y que son totalmente insolubles en agua (Singh y Reddy, 2011). Las características químicas del suelo y la fuente de fertilizante fosfatado son determinantes en la reacción y transformación del fósforo, lo cual influye en su disponibilidad para las plantas. Todos los fertilizantes aplicados al suelo inicialmente aumentan el P en solución, pero este disminuye subsecuentemente debido a la influencia del tipo de suelo (Havlin *et al.,* 2005). Los factores más importantes que pueden influir y/o afectar en la disponibilidad del P en solución es la interacción de N con P, tamaño del granulo y solubilidad, humedad del suelo (> efectividad cuando el suelo está a capacidad de campo) y dosis de aplicación (> solubilidad con dosis bajas).

1.2.6. Rizobacterias promotoras de crecimiento vegetal (PGPR).

Las rizobacterias promotoras de crecimiento (PGPR, por sus siglas en inglés) son un grupo de bacterias de vida libre que habitan en la rizósfera y pueden estimular el crecimiento y la salud vegetal (Kloepper *et al.,* 1991). Estas se caracterizan por ser proficientes en colonizar la superficie radicular y tienen capacidad para sobrevivir, multiplicarse y competir con otros microorganismos, al menos por el tiempo que se necesite para expresar la promoción del crecimiento vegetal o actividades de protección (Kloepper, 1994).

Somers *et al.* (2004) clasificaron a estas bacterias de acuerdo con su actividad funcional como: 1) biofertilizantes (fomentan la disponibilidad de nutrientes), 2) fitoestimuladores (promueven el crecimiento vegetal), 3) rizoremediadores (degradan contaminantes orgánicos) y, 4) bioplaguicidas (controlan plagas y enfermedades mediante la producción de antibióticos y metabolitos antifúngicos.

Ciertamente, se documenta que las rizobacterias son más versátiles en transformar, movilizar y reciclar nutrientes, en comparación con aquellas que están fuera del área radicular (Hayat *et al.,* 2010), por lo tanto, su efecto es crucial en la fertilidad del suelo (Glick, 2012).

Dakora y Phillips (2002) mencionan que la exudación de algunos compuestos químicos modifica las propiedades fisicoquímicas del suelo. De esta manera pueden regular la estructura de la comunidad microbiana en la zona próximal de las raíces. De hecho, otros autores como Kang *et al.* (2014) afirman que algunos de esos exudados actúan como repelentes contra ciertos microorganismos mientras, otros actúan como atrayentes. Los exudados dependen fuertemente de la especie huésped, de su estatus fisiológico, y de los microorganismos hospedantes.

Existe abundante evidencia de que este grupo de microorganismos colonizadores de las raíces promueven el crecimiento vegetal (Dashti *et al.,* 1998; Remans *et al.,* 2008). Esto se hace a través de diferentes mecanismos como la fijación de nitrógeno, la producción de reguladores de crecimiento, el control de enfermedades causadas por patógenos (Vassilev *et al.,* 2006), así como la solubilización del fósforo (Gray y Smith, 2005; Khan *et al.*, 2009) y otros nutrientes. En particular, la solubilización es resultado de que excretan al medio ácidos orgánicos de bajo peso molecular, los cuáles a través de sus grupos hidroxilo y carboxilo quelatan cationes (Chen *et al.*, 2006) y permiten una mayor absorción nutrimental, a la vez que acidifican más la rizósfera (Dey *et al.,* 2004; Aseri *et al.*, 2008). Las PGPR tienen también la capacidad de vivir como endófitos en tallos, hojas, tubérculos y otros órganos (Compant *et al.*, 2005), ya que pueden trascender barreras de la endodermis, corteza y sistema vascular sin causar daño a la planta.

De acuerdo con Whitelaw (2000), los microorganismos solubilizadores de fósforo más potentes son cepas de bacterias de los géneros *Pseudomonas*, *Bacillus*, *Rhizobium* y *Enterobacter;* estas, junto con las cepas de hongos *Penicillium* y *Aspergillus*, son los microorganismos que se han recomendado con más frecuencia como inoculantes (Richardson *et al.,* 2011, Oliveira *et al.,* 2009).

1.2.6.1. *Bacillus spp.*

El género *Bacillus* (Bacillaceae) fue descubierto por Cohn en 1872, y de él se conocen más de 100 especies filogenética y fenotípicamente heterogéneas. En lo general se consideran ubicuas y hoy en día tienen un rol principal en la agricultura sustentable.

Bacillus spp. es una bacteria Gram positiva formadora de endospora. Tiene la característica de que sus bacilos son fermentadores, principalmente de caseína y almidón; generalmente crecen bien en medios que contienen azucares, ácidos orgánicos y alcoholes como únicas fuentes de carbono y amonio como única fuente de nitrógeno. Además, viven dentro de los límites de temperatura de 55 a 80 ºC y su límite inferior de pH es de 2 a 3 (Butt *et al*., 1999).

1.2.6.2. Mecanismos de acción de PGPR.

Regulación del crecimiento vegetal

De acuerdo con Kloepper *et al.* (1980), la promoción de crecimiento vegetal ocurre por la alteración de toda la comunidad microbiana en la rizosfera. Normalmente, esto se consigue mediante la adquisición de nutrientes o la modulación de los niveles de fitohormonas (Glick, 2012). Los mecanismos específicos de estas bacterias para promover el crecimiento vegetal aún no estan del todo claros, sin embargo, hay numerosos estudios que aportan evidencias de este vínculo (Khan *et al.,* 2009; Zaidi *et al.,* 2009).

Se informa que aproximadamente el 80% de los microorganismos aislados de la rizosfera de varios cultivos poseen la capacidad de sintetizar y liberar auxinas (IAA-Acido Indolacetico) en metabolitos secundarios (Glick, 2012), implicadas en el crecimiento o diferenciación. Además, el IAA producido por las PGPR incrementa el área y longitud radical, lo cual significa mayor acceso a los nutrientes (Ahemad y Kibret, 2014). También se asocia con menor rigidez de la pared celular y como resultado se facilita la cantidad de exudados que a su vez proporcionan nutrientes adicionales para soportar el crecimiento de las bacterias en la rizosfera (Glick, 2012).

Ciertas cepas de *Bacillus subtilis* estimulan el crecimiento de las raíces en el cultivo de cacahuate, lo cual a su vez incrementa el contenido de nitrógeno, potasio y boro en la planta. La producción de fitohormonas en aislados de *Bacillus subtilis* sobre *Dioscorea rotundata* L. ejerce un efecto positivo sobre el crecimiento vegetal (Swain *et al.,* 2007).

Producción de ácidos orgánicos

En plantas y microorganismos, los mecanismos de solubilización de fósforo y otros nutrientes implican la producción de ácidos orgánicos. Bajo condiciones naturales o *in vitro,* los ácidos orgánicos tienen acción directa en la acidificación, quelación y reacciones de óxidación-reducción (Pradhan y Sukla, 2009).

El género *Bacillus* se ha revelado con capacidad para solubilizar fósforo, debido a la liberación de metabolitos (ácidos orgánicos) como el ácido gluconico y keto-gluconico (Rodriguez-Hernandez *et al.,* 2006; Rajankar *et al.,* 2007); también participan enzimas fitasas (Idriss *et al.,* 2002).

La acción solubilizadora de los ácidos orgánicos se debe a los grupos hidroxilo y carboxilo que quelatan cationes (Al, Fe, Ca), compiten con el fosfato en sitios de adsorción y disminuyen el pH en suelos alcalinos (Trivedi *et al.,* 2012). La liberación de exudados radicales como ligandos orgánicos pueden también alterar la concentración de fósforo en la solución del suelo (Hinsinger, 2001).

Fijación biológica de nitrógeno

El nitrógeno (N) es el elemento más vital que las plantas requieren para su productividad (Ahemad y Kibret, 2014). La fijación biológica de nitrógeno atmosférico constituye, después de la fotosíntesis, la ruta metabólica más importante (Tejera-Hernández *et al.,* 2011), realizada por microorganismos fijadores de N ampliamente distribuidos en la naturaleza (Raymond *et al.,* 2004). Es, además, una alternativa económica sustentable para disminuir el uso de fertilizantes (Ladha *et al.,* 1997).

Los organismos fijadores de N son categorizados como bacterias simbióticas fijadoras de N_2 (*Rhizobium*), plantas no leguminosas y bacterias no simbióticas, como cianobacterias (*Anabaena-Nostoc*), *Azospirillum, Azotobacter* etc (Bhattacharyya y Jha, 2012).

La simbiosis involucra una interacción compleja entre el simbionte y la planta huésped, que da por resultado la formación de nódulos en donde el *rizobium* coloniza de forma intracelular (Giordano y Hirsch, 2004).

El género *Bacillus* presenta una gran versatilidad y ha demostrado su capacidad de llevar a cabo el proceso de fijación biológica de nitrógeno (Rozycky *et al.,* 1999). Se ha informado que algunas especies como *Bacillus fusiformis*, aislada de maíz, trigo y arroz, presenta una gran actividad nitrogenasa (Park *et al.,* 2010). Otros estudios sugieren que co-inoculaciones con bacterias del género *Azospirillum* en plantas de soya han posibilitan un aumento en la actividad nitrogenasa, lo que estaría relacionado con un aumento en la fijación del nitrógeno atmosférico (Fernández *et al.,* 2007).

Producción de sideróforos

El hierro (Fe^{3+}) es un elemento muy insoluble, esencial en el crecimiento de los organismos bajo condiciones limitantes; algunas bacterias producen sideróforos para secuestrar el hierro de su entorno (Tejera-Hernández *et al.,* 2011). Los sideróforos (portadores o acarreadores de hierro) son moléculas de bajo peso molecular sintetizadas principalmente por bacterias Gram negativas, hongos, levaduras y algunas plantas (fitosideróforos), particularmente gramíneas. Estas moléculas actúan como agentes quelantes específicos de Fe^{3+} (Aguado-Santacruz *et al.,* 2012), cuya característica principal es que poseen una elevada constante de disociación del hierro (Neilands, 1995), incrementada cuando el microorganismo se encuentra en condiciones limitantes (Ratledge y Dover, 2000).

Los complejos de hierro formados en los sideróforos son asimilados eficientemente, tanto por el microorganismo que los producen como por otros que están en su entorno (Winkelmann, 1991). La concentración de estos complejos es en ocasiones lo suficientemente alta para beneficiar la nutrición de las plantas (Crowley *et al.,* 1987). Los exudados radicales, particularmente los compuestos fenólicos, tienen un efecto importante en la proliferación de microorganismos productores de sideróforos en la rizosfera de las plantas (Jin *et al.,* 2010).

Crowley *et al*. (1991) mencionan que el grado de disponibilidad de hierro para una planta dependerá de las afinidades y concentraciones relativas de los quelatores microbianos y vegetales. Los sideróforos forman también complejos estables con metales contaminantes (Al, Cd, Cu, Pb y Zn), incrementando su solubilidad y aliviando el estrés en las plantas (Ahemad y Kibret, 2014).

El hierro de los sideróforos es asimilado por las plantas por medio de mecanismos como la quelación (Fe^{3+}- sideróforo), a través de un receptor específico localizado en la membrana bacteriana. Así, el metal no se encuentre disponible para otros microorganismos que carezcan del sistema de asimilación específico para reconocer dicho complejo (Compant *et al.,* 2005); también es a través de reacciones de intercambio de ligandos (Schmidt, 1999).

1.2.7. Fenologia en cultivos.

El termino fenología se deriva de la palabra griega *phaino* que significa mostrar o aparecer. Puede definirse como el estudio del tiempo en el que recurren los eventos fisiológicos, las correlaciones del tiempo con fuerzas bióticas y abióticas y su interrelación entre fases de una misma especie o diferentes (Lieth, 1974).

Para las plantas en estado natural, el tiempo estacional de tales eventos puede ser crítico para la supervivencia y reproducción, mientras que, para las plantas en agroecosistema, la fenología puede ser crítica para el manejo. Típicamente, después de la siembra o plantación, las plantas forman raíces, hojas y tallos durante la etapa vegetativa, posteriormente, florean, las semillas se forman y se llenan y el cultivo madura en su etapa reproductiva.

En este punto es importante reconocer la distinción entre crecimiento y desarrollo. El crecimiento es el incremento en peso o volumen de toda la planta o de varios de sus órganos. El desarrollo se refiere al paso consecutivo de fases fenológicas y se caracteriza por el orden y velocidad de aparición de órganos vegetativos y reproductivos (Hopkins y Hüner, 2004).

El orden de aparición de los órganos es característico de la especie y casi independiente de las circunstancias. El tiempo y la velocidad de aparición de órganos dependen de las condiciones ambientales como la temperatura, estatus nutrimental, estrés hídrico etc., como consecuencia es altamente variable.

De acuerdo con el concepto básico de tiempo térmico, en la mayoría de los cultivos, los procesos fenológicos y de crecimiento proceden en relación directa con la temperatura acumulada que experimenta el cultivo. Por debajo de una temperatura basal, no se acumula tiempo térmico y el desarrollo cesa. Por encima de una temperatura optima, el desarrollo no se acelera. Se asume que, entre estos dos umbrales de temperatura, la planta debe acumular una cierta cantidad de calor que le permita completar una etapa de desarrollo (Roberts y Summerfield, 1987).

1.2.8. Acumulación de materia seca.

A medida que las plantas crecen y se desarrollan, el carbono asimilado es redistribuido a los tejidos para su uso y almacenamiento. De acuerdo con ello, las estructuras vegetales pueden dividirse en dos grupos (Sonnewald y Willmitzer, 1992): A) sitios de producción de asimilados (“fuente”), que son las porciones fotosintéticamente activas, tales como las hojas maduras que son exportadores netos de foto-asimilados; B) regiones que reciben los asimilados (“receptores”), los cuales son importadores netos del carbono fijado para crecimiento estructural vegetativo y reproductivo, así como también su mantenimiento.

Los tejidos receptores pueden ser clasificados en receptores de utilización o de almacenamiento. Los primeros son activos, altamente metabólicos, en tejidos de rápido crecimiento, como los meristemos y hojas inmaduras. Mientras que los segundos depositan los carbohidratos entrantes en componentes de almacenamiento, tales como almidón, sacarosa, ácidos grasos y proteínas. La interacción fuente-receptor evoca la pregunta a cuál de ambos procesos se le atribuye la causa de aquellos cambios en la distribución de asimilados (Gifford y Evans, 1981).

Durante el curso del crecimiento, la estructura de los órganos varia en el comportamiento de asimilación de fotosintatos. Por ejemplo, una hoja nueva es un importador neto de carbono antes de madurar y convertirse en un exportador neto (Hay y Walker, 1992).

La translocación de asimilados de la fuente al receptor es controlada por procesos que operan tanto en la fuente como en el receptor. Durante el desarrollo de un receptor, su relación con la fuente cambia, de tal manera que el rendimiento económico puede ser atribuido a una serie secuencial de los componentes de rendimiento, siendo cada uno producto de la interacción fuente-receptor ocurrida durante fases sucesivas de desarrollo (Hay y Walker, 1992). La mayoría de los cultivos no son cultivados por una biomasa total sino por órganos de almacenamiento como tubérculos, granos o vainas.

En la historia del mejoramiento genético del potencial de rendimiento en cultivos, el aumento en la asignación de asimilados a órganos cosechables ha sido un paradigma (Gifford *et al.,* 1984). Un ciclo de cultivo corto resulta en baja producción de biomasa, especialmente de hojas las cuales son responsables de la intercepción de luz. Cuando una gran proporción de asimilados son distribuidos a los frutos, las raíces dejan de crecer e incluso pueden morir (De Stitger, 1969), lo cual sería contraproducente. Es deseable una amplia relación de peso seco vegetativo: reproductivo, ya que los órganos vegetativos deben desarrollarse suficientemente para interceptar la radiación y acumular agua y nutrientes. Por lo tanto, el análisis de crecimiento se ha usado extensamente para cuantificar los patrones de producción de materia seca, para elucidar un buen índice de la fisiología intrínseca del crecimiento y desarrollo.

La partición de la materia seca entre los órganos depende del número de órganos por grupo y de la fuerza del órgano receptor o vertedero (Marcelis, 1996). La fuerza del vertedero es la capacidad potencial del mismo para acumular asimilados (Ho, 1988). Esta capacidad potencial puede ser cuantificada por la tasa de crecimiento potencial (bajo condiciones no limitantes).

Por consiguiente, la tasa de crecimiento de una planta es fuertemente influenciada por la temperatura del aire, la cual afecta tanto a la tasa de producción de materia seca como a la distribución hacia los tejidos de las hojas.

En el caso de cultivos hortícolas, el desarrollo de frutos es un evento importante que trae cambios significantes en la carga del vertedero. También, se ha observado que muchas especies acumulan asimilados a expensas del crecimiento vegetativo (Fontes *et al.,* 2005).

En plantas de floración indeterminada, un incremento descontrolado de la demanda de asimilados conduciría a una fructificación lenta, pocos frutos llegarían a madurez. Eso pudiera ser controlable por auto-regulación del número de frutos que pudieran estar creciendo simultáneamente (Bauer *et al.,* 1984). Por lo tanto, no únicamente la producción de materia seca es importante, también su distribución entre los órganos.

1.2.9. Absorción nutrimental.

Las plantas necesitan energía solar, bióxido de carbono y agua para producir materia seca. Sin embargo, los tejidos de las plantas no contienen únicamente oxigeno (O), hidrogeno (H) y carbono (C), sino además otros elementos como nitrógeno, fósforo y azufre en proteínas, así como potasio en aniones orgánicos acompañantes. Muchos elementos se encuentran en pequeñas cantidades como constituyentes (cofactores) de enzimas. Estos elementos deber ser tomados del suelo a través del sistema radical.

En la mayoría de los casos, el suelo en su condición natural no suministra suficiente nivel de nutrientes para satisfacer las necesidades del cultivo. El nivel de rendimiento es entonces determinado por la cantidad del nutriente limitante que puede ser absorbido por las plantas (Marschner, 1995).

La composición mineral de las plantas se caracteriza por su estatus nutrimental e indica el potencial de rendimiento. Las demandas nutrimentales son estimadas de acuerdo con las cantidades de nutrientes removidas durante todo su ciclo en relación con la acumulación de materia seca. La absorción nutrimental es determinada por los requerimientos del cultivo, por el efecto que tiene la rizósfera en la función de las raíces, los procesos de absorción, así como los efectos del ambiente aéreo sobre la tasa de transpiración (Adams y Ho, 1995), también depende de la etapa fenológica de la planta y se incrementa durante floración y formación de fruto.

La dinámica de acumulación de nutrientes puede ser evaluada si toda la planta se divide regularmente durante todo su ciclo (Gifford y Evans, 1981). Correlacionar la absorción de nutrientes con las etapas fisiológicas durante el crecimiento y desarrollo es esencial para proponer una estrategia de manejo de fertilización que maximice la absorción y acumulación, tener mayor eficiencia de la fertilización, así como prevenir estrés nutrimental durante el ciclo del cultivo. La curva de absorción de nutrientes permite determinar la cantidad de fertilizante para evitar posible deficiencia o exceso, así como administrar la cantidad total de nutrientes del cultivo (Marcussi *et al.,* 2004).

1.2.10. El cultivo de frijol.

El frijol común es una especie termófila cultivada desde el trópico hasta las zonas templadas. Sus semillas tienen un alto contenido proteico, aproximadamente del 22%.

México es un importante productor de frijol en el mundo (SIAP, 2018). En Sinaloa es uno de los principales cultivos durante el ciclo otoño-invierno, por la duración de su ciclo productivo, que permite un segundo cultivo. Aquí se siembran genotipos de alto rendimiento, principalmente Azufrado Noroeste, Azufrado Higuera, Azufrado Regional 87, Azufrado Peruano 87, Peruano P80, Negro Sinaloa, Negro Pacífico, Negro Tacaná y Negro Sahuatoba (INIFAP, 2010).

El frijol se desarrolla en altitudes que van de los 0- 2400 msnm, con una precipitación media anual de 1,000-1,500 mm y humedad ambiental moderada (50-60%). La temperatura óptima para su crecimiento está comprendida entre 18 y 25 °C con alta luminosidad. El suelo debe ser preferentemente franco-arcilloso o franco-arenoso, con profundidad suficiente para un sistema radical de 0.6 a 1 m; el cultivo puede tolerar hasta 1 $dS \cdot m^{-1}$ de salinidad y su rango de pH varía de 5.3 a 7.5 (Villalpando, 2001). Su requerimiento hídrico oscila entre 30 y 40 cm, incluidos riego y precipitación, mismos que son suficientes para un rendimiento aceptable (Ríos y Quiroz, 2002).

La temperatura es uno de los factores que más afectan el desarrollo de los cultivos. Incrementos en la temperatura ambiental pueden afectar su tasa de crecimiento (acortamiento del ciclo), limitar su actividad fotosintética y aumentar su respiración (Lawlor, 2005), lo cual afecta drásticamente el potencial productivo. El cultivo de frijol comprende diez fases dentro de todo su ciclo, representadas a continuación.

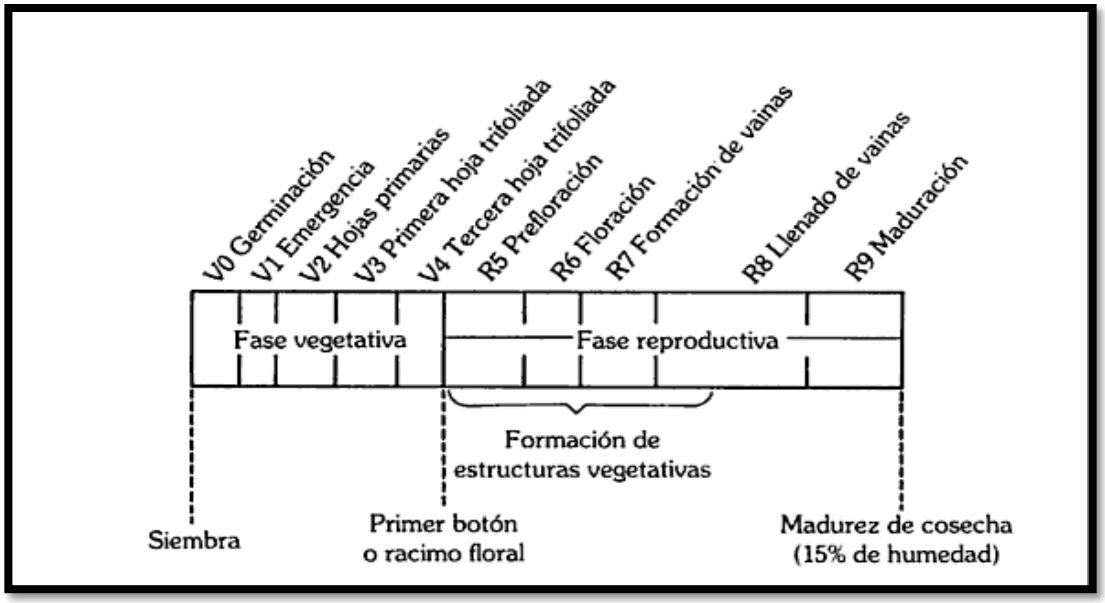

Figura 1. Fases de desarrollo de frijol común (CIAT, 1983).

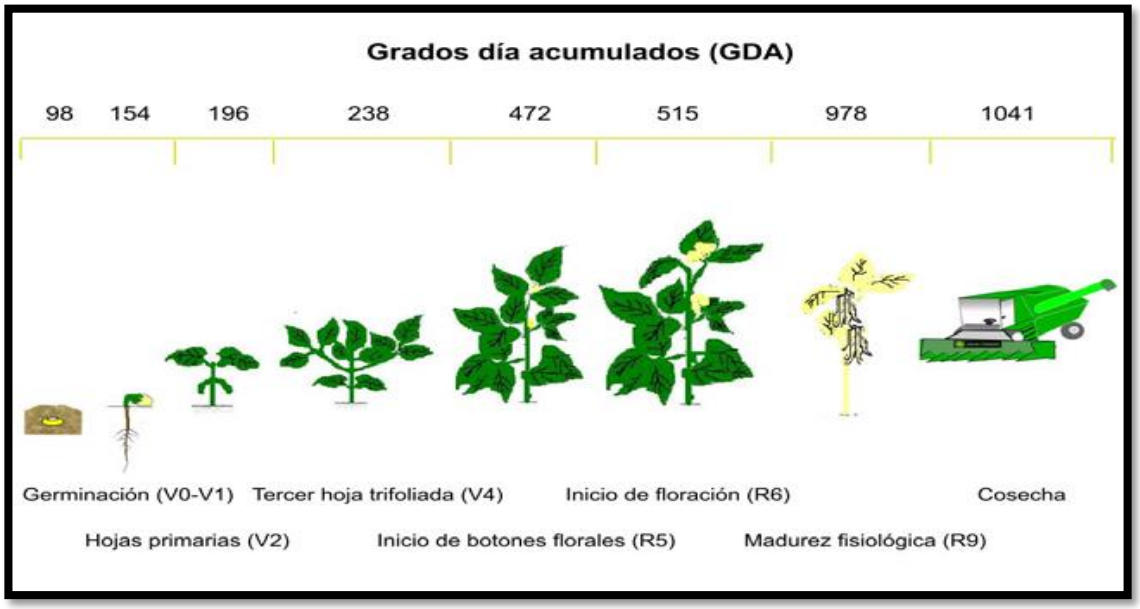

Figura 2. Fenología del cultivo de frijol (°GDA) en Sinaloa (INIFAP, 2010).

1.2.11. Uso de inoculantes en la agricultura.

De acuerdo a informes previos, el uso de microorganismos solubilizadores de nutrientes puede incrementar la productividad de cultivos sin deteriorar el suelo (Reyes- Ramírez *et al.,* 2014). La inoculación combinada de micorriza y rizobacterias proporcionan una mejor absorción de fósforo (Goenadi *et al.*, 2000; Cabello *et al.*, 2005). Son *et al.* (2006) informan que *Pseudomonas* spp. incrementa el número y peso seco de nódulos en soya, así como la disponibilidad de nutrientes, componentes de rendimiento y el rendimiento de grano.

Sharma *et al.* (2007) que las rizobacterias incrementaron la longitud de las plántulas de *Cicer arietinum,* y la co-inoculación de bacterias solubilizadoras de P y rizobacterias redujo la aplicación de fósforo al 50% sin afectar el rendimiento de maíz (Yazdani *et al.*, 2009).

Otras investigaciones mostraron un incremento en rendimiento de caña de azúcar al 12.6% (Sundara *et al*., 2002), mientras que la aplicación individual de las mismas bacterias junto con micorriza logró los máximos rendimientos en peso de grano (Mehrvarz *et al.*, 2008).

Afzal y Bano (2008) encontraron que la inoculación individual o combinada con fertilizantes fosfatados fue mejor al aumentar el rendimiento de trigo 30-40%. Zaidi y Khan (2006) registraron el incremento de 24% en el rendimiento de chícharo con la triple inoculación de *Bradyrhizobium* + *Glomus fasciculatum* + *Bacillus subtilis*. El tratamiento de micorriza + *Pseudomonas putida* incrementó el contenido de clorofila en cebada (Mehrvarz *et al.*, 2008).

Ensayos realizados con diversas rizobacterias en diferentes suelos, regiones climáticas y diversos cultivos, demostraron incrementos de rendimiento del orden del 5 al 30%, así como la disminución del 25 al 50% de la dosis del fertilizante químico (Aguado y Moreno, 2008; Adesemoye *et al*., 2009; García-Crespo *et al*., 2012; Hernández-Escareño *et al*., 2015).

Otros estudios han mostrado que las bacterias del género *Bacillus* spp. promueven incrementos en la emergencia, vigor, peso seco de plántula, volumen de raíces y aumentos del 25 al 30 % del rendimiento en cultivos como papa (*Solanum tuberosum* L.), rábano (*Raphanus sativus* L.), tomate (*Solanum lycopersicum* L.), trigo (*Triticum aestivum* L.), soya (*Glycine max* L.) y chile (*Capsicum annum* L.) (Altamirano *et al*., 2002; Hernández-Castillo *et al*., 2014).

Recientemente, Armenta-Bojórquez *et al*. (2015) evaluaron aislados de *Rhizobium* y *Bacillus* nativos como biofertilizantes en invernadero y campo en el cultivo de frijol. La combinación de ambos condujo a mayor rendimiento de grano, producción de biomasa, número de nódulos por planta y peso seco de nódulos en comparación con el control.

Los acontecimientos de nodulación escasa y respuestas variables a la inoculación son principalmente atribuidos a las características intrínsecas de la planta hospedera (Michiels *et al.,* 1998), o la gran sensibilidad a factores limitantes entre los que se encuentran las altas dosis de fertilizante nitrogenado, altas temperaturas y la capacidad de secado del suelo (Giller y Cadisch, 1995).

La evaluación de los beneficios de la fertilización orgánica y su efecto en la disponibilidad de nutrientes es complicada, ya que la transformación de compuestos orgánicos y la liberación de nutrientes son procesos complejos. Dependen fuertemente de la estabilidad de las sustancias orgánicas (Gutser *et al.,* 2005), las condiciones climáticas (Dorado *et al.,* 2003), las propiedades físicas del suelo (Huffman *et al.,* 1996), el tipo de cultivo (Van den Bossche *et al.,* 2005) y la interacción con fertilizantes minerales (Kaur *et al.,* 2005) ya que la relación planta–suelo–microorganismo-ambiente son variadas, por lo que una comprensión completa de todo el conjunto es poco probable.

CAPITULO 2

Effect of Phosphorus Rates and *Bacillus subtilis* on Growth Components and Yield of Common Bean.

Jesús Del Rosario Ruelas Islas[1], Gabriel A. Lugo García[1], Fidel Núñez-Ramírez[2*], Jorge Flores Velázquez[3], Federico Villarreal Guerrero[4]

[1]School of Agriculture, Autonomous University of Sinaloa, Sinaloa, México.
[2]Agricultural Science Institute. Autonomous University of Baja California. Baja Califronia, México.
[3]Mexican Institute of Water Technology. Morelos, México.
[4]School of Animal Sciences and Ecology. Autonomous University of Chihuahua, Chihuahua, México.

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an important legume that constitutes part of the daily feeding in countries like Mexico. In Northern Sinaloa, yield of this crop remains low due to management and environmental factors that affect yield potential. Therefore, a field experiment was conducted in this region in order to investigate the response of common bean to different rates of phosphorus fertilizer and the role of *Bacillus subtilis* strain Q11 on overall growth and yield potential. The experiment was conducted as a split plot in a randomized complete block design with three replicates. The main plot consisted of the P rates whereas the subplots consisted of the inoculation of *Bacillus subtilis* Q11 and the control. Each main plot had a dimension of 64 m^2 while the subplots were 32 m^2 within the main plot. Variables such as height, canopy closure, growth index, yield and its components were evaluated. Based on the results obtained, P rates significantly influenced parameters of growth as a function of time, growth components of shoot and roots and yield. Rate of 50 kg ha^{-1} efficiently enhanced the maximum growth and maximizing yield potential as compared to the highest (100 kg P ha^{-1}) under the environment and soil conditions in which the trial was evaluated.

Keywords: *Common bean, Fertilizer rates, yield potential*

2.1 INTRODUCTION

Legume crops have become an important source for human nutrition worldwide after cereals (Anderson *et al*., 2004). In Mexico, common bean ranks the fourth position due to land surface established. Recently, one of the most concerning factors that cause declines in yield is related to water deficit and improper fertilization and/or irrigation.

The average yield of this crop under irrigated agriculture is between a range of 1.5 and 2.0 Mg ha^{-1}. Under rain-fed agriculture, the range is of 0.4 through 0.6 Mg ha^{-1} (SIAP, 2018). Despite the fact that common bean has the ability to fix atmospheric nitrogen regardless of soil fertility and mineral nutrition, it requires high nitrogen inputs for good growth, development and yield (Whiters *et al.,* 2014; Escalante-Estrada *et al.,* 2013).

Diverse studies have shown that increases in yield are due to higher N rates which ultimately lead to greater number of grains and pods (Fageria and Santos, 2008; Escalante-Estrada *et al.,* 2013). Besides, they also mention that decreases in N accumulation in grains offers less protein content (Escalante and Rodríguez, 2010). Other such as Eckert *et al.* (2009) stated that an acceptable yield could be achieved through an appropriate combination of the genotype, environment application timing, water availability and agronomic practices.

In that respect, García-Esteva *et al.* (2003) mentioned that yield of common bean (var Cacahuate-72) under rain-fed agriculture reached a yield potential of 25.35 g m^{-2} with a high plant density (8900 plants) and a fertilization rate of 40-40-10 as contrast with previous works where they employed a higher plant density and higher N fertilization rate.

Most of the reports only relate N application without considering other major nutrients such as P. Despite its low availability, it becomes necessary an understanding of the behavior of P fertilizers in soils including those properties that can influence or affect the growth and/or yield as a crucial process in fertilization management programs.

The majority of P is present as low available forms due to reactions that occurs in soils (Ryan *et al.,* 2012). One of them is precipitation in calcareous soils with high pH and low organic matter content (Amanullah and Khan, 2015). Therefore, some researchers suggest more efficient fertilization practices as well as the use of microorganisms to improve P availability, crop productivity and soil sustainability (Patel *et al.,* 2010; Wang *et al.,* 2011; Heppell *et al.,* 2015; Simpson *et al.,* 2011; Whiters *et al.,* 2014).

According to it, the most studied plant growth promoting rhizobacteria (PGPR) are *Bacillus, Pseudomonas, Rhizobium* and *Enterobacter* (Araújo, 2008). However, their action could be affected by the sorption capacity of the growing medium and the solubility of nutrients in it (Rodríguez *et al.,* 2006. Their mode of action occurs indirectly through reduction of plant pathogens agents (Yuan *et al.,* 2012; De Santiago *et al.,* 2011) and directly by production of organic acids and acid phosphatases which solubilize nutrients especially phosphates (Khan *et al.,* 2013; Mena and Portugal, 2007; Sarwar *et al.,* 2016).

Actually, the information concerning accurate rates of P as well as the use of the inoculant *Bacillus subtilis* Q11 as nutrient solubilizing agent of common bean in northern Sinaloa is scarce. So far, the reports of this strain have shown to have an antagonistic effect against *Sclerotium rolfsii* and other soil borne pathogens (Hernández *et al.,* 2016). Therefore, the aim of this research was to evaluate the response of the crop to P fertilizer rates and inoculation with *Bacillus subtilis* Q11 on parameters of growth and yield of common bean.

2.2 MATERIALS AND METHODS

The field experiment was conducted during the winter season at Valle del Fuerte located northern Sinaloa, Mexico (25° 45´49” N, 108° 51´41” W). The weather is hot during summer (maximum temperatures up to 40ºC) and moderate cold during winter (12-30ºC). The annual precipitation ranges between 700-750 mm and its distribution is highly variable. Soil tilling techniques were realized following the guideline provided by the Forestry, Agriculture and Livestock National Research Institute (SIAP, 2013). Soil physical and chemical properties were determined before fertilizer application (Table 1).

Table 1. **Physical and chemical properties of soil.**

Soil parameter	**(Meq L^{-1})**	
Saturation (%)	50	
pH (1:2 ratio)	7.6alkaline	
ECe 25 ºC (dS m^{-1})	0. 69	
Carbonates	TRACE	
Bicarbonates	1.60	
Calcium	3.00	
Magnesium	1.50	
Sodium	1.58	
Potasium (PPM)	32	
Chlorine	1.80	
Sulfates (PPM)	55	
Sodium absorption ratio	1.05	
MACRONUTRIENTS	mg L^{-1}	
Nitrogen	145	High
phosphorus	32	Medium
Potasium	760	High
Calcium	3950	High
Magnesium	590	High
Sodium	180	Low
MICRO-NUTRIENTS	mg L^{-1}	
Iron	6.40	Low
Manganese	11.96	High
Copper	1.82	Low
Zinc	1.48	Low

Nitrogen (N) was applied at a rate of 150 kg ha^{-1}. Total N and P were applied pre-plant using highly soluble Blaukorn® Classic (12-8-16). The variety planted was Azufrado Higuera. The experiment was carried out as a split plot in a randomized complete block design with three replicates. The main plot consisted of four P rates (0, 25, 50 and 100 kg ha^{-1}) whereas the subplots consisted of the inoculation of *Bacillus subtilis* Q11 and the control. Each main plot had a dimension of 64 m^2 while the subplots were 32 m^2 (4 rows wide arranged linearly head to tail of the field) within the main plot.

The planting was done on moistened soil and pest management was successfully controlled throughout the season. Irrigation scheduling was managed by the water balance method with the use of IrriModel software (Sifuentes *et al.*, 2012) which estimates the depletion levels according to the methodology proposed by Ojeda *et al*., (2004). Irrigation targets were set as a 50% depletion of plant available water.

Measurements

Plant growth measurements were done from five plants of each treatment. Such parameters were plant height and canopy closure. Those measurements were realized every other week and the following stages of growth were identified in relation to plant data collection: third trifoliate leaf, flowering, pod filling, and physiological maturity.

Growth stages were recorded when more than 50% of the plants in a representative area developed the following characteristics. Stage V4 when third trifoliate leaf was totally expanded. In this stage, some structures such as stem, branches and other trifoliate leaves were clearly observed. Flowering when most of the blossoms were present, pod filling when all vines had reached a size and number of grains, physiological maturity when the plant itself had reached senescence as well all pods and grains inside had gained sufficient weight.

Growth index was realized following the methodology proposed by Atland *et al.* (2003) and Torres *et al.* (2017) which is formed by the variables of height and canopy closure as described in the following formula:

$$[(H + Cc + Cc)/3]$$

Additionally, at the beginning of pod filling stage, other crop growth parameters such as root length, stem diameter, stem dry weight, root fresh and dry weight were also measured. At the end of experiment, yield and its components were determined from two central rows. Data of growth were subject to an appropriate analysis of variance. Treatment mean differences were separated using Fishers' least significant difference (LSD) test at P≤ 0.05 (Minitab, 2017).

2.3 RESULTS AND DISCUSSION

Plant height, Canopy Closure and Growth index

In this study, the applied P rates by themselves revealed significant differences (P<0.05) on plant height as a function of time (days after planting-DAP). The maximum heights were achieved in a period from 60 to 75 DAP (Table 2) which corresponded to the most intense stage of growth (pod filling) and decreased at the end of the season.

These results agree with data reported by Blanco *et al.* (1995) who obtained values from 45 up to 51 cm in the variety RV81 grown along with coffee (*Cofeen Arabica* L.) plantings. However, this differs from Meseret and Amin (2014) who found a non-significant response of plant height to different P rates in a humid environment. They indicated that part of the P applied had been taken up by plants, adsorbed on mineral surfaces or precipitated as secondary P minerals.

Canopy closure was significantly enhanced by P rates as a function of time ($P<0.05$). No differences ($P>0.05$) were found between treatments at 15 and 45 DAP. However, the crop had a rapid closure on treatments that received 50 and 100 kg ha^{-1}, reaching its maximum canopy at pod filling stage. As the crop reached maturity, it was also observed that plants on the same rates had delayed senescence as compared to the lower rates that had been mostly defoliated (Table 2).

Regarding the growth index pattern, the overall phosphorus rates significantly influenced this parameter as a function of time ($P<0.05$). It was observed an initial period of slow growth at third trifoliate leaf (25 DAP) increasing rates at flowering (45 DAP) reaching a plateau at pod filling stage (from 60 to 75 DAP) and then declining as the crop progressed to maturity (100 DAP).

In addition, it was found series of interaction effects between P rates and the bacteria on plant height (30, 60 DAP), canopy closure (30, 75, 90, 105 DAP) and growth index (30, 75, 90 DAP) related in all cases from flowering through pod filling stage, in which the crop exhibited the most active stages for growth and development (Figure 3).

Overall, these data showed that rate 50 kg ha^{-1} performed better on those parameters over the course of the season. The slow response of the crop to the highest P rate (100 kg ha^{-1}) could have been possibly attributed to changes in soil solution pH after fertilizer application (Sánchez, 2007) or low efficiency of plant uptake related to NH_4^+ toxicity affecting plant growth (Havlin *et al.,* 2005). Another cause could be that N_2 fixation was limited as observed with poor root nodulation (data not shown). Some other factors could have been related to interactions between the different phases in the porous media affecting the availability of nutrients for plants, or that part of phosphorus was strongly retained in the area of contact (Sánchez, 2007; Havlin *et al.,* 2005).

Inoculation with *B. subtilis* showed slightly influence on parameters of growth only at specific time intervals (active stages) when compared to non-inoculated treatments. A possible cause could have been attributed that some bacteria are preferred for certain crops (Bashan, 1998), some others are more or less efficient in competing with other microorganisms (Khalid *et al*., 2004), while others are affected by soil physical properties and conditions in the rhizosphere (Kannan *et al*., 2005; Lalfakzual *et al*., 2008). These results are different from those reported by Joe and Sivakumaar (2009) who found that inoculating with PGPR positively enhanced growth of sunflower crop. Others as Sarwar *et al.* (2016) reported a better performance of P fertilizers and *Pseudomonas* isolates rather than *Bacillus.*

Table 2. **Plant height, canopy closure and growth index as affected by phosphorus rates and *Bacillus subtilis*.**

Plant height	**Days after planting**						
P rates	15	30	45	60	75	90	105
0 kg ha^{-1}	12.50 b	36.44 b	38.33 b	54.28 c	50.84 b	36.95 b	31.00 c
25 kg ha^{-1}	14.00 ab	37.27 ab	38.27 b	61.72 b	54.33 a	40.28 b	30.00 c
50 kg ha^{-1}	15.27 a	38.00 ab	42.55 a	65.17 ab	56.11 a	51.39 a	40.83 b
100 kg ha^{-1}	15.05 a	40.39 a	40.89 a	68.44 a	55.27 a	55.00 a	45.00 a
OSL	0.007	0.02	0.004	<0.0001	0.001	<0.0001	<0.0001
inoculation	14.22 a	38.2 a	40.33 a	62.67 a	54.44 a	46.67 a	37.3 a
No inoculation	14.19 a	37.2 a	39.69 a	62.14 a	53.83 a	45.14 a	36.1 a
OSL	0.9	0.7	0.4	0.6	0.4	0.2	0.2
P*I OSL	0.3	0.005	0.9	0.009	0.09	0.2	0.05
Canopy closure							
0 kg ha^{-1}	21.11 a	54.39 bc	66.89 a	80.22 b	53.07 c	43.61 c	34.73 c
25 kg ha^{-1}	20.66 a	52.00 c	66.11 a	80.22 b	56.66 bc	42.50 c	33.22 c
50 kg ha^{-1}	21.77 a	57.27 a	66.11 a	84.28 a	62.778 a	50.56 b	41.11 b
100 kg ha^{-1}	21.05 a	56.72 ab	70.83 a	82.28 ab	58.60 b	55.83 a	45.83 a
OSL	0.3	<0.001	0.3	0.01	<0.0001	<0.0001	<0.0001
Inoculation	21.47 a	55.5 a	67.64 a	82.63 a	57.8 a	48.20 a	38.75 a
No inoculation	20.83 a	54.6 a	67.33 a	80.86 a	57.7 a	48.05 a	38.70 a
OSL	0.1	0.1	0.8	0.1	0.9	0.9	0.9
P*I OSL	0.1	0.002	0.2	0.9	0.001	0.02	0.04
Growth index							
0 kg ha^{-1}	18.24 b	48.40 b	57.37 a	74.28 b	52.31c	41.39 c	33.48 c
25 kg ha^{-1}	18.44 ab	47.09 b	56.83 a	74.06 b	55.889 b	41.76 c	32.15 c
50 kg ha^{-1}	19.61 a	50.85 a	58.26 a	75.20 ab	60.556 a	50.83 b	41.02 b
100 kg ha^{-1}	19.05 ab	51.27 a	60.852 a	77.66 a	57.500 b	55.55 a	45.56 a
OSL	0.08	<0.001	0.2	0.09	<0.001	<0.001	<0.001
Inoculation	19.04 a	49.66 a	58.54 a	75.80 a	56.67 a	47.69 a	38.26 a
No inoculation	18.63 a	49.14 a	58.12 a	74.79 a	56.46 a	47.08 a	37.84 a
OSL	0.2	0.3	0.7	0.3	0.7	0.5	0.7
P*I OSL	0.2	0.001	0.2	0.4	0.001	0.06	0.05

- Means followed by different letter are statistically different (Fisher Mean separation Test LSD ($P \leq 0.05$),
- Observed significance level (OSL Pr> 0.05).

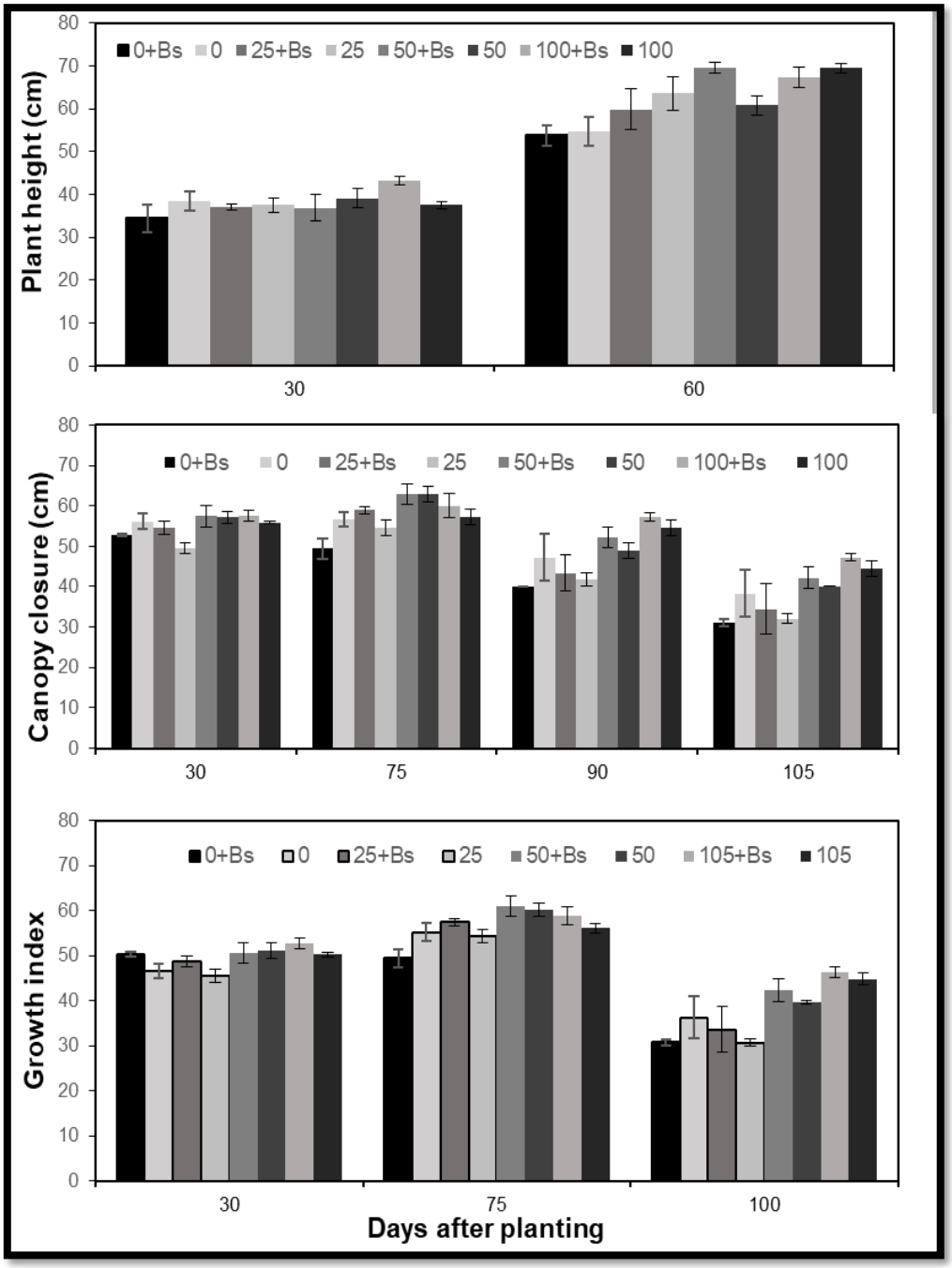

Figure 3. Interaction effects of plant height, canopy closure and growth index as affected by phosphorus rates and *Bacillus subtilis.*

Growth components of shoots and roots

Table 3, shows the plant growth parameters measured at pod filling stage and its effect by P rates. Application of different P rates resulted in significant differences for almost all parameters except for root length ($P>0.05$). The stem diameter, root fresh and dry weight, as well as stem dry weight were in the order of 5.0 mm plant^{-1}, 15, 4.6 and 30 g plant^{-1} approximately.

In all cases, the rate of 50 kg ha^{-1} showed the highest values ($P<0.05$) whereas the rate of 100 kg ha^{-1} slightly decreased by 25, 18 and 13.5% for the last three parameters measured. These results are higher as compared with those presented by Araújo *et al.* (2000) who attained lower values of shoot dry weight (from 5.34 to 7.10 g plant^{-1}) and root dry weight (from 0.41 to 0.51 g plant^{-1}).

Kouas *et al.* (2009) reported that limited P supply decreased significantly root dry weight of two common bean cultivars and the decrease ranged between 58 and 70% as compared to the control. Similarly, Elkoca *et al.* (2010) reported that bacterial inoculations as single, dual and triple with *Rhizobium*, *N_2-fixing Bacillus subtilis* (OSU-142) and P solubilizing *Bacillus megaterium* (M-3) significantly increased shoot dry weight. While, Zafar *et al.* (2011) demonstrated that bacterial inoculations enhanced shoot dry weight by 19.7 to 54.3% over control.

At the same time, Abbasi *et al*. (2011) reported that application of PGPR significantly increased wheat plant height, shoot fresh weight and shoot dry weight by 25, 45 and 86%, respectively, while increase in root length, root fresh and dry weight was 27, 102 and 76%, respectively, over the non-inoculated treatments. Some other works presented by Remans *et al.* (2007); Yadegari *et al.* (2010) and Stajkovic *et al.* (2011) reported similar findings for improved bean growth parameters.

Table 3. **Effect of phosphorus rates on growth components at pod filling stage.**

Phosphorus rate (P)	Root length (cm)	Stem width (mm plant^{-1})	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	Stem dry weight (g plant^{-1})
0 kg ha^{-1}	16.40 a	4.26 b	13.80 b	3.93 c	24.33 c
25 kg ha^{-1}	18.1 a	5.23 a	13.90 b	5.06 ab	27.33 c
50 kg ha^{-1}	19.6 a	5.05 a	19.26 a	5.33 a	37.00 a
100 kg ha^{-1}	18.2 a	5.51 a	14.35 b	4.38 bc	32.00 b
OSL	0.5	0.01	0.002	0.01	<0.0001
Inoculation	18.1 a	5.2 a	15.7 a	4.8 a	32.3 a
No inoculation	17.8 a	4.9 a	15.0 a	4.5 a	28.0 b
OSL	0.8	0.2	0.5	0.6	<0.0001
P*I	0.07	0.5	0.2	0.2	0.06

- Means followed by different letter are statistically different (Fisher Mean separation Test LSD P ≤ 0.05), Observed significance level (OSL Pr> F).

Yield and its components

Table 4 shows the response of pod dry weight, shelling percent, grain weight, and weight of 100 grains by effect of four P rates. The application of 50 kg ha^{-1} was the optimal rate that significantly (P<0.05) increased pod dry weight, grain dry weight and 100 grain weight which were responsible of maximizing yield.

The highest rate (100 kg ha^{-1}) decreased by 19 %, 5% and 7.5% over the medium rate (50 kg ha^{-1}) for each parameter. No statistical differences were found in shelling percentage (ratio of grain dry weight and pod dry weight).

The highest yield (3.23 Mg ha^{-1}) was achieved on the medium P rate (50 kg P ha^{-1}) but at the same time statistically equal to the highest rate (100 kg P ha^{-1}) (Fig 4). This result agrees with Araújo *et al.* (2000) who reported that this crop exhibited a generalized response to P fertilization. In addition, that many combined experiments needed a P rate of 47 kg ha^{-1} to reach the maximal economic yield.

Rodríguez-Rodríguez *et al.* (2014) found that planting dates from the last week of September (2940 kg ha^{-1}) to first week of October (2850 kg ha^{-1}) attained the maximum yields for common bean in Tabasco. Furthermore, Zafar *et al.* (2011) reported that a combined application of P sources and PGPR further increased seed yield ranging from 27 to 115% over the control treatments. They attributed this positive response to an increase in number of pods per plant and individual seed weight due to P nutrition. Their reports also coincide with Chiezey and Odunze, (2009) who reported that pod yield and 100-seed weight enhanced with P application. All these findings agree with our results in that P rates promoted positively the parameters of growth and yield.

Table 4. Effect of phosphorus rates and *Bacillus subtilis* on yield components of common bean.

Phosphorus rates (P)	Pod dry weight (g)	Shelling (%)	Grain dry weight (g)	Dry weight (100 grains)
0 kg ha^{-1}	220.3 b	77	161.6 c	37.67 c
25 kg ha^{-1}	231.2 b	74	168.0 bc	42.06 b
50 kg ha^{-1}	289.0 a	79	195.4 a	46.58 a
100 kg ha^{-1}	234.6 b	70	185.7 ab	43.02 ab
OSL	0.02	0.7	0.02	0.001
No inoculation	222 a	69 a	174.5 a	41 b
Inoculation	235.5 a	84 a	181 a	44 a
OSL	0.1	0.4	0.4	0.002
P*I	0.7	0.1	0.7	0.01

- Means followed by different letter are statistically different (Fisher Mean separation Test (LSD $P \leq 0.05$).

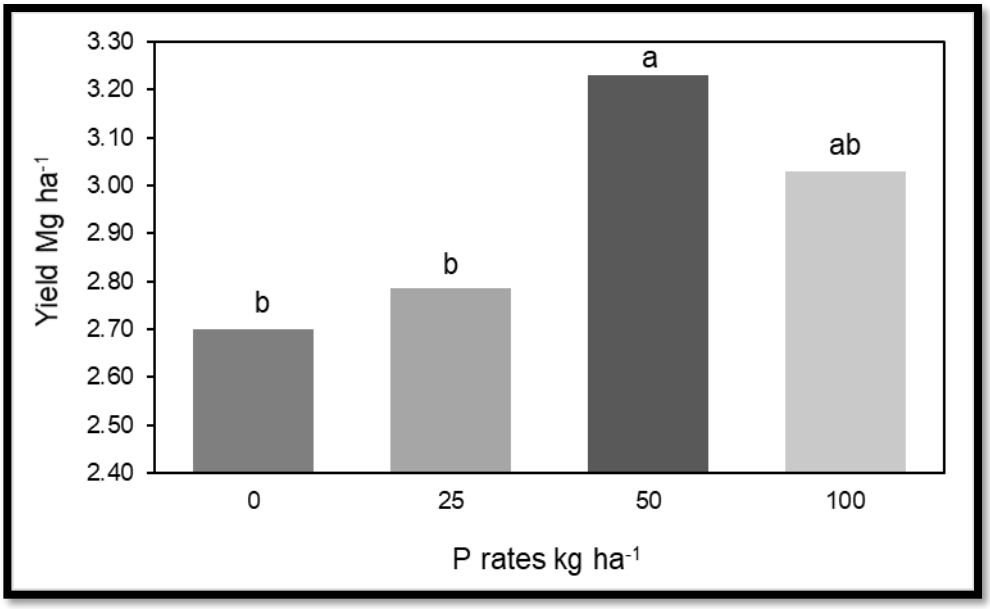

Figure 4. Yield of common bean.

2.4 CONCLUSIONS

Application of 50 kg ha^{-1} P rates positively influenced almost all parameters of growth, yield components and overall yield potential as compared to a higher rate (100 kg ha^{-1}).

The peak period of maximum growth (height-canopy-growth index) started at 60 DAP and remained relatively constant up to 75 DAP (end of pod filling stage).

The maximum yield attained was 3.2 Mg ha^{-1} on medium rate (50 kg ha^{-1} P) but at the same time statistically equal to the highest rate (100 kg P ha^{-1}).

The highest rate (100 kg P ha^{-1}) only showed delayed senescence and defoliation.

Inoculation with *Bacillus subtilis* showed no influence as growth promoter, response mainly attributed to interactions between the soil and bacteria.

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CAPITULO 3

Effect of Phosphorus Rates and *Bacillus subtilis* on Dry matter accumulation and partitioning of Common Bean.

Jesús Del Rosario Ruelas Islas[1], Gabriel A. Lugo García, Fidel Núñez-Ramírez[2*], Jorge Flores Velázquez[3], Federico Villarreal Guerrero[4]

[1]School of Agriculture, Autonomous University of Sinaloa, Sinaloa, México.
[2]Agricultural Science Institute. Autonomous University of Baja California, Baja California, Mexico.
[3]Mexican Institute of Water Technology, Morelos, México.
[4]School of Animal Sciences and Ecology. Autonomous University of Chihuahua, Chihuahua, México.

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an excellent source of proteins, fiber and folic acid, which constitutes part the feeding in many developing countries. Up to now, little is known about the behavior of dry matter (DM) accumulation and distribution as tool for quantifying the effect on yield potential. Therefore, a field experiment was conducted in Northern Sinaloa to investigate the response of common bean to different rates of phosphorus fertilizer and the role of *Bacillus subtilis* strain Q11 on DM accumulation and partitioning. The experiment was set as a split plot in a randomized complete block design with three replicates. The main plot consisted of the P rates whereas the subplots consisted of the inoculation of *Bacillus subtilis* Q11 and non-inoculated treatments. Each main plot had a dimension of 64 m^2 while the subplots were 32 m^2 within the main plot. Based on the results, P rates significantly influenced DM accumulation and distribution pattern as a function of time. Seasonal DM accumulation was higher on rates of 50 and 100 kg P and on inoculated treatments. The partition process was 56% of total DM in vegetative organs. The most intense periods for the evaluated parameters were pod filling and physiological maturity as indicated by their crop growth rate.

Keywords: *Crop growth rate, Fertilizer rates, Dry matter accumulation.*

3.1 INTRODUCTION

Dry matter (DM) accumulation is employed as an important indicator to characterize intrinsic growth. Higher seed yields over the past half century can simply be explained as the product of increased total plant DM, greater harvest index (HI), or both through better management practices like irrigation, physiological changes like a longer reproductive growth period (Rowntree *et al.,* 2014) and genetic improvement through breeding (Rincker *et al.,* 2014).

The presence of plant organs with a net demand for assimilates can strongly influence the pattern of production and distribution of dry matter. The metabolism of source (sites of assimilate production) and sink (regions that draw assimilate) are bounded together since the information about availability of assimilates in each organ is perceived and used to orchestrate gene expression.

This coordination is necessary to avoid ample fluctuations and imbalances between the sources and sinks (Foyer and Paul, 2001). The partitioning of dry matter among various groups of organs depends on the number of organs per group and on their sink strength, (potential capacity of a sink to accumulate assimilates).

This potential capacity can be quantified by the potential growth rate of the sink, (i.e. the growth rate under conditions of non-limiting assimilate supply) (Marcelis, 1996). In addition to environmental conditions, crop management and genotype (Nustez-López *et al.,* 2009; Jerez-Mompie *et al.,* 2015).

Koester *et al.* (2014) found that HI increased by 0.0022% each year up to 2007 for commercially available bean varieties. Greater DM partitioning to the seed (>HI) may also affect partitioning to other plant parts. Bender *et al.* (2015) reported an average total DM accumulation of 9775 kg ha^{-1} for a yield of 3480 kg ha^{-1}, resulting in a HI of 37% within a modern production environment, with a peak accumulation rate of ~162 kg ha^{-1} day^{-1}.

In the case of common bean, mobilization of assimilates to the grain is very intense at the end of the season. This organ concentrates the most significant portion of dry weight of plants (Coelho and Benedito, 2008). One efficient measure of dry matter distribution is the harvest index (ratio of yield relative to total weight). It also indicates the percent of aboveground biomass that the crop utilizes for grain production regarding the rest of plant structures (Araújo and Teixeira, 2012).

It is mentioned that an appropriate balance between the source-sink is of great importance to optimize production and quality. Nevertheless, this process is not usually optimum in most crops leading to the following situations: A short growing period, resulting in low vegetative biomass, especially of leaves responsible for light interception, leads inevitable to a poor crop. On the other hand, when too large proportion of assimilates is distributed to the fruits, roots stop growing or even die (De Stitger, 1969) which can be counterproductive. Therefore, a large reproductive: vegetative dry weight ratio is desirable, yet vegetative organs must develop sufficiently to intercept light and accumulate water and nutrients which can be attained only by maximizing both processes of photosynthesis and respiration (crop grown under non-limiting conditions) (Verdoot *et al.,* 2004; Amanullah and Asim, 2011).

In that sense, previous studies by García-Esteva *et al.* (2003) showed significant differences in total biomass (1390 g m^{-2}) and yield (692 g m^{-2}) of common bean grown under hydroponics as compared with 1116 g m^{-2} and 605 g $m^{-}2$, for the same crop grown in soil. They also found that most of the allocation of dry matter in different organs was greater in the hydroponic system than in soil.

On the other hand, Escalante-Estrada *et al.* (2014) found that genotype species affect the pattern of dry matter accumulation and partitioning with no influence of N fertilization. The highest accumulation was in the order of 552 g m^{-2} while the lowest was of 437 g m^{-2} for different cultivars.

Finally, information dealing with the dynamics of dry matter production and partitioning on common bean is scarce. This is why plant growth analyses have been extensively used for quantifying patterns of dry matter production in many crops. Therefore, the objectives of this study were to determine the maximum dry matter accumulation associated with optimum yield, the rate of dry matter accumulation throughout the life span of the plant, growth stages of maximum dry matter accumulation, and the distribution of assimilates within the plant parts.

3.2 MATERIALS AND METHODS

The field experiment was conducted during the winter season at Valle del Fuerte located northern Sinaloa, Mexico (25° 45´49” N, 108° 51´41” W). The weather is hot during summer (maximum temperatures up to 40°C) and moderate cold during winter (12-30°C). Soil tilling techniques were realized following the guideline provided by the Forestry, Agriculture and Livestock National Research Institute (SIAP, 2016).

Soil physical and chemical properties were determined before fertilizer application (Table 1). Nitrogen (N) was applied at a rate of 150 kg ha^{-1}. Total N and P were applied pre-plant using highly soluble Blaukorn® Classic (12-8-16). The variety planted was Azufrado Higuera.

The experiment was carried out as a split plot in a randomized complete block design with three replicates. The main plot consisted of four P rates (0, 25, 50 and 100 kg ha^{-1}) whereas the subplots consisted of the inoculation of *Bacillus subtilis* Q11 and the control. Each main plot had a dimension of 64 m^2 while the subplots were 32 m^2 (4 rows wide arranged linearly head to tail of the field) within the main plot.

The planting was done on moistened soil and pest management was successfully controlled throughout the season. Irrigation scheduling was managed by the water balance method with the use of IrriModel software (Sifuentes *et al.*, 2012) which estimates the depletion levels according to the methodology proposed by Ojeda *et al*., (2004). Irrigation targets were set as a 50% depletion of plant available water.

For tracking dry matter production, destructive sampling was conductive in each phenological stage. The method of sampling involved removal of the entire aboveground biomass at one randomly selected location (1 m of row length) in each plot.

Plant samples were taken to the laboratory where all structures were separated. Both vegetative and fruit samples were oven dried at 70ºC until constant dry weights were achieved before taking final weights. Also, crop growth rate was estimated with the purpose of tracking variations all over the season.

According to Hunt (1978), this important analysis is determined as:

$$CGR= W_2-W_1/A\ (t_2-t_1)$$

Where:

CGR= kg ha^{-1} day^{-1} (measures the increase in biomass per unit surface and time).

W_1- W_2= initial and final weight.

t_1- t_2= initial and final time.

At the end of the season, data of DM was subject to an appropriate analysis of variance. Treatment mean differences were separated using Fishers' least significance difference (LSD) test at $P \leq 0.05$ (Minitab, 2017).

3.3 RESULTS AND DISCUSSION

3.3.1 Dry matter production in stages of growth.

Dry matter production is a very important process that greatly influences crop productivity. As expected, all P rates, inoculation with *Bacillus subtilis* and plant age affected differently this pattern. The natural variation in production of DM greatly contributed to the final accumulation in which the most intense stages occurred at pod filling and physiological maturity.

The production of DM was low at the beginning of the season due to poor development of vegetative structures. By flowering, that production increased about twice the amount found in the previous stage. At pod filling, the net production dramatically enhanced in high P rates (6497 and 6331 kg ha^{-1}) approximately. At physiological maturity, rate of 50 kg ha^{-1} efficiently enhanced DM with 6341 kg ha^{-1}, which also showed a strong correlation with final yield (Table 5 and Figure 5).

These results coincide from those of Shubbhashre (2007) and Veeresh (2003) who reported that dry matter accumulation increased linearly with application of phosphorus rates, as well as Meseret and Amin (2014) who achieved the greatest dry matter production with medium P rate as compared to the highest rate. Other findings by Rodríguez *et al.* (2014) found that the highest dry matter was achieved in planting dates of September (4120 kg ha^{-1}) and October (4150 kg ha^{-1}), parameter that was strongly correlated with yield. On the other hand, the average DM on the inoculated treatments was of 6143 kg ha^{-1}, which represented an increase of 10.5% over non-inoculated treatments (Figure 6).

This finding is similar to that reported by Joe and Sivakumaar (2009) who found that dual inoculation of PGPR positively increased dry matter production of sunflower crop. In the same manner, Sharma and Prasad (2003) recorded those PS bacteria when inoculated with phosphorus fertilization, effectively enriched dry matter of rice and wheat.

Gupta *et al*. (2009) also found that dry matter yield increased with application of medium P rate (40 kg P_2O_5 ha^{-1}) along with *Bacillus polymyxa* and *B. megaterium.* Finally, the dynamics of DM in this trial also coincides with the pattern reported by Sanaz-Zardari *et al.* (2013) who reported that total DM in common bean increased in the last two final stages of growth.

Table 5. Dry Matter Production (kg ha^{-1}) in phenological stages of Common Bean

P Rates	Third Trifoliate leaf	Flowering	Pod filling	Physiological Maturity
0 kg ha^{-1}	352.5	1054	5089 b	4494 b
25 kg ha^{-1}	360.0	1221	5729 ab	5547 ab
50 kg ha^{-1}	413.1	1288	6497 a	6341 a
100 kg ha^{-1}	397.3	1382	6331 a	5611 ab
OSL	0.6	0.3	0.006	0.007
Inoculation	388.3	1319	6233 a	6143 a
No inoculation	373	1153	5590 b	4854 b
OSL	0.6	0.2	0.02	0.001
P*I	0.4	0.8	0.01	0.02
Average	380.7	1258.5	5911.5	5498.2

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

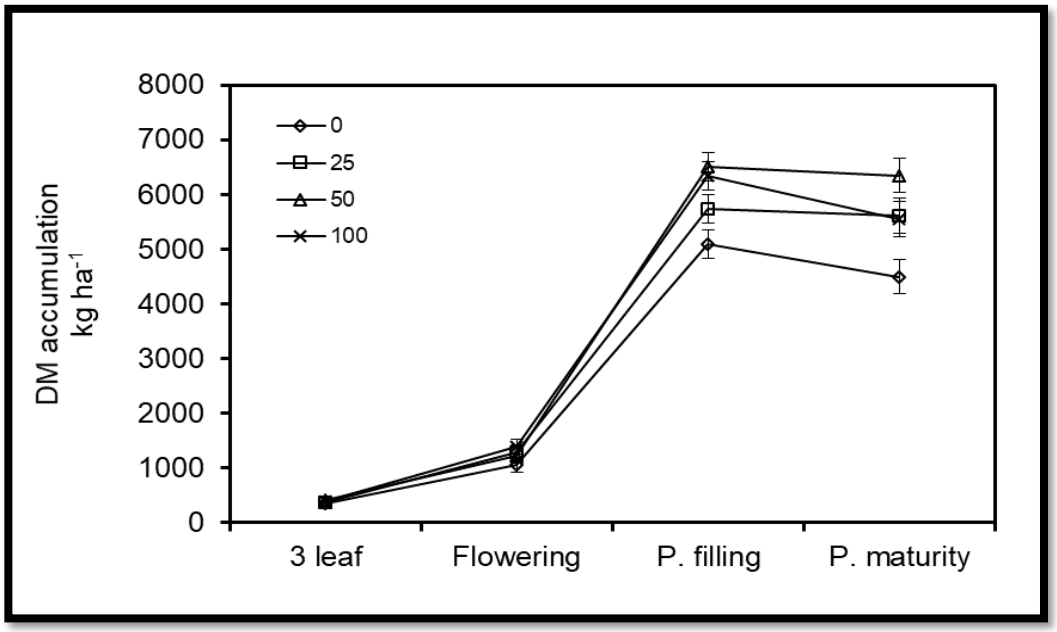


Figure 5. Pattern of DM dynamics as a function of P rates.

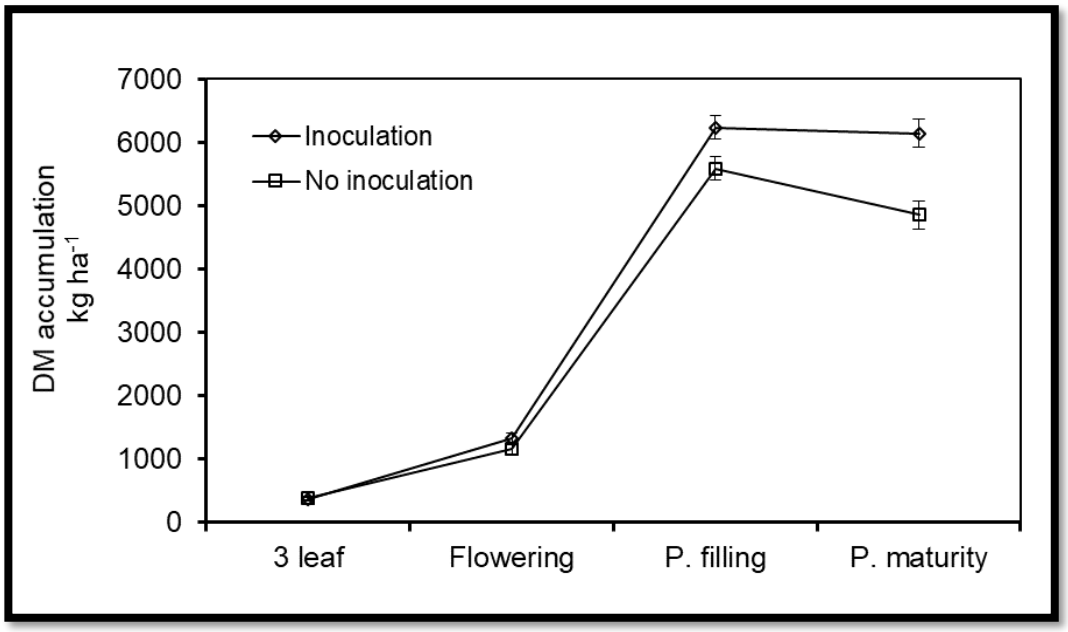


Figure 6. Pattern of DM dynamics as a function of time.

3.3.2 Dry matter partitioning

As observed, there was only a response by the effect of P rates at the end of the season (Table 6). In this case, crop vegetative structures (leaves and stalks) were consistently implicated as the main source for dry matter accumulation up to flowering period (100% of total).

By pod filling stage, the average distribution for all treatments was balanced between the vegetative and reproductive organs. However, by physiological maturity, the allocation of DM was slightly lower (44% of total) in reproductive structures. At that point, there was a lesser demand of the pods for assimilates in order for the grains to lose moisture (Figure 7). These results agree with those findings by Padilla *et al.* (2008) and Chavarín *et al.* (2008), both reported that increases in DM caused a higher percent in distribution on different structures.

Table 6. Dry Matter Partitioning (% of total) in Vegetative (V) and Reproductive (R) organs as a function of P rates and inoculation.

P Rates	Third trifoliate leaf		Flowering		Pod filling		Physiological Maturity	
	V (%)	R (%)	V (%)	R (%)	V (%)	R (%)	V (%)	R (%)
0 kg ha^{-1}	100	0	100	0	52.55	47.45	60.06 a	39.94 b
25 kg ha^{-1}	100	0	100	0	49.89	50.11	57.22 a	42.78 ab
50 kg ha^{-1}	100	0	100	0	45.88	54.12	47.61 b	52.39 a
100 kg ha^{-1}	100	0	100	0	46.84	53.16	59.51 a	40.49 b
OSL	NS	NS	NS	NS	0.2	0.2	0.1	0.1
Inoculation	100	0	100	0	50.30	52.72	58.89	46.69
No inoculation	100	0	100	0	47.28	49.70	53.31	41.11
OSL	NS	NS	NS	NS	0.2	0.2	0.1	0.1
P*I	NS	NS	NS	NS	0.4	0.4	0.4	0.4
Average	100	0	100	0	49	51	56	44

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

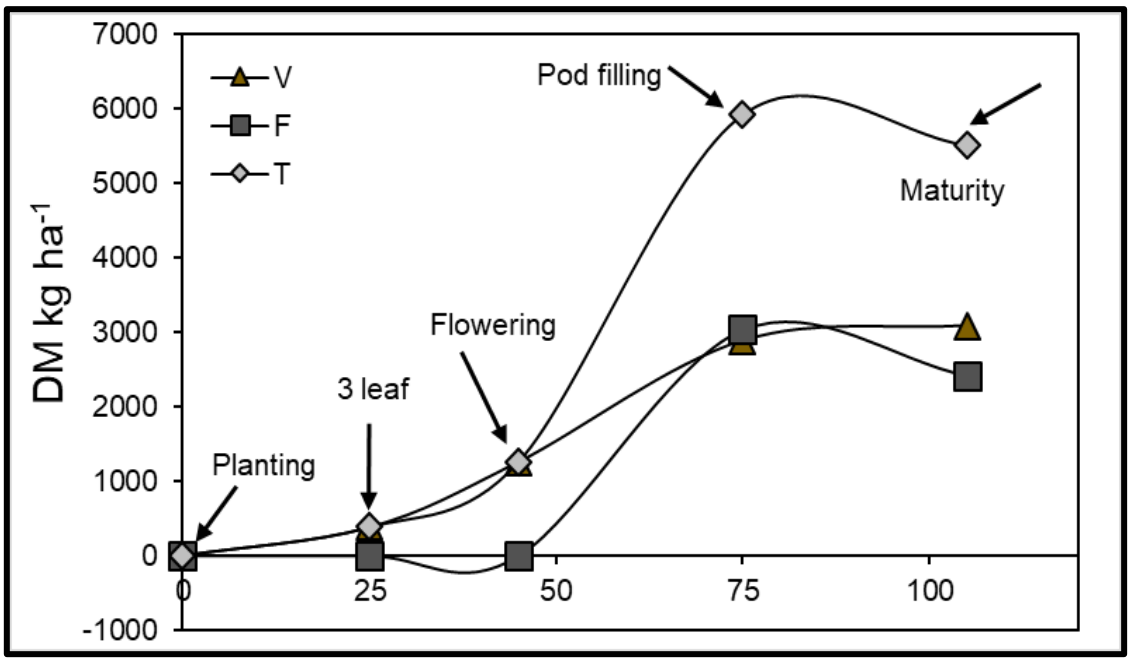


Figure 7. Overall, dry matter-partitioning pattern as a function of time.

Regression equation	R^2
T= -0.0298 DAP^3 + 4.6706 DAP^2 - 109.59 DAP + 122.92	0.97
F = -0.02 DAP^3 + 3.1905 DAP^2 - 91.815 DAP + 113.09	0.91
V = -0.0098 DAP^3 + 1.4801 DAP^2 - 17.77 DAP + 9.8284	0.99

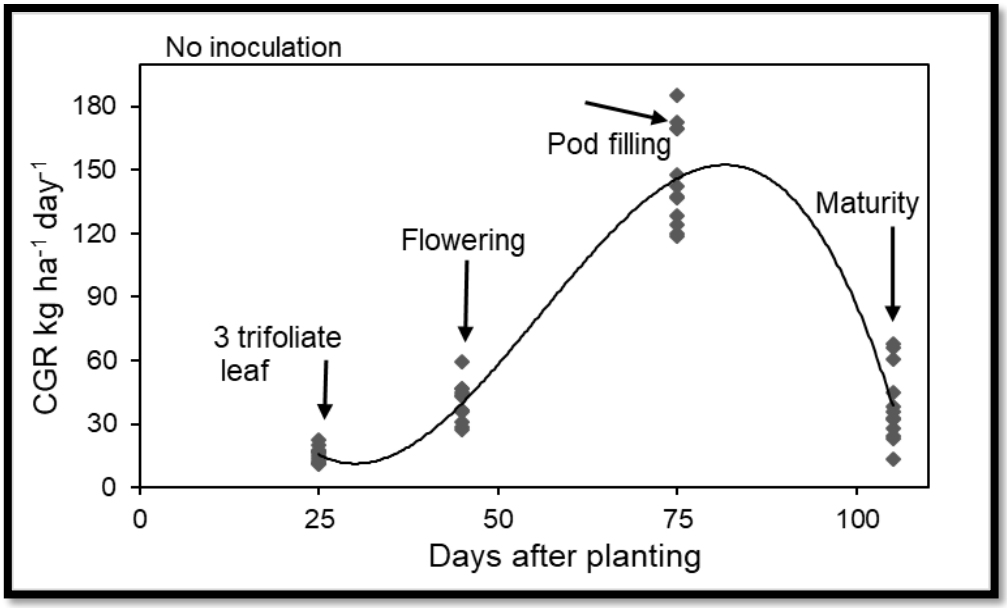
3.3.3 Crop Growth Rate (CGR)

Crop growth rate in early stages of growth was low due to complete absence of vegetation and lesser light absorption. Once the crop started to develop more structures up to flowering (45 DAP) this rate increased from approximately 36 kg ha^{-1} day^{-1} up to 59 kg ha^{-1} day^{-1} on non-inoculated treatments and 50 kg ha^{-1} day^{-1} up to 59 kg ha^{-1} day^{-1} on inoculated treatments.

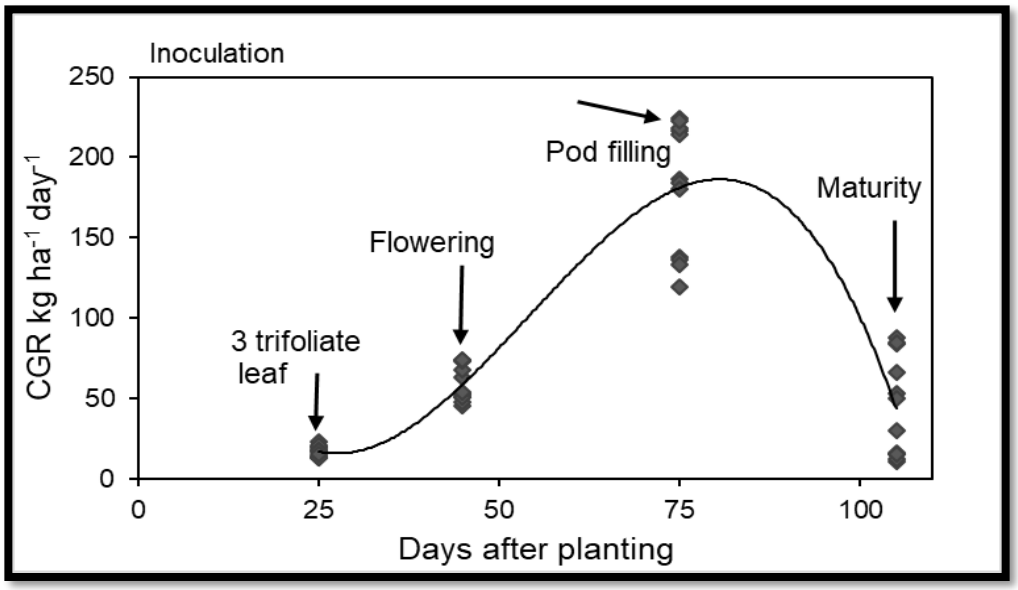
By pod filling (75 DAP) the growh rate was maximized with 146 kg ha^{-1} day^{-1} on non-inoculated treatments and 181 kg ha^{-1} day^{-1} on inoculated treatments. At the end of the season, the crop exhibited a decrease in DM production because there was less leaf area. Consequently, that process strongly produced a shift in CGR with 75 kg ha^{-1} day^{-1} and 60 kg ha^{-1} day^{-1} for inoculated and non-inoculated treatments respectively (Figure 8).

These findings are similar with those presented by Sanaz-zardari *et al.* (2013) who stated that CGR in common bean under different intercropping systems was relatively low at the beginning of the season with increasing rates in later stages.

Escalante-Estrada *et al.* (2014) mentioned that CGR had a similar trend pattern (sine curve form) among cultivars and N rates. They also stated that maximum CGR was attained at 55 DAP in cacahuate cultivar (determinate growth habit), 65 DAP in Michoacan and Mayo cultivars (bushy indeterminate growth habit). In cultivars with N, the CGR was maximized from 70 to 80 DAP while the máximum CGR ranged from 80 to 90 kg ha^{-1} day^{-1} indicating that crop management practices for this crop must be focused on increasing canopy and CGR in order to obtain higher DM production.



Regression equation	R^2
$Y= -0.0021\ DAP^3 + 0.3449\ DAP^2 – 15.148\ DAP + 210.86$	0.92



Regression equation	R^2
$Y= -0.0023\ DAP^3 + 0.3726\ DAP^2 – 15.319\ DAP + 203.04$	0.86

Figure 8. Crop Growth Rate as a function of time.

No statistical differences in the harvest index (HI- ratio of biomass relative to yield) were found neither the different P rates nor the inoculation process evaluated in the trial (Figure 9). This process can be attributable that treatments did not have any contrasting environmental conditions (precipitation, temperature either extreme heat or cold), soil types (acidic, alkaline, saline, organic, clay type) or management practices (irrigation, fertilizer source, genotype, etc). Thus, it was expected not to have such differences that could increase the final yield.

In that respect, Reynolds and Langridge, (2016) mention that HI is the cumulative result of allocation of acquired resources and reflects the balance between source and sink in which the fundamental basis on seed crops is carbon centric and dictates that total dry matter determines “sources” of photoassimilate and harvested grain represents the “sinks”. Some reports mention that all improvements to HI have historically increased yield potentials in major food crops (Long *et al.,* 2015) leading to broad economic gains for farmers. However, its measurement does not capture the efficiency of resource investment and confounds the processes and pathways that regulate the transfer of those resources from the total biomass into grain (Luo *et al.* (2015).

Smith *et al.* (2018) state that much of the variation observed in HI values results from the diverse range of climates and soils. Factors that influence those variations include the energy and protein content of seeds, extreme temperatures (either hot or cold) during crop reproductive development.

Numerous studies across many species (Li et *al.,* 2012; Cui *et al.,* 2008; Quarrie *et al.,* 2006) are unable to identify specific trait directly associated with HI. Nevertheless, they conclude that it is integrative and therefore affected by many factors, which influence the overall source-sink dynamics (Hay, 1995; Luo *et al.,* 2015). While, Yang and Zhang (2010) mention that as HI varies with differences in crop management, it is likely to assure a high yield potential only under the environment for which is selected.

Actually, all the efforts to improve yield in crops have been based on luxurious supplementation of resources such as nutrients and water (Korner, 2015). Generally, the number and size of grain tissue limit increases in yield (Borrás *et al.,* 2004; Rao et *al.,* 2017).

Finally, some authors suggest that HI may be used as a measure to improve yields of some crops, while still recognizing that the interaction between HI and environmental variation is complex and may not scale accordingly with total yield (Smith *et al.,* 2018). Consequently, some crops improve yield at the expense of reducing the nutritional quality of that yield (Fan *et al.,* 2008)

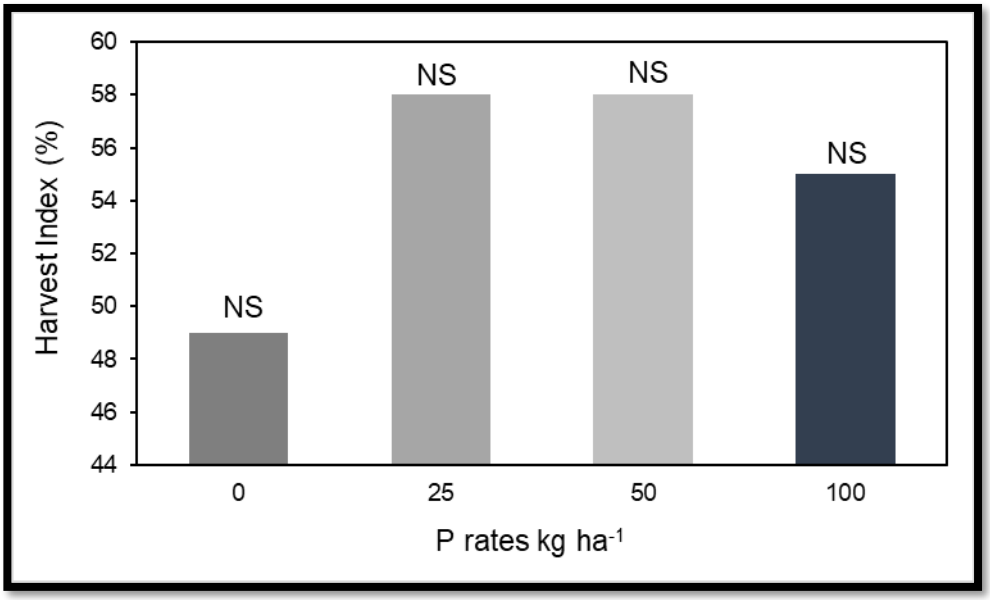


Figure 9. Harvest index for P rates.

3.4 CONCLUSIONS

The most active stage for DM accumulation (CGR) was pod filling which likely corresponded to physiological peak in photosynthesis, water use and nutrient uptake.

Rate of 50 kg P accumulated the highest amount of dry matter.

The partitioning process of DM in reproductive and vegetative phases was relatively equal at pod filling. By the end of the season, the distribution process in the pods was slightly lower than that of vegetation.

Even though, inoculation with *Bacillus subtilis* had an influence on DM production at the second half of the season, it did not exert any effect in the partitioning process.

Further validation on other cultivars and growth habits is needed.

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CAPITULO 4

Performance of Phosphorus and *Bacillus subtilis* on nutrient uptake of Common Bean.

Jesús Del Rosario Ruelas Islas[1], Gabriel A. Lugo García [1], Fidel Núñez-Ramírez[2*], Jorge Flores Velázquez[3], Federico Villarreal Guerrero[4]

[1]School of Agriculture, Autonomous University of Sinaloa, Sinaloa, México.
[2]Agricultural Science Institute. Autonomous University of Baja California, Baja California, México.
[3]Mexican Institute of Water Technology, Morelos, México.
[4]School of Animal Sciences and Ecology. Autonomous University of Chihuahua, Chihuahua, México.

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an important legume that is highly consumed in Latin America and other countries as a part of feeding. In Northern Sinaloa, bean yield remains low due to management and environmental factors that affect yield potential. Therefore, a field experiment was conducted in order to investigate the response of common bean to different rates of phosphorus (P_2O_5) fertilizer (0, 25, 50, 100 kg ha^{-1}) and the role of *Bacillus subtilis* strain Q11 on nutrient uptake of the crop. The experiment was conducted as a split-plot in a randomized complete block design with three replicates. Based on the results, all P rates and inoculation with *B. subtilis* significantly influenced tissue concentration and uptake in each phenological stage. For all treatments, total macro and micronutrient uptake were in the following order K> N> Ca> Mg>P, Fe>Mn>Zn>B>Cu in which the most intense period occurred at 75 DAP (pod filling). At the end of the season, nutrient flux was N> K> Ca> Mg > P with 3.5, 2.4, 1.5, 0.9 and 0.18 kg ha^{-1} day^{-1} for mayor nutrients, whereas Fe> Mn> Zn> B> Cu with 284, 56, 20, 8 and 4 g ha^{-1} day^{-1} for micronutrients. Inoculation with *B*. *subitilis* Q11 favored positively the uptake process from approximately 15 to 30% with respect the non-inoculated treatments. Also, it substantially influence nutrient concentration in seeds

Keywords: *Common Bean, Bacillus subtilis, Fertilizer rates, Macro and micronutrients*

4.1 INTRODUCTION

Common bean is one of the most important grain legumes cultivated worldwide (Terán *et al.,* 2009; Urrea *et al.,* 2009) and that represents a way of supplying protein source as a part of the diet (Fageria *et al.,* 2010). Domestic consumption has not been covered in the last decade (Escalante-Estrada *et al.,* 2014) even though this crop is extensively grown in México (FIRA, 2015).

Constraints about yield potential usually arise from biotic (soil-borne pathogens) and abiotic factors such as low P availability in various soil types (Banerjee *et al.,* 2010), water deficit, temperature variability, improper fertilization (Mweetwa, 2011) and interaction with mineral fertilizers (Hidayatullah *et al.,* 2013; Iqbal *et al.,* 2015).

Phosphorus (P) has become more limiting despite the large amounts present in most soils (Khan *et al.,* 2009). Its high fixing capacity with soil mineral fraction makes it unavailable for plant uptake. Many studies have shown that excessive P rates can result in plant toxicity (poor growth, leaf chlorosis, and micronutrient deficiency) and soil P build up.

According to Westerman *et al.* (2011), the way of increasing productivity is related not only to high nutrient availability in soil solution but also to the absorption capacity by the plant and seeds. In that aspect, Fageria *et al.* (2011), Pagani and Mallarino (2012) as well as Crusciol (2013) mention that nutrient uptake patterns are a good tool to better define fertilization programs.

In recent years, the application of new field technologies like inoculants based on Plant Growth Promoting Rhizobacteria (PGPR) are considered efficient in increasing nutrient availability (Ahemad *et al.,* 2009; Chandler *et al.,* 2008), water uptake, higher yields (Stajkovic *et al.,* 2011) and recycling soil nutrients by enhancing natural soil fertility (Glick, 2012).

However, the success of inoculation depends on favorable environmental conditions for its establishment and performance (Ahemad and Kibret, 2014).

Studies show that PGPR isolates possess the ability to transform its surroundings by secreting different array of compounds (Walker *et al.,* 2003) which modifies chemical and physical properties of soils (Dakora and Phillips, 2002), influences growth by stimulating biological nitrogen fixation and enhancing the availability of trace elements (Zaidi *et al.,* 2009).

Some other works show that certain strains of *Rhizobium* inoculation lead to siderophore production (secretion of low molecular iron chelators) (Sridevi *et al.,* 2007) increasing the availability of Fe (Fabiano *et al.,* 1994), Zn (Wani *et al.,* 2008), P (Sridevi *et al.,* 2007) rendering more available for plant uptake.

In a similar manner, Rengel *et al.* (1999) found that inoculation with *Rhizobium* in legumes had a positive influence on plant growth and micronutrient concentration of plant parts. Meanwhile, reports on *B. subtilis* mention that environmental challenges (temperature changes) and nutrient scarcity limits its survival in soils (Qiao *et al.,* 2017), being those the key factors for PGPR inoculants to be effective (Compant *et al.,* 2010).

Other findings by Zhang *et al.* (2007) showed that volatile organic compounds (VOCs) produced by *B. subtilis* GB03 induced auxin biosynthesis, regulated cell expansion in Arabidopsis plants while in others promoted Fe absorption (Zhang *et al.,* 2009). Therefore, there is evidence that a wide range of bacterial species like *Paenibacillus* (Lee *et al.,* 2012), *Bacillus* (Zhang *et al.,* 2007) and *Pseudomonas* (Park *et al.,* 2015) can release different kinds of VOCs to regulate nutrient uptake, abiotic and biotic stress responses in plants. Finally, Fageria (2002) reports that all nutrient interactions in plants can either be positive, negative or neutral measuring them by their crop growth and nutrient concentration in tissues.

Despite the importance of common bean in Mexico, there is only limited information regarding the role of *Bacillus* Q11 inoculant on the availability of nutrients. Based on that, it is important to determine the amounts of nutrients related to best yield, rates of nutrient uptake all over the season, stages of growth related to maximize nutrient uptake and partitioning in plant organs in order to develop a rational P fertilization program. Therefore, the objectives of this study consisted of evaluating the performance of *Bacillus subtilis* Q11 and different phosphorus rates on nutrient concentration and uptake patterns for common bean.

4.2 MATERIALS AND METHODS

A field experiment was conducted during the winter season at Valle del Fuerte located northern Sinaloa, Mexico (25° 45´49” N, 108° 51´41” W). The soil at the study site is classified as clay loam (50% clay, 30% silt and 20% sand), low in organic matter content (< 1%), bulk density of 1.15 g cm^{-3} and volumetric water content of 0.155 cm^3 cm^{-3}, other physical and chemical properties are described in the following table.

Table 7. **Physical and chemical properties of soil.**

Soil parameter	**(Meq L^{-1})**	
Saturation (%)	50	
pH (1:2 ratio)	7.6 alkaline	
ECe 25 ºC (dS m-^{1})	0. 69	
Carbonates	TRACE	
Bicarbonates	1.60	
Calcium	3.00	
Magnesium	1.50	
Sodium	1.58	
Potasium (PPM)	32	
Chlorine	1.80	
Sulfates (PPM)	55	
Sodium absorption ratio	1.05	
MACRONUTRIENTS	mg L^{-1}	
Nitrogen	145	High
phosphorus	32	Medium
Potasium	760	High
Calcium	3950	High
Magnesium	590	High
Sodium	180	Low
MICRO-NUTRIENTS	mg L^{-1}	
Iron	6.40	Low
Manganese	11.96	High
Copper	1.82	Low
Zinc	1.48	Low

Soil tilling techniques were realized following the guideline provided by the National Institute of Agricultural, Forestry and Livestock Research (SIAP, 2013). A composite surface 30 cm soil samples were collected before fertilization.

Treatments consisted of four phosphorus rates (0, 25, 50 and 100 kg ha^{-1} of P_2O_5) and the inoculation of seed Azufrado Higuera with *Bacillus subtilis* Q11®. Five mL of inoculant at $2x10^8$ CFU mL^{-1} was sprayed for each kilogram of seed. Nitrogen rate consisted of 150 kg ha^{-1}. Total N and P rates were pre-plant applied using highly soluble Blaukorn Classic ® (12-8-16).

The experiment was carried out as a split-plot in a randomized complete block design with three replicates. The main plot consisted of the P rates whereas the subplots consisted of the inoculation of *Bacillus subtilis* Q11 and the control. Each main plot had a dimension of 64 m^2 while the subplots were 32 m^2 within the main plot.

Destructive sampling of different plant parts (leaves, stems, and pods) was performed in each phenological stage (third trifoliate leaf, flowering, pod filling, and physiological maturity). The method of sampling involved the removal of the entire aboveground biomass at one randomly selected location (1 m of row length) in each plot. Plant samples were taken to the laboratory where all structures were separated. Both vegetative and fruit samples were oven-dried at 70ºC until constant dry weights. Nitrogen was determined by wet digestion with the Kjeldahl method, phosphorus by wet digestion with nitric acid (HNO_3) + perchloric acid ($HClO_4$) + sulfuric acid (H_2SO_4). Thereafter, all the filtered material obtained by wet digestion for P was used to determine the concentration of K (flame method), Ca, Mg (dilutions), Fe, Mn, Zn, Cu (no dilutions) using spectrophotometer following the guideline on the Official Mexican regulation (NOM-021-RECNAT-2000).

The amount of each nutrient taken up and distributed in plant parts was calculated by multiplying the dry weight by the elemental concentration (kg ha^{-1}). Nutrient flux by taking the first derivative of nutrient accumulation with respect to time [d NA/d t].

Data on total nutrient concentration and uptake was subject to repeated measures analysis of variance treating the stage of growth as the repeated factor while phosphorus rates and Bs as the subject variable. Treatment mean differences within each phenological stage were separated using Fishers' least significance difference (LSD) test at $P \leq 0.05$ (Minitab, 2017). Regression models were fitted to each experimental unit and tested on significance levels ($\alpha = 0.05$) and R^2 values.

4.3 RESULTS AND DISCUSSION

4.3.1 Macronutrient concentration in plant tissues.

The concentration of macronutrients in phenological stages significantly differed between the vegetative and reproductive organs ($P \leq 0.05$) and is presented in Tables 8 through 12. It was observed that the highest concentration was intensified at pod filling stage (75 DAP).

According to Urzúa (2005), common bean is a plant that usually has high N concentrations in its tissues and grains. It is, therefore, that N was especially concentrated in leaves all over the season, which indicated its mobility inside the plant. Besides, that increase corresponded from flowering up to pod filling stage, to then decline at maturity. Total N concentration in each respective organ (leaves-stems-pods) was that of 39, 36 and 29 g kg^{-1} of DM for inoculated treatments and 29, 27 and 26 g kg^{-1} of DM for non-inoculated treatments (Table 8).

Maximum phosphorus (P) concentration occurred at flowering with a slight decrease in maturity. Total P concentration for the same parts was 3, 3.5 and 3.5 g kg^{-1} of DM for inoculated treatments and 1.7, 2 and 2.4 g kg^{-1} of DM for non-inoculated treatments (Table 9). This result coincides with reports of Puentes *et al.* (2014) who found low concentrations of P in tissues.

Potassium (K) was concentrated in a higher amount as compared to N. However, the higher concentration was on stems maximizing that range up to flowering. This result coincides with previous reports by Padilla (2008), which mentioned that in some cases, K is uptaken earlier by the crop as compared to N or P, but soon after it can be translocated to other plant parts. At the end of the season, total concentration was 51, 54 and 22 g kg^{-1} of DM for inoculated treatments and 33, 40 and 17 g kg^{-1} of DM for non-inoculated treatments (Table 10).

Table 8. Nitrogen concentration in vegetative and reproductive organs of common bean.

Phosphorus	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
(kg ha^{-1})	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
				g kg^{-1} of dry matter						
0	27.50 b	20.90 b	31.83 b	30.73 b	30.73 b	29.97 b	22.68 b	26.06 b	29.07 b	26.06 b
25	31.50 b	25.33 ab	37.33 ab	33.80 ab	28.37 b	34.07 ab	26.78 a	27.23 b	31.07 b	27.23 b
50	38.83 a	28.95 a	42.10 ab	39.93 a	39.67 a	38.37 a	26.95 a	33.66 a	41.87 a	33.66 a
100	40.50 a	25.77 ab	45.17 a	31.53 b	40.17 a	27.37 b	24.83 ab	32.53 a	37.40 ab	32.53 a
OSL	0.001	0.002	0.009	0.03	0.02	<0.001	0.04	0.001	0.05	<0.001
Inoculation	39.17 a	29.29 a	46.83 a	37.05 a	40.63 a	37.53 a	26.23 a	30.93 a	41.08 a	30.93 a
No inoculation	30.02 b	21.18 b	31.38 b	30.95 b	28.83 b	27.35 b	24.39 a	28.82 a	28.61 b	28.82 a
OSL	<0.001	<0.001	<0.001	0.01	<0.001	<0.001	0.06	0.06	<0.001	0.2
0 + Bs	30.0 e	22.56 bc	38.66 c	31.06 c	35.13 b	36.66 b	24.67 bc	27.13 de	32.02 d	24.67 bc
0	25.0 f	19.23 c	25.00 e	30.40 c	26.33 d	23.27 d	20.70 d	25.0 e	26.13 e	20.70 d
25 + Bs	38.0 c	27.01 b	42.00 c	37.02 b	30.40 c	36.66 b	27.43 ab	29.46 cd	34.01 c	27.43 ab
25	25.0 f	23.67 bc	32.66 d	30.60 c	26.33 d	31.47 c	24.60 bc	25.0 e	26.13 e	24.60 bc
50 + Bs	42.6 b	35.52 a	49.67 b	49.01 a	48.01 a	45.33 a	29.3 a	32.33 ab	54.33 a	29.32 a
50	35.0 d	22.40 bc	34.53 d	30.86 c	31.33 c	31.40 c	26.13 abc	35.0 a	29.40 de	26.13 abc
100 + Bs	46.0 a	32.10 a	57.00 a	31.13 c	49.01 a	31.47 c	26.13 abc	34.80 a	42.01 b	26.13 abc
100	35.0 d	19.43 c	33.33 d	31.93 c	31.33 c	23.27 d	23.53 cd	30.27 bc	32.80 cd	23.53 cd
OSL	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.01	<0.001	<0.001	0.01
Average	34.5	25.2	39.1	33.4	34.8	32.4	25.3	29.9	34.9	29.8

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

Table 9. Phosphorus concentration in vegetative and reproductive organs of common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0	1.71 a	1.80 b	2.38 b	2.41 b	2.21 ab	2.91 a	2.85 a	1.56 a	1.51 b	2.85 a
25	2.13 a	2.25 ab	2.85 ab	3.28 b	1.95 b	3.03 a	3.06 a	1.95 a	2.46 ab	3.06 a
50	2.58 a	3.26 a	3.76 a	5.25 a	2.91 a	2.90 a	3.10 a	2.33 a	3.65 a	3.10 a
100	2.58 a	3.01 a	3.18 ab	2.76 b	2.90 a	1.85 a	2.93 a	2.21 a	2.86 a	2.93 a
OSL	0.06	0.004	0.005	0.001	0.01	0.06	0.9	0.07	0.01	0.9
Inoculation	3.50 a	3.37 a	3.62 a	4.09 a	2.90 a	3.55 a	3.55 a	2.78 a	3.25 a	3.55 a
No inoculation	1.00 b	1.79 b	2.46 b	2.76 b	2.09 b	1.79 b	2.41 b	1.25 b	2.0 b	2.41 b
OSL	<0.001	<0.001	0.001	0.030	0.002	<0.001	0.001	<0.001	0.009	0.001
0 + Bs	2.43 c	2.43 d	3.0 bc	2.76 cd	2.50 b	3.90 ab	3.36 abc	2.13 c	2.33 b	3.36 abc
0	1.01 d	1.16 e	1.76 d	2.06 d	1.93 c	1.93 cd	2.06 d	1.0 d	0.70 c	2.06 d
25 + Bs	3.26 b	2.96 c	3.01 bc	3.90 b	2.01 bc	3.40 ab	3.63 ab	2.40 bc	3.0 b	3.63 ab
25	1.01 d	1.53 e	2.70 c	2.61 d	1.90 c	2.61 bc	2.50 bcd	1.50 d	1.93 b	2.50 bcd
50 + Bs	4.16 a	4.43 a	4.83 a	7.01 a	3.53 a	4.23 a	3.83 a	3.66 a	4.80 a	3.83 a
50	1.02 d	2.10 d	2.70 c	3.50 bc	2.30 bc	1.57 cd	2.80 abcd	1.0 d	2.50 b	2.80 abcd
100 + Bs	4.16 a	3.61 b	3.66 b	2.70 cd	3.56 a	2.70 bc	3.40 abc	2.93 b	2.86 b	3.40 abc
100	1.03 d	2.31 d	2.70 c	2.83 cd	2.23 bc	1.01 d	2.30 cd	1.50 d	2.86 b	2.30 cd
OSL	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.03	<0.001	0.04	0.03
Average	2.25	2.6	3.0	6.9	2.5	5.3	2.9	2.0	2.6	2.9

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

Table 10. Potassium concentration in vegetative and reproductive organs of common bean.

Phosphorus	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
(kg ha^{-1})	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
					g kg^{-1} of dry matter					
0	24.0 b	9.33 c	29.43 b	52.67 a	41.07 a	60.16 a	19.03 a	38.40 c	39.07 b	19.03 a
25	33.80 ab	20.50 bc	42.0 ab	52.40 a	42.90 a	60.16 a	19.15 a	41.13 bc	39.80 b	19,15 a
50	38.08 a	25.67 b	49.67 a	57.33 a	43.33 a	66.33 a	20.32 a	53.17 ab	68.80 a	20.32 a
100	39.13 a	38.0 a	55.27 a	58.52 a	49.83 a	67.97 a	19.58 a	56.50 a	68.80 a	19.58 a
OSL	0.02	0.001	0.005	0.2	0.4	0.7	0.8	0.01	<0.001	0.8
Inoculation	41.23 a	31.33 a	52.97 a	62.79 a	55.48 a	68.65 a	22.01 a	55.18 a	52.43 a	22.01 a
No inoculation	26.28 b	15.42 b	35.22 b	47.67 b	33.08 b	58.66 b	17.03 b	39.42 b	42.65 a	17.03 b
OSL	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	0.001	0.001	0.4	0.001
0 + Bs	31.33 c	11.33 de	42.20 c	62.33 a	51.41 c	61.67 b	21.01 abcd	41.47 bcd	41.04 de	21.02 abcd
0	16.67 d	7.33 e	16.67 d	43.03 c	30.62 e	58.61 b	15.87 d	35.33 e	37.13 e	15.87 d
25 + Bs	38.60 b	27.01 c	44.01 c	61.80 a	55.15 b	61.67 b	22.20 ab	45.13 b	42.47 cd	22.20 ab
25	29.02 c	14.02 d	40.02 c	43.01 c	30.61 e	58.62 b	17.50 bcd	37.13 de	37.13 e	17.50 bcd
50 + Bs	47.17 a	50.01 a	57.33 b	63.02 a	52.01 bc	74.03 a	23.13 a	66.13 a	80.26 a	23.13 a
50	29.01 c	26.02 c	42.00 c	51.67 b	34.67 d	58.61 b	18.13 abcd	40.20 cde	57.33 b	18.13 abcd
100 + Bs	47.83 a	37.01 b	68.33 a	64.03 a	63.34 a	77.27 a	21.70 abc	68.01 a	46.01 c	21.70 abc
100	30.43 c	14.33 d	42.20 c	53.01 b	36.34 d	58.63 b	16.60 cd	45.02 bc	39.02 de	16.60 cd
OSL	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.05	<0.001	<0.001	0.05
Average	15.2	46.8	44.0	55.2	44.2	63.7	19.5	47.3	47.6	19.5

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

Calcium (Ca) concentration dramatically decreased at flowering with respect to the initial stage (third trifoliate leaf). Thereafter, it increased at pod filling by then decline at the end of the season. The concentration was 38, 16 and 10 g kg^{-1} of DM for Bs treatments and 27, 12 and 9 g kg^{-1} of DM for non-Bs treatments (Table 11). In addition, it was observed that the reduction for Ca accumulated in leaves and stems at the end of the season could have been attributed to leaf abscission and senescence since removilization of this nutrient to foliar tissues is low. Finally, magnesium (Mg) concentration had a similar pattern of Ca, which was highly variable during the season (Table 12). The same author stated that Mg could also vary depending upon the species, cultivars or stages of growth. Total concentration was 13, 4.5 and 9 g kg^{-1} of DM for Bs treatments and 8.5, 1.8 and 7.5 g kg^{-1} of DM for non-Bs treatments.

All exposed results regarding mayor nutrient concentration in plant tissues are very similar to those found by Lata-Tenesaca *et al*. (2017) who reported that leaves registered the highest macronutrient concentration. However, the maximum concentration occurred at similar times during the season, being the most intense, the period between flowering (45 DAP) and pod filling (75 DAP). However, they differed from those of Peres-Soratto *et al*. (2013) which found greater amounts of N-K-Ca in stems. The order of preference with respect to concentration was K>N>Ca>Mg>P which is comparable to those of Puentes *et al.* (2014) who found low P and Mg concentration, and that Mg tended to be accumulated in foliar tissues depending on plant age.

Inoculation with *B. subtilis* favored the increase in concentration of these elements in different organs such as leaves, stems, and pods. According to the behavior observed, this process can be attributed to that reported by Zaidi *et al.* (2009) who stated that nutrient solubilization typically occurs consequently by the action of low molecular weight organic acids that are synthesized by different bacteria. On the other hand, these data are like that of Zafar *et al.* (2011), Oliveira (2009), Wang *et al.* (2011), Yasmin and Bano (2011) who recorded that nutrient in tissues increased by the application of P and inoculation with rhizobacteria.

Table 11. Calcium concentration in vegetative and reproductive organs of common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0	20.90 b	11.98 a	7.83 a	4.96 b	54.20 b	12.61 b	9.28 a	11.01 c	9.93 b	9.28 a
25	23.93 ab	13.52 a	9.50 a	5.61 b	58.03 b	12.61 b	9.55 a	14.98 c	22.13 a	9.55 a
50	27.62 a	13.08 a	14.07 a	7.63 a	65.02 ab	22.47 a	10.06 a	59.83 a	25.50 a	10.06 a
100	26.02 a	12.68 a	13.35 a	4.61 b	74.40 a	18.12 ab	9.60 a	42.83 b	27.40 a	9.60 a
OSL	0.03	0.8	0.2	0.004	0.01	0.04	0.2	<0.001	0.002	0.2
Inoculation	27.50 a	14.93 a	15.90 a	6.62 a	71.77 a	20.67 a	10.03 a	38.49 a	21.98 a	10.03 a
No inoculation	21.72 b	10.70 b	6.47 b	4.79 b	54.05 b	12.24 b	9.21 b	25.83 a	20.50 a	9.21 b
OSL	<0.001	<0.001	<0.001	0.003	<0.001	0.003	0.005	0.7	0.2	0.005
0 + Bs	24.26 bcd	14.96 a	12.43 bc	5.33 cd	63.07 c	13.01 c	9.66 abc	14.02 de	11.33 bc	9.66 abc
0	17.53 e	9.03 c	3.23 d	4.63 de	45.33 e	12.22 c	8.90 c	8.03 e	8.53 c	8.90 c
25 + Bs	26.50 bc	15.76 a	18.13 a	6.63 b	63.53 c	13.01 c	10.10 ab	18.32 d	23.03 ab	10.10 ab
25	21.37 de	11.26 b	0.86 d	4.02 e	52.53 d	12.21 c	9.43 bc	11.63 e	21.27 ab	9.43 bc
50 + Bs	31.23 a	14.90 a	18.13 a	9.30 a	76.67 b	32.33 a	10.60 a	74.67 a	27.66 a	10.60 a
50	24.02 cd	11.26 b	10.01 c	5,96 bc	53.33 d	12.63 c	9.53 abc	45.03 b	23.33 a	9.53 abc
100 + Bs	28.03 ab	14.10 a	14.93 b	5.23 cde	83.80 a	24.33 b	9.76 abc	47.02 b	25.93 a	9.76 abc
100	24.01 cd	11.26 b	11.76 c	4.63 de	65.02 c	11.92 c	9.03 bc	38.67 c	28.86 a	9.03 bc
OSL	<0.001	0.05	<0.001	<0.001	<0.001	<0.001	0.05	<0.001	0.01	0.05
Average	24.6	12.8	11.2	5.7	62.9	16.5	9.6	32.1	21.2	9.6

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

Table 12. Magnesium concentration in vegetative and reproductive organs of common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0	6.21 a	4.80 a	3.73 a	3.45 a	13.23 b	3.45 a	7.75 a	10.73 b	1.91 a	7.75 a
25	5.85 a	3.30 a	2.96 a	2.41 a	15.50 b	2.41 a	8.16 a	12.33 b	2.16 a	8.16 a
50	6.45 a	3.71 a	4.30 a	3.16 a	17.27 b	3.76 a	8.80 a	17.27 ab	3.16 a	8.80 a
100	6.13 a	3.21 a	2.66 a	2.40 a	23.63 a	4.63 a	8.71 a	24.53 a	2.40 a	8.71 a
OSL	0.4	0.2	0.4	0.6	0.005	0.4	0.1	0.003	0.2	0.1
Inoculation	6.92 a	5.24 a	3.73 a	4.06 a	21.02 a	5.48 a	9.14 a	21.13 a	3.17 a	9.14 a
No inoculation	5.40 b	2.27 b	3.10 a	1.65 b	13.80 b	1.65 b	7.57 b	11.30 b	1.65 b	7.57 b
OSL	<0.001	<0.001	>0.05	<0.001	0.001	<0.001	<0.001	0.001	<0.001	<0.001
0 + Bs	7.03 ab	5.50 a	2.73 ab	5.56 a	16.47 c	5.56 a	8.33 bcd	16.47 cd	2.50 bc	8.33 bcd
0	5.40 c	4.11 ab	4.73 ab	1.33 e	10.01 d	1.33 c	7.16 d	5.02 e	1.33 d	7.16 d
25 + Bs	6.30 bc	5.60 a	3.60 ab	3.43 bc	21.02 b	3.43 b	9.50 ab	19.67 bc	2.93 b	9.50 ab
25	5.40 c	1.02 c	2.33 ab	1.40 e	13.40 cd	1.40 c	7.76 cd	5.01 e	1.40 d	7.76 cd
50 + Bs	7.50 a	5.43 a	5.26 a	4.60 ab	21.13 b	5.80 a	9.83 a	21.133 b	4.60 a	9.83 a
50	5.40 c	2.02 bc	3.33ab	1.73 de	10.02 d	1.73 c	7.93 cd	13.40 d	1.73 cd	7.93 cd
100 + Bs	6.86 ab	4.43 a	3.33 ab	2.66 cd	25.47 a	7.13 a	8.90 abc	27.27 a	2.66 b	8.90 abc
100	5.40 c	2.01 bc	2.0 b	2.13 de	21.80 b	2.13 bc	7.43 d	21.80 b	2.13 bcd	7.43 d
OSL	0.002	0.003	0.05	<0.001	<0.001	<0.001	0.01	<0.001	0.01	0.01
Average	6.1	3.8	3.4	2.9	17.4	3.5	8.3	16.2	2.4	8.3

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

4.3.2 Macronutrient uptake in stages of growth.

Nutrient uptake was significantly influenced in response to P rates and inoculation with *B. subtilis.* The uptake process gradually increased for all treatments during the season. Additionally, rates of 50 and 100 kg P exhibited the range of higher uptake.

Nitrogen uptake was low at the beginning of the season. At flowering, it significantly increased with 36 kg ha^{-1}, a process that was relatively short to make way to the beginning of pod formation. Therefore, the uptake was maximized in pod filling with 114 kg ha^{-1}, to then decline at physiological maturity (Table 13). The uptake pattern showed that N uptake remained almost constant especially in high P rates (Figure 10).

This result differed from that of Brito *et al.* (2009) who reported that approximately one third of applied N was absorbed in pre-bloom, but coincides with those of Lata-tenesaca *et al.* (2017) who reported a continuous increase of N uptake in the last stage of growth. While Peres-Soratto *et al*. (2013) stated that N absorption was higher during pod formation as compared to others stages of growth. They also mentioned that the uptake was even greater on those treatments with 100 % of recommended NPK fertilization.

Inoculation treatments also increased the uptake process all over the season as compared to non-treated plants (Figure 11) which coincides with results of Figueiredo *et al.* (2008) who reported that inoculation with *Rhizobium* and *P. polymyxa* strain Loutit (L) stimulated nodulation as well as nitrogen fixation and that PGPR stimulated specific-nodulation increasing into higher levels of accumulated nitrogen.

Table 13. Nitrogen uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	7.09 b	26.65 b	81.72 b	65.52 b
25	7.98 b	37.19 ab	94.26 b	108.7 a
50	11.99 a	42.69 a	147.4 a	140.7 a
100	11.07 a	38.78 ab	133.1 a	120.82 a
OSL	0.002	0.08	<0.001	0.008
Inoculation	10.20 a	41.66 a	130.5 a	124.0 a
No inoculation	8.86 a	30.99 b	97.74 b	93.86 a
OSL	0.2	0.02	0.004	0.08
0 + Bs	7.69 bc	32.7 abc	85.9 c	70.5 cd
0	6.49 c	20.5 c	76.9 c	60.4 d
25 + Bs	9.60 abc	3808 ab	102.5 c	123.1 b
25	6.37 c	29.2 bc	86.5 c	88.9 bcd
50 + Bs	12.83 a	50 a	179.4 a	178.4 a
50	11.16 ab	35.5 abc	115.3 bc	102.9 bcd
100 + Bs	12.37 a	45.17 ab	153.3 ab	128.6 b
100	9.77 abc	38.7 ab	112.8 bc	118.5 bc
OSL	0.006	0.07	0.001	0.002
Average	9.53	36.32	114.1	108.9

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

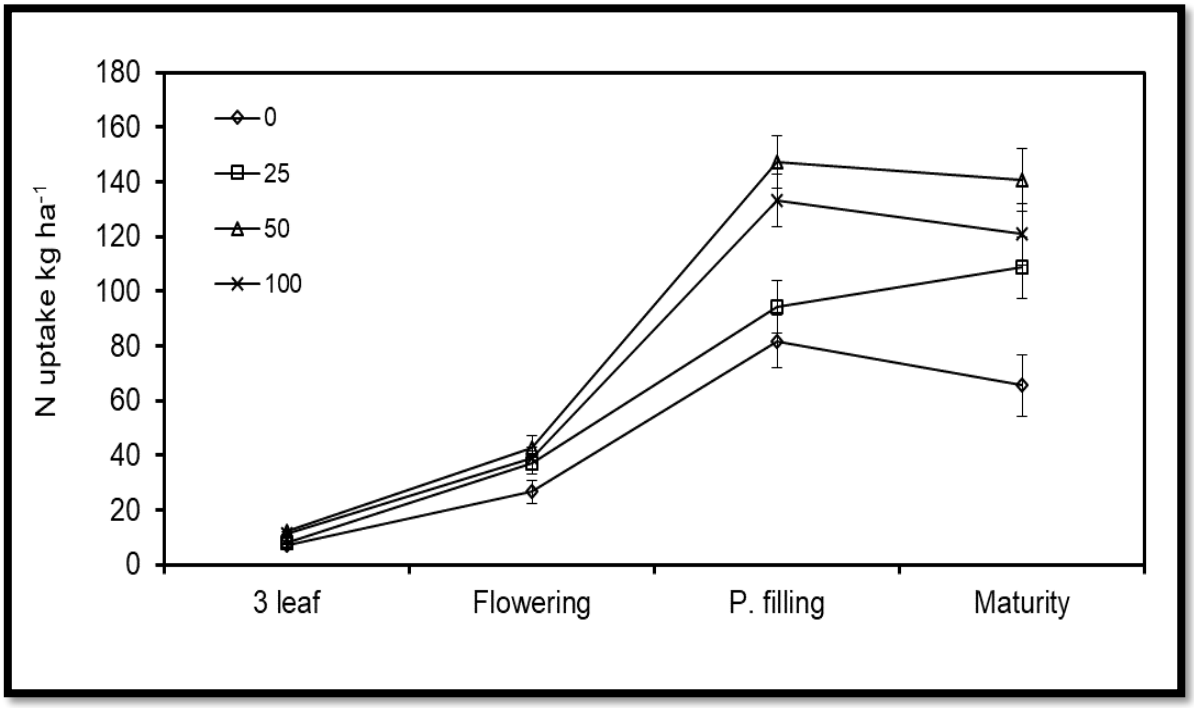

Figure 10. Nitrogen uptake pattern as a function of P rates.

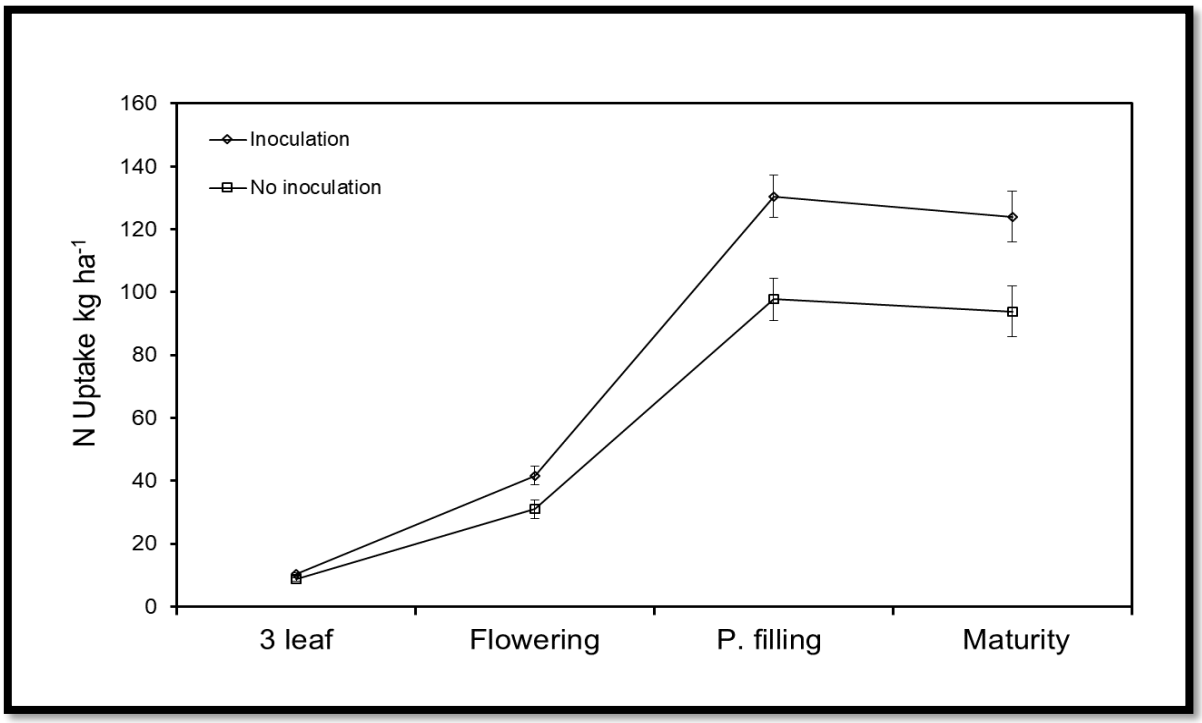

Figure 11. Nitrogen uptake pattern as a function of time.

Phosphorus uptake had the same tendency as N. The last stages exhibited higher uptake respectively. Nevertheless, it was observed that at third trifoliate leaf, P uptake was higher than in flowering, a process that was closely linked to root development (Table 14). This report contrasts with that of Peres-Soratto *et al.* (2013) who mentioned that P uptake increased only at the end of the cycle. However, Lata-Tenesaca *et al.* (2017) mentioned that P uptake was relatively low at flowering (3 kg ha^{-1}) increasing at pod filling and maturity with approximately 13 kg ha^{-1}.

The uptake pattern showed a linear increase up to the pod filling stage to decrease by almost half the amount at athe end of the season. In addition, high rates of P (50 and 100 kg ha^{-1}) absorbed more P as compared to low rates (Figure12). Furthermore, Bs treated plants exerted higher P uptake with respect to non-treated plants (Figure 13).

This finding coincides with works by García-López and Delgado (2016) who proved that *Bacillus subtilis* QST713 was effective in increasing P uptake by plants irrespective of the solubility of the P source applied (fertilizer) or the adsorption capacity of the growing medium. They also mentioned that the benefit on P uptake could be attributed to an increased P mobilization from growing media and to enhanced root growth.

Table 14. Phosphorus uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	0.50 a	2.08 b	7.97 b	3.36 b
25	0.60 a	3.22 ab	8.30 b	5.70 a
50	0.88 a	4.73 a	12.66 a	6.85 a
100	0.84 a	3.01 ab	11.34 a	5.56 a
OSL	0.2	0.03	0.004	0.003
Inoculation	0.99 a	3.88 a	10.90 a	5.81 a
No inoculation	0.41 b	2.63 a	9.24 a	4.93 a
OSL	<0.0001	0.06	0.1	0.2
0 + Bs	0.7 bc	2.7 bc	8.0 c	3.5 cd
0	0.30 d	1.4 c	7.8 c	3.2 d
25 + Bs	0.89 ab	2.78 bc	8.4 bc	5.6 bc
25	0.31 d	2.4 bc	8.1 c	5.2 bcd
50 + Bs	1.19 a	6.0 a	15.3 a	8.2 a
50	0.56 bcd	3.4 bc	10.0 bc	5.4 bcd
100 + Bs	1.20 a	3.9 ab	11.2 ab	6.1 ab
100	0.47 cd	3.2 bc	10.7 bc	5.5 bc
OSL	<0.0001	0.01	0.008	0.009
Average	0.70	3.2	10	5.3

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

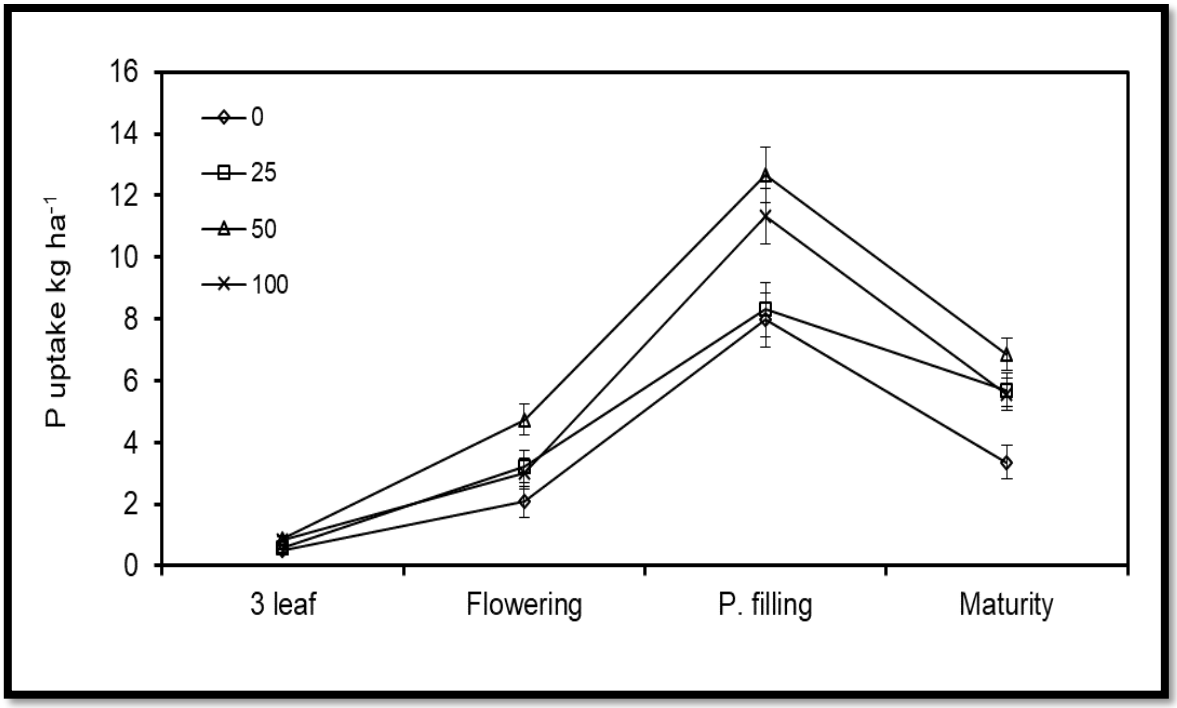

Figure 12. Phosphorus uptake pattern as a function of P rates.

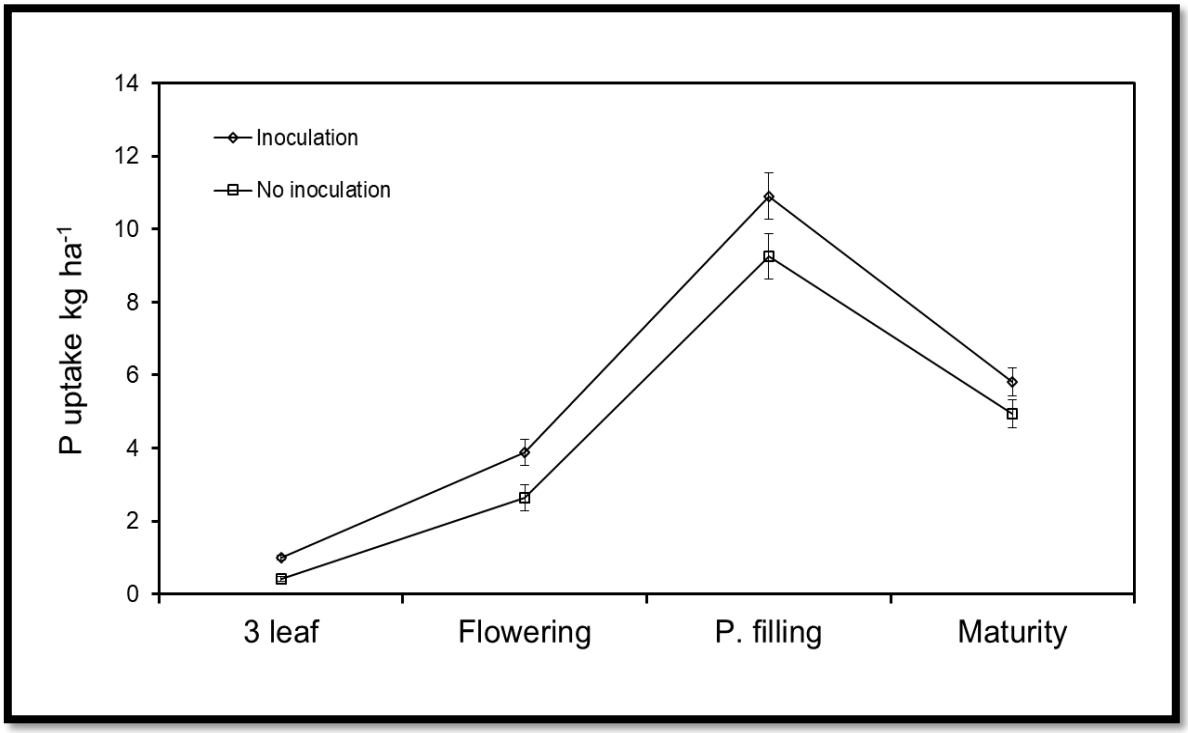

Figure 13. Phosphorus uptake pattern as a function of time.

Potassium uptake process was relatively low at the beginning with increasing uptake in later stages, reaching a peak demand at 75 DAP (Table 15). These findings are like those reported by Bertsh (2009) indicating when K uptake is higher than N especially early in the season, that can enhance dry matter production.

In this trial, the uptake pattern exhibited the same trend as in P in that maximum K acquisition was at pod filling with an average of 151 kg ha^{-1}, decreasing by half the amount at the end of the season. As in previous cases, high rates of P had greater absorption with respect to low rates of P (Figure 14).

Bs treated plants had a remarkable effect on K uptake over the season and especially at pod filling with a slight decrease at maturity (Figure 15). This indicated that Bs Q11 was effective in solubilizing not only K from the rhizosphere to plants roots but also some other nutrients that in a certain time could replenish soil solution.

As indicated by Murugan *et al.* (2011), application of biofertilizers *Rhizobium leguminosarum-phaseoli* and/or *Pseudomonas fluorescence* combined with neem cake improved nutrient availability (C, N, P) and productivity of black beans (*Vigna mungo* L.) but the combination with the same biofertilizers with farmyard manure improved microbial population in the root zone.

Table 15. Potassium uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	5.34 c	35.55 b	111.66 b	40.97 b
25	7.80 bc	49.47 ab	125.27 b	75.62 a
50	12.45 a	56.24 a	197.2 a	84.8 a
100	10.79 ab	57.33a	172.2 a	81.97 a
OSL	0.001	0.06	0.001	0.003
Inoculation	10.81 a	57.66 a	171.7 a	79.01 a
No inoculation	7.38 b	41.63 b	131.43 b	62.69 a
OSL	0.002	0.02	0.01	0.1
0 + Bs	6.9 cd	48.9 a	107.5 d	41.0 c
0	3.8 d	22.2 b	106.7 d	40.8 c
25 + Bs	9.7 abc	58.3 a	143.0 bcd	76.8 ab
25	5.9 cd	37.5 ab	116.3 cd	65.5 bc
50 + Bs	13.36 a	62.0 a	226.7 a	104.1 a
50	11.55 ab	50.5 a	143.5 bcd	74.3 b
100 + Bs	13.35 a	61.4 a	210 ab	94.2 ab
100	8.2 bc	56.3 a	167.6 bc	69.7 bc
OSL	<0.0001	0.4	0.003	0.003
Average	9.0	49.64	151.5	74.1

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

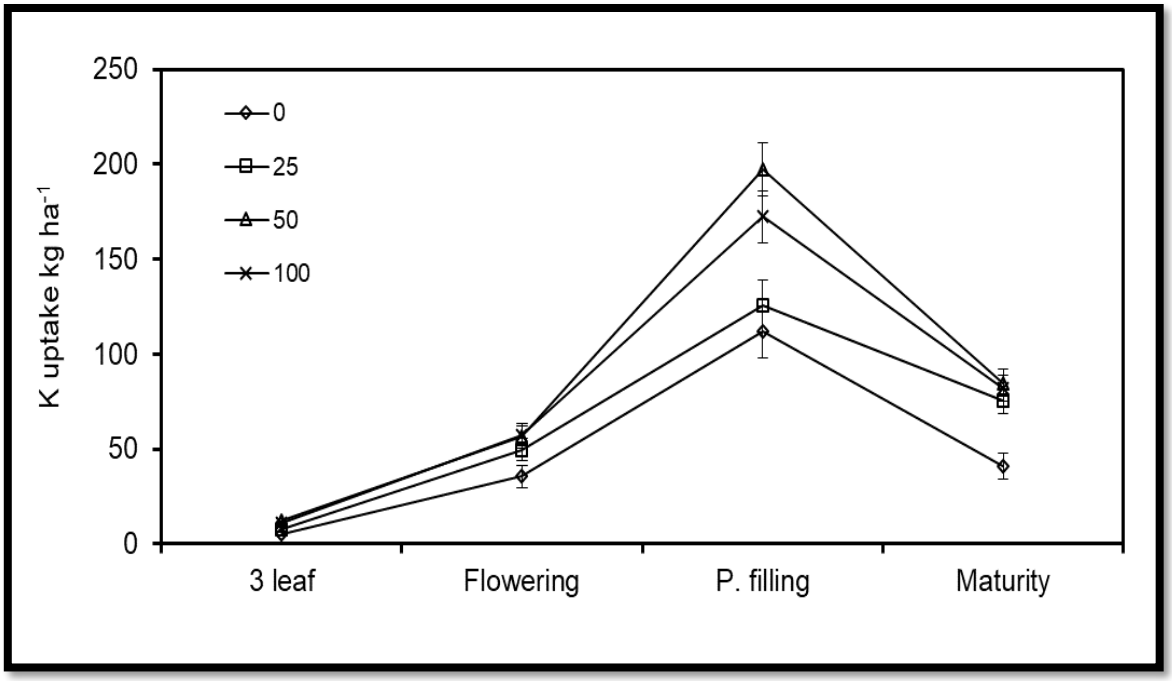

Figure 14. Potassium uptake pattern as a function of P rates.

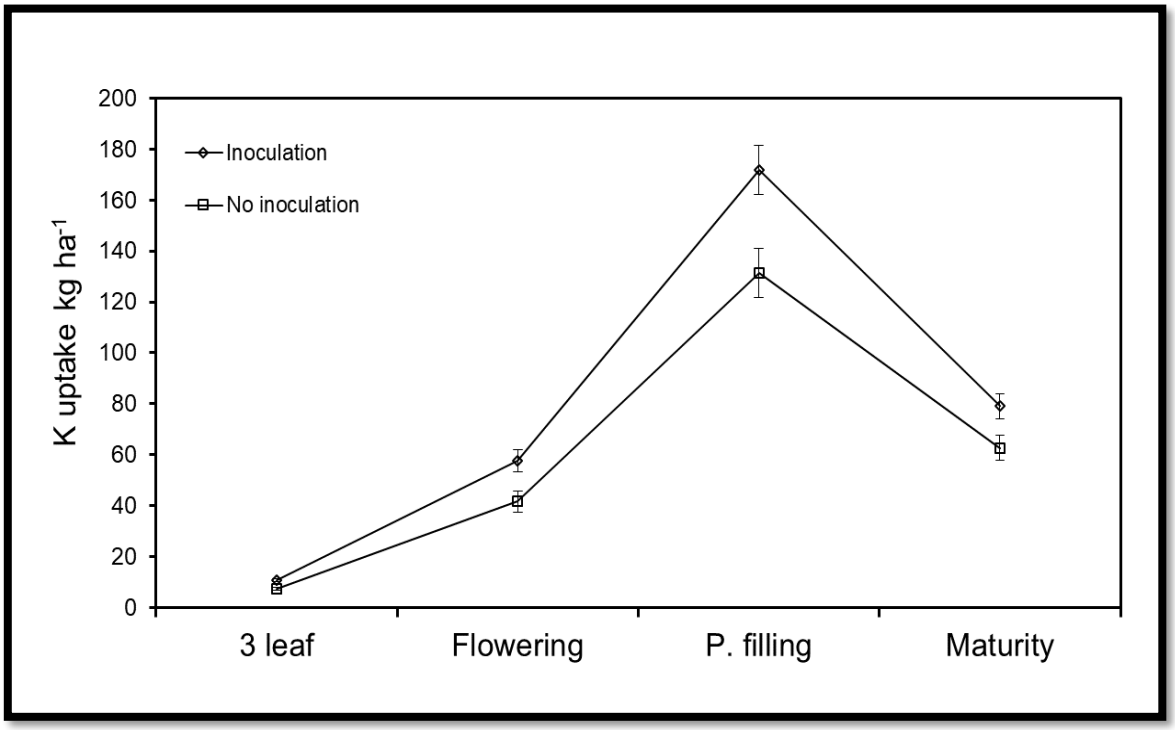

Figure 15. Potassium uptake pattern as a function of time.

Calcium uptake was relatively constant up to flowering, increasing exponentially at pod filling (116 kg ha^{-1}) and declining at maturity (Table 16). High rates of P exhibited the higher Ca uptake (Figure 16). On the other hand, inoculation with Bs exerted a positive effect on the uptake process all over the season. At pod filling, treated plants absorbed up to 133 kg ha^{-1} as compared to non-treated plants (Figure 17).

According to some reports, Ca content can be very variable depending upon variety and species (Padilla, 2005). Other like Peres-Soratto *et al.* (2013) stated that high amounts of Ca uptaken and accumulated in reproductive structures was strongly related to formation of calcium pectate in cell walls, thus the supply of this nutrient is essential for good development of fruit and seeds (Marschner, 1995).

Magnesium uptake also occurred in a consistent manner at pod filling and slightly declined at physiological maturity (Table 17 and Figure 18). In addition, inoculation favored Mg uptake during the season especially in the last two stages of growth (Figure 19). In that manner, Yao *et al.* (2006) reported that biofertilizing with *Bacillus subtilis* FZB 241® can enhance overall growth by mobilizing and taking up more nutrients and substances. However, they stated that the effectiveness of the product depends on climatic conditions, soil type, fertility level as well as presence and availability of plant nutrients.

Table 16. Calcium uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	5.01 b	5.74 b	75.39 b	15.09 d
25	5.47 b	8.13 ab	90.30 b	35.33 c
50	7.65 a	11.21 a	154.8 a	72.47 a
100	6.75 ab	9.06 ab	143.7 a	58.15 b
OSL	0.05	0.01	0.001	<0.0001
Inoculation	6.61 a	11.27 a	133.0 a	51.32 a
No inoculation	5.83 a	5.80 b	99.11 b	39.20 a
OSL	0.2	<0.0001	0.05	0.2
0 + Bs	5.95 abc	3.1 c	78.9 c	16.9 d
0	4.0 c	2.3 c	72 c	13.2 d
25 + Bs	6.35 abc	8.9 b	100 bc	63.7 ab
25	4.61 bc	8.3 b	80.5 c	24.9 d
50 + Bs	8.16 a	14.0 a	178.6 a	78.8 a
50	7.15 a	8.6 b	108.8 bc	45.7 c
100 + Bs	7.02 ab	13.8 a	174.3 a	66.1 ab
100	6.5 abc	9.2 b	135.2 ab	52.5 bc
OSL	0.1	0.01	0.001	<0.0001
Average	6.22	8.53	116.4	90.5

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

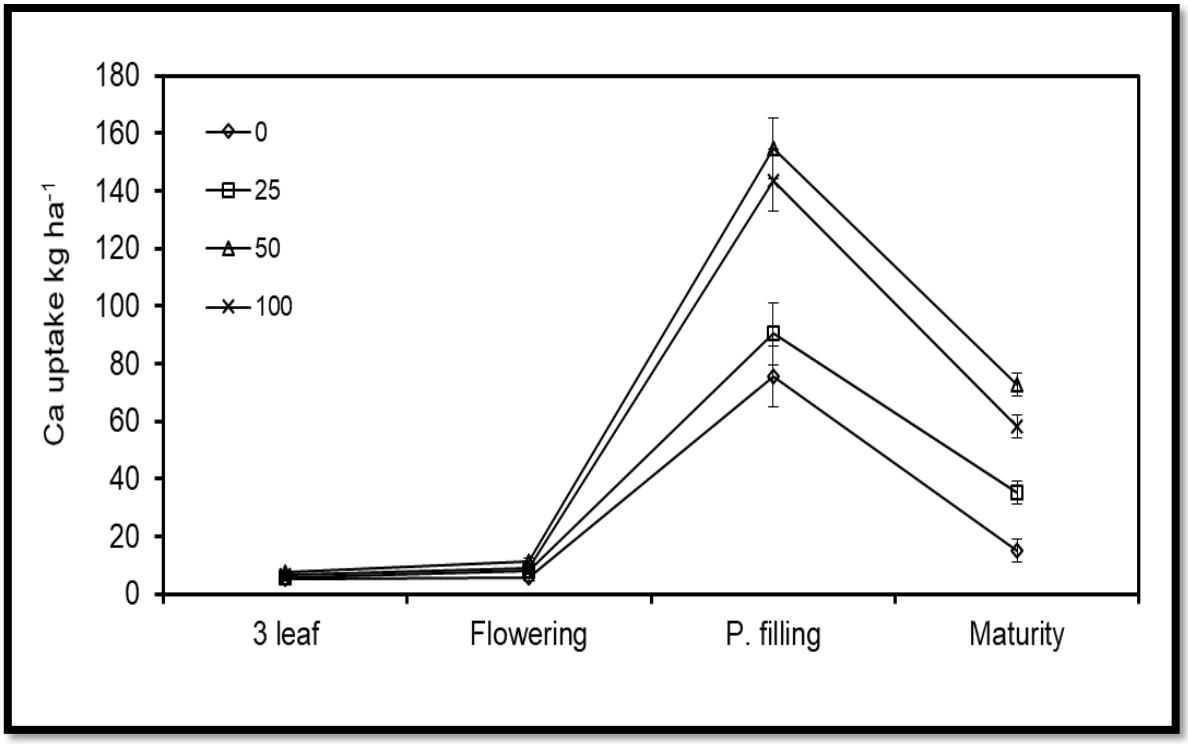

Figure 16. Calcium uptake pattern as a function of P rates.

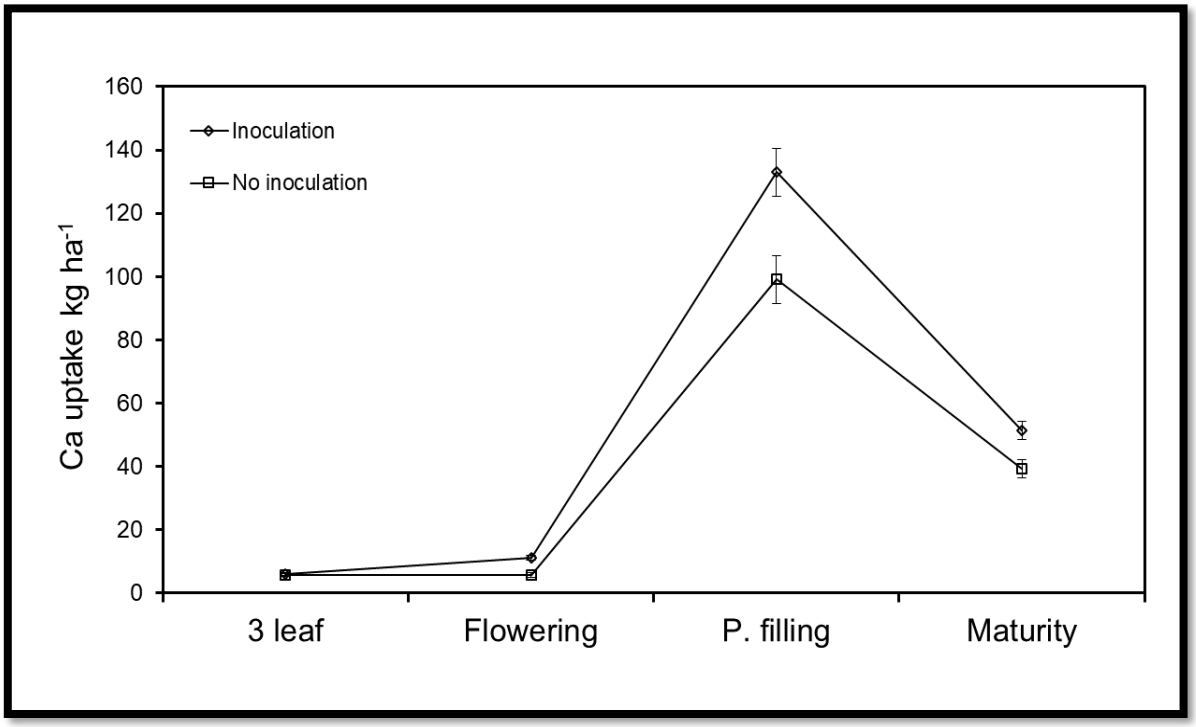

Figure 17. Calcium uptake pattern as a function of time.

Table 17. Magnesium uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	1.62 a	3.01 a	25.33 b	13.95 b
25	1.32 a	3.05 a	29.76 b	26.15 ab
50	1.80 a	3.87 a	55.76 a	35.66 a
100	1.59 a	2.54 a	44.61 a	30.14 a
OSL	0.2	0.4	<0.001	0.01
Inoculation	1.79 a	3.89 a	44.52 a	31.82 a
No inoculation	1.38 b	2.34 b	33.21 b	21.12 b
OSL	0.02	0.01	<0.001	0.04
0 + Bs	1.39 ab	2.2 b	23.1 c	15.1 cd
0	0.96 b	1.8 b	22 c	10.5 d
25 + Bs	1.80 a	3.8 ab	35.5 bc	32.6 ab
25	1.7 a	2.4 b	27.5 bc	17.3 cd
50 + Bs	1.87 a	5.0 a	59.4 a	45.1 a
50	1.73 a	2.7 ab	37.4 b	26.1 bc
100 + Bs	1.81 a	4.1 ab	53.7 a	38.6 ab
100	1.4 ab	2.6 b	52.1 a	26.1 bc
OSL	0.1	0.1	0.001	<0.0001
Average	1.58	3.12	38.8	26.4

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

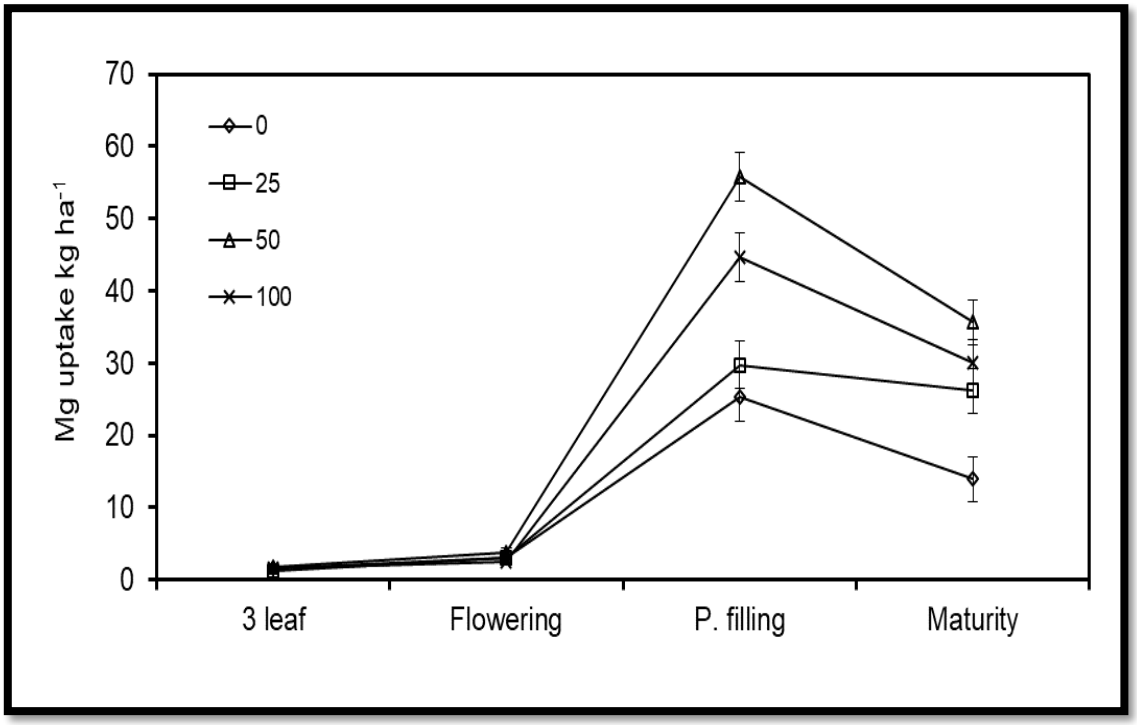

Figure 18. Magnesium uptake pattern as a function of P rates.

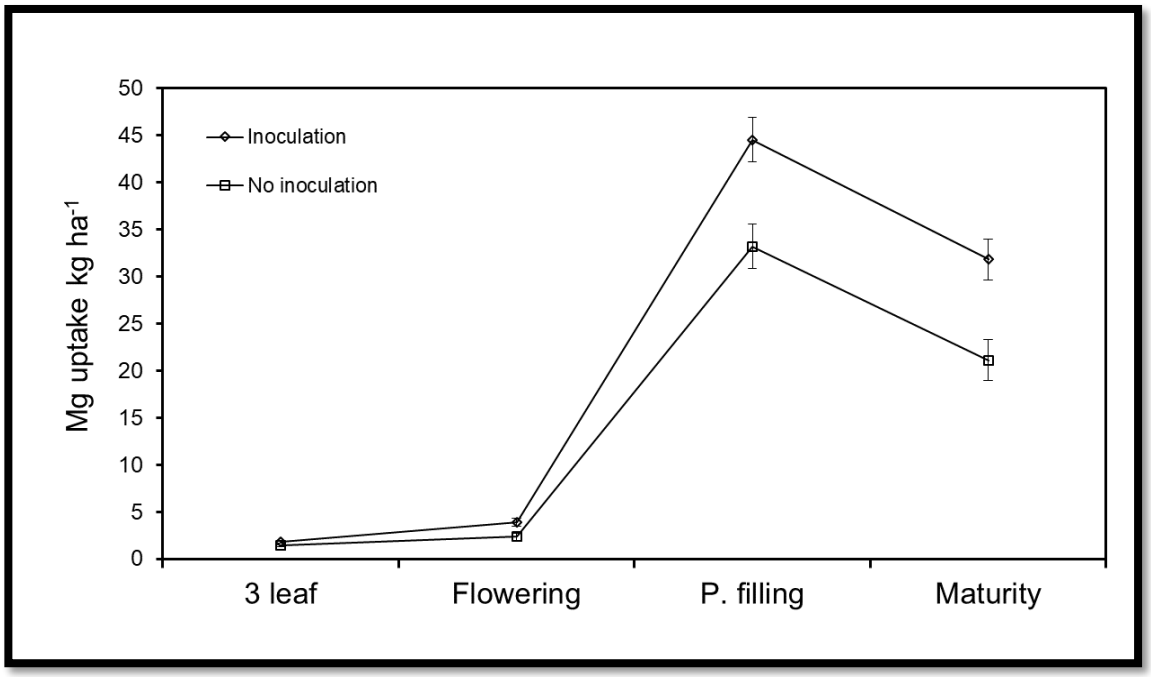

Figure 19. Magnesium uptake pattern as a function of time.

4.3.3 Seasonal uptake and flux.

Total macronutrient uptake increased gradually in response to P rates and inoculation with *B. subtilis* in which the higher P rates exhibited the higher absorption. The uptake preference at pod filling stage was in the following order: K> Ca> N> Mg> P. At physiological maturity was N> Ca> K> Mg> P approximately (Tables 13 through 17). At the end of the season total uptake was K> N> Ca> Mg> P in which the last stages of growth were the most active for nutrient uptake (Table 18). The inoculated treatments had an increase on the uptake process of 27, 27, 24, 34 and 13% with respect to non-inoculated plants for each nutrient respectively.

The overall uptake pattern showed that at pod filling stage all nutrients were uptaken in higher amounts due to the greater demand of pods for size and weight. However, by physiological maturity the uptake process slightly declined for most nutrients except for nitrogen that remained relatively constant (Figure 20).

The uptake process of these nutrients was very similar to that reported by Lata-Tenesaca *et al.* (2017) that found a total uptake of N>K>Ca>Mg>P with approximately 221, 186, 163, 29 and 24 kg ha^{-1}. On the other hand, Vieira *et al.* (2009) also found that the maximum uptake was N>K>Ca>Mg>P in different bean cultivars grown under different tilling techniques and plant densities, while Pergoraro *et al.* (2014) reported that nutrient uptake was K>N>Ca>P>Mg with 138, 112, 66, 16, 7 kg ha^{-1} respectively.

Peres-Soratto *et al.* (2013) reported that uptake preference was in the following order: N> K> Ca> P> Mg> S. Finally, Bray (1954) proposed that nutrient mobility in soils is largely determined by the ionic state at which nutrients reach absorbing root surfaces and that partially influence the effectiveness of nutrient applications (Havlin *et al.,* 2005).

Table 18. Seasonal macronutrient uptake (kg ha^{-1}) as a function of phosphorus rates and *Bacillus subtilis*.

Rates (P_2O_5) kg ha^{-1}	N	P	K	Ca	Mg
0	180.98 b	13.92 b	193.53 c	101.24.b	43.95 c
25	280.1 a	18.99 a	267.4 b	139.2 b	64.2 bc
50	322.9 a	23.98 a	316.6 ab	228.8 a	76.44 ab
100	291.7 a	20.75 a	347.4 b	235 a	95.66 a
OSL	0.002	0.004	<0.0001	<0.0001	<0.0001
Inoculation	306.4 a	21.60 a	319.2 a	202.2 a	82.03 a
No inoculation	231.5 b	17.23 b	243.1 b	150 b	58.06 b
OSL	0.01	0.04	0.01	0.001	0.02

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), Observed significance level (OSL Pr> F).

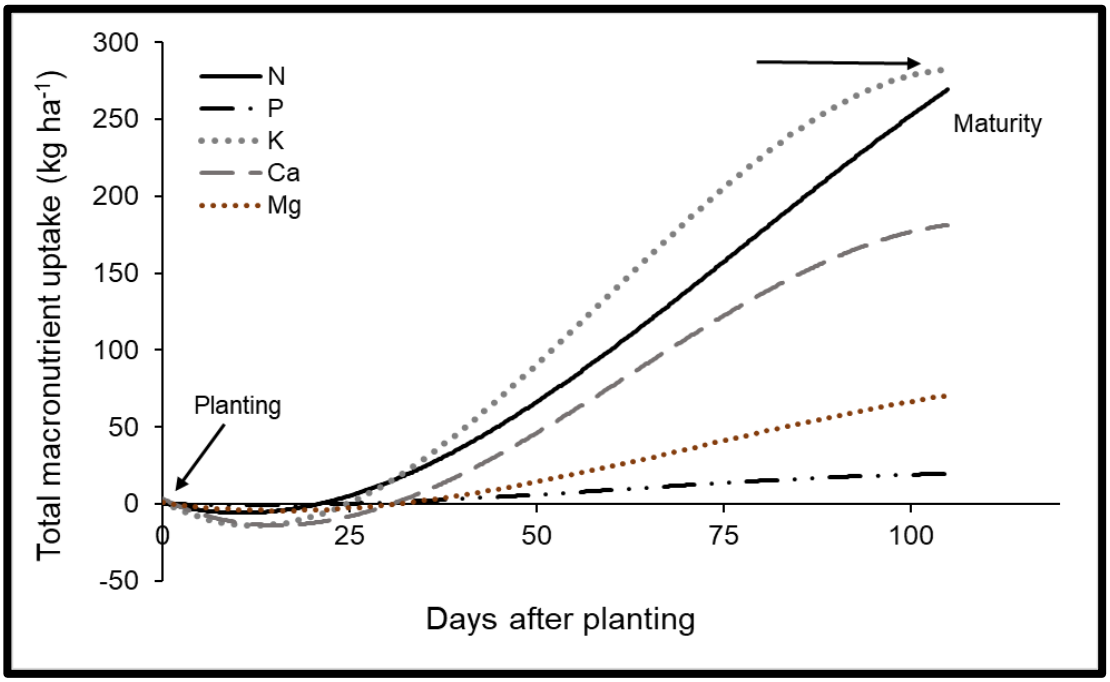

Figure 20. Seasonal macronutrient uptake pattern.

Regression equation	R^2
$N = -0.0003\ DAP^3 + 0.0663\ DAP^2 - 1.3032\ DAP + 0.9313$	0.99
$P = 0.0012\ DAP^2 + 0.0792\ DAP - 0.8443$	0.96
$K = -0.0007\ DAP^3 + 0.1257\ DAP^2 - 2.7366\ DAP + 1.8419$	0.99
$Ca = -0.0005\ DAP^3 + 0.0871\ DAP^2 - 2.3346\ DAP + 3.203$	0.97
$Mg = -0.0001\ DAP^3 + 0.0251\ DAP^2 - 0.6977\ DAP + 0.9456$	0.98

Total maximum uptake rate pattern for all nutrients showed an increase during the season in which the peak demand coincided with the most critical stage of development and maximum rates of dry weight production (Figure 21).

The average rate for all treatments at pod filling (75 DAP) was K> N~ Ca> Mg> P with 5, 3.8, 3.8, 1.3 and 0.3 kg ha^{-1} day^{-1} in the same order. At maturity the uptake preference was N> K> Ca> Mg> P with 3.5, 2.4, 1.5, 0.9 and 0.2 kg ha^{-1} day^{-1} respectively.

As seen from the pattern, the uptake process early in the season was relatively low by then increase in later stages of growth, a process that was related to nutrient's movement in the soil from its initial deposition, concentration in soil solution and moisture level. This trend pattern was consistent to that reported by Peres-Soratto *et al.* (2013) in which the highest demand of macronutrients occurred in a period between 45 and 70 DAP.

In order to explain that process clearly, Jungk and Claassen (1997) state that nutrients reach root surfaces for uptake via morphological growth of roots and the physical and chemical transport of nutrients to root sorbing surfaces. These principal methods explain the three specific mechanisms of nutrient uptake: i) root interception, ii) mass flow of nutrients in solution, and iii) diffusion of nutrients in solution. The first major mechanism is the primary mode of nutrient uptake resulting from root growth through ion exchange between soil and root surfaces (Havlin *et al.,* 2005). Thus physical and chemical properties of soil play a key role in the extent of reactions that favor or inhibit the uptake by plants.

Accordingly, some other important factors are related to agronomic management practices including planting density, hybrid and trait background selection, and soil fertility level that can affect nutrient removal, uptake and subsequent maintenance of fertilizer application rates (Below *et al.,* 2010; Raymond *et al.,* 2009). Extreme planting populations, those above and below recommended levels, have reduced nutrient uptake despite continued increases in yield with increasing plant populations (Raymond *et al.,* 2009).

Under optimal management and environmental conditions, nutrient mineralization, nutrient supply and applied fertilizer meet crop needs with nutrient availability. However, the ability of the root systems to acquire these supplied nutrients is influenced by abiotic (salt-compaction-dehydration) and biotic (pest feeding) problems in the rhizosfere.

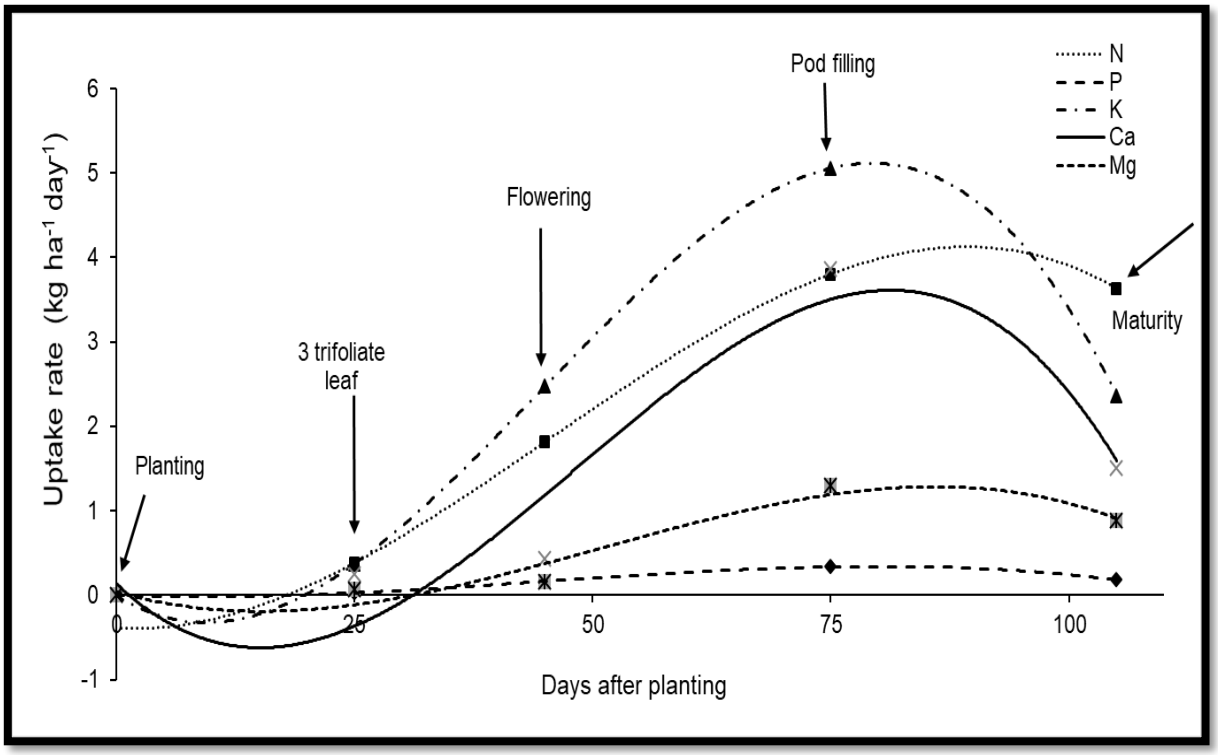

Figure 21. Seasonal macronutrient uptake rate pattern.

Regression equation	R^2
N = -1E-05 DAP^3 + 0.0019 DAP^2 - 0.0074 DAP - 0.3863	1
P = -2E-06 DAP^3 + 0.0003 DAP^2 - 0.0043 DAP + 0.0002	1
K = -3E-05 DAP^3 + 0.0042 DAP^2 - 0.0712 DAP - 0.0014	1
Ca = -3E-05 DAP^3 + 0.0042 DAP^2 - 0.1076 DAP + 0.1392	0.88
y = -8E-06 DAP^3 + 0.0013 DAP^2 - 0.0325 DAP + 0.04	0.93

4.3.4 Macronutrient acquisition timing.

Considering the amount of nutrients, it was found that the crop took up only 20% of the total up to flowering for primary nutrients. For secondary nutrients, around 7% was taken up at the same stage. This indicated that the most intense period of nutrient acquisition was the second half of the season. Therefore, between flowering and pod formation, the supply of nutrients in soil solution could be critical to prevent nutrient stress during the season (depending upon the nutrient source and irrigation management) (Figure 22).

The acquisition process was very similar to that reported by Lata-Tenesaca *et al.* (2017) that found that the greater demand for macronutrients occurred between 65 and 89 DAP. Vieira *et al.* (2009) also found the maximum uptake at the end of the season in different bean cultivars grown under different tilling techniques and plant densities. Besides, Peres-Soratto *et al.* (2013) reported no K uptake after 50-55 DAP. They also reported that the period of higher demand for N-P-Ca-Mg occurred between 45 and 55 DAP.

This result differed from that of Brito *et al.* (2009) who reported that approximately 80% of N was absorbed in pre-bloom, but agrees from those of Lata-tenesaca *et al.* (2017) who reported a continuous increase of N uptake in the last stage of growth. While Peres-Soratto *et al*. (2013) stated that N absorption was higher during pod formation (45 to 55 DAE) and on those treatments with 100 % of recommended NPK fertilization.

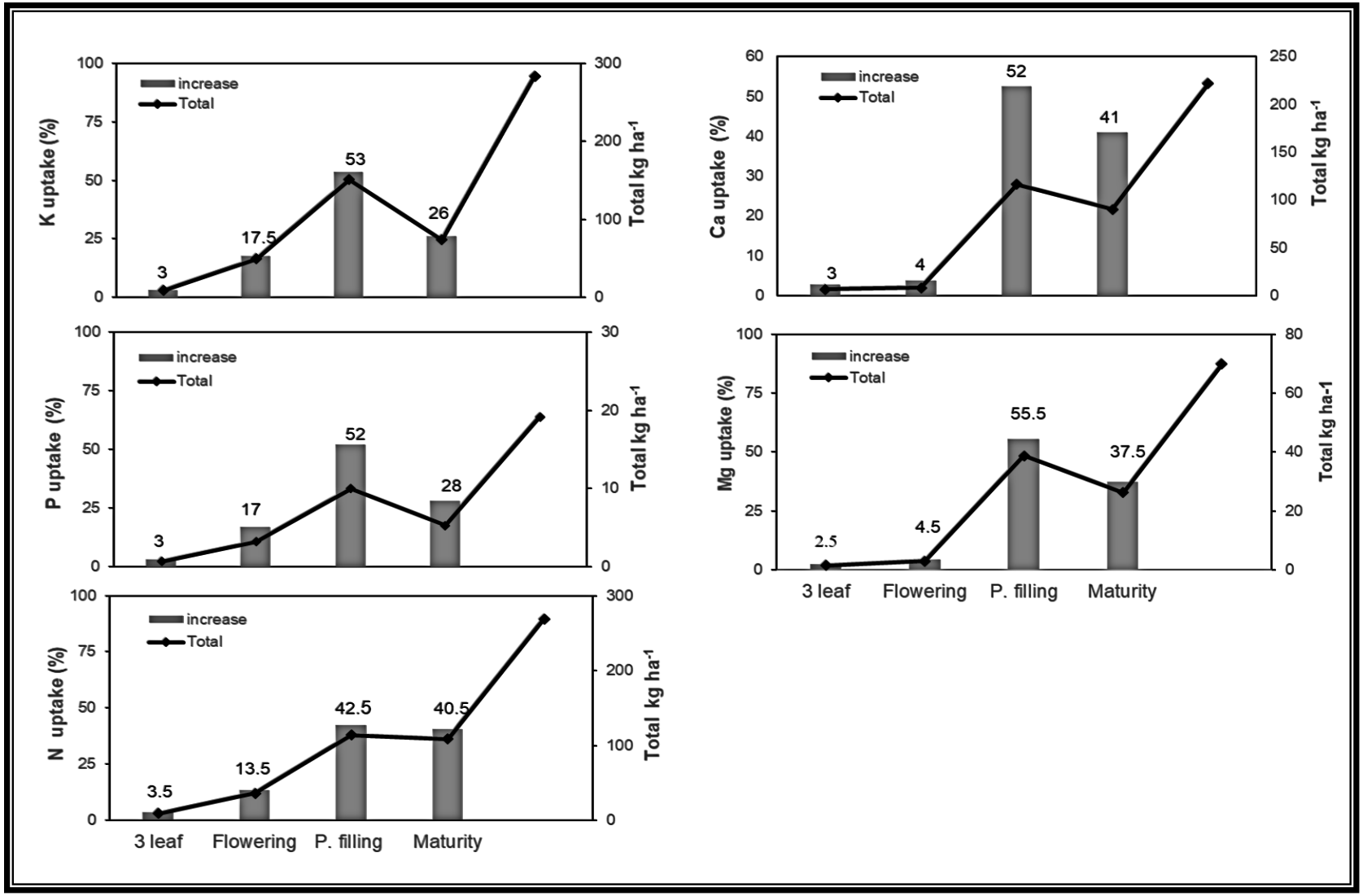

Figure 22. Stages of maximum macronutrient demand (% of total).

4.3.5 Macronutrient partitioning.

Distribution of nutrients in vegetative organs was 100% up to flowering, in later stages around 30% was partitioned in reproductive organs. At pod filling stage, distribution was in the following manner: Ca> K> N> Mg> P in vegetative organs, whereas in reproductive organs was P> Mg> N> K> Ca. At physiological maturity, distribution was registered as N> Ca~ K> Mg> P in vegetative parts and P> Mg> K~ Ca> N in reproductive organs (Tables 19 through 23). In these last two stages, N was distributed approximately 70% in vegetative organs whereas the rest was allocated in the reproductive structure (Table 19).

During pod filling, 40% of P was translocated to pods and 60% in vegetation. At maturity, there was a shift in the distribution pattern with a higher percent of translocation to pods (Table 20). Approximately 82 and 70% of K were distributed in vegetative organs in the last two stages (Table 21). Some works by Vargas *et al.* (2013) mentioned that K was usually uptaken early during vegetative growth and then translocated to other plant parts. 88% of calcium was taken up at pod filling and 70% at the end of the season while 30% was taken up by pods only (Table 22). Magnesium distribution in vegetation was around 65% whereas the rest was allocated in pods (Table 23).

Considering the total uptake, approximately 200 kg ha^{-1} of N were partitioned to vegetation and only 68 kg ha^{-1} were allocated in fruits (Figure 23). P distribution in fruits was of 11 kg ha^{-1} and 8 kg ha^{-1} in vegetation (Figure 24). K distribution in vegetation was similar to nitrogen whereas only 86 kg ha^{-1} were distributed in fruits (Figure 25). Calcium partitioning process was 157 kg ha^{-1} in vegetation and 65 kg ha^{-1} in fruits (Figure 26). Finally, Mg allocation was of 4 kg ha^{-1} in vegetation and 0.2 kg ha^{-1} in fruits (Figure 27).

Table 19. Nitrogen distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	73.30 a	26.70 b	77.17 a	22.83 b
25	100	0	100	0	70.24 ab	29.76 ab	77.0 a	22.99 b
50	100	0	100	0	66.44 b	33.55 a	72.84 b	27.16 a
100	100	0	100	0	69.02 ab	30.98 ab	72.87 b	27.13 a
OSL	NS	NS	NS	NS	0.003	0.003	0.003	0.003
Inoculation	100	0	100	0	66.72 b	33.28 a	73.2 b	26.81 a
No inoculation	100	0	100	0	72.78 a	27.22 b	76.7 a	23.25 b
OSL	NS	NS	NS	NS	<0.001	<0.001	0.001	0.001
Average	100	0	100	0	70	30	75	25

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

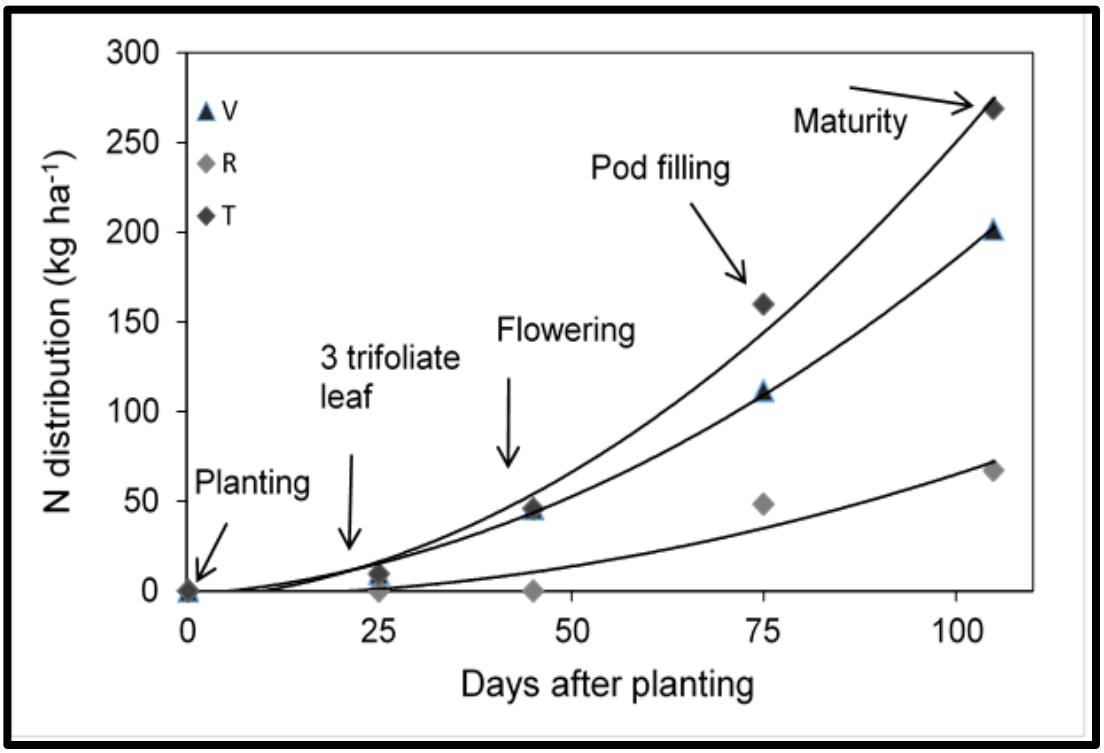

Figure 23. Total nitrogen distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 20. Phosphorus distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	60.35 a	39.65 a	45.35 a	54.65 a
25	100	0	100	0	59.50 a	40.50 a	41.56 a	58.44 a
50	100	0	100	0	58.83 a	41.17 a	35.76 a	64.24 a
100	100	0	100	0	58.88 a	41.12 a	40.71 a	59.29 a
OSL	NS	NS	NS	NS	0.9	0.9	0.8	0.8
Inoculation	100	0	100	0	48.65 b	51.35 a	26.95 b	73.05 a
No inoculation	100	0	100	0	70.13 a	29.9 b	54.74 a	45.26 b
OSL	NS	NS	NS	NS	<0.001	<0.001	<0.001	<0.001
Average	100	0	100	0	59	41	41	59

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

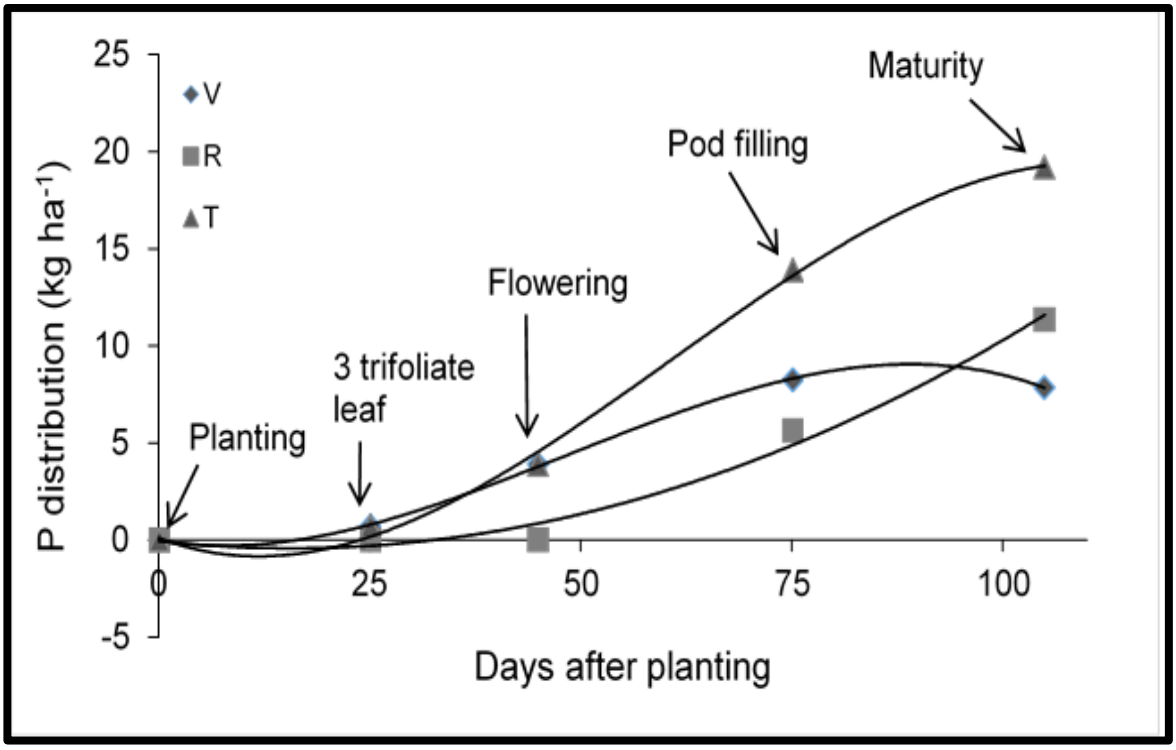

Figure 24. Total phosphorus distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 21. Potassium distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	84.14 a	15.86 a	74.06 a	25.94 a
25	100	0	100	0	82.42 a	17.58 a	71.34 a	28.66 a
50	100	0	100	0	80.47 a	19.53 a	66.94 a	33.06 a
100	100	0	100	0	81.21 a	18.79 a	67.65 a	32.35 a
OSL	NS	NS	NS	NS	0.2	0.2	0.3	0.3
Inoculation	100	0	100	0	77.5 b	22.44 a	64.0 b	35.96 a
No inoculation	100	0	100	0	86.55 a	13.44 b	75.6 a	24.04 b
OSL	NS	NS	NS	NS	<0.001	<0.001	0.001	0.001
Average	100	0	100	0	82	18	70	30

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

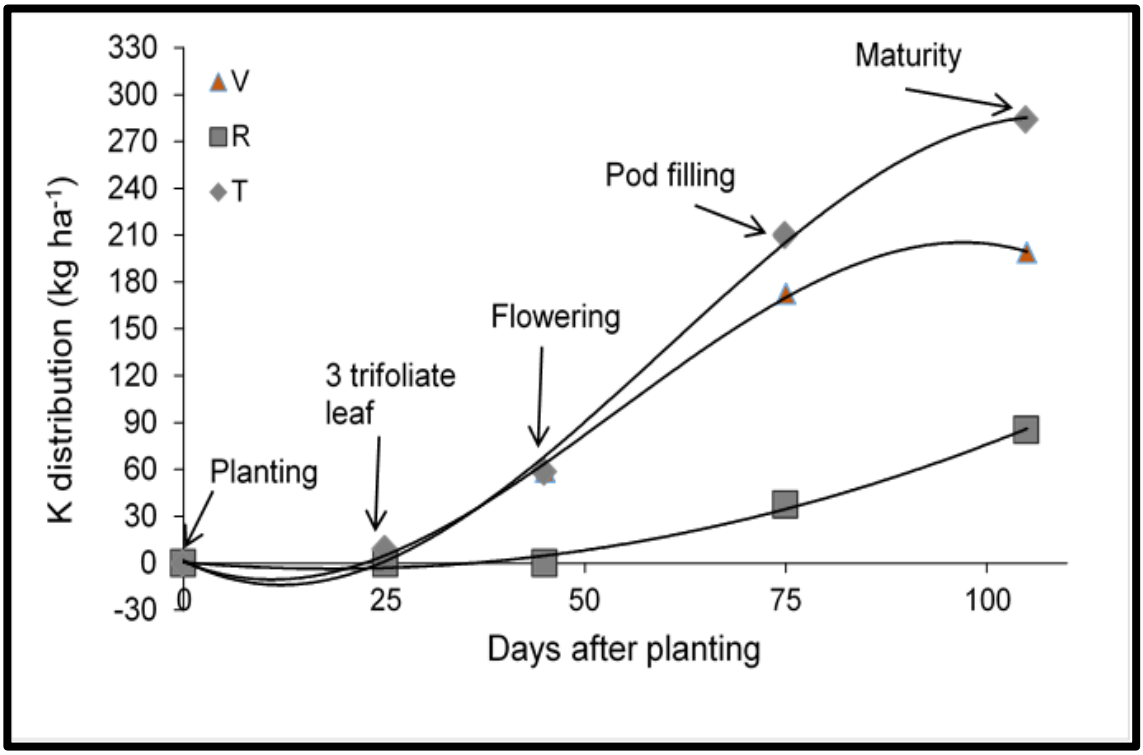

Figure 25. Total potassium distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 22. Calcium distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	89.61 a	10.39 a	85.75 a	14.25 c
25	100	0	100	0	88.77 a	11.23 a	81.16 a	18.84 c
50	100	0	100	0	86.92 a	13.08 a	53.68 c	46.32 a
100	100	0	100	0	87.77 a	12.22 a	61.98 b	38.02 b
OSL	NS	NS	NS	NS	0.1	0.1	0.001	0.001
Inoculation	100	0	100	0	86.2 b	13.81 a	65.72 a	34.28 a
No inoculation	100	0	100	0	90.35 a	9.64 b	75.56 a	24.44 a
OSL	NS	NS	NS	NS	<0.0001	<0.0001	0.1	0.1
Average	100	0	100	0	88	12	71	29

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

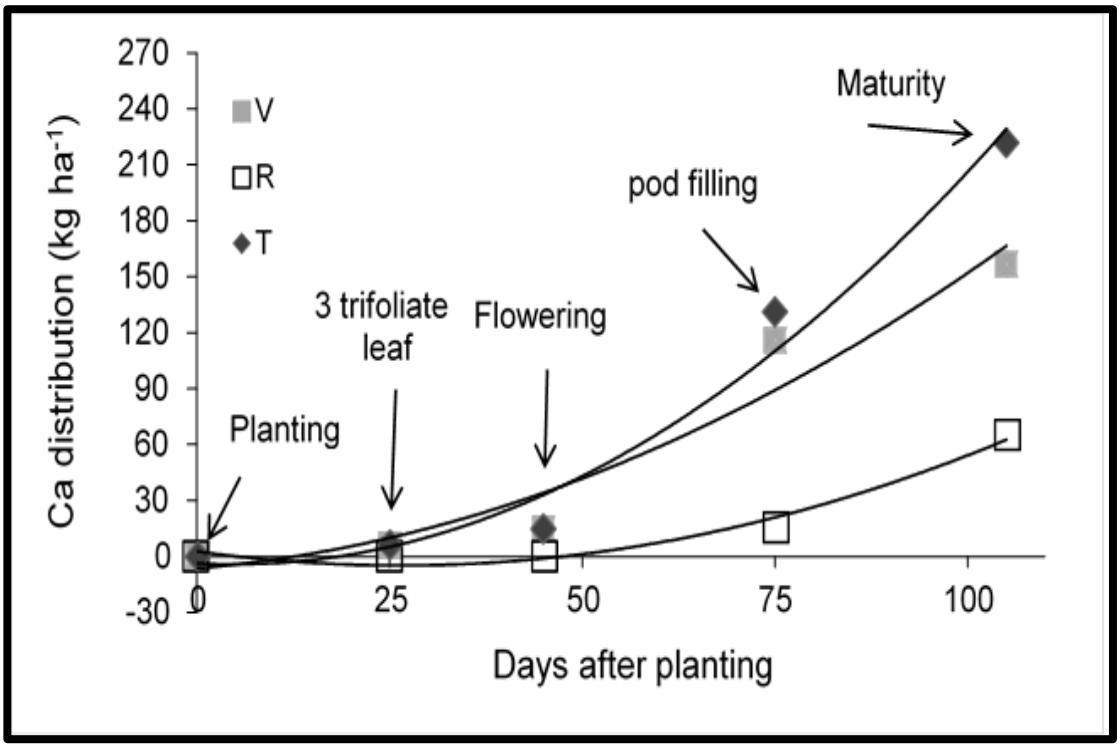

Figure 26. Total calcium distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 23. Magnesium distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	75.52 a	24.48 b	73.57 a	26.43 a
25	100	0	100	0	69.23 ab	30.77 ab	66.74 ab	33.26 ab
50	100	0	100	0	63.61 b	36.39 a	50.95 b	49.05 a
100	100	0	100	0	66.76 ab	33.24 ab	55.30 b	44.70 a
OSL	NS	NS	NS	NS	<0.001	<0.001	<0.001	<0.001
Inoculation	100	0	100	0	60.73 b	39.27 a	50.10 b	49.5 a
No inoculation	100	0	100	0	76.82 a	23.17 b	73.17 a	26.83 b
OSL	NS	NS	NS	NS	<0.001	<0.001	<0.001	<0.001
Average	100	0	100	0	69	31	62	38

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

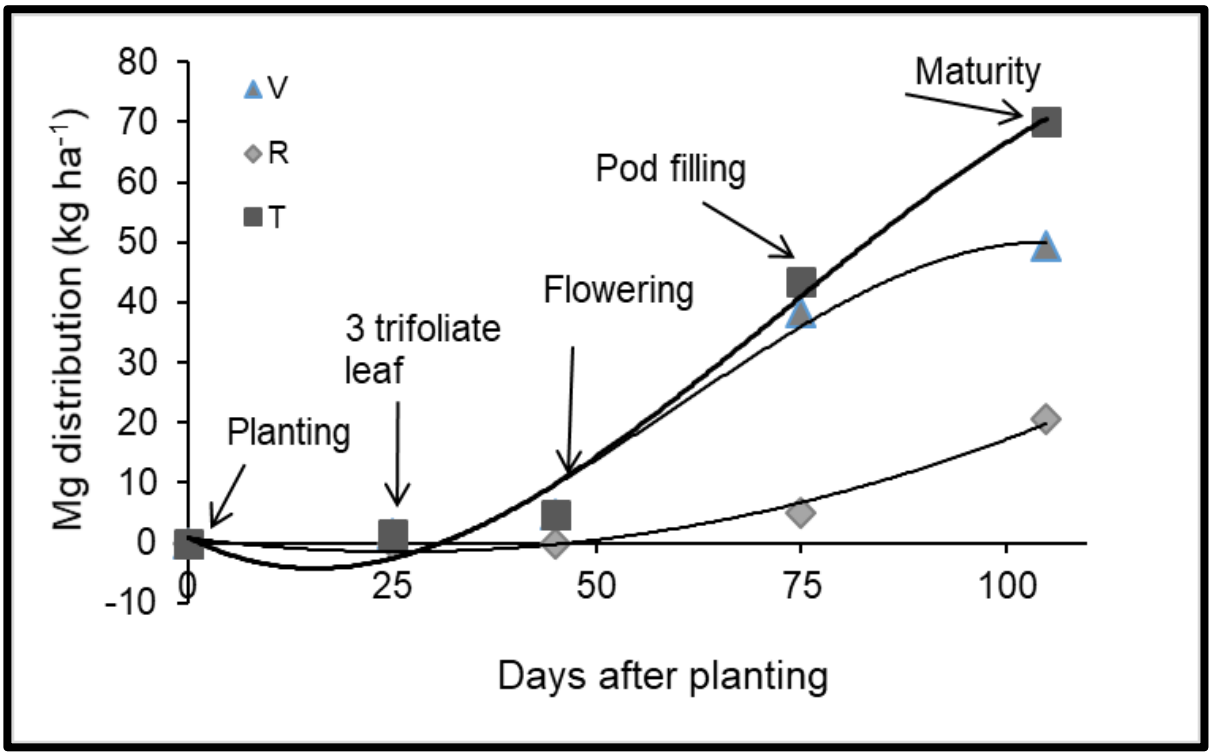

Figure 27. Total magnesium distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

4.3.6 Micronutrient concentration in plant tissues.

The concentration of micronutrients in vegetative and reproductive structures was significantly different ($P \leq 0.05$) in response to the application of phosphorus and inoculation with *Bacillus subtilis* (Bs). Also, it was observed that the highest concentrations were synchronized with stages of maximum demand and were also higher in leaves than stems throughout the season.

Iron (Fe) concentration in leaves started to increase as early (third trifoliate leaf) until the end of the season (physiological maturity). Rates of 50 and 100 kg ha^{-1} exhibited the highest concentration range compared to 0 kg ha^{-1} in each stage. Total concentrations in leaves reached values from 2.0 up to 4.6 g kg^{-1} of dry matter (DM) in the second half of the season while the concentration range in stems was much lower (0.05, through 0.18 g kg^{-1} of DM). The effect of Bs promoted an increase of 46% of Fe concentration in tissues compared to non-inoculated plants (Table 24). According to it, Tofiño-Rivera *et al.* (2016) mentioned that P fertilization can increase Fe concentration but not that of Zn in plants and/or seeds, which agrees with the results of this work in that Fe, was the nutrient highly concentrated.

The concentration of manganese (Mn) increased over time. At physiological maturity, the concentration in leaves was approximately twice greater than that found in stems. Besides, rates of 50 and 100 kg ha^{-1} accumulated the greatest amount. Inoculated treatments enhanced approximately 26% of Mn in all tissues, whereas the non-inoculated treatments decreased 15% regarding the total concentration (1.5 g kg^{-1} of DM) at the end of the season (Table 25).

Copper (Cu) concentration in leaves was relatively stable and higher than concentration in stems throughout the season. Rates of 50 and 100 kg ha^{-1} exhibited the highest concentrations. At physiological maturity, total concentration on Bs inoculated plants enhanced 42% while non-inoculated plants decreased by 17% with respect to overall concentration (0.12 g kg^{-1} of DM) (Table 26).

Table 24. Iron concentration in vegetative and reproductive organs of common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0	2.23 b	0.16 b	3.1 b	0.08 a	1.5 c	0.048 bc	0.56 a	3.5 b	0.09 c	0.560 a
25	2.51 b	0.15 b	3.2 ab	0.09 a	2.0 b	0.042 c	0.59 a	4.3 ab	0.15 b	0.597 a
50	5.12 a	0.25 a	3.1 ab	0.11 a	2.1 b	0.060 ab	0.70 a	5.5 a	0.24 a	0.707 a
100	4.49 a	0.20 ab	4.3a	0.09 a	2.7 a	0.066 a	0.67 a	5.5 a	0.24 a	0.677 a
OSL	0.002	0.004	0.001	0.4	<0.001	0.01	0.7	0.03	<0.001	0.7
Inoculation	4.48 a	0.22 a	4.3 a	0.11 a	2.3 a	0.062 a	0.80 a	5.7 a	0.20 a	0.80 a
No inoculation	2.69 b	0.15 b	2.5 b	0.076 b	1.8 b	0.046 b	0.46 b	3.6 b	0.16 a	0.46 b
OSL	0.009	0.006	<0.001	<0.001	0.005	0.003	0.001	<0.001	0.1	0.001
0 + Bs	2.8 c	0.19 bc	3.8 d	0.107 c	1.7 de	0.053 c	0.687 ab	4.6 c	0.10 e	0.687 ab
0	1.5 d	0.14 cd	2.4 e	0.072 e	1.2 f	0.043 e	0.416 b	2.3 e	0.07 f	0.416 b
25 + Bs	3.2 c	0.18 bcd	4.0 c	0.11 b	2.3 c	0.043 e	0.777 ab	6.1 a	0.15 c	0.777 ab
25	1.8 d	0.12 d	2.4 e	0.067 f	1.7 e	0.042 e	0.433 b	2.5 d	0.14 d	0.433 b
50 + Bs	7.0 a	0.32 a	4.6 b	0.14 a	2.5 b	0.069 b	0.883 a	6.1 a	0.27 a	0.883 a
50	3.1c	0.18 bcd	1.6 f	0.082 d	1.8 d	0.051 c	0.530 ab	4.8 b	0.21 b	0.530 ab
100 + Bs	4.7 b	0.21 b	4.9 a	0.11 b	2.9 a	0.085 a	0.880 a	6.1 a	0.27 a	0.880 a
100	4.2 b	0.18 bcd	3.8 d	0.085 d	2.5 b	0.047 d	0.473 b	4.8 b	0.21 b	0.473 b
OSL	0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.1	<0.001	<0.001	0.1
Average	3.5	0.19	3.4	0.009	2.0	0.05	0.63	4.6	0.18	0.63

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

Table 25. Manganese concentration in vegetative and reproductive organs of common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0 kg ha^{-1}	0.72 b	0.03 a	0.50 b	0.03 a	0.88 b	0.02 c	0.14 a	1.2 b	0.026 b	0.14 a
25 kg ha^{-1}	0.80 b	0.03 a	0.59 b	0.03 a	0.83 b	0.03 bc	0.18 a	1.3 a	0.030 b	0.18 a
50 kg ha^{-1}	1.32 a	0.04 a	0.75 a	0.03 a	0.92 ab	0.04 ab	0.23 a	1.4 a	0.035 ab	0.23 a
100 kg ha^{-1}	1.0 ab	0.03 a	0.79 a	0.03 a	1.0 a	0.06 a	0.23 a	1.3 a	0.060 a	0.23 a
OSL	0.002	0.3	0.001	0.9	0.006	0.01	0.2	0.1	0.001	0.2
Inoculation	1.09 a	0.045 a	0.75 a	0.045 a	0.98 a	0.05 a	0.25 a	1.4 a	0.053 a	0.25 a
No inoculation	0.85 a	0.024 b	0.57 b	0.018 b	0.85 b	0.02 b	0.15 b	1.2 b	0.022 b	0.151 b
OSL	0.103	<0.001	0.003	<0.001	0.003	<0.001	0.004	<0.001	<0.001	0.004
0 + Bs	0.83 bc	0.04 b	0.57 d	0.043 b	0.92 c	0.037 d	0.19 ab	1.2 d	0.037 bc	0.19 ab
0	0.61 c	0.018 e	0.42 e	0.018 c	0.85 d	0.015 e	0.11 b	1.1 e	0.015 d	0.11 b
25 + Bs	0.84 bc	0.04 b	0.76 b	0.043 b	0.84 d	0.046 c	0.236 ab	1.4 b	0.046 b	0.23 ab
25	0.76 bc	0.02 d	0.42 e	0.018 c	0.83 d	0.019 e	0.126 b	1.3 c	0.015 d	0.12 b
50 + Bs	1.63 a	0.05 a	0.83 a	0.054 a	1.03 b	0.065 b	0.300 a	1.6 a	0.049 b	0.30 a
50	1.02 bc	0.03 c	0.68 c	0.018 c	0.82 d	0.033 d	0.176 ab	1.3 cd	0.022 cd	0.17 ab
100 + Bs	1.07 b	0.04 b	0.84 a	0.043 b	1.12 a	0.083 a	0.286 a	1.4 b	0.083 a	0.28 a
100	1.02 bc	0.02 de	0.75 b	0.018 c	0.92 c	0.038 d	0.180 ab	1.2 d	0.038 bc	0.18 ab
OSL	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	0.05	<0.001	<0.001	0.05
Average	0.9	0.03	1.32	0.03	0.9	0.04	0.20	1.3	0.03	0.20

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

Table 26. Cupper concentration in vegetative and reproductive organs of common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0 kg ha^{-1}	0.06 b	0.007 c	0.07 a	0.007 a	0.07 a	0.009 b	0.038 b	0.05 b	0.008 b	0.038 b
25 kg ha^{-1}	0.08 b	0.008 bc	0.08 a	0.007 a	0.08 a	0.011 ab	0.043 ab	0.06 b	0.011 ab	0.043 ab
50 kg ha^{-1}	0.12 a	0.011 ab	0.09 a	0.007 a	0.08 a	0.014 a	0.051 a	0.08 ab	0.014 a	0.051 a
100 kg ha^{-1}	0.14 a	0.012 a	0.08 a	0.007 a	0.09 a	0.013 a	0.046 ab	0.1 a	0.013 a	0.046 ab
OSL	<0.001	0.001	0.2	1.0	0.3	0. 03	0.05	0.01	0. 01	0.05
Inoculation	0.12 a	0.012 a	0.09 a	0.01 a	0.09 a	0.014 a	0.049 a	0.09 a	0.014 a	0.049 a
No inoculation	0.08 b	0.007 b	0.07 b	0.005 b	0.07 b	0.009 b	0.040 b	0.06 b	0.008 b	0.040 b
OSL	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	0.03	0.005	<0.001	0.03
0 +Bs	0.08 f	0.009 d	0.07 c	0.010 a	0.07 c	0.011 c	0.046 ab	0.05 b	0.011 c	0.046 ab
0	0.05 h	0.005 f	0.07 c	0.005 b	0.07 c	0.006 e	0.033 b	0.05 b	0.004 e	0.033 b
25 + Bs	0.1 d	0.010 c	0.09 b	0.010 a	0.09 b	0.013 b	0.050 a	0.09 a	0.013 b	0.050 a
25	0.07 g	0.007 e	0.07 c	0.005 b	0.07 c	0.009 d	0.040 ab	0.04 b	0.009 d	0.040 ab
50 + Bs	0.16 b	0.014 b	0.11 a	0.010 a	0.11 a	0.017 a	0.053 a	0.11 a	0.017 a	0.053 a
50	0.09 e	0.009 d	0.07 c	0.005 b	0.07c	0.011 c	0.043 ab	0.05 b	0.011 c	0.043 ab
100 + Bs	0.17 a	0.016 a	0.1 b	0.010 a	0.1 b	0.016 a	0.053 a	0.11 a	0.016 a	0.053 a
100	0.12 c	0.009 d	0.07 c	0.005 b	0.07c	0.011 c	0.040 ab	0.1 a	0.011 c	0.040 ab
OSL	<0.001	<0.001	0.01	1.0	<0.001	<0.001	0.09	<0.001	<0.001	0.09
Average	0.1	0.009	0.08	0.007	0.08	0.01	0.044	0.03	0.01	0.044

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F)

No variable differences of zinc (Zn) concentration in leaves during the season. At the end of the season, the total average concentration in tissues was 0.59 g kg^{-1} of DM. However, Bs inoculation contributed to accumulate about 41% of Zn over the non-inoculated treatments (Table 27).

Boron (B) concentration substantially increased in stages of flowering and pod filling, decreasing its range as the crop reached maturity. The highest B concentration registered was 0.4 g kg^{-1} of DM at third trifoliate leaf. Bs inoculation treatments increased by 54% when compared to non-treated plants (Table 28).

According to many studies, soil interactions and environmental conditions are highly unpredictable and can lead to unexpected outcomes regarding the behavior of a fertilizer and the performance by microorganisms under lab, greenhouse or field conditions affecting the uptake of nutrients by plants.

Generally, low availability of micronutrients is present in soils with alkaline pH. This study showed that micronutrient concentration in plant tissues was higher in the latest stages of growth (pod filling and physiological maturity) which is in accordance with works of Vieira *et al.* (2009) who found a maximum accumulation of nutrients at the end of the crop cycle. In addition, there was a gradual increase in different plant organs, which were higher and relatively constant in vegetation rather than fruit all over the season. That also agrees with the reports by Pergoraro *et al.* (2014) who mentioned that phases of higher demand and accumulation of nutrients in common bean were indicated by the uptake of leaves.

Reports by Ndakidemi *et al.* (2011) stated that supplying plants of *P. vulgaris* with Rhizobium inoculants significantly altered the uptake of Mn, Fe, Cu Zn, B and Mo in different organs (shoot, root, pod and whole plant).

Table 27. Zinc concentration in vegetative and reproductive organs in common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0 kg ha^{-1}	0.2 c	0.027 c	0.2 c	0.020 a	0.2 c	0.023 ab	0.220 a	0.22 c	0.026 c	0.220 a
25 kg ha^{-1}	0.25 b	0.037 bc	0.25 b	0.021 a	0.25 b	0.021 b	0.225 a	0.28 bc	0.037 bc	0.225 a
50 kg ha^{-1}	0.3 a	0.050 ab	0.3 a	0.024 a	0.3 a	0.026 a	0.256 a	0.37 ab	0.050 ab	0.256 a
100 kg ha^{-1}	0.3 a	0.058 a	0.3 a	0.023 a	0.3 a	0.024 a	0.243 a	0.41 a	0.058 a	0.243 a
OSL	0.001	0.008	0.001	0.71	0.001	0.02	0.7	0.003	0.007	0.7
Inoculation	0.29 a	0.055 a	0.29 a	0.028 a	0.29 a	0.024 a	0.274 a	0.39 a	0.055 a	0.274 a
No inoculation	0.23 b	0.030 b	0.23 b	0.016 b	0.23 b	0.023 a	0.198 b	0.25 b	0.030 b	0.198 b
OSL	0.005	<0.001	0.005	<0.001	0.005	0.1	0.01	<0.001	<0.001	0.01
0 + Bs	0.24 d	0.036 e	0.24 d	0.024 d	0.24 d	0.023 c	0.250 abc	0.32 c	0.036 e	0.250 abc
0	0.16 f	0.018 g	0.16 f	0.016 e	0.16 f	0.023 bc	0.146 c	0.12 e	0.016 g	0.146 c
25 + Bs	0.29 b	0.047 c	0.29 b	0.027 c	0.29 b	0.020 c	0.270 ab	0.37 b	0.047 c	0.270 ab
25	0.2 e	0.027 f	0.2 e	0.016 e	0.2 e	0.022 c	0.166 bc	0.19 d	0.027 f	0.166 bc
50 + Bs	0.34 a	0.067 b	0.34 a	0.033 a	0.34 a	0.028 a	0.293 a	0.43 a	0.067 b	0.293 a
50	0.26 c	0.033 e	0.26 c	0.016 e	0.26 c	0.023 bc	0.243 abc	0.32 c	0.033 e	0.243 abc
100 + Bs	0.3 b	0.072 a	0.3 b	0.03 b	0.3 b	0.026 ab	0.283 a	0.45 a	0.072 a	0.283 a
100	0.3 b	0.043 d	0.3 b	0.016 e	0.3 b	0.022 c	0.236 abc	0.37 b	0.043 d	0.236 abc
OSL	<0.001	<0.001	0.005	<0.001	<0.001	0.001	0.09	<0.001	<0.001	0.09
Average	0.26	0.04	0.26	0.02	0.26	0.02	0.235	0.32	0.04	0.235

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr> F).

Table 28. Boron concentration in vegetative and reproductive organs in common bean.

Phosphorus (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling			Physiological maturity		
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Pods	Leaves	Stems	Pods
	g kg^{-1} of dry matter									
0 kg ha^{-1}	0.36 b	0.025 a	0.2 b	0.030 b	0.14 a	0.020 b	0.038 a	0.14 a	0.029 b	0.038 a
25 kg ha^{-1}	0.42 b	0.026 a	0.22 b	0.038 ab	0.16 a	0.023 ab	0.041 a	0.16 a	0.038 ab	0.041 a
50 kg ha^{-1}	0.51 a	0.029 a	0.28 a	0.051 a	0.2 a	0.027 a	0.043 a	0.2 a	0.051 a	0.043 a
100 kg ha^{-1}	0.51 a	0.032 a	0.28 a	0.038 ab	0.2 a	0.025 ab	0.041 a	0.2 a	0.038 ab	0.041 a
OSL	0.001	0.6	0.006	0.03	0.7	<0.001	0.7	0.7	0.02	0.7
Inoculation	0.5 a	0.038 a	0.27 a	0.049 a	0.27 a	0.028 a	0.045 a	0.27 a	0.049 a	0.045
No inoculation	0.39 b	0.018 b	0.21 b	0.030 b	0.08 b	0.020 b	0.037 a	0.08 b	0.029 b	0.037
OSL	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	0.1	<0.001	<0.001	0.1
0 + Bs	0.42 d	0.036 b	0.21 cd	0.036 de	0.21 c	0.020 c	0.043 ab	0.21 c	0.036 de	0.043 ab
0	0.29 f	0.014 e	0.2 d	0.0243 f	0.08 d	0.021 c	0.030 b	0.08 d	0.022 g	0.030 b
25 + Bs	0.47 c	0.036 b	0.24 b	0.044 c	0.24 b	0.030 b	0.043 ab	0.24 b	0.044 c	0.043 ab
25	0.36 e	0.016 d	0.21 cd	0.033 e	0.08 d	0.017 d	0.036 ab	0.008 d	0.033 e	0.036 ab
50 + Bs	0.58 a	0.044 a	0.33 a	0.066 a	0.08 d	0.033 a	0.053 a	0.33 a	0.066 a	0.053 a
50	0.44 d	0.021 c	0.23 bc	0.037 d	0.33 a	0.021 c	0.040 ab	0.08 d	0.037 d	0.040 ab
100 + Bs	0.54 b	0.036 b	0.33 a	0.051b	0.33 a	0.029 b	0.043 ab	0.33 a	0.051 b	0.043 ab
100	0.48 c	0.021 c	0.23 bc	0.026 f	0.08 d	0.021 c	0.040 ab	0.08 d	0.026 f	0.040 ab
OSL	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.2	<0.001	<0.001	0.2
Average	0.4	0.02	0.24	0.03	0.17	0.02	0.041	0.1	0.03	0.041

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05),
- Observed significance level (OSL Pr>).

4.3.7 Micronutrient uptake in stages of growth.

Fe uptake increased all over the season (Table 29). High rates of P exhibited the greatest uptake as compared to low rates (Figure 28). The higher uptake occurred at maturity in which Bs inoculated plants increased by 35% over non-treated plants (Figure 29).

Manganese uptake was influenced by phosphorus rates during the season (Table 30). Rate of 50 kg ha^{-1} had greater uptake (0.340 kg ha^{-1}) whereas the rate of 100 kg ha^{-1} decreased 26% in its uptake process compared to the previous rate and from that of 0 kg ha^{-1}. The highest uptake occurred at pod filling stage with a slight decrease in physiological maturity (Figure 30). Besides, Bs treated plants increased Mn by 12% with respect to non-treated plants (Figure 31).

Copper uptake was widely influenced by P rates and consistently increased over the course of the season. Again, rates of 50 and 100 kg ha^{-1} had the highest uptake respectively (Table 31 and Figure 32). The highest uptake occurred at maturity stage. Bs inoculation enhanced by 43% Cu uptake (Figure 33).

Zinc uptake started to increase early in the season reaching a peak period at the pod filling stage and then declining at the end of the season where the highest P rates exhibited greater uptake (Table 32 and Figure 34). Inoculated treatments increased by 14% Zn uptake (Figure 35).

Boron uptake followed the same trend as Zn, the highest concentration occurred at pod filling in which the high P rates exhibited the greatest uptake (Table 33 and Figure 36). Besides, Bs treatments accounted for an increase of 94% over non-treated plants (Figure 37).

Table 29. Total iron uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	0.585 b	1.743 a	2.669 b	3.924 b
25	0.590 b	2.241 a	3.568 b	9.817 a
50	1.355 a	2.164 a	6.010 a	10.425 a
100	1.143 a	2.716 a	4.984 a	9.857 a
OSL	<0.0001	0.07	<0.0001	0.006
Inoculation	1.059 a	2.738 a	4.561 a	10.01 a
No inoculation	0.778 b	1.693 b	4.055 a	7.00 a
OSL	0.1	0.002	0.2	0.06
0 + Bs	0.73 d	1.3 bc	2.71 cd	4.85 de
0	0.41 d	1.1 c	2.62 d	2.99 e
25 + Bs	0.76 cd	2.3 ab	3.82 cd	10.42 b
25	0.43 d	1.5 ab	3.31 cd	6.35 cde
50 + Bs	1.58 a	3.0 a	6.16 a	14.49 a
50	1.12 bc	2.7 a	4.33 bc	8.22 bcd
100 + Bs	1.15 b	2.9 a	5.86 ab	11.48 ab
100	1.13 bc	2.7 a	5.63 ab	9.21 bc
OSL	<0.0001	0.005	0.001	<0.0001
Average	0.91	2.21	4.3	8.5

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

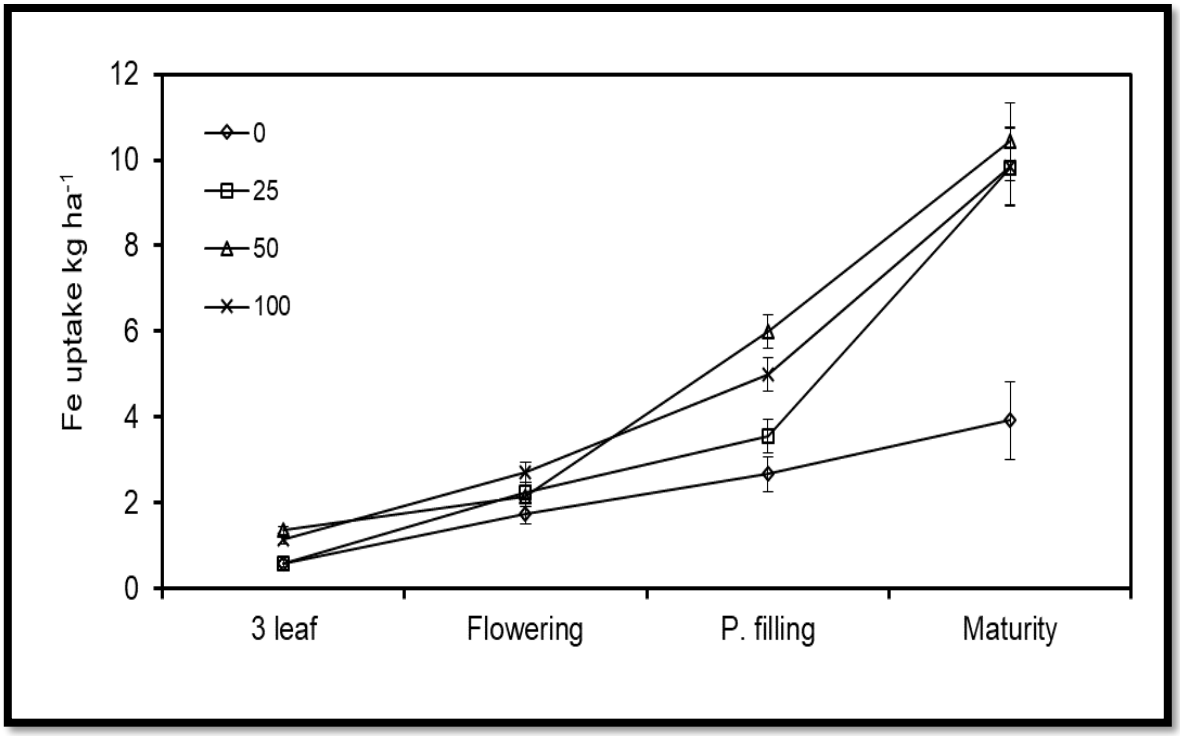

Figure 28. Iron uptake pattern as a function of P rates.

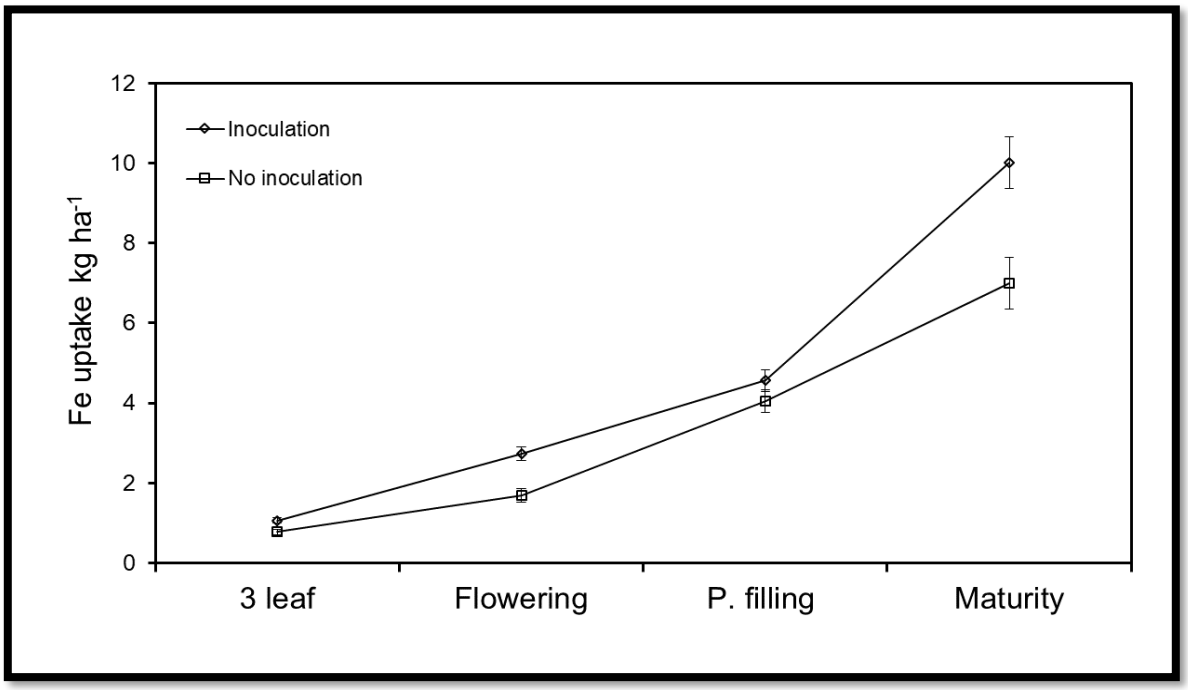

Figure 29. Iron uptake pattern as a function of time.

Table 30. Total manganese uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	0.161 c	0.348 b	1.278 b	0.980 b
25	0.169 c	0.487 ab	1.547 b	1.731.8 a
50	0.340 a	0.580 a	2.771 a	2.248 a
100	0.250 b	0.553 ab	2.297 a	1.750 a
OSL	0.001	0.04	<0.0001	0.001
Inoculation	0.24 a	0.60 a	2.17 a	1.790 a
No inoculation	0.21 a	0.38 b	1.77 b	1.565 a
OSL	0.2	0.001	0.05	0.3
0 + Bs	0.145 bc	0.29 bc	1.28 d	1.0 cd
0	0.128 c	0.22 c	126 d	0.95 d
25 + Bs	0.194 bc	0.47 ab	1.65 cd	1.82 b
25	0.193 bc	0.46 ab	1.43 d	1.64 bcd
50 + Bs	0.355 a	0.7 a	3.0 a	2.66 a
50	0.324 a	0.54 a	1.94 bcd	1.65 bc
100 + Bs	0.251 ab	0.68 a	2.64 ab	1.84 b
100	0.249 ab	0.56 a	2.44 abc	1.81 b
OSL	0.009	0.007	0.001	0.002
Average	0.230	0.492	1.97	1.67

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), (OSL Pr> F).

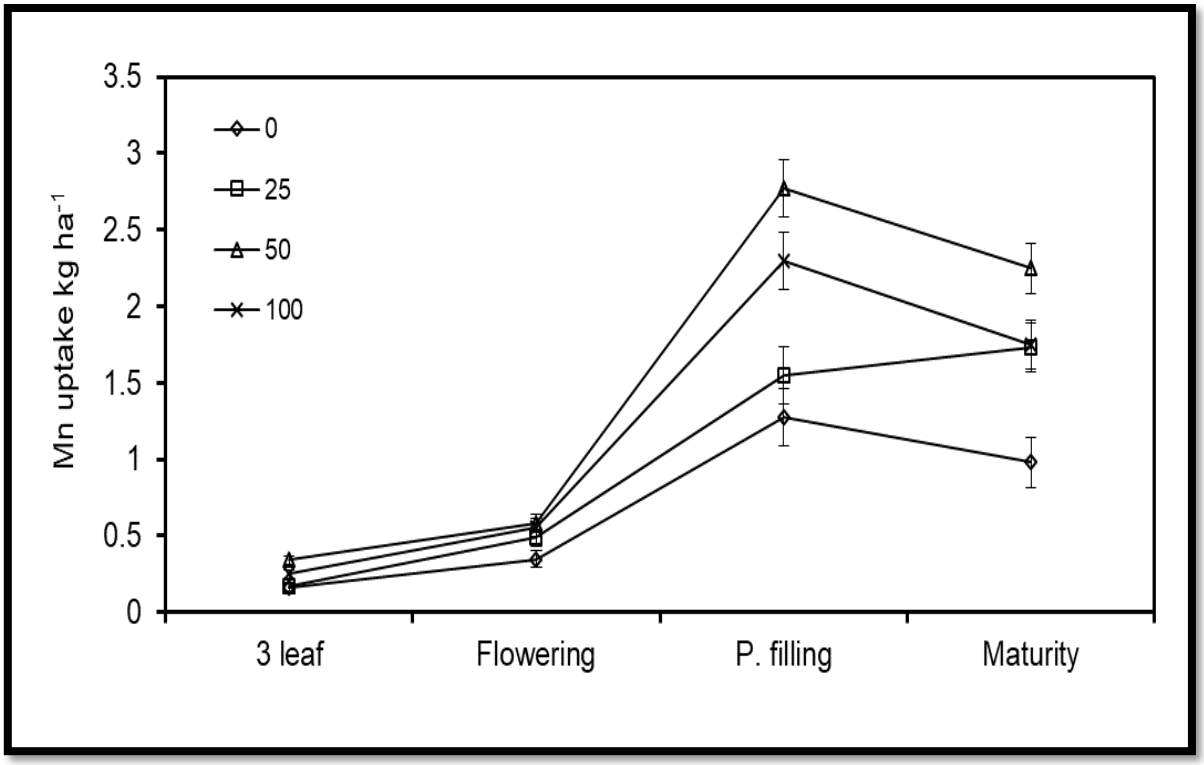

Figure 30. Manganese uptake pattern as a function of P rates.

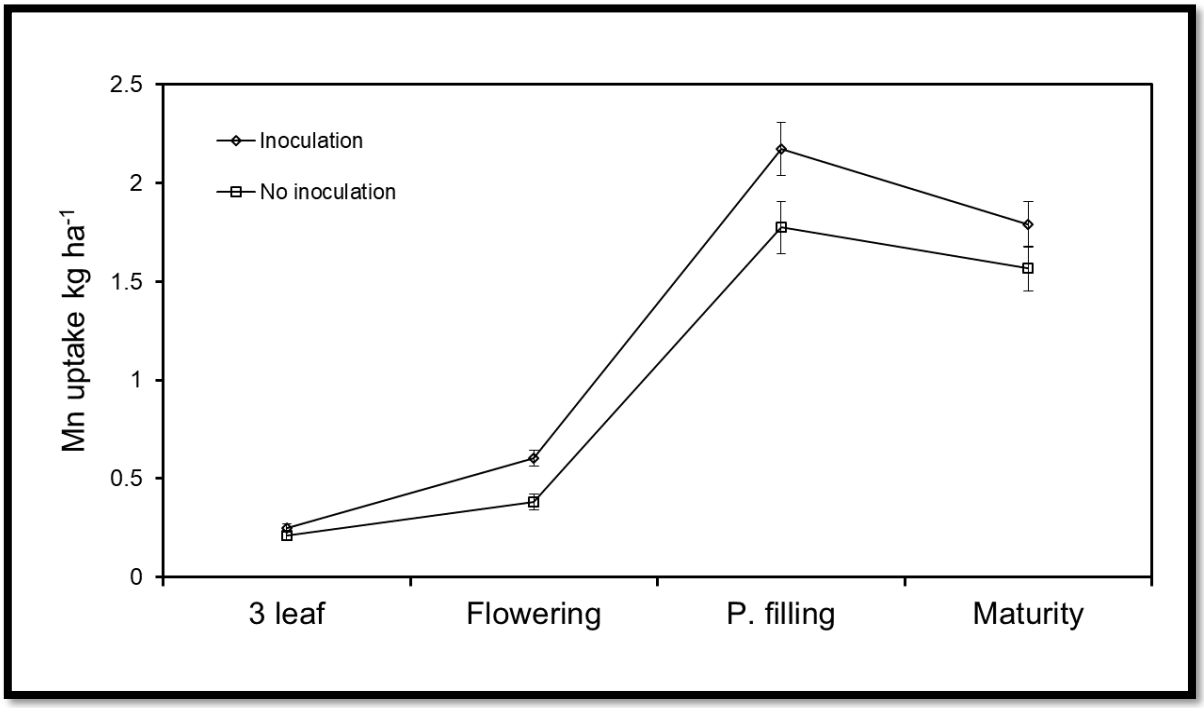

Figure 31. Manganese uptake pattern as a function of time.

Table 31. Total cupper uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	0.0187 b	0.065 a	0.2 b	0.073 b
25	0.0234 b	0.085 a	0.246 b	0.137 ab
50	0.040 a	0.089 a	0.381 a	0.162 a
100	0.0438 a	0.082 a	0.374 a	0.158 a
OSL	<0.0001	0.4	<0.0001	0.003
Inoculation	0.0367 a	0.1 a	0.357 a	0.161 a
No inoculation	0.0264 b	0.061 b	0.244 b	0.104 b
OSL	0.004	0.002	<0.0001	0.02
0 + Bs	0.023 def	0.57 b	0.20 bc	0.079 de
0	0.018 ef	0.46 b	0.17 c	0.067 e
25 + Bs	0.028 cde	0.9 ab	0.28 bc	0.15 bc
25	0.014 f	0.66 b	0.22 bc	0.095 cde
50 + Bs	0.051 a	0.11 a	0.47 a	0.23 a
50	0.037 bc	0.84 ab	0.29 b	0.11 bcde
100 + Bs	0.043 ab	0.11 a	0.44 a	0.17 ab
100	0.036 bcd	0.74 ab	0.30 b	0.13 bcd
OSL	<0.0001	0.6	0.001	0.002
Average	0.031	0.08	0.30	0.132

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), (OSL Pr> F).

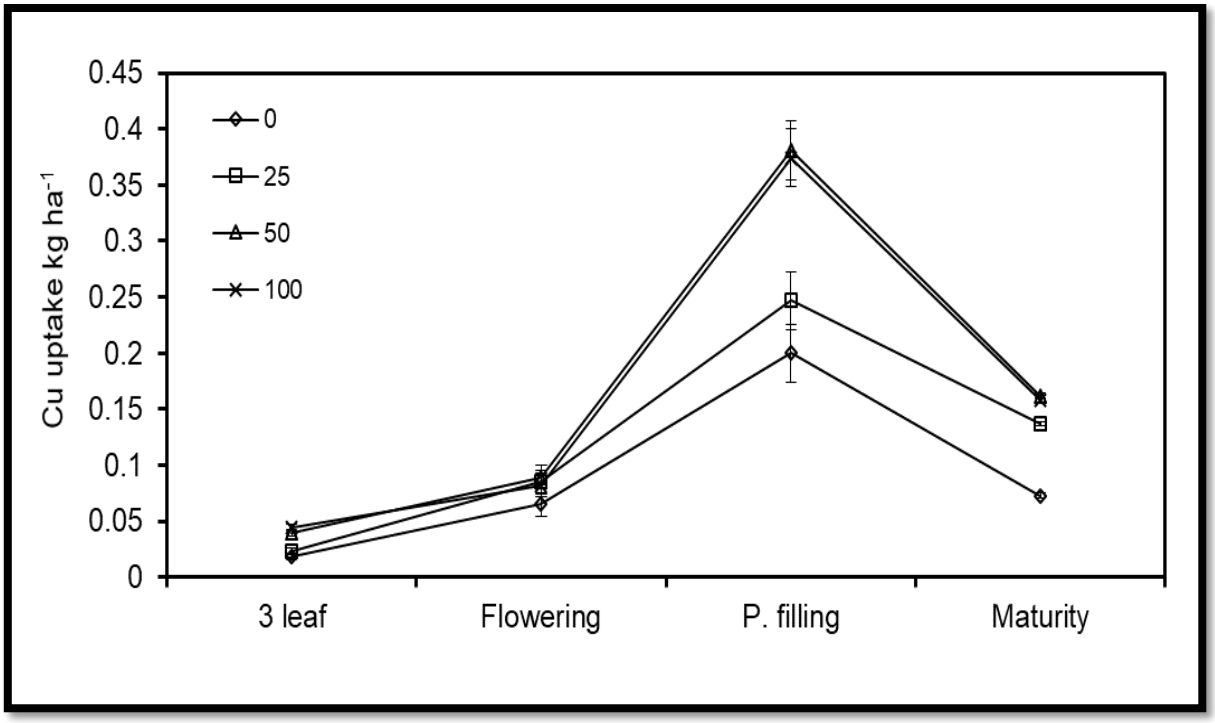

Figure 32. Cupper uptake pattern as a function of P rates.

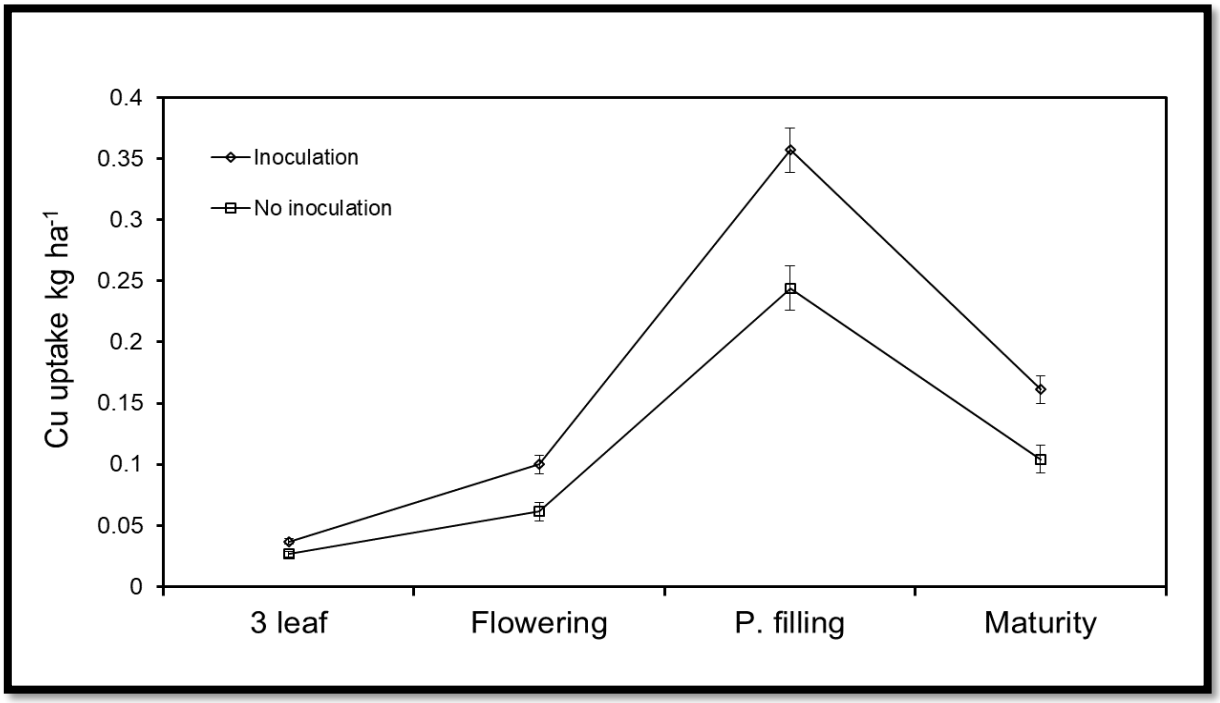

Figure 33. Cupper uptake pattern as a function of time.

Table 32. Total zinc uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	0.063 b	0.174 b	0.638 b	0.326 b
25	0.082 b	0.249 ab	0.778 b	0.675 a
50	0.123 a	0.287 a	1.164 a	0.778 a
100	0.129 a	0.272 ab	1.156 a	0.715 a
OSL	<0.001	0.005	0.002	0.005
Inoculation	0.113 a	0.290 a	0.95 a	0.69 a
No inoculation	0.085 b	0.201 b	0.91 a	0.55 a
OSL	0.005	0.001	0.8	0.2
0 + Bs	0.8 cd	0.17 bc	0.71 cd	0.34 c
0	0.047 d	0.12 c	0.56 d	0.30 c
25 + Bs	0.103 bc	0.23 abc	0.83 bcd	0.70 b
25	0.061 d	0.22 abc	0.71 cd	0.52 b
50 + Bs	0.143 a	0.34 a	1.30 a	1.0 a
50	0.118 ab	0.26 ab	1.0 abc	0.66 b
100 + Bs	0.128 ab	0.32 a	1.23 ab	0.73 b
100	0.114 abc	0.27 ab	1.0 abc	0.68 b
OSL	0.001	0.02	0.01	0.002
Average	0.99	0.246	0.934	0.623

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), (OSL Pr> F).

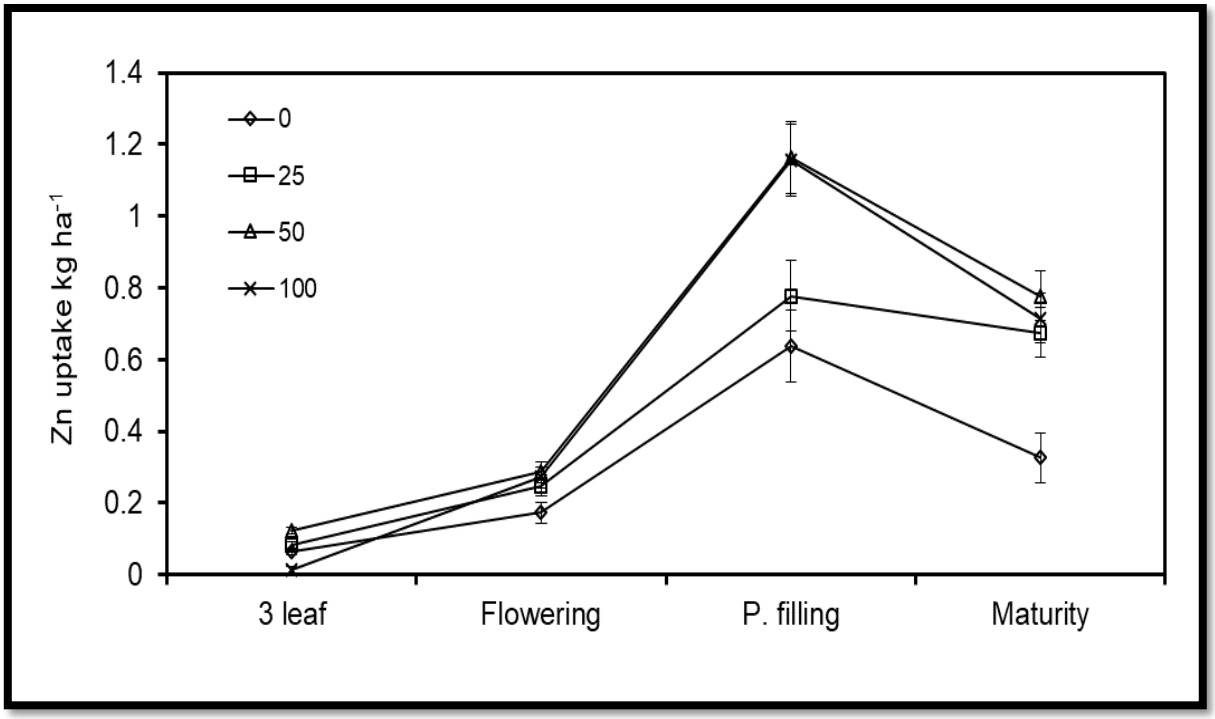

Figure 34. Zinc uptake pattern as a function of P rates.

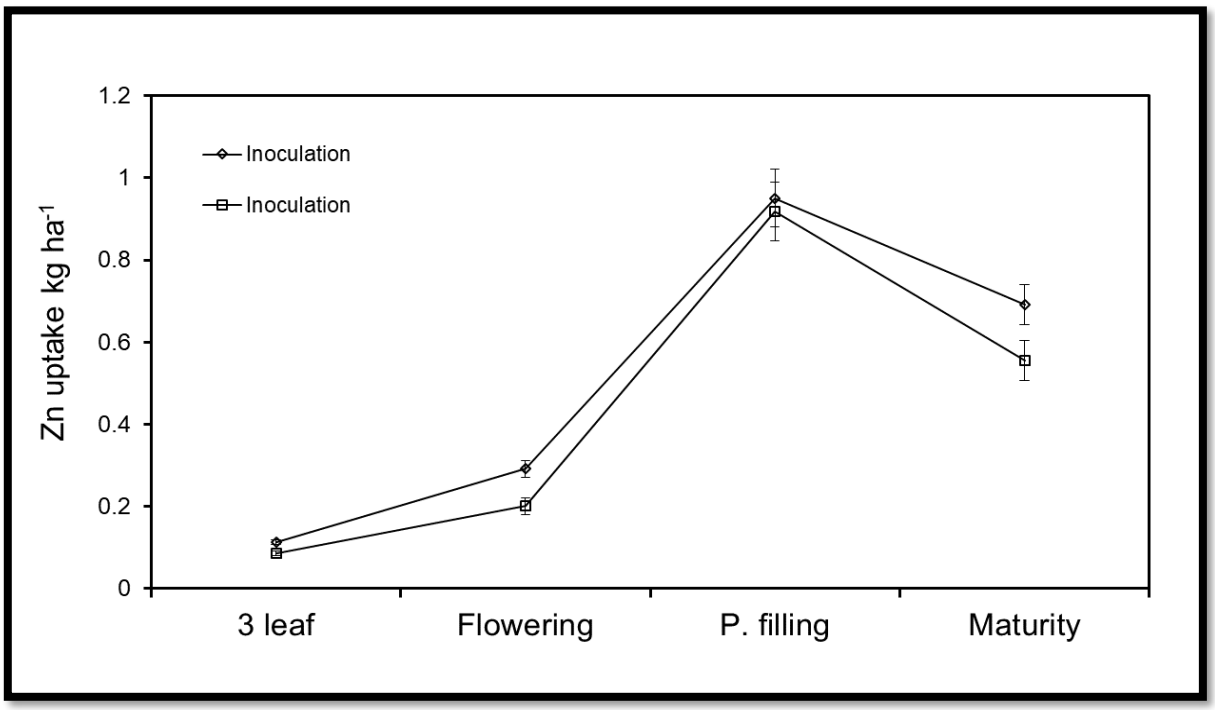

Figure 35. Zinc uptake pattern as a function of time.

Table 33. Total boron uptake (kg ha^{-1}) in phenological stages of growth.

P (kg ha^{-1})	Third trifoliate leaf	Flowering	Pod filling	Physiological maturity
0	0.089 b	0.217 b	0.364 b	0.141 a
25	0.099 b	0.317 ab	0.447 ab	0.257 a
50	0.149 a	0.423 a	0.708 a	0.313 a
100	0.136 a	0.333 ab	0.691 a	0.276 a
OSL	0.006	0.04	<0.001	0.2
Inoculation	0.132 a	0.387 a	0.742 a	0.361 a
No inoculation	0.105 a	0.258 b	0.363 b	0.133 b
OSL	0.08	0.01	0.003	<0.0001
0 + Bs	0.112 ab	0.24 bc	0.32 c	0.13 b
0	0.067 b	0.16 c	0.28 c	0.09 b
25 + Bs	0.123 a	0.36 ab	0.40 bc	0.36 a
25	0.075 b	0.27 bc	0.37 c	0.14 b
50 + Bs	0.150 a	0.51 a	1.0 a	0.47 a
50	0.143 a	0.29 bc	0.46 bc	0.15 b
100 + Bs	0.149 a	0.39 ab	094 a	0.41 a
100	0.128 a	0.33 abc	0.60 b	0.18 b
OSL	0.006	0.04	0.001	0.001
Average	0.118	0.322	0.552	0.247

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), (OSL Pr> F).

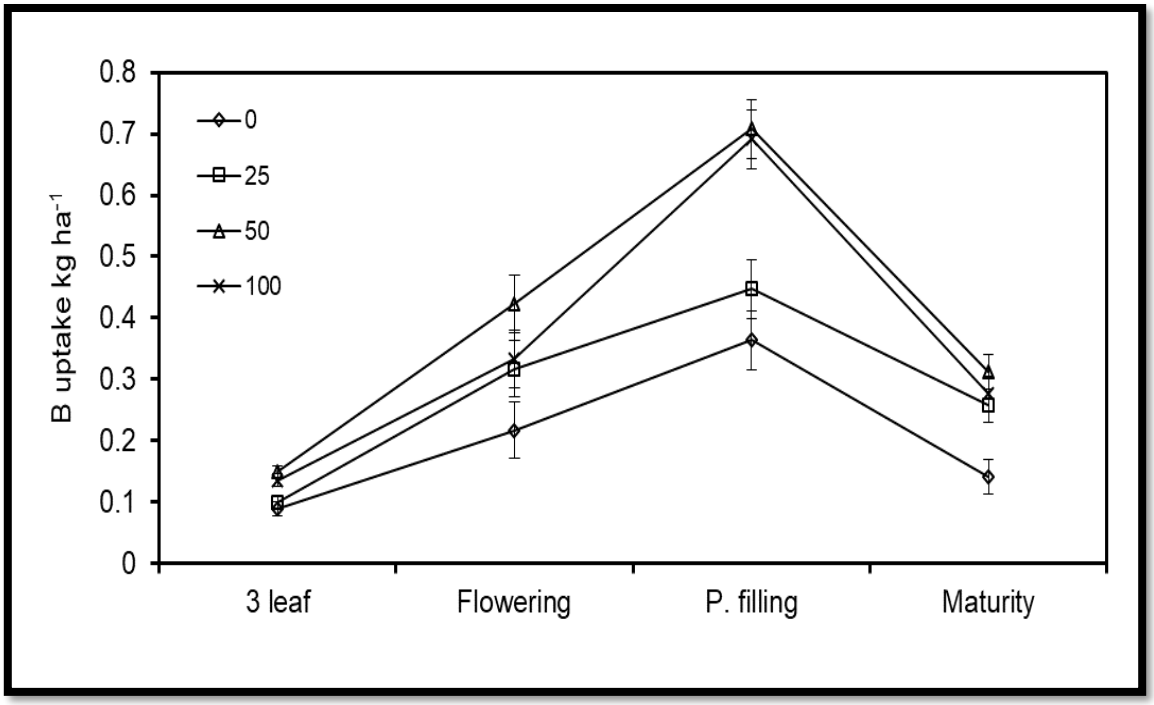

Figure 36. Boron uptake pattern as a function of P rates.

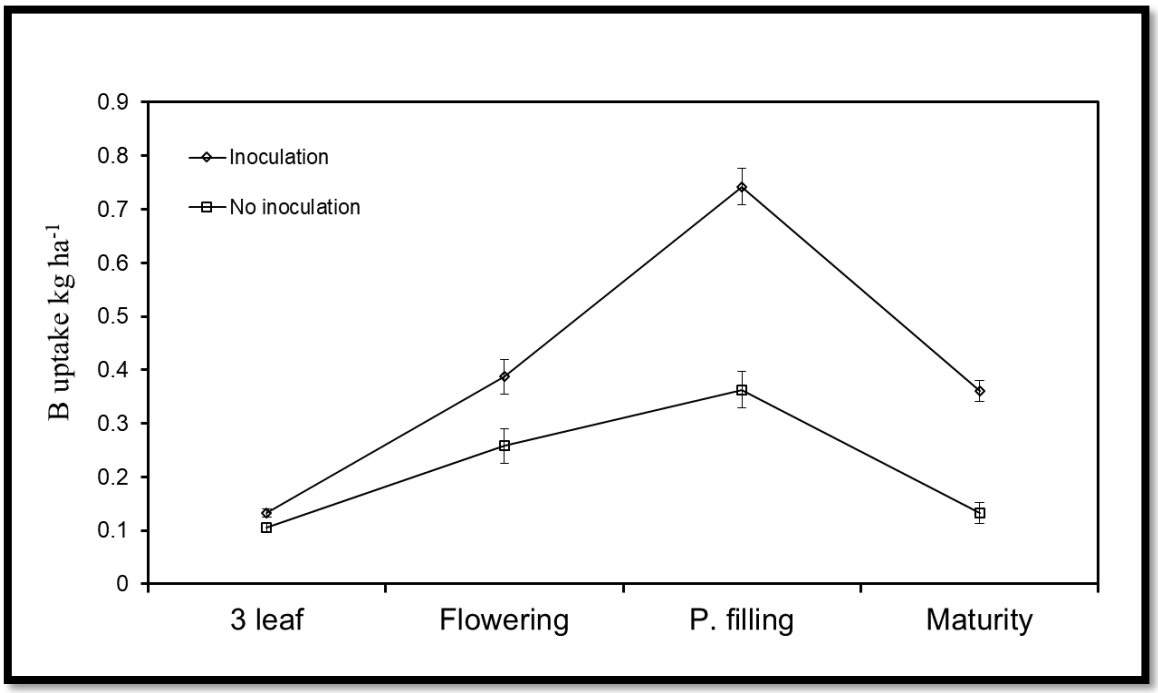

Figure 37. Boron uptake pattern as a function of time.

According to results presented previously, the process of uptake exhibited a significant response to phosphorus rates with the progression of the season. This positive influence was related to an increase in total dry matter accumulation due to P application and inoculation treatments as well (Table 5, Figure 5 and 6, Chapter 3).

The uptake for all these nutrients was higher from those found by Westermann *et al.* (2011) who reported that Zn, Cu and Mn uptake differed over production systems and 16 bean genotypes. These researchers found that the greatest Zn uptake (0.131 kg ha^{-1}) was on a conventional bean production and the lowest (0.127 kg ha^{-1}) on organic high input production, while the highest Cu and Mn uptake (0.043 kg ha^{-1}) and (0.179 kg ha^{-1}) were on a conventional irrigated system. They also mentioned that greater uptake occurred in a high input production system (no water scarcity and high input-fertility) which coincides with the results of this work.

The variable response of the crop and its low agronomic efficiency to the highest P rate (100 kg ha^{-1}) could be possibly attributed to changes in soil solution pH after fertilization (Sánchez, 2007) resulting in low plant uptake and/or a limiting effect of other nutrients with increasing P rates (Mengel and Kirkby, 2001). This process has been also reported on maize *(Zea mayz)* (Hussein, 2009), common bean (*Phaseolus vulgaris* L.) (Gidago *et al*., 2011; Girma *et al*., 2014) and soybean *(Glycine max)* (Devi *et al*., 2012).

Similarly, studies on soybean reported an increase of 40% in nutrients due to application of P (Chaudhary *et al.,* 2008) reaching maximum yields of 3.5 Mg ha^{-1} in different varieties (Bender *et al.,* 2015). Those findings are consistent with the results of this trial in that micronutrient content and best yield enhanced (3.23 Mg ha^{-1}) as P fertilization increased.

Inoculation with *Bacillus subtilis* exerted a positive response in the process of uptake and concentration in the plant compared to that of non-treated plants. This effect could be mainly brought about by ligand production known as siderophores (molecules secreted by microorganisms under Fe deficiency) (Harrington and Crumbliss, 2009) and/or production of natural organic compounds (chelates) that bound metals such as Fe, Zn, Cu and Mn increasing their availability for plant uptake (Wani *et al.,* 2008; Sridevi *et al.,* 2007; Havlin *et al.,* 2005).

The results are similar from those of Ndakidemi *et al.* (2011) suggesting that the simulative effect of *Rhizobium* on the uptake could be due to their activities on the solubilization process. Plenty of research evidence suggests that different nitrogen-fixing organisms may produce siderophores, which may facilitate the solubility of nutrients from different sources (Sridevi *et al.*, 2008).

Works by Ndakidemi *et al.* (2011) reported that sole application of *Rhizobium* inoculants to *P. vulgaris* promoted greater nutrient uptake (Mn, Fe, Cu, Zn B and Mo) and accumulation in their tissues, while the combination of the inoculant with other nutrients such as Molybednum and lime had some antagonistic influences by reducing the availability of those micronutrients.

Other works have shown that an increase of nutrients in plants have been due to application of P and inoculation with rhizobacteria (Abbasi *et al.,* 2010; Sarwar *et al.*, 2016; Wan et al., 2011; Yasmin and Bano (2011). Therefore, P application alone or in combination with rhizobacteria contributed to enhance micronutrient levels in plant tissues (leaves, stems, pods) which could also promote higher quality parameters in seeds especially with Fe and Zn.

4.3.8 Seasonal uptake and nutrient flux.

Seasonal uptake preference was in the following order: Fe> Mn> Zn> B> Cu with 16, 4.3, 2.8, 1.2 and 0.540 kg ha^{-1} (Table 34), which was the average requirement for all treatments. Accordingly, micronutrient uptake was greater in rates of 50 and 100 kg ha^{-1}. The inoculated treatments had an increase of 27, 27, 24, 34 and 13% with respect to non-inoculated plants. Total uptake pattern showed that all micronutrientes increased gradually from flowering maximizing the uptake at pod filling remaining relatively constant at the end of the season (Figure 38).

Fe uptake increased all over the season remaining high at maturity. This indicated that soil conditions such as carbonate content, high P level or interaction between Fe and metals (Mn, Cu, Zn high content) did not interfere in the uptake by plants. These results coincide with those reported by Biddulfph and Woodbridge (1952) who determined that plants absorbed phosphorus in direct relationship with Fe, while, previous works by Dehner *et al.* (2010) showed that inoculation with rhizobacteria enhanced siderophore formation increasing Fe uptake in crops. In this study, the average phosphorus uptake was 14 kg ha^{-1}, slightly lower as compared to iron (16 kg ha^{-1}). In most cases, immovilization processes rather than scarcity in soil affect Fe supply by plants.

Zinc uptake was much lower than Mn, which was attributed that high P levels in soils reduces Zn availability causing a physiological inhibition of Zn from roots to aerial parts (Dominguez-Vivancos, 1997). Other works by Astudillo and Blair (2008), Joshi *et al.* (2010), Velu *et al.* (2014) mentioned that increasing P content in the plant decreases Zn content.

Mn uptake pattern increased as a function of time since its behavior in the soil-plant system is very similar to that of Fe in which its solubility is totally pH dependent unless another sources such as those produced from microbial metabolism promote solubilization. High levels of Fe, Zn or Cu can reduce its uptake by plants.

As observed from the pattern, Cu uptake process was the most limited as compared the rest of the metals which was due to the well known interaction between P and Cu, in which high P levels decreases Cu uptake by plants, a behavior similar in a lower extent to that reported on Zn.

B uptake was slightly higher than Cu, usually because the process of uptake is reduced in well-drained soils, hot weather and/or low soil moisture conditions. This behavior is usually related to restricted release of B from organic matter and low B uptake due to lack of moisture in root zone (Havling *et al.,* 2005). Another factor that reduces B uptake is the low Ca supply.

Finally, reports presented by Ciampitti and Prasad (2016), Guttieri *et al.* (2017) and Sadras (2006) stated that all variations in nutrient uptake and efficiencies had implications in soil management, crop performance, and seed quality.

Table 34. Seasonal micronutrient uptake (kg ha^{-1}) as a function of phosphorus rates and *Bacillus subtilis*.

Rates (P_2O_5) kg ha^{-1}	Fe	Mn	Cu	Zn	B
0	8.91 b	2.76 b	0.358 b	1.20 b	0.81 b
25	16.82 a	4.45 a	0.519 ab	1.88 a	1.17 ab
50	19.73 a	5.32 a	0.659 a	2.24 a	1.52 a
100	19.32 a	4.94 a	0.647 a	2.28 a	1.45 a
OSL	0.001	0.001	0.001	0.001	0.001
Inoculation	18.37 a	4.81	0.655 a	2.0	1.63 a
No inoculation	13.53 b	3.93	0.436 b	1.7 b	0.86 b
OSL	0.03	0.008	0.002	0.008	0.02
Average	16	4.3	0.540	2.8	1.2

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), (OSL Pr> F).

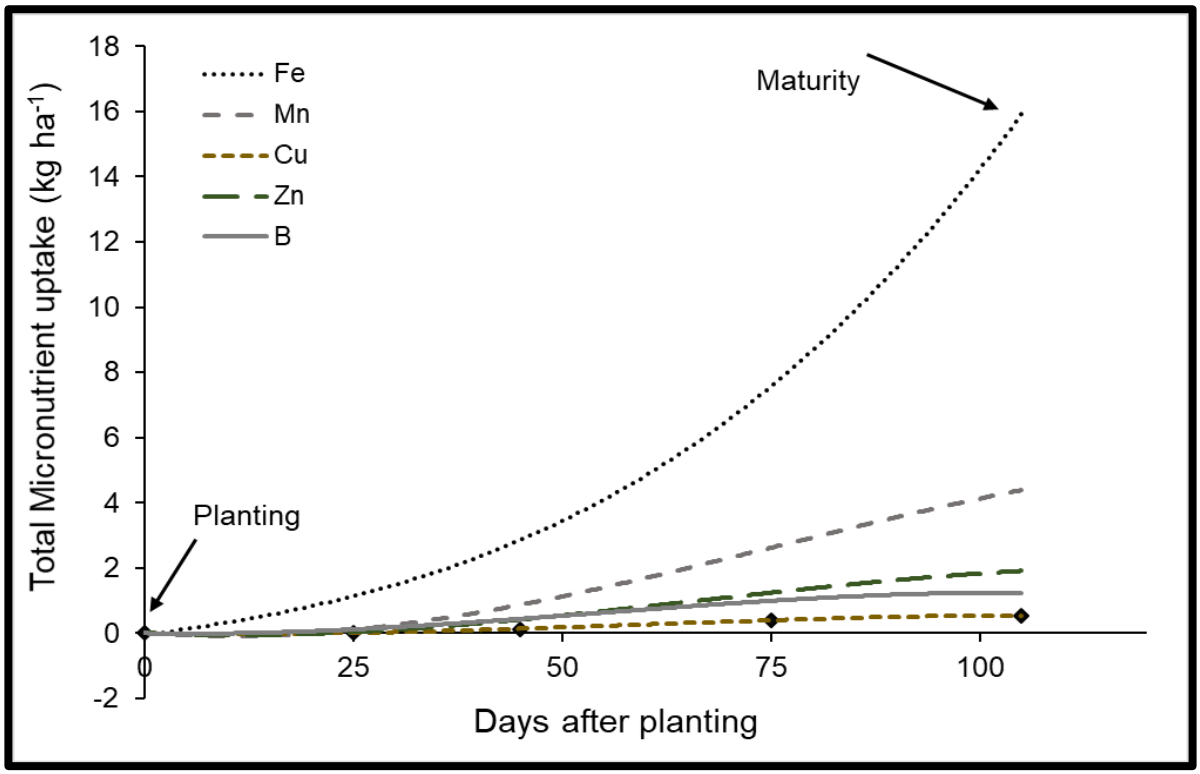

Figure 38. Seasonal micronutrient uptake pattern.

Regression equation	R^2
Fe = 0.0015 DAP^2 - 0.0082 DAP + 0.1162	0.99
Mn = 0.0004 DAP^2 + 0.0065 DAP - 0.0823	0.98
Cu = 3E-05 DAP^2 + 0.0026 DAP - 0.0239	0.96
Zn = 0.0001 DAP^2 + 0.0054 DAP - 0.0561	0.97
B = 2E-05 DAP^2 + 0.0109 DAP - 0.0604	0.97

The uptake rate patterns for all nutrients showed an increase during the season in which the peak demand coincided with the most critical stage of development (Figure 39). The average rate for all treatments at pod filling (75 DAP) was Fe> Mn> Zn> B> Cu with 144, 66, 31, 18 and 10 g ha^{-1} day^{-1} in the same order. At maturity the uptake preference was Fe> Mn> Zn> B> Cu with 284, 56, 20, 8 and 4 g ha^{-1} day^{-1} respectively.

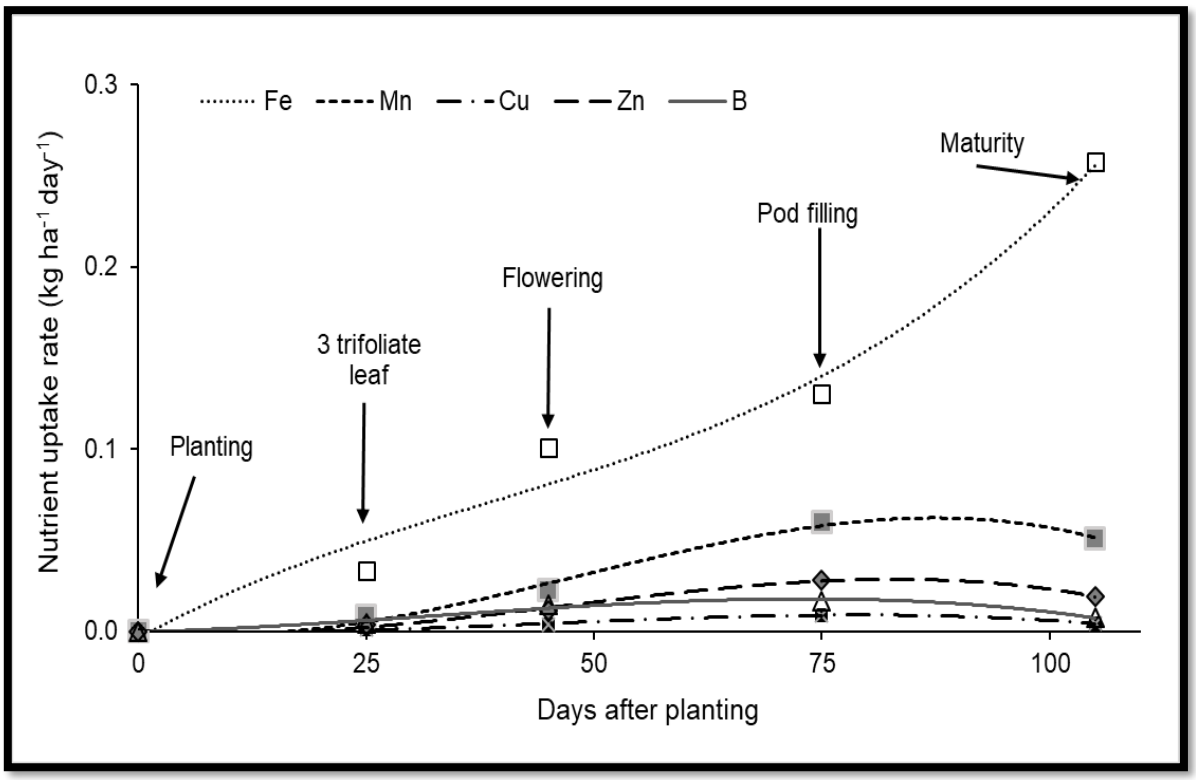

Figure 39. Seasonal micronutrient uptake rate pattern.

Regression equation	R^2
Fe = 3E-07 DAP^3 - 4E-05 DAP^2 + 0.003 DAP - 0.004	0.98
Mn = -3E-07 DAP^3 + 4E-05 DAP^2 - 0.0007 DAP + 0.0008	0.98
Cu = -6E-08 DAP^3 + 8E-06 DAP^2 - 0.0001 DAP + 0.0001	0.98
Zn = -2E-07 DAP^3 + 2E-05 DAP^2 - 0.0004 DAP + 0.0003	0.98
B = -7E-08 DAP^3 + 7E-06 DAP^2 + 0.0001 DAP - 0.0004	0.95

4.3.9 Micronutrient acquisition timing.

Knowledge of interactions between the soil, plant and environment ensure optimum nutrient availability through better management practices. The quantity of nutrient that crop requires depends on its characteristics (yield level, variety), environment conditions (moisture and temperature), soil factors (fertility, soil type and landscape position) and crop management.

The accumulation of nutrients during the growing season generally follows a plant growth pattern. Obviously, the shape of the patterns varies among crops, but nearly all plants exhibit a rapid increase followed by a period of decline. Some plants exhibit a rapid nutrient uptake and growth early in the season while others exhibit maximum growth rates much later. It is therefore important identifying the stages of growth in which the peak nutrient demand occurs in order to apply and choose the correct amount of nutrients.

As observed in this trial and considering the total micronutrient requirement, it was found that the crop took up until flowering only 20% of the total Fe and Cu, 44% of Zn, 35.5% of B, 16% of Mn taking the rest on later stages. This indicated that the most intense period of the nutrient acquisition was the second half of the season (Figure 40). This work coincides with that of Vieira *et al.* (2009) who reported a maximum accumulation of nutrients at the end of the crop cycle in different cultivars, populations and farming systems.

Summarized by International Plant Nutrition Institute, optimizing nutrient management includes using the right source and right rate at the right time and right place, the 4R approach (IPNI, 2012). Estimation of this “right rate,” is greatly influenced by numerous factors including hybrid background, transgenic insect protection, agronomic management practices, and soil fertility.

Although nutrient management is a complex process, improving our understanding of when, where, and how plants use nutrients, provides opportunities to optimize fertilizer rates and application timing. Earlier research pertaining to primary macro and micronutrient uptake, partitioning and timing (Sayre, 1948; Hanway, 1962b; Karlen *et al.,* 1988) mention that is rellatively unrepresentative of modern genotypes grown under current management practices.

Due to the immobility of micronutrients, application timing and placement should include practices that favor uptake through bean roots. Since common bean is relatively short crop, that implies the full nutrient requirement as one sole application as the most convenient tool for grower when surface irrigation system is used. In those cases with drip irrigation system, fertilization can be split in several events.

Thus, fertilizer placement decisions will include factors such as efficient use of nutrient (plant emergence to maturity), prevention of salt injury to the seedling and always convenience for the grower.

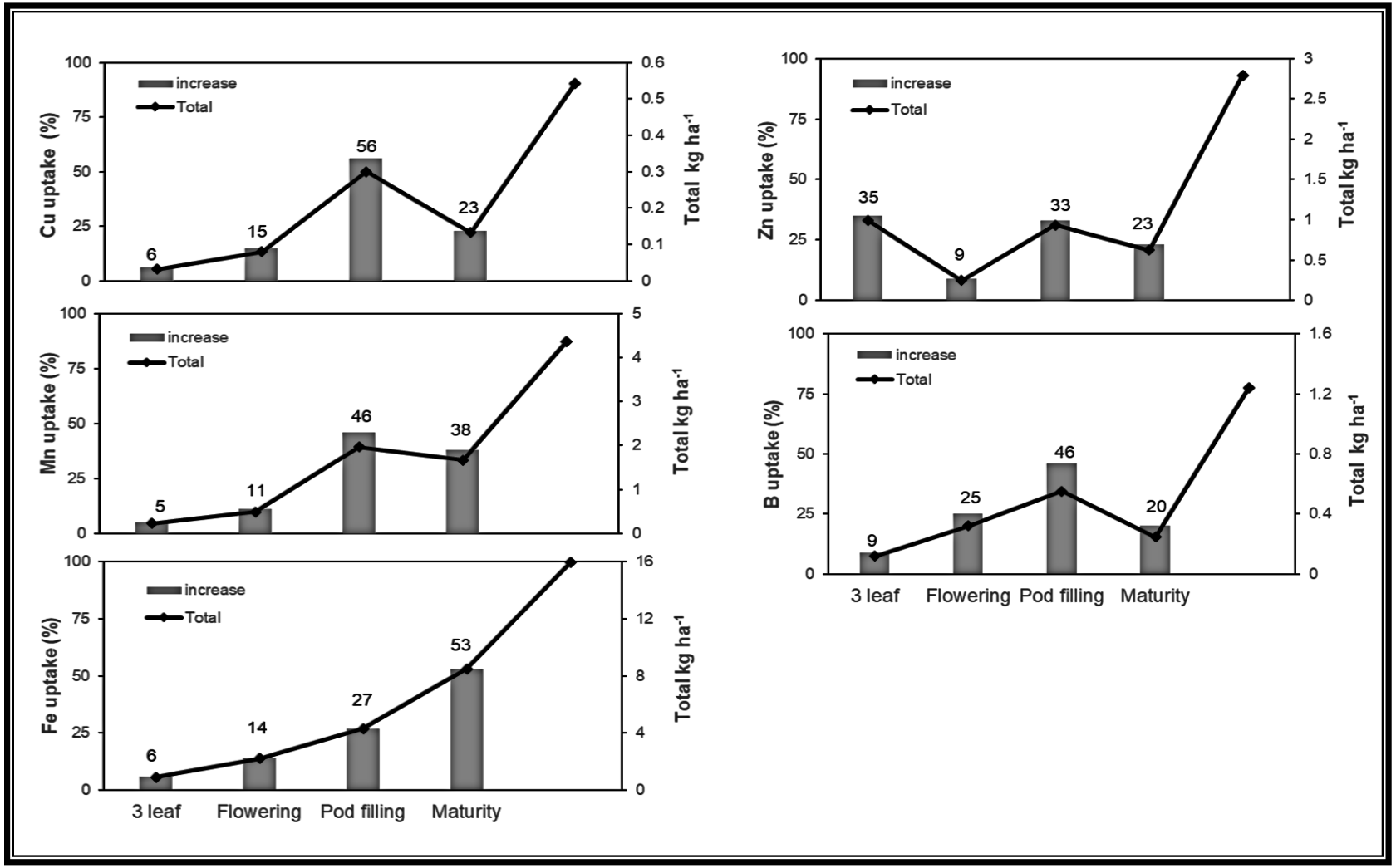

Figure 40. Stages of maximum micronutrient demand (% of total).

4.4 Micronutrient partitioning.

It was found that Fe was mostly allocated in vegetative organs so that rate of 50 kg ha^{-1} had the highest accumulation in pods (27 through 14%) with respect to 0 kg ha^{-1} (15 through 6%) in the final stages respectively (Table 35).

The distribution of Mn was higher in vegetation rather than fruit with approximately 85% of total uptake (Table 36). The average percentage in the allocation of Cu in pods was 21 to 38% of total uptake where 61 through 79% was concentrated in vegetative parts (Table 37). In the same manner, about 58 and 85% of total uptake was mainly distributed in vegetative organs for Zn and B respectively (Table 38 and 39).

Considering the total uptake, approximately 14 kg ha^{-1} of Fe were partitioned to vegetation and only 1.5 kg ha^{-1} were allocated in fruits (Figure 41). Mn distribution in fruits was of 0.55 kg ha^{-1} and 4 kg ha^{-1} in vegetation (Figure 42). Cu distribution in vegetation was the lowest (0.33 kg ha^{-1}) whereas only 0.2 kg ha^{-1} were distributed in fruits (Figure 43). Zinc partitioning process was 1.7 kg ha^{-1} in vegetation and 1 kg ha^{-1} in fruits (Figure 44). Finally, B allocation was of 0.9 kg ha^{-1} in vegetation and 0.2 kg ha^{-1} in fruits (Figure 45).

It is understood that only 2-4% of available radiation is converted into growth (Zhu *et al.,* 2010) and typically, that 50-80% of photoassimilates from a single mature leaf are transported into the phloem (Ainsworth and Bush, 2011). However, less is known about the influence of changing environmental conditions on the export of carbon from leaves and import to sinks, partitioning between heterotrophic tissues and remobilization of carbohydrates into reproductive structures.

According to Lemoine *et al.* (2013) it is important to have a better understanding of the way energy and nutrients move through a plant and into a developing seed to ensure the efficiency of yield production, particularly under abiotic stress.

On the other hand, Smith *et al.* (2018) mention that nutritional quality may not necessarily be flexible under varying resource availabilities. As supply of water and nutrients changes, plants may be predisposed to a set nutritional content for individual seeds, guided by a threshold for reproductive viability before then allocating resources to the next seed.

Under these circumstances, nutritional quality is less coupled with resource availability, so that plants respond quickly to stress by altering physiology or morphology in order to survive, often enhancing reproductive fitness and yields in stressful environments.

Nonetheless, ambiguity surrounding sink strength occurs due to an inability to directly measure it (quantify) and lack of understanding of the processes that drive sink activity. Given the number of factors that relate to sink activity (growth, metabolism); it is incongruous that sink size has a proportional influence on sink activity over sink strength.

Finally for agricultural activities, the net cumulative result of sink strength under a given source availability is yield, so the relationship between sources and sinks is undoubtedly complex and changes with changing environment.

Table 35. Iron distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	84.50 a	15.50 b	94.05 a	5.95 b
25	100	0	100	0	81.85 ab	18.15 ab	93.06 a	6.94 b
50	100	0	100	0	72.78 b	27.22 a	85.62 b	14.38 a
100	100	0	100	0	77.83 ab	22.17 ab	89.63 ab	10.37 ab
OSL	NS	NS	NS	NS	0.02	0.01	0.002	0.002
Inoculation	100	0	100	0	85.9 a	14.0 b	86.5 b	13.45 a
No inoculation	100	0	100	0	72.5 b	27.4 b	94.6 a	5.36 b
OSL	NS	NS	NS	NS	<0.0001	<0.0001	<0.0001	<0.0001
Average	100	0	100	0	79	21	90.5	9.5

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

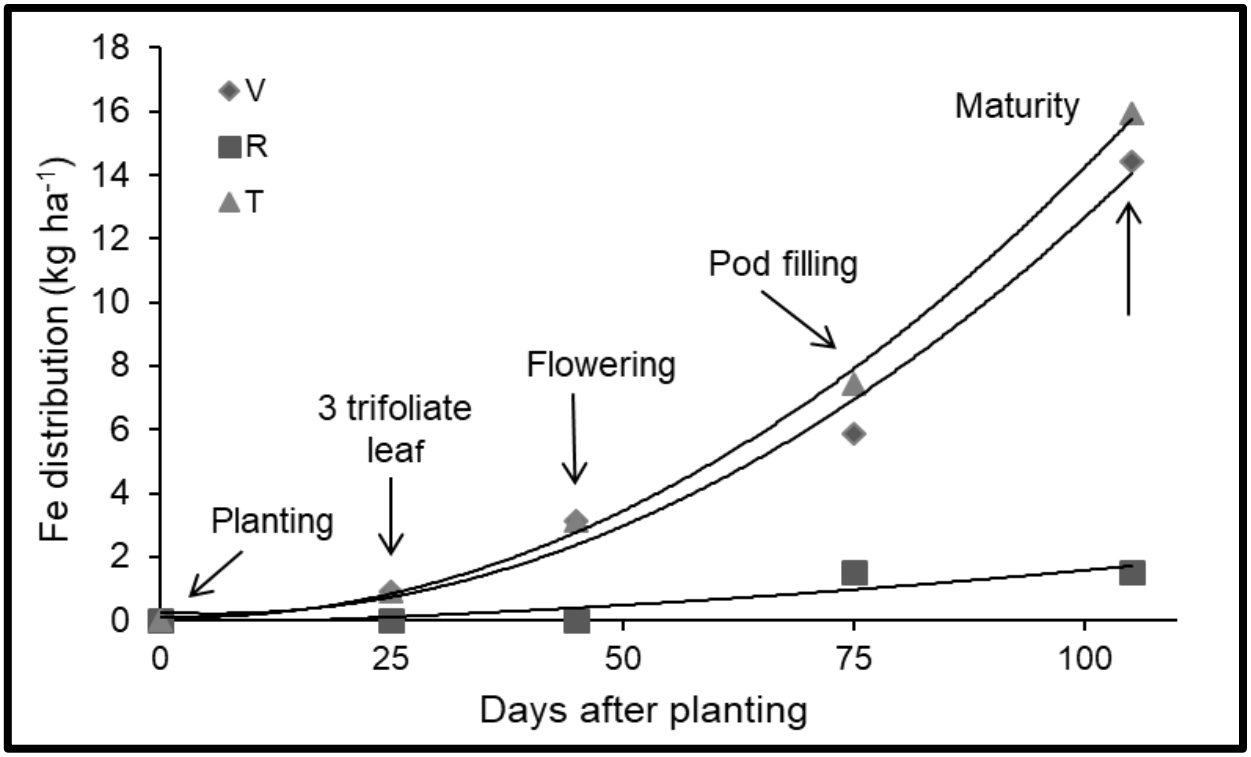

Figure 41. Total Iron distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 36. Manganese distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	88.26 a	11.74 a	89.42 a	10.58 a
25	100	0	100	0	86.88 a	13.12 a	88.99 a	11.01 a
50	100	0	100	0	81.78 a	18.22 a	85.30 a	14.70 a
100	100	0	100	0	85.95 a	14.05 a	85.42 a	14.58 a
OSL	NS	NS	NS	NS	0.1	0.1	0.1	0.1
Inoculation	100	0	100	0	81.0 b	18.94 a	83.9 b	16.13 a
No inoculation	100	0	100	0	90.37 a	9.62 b	90.7 a	9.30 b
OSL	NS	NS	NS	NS	<0.0001	<0.0001	0.001	0.001
Average	100	0	100	0	86	14	87	13

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

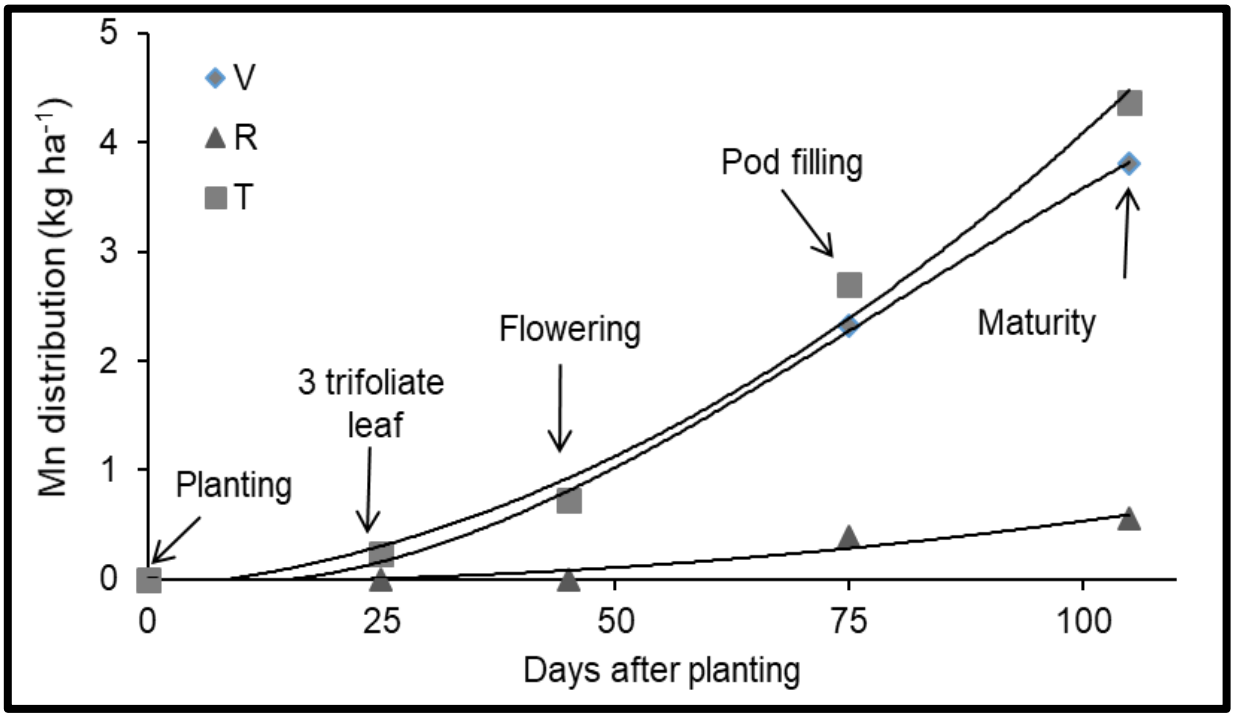

Figure 42. Total manganese distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 37. Cupper distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	83.93 a	16.07 b	74.10 a	25.90 b
25	100	0	100	0	78.91 ab	21.09 ab	59.45 b	40.55 a
50	100	0	100	0	76.70 b	23.30 a	54.38 b	45.62 a
100	100	0	100	0	77.55 b	22.45 a	59.38 b	40.62 a
OSL	NS	NS	NS	NS	0.03	0.03	0.002	0.002
Inoculation	100	0	100	0	77.28 a	22.8 a	58.7 a	35.02 a
No inoculation	100	0	100	0	81.26 a	18.74 a	64.9 a	41.3 a
OSL	NS	NS	NS	NS	0.05	0.05	0.1	0.1
Average	100	0	100	0	79	21	62	38

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

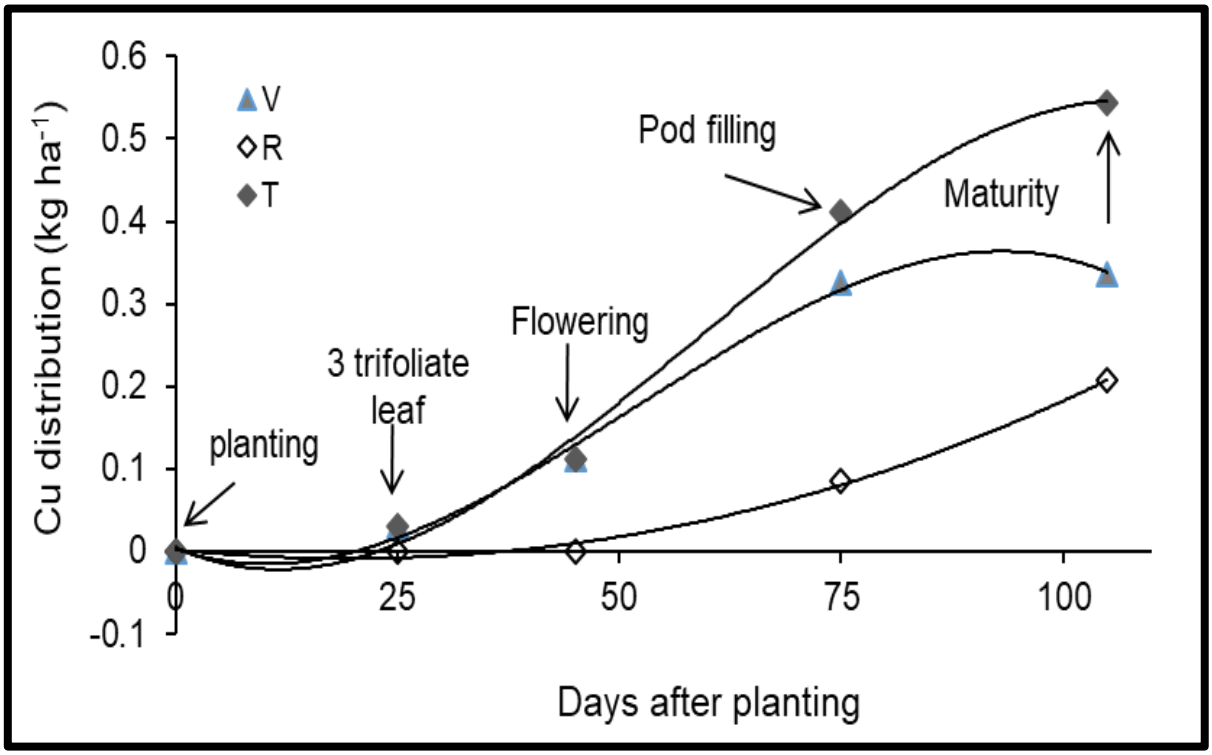

Figure 43. Total cupper distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 38. Zinc distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	70.60 a	29.40 a	66.56 a	33.44 a
25	100	0	100	0	66.35 a	33.65 a	63.22 a	36.78 a
50	100	0	100	0	61.51 a	38.49 a	50.93 b	49.07 a
100	100	0	100	0	65.34 a	34.66 a	52.07 b	47.93 a
OSL	NS	NS	NS	NS	0.2	0.2	0.1	0.1
Inoculation	100	0	100	0	59.72 b	40.3 a	47.52 b	52.48 a
No inoculation	100	0	100	0	72.2 a	27.81 b	68.9 a	31.13 b
OSL	NS	NS	NS	NS	0.001	0.001	<0.001	<0.001
Average	100	0	100	0	56	44	58	42

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL Pr> F).

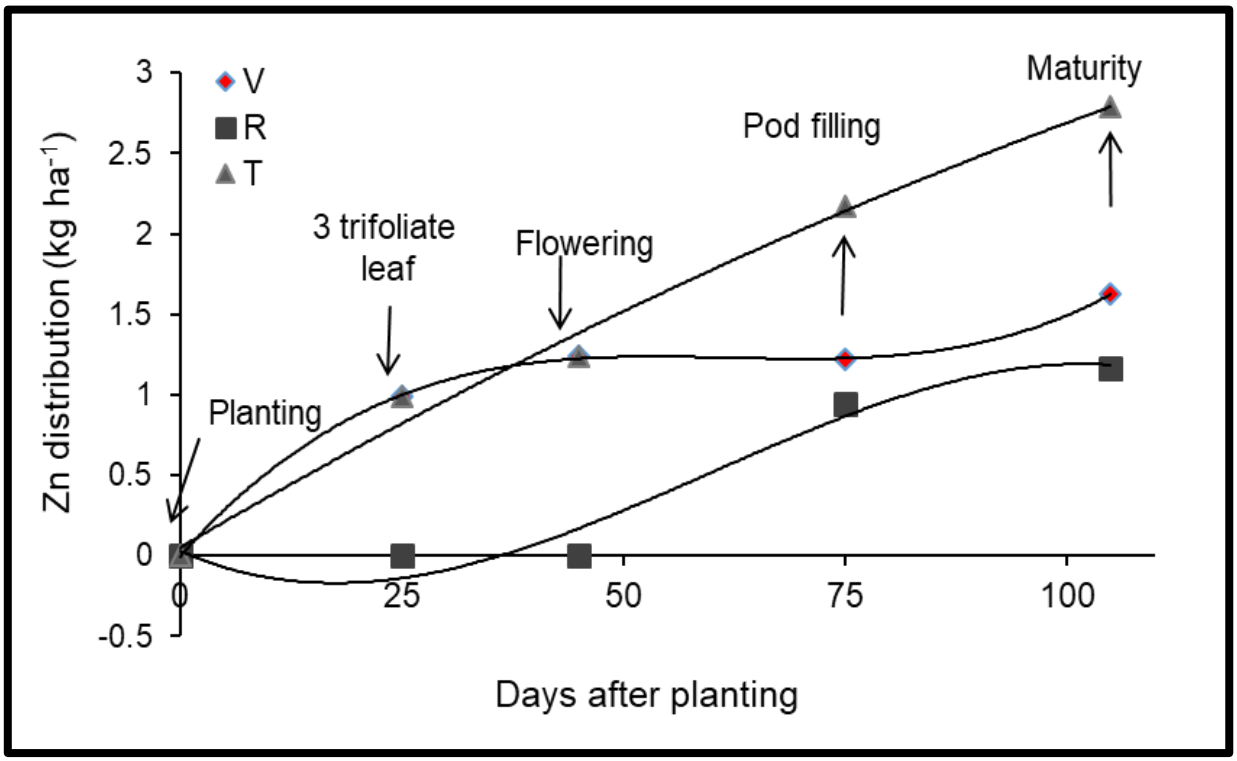

Figure 44. Total zinc distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

Table 39. Boron distribution (% of total) in vegetative (V) and reproductive organs (F).

P (kg ha^{-1})	Third trifoliate leaf		Flowering		Pod filling		Physiological maturity	
	V	F	V	F	V	F	V	F
0	100	0	100	0	90.2 a	9.7 a	81.21 a	18.79 a
25	100	0	100	0	89.4 a	10.5 a	81.03 a	18.97 a
50	100	0	100	0	87.7 a	12.2 a	75.48 a	24.52 a
100	100	0	100	0	87.8 a	12.1 a	77.32 a	22.68 a
OSL	NS	NS	NS	NS	0.5	0.5	0.2	0.2
Inoculation	100	0	100	0	86.1 b	13.8 a	71.3 b	28.67 a
No inoculation	100	0	100	0	91.6 a	8.4 b	86.19 a	13.81 b
OSL	NS	NS	NS	NS	0.002	0.002	<0.001	<0.001
Average	100	0	100	0	89	11	79	21

- Values with different letters are significantly different. Fisher's mean separation test (P≤ 0.05), (OSL>F).

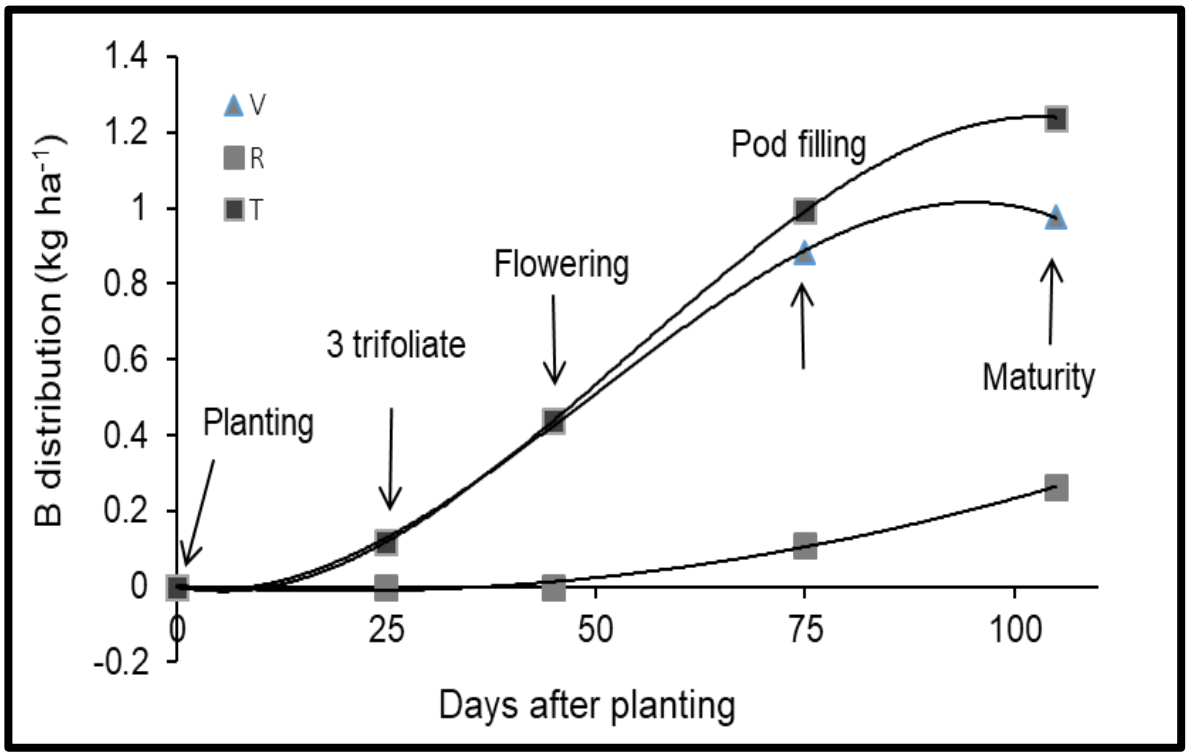

Figure 45. Total boron distribution (kg ha^{-1}) in vegetative (V) and reproductive organs (R).

4.5 Nutrient removal

Remobilization is a process-describing nutrient's movement from its initial location of nutrient deposition (leaf, stalk) to a new area of reuse, typically the grain. Accordingly, the bean grain represents an excellent source of proteins, aminoacids and iron. It is considered that has twice the amount of proteins, about equal amount of carbohydrates and micronutrients as compared to cereals (McClean *et al.*, 2011).

Recently, quality characters of grain along with high yield and pest resistance are very important for consumers as well as growers (Mederos, 2006). Among *Phaseolus vulgaris* L, there are different types of grains, and market value is strongly influenced by characters such as size, color, grain uniformity, cooking time, taste and broth thickness (Acosta *et al.,* 1993).

On the other hand, nutritional value of this legume is determined by its protein content and digestibility, as one of the main nutrient source for low-income population (Perez-Herrera *et al.,* 2002). In addition, it is a good source of iron, potassium, magnesium, zinc, fiber, starch, folic acid, thiamine (Mune *et al.,* 2013; Santalla *et al.,* 1999). Thus, it could also be used as a substitute for meats and other protein sources (Oliveira *et al.,* 2002).

The vast majority of literature indicates that bean materials have raw protein values on dry basis from 16 and 30% (Soris and Mohan, 2011). The most consumed *Phaseolus vulgaris* L. varieties in Latin America have an average protein content of 20%, with a variation interval from 19.3 to 35.2% (Nabhan *et al*., 1985).

Vegetable proteins can be classified according to solubility as water-soluble (albumins), dilute on salt solution (globulin), dilute on acid and basic solution (glutenin), dilute on alcohol solution (prolamin) (Mederos, 2006).

Overall, the higher amount of protein found in legumes is in the form of globulins followed by albumis while glutenins and prolamins in lesser amounts (Nikokyris and Kandylis, 1997). It is also found that nutritional quality of total protein is dependent on the amounts present in grains.

In this study, protein content was significantly ($P \leq 0.05$) affected by P rates and inoculation. As observed from data, the greatest percentage was recorded on rate of 50 kg ha^{-1} (22.27%). The mean concentration was 20.5%, inoculated treatments increased by 5% over the non-inoculated.

According to the results found, protein content varied between 22 and 20%, which is in accordance with Aramendiz-Tatis *et al.* (2016) who reported 22% on protein content. Some other studies report 21 to 27% protein in seeds of cowpea beans (*Vigna unguiculata*) (Gupta *et al.,* 2010; Antova *et al.,* 2014).

Bean protein has high lysine content (6.4-7.6 g* 100 g^{-1} of protein), phenylalanine + tyrosine (5.3-8.2 g*100 g^{-1} of protein) which means that it satisfies the minimum required by the Food and Agriculture Organization (FAO) and World Health Organization (Guzmán-Maldonado *et al.,* 2002; Miquilena and Higuera, 2012). However, Azufrado beans are deficient in cysteine and methionine (Reyes and Paredes, 1993).

As mentioned previously, legumes contain great amount of iron and other minerals such calcium, phosphorus and zinc. Even though legumes are cited as a complement of cereals in terms of aminoacid content. They also play an important role in nutrition due to micronutrient and vitamin inputs (Guzmán-Maldonado *et al.,* 2002).

The range of P was of 4.5 g kg^{-1} of seed, a similar value to those reported by Aramendiz-Tatis *et al.* (2016), Mesquita *et al.* (2007) and Frota *et al.* (2008). Araujo (2007) found 3.0 and 5.4 mg g^{-1} p, values similar (2.6 to 5.1 mg g^{-1} p) to those reported by Teixeira *et al.* (2009) for cultivar Carioca at different levels of soil P supply.

Espinoza-García *et al.* (2016) found high values of P content in several landraces of common bean (4.9 g kg^{-1} of seed) grown at different locations of Oaxaca. They reported that those values were higher from those found by Martínez-Meyer *et al.* (2013) and Prolla *et al.* (2010) from 4.7 and 3.6 g kg^{-1} of seed respectively.

Potassium in seed had a mean concentration of 18 g kg^{-1}, which was higher from those found by Avanza *et al.* (2013), Aramendiz-Tatis *et al.* (2016) and Mesquita *et al.* (2007) on some species. Furthermore, Bruuselma *et al.* (2012) K demand in human nutrition is of 2.7 g day^{-1}.

No differences were found regarding K, Ca, Mg, Mn, Cu, Zn and B concentration in response to P rates. However, the highest values registered were on rate 50 kg ha^{-1}, followed by rate of 25 and 100 kg ha^{-1}. Besides, inoculation with Bs favored significantly the availability of nutrients present in soils. Thus, the total concentrations were in the following order: Zn> Mn> B> Cu> K> Ca> Mg with 318, 139.1, 114.5, 92.91, 18.19, 6.71, 3.97 in which Bs had an increase of 2.2, 19, 14.2, 13, 5, 21 and 38% over non-treated treatments for the above nutrients.

Ca concentration in seeds was high (6.7 g kg^{-1} of seed) in contrast to those of Frota *et al.* (2008) who reported 1.4 g kg^{-1} of seed, Espinoza-García *et al.* (2016) found from 1 to 1.5 g kg^{-1} of seed, Prolla *et al.* (2010) reported 4 g kg^{-1} of seed, while Meyer *et al.* (2013) found 1.41 kg^{-1} of seed.

It is mentioned that calcium content differences are attributed to genetic variation that can influence in the capacity to form new roots, which are determinants in uptake and accumulation in seed (Ribeiro *et al.,* 2012). Possibly, the accumulation of Ca in this study was closely related to soil fertility where the experiment was conducted, due to its optimum concentration to meet plant requirement. Meanwhile, Mg content was around 4 g kg^{-1} of seed, values that were above from those reported by Avanza *et al.* (2013) in cultivars of beans.

Fe content also significantly differed ($P \leq 0.05$) in response of P rates and inoculation exhibiting the highest values on rate 50 kg ha^{-1} with 420 mg kg^{-1} in contrast to the lowest rates. The mean concentration was of 372 mg kg^{-1} of seed respectively in which Bs increased by 18% over the non-treated plants. These values were superior than those reported by Espinoza-García *et al.* (2016), Prolla *et al.* (2010), Martínez-Meyer *et al.* (2013) and Gouveia *et al.* (2014) who found 37, 74, 33, 61 mg kg^{-1}.

Zn content had a mean concentration of 318 mg kg^{-1}, higher value than those found by Astudillo and Blair (2008) in 40 cultivars of common bean. Some other studies suggest that application of P to soil can increase Fe concentration but at the same time it can decrease Zn concentration en bean seeds (Astudillo and Blair, 2008); while Gianquinto *et al*. (2000) found that Zn levels in the whole plant decreases as increasing P in soil.

Studies show that zinc content ranges between 21 a 54 mg L^{-1} and that is one of the highest among legume sources, almost equal to those in dairy products but less than meats.

As reported in some trials, Azufrado beans have a very important source of proteins. However, they possess a low nutritional value due to low digestibility and bioavailability of aminoacids; factors that are related to protein's own structure (Mederos, 2006).

It is also mentioned that bean crops provide about 40% of iron to human diet. Nevertheless, it is estimated that only 20% is assimilated with respect to total iron present in grains, thus its contribution is quite low and has been related to anemia (Guzmán-Maldonado *et al.,* 2002); so that, this micronutrient forms a great number of enzymes that intervene in energy production and good response to immune system (Mederos, 2006).

On the other hand, Frossard *et al.* (2000) state that aminoacid deficiency in Azufrado beans is a non-nutritional factor factor due to low use of iron and zinc. Besides, that it can be considered as a negative character of grains.

Finally, numerous studies have demonstrated the impact of crop management on nutrient removal due to hybrid variation in grain nutrient concentrations and yield components while others report that weather and agronomic management influences and/or affect nutrient removal.

Table 40. Protein and seed nutrient content of common bean (*Phaseolus vulgaris*).

(P_2O_5) kg ha^{-1}	Protein	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B
	(%)		g kg^{-1} of seed							
0	18.59 b	4.45 ab	17.55 a	6.40 a	3.65 a	0.30 b	0.133 a	0.087 a	0.30 a	0.108 a
25	20.12 ab	4.83 ab	18.73 a	6.73 a	3.88 a	0.41 ab	0.136 a	0.097 a	0.33 a	0.108 a
50	22.27 a	5.25 a	18.85 a	7.06 a	4.18 a	0.42 a	0.150 a	0.097 a	0.33 a	0.113 a
100	21.14 ab	4.10 b	17.66 a	6.65 a	4.18 a	0.36 ab	0.136 a	0.092a	0.30 a	0.128 a
OSL	0.05	0.03	0.6	0.6	0.6	0.005	0.8	0.7	0.4	0.4
No inoculation	19.40 b	4.23 b	17.15 b	5.30 b	2.47 b	0.30 b	0.112 b	0.080 b	0.31 a	0.099 b
Inoculation	21.65 a	5.08 a	19.24 a	8.12 a	5.47 a	0.44 a	0.166 a	0.105 a	0.32 a	0.130 a
OSL	0.02	0.005	0.03	0.01	0.01	0.001	0.004	0.004	0.4	0.005

- Values with different letters are significantly different. Fisher's mean separation test ($P \leq 0.05$), (OSL Pr> F).

4.7 CONCLUSIONS

The growth region between pod filling stages until physiological maturity represented the peak periods for nutrient acquisition which accounted for a seasonal uptake preference in the following order: K> N> Ca> Mg> P and Fe> Mn> Zn> B> Cu.

Nutrient supply in the soil solution for optimum yield during these growth stages is proposed as critical window. As a result, growers must assure the requierements before that time especially in fields with surface irrigation systems.

Despite, inoculation with *B. subitilis* Q11 favored positively the uptake process (approximately 15 to 30%) with respect to non-inoculated plants. It did not enhance the final yield as indicated by a great number of reports. This behavior showed a marked luxury nutrient consumption by the crop. However, this mecanism could be used as a potential strategy for nutrient solubility and acquisition by plants in other soil types, crops and environmental conditions.

Approximately 30% of uptake process was partitioned on reproductive organs during the season, while the rest was allocated in vegetative organs.

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CAPITULO 5

MAIN CONCLUSIONS

Rate of 50 kg ha^{-1} P influenced positively the growth and yield potential as compared to a higher rate (100 kg ha^{-1}). The peak period of maximum growth rate and growth parameters (height-canopy) started at 60 DAP and was maximized up to 75 DAP (end of pod filling stage).

The most active stage for DM accumulation was pod filling which likely corresponded to physiological peak in photosynthesis, water use and nutrient uptake attaining a maximum yield of 3.2 Mg ha^{-1} on medium rate (50 kg ha^{-1} P).

DM partitioning in the reproductive and vegetative phases was relatively equal at pod filling. At the end, there was only a small shift in the distribution process, which allowed the vegetative organs accumulate a slightly higher amount of DM.

Total macronutrient uptake preference was that of K>N>Ca>Mg>P. Among some micronutrients, mobility showed to be less consistent. The order of preference was that of Fe>Mn>Zn>B>Cu.

Macro and micronutrient distribution for all treatments in vegetative organs was of 100% up to flowering stage. About 30% was distributed in reproductive organs and 70% in vegetative organs in later stages.

Even though, the results revealed that application of P fertilizer in combination with *Bacillus subtilis* significantly increased nutrient concentration and uptake, it did not contribute to enhance the final yield over 3.0 Mg ha^{-1}. This process clearly demonstrated that plants were absorbing nutrients above those needed for maximum yield (luxury consumption).

It is therefore important to supply nutrient requirement as one or split applications depending upon P sources and irrigation system to be applied. The supply of *Bacillus subtilis* inoculants to *P. vulgaris* promoted greater nutrient uptake and accumulation in their tissues.

This work constitutes only a background for further studies in evaluating different methods of fertilizer application and sources as well as types of inoculants on nutrient uptake. Therefore, this experiment provide a basis from which to build or modify a nutrient strategy to render an optimal efficiency.

Finally, farmers' management decisions such as type of hybrids and agronomic management will influence and/or affect nutrient management and final quality of grains. It is recogizing that not all varities are not the same in terms of yield, nutrient uptake, removal, and partitioning.

CAPITULO 6

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