

**UNIVERSIDADE DO ESTADO DE SANTA CATARINA – UDESC**  
**CENTRO DE CIÊNCIAS AGROVETERINÁRIAS – CAV**  
**PROGRAMA DE PÓS-GRADUAÇÃO EM PRODUÇÃO VEGETAL – PPGPV**

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**INTEGRATION OF ROOT PHENOTYPING METHODS AND PHENOLOGICAL  
STAGES IN COMMON BEAN BREEDING FOR SELECTION OF SUPERIOR  
GENOTYPES**

**LAGES**

**2024**

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Thesis presented as a partial requirement to obtain the title of Doctor in Plant Production by the Postgraduate Program in Plant Production, under the line of research in Genetic Improvement and Resources, at the Centro de Ciências Agroveterinárias – CAV, at the Universidade do Estado de Santa Catarina.

Advisor: Prof. Dr. Jefferson Luís Meirelles

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**Ficha catalográfica elaborada pelo programa de geração automática da  
Biblioteca Universitária Udesc,  
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CERUTTI, PAULO HENRQUE  
INTEGRATION OF ROOT PHENOTYPING METHODS  
AND PHENOLOGICAL STAGES IN COMMON BEAN  
BREEDING FOR SELECTION OF SUPERIOR GENOTYPES /  
PAULO HENRQUE CERUTTI – 2024.  
95 p.

Orientador: JEFFERSON LUIS MEIRELLES COIMBRA  
Tese (doutorado) – Universidade do Estado de Santa Catarina,  
Centro de Ciências Agroveterinárias, Programa de Pós-Graduação  
em Produção Vegetal, Lages, 2024.

1. Phaseolus vulgaris L. 2. Root Evaluation. 3. Shovelomics. 4.  
WinRHIZO. 5. Path Analysis. I. COIMBRA, JEFFERSON LUIS  
MEIRELLES. II. Universidade do Estado de Santa Catarina, Centro  
de Ciências Agroveterinárias, Programa de Pós-Graduação em  
Produção Vegetal. III. Título.

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Lages, 16<sup>th</sup> of february 2024

To students of agricultural sciences, especially those involved in the fantastic area of plant breeding.

## ACKNOWLEDGMENTS

I thank God for the opportunity to live and learn every day.

To my family, who spared no effort to support me at all stages of postgraduate studies.

To my friend, professor, advisor and scientific father Prof. Dr. Jefferson Luís Meirelles Coimbra, for literally changing my life. There were so many teachings and experiences that will be forever etched in my memory.

To the professor and friend, Prof. Dr. Altamir Frederico Guidolin, for his extensive help in my personal and professional life.

To colleagues from IMEGEM group, Luan, Pedro, Carlos, Arthur, Felipe, Rita and Nicole, for exchange of knowledge, mutual help and partnership in carrying out the activities.

The Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, and funding agencies, both teaching scholarships and resources for project execution, namely the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC).

Thank you very much!

A ship in port is safer, but that's not what ships were made for."

John A. Shedd.





## RESUMO

A cultura do feijão se destaca no cenário de produção agrícola. Isso porque os grãos dessa leguminosa são uma das fontes de proteínas mais acessíveis a população. Com base nisso, programas de melhoramento genético tem desenvolvido genótipos com elevada produtividade (acima de 4000 kg ha<sup>-1</sup>), e com caracteres adaptativos. Dentre os caracteres adaptativos, se destaca o aprimoramento do sistema radicular. Desse modo, esta tese está dividida em quatro capítulos, que contemplam experimentos executados com a cultura do feijão, visando aprimorar a seleção de genótipos promissores para o sistema radicular. Os capítulos um, dois e três apresentam a integração de métodos de fenotipagem de raízes (“Shovelomics e WinRHIZO”), e estádios de desenvolvimento (V<sub>4-4</sub>; V<sub>4-8</sub>; R<sub>5</sub>; R<sub>6</sub>; R<sub>7</sub> e R<sub>8</sub>) na avaliação do sistema radicular de populações fixas e segregantes de feijão. Já o capítulo quatro, relata a associação entre caracteres da parte aérea com os radiculares, no intuito de favorecer a seleção indireta de duas ou mais características nas etapas de condução e seleção de populações segregantes de feijão. Os resultados dos três primeiros capítulos salientaram que métodos específicos de fenotipagem foram indicados para melhoria da avaliação radicular de acordo com o grupo gênico dos genótipos (Mesoamericanos e Andinos). O método Shovelomics foi útil para fenotipar genótipos Andinos, pela sua particularidade de medida (manualmente e de forma superficial no solo). Já o método WinRHIZO, foi recomendado para fenotipagem de genótipos Mesoamericanos, pois estes apresentam um sistema radicular mais ramificado e profundo, comparativamente ao Andino. Dessa forma, medições automatizadas obtidas pelo método WinRHIZO facilitaram a medição radicular destes genótipos. Além disso, foram diagnosticadas associações significativas entre caracteres do sistema radicular e de parte aérea, com destaque para as características: teor de clorofila b *versus* comprimento horizontal esquerdo de raízes ( $\tau = -0,22$ ) e teor de clorofila a *versus* comprimento total de raízes ( $\tau = 0,24$ ). O desdobramento desses valores em efeitos diretos e indiretos pela análise de trilha indicaram a elevada contribuição dos teores de clorofila sobre o desenvolvimento radicular. Este resultado facilita a seleção indireta, com melhorias simultâneas entre caracteres radiculares e de parte aérea, já que avaliações de caracteres acima do solo são fáceis de serem executadas.

**Palavras-chave:** *Phaseolus vulgaris* L; Avaliação radicular; Shovelomics; WinRHIZO, Análise de trilha.

## ABSTRACT

Common bean cultivation stands out in the agricultural production scenario. This is because the grains of this legume are one of the most accessible sources of protein for the population. Based on this, genetic improvement programs have developed genotypes with high productivity (above 4000 kg ha<sup>-1</sup>), and with adaptive characters. Among the adaptive characters, the improvement of the root system stands out. Therefore, this thesis is divided into four chapters, which include experiments carried out with common bean crop, aiming to improve the selection of promising genotypes for root system. Chapters one, two and three present the integration of root phenotyping methods (“Shovelomics and WinRHIZO”), and phenological stages (V<sub>4-4</sub>; V<sub>4-8</sub>; R<sub>5</sub>; R<sub>6</sub>; R<sub>7</sub> and R<sub>8</sub>) in the evaluation root system in fixed and segregating common bean populations. Chapter four reports the association between aerial and root characters, with the aim of favoring the indirect selection of two or more characteristics in the stages of conduction and selection of segregating populations. The results of the first two chapters highlighted that specific phenotyping methods were indicated to improve root evaluation according to the genotype gene group (Mesoamerican and Andean). The Shovelomics method was useful for phenotyping Andean genotypes, due the particularity of measurement (manually and superficially in the soil). The WinRHIZO method was recommended for phenotyping Mesoamerican genotypes, as they have a more branched and deeper root system, compared to Andean. Thus, automated measurements obtained by the WinRHIZO method facilitated the root measurement of these genotypes. Furthermore, significant associations were diagnosed among characters of the root system and shoots, with emphasis on the characteristics: chlorophyll b content versus root left horizontal length ( $\tau = -0.22$ ) and chlorophyll a content versus total root length ( $\tau = 0.24$ ). The breakdown of these values into direct and indirect effects by path analysis indicated the high contribution of chlorophyll levels to root development. This result facilitates indirect selection, with simultaneous improvements between root and shoot traits, since evaluations of above-ground traits are easy to perform.

**Keywords:** *Phaseolus vulgaris* L; Root Evaluation; Shovelomics; WinRHIZO, Path Analysis.

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## 1 INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the main foods that make up the diet of population of South America and the African continent. The main reason associated with the consumption of this legume is associated with the quality and quantity of protein present in its grains. (Souter *et al.*, 2017). At a national level, according to information provided by the Companhia Nacional de Abastecimento (CONAB), the area currently cultivated with common bean in Brazil is 2.775,900 hectares, with grain productivity of 1091 kg ha<sup>-1</sup> in the 2023/24 harvest (Conab, 2024).

Among the various areas of research aimed at improving the productivity of common bean grains, plant breeding, associated with the areas of genetics, biochemistry, plant physiology and crop plants, were the main responsible for the increase in production over the years. In particular, plant breeding has sought, through art and science, the development of bean genotypes with agronomic characteristics superior to those already cultivated today.

However, even with the development and use of genetically improved cultivars, in many cultivation situations the maximum productive potential of the common bean crop is not reached. This is because the expression of a certain characteristic is due to the genetic effect, added to the environmental effect (Allard, 1971; Fritsche-Neto; Borém, 2013). The cultivation environment can in some situations inhibit the maximum expression of the genetic effect (Fageria *et al.*, 2005). The lack or excess of some environmental resource can develop a stress condition (scarcity or abundance of water, low availability of nutrients or inadequate temperatures).

One of the ways to mitigate environmental stress is the development of common bean genotypes with an improved root system, capable of capturing soil resources (water and mineral elements) in limiting cultivation conditions. Common bean plants with an improved root system (greater root length and volume) enhance water acquisition in environments with water stress. This minimizes the negative effects that a lack of water can cause at some critical moments in the crop, such as flowering (Polania *et al.*, 2016).

Despite the vast importance of the root system, there are still gaps to be filled in literature, such as, which phenotyping method is the best to quantify populations in common bean breeding? And at what phenological moment in the culture is this phenotyping effective?

Therefore, this work aims to study the expression of the root system in common bean genotypes, seeking to integrate root phenotyping methods and phenological development

stages, to improve the evaluation of roots in the stages of conduction and selection of agronomically superior genotypes.

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## **6 FINAL CONSIDERATIONS**

The genetic improvement of bean crops focused on root traits is essential for the development of plants with potential tolerance to stress caused by the environment.

By carrying out work focused on root phenotyping, the high effect of environment on root expression was observed, that is, root characters show plasticity to environmental conditions. This fact must be considered when selecting superior plants, seeking to eliminate non-genetic effects that hinder selection.

The improvement of root evaluation in common bean crop, considering fixed and segregating genotypes, was obtained through the integration between specific development stages with the evaluated root phenotyping methods. This integration helps breeders choose the best bean genotypes considering root variables.

The association between root and shoot traits stands out as a useful tool for the facilitated selection of genotypes simultaneously improved for root system and above-ground traits.