GABRIEL LEMES JORGE

SELECTION OF SOYBEAN LINES UNDER BIOTIC STRESSES AND ENDOPHYTIC BACTERIA AS AN ALLEVIATING FACTOR OF DROUGHT DURING LENTIL CULTIVATION

Master thesis submitted to the faculty of Federal University of Uberlândia, in partial fulfillment of the requirements of the Graduate Program in Agronomy – Master of Science degree, specialization in Crop Science, to obtain the title of "Master".

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UBERLÂNDIA
MINAS GERAIS – BRAZIL
2018

Dados Internacionais de Catalogação na Publicação (CIP)
Sistema de Bibliotecas da UFU, MG, Brasil.

J82s
2018

Jorge, Gabriel Lemes, 1992

Selection of soybean lines under biotic stresses and endophytic bacteria as an alleviating factor of drought during lentil cultivation / Gabriel Lemes Jorge. - 2018.

77 f. : il.

Orientadora: Ana Paula Oliveira Nogueira.

Coorientador: Osvaldo Toshiyuki Hamawaki.

Dissertação (mestrado) - Universidade Federal de Uberlândia, Programa de Pós-Graduação em Agronomia.

Disponível em: http://dx.doi.org/10.14393/ufu.di.2018.735

Inclui bibliografia.

1. Agronomia - Teses. 2. Soja - Melhoramento genético - Teses. 3. Soja - Doenças e pragas - Teses. 4. Ferrugem asiática - Teses. I. Nogueira, Ana Paula Oliveira. II. Hamawaki, Osvaldo Toshiyuki. III. Universidade Federal de Uberlândia. Programa de Pós-Graduação em Agronomia. IV. Título.

CDU: 631

Angela Aparecida Vicentini Tzi Tziboy – CRB-6/947

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APPROVED in January 29th, 2018

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Aos meus pais, Luciane e Cesar.
Ao meu irmão, Vinicius.

DEDICO

AGRADECIMENTOS

Primeiramente, agradeço a Deus, por ser o meu guia e ensinar que qualquer conquista vem acompanhada de bastante perseverança.

À minha orientadora, Ana Paula Oliveira Nogueira, pela confiança, oportunidade, orientação e ensinamentos, que foram essenciais para a conclusão deste trabalho.

Aos meus Orientadores Canadenses, Neil Emery e Anna Kisiala, pela confiança e parceria em tornar parte deste trabalho possível.

Ao professor Osvaldo Toshiyuki Hamawaki, pela oportunidade de trabalhar no Programa de melhoramento genético de soja da Universidade Federal de Uberlândia.

Ao professor Ednaldo Carvalho Guimarães pelo auxílio em estatística.

Aos membros do programa de Melhoramento Genético de Soja - UFU, pela fundamental ajuda na condução deste estudo.

À Universidade Federal de Uberlândia e à Trent University por serem os intermediadores deste trabalho.

À CAPES/ CNPq pela bolsa de estudo.

À professora de basquete Rita de Cássia, por me ensinar que não é possível chegar ao fim de uma escada, sem que tenhamos subido degrau por degrau.

Finalmente, mas não menos importante, aos meus pais Cesar Jorge e Luciane Maria Lemes Jorge, ao meu irmão Vinicius Lemes Jorge, e minha família que me ensinam valores e quão melhor podemos nos tornar a cada dia. Também, pelo estímulo, apoio, suporte, carinho, compreensão e motivação para realizar meus sonhos.

E àquelas pessoas que surgem em nossas vidas, e não por acaso, permanecem, vocês amigos, foram fundamentais nesta conquista.

ABSTRACT

JORGE, GABRIEL LEMES. **Selection of soybean lines under biotic stresses and endophytic bacteria as an alleviating factor of drought during lentil cultivation**, 2018, 77p. Dissertation (Masters on Agronomy/crop science) – Federal University of Uberlândia, Minas Gerais state, Brazil[1].

Biotic and abiotic stresses are predicted to raise in the future scenario of agricultural production due to escalated climate change. Those factors are going to be responsible for increasing challenges in crop production, and can possibly lower grain yield worldwide. Consequently, plant exposition to a combination of both biotic and abiotic stresses is more likely to occur in the future. Higher incidence of stink bugs and drought stresses are common issues that pose an additional threat to soybean and lentil crop productions. In this scenario, breeding programs have a crucial role in developing tolerant lines to main pests. Due to the significant attack of stink bugs on soybeans, especially *Euschistus heros,* which can considerably compromise grain yield and seed quality, the development of superior lines is crucial. The selection based on positive correlations between grain yield and a higher number of pods and weight of seeds has demonstrated to be an important indicator of soybean resistance to stink bug attack. Twenty-three soybean F8 lines developed by the Soybean Breeding Program of Federal University of Uberlândia and four cultivars (Msoy 8527, UFUS Xavante, Msoy 8787, and UFUS Milionária) were evaluated during the growing season of 2015/2016 and 16 agronomic traits were accessed. The lines G1, G2, and G24 are very promising genotypes as they have shown valuable agronomic traits for stink bug resistance. Besides, another concern that can drastically impact crop yields is the occurrence of drought stress. An alternative to alleviate the negative effect of drought, other than genetic breeding, is the association of crop species with beneficial growth-promoting bacteria. Thus, the direct benefit of plant growth and tolerance to drought stress by bacterially produced phytohormones could minimize the negative effect of water scarcity on lentil production. A vegetative stage experiment was aimed to evaluate the performance of lentil plants during 25 day-long period of complete water withdrawal (terminal drought). Lentil seeds were inoculated with *Methylobacterium* at the time of planting by adding bacterial solution to half of the pots of the experiment. Through the analysis of morphological, biometrical, and physiological parameters, the positive effect of inoculated lentils was to a high extent a result of visibly improved plant vigor, plant growth, nutrient uptake (electrolyte content), as well as optimized water management parameters (WUE, LWL). Consequently, a sustainable growth enhancer of bacterial origin could lower the negative effect of drought on lentil cultivation. Thus, plant breeding and sustainable technologies together could minimize concerning issues in the agricultural scenario such as the high incidence of stink bugs in soybeans and drought stress in lentils.

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RESUMO

JORGE, GABRIEL LEMES. **Seleção de linhagens de soja sob estresses bióticos e bactéria endofítica como fator mitigador do estresse hídrico em lentilha**, 2018, 77p. Dissertação (Mestrado em Agronomia/Fitotecnia) - Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil.

A ocorrência de estresses bióticos e abióticos cada vez mais frequentes no cenário agrícola, provavelmente serão agravadas em função de mudanças climáticas. Estes fatores serão responsáveis por aumentar desafios na produção de alimentos podendo reduzir a produtividade de grãos no mundo. A alta incidência de percevejos e estresses hídricos são fatores que representam uma ameaça na produção comercial de soja e lentilha. Nesse cenário, programas de melhoramento têm um papel crucial no desenvolvimento de linhagens tolerantes às principais pragas em leguminosas. Devido ao significante ataque de percevejos na cultura da soja, principalmente o percevejo *Euschistus heros,* que compromete a produtividade de grãos e a qualidade de sementes da cultura, o desenvolvimento de linhagens superiores é necessário. A seleção baseada em correlações positivas entre a produtividade de grãos e maior número de vagens e peso de sementes tem demonstrado ser um importante indicativo da resistência da cultura da soja ao ataque de percevejos. Foram avaliadas 23 linhagens de soja na geração F8 desenvolvidas pelo Programa de Melhoramento Genético de Soja da Universidade Federal de Uberlândia e quatro cultivares (Msoy 8527, UFUS Xavante, Msoy 8787 e UFUS Milionária) durante a safra 2015/2016 e 16 caracteres agronômicos foram mensurados. As linhagens G1, G2 e G24 apresentaram caracteres agronômicos promissores para a resistência ao percevejo marrom da soja. Outro fator preocupante que pode drasticamente impactar a produtividade de grãos é a ocorrência de estresse hídrico em leguminosas. Uma alternativa para aliviar o efeito negativo da seca, além do emprego do melhoramento genético, é a associação de espécies de plantas com bactérias promotoras de crescimento. Assim, o benefício direto no crescimento de plantas e aumento da tolerância ao déficit hídrico em função de fito hormônios produzidos por bactérias podem minimizar o efeito negativo da falta de água na produção comercial de lentilha. Um experimento no estádio vegetativo foi desenvolvido para avaliar o desempenho de lentilha durante 25 dias de seca. Partes das sementes de lentilha foram inoculadas com a estirpe selecionada de *Methylobacterium* no momento da semeadura adicionando a solução contendo as bactérias diretamente no solo. Pelas análises de parâmetros morfológicos, biométricos e fisiológicos, o efeito positivo da inoculação em lentilha foi constatado na melhoria do vigor e crescimento de plântulas, absorção de nutrientes (conteúdo de eletrólitos), bem como um melhor aproveitamento de água pelas plantas (maior eficiência no uso da água e menor perda de água nas folhas). Logo, um sustentável inoculante promotor de crescimento, poderia minimizar o efeito negativo da seca no cultivo de lentilha. Assim, o melhoramento de plantas junto ao emprego de tecnologias sustentáveis, poderia minimizar problemas no cenário agrícola como a alta incidência de percevejos na cultura da soja e o estresse hídrico em lentilha.

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1. GENERAL INTRODUCTION

The future scenario of agricultural production is expected to face challenges in supplying worldwide food demand with the rising population, diet shifts, and increasing biofuels consumption (ODEGARD; VAN DER VOET, 2014). Estimates have demonstrated that the total agricultural production, despite all challenges, in order to meet the global crop demand has to increase by 100 to 110% rate by 2050 (TILMAN et al., 2011).

Biotic and abiotic stresses caused by changing climatic factors are going to be responsible for increasing challenges in crop production, which can possibly lower grain yield standards worldwide (RAMEGOWDA; SENTHIL-KUMARB, 2015). Some evidence also suggests that the climate change will enlarge the host range of virulent pathogens (BEBBER; HOLMES; GURR, 2014). Consequently, simultaneous exposition of plants to a combination of both biotic and abiotic stresses is more likely to occur in the future, which can negatively impact crop yields (RAMEGOWDA; SENTHIL-KUMARB, 2015).

Raises in abiotic and biotic stresses, such as the higher incidence of stink bugs can damage a very important commodity, the soybean crop, by reducing the soybean grain yield, lowering the overall production to very low levels and posing an additional threat to food security (BEBBER; HOLMES; GURR, 2014). Stink bug damage can reach up to 30% of soybean grain yield losses representing an expressive damage potential on the soybean crop (VIVAN; DEGRANDE, 2011).

Another issue that is more likely to occur is the water scarcity in agricultural fields. Drought stress may occur at different stages in plant development with varying intensities across the years and may cause substantial losses depending on its severity (IDRISSI et al., 2015). Drought stress can potentially decrease average yields by more than 50% (WU et al., 2011).

Breeding programs have a crucial role in developing new cultivars and assessing the potential of lines that show valuable parameters for selection, such as the resistance to main pest and diseases, high standards of grain yield, drought tolerance, among others (RAMALHO, 2012). According to Ray et al. (2013), the production increase should rather come from yield improvement than extending the cultivated area, which in turn, highlights the importance of breeding programs. For instance, soybean, together with three other crops: maize, rice, and wheat, are responsible for providing two-thirds of current harvested global crop calories (RAY et al., 2013).

Beyond soybeans, another important source of protein for human diet that can help improving future perspectives on food security is lentil. Drought can drastically impact this crop yield by reducing its biomass for animal feed and grains for human food (POPELKA; TERRYN; HIGGINSA, 2004).

An alternative to alleviate the negative effect of drought, besides genetic breeding, is the association of many crop species with beneficial growth-promoting microbes (KUMAR et al., 2016). They are often successfully used to minimize the risk of limited water availability and improve yield levels and seed quality (QIAO et al., 2011). Furthermore, the direct enhancements of host growth and development, as well as abiotic stress alleviation, have been previously attributed to bacterially produced phytohormones (TSAVKELOVA et al., 2006; FIGUEIREDO et al., 2008).

Plant breeding is an important tool to achieve these goals, as it generates superior, high-yielding genotypes well adapted to a wide range of environmental conditions (ROCHA et al., 2015). Therefore, an agricultural scenario that creates adapted genotypes and uses new technologies such as bacterial inoculants that together can better perform against escalated biotic and abiotic stresses become crucial in order to guarantee food security.

2. LITERATURE REVIEW

2.1 Soybean economic importance

The soybean (*Glycine max* L. Merrill) top world producers are United States, Brazil, and Argentina; they together are responsible for over 80% of the global soybean grain production (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2017). Brazil is the second largest worldwide producer of soybean and the cultivated area during the growing season of 2016/2017 was estimated at 33.91 million of hectares. The total grain production over the same season was estimated at 114 million tons of grains (CONAB, 2017).

Although it is not usually considered to be a basic food for direct consumption, soybeans are one the most important crops worldwide mainly because of their high content of protein and vegetal oil (BEZERRA et al., 2015). The soybean grain is rich in protein and oil, which average contents in Brazilian cultivars oscillate around 40% and 20%, respectively (BEZERRA et al., 2015).

Beyond that, the wide adaptation of cultivars to tropical and subtropical climate allowed the development of the crop and the establishment of an effective industrial complex designed for its processing (BEZERRA et al., 2015). Due to its versatility, profitability, and huge potential for animal and human feed, the soybean crop reached approximately 56% of the total land occupied with grain crops in Brazil over the growing season of 2016/2017 (CONAB, 2017).

2.2 Soybean genetic breeding

The soybean crop expansion in Brazil should be attributed to a great extent to the public and private investments in genetic breeding research programs, which in turn allowed the constant availability of adapted cultivars to nearly all regions of the country. In this scenario, soybean breeding programs have aimed on the development of cultivars resistant to main pests and diseases, as well as the adaptation to different environmental conditions and cultivation systems (NOGUEIRA; SEDIYAMA; GOMES, 2015).

The development of superior cultivars is a challenge for soybean breeding programs as the market requires beyond high-yielding standards, the resistance to main pests and diseases, and at the same time, wide adaptation to diverse environmental conditions (RAMALHO, 2012).

Breeding process involves a lot of stages i.e. the selection of parental lines in order to achieve segregating populations, advancement of generations, establishment of selection criteria, segregating population management methods, correlations studies, and cultivar recommendation (ALMEIDA; KIIHL, 1998). As there are many desirable agronomic traits to be improved, the study of the correlation between traits is indeed important since it helps the breeder to establish the appropriate selection criteria (CRUZ; REGAZZI; CARNEIRO, 2012).

Phenotypic, genotypic and environmental correlations are important tools for criteria selection (FALCONER; MACKAY, 1996). Phenotypic correlation is estimated through the direct data measurements, which could be of genetic or environmental causes. Moreover, the occurrence of genotypic correlation is mainly due to pleiotropism, a phenomenon where a single gene can influence 2 or more characteristics. Genetic linkage can also be the cause for genotypic correlation occurrence. However, this is a transitory correlation that often happens in populations derived from divergent lines (FALCONER; MACKAY, 1996).

In this context, phenotypic and genotypic correlations are essential for breeding programs as they can create a helpful association among agronomic traits, which may be negatively correlated with others (RAMALHO, 2012). Furthermore, since many agronomic traits of interest are of quantitative nature, influenced by more than two genes, it can be hard to identify the lines that may stand out based only on one trait (CRUZ; REGAZZI; CARNEIRO, 2012).

Through the correlation and path analysis in 90 soybean genotypes over two sowing seasons, Nogueira et al. (2012) affirmed that the total number of pods per plant and number of nodes of the main stem can be used as a useful base for soybean grain yield indirect selection.

When analyzing genotypic and phenotypic correlations in soybean genotypes, moderate to high correlations were found between total number of pods and grain yield, indicating that the selection for total number of pods at maturity directly influences others characters (LEITE et al., 2016).

Rocha et al. (2015), while studying genotypic and phenotypic correlations in soybean genotypes infected by naturally occurring stink bug infestation, have concluded that the total number of pods is a useful trait for indirect selection of soybean genotypes with high grain yield.

2.3 Stink bug damage on soybeans

In Brazil, three species of the stink bug complex are considered to be predominant; the small green stink bug (*Piezodorus guildinii*), green stink bug (*Nezara viridula*), and brown stink bug (*Euschistus heros*) (GUEDES et al., 2012). The brown stink bug is the predominant species in soybean growing areas as it is better adapted to neotropical regions and commonly found in higher densities (CORRÊA- FERREIRA et al., 2009).

Starting from the 3rd to 5th instar nymphs, stink bugs can cause direct and indirect irreversible damages to seed development (PRADO et al. 2010). The insects feed directly from the pods by inserting their sucking mouth apparatus that reaches the grains (CORRÊA- FERREIRA 2000). Plant maturation can also be delayed when the seeds are significantly injured (LEONARD et al., 2011). Other important injuries are associated with the injection of digestive enzymes that lead to deformation, abortion, loss in germination and seed vigor (OLIVEIRA, 2010). Moreover, stink bugs can cause foliar retention, impairing mechanical harvesting of the crop (SILVA; CANTERI; SILVA, 2013). In fact, stink bug damage can cause up to 30% of soybean grain yield losses according to Vivan and Degrande (2011), which represent an expressive damage potential of the soybean crop.

The control level is reached when 2 adults or third instar nymphs per linear meter are found at any time during all reproductive stages (CORRÊA-FERREIRA; PANIZZI, 1999). As a mean to mitigate the effects of these insect pests on soybeans, insecticides have been intensively used (ROCHA et al., 2014). Nevertheless, this control method is harmful to the environment, producing waste and promoting the selection of bug populations resistant to certain molecules (MAIA et al., 2009). To this end, the development of soybean cultivars resistant to the stink bug complex is extremely meaningful for the maintenance and/or increase in yield levels of the crop (ROCHA et al., 2014).

2.4 Abiotic and biotic stresses under climate change

Extreme agronomic conditions, including variations in temperature, precipitation, drought, and floods can have devastating effects on food security across the world. Therefore, reliable food production as a consequence of climate change will require considerable changes in crop breeding, farming practices and infrastructure (ABBERTON et al., 2015).

The frequency and intensity of drought episodes (the major abiotic stress affecting crop yield globally) have increased in the last decades (ABBERTON et al., 2015). Likely, global climate change and temperature increases in some regions of the planet, as well as the rapid growth of the human population will demand higher yields to feed the growing population (ABBERTON et al., 2015).

Some examples of main causes of yield loss are: global warming (especially as the effect of night temperature that increases plant respiration and also, higher day temperature events triggering decreased fertility), changes of rainfall pattern, which often result in droughts and floods, higher incidence of pests and diseases, and other extreme weather events (ABBERTON et al., 2015). Their frequency will potentially be increased due to the effect of climate change (ABBERTON et al., 2015).

Globally, the area under drought is generally projected to increase and this pressure could be further aggravated by the changing climate, especially in the longer-term (FALLOON et al., 2015). Plant growth and development will be adversely influenced causing crop failure and decreasing average yields by more than 50% (WU et al., 2011). Furthermore, this scenario indicates that many countries will experience water stress in 2050 (ODEGARD; VAN DER VOET, 2014).

Changing climate is expected to cause longer growing seasons in many regions of the world, and therefore, it will also affect the adaptation (STOECKLI et al., 2012) and behavior (MA; MA, 2012) of insects, which are likely to be spread into areas where they did not exist before. Insects are globally widespread, and often limit crop yields (ABBERTON et al., 2015). They carry highly adaptable virulence genes that can adapt to changing habitat and to the deployment of new resistant varieties forcing insects to develop new virulence genes to survive in such environment (HALEY et al., 2004; TOLMAY; LINDEQUE; PRINSLOO, 2007).

2.5 Food demand and other sources of protein

At the current rate of crop yield increase, approximately only 67%, 42%, 38%, and 55% of what is needed for maize, rice, wheat, and soybean respectively, will be produced by the year of 2050 in order to meet worldwide food demand (RAY et al., 2013). Therefore, as the current rate of global yield increase per year may not supply all the food demand by the year of 2050, other sources of food and protein have to be included in human diet (RAY et al., 2013).

Lentil (*Lens culinaris* L.), one of the oldest domesticated plants in the world, originates from the Near East and central Asia (ZOHARY, 1972). Seeds of this species are an important source of protein for human diet and the entire biomass of plant is appropriate for animal feed (MUSCOLO et al., 2014). The top three world producers of lentils are Canada, India, and Turkey with a total production of 2.17, 1.13, and 0.42 million tonnes, respectively, over the year of 2013 (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAOSTAT, 2013). They together produce over 75% of the global lentil production (FAOSTAT, 2013).

Being a major class of cash crops, increase in the global yield of oilseeds and pulses with improved protein, carbohydrate, and oil for food, feed, and industrial applications are needed (ABBERTON et al., 2015). However, biotic and abiotic stresses are the most concerning production constraints for global oilseed and pulse production, and they are predicted to worsen with the adverse effect of climate change (ABBERTON et al., 2015).

Drought conditions have emerged as the major yield constraint in lentil over its production localities, which can lead to forced maturity and, consequently, lower yield levels (FOUAD et al., 2011). For instance, in semiarid environments where lentils are widespread, unfavorable soil moisture at sowing often compromise severely seed germination, and as a result, it leads to irregular seedling emergences hindering the establishment of an appropriate stand and grain yield (OKCU; KAYA; ATAK, 2005).

2.6 Methylobacteria as a stress alleviating factor under drought conditions

Various microorganisms reside on plants (WHIPPS et al., 2008). A very important type of microorganisms is called endophytic bacteria. They live as biotrophic symbionts and can be either obligate or facultative plant-associated bacteria living inside plant tissues without causing any harm to plants (JOSHI; KULKARNI, 2014). Endophytic bacteria can be detected inside surface-sterilized tissues or extracted from inside plants, which are not harmed by the presence of the bacteria (HALLMANN et at., 1997).

To maximize crop yield by manipulating plant-associated microorganisms, the optimal selection of growth-promoting microbes has to be done in order to identify the most effective strains for the particular planting situation (FORCHETTI et al., 2007; TANK; SARAF, 2010; WILSON; WALKER, 2010; MARASCO et al., 2012; PATEL et al., 2012; VERMA et al., 2014).

Methylobacteria, often called Pink Pigmented Facultatively Methylotrophs (PPFMs), are naturally occurring endophytic microorganisms found in plants, soil, and freshwaters (MADHAIYAN; POONGUZHALI; SA, 2007; FERREIRA et al., 2008; ASSUMPÇÃO et al., 2009; ANDA et al., 2011; MEENA et al., 2012). They thrive on the methanol released from leaves as the source of carbon and energy and in response, they secrete vital elements for plant development such as phytohormones (Cytokinins) (HOLLAND, 1994; HOLLAND, 1997). *Methylobacteria* have been reported to enhance seed germination, stimulate seedling growth, and increase systemic resistance in numerous plant species (MADHAYIAN et al., 2005; ABANDA-NKPWATT et al., 2006; KUMAR et al., 2016).

Plant hormones Cytokinins (CKs), produced in great quantities also by *Methylobacteria*, are responsible for many crucial benefits for plant growth and development. They act to regulate "source-sink" relationships among organs within the plant itself, guiding and diverting resources (sugars, amino acids, minerals, among others) (MOK; MOK, 2001). The direct enhancement of host growth and development, as well as abiotic stress alleviation, have been previously attributed to bacterially produced phytohormones (IVANOVA et al., 2000; TSAVKELOVA et al., 2006; FIGUEIREDO et al., 2008).

Importantly, CKs are remarkably beneficial to crops not only in terms of promoting seed growth and yield but also increasing tolerance to environmental stresses (O'BRIEN; BENKOVA, 2013; ZWACK; RASHOTTE, 2015). They strongly counteract many processes induced by water stress such as stomata closure, lower photosynthesis and transpiration capacity, growth inhibition and senescence acceleration, acting as strong drought resisting agents (NOVAKOVA et al., 2007; RIVERO; SHULAEV; BLUMWALD, 2009).

The direct enhancements of host growth and development, as well as abiotic stress alleviation, have been previously attributed to bacterially produced phytohormones (IVANOVA et al., 2000; TSAVKELOVA et al., 2006). Therefore, the end result is the plant benefit by increasing appropriately concentrated CK levels delivered internally to stimulate plant growth and to improve water stress resilience (FIGUEIREDO et al., 2008).

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CHAPTER 1. LINE SELECTION AND CORRELATION BETWEEN TRAITS OF SOYBEAN GENOTYPES UNDER HIGH NATURALLY OCCURRING STINK BUG INFESTATION

ABSTRACT

The soybean crop is undoubtedly important to not only Brazil but also for most parts of the globe, as economic and social dependency upon this crop becomes evident. However, the scenario of the soybean crop production has faced challenges with environmental changes, which have escalated the incidence of pests. Due to the abundance of stink bugs, especially *Euschistus heros* in tropical regions, they can considerably impact the productivity of the crop compromising total grain yield and seed quality. Consequently, developing resistant genotypes to this species becomes a crucial need. Studies on the correlation between traits are indeed important to breeding programs in order to identify indirect selection criteria that benefit other quantitative traits such as the resistance to stink bugs. Therefore, this research was aimed to evaluate soybean lines with desirable agronomic traits under high natural infestation of *Euschistus heros* and to access the genotypic and phenotypic correlations between important agronomic traits for soybean breeding. Twenty-three soybean F8 lines developed by the Soybean Breeding Program of Federal University of Uberlândia and four cultivars (Msoy 8527, UFUS Xavante, Msoy 8787, and UFUS Milionária) were evaluated under randomized complete block design with three repetitions during the growing season of 2015/2016 and 16 agronomic traits were accessed. There was genetic variability for all traits but Asian soybean rust severity at 1% probability level through F test. For all traits in the study but the total number of pods and Asian soybean rust severity, the coefficient of genotypic determination was superior to 70%, indicating that the most part of phenotypic variability was due to genetic differences among the genotypes in study. Grain yield was demonstrated to be a useful trait for indirect selection of soybean genotypes with resistance to brown stink bugs. Also, early cycle genotypes, heavy seeds and a higher number of pods revealed a positive correlation with grain yield over the same conditions. The lines G1, G2, and G24 are very promising genotypes as they have shown valuable agronomic traits for stink bug resistance.

KEYWORDS: *Glycine max, Euschistus heros,* correlation, breeding.

SELEÇÃO DE LINHAGENS E CORRELAÇÃO ENTRE CARACTERES EM GENÓTIPOS DE SOJA SOB ALTA INFESTAÇÃO NATURAL DE PERCEVEJOS

RESUMO

A cultura da soja é sem dúvida de grande importância não somente para o Brasil, mas também para grande parte do mundo, onde a dependência econômica e social sobre essa cultura se torna evidente. Porém, o cenário agronômico da cadeia produtiva da soja tem encontrado crescentes desafios com as mudanças climáticas, o que tem aumentado a incidência de pragas. Devido aos grandes danos ocasionados por percevejos, especialmente *Euschistus heros,* em regiões tropicais, eles podem consideravelmente impactar a produtividade da cultura comprometendo a produção total de grãos e a qualidade de sementes. Consequentemente, o desenvolvimento de genótipos resistentes a essa praga torna-se de crucial importância. Estudos de correlações entre caracteres são de fato essenciais para programas de melhoramento que buscam identificar critérios de seleção indireta que beneficiam outras características de natureza quantitativa como, por exemplo, a resistência ao complexo de percevejos. Portanto, esta pesquisa teve como objetivo avaliar linhagens de soja com caracteres agronômicos desejáveis em condições de alta infestação natural de *Euschistus heros* e acessar correlações fenotípicas e genotípicas entre importantes caracteres agronômicos para o melhoramento genético de soja. Foram avaliadas 23 linhagens de soja na geração F8 desenvolvidas pelo Programa de Melhoramento Genético de Soja da Universidade Federal de Uberlândia e quatro cultivares (Msoy 8527, UFUS Xavante, Msoy 8787 e UFUS Milionária) em delineamento de blocos completos casualizados com três repetições durante a safra 2015/2016 e 12 caracteres agronômicos foram mensurados. De acordo com o teste F a 1% de probabilidade, existiu variabilidade genética para todos os caracteres agronômicos avaliados, com exceção para a severidade da ferrugem asiática. Também, excetuando-se a última e ao número total de vagens por planta, todos os caracteres apresentaram o coeficiente de determinação genotípico superior a 70%, indicando que a maioria da variabilidade fenotípica foi atribuída às diferenças genéticas entre os genótipos em estudo. A produtividade de grãos demonstrou ser um caráter útil na seleção indireta de genótipos de soja com resistência ao percevejo marrom. Ainda, genótipos de ciclo precoce, sementes pesadas e um maior número de vagens por planta apresentaram uma correlação positiva com a produtividade de grãos nas mesmas condições de cultivo. As linhagens G1, G2 e G24 apresentaram caracteres agronômicos promissores para a resistência ao percevejo marrom da soja.

Palavras-chave: *Glycine max, Euschistus heros,* correlação, melhoramento.

1. INTRODUCTION

As the second largest worldwide producer of soybeans, the cultivated area in Brazil during the growing season of 2016/2017 was estimated at 33.91 million of hectares and an overall production at 114 million tons of grains (CONAB, 2017). The soybean crop reached approximately 56% of the total land occupied with grain crops in Brazil over the same period, because of its versatility, profitability, and huge potential for animal and human feed (CONAB, 2017).

Current issues in the productive scale of the crop have been escalated by global warming and climate change that make the agricultural scenario of cropping production even more prone to failure (NELSON et al., 2014). Consequently, raises in abiotic and biotic stresses, such as the higher incidence of bugs can damage the crop by reducing the potential of soybean grain yield, which can lower the overall production to very low levels and pose an additional threat to food security (BEBBER; HOLMES; GURR, 2014).

Breeding programs have a crucial role in developing new cultivars and assessing the potential of soybean lines that show valuable parameters for selection, such as resistance to main pest and diseases of the crop, high standards of grain yield, heavy seeds, efficiency of water and nutrient uptake, among others (RAMALHO, 2012).

Stink bugs represent a hazard to many crops, especially to soybeans as they can considerably impact the productivity of the crop by the occurrence of stunted plants, empty pods, and foliar retention, which can compromise total grain yield and seed quality (GUEDES et al., 2012). Among the soybean stink bug complex, the species *Euschistus heros,* known as the brown stink bug, is one of the most damaging pests (SOUZA et al., 2017) as it is generally associated with the highest population density among other species of stink bugs; therefore, it has a greater potential to cause injuries to soybeans (KUSS et al., 2012).

Phenotypic and genotypic correlations are indeed important in this scenario as they can establish a helpful association among agronomic traits for breeding programs. Furthermore, since many agronomic traits of interest are of quantitative nature, which is influenced by more than two genes, it can be hard to identify the lines that may stand out basing only on one trait (CRUZ; REGAZZI; CARNEIRO, 2012).

Those types of correlations can infer a positive or negative linear relationship among two agronomic traits (CRUZ; REGAZZI; CARNEIRO, 2012). Correlation studies on soybeans, mainly under field conditions with natural infestation of stink bugs, have suggested significant positive correlations among important agronomic traits (ROCHA et al., 2014; ROCHA et al., 2015; MOREIRA, 2015). Consequently, to achieve such approach, identifying lines that have tolerance the attack of stink bug complex, as well as have desirable phenotypic and genotypic correlations is very important for the success of soybean cultivars (KURASCH, et al. 2017).

This research is aimed at selecting soybean lines with desirable agronomic traits under high natural infestation of *Euschistus heros*, evaluating the genotypic and phenotypic correlations between important agronomic traits for soybean breeding, as well as estimating important genetic parameters for the soybean crop.

2. MATERIAL AND METHODS

The experiment was carried out during the growing season of 2015/2016 in an experimental area located at Capim Branco farm (18º52'S; 48º20'W and 805 meters of altitude), belonging to the Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil. The meteorological data for the experimental field during the vegetation season of the experiment can be seen in Figure 1.

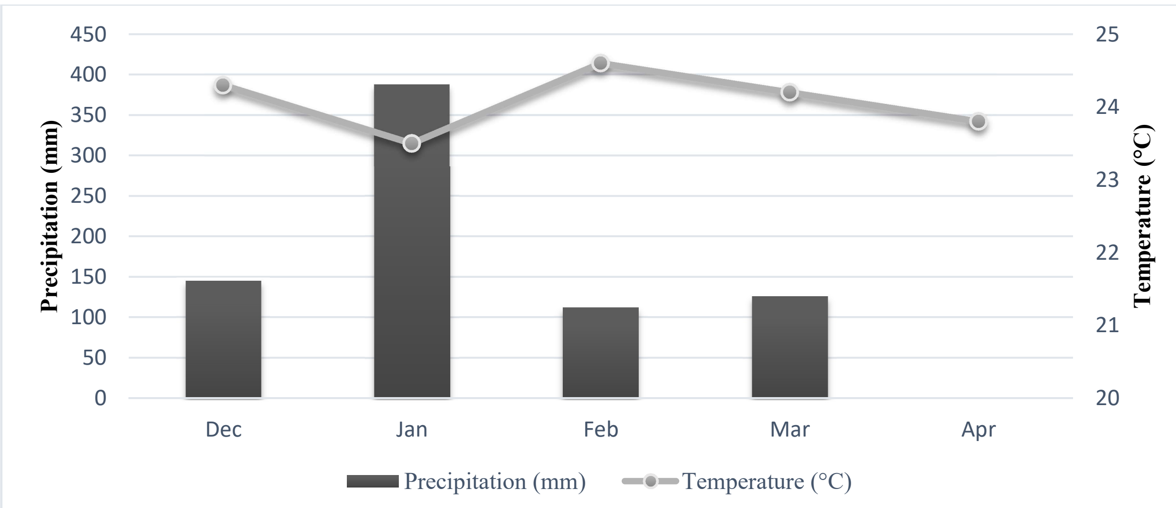

FIGURE 1. Temperature and rainfall averages at Capim Branco farm in Uberlândia MG, growing season 2015/16, from December 2015 to April 2016. Source: Meteorological station of Capim Branco farm and National Institute of Meteorology (INMET).

Climatic conditions such as the rainfall and temperature were classified as satisfactory during the development of soybeans according to the soybean crop necessity of water and temperature, which depends on the genotype and it varies from 450 to 800 millimetres of water per cycle with temperatures oscillating between 20°C and 30°C (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA, 2014).

The experimental field is situated on a dystrophic Dark Red Latosol. The soil preparation for seeding was done over conventional tillage system with one plowing and two harrowing. Before sowing, the field was grooved and fertilized according to soil analysis (Table 1).

TABLE 1. Chemical characteristics of the soil, samples collected from 0 to 20 cm depth, in Uberlândia, MG.

pH in H_20	O.M (dag kg^{-1})	P Meh $^{-1}$ (mg dm^{-3})	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+ and Al^+	V (%)
			cmol$_c$ dm^{-3}					
6.2	2.5	16.6	0.4	3.4	1,3	0	1.5	77

O.M: Organic matter; V: Base saturation.

It was evaluated 23 soybean F8 lines developed by the Soybean Breeding Program of Federal University of Uberlândia and four cultivars (Msoy 8527, UFUS Xavante, Msoy 8787, and UFUS Milionária) under randomized complete block design with three repetitions.

The experimental plot was formed by 4 soybean plant rows with 5 m length, spaced at 0.5 m within rows. The useful area was composed of the 2 central lines, wherein 0.5 m from each edge was discarded, resulting in a useful plot of 4 m^2.

Before sowing, the seeds were treated with fungicide Carbendazim and Tiram (Protreat®) and inoculated with liquid inoculant containing *Bradyrhizobium japonicum.* The sowing occurred manually on December 5th, 2015, adopting 16 seeds per linear meter as an average. Regarding crop operations for weed control, pre-emergent and post-emergent herbicides were used. Firstly, the herbicide application of S-Metolachlor (Dual Gold®) was done right after sowing and Haloxyfop-P-Methyl (Verdict®) 20 days after sowing. Meanwhile, complementary weeding was done as often as necessary. Also, at the 30th day after emergence, 100 mL ha^{-1} dose of cobalt and molybdenum (Nectar®) was applied, which is approximately 3 grams of cobalt and 22 grams of molybdenum per hectare (EMBRAPA, 2014).

In order to control *Phakopsora pachyrhizi* fungi, field application of Trifloxystrobin and Prothioconazole (Fox®) was done in a dose of 0.4 L ha^{-1}, as well as Strobilurin and Triazol (APROACH® PRIMA) were applied in a dosage of 0.3 L ha^{-1}. Furthermore, to control pests, two applications, one with Acephate (Achero®), 0.4 kg ha^{-1} dose, and another with Thiamethoxam and Lambda-Cyhalothrin (Platinum Neo®), 200 mL ha^{-1} dose, was done. However, the population of brown stink bugs was higher than the control level, which is 2 adults or third instar nymphs per linear meter, during all reproductive stage (CORRÊA-FERREIRA; PANIZZI, 1999).

By means of visual observation, measurements were performed in accordance with the stages of soybean development proposed by Fehr and Caviness (1977) and the following traits were accessed:

a) Plant height at flowering (PHF) and at maturity (PHM): the distance, in centimeters, from the soil surface up to the end of the main stem was measured when plants were at reproductive stage R1 and R8, respectively.

b) Number of nodes on the main stalk at flowering (NNF) and at maturity (NNM): the number of nodes on the main stem was counted when 50% of the plot was identified at the R1 and R8 stage, respectively.

c) Number of days to bloom (NDB) and to maturity (NDM): These parameters are defined as the number of days from the emergence to flowering, when 50% of plants in the useful plot have at least one flower fully opened (R1), and 95% of the pods of the useful area are ripe (R8), respectively.

d) Number of seeds per pod (NSP): the average number of seeds per pod from randomly collected plants from each useful plot was counted after harvesting.

e) Total number of pods per plant (TNP): the average number of pods was counted in five randomly collected plants of the useful plot.

f) Number of empty pods (NEP): the average number of empty pods (without at least one seed) caused by the attack of stink bugs was randomly sampled in each useful plot.

g) One hundred seed weight (HSW): after plants of the useful area were harvested and processed, a hundred-seed weight was determined according to the methodology proposed by Rules For Seeds Analysis (MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO - BRASIL, 2009). The weight of each sample was adjusted to a moisture content of 13% according to the formula below:

$$\mathrm{PF} = \mathrm{PI}\,\frac{100\text{-UI}}{100\text{-UF}}$$

Where:

PF: corrected weight of the sample;

PI: initial weight of the sample;

UI: initial moisture content of the sample;

UF: final moisture of the sample (13%).

h) Asian soybean rust severity (SEV): the Asian soybean rust severity was measured according to the Godoy diagrammatic scale from the average of three samplings, the first occurred 20 days after the first fungicide application followed by the second, which was done seven days after the second fungicide application (GODOY; KOGA; CANTERI, 2006).

i) Grain yield (Y): Accomplished through harvesting, threshing, and weighing the soybean seeds from the useful plot. Data obtained (grams per useful plot) were transformed into kg ha^{-1}.

j) Stink bug damage to seeds: The stink bug damage to soybean seeds was visually classified in percentages according to the criteria proposed by Panizzi et al. (1979). The classification was based on a sample size of 50 seeds per each experimental plot randomly collected and sorted into four different groups, which were: A) healthy seeds, without discoloration; B) slightly damaged, normal form, discoloration caused by punctures; C) damaged seeds, deformed, partially wrinkled seeds with discoloration caused by punctures; D) highly damaged seeds, completely deformed and discolored seeds (Figure 2).

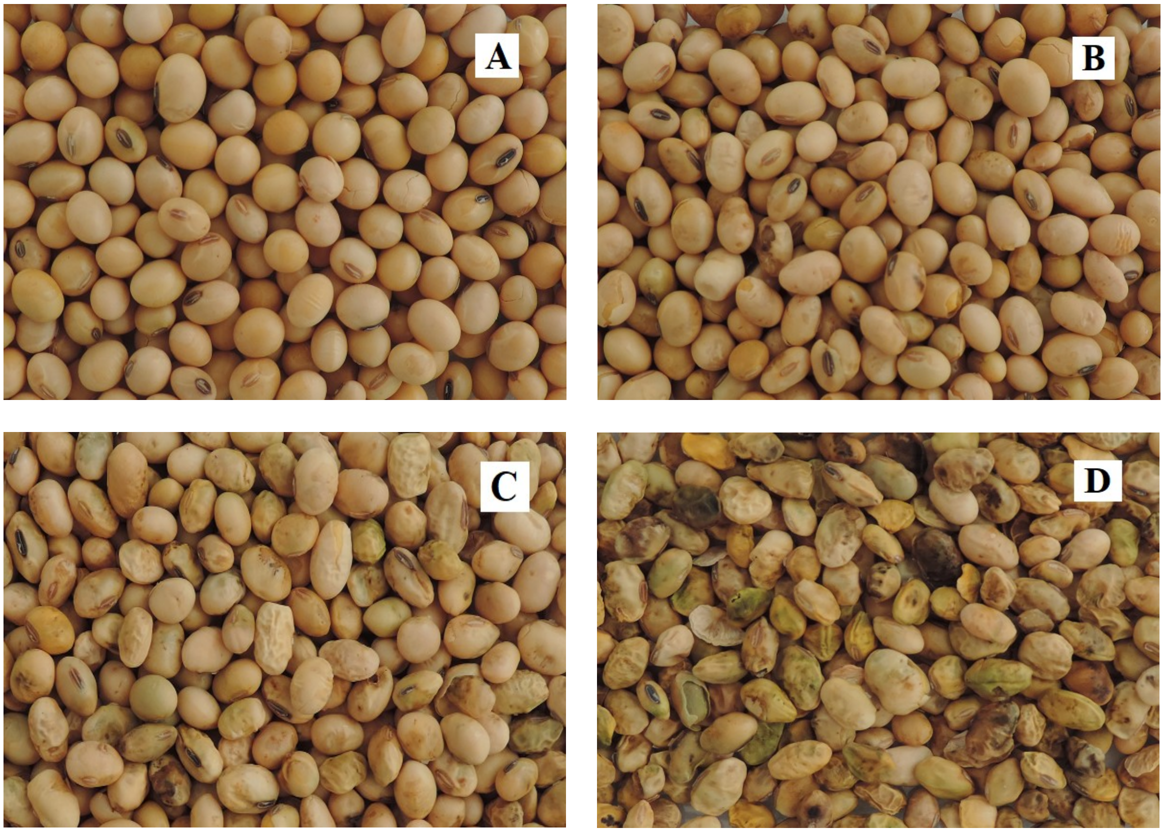

FIGURE 2 –Visual classification criteria for stink bug damage to seeds of soybean genotypes. A) Healthy seeds; B) slightly damaged seeds; C) damaged seeds; D) highly damaged seeds. Each group represents a joint sample collected from more than one genotype.

For statistical analysis, having met all the ANOVA presuppositions such as casualization, variance homogeneity, residual normality, and block additivity for the agronomic traits evaluated using SPSS software, analysis of variance was carried out for the assessed parameters with Sisvar program (FERREIRA, 2014). When differences in the effect of treatment were observed by F test (P<0,05), it was used Scott-Knott test for grouping the averages.

After tabulating all the data, phenotypic and genotypic correlation analyses were done adopting the effect of genotype as fixed, conforming to the following estimators:

Phenotypic correlation

$$r_p = \frac{PMGxy}{\sqrt{MSGx\ MSGy}}$$

Where:

r_p: estimator of phenotypic correlation;

PMGxy: average product associated with the effects of genotypes for x and y traits;

MSGx: mean square among the genotypes for the trait X;

MSGy: mean square among the genotypes for the trait Y.

Genotypic correlation

$$r_g = \frac{(PMG_{xy} - PMR_{xy})/r}{\sqrt{\hat{\phi}_{g(X)}\hat{\phi}_{(Y)}}} = \frac{\hat{\phi}_{g(XY)}}{\sqrt{\hat{\phi}_{g(X)}\hat{\phi}_{(Y)}}}$$

$$\hat{\phi}_{g(X)} = \frac{MSG_x - MSE_x}{r}$$

$$\hat{\phi}_{g(Y)} = \frac{MSG_y - MSE_y}{r}$$

Where:

r_g: estimator of genotypic correlation;

$\hat{\phi}_{g(XY)}$: estimator of genotypic covariance;

$\hat{\phi}_{g(x)}$ e $\hat{\phi}_{g(Y)}$: estimators of the quadratic components associated with genotypic variability for characters X and Y, respectively.

The phenotypic correlation significance was estimated by t-test with n-2 degrees of freedom, where the “n” corresponds to the number of genotypes evaluated. The significance of genotypic correlations was evaluated through the *bootstrap* method with 5000 simulations. Those statistical analyses were accomplished by Genes program (computational application in genetics and statistics) (CRUZ, 2016).

3. RESULTS AND DISCUSSION

For all agronomic traits, the existence of genetic variability was observed at 1% probability level by F test, with the exception of Asian soybean rust severity (Table 2). The coefficient of variation (CV) fluctuated from 2.54 % (NDM) to 54.13 % (HDS) (Table 2). In another study with soybeans, the coefficient of variation (CV) ranged from 19.88 to 51.27%. These high CV values, beyond genetic variability, can be explained as some genotypes are more affected than others by the stink bug attack (ROCHA et al., 2014).

TABLE 2 – Genotypic mean square, genotypic determination coefficient, quotient between genotypic and environmental variation coefficients, and coefficient of variation of soybean agronomic traits evaluated from 27 genotypes over the growing season of 2015/2016, Uberlândia- MG.

Traits	MSG	H^2(%)	CV/Cve	CV(%)
Number of days to bloom (NDB)	74.32**	84.04	1.32	6.28
Number of days to maturity (NDM)	64.67**	85.37	1.39	2.54
Plant height at flowering (PHF)	239.33**	88.27	1.58	7.19
Plant height at maturity (PHM)	281.64**	92.64	2.04	5.22
Number of nodes at flowering (NNF)	4.13**	89.50	1.68	5.28
Number of nodes at maturity (NNM)	9.65**	87.31	1.51	6.93
Total number of pods (TNP)	747.82**	63.16	0.75	28.19
Number of empty pods (NEP)	307.27**	72.93	0.94	22.83
Number of seeds per pod (NSP)	0.09**	94.95	2.50	2.93
One hundred seed weight (HSW)	5.81**	80.23	1.16	10.91
Asian soybean rust severity (SEV)	117.07^{ns}	32.38	0.40	38.16
Grain yield (Y)	2280853.64**	74.20	0.98	31.77
Healthy seeds (HS)	296.57**	91.45	1.89	37.65
Slightly damaged seeds (SDS)	558.78*	69.46	0.87	31.49
Damaged seeds (DS)	452.37*	68.43	0.85	38.04
Highly damaged seeds (HDS)	602.16**	91.10	1.84	54.13

*, **: significant at 5% and 1% probability level, respectively, by F test; ^{ns}: non-significant.

The existence of phenotypic variability that is mainly attributed to genetic causes is crucial for establishing an outstanding base for selection (CRUZ; REGAZZI; CARNEIRO, 2012). In advanced generations through genetic breeding, the H^2 parameter is called coefficient of genotypic determination as the genotypes are already fixed and pre-selected (VASCONCELOS et al., 2012).

For all the traits in the study, with exception of the total number of pods (63.16%), Asian soybean rust severity (32.38%), slightly damaged seeds (69.46%), and damaged seeds (68.43%), the coefficient of genotypic determination was superior to 70%, indicating that the most part of phenotypic variability was due to genetic differences among the genotypes (OLA MOREIRA, 2015). Similar results were accomplished by Glasenapp et al. (2015), which found H^2 values superior to 70% for some traits in the study. Besides, most of the evaluated traits (NDB, NDM, PHF, PHM, NNF, NNM, NSP, HSW, HS, and HDS) showed a quotient between the genotypic and environmental variation coefficient higher that 1, which is another indicator that suggests successful possibility of selection (CRUZ; REGAZZI; CARNEIRO, 2012).

The number of days to bloom and maturity are important parameters as the longer time soybean genotypes need to complete their cycle, the more time of exposure to the attack of stink bugs there is (ROCHA et al., 2015). Therefore, in a natural occurring infestation scenario early genotypes tend to escape from the attack of pests by a host evasion type of resistance, and consequently, maintain higher grain yield by shortening the most susceptible stages of the crop, R5 to R7 (ROCHA et al., 2015; CORRÊA- FERREIRA et al., 2013; FERNANDES et al., 2017).

One indirect symptom that is also caused by the attack of stink bugs and may have increased the life cycle of genotypes is the foliar retention that can delay the physiological maturity, impairing harvesting of the crop (SILVA; CANTERI; SILVA, 2013). Also, it is important that soybeans should not exceed a height of 100 cm according to Sediyama et al. (2015) in order to avoid large lodging indexes, as well as to enhance mechanical harvesting efficiency. In the current experiment, only one line reached over 100 cm height at maturity (G23 – Table 3).

TABLE 3- Soybean agronomic traits evaluated among 27 soybean genotypes during the growing season of 2015/2016 in Uberlândia- MG.

Genotypes	Traits					
	NDB	NDM	PHF	PHM	NNF	NNM
G1	55.4 b	116.0 a	62.1 d	75.1 d	13.3 b	17.4 b
G2	58.7 c	116.0 a	66.4 d	77.5 d	13.8 a	16.0 b
G3	60.3 c	124.5 c	81.2 b	81.4 c	13.1 b	15.3 c
G4	57.5 c	116.5 a	68.4 c	75.8 d	12.8 b	16.2 b
G5	58.7 c	116.0 a	87.8 a	98.1 b	13.0 b	16.4 b
G6	47.0 a	127.5 c	63.1 d	97.3 b	11.7 c	21.6 a
G7	60.3 c	127.5 c	82.9 b	94.0 b	13.4 b	17.1 b
G8	58.7 c	118.5 b	83.7 b	91.1 b	14.6 a	16.6 b
G10	47.7 a	118.0 a	70.8 c	88.4 b	11.3 c	14.4 c
G11	49.0 a	116.0 a	83.6 b	97.7 b	11.5 c	15.5 c
G12	56.0 b	117.0 a	72.2 c	81.3 c	13.0 b	16.7 b
G13	57.0 b	120.0 b	69.2 c	75.7 d	12.7 b	15.0 c
G15	55.3 b	121.0 b	74.3 c	91.9 b	11.8 c	16.7 b
G17	51.0 a	123.5 c	69.4 c	89.5 b	11.4 c	15.7 b
G18	48.3 a	120.0 b	57.2 d	71.2 d	10.6 c	13.3 c
G19	57.0 b	116.0 a	73.7 c	90.6 b	12.7 b	14.5 c
G20	58.7 c	120.5 b	80.9 b	92.2 b	13.9 a	16.2 b
G21	60.3 c	123.5 c	80.5 b	84.1 c	13.6 a	14.6 c
G22	53.7 b	126.5 c	73.9 c	88.7 b	12.5 b	16.3 b
G23	54.3 b	130.0 c	96.1 a	112.9 a	14.8 a	17.2 b
G24	46.3 a	120.0 b	62.0 d	75.3 d	10.8 c	13.3 c
G25	46.3 a	114.0 a	63.3 d	72.8 d	11.1 c	14.0 c
Msoy 8527	50.0 a	120.0 b	76.8 b	92.7 b	11.4 c	15.2 c
G27	55.3 b	120.5 b	74.1 c	87.5 b	12.8 b	17.1 b
UFUS Xavante	54.3 b	127.5 c	71.4 c	92.5 b	13.0 b	14.3 c
Msoy 8787	62.0 c	128.0 c	75.3 c	94.5 b	11.3 c	17.4 b
UFUS Milionária	62.0 c	126.0 c	69.0 c	82.8 c	10.9 c	16.0 b

L' Means followed by the same lowercase letter (column) belong to the same group at 5% level of significance by Scott and Knott test. NDB and NDM: number of days to bloom and to maturity, respectively; PHF and PHM: plant height at flowering and at maturity (cm), respectively; NNF and NNM: number of nodes on the main stalk at bloom and at maturity, respectively.

Regarding the number of nodes, both at flowering and at maturity, a higher number of nodes is a valuable soybean characteristic, as they will potentially become reproductive nodes. According to some authors, for soybean plant to accomplish a large productive potential, it would need an average of 17 to 18 nodes on the main stalk (SEDIYAMA et al., 2015). In the current study, most of genotypes achieved numbers of nodes at maturity close to this average, including the genotypes G1, G7, G8, G12, G15, G23, G27, Msoy 8787, and the genotype G6 that overcame those values with an average of 21.6 nodes on the main stalk at maturity (Table 3). In another study, number of nodes at soybean maturity oscillated from 12.03 to 16.61 (PERINI JUNIOR et al., 2012).

According to Camâra (1998), a soybean plant can reach up to 400 pods; nevertheless, typical Brazilian cultivars usually have an average of 30 to 80 pods per plant. As it can be seen in table 4, all genotypes, except G1, have shown averages in between this interval.

TABLE 4 – Soybean agronomic traits evaluated among 27 soybean genotypes during the growing season of 2015/2016 in Uberlândia- MG.

Genotypes	Traits					
	TNP	NEP	NSP	HSW	SEV	Y
G1	114.3 a	52.5 c	2.2 c	10.4 a	15.4 a	4113.1 a
G2	69.7 a	30.8 a	2.2 c	10.9 a	14.9 a	4426.6 a
G3	40.2 a	37.7 b	2.5 b	10.9 a	23.0 a	1993.6 c
G4	52.6 a	34.4 b	2.7 a	10.3 a	22.5 a	3253.6 b
G5	33.5 a	24.7 a	2.6 a	10.4 a	35.4 a	2197.4 c
G6	59.5 a	54.8 c	2.1 d	8.4 b	10.2 a	1062.5 c
G7	53.9 a	44.3 c	2.2 c	9.5 a	30.7 a	1487.5 c
G8	50.1 a	25.7 a	2.1 d	11.1 a	15.8 a	2316.8 c
G10	49.2 a	30.4 a	2.3 c	11.6 a	26.7 a	2616.2 b
G11	54.2 a	39.9 b	2.2 c	10.7 a	28.2 a	2705.2 b
G12	57.1 a	40.0 b	2.4 b	10.7 a	21.7 a	2571.4 b
G13	61.6 a	41.5 b	2.3 c	9.4 a	31.8 a	2836.0 b
G15	67.1 a	54.8 c	2.2 c	10.1 a	31.8 a	1635.3 c
G17	57.0 a	41.6 b	2.3 c	8.3 b	27.4 a	2565.4 b
G18	48.9 a	24.5 a	2.1 d	9.2 a	22.7 a	1753.8 c
G19	57.9 a	36.8 b	2.3 c	10.3 a	28.7 a	2819.3 b
G20	51.7 a	35.0 b	2.6 a	11.0 a	8.2 a	2665.0 b
G21	44.4 a	29.4 a	2.2 c	12.0 a	27.0 a	2378.1 c
G22	71.7 a	49.5 c	2.3 c	9.5 a	25.7 a	1881.2 c
G23	72.1 a	59.2 c	2.4 b	8.3 b	20.2 a	1451.3 c
G24	38.9 a	24.0 a	2.2 c	10.78 a	18.1 a	3550.8 a
G25	85.6 a	47.1 c	2.0 d	10.0 a	19.0 a	2766.5 b
Msoy 8527	50.5 a	39.2 b	2.2 c	10.8 a	25.0 a	3695.9 a
G27	62.1 a	41.4 b	2.4 b	9.7 a	30.8 a	2289.7 c
UFUS Xavante	61.2 a	52.5 c	2.3 c	7.1 c	18.5 a	1650.2 c
Msoy 8787	66.6 a	48.8 c	2.5 b	7.9 b	23.0 a	1173.8 c
UFUS Milionária	57.9 a	38.0 b	2.4 b	6.1 c	17.1 a	1329.4 c

L' Means followed by the same lowercase letter (column) belong to the same group at 5% level of significance by Scott and Knott test. TNP: Total number of pods; NEP: Number of empty pods; NSP: Number of seeds per pod; HSW: One hundred seed weight (g); SEV: Asian soybean rust severity (%); Y: Grain yield (kg ha^{-1}).

Along with that, the one hundred seed weight can vary from 2 to 30 grams (SEDIYAMA et al., 2013). As an expected symptomatology of the attack of stink bugs, the weight of seeds has been negatively affected. They oscillated from 6.1 (UFUS MILIONÁRIA) to 12 grams (G21) (Table 4), which shows the impact of stink bugs on reducing soybean seeds weight. A pseudo- resistance mechanism, defined as damage dilution type, may reduce the proportional number of damaged seeds due to insect attack by increasing the number of seeds per plant (ROCHA et al., 2015).

The Asian soybean rust severity did not statistically differ among the evaluated genotypes, showing that it has not interfered in differentiating the resistance of soybean genotypes to stink bugs. Overall, those genotypes have shown a lower disease severity compared to other studies that have identified potential sources of genetic resistance to this disease (RIBEIRO; TOLEDO; RAMALHO, 2008; GLASENAPP et al., 2015). Still, the soybean lines' resistance to soybean rust severity may vary over time and it is associated with the genotype, the type of resistance mechanism according to the environmental factors or physiological effects (MARTINS; JULIATTI, 2014).

The soybean grain yield fluctuated from 1.062 kg ha^{-1} to 4.427 kg ha^{-1} (lines G6 and G2, respectively) (Table 4). The observed variations, beyond genetic variability, may be attributed to the differential levels of resistance of genotypes to attack of stink bugs, mainly *Euschistus heros*, which were found in the highest densities in the experiment. The lines G1, G2, G24, and the cultivar Msoy 8527 achieved the highest yielding standards, staying above the national average of 3.037 kg ha^{-1} during the same growing season of 2015/2016 (CONAB, 2016). In addition, the grain yield is considered to be a good indicator parameter towards the selection of genotypes resistant to the attack of stink bugs (LOURENÇÃO et al., 2010; ROCHA et al., 2014). Another indicator of soybean stink bug resistance can be approached through the visual analyses of seeds. In the current research, most seeds were affected by the stink bug attack indicating a high damage potential to soybean seeds in nearly all genotypes (Table 5).

TABLE 5- Damage rates of stink bug infestation in percentages (± standard error) on soybean seeds according to visual classification into four groups over the growing season of 2015/2016 in Uberlândia.

Genotypes	Seeds (%)			
	Healthy	Slightly damaged	Damaged	Highly damaged
G1	25 ± 6.1 a	43 ± 1.4 b	23 ± 3.3 a	9 ± 4.2 a
G2	14 ± 2.8 c	51 ± 0.5 b	30 ± 1.9 a	5 ± 0.5 a
G3	9 ± 4.2 c	34 ±1 3.2 a	50 ± 15.1 b	7 ± 2.4 a
G4	22 ± 1 b	52 ± 1 b	29 ± 1.4 b	0 ± 1 a
G5	23 ± 2.4 b	57 ± 2.4 b	20 ± 4.7 a	0 ± 1 a
G6	3 ± 1.4 d	24 ± 2.8 a	36 ± 1.9 b	37 ± 6.1 c
G7	2 ± 1 d	18 ± 1 a	58 ± 1 a	22 ± 1 b
G8	26 ± 1.9 a	66 ± 1 b	8 ± 1.9 a	0 ± 1 a
G10	13 ± 3.3 c	45 ± 9.9 b	27 ± 8.0 a	15 ± 5.2 a
G11	10 ± 0.9 c	50 ± 1.9 b	33 ± 0.5 a	7 ± 3.3 a
G12	17 ± 5.2 b	47 ± 9.0 b	29 ± 10.8 a	7 ± 3.3 a
G13	27 ± 2.4 a	56 ± 3.8 b	16 ± 0.9 a	1 ± 0.5 a
G15	8 ± 1.9 c	33 ± 6.1 a	40 ± 2.8 b	19 ± 5.2 b
G17	5 ± 1.4 d	32 ± 9.4 a	32 ± 1.9 a	31 ± 9.0 c
G18	8 ± 3.8 c	38 ± 11.3 a	37 ± 9.9 b	17 ± 5.2 a
G19	35 ± 2.4 a	50 ± 0.9 b	15 ± 1.4 a	0 ± 1 a
G20	15 ± 3.3 c	60 ± 5.7 b	25 ± 2.7 a	1 ± 0.5 a
G21	16 ± 1.9 b	35 ± 1.4 a	43 ± 0.5 b	6 ± 2.8 a
G22	1 ± 0.5 d	19 ± 2.4 a	37 ± 1.4 b	33 ± 0.5 c
G23	2 ± 0.9 d	24 ± 2.8 a	27 ± 1.4 a	47 ± 3.3 c
G24	10 ± 3.8 c	34 ± 8.5 a	55 ± 12.7 b	1 ± 0.5 a
G25	31 ± 0.5 a	54 ± 0.9 b	15 ± 1.4 a	0 ± 1 a
Msoy 8527	21 ± 0.5 b	61 ± 6.1 b	18 ± 6.6 a	0 ± 1 a
G27	11 ± 0.5 c	48 ± 0.9 b	32 ± 1.9 a	9 ± 1.4 a
UFUS Xavante	3 ± 1.4 d	27 ± 12.7 a	44 ± 3.8 b	26 ± 10.4 b
Msoy 8787	3 ± 0.5 d	36 ± 11.3 a	30 ± 4.7 a	31 ± 6.1 c
UFUS Milionária	1 ± 0.5 d	26 ± 6.6 a	39 ± 7.1 a	34 ± 1 c

L' Means followed by the same lowercase letter (column) belong to the same group at 5% level of significance by Scott and Knott test.

Feeding activity of *E. heros* during seed filling stage can considerably cause direct damage to soybean seeds resulting in more often wrinkled or cracked seed coats (WEVERTON et al., 2011) and reduce soybean yield (CORRÊA-FERREIRA, 2005). The genotypes G6, G17, G22, G23, and the cultivars Msoy 8787 and UFUS Milionária have presented the largest percentage of highly damaged soybean seeds. Also, the effect of *E. heros* population on escalating the number of damaged seeds corroborates with the results found by Bridi (2012). Seeds attacked by stink bugs, especially *Euschistus heros*, show higher physical damage as well as an inferior seed weight, suggesting that the damage caused by *E. heros* is more severe than other species such as *Edessa meditabunda* (WEVERTON et al., 2011). On the other hand, the genotypes G1, G8, G13, G19, and G25 have shown the highest percentage of healthy seeds, which reinforce a superior behavior of those lines in respect to avoiding damage occasioned by the vast presence of brown stink bugs.

An important parameter to assess the relationship between agronomic traits and identify beneficial characteristics for soybean resistance to stink bug is the establishment of correlations. To correctly interpret correlations, beyond significance, the magnitude and direction has also to be considered (CRUZ; REGAZZI; CARNEIRO, 2012). Regarding direction, the correlations can be positive, which indicates a trend of increasing a variable while another also increases; on the other hand, when negative, it suggests a possibility of increasing a variable value while reducing another (NOGUEIRA et al., 2012). It is important to know the association among agronomic traits for selection of genotypes as they can infer a better understanding of the correlated traits and help to avoid selecting one trait that leads to an undesirable selection of another (RAMALHO, 2012).

For most agronomic traits, the genotypic correlations were higher than the phenotypic ones, with the same direction. This aspect demonstrates that the genotypic factors have contributed more to the correlations than the environmental factors (ALMEDIA et al., 2010). Correlation coefficients whose magnitudes are above 0.7 indicate variables that can be considered highly correlated (CRUZ; REGAZZI; CARNEIRO, 2012). Still, researchers tend to value mostly the signal and magnitude estimates on interpreting correlation values when they are above 0.5 in module (LOPES et al., 2002).

The increase in the number of days to maturity led to a rise in the number of empty pods (genotypic correlation of 0.57), as well as a reduction on the weight of a hundred seed and grain yield (Table 6). The association between the number of days to maturity and both grain yield and weight of one hundred seeds were highly negatively correlated, -0.65 and -0.63, phenotypically, and -0.74 and -0.69, genotypically, respectively. Similar results were observed by Rocha et al. (2015), as the longer plant cycles are, the exposure of the plant to the attack of pests increases.

TABLE 6- Coefficient of phenotypic (r_p) and genotypic (r_g) correlations between fifteen traits from 27 soybean genotypes over the growing season of 2015/2016 in Uberlândia.

Traits		NDM	PHF	PHM	NNB	NNM	TNP	NEP	NSP	HSW	Y	HS	SDS	DS	HDS
NDB	r_p	0.21^{ns}	0.44*	0.98^{ns}	0.55**	0.13^{ns}	-0.08^{ns}	-0.06^{ns}	0.51**	-0.08^{ns}	-0.17^{ns}	-0.01^{ns}	0.01^{ns}	0.03^{ns}	-0.02^{ns}
	r_g	0.21^{ns}	0.45^{+}	0.10^{ns}	0.60^{++}	0.11^{ns}	-0.12^{ns}	-0.07^{ns}	0.55^{++}	-0.07^{ns}	-0.21^{ns}	0.01^{ns}	0.03^{ns}	0.02^{ns}	-0.05^{ns}
NDM	r_p		0.25^{ns}	0.49**	0.06^{ns}	0.35^{ns}	-0.05^{ns}	0.49**	0.08^{ns}	-0.63**	-0.65**	-0.79**	-0.79**	0.52**	0.83**
	r_g		0.31^{ns}	0.57^{++}	0.04^{ns}	0.37^{+}	-0.06^{ns}	0.57^{++}	0.09^{ns}	-0.69^{++}	-0.74^{++}	-0.85^{++}	-0.88^{++}	0.56^{++}	0.87^{++}
PHF	r_p			0.76**	0.59**	0.12^{ns}	-0.29^{ns}	0.07^{ns}	0.36^{ns}	0.14^{ns}	-0.45*	-0.09^{ns}	0.04^{ns}	-0.07^{ns}	0.08^{ns}
	r_g			0.77^{++}	0.62^{++}	0.12^{ns}	-0.37^{ns}	0.38^{ns}	0.41^{+}	0.14^{ns}	-0.53^{++}	-0.12^{ns}	0.02^{ns}	-0.06^{ns}	0.10^{ns}
PHM	r_p				0.29^{ns}	0.44*	-0.14^{ns}	0.38*	0.19^{ns}	-0.21^{ns}	-0.59**	-0.36^{ns}	-0.21^{ns}	-0.04^{ns}	0.48*
	r_g				0.33^{ns}	0.48^{+}	-0.14^{ns}	0.49^{+}	0.26^{ns}	-0.25^{ns}	-0.65^{++}	-0.42^{+}	-0.30^{ns}	-0.01^{ns}	0.55^{++}
NNF	r_p					0.23^{ns}	0.09^{ns}	0.07^{ns}	0.27^{ns}	0.26^{ns}	0.07^{ns}	0.17^{ns}	0.14^{ns}	-0.17^{ns}	-0.10^{ns}
	r_g					0.22^{ns}	0.09^{ns}	0.05^{ns}	0.28^{ns}	0.32^{ns}	0.09^{ns}	0.19^{ns}	0.22^{ns}	-0.24^{ns}	-0.16^{ns}
NNM	r_p						0.24^{ns}	0.50**	0.09^{ns}	-0.24^{ns}	-0.23^{ns}	-0.28^{ns}	-0.23^{ns}	-0.02^{ns}	0.44*
	r_g						0.19^{ns}	0.52^{+}	0.07^{ns}	-0.24^{ns}	-0.33^{ns}	-0.30^{ns}	-0.25^{ns}	-0.04^{ns}	0.45^{+}
TNP	r_p							0.66**	-0.25^{ns}	-0.21^{ns}	0.58**	0.11^{ns}	-0.10^{ns}	-0.26^{ns}	0.22^{ns}
	r_g							0.57^{+}	-0.37^{ns}	-0.29^{ns}	0.48^{+}	0.07^{ns}	-0.24^{ns}	-0.26^{ns}	0.30^{ns}
NEP	r_p								-0.06^{ns}	-0.50**	-0.15^{ns}	-0.34^{ns}	-0.48**	0.06^{ns}	0.61**
	r_g								-0.94^{ns}	-0.58^{+}	-0.38^{ns}	-0.44^{+}	-0.71^{++}	0.15^{ns}	0.74^{++}
NSP	r_p									-0.06^{ns}	-0.13^{ns}	-0.08^{ns}	0.75^{ns}	0.01^{ns}	-0.01^{ns}
	r_g									-0.06^{ns}	-0.19^{ns}	-0.07^{ns}	0.14^{ns}	-0.02^{ns}	-0.03^{ns}
HSW	r_p										0.40*	0.54**	0.55**	-0.18^{ns}	-0.74**
	r_g										0.45^{+}	0.61^{++}	0.60^{++}	-0.16^{ns}	-0.80^{++}
Y	r_p											0.60**	0.59**	-0.32^{ns}	-0.69**
	r_g											0.69^{++}	0.77^{++}	-0.37^{ns}	-0.82^{++}
HS	r_p												0.80**	-0.72**	-0.80**
	r_g												0.91^{++}	-0.77^{++}	-0.85^{++}
SDS	r_p													-0.76**	-0.82**
	r_g													-0.72^{++}	-0.87^{++}
DS	r_p														0.35^{ns}
	r_g														0.37^{ns}

**, *: significant at 1% and 5% probability level by t test; ++, +: significant at 1% and 5%, respectively, by *bootstrap* method with 5 thousand simulations. NDB and NDM: number of days to bloom and to maturity, respectively; PHF and PHM: plant height at flowering and at maturity, respectively; NNF and NNM: number of nodes on the main stalk at bloom and at maturity, respectively; TNP: Total number of pods; NEP: Number of empty pods; NSP: Number of seeds per pod; HSW: One hundred seed weight; Y: Grain yield; HS: Healthy seeds; SDS: Slightly damaged seeds; DS: Damaged seeds; HDS: Highly damaged seeds.

Significant positive correlations of medium magnitude were found between one hundred seed weight (HSW) and grain yield (Y) (Table 6). Rocha and collaborators (2014) emphasized the weight of a hundred seeds as a useful trait in simultaneous selection for high yield and resistance to the stink bug complex, as well as the importance of those types of resistance associations as a strategy to lower costs for farmers.

Leite and collaborators found 0.57 (phenotypic) and 0.78 (genotypic) correlations between grain yield and total number of pods, which agrees with the results found in the current study and reinforces the importance of number of pods for achieving high yielding soybean genotypes (ALCANTARA NETO et al., 2011; LEITE et al., 2016; ROCHA et al., 2015).

The correlations between damage rates of stink bug to soybean seeds according to visual classification and other agronomic traits are indeed important to assess genotype's resistance to stink bug. For instance, an increase in the number of days to maturity has significantly escalated the percentage of damaged and highly damaged seeds (Table 6). On the other hand, phenotypic and genotypic correlations towards a larger number of healthy (-0.79, -0.85, respectively) and slightly damaged seeds (-0.79, -0.88, respectively) were achieved with shorter number of days to maturity. Another study suggests the importance of a shorter pod-filling period as an indicator of soybean genotypes' resistance to stink bug (SANTOS et al., 2017). Also, there was a significant positive correlation between the number of empty pods and the percentage of highly damaged seeds (0.61 and 0.74, phenotypically and genotypically, respectively), which reinforces that the occurrence of empty pods were associated with stink bug damage. Furthermore, both yield and one hundred seed weight parameters similarly correlated with the percentage of healthy and slightly damaged seeds (positive correlations of medium to high magnitude). Yet, the opposite can be inferred regarding the correlation of yield and one hundred seed weight with the number of damaged and highly damaged seeds, which were negatively correlated.

The genotypes Msoy 8527, G1, G2, and G24 have indicated higher resistance to the attack of stink bugs by withstanding or recovering from damage caused by insect and probably exhibiting antixenosis resistance (usually expressed as non-preference of the insect) to *E. heros*, which can be a valuable characteristic for soybean breeding programs that aim to develop cultivars resistant to this species (SOUZA et al., 2017). Also, the genotypes G1 and G2 have demonstrated great tolerance concerning stink bug damage to seeds associated with a high grain yield standard, which reveals a great potential source of genetic resistance to stink bugs.

4. CONCLUSION

Regarding the selection of the main traits of assessment in soybeans, grain yield and the evaluation of stink bug damage to seeds have demonstrated to be useful traits for indirect and direct selection, respectively, of soybean genotypes with resistance to brown stink bugs. The traits HSW and TNP are useful for indirect selection of soybean lines with high yield potential under natural infestation of brown stink bug. Also, early cycle genotypes have demonstrated a positive correlation to grain yield and lower damage percentage to seeds over the same conditions. Finally, the lines G1, G2, and G24 are very promising lines as they have shown high grain yield standards, as well as valuable agronomic traits for stink bug resistance.

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CHAPTER 2. PLANT SYMBIOTIC *METHYLOBACTERIUM* CAN REDUCE THE NEGATIVE EFFECT OF LIMITED WATER SUPPLY DURING LENTIL CULTIVATION

ABSTRACT

Climate change has challenged agriculture production by increasing abiotic stresses, which cause significant reduction in crop yields. The most concerning abiotic constraint to global crop production is water stress. Drought can adversely affect plant development causing negative consequences on plant physiology and escalated economic losses in agriculture. In this scenario, alternative strategies to mitigate the negative effect of drought are often used. Plant-associated microorganisms can promote seed growth, yield, and more importantly, increase tolerance to drought. However, these plant-association relationships on lentils are not well known. Therefore, sustainable alternatives that could minimize the negative effect of water scarcity on lentil production such as *Methylobacteria* inoculation are crucial. A *Methylobacterium* strain, highly active producer of plant hormones (Cytokinins), was selected as a plant growth promoter in dry environments. A vegetative stage experiment was aimed to evaluate the performance of lentil plants during 25 day-long period of complete water withdrawal (terminal drought). Lentil seeds were inoculated with *Methylobacterium* at the time of planting by adding bacterial solution to half of the pots of the experiment. The water supply was limited at the beginning of the 3rd week old-plant growth and lentils were harvested in the 7th week of vegetation. Lentil biometrical and physiological traits, as well as water management parameters under drought conditions were evaluated. The analysis of all the measured plant morphological and physiological parameters suggests that the beneficial effect of inoculation was to a high extent a result of visibly improved plant vigour and general performance (faster growth, elevated chlorophyll (SPAD) and nutrient uptake (electrolyte content), as well as optimized water management parameters (WUE, LWL). Therefore, a sustainable growth enhancer of bacterial origin could lower the negative effect of drought in lentil cultivation.

Key Words: Drought; Cytokinins; Methylobacteria inoculation.

AGENTE SIMBIÓTICO DO GÊNERO *METHYLOBACTERIUM* PODE REDUZIR O EFEITO NEGATIVO DA SECA DURANTE O CULTIVO DE LENTILHA

RESUMO

Mudanças climáticas têm desafiado a produção agrícola por aumentar estresses de origem abiótica, o que por sua vez, causa significante perda em produtividade nas culturas. O principal fator limitante para a produção agrícola no mundo é o estresse hídrico. A seca pode causar efeitos adversos no desenvolvimento das plantas, gerando consequências negativas em sua fisiologia e consequentemente altas perdas em produtividade. Nesse cenário, estratégias alternativas para minimizar o efeito negativo da seca são geralmente adotadas. Microrganismos associados às plantas podem promover o desenvolvimento de sementes, aumento de produtividade e, o mais importante, aumentar a tolerância à seca. Porém, essas associações entre planta-hospedeiro em lentilha são pouco conhecidas. Assim, alternativas sustentáveis que possam minimizar o efeito negativo da falta de água na produção de lentilha por meio de inoculações com bactérias são cruciais. Uma estirpe do gênero *Methylobacterium*, altamente ativa na produção do fito hormônio (Citocinina), foi selecionada como uma bactéria promotora de crescimento em ambientes secos. Um experimento no estádio vegetativo foi desenvolvido para avaliar o desempenho de lentilha durante 25 dias de seca. Partes das sementes de lentilha foram inoculadas com a estirpe selecionada de *Methylobacterium* no momento da semeadura adicionando a solução contendo as bactérias diretamente no solo. O fornecimento de água foi interrompido no começo da terceira semana após a semeadura e o experimento foi colhido na sétima semana de cultivo. Caracteres biométricos e fisiológicos, bem como parâmetros de eficiência no uso da água foram mensurados em condições de déficit hídrico. Por meio das análises de todos os aspectos morfológicos e fisiológicos, foi notado um positivo efeito do inoculante no aumento de vigor, crescimento, unidade de clorofila (SPAD), absorção de nutrientes (conteúdo de eletrólitos), bem como um melhor aproveitamento de água pelas plantas. Portanto, um sustentável bioinoculante de origem bacteriana mostrou-se capaz de minimizar os impactos negativos da ocorrência de seca no cultivo de lentilha.

Palavras - Chave: Estresse hídrico; Citocinina; bionoculantte; Metilobactéria.

1. INTRODUCTION

With rapid population growth, projected to be 9.4 billion by 2050, worldwide food production needs to be significantly increased in order to meet food demand in the coming years (PASSIOURA, 2007). Current issues in the productive scale of agriculture have been escalated by global warming and climate change, which both cause stagnation or reduction in crop yields (NELSON et al., 2014). Abiotic stresses are one of the most concerning production constraints for global oilseed and pulse production and they are predicted to worsen with the adverse effect of climate change (ABBERTON et al., 2015).

Various abiotic stresses can affect plants and water stress is considered to be the most important limiting factor for crop production in many parts of the world (PASSIOURA, 2007). Drought stress can adversely affect plant development causing negative consequences on plant physiology and huge economic losses in agriculture (PEREYRA et al., 2009; ARZANESH et al., 2011). Therefore, plant growth and development is adversely influenced by drought stress causing crop failure and decreasing average yields by more than 50% (WU et al., 2011).

Drought stress can damage an important crop: lentil (*Lens culinaris* L.), one of the oldest domesticated plants in the world. The top 3 worldwide producers of lentils are Canada, India and Turkey with 2.17, 1.13, and 0.42 million tonnes, respectively. They together represent approximately 75% of the world total production of the crop (FAOSTAT, 2013). In fact, this major class of cash crops is often affected by drought stress (ABBERTON et al., 2015). For instance, in semiarid environments where lentils are widespread, unfavorable soil moisture at sowing often damages severely seed germination, and hence, it leads to irregular seedling emergences impeding the establishment of an appropriate stand and grain yield (OKCU; KAYA; ATAK, 2005).

As an alternative to mitigate the negative effect of drought, plant-associated microorganisms are often used (KUMAR et al., 2016). Plant growth-promoting bacteria benefit plant growth in several ways i.e. by direct enhancing plant metabolism (e.g. nitrogen fixation, phosphorus and iron solubilization), improving plant tolerance to environmental stresses such as drought and salinity by increasing phytohormones levels, and indirectly promoting plant growth by mitigating the harmful effects of phytopathogenic bacteria, fungi, nematodes, and viruses (WEYENS et al., 2009; ABBASI et al., 2011; KARAKURT; KOTAN, 2011).

The soil-plant interaction is highly complexed and, in order to maximize crop productivity by manipulating plant-associated microorganisms, optimal selection of growth-promoting microbes has to be done for the identification of the most effective strain for particular planting situation (FORCHETTI et al., 2007; TANK; SARAF, 2010; WILSON; WALKER, 2010; MARASCO et al., 2012; VERMA et al., 2014). In this context, *Methylobacterium*, a bacterial genus associated with plant phyllosphere, has been identified as an important plant growth promoting microbes **(**HOLLAND, 1997; PÝRLAK; KOSE, 2009).

The direct enhancements of host growth and development, as well as abiotic stress alleviation, have been previously attributed to bacterially produced phytohormones (IVANOVA et al., 2000; TSAVKELOVA et al., 2006). Cytokinins are remarkably known for their beneficial role in promoting seed growth, yield, and more importantly, increasing tolerance to environmental stresses (ZWACK; RASHOTTE, 2015). Cytokinins strongly counteract many processes induced by water stress such as stomata closure, lower photosynthesis and transpiration capacity, and senescence acceleration, acting as a robust drought resisting agent (NOVAKOVA et al., 2007; RIVERO; SHULAEV; BLUMWALD, 2009).

Drought stress has always been a complex issue on plant cultivation and finding sustainable alternatives that could minimize the negative effect of water scarcity on worldwide agriculture is crucial. Therefore, this research was aimed at testing the beneficial effect of *Methylobacteria* inoculation on lentil biometrical and physiological traits, as well as water management parameters under drought conditions.

2. MATERIAL AND METHODS

Selection of a drought tolerant *Methylobacterium* strain

A *Methylobacterium* strain was selected based on two main characteristics of bacteria: 1) tolerance to the drought stress induced by Polyethylene Glycol (PEG6000), a compound commonly used to imitate drought conditions during *in vitro* culture (LARHER et al., 1993); 2) production of growth hormone cytokinin (CK) – and in particular - the most active CK called free base Zeatin (LOMIN et al., 2015).

Seven *Methylobacterium* strains previously selected as the highly active producers of plant hormones (cytokinins) were cultured for 5 days in the incubator shaker ($28^{o}C$/180 rpm) in 50 mL selective minimal medium (125DSM + 0.5% MeOH) in the presence of Polyethylene Glycol 6000 (PEG6000). To assess bacterial tolerance to drought conditions, growth of control cultures (0% PEG6000) was compared with bacterial performance in the medium supplemented with 20% PEG6000 (w/v). At the 5^{th} day of the culture, bacteria growth was measured by optical density analysis (OD_{600}). The experiment was performed in four replicates for each strain and factorial analyses were carried out for further testing. The *Methylobacterium* strain with the highest OD_{600} at 20% PEG6000 concentration was selected as a plant growth promoter in dry environments.

Plant materials and bacteria treatments

The experiment was designed under completely randomized design. A small red lentil cultivar, CDC Maxim, was used in all the experiments to test the beneficial effect of plant inoculation with drought tolerant *Methylobacterium* strain on alleviating the negative effect of limited water supply at different stages of plant development.

Lentils were cultivated in 7L pots in the environmental growth rooms under fully controlled growth conditions: photoperiod (16h/8h), light (Sunblaster T5HO 54W 6400K Light Reflector with NanoTech T5 Reflection) and temperature (22±1°C) to assure replicability of growth conditions in subsequent experiments. The soil used in the experiment was a mix of Canadian Sphagnum peat moss and perlite (Sunshine Mix #1; Sungro, Agawam, MA, USA). The field water capacity (FWC) of the soil had been determined prior to planting through gravimetric method in order to establish appropriate water delivery rates to the pots (SCHMUGGE; JACKSON; MCKIM, 1980).

Soil water potential was automatically read four times a day by tensiometers (Irrometer Model MLT, Irrometer Company, Inc., Riverside, USA) and compared to a preset threshold. When the actual value decreased below the threshold of 80% FWC (field water capacity) for individual pots, they were watered by micro sprinklers with a magnetic valve–activated watering system. The amount of water added per period was proportional to the deviance from the threshold. This watering regime was applied throughout the experiments for all watered pots and until some pots reached the drought stress phase.

Plants were fertilized once a month with Fertilizer Shake & Grow® 12-12-12 formula plus microelements according to the manufacturer instructions. Lentil seeds were inoculated with *Methylobacterium* at the time of planting by adding 20 ml of the bacterial solution in each pot (approximately 10^9 CFU /mL; OD_{600} = 0.6) to half of the pots in each of the experiments.

Vegetative growth stage experiment

The vegetative stage experiment was aimed at evaluating the performance of lentil plants during the 25 day-long period of complete water withdrawal (terminal drought). The water supply was limited at the beginning of the 3rd week old plant growth. Lentils were harvested in the 7th week of vegetation. Vegetative stage experiment was conducted with 3 plants per pot. Each experimental plot was consisted of one pot, total of 10 pots per treatment.

The following plant parameters were evaluated during the experiment:

Biometrical traits

a) *Germination rate*: percentage of germinated seeds counted from day 3 to day 7 after sowing;

b) *Growth rate*: height of plants measured daily over the period of drought stress (5 replications);

c) *Chlorophyll level (SPAD)*: chlorophyll level measured at 23rd day of drought by the chlorophyll meter (SPAD 502, Minolta, Japan) (10 replications);

d) *Shoot/root length, fresh weight [FW], dry weight [DW]*: parameters assessed at harvest in the 7th week of vegetation (10 replications).

Physiological traits

a) *Photosynthesis, Transpiration and Water Usage Efficiency (WUE)*: photosynthesis and transpiration rate were determined using the Portable Photosynthesis System Li-Cor, LI-6400XT (Li-Cor, Lincoln, NE, USA). Each parameter was measured for 3 min at a photosynthetically active radiation of 2000 µmol m^{-2} s^{-1}, CO_2 concentration of 400 µmol mol^{-1}, and air flow of 400 µmol s^{-1}. In total, 4 plants of each treatment were tested at 5 different time points during the drought stress period. The protocol used to perform these measurements was the one proposed by Evans and Santiago (2014). Additionally, the water use efficiency (WUE) was calculated based on the equation:

$$\mathrm{WUE} = \frac{\text{Photosynthesis rate}}{\text{Transpiration rate}}$$

b) *Electrolyte conductivity (EC)*: Leaf samples were cut into fragments of uniform size, weighted and rinsed with demineralised water, for immersion overnight in 30 mL deionized water at room temperature. Total conductivity was obtained after autoclaving the samples (AWAD; SOAUD; EL-KONAISSI, 2006). A conductivity meter (Omega Conductivity Meter - CDH-5022, Laval, Canada) was used for all the analysis performed at 6 different time points in 6 replications.

c) *Hormone levels (Cytokinin):* Plant growth regulators were measured by the highly precise analytical techniques of High Performance Liquid Chromatography – Electrospray Ionization - Tandem Mass Spectrometry (HPLC-(ESI)-MS/MS; ABI Sciex QTrap5500, Concord, Canada, coupled with Shimadzu LC10ADvp, Kyoto, Japan) from randomly sampled lentil leaves harvested at 4 different time points during the drought stress period in 3 replications.

Water Management Parameters

a) *Relative water content (RWC):* Measurements of relative water content were determined by recording fresh weight, saturated weight, and dry weight of leaves (TEULAT et al., 2003). Cut out leaf fragments were individually weighed (FW) and kept in the petri dishes with deionized water until they reached full turgidity at 4°C (2h). The samples were then removed from water, residual leaf moisture was removed with filter paper, and samples were immediately weighed to obtain a fully turgid weight (TW). Subsequently, the plants were dried in an oven at 65°C for 24 h, and dry weight was recorded (DW). RWC was calculated in 6 replications and at 6 different time points during the drought stress period according to the equation:

RWC (%) = [(FW-DW) / (TW-DW)] × 100.

b) *Leaf Water Loss (LWL)*: Leaf water loss rate was calculated through the measurements of leaf fresh weight right after harvest (Leaf FW) and the weight loss of the detached leaf after 2 hours in room temperature (Leaf 2HW) (XING et al., 2004). All the tests were performed in 6 repetitions at 6 different time points during the period of drought stress and the LWL was calculated according to the equation:

$$\mathrm{LWL} = \frac{\mathrm{Leaf\ FW} - \mathrm{Leaf\ 2HW}}{\mathrm{Leaf\ FW}}$$

Statistical analyses

Equal numbers of irrigated and drought-stressed plants were randomly selected for each taken measurement. Before statistical analyses, ANOVA presuppositions such as casualization, variance homogeneity, and residual normality, were accessed through SPSS software.

The effects of treatments on watering regimes and days were examined by analyses of variance in a slip-plot arrangement in time. The watering regimes were used as the main plot and days of sampling as the subplot. When interactions or repeated measures over time were significant, regression models were adjusted by Sisvar Software (FERREIRA, 2014). When only qualitative factors were significant, treatment means were assessed through Tukey's test ($\alpha < 0.05$) in order to confirm significant differences between the treatment levels using Sisvar Software. Also, for Cytokinin and growth rate comparisons, ninety-five percent confidence intervals of linear regressions were calculated and checked for overlapping coefficients.

Additionally, individual analyses of variance were carried out for measurements taken only once (at harvest and SPAD on the 23rd of drought stress). Once the proportion of the highest and the lowest mean square error was lower than 7, joint analyses were proceeded in order to test the effect of bacteria on the evaluated parameters by Genes Software (CRUZ, 2016).

3. RESULTS AND DISCUSSION

Drought tolerant *Methylobacterium* strain

The selection of a drought tolerant *Methylobacterium* strain was based on a 2x7 factorial analyses, where the first factor was related to the concentration of PEG (0% and 20%), and the second was related to the 7 selected bacterial strains of highly active Cytokinin profiles. The evaluated variable was based on the optical density of the strains. As expected, the performances of all strains were superior under growth of control cultures (0% PEG6000) (Table 1).

TABLE 1- Optical density of 7 *Methylobacterium* strains at 0% and 20% PEG6000 concentration.

Strains	**PEG concentration**	
	0 %	**20 %**
M. gossipicola	0.267A h*	0.027B d
M. radiotolerans	0.767 A f	0.048 B d
M. jeotgali	1.005 A c	0.061 B d
M. phylosphaerae	0.634 A g	0.099 B d
M. organophillum	0.858 A e	0.241 B c
M. organophillum	1.185 A a	0.365 B b
M. oryzae	1.088 A b	0.479 B a

*Means followed by the same uppercase letter (line) and lowercase letter (column) belong to the same group at 5% level of significance by Scott and Knott test.

In the presence of 20% Polyethylene Glycol 6000 (PEG6000), bacteria growth measured by the optical density at the 5th day of the culture was reduced. Bacteria strain able to grow in this condition is considered to be drought tolerant (ALI; SANDHYA; RAO, 2014). Therefore, the *Methylobacterium oryzae* strain, which reached the highest OD_{600} at 20% PEG6000 concentration, was selected to be used as a plant growth promoter in dry environments.

Biometrical traits

Uniformity of germination and initial seedling establishment are indeed important plant characteristics especially in regions where drought stress is most likely to occur. Germination rate of vegetative stage experiment was higher at Day 4 when inoculated with *Methylobacterium* (Figure 1). Likewise, most seeds were germinated by Day 4 after sowing in the presence of the bacteria, whereas in the bacteria absence, non-treated lentils reached similar values only at Day 6.

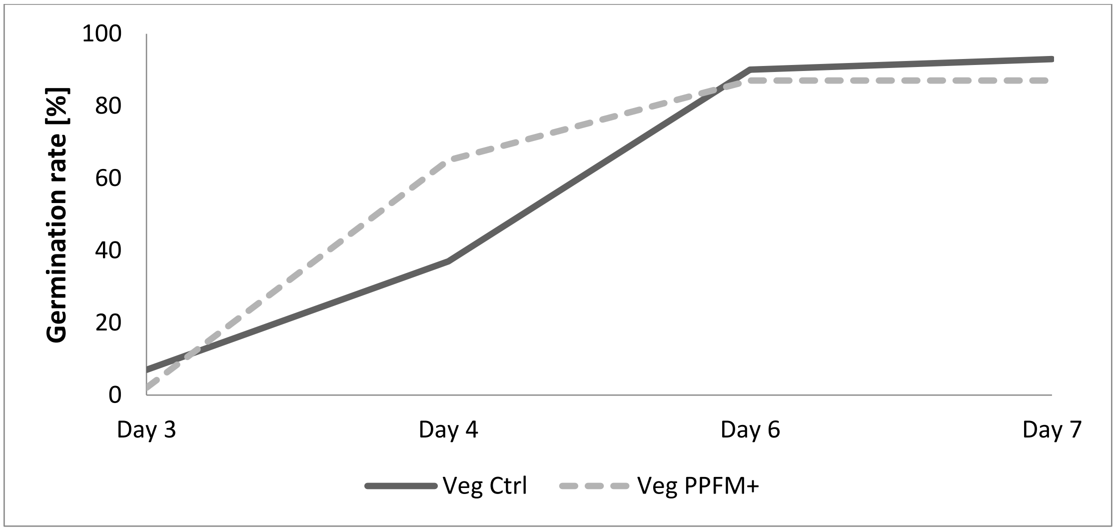

FIGURE 1- Germination rate of lentil seeds in vegetative growth stage experiment in the presence and absence of bacteria. Abbreviations for the figure: Veg Ctrl: vegetative experiment, non-inoculated seeds; Veg PPFM+: vegetative experiment, inoculated seeds. The data are presented as total percentage values based on daily readings.

A higher germination rate and faster seedling growth was observed also by Meena et al. (2012) as the effect of beneficial symbiotic relationships between *Methylobacteria* and wheat. Also, the positive effect of inoculation with plant growth-promoting *Methylobacteria* on maize and sorghum-sudangrass germination has been previously affirmed (KIM et al., 2012).

Bacteria inoculants have promising roles in integrated solutions to environmental issues i.e. the occurrence of drought stress in plants. Beneficial effects of plant growth-promoting rhizobacteria (PGPR) include enhancing plant growth, improving nutrient availability and uptake, and supporting the health of plants (MANTELIN; TOURAINE, 2004; ARDANOV et al., 2012). Since the discovery of beneficial role of PGPR, bacteria strains have been investigated for their usage in sustainable plant cultivation (MADHAIYAN et al., 2005).

In the present study, the growth rate of lentils over 25 day-long period of complete water withdrawal was measured daily in order to evaluate the bacteria performance in the inoculated plants. Linear equations were significant for all growth rate treatments at 5% probability level by F test (Figure 2).

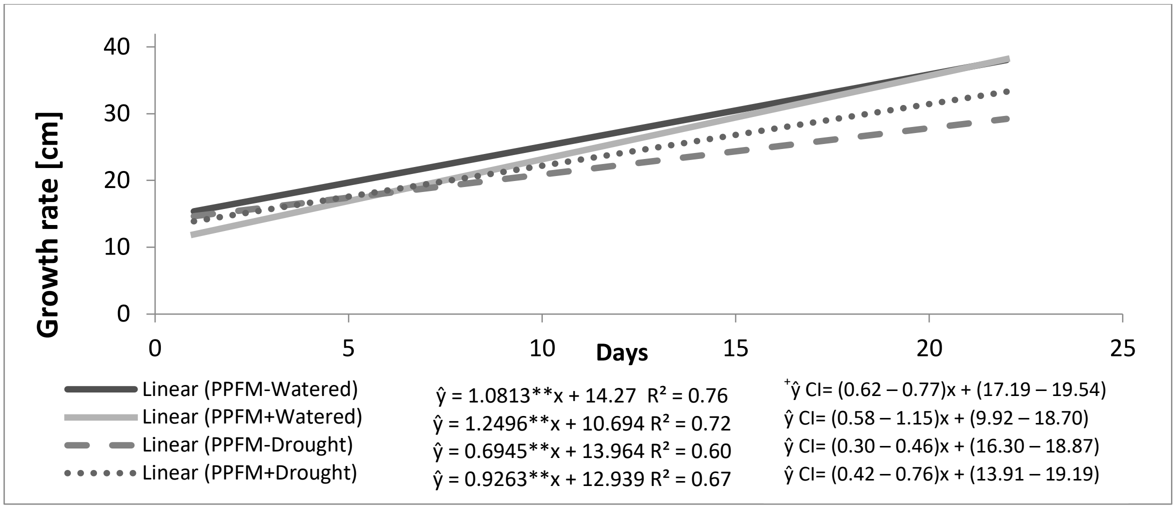

FIGURE 2- Lentil growth rate over 25 days of drought. Abbreviations for the figure: PPFM-Watered – well-watered, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Drought – drought stressed, inoculated. Data are presented as significant linear trend lines based on the daily measurements of plant height at 5% probability level by F test. Ninety-five percent confidence intervals of linear regressions were calculated and checked for overlapping coefficients according to the formula: $CI(b_0)=b_0 \pm 1.96x\ SE(b_0)$ and $CI(b_1)=b_1 \pm 1.96x\ SE(b_1)$. $^{+}\hat{y}$ CI: Estimated equation of confidence interval; SE: standard error.

When comparing both well-watered treatments (with and without bacteria) during the 25 days of measurements, the two linear (b0) and angular (b1) coefficients showed overlapping coefficients; therefore, they indicated similar growth rate patterns according to their superior and inferior linear confidence intervals. The same occurred when plants were exposed to drought stress. However, a steeper angle of growth curve for PPFM+Watered and PPFM+Drought can be seen as an indicator of superior plant height at the later stages. In fact, other studies with plant growth promoting bacteria have highlighted the ability of such microorganisms to promote plant development (ERDOGAN; DONMEZ; CAKMAKCI, 2007; WOYESSA; ASSEFA, 2011; VERMA et al., 2013).

Plant growth-promoting bacteria can also stimulate other biometrical traits. *Methylobacterium oryzae* had a positive effect on lentils under both watering regimes (watered and drought stressed plants) as it significantly increased root length, shoot length, root dry weight, and shoot dry weight by Tukey test at 5% significance level (Figure 3). For all shoot parameters and root fresh weight, watered lentils at harvest have demonstrated higher averages when compared to drought stressed plants.

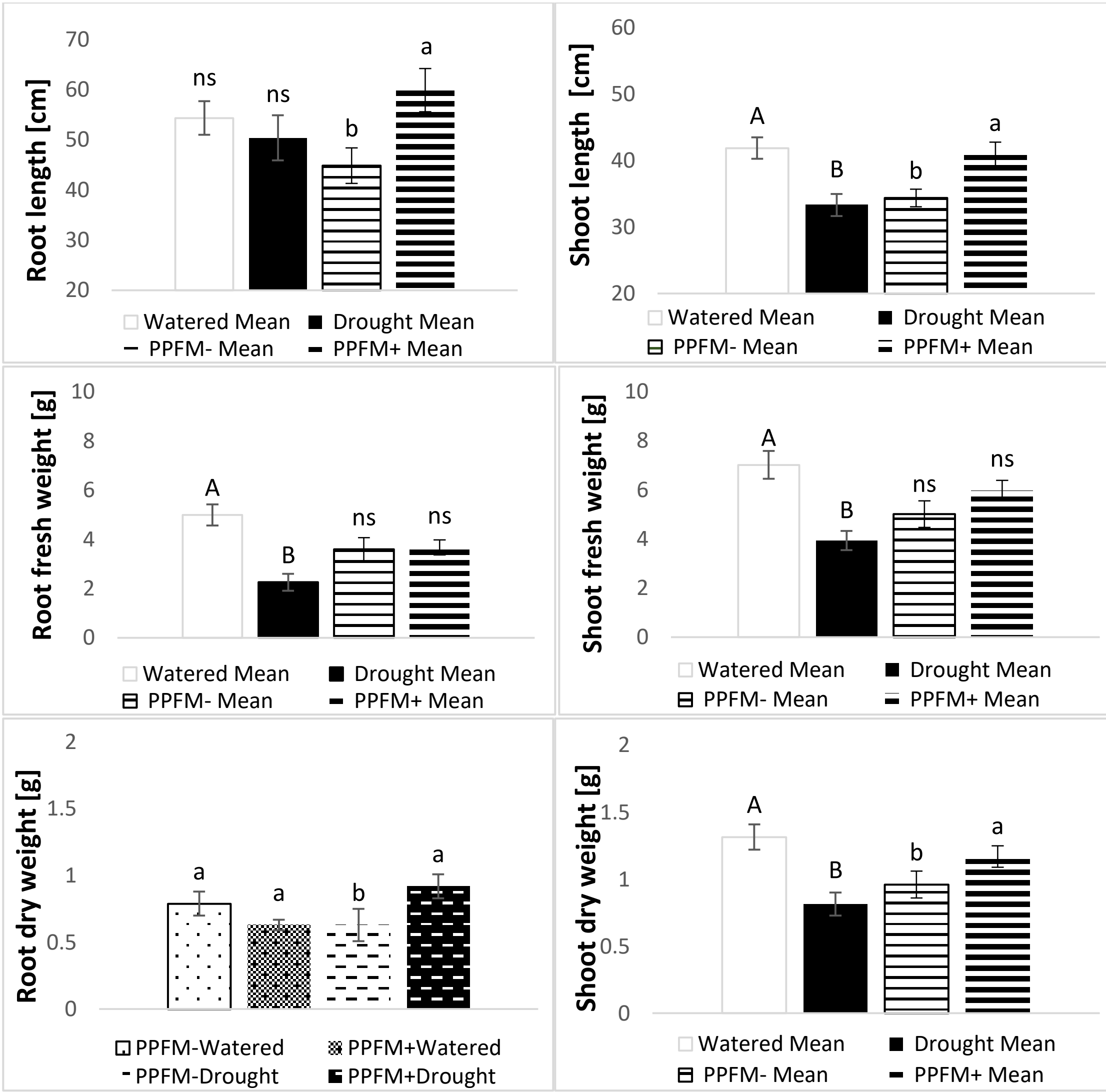

FIGURE 3 - Shoot/root length, fresh weight, dry weight assessed at harvest on the 7th week of vegetation. Abbreviations for the figure for: PPFM-Watered – well-watered, non-inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM+Drought – drought stressed, inoculated; Watered Mean – well-watered, inoculated and well-watered non-inoculated average; Drought Mean – drought stressed, inoculated and drought stressed non-inoculated average; PPFM-Mean – non-inoculated, watered and drought stressed average; PPFM+Mean – inoculated, watered and drought stressed average. In the absence of significant interactions (bacteria treatment vs. watering regime), results were presented as averages for watering regimes and bacteria treatment, respectively. Values are means ± SE of 10 replicates. Different uppercase letters above columns indicate significant difference among watering regimes and lowercase letters between bacteria treatments by Tukey test at P<0.05.

Increases by 18% (6.4 cm) for shoot length and 33% (15 cm) for root length were verified on inoculated lentils, regardless of watering regimes (Figure 3). Also, there was a significant interaction for root dry weight as inoculated lentils when exposed to drought achieved averages similar to the watered plants both inoculated and non-inoculated. In another study, plant growth-promoting bacteria were found to increase the dry weights of above-ground tissues and roots of rapeseed (ZHANG et al., 2011). Furthermore, inoculation with PGPR had significant effect on increasing fresh and dry weight of shoot (LIU et al., 2013), as well as increasing the shoot dry weight, root dry weight, and total dry matter of maize by up to 38% (EGAMBERDIYEVA, 2007).

Drought stress activates highly energy-demanding processes in order to tolerate water shortage within the plant and the preservation of photosynthetic machinery contributes significantly to plant ability to withstand drought stress (MACKOVÁ, 2013). Consequently, the protection of higher relative levels of chlorophyll, the main light-harvesting pigment, is crucial for a better plant physiological status (MACKOVÁ, 2013). Both watering regimes when inoculated with the bacteria behaved similarly regarding measurements taken on the 23rd day of water withdrawal by a chlorophyll meter (non-significant interaction) (Figure 4). *Methylobacterium* had a significant, positive effect on SPAD readings in drought stressed and watered lentils.

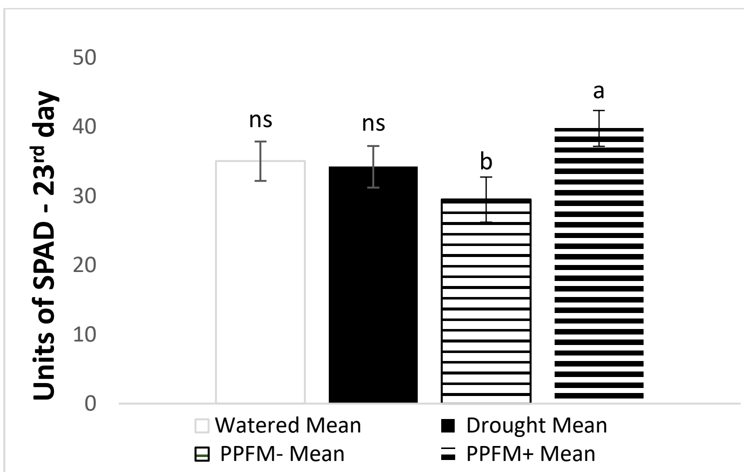

FIGURE 4 – Level of chlorophyll (in SPAD units) measured on the 23rd day of water withdrawal in non-inoculated and inoculated plants. Values are means ± SE of 10 replicates. Abbreviations for the figure: Watered Mean – well-watered, inoculated and non-inoculated averages; Drought Mean – drought stressed, inoculated and non-inoculated averages. Different lowercase letters above columns indicate significant difference between bacteria treatments by Tukey test at P<0.05. ns: not significant.

Physiological traits

As an important physiological trait, the photosynthesis rate, is a useful parameter to determine how drought stress affects carbon fixation, and thereby biomass accumulation in plants. Both inoculated and non-inoculated watered plants maintained higher photosynthesis averages (7.75 and 6.21 umol $CO_2/m^2/s$, respectively) when compared to inoculated and non-inoculated, drought stressed lentils (4.87 and 3.85 umol $CO_2/m^2/s$, respectively). Additionally, photosynthesis rates did not statistically varied throughout the days for both drought and watered experiments, with and without bacteria. Therefore, no regression models could be adjusted and real values are shown in Figure 5. Nevertheless, it is worth to mention that both watered and drought stressed plants revealed higher photosynthesis rates when they were inoculated with *Methylobacterium.*

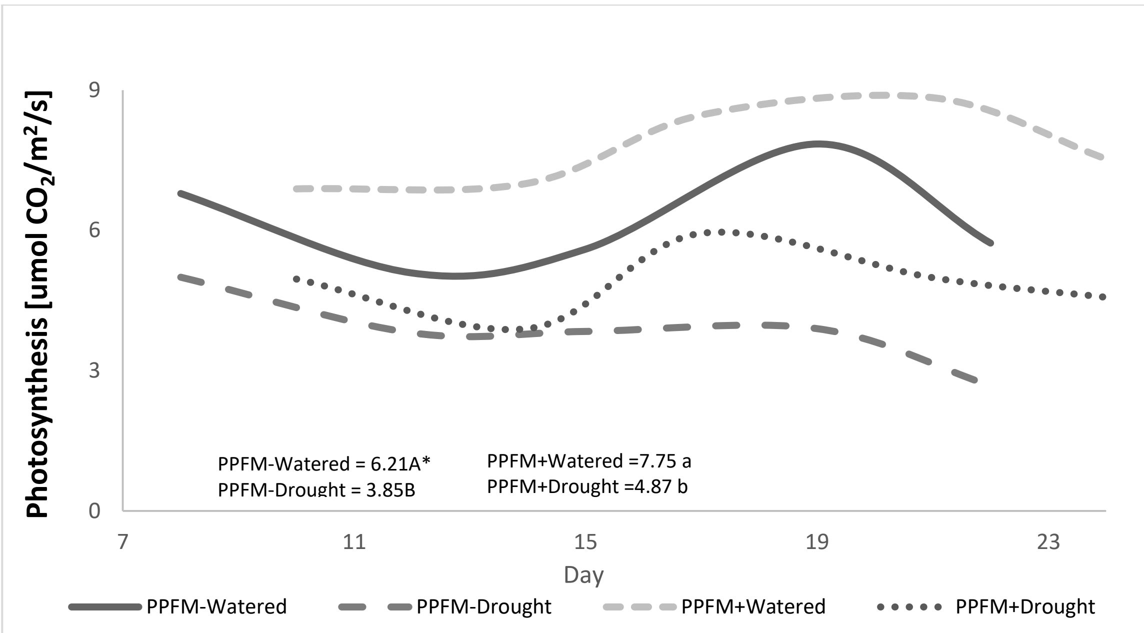

FIGURE 5 – Photosynthesis rate in inoculated and non-inoculated lentils over the period of 25 days of complete water withdrawal. Abbreviations for the figure: PPFM-Watered – well-watered, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Drought – drought stressed, inoculated. Raw data are presented.*Means followed by different uppercase letter (non-innoculated) and lowercase letter (innoculated) are significantly different at 5% level of significance by Tukey test.

The presence of *Methylobacterium* resulted in the highest photosynthesis rates observed for inoculated, well-watered plants. Drought stress induced a more significant decrease in photosynthesis of non-inoculated plants, while bacteria-treated plants maintained a stable and higher rate of photosynthesis. Another research also suggests the beneficial role of bacteria in enhancing photosynthetic activity of grapevine under drought (ROLLI et al., 2015).

Transpiration rate is an important parameter that indicates the loss of water through the leaves and can be an indicator of the plant water status under drought. A significant interaction between watering regimes and days of drought was found for non-inoculated lentils. Watered plants showed a cubic model distribution; however, for non-inoculated, drought plants, no significant model was found for such data distribution (Figure 6).

In the presence of bacteria, no interaction was found and only individual effects (watering regimes and days of drought) were significant. In this case, watered plants kept a higher overall transpiration average (1.44 mmol $H_20/m^2/s$) when compared to drought stressed plants (0.79 mmol $H_20/m^2/s$). Over the period of water withdrawal, both watering regimes were adjusted in a cubic regression (Figure 6).

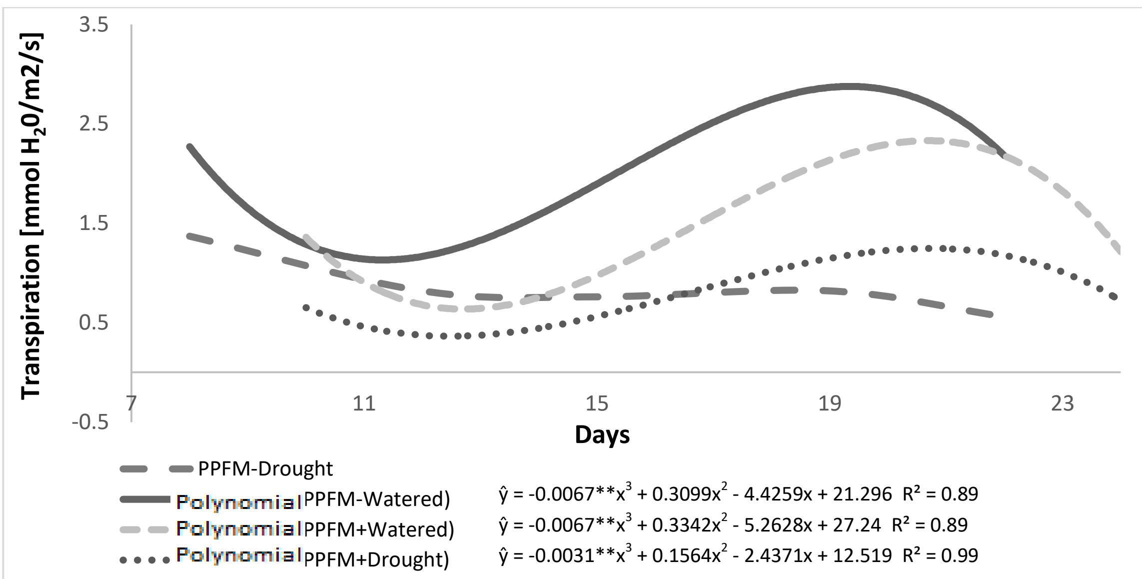

FIGURE 6 – Transpiration rate in inoculated and non-inoculated lentils over the period of 25 days of complete water withdrawal. Abbreviations for the figure: PPFM-Watered – well-watered, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Drought – drought stressed, inoculated. Raw data are presented and significant regression models based on analyses of variance in a slip-plot arrangement in time of 5 days' transpiration rate measurements at 5% probability level by F test.

When comparing transpiration levels of inoculated and non-inoculated, watered plants, a better water management can be inferred when the bacteria were present since a lower pattern of water loss by leaves throughout the experiment was observed when lentils were inoculated with *Methylobacterium oryzae*. Similarly, plant photosynthetic activity has been highlighted to be improved as an effect of bacteria inoculation under drought conditions in wheat (TIMMUSK et al., 2014).

Regarding the Water Usage Efficiency (WUE), interaction was significant for all treatments. Quadratic regression models were significant for non-inoculated lentils. In contrast, inoculated lentils had a significant cubic distribution over the days of drought. Overall, plants in both well-watered and drought stressed conditions, especially at the early stages of drought stress have demonstrated a better water management and superior WUE across the whole course of the experiment when inoculated with the bacteria (Figure 7).

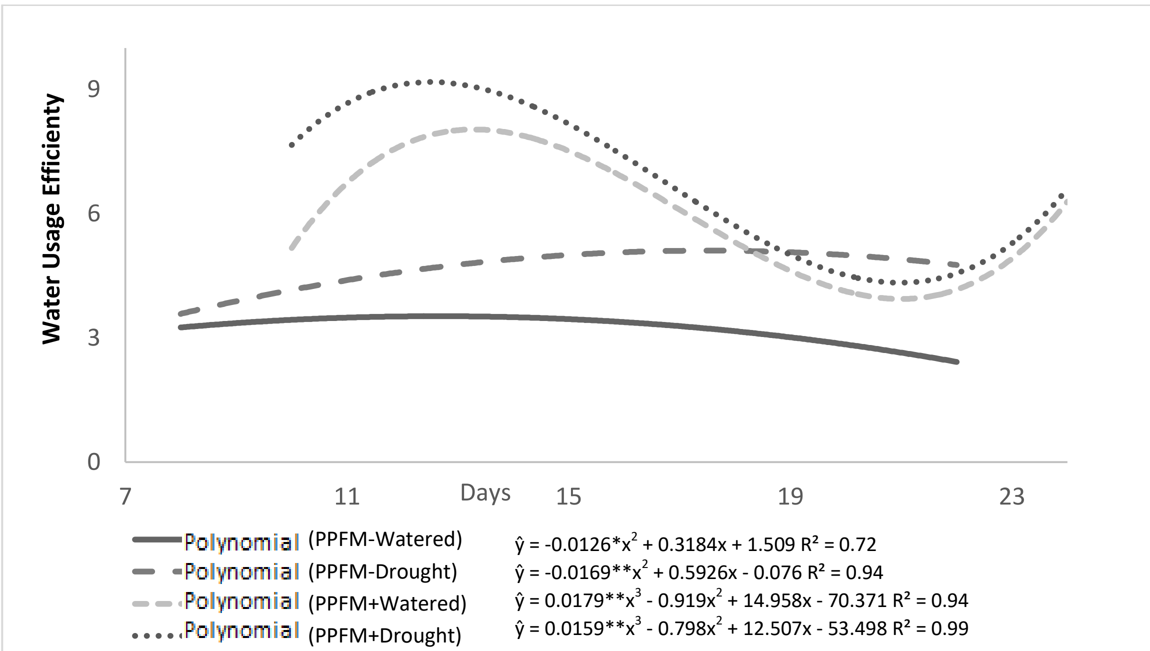

FIGURE 7– Water Usage Efficiency (WUE) in inoculated and non-inoculated lentils over the period of 25 days of complete water withdrawal. Abbreviations for the figure: PPFM-Watered – well-watered, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Drought – drought stressed, inoculated. Data are presented as significant regression models based on analyses of variance in a slip-plot arrangement in time of 5 days' transpiration rate measurements at 5% probability level by F test.

In another research, root-associated microbiome has improved WUE and grapevine resistance to drought (ROLLI et al., 2015). Therefore, a better water management can be achieved as a reflection of bacteria inoculation that alleviates the negative effect of drought on plant physiology.

Another physiological parameter assessed in this work was the total electrolyte conductivity (EC; Figure 8). This parameter arises as part of an osmotic adjustment process that usually involves accumulation of compatible solutes such as sugars, ions and amino acids by plant in response to drought conditions. The enlarged accumulation of solutes can lower leaf osmotic potential and allow movement of water into the leaf cells; therefore, help maintaining turgor potential and increasing tissue tolerance to drought conditions (MIDEGA et al., 2017).

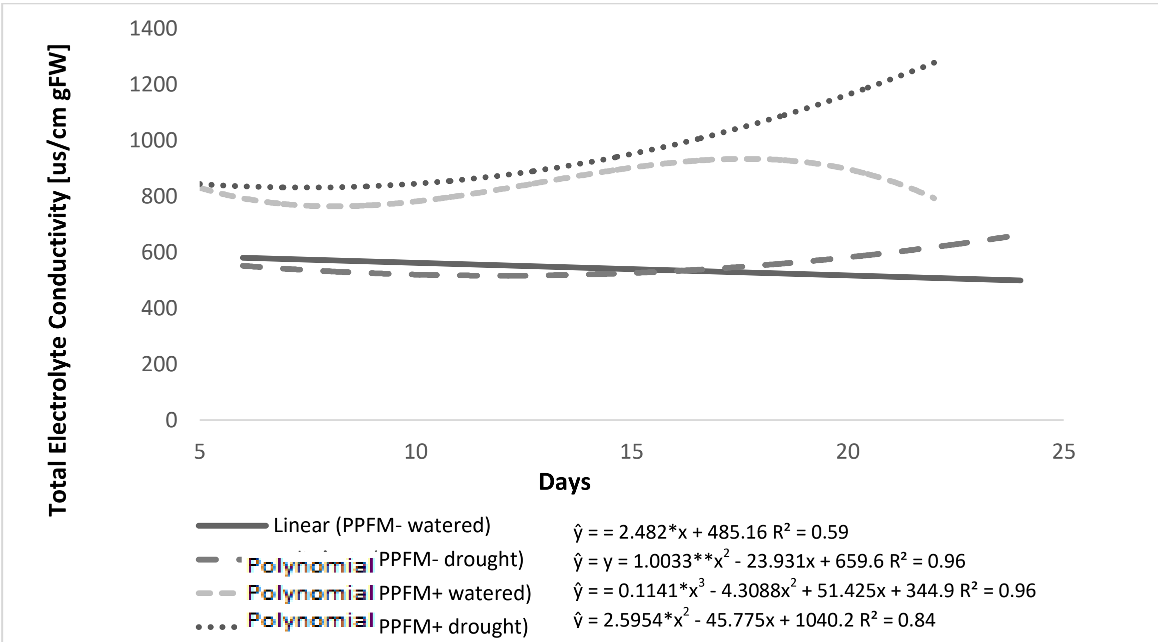

FIGURE 8– The effect of *Methylobacterium* on total electrolyte conductivity (EC; us/cm gFW) in leaves of well-watered and drought stressed lentils during 25 days of drought stress. Abbreviations for the figure: PPFM-Watered – well-watered, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Drought – drought stressed, inoculated. Data are presented as significant regression models based on analyses of variance in a slip-plot arrangement in time of 6 days' total electrolyte conductivity measurements at 5% probability level by F test.

Significant interactions were found for watering regimes and days of drought for both inoculated and non-inoculated lentils. A superior total eletroctrolyte conductivity was confirmed troughout drought stress imposition for inoculated lentils under both watering regimes (Figure 8). In other words, this confirms the benefitial effect of *Methylobacterium* on enhancing eletrolyte conductivity levels regardless of watering regimes. The accumulated solutes are also responsible for sequestering water molecules, protecting cell membranes and protein complexes (CHAVES; MAROCO; PEREIRA, 2003).

Water Management Parameters

For the Relative Water Content parameter (RWC), none of the treatments was significantly different between both watered and drought stressed plants in the presence or absence of the bacteria. Also, the obtained data did not fit in any significant model regression. The only statistical difference was found for watering regimes in which PPFM-Watered plants showed a higher average of RWC (95.17%) when comparing to the PPFM-Drought plants (91.68%) (Figure 9).

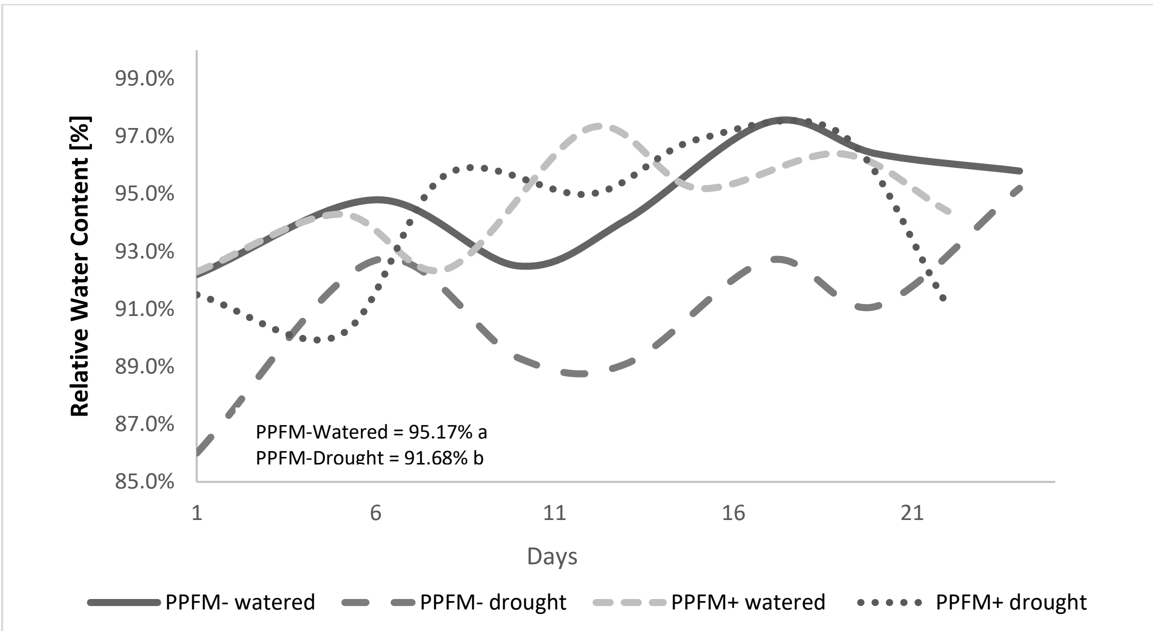

FIGURE 9 – The effect of *Methylobacterium* on Relative Water Content (RWC; %) in leaves of well-watered and drought stressed legume plants during the period of complete water withdrawal. Abbreviations for the figure: PPFM-Watered – well-watered, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Drought – drought stressed, inoculated. Raw data are presented. *Means followed by different lowercase letter are significantly different at 5% level of significance by Tukey test.

The relative degree of hydration of leaf tissues of PPFM-Drought plants had the lowest relative water content throughout the imposition of drought stress (Figure 9). Besides, PPFM+Drought lentils have shown a superior water plant status, similarly to the well-watered plants. A greater tolerance to water stress was also evident as increase in relative water content (RWC) of wheat following bacteria treatment in relation to drought-stressed, untreated plants (CHAKRABORTY et al., 2013).

In regard to Leaf Water Loss (LWL) during complete water withdrawal, significant interactions were found for both watering regimes in inoculated and non-inoculated lentils.

Water withdrawal caused a drastic reduction on leaf water loss overall. A higher loss in the presence of the bacteria have indicated a higher water content in cells and so, a better potential to maintain leaf turgor under the occurrence of drought (Figure 10). Also, non-inoculated, drought stressed lentils had a steady decline to very low levels of water in the leaves by the end of complete water withdrawal.

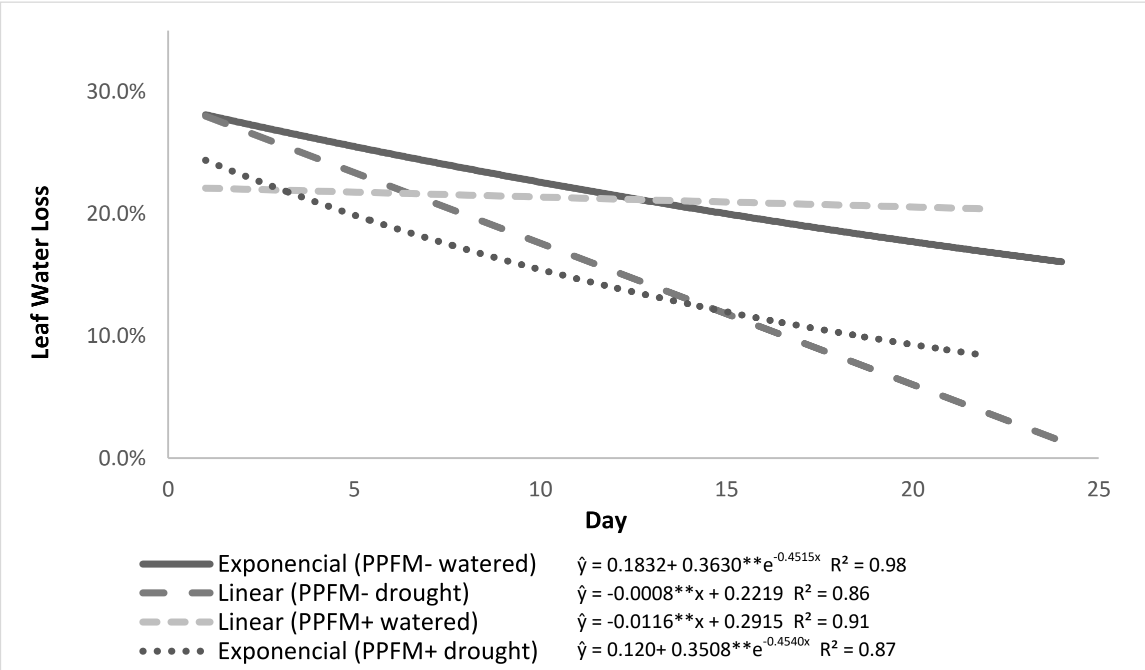

FIGURE 10 – The effect of *Methylobacterium* on Leaf Water Loss (LWL; %) measured in leaves of well-watered and drought-stressed lentils during the period of complete water withdrawal. Abbreviations for the figure: PPFM-Watered – well-watered, non-inoculated; PPFM+Watered – well-watered, inoculated; PPFM-Drought – drought stressed, non-inoculated; PPFM+Drought – drought stressed, inoculated. Data are presented as significant regression models based on analyses of variance in a slip-plot arrangement in time of leaf water loss measurements at 5% probability level by F test.

Hormone levels (Cytokinin)

The HPLC-MS/MS analysis of the Cytokinin levels in leaf samples collected from lentils at the vegetative developmental stages revealed considerably higher levels of active forms of Cytokinin in inoculated plants as compared to non-inoculated controls. The increased Cytokinin level is considered as an additional symptom of the presence of the population of growth promoting bacteria in plants (TIRICHINE et al., 2007).

For total Cytokinins (CKs), the interaction between bacteria and watering regimes was not significant. On the other hand, there was a significant difference over the days for bacteria treatments on Cytokinin levels of both drought and watered plants (Figure 11).

Plants inoculated with bacteria had superior levels of Cytokinin during the whole evaluation period reinforcing the beneficial effect of bacteria on enhancing Cytokinin levels of the host plant (no overlapping coefficients). With and without bacteria, all treatments regardless of water availability, have demonstrated similar behavior on Cytokinin levels over time (Figure 11). Other researchers have found similar results on improving CK levels in plants inoculated with *Methylobacterium* (IVANOVA, et al., 2008).

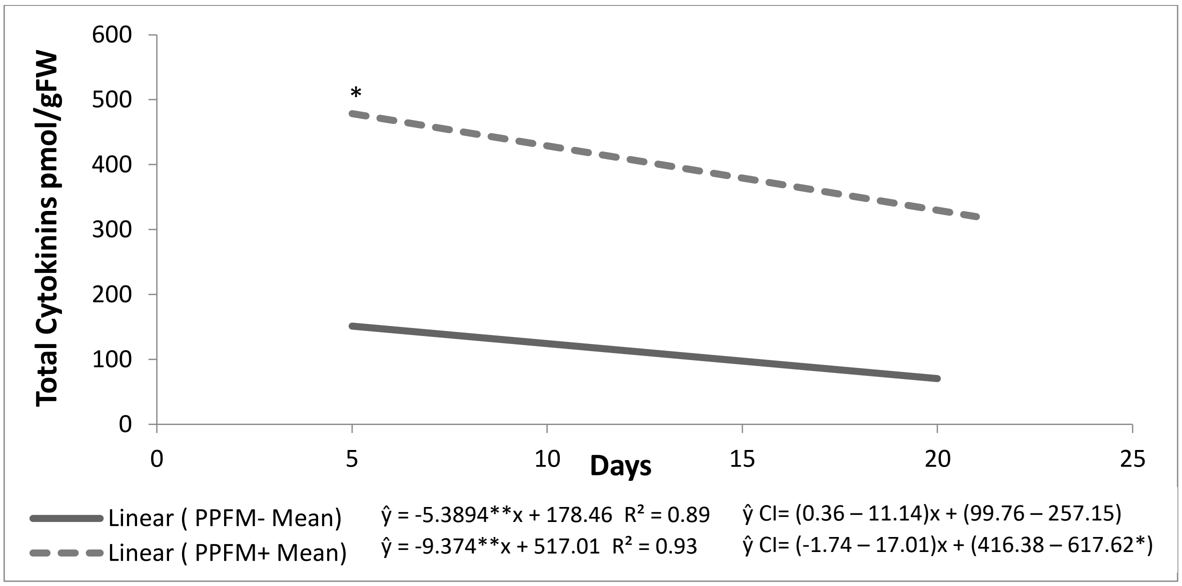

FIGURE 11. Total Cytokinin content in inoculated and control plants over the period of complete water withdrawal. Abreviations: PPFM-Mean – non-inoculated, watered and drought stressed average; PPFM+Mean – inoculated, watered and drought stressed average. Data are presented as significant linear trend lines based on analyses of variance in a slip-plot arrangement in time at 5% probability level by F test. Ninety-five percent confidence intervals of linear regressions were calculated and checked for overlapping coefficients according to the formula: CI(b_0)=b_0 ± 1.96x SE(b_0) and CI(b_1)=b_1 ±1.96x SE(b_1). CI: Confidence interval. *: Indicates no overlap for the confidence interval of at least one regression coefficient.

Regardless of water supply levels, significant effects of *Methylobacterium* inoculation on Cytokinin concentrations in lentil leaves were observed. These results are in accordance with the beneficial effect of plant growth-promoting rhizobacteria (PGPR) on increasing plant growth associated hormone in poplar trees (TAGHAVI et al., 2009). Furthermore, Cytokinins stimulated cell division, cell enlargement, and tissue expansion in certain plant parts enhancing shoot growth in food crops (WEYENS et al., 2009). In another study, increased Cytokinin content improved photosynthetic capacity, and therefore, benefited plant growth and dry matter accumulation (LIU et al., 2013).

The analysis of all the measured plant morphological and physiological parameters suggests that the beneficial effect of inoculation was to a high extent a result of visibly improved plant vigor and general performance (faster growth, higher photosynthesis rate, elevated chlorophyll (SPAD), nutrient uptake (electrolyte content)), and optimized water management parameters (WUE, LWL). Better plant fitness can lessen the negative effect of water withdrawal and therefore, help improving plant recovery in case of temporary limited water supply during vegetation season. Consequently, the introduction of *Methylobacterium* inoculant to farmer practices could facilitate expansion of legume production over areas currently challenged by dry soils, and at the same time, guarantee food security.

4. CONCLUSION

The inoculation with *Methylobacterium oryzae* had a beneficial effect on lentil biometrical and physiological traits, as well as water management parameters. It could be effectively employed in agricultural practices to stimulate growth, improve physiological parameters, and increase legume tolerance to water stress. Therefore, a promising bio inoculant formula of bacterial origin, able to release significant amounts of beneficial CKs, could lower the negative effect of drought on lentil cultivation.

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