



DEPARTMENT OF BIOLOGICAL AND
ENVIRONMENTAL SCIENCES

CAN LEAF LEVEL GAS EXCHANGE BE USED AS A PREDICTOR FOR DROUGHT TOLERANCE IN COMMON BEAN (PHASEOLUS VULGARIS)?

Villemo Holmberg

Degree project for Bachelor of Science with a major in biology

BIO602 Examenskurser i biologi

First cycle

Semester/year: Spring 2022

Supervisor: Mats Andersson, Department of Biological and Environmental Sciences

Examiner: Lasse Tarvainen, Department of Biological and Environmental Sciences

Table of Contents

Sammanfattning	2
Abstract	2
Introduction	2
Climate	3
Origins of the common bean	3
Drought responses in plants	4
Stomatal conductance	4
Chlorophyll fluorescence	4
Aim	4
Materials and methods	5
Growth conditions and plant material	5
Leaf-level gas exchange experiment	5
Drought experiment	5
Data analysis	6
Results	6
Leaf level gas analysis	6
Drought experiment	7
Correlations between conductance and drought tolerance data	10
Discussion	12
Variation in photosynthesis and stomatal conductance	12
Chlorophyll fluorescence as a predictor for wilting	13
Drought sensitivity	13
Further studies	14
Conclusion	15
Acknowledgement	15
Reference list	15

Sammanfattning

Syftet med studien var att undersöka torktoleransen hos en mängd landraser tillhörande arten *Phaseolus vulgaris* för att upptäcka om gasutbyte på bladnivå kan användas som en prediktor för torktolerans. Olika landraser av bönor från Rwanda planterades och när de vuxit i ungefär fyra veckor utfördes en analys av gasutbytet på bladnivå genom att mäta på ett blad från varje planta både under dagen och natten. Hälften av växterna slutade sedan att vattnas. När dessa växter tappat sitt turgortryck blev de rehydrerade. Mätningar av klorofyllfluorescens utfördes varje dag under torkbehandlingen och även bladarean mättes. Resultaten visade skillnader mellan landraserna gällande den genomsnittliga sloktiden samt den genomsnittliga fältkapaciteten, vattenmängden, kvar i jorden vid vattning. De flesta böntyper slokade mellan dag fem och sju men typ 2 och 3 slokade något tidigare. De flesta typer hade en genomsnittlig fältkapacitet på 21% vid tiden de slokade. Typ 20 hade högst fältkapacitet. En stark korrelation mellan bladarea och sloktid fanns, dock hittades ingen korrelation mellan bladarea och procent av fältkapacitet. Mellan sloktid och bladarea multiplicerat med konduktans fanns en stark korrelation. Baserat på resultaten konkluderades det att mätningen av klorofyllfluorescens inte förutsåg slokning, medan gasutbyte på bladnivå är en någorlunda bra prediktor när det gäller förutsägelsen av taken på vattenförlust, men inte när det gäller hur låg procenthalt av fältkapacitet växterna tål.

Abstract

This study aimed to examine drought tolerance among a variety of landraces of the species *Phaseolus vulgaris* to discover whether leaf level gas exchange can be used as a predictor for drought tolerance. Beans of different landraces from Rwanda were planted and when they had grown for four weeks a leaf-level gas exchange analysis was carried out, measuring one leaf from each plant both during the day and night. Half of the plants then stopped being watered. Once these plants had lost turgor pressure they were rehydrated. Chlorophyll fluorescence measurements were taken every day during the drought treatment and leaf area was measured. The results showed differences between the land races regarding the average wilting time and the average percentage of field capacity, the amount of water, left in the soil at wilting. Most types wilted between day five and seven although type 2 and 3 wilted somewhat sooner. Most types had an average field capacity of 21% at the time of rewatering. Type 20 had the highest field capacity. A strong correlation between leaf area and wilting time was found but no correlation between leaf area and percentage of field capacity. Between wilting time and leaf area times conductance a strong correlation was found. Based on the results, it was concluded that measuring chlorophyll fluorescence did not predict wilting, while leaf level gas exchange is a somewhat good predictor when it comes to predicting the rate of water loss, not, however, when it comes to how low of a percentage of field capacity the plants can withstand.

Introduction

Climate change is a global phenomenon that is becoming increasingly problematic. Availability of fresh water, an essential resource for life, is negatively affected by this process. Investment is needed to secure water supplies, leaving nations without enough resources vulnerable. Around 80% of the world's population is living under conditions where water security is threatened on a high level [1]. One of the problems associated with freshwater access is caused by rainfall and drought patterns that are becoming increasingly unreliable because of climate

change, in turn causing concerns for farmers [2]. Temperature rises, and precipitation patterns change, resulting in an increased frequency and intensity of droughts, threatening global food security [3]. Countries that rely heavily on rainfall to maintain production are especially susceptible to drought. Despite this, most legume production is located where water shortage is experienced often, namely in India, China, and Africa. Droughts are among the abiotic factors that have the biggest effect on legume crop productivity through one or some of these processes: the time for reproductive development is shortened, branching is reduced leading to fewer pods forming, and/or a reduction in seed weight as well as the number of seeds produced per pod [4].

Plants are most sensitive to heat and drought during their reproductive phase. They have evolved mechanisms that speed up this phase, causing it to occur before the dry season has arrived. Breeders are utilizing this feature by crossbreeding these crops to improve yields. This process, however, is slow, and science is therefore researching genomic regions, trying to identify which regions are associated with improved productivity during a period of access to a limited amount of water. Once found, these loci can be bred to improve yields. So far, though, the improvements regarding water use have not led to any significant results [2]. Because of this, research is needed to see how plants survive droughts by using avoidance and tolerance processes. This means that plants can modulate the ability of harvesting water and maintaining healthy function when water is scarce to avoid becoming too dry. The goal is finding a plant that can balance the tradeoff between focusing on fruit per grain production at the expense of maintenance, which may lead to premature death, and stopping production completely in drought conditions. Drought conditions vary between regions, which means that no single trait will be ideal for all farmers [2]. This study focuses on drought tolerance in legumes grown in Rwanda, specifically beans of the common bean species, *Phaseolus vulgaris*. The cultivation of beans brings positive impacts to the environment and to the sustainable intensification of agriculture due to their nitrogen fixation, their control on weeds and their effects on the soil. Beans in general, and especially the common bean, are a major protein and micronutrient source worldwide as they represent the most important grain legume consumed [5]. Despite this, the amount of production of grain legumes is static or decreasing, threatening food security [6].

Climate

The common bean grows in climates where rainfall ranges between 350 mm and 500 mm during the plant's growth period and with temperatures ranging from 15 to 27° C [7]. The country Rwanda is mountainous and located in the Great Lakes region of Africa with a moderate and tropical climate. The temperature varies throughout the year, with an annual average temperature of 20 degrees Celsius and the minimum temperature occurring in February and the maximum in August. Annual precipitation is dispersed throughout the year but varies around the country [8]. The Rwandan climate meets the requirement for the common bean's growth, reflected in the fact that Rwanda has the highest per capita bean consumption in the world [9].

Origins of the common bean

The species did not, however, originate in Africa, instead it can be traced back to Mexico. The *Phaseolus* genus is important both for its high consumption but also for a quite unique property, namely that five of the around 70 species have been domesticated, making it a unique model for understanding crop evolution. The many independent domestication events make it possible to investigate how the domestication process occurs both between gene pools of the same species and between different species of the same genus. The crop is adapted to different environments as they have spread throughout different parts of the world. In the focus species of this study, *P. vulgaris*, this spreading removed the spatial isolation of the Mesoamerican and Andean gene pools causing an increase in the possibility for spontaneous hybridization and

therefore new genotypes and phenotypes [5]. The historical knowledge about the genetics of the genus is important in the study of how to preserve and evaluate the diversity within the genus, as well as in developing new varieties to combat climate change and increase food security [5]. Through evolution the wild types a species can become more adapted to the environment, but there can also exist a wide genetic variability within a species, for example types that are more drought resistant than others. These types could potentially be found, bred, and spread to help ensure a more stable food supply [4].

Drought responses in plants

In response to drought, plants will regulate their stomata, pores in the epidermis of a leaf, as a drought stress avoidance mechanism. By partially closing their stomata, plants attempt to maintain their leaf water content by reducing transpiration. This means the amount of gas exchange is modified, leading to a limitation of shoot growth and photosynthesis due to the decrease of CO₂ absorbed. To tolerate drought, they will use osmotic regulation. By increasing the solute concentration, water potential is lowered, causing water uptake in the roots through osmosis. By using this process, carbon dioxide uptake continues, which therefore leads to a continued photosynthesis. The common bean uses both methods to minimize yield reduction during droughts [4].

Stomatal conductance

The rate at which water vapor exits through the stomata of the leaf or the rate at which carbon dioxide enters it, is measured using stomatal conductance (g_s) [10]. g_s is therefore dependent on stomatal aperture and stomatal density [11]. When conducting gas-exchange studies, results have shown that g_s has increased in the last decades as plant breeding has resulted in higher yields, pointing to the importance of higher g_s rates when seeking optimum growth and yield. During the day, plants require stomata to be open for gas exchange to take place. When water exits through transpiration, carbon dioxide diffuses into the mesophyll. Because of this, the rate of photosynthesis and of g_s have been seen to correlate, as the stomata are considered gateways to both carbon dioxide and water. Under drought stress the level of g_s tries to be maintained as a form of drought avoidance to try to not lose yield [10]. High levels of conductance are seen in plants with large stomatal aperture, resulting in the loss of water as well as the uptake of larger amounts of carbon dioxide. Closing the stomata therefore lowers g_s and saves water, but at the same time limits photosynthesis, affecting yield [11]. It is when photosynthesis is strongly limited, for example during droughts or in low light, it seems to be of value for plants to close their stomata. Although this minimizes water loss, it does not stop it completely. Minimum leaf conductance (g_{min}) is a measurement of water lost through the cuticle and incompletely closed stomata [12].

Chlorophyll fluorescence

Chlorophyll fluorescence is the light that gets re-emitted by chlorophyll molecules during the return from excited to non-excited states. For this reason, it can be used to measure changes in the efficiency of photochemistry, the photosynthetic energy conversion [13]. Chlorophyll fluorescence is calculated by taking the variable fluorescence and dividing that by the maximum fluorescence (F_v/F_m). Chlorophyll fluorescence is commonly used in drought stress studies, as it reveals the efficiency of PSII under stress conditions [14].

Aim

The aim of this study is to examine drought tolerance among a variety of landraces of the species *P. vulgaris* collected during the 1980s in different regions of Rwanda. The physiological traits

related to drought tolerance, in particular the leaf gas exchange, as well as their relatedness to drought stress will be studied. The study further aims to discover whether leaf level gas exchange can be used as a predictor for drought tolerance, and how leaf-level gas exchange varies among landraces.

Materials and methods

Growth conditions and plant material

Beans of eighteen different landraces were obtained from the seed data bank of the International Centre for Tropical Agriculture (CIAT; <https://genebank.ciat.cgiar.org>). Two-liter pots with three sticks (for the plants to climb) were weighed and filled with soil (S-jord, Hasselfors Garden), weighted again, then watered and weighted a third time once excess water had run off. In these pots, bean seeds were planted, and each pot was marked with a pot number as well as with which line and replicate of bean was planted. Eight seeds of each landrace was planted, a total of 144 plants. Vermiculite was placed on top of the soil. The beans were left to grow for two weeks in a plant room where the relative humidity was 30-40% and the temperature 27° C. The lights were on for twelve hours during the day, and off during the night for twelve hours. The light intensity was 150 $\mu\text{mol m}^{-1} \text{s}^{-1}$. After germination, the plants were rotated and watered every other day. The rotation was done in order to avoid having the differences in light intensity in the room as a source of error. Once a week the plants were fertilized with Trädgårdsnäring (Vitagro, Blomsterlandet, Gothenburg, Sweden). Because some plants did not germinate, or germinated very slowly, they were removed from the experiment. Five aluminum trays with two liters of soil were watered, then dried in a drying cabinet at 20° C, and weighed once dry.

Leaf-level gas exchange experiment

When the plants had grown for four weeks a leaf-level gas analysis was carried out, measuring one leaf from each plant using the photosynthesis system Li-6400 XT (LI-COR; Lincoln (Nebraska), USA) with a leaf-level cuvette (2x3 cm leaf chamber with a 6400-02B LED light source) attached. The settings used were the following: the block temperature in the cuvette was set to 27° C, the CO₂-level to 420ppm, flow rate to 400 mmol s^{-1} , and PARin to 300 $\text{mmol m}^{-2} \text{s}^{-1}$, which was similar to the levels in the plant room. The rate of photosynthesis and stomatal conductance was taken note of after the curves had stabilized (after approximately two minutes). The measurements were taken in a different room to make sure that the CO₂ levels did not rise due to breathing in the plant room.

The same measurements were taken during nighttime, to measure nighttime stomatal conductance. The measurements were taken two hours after the lights had been switched off, to allow for the stomata to adjust to darkness. New specifications were used during these measurements; temperature was changed to 20° C and relative humidity was set to 30-50%. After a leaf had been clamped on, around two to three minutes passed, during which the curves stabilized, before any values were registered.

Drought experiment

After five weeks, once the leaf-level gas exchange experiment was finished, the beans were sorted into a control and drought-treatment group so that half of them were to be experiencing drought stress. During this period, the plants in the drought-treatment group completely stopped being watered. They were weighed every day. Chlorophyll fluorescence was also measured every day on the selected beans and every three days on the other plants. This was done using the PAM (Pulse Amplitude Modulation) chlorophyll fluorometer (Handy PEA+, Amesbury,

(Massachusetts), USA). The leaf-clips were clamped onto a leaf to make them adapt to darkness. Measurements were taken once half an hour had passed. Measuring chlorophyll fluorescence and weight was done until the plant had wilted, meaning it had completely lost turgor pressure in the first set of trifoliate leaves, and some of the turgor pressure in the next set of trifoliate leaves.

Leaf area was also measured, using the app Easy Leaf Area and instructions by Easlon and Bloom [15]. By putting the leaves against a white sheet of paper with a red 2x2cm square, each leaf photographed, and the area measured. All data was recorded in Microsoft Office 365 Excel. When sorting, only one plant of type 6 was placed in the drought experiment group, making the results for this type unreliable and not statistically significant.

Data analysis

The software GraphPad Prism v 9.4 (GraphPad Software Inc., La Jolla, USA) was used for ANOVA-analyses and Turkeys parvist tests. Significant differences between genotypes were found for each test, except for when testing the percentage of field capacity between genotypes, where no statistically significant differences where found.

Results

Leaf level gas analysis

Eighteen different land races of *P. vulgaris* obtained from Rwanda were cultivated in a growth room and gas exchange measurements were taken after four weeks. In figure 1, a small variation in photosynthesis can be seen among the *P. vulgaris* cultivars. The average photosynthetic rate was lowest in type 6 with $10.7 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$. The highest photosynthetic rate was found in type 10 with $13.5 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$. The total average for all types was $12.2 \text{ CO}_2 \text{ m}^{-2}\text{s}^{-1}$.

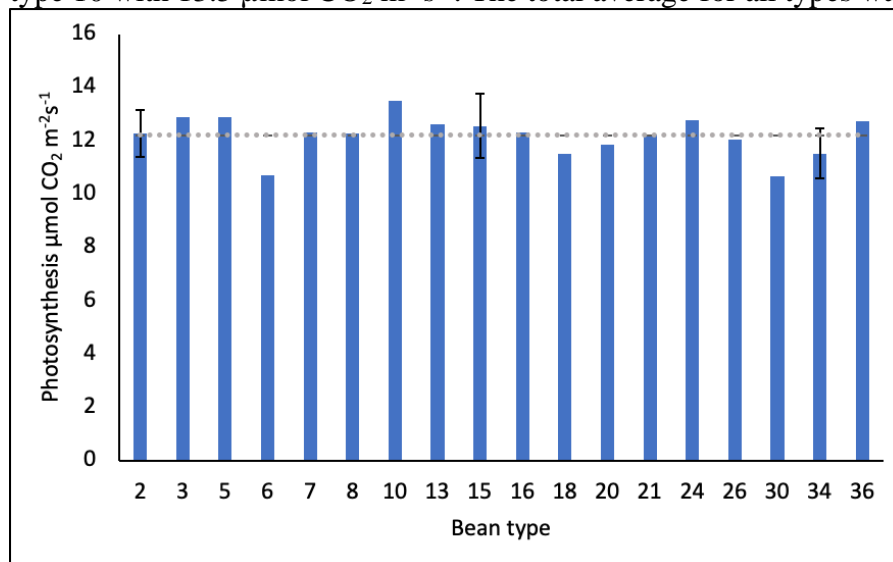


Figure 1. Average net photosynthetic rate for each cultivar of *P. vulgaris*. The black bars with serifs indicate standard error; the dotted line indicates the mean value for all types.

A large variation regarding daytime stomatal conductance was found, seen in figure 2, with an average of $0.34 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$. The highest stomatal conductance was found in type 10 with $0.52 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ and the lowest in type 30 with $0.22 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$.

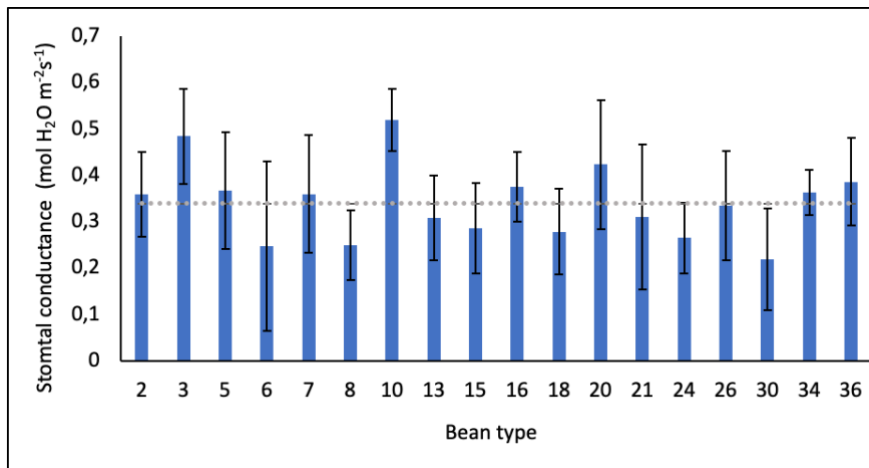


Figure 2. Average daytime stomatal conductance for each cultivar of *P. vulgaris*. The black bars with serifs indicate standard error; the dotted line represents the mean value for all types.

The average nighttime conductance, seen in figure 3, was $0.0086 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$. The highest nighttime conductance was measured in type 16 at $0.13 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$, and the lowest in type 18 at $0.005 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$.

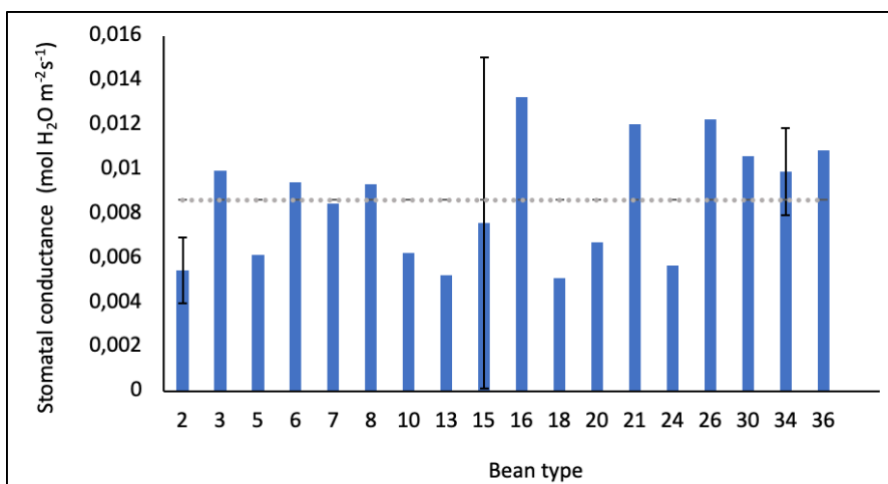


Figure 3. Average nighttime stomatal conductance for each cultivar of *P. vulgaris*. The black bars with serifs indicate standard error; the dotted line represents the mean value for all types.

Drought experiment

Half of the plants were put into a drought experiment group that stopped being watered. The landraces wilted after different periods of time. A wilted plant had at least lost its turgor pressure completely in the first set of trifoliate leaves and some of the turgor pressure in the next set of trifoliate leaves, seen in figure 4A.



Figure 4: Unwilted plant to the left (A). Wilted plant to be rewatered to the right (B)

In figure 5A, pot 10 lost water more rapidly than pot 89, which had a less steep water loss over time. Pot 10 weighed 1525 g on day zero and 836 g on day four, when it was rewatered. Pot 89 weighed 1569 g on day zero and 1125 g on day seven, when it was rewatered. In total, pot 10 lost 689 g of water in four days, while pot 89 lost 444 g of water in seven days. Graph A is used to illustrate a plant losing water quickly, represented by pot 10, and one that does not, pot 89.

No apparent changes in chlorophyll fluorescence over time was found, even after multiple days of drought, although the value for pot 89 dropped somewhat on day four, as seen in graph B.

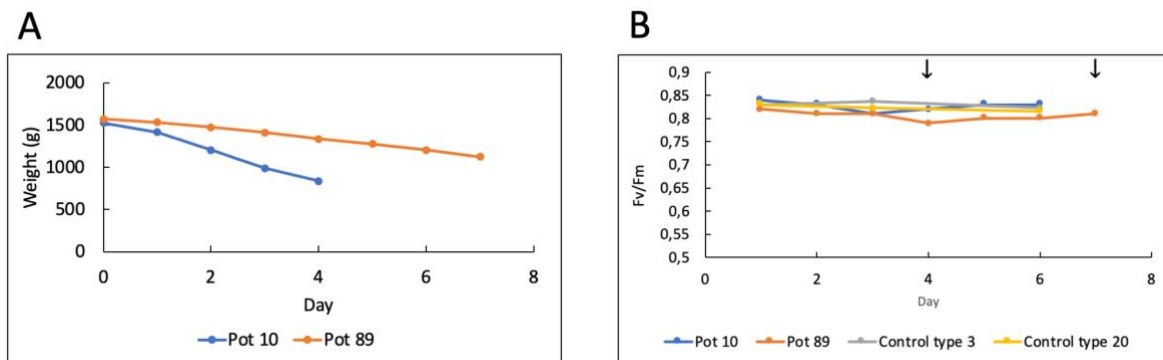


Figure 5: A shows the average weight loss in grams per day for pot 10 (type 3, replicate 2) and pot 89 (type 20, replicate 1) respectively. B shows the chlorophyll fluorescence measurements (F_v/F_m) for each day for pot 10, pot 89 and their respective controls. The arrows in B show when the plants were rewatered, pot 10 on day four and pot 89 on day seven.

There is some variety among the different seed races regarding wilting time, seen in figure 6. Type 2 and 3 wilted somewhat sooner than the other types; type 2 between three and four days; type 3 at four days. Most types wilted between day five and seven and the average wilting time for all types was 5.86 days. The last type to wilt was type 20 on day seven.

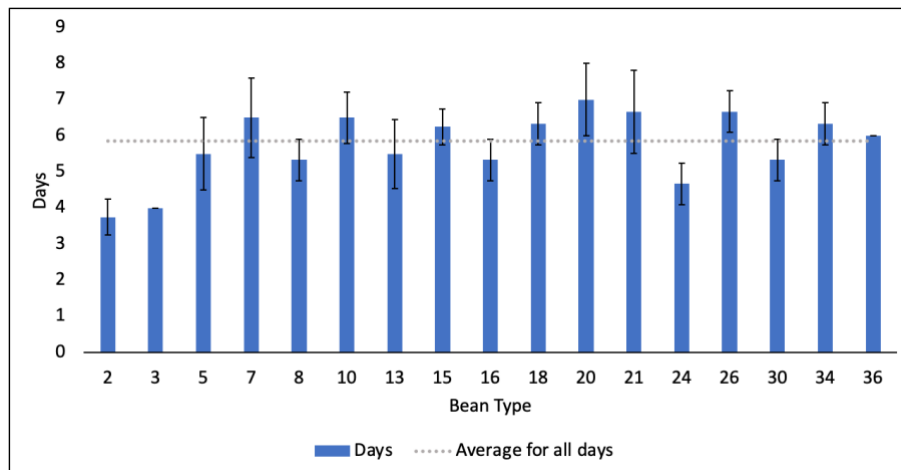


Figure 6: The number of days it took for each type to wilt. The dotted line shows the mean for all types; black bars with serifs represent standard error.

A variation of the amount of water left in the soil at rewatering, expressed as percentage of field capacity, was found. The average for all types was 21%. Type 20 had the highest percentage of field capacity left, 33%, while type 26 had the lowest, 15%.

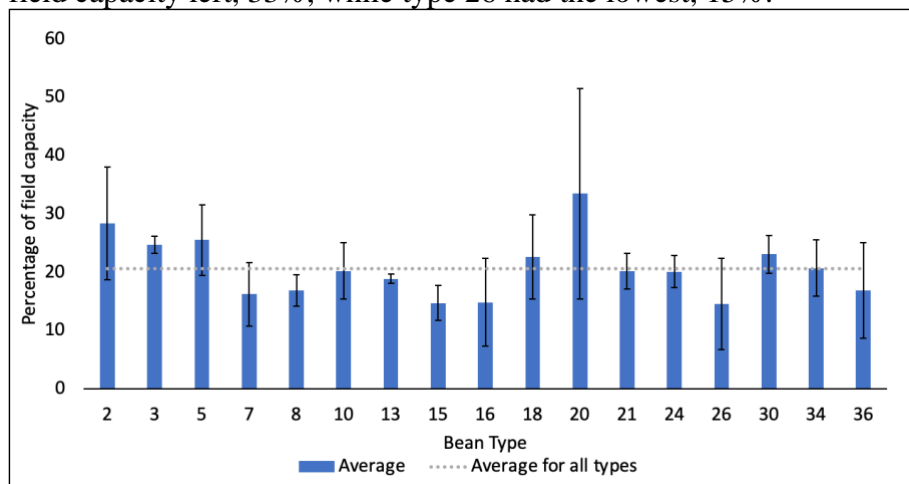


Figure 7: The average percentage of field capacity left at rewatering in percentage for each bean type. The dotted line shows the average for all types; black bars with serifs represent standard error.

The results show a large variety in water loss per day among the land races, showed in figure 8. Type 20 had the lowest water loss per day, at 66 g. Type 2 had the highest water loss per day at 189 g, followed by type 3 at 159 g. The average for all types was 125 g.

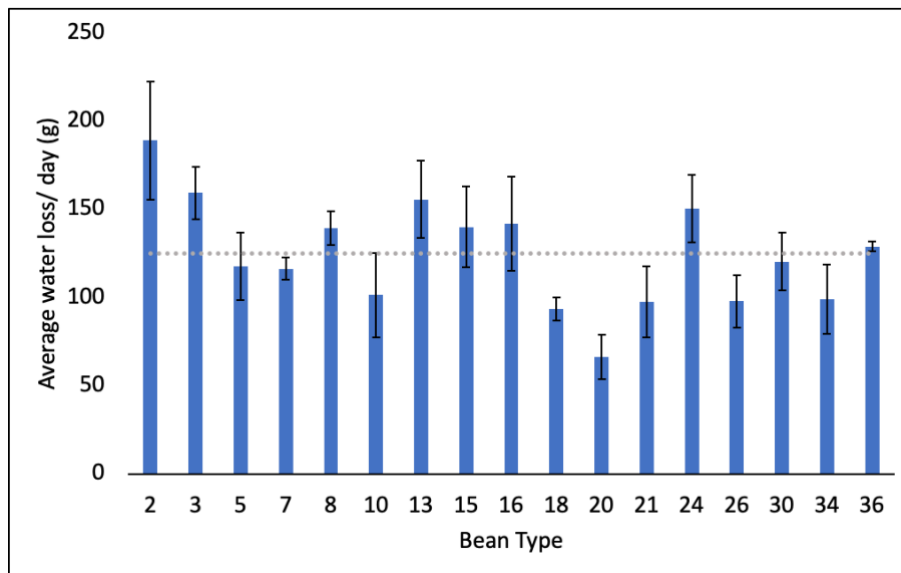


Figure 8: Average water loss per day in grams for each land race. The dotted line shows the average for all types; black bars with serifs represent standard error.

Correlations between conductance and drought tolerance data

Figure 9A shows no correlation between field capacity and leaf area, while figure 9B shows a correlation between leaf area and the number of days to wilting. Plants with a larger leaf area wilted sooner than plants with a smaller leaf area.

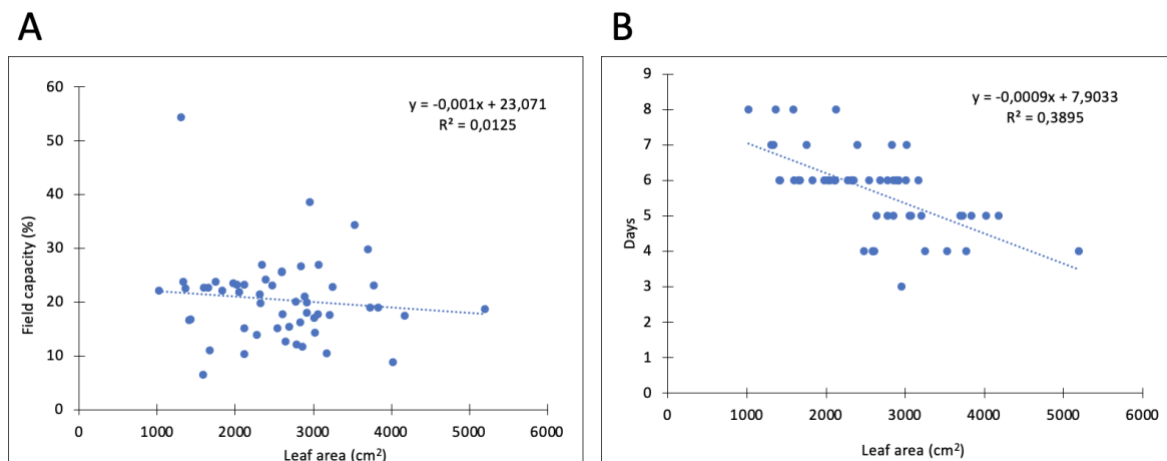


Figure 9: A shows leaf area and the percentage of field capacity left at rewetting for all plants in the drought experiment group. B shows the leaf area and the number of days to wilting for all plants in the drought experiment group. The dotted line shows a linear trend line.

No correlation was found for either nighttime stomatal conductance and the number of days to wilting (10B), or daytime stomatal conductance and days to wilting (10A). No correlation between daytime and nighttime stomatal conductance was found.

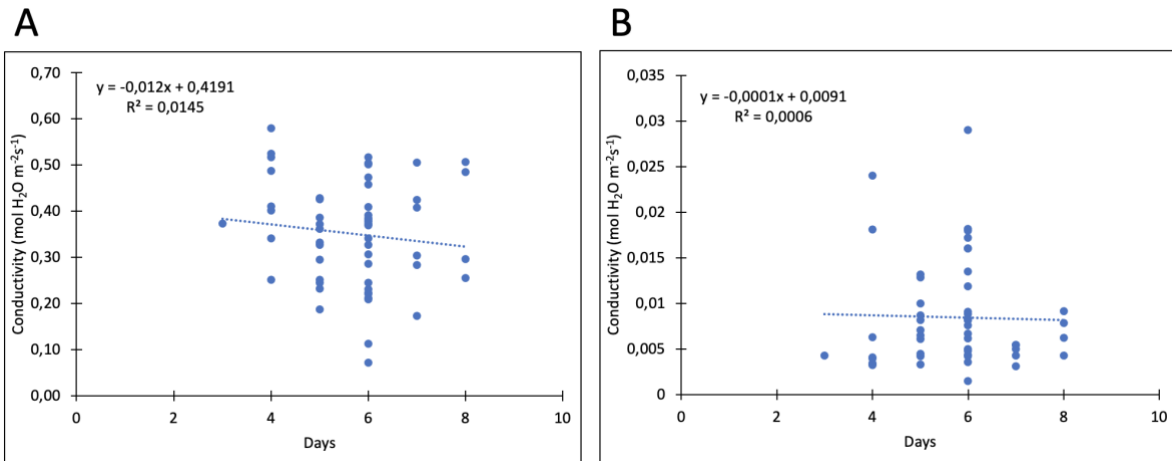


Figure 10: A shows the daytime conductivity and the number of days to wilting for each land race. B shows the nighttime conductivity and the number of days to wilting for each land race. The dotted lines show a linear trend line.

Figure 11 shows A correlation found between the number of days to wilting and the leaf area times conductance prior to drought exposure. Leaves with a lower conductance per leaf area wilted later.

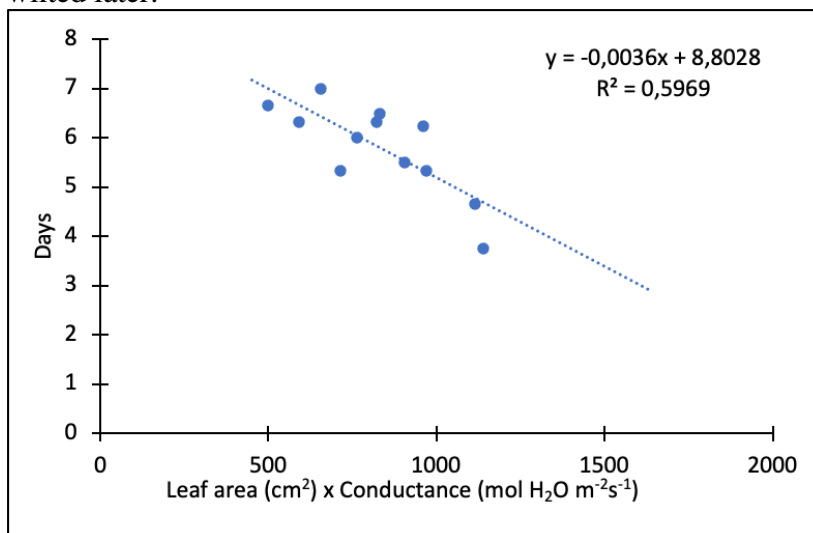


Figure 11: The graph shows the average leaf area times conductance and the number of days to wilting for each type of *P. vulgaris* land race. The dotted line shows a linear trend line.

No correlation was found for either nighttime stomatal conductance and the number of days to wilting (11B), or daytime stomatal conductance and days to wilting (11A).

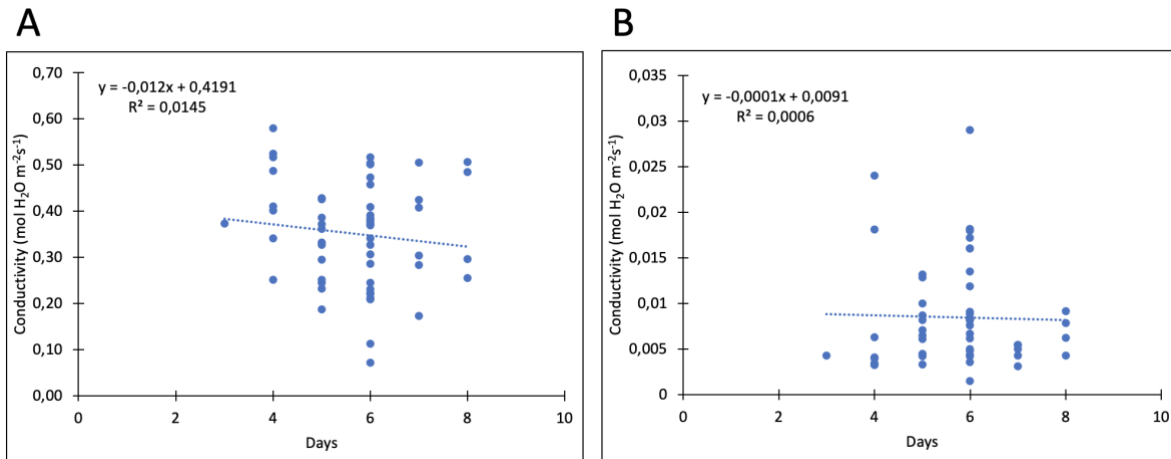


Figure 11: A shows the daytime conductivity and the number of days to wilting for each land race. B shows the nighttime conductivity and the number of days to wilting for each land race. The dotted lines show a linear trend line.

In figure 12, a correlation between stomatal conductance and photosynthesis is shown. Plants with a lower rate of photosynthesis have a lower stomatal conductance.

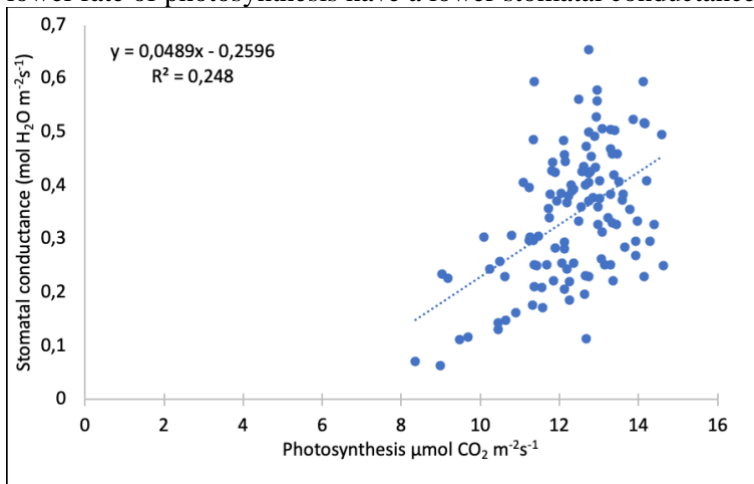


Figure 12: The rate of photosynthesis and the stomatal conductance for each plant.

Discussion

Variation in photosynthesis and stomatal conductance

Not a lot of differences in photosynthetic rate between the cultivars was found. However, a large variation between daytime conductivity and therefore water-use efficiency was found. A higher stomatal conductance means more water leaving the plant as the stomata are more open. This leads to a higher gas exchange, and therefore more photosynthesis, increasing yields [10]. A higher stomatal conductance in combination with a low resistance to a low amount of water in the soil, however, means a land race is not using water efficiently and should therefore not be selected in future breeding programs. Nighttime stomatal conductance was found to be lower than daytime stomatal conductance, but the values were never zero, meaning the stomata were not closed completely. When selecting plants for further breeding, it should be taken into consideration why *P. vulgaris* does not close its stomata. It is not certain why some plants have a high nighttime stomatal conductance, although several theories have been put forth. One theory is that water loss during nights is not large enough for nighttime stomatal conductance to be under selective pressure [16]. In another study a positive correlation was found between nighttime conductance and relative growth rate, suggesting that nighttime conductance acts as

a primer for the stomata, enhancing predawn stomatal conductance to increase photosynthesis in the early mornings, increasing growth rate [17]. However, in this study night time conductance measurements were quite low. No correlation between daytime and nighttime stomatal conductance was found. The same result was found in another study by Janacke in 2020 [18]. This means that *P. vulgaris* regulates daytime and nighttime conductance independently. As the study mentioned also suggests, nighttime stomatal conductance should therefore be able to be decreased without affecting daytime stomatal conductance to help save more water during nights. During the leaf level gas exchange measurements, plants were taken out of the plant room and moved through a corridor to another room where the leaf was clamped. This took some time and could have been optimized better to get more valid measurements. During the nighttime measurements, a dim light was turned on in the corridor which could have had a small effect on the results. Having the measurements taken in a room connected to the plant room would have removed this source of error and sped up the process of taking the plants and clamping the leaf.

Chlorophyll fluorescence as a predictor for wilting

As mentioned before, when faced with drought, plants will partially close their stomata to maintain the leaf water content [4]. A decrease in chlorophyll fluorescence after the drought process was initiated was to be expected as stomata would be more closed before turgor pressure was lost. However, no changes in chlorophyll fluorescence were found even after multiple days of drought, therefore concluding that this measurement does not predict wilting. A possible reason as to why measuring chlorophyll fluorescence did yield any results in this study is that the plants were not stressed enough to react. In further studies, having a higher light intensity directed at the wilted leaf could be tested to see if this would trigger a response in chlorophyll fluorescence. In another study a slight decrease in F_v/F_m was found. The plants had at that point not been watered for seven days [19]. A longer stress period could potentially have resulted in a similar decrease in F_v/F_m , however, on day seven in this study, the plants had already wilted and measurements had therefore stopped being taken.

Drought sensitivity

The different landraces of *P. vulgaris* lose water at different rates and need different amounts of water in the soil to survive. A variety in wilting time between the landraces was found. However, one source of error quickly discovered regarding this was observer bias. Determining when a plant had lost turgor pressure was done by two different people who could have had different opinions on this question. This was solved after on the second day of the treatment, by determining together when plants had lost turgor pressure.

The variety in wilting time is explained by the difference in water-use efficiency between the different land races. The time to wilting is determined by conductance and leaf area. If a plant has a low conductance or a smaller leaf area, less water will be transpired, and the plant will therefore take longer to wilt. Plants with a higher water loss per day wilted sooner. Type 20 had the lowest water loss per day and was also the last type to wilt. It must also be considered that this type had a below average leaf area so it would not use as much water when transpiring. Factoring in the percentage of field capacity left at rewatering is also important for determining a land race's water-use efficiency. A relatively high percentage left at the day of rewatering means that a plant requires a higher amount of water in the soil to survive. This in combination with a high water loss per day and therefore fewer days until wilting occurs, is indicative of high sensitivity to drought. Looking again at type 20, it had the highest percentage of field capacity left at rewatering, meaning that although it might not lose water that fast, it also requires a lot of water in the soil to survive, making it unsuitable for further breeding programs.

Other types not suitable are type 2 and 3, although this result is not certain because when sorting the plants into a drought experiment group and a control group, only the placement of the control group was randomized. This led to types 2 and 3 being placed on the same table. These types were also the ones to wilt the soonest (see figure 6) and they had the highest water loss per day (see figure 8). Both type 2 and 3 had a high percentage of field capacity left at wilting. If the wilting time was affected by the placement of the plants (there might have been stronger lighting over that table causing a higher water loss) or if it was due to genetics is not possible to know, one can only speculate. In further studies, randomization should therefore be included.

More ideal land races are types 26, 34 and 36. These types have a low percentage of field capacity left at rewatering, meaning they can survive on a small amount of water in the soil, while losing less than the average amount of water per day. They were also among the latest land races to wilt, between day six and seven. When selecting landraces for future breeding programs, another factor taken into consideration should be its productivity. It is important finding landraces that can balance productivity with drought tolerance. This could be looked at in further studies.

Other factors playing a role in water-use is the total leaf area. Plants with a larger total leaf area will transpire more and therefore also not be able to retain water as well [20]. If the plant is also sensitive to low amounts of water in the soil it will wilt more quickly. As seen in the results, landraces with larger leaves did indeed wilt sooner, although no correlation between field capacity and leaf area was found in this study. It could be that some land races with larger leaves can endure a lower amount of water in the soil, as an adaptation to their increased transpiration, and others with smaller leaves do not as they do not transpire as much and therefore do not need as much water. When selecting for breeding, a combination of the two would be optimal, selecting for a plant with smaller leaves but which can also survive with a lower water potential in the soil.

There was no correlation found between stomatal conductivity and the number of days to wilting. Conductance does therefore not give much information. This could be a result of the fact that stomatal conductance was measured on well-watered plants. If stomatal conductance instead had been measured when the plants experienced drought stress, the correlation to wilting time could have been stronger as some landraces could be more effective in their stomatal regulation than others.

A correlation was, however, found between stomatal conductance times leaf area and wilting time, showing how much water is used by a plant each day. Although this was the best measurement found for predicting how much water a plant will lose during droughts, and therefore predicting wilting, this method did not predict the amount of water in the soil, expressed as percentage of field capacity, left at rewatering as the landrace that wilted the slowest, type 20, also had a high percentage of field capacity left at the time it was rewatered. It is therefore concluded that leaf level gas exchange is a somewhat good predictor and can be used when it comes to prediction wilting time when using conductance times leaf area, but not when it comes to predicting the percentage of field capacity left in the soil at the time of wilting.

Further studies

The results found in this study could be used to potentially develop new landraces of the common bean more resistant to drought, although further studies are needed prior to this. More research on the common bean is important as this species is highly sensitive to drought whilst being the third most produced legume in the world, as well as being second in world trade [4].

Studies focusing on the gene mapping of genes related to drought tolerance could be useful in the development of new land races, as well as repeated studies focusing on leaf level gas exchange to see if there is validity or variety in the results. Studies focusing on osmolarity as part of the drought experiment could also be useful as a lower osmotic potential in the plant should mean that it can withstand a lower water potential in the soil.

Conclusion

The aim of this study was to see if leaf level gas exchange can be used as a predictor for drought tolerance in the Rwandan common bean. The results showed that nighttime conductance and daytime conductance are regulated independently and could be used to breed new landraces with a more conservative water-use. Measuring chlorophyll fluorescence was not found to predict wilting, as the values measured (F_v/F_m) were consistent throughout the drought treatment. The results showed a very stable rate of photosynthesis between landraces, but variation in conductance. A strong correlation was found between conductance times leaf area and the number of days until a plant wilted, indicating that this measurement could be used to predict wilting time. As the method was not found to predict field capacity, a conclusion drawn is that leaf level gas exchange is a somewhat good predictor when predicting wilting time, however not when predicting the percentage of field capacity left in the soil at the time of wilting. Landraces suggested for further breeding are type 26, 34, and 36 as took a long time to wilt and they can survive on a low amount of water in the soil as well as lose a below average amount of water per day.

Acknowledgement

I would like to thank my supervisors Mats Andersson and Karin Johansson for all of their help and advice throughout this process. I also thank Ella Köster for being a great partner and friend. Without her support, finishing this process would have been much more difficult.

Reference list

- [1] Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Liermann, C. R., & Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*, 467(7315), 555–561.
- [2] Eisenstein, M. (2013). Plant breeding: discovery in a dry spell. *Nature*, 501(7468), S7-S9.
- [3] Hardy, J. T. (2003). *Climate change: causes, effects, and solutions*. John Wiley & Sons.
- [4] Daryanto, S., Wang, L., & Jacinthe, P. A. (2015). Global synthesis of drought effects on food legume production. *PLoS ONE*, 10(6).
- [5] Bitocchi, E., Rau, D., Bellucci, E., Rodriguez, M., Murgia, M. L., Gioia, T., Santo, D., Nanni, L., Attene, G., & Papa, R. (2017). Beans (*Phaseolus* spp.) as a model for understanding crop evolution. In *Frontiers in Plant Science* (Vol. 8). Frontiers Media S.A.
- [6] Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H. M., Varshney, R. K., Colmer, T. D., Cowling, W., Bramley, H., Mori, T. A., Hodgson, J. M., Cooper, J. W., Miller, A. J., Kunert, K., Vorster, J., Cullis, C., Ozga, J. A., Wahlqvist, M. L., Liang, Y., Shou, H., ... Considine, M.

J. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature Plants*, 2(8).

[7] Salcedo J.M. (2008). Regeneration guidelines: common bean. In: Dulloo M.E., Thormann I., Jorge M.A. and Hanson J., editors. *Crop specific regeneration guidelines [CD-ROM]*. CGIAR System-wide Genetic Resource Programme, Rome, Italy. 9 pp.

[8] Bonfils, S. (2012). Trend analysis of the mean annual temperature in Rwanda during the last fifty-two years. *Journal of Environmental Protection*, 2012.

[9] FAO (Ed.), 2016. *Food Outlook - Biannual Report on Global Food Markets*, Rome.

[10] Roche, D. (2015). Stomatal Conductance Is Essential for Higher Yield Potential of C3 Crops. *Critical Reviews in Plant Sciences*, 34(4), 429–453.

[11] Rosales, M.A., Ocampo, E., Rodríguez-Valentín, R., Olvera-Carrillo, Y., Acosta-Gallegos, J., Covarrubias, A.A., 2012. Physiological analysis of common bean (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. *Plant physiology and biochemistry: PPB* 56, 24–34.

[12] Duursma, R. A., Blackman, C. J., López, R., Martin-StPaul, N. K., Cochard, H., & Medlyn, B. E. (2019). On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytologist*, 221(2), 693-705.

[13] Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of experimental botany*, 51(345), 659-668.

[14] Khaleghi, E., Arzani, K., Moallemi, N., & Barzegar, M. (2012). Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content index under water stress in *Olea europaea* cv. Dezful. *World Acad. Sci. Eng. Technol*, 68, 1154-1157.

[15] Easlon, H. M., & Bloom, A. J. (2014). Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in plant sciences*, 2 (7), 1400033.

[16] Christman, M.A., Richards, J.H., McKay, J.K., Stahl, E.A., Juenger, T.E., Donovan, L.A., 2008. Genetic variation in *Arabidopsis thaliana* for night-time leaf conductance. *Plant, cell & environment* 31 (8), 1170–1178.

[17] Resco de Dios, V., Chowdhury, F. I., Granda, E., Yao, Y., & Tissue, D. T. (2019). Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. *New Phytologist*, 223(4), 1696-1706.

[18] Janecke, N. (2020). WATER ECONOMY OF TRADITIONAL RWANDAN COMMON BEAN CULTIVARS (*PHASEOLUS VULGARIS*). GUPEA.
https://cms.it.gu.se/infoglue/DeliverWorking/digitalAssets/1791/1791308_es2521-nicolas-janecke_2020.pdf

- [19] Miyashita, K., Tanakamaru, S., Maitani, T., & Kimura, K. (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environmental and experimental botany*, 53(2), 205–214.
- [20] Maylani, E. D., Yuniati, R., & Wardhana, W. (2020, July). The Effect of leaf surface character on the ability of water hyacinth, *Eichhornia crassipes* (Mart.) Solms. to transpire water. In *IOP Conference Series: Materials Science and Engineering* (Vol. 902, No. 1, p. 012070). IOP Publishing.