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Can cellular osmolarity be used as a predictor for drought tolerance in common bean?

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Sammanfattning

Phaseolus Vulgaris, även känd som bönan, är en protein- och näringsrik livsmedelskälla som odlas över hela världen. Rwanda har en av de högsta konsumtionsnivåerna per capita i världen. Eftersom klimatförändringarna hotar livsmedelssäkerheten ökar behovet av mer anpassningsbara grödor. Syftet med denna studie är att utforska egenskaper som är relaterade till torktolerans, särskilt osmolaritet, för att bidra till att skapa mer torktoleranta bönor i framtiden. 18 Rwandiska landraser av *P. vulgaris* från 1980-talet valdes ut från en fröbank i Colombia och för var och en av dem planterades 8 replikat. Mätningar av gasutbytet och ett torkexperiment utfördes. De flesta sorter vissnade inom 5-7 dagar och hade omkring 15-20 % av fältkapaciteten kvar när de vissnade. Klorofyllfluorescens visade inga större skillnader mellan den torkstressade gruppen och kontrollgruppen, eller mellan vissnade plantor och friska plantor. Osmolariteten hade endast svaga korrelationer med vissningstid, procentuell andel av fältkapaciteten kvar vid vissningstidpunkten och konduktivitet. Därför var osmolariteten i denna studie inte en bra prediktor för torktolerans. Det finns dock ett antal felkällor som kan ha påverkat resultatet, varav en är det mycket begränsade antalet osmolaritetsmätningar.

Abstract

Common bean, Phaseolus vulgaris, is a protein and nutrient rich food source grown around the world. Rwanda has one of the highest per capita consumption rates in the world. With climate change threatening food security, the need for more adaptable crops is rising. This study aims to explore traits related to drought tolerance, in particular cellular osmolarity, to help create more drought tolerant beans in the future. Eighteen Rwandan landraces of *P. vulgaris* collected in the 1980's were chosen from a seed bank in Columbia and for each 8 replicas were planted. Gas exchange measurements and a drought experiment was performed. Most varieties wilted within 5-7 days after termination of watering, and had a water content of around 15-20% at the time of wilting. Chlorophyll fluorescence did not show major differences between the drought stressed group and the control group, nor between wilted plants and vital plants. Osmolarity had only weak correlations with wilting time, percentage of field capacity left at the wilting point and conductivity. Therefore, in this study osmolarity was not a good predictor of drought tolerance. Though there are several sources of error that could have affected the result, one being a very limited amount of osmolarity measurements.

Introduction

The common bean (*Phaseolus vulgaris*) is considered one of the most important crops for humans worldwide because they are rich in both protein and micronutrients such as iron [1]. Today, beans are grown around the world, but the per capita consumption is the highest in east African regions, in particular Kenya and Rwanda [2]. In Rwanda beans make up 65% of national protein intake and 32% of calorie intake and are hence often dubbed “the meat of the poor” [3].

P. *vulgaris* wild type can be traced back to Mesoamerica and South America. Due to reproductive isolation caused by a geographic barrier, the species was able to undergo two independent domestications [1]. This allowed for different adaptations to take form and led to the formation of two different gene pools: Mesoamerica and the Andes [1].

Rwanda-Climate

Rwanda’s hilly topography has led to its nickname “the land of a thousand hills", with the highest elevations (>2000 m) found in west and decreasing toward the center (1500-2000 m) and eastern parts of the country (<1500 m) [4]. Despite Rwanda's proximity to the equator, the country has a tropical temperate climate. The climate is cooler than one might expect because of the country's high elevation [4]. Due to the hilly topography the annual average temperature varies throughout the country. In the high, southern parts the temperature ranges between 15-17°C, while in the northern, volcanic parts the temperature can drop below 0 degrees [5]. Lastly, in the lower, eastern areas the temperature can reach up to 30 degrees [5].

Rwanda's average annual rainfall is around 1000 mm distributed over one short and one long rain season, stretching from September to December and March to May respectively [6]. It has also been shown that higher altitude regions tend to get more precipitation than lower altitude regions [6]. The Rwandan temperatures support bean crops well as they prefer a range between 15-27°C but can tolerate up to 30°C. The Common bean requires around 350-500mm of rainfall during the growing season and a low relative humidity [7]

One substantial consequence of climate change people face more and more is food insecurity [8]. Though this affects most people on earth, small scale farmers are often extra exposed due to the lack of alternative food sources and their limitations of resources allowing them to adapt to new conditions [8]. Rwanda, amongst many other African countries, now faces increased temperatures and less predictable weather patterns such as droughts and floods [8]. Müller et al state that there is a consensus that the changes in climate will have a negative impact on most African agricultural areas [9]. The crops might not be adapted to a higher temperature, irregular rainfall or periods of drought and hence it is vital for many people that science provides a solution to ensure future food security [9]. Beans are sensitive to abiotic stress factors such as drought, and improvements in this aspect could increase bean production areas up to 31% [10].

There are multiple ways plants can handle drought stress, stomatal regulation, and osmotic adjustment being two examples of leaf scale responses. Some plants use one

method while others use both. The common bean tends to use stomatal regulation during light drought but is also capable of osmotic regulation under certain conditions [11]. Osmotic regulation requires less energy and is therefore beneficial as it can lead to less yield loss [11].

Stomatal Regulation

Stomata consist of two specialized cells called guard cells that regulate the opening and closing of the pore. If the guard cells have high turgor pressure the stomata opens and when the turgor pressure is low the stomata close. The opening and closing mechanism is regulated by multiple environmental factors such as light, water availability, CO_2 concentration, and temperature, as well as the plant hormone ABA (Abscisic Acid) [12,13].

When stomata are open CO_2 can enter the plant in order for the plant to perform photosynthesis, but at the same time water vapor exits the plant. These types of movements through the stomata can be regulated and the level of resistance can be referred to as stomatal conductance (g_s) and is usually measured in mmol m^{-2} s^{-1} [14]. If not specified otherwise stomatal conductance will in this paper refer to water vapor exiting the stomata.

Osmotic Regulation

As mentioned above, another way for plants to handle drought stress is osmotic adjustment [11]. Contrary to water retention through the closing of stomata, osmotic regulation allows for continued uptake of carbon dioxide and therefore ongoing photosynthesis [11]. Water retention through osmotic adjustment involves an increased concentration of solutes in the plant cells so that water keeps flowing in and turgor is maintained. The solutes in question are sugars, salts, certain ions, and some amino compounds [11,15]. During drought stress these solutes lower the osmotic potential of the plant which will lead to continued water uptake [11].

Chlorophyll fluorescence

A plant can only perform a limited amount of photochemistry i.e., use a limited amount of light energy for its internal processes. The excess is either disposed of as heat or re-emitted as light, and the latter example is what is known as chlorophyll fluorescence. Since a decrease in one of these processes would lead to an increase in the other two, chlorophyll fluorescence can give an indication about changes in photosynthesis as well as re-emission of heat. The amount of re-emitted light depends on different environmental factors and stresses. This measurement is taken on drought adapted leaves and the variable used in this study was the variable fluorescence (F_v) divided with the maximum fluorescence (F_m) [16].

Aim

The aim of this study is to find a connection between osmolarity and drought tolerance in beans and if there are genotypic differences between varieties. This knowledge will be

helpful in creating more drought tolerant plants for consumption in Rwanda amongst other east African countries.

Materials and Methods

Growth conditions and plant material

A total of 144 plants were potted, 8 replicates of 18 varieties of Common bean (Phaseolus *vulgaris*). The varieties are landraces collected in Rwanda (from the 1980's) and were obtained from the seed data bank of the International Center for Tropical Agriculture in Columbia (CIAT; https://genebank.ciat.cgiar.org). At the start of the experiment all pots were weighed together with 3 wooden sticks (for the beans to climb on). Each pot was filled with 2 Liters of soil (S-Jord, Yrkeskvalité, Hasselfors Garden; Gothenburg, Sweden) and saturated with water. After letting excess water run off, the weight was noted again, and seeds were placed in the soil. A small amount of vermiculite was also placed on top of the soil. Thereafter, the plants were randomized and left to grow for approximately 4 weeks in a plant room until they were big enough to conduct the gas exchange experiment. They were considered big enough when at least one trifoliate leaf had reached the size of the Li-6400XT cuvette (see below) The plant room's temperature varied between 20-27 degrees and the humidity was 30-40%. The plants were exposed to a cycle of 12h light and 12h darkness. The beans were moved around in the plant room three times a week to ensure that each pot was exposed to the same conditions. On the same days they were also watered and once a week a fertilizer (Trädgårdsnäring, VitaGro, Gothenburg, Sweden) was added.

Five aluminum trays were filled with 2 liters of water saturated soil and placed in a drying cabinet at 60°C until fully dried (constant weight) so that the field capacity could be determined.

The plants that did not germinate after approximately 2 weeks, or the ones that germinated very late were removed.

Gas exchange measurements

Once the beans had grown big enough (approximately 4 weeks) gas exchange measurements were carried out with the Li-6400XT (LI-COR; Lincoln (Nebraska), USA) machine both during the day and night. A 2x3 cm leaf-level cuvette was attached with a 6400-02B LED light source to measure photosynthesis and stomatal conductance.

The settings of the Li-6400XT were adjusted to the conditions the plants experienced in the plant room. The following settings were used during the day measurements: CO_2 = 420 ppm, light intensity (ParIn)= 300 mmol m^{-2} s^{-1}, temperature (TBlock)= 27°C, flow= 400. As the rates of photosynthesis and stomatal conductance stabilized, three values were logged with 10 second intervals.

During the nighttime measurements the following settings were used: CO_2 = 420 ppm, light intensity (ParIn)= 0, temperature (TBlock)= 20 °C, relative humidity=30-50%, flow= 400 and leaf fan= 5 (max). During these measurements the room in which they were performed was dark to match the conditions to the plant room from where the beans were taken. Like the day-time experiment three values were logged with 10 second intervals once the gas exchange rates had stabilized.

Drought experiment

Half of the replicates for each bean variety were randomly selected for the drought stress treatment. If the remaining number of replicates was uneven the majority was chosen to be in the control group. The chosen plants were then left without water until the first two trifoliate leaves lost turgor completely. The plant was then rewatered. The plants selected for drought stress were weighed every day until rewatering to determine the percentage of field capacity that was left at the time of wilting. Chlorophyll fluorescence (PAM: Pulse Amplitude Modulation) measurements were also performed every day on all plants under drought stress. PAM measurements for the control group were done every 3 days. The handy PEA+ portable analyzer was used for these measurements (Handy PEA+, Amesbury, Massachusetts, USA) and clips were attached to each leaf to darken the measuring area. The clips were left on the leaves for 30 minutes before measuring to ensure adaptation to the dark.

Once a drought-stressed plant had been re-watered for approximately 24h one trifoliate leaf was cut off and quickly placed into a 15 ml falcon tube filled with water, marked with its pot number and put in incubation under a glass hood. A wet towel was also placed under the hood to ensure maximum rehydration. The rehydration period lasted 24h in a dark room with a temperature of 16°C. In the next step the leaf was put into a zip-lock bag in which humidity was added by a few breaths out. The bag was then put into a bigger zip-lock bag in which a little wet paper was placed. It was then marked with the pot number and put into a 4°C room. For every plant under drought stress one control of the same bean type was taken and prepared in the same way.

Figure 1: Incubation during the rehydration period.

In the beginning of the drought experiment the total leaf area was measured for all the drought stressed plants. One leaf at a time was held against a white paper next to a 4cm^2 red square and photographed with the “Easy Leaf Area” app [17].

Osmolarity Measurements

For the osmolarity measurements a vapor pressure osmometer (Wescor, Vapro 5600, Logan, Utah, USA) was used. After calibration with 100, 290 and 1000 mmol/kg solutions, each leaf was taken out of its plastic bag, quickly wiped down to remove moisture and trichomes and small discs were cut out with a cork-borer. These were then as quickly as possible put in an aluminum foil envelope and frozen in liquid nitrogen for at least two minutes. The envelope was removed and the leaf disc taken out. With a pipette tip approximately five holes were made in the disc to speed up evaporation. When the measurements became stable (the concentration changed less than 5 mmol/kg between a measurement) the disc was removed, and the chamber cleaned with a kimwipe until completely clean and lint-free [18]

After 5-6 measurements the chamber was closed without a disc to allow for corrections of small shifts in temperature.

Result

Drought experiment

Half of each bean type was subjected to drought stress and no longer received any water. The different types experienced a variety of wilting times. A plant was determined as wilted when it had lost turgor completely in the first trifoliate leaf and following trifoliate leaves had lost at least some turgor (Fig. 2). The decision was made to exclude bean type 6 from

all the graphs because there was only one replica that was drought stressed. Many type 6 beans were removed earlier in the experiment because they took too long to germinate or did not germinate at all.

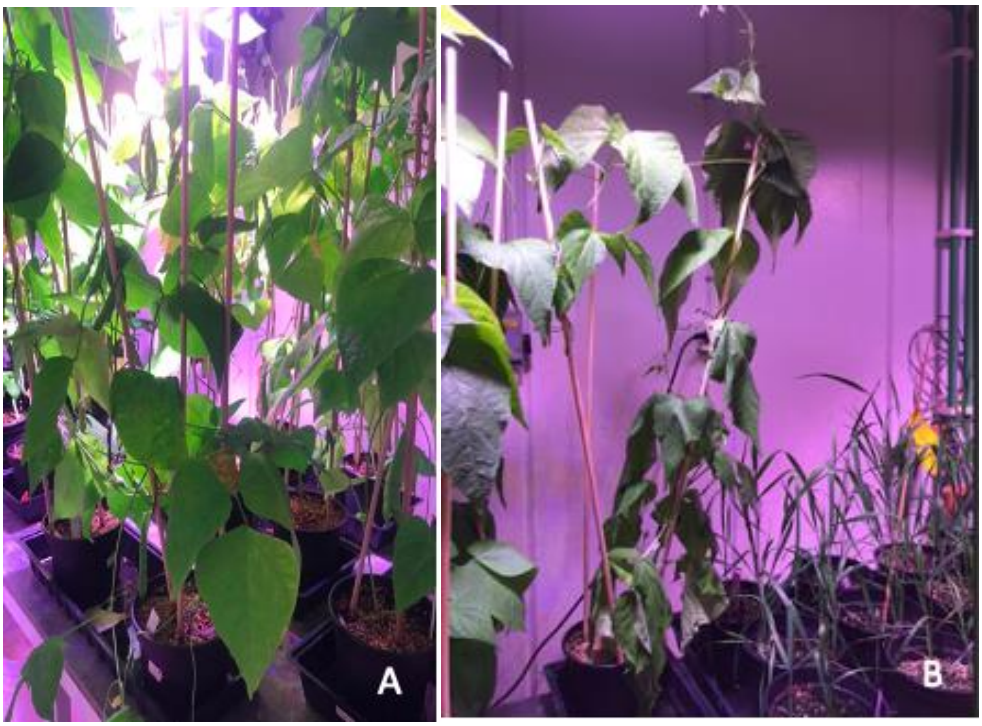


Figure 2: Picture of a vital plant to the left (A) and a wilted plant to the right (B).

Chlorophyll fluorescence (F_v/F_m), which was taken as an indicator of photosynthetic stress was a very stable variable for both drought stressed and control plants (Fig 3). It stayed within the range of 0.80-0.84 for both drought stressed and control plants all days it was measured. In figure 3 below, pots 10 and 89 are depicted because they belong to bean types that had very different wilting times.

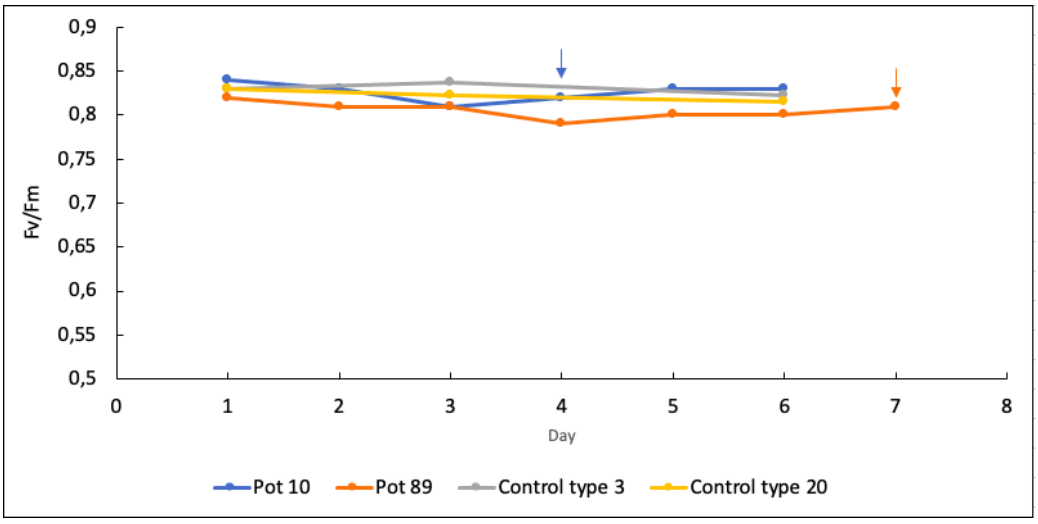


Figure 3: Chlorophyll fluorescence measurements (PAM) for pot 10 and 89 as well as the average for all plants in type 3 and 20. Pot 10 belongs to type 3 and pot 89 belongs to type 20. The blue and orange arrows show the days of rewatering for pot 10 and 89 respectively.

All bean genotypes had a wilting time within the range of 3 and 8 days but most of them wilted between day 5 and day 7. Bean type 2 and 3 had the lowest average wilting times while type 20 had the longest (Fig. 4).

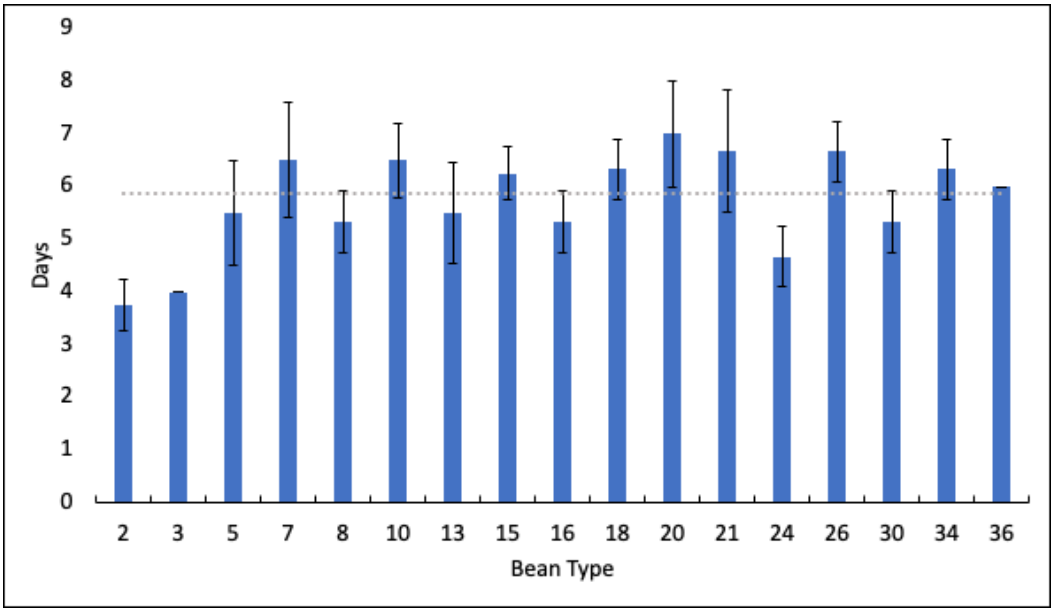


Figure 4: Half of each type was selected for the drought experiment. The ones subjected to drought were checked every day for signs of wilting. When the first trifoliate leaf had lost turgor completely the plant was rewatered (fig. 3). This Graph shows the average wilting time for each bean type. Error bars represent standard deviation and the dotted line represents the average for all types which was 5,89 days. The replicates in each group ranged from n=2 to n=4.

Figure 5 shows the average percentage that is left of the field capacity in the soil for each bean type at the rewatering stage i.e., when they wilted. Type 20 contains the most amount of water at the wilting stage while type 6 contains the least. Most types wilted when they have an average of around 20% of the field capacity left.

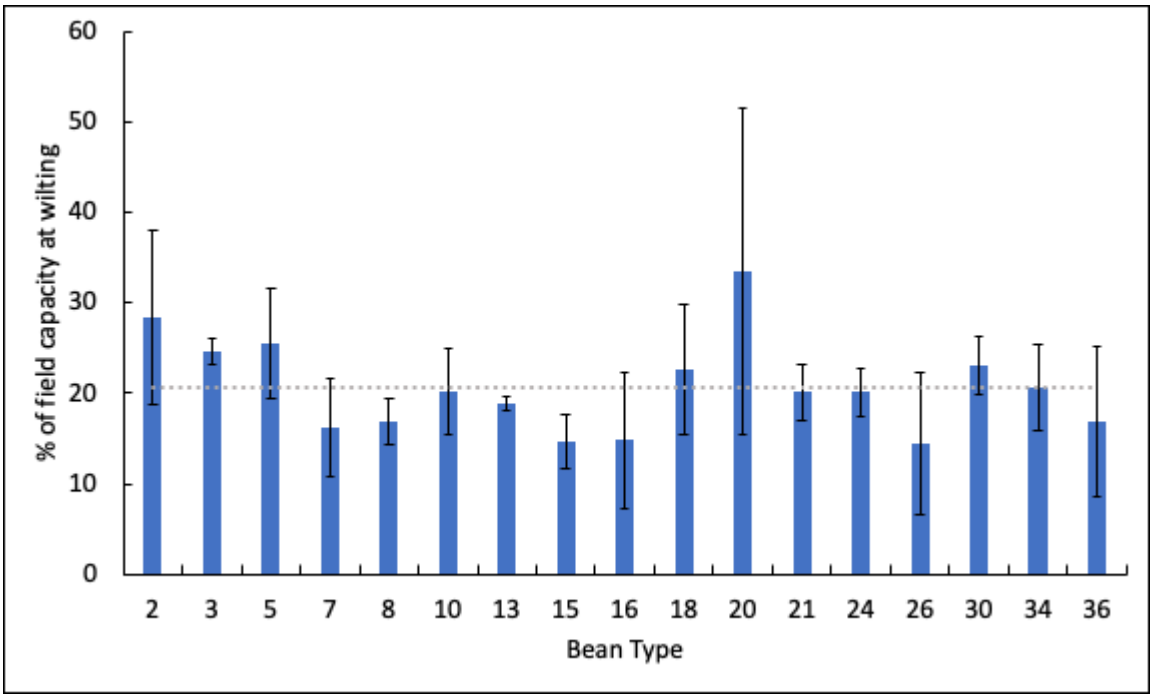
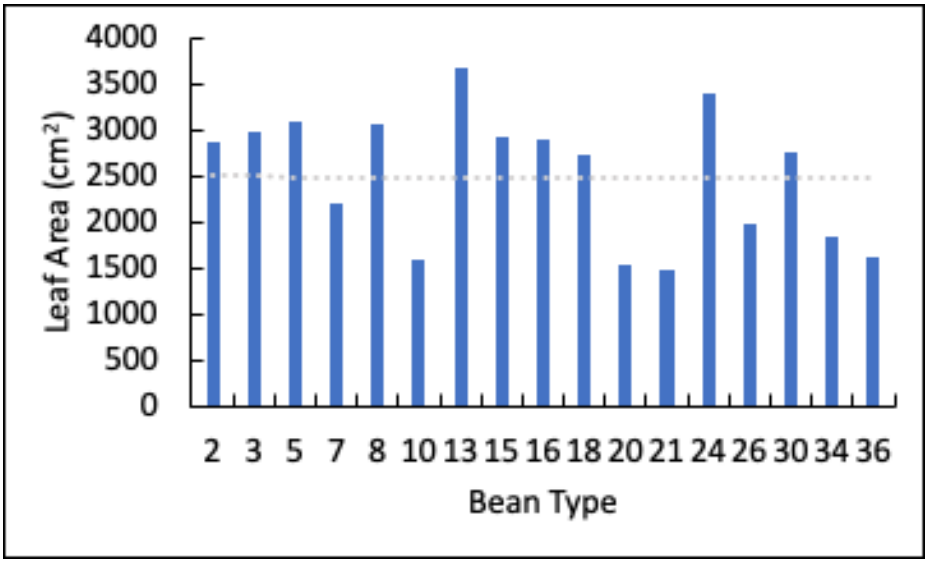


Figure 5: On day zero of the drought experiment the weight for fully saturated plants was taken and field capacity was determined. By weighing the plants every day until rewatering the percentage of the field capacity that was left at the point of wilting could be determined. In this graph the average percentage of field capacity that was left at rewatering (wilting) for each type is depicted. Error bars represent standard deviation and the dotted line represents the average of all types (20,61%).

The total leaf area varied a lot between the different bean types. The values ranged between 1494 cm^2 and 3690 cm^2. Types 13 and 24 had the highest values while types 20 and 21 had the lowest.



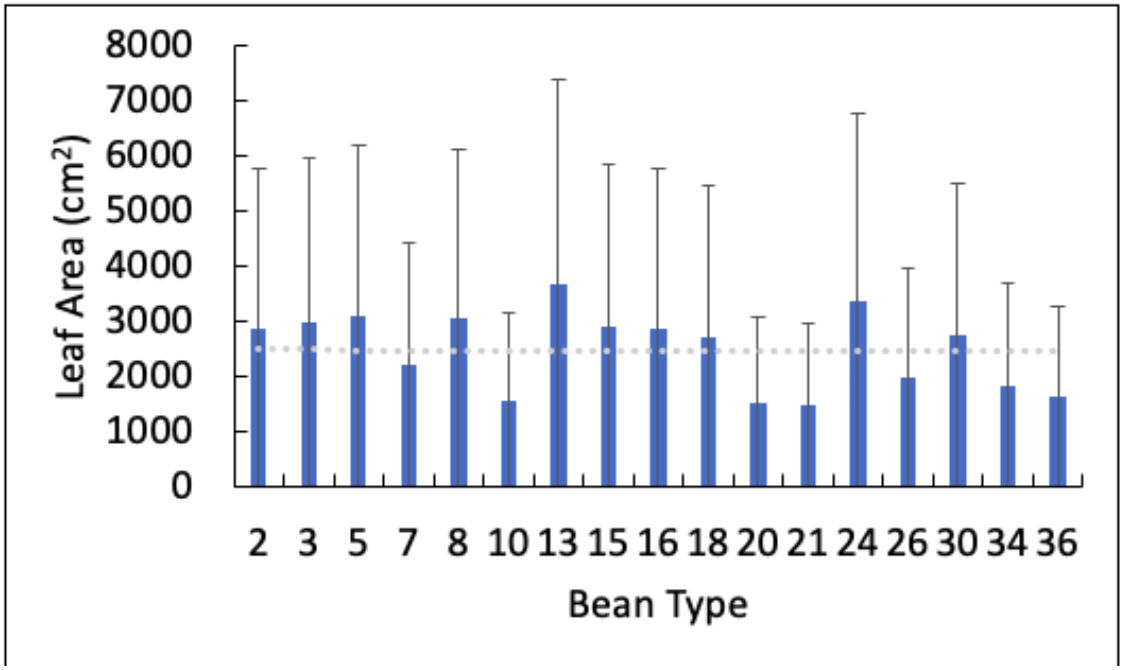


Figure 6: The size of all leaves for a plant were determined with the help of the app “Easy Leaf Area” and then added together. This resulted in the total leaf area for each plant as shown in the graph above.

As figure 5 indicates, bean type 20 has the highest percentage of field capacity left at the point of wilting which goes hand in hand with it being the type with the least water loss per day (Fig. 7). Type 2 has the highest amount of water loss per day as depicted in the graph below and is also one of the first to wilt (Fig. 4).

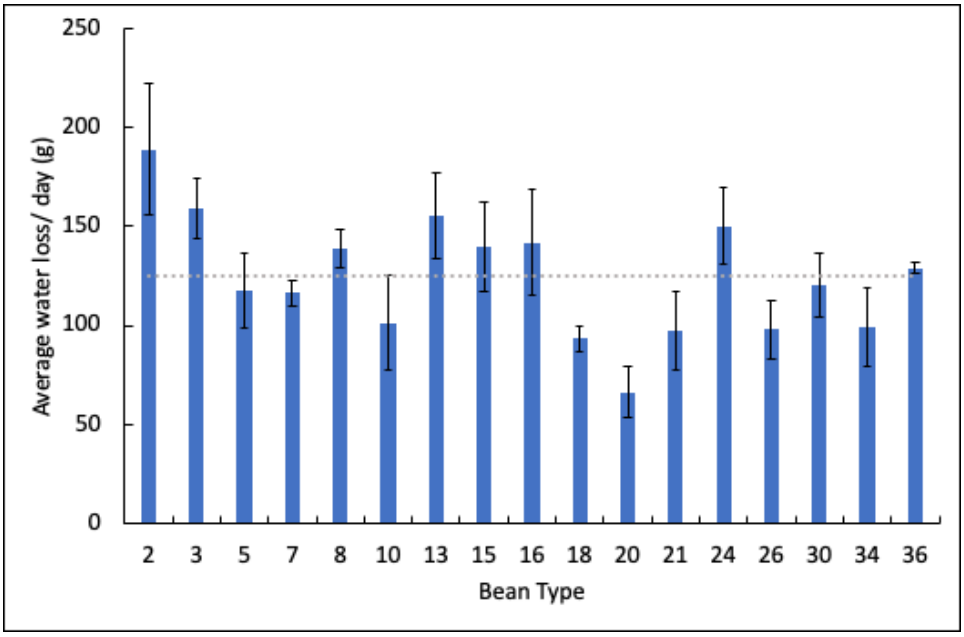


Figure 7: Average water loss per day for each bean type. Error bars represent the standard deviation and the dotted line represents the average water loss per day for all types.

Correlations between osmotic potential and drought experiment data

A very weak correlation was determined between leaf osmotic potential and daytime conductance both for the drought stressed and control plants. The correlation coefficient (R^2) was 0,0656 and 0,1059 respectively.

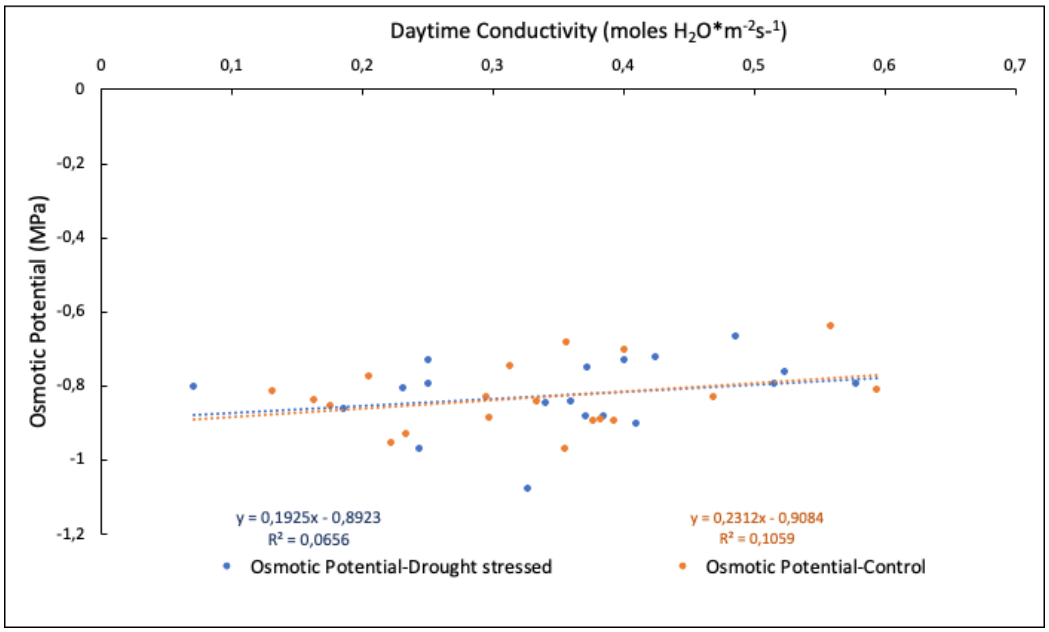


Figure 8: Correlation between osmotic potential and daytime conductivity

No major differences could be determined between the osmotic potential in drought stressed plants and control plants, which means that the beans did not perform an osmotic adjustment as a response to drought stress (Fig. 9).

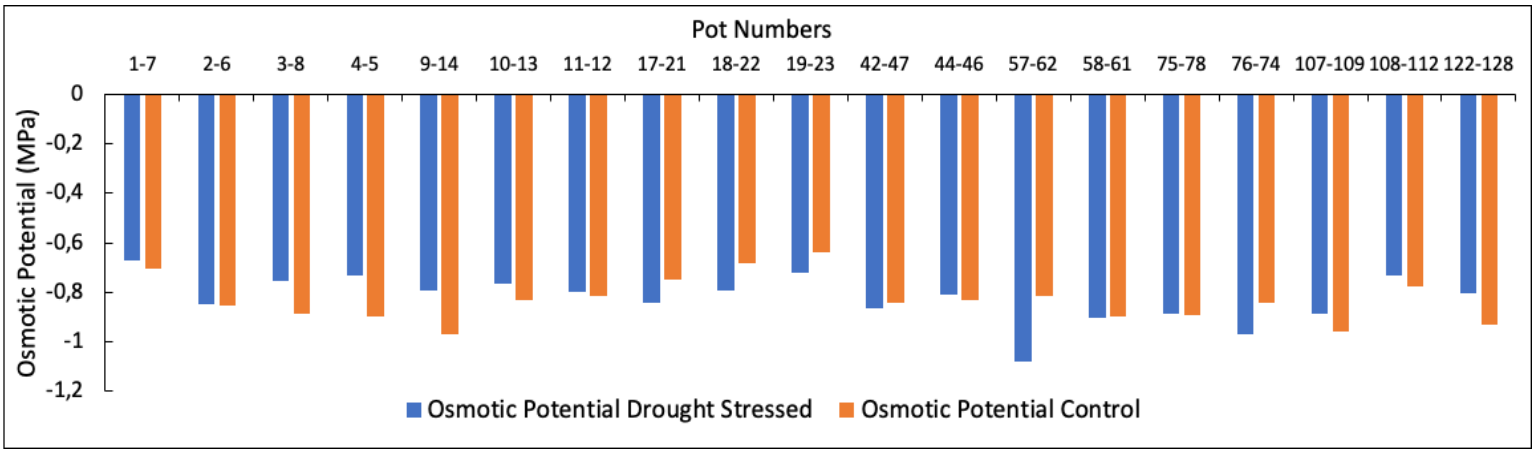


Figure 9: Osmotic potential was determined for 19 drought stressed plants as well as one control from the same genotype for each.

Osmotic potential and the number of days it took for the plants to wilt also only correlated weakly with the correlation coefficient being 0,1515 (fig. 10). Similarly, osmotic potential and water content at the point of wilting had a weak correlation (R^2= 0,1108) (fig. 11).

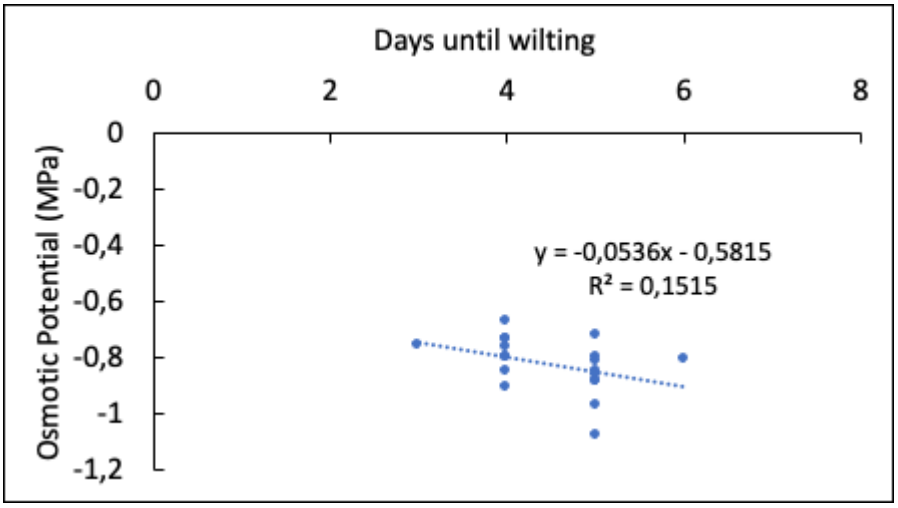


Figure 10: Osmotic potential did not correlate strongly with wilting time

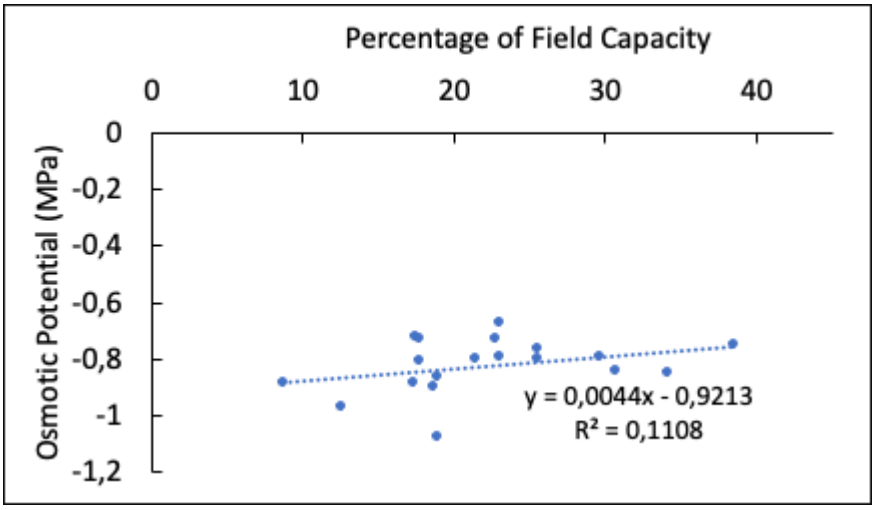


Figure 11: Osmotic potential did not correlate strongly with water content at the wilting point.

Discussion

Drought experiment

The time it takes for a plant to wilt can depend on two things. Firstly, the rate at which it loses water, which is dependent on the total leaf area and transpiration The more leaves a plant has with open stomata the faster it loses water. In response to drought stress the concentration of the hormone ABA increases and promotes closing of the stoma [13]. Secondly, the degree to which plants tolerate a low water content in the soil also affects wilting. Therefore, a plant with good drought tolerance would ideally have little loss of water through transpiration and the ability to withstand a low water content in the soil. As depicted in figures 4 and 5, bean type 20 took the longest to wilt but also contained the most amount of water in the soil at the time of wilting. This indicates that this type loses little water through transpiration but does not tolerate a low water content in the soil compared to other varieties. It would have been interesting to connect this to a high osmotic potential value but unfortunately there was not enough time to test all types.

Types 26, 34 and 36 are examples of better performing types. They all have above average wilting time (fig.4), withstand relatively low soil water contents (fig. 5), have below average leaf areas (fig.6) and they all lose relatively little water per day (fig. 7).

Types 2 and 3 wilted quickly but had an above average water content left at wilting, which could indicate that they require a high amount of soil water content to survive. On the other hand, a possible source of error regarding types 2 and 3, is that they were all situated on the same table, potentially with more light. They wilted faster than expected and randomization at this point should have been performed but was missed.

As figure 5 shows, there is quite a bit of variation regarding the water content at the point of wilting between the different bean types. This could be linked to the total leaf area as a bigger leaf area means a higher amount of transpiration. To breed a drought tolerant bean, it would ideally have a small total leaf area, low conductance, and withstand a low water potential in the soil. When comparing e.g., type 36 in graphs 5 and 6 we can see that it had a below average water content when it wilted (fig. 5) and a very small total leaf area (fig. 6), which is a desirable combination. Types 26 and 34 show similar trends.

Chlorophyll fluorescence was not a good response variable for drought stress in this study. It was expected that the values would decrease as the plants experienced stress and closed their stoma, but they did not. A reason for this could be the relatively short amount of time the plants experienced drought stress. It is possible that there would have been a clearer change in F_v/F_m if the wilted leaves had been subjected to more light. Interestingly, studies performed by Saglam et al and Santos et al showed similar results with different common bean cultivars [19, 20].

Osmotic potential and conductivity (fig. 8) did not show a strong correlation for the drought stressed group nor the control group. The stomatal conductance (g_s) is affected by the osmotic potential in the guard cells but not by other leaf cells. Therefore, it was expected that conductance did not correlate with our general measurements of osmotic potential in a leaf. An ideal genotype would have high water use efficiency, the ability to close the stomata during drought stress and have a low osmotic potential to keep a positive turgor pressure. The fact that osmotic potential and conductivity did not strongly correlate indicates that a combination like this is possible.

Osmolarity measurements

Due to the limited time for this project, the osmolarity measurements were only performed on a limited amount of leaves. The ones that wilted and went through the rehydration period first were the ones that were prioritized. Though there are some pairs in figure 9 that show a slight osmotic adjustment, some also show the opposite where the control plant has a lower osmotic potential value. There were also no major differences between the genotypes we were able to compare. Similar to the chlorophyll fluorescence measurements, osmotic adjustment is a process that takes time, and it is likely that the drought stress period for the beans in this project was not long enough to see significant changes [21]. Other studies have similarly reported that the common bean did not show an osmotic adjustment [22] while others have shown that certain cultivars were osmotically adjusted [23] but in that study the drought stress period was significantly longer.

Furthermore, osmolarity measurements require precision and speed which led to a few sources of error. It was very challenging to take the leaf out of the plastic bag, wipe away the trichomes, cut out the disk, put it in the envelope and into the liquid nitrogen in only 20 seconds. Often the disc stuck to the cork-borer or the tweezers which led to a few seconds delay. Similarly, after the aluminum envelope was taken out of the liquid nitrogen it was difficult to get the disk out and get it into the osmometer within the recommended time frame.

Osmolarity did not show a strong negative correlation with wilting time (fig. 10), but the small trend indicates that the osmolarity values might have differed in the different types from the beginning. It is possible though that if an osmotic adjustment would have taken place that lower osmotic potential values (higher concentration of solutes) would correlate stronger with longer wilting time and vice versa. Similarly, osmotic potential did not strongly correlate with the percentage of field capacity that was left at the point of wilting but there is a small positive trend. This means that with decreased osmotic potential (higher concentration of solutes) the beans seem to wilt with a lower percentage of the field capacity which shows that a lowered osmotic potential can lead to better water retention. Drawing conclusions from this data should only be done cautiously though due to the low number of measurements.

The results of this study show that there are genotypic differences in drought tolerance among the bean varieties. In the future it would be interesting to link more traits of the common bean to drought tolerance and gain further knowledge about how to optimize drought tolerance. It would also be interesting to investigate more landraces and how they would respond to a longer drought period. There is still much more research that needs to be done regarding drought tolerance in the common bean, but the results of this study indicate that an informed selection of which bean varieties are planted has the potential to reduce the food insecurity in e.g., Rwanda.

Conclusion

In conclusion, osmolarity showed no strong correlations with stomatal conductance, wilting time nor water content at wilting. Therefore, osmolarity was not a good predictor of drought tolerance in this study. It was found that types 26, 34 and 36 were amongst the best performing in regard to drought tolerance.

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